Universidad de La Laguna

Escuela de Doctorado y Estudios de Posgrado

TÍTULO DE LA TESIS DOCTORAL

Desarrollo y caracterización de formulaciones farmacéuticas adaptadas para pediatría: medicamentos antituberculosos

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FECHA DE LECTURA

12/06/20

UNIVERSIDAD DE LA LAGUNA

DESARROLLO Y CARACTERIZACIÓN DE FORMULACIONES FARMACÉUTICAS ADAPTADAS PARA PEDIATRÍA: MEDICAMENTOS ANTITUBERCULOSOS

JAVIER SUÁREZ GONZÁLEZ

SAN CRISTOBAL DE LA LAGUNA, 2020

Desarrollo y caracterización de formulaciones farmacéuticas adaptadas para pediatría: medicamentos antituberculosos.

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San Cristóbal de La Laguna, 2020

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DOCTORADO
CIENCIAS DE LA SALUD
Verificado ANECA, Resol. 19-3-2014

Ana Mª Santoveña Estévez, profesora titular del área de Farmacia y Tecnología Farmacéutica del Departamento de Ingeniería Química y Tecnología Farmacéutica,

Certifica:

1. Que D. **Javier Suárez González**, Graduado en Farmacia ha realizado este trabajo de Tesis Doctoral que lleva por título **Desarrollo y caracterización de formulaciones farmacéuticas adaptadas para pediatría: medicamentos antituberculosos.**
2. Que una vez revisada la memoria de Tesis Doctoral y, previo informe favorable de la Comisión Académica del Programa de Doctorado en Ciencias de la Salud, expresa su conformidad para que sea defendida ante el Tribunal correspondiente designado al efecto, ya que la misma reúne los requisitos necesarios para optar al título de Doctor.

Para que conste y surta los efectos oportunos, firma el presente certificado en San Cristóbal de La Laguna, a 4 de marzo de 2020.

Dra. Ana Mª Santoveña Estévez
Directora

“La ciencia no es perfecta, con frecuencia se utiliza mal, no es más que una herramienta, pero es la mejor herramienta que tenemos, se corrige a sí misma, está siempre evolucionando y se puede aplicar a todo. Con esta herramienta conquistamos lo imposible”

Carl Sagan

Durante el Grado en Farmacia constantemente rondaba la incertidumbre de qué hacer en un futuro. Entre las opciones siempre estaba la realización de una tesis doctoral. No obstante, cuando se aludía a la realización de un doctorado en cualquier área, mi respuesta solía ser la misma: “no me veo capaz y nunca lo haré, me gustaría trabajar en una farmacia”.

El doctorado me ha enseñado muchas cosas, y no solo en el ámbito científico, pero una que nunca olvidaré es: “nunca digas nunca”.

Por ello, quisiera agradecer a *José B. Fariña* y a mi directora de tesis *Ana Mª Santoveña* por introducirme en este mundo de la investigación y hacerme descubrir nuevas posibilidades. Además, quisiera agradecerles su apoyo incondicional y todos y cada uno de los consejos que me han dado desde el primer momento.

Agradecer también a *Mabel Soriano*, no solo por los consejos, sino también por haber sido un gran apoyo y por su buen humor en todo momento. Estoy seguro de que, sin ella, nada hubiera sido igual.

A *Esther Sánchez* y a *Magdalena Echezarreta* por tener siempre una palabra amable, un consejo y una solución para casi todo.

Asimismo, muchas gracias a todos los profesores que conforman el Área de Tecnología Farmacéutica: *Matías Llabrés, Carmen Évora, Obdulia Munguía, Alexis Oliva, Araceli Delgado, Edgar Pérez* y a*, Patricia Díaz* por su disponibilidad siempre que lo he necesitado.

A *Margarita de la Rosa*, *Diego Molina* y a *Laura Barroso* por tener siempre una solución para cualquier problema.

A *Ángel Concheiro* y a *Carmen Álvarez,* de la Universidad de Santiago de Compostela, por permitirme pasar una pequeña temporada en su laboratorio y hacer posible la realización de una estancia en el extranjero.

A *Peter Kleinebudde* y a *Jörg Breitkreutz*, de la Heinrich-Heine-Universität Düsseldorf (Alemania), por su amabilidad y dejarme realizar una estancia de investigación en su laboratorio. Al igual que a *Ard Lura*, *Olga Kiefer*, *Philipp Kiefer*, *Bastian Hahn*, *Dina Kottke*, *Hannah Lou* e *Ili As* por introducirme en el ambiente alemán. Asimismo, a Ana Gago por ser mi familia española en la ciudad.

A mis compañeros de laboratorio, *Ceci, Iris, Diego, Martín, Marina, Zuleima* y *Lorena,* por todo su apoyo, opiniones, buenos momentos, charlas y compañía. Especial mención se merecen *Amor*, *Eduardo* y *María,* que comienzan su andadura en el laboratorio y a los que les deseo todo lo mejor.

A *Patri* por ser la compañera de al lado con la que siempre he podido compartir experiencias, opiniones, frustraciones, risas y tableros de juegos.

A mis amigas *Gemma* y *M. Jesús* por aguantarme durante todos estos años sin abandonarme.

A *Míriam*, por estar siempre ahí y por ser la compañera de viaje que cualquiera desearía.

A mis padres, *Gaspar* y *Nereida*, por que, aunque no saben exactamente qué es lo que hago, siempre me han arropado, me han enseñado a no rendirme y nunca me han puesto límites.

A *Blanca* por ser ella.

Al servicio de farmacia del Hospital Universitario Nuestra Señora de la Candelaria, Farmacia Feria, Instituto Tecnológico del Medicamento Individualizado (ITMI) y la Comisión Nacional de Unificación de Criterios en Formulación Pediátrica por su apoyo.

Finalmente, agradezco a la Universidad de La Laguna, Fundación CajaCanarias (BIO27), CajaSiete y al Gobierno de Canarias (ProID2017010094) por los sucesivos contratos y financiación que han posibilitado realizar esta tesis doctoral.

A todos, muchas gracias.

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1. ABSTRACT

Appropriate medicines are need for pediatric requirements. However, the development of these medicines is challenging due to the composition of this group (from birth to 13-17 years old) which implies differences regarding absorption, distribution, metabolism and excretion of drugs. In addition, these medicines should be easy to swallow, have an adequate dose volume/content, correct composition for pediatrics and good organoleptic properties, etc.

For this reason, individualized medicines for pediatrics are a useful alternative if there is no correct market dosage for this segment of population. However, a thorough investigation must be carried out in order to ensure quality testing, content uniformity, physical (homogeneity after shaking, in the case of liquid dosage forms), chemical, and microbiological stability.

The quality of individualized medicines, used daily in pharmacy services (hospitals and community pharmacies), have been tested. These have been selected based on their demand, problems that arose during elaboration and the excipients used. In all cases, a new standard operative procedure, which ensures the quality of the individualized medicine, was developed for each active pharmaceutical ingredient studied and disseminated in the different services.

The data obtained from the validation of the formulations previously mentioned, was used to elaborate a high-demanding strategy to ensure the highest quality standards of oral liquid individualized medicines for pediatric use. This included a first part related to the validation of the analytical method used, and a second part focused

on the critical quality attributes that should be checked, some of them recommended by Pharmacopoeia.

Following the European initiative to enhance the research in the pediatric field, the World Health Organization, the National Institutes of Health and the European Medicine Agency have been publishing lists about pediatric needs regularly (dosing, efficacy, pharmacokinetics, safety and formulation development).

Active pharmaceutical ingredients used for the treatment of Tuberculosis were selected to develop medicines appropriate for children, based on the list previously mentioned. They were selected because of the number of children that died every year due to the lack of an appropriate dosage form. The treatment it is based on at least three drugs: Isoniazid, Pyrazinamide and Rifampicin, which are used as first-line treatment for treating tuberculosis in the intensive and in the continuation phase.

This last phase is based on the combination of Isoniazid and Rifampicin. This last active pharmaceutical ingredient is the only drug which has an appropriate dosage form for pediatrics. For this reason, a formulation of isoniazid of 50 mg/ml was validated using the strategy previously mentioned; the continuation phase is covered.

The treatment for the intensive phase is based on the combination of Isoniazid, Pyrazinamide and Rifampicin and, in resistant cases, Ethambutol. Therefore, for this phase a fixed-dose combination of the three active pharmaceutical ingredients is required. However, this is difficult to achieve in a liquid dosage form, which it is more

accepted by the pediatric patient, due to the chemical incompatibility between Isoniazid and Rifampicin. Subsequently, a solid dosage form is preferred.

Hence, a high-quality, child-friendly, fixed dose combination water-dispersible tablet of Isoniazid, Rifampicin and Pyrazinamide was developed according to international recommendations in terms of excipients for pediatrics and meeting pharmacopoeia requirements. In addition, its production has been optimized to be elaborated at maximum press speed while maintaining quality requirements.

Stability studies according to the International Conference of Harmonization were carried out at accelerated, long-term and low relative humidity conditions. Moreover, the influence of light on the stability of the tablets was also tested. Tablets offred more stability when the humidity was low. No property, chemical or physical, suffered any significant change under this condition after three months storage. According to the results, a packaging which protects the tablets from moisture is needed.

2. OVERVIEW

The appropriate medicine is essential in order to provide optimal treatment for each specific disease. However, it is difficult to develop medicines for certain population groups as is the case for pediatrics.

The development of child-friendly medicine is very challenging due to several facts. In the first place, pediatrics is a heterogeneous group which extends a period that goes from birth to 13-17 years old. It is divided into various stages following pharmacokinetic changes during growth ((absorption, disposition, metabolism and excretion (ADME)): preterm neonates (<36 weeks of gestation), full-term neonates or newborn (0–27 days), infants and toddlers (28 days–23 months), children (2–11 years), adolescents (12–17 years) [1]. There are differences between authors regarding this division due to the fact that growth is a continuous process which is difficult to classify. However, the European Medicine Agency (EMA) and the International Conference for Harmonization (ICH) have published documents about this topic in order to unify criteria [2 – 5].

Drug absorption could be altered during the first days of life due to changes in some factors like gastric pH. It is neutral at birth and decreases during the following 24–48 h till reaching pH 3 with a further rise to neutral after 72 h; or 10 days at neutral followed by a decrease in acidic values comparable to adults at 2 years [6]. Also, gastric emptying and peristalsis are irregular during the early months of life. Another factor that must be taken into account is the diet of the neonate, which is based on milk and may alter the absorption of certain drugs [4].

Weight increases rapidly from birth to adolescence meaning changes in disposition. In addition, the production of proteins during the first months of life is reduced which means an increase of unbound drug and a higher possibility of secondary effects [4].

Clearance normalized per kilogram of body weight is depressed in newborn, and even more so in the case of premature infants, reaching its maximum value at 6 months (being more than twice in the 60-year-old patient). Then it starts to decrease till reaching adult values. The ability of neonates to metabolize drugs and excipients matures within the first 6 to 12 months of life which means lower metabolization but also smaller first-pass loss in this period of life [4, 7]. Due to the low metabolic function certain excipients could be toxic to newborns and children and therefore should be avoided: ethanol, propylene glycol, benzyl alcohol…

There is a good correlation between body weight/surface area and the maturity of the renal and liver function. Therefore, these physical characteristics need to be used to adjust the dose [4].

As may be seen, there are a great number of factors that must be taken into account when a medicine aimed at pediatrics requires development.

For this reason, pharmaceutical industries do not usually focus on the development of pediatric medicines. They need to take into account the particularities of this population group according topharmacokinetics and metabolism maturity. These difficulties

result in extensive and deep pre-marketing medicine research, reducing their profit [8].

During pharmaceutical development, one of the most important characteristic is the classification of the active pharmaceutical ingredient (API) in the biopharmaceutical classification system (BCS). This classification organized each API based on their solubility and permeability, which could be limiting steps in oral absorption [9]. In addition, this system is useful in order to obtain waivers for *in vivo* bioequivalence studies of APIs from classes I and III formulated in immediate release dosage forms [10].

However, this classification is based on adults' gastrointestinal track and it is unknow how this could be translated for pediatrics. As mentioned above, there are plenty of changes from birth to adolescence which could produce variation in ADME. For example, it is specified that high soluble APIs are those whose highest dose strength is soluble in 250 ml of aqueous medium with a pH from 1 to 7.5. This 250 ml is related to the initial gastric volume for adults but in pediatrics this would depend on age and body size. With this in mind, a pediatric BCS (PBCS) was developed in order to improve this classification and to be adapted to pediatric population [11 – 13].

The most commonly used administration method in pediatrics is orally [2, 13]. There are plenty of dosage forms that could be used in the administration of medicines orally. However, there is not a single one that could be used for all age groups acceptability, which is one of the most important items during pharmaceutical development for the patient, parents or caregivers.

Acceptability is defined as an overall ability of the patient and caregiver to use a medicinal product as intended. It will be influenced by 4 principal factors [2, 14, 15]:

1. Suitability of the dosage form for a specific age group:

The most used dosage forms in pediatrics are liquids and solids [16].

In liquid dosage forms the taste, mouthfeel (texture) and after taste are important parameters to ensure a good palatability of the medicine. This means that sweeteners and taste-masked excipients are needed. In addition, as the API is formulated in solution, a solvent will be needed (water, syrup…), thus the formulation will need preservatives and might have limited stability. Moreover, if the API is formulated as a suspension it might be not homogenously distributed in the formulation and more excipients are needed (surfactant and suspending agents). Other disadvantages are the volume of dose, the need of a dosing device and, in some cases, the requirement of refrigeration. However, this kind of formulation allows dosage flexibility and it is one of the most accepted forms in pediatrics.

Solid dosage forms are generally preferred over liquid dosage forms; minitablets (MT) in comparation with syrups [17]. They are more stable and easier to transport than the liquid dosage forms. However, with these formulations there are a high risk of choking, chewing and aspiration.

In order to avoid these problems some dosages forms are preferred depending on the age group.

- **Liquid dosage forms.** They are normally accepted from full term birth and from pre-term neonates if they are able to accept enteral feeding [2].

- **Solid dosage forms.**

  Powders and granules: They can be given from birth, if they can be administered as a liquid preparation, and from 6 months, if they need to be administered with semi-solid food. The risk of aspiration, choking and chewing will depend on the age group and the characteristics of the formulation (size, shape and quantity).

  Tablets and Capsules: The size and shape of the tablet will influence the ability of the children to swallow it. Dispersible tablets are a good option in order to mix the advantages of solid preparations with liquid ones as they are formulated to be dispersed in water before being taken, so they can be used as soon as the patient is able to drink water.

  Orodispersible films: They are defined as polymers with one or multiple layers which are placed in mouth in order to be dispersed before being swallowed. Hence, they may be used from 6 months onwards.

2. The dosing devices used for liquid formulations.

The Food and Drug Administration (FDA) of USA and EMA in their guidelines on the development of oral liquid formulations recommend the incorporation of a dosing device in the packaging. In 2016 an evaluation about the presence of these dosing devices was carried out in USA with a total of 382 medicines. It was concluded that 12.8% of medicines were packaged without any device and calibrated droppers and oral syringes with adapters were the most used [18].

In table 2-1 is shown a summary of the main advantages and disadvantages of the most commonly used devices for oral liquid preparations [19].

Device	Advantages	Disadvantages
Spoon	- Easy to use	- Variability. - Graduation and shape can affect accuracy. - Splitting of dose during dosing.
Cup	- Larger volumes can be used (> 5 ml)	- Graduation can affect accuracy and be confused. - Residual volume after dosing. - Splitting of dose during dosing.
Droppers	- Useful to administer small volumes (drops)	- Drop size is affected by physical properties of the liquid. - Accuracy and consistency of dose depends of the dropper and how it is handled.
Syringe for oral use	- Accuracy - Flexibility of dose - Various size available - Spillage of dose improbable	- Cost - Measurement could be confused

Table 2-1. Advantages and disadvantages of the most commonly used devices for oral liquid preparations.

3. Sized or dose volume to be administered

As already mentioned, for solid dosage forms the size and shape of the tablet will be fundamental for children. In 2016 Punam Mistry and Hannah Batchelor published a review about the acceptability of oral pediatric medicines paying special attention to the size and shape of tablets. According to this review it is accepted that children up to 6 months are able to swallow tablets smaller than 2 mm. These tablets are named MT and orodispersible minitablets (ODMT), if they are formulated to be dispersed in the mouth, and they can be used in pre-term age [20, 21]. Even more, up to 10 MT can be given with semi-solid food (yoghurt, jelly etc.) to children from 2 years old [22].

From 2 to 5 years old, children are able to swallow tablets under 4 mm and could get used to tablets up to 7 – 8 mm. Children over 5 years of age are able to swallow tablets measuring 7 mm and from 12 years onwards they are able to take tablets with bigger than 10 mm [23 - 27]. The most accepted shape is oblong [24].

For oral liquid formulation the EMA published a guideline to establish the maximum volume of dose according to patient's age, 5 and 10 ml maximum for children of 5 and 10 years old respectively [28].

4. Palatability

It is defined as the organoleptic properties which makes a patient respond positively to a product and includes smell, taste, aftertaste, dose volume or size and texture [2, 13]. Palatability depends mainly on the API used and is important for the development process. Most of the APIs have a bitter taste that needs to be masked using excipients and selecting the correct dosage form (tablets, capsules…). This is studied in several ways: *in vitro* (analytical techniques and electronic tongue) and *in vivo* (animal models). Although these methods have proved successful, the acceptability of the formulation has to be confirmed by a clinical trial [25].

The excipients used during pharmaceutical development are very useful to improve the characteristics of the API in order to be formulated better. For example, the use of sweeteners to improve the palatability of oral liquid formulations. However, although they are usually defined as inert substances, their use needs to be justified through a risk-based assessment taking into account: age group, frequency of dosing and duration of treatment [13].

One of the biggest problems is that children are more sensitive than adults due to their physiological and metabolic development [4, 7]. This explains why excipients like benzyl alcohol, azo-dyes, propylene glycol, ethanol and propyl paraben produce adverse reactions in children but not in adults [13]. Due to the possibility of an adverse reaction, the EMA and the World Health Organization (WHO) recommends using the lowest number and quantity of

excipients in each formulation [2, 13, 29]. The choice of the correct excipient will depend on [13]:

- Safety profile of the excipient for children of the target age groups
- Route of administration
- Single and daily dose of the excipient
- Duration of the treatment
- Acceptability for the intended pediatric population
- Potential alternatives
- Regulatory status in the intended market

The table 2-2 shows the relation of some excipients and the adverse reactions in pediatrics according to literature [13, 30-33].

Function	Excipients	Comments
Colouring agents	Sunset yellow Tartrazine Indigo Carmine	Hypersensitivity Some colorants were banned in some countries
Preservatives	Sulphites Benzyl alcohol	Bronchospasm Cardiogenic shock Neurological effect
Sweeting agents	Aspartame Sorbitol Saccharin	Laxative effect Special conditions: diabetes, intolerance, phenylketonure
Solubility Enhancers	Ethanol Propylene glycol Glycerol (>40%)	Neurological effect Pharmacological interactions Gastrointestinal reactions
Surfactant	Polysorbates	Serious toxicity in children administered with Vit E
Diluents	Lactose Starch	Intolerance Alergic reactions

Table 2-2. Examples of excipients where side effects have been detected.

In order to avoid side effects, the EMA published a roadmap concerning points to consider in the evaluation of the safety profile of excipients in pediatric formulations for a specific target age group [2].

Moreover, the EMA recommends some information sources to assess the safety profile of the excipient used. This list, apart from the respective guidelines and reflection papers published by WHO, EMA and FDA, is an information source for the quantitative composition of currently authorized products for children, the food legislation or the European Food Safety Scientific Opinions. Other sources could be the Safety and Toxicity of Excipients for Pediatrics (STEP) Database created as part of the European Pediatric Formulation Initiative (EuPFI) [29, 34].

As it can be seen, there are aspects that must be taken into account in order to develop a formulation for pediatric use when no commercial drug product is available. In order to facilitate the development and accessibility of commercial medicinal products for use in pediatrics, the European Parliament approved the Regulation (EC) No 1901/2006 in 2006. A system of obligations, rewards and incentives was set to ensure that medicinal products are appropriately authorized for use in pediatrics and have been investigated according to ethical research of high quality. [35].

In addition, a pediatric committee was created which is responsible for the scientific assessment and agreement of pediatric investigation plans (PIPs). The aim of such a plan is to ensure that the development of a medicinal product, which could be used with children, becomes an important part integrated into the development program for adults [36].

A new type of marketing authorization was created called the Pediatric Use Marketing Authorization (PUMA). This was created specifically for medicinal products developed exclusively for pediatrics. One of the most important incentives is 10 years of market exclusivity that could be extended to twelve if the requirement for data on use in the pediatric population is fully met [35].

The European Commission published a ten-year report about the implementation of the pediatric regulation in 2017. This shows an increase in medicine for children but shows little progress in the development of medicines that only affect children [36].

The EMA and the European Commission published an action plan to increase the development of medicines for pediatrics focused on: identifying pediatric medical needs, strengthening of cooperation of decision makers, ensuring timely completion of PIPs, improving the handling of PIP applications and increasing transparency in pediatric medicines [37].

It could take 10 years till a medicinal product for pediatrics reaches the market and becomes available for children. In the meantime, there are alternatives such as:

- Off-label use. This term means the use of a drug for one disease, dose, age, weight, route of administration that is not included on its technical sheet. Safety and efficacy data are needed in order to approve such off-label use [38]. A study by Yackey et al revealed that 1 out of every 4 children were associated with off-label prescriptions [39].

- Compassionate use. Under certain conditions products in development can be made available to groups of patients with life-threatening, long-lasting or seriously debilitating illnesses which cannot be treated with any currently authorized medicines. This medicine must be undergoing clinical trials or have entered the marketing-authorization process [40].

- Use of non-authorized medicines in the country. Use of medicines non-authorized in one country which are used in another one prior to authorization by sanitary authorities [41].

- Pharmaceutical compounding. Preparation of individualized medicines using APIs and excipients for treating a specific disease which has no medicine available on the market.

This last alternative is one of the fastest options when there is no available medicine for pediatrics This technique could be useful in the following situations: the medicine is not available on the market, if the excipients might produce allergies or if modified doses are required… [41, 42]. Modified doses could be useful but they would be understood as adapted medicines, instead of individualized medicines; as a commercially available formulation for adults is adjusted in order to be suitable for pediatrics. An example of this would be to dilute the content of a capsule using simple syrup.

The compounding process is mentioned in the European Union's regulatory framework for medical product as a "pharmacy preparation" but these do not apply to this kind of preparation [43, 44].

In 2016 the European Committee of Ministers updated the resolution published in 2011 about quality and safety assurance requirements for medicinal products prepared in pharmacies for the special needs of patients. This resolution is a guideline regarding the preparation process, labelling, compliance with pharmacopoeia requirements, authorization for pharmacies, communication and information for patients and distribution of pharmacy preparations [45, 46].

Every year the "WHO Expert Committee on Specifications for Pharmaceutical Preparations" is published. In the annex 6 of this document there is a summary regarding the most important items in pharmaceutical compounding: identification test, assays, monograph development etc. [47]

In order to elaborate an individualized medicine a Standard Operative Procedure (SOP) must be followed and have been previously validated in order to ensure safety and efficacy. However, the information that endorses this SOP should be available (formulation design, homogeneity and stability studies).

During the development of an individualized medicine for children there are some items that must be taken into account. First of all, they must be adapted to specific needs and physiological condition: age, size, ability to swallow etc. In addition, the use of the API is preferable to commercial formulations as when the API it is used a low number of excipients (and in smaller quantities) can be added. Moreover, complex excipients could change the distribution of the API when it is formulated at a low proportion [48]. When a commercial formulation is used to elaborate a new one, the first formulation is usually adapted (adapted medicines). In Spain this procedure is controlled by the government and only hospital pharmacies services are authorized to use this procedure [49].

The excipients are very useful in pharmaceutical technology but some of them are toxic for pediatrics when they are administered in high doses or during a long period of time, as mentioned previously [2, 29].

Although there are sources where SOPs for the elaboration of individualized medicines for pediatrics can be obtained, some of this SOPs do not follow the recommendation of international agencies for pediatrics: the volume of doses recommended, 5 and 10 ml for 5 and 10 years old respectively [28].

In addition, most of them lack important information like the conditions used for the stability studies or if the homogeneity have been checked in the case of suspensions. However, this information and tests are not needed according to the European Pharmacopoeia (Ph. Eur.). The only test recommended is mass uniformity test and checking pH and organoleptic properties.

Following the European initiative to enhance research in the pediatric field, WHO, the National Institutes of Health (NIH) and EMA have been publishing lists about pediatric needs (dosing, efficacy, pharmacokinetics, safety and formulations of APIs).

Based on these lists, this thesis focuses on the development of child-friendly formulations of different APIs [7] that where selected based on the list of pediatric needs previously mentioned and by request from Spanish community pharmacies and hospitals. The validation of these formulations was carried out following the recommended test from international compendial. However, more tests were done in order to evaluate the quality of such formulations in terms of homogeneity, physical and chemical stability etc...

Other APIs listed as necessary for children are; isoniazid (INH), pyrazinamide (PZA) and rifampicin (RFP) to treat Tuberculosis (TB) disease. First line treatment of TB in pediatrics is based on the combination of these 3 APIs. However, there is no appropriate child-friendly formulation available which combines these APTs in a single formulation. Moreover, INH and PZA do not have an appropriate formulation as a single drug [2, 50 – 53].

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3. AIM

1. Select APIs listed for child-friendly formulations required by international organization and pharmacy services of hospitals and community pharmacies, as well as those which, during formulation, showed problems related to elaboration, doses administration, stability etc.

2. Analyze those problematic liquid oral individualized medicines and design, develop and optimize a new one if necessary.

3. Design, develop and optimize the elaboration of the most useful individualized medicines of INH, PZA and RFP for oral treatment of tuberculosis in pediatrics.

4. Ensure the quality and stability (chemical, physical and microbiological) of individualized medicines following the recommendation of international pharmacopoeias.

5. Elaborate a strategy to ensure the highest quality standards of individualized medicines for pediatric use.

6. Disseminate the validated SOPs for each API studied.

4. DEVELOPMENT AND CHARACTERIZATION OF FORMULATIONS FOR PEDIATRICS

4.1. Introduction.

Individualized medicines for pediatrics are a useful alternative when there is not marketed a correct dosage form for this population (easy to swallow, adequate volume and content, correct composition, good organoleptic properties, etc.). In the development of individualized medicines for pediatric use, a good validation strategy is essential to ensure a quality target product profile (QTTP) during the stability period of the formulation.

As it was seen previously, liquid formulations are one of the most accepted dosage form for pediatrics as its administration is less traumatic than others such as tablets or capsules. In addition, they can be easily elaborated by pharmacy services following a good SOP.

In individualized liquid medicines for oral use one of the most important critical quality attributes (CQA) is content uniformity; each dose must have the amount of API label, declared or prescribed. This is especially important in the case of suspensions, where the API might not be homogenously distributed in the multidose formulation. According to the Ph. Eur. these individualized liquid medicines stored in multidose containers are only required to comply with the test for uniformity of mass delivered from multidose containers [1]. However, this test evaluates the uniformity of the weight of each dose, assumes a homogeneous distribution of the API in the whole formulation [2]. In 2017 Schlatter et al. published an article where the uniformity of doses of a suspension was not tested following Ph. Eur. recommendations. Therefore, content uniformity cannot be

ensured, it is unknow if each dose have the label amount of API or if the decrease of API content it is related to a loss of homogeneity [3].

Physical stability is another important attribute, even more in the case of suspensions. During storage there must be no crystallization, sedimentation, or other physical processes that could affect the stability (quality) of the disperse system. In addition, if some of these processes take place during storage it must be assured that, after shaking the multidose container, a homogenous formulation and the declared doses are obtained. Although this is important, it becomes even more so when the formulations are stored at 5 ºC as they are not usually tested [3 - 7]. A flocculated system it is preferred as it provide a rapid sedimentation rate being the system resuspended easily.

Chemical stability is another point that must be checked as a CQA in individualized medicines for pediatrics. The medium used to dissolve API, the excipients, the pH or the storage conditions are elements which can affect stability and so must be tested.

Last but not least, microbiological stability is as significant as the other points. The growth of microorganisms produces changes in the pH that could produce a reduction in the stability of the molecule, apart from producing health problems in the patient. However, there are some articles without microbial stability testing, even when there were no preservatives in the composition [3 – 7]. Moreover, in some cases the pH variation was not followed during the stability test, despite the importance of this parameter as an indicator of the API stability or microbial contamination [8, 9].

In addition, a stability test must be carried out following the ICH guidelines. Some National Formularies recommend checking their organoleptic properties and pH [10, 11]. All these tests would cover two of the five CQAs proposed.

As pointed out, most authors validate each formulation taking into account different CQAs. That is the reason why a high-demanding strategy is needed in order to unify criteria and ensure the quality of liquid dosage forms.

The physicochemical properties of the API (solubility, BCS class etc.) will have an enormous influence on these CQA. Solubility and dose will affect the content uniformity of the formulation because they determine if a certain formulation will be a solution or a suspension. The antimicrobial activity of the API or if it is photosensitive will also have an influence in the stability of the formulation.

The excipients used in individualized medicine will influence the quality, so it is another important point during its design. As it was mentioned before, a very common way to prepare these formulations is to manipulate or compound the authorized and marketed tablets. This means that the API and excipients used will be transferred from them. These might not be suitable for children [12], then may not be soluble in water or even interfere with the distribution of the API [2]. In this sense, it is suggested to start from the API (as raw material) and add the least number of excipients and in the lowest proportion to produce their effect in the formulation [13, 14].

Certainly, the elaboration of a good SOP is essential to ensure the quality of the final formulation. The SOP must include information about packaging. The Guideline regarding packaging for pharmaceutical products published by WHO in 2002 must be followed [15]. Quality packaging selection is essential to ensure protection of the API in the formulation (light, moisture, oxygen). In addition, the compatibility of the packaging with the API is very important: interaction between container and substances, release of chemicals from packaging materials, absorption or adsorption of substance by packaging materials, degradation of packaging materials etc.

The aim of this chapter is to develop a high-demanding strategy, based on the QTTP and CQAs, to be used during the validation process of multidose oral liquid dosage forms, solutions or suspensions, and thus ensure its quality in order to be use in pediatrics.

4.2. Effectiveness of antimicrobial preservation in liquid formulations

The lack or scarcity of a marketed API at pediatric doses is the main reason to formulate or at worst reformulate adult dosage forms of APIs. This practice gives rise to off-label or unlicensed use [16]. On formulating these APIs in liquid form for oral administration it is common practice to use syrup as vehicle. In general, there are three types of syrups: simple syrup containing only sucrose and purified water, flavoring syrup containing flavored substances, and medicinal syrups to which other therapeutic compounds have been added [17].

These syrups are used to enhance palatability or to increase viscosity of the formulation and also to create adequate osmotic pressure to inhibit microbial contamination (>60% w/w) [18]. At present the availability of marketed suspending vehicles such as Ora (Perrigo, Dublin, Ireland) products simplifies the compounding of oral syrups. These products have a high cost and complex composition owing to their constituent suspending agents, preservatives, sweeteners, and buffers [19]. This makes them non-ideal candidates for pediatric formulation, where the number and quantity of excipients in a formulation should be the minimum necessary to support product quality [20]. Therefore, at least in pediatrics, it is necessary to use simple vehicles that can be prepared by using traditional compounding techniques [21].

In pediatrics, simple syrup is often diluted with carriers such as water, or other excipients for different purposes, such as to reduce the amount of sucrose administered or adjust the viscosity of the final

preparation [22]. The influence of dilution on the efficacy of antimicrobial preservation is insufficiently studied in the literature and should be further studied, since it is a habitual practice in the preparation of non-sterile multidose formulations [23].

In some countries the Pharmacopoeias or the National Formularies of Pharmaceutical Compounding include a monography of water used in compounding named *aqua conservans* (conserved water), that is prepared with a hydroxybenzoates (parabens) solution [24-26]. The propylene glycol habitually used as drug solvent in this hydroxybenzoate solution is toxic at least for infants, since it can accumulate and cause lactic acidosis, central nervous system depression, coma, hypoglycemia, seizures, and hemolysis [27]. As EMA indicates however, owing to insufficient clinical evidence of comparable effects in humans, continued use of parabens as antimicrobial preservatives appears to be justified, particularly in the case of pediatric formulations [28]. Nevertheless, the concentration should be at the lowest feasible level (0.015% and 0.01% for methylparaben and propylparaben, respectively) [28]. For these reasons, quantities added to the water must be just sufficient for the desired preservative effect, without being in excess.

This study assesses the effectiveness of antimicrobial conservation in vehicles prepared with diluted simple syrup. For this dilution, purified water and *aqua conservans* were used, and in the latter, the proportion of propylene glycol and parabens used as excipients was eliminated or reduced, respectively.

- Materials and Method

Vehicles. We studied five vehicles (Table 4-1). Vehicle Nº1 was simple syrup and the others were dilutions of it with other solutions in the proportion 50:50 (v/v). The simple syrup was prepared as a solution of 64% sucrose w/w in purified water, which is roughly equivalent to 85% w/v (29).

A concentrated solution of hydroxybenzoates was prepared by mixing 8 g of methyl p-hydroxybenzoate (methylparaben) and 2 g of propyl p-hydroxybenzoate (propylparaben) with propylene glycol qs 100 g [26]. The *aqua conservans* was made up of 1 g of this solution in purified water qs 100 ml [25].

In vehicle Nº4, the hydroxy benzoates were diluted at the same concentration as in the aqua conservans, without the addition of propylene glycol.

Finally, vehicle Nº5 was prepared by using the lowest proportion of each hydroxybenzoate recommended for oral solutions and suspensions (0.015% methyl p-hydroxybenzoate and 0.01% propyl p-hydroxybenzoate), without the addition of propylene glycol.

Nº.	Vehicle	Sucrose (%)	Methylparaben (%)	Propylparaben (%)	Propylene Glycol (%)
1	Simple syrup	64	-	-	-
2	Simple syrup: purified water (50:50 v/v)	32	-	-	-
3	Simple syrup: aqua conservans (50:50 v/v)	32	0.04	0.01	0.45
4	Simple syrup: aqua conservans without propylene glycol (50:50 v/v)	32	0.04	0.01	-
5	Simple syrup: aqua conservans diluted without propylene glycol (50:50 v/v)	32	0.008	0.005	.

Table 4-1. Composition of each vehicle studied % (w/w)

All excipients were prepared from pharmacopoeia-grade raw materials, provided by Acofarma (Madrid, Spain). The culture media was prepared from commercial media (Scharlab, Barcelona, Spain).

Efficacy of Antimicrobial Preservation. In this study the Ph. Eur. test of efficacy of antimicrobial preservation was applied, which is stricter than the antimicrobial effectiveness test of United States Pharmacopoeia (USP) [30,31]. This test must show that the formulation provides adequate protection against adverse effects due to contamination or microbial growth during storage and use. The test consists of deliberate contamination of the preparation in the final container with a prescribed inoculum of suitable microorganisms, conservation of the inoculated preparation at a set temperature, withdrawing samples from the container at specified time intervals, and counting microorganisms in the samples taken. The preservative

properties of the preparation are adequate if a significant decrease or no increase in the number of microorganisms occurs in the inoculated preparation after the prescribed times and temperatures. The acceptance criteria vary depending on the type of preparation (parenteral, ophthalmic, intra- uterine, intramammary, otic, nasal, cutaneous, inhaled, oral, or rectal) and the degree of protection required.

The microorganisms used were *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Candida albicans*, *Aspergillus brasiliensis*, *Escherichia coli* (for oral administration vehicles), and *Zygosaccharomyces rouxii* (for oral administration vehicles with high sugar content), all of which were obtained from the Spanish Type Culture Collection in Valencia, Spain.

The inoculum for each microorganism was prepared on the surface of soybean casein digest agar for bacteria, or Sabouraud dextrose agar without the addition of antibiotics for fungi. Incubation is at 30°C to 35°C for 18 to 24 hours in the case of bacteria, at 20°C to 25°C for 48 hours with *C. albicans* and *Z. rouxii*, and at 20°C to 25 °C for one week or until good sporulation is achieved with *A. brasiliensis*. A minimum subculturing was sometimes required. A sterile liquid suspension containing sodium chloride 9 g/l was used to collect bacterial, *C. albicans*, and *Z. rouxii* cultures. For *A. brasiliensis* the sterile liquid suspension must also contain 0.5 g/l polysorbate 80. Enough liquid should be used to reduce the suspended microbial count to about 10^8 organisms per milliliter. Subsequently, appropriate samples were removed from each suspension (0.1 ml from serial

dilutions) and the number of colony-forming units per milliliter was determined in each suspension by plate count. This value was used to determine the inoculum and the reference values used in the assay. The suspensions should be used immediately.

The test then began with inoculation of the studied vehicles. Inoculate is done in the vehicles' final package with each of the test microorganisms in order to obtain an inoculum of 10^5 to 10^6 organisms per milliliter or per gram of preparation. The volume of the inoculum suspension did not exceed 1% of the volume of the product. It was thoroughly mixed to ensure homogeneous distribution. The inoculated product was maintained at 20°C to 25°C and protected from light. At time zero and at suitable intervals, depending on the type of product (e.g., oral preparations at 14 and 28 days), a sample of each package (1 ml) is removed and the number of viable microorganisms determined by plate count. Results are the average of duplicate readings.

- Results and Discussion

To meet the Ph. Eur. criteria for oral preparations, the antimicrobial activity of a preservative must result in a 3 log reduction in the inoculated dose of bacteria after 14 days and no increase as compared to the previous reading at 28 days. For fungi, these criteria change to 1 log reduction at 14 days and no increase as compared to the previous reading at 28 days.

Table 4-2 shows the results of the assays. As can be seen, simple syrup (vehicle Nº1) was microbiologically stable during 15 days (a

log reduction above 3 was detected at 14 days for every microorganism). *A. brasiliensis* growth was detected at 28 days (a negative log reduction at 28 days). When this vehicle was diluted with purified water (vehicle Nº2), at each sampled time, the quality criteria were not met (log reduction was less than the desired one or an increase in growth was detected).

Vehicle Nº3, simple syrup diluted with *aqua conservans*, met the quality criteria (log reduction was above 3 or 1 for bacteria or fungi, respectively, at 14 days and no increase at 28 days) owing to its containing propylene glycol and parabens in a final proportion of 0.04% and 0.01% w/w for methylparaben and propylparaben, respectively.

Vehicle Nº4, *aqua conservans* without propylene glycol, met the microbiological quality criteria too. When vehicle Nº5 was assayed, in which *aqua conservans* was prepared without propylene glycol and lower proportions of parabens, bacterial growth (*E coli*) was detected at 14 days, and vehicle Nº5 did not meet quality criteria.

Organism		*S. aureus*		*P. aeruginosa*		*C. albicans*		*A. brasiliensis*		*E. coli*		*Z rouxii*	
Incubation time (days)		14	28	14	28	14	28	14	28	14	28	14	28
Sample Nº	1	6.0	NI	5.0	NI	4.0	NI	3.0	-1.0	5.0	NI	4.0	NI
	2	0.2	-0.3	-1.1	0.3	-1.3	0.2	-0.6	-0.6	0.7	CM	-2.1	CM
	3	6.0	NI	5.0	NI	2.0	2.0	2.0	NI	5.0	NI	4.0	NI
	4	6.0	NI	5.0	NI	4.0	NI	2.0	NI	5.0	NI	4.0	NI
	5	6.0	NI	6.0	NI	4.0	NI	1.0	1.0	-1.0	2.0	4.0	NI

Table 4-2. Test results for the five vehicles studied*†‡. CM, growth of countless microorganisms; NI, no increase in number of viable microorganisms as compared to the previous reading. *Positive numbers: log reduction in the inoculated dose of microorganisms after different incubation times (14 and 28 days). † Negative numbers: increase in microorganism count after initial time of incubation. ‡ To meet the European Pharmacopoeia criteria for oral preparations, the antimicrobial activity of the vehicles studied must result in a 3 log reduction (equal or more) in the inoculated dose of bacteria after 14 days and NI as compared to the previous reading at 28 days. For fungi, these criteria change to 1 log reduction (equal or more) at 14 days and NI as compared to the previous reading at 28 days.

Simple syrup (vehicle Nº1) was microbiologically stable during its declared validity period of 15 days even though fungal growth was later detected [29]. The quality criteria for vehicle Nº2 were not met since it lacked preservatives, except sucrose itself in too low a proportion (32% w/w) to prevent microbial contamination (<60% w/w).

The proportions of parabens in vehicle Nº3 (above or equal to the minimum proportion recommended, 0.015% and 0.01% for methylparaben and propylparaben, respectively [28]) prevent the growth of the inoculated microorganism. With the aim of eliminating the propylene glycol and reducing the parabens' proportions to the minimum, vehicles Nº4 and Nº5 were assayed.

Vehicle Nº4, in which *aqua conservans* is prepared without propylene glycol to prevent its toxic action, met the microbiological quality criteria. Therefore, the elimination of propylene glycol is possible when the *aqua conservans* is prepared with parabens above the lowest feasible level, in the final proportion (equal to vehicle 3).

On the other hand, when vehicle Nº5 was assayed, prepared with *aqua conservans* without propylene glycol and the lower quantity of parabens recommended as preservatives for oral liquid formulations, bacterial growth was detected at 14 days. This minimum quantity of parabens in vehicle Nº5 is evidently insufficient to protect it from microbial contamination; indeed the final proportions (0.008% and 0.005% for methylparaben and propylparaben, respectively) in the diluted syrup were less than those recommended.

For all the above, when APIs are formulated with simple syrup diluted 50:50 v/v with *aqua conservans* (vehicle Nº3) or with *aqua conservans* without propylene glycol as solvent (vehicle Nº4), these vehicles are able to inhibit microbial growth with and without the use of propylene glycol, respectively, which is not recommended for pediatrics owing to its toxic effects. Thus, less toxic formulations can be used to administer APIs to this vulnerable group. But, if it is used,

simple syrup diluted 50:50 v/v with purified water (vehicle Nº2) or dilute aqua conservans prepared with the lowest feasible proportion of hydroxybenzoates recommended to exert a preservative effect without propylene glycol (vehicle Nº5) is not able to inhibit growth if these vehicles become contaminated by microorganisms before or during API administration.

Taking into account that this study is made as the Pharmacopoeia test indicates, and the vehicles are tested without the API incorporation and are incubated not during the real administration of the doses, the microorganism contamination can be greater if adequate hygienic measures are not considered.

In conclusion, when diluted simple syrup is necessary to use in the formulation of an API in pediatrics, it is possible to use water with parabens at adequate proportions without being in excess to assure the effectiveness of its antimicrobial preservation and without propylene glycol used as solvent. Thus, if during the oral administration of the formulation, it is contaminated, the preservatives will be able to inhibit their growth.

Adapted for thesis from paper:

Effectiveness of Antimicrobial Preservation of Extemporaneous Diluted Simple Syrup Vehicles for Pediatrics. Santoveña A, Suárez-González J, Martin V, González-Martín C, Soriano M, Fariña JB.

Published in: J Pediatr Pharmacol Ther. 2018 Sep-Oct; 23(5): 405–409.
doi: 10.5863/1551-6776-23.5.405

4.3. Quality assurance: materials and methods.

All APIs and excipients were pharmacopoeia grade and provided by Acofarma (Madrid, Spain). All other reagents were analytical grade (Sigma-Aldrich, Madrid Spain).

This global strategy has been developed based on the data obtained during the validation of the following APIs: Flecainide, Dexamethasone, Furosemide and Acetazolamide. Data from APIs previously studied by the investigation group were also used (Ursodeoxycholic acid and Carbamazepine) [32 - 35]. These have different physicochemical characteristics (solubility, permeability, dose, particle size…) so a general strategy can be reached.

The selections of the APIs took into account several aspects. One of these aspects, as it was said previously, was the most commonly demanded APIs in hospitals and community pharmacies to treat ailments in pediatrics which did not have a commercial formulation. Another aspect was the problems that arose during the elaboration of several formulations using previously proposed SOPs. And finally, the excipients used, they must be adequate for pediatric use and have sufficient data regarding safety.

- <u>UPLC Validation</u>

Each API was analyzed using an Ultra Performance Liquid Chromatography (UPLC) in an Acquity® H-Class System (Waters Corporation, Milford, MA). The data acquisition software was Astra 6.0.1 (Chromatographic Manager, Water Corporation). All chemical

and reagents were HPLC grade, samples and solvents were filtered with 0.2 μm pore-size filters (Milipore, Bilerica, MA) before using.

In order to validate the analytical method several standards solutions were prepared for each API and analyzed several times. The variance analysis (ANOVA) of the linear regression confirmed the linearity of the method, through rejection of the null hypothesis of deviation from linearity for a significance level of 5% ($\alpha = 0.05$). Also, precision (six-fold analysis of the same sample), accuracy (expressed as a percentage recovery by the assay of a known added amount of drug) and detection and quantification limits were also tested (based on the standard deviation of the response and slope).

To calibrate the UPLC system and monitor its performance, a solution sample of each API was analyzed daily as standard. The chromatographic conditions and column performance were checked, especially the tailing factor and column efficiency. When necessary, corrective action was taken.

- Stability marker

One standard solution was storage under stress conditions (for example high temperature) to test the ability of the method to follow the degradation of the API, and check if it was able to detect and quantify any degradation product.

- Extraction

To determine the capability of the method to extract the API of every formulation studied, several samples of 5 ml were prepared. API and excipients of each formulation were weighed in the right amount for this volume. Then, they were analyzed in UPLC. The average extraction yield should be around 100%.

- Quality control
  - Organoleptic properties and pH

Quality control of each formulation was done starting with the evaluation of organoleptic properties and pH control. The pH of each formulation was measured in a Crison GLP 21 pHMeter previously calibrated. A 10 ml sample was taken from each solution during the assays. The measurement was done in duplicate at 25 ºC.

- Mass Uniformity test

Ph. Eur. only recommends as quality test for oral liquid formulations to meet the uniformity of mass of delivered doses from multidose containers (Ph.Eur. - 2.9.27): “Weigh individually 20 doses taken at random from one or more containers with the measuring device provided and determine the individual and average masses. Not more than 2 of the individual masses deviate from the average mass by more than 10 per cent and none deviates by more than 20 per cent.” [1]. The individual masses were weighted in a Sartorius analytic balance.

- Content Uniformity test:

Content uniformity test should be done in the case of suspensions. For this task a test to check this content uniformity have been elaborated. In this test, 20 individual doses have to be taken at random from one or more containers with the measuring device provided, an oral syringe [36], and its content must be analyzed. The individuals and average content have to be calculated. Not more than 2 of the individual contents deviate from the average mass by more than 10 per cent and none deviates by more than 20 per cent. [37]

As an alternative of previous test, uniformity of dosage units (Ph. Eur. - 2.9.40) can be used. Analyze the content of 10 dosage units and calculate the acceptance value (AV) of each formulation. AV should be less than 15 for these 10 dosages. Samples were analyzed by UPLC.

- Physical Stability Test

In case of suspensions, due to the relevance of the viscosity to avoid API sedimentation and ensure dose uniformity, this was tested in a programmable viscometer LVDV-II Brookfield (Essex, England) at 25 ºC. A spindle SC4-18 was used to determine viscosities between 1.5 and 30,000 mPa·s, with 8 ml as sample volume. The data were processed with the Wingather ® 32 program Brookfield (Essex, England). All the measurements were made by triplicate with a torque between 10 and 90%.

Due to the possibility of sedimentation of the API (mostly in the case of suspensions), stirring before removing the dose is very important.

Each formulation was placed in a suitable container (100 ml graduated cylinder) and before and after stirring (10 times inverted 180º) doses (5 ml) were taken from Z1 (top of the formulation) and Z3 (bottom of the formulation) and their API content were measured by UPLC calculating the maximum difference between zones (Dmax).

- Chemical Stability Test

The formulations were placed at different temperatures and storage conditions following the ICH guidelines (Q1A and Q1B) [38, 39]. At 5 ± 0.1 ºC/10 ± 5 Relative Humidity (RH) (Fridge-stove P-selecta Welidow type, Spain), 25 ± 0.5 ºC/45 ± 5 RH (Memmert ULP500, Spain), 40 ± 0.1 ºC/20 ± 5 RH (Heraeus UT6060, Spain). Samples of 5 ml were taken from each batch at predetermined intervals and measured by UPLC.

- Microbiological Stability Test

Ph. Eur. recommends testing the microbiological stability of the formulation if there is not any preservative in its composition or if the API does not have an antimicrobial activity. This test is performed according to the Microbiological Examination of Non-Sterile products: Total Viable Aerobic Count (Ph.Eur. – 2.9.16) [40]. The microbial count was considered to be the average number of colonies forming units (cfu) found in the appropriate medium.

Oral liquid formulations meet requirement if the aerobic microbial count is less than 10^2 cfu/ml, the total combined yeast/mold count are less than 10^1 cfu/ml, and the absence of *Escherichia coli* is confirmed.

4.4. Flecainide.

Flecainide acetate (FA) is an antiarrhythmic used in the prevention and treatment of several diseases related with the heart (tachycardia, fibrillations and arrhythmias) [41]. It is an API which has narrow therapeutic index, that is the reason why, dose must be stablished by a cardiologist with experience [42]. Doses for pediatrics are usually 1 - 6 mg/kg/day distributed in three times. It can be given with food, but dairy products may inhibit its absorption [43].

FA has a solubility in water at 37 ºC of 48.4 mg/ml [44], belongs to class I in the BCS due to its high solubility and permeability [45]. In Spain there is not commercially available a formulation for pediatric use, it is marketed as 100 mg tablets and a 10 mg/ml intravenous injectable solution, whereas in USA it is only commercialized as tablets [46, 47].

There are some SOPs about the development of formulation for pediatric use in the literature but in most of the cases they use complex vehicles or commercial tablets in order to prepare an adapted formulation of FA for children. In these cases, despite of being a highly water-soluble API, the formulations were prepared as suspensions which may be due to the incorporation of non-soluble excipients [48, 49]. In Spain there are some SOP which use the API in order to prepare the formulation with a shelf life of 30 days at 2 - 8 ºC [50]. However, during storage it have been notice the appearance of a precipitate due to the insolubility of FA to those temperatures.

In order to solve this problem four different formulations, of 20 mg/ml, were made and tested according to pharmacopoeia tests, table 4-3. F1 and F2 are an adaptation of the SOPs that are often used at pharmacies and hospitals.

	Ingredient	
Formulation	**Wetting agent (v/v)**	**Diluent-swetener (qs 100 ml)**
F1	-	Simple Syrup
F2	Glycerol 10%	Simple Syrup
F3	-	Water:Simple syrup (50:50, v/v)
F4	Glycerol 25%	Water:Simple syrup (25:75, v/v)

Table 4-3. Composition of the different formulations. qs: amount which is enough to complete 100 ml.

SOPs for the elaboration of the different formulations are placed in the annex.

- UPLC Validation.

FA was analyzed by UPLC using an adapted HPLC method [51]. The mobile phase was a 0.05 M 1-pentanesulfonate monohydrate/acetic acid (99:1, v/v) and acetonitrile in a 45:55 mixture (v/v), at a flow rate of 0.3 ml/min. The UV detection was at 298 nm. In order to validate the analytical method, five FA standard solutions were prepared at concentrations of 1 - 11 µg/ml. ANOVA of the linear regression confirmed the linearity of the method, through rejection of the null hypothesis of deviation from linearity for a significance level of 5% ($\alpha = 0.05$). The results are shown in tablet 4-4.

Area = 2,843+5,396*C; R = 0.995 (n = 30)	
RSD	2,418
Precision, (%) (n=6)	0.21
Accuracy, % (n=9)	99.1
Detection limit (µg/ml)	1.21
Quantification limit (µg/ml)	3.68

Table 4-4. Data from the UPLC method validation.

A robustness test was performed to examine the effect of operational parameters on the analysis results. The flow rate (0.3 ± 0.01 ml/min), injection volume (5 ± 0.3 µL), temperature (40.0 ± 1.5 ºC), mobile phase composition (45.0 ± 1/55 ± 0.5), and column performance over time were determined in order to confirm the method.

The estimated area for the standard concentration was 56803 µV·sec with an RSD of 5.9%. FA is detected at 0.5 min of elution time, at 0.35 min there is a noise signal, but it is also detected when the mobile phase is injected. In the figure 4.1 is shown a chromatogram of a pure FA and a sample extracted from F3. The average extraction yield of FA from the formulations was 111.2 ± 10.2%.

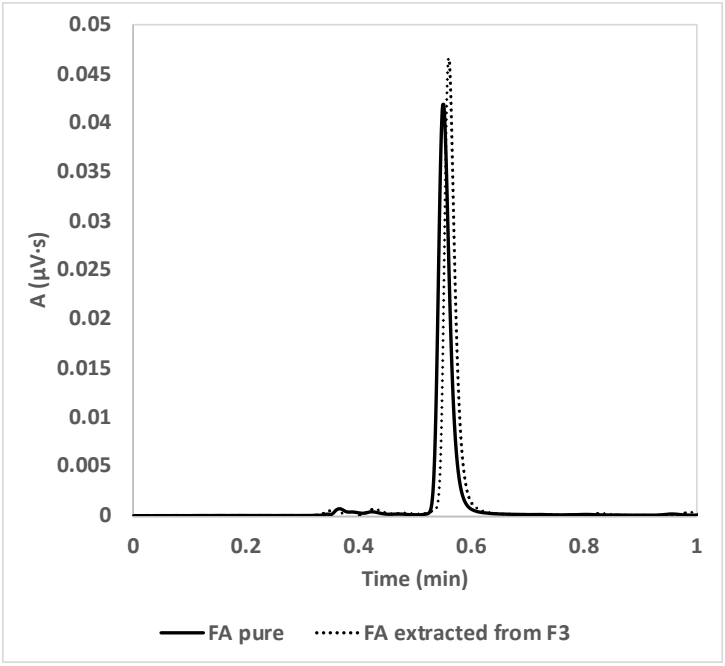

Figure 4-1. FA pure pattern chromatographic peak (continuous line), FA after extracted form F3 formulation (dashed line), and a sample of mobile phase (dotted line)

- Quality Control

F3 is a transparent solution. However, the other formulations present different degrees of opalescence due to the incomplete dissolution of FA. This is a very important fact because FA could act as nuclei for particle growth, promoting sedimentation in certain storage conditions.

All FA formulations studied met the Ph.Eur. test requirements for mass uniformity of dose from multi-dose containers, no individual masses deviated from the average by more than 10% (Table 4-5 and 4-6)

	Dw (g)															
	F1														F1-CP	
	25 ºC		5 ºC				25 ºC				40 ºC				25 ºC	
	0 days		30 days		60 days		30 days		60 days		30 days		60 days		0 days	
1	6.51		6.52		6.62		6.62		6.59		6.63		6.58		6.52	
2	6.52		6.59		6.59		6.63		6.52		6.67		6.57		6.53	
3	6.51		6.57		6.66		6.61		6.56		6.64		6.56		6.57	
4	6.55		6.54		6.64		6.58		6.55		6.63		6.60		6.57	
5	6.43		6.56		6.63		6.58		6.59		6.61		6.59		6.57	
6	6.53		6.56		6.63		6.60		6.54		6.61		6.61		6.61	
7	6.48		6.53		6.64		6.68		6.58		6.64		6.58		6.54	
8	6.54		6.54		6.60		6.53		6.53		6.62		6.61		6.57	
9	6.51		6.56		6.61		6.60		6.56		6.62		6.59		6.54	
10	6.53		6.56		6.59		6.59		6.57		6.70		6.63		6.56	
11	6.54		6.56		6.58		6.65		6.60		6.61		6.57		6.5	
12	6.54		6.56		6.70		6.59		6.64		6.67		6.60		6.58	
13	6.51		6.58		6.63		6.61		6.58		6.61		6.57		6.5	
14	6.51		6.57		6.69		6.69		6.58		6.66		6.57		6.59	
15	6.50		6.56		6.61		6.66		6.55		6.59		6.57		6.56	
16	6.53		6.55		6.65		6.66		6.56		6.61		6.59		6.53	
17	6.55		6.57		6.56		6.66		6.48		6.57		6.59		6.58	
18	6.56		6.54		6.64		6.64		6.61		6.62		6.54		6.57	
19	6.55		6.57		6.56		6.59		6.54		6.62		6.53		6.55	
20	6.55		6.58		6.63		6.60		6.54		6.60		6.55		6.54	
A	6.52		6.59		6.62		6.62		6.56		6.63		6.58		6.55	
	LL	UL	LL	UL	LL	UL	LL	UL	LL	UL	LL	UL	LL	UL	LL	UL
10%	5.87	7.17	5.93	7.25	5.96	7.28	5.96	7.28	5.91	7.22	5.96	7.29	5.91	7.22	5.90	7.21
20%	5.22	7.83	5.27	7.91	5.30	7.95	5.29	7.94	5.25	7.88	5.30	7.95	5.25	7.88	5.24	7.86

Table 4-5. Mass uniformity test for F1 formulation prepared in our laboratory and in a compounding pharmacy (CP) in all conditions studied. ***Dw: dose weight; A: average; LL: lower limit; UL: upper limit.***

	Dw (g)													
	F2						F3		F3-H		F3-CP		F4	
	25 ºC		5 ºC				25 ºC		25 ºC		25 ºC		25 ºC	
	0 days		30 days		60 days		0 days		0 days		0 days		0 days	
1	6.65		6.55		6.57		5.84		5.85		5.81		6.11	
2	6.59		6.56		6.60		5.84		5.83		5.82		6.17	
3	6.62		6.56		6.59		5.83		5.85		5.84		6.12	
4	6.58		6.53		6.60		5.87		5.84		5.84		6.21	
5	6.61		6.55		6.58		5.84		5.83		5.81		6.11	
6	6.64		6.54		6.58		5.85		5.81		5.84		6.24	
7	6.60		6.53		6.56		5.87		5.89		5.85		6.14	
8	6.57		6.54		6.62		5.84		5.86		5.84		6.16	
9	6.59		6.56		6.55		5.83		5.84		5.81		6.10	
10	6.58		6.52		6.56		5.86		5.83		5.82		6.14	
11	6.64		6.61		6.53		5.81		5.83		5.81		6.13	
12	6.61		6.60		6.61		5.84		5.85		5.83		6.09	
13	6.58		6.57		6.51		5.85		5.84		5.85		6.20	
14	6.58		6.60		6.60		5.83		5.85		5.83		6.10	
15	6.68		6.59		6.55		5.85		5.85		5.81		6.11	
16	6.68		6.54		6.60		5.87		5.85		5.83		6.12	
17	6.65		6.58		6.61		5.85		5.87		5.82		6.20	
18	6.64		6.60		6.63		5.88		5.86		5.83		6.22	
19	6.64		6.55		6.54		5.82		5.87		5.83		6.12	
20	6.64		6.52		6.62		5.86		5.84		5.82		6.21	
A	6.62		6.56		6.58		5.85		5.85		5.81		6.15	
	LL	UL	LL	UL	LL	UL	LL	UL	LL	UL	LL	UL	LL	UL
10%	5.96	7.28	5.90	7.21	5.92	7.24	5.26	6.43	5.26	6.43	5.24	6.04	5.54	6.77
20%	5.29	7.94	5.25	7.87	5.26	7.90	4.67	7.02	4.68	7.02	4.66	6.99	4.92	7.38

Table 4-6. Mass uniformity test for F2 (after 30 and 60 days at 5 ºC), F3 (prepared at our laboratory, at a hospital (H) and at a compounding pharmacy (CP) and F4 formulation. ***Dw: dose weight; A: average; LL: lower limit; UL: upper limit.***

Content uniformity is not met for F1, F2 and F4 at time zero (see tables 4-7 and 4-8). However, F1 fulfills criteria for 25 and 40 ºC after 60 days of storage, this should be related to the increase of the solubilization of FA at these temperatures. This does not occur at 5ºC. F3 pass uniformity content test with an average content uniformity near to 100 % of DV for hospital pharmaceutical service and compounding pharmacist.

	DV (%)															
	F1														F1-CP	
	25 ºC		5 ºC				25 ºC				40 ºC				25 ºC	
	0 days		30 days		60 days		30 days		60 days		30 days		60 days		0 days	
1	116		103		85.1		99.9		97.4		107.2		111		75.2	
2	104		95.6		86.0		83.3		106		75.0		107		78.4	
3	110		98.1		90.3		96.9		102		98.2		108		79.7	
4	110		102		89.3		98.0		110		104.3		107		81.8	
5	95.8		76.1		93.1		104.0		102		114.9		109		75.5	
6	58.2		99.2		93.6		68.2		110		106.3		114		82.5	
7	99.5		109		103		94.5		103		109.4		109		82.3	
8	109		114		97.3		104.1		110		104.6		114		83	
9	73.8		92.9		98.0		83.6		111		108.6		108		86.1	
10	63.8		107		104		70.9		107		100.1		113		83.5	
11	100		102		109		93.6		112		111.2		111		93.4	
12	101		96.5		77.7		96.6		107		108.1		113		86.9	
13	115		104		97.1		99.1		106		105.7		107		85.2	
14	112		87.9		81.2		101.6		108		107.9		113		95.4	
15	109		108		102		103.4		106		116.0		107		87.7	
16	107		113		85.6		106.4		110		118.3		105		100	
17	107		100		115		95.7		110		110.7		108		101	
18	109		110		95.6		106.0		114		110.0		115		104	
19	69.2		97.0		106		91.9		108		108.7		108		92.9	
20	106		111		110		103.9		114		105.8		115		102	
A	98.7		101		96.0		95.2		108		107		110		87.8	
	LL	UL	LL	UL	LL	UL	LL	UL	LL	UL	LL	UL	LL	UL	LL	UL
10%	88.8	109	91.2	112	86.4	106	85.7	105	96.9	118	95.9	117	98.9	121	79.0	96.6
20%	79.0	118	81.1	122	76.8	115	76.1	114	86.1	129	85.2	128	87.9	132	70.3	105

Table 4-7. Compounded preparation test (USP) and content uniformity of unidose preparations test (Ph.Eur.) for F1 formulation prepared in our laboratory and in a compounding pharmacy (CP) in all conditions studied. ***DV (%): percentage of declared value; A: average; LL: lower limit; UL: upper limit; Soft shading values: outside the limits of 10%; Strong shading values: outside the limits of 20%.***

	DV (%)													
	F2						F3		F3-H		F3-CP		F4	
	25 ºC		5 ºC				25 ºC		25 ºC		25 ºC		25 ºC	
	0 days		30 days		60 days		0 days		0 days		0 days		0 days	
1	105		109		99.5		111.0		101.0		103.0		101.0	
2	118		97.0		102.2		115.0		108.0		104.0		93.1	
3	131		98.9		97.5		106.0		93.2		99.7		98.9	
4	124		102.5		109.4		109.0		98.6		103.0		101.0	
5	131		99.2		108.3		113.0		99.1		103.0		90.9	
6	130		86.1		111.8		120.0		99.9		104.0		94.1	
7	123		101.8		104.6		108.0		106.0		101.0		91.5	
8	116		108.4		117.1		107.0		103.0		99.1		104.0	
9	115		72.5		106.7		111.0		101.0		98.1		99.7	
10	113		100.1		114.9		100.0		97.7		99.3		97.8	
11	119		92.9		111.1		107.0		103.0		101.0		96.8	
12	112		99.0		118.2		110.0		101.0		102.0		96.3	
13	124		98.7		119.4		109.0		116.0		106.0		92.4	
14	124		102.5		103.1		119.0		102.0		98.5		101.0	
15	106		105.8		121.4		121.0		102.0		100.0		101.0	
16	130		106.4		109.1		108.0		103.0		103.0		108.0	
17	113		103.5		119.6		112.0		98.5		102.0		98.1	
18	109		99.1		104.3		111.0		99.3		104.0		106.0	
19	107		90.4		116.1		113.0		101.0		98.4		75.6	
20	98.6		94.7		114.4		111.0		97.0		101.0		103.0	
A	117		98.4		110		111		102		101		97.50	
	LL	UL	LL	UL	LL	UL	LL	UL	LL	UL	LL	UL	LL	UL
10%	106	129	88.6	108	99.3	121	99.8	122	91.3	112	91.3	111	87.8	107
20%	94	141	78.8	118	88.3	132	88.7	133	81.2	122	81.2	121	78.0	117

Table 4-8. Compounded preparation test (USP) and content uniformity of unidose preparations test (Ph.Eur.) for F1 formulation prepared in our laboratory and in a compounding pharmacy (CP) in all conditions studied. ***DV (%): percentage of declared value; A: average; LL: lower limit; UL: upper limit; Soft shading values: outside the limits of 10%; Strong shading values: outside the limits of 20%.***

AV for F1 was 54.8 and for F2, 5.35; 13.0 and 10.85 for F3 and F4 respectively.

All formulations that were developed were easily redispersible. However, due to the variability of dose uniformity in F1 and F2 a sedimentation test was done to check the influence of sedimentation on FA distribution. The FA content were determined in samples taken at different heights from formulations storage in 100 ml graduated cylinder at different temperatures. As it can be seen in table 4-9 after stirring the formulation the percentage dose recovery increased in most cases.

Formulation	**F1**			**F2**	**F1**			**F2**
Temperature (ºC)	5	25	40	5	5	25	40	5
Time (days)	30				60			
Z1 (DV, %)	82.2	74.7	104.5	108.6	72.2	75.9	94.9	80
Z3 (DV, %)	99.4	89.7	24.9	80.3	64.8	74.7	87.4	74.3
Dmax	17.2	15	79.7	28.2	7.4	1.2	7.5	5.7

Table 4-9. FA percentage of declared value (DV (%)) determined for F1 and F2 at Z1 and Z3 heights and evolution of the maximum observed difference (Dmax) at different storage condition.

Although F3 could be the best formulation according to content uniformity, chemical stability test was also done for the other formulations, table 4-10. As it was seen from the other assays, when the formulations are storage at 25 and 40 ºC the concentration raises due to the improvement of the solubility. F3 and F4 kept their initial FA concentrations between 90 and 100% for 60 days of storage at 5 and 40 ºC. F1 and F2 shows differences exceeding 10% when they are stored at 5 ºC. The was no evidence of chromatographic degradation.

F1	DV (% average ± SD)				
	Time				
Temperature	**0 days**	**15 days**	**30 days**	**45 days**	**60 days**
5 ºC	98.7 ± 17.6	88.8 ± 25.7	101 ± 9.2	77.1 ± 0.1	96 ± 10.1
25 ºC	98.7 ± 17.6	95.6 ± 0.8	95.2 ± 10.9	102 ± 6.9	108 ± 4.3
40 ºC	98.7 ± 17.6	97.9 ± 9.4	107 ± 8.9	107 ± 7.5	110 ± 3.1
F2	**DV (% average ± SD)**				
	Time				
Temperature	**0 days**	**15 days**	**30 days**	**45 days**	**60 days**
5 ºC	117 ± 9.6	89.1 ± 2.6	98.4 ± 8.4	107 ± 15.5	110 ± 7.2
25 ºC	117 ± 9.6	ND	ND	ND	ND
40 ºC	117 ± 9.6	ND	ND	ND	ND
F3	**DV (% average ± SD)**				
	Time				
Temperature	**0 days**	**15 days**	**30 days**	**45 days**	**60 days**
5 ºC	110 ± 5.0	100 ± 3.4	103 ± 8.2	102 ± 2.8	103 ± 8.2
25 ºC	ND	ND	ND	ND	ND
40 ºC	ND	ND	ND	ND	ND
F4	**DV (% average ± SD)**				
	Time				
Temperature	**0 days**	**15 days**	**30 days**	**45 days**	**60 days**
5 ºC	97.5 ± 7.0	102 ± 1.4	106 ± 2.3	ND	107 ± 2.0
25 ºC	ND	ND	ND	ND	ND
40 ºC	97.5 ± 7.0	108 ± 0.1	110 ± 4.6	ND	96.5 ± 0.1

Table 4-10. Evolution of FA percentage of declared value (DV (%)) in time for F1, F2, F3 and F4 formulations at different storage conditions. ***ND: not determined; SD: standard deviation. n=3.***

F3 complied with the Ph.Eur. specifications for microbial examination of non-sterile products during a 30 day period.

According to the results obtained, F3 is the one selected for this API. Fulfills all criteria of the Pharmacopoeias, including microbiological tests, and can be used till 30 days after it is made.

Adapted for thesis from paper:

Development of a novel physico-chemically and microbiologically stable oral solution of flecainide for pediatrics. Santoveña A, Charola I, Suárez-González J, Teigell-Pérez N, García-van Nood S, Soriano M, Fariña JB.

Published in Pharm Dev Technol. 2018 Dec;23(10):978-985.
doi: 10.1080/10837450.2016.1238484

Factor Trend (JCR 2016): 1.860
Quartile in Pharmacology & Pharmacy: Q3 (175/257)

4.5. Dexamethasone.

Dexamethasone (Dexa) is a glucocorticoid with high anti-inflamatory activity and a slight mineralocorticoid effect used in pediatrics in treatment of croup and bronchopulmonary dysplasia at doses of 0.5 - 0.6 mg/kg/day [52, 53].

Dexa is a drug which can be classified in the BCS classification system in the Class I/III [45, 54].

It is commercialized as tablets, otic and eye drops and also as injectable solutions. In addition, it can be found formulations made of Dexamethasone Sodium Phosphate (Dexa-P) for parenteral, ophthalmic and otic routes [46, 47]. There is just one liquid formulation for oral administration, available in USA, but it is an elixir so it is not suitable for pediatric use. Then, in Spain it is not commercially available a dosage form appropriate for pediatrics.

Although there are SOPs regarding to the development of formulations of Dexa for the pediatric use, they not only use the commercialized formulations but they even use Dexa-P as API when it is not authorized for oral administration, just in France [55 - 58]. It has been studied the bioequivalence between Dexa and Dexa-P when they are administered orally, and it has been concluded that they are not bioequivalent [59]. Two different formulations of 1 mg/ml were tested, they are shown in table 4-11.

	Ingredient	
Formulation	**Citric/citrate buffer (v/v)**	**Diluent-sweetener (qs 100 ml)**
F1	-	Simple Syrup
F2	20	Simple Syrup

Table 4-11. Composition of the different formulations. ***qs: amount which is enough to complete 100 ml.***

As it can be seen, the difference is the incorporation of a buffer solution to control the pH ensuring the stability of the API (pH 4.00) [60]. Formulations were developed as it is shown in the corresponded annex.

- UPLC Validation.

DEX was analyzed by UPLC using an adapted method [61]. The mobile phase was acetonitrile/water (40:60, v/v) at a flow rate of 0.4 ml/min. The UV detection was at 240 nm. Six standards solutions were prepared at concentrations of 1 - 8 µg/ml and they were analyzed five times. The results of the validation are shown in table 4-12.

Area = 53,995*C; R = 0.99 (n = 36)	
RSD	4,905
Precision (%) (n=6)	0.48
Accuracy, % (n=9)	98.7
Detection limit (µg/ml)	0.4
Quantification limit (µg/ml)	1.2

Table 4-12. Data from the UPLC method validation

A robustness test was performed to examine the effect of operational parameters on the analysis results. The flow rate (0.4 ± 0.01 ml/min), injection volume (10 ± 0.3 µL), mobile phase composition (40.0 ± 1/60.0 ± 0.2), and column performance over time were determined in order to confirm the method.

The estimated area for the standard concentration was 272,562 µV·sec with an RSD of 2.3%.

DEX is detected at approximately 1.4 min of elution time. In the figure 4-2 is shown a chromatogram of a pure DEX and a sample extracted from suspension. As it can be seen there is no interference with the excipients. Also, to test the ability of the method to separate degradation products a sample was storage at 60 ºC and pH 6.14. The average extraction yield of DEX from the formulations was 102.5 ± 4.3%. Then, this chromatographic method is a stability-indicating method, which allowed us to detect and quantify DEX accurately and precisely.

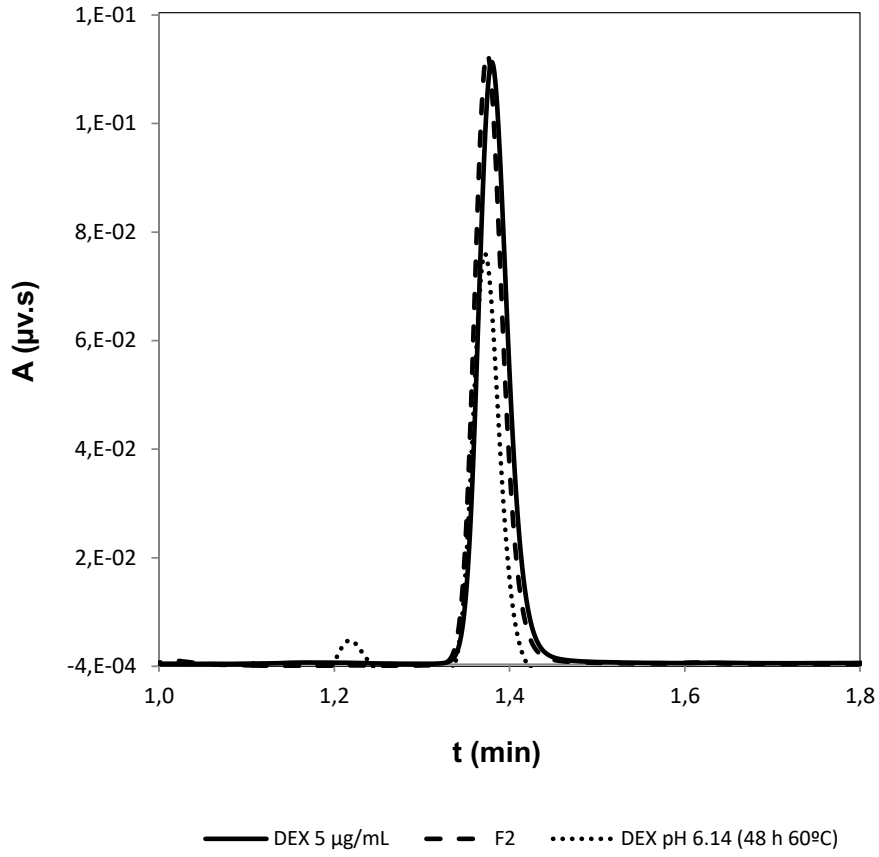

Figure 4-2. Dexamethasone pure patter chromatographic peak as standard of 5 µg/ml (continuous bold line), Dexamethasone extracted from F2 suspension (discontinuous bold line) and Dexamethasone in pH 6.14 solution, after 48 h stored at 60 °C (discontinuous thin line).

- Accelerated Stability Test:

Previous to the development of the formulations, to know the chemical stability of the API, an accelerated stability test was carried out at 60 ± 0.1 ºC (Heraeus UT 6060, Spain) and different pH conditions (citric/citrate buffer, pH 2 - 8). 50 µg/ml solutions were prepared, and samples were taking at times 0, 1, 18, 24, 42 and 48 h and then analyzed by UPLC.

As it can be seen from the plot (figure 4-3) the pH with maximum stability for the Dexa was the most acidic, with 100% remaining at 42 h [62].

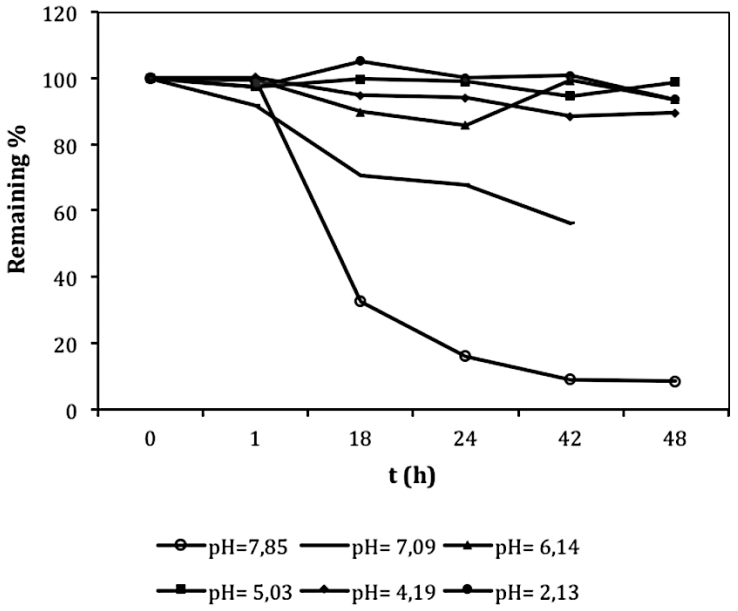

Figure 4-3. Remaining percentage of Dexamethasone in different pH solutions stored at 60 °C.

- Quality Control

Viscosity and pH value for each formulation are presented in table 4-13. As it can be seen, F2 shows viscosity and pH value lower than F1 because of its composition. F2 has citric/citrate buffer, which reduce pH value and also dilute the simple syrup making the suspension more liquid.

Formulation	pH	Viscosity (mPa·s) at 20 rpm
F1	7.06 ± 0.05	98.3 ± 1.4
F2	4.43 ± 0.01	21.7 ± 0.4

Table 4-13. Characterization of 1 mg/ml DEXA suspensions. They are expressed as mean value ± SD. ***SD: Standard Deviation***

pH variation along time for both formulations at different temperatures is shown in figure 4-4. F2 remained with a more constant pH value than F1 for 60 days. At 14 and 21 days, for F1 and F2 respectively, pH value starts to decrease making it more acid.

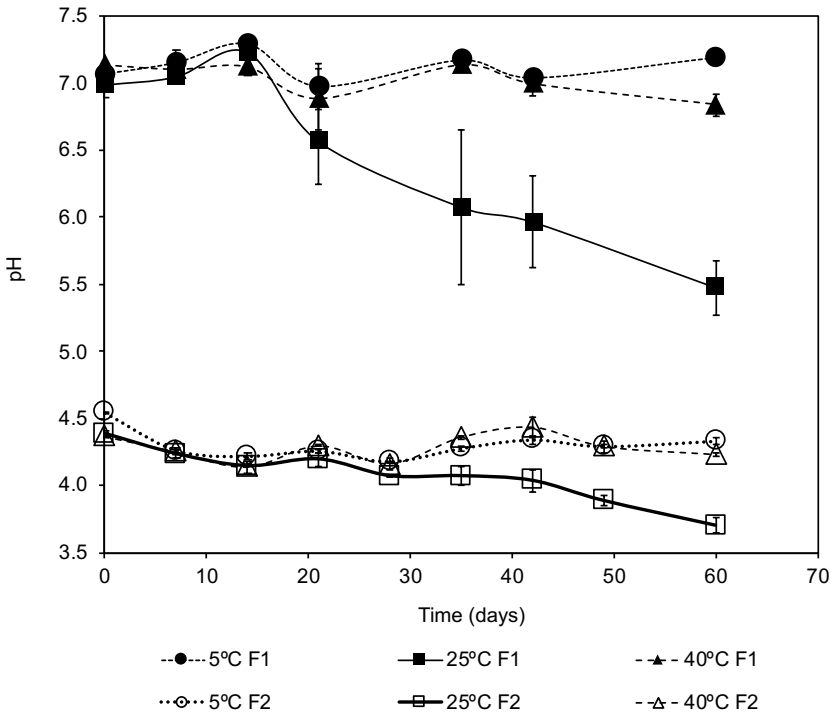

Figure 4-4. pH evolution at F1(symbols with filler) and at F2 (symbols without filler) during the 60 days storage at 5, 25 and 40 °C.

Both suspensions showed Newtonian behavior, wherein the viscosity of each system remained constant with shear rate and the shear stress increased linearity with shear rate, figure 4-5.

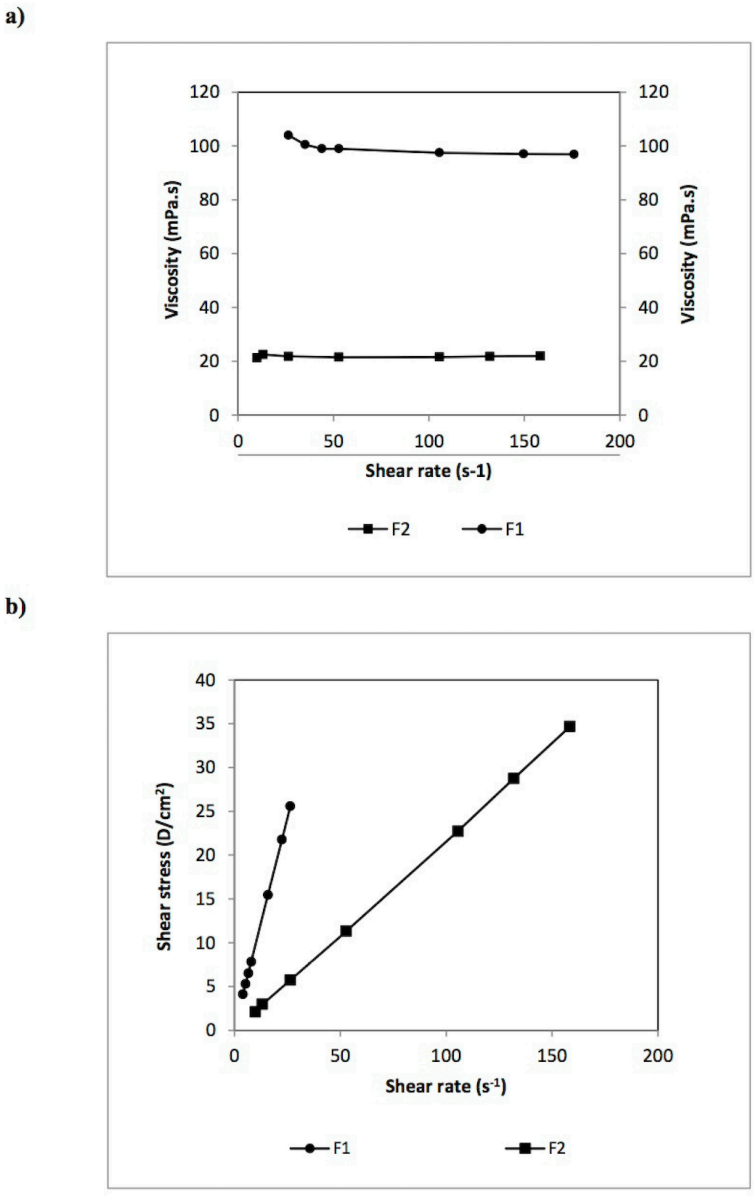

Figure 4-5. Variation in viscosity with shear rate (a), and in shear stress with shear rate (b) of F1 and F2.

Both formulations fulfill pharmacopoeia criteria for mass and content uniformity, tables 4-14; 4-15. These criterions were also fulfilled at 30 days of storage at 5 ºC.

Dw (g)				
Dose	**F1**		**F2**	
1	6.75		6.40	
2	6.70		6.40	
3	6.67		6.39	
4	6.65		6.40	
5	6.70		6.40	
6	6.70		6.39	
7	6.65		6.38	
8	6.61		6.41	
9	6.68		6.40	
10	6.62		6.43	
11	6.63		6.40	
12	6.66		6.42	
13	6.67		6.38	
14	6.70		6.38	
15	6.64		6.40	
16	6.67		6.38	
17	6.63		6.41	
18	6.61		6.41	
19	6.59		6.41	
20	6.65		6.39	
A	6.66		6.40	
	LL	**UL**	**LL**	**UL**
10%	5.99	7.32	5.76	7.04
20%	5.33	7.99	5.12	7.68

Table 4-14. Mass uniformity test of the doses (5 ml) of F1 and F2 at time 0.

Dw (g)								
Dose	**F1**				**F2**			
	0 days		**30 days**		**0 days**		**30 days**	
1	102.0		92.6		95.3		84.8	
2	106.0		90.9		98.0		86.1	
3	96.3		92.5		97.0		85.1	
4	101.0		90.1		99.6		86.3	
5	104.0		90.9		98.2		86.2	
6	97.9		94.2		97.6		86.3	
7	97.0		92.3		99.1		87.3	
8	98.3		95.5		101.0		85.1	
9	106.0		93.2		98.4		88.5	
10	95.5		93.7		102.0		86.3	
11	95.7		93.3		97.9		84.0	
12	93.0		97.5		98.2		85.8	
13	89.4		95.4		96.6		86.4	
14	95.0		99.4		95.6		86.3	
15	95.0		96.6		99.2		87.4	
16	88.2		99.6		97.8		86.0	
17	101.0		97.9		97.2		84.7	
18	96.9		98.0		98.3		86.6	
19	96.4		97.9		96.6		87.5	
20	89.3		100.0		96.6		86.5	
A	97.2		95.1		98.0		86.1	
	LL	**UL**	**LL**	**UL**	**LL**	**UL**	**LL**	**UL**
10%	87.4	107	85.6	105	88.2	108.0	77.5	94.8
20%	77.7	117	76.1	114	78.4	118.0	68.9	103.4

Table 4-15. Content uniformity test of the doses (5 ml) of F1 and F2 at time 0 and after storage at 5 ± 0.1°C for 30 days.

AV at 0 days were 7.62 and 4.63 for F1 and F2 respectively.

In table 4-16, the values for the physical study are shown. This test is very useful to know the importance of stirring before taking a dose, due to the fact that we took doses from different heights of the formulation and analyzed its drug content.

Formulation	F1	F2
Z1 (% DV ± SD)	87.7 ± 3.7	15.3 ± 0.1
Z3 (% DV ± SD)	96.5 ± 9.3	23.1 ± 2.1
Dmax	17.9	9.4
Time (min)	70	90
Z1 (% DV ± SD)	93.4 ± 0.2	96.1 ± 3.5
Z3 (% DV ± SD)	92.8 ± 0.9	95.9 ± 4.0
Dmax	1.4	5.6
Dmax/min	0.02	0.06

Table 4-16. Results of physical stability studies at 30 days of storage at 5 ± 0.1°C with two samples per group. *DV (%): declared value expressed as percentage; F: formulation; Z: heights; Dmax: maximum observed difference; Dmax/t: Dmax corrected for the standing time after shaking*

In this case the formulation with the highest Dmax (%) before stirring was F1. F2 has a sediment, which although it was readily resdispersable after shaking, made that DV was so low.

After storage of the two formulations under different temperature conditions, Dexa in F2 was observed to maintain its average remaining percentage above 90%, regardless of temperature. In addition, at 25 ºC the average remaining percentage detected was higher than 95% for 21 days, figure 4-6.

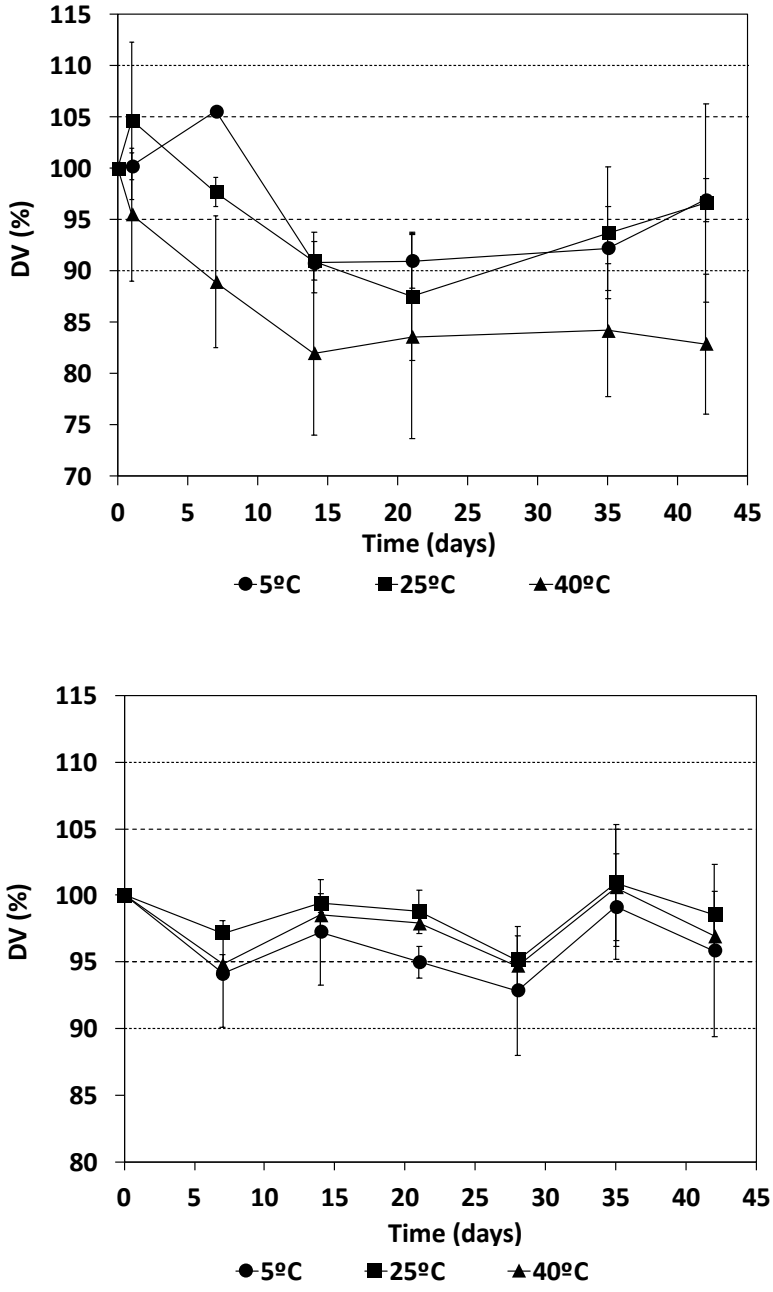

Figure 4-6. Percentage of Dexamethasone remaining in F1 (Top) and F2 (Bottom) after 60 days storage at 5, 25 and 40 °C. Dashed lines are the upper and lower limits established at 100 ± 5 % and 100 ± 10 %.

Two formulations were prepared, following strictly the SOPs proposed, at two pharmaceutical compounding pharmacies. They were used to check the microbiological stability. The total aerobic microorganism count was less than 10^2 cfu/ml and total combined yeast/mold was also less than 10 cfu/ml at 15 days of storage and *Escherichia coli* contamination was not detected.

In conclusion, F2 is the one selected for this API. Fulfills all criteria of the Pharmacopoeias, including microbiological tests, and can be used till 15 days after it is made when it is storage at 5 or 25 ºC.

Adapted for thesis from paper:

Safe use of Dexamethasone in pediatrics: design and evaluation of a novel stable oral suspension. Santoveña-Estévez A, Dorta-Vera D, González-García I, Suárez-González J, Teigell-Pérez Nuria, Fariña JB.

Published in Pharmaceutical Technology in Hospital Pharmacy. 2018;3(2):59-70. doi: 10.1515/pthp-2018-0003

4.6. Furosemide.

Furosemide is a loop diuretic indicated for the treatment of cardiac and renal edema in pediatrics; it blocks the co-transport system $Na^+K^+2Cl^-$ which is placed on the ascending limb of the loop of Henle. The diuretic action is the result of the inhibition of the reabsorption of sodium chloride in this segment of the loop of Henle [63, 64].

The oral dose for a newborn child is 1 - 4 mg/kg body weight each 12 - 24 h and 1 - 2 mg/kg body weight each 6 - 12 h in nurslings and older children [63].

The solubility of Furosemide is pH-dependent, its maximum solubility is to be reached at pH greater than 8, 21.9 mg/mL at 30 ºC [65]. In addition, bioavailability is very low, near to 20 - 60%, due to its low permeability [66].

According to the BCS, Furosemide is a class IV due to its low solubility and low permeability. Nevertheless, the amount of API required to develop a formulation of 2 mg/ml allow to have the dose dissolved in our formulation. Furosemide is a good example which proves that the BCS needs to be adapted for pediatrics. BCS is focused on adults because in this classification, a high solubility drug means that the highest dose permitted (for adults), is dissolved in 250 ml of purified water. However, in pediatrics this dose lower, permeability it is not the same than in adults and the initial volume gastric is 40 ml instead of 250 ml for adults. For that reason, a PBCS could be useful in order to select the appropriate amount and number

of excipients during the design of individualized medicines for pediatric use [67, 68].

Furosemide is marketed in USA and France as tablets and oral solution [47, 69]. In other countries such as Belgium, Spain, Norway or Sweden it is only available in tablet form for adults so, an oral formulation for pediatric use is required in community pharmacies and hospitals [46, 70 - 72].

Although there are SOPs in the literature for the elaboration of oral formulations of Furosemide (some of them using already commercial dosage forms) there is a deficiency of published data related to dose homogeneity, stability and, in general, about the steps to ensure its quality [73 – 79].

Formulations obtained in the literature with a high number of excipients or not accepted for pediatric used were discard. Three different formulations of Furosemide (2 mg/ml), contained in multidose containers, were chosen to evaluate its quality. F1 is the most used formulation until 2017 [79], F2 is the formulation proposed by ISPHC (International Society of Pharmaceutical Compounding) [77] and F3 is the one which was published in 2018 in the Spanish National Formulary [76], see table 4-17.

	Formulation		
Ingredient	**F1**	**F2**	**F3**
Furosemide (mg)	200	200	200
$Na_2HPO_4 \cdot 2\ H_2O$ (g)	-	6.96	-
$Na_2HPO_4 \cdot 12\ H_2O$ (g)	-	-	1.50
Sörensen Buffer, pH 7.4 (ml) (19.2% of KH_2PO_4 v/v and 80.8% of Na_2HPO_4 v/v in purified water)	70	-	-
Monohydrate Citric Acid (mg)	-	63	-
Diluent (qs 100 ml)	Symple syrup with ACWP	ACWP	ACWP
pH	6.92 ± 0.01	7.90 ± 0.01	7.87 ± 0.01

Table 4-17. Composition of the different formulations. ***qs: amount which is enough to complete 100 ml.*** ***ACWP: Aqua conservans without propyleneglycol.***

- UPLC Validation.

Furosemide was analyzed by UPLC applying an adapted HPLC method to UPLC using a X-Select® C18 reversed phase column 2.5µm XP (2.1x75 mm) (Waters, Milford, MA, USA) [65, 80]. The mobile phase was ammonium phosphate buffer 0.01M:Methanol (57:43, v/v), at a flow rate of 0.4 ml/min. The UV detection was at 273 nm. The injection volume was 10 µl.

The validation of the analytical method was done following the ICH guideline for this purpose [81], 8 standard solutions were prepared. These were prepared weighting 10 mg of Furosemide and adding 10 ml of a solution to promote the solubility of the API, diluting solution. Such solution was made of 50% v/v of acetonitrile, 2.2% v/v of acetic acid and purified water in quantity sufficient to 100 ml [80]. This first standard solution was diluted with mobile phase to a concentration interval 6 – 20 µg/ml.

Area (µV·sec-1) = 80,135 · C (µg/ml); R = 0.99 (n = 32)	
RSD	2,418
Precision (%) (n=6)	0.70
Accuracy, % (n=9)	99.3
Detection limit (µg/ml)	1.78
Quantification limit (µg/ml)	5.41

Table 4-18. Data from the UPLC method validation.

The flow rate (0.4 ± 0.5 ml/min), injection volume (10 ± 0.3 µl), mobile phase composition (57 ± 5/43 ± 5, v/v), and column performance over time were determined to confirm the method's robustness. To calibrate the UPLC system and monitor its performance, we analyzed a Furosemide solution sample daily as standard.

A 20 µg/ml solution of furosemide was stored at 80 ºC (Heraeus UT 6060, Spain) to test the ability of the method to follow the degradation of the API and check if it was able to detect and quantify any product of degradation. This solution was analyzed at 1, 2, 24 and 48h. In figure 4-7 the chromatogram for Furosemide as pure pattern is shown (1.65 min of retention time). It also shown who the peak changes along time when this standard solution it is placed in a chamber at 80 ºC. Moreover, at 0.6 min it can be seen another peak which area increases along time, maybe a degradation product of the furosemide.

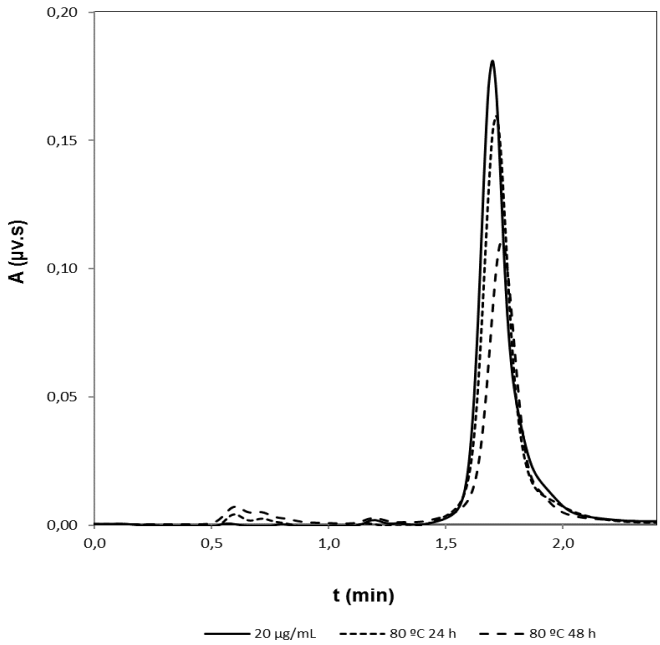

Figure 4-7. Furosemide pure patter chromatographic peak as standard of 20 µg/ml (continuous bold line), Discontinuous line represent how the area of the API changes along time when it is placed at 80 ºC.

The average extraction yield of the Furosemide for F1 is 103 ± 3.0% and 100 ± 1.8% for F2. Then, this chromatographic method is a stability-indicating method which allowed us to detect and quantify the API accurately and precisely.

- Quality Control

All solutions showed a pH near to 7 and a transparent aspect when they were elaborated. They were odorless and insipid. Table 4-19 shows the mass uniformity test performed for the three formulations. All formulations met the Ph. Eurp. test for mass uniformity of multidose containers, all individual values are inside the limit of 10% of the average mass.

	Dw (g)					
Dose	**F1**		**F2**		**F3**	
1	5.46		5.22		5.09	
2	5.48		5.25		5.12	
3	5.50		5.28		5.17	
4	5.50		5.23		5.17	
5	5.48		5.25		5.11	
6	5.51		5.24		5.12	
7	5.51		5.24		5.11	
8	5.50		5.24		5.12	
9	5.51		5.25		5.12	
10	5.49		5.25		5.20	
11	5.50		5.25		5.16	
12	5.51		5.27		5.12	
13	5.48		5.23		5.14	
14	5.50		5.27		5.15	
15	5.48		5.26		5.13	
16	5.50		5.28		5.16	
17	5.51		5.27		5.14	
18	5.50		5.24		5.14	
19	5.49		5.27		5.18	
20	5.48		5.22		5.13	
Average	5.49		5.25		5.14	
	LL	**UL**	**LL**	**UL**	**LL**	**UL**
10%	4.94	6.04	4.73	5.78	4.62	5.65
20%	4.40	6.59	4.20	6.30	4.11	6.17

Table 4-19. Mass uniformity test of the doses (5 ml) of F1, F2 and F3.

In the case of Furosemide, as a solution, the API is dissolved in the entire formulation, so the weight of each dose could be a good indicator to know if the right amount of API is dosed each time. So, it is not needed a content uniformity test.

Table 4-20 shows the stability of furosemide in the different formulations when they are placed at different conditions of storage.

		DV (% average ± SD)			
Formulation	**Time**	**0 days**	**15 days**	**30 days**	**60 days**
F1	5ºC	100.0 ± 0.0	ND	94.3 ± 20.8	101.1 ± 0.4
	25ºC	100.0 ± 0.0	ND	94.3 ± 29.7	98.1 ± 2.5
	40ºC	100.0 ± 0.0	ND	107.9 ± 11.8	101.0 ± 1.0
F2	5ºC	100.0 ± 0.0	ND	119.0 ± 2.7	106.1 ± 0.6
	25ºC	100.0 ± 0.0	ND	107.4 ± 4.3	102.3 ± 1.7
	40ºC	100.0 ± 0.0	ND	105.6 ± 0.3	99.1 ± 0.5
F3	5ºC	100.0 ± 0.0	104.0 ± 1	98.9 ± 2.9	ND
	25ºC	100.0 ± 0.0	104.5 ± 1.9	98.7 ± 0.3	ND
	40ºC	100.0 ± 0.0	105.9 ± 0.3	98.9 ± 3.1	ND

Table 4-20. Percentage of Furosemide remaining in F1, F2 and F3 after 60 days of storage at 5, 25 and 40 °C. ***ND: Not Determined; SD: Standard deviation.***

At 30 and 60 days of storage at 5 ºC, maybe because of the mayor concentration of salts and the temperature, F2 showed the formation of crystals. These crystals were studied by X-ray refraction. Powder XRD spectra were acquired from X'Pert PRO X-ray diffractometer (PANalytical, Madrid, Spain) to determine the structure of the

crystals formed in the formulations during storage. CuKα radiation (λ= 1.5406 Å) was employed, and 20 data were collected from 5.00º to 100º with a scanning rate of 0.03 s^{-1}. Crystalline phases were identified by comparing the experimental diffraction patterns with a furosemide pure pattern and using the Joint Committee on Powder Diffraction Standards (JCPDS). There was not Furosemide in its composition (halite, cristobalite y magnetite). Then, F2 does not fulfill the standards of quality.

F1 and F2 are chemically stable for 60 days at 5 ºC and 25 ºC, respectively. In the case of F3 the results obtained agreed with the stability period stablished by the Spanish National Formulary, it is stable for 30 days at 25 ºC [76].

In table 4-21 are shown the results of the variation of pH along time. In all formulations and storage condition, except for F1 at 25 ºC, it produces a decrease in the pH value.

	pH				
Formulation	**Time (days)**	**0**	**15**	**30**	**60**
F1	5 ºC	6.92	ND	6.90	6.40
	25 ºC	6.92	ND	5.80	6.80
	40 ºC	6.92	ND	6.90	6.30
F2	5 ºC	7.90	ND	ND	7.50
	25 ºC	7.90	ND	ND	7.80
	40 ºC	7.90	ND	ND	7.60
F3	5 ºC	7.87	7.61	6.98	ND
	25 ºC	7.87	7.54	7.13	ND
	40 ºC	7.87	7.70	7.09	ND

Table 4-21. pH evolution at F1, F2 and F3 during the 60 days storage at 5, 25 and 40 °C.

pH variation is an important tool which could indicate degradation of the API or microbial contamination. In this case pH value is an important parameter for Furosemide because it will have an influence in the solubility and stability [82].

Recently, Zahalka et al examined the stability of an oral formulation of furosemide which had a similar composition to F3, with a good stability period [83]. Saccharin was included in their formulation to improve palatability; a color change and a pH decrease was detected when sucrose was used. In F1 a pH decrease was detected but did not affect the chemical stability when stored at 5 ºC. In addition, methylparaben was included to ensure antimicrobial preservation but, according to the EMA, there is not sufficient clinical evidence regarding the effect of methylparaben and propylparaben as preservatives in children. Due to the importance of preventing microbial contamination in pediatrics formulations, a concentration range has been agreed on for both preservatives to ensure good antimicrobial activity and safety [28].

Therefore, as the case of our formulation, using methylparaben and propylparaben ensured that there would be no microbial contamination and thus safe for children.

In conclusion, three individualized medicines of 2 mg/ml of Furosemide have been tested. F1 when it is storage at 5 ºC for 60 days and F3 at 25 ºC for 30 days are good options when a treatment for children is required. Our studies for F3 agrees with the stability period published for this formulation in the Spanish National Formulary.

Adapted for thesis from paper:

A high demanding strategy to ensure the highest quality standards of oral liquid individualized medicines for pediatric use. Suárez-González J, Santoveña-Estévez A, Armijo-Ruíz S, Castillo A, Fariña JB.

Published in AAPS PharmSciTech (2019) 20: 208.
doi:10.1208/s12249-019-1432-x.

Factor Trend (JCR 2018): 2.608
Quartile in Pharmacology & Pharmacy: Q2

4.7. Acetazolamide.

Acetazolamide (AZM) is an inhibitor of the carbonic anhydrase which is an enzyme in charge of maintain the balance of water-salts. It is use due to its diuretic effect in the treatment of edema and glaucoma, whether open or close angle [84, 85]. As a diuretic the posology for children is 250 mg/day and, in the treatment of glaucoma disease, 250 mg can be given once or four times a day [86].

Is a white crystalline powder, very slightly soluble in water, slightly soluble in 96% ethanol, and dissolves readily in dilute solutions of alkaline hydroxides [85]. This API has two polymorphic forms, both with low solubility [87 – 89]. Due to its low solubility and permeability it is classified as class IV in the BCS [75, 76]. It is an API whose stability is pH dependent being the maximum stability in the pH range of 4 to 5 [92].

AZM is commercialize in Spain as 250 mg tablets [46]. In USA it is available as tablets, capsules and injectable injection (acetazolamide sodium) [47]. As it can be seen, there is not available a commercial pediatric formulation but there are SOPs for the development of them. However, all of them use the commercial tablets to the elaboration of the formulation and some of them use complex excipients, flavoring agents and preservatives [73, 93 – 95].

AZM is a perfect candidate to study and develop a child-friendly formulation due to it stability is influenced by pH and is a class IV. All the AZM formulations that were studied are shown in table 4-22.

Formulation	Wetting agent (v/v)	Suspending agent	Buffer (v/v)	Diluent-sweetener (qs 100 ml)
F1	-	Methylcellulose 1000 1% w/w, 50 ml	-	Simple Syrup
F2	Glycerol 20%	Methylcellulose 1000 1% w/w, 20 ml	Citrate 30%	Simple Syrup
F3	Glycerol 20%	Hydroxypropylmethylcellulose 4500 2% w/w, 20 ml	Citrate 30%	Simple Syrup
F4	Glycerol 20%	Hydroxypropylmethylcellulose 4500 2% w/w, 15 ml	Citrate 35%	Simple Syrup

Table 4-22. Composition of the different formulations. ***qs: amount which is enough to complete 100 ml.***

F1 is the formulation that it has been done till know in the pharmacy services. In F2 a wetting agent (glycerol) and a citrate buffer were added to improve content uniformity and chemical stability of the API, respectively. In F3 and F4 it was added another suspending agent in order to obtain different viscosities than with methylcellulose.

- UPLC Validation.

AZM was analyzed by UPLC using an adapted method of HPLC that can be found in the USP pharmacopoeia [96]. The mobile phase was 0.5 M anhydrous sodium acetate in water (95%, v/v) and acetonitrile:methanol (60:40, v/v) (5%, v/v) adjusted to pH 4.0 with glacial acetic acid, at a flow rate of 0.3 ml/min. The UV detection was at 254 nm. Five standards solutions were prepared at concentrations of 2 - 10 µg/ml and they were analyzed five times. The results of the validation are shown in table 4-23.

Area = 42,550 +59,360*C; R = 0.989 (n = 30)	
RSD	29,880
Precision, (%) (n=6)	0.71
Accuracy, % (n=9)	102.4
Detection limit (µg/ml)	1.36
Quantification limit (µg/ml)	4.13

Table 4-23. Data from the UPLC method validation

A robustness test was performed to examine the effect of operational parameters on the analysis results. The flow rate (0.3 ± 0.01 ml/min), injection volume (5 ± 0.3 µL), temperature (21.0 ± 0.5 ºC), mobile phase composition (95.0 ± 1/5.0 ± 0.2), and column performance over time were determined in order to confirm the method.

The estimated area for the standard concentration was 339,352 µV·sec with an RSD of 7.9%.

AZM is detected at approximately 2.0 min of elution time. In the figure 4-8 is shown a chromatogram of a pure FA and a sample extracted from suspension. The average extraction yield of FA from the formulations was 105.1 ± 6.5%. Then, the chromatographic method allowed us to detect and quantify AZM accurately and precisely.

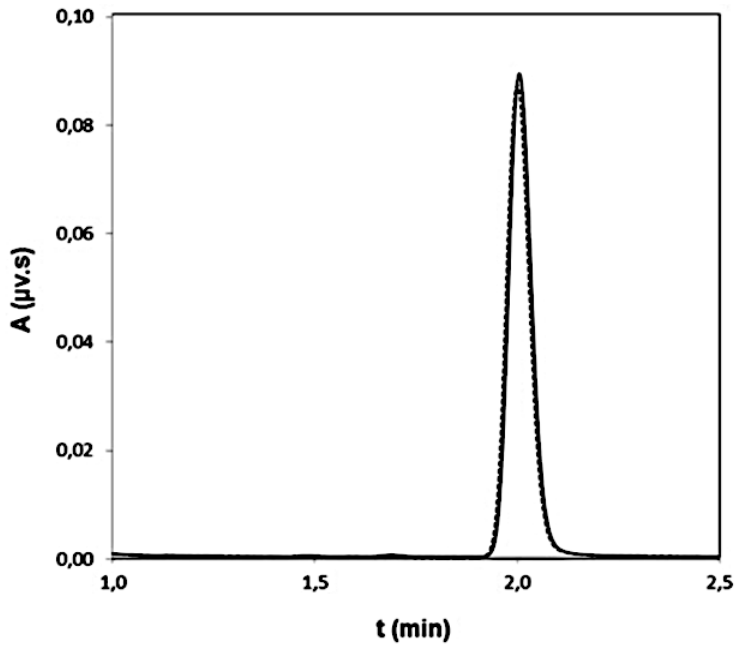

Figure 4-8. AZM pure pattern chromatographic peak (continuous line), AZM after extracted from F3 formulation (discontinuous line).

- Quality Control

Glycerol was added in formulations F2 - F4 to improve the wettability of AZM and suspension homogeneity. No sediment was observed in formulations F1, F3 and F4.

F2 was discarded from the assays because of the formation of a deflocculated system after a couple of days of storage, figure 4-9. This must be due to the interaction between buffer citrate and the suspending agent at higher temperature [18]. That is the reason why, this last was changed to hydroxipropylmethylcellulose for F3 and F4.

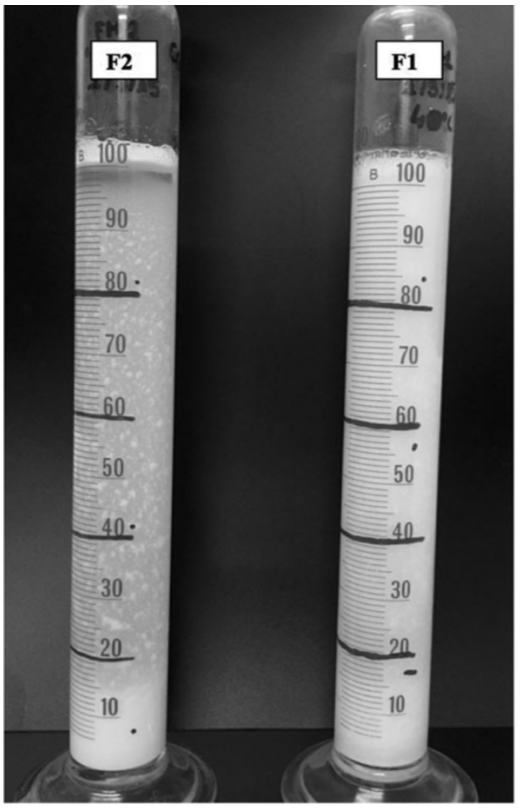

Figure 4-9. Aspect of F1 and F2 after 15 days of storage at 40 ºC.

Figure 4-10 shows the variation of viscosity versus shear rate of the formulation studied. F1, F4 and the methylcellulose 1000 dispersion (1%, w/v) behave as a Newtonian fluid, otherwise, F3 and the dispersion of hydroxypropylmethylcellulose 4500 (2%, w/v) behave as a plastic fluid. This agree with the shear stress versus shear rate profile. F3 and the hydroxypropylmethylcellulose dispersion were fitted as Bingham materials with a yield value of about 2.99 and 3.39 D/cm^2, respectively.

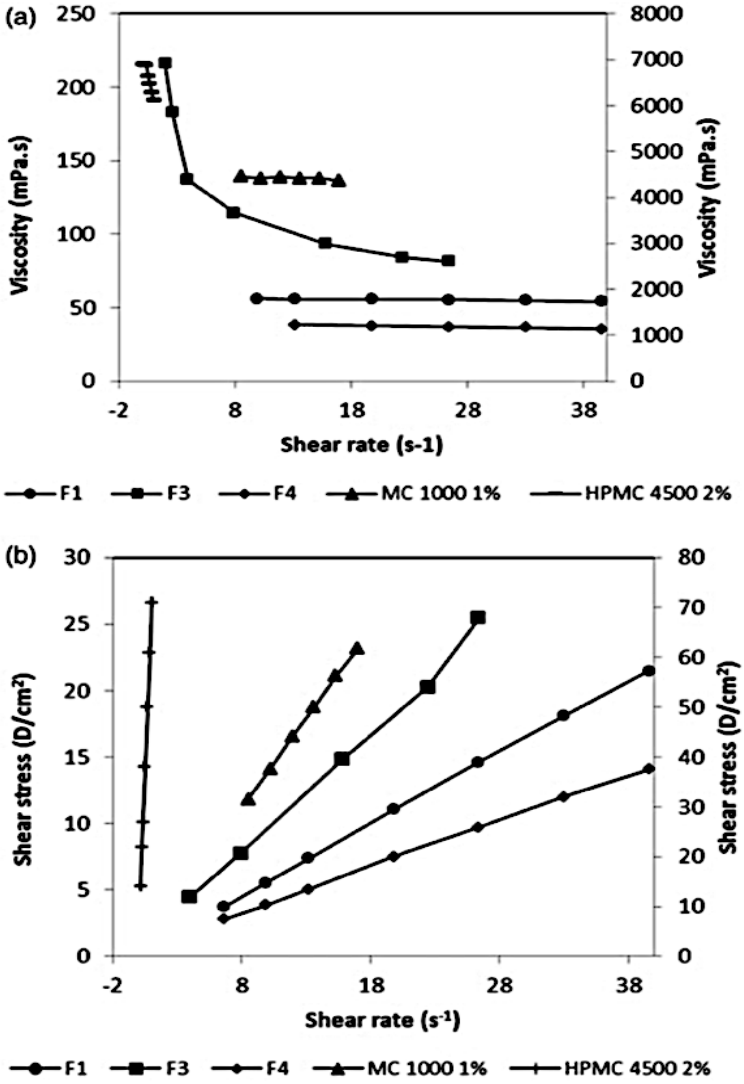

Figure 4-10. Viscosity versus shear rate (a) and shear stress versus shear rate; (b) of F1, F3, F4 and the dispersions of methylcellulose 1000 and hydroxypropylmethylcellulose 4500. The viscosity and shear stress values of hydroxypropylmethylcellulose 4500 are represented on the second y-axis of (a) and (b), respectively.

All the AZM formulations meet the mass uniformity of dose obtained for multi-dose containers test; not more than two of the individual masses deviate from the average more than 10% (table 4-24).

Dw (g)						
	F1		**F3**		**F4**	
1	5.74		4.56		5.80	
2	5.77		4.46		5.79	
3	5.88		4.39		5.89	
4	5.74		4.34		5.45	
5	5.70		4.25		6.15	
6	5.64		4.31		5.60	
7	5.59		4.11		5.71	
8	5.56		4.44		5.68	
9	5.57		4.48		6.05	
10	5.46		3.97		6.23	
11	5.70		4.74		5.70	
12	5.66		4.41		5.69	
13	5.65		4.42		5.91	
14	5.62		4.54		6.10	
15	5.73		4.20		5.75	
16	5.60		4.18		6.05	
17	5.74		4.29		5.78	
18	5.62		4.32		5.89	
19	5.58		4.19		5.66	
20	5.37		4.19		6.10	
A	5.65		4.34		5.85	
	LL	**UL**	**LL**	**UL**	**LL**	**UL**
10%	5.08	6.21	3.91	4.78	5.08	6.21
20%	4.52	6.78	3.47	5.21	4.52	6.78

Table 4-24. Mass uniformity test (Ph.Eur.) for F1, F3 and F4. Soft shading values: outside the limits of 10%; Strong shading values: outside the limits of 20%.

None of the formulations studied meet the pharmacopoeia criteria for content uniformity. All formulations showed up to till 30 % of deviations of the dose contents from the theoretical declared value, table 4-25.

DV (%)						
	F1		**F3**		**F4**	
1	77.8		89.3		95.7	
2	90.9		105.0		73.1	
3	94.5		111.0		72.9	
4	88.4		111.0		89.7	
5	85.2		109.0		90.8	
6	84.9		109.0		60.3	
7	84.2		103.0		86.8	
8	84.8		109.0		66.1	
9	87.0		119.0		79.9	
10	89.7		125.0		92.3	
11	79.1		131.0		86.6	
12	78.1		133.0		91.4	
13	81.9		137.0		88.5	
14	90.3		97.9		69.4	
15	101.0		103.0		74.2	
16	92.6		93.9		71.3	
17	105.0		109.0		93.5	
18	112.0		93.0		87.5	
19	115.0		102.0		ND	
20	111.0		92.9		ND	
A	91.7		109.2		81.7	
	LL	**UL**	**LL**	**UL**	**LL**	**UL**
10%	82.5	101	98.2	120	73.5	89.8
20%	73.3	110	87.3	131	65.3	98.0

Table 4-25. Compounded preparation test (USP) and content uniformity of unidose preparations test (Ph.Eur.) for F1, F3 and F4. ***DV (%): percentage of declared value; Soft shading values: outside the limits of 10%; Strong shading values: outside the limits of 20%.***

AV for these formulations were: 22.6, 30.2 and 12.6 for F1, F3 and F4 respectively.

In order to observe the influence of sedimentation in the non-content uniformity a physical stability study was carried out. After 30 days of storage the AZM content at different heights were determined for formulations F1 and 3 before and after stirring, values are shown in tablet 4-26.

The maximum difference (Dmax) it is observed before stirring, and it decrease after it for both formulations, being F1 the one with the lowest value. This proof, once more, that suspensions must be stirred before taking a dose.

Formulation	F1	F3
Z1 (% DV ± SD)	42.6 ± 10.3	102 ± 20.9
Z3 (% DV ± SD)	259 ± 21.9	88.5 ± 8.7
Dmax	239	34.8
Time (min)	35	45
Z1 (% DV ± SD)	70.2 ± 2.2	107 ± 37.6
Z3 (% DV ± SD)	71.1 ± 2.1	92.3 ± 31.1
Dmax	3.88	73.57
Dmax/min	0.11	1.63

Table 4-26. Results of physical stability studies at 30 days of storage at 40 ± 0.1°C with two samples per group. *DV (%): declared value expressed as percentage; F: formulation; Z: heights; Dmax: maximum observed difference; Dmax/t: Dmax corrected for the standing time after shaking*

Also, in both formulations can be seen, in table 4-26, how after 30 days the DV are far from 100%. To know if the reason was an AZM degradation a chemical stability test was carried out, figure 4-11. At 30 days F1 and F4 had DV between 90 and 100%, F3 at 40 ºC had a DV less than 90%. After 60 days of storage, F1 is the only one which showed a variation less than 10% for all temperatures, the rest keeps stable at 25 ºC. However, at 5 ºC and 40 ºC the DV variation is higher than 10% for F3 and F4 being the highest variation at the lowest temperature. Refrigerated storage leads to physical instability of the dispersed system formed, formation of the sediment most difficult to re-disperse and therefore, the recovery of less AZM to provide dose. The room temperature is the best for the better physical stability of the AZM formulation.

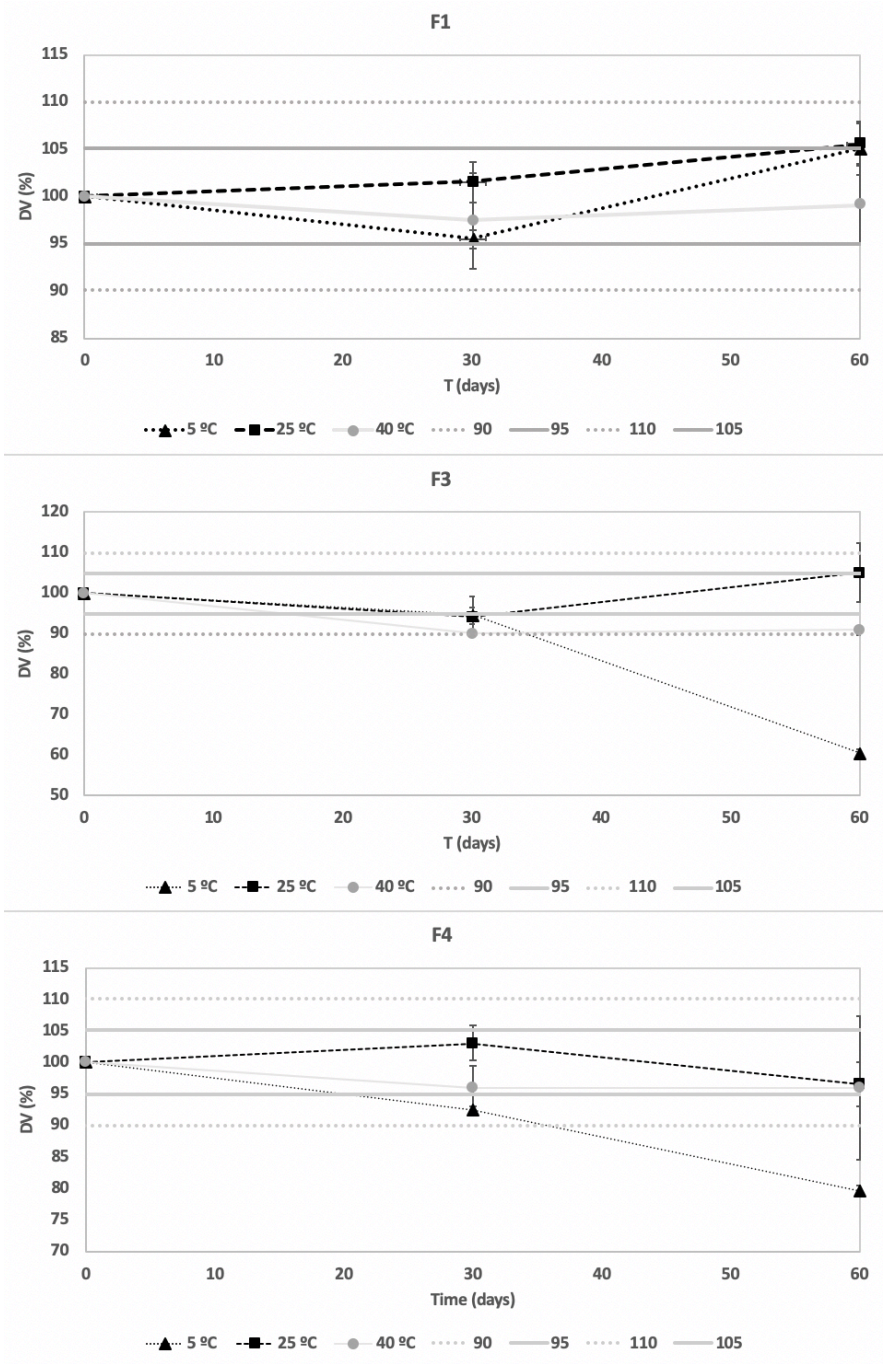

Figure 4-11. Evolution of DV (%) of AZM in time for F1, F3 and F4 at different storage conditions. Dashed lines are the upper and lower limits established at 100 ± 5 % and 100 ± 10 %.

As it had been shown, the development of a child-friendly oral formulation of AZM which contains less excipients and all of them accepted for pediatric population it is not easy. More excipients like complexing agents, surfactants, etc must be added to ensure the solubilization of the API and then the content uniformity. That is the reason why other solutions must be carried out.

Although liquids oral formulations are the most used dosage form for pediatric use due to the easy of dosing and swallowing, this is not an option for some APIs, AZM for example. For this kind of APIs, with low solubility (class IV) and problems in content uniformity in the case of liquid formulation, a solid dosage form could be a solution [12]. There are several options like dispersible tablets, ODT, MT or ODMT suitable for pediatrics [97 – 100]. In this case, due to the dose of AZM (250 mg/day) it might not be advantageous the use of an ODT or MT but it could solve the situation the development of a dispersible tablet

In conclusion, the development of a child-friendly suspension of AZM with the lowest number of excipients and accepted for pediatric use, which fulfills pharmacopoeia recommendations, it was not reach due to the physicochemical characteristics of the API. That it is the reason why, for pediatric use, it is suggested the development of an oral solid dosage form like dispersible tablets.

Adapted for thesis from paper:

Formulation design of oral pediatric Acetazolamide suspension: dose uniformity and physico-chemical stability study. Santoveña A, Suárez-González J, Martín-Rodríguez C, Fariña JB.

Published in: Pharm Dev Technol. 2017 Mar;22(2):191-197.
doi: 10.1080/10837450.2016.1175475.

Factor Trend (JCR 2017): 1.945
Quartile in Pharmacology & Pharmacy: Q3 (177/261)

4.8. Results: strategy development.

Develope a good analytical method is essential to ensure that it is efficient to analyze all the CQAs of the formulation. Table 4-27 shows a summary of the main properties of different analytical methods used to study the different APIs, some of them where seen previously and Ursodeoxycholic acid was obtain from literature. The method must be precise and accurate. In addition, it should follow the degradation of the API and quantify degradation products, as this might be dangerous above a certain limit [101].

API	System	Analysis time (min)	Precision (%, <1%)	Accuracy (%, 97 – 103%)	Degradation products	
					D	Q
Flecainide	UPLC	0.80	0.21	99.1	Y	N
Dexamethasone	UPLC	1.70	0.48	98.7	Y	Y
Furosemide	UPLC	2.70	0.70	99.3	Y	N
Acetazolamide	UPLC	2.50	0.71	102	Y	N
Ursodeoxycholic acid [32]	HPLC	8.00	0.93	102	Y	N

Table 4-27. Summary of the main characteristics of different analytical method used to validate oral liquid individualize medicines. D: detect degradation products; Q: quantify degradation products; Y: yes; N: no. Ursodeoxycholic acid was study by the working group previously to the elaboration of this thesis.

As may be seen, all the APIs were analyzed over a short period of time when the UPLC system was used. All methods showed values of precision and accuracy within the limits and they were all able to detect the degradation of the API; fundamental when testing their chemical stability.

A fundamental aspect to check is that the excipients of the formulation do not interfere with the analysis of the API and that it possible to extract the right amount from a complex matrix as the formulation (simple syrup, glycerol, cellulose etc.).

In this case, for all APIs studied, correct extraction was possible for each method, table 4-28.

API	Formulation	Extraction Yield (%)
Flecainide	F1	111 ± 10.2 (n=20)
Acetazolamide	F1	105 ± 6.5 (n=6)
Dexamethasone	F1	103 ± 4.3 (n=5)
	F2	100 ± 3.4 (n=5)
Furosemide	F1	103 ± 3.0 (n=10)
	F2	100 ± 1.8 (n=10)
Ursodeoxycholic acid [32]	F1	95.1 ± 0.06 (n=20)

Table 4-28. Extractions yields (%) for the API studied

As it can be seen from previously presented data, all formulations fulfill the only test that Ph.Eur. recommends test of uniformity of mass of delivered doses from multidose containers, independently if it is a suspension or a solution. In the case of Furosemide, prepared as a solution, the API is homogeneously distributed in the

formulation. However, in the case of suspensions (Flecainide, Acetazolamide, Dexamethasone, Carbamazepine or Ursodeoxycholic acid) this does not happen which means that the weight of the doses could be not good correlated with the content in API.

In table 4-29 it is shown some of the formulations studied for each API. Must be remember that all of them fulfill the uniformity of mass test according to Ph.Eur. recommendations. However, it can be seen that only five formulations from ten pass the content uniformity test, which means that five formulations would have problems with dose uniformity.

Formulation		Content Uniformity test				Dmax/t
		± 10%	± 20%	RSD	AV	
Flecainide	F1	8	4	17.9	54.8	ND
	F3	0	0	2.2	4.4	ND
Acetazolamide	F1	4	3	12.4	22.6	0.11
	F3	7	2	12.6	30.2	1.63
Dexamethasone	F1	0	0	5.2	7.6	0.02
	F2	0	0	1.7	4.6	0.06
Carbamazepine MTP	F1A	0	0	3.5	8.7	0.30
	F3A	1	0	4.1	9.6	0.42
Ursodeoxycholic acid [32]	F1	0	0	7.3	28.8	5.93
	F2-H3	1	19	65.4	132.8	2.20

Table 4-29. Content uniformity test and Dmax/t value. ***ND: Not determined. MTP: Manuscript to be prepared. Shading row: none individual value greater than ± 10% of the average content but with AV > 15.***

For this reason, a content uniformity test is suggested in the validation of suspensions; to ensure that each dose contents have the right amount of API. Mass uniformity test of multidose containers could be adapted to this task and individual content of 20 doses and its average could be measured. As limits: no more than 2 of the individual content deviates from the average content by more than 10 percent and none deviate by more than 20 percent. RSD could be calculated to translate this deviation, in more than 10 or 20 percent, into a numerical meaning.

In addition, uniformity of dosage units test could be used to calculate content uniformity. Individual content of 10 doses could be used to calculate the acceptance value of each formulation. This would be more precise than the first test and could detect formulations with individual values within the limits ± 10% but with an AV higher than 15 (AV limit for 10 doses), see table 4-29.

Generally speaking, when mass uniformity limits are used to check content uniformity, if a formulation does not meet this test, it will not meet the test for uniformity of dosage units (for example, Flecainide F1). However, in the case of Ursodeoxycholic acid-F1 there is no individual value which deviates by ± 10% of the average content but its AV value is greater than 15. So, as explained before, the determination of AV is stricter than knowning how many individual values deviate ± 10/20% of average content.

Another important point to consider in validation of liquid formulation is the physical stability of suspensions. Understanding the behavior of the formulation is essential to obtain a homogeneous

suspension once it is shaken after several hours of standing. For this reason, rheological studies should be carried out during the validation of suspension. Differentiating between a Newtonian and a non-Newtonian fluid is basic to know how a liquid individualized medicine should be shaken. In the first type, viscosity is independent of shear rate, which means that it does not matter how much a formulation is shaken; its viscosity will not change, like Dexamethasone and Acetazolamide. In the case of the non-Newtonian fluid, viscosity changes with shear rate, if the formulation is shaken vigorously its viscosity decreases and would be easier to re-suspend the API and get a homogeneous formulation after standing; for example, Ursodeoxycholic acid and Acetazolamide [32].

Obtaining a homogeneous suspension after standing can be checked, as done in the case of Dexamethasone, Ursodeoxycholic Acid or Acetazolamide. Each formulation was placed into a 100 ml graduated cylinder and deposited in a 5 ± 0.1 ºC (Fridge-stove P-selecta Welidow type, Spain) for 30 days. Doses (5 ml) were taken from Z1 (top of the formulation) and Z3 (bottom of the formulation) after shaking (10 times inverted 180º) and left to stand for several minutes. Then, their content, expressed as % DV, was studied in order to see how homogenous the suspension was.

Maximum difference between Z1 and Z3, divided by the resting time (Dmax/t), was used as an indicator of homogeneity of a suspension and to know how fast the sedimentation process takes place. Table 4-29 shows Dmax/t values for all the formulations studied by

our group. Although it is not possible to establish a solid correlation between Dmax/t and AV, it can be seen that when Dmax/t has values below 1, AV is less than 15 (pharmacopeia limit for 10 samples). Acetazolamide-F1 formulation, is the only one which, having values for Dmax/t < 1 but shows AV > 15. In this case, another 20 doses should be analyzed to confirm this AV value. More data are needed to confirm this limit value of 1 for Dmax/t to meet content uniformity and confirm homogeneous of suspensions after shaking.

Chemical stability test, following the ICH guideline, should be done during the validation of a liquid formulation either solution or suspension.

Ph. Eur. recommends doing the test of microbiological examination of non-sterile products in case the API does not have antimicrobial activity or there is not preservative in their composition [102]. That was the case of already validated formulations like Flecainide or Dexamethasone which, although their chemical stability was higher than 60 and 40 days respectively, due to the microbiological contamination their stability period was fewer.

According to everything exposed above, a high demanding strategy to validate liquid individualized medicines has been elaborated to ensure QTTP and control CQAs, see figure 4-12.

This strategy can be divided in two different parts, the first is to discern the suitability of the method to validate liquid formulations. The analytical method used needs to comply with some tests according to ICH guideline: precision, accuracy, detections and

quantifications limits; it should be able to detect and quantify degradation products (especially if it could produce health problems above a certain limit). Moreover, the capability of the method to extract the right amount of API from the formulation must be checked too.

The second part concerns the validation of liquid formulations, which should start with its organoleptic properties and pH. If a SOP it is available, organoleptic properties and pH should agree with it. If it is not, this must be taken into account in order to establish the stability period.

Then, once mass uniformity of the liquid formulation has been checked, a distinction between suspension and dissolution must be carried out. In the first case, content uniformity and physical stability must be ensured. Finally, a chemical and if needed, a microbiological stability test should be done. If the results of any these tests, do not meet the requirement standards of quality for any formulation this should be re-designed in order to improve the quality.

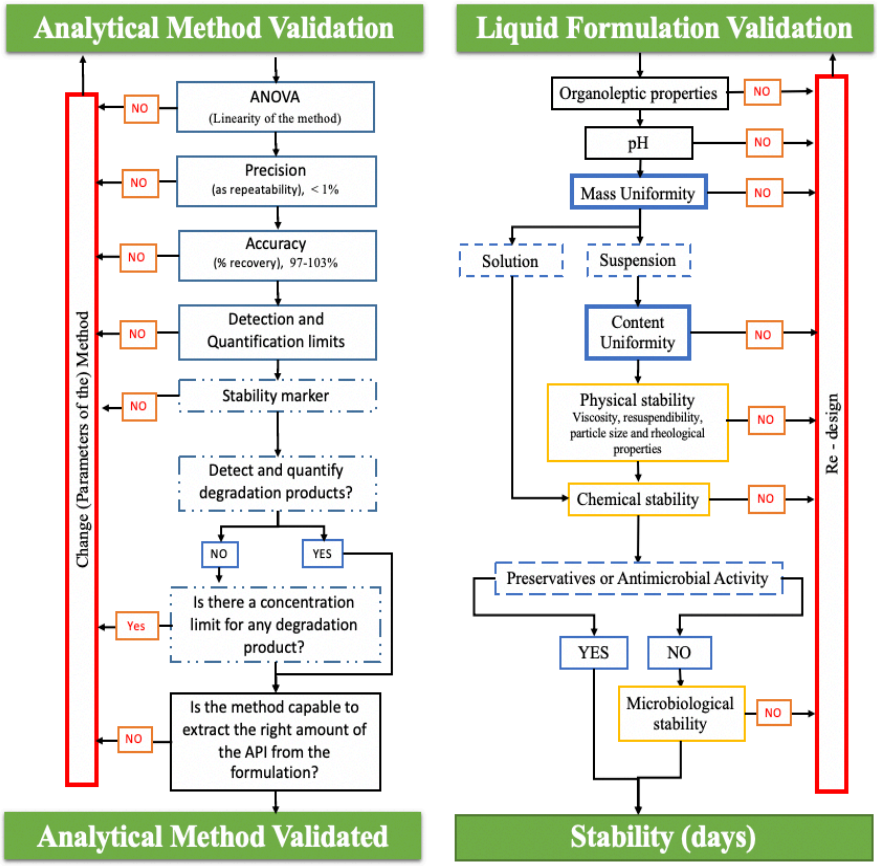

Figure 4-12. A general High-demand strategy to validate liquid oral formulation for pediatric use.

Adapted for thesis from paper:

A high demanding strategy to ensure the highest quality standards of oral liquid individualized medicines for pediatric use. Suárez-González J, Santoveña-Estévez A, Armijo-Ruíz S, Castillo A, Fariña JB.

Published in AAPS PharmSciTech (2019) 20: 208.
doi: 10.1208/s12249-019-1432-x.

Factor Trend (JCR 2018): 2.608
Quartile in Pharmacology & Pharmacy: Q2

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5. ANTI-TUBERCULOSIS MEDICINES

5.1. Introduction.

TB is one of the top 10 causes of death from a single infection agent. It caused approximately 1,3 million deaths; 233,000 were children. In 2017, a total of 292,182 children under 5 years old were reported to have initiated TB preventive treatment [1, 2].

One of the reasons of mortality was the lack of child-friendly formulations for treatment [2]. Since 2015 WHO, NIH and EMA have been publishing articles and guidelines regarding the need for efficient studies for global health and formulations focused on pediatrics TB [3 - 7].

The first-line treatment is based on the combination of three APIs: INH, PZA and RFP, used in the intensive and in the continuation phase. In 2014, WHO increased its daily doses to 10 (7 - 15) mg/kg of INH, 35 (30 – 40) mg/kg of PZA and 15 (10 - 20) mg/kg of RFP based on previous experience and the increase of resistance and dose inefficiency [8].

The first phase usually takes three months where a combination of INH, PZA and RFP is used. In some cases, Ethambutol could be added in this phase to prevent resistances. Then, INH and RFP are used in the second phase for six months.

INH is a prodrug which must be activated by bacterial catalase. Once it is activated, it inhibits the synthesis of mycoloic acids. At therapeutic levels it is bacteriocidal against actively growing intracellular and extracellular *Mycobacterium tuberculosis* microorganisms, responsible of the disease [9]. Its absorption is

influenced by food and therefore it should be taken on an empty stomach. It has a solubility in water of $1.4 \cdot 10^5$ mg/L (at 25 °C) and it belongs to BCS Class I/III [10, 11].

The mechanism of action of PZA is under discussion, as there is no agreement on this issue. PZA diffuses into the bacteria where it is transformed into pyrazinoic acid, the active form of the API, and accumulates inside the bacilli. Some authors thought that this compound inhibited the enzyme fatty acid synthase, which is in charge of producing fatty acids, and others suggest that the accumulation disrupts membrane potential and interferes with energy production [9]. Its solubility is very similar to INHs, $1.5 \cdot 10^4$ mg/L (at 25 °C), and it belongs to class I in the BCS [10, 11].

RFP is an API classified as class IV in the BCS due to its low solubility, pH-dependent (1400 mg/L (at 25 °C)), and low permeability. It is an antibiotic which acts via inhibition of DNA-dependent RNA polymerase, leading to a suppression of RNA synthesis and cell death [9 – 11]. In this case should be taken on an empty stomach: 1 hour before or 2 hours after meals.

For pediatrics, at this moment, there is just one marketed formulation appropriate for children, a 20 mg/ml oral suspension of RFP [12, 13]. That is the reason why a formulation of INH should be validated, thus continuation phase would be covered.

Even though there are SOPs about the development of liquid formulations of INH and PZA, there is a lack of data in terms of dose homogeneity and stability. In addition, the doses of these

formulations have to be checked due to the increase of daily doses. In this regard, the concentration must be high enough to allow the administration of the three APIs in a rational volume for pediatrics (< 10 ml), if they are formulated separately.

These APIs were selected in order to elaborate oral formulations for pediatrics with good standards of quality excipients more suitable for children.

The aim of this chapter is to develop, using the strategy previously presented in chapter 4, child-friendly dosage forms of anti TB drugs to improve adherence and effectiveness.

5.2. Validation of the analytical method.

INH, PZA and RFP were analyzed by reversed phase UPLC in an Acquity UPLC® H-Class System (Waters Corporation, Milford, MA) using two different methods: INH and PZA were analyzed with a method based on an UHPLC gradient method [14] and the RFP was analyzed using a method adapted from HPLC [15].

The phase reserved column used in this study was a XSelect™ CSH™ C18 (75 mm x 2.1 mm id, 2.5 µm) provided by Waters. The mobile phase used for both methods was Acetonitrile and Phosphate buffer 50 mM, pH 3.77 (Acetonitrile: Phosphate buffer, 2:98 (v/v) for INH and PZA, and 38:62 (v/v) for RFP) at a flow rate of 0.5 ml/min and 30 ºC. The UV detection was done at 242 nm.

The validation of the analytical method was done according to the strategy previously shown in chapter 4. Standard solutions with concentrations from 10.0 to 27.0 µg/ml for INH, PZA and RFP were used.

ANOVA was carried out for each API to confirm the linearity of the method, which was studied through rejection of the null hypothesis of deviation from linearity for a significance level of 5%. Characteristics of the method for each API is shown in table 5-1.

API	INH	PZA	RFP
Calibration curve	A = 31,925.3·C	A = 35,181·C	A = - 150,190 + 49,378·C
Correlation coefficient	0.99	0.99	0.98
CV (%)	3.11	3.32	5.89
Precision (%, <1%)	0.28	0.16	0.23
Accuracy (%, 97-103%)	98.0	97.7	97.7
Detection limit (µg/ml)	1.70	1.74	3.10
Quantification limit (µg/ml)	5.16	5.28	9.40

Table 5-1. Characteristic of the method used to the analysis by UPLC of each API. ***C: Concentration. CV: coefficient of variation.***

It must be ensured that the method used is able to follow the degradation of the APIs and check if it is able to detect and quantify degradation products. For that reason, a solution of INH, PZA and RFP with a pH 7 was storage at 50 ºC (Heaeus UT 6060, Spain) during 72 h.

Figure 5-1 shows the chromatogram for each API obtained by the UPLC method as pure patterns and also how these peaks change along time under 50 ºC of storage in a pH of 7: INH (0.6 min); PZA (1 min); RFP (1.6 min). As can be seen, there were a decrease of signals for each API under these storage conditions. Therefore, this method is able to follow the degradation of the different APIs.

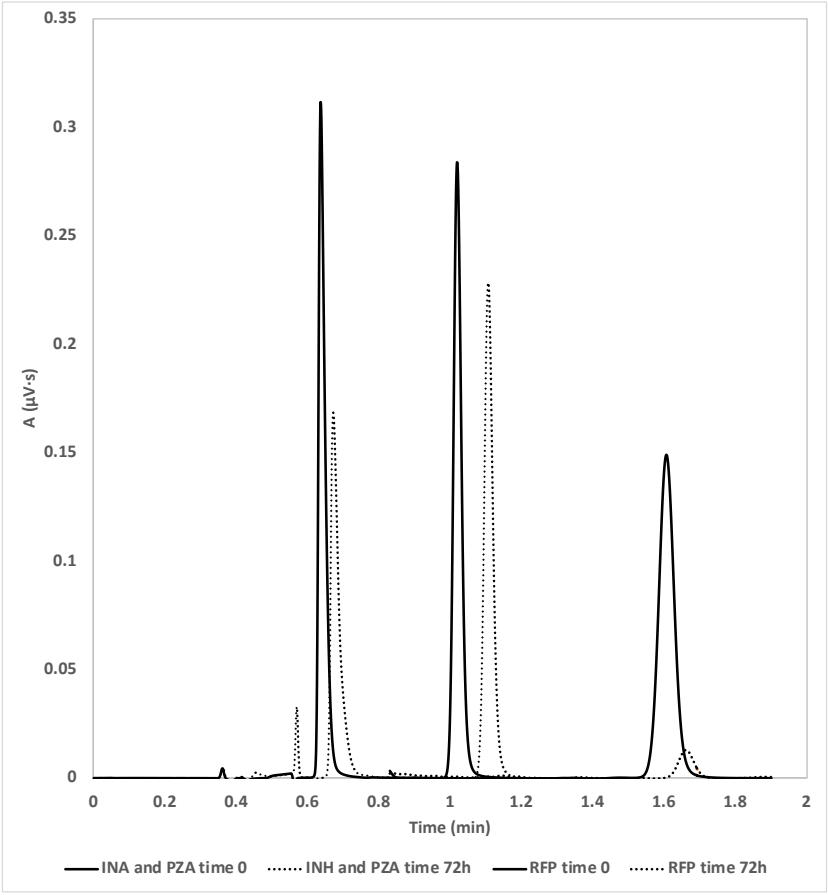

Figure 5-1. INH (0.6 min), PZA (1 min) and RFP (1.6 min) as pure patter chromatographic peaks (continuous bold line); discontinuous line represents the decrease of signals for each API after 72h of storage at 50 ºC in a medium with a pH of 7.

The final step is to check that the method is able to extract the declared amount of each API in each dosage form studied.

The average extraction yield for INH from its solution was 97.6 ± 2.5% so, it can be said that the method is able to extract the right amount of INH.

In the case of tablets, each ingredient of one tablet was weighted, dissolved in 50 ml of methanol and filled with water till 250 ml. Then, its content was filtered using 110 mm filter paper (Albet LabScience, Spain) and was diluted till a concentration which could be measure in the UPLC system. The average extraction yield of each API from the tablets are: 103 ± 2.07% for INH, 98.4 ± 1.95% for PZA and 98.3 ± 0.95% for RFP.

In conclusion, the UPLC method is able to analyze INH, PZA and RFP correctly, with good precision and correct detection and quantification limits. In addition, the degradation of the APIs can be followed (qualitatively), and it is able to extract the right amount of every API from a complex matrix as its average extraction yield is always near 100%.

5.3. Liquid Stability Studies.

One of the main disadvantages of the combination of these three APIs is the chemical interaction between them.

The interaction of RFP with INH it is well described in the literature being this higher in solution. RFP is hydrolyzed under acidic conditions to 3-formylrifamycin, which reacts with INH to form Hydrazine (HYD). HYD converts back to INH and 3-formylrifamycin. As result, INH is recovered but RFP is lost [16]. This is the reaction which could explain the poor bioavailability of RFP [17, 18].

HYD is a degradation product which concentration needs to be checked thus it is considered as a mutagenic and genotoxic degradation product. HYD is included in the ICH guideline M7(R1) as an impurity that must be controlled in pharmaceutical products being 39 µg/day as the acceptable intake [19].

The aim of this section is to check the API content using the new recommended doses, and selecting the pH where the combination at liquid form could be more stable.

- Materials and Method

An *in vitro* study of the stability of the APIs at a temperature of 37 ± 0.1 ºC (Heraeus UT 6060, Spain) and at different pH conditions of the digestive tract (1.5; 3.0; 6.3; 7.4) was performed to know the evolution of its stability along gastrointestinal when they are orally

administered, individually or at FDC without coating at pediatric doses.

The vehicle used as artificial gastric juice was prepared with sodium chloride (Sigma®), pepsin (Vinyals®), purified water and hydrochloric acid (Merck®), the final pH was adjusted with sodium hydroxide (Panreac®) to 1.5 or 3.0 [20]. To simulate the pHs of intestinal tract it was used and artificial intestinal juice prepared with sodium hydroxide (Panreac®), pancreas powder (Escuder®), water and dihydrogen potassium phosphate (Merck®) and finally pH was adjusted to 6.3 or 7.4 with sodium hydroxide (Panreac®) [20]. The pHs were adjusted using a pHmeter at 25 ºC (Crison®).

For every pH, the stability of antiTBs were studied separately (individual samples) and in combination (combined samples) at the minimum doses currently recommended by WHO: 7, 30, and 10 mg/kg/day for INH, PZA and RFP respectively [6, 21].

A body weight of 10 kg (one year old child) was considered to calculate the required doses. AntiTBs concentrations were calculated taking into account the normalized gastric volume of 40 mL for an infant with 10 kg on body weight [22].

Then, the concentrations studied were 1.75 and 7.5 mg/ml for INH and PZA respectively. RFP was studied at 2.5 mg/ml at pH 1.2 and at 0.5 mg/ml at pH 3 - 7.4 due to its pH dependent solubility, which decrease with increase in pH [10, 23].

At different times, the samples were diluted to a concentration into the linear interval of concentrations studied and were analyzed

immediately by UPLC. The evolution of the chromatographic areas was fitted to different kinetic orders to calculate the shelf life at which the 5% of the initial dose is degraded (t5%).

- Results and discussion

Table 5-2 shows the remaining percentage at 0.75 h, mean gastric residence time for aqueous solutions in infants, of every API stored at simple or combined doses at pH interval between 1.25 and 7.4 and 37 ºC [24].

	Individual	**Combined**
	INH	
pH	**DV (% average ± SD)**	**DV (% average ± SD)**
1.25	100.1 ± 2.84	97.2 ± 1.08
3.00	95.7 ± 1.10	99.8 ± 0.00
6.30	98.3 ± 5.25	103.2 ± 5.22
7.40	104.4 ± 4.00	99.8 ± 2.00
	PZA	
pH	**DV (% average ± SD)**	**DV (% average ± SD)**
1.25	112.6 ± 3.03	99.8 ± 0.20
3.00	99.2 ± 1.76	99.8 ± 0.00
6.30	95.0 ± 2.14	96.2 ± 1.99
7.40	97.4 ± 1.20	99.7 ± 4.10
	RFP	
pH	**DV (% average ± SD)**	**DV (% average ± SD)**
1.25	94.7 ± 0.18	89.4 ± 2.28
3.00	89.0 ± 0.82	88.7 ± 3.90
6.30	96.6 ± 5625	95.05 ± 12.8
7.40	103.4 ± 1.40	100.8 ± 3.50

Table 5-2. % of API, express as DV, after 0.75h storage at different pH and 37 ºC. (n=2). ***DV: declared value; API: active pharmaceutical ingredient; Soft shading values: outside the limits of ± 5% of DV.***

As it can be seen, INH and PZA are above 95% of initial dose at all pH conditions at simple or combined doses. RFP maintain it initial percentage above 95% at pH 6.3 and 7.4 for both type of samples, simple or combined. But, at more acidic pHs (1.25 and 3.0) its initial percentage is below 95% at both type of samples.

The time at which the t5% of initial drug dose has been degraded has been calculated through this degradation kinetics. The best adjustment to a kinetic of degradation for every API analyzed at simple or at combination samples is shown in table 5-3.

	Individual			**Combined**		
INH						
pH	**Order**	**t5% (h)**	**R**	**Order**	**t5% (h)**	**R**
1.25	2	6.6	0.98	0	7.6	0.99
3.00	1	6.2	0.90	2	6.6	0.69
6.30	2	3.9	0.91	2	11.2	0.92
7.40	0	18.0	0.99	2	9.4	0.97
PZA						
pH	**Order**	**t5% (h)**	**R**	**Order**	**t5% (h)**	**R**
1.25	2	> 24	0.97	0	> 24	0.92
3.00	2	8.2	0.88	-	> 24	-
6.30	2	> 24	0.94	-	> 24	-
7.40	2	> 24	0.84	1	> 24	0.99
RFP						
pH	**Order**	**t5% (h)**	**R**	**Order**	**t5% (h)**	**R**
1.25	1	1.2	0.99	1	0.5	0.97
3.00	1	2.1	0.99	1	0.7	0.99
6.30	1	2.5	0.95	2	1.8	0.94
7.40	1	7.8	0.97	1	5.8	0.99

Table 5-3. Kinetic orders to calculate the shelf life at which 5% of the initial dose is degraded (t5%). ***R: correlation coefficient.***

As can be shown, INH and PZA have a t5% above 0.75 h at every pH, when they were administered at simple or combined doses. RFP, at simple doses, maintain above 95% of initial dose more than 1 h, but in combination, at pH 1.25 and 3.0, it is degraded in more percentage than 5% before the stomach empties out.

If an API has a t5% below mean gastric residence pediatric time for aqueous solutions, 0.75 h, a 5% of the initial dose is degraded before reaching to duoden for its absorption. Only in case of RFP, at pH 1.25 and 3.0 at FDC doses, t5% is below 0.75 h. Our results show that RFP at pediatric doses is the antiTBs more unstable during their residence at stomach. This instability increases when it is administered at combined dose probably due to, as literature postulates, the interaction with INH when they are administered in combination below pH 2, among other reasons [25].

- Conclusion

AntiTBs of first-line treatment in pediatrics can be administered in combination at liquid forms, however it does not ensure complete bioavailability of RFP due to it instability at acid conditions and the presence of INH. Owing to the gastric pH in infants is more basic than adults, the combination as liquid oral formulation must be formulated with a buffer at pH between 6.3 - 7.4 to increase the stability of RFP, which would condition its better absorption in duoden and its higher bioavailability [26].

Adapted for thesis from paper:

Stability study of Isoniazid and Rifampicin oral solutions using hydroxypropyl-β-cyclodextrin to treat tuberculosis in paediatrics. Santoveña-Estévez A, Suárez-González J, Cáceres-Pérez AR, Ruíz-Noda Z, Machado-Rodríguez S, Echezarreta M, Soriano M, Fariña JB.

Published in Pharmaceutics 2020, 12(2), 195.
doi: 10.3390/pharmaceutics12020195

Factor Trend (JCR 2018): 4.773
Quartile in Pharmacology & Pharmacy: Q1

5.4. Dosage Forms.

5.4.1. Liquid Dosage Forms: Isoniazid.

The medium oral dose of INH for children till 25 kg is 10 mg/kg (7 – 15 mg/kg). The pH where the INH presents the maximum stability is 6 [28].

INH is available in France, Spain and USA in tablet form and as an injectable solution. USA is the only country where a syrup of INH is available with a concentration of 50 mg/5 ml [12, 13, 29].

Up until now, hospital pharmacies or community pharmacies in Spain have been using SOPs in order to prepare oral liquid formulations of INH to treat the pediatric population [30]. However, this formulation has a concentration of 10 mg/ml which means that a child of 10 kg of body weight will need 100 mg of INH, 10 ml of the solution. This volume is greater than the recommended dose for children weighing 10 kg (two years according to WHO tables), 5 ml. [31, 32]

Therefore, a new formulation of 50 mg/ml of INH, recommended by Piñeiro et al. published in 2016, with a higher concentration was tested following the strategy previously described: see table 5-4 [28].

Ingredient	F1
Isoniazid (g)	5
Sorbitol solution 70% (ml)	50
ACWP (qs, ml)	100

Table 5-4. Composition of formulations of INH. ***ACWP: Aqua conservans without propyleneglycol. Qs: amount which is enough to complete.***

SOP for the elaboration of the formulation can be found in the annex.

The use of a water solution of sorbitol at 70% instead of a simple syrup was to avoid the inactivation of isoniazid due to condensation process of sucrose. [33].

- Quality Control

The validation of this formulation was done using the strategy mentioned in chapter 4.

The solution was odorless and presented a transparent aspect when elaborated with a pH of 6.34. The following step was to test that the formulation meets the mass uniformity test of the Ph.Eur., see table 5-5. As can be seen, not one of the individual values deviate more than ± 10% and therefore complies with the requirements. As it is a solution, it is understood that the API is homogeneously distributed in the formulation, thus ensuring the content uniformity of the formulation.

Dose	Dw (g)	
	F1	
1	2.21	
2	2.23	
3	2.22	
4	2.22	
5	2.22	
6	2.21	
7	2.21	
8	2.22	
9	2.22	
10	2.23	
11	2.23	
12	2.22	
13	2.21	
14	2.23	
15	2.22	
16	2.22	
17	2.22	
18	2.22	
19	2.20	
20	2.21	
Average	2.22	
	LL	**UL**
10%	1.99	2.44
20%	1.77	2.66

Table 5-5. Mass uniformity test of the doses (5 ml) of F1 at time 0.

Finally, the last step was to check the chemical stability of the formulations. As it can be seen in figure 5-2, the formulation is chemically stable up to 40 days regardless of the storage condition.

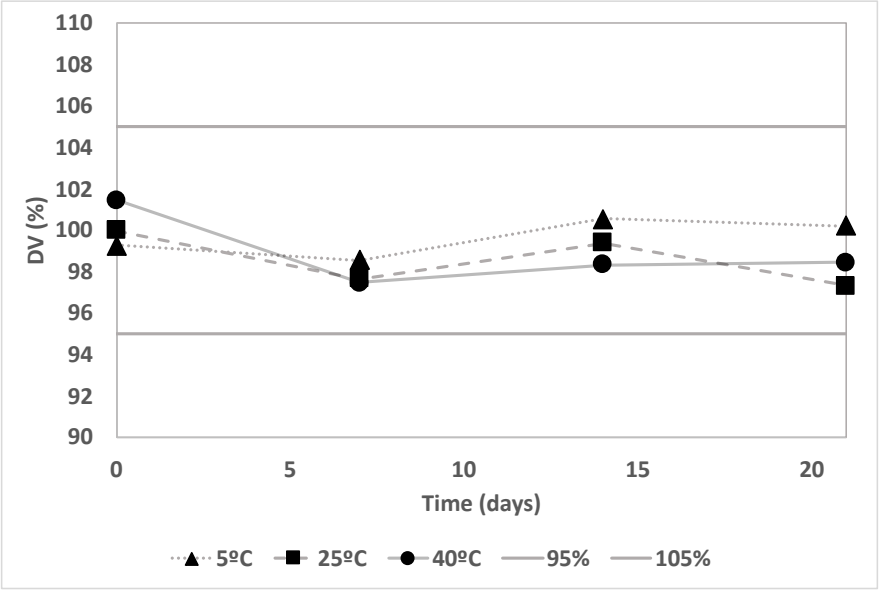

Figure 5-2. Percentage of Isoniazid remaining in F1 after 40 days of storage at 5, 25 and 40 °C. Continued lines are the upper and lower limits established at 100 ± 5 %.

At 40 ºC and 40 days of storage a new peak was observed at 0.58 minutes with a very low signal. In order to increase such a signal, a new formulation was made and stored at 60 ºC for 40 days, figure 5-3.

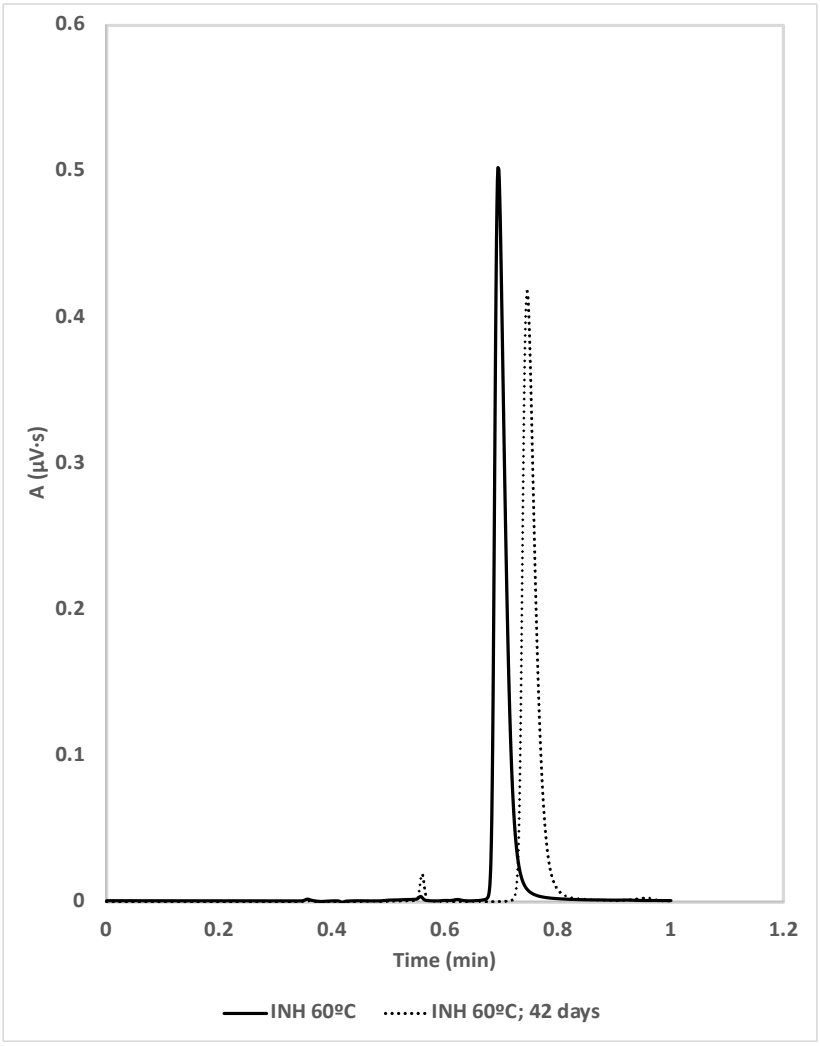

Figure 5-3. INH individualized medicine at time 0 (continuous bold line), Discontinuous line represent how the area of the API changes along time when it is placed at 60 ºC. A new peak appears at 0.58 min.

This degradation could be the isonicotinic acid due to the degradation mechanism of INH. HYD could produce health problems in pediatrics but it cannot be detected using liquid chromatography without previous steps of derivatization.

Merino-Bohorquéz et al. studied the amount of HYD intake in one dose of a formulation of INH with the same composition. The amount of HYD found in a dose of 6 ml of this formulation stored for 90 days at 5 and 25 ºC was 3.23 and 20.34 µg, below the daily intake established by ICH (39 µg/day) [19, 34].

In addition, the pH was measured during the chemical stability test, table 5-6. It was checked to find out if its value deviates during storage from the pH where the API is most stable; around 6. As may be seen, the pH of our formulation remains near 6 during 40 days of storage.

		Time (days)				
	Temperature (ºC)	**0**	**7**	**14**	**21**	**40**
F1	5 ºC	6.31	6.04	6.24	7.01	6.56
	25 ºC	6.32	6.28	6.11	6.72	6.29
	40 ºC	6.34	6.23	5.83	6.27	5.84

Table 5-6. pH evolution at F1 during the 40 days storage at 5, 25 and 40 °C.

In conclusion, a high-quality formulation of INH with a concentration of 50 mg/ml has been validated. This new formulation ensures a good volume of dose, meeting EMA recommendations in this matter. It is stable for 40 days when stored at 5 or 25 ºC. Therefore, the continuous phase is completed for pediatrics; an appropriate RFP medicine is available on the market.

5.4.2. Solid Dosage Forms.

The development of a fixed-dose combination (FDC) dosage form which combined the three APIs in one formulation it is preferred for the intensive phase of treatment [35 – 37].

The development of a rational FDC should be based in [38]:

- APIs should have different mechanism of action.
- Pharmacokinetics should be similar.
- The combination of APIs should not have supra-additive toxicity.

The main advantages of using FDCs is that treatment is simplify increasing adherence and patient outcomes, a greater efficacy it is obtained and less adverse reaction in comparison with monotherapy at higher doses are detected [39, 40].

There is not enough information about the stability of this APIs in solid dosage forms at the previously mention new doses. Singh et al. in 2004 did a stability test using different commercially available FDC for adults (packaged and unpackaged) at different conditions of storage. At 3 months of study a 30% of degradation was detected for INH, PZA and RFP at ambient conditions. Under accelerated conditions the degradation increase till 40 - 90% for INH and RFP. In the case of PZA such degradation it is similar to ambient conditions [17, 41].

Due to the fact that there is not an adequate FDC for pediatrics in the market, TB alliance presented a FDC dispersible tablet which has been prequalified by the WHO. This new formulation is made with the recent recommended doses of APIs, but contains excipients such as: povidones, aspartame and flavors which may not be suitable for pediatrics [42 – 44]. For example, povidone have been responsible of serious allergic reactions in children [45] and there is literature about seizures and increase of body temperature produced by aspartame [46]. In addition, this cannot be used in children with phenylketonure.

The aim is to develop a new FDC which takes into account the recommendations of the EMA about the use of excipients accepted for pediatrics and in their right amount in order to prevent side effects [3]. In addition, other recommendations regarding dose accuracy [28].

- Materials and Method

INH (Acofarma®), PZA (Sygma-Aldrich®) and RFP (Fagron®) has been used as the API to develop a FDC Tablet for TB treatment. The following excipients were used: AcDiSol® (Croscarmellose Sodium, FMC Corp.), Avicel® PH102, (Microcrystalline Cellulose, FMC Corp.), Explosol® (Sodium Starch Glycolate, Blanver), CompactCel® (Isomalt, sucralose, betadex, carboxymethylcellulose sodium, Biogrund GmbH), Luzenac® (talc, Imerys Talc) and CabOSil® (fumed silica, Cabot CorporaFon). Purified water was obtained from a water purification system (Puranity TU 12, VWR, USA).

APIs and excipients were weighted and blended in a V-Type Blender (FTLMV-0,5, FILTRA® VIBRACIÓN, Spain) with a mixing power of 0.12 kW for 5, 10 and 15 minutes. At each time the powder mix was placed in a rectangular container which was divided in 5 different zones and a sample of 200 mg was taken. Finally, its content in API was determined as described above.

Process Capability index (CpK in equation 1) was used to know if the mixing process satisfied quality specification in terms of content uniformity.

$$CpK = \min\left(\frac{USL - \mu}{3\sigma}, \frac{\mu - LSL}{3\sigma}\right) \quad (1)$$

where μ and σ are average and standard deviation respectively, and USL/LSL are upper and lower specification limits using ±15% as limits for the theoretical content that should be in these samples.

Flow properties of the powder mix were evaluated according to Ph. Eur. tests: angle of repose (Granulate Tester GTB, Erweka, Germany), Carr's Index and Hausner's Ratio (Tapped Density Tester SVM 223, Erweka, Germany). Other flow properties such as: flow rate, volume flow rate, mass flow rate and flow angle were tested using a 100 ml steel hopper and a 15 mm cylindrical nozzle [47, 48].

Tablets were obtained by direct compression of the powder mix in an instrumented eccentric tablet machine XP1, Research Tablet Press (Korsch, Germany) using 15-mm flat-faced bisect punches (FFBP) and 12-mm flat-faced with beveled edge (FFBE). Tablets were produced with different compressions forces and press speed.

Compression force and press speed were controlled by PharmaReseach® software (Korsch, Germany).

The variables selected for the experimental design of dispersible tablets were the levels of excipients with function as disintegrate (AcDiSol® and Explosol®) and compression forces. These were chosen in order to evaluate their influence on disintegration time and friability on 15-mm tablets. For this purpose, a factorial design based on 3 quantitative factors (compression force and concentration of AcDiSol® and Explosol®) at three different levels each was used.

Table 5-7 shows the coded levels and values of the design variables. Therefore, a 3^3-factorial design was performed with 27 different combinations of variables and replicating the center point three times, which meant the elaboration of 30 batches. Sodium starch glycolate shows better properties than croscarmellose sodium according to the literature [49 – 53]. For this reason, percentages from 2-9% w/w of Explosol® where used and 0-5% w/w of AcDiSol® in order to verify if the second one improves disintegration time or friability.

Factor	-1	0	+1
% AcDiSol® (A, w/w)	0.00	2.50	5.00
% Explosol® (B, w/w)	2.00	6.00	9.00
Compression Force, kN (C)	11.0	14.0	16.0

Table 5-7. Coded levels and values of design variables to the development of dispersible tablets.

Table 5-8 shows the final composition of formulation 1 to 9, each of which was compressed at three compression forces to develop the dispersible tablets.

	Formulation								
Ingredient (mg)	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Isoniazid	50	50	50	50	50	50	50	50	50
Pyrazinamide	150	150	150	150	150	150	150	150	150
Rifampicine	75	75	75	75	75	75	75	75	75
Ac-Di-Sol®	0	0	0	23	23	23	46	46	46
Avicel®	513	476	450	490	453	427	467	430	404
Explosol®	18	55	81	18	55	81	18	55	81
CompactCel®	63	63	63	63	63	63	63	63	63
CabOSil®	9	9	9	9	9	9	9	9	9
Luzenac®	22	22	22	22	22	22	22	22	22
Total (mg)	**900**	**900**	**900**	**900**	**900**	**900**	**900**	**900**	**900**

Table 5-8. Composition in mg of formulations 1 to 9, each of which was compressed to the three compressions forces, to develop the dispersible tablets following the experimental design.

A statistical approach is used to fit a model using Design-Expert 9.0.3 (Stat-Ease Inc., Minneapolis, MN, USA). Logarithmic values for disintegration time and inverse of square root for friability were used to improve the quality of the model. P-value was used in each case to know which terms were significant for each response and R-squared

(R^2), adjusted R-squared (R^2adj) and predicted R-squared (Q^2) were used to measure the goodness of the model [54]. All tests were performed at 5% level of significance ($\alpha = 0.05$). The complete model equation is as follows:

$$y = \beta_0 + \beta_A X_A + \beta_B X_B + \beta_C X_C + \beta_{AB} X_A X_B + \beta_{AC} X_A X_C + \beta_{BC} X_B X_C + \varepsilon \quad (2)$$

where A is AcDiSol® (%, w/w), B is Explosol® (%, w/w) and C is compression force (kN).

When a formulation complied with the requirements of dispersible tablets in terms of friability and disintegration time the influence of press speed is tested tableting at 10, 25 and 50 cycles/minute. Tablets are then characterized testing disintegration time, friability, tensile strength, content uniformity, fineness of dispersion and effectiveness of score lines as CQAs.

Disintegration time: Disintegration time of 6 tablets was determined using a disintegration tester (Disintegrator Tester ZTx20, Erweka, Germany) following the Ph. Eur. recommendations [55]. The time that all the tablets disintegrated was used or accepted for the study.

Friability: It was studied using a friability test (Tablet Friability/Abrasion Tester TAR Series, Erweka, Germany) following the Ph. Eur. guideline [56].

Tensile strength, TS: This was measured for each batch (Hardness Tester TBH 125 Series, Erweka, Germany), following the recommendations given by Ph. Eur. and USP, by equation 3 [57, 58].

$$TS = \frac{2 \cdot p}{\pi \cdot d \cdot l} \tag{3}$$

where p, d and l are: tablet breaking force, tablet diameter and tablet thickness, respectively.

Content Uniformity: This was tested according to the uniformity of dosage units test by Ph. Eur. [59]. The content of 10 dispersible tablets for each batch were analyzed using a UPLC system and their acceptance value was calculated.

Fineness of dispersion: Two dispersible tablets dissolved in 100 ml of purified water must pass through a sieve with 710 μm of nominal mesh aperture [60].

Effectiveness of scoring lines: As 15-mm tablets have breaking marks suitability must be tested in terms of mass uniformity. First, 30 tablets were choosen randomly and broken by hand. One half was used for the test and the other half were rejected. 30 parts were weighted, and the average mass was calculated.

Critical Process parameters, such as compression force and press speed, were controlled and signals were imported from Extended Data Analysis® (EDA) (Korsch, Germany) and analyzed using a macro for MS Excel (Microsoft Corporation, USA). Compression process were controlled using a control chart of compression forces

and establishing stop reasons when the compression force was greater than 3% of target force.

K value was obtained from the slope of straight-line interval of the Heckel plot using the data from the space between the upper and lower punch and matrix diameter to calculate the relative density of the material (D) according to equation 4 [61, 62].

$$\ln\left(\frac{1}{1-D}\right) = K \cdot F + A \tag{4}$$

where D is relative densitity, F is compression force and K and A are constant.

Mean yield pressure (Py) and strain-rate sensitivity (SRS) were calculated using K following equations 5 and 6.

$$Py = \frac{1}{K} \tag{5}$$

$$SRS = \frac{Py1 - Py2}{Py1}.100 \tag{6}$$

where Py1 and Py2 are the yield pressure at low (10 strokes/min) and high speed (50 strokes/min), respectively.

Plasticity, equation 7, was estimated from the force-displacement compression profile using the average energy consumption within the different compaction phases: W1 (friction work), W2 (net work) and W3 (elastic work). [61, 63 – 65].

$$PL = \left(\frac{W2}{W2+W3}\right).100 \tag{7}$$

- Results and Discussion

Selection of excipients and previous tests.

The selection of excipients was carried out taking into account the complexity of our ideal formulation. All excipients need to be suitable for direct compression and provide good flow properties to ensure API's content. A taste-masking excipient is needed as INH has a bitter taste and they have to be accepted for pediatrics. In addition, the tablets must disintegrate in less than 3 minutes and have a friability below 1% [56, 66].

The first selection of excipients was done taking into account the most common excipients used in published papers related to the development of dispersible tablets: croscarmellose sodium, sodium starch glycolate, crospovidone, microcrystalline cellulose, magnesium stearate and talc [67-71]. Therefore, we selected the excipients according to their function (lubricant, (super)disintegrant, glidant, etc.), physical characteristics (water-solubility, particle size and shape) and safety.

All of these excipients are generally recognized as safe (GRAS). However, due to the numbers of tablets which have to be taking to treat TB, some excipients were preferred instead of others. Crospovidone was not included in the formulation due to the lack of data in terms of acceptable daily intake and safety in children. In addition, as lubricant, talc was preferred instead of magnesium stearate because of its laxative effect and mucosal irritation when large quantities are taken [51].

Previous test of powder flow, mixing time to obtain a homogenous powder and tableting process were done to find the right number and percentage of each excipient.

Our objective was to obtain dispersible tablets with a disintegration time below than 3 minutes, according to WHO requirements, so we need a high disintegration power with the lower amount of excipient. For that reason, superdisintegrants were preferred than ordinary disintegrants.

Explotab® and AcDiSol® were selected as theses excipients have a high disintegration power at low concentrations and physical properties useful to develop these tablets. The disintegration power of Explotab® does not seem to be affected by concentration of lubricant or compression force. AcDiSol® also has a good disintegration power and imparts exceptional long-term dissolution stability in comparison to other superdisintegrants. However, at high concentrations of excipient, tablets could become soft when stored with an elevated RH [49 – 53].

The relationship between concentrations of excipient and disintegration time and friability are very important and therefore studied carefully.

During the first trials, adherence of powder mix to the surface of punches was noticed which made the tableting process difficult. To reduce such adherence, talc (Luzenac®) was increased from 1 to 2.5% w/w improving the situation.

CompactCel® was added to the formulation in order to mask the bitter taste of INH; one of the problems of patient's poor adherence to treatment [72]. This complex excipient was chosen instead of other excipients due to the composition (isomalt, sucralose, betadex, carboxymethylcellulose sodium), and also because of the superior performance in terms of disintegration time and friability. It was added at 7% w/w because, along with microcrystalline cellulose (Avicel®), reduced powder adherence to punch surfaces [73].

The flow properties according to Carr's Index, Hausner's Ratio and flow angle were very poor when no glidant was used. Although the incorporation of 1% w/w CabOSil® did not improve the value of these parameters it produced a relevant improvement in flow rate, from 95.8 to 28.8 s/100 g [47, 48, 51].

When 50% w/w of Avicel® was added, any punch surfaces adherence was observed, regardless of type (FFBP or FFBE), and disintegration time and friability were near to the recommendations established by EMA and WHO for dispersible tablets, 2.33 min and 0.87%. Moreover, the use of this concentration of Avicel® reduced the blending process from 20 to 15 minutes due to its particle size and shape.

Therefore, taking into account the results of the previous test, we adjusted the excipients and their concentrations as follows: 2.5% w/w Luzenac®, 1% w/w CabOSil®, 7% w/w CompactCel® and 50% w/w of Avicel®.

Optimization of blending process

Cpk value could be used to classified production process, according to USP: “exceeding 1.33 show that the process is adequate to meet specifications” [74].

To establish an optimum mixing time, Cpks values were estimated. Table 5-9 shows the evolution over time for each API. As can be seen, at 15 minutes the blending process is under control (CpK > 1.33). INH is the only one that required more time to reach this CpK value, due to the lower proportion in the mixture. The other APIs showed CpK > 1.33 after 5 minutes of mixture.

Cpk			
Time (min)	**5**	**10**	**15**
INH	0.89	0.61	3.79
PZA	3.05	2.63	2.14
RFP	3.18	1.98	2.53

Table 5-9. Evolution of CpK over time for each API.

According to Hausner’s ratio and Carr’s index, the flow properties of the powder can be classified as acceptable, which agrees with angle of repose (39.3, fair). Mass flow rate, volume flow rate, flow rate and flow angle were: 4.59 ± 0.99 g/s, 10.1 ± 0.40 s/100 ml, 20.0 ± 0.87 s/100 g and 78.2 ± 1.72º, respectively.

Experimental design

As already stated, we wanted to evaluate the influence of the concentration of excipients (AcDiSol® and Explosol®) and compression force on the disintegration time and friability of 15-mm water-dispersible tablets.

The results obtained with the different batches of tablets produced according to the experimental design are shown in table 5-10.

	Factors			Responses	
Batch Nº	**A (%, w/w)**	**B (%, w/w)**	**C (kN)**	**Disintegration time (seconds)**	**Friability (%)**
1	0.0	2.0	11	69	1.44
2	0.0	2.0	14	145	0.95
3	0.0	2.0	16	270	0.82
4	0.0	6.0	11	80	1.36
5	0.0	6.0	14	141	1.02
6	0.0	6.0	16	195	0.83
7	0.0	9.0	11	100	1.43
8	0.0	9.0	14	132	0.97
9	0.0	9.0	16	170	0.74
10	2.5	2.0	11	124	1.09
11	2.5	2.0	14	128	0.79
12	2.5	2.0	16	140	0.75
13	2.5	6.0	11	86	1.31
14	2.5	6.0	14	120	0.91
15	2.5	6.0	16	146	0.84
16	2.5	9.0	11	85	1.79
17	2.5	9.0	14	107	1.17
18	2.5	9.0	16	130	0.90
19	5.0	2.0	11	145	1.05
20	5.0	2.0	14	155	0.78
21	5.0	2.0	16	175	0.64
22	5.0	6.0	11	124	1.53
23	5.0	6.0	14	132	1.07
24	5.0	6.0	16	141	0.88
25	5.0	9.0	11	113	1.66
26	5.0	9.0	14	121	1.10
27	5.0	9.0	16	135	0.84
28	2.5	6.0	14	128	1.02
29	2.5	6.0	14	142	0.91
30	2.5	6.0	14	139	0.93

Table 5-10. Experimental Results: disintegration time and friability obtained with different batches of tablets according to the experimental design. *A: AcDiSol® (%, w/w). B: Explosol® (%, w/w). C: Compression Force (kN)*

Using a regression analysis, the relation between the studied factors (excipients and compression force) with the changes produced in tablet properties (disintegration time and friability) were studied. The statistical parameters to evaluate the goodness of the model is shown in table 5-11.

	Disintegration time (min)	**Friability (%)**
Model (p-value)	< 0.0001	< 0.0001
R-Squared (R^2)	0.81	0.94
Adjusted R-Squared (R^2_{adj})	0.76	0.92
Predicted R-Squared (Q^2)	0.57	0.88
Lack of Fit (p-value)	0.19	0.36

Table 5-11. Quality of the experimental design using regression analysis.

Values for R^2, R^2adj and Q^2 are greater than 0.5, and their difference is not less than 0.3. Therefore, the indicators suggest a high quality of the model for fitting and predicting the effects on disintegration time and friability [54]. This lack of Fitting in both responses were not significant.

Once the non-statistically significant terms were removed, the model equation for each response was:

$$\text{Log (disintegration time)} = 2.11 - 0.04 \cdot \text{B} + 0.11 \cdot \text{C} - 0.09 \cdot \text{AC} \quad (8)$$

$$\frac{1}{\sqrt{Friability}} = 0.99 - 0.06 \cdot B + 0.13 \cdot \text{C} - 0.05 \cdot AB \quad (9)$$

where: A is AcDiSol® (%, w/w), B is Explosol® (%, w/w) and C is compression force (kN).

As may be seen from the equations, the concentration of A does not have any statistically significant influence over disintegration time (p-value: 0.38) or friability (p-value: 0.37). The concentration of B has a negative influence over disintegration and a positive one over friability, mainly because of its properties as a superdisintegrant (p-value: 0.0109 and < 0.0001, respectively) [51]. C, as expected, increase disintegration time and reduce friability of the tablet (p-value <0.0001 for both responses).

There are two interactions which are statistically significant (p-value < 0.0001) and both showed a negative effect over their response: AC in the case of disintegration time and AB for friability. Such negative effect means that the effect of one parameter is lower when the value of the other is high.

Figure 5-4 shows the 3D response surface for the predicting model. In red, highest desirability, the conditions where the minimum disintegration time and friability is obtained using the lowest number of excipients. Therefore, the tablets that meet these conditions are those corresponding to formulation 3 (table 5-8) produced without AcDiSol® with 9% w/w of Explosol® and a compression force of 16 kN (batch number 9 in table 5-10).

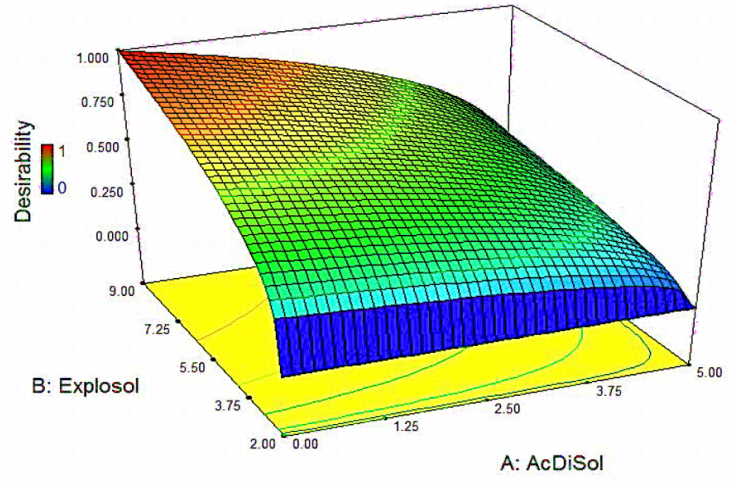

Figure 5-4. Response 3D-surface for factors A and B when C is 16 kN.

This batch was also compressed using the 12-mm FFBE punches with the same compression pressure (9 kN/cm^2). As can be seen, in table 5-12, when the 12-mm punches were used, the weight of the tablets was reduced by 50% and they meet disintegration time (< 3 min) and friability test (<1% less of initial weight)

Formulation 3 (Batch Nº9)		
Punches	**12-mm FFBE**	**15-mm FFBP**
Compression Pressure (kN/cm^2)	9.00	
Compression Force (kN)	10.0	16.0
Tablet weight (mg)	450	900
Disintegration time (seconds)	150	170
Friability (%)	0.09	0.74

Table 5-12. Comparation of tablet properties using the same compression pressure and composition but different punches.

Since the previous tableting process were done at 10 cycles/min of press speed, the influence of this on CQAs using 15- and 12-mm punches at a compression force of 16 and 10 kN respectively was tested. Tables 5-13 and 5-14 shows how quality attributes changes with press speed for both punches.

Punches			**12-mm FFBP**		
Cycles/minute			**10**	**25**	**50**
Mass variation (g)	Average ± SD		0.45 ± 0.002	0.44 ± 0.003	0.41 ± 0.002
	RSD		0.53	0.57	0.53
Friability (%)			0.09	0.14	0.17
Disintegration time (sec)			150	136	125
Tensile Strength (N/cm^2)	Average ± SD		165 ± 5.61	151 ± 4.10	145 ± 5.69
	RSD		3.39	2.71	3.93
Content Uniformity	INH	% DV ± SD	102 ± 3.37	99.1 ± 4.41	98.6 ± 11.1
		AV	8.10	10.59	11.09
	PZA	% DV ± SD	93.7 ± 1.10	92.9 ± 1.02	98.5 ± 8.11
		AV	7.46	7.99	8.12
	RFP	% DV ± SD	92.75 ± 1.17	90.4 ± 2.63	98.5 ± 10.6
		AV	8.56	14.40	10.60
Fineness of dispersion			Ok	Ok	Ok

Table 5-13. Variation of CQAs according to press speed for 12-mm punches. ***SD: standard deviation; RSD: relative standard deviation; DV: declared value; AV: acceptance value.***

Due to the improved strength transmission at slower press speed, at 10 cycles/min TS showed the highest value, whereas at 50 cycles/min showed the lowest thus this will have an influence on friability and disintegration time. At the slowest press speed, as the TS increases

friability decreases and so will require a longer period to disintegrate. 12-mm tablets showed a 6 times lower friability than the larger ones. This could be explained by the best strength transmission when a flat face is used compared to when score lines are present. Furthermore, due to the beveled edge in these tablets the possibility of chipping during the friability test is reduced [75, 76]. Acceptance value (VA) was always below 15, regardless of press speed or the type of punches used.

Punches			**15-mm FFBE**		
Cycles/minute			**10**	**25**	**50**
Mass variation (g)	Average ± SD		0.92 ± 0.004	0.90 ± 0.004	0.89 ± 0.012
	RSD		0.43	0.44	1.32
Friability (%)			0.85	0.87	1.01
Disintegration time (sec)			160	155	132
Tensile Strength (N/cm^2)	Average ± SD		171 ± 9.82	165 ± 9.39	159 ± 3.64
	RSD		5.73	5.68	2.29
Content Uniformity	INH	% DV ± SD	102 ± 2.64	97.2 ± 3.21	101 ± 3.06
		AV	6.74	9.05	7.35
	PZA	% DV ± SD	100 ± 0.87	99.1 ± 1.62	97.7 ± 1.45
		AV	2.08	3.88	4.27
	RFP	% DV ± SD	100 ± 1.86	99.5 ± 2.35	99.6 ± 1.33
		AV	3.99	5.63	3.19
Fineness of dispersion			Ok	Ok	Ok

Table 5-14. Variation of CQAs according to press speed for 15-mm punches.
SD: standard deviation; RSD: relative standard deviation; DV: declared value; AV: acceptance value.

In the case of 15-mm tablets, the tableting process could be done up to 25 cycles/minute ensuring good quality attributes since at 50 cycles/minute friability is greater than 1%. The highest press speed could be used for the 12-mm tablets since it showed good quality attributes at this speed. In this sense, this could be an alternative, in terms of industrial development, due to the improved friability in comparison with 15-mm tablets. However, as these tablets have 50% of the required daily dose, two tablets would need to be taken instead of one.

RSD values from TS are always higher in 15-mm tablets than 12-mm which means a higher variability in compression pressure during production process. This could be related to the differences in the filling of the die and punches shape.

Finally, effectiveness of score lines: 15-mm tablets produced at 25 cycles/minute fulfilled this test since none of the 30 half tablets deviate in more than ± 15% of the average mass, which means that they could be split correctly. Moreover, the subdivision of these tablets could be useful to improve the dose scheme.

Compaction data obtained from an instrumented tableting machine enables rationale scientific designing of a tablet formulation with the desired quality attributes. Additionally, the parameters derived from Heckel plot like mean yield pressure and SRS or those obtained from compression curves, like plasticity, give us information which is important for production efficiency and the final tablet quality [62, 77, 78].

The material had a plasticity of 92.0 ± 0.20 (n = 26) and it is independent of matrix diameter and press speed. Mean yield pressure is not influenced by press speed but depends on the diameter of the matrix: 12-mm (3.59 ± 0.68 kN) and 15-mm (81.0 ± 1.75 kN), n = 5. The SRS value could be useful in order to catalogue our product according to Robert and Rowe classification which goes from very soft to a moderately hard/brittle material [65]. Taking into account the low values obtained for SRS, 21.8 and 3.5 and for 12-mm and 15-mm respectively, the material seems not to be affected by press speed.

According to the results obtained, a high-quality child-friendly water-dispersible tablet containing INH, PZA and RFP for TB treatment has been developed in a design space using the lowest number of excipients and in the lowest proportion; all of them accepted by pediatrics (as EMA recommends). This new dosage form meets compendial requirements in terms of friability, disintegration time and content uniformity and could be an alternative for treating tuberculosis in pediatrics.

Adapted for thesis from paper:

Design and optimization of a child-friendly dispersible tablet containing Isoniazid, Pyrazinamide and Rifampicin for treating Tuberculosis in pediatrics. Suárez-González J, Santoveña A, Soriano M, Fariña JB.

Published in Drug Dev Ind Pharm. 2020 Jan 26:1-9.
doi: 10.1080/03639045.2020.1717516

Factor Trend (JCR 2019): 2.367
Quartile in Pharmacology & Pharmacy: Q3 (152/267)

5.4.2.1. Pre-stability studies

According to the Ph. Eur., friability must be below 1% of weight loss to meet requirements for dispersible tablets. Due to the number of tablets needed to evaluate the friability, a correlation between TS and friability was carried out for both sizes in order to have an approximation of friability value.

Four batches for each tablet size were obtained at different compression forces by direct compression of the powder mix in an instrumented eccentric tablet machine XP1, Research Tablet Press (Korsch, Germany) using 15-mm FFBP punches and 12-mm FFBE. Compression force and press speed were controlled by PharmaReseach® (Korsch, Germany).

Then, friability was carried out for each batch using a friability tester (Tablet Friability/Abrasion Tester TAR Series, Erweka, Germany) following the Ph. Eur. guideline [56, 79].

In figure 5-5, linear correlation for TS and friability for 12- and 15-mm tablets is shown. In both cases a statistically significant relation can be set ($r > 0.90$; ρ-value < 0.05). 70.43 and 149.53 N/cm^2 is set as the minimum values to pass the friability test for 12- and 15-mm tablets respectively.

As can be seen, there is a clear difference between limit values for both sizes. For the same TS, friability is higher in 15-mm than in 12-mm. However, they cannot be compared as different punches, with dissimilar geometrics, were used (FFBP and FFBE).

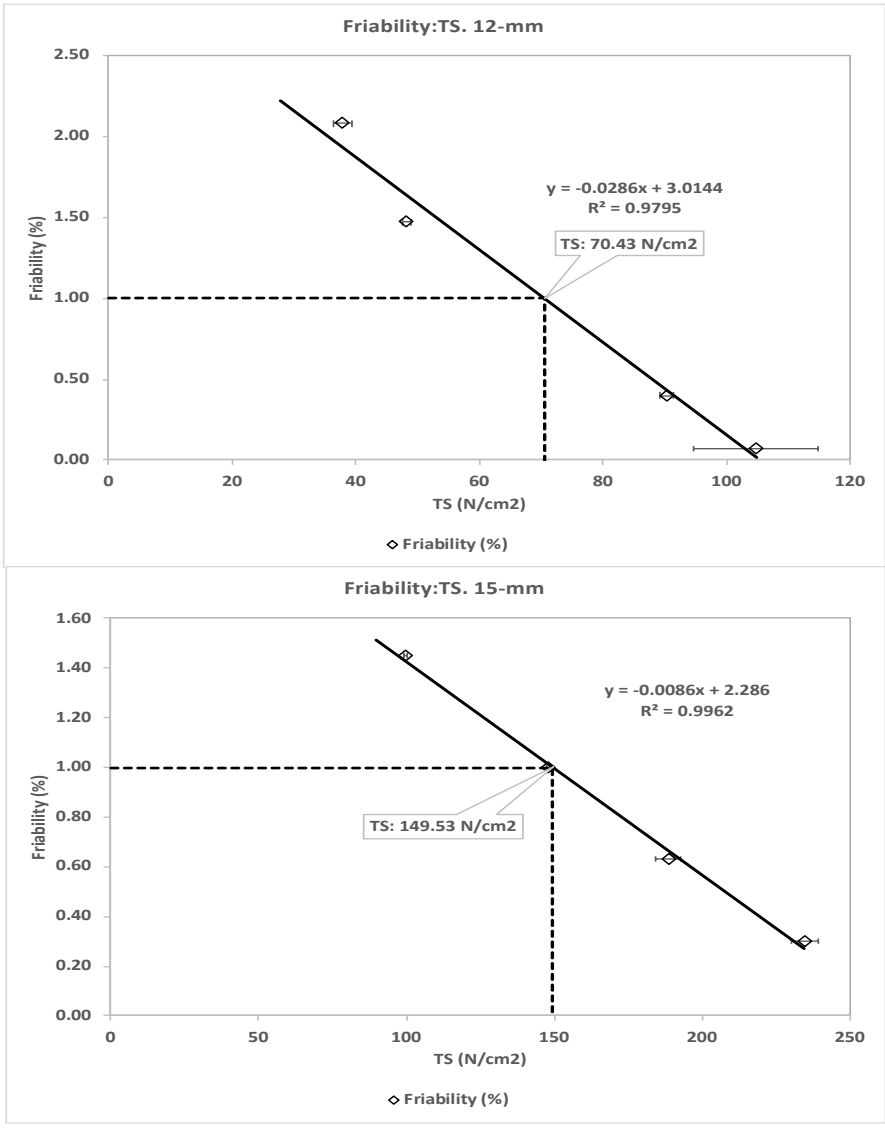

Figure 5-5. Relationship between friability and Tensile Strength (TS) for 12- and 15-mm tablets.

5.4.2.2. Stability Studies

The influence of temperature, RH and light over critical quality attributes (physical and chemical factors) were tested for tablets elaborated with 9% of Explosol® and compressed at 9.00 kN/cm^2 using 12- and 15-mm punches with a press speed of 50 and 25 cycles/min respectively (formulation 3 (Batch Nº9)) [80, 81].

The physical properties evaluated during the stability study were weight, TS and disintegration time. In the case of chemical properties, the content of each API (INH, PZA and RFP) was tested.

Tablets were placed under accelerated (Heraeus UT 6060, Spain) and long-term conditions (Memmert ULP500, Spain) to evaluate the influence of temperature and RH. ICH recommends conducting accelerated studies looking for significant changes during 6 months' testing. In general, significant change is defined as:

- A 5% change in assay from its initial value.
- Any degradation product's exceeding its acceptance criteria
- Failure to meet the acceptance criteria for appearance, physical attributes and functionality test.

In addition, the influence of light and the stability on the tablets was also tested [80, 81]. Due to the fact that hygroscopic excipients were used, tablets were stored under low relative humidity conditions in order to see its influence on physical and chemical properties.

In table 5-15 a summary of all conditions it is shown.

Condition	Storage Condition
Accelerated	40 ± 2ºC/75 ± 5% RH
Long-term	30 ± 2ºC/65 ± 5% RH
Low RH	25 ± 2ºC/11 ± 5% RH
Photostability	30 ± 2ºC/30 ± 5% RH

Table 5-15. Storage conditions for the stability studies. RH: Relative humidity (%).

Tablets formulations were packaged in SPD® Venalink system which was classified as class B according to the permeation test for containers recommended by United States Pharmacopoeia (USP) [82]. In the photostability test, an SPD® Venalink system for photosensitive APIs was used and the results were compared with tablets without this protection.

In the case of accelerated, photostability and low RH storage condition, tablets were analyzed every 1.5 month and for long term condition every 3 months. The aspect of the tablet was checked as well as weight (mg), TS (N/cm^2), disintegration time (min) and API content (% declared value), by triplicate (n=3).

Nitrogen adsorption/desorption isotherms determined at 77 K were used to calculate the surface area, pore volume and pore size of 12- and 15-mm tablet at accelerated conditions (ASAP 2020, Micromeritics Instrument Co.). BET and BJH methods were used to calculate surface area and pore distribution respectively [83, 84].

Regression analysis and ANOVA were performed to check the correlation between every property tested and time. In addition, a F-test of equality of variances and Student's t-test were carried out

control the influence of light. All tests were performed at 5% level of significance ($\alpha = 0.05$) by MS Excel (Microsoft Corporation, USA).

- Results and Discussion

Figure 5-6 pictures shows the evolution of the tablets under different conditions at 6 months of storage. Only pictures with noticeable changes were included.

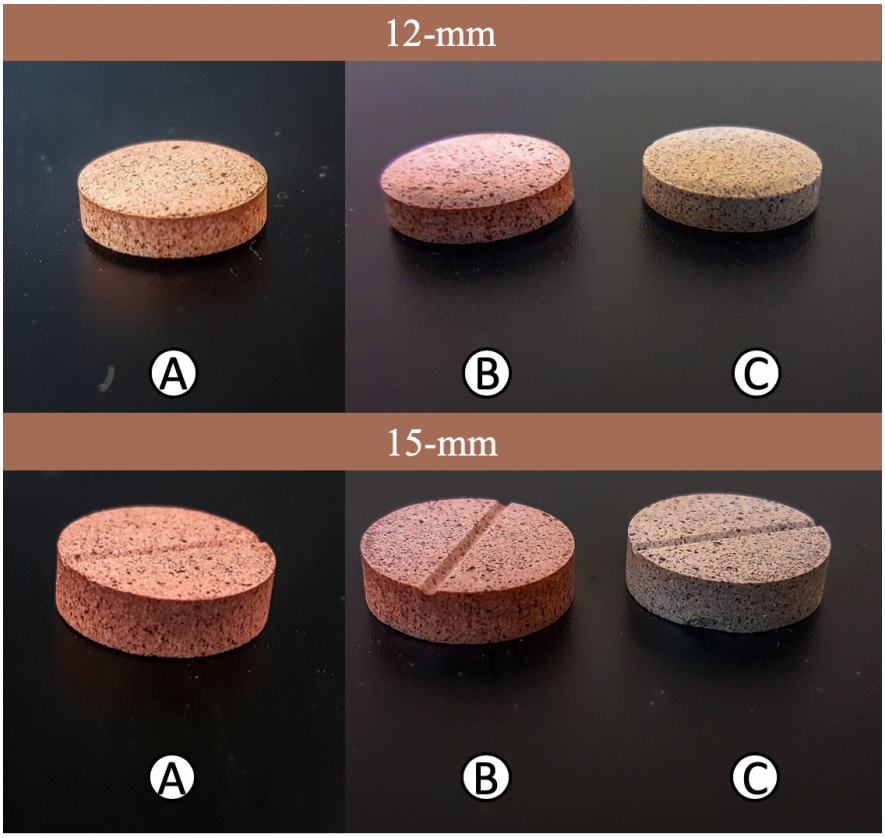

Figure 5-6. Pictures of tablets of 12- (top) and 15-mm (bottom) 6 months of storage at different conditions: A. accelerated; B. initial aspect; C photostability.

Physical properties were adjusted to a polynomial model, and content values of APIs were adjusted to a zero kinetic order as it provided better adjustment.

- Accelerated condition:

As can be seen in figure 5-6, 12- and 15-mm tablets increase in size. This increase was quantified by measuring thickness and diameter; approximately 0.05 cm. Table 5-16 shows data for physical properties.

Accelerated Condition					
12-mm					
Time (months)	**0**	**1.5**	**3**	**4.5**	**6**
Weight (mg)	458.3 ± 2.1	470.7 ± 5.0	472.0 ± 4.6	465.3 ± 7.6	467.7 ± 5.0
TS (N/cm^2)	148.9 ± 6.8	49.0 ± 2.8	39.3 ± 4.3	39.1 ± 2.3	40.5 ± 6.6
Disintegration time (seconds)	85.7 ± 9.7	43.3 ± 5.7	56.7 ± 5.7	40.0 ± 6.0	43.0 ± 0.1
15-mm					
Time (months)	**0**	**1.5**	**3**	**4.5**	**6**
Weight (mg)	897.7 ± 5.7	930.7 ± 6.0	921.0 ± 10.6	919.7 ± 1.2	930.7 ± 4.7
TS (N/cm^2)	163.4 ± 1.4	58.1 ± 4.3	46.9 ± 1.9	44.6 ± 1.9	42.6 ± 0.9
Disintegration time (seconds)	162.7 ± 3.1	56.5 ± 9.5	54.3 ± 4.7	72.7 ± 15.0	51.0 ± 2.6

Table 5-16. Results for stability of tablets under accelerated conditions of storage.

Tablet weight remained inside ± 5% of initial value independently of size, the highest increase was 103 and 104% for 12- and 15-mm tablets respectively. This variation is statistically significant in bigger sizes (r: 0.91; ρ-value < 0.05), but not enough to be classified as a significant change. On the other hand, the analysis of the data from the weight variation of 12-mm tablets showed a non-statistically

significant variation because of its low correlation coefficient (r: 0.75; ρ-value > 0.05).

TS and disintegration time showed a statistically significant variation with time for both sizes (r > 0.90; ρ-value < 0.05). As can be seen in table 5-158, tablets achieved a 33% of the initial TS value at 1.5 months of storage. TS value at this time was below the minimum found in figure 5-5 to pass friability test.

Accelerated studies were performed at the highest levels of temperature and HR. The absorption of humidity could be responsible for the weight increase and, consequently, the reduction of TS and disintegration time. Excipients that function as a disintegrant have a high moisture absorption capacity as their function is to uptake water, increase volume and break the tablet [85, 86]. In this case, sodium starch glycolate and cellulose derivates are classified as class II in the Hygroscopicity classification system; sodium starch glycolate is slightly higher than the first one [51, 87, 88]. Fumed silica is considered as hygroscopic by some authors, but others classify it as class I (non-hygroscopic) [88]. Hardness changes are due to the incorporation of water molecules between interparticle and intermolecular bounds which makes tablet soft [89].

API variation, express as declared value, for 12- and 15-mm are shown in figure 5-7. PZA it is stable at this condition, % DV is always greater than 95%, regardless of tablet size (k = 0; r > 0.10; ρ-value > 0.05). RFP shows a similar degradation in 12- and 15-mm tablets, its % DV decreases till reaching 90% at 6 months (k ≠ 0; r > 0.60; ρ-value < 0.05). The biggest difference INH, as its % DV decreases

more in 12-mm (79.0 ± 1.3%) than 15-mm (90 ± 2.0%) tablets, for both sizes there is a statistically significant time variation ($k \neq 0$; $r > 0.60$; ρ-value < 0.05).

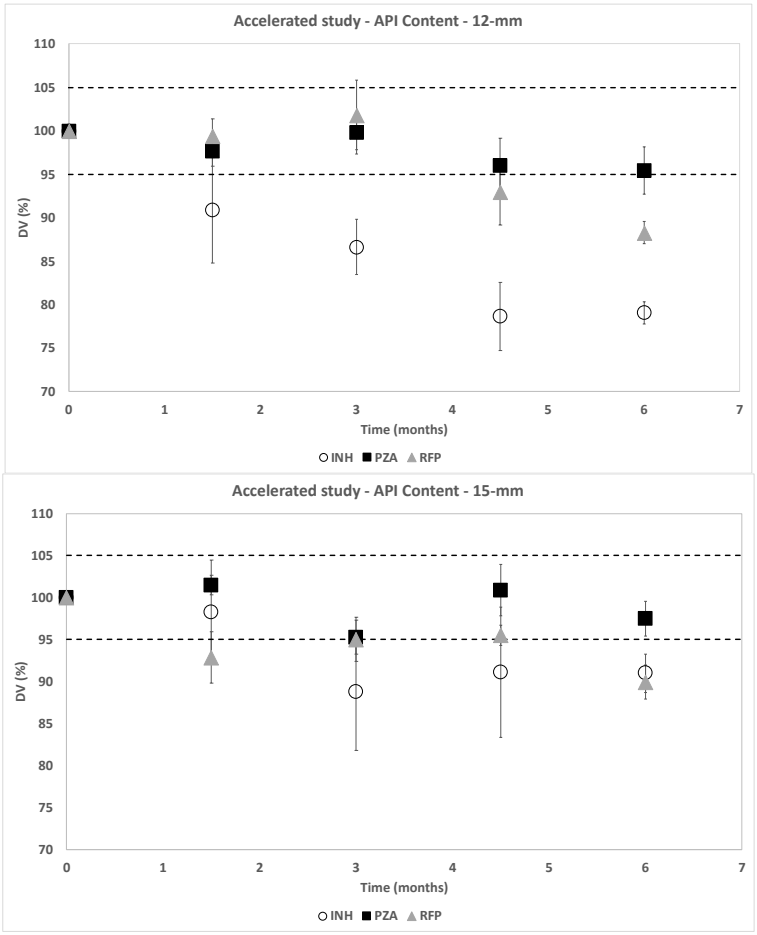

Figure 5-7. API variation for 12- and 15-mm tablets storage under accelerated conditions of storage.

In literature regarding the stability of FDC of antiTB for adults, the authors studied the same storage conditions during 3 months. It must be highlighted that the doses of API in these formulations are, in some cases, double the new recommended doses. According to their

results, there is a large variability of data in terms of degradation of APIs: 10 - 60%, 40 - 75% and 17 - 60% for INH, PZA and RFP in that order remains after 3 months of storage [17, 41]. According to this, our formulations seem to be stable.

In table 5-17 results for surface area and pore size are shown.

Tablet	**12-mm**	
Time	**Initial**	**6 months of storage**
Surface Area (m^2/g)	2.34	1.09
Volume of pores (cm^3/g)	0.026	0.014
Average Pore diameter (Å)	405.63	596.64
Tablet	**15-mm**	
Surface Area (m^2/g)	2.87	1.70
Volume of pores (cm^3/g)	0.027	0.019
Average Pore diameter (Å)	369.43	504.82

Table 5-17. Results from the determination of surface area and pore size from tablets of 12- and 15-mm size.

A reduction in surface area is detected at 6 months of storage at 75% HR and 40ºC for both sizes. This could be related to the absorption of water in these storage conditions by disintegrants which produce the dissolution of the small particles, as described by Leeson and Mattocks [90].

Before analyzing the samples in the ASAP, this must be dry and therefore small particles which are dissolved in water molecules were lost, see figure 5-8.

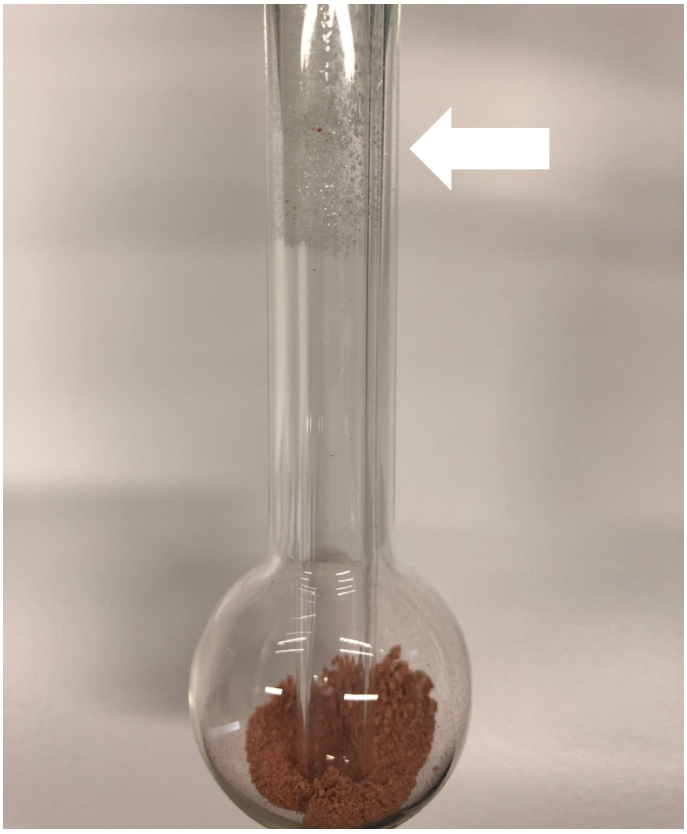

Figure 5-8. Water and particles attached in the glass surface.

Hence, as particle number decreases the surface area reduces too. The loss of this surface area was 54% and 40.7% for 12- and 15-mm respectively, which could be explained due to the higher pore diameter in the case of smaller tablets. This alteration is related to the different punches used, FFBP and FFBE.

This could explain why the declared value of INH reduces faster in the case of 12-mm tablets compared to the other sizes; these tablets absorbed more water because of their bigger pore diameter.

In conclusion, significant changes were found for physical and chemical properties as a decrease of 5% of their initial value was detected: TS, disintegration time, INH and RFP. Therefore, following ICH recommendations, long-term studies are needed in order to establish shelf life and label storage instructions.

- *Long-term condition:*

Tablets under long-term conditions increased their dimensions, thickness and diameter by 0.02 cm. Such increase was lower than the noticed under the accelerated condition, 0.05 cm, due to the higher RH.

In table 5-18 the results for long-term studies are shown.

Long-term condition				
12-mm				
Time (months)	**0**	**3**	**6**	**9**
Weight (mg)	458.3 ± 2.1	459.0 ± 3.6	467 ± 7.2	460 ± 7.6
TS (N/cm^2)	149.0 ± 6.8	59.3 ± 3.7	54.9 ± 3.2	53.9 ± 5.3
Disintegration time (seconds)	85.7 ± 9.7	50.3 ± 13.0	34.7 ± 1.5	35.0 ± 5.0
15-mm				
Time (months)	**0**	**3**	**6**	**9**
Weight (mg)	897.7 ± 5.7	909.6 ± 6.0	905.0 ± 7.2	894.3 ± 3.2
TS (N/cm^2)	163.4 ± 1.4	56.9 ± 3.3	50.4 ± 2.3	48.2 ± 1.3
Disintegration time (seconds)	162.7 ± 3.1	62.0 ± 3.0	43.7 ± 0.6	47.3 ± 1.2

Table 5-18. Results for stability of tablets under accelerated conditions of storage.

Tablet weight remains within ± 5% of initial value regardless of size. However, as this happened during accelerated studies, the variation of the tablet's weight with time is statistically significant in the 15-mm size (r: 0.79; ρ-value < 0.05), but not enough to be classified as a significant change. On the other hand, the analysis of the data from the weight variation of 12-mm tablets showed a non-statistically significant variation which might be due to its low correlation coefficient (r: 0.56; ρ-value > 0.05).

TS and disintegration time showed a statistically significant time variation for both sizes (r > 0.80; ρ-value < 0.05). As can be seen in table 5-18, tablets achieved a 55% of the initial TS value at three months of storage. This is below the minimum found in figure 5-5 to pass friability test. The loss of hardness is lower in comparison with accelerated studies due to the lower RH.

API variation over time is statistically significant for all API and regardless of tablet size, see figure 5-9 ($k \neq 0$; r > 0.60; ρ-value < 0.05). As was observed in accelerated conditions, the reduction in INH content was higher in 12mm tablet than in 15-mm tablet.

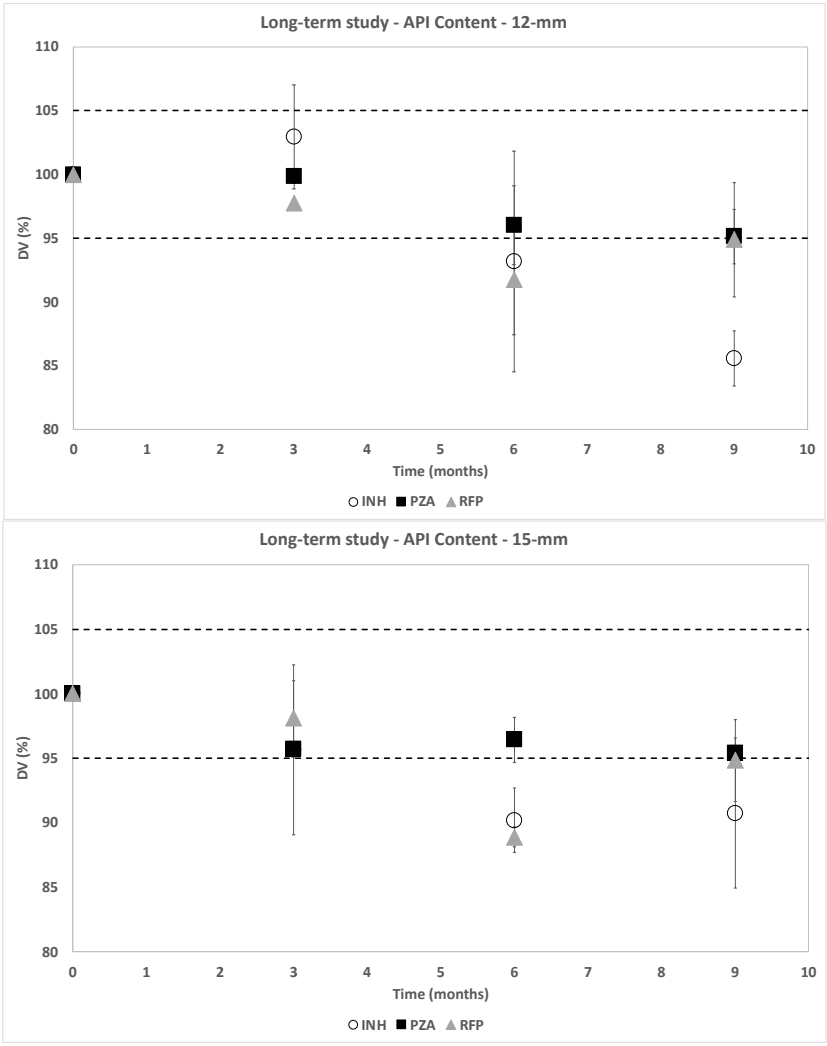

Figure 5-9. API variation for 12- and 15-mm tablets storage under long-term conditions of storage.

At three month of storage a significant change was detected for physical properties (disintegration time, TS), and at six months of storage for INH content.

These tablets were storage in a class B container, 5-10 mg per day in moisture permeation rate, which means that a high amount of water can be absorbed by tablets. Then, a better packaging it is needed. That is the reason why tablets were storage in optimal conditions of RH and temperature to evaluate the stability of the tablets in conditions of low RH.

- *Low RH:*

Tablets under these conditions did not show significant changes after three months of the study; neither physical nor chemical, see table 5-19.

Low RH			
12-mm			
Time (months)	**0**	**1.5**	**3**
Weight (mg)	436.0 ± 1.8	437.3 ± 2.9	431.0 ± 1.0
TS (N/cm^2)	132.4 ± 0.2	134.0 ± 4.2	138.2 ± 2.9
Disintegration time (seconds)	80.0 ± 0.7	73.30 ± 0.0	68.7 ± 2.3
15-mm			
Time (months)	**0**	**1.5**	**3**
Weight (mg)	857.7 ± 2.0	855.6 ± 6.4	858.6 ± 5.7
TS (N/cm^2)	155.7 ± 4.4	156.1 ± 13.2	156.7 ± 13.9
Disintegration time (seconds)	143.0 ± 1.2	140.0 ± 26.5	129.3 ± 8.1

Table 5-19. Results for stability of tablets under low RH conditions of storage.

All APIs remained between ± 5% of their initial content during the time of study, figure 5-10. In addition, average content for each API is similar between the different sizes ($k = 0$; $r > 0.60$; ρ-value > 0.05).

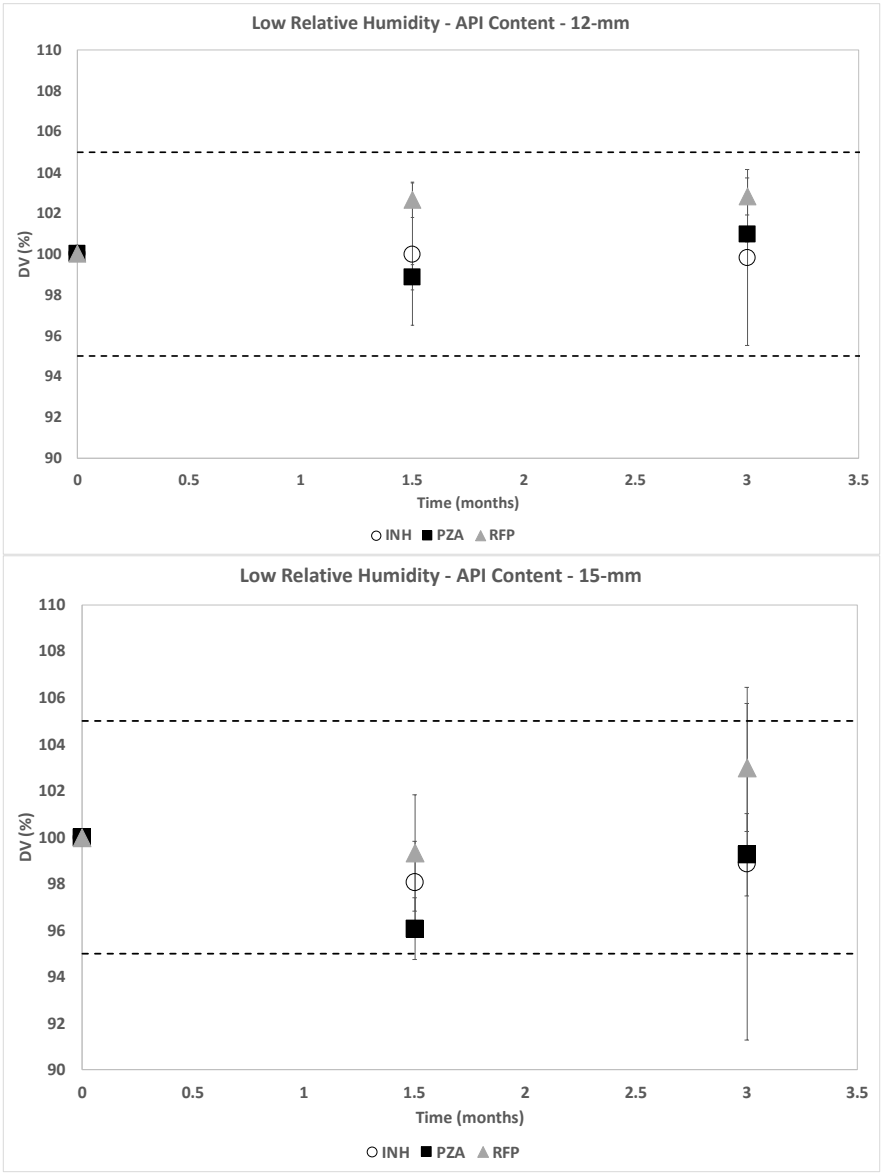

Figure 5-10. API variation, express as % of declared value (DV), for 12- and 15-mm tablets storage under low RH storage conditions of storage.

This storage condition revealed the best results after 3 months as no physical or chemical properties showed any significant change. This which could be explained based on the low RH.

In order to improve storage, a better packaging system should be found. For example, polyvinyl chloride films (PVC) laminated with high barrier plastics like polyvinylidene chlorid (PVDC) which protect the dosage form from moisture. In addition, aluminum could be added to improve protection. Another alternative could be the use of tubes and desiccant closures similar to those used to preserve effervescent tablets from moisture [91].

- *Photostability condition:*

The most important variation concerning the aspect of the tablet was colour, figure 5-6. In addition, this change in colour only affected the surface as it can be seen in figure 5-11.

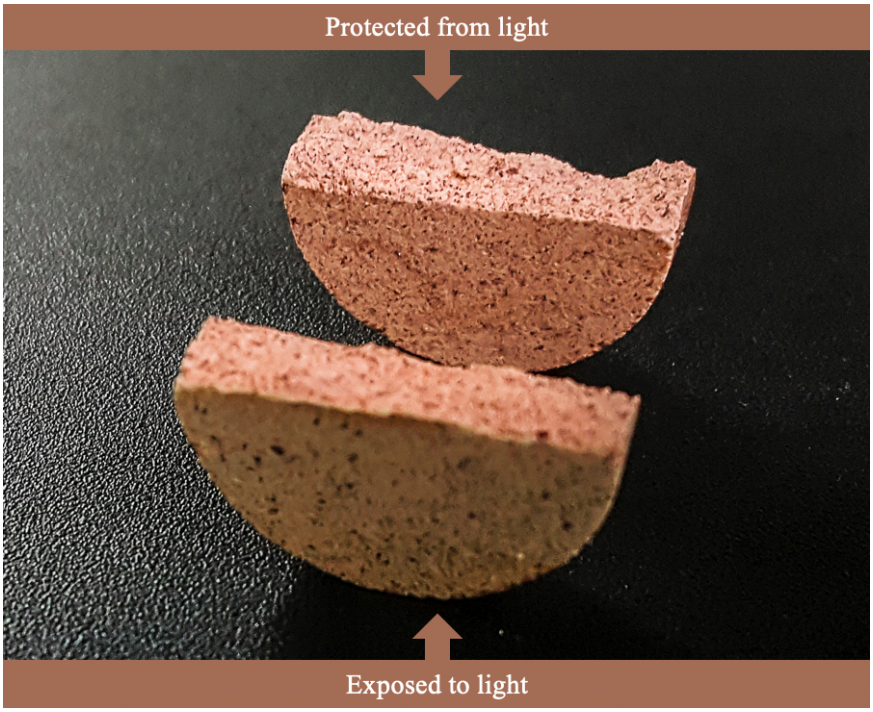

Figure 5-11. Transverse cut of the tablet exposed and protected from light.

The equality of variance was confirmed by F-test between both groups: exposed and protected from light. Moreover, a T-test determined that there is no statistically significant difference between them, regardless the property study. This concurs with the conclusions obtained by other authors during their research under similar conditions [17, 41].

Weight did not suffer a significant change during the six months of study as it is always near 100% of initial weight, table 5-20. In the case of 12 mm tablet a statistically significant variation with time was detected for both groups, light and protected from light, ($r > 0.85$; ρ-value < 0.05), in the case of 15-mm tablet this was not achieved ($r < 0.17$; ρ-value > 0.05).

Photostability Condition					
12-mm					
Light					
Time (month)	**0**	**1.5**	**3**	**4.5**	**6**
Weight (mg)	447.0 ± 1.0	441.7 ± 0.6	442.3 ± 0.6	439.0± 1.7	441.7 ± 0.6
TS (N/cm^2)	141.0 ± 3.2	137.6 ± 5.5	95.9 ± 2.0	131.2 ± 3.5	129.6 ± 3.4
Disintegration time (seconds)	90.7 ± 9.3	73.7 ± 10.0	68.7 ± 3.7	52.0 ± 1.0	42.0 ± 0.0
Protected from Light					
Weight (mg)	447.0 ± 1.0	443.0 ± 2.0	443.0 ± 2.0	441.0 ± 2.0	442.0 ± 0.0
TS (N/cm^2)	141.0 ± 3.2	130.9 ± 6.3	129.4 ± 1.8	131.5 ± 7.2	122.1 ± 4.2
Disintegration time (seconds)	90.7 ± 9.3	78.7 ± 2.1	63.3 ± 1.5	53.0 ± 2.6	43.0 ± 0.1
15-mm					
Light					
Time (month)	**0**	**1.5**	**3**	**4.5**	**6**
Weight (mg)	885.3 ± 22.6	875.3 ± 14.8	879.3 ± 3.2	879.0 ± 8.9	879.3 ± 4.5
TS (N/cm^2)	166.6 ± 10.5	153.5 ± 12.7	154.5 ± 6.7	157.2 ± 8.6	153.4 ± 4.8
Disintegration time (seconds)	149.7 ± 16.6	166.3 ± 18.9	91.6 ± 4.7	106.3 ± 9.1	96.0 ± 21.8
Protected from Light					
Weight (mg)	885.3 ± 22.6	886.7 ± 5.1	887.3 ± 7.2	886.3 ± 5.7	882.3 ± 8.7
TS (N/cm^2)	166.6 ± 10.5	158.5 ± 2.6	155.9 ± 3.3	154.8 ± 6.2	142.0 ± 3.9
Disintegration time (seconds)	149.7 ± 16.6	128.3 ± 26.6	131.0 ± 3.6	108.0 ± 8.7	95.3 ± 18.0

Table 5-20. Results for stability of tablets under photostability conditions of storage.

TS value decreases with time for 12- and 15-mm tablets, being statistically significant ($r > 0.80$; ρ-value < 0.05) just for the smaller size, see table 5-20. TS's values for 12- and 15-mm tablets at 6 months are outside of ± 5% of its initial value. However, tablets of 12-mm showed TS values higher than the minimum needed to pass friability test at 6 months of study, figure 5-5. In the case of the size of 15-mm, at 4.5 months tablets would pass friability test but at 6 months the values are very near to the minimum needed.

In all cases, disintegration time suffer a statistically significant decrease with time ($r > 0.84$; ρ-value < 0.05).

Using this storage condition, a difference in API degradation was noticed between 12-mm and 15-mm tablets. The last case was stabler; always within ± 5% DV, figure 5-11. This could be the reason why a statistically significant relation between API content and time could not be obtained ($k = 0$; $r < 0.45$; ρ-value > 0.05). However, in the case of 12-mm tablet all APIs are outside 95% at 3 months of storage which is a statistically significant decrease ($k \neq 0$; $r > 0.5$; ρ-value < 0.05).

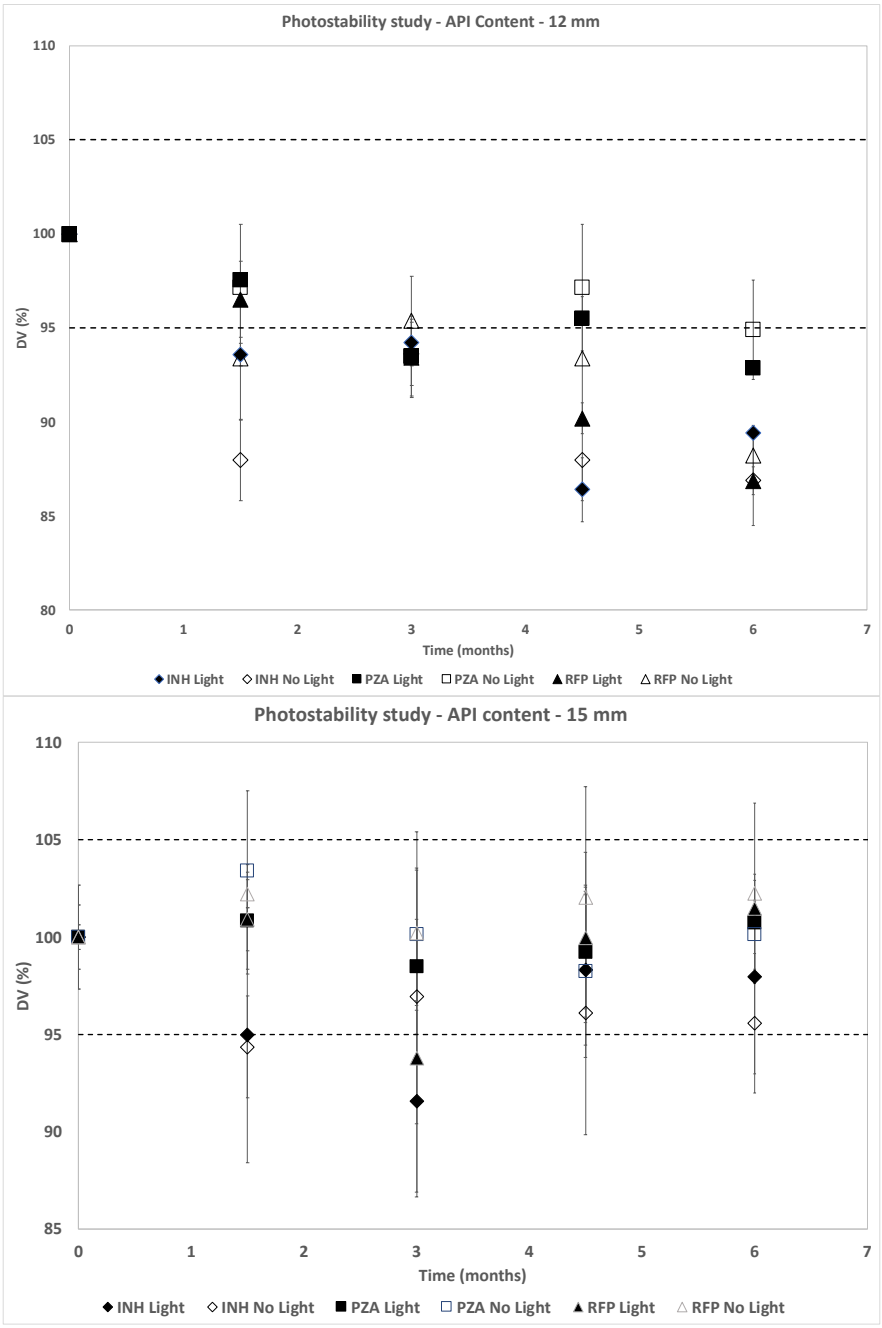

Figure 5-12. API variation, express as % declared value (DV), for 12- and 15-mm tablets storage under photostability conditions of storage.

According to the t-test previously mention there is no statistically significant difference between tablets exposed and protected from light.

5.5. References

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6. GENERAL DISCUSSION

Como se expone en el tercer apartado de esta memoria, el objetivo de la tesis es desarrollar, optimizar y evaluar la calidad de medicamentos individualizados para su uso en pediatría. Se pretende, de esta manera, mejorar el tratamiento de las enfermedades que afectan a los niños. Así, mediante el desarrollo de este tipo de formulaciones se consigue mejorar las situaciones en las que se carece de medicamentos comerciales adaptados a esta población, ya sea porque este no esté comercializado, esté en desabastecimiento o no cumpla con los criterios de calidad recomendados (excipientes aprobados para pediatría, intolerancia a algún excipiente, volúmenes de dosis excesivos para la edad, dispositivos de dosificación inadecuados, palatabilidad mejorable etc).

Actualmente existen 229 principios activos expuestos por organismos internacionales, como la Agencia Europea del Medicamento, que necesitan de una forma farmacéutica adaptada a pediatría [1]. Esta falta de medicamentos comerciales se ve reflejada en las oficinas de farmacia y servicios de farmacia hospitalaria donde deben de acudir a fuentes bibliográficas en busca de procedimientos normalizados de trabajo que permitan elaborar una fórmula adecuada para cada paciente [2-6].

De estas fuentes bibliográficas en España destaca el Formulario Nacional donde se aglutinan las fórmulas mas utilizadas y con mayor demanda en el ámbito español [6). No obstante, este no cubre todas las necesidades del sector. Por tanto, el farmacéutico debe acudir a otras fuentes bibliográficas, en ocasiones de menor calidad y

seguridad, en busca de un procedimiento normalizado de trabajo para el desarrollo de una formulación de un principio activo en particular.

Estos procedimientos llevan a la elaboración de formulaciones cuya calidad no siempre está asegurada. Por ello, uno de los objetivos de esta tesis es la de asegurar la calidad de aquellas formulaciones donde se han detectado problemas durante su elaboración en los diferentes servicios. Esta es la razón por la cual principios activos como la Dexametasona, la Acetazolamida, la Furosemida y la Flecainida fueron seleccionados. Por ejemplo, en las formulaciones elaboradas a partir de Acetazolamida y la Dexametasona se detectó una pérdida de la homogeneidad de la dosis ya que se formulan en suspensión.

Esto llevó a la evaluación de los atributos de calidad de dichas formulaciones (propiedades organolépticas, pH, uniformidad de masa, contenido, viscosidad, estabilidad física, química y micriobiológica) y a plantear formulaciones nuevas en el caso de que las iniciales no cumplieran los criterios de calidad establecidos [7-11].

Se evaluó la calidad de un total de 13 formulaciones líquidas para los cuatro principios activos estudiados, ninguno de ellos con formas de dosificación comercializadas aptas para pediatría, ver tabla 6-1.

Principio activo	Formas de dosificación		Formulaciones evaluadas
	Comercializadas en España	¿Apta para pediatría en España?	
Flecainida	Comprimidos Solución inyectable	No	4
Dexametasona	Comprimidos Gotas óticas Solución inyectable (Dexa-P)	No	2
Furosemida	Comprimidos	Solución oral (EEUU y Francia)	3
Acetazolamida	Comprimidos Cápsulas Solución inyectable	No	4

Tabla 6-1. Principios activos estudiados, formas de dosificación comercializadas y número de formulaciones evaluadas.

Tras la evaluación de la calidad y estabilidad de cada formulación se seleccionó aquella con mejores atributos y se elaboró un procedimiento normalizado de trabajo para su posterior difusión a los servicios de farmacia hospitalaria y oficinas de farmacia, ver tabla 6-2.

Formulación	Excipiente	Estabilidad
Flecainida 20 mg/ml	Agua:jarabe simple (50:50, v/v) csp. 100 ml	30 días, 5ºC
Dexametasona 1 mg/ml	20 ml tampón cítrico/citrato Jarabe simple csp. 100 ml	15 días, 5 - 25ºC
Furosemida 2 mg/ml	1.50 g $Na_2HPO_4 \cdot 12\ H_2O$ ACWP qs. 100ml	30 días, 25ºC
Acetazolamida 20 mg/ml	Se sugiere el desarrollo de una forma de dosificación sólida	

Tabla 6-2. Formulaciones finalmente obtenidas para cada principio activo estudiado y cuyo procedimiento normalizado de trabajo ha sido difundido. ACWP: Agua conservante sin propilenglicol.

En el caso de la Acetazolamida, debido a la falta de homogeneidad de dosis, no ha sido posible la realización de una formulación líquida y es necesario optar por formas de dosificación alternativas como pueden ser los minicomprimidos o los comprimidos dispersables. Los primeros, al ser muy pequeños (< 5 mm), la posibilidad de asfixia o masticar los comprimidos se ve reducida, al igual que en el caso de los segundos ya que estos se dispersan en un pequeño volumen de agua previo a su ingesta.

Como se puede observar de la tabla anterior dos de las tres formulaciones contienen jarabe simple en su composición. Este está presente en un gran número de medicamentos comerciales e

individualizados ya que este excipiente aporta un ligero sabor dulce que mejora la aceptación por parte del paciente [12]. No obstante, este es un medio de cultivo ideal para el crecimiento de microorganismos debido al alto contenido en azúcares [13]. Por ello, es común la utilización de conservantes en estas formulaciones con el fin de disminuir este crecimiento. Sin embargo, hasta el momento no existía documentación científica sobre la cantidad mínima de conservantes que asegurara la estabilidad microbiológica de la formulación, algo esencial para cumplir con las recomendaciones de la EMA sobre el uso racional de excipientes [14,15].

De esta manera se realizó el ensayo de efectividad de la actividad antimicrobiana recogido en la Eur. Ph. a un total de cinco vehículos con diferentes concentraciones de metilparabeno, propilparabeno y propilenglicol, componentes del *aqua conservans*, ver tabla 6-3 [14,15].

Número	**Vehículo**
1	Jarabe Simple
2	Jarabe simple:agua purificada (50:50 v/v)
3	Jarabe simple:*aqua conservans* (50:50 v/v)
4	Jarabe simple:*aqua conservans* sin propilenglicol (50:50 v/v)
5	Jarabe simple:*aqua conservans* diluída sin propilenglicol (50:50 v/v)

Tabla 6-3. Vehículos sometidos al ensayo de efectividad de la actividad antimicrobiana.

Este ensayo permitió la eliminación del propilenglicol del *aqua conservans* ya que se concluyó que su presencia no mejora la capacidad antimicrobiana. Es más, este es un excipiente tóxico en pediatría ya que se acumula en el organismo produciendo coma, acidosis láctica hemolisis etc. Asimismo, esta solución cuando se ve diluida con jarabe simple mantiene su efectividad antimicrobiana superando el ensayo recogido en la farmacopea.

A partir de los datos obtenidos de la validación y el control de calidad realizado a las 13 formulaciones líquidas se elaboró una estrategia que asegure la calidad de estas, incluyendo atributos críticos de calidad no contemplados en Farmacopeas y Formularios Nacionales. En este sentido, se han adaptado pruebas enfocadas a la determinación de la calidad de medicamentos que se elaboran por lotes en la industria farmacéutica al desarrollo y control de medicamentos individualizados.

Dicha estrategia recoge las recomendaciones en cuanto a la evaluación de las características organolépticas, pH y uniformidad de masa de las dosis obtenidas de envases multidosis pero las amplía añadiendo ensayos específicos para las formulaciones líquidas de principios activos en suspensión como la determinación de la uniformidad en contenido de las dosis, la uniformidad tras su agitación y entre tomas, comportamiento reológico etc. Así como la evaluación de la estabilidad química y microbiológica.

Realizados estos ensayos se establece el periodo de validez dentro del cual se cumplen los criterios de calidad. No obstante, al ser la mayoría de estas pruebas destructivas, es esencial la elaboración de

un procedimiento normalizado de trabajo el cual asegure que, siguiendo los pasos, se obtenga una formulación líquida que tenga las propiedades organolépticas adecuadas, sea homogénea en cuanto al peso y al contenido de la dosis y sea estable (microbiológica, física y químicamente)

Además de estos cuatro principios activos hay 229 más que necesitan de un medicamento adaptado para pediatría, entre ellos, 29 corresponden a principios activos para el tratamiento de enfermedades infecciosas. De estas destaca la Tuberculosis por ser una de las diez primeras causas de muerte en el mundo y producir un gran número de defunciones en niños. La principal causa a esta situación es la falta de medicamentos adaptados para el tratamiento de la Tuberculosis en pediatría y la presencia de resistencias a estos principios activos [16].

El tratamiento de la Tuberculosis se divide en dos fases, una intensiva y otra de mantenimiento. En ambas fases se combinan diferentes antimicrobianos para eliminar la bacteria responsable, *Mycobacterium tuberculosis.* El tratamiento de primera línea combina hasta tres principios activos: INH, PZA y RFP. En la primera fase se administran los tres principios activos y en la fase de mantenimiento dos de ellos, INH y RFP [17]. Para el tratamiento de la esta enfermedad solo existe un único medicamento comercializado el cual está adaptado para ser usado en pediatría, un jarabe oral de RFP [18].

Por tanto, se optó por elaborar y realizar el control de calidad a una formulación oral de INH la cual se mantiene estable durante unos 40 días manteniendo el contenido de HYD (producto de degradación tóxico) por debajo de los niveles máximos diarios tolerables, 39 µg/día [19-21]. Con el desarrollo de esta formulación, junto con la ya comercializada de RFP, se consigue cubrir por completo la fase de mantenimiento.

No obstante, lo ideal sería la obtención de una misma forma farmacéutica que contenga INH, PZA y RFP en una única dosis y que, a la vez, sea apta para pediatría y estable. La combinación de estos tres mejoraría el tratamiento ya que se incrementaría la aceptación por parte del paciente y se reduciría la posibilidad de resistencias [22,23].

La combinación de estos tres principios activos en disolución es difícil ya que existe una interacción entre INH y RFP que produce una reducción de la cantidad disponible de la segunda. En los estudios de estabilidad se confirmó esta interacción reduciendo el t5% (tiempo necesario para que el contenido declarado se reduzca en un 5%) de la RFP a más de la mitad cuando la INH está presente, ver tabla 6-4.

RFP		**INH + PZA + RFP**	
t5% (h)	**R**	**t5% (h)**	**R**
1,2	0,99	0,5	0,97

Tabla 6-4. Comparación de los t5% relativo al contenido de RFP cuando esta se encuentra sola y en combinación.

Debido a esta inestabilidad el desarrollo de una formulación líquida de administración oral de los tres principios activos fue descartada. Entonces, se optó por la elaboración de una forma de dosificación oral sólida dispersable que permite una mayor estabilidad química sin afectar a la aceptabilidad del paciente ya que esta se disuelve en un pequeño volumen de agua [24].

El desarrollo de estos comprimidos comenzó con la selección de los excipientes necesarios para obtener un comprimido dispersable, el cual se debe disgregar en menos de tres minutos y tener una friabilidad inferior al 1%, como recomienda la Farmacopea [24]. Asimismo, todos los excipientes usados están aceptados para pediatría y corrigen aspectos tales como las propiedades de flujo, la adherencia a la superficie de la matriz y punzones, la mejora del sabor etc. Así, los excipientes usados fueron los siguientes: AcDiSol® (Croscarmellose Sodium, FMC Corp.), Avicel® PH102, (Microcrystalline Cellulose, FMC Corp.), Explosol® (Sodium Starch Glycolate, Blanver), CompactCel® (Isomalt, sucralose, betadex, carboxymethylcellulose sodium, Biogrund GmbH), Luzenac® (talc, Imerys Talc) and CabOSil® (fumed silica, Cabot CorporaFon).

Dado el amplio número de excipientes, todos ellos aceptados en pediatría, y la existencia de tres principios activos en la mezcla fue necesaria la optimización del tiempo de mezclado mediante el cálculo del índice de capacidad de mezclado a diferentes tiempos concluyendo que 15 minutos de mezclado en una mezcladora en “V” es suficiente para conseguir la homogeneidad de la mezcla y asegurar la uniformidad de los comprimidos.

Los comprimidos fueron elaborados en una máquina de comprimir excéntrica usando punzones planos y ranurados de 15 mm. Además, se trazó un espacio de diseño donde se estudió la influencia de tres variables (cantidad de AcDiSol®, Explosol® y la fuerza de compresión) sobre el tiempo de disgregación y friabilidad [25,26]. A partir de los resultados se concluyó que el lote el cual cumplía todos los criterios establecidos (mínima cantidad y número de excipientes, menor tiempo de disgregación y menor friabilidad) era el que estaba elaborado a 16 kN, sin Explosol® y con un 9% p/p de AcDiSol®.

Dicho sólido pulverulento también fue comprimido usando punzones bicóncavos y biselados de 12 mm. Debido a la propia geometría de los comprimidos resultantes estos comprimidos más pequeños mostraron mejores propiedades en cuanto a tiempo de disgregación y friabilidad. En ambos casos se aseguró la homogeneidad de dosis de los tres principios activos en los comprimidos, así como la finura de la dispersión [24,27,28]. Asimismo, la producción de los comprimidos fue optimizada estableciendo las velocidades máximas de compresión para cumplir especificaciones, 25 y 50 ciclos/minuto para los punzones de 12 y 15 mm respectivamente.

Finalmente, se estudió la estabilidad de estos comprimidos a diferentes condiciones de almacenamiento: acelerado, largo plazo, fotoestabilidad y baja humedad, ver tabla 6-5 [24,27,28].

Condición	Condiciones de almacenamiento
Acelerado	40 ± 2ºC/75 ± 5% HR
Largo Plazo	30 ± 2ºC/65 ± 5% HR
Baja HR	25 ± 2ºC/11 ± 5% HR
Fotoestabilidad	30 ± 2ºC/30 ± 5% HR

Table 6-3. Condiciones de almacenamiento para los estudios de estabilidad. HR: Humedad Relativa (%).

Los comprimidos muestran una mayor estabilidad cuando se almacenan en condiciones de baja humedad. Sin embargo, en condiciones extremas de almacenamiento, es decir alta humedad (> 65%) y temperatura (> 30 ºC) los excipientes absorben agua haciendo que estos sean más blandos (menores tiempos de disgregación y friabilidad > 1%) y se produzca una mayor degradación química de los principios activos por el aumento de la cantidad de agua adsorbida. Además, de acuerdo con los resultados obtenidos, la presencia de una fuente de luz no afecta a la estabilidad de los principios activos.

Por tanto, se concluye que los comprimidos deben ser almacenados en un acondicionamiento primario el cual los proteja de la humedad ambiental. Por ejemplo, materiales elaborados con policloruro de vinilo (PVC), cloruro de polivinilideno (PVDC) o aluminio. Otra alternativa podría ser el uso de tubos y agentes secantes similares a los encontrados en los acondicionamientos primarios usados para la conservación de comprimidos esfervescentes [29].

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7. CONCLUSIONS

1. Different compounding problems have been detected during the elaboration of formulations made using four active pharmaceutical ingredients (Flecainide, Dexamethasone, Acetazolamide and Furosemide) in pharmacy services

2. Individualized medicines of Flecainide, Dexamethasone and Furosemide have been developed with the highest standards of quality for pediatrics. All were made using pure active pharmaceutical ingredients, the least number of excipient and in the lowest quantity.

3. A general strategy to validate the final quality of oral liquid individualized medicine (solutions or suspensions) was developed. This strategy included two different actions: analytical method and liquid formulation validation.

4. An oral individualized medicine appropriate for children of Isoniazid was validated. In this sense, continuous phase for children is covered as an RFP suspension is available in the market for pediatrics.

5. A high-quality child-friendly water-dispersible tablet containing Isoniazid, Pyrazinamide and Rifampicin for Tuberculosis treatment has been developed by quality by design. Excipients authorized for pediatrics (in the stipulated amount) were used and international compendia to ensure the quality of the formulation was met.

6. The caption of moisture by the excipients produced was identified as the main reason for the instability of the tablet, causing significant changes in physical and chemical properties at accelerated and long-term conditions.

7. Light had no influence on the stability of the tablet. Therefore, packaging which protects the API from it is not needed.

8. Tablets storage at low relative humidity showed to be stable up to three months. Accelerated stability tests need to be done with an appropriate packaging (PVC, PVDC and aluminum) which protect the APIs from moisture.

9. Hydrazine is the most important degradation product of Isoniazid due to its potential carcinogenic risk. Thus, the formation of this product should be followed during stability studies.

8. ANNEX.

8.1. STANDARDS OPERATING PROCEDURES.

8.1.1. Flecainide.

8.1.1.1. **F1 and F2**

1. Firstly, the simple syrup is prepared.
2. All the solid components are pulverized and weighed.
3. The FA (2.0 g) is added to a 250 ml beaker and then 10 ml of glycerol (F2 only) is added with constant shaking for 15 minutes, until a homogeneous paste is formed.
4. Then, slowly incorporate the simple syrup previously prepared with continuous magnetic stirring in 3 periods of 10 min. For F1: 20 ml + 40 ml + 20 ml. For F2: 10 ml + 40 ml + 20 ml.
5. Transfer the contents of the beaker to the 100 ml graduated cylinder.
6. Wash the beaker with approximately 15 ml of simple syrup and transfer it to he100 ml graduated cylinder.
7. Add the simple syrup to the 100 ml graduated cylinder to the full volume of 100 ml.
8. Transfer to the beaker with stirring for 10 min to homogenize the mixture again.
9. It is then packaged in a 125 ml amber bottle with dispenser closure.

8.1.1.2. **F3.**

1. Firstly, the simple syrup is prepared.
2. All the solid components are pulverized and weighed.
3. The FA (2.0 g) is added to a 250 ml beaker.
4. In another beaker, approximately 60 ml of purified water is heated to 37 ° C.
5. Measure 50 ml of water at 37° C with a 100 ml graduated cylinder and add it into the beaker containing the FA.
6. Place the solution under magnetic stirring and heating to reach a transparent solution (10 min), making sure that the temperature does not exceed 37 ºC.
7. Measure 35 ml of simple syrup with the 100 ml graduated cylinder, and slowly incorporate to the solution with continuous magnetic stirring for 10 min, not exceeding 37°C.
8. Transfer to the 100 ml graduated cylinder, the contents of the beaker.
9. Wash the beaker with approximately 10 ml of simple syrup and transfer it to the100 ml graduated cylinder.
10. Add the simple syrup to the 100 ml graduated cylinder to the full volume of 100 ml.
11. Transfer to the beaker with stirring for 10 min to homogenize the mixture again and packaged in a 125 ml amber bottle.

8.1.1.3. **F4.**

1. Firstly, the simple syrup is prepared.
2. All the solid components are pulverized and weighed.
3. The FA (2.0 g) is added to a 250 ml beaker
4. In another beaker place approximately 35 ml of purified water and heat to 37 °C.
5. Measure 25 ml of water at 37 °C with a 100 ml graduated cylinder and add it into the beaker containing the FA.
6. Place the solution under magnetic stirring and heating to reach a transparent solution (10 min), making sure that the temperature does not exceed 37 ºC.
7. Then 25 ml of glycerol is added with constant shaking, for 15 min, until a homogeneous paste is formed.
8. Measure 35 ml of simple syrup with the 100 ml graduated cylinder, and slowly incorporate to the solution with continuous magnetic stirring for 10 min.
9. Transfer the contents of the beaker to the 100 ml graduated cylinder.
10. Wash the beaker with approximately 10 ml of simple syrup and transfer it to the100 ml graduated cylinder.
11. Add the simple syrup to the 100 ml graduated cylinder to the full volume of 100 ml.
12. Transfer to the beaker with stirring for 10 min to homogenize the mixture again.
13. It is then packaged in a 125 ml amber bottle with dispenser closure.

8.1.2. Dexamethasone.

8.1.2.1. **F1.**

1. Firstly, the simple syrup vehicle is prepared.
2. 100 mg of Dexamethasone are weighed and transferred to a mortar where the product is pulverized.
3. Measure approximately 70 ml of simple syrup in a 100 ml graduated cylinder and then 5 ml of this volume are transferred to the mortar and mixing with a pestle until a homogeneous paste is formed with the total volume measured of simple syrup.
4. The contents of the mortar are transferred to a 100 ml Erlenmeyer with constant shaking using a magnetic stirrer. Recover the total paste of the mortar with approximately 10 ml of simple syrup.
5. Maintain under magnetic stirring at medium power without foaming until a suspension of homogeneous appearance forms.
6. The suspension is then transferred to a 100 ml graduated cylinder and washed with approximately 20 ml of simple syrup.
7. Complete the 100 ml volume with simple syrup.
8. It is then packaged in a 125 ml amber bottle with dispenser closure.

8.1.2.2. **F2.**

1. Firstly, the simple syrup vehicle and the citric/citrate buffer are prepared.
2. 100 mg of Dexamethasone are weighed and transferred to a mortar where the product is ground to powder.
3. Measure 20 ml of the buffer in a 25 ml graduated cylinder.
4. Add approx. 5 ml of the buffer to the mortar and mixing with a pestle.
5. Transfer the contents of the mortar to a 100 ml Erlenmeyer with constant shaking using a magnetic stirrer.
6. Recover the total suspension from the mortar with the rest of the buffer.
7. Measure approximately 60 ml of simple syrup in a 100 ml graduated cylinder.
8. Wash the mortar with this volume of simple syrup and transfer to the Erlenmeyer, recovering the total suspension of the mortar.
9. Maintain under magnetic stirring at low power without foaming until a suspension of homogeneous appearance forms.
10. The suspension is then transferred to a 100 ml graduated cylinder and washed with approx. 20 ml of simple syrup.
11. Complete the 100 ml volume with simple syrup
12. It is then packaged in a 125 ml amber bottle with dispenser closure.

8.1.3. Furosemide.

8.1.3.1. **F1.**

1. 200 mg of Furosemide was weighted.
2. Measure 70 ml of Sörensen Buffer in a 100 ml graduated cylinder and transferred it to a 250 ml beaker.
3. Furosemide was transferred to the beaker with constant magnetic stirring.
4. Maintain under magnetic stirring at medium power until getting a solution.
5. The solution is then transferred to a 100 ml graduated cylinder and complete with simple syrup with ACWP.
6. Finally, it is packaged in a 125 ml amber bottle with dispenser closure.

8.1.3.2. **F2.**

1. 200 mg of Furosemide, 6.96 g of $Na_2HPO_4 \cdot 2\ H_2O$ and 63 mg of monohydrated citric acid were weighted.
2. 80 ml of ACWP were measured in a 100 ml graduated cylinder and transferred to a 250 ml beaker.
3. Na2HPO4 · 2 H2O and the monohydrated citric acid were transferred to the beaker with constant magnetic stirring. Maintain under magnetic stirring at medium power until getting a solution.
4. Furosemide was transferred to the beaker with constant magnetic stirring.
5. Maintain under magnetic stirring at medium power until getting a solution.
6. The solution is then transferred to a 100 ml graduated cylinder and complete with ACWP.
7. Finally, it is packaged in a 125 ml amber bottle with dispenser closure.

8.1.3.3. **F3.**

1. 200 mg of Furosemide, and 1.5 g of $Na_2HPO_4 \cdot 12\ H_2O$ were weighted.
2. 80 ml of ACWP were measured in a 100 ml graduated cylinder and transferred to a 250 ml beaker.
3. $Na_2HPO_4 \cdot 12\ H_2O$ was transferred to the beaker with constant magnetic stirring. Maintain under magnetic stirring at medium power until getting a solution.
4. Furosemide was transferred to the beaker with constant magnetic stirring.
5. Maintain under magnetic stirring at medium power until getting a solution.
6. The solution is then transferred to a 100 ml graduated cylinder and complete with ACWP.
7. Finally, it is packaged in a 125 ml amber bottle with dispenser closure.

8.1.4. Acetazolamide.

8.1.4.1. **F1.**

1. The simple syrup and the methylcellulose 1000 at 1% w/w are prepared.
2. All the solid components are pulverized and weighed.
3. AZM (2.0 g) is placed in a small mortar and then the right amount of methylcellulose 1000 at 1% w/w is added with constant manual stirring for 10 min, until a homogeneous paste is formed.
4. Then, slowly add the homogeneous paste to a 100 ml Erlenmeyer flask with continuous magnetic stirring.
5. Gradually add half of the simple syrup, maintaining vigorous stirring.
6. Transfer the content of the flask into a 100 ml graduated cylinder.
7. Wash the flask twice with the remaining half of the simple syrup to recover the remains of the suspension, and add this to the beaker to complete the volume of 100 ml.
8. It is then package in a 125 ml amber bottle with dispenser closure

8.1.4.2. **F2, 3 and 4.**

1. The simple syrup, the methylcellulose 1000 at 1% w/w (F2) or the hydroxypropylmethylcellulose at 2% w/w (F3 and F4) and the cit- rate buffer (0.1 M citric acid and 0.1 M sodium citrate) are prepared.
2. All the solid components are pulverized and weighed.
3. The AZM (2.0g) is added to a small mortar and then the right amount of glycerol is added with constant manual stirring for 5 min, until a homogeneous paste is formed.
4. Then, slowly add the homogeneous paste to a 100 ml Erlenmeyer flask with continuous magnetic stirring.
5. Wash the mortar with a small amount of the citrate buffer and add the rest to the Erlenmeyer flask (30ml for F2 and F3, and 35 ml for F4), maintaining vigorous stirring.
6. Slowly add the right amount of methylcellulose 1000 at 1% w/w (F2) or hydroxypropylmethylcellulose 4500 at 2% w/w (F3 and F4), maintaining vigorous stirring for 10 min.
7. Gradually add half of the simple syrup, maintaining vigorous stirring.
8. Transfer the contents of the flask to the 100 ml graduated cylinder.

9. Wash the flask twice with the remaining half of the simple syrup to recover the remains of the suspension, then add this to the beaker to complete the volume of 100 ml.
10. It is then packaged in a 125ml amber bottle with dispenser closure.

8.1.5. Isoniazid

1. 5 g of INH was weighted.
2. Measure 50 ml of a solution of 70% Sorbitol in a 100 ml graduated cylinder and transferred it to a 250 ml beaker.
3. INH was transferred to the beaker with constant magnetic stirring.
4. Maintain under magnetic stirring at medium power until getting a solution.
5. The solution is then transferred to a 100 ml graduated cylinder and complete with simple syrup with ACWP.
6. Finally, it is packaged in a 125 ml amber bottle with dispenser closure.

8.1.6. Excipients.

8.1.6.1. **ACWP**

It was prepared by mixing 0.8 g of methyl p-hydroxybenzoate (methylparaben) and 0.2 g of propyl p-hydroxybenzoate (propylparaben) in 1 liter of purified water at 50 ºC.

8.1.6.2. **Simple Syrup.**

The simple syrup is prepared with 64 % w/w of sucrose and 36 % w/w of purified water at constant shaking until getting a homogeneous solution, then, it was filtered.

8.1.6.3. **Simple Syrup with ACWP.**

The simple syrup with ACWP was prepared as a solution of 64% sucrose w/w and 36% w/w of ACWP at constant shaking until getting a homogeneous solution, then, it was filtered.

8.1.6.4. **Methylcellulose 1000 at 1% w/w.**

Heat up the amount of water needed at 70-80 ºC. Without heating up, the methylcellulose 1000 is added while using magnetic stirring in order to obtain a homogeneous dispersion. Let the dispersion cold down during 24 h.

8.1.6.5. **Hidroxipropilmethylcellulose 4500 at 2% w/w.**

Heat up the amount of water needed at 70-80 ºC. Without heating up, the hidroxipropilmethylcellulose 4500 is added while using magnetic stirring in order to obtain a homogeneous dispersion. Let the dispersion cold down during 24 h.

8.1.6.6. **Citric/Citrate Buffer.**

The citric/citrate buffer are prepared with 33 ml of 0.1 M citric acid solution and 17 ml of 0.1 M sodium citrate solution completed 100 ml with water. The pH of this buffer is adjusted to pH 4 with approximately 5 ml of sodium citrate solution.

8.1.6.7. **Sörensen Buffer.**

A solution of 19.2% of KH2PO4 v/v and 80.8% of Na2HPO4 v/v in purified water.

8.1.6.8. **Sorbitol Solution at 70% w/v**

A solution of 70% w/v of sorbitol in purified water.

8.2. ABBREVIATIONS

ADME. Absorption, Distribution, Metabolism and Excretion.
ACWP. Aqua Conservans without Propylene glycol
ANOVA. Analysis of Variance.
API. Active Pharmaceutical Ingredient.
AV. Acceptance Value.
AZM. Acetazolamide
BCS. Biopharmaceutical Classification System.
CQA. Critical Quality Attributes.
CpK. Process Capability index.
Cfu. Colony-forming units.
D. Density.
Dexa-P. Dexamethasone Sodium Phosphate.
Dexa. Dexamethasone.
DV. Declare Value.
EMA. European Medicine Agency.
EuPFI. European Pediatric Formulation Initiative.
FA. Flecainide Acetate.
FDA. Food and Drug Administration.
FDC. Fixed-dose Combination
FFBE. Flat-Faced with Beveled Edge.
FFBP. Flat-Faced Bisect Punches.
GRAS. Generally Recognize as Safe.
HPLC. High Performance Liquid Chromatography.
HYD. Hydrazine.
LSL. Lower Specification Limit.
ICH. International Conference of Harmonization.
INH. Isoniazid.

JCPDS. Joint Committee on Powder Diffraction Standards

K. Slope.

MT. MiniTablet.

ND. Not Determined.

NIH. National Institutes of Health.

ODMT. Orodispersible MiniTablet.

ODT. Orodispersible Tablet.

Ph.Eur. European Pharmacopoeia.

PBCS. Pediatric Biopharmaceutical Classification System.

PL. Plasticity.

PZA. Pyrazinamide.

PIP. Pediatric Investigation Plan.

PUMA. Pediatric-use marketing authorization.

Py. Mean Yield Pressure.

QTTP. Quality Target Product Profile.

R. Correlation coefficient.

R^2. R-Squared.

$Radj^2$. Adjusted R-Squared.

Q^2. Predicted R-Squared.

RFP. Rifampicin

RH. Relative Humidity.

RSD. Relative Standard Deviation.

SD. Standard Deviation.

SRS. Strain-rate Sensitivity.

SOP. Standard Operating Procedure.

STEP. Safety and Toxicity of Excipients for Paediatrics.

TB. Tuberculosis

TS. Tensile Strength.

UHPLC. Ultra-High-Performance Liquid Chromatography.

USL. Upper Specification Limit.

UPLC. Ultra-Performance Liquid Chromatography.

USA. United States of America.

USP. United States Pharmacopoeia.

W_1. Friction Work.

W_2. Net Work.

W_3. Elastic Work.

WHO. World Health Organization.

9. PUBLISHED PAPERS AND AWARDS

Effectiveness of Antimicrobial Preservation of Extemporaneous Diluted Simple Syrup Vehicles for Pediatrics

Ana Santoveña-Estévez, PhD; Javier Suárez-González, PhD student; Martín Vera, Hospital Pharmacy Resident; Cristina González-Martín, PhD; Mabel Soriano, PhD; and José B. Fariña, PhD

OBJECTIVES Extemporaneous or magistral formulation of active pharmaceutical ingredients using traditional compounding techniques is a common practice when no commercial form is available for pediatrics. For this vulnerable group of patients, the formulation must be prepared with the minimum quantity and lowest proportion of excipients approved for pediatrics, avoiding the use of preservatives. Often the vehicles used for these preparations are dilutions of simple syrup with water. The objective of this study is to assess the effectiveness of antimicrobial preservation in simple syrup diluted with aqua conservans (conserved water), without propylene glycol or with a reduced proportion of parabens.

METHODS The European Pharmacopoeia test of efficacy of antimicrobial preservation was applied to 5 trial vehicles prepared with simple syrup diluted with water.

RESULTS Simple syrup is stable during 14 days. Vehicles prepared with simple syrup diluted with purified water did not meet the microbiological quality criteria, but when they are diluted with water that incorporates propylene glycol and parabens (aqua conservans), then they meet the criteria. In addition, if the water is prepared with parabens and without propylene glycol, the criteria for the dilution are met. Nevertheless, if the dilution is done with water prepared with an insufficient proportion of parabens to act as preservatives, the dilution does not meet the pharmacopoeia microbiological criteria.

CONCLUSIONS Dilution of simple syrup (50:50 v/v) to prepare a vehicle for extemporaneous or magistral preparation is microbiologically safe when water with methylparaben and propylparaben is used in a proportion of 0.08% and 0.02% (w/w), respectively, avoiding the use of propylene glycol as a solvent and thus its toxic effects in pediatrics.

ABBREVIATIONS API, active pharmaceutical ingredient; CM, growth of countless microorganisms; NI, no increase

KEYWORDS antimicrobial preservation; conserved water; parabens; propylene glycol; simple syrup

J Pediatr Pharmacol Ther 2018;23(5):405–409

DOI: 10.5863/1551-6776-23.5.405

Introduction

The lack or scarcity of a marketed active pharmaceutical ingredient (API) at pediatric doses is the main reason to formulate or at worst reformulate adult dosage forms of APIs. This practice gives rise to off-label or unlicensed use.[1] On formulating these APIs in liquid form for oral administration it is common practice to use syrup as vehicle. In general, there are 3 types of syrups: simple syrup containing only sucrose and purified water, flavoring syrup containing flavored substances, and medicinal syrups to which other therapeutic compounds have been added.[2] These syrups are used to enhance palatability or to increase viscosity of the formulation and also to create adequate osmotic pressure to inhibit microbial contamination (>60% w/w).[3] At present the availability of marketed suspending vehicles such as Ora (Perrigo, Dublin, Ireland) products simplifies the compounding of oral syrups. These products have a high cost and complex composition owing to their constituent suspending agents, preservatives, sweeteners, and buffers.[4] This makes them non-ideal candidates for pediatric formulation, where the number and quantity of excipients in a formulation should be the minimum necessary to support product quality.[5] Therefore, at least in pediatrics, it is necessary to use simple vehicles that can be prepared by using traditional compounding techniques.[6]

In pediatrics, simple syrup is often diluted with carriers such as water, or other excipients for different purposes, such as to reduce the amount of sucrose administered or adjust the viscosity of the final preparation.[7] The influence of dilution on the efficacy of antimicrobial preservation is insufficiently studied in the literature and should be further studied, since it

Table 1. Composition of Each Vehicle Studied % (w/w)

No.	Vehicle	Sucrose (%)	Methylparaben (%)	Propylparaben (%)	Propylene Glycol (%)
1	Simple syrup	64	–	–	–
2	Simple syrup: purified water (50:50 v/v)	32	–	–	–
3	Simple syrup: aqua conservans (50:50 v/v)	32	0.04	0.01	0.45
4	Simple syrup: aqua conservans without propylene glycol (50:50 v/v)	32	0.04	0.01	–
5	Simple syrup: aqua conservans diluted without propylene glycol (50:50 v/v)	32	0.008	0.005	–

is a habitual practice in the preparation of non-sterile multidose formulations.[8] In some countries the Pharmacopoeias or the National Formularies of Pharmaceutical Compounding include a monography of water used in compounding,[9,10] named *aqua conservans*[11] (conserved water), that is prepared with a hydroxybenzoates (parabens) solution.[12,13] The propylene glycol habitually used as drug solvent in this hydroxybenzoate solution is toxic at least for infants, since it can accumulate and cause lactic acidosis, central nervous system depression, coma, hypoglycemia, seizures, and hemolysis.[14] As European Medicines Agency[15] indicates however, owing to insufficient clinical evidence of comparable effects in humans, continued use of parabens as antimicrobial preservatives appears to be justified, particularly in the case of pediatric formulations. Nevertheless, the concentration should be at the lowest feasible level (0.015% and 0.01% for methylparaben and propylparaben, respectively).[15] For these reasons, quantities added to the water must be just sufficient for the desired preservative effect, without being in excess.

This study assesses the effectiveness of antimicrobial conservation in vehicles prepared with diluted simple syrup. For this dilution, purified water and aqua conservans were used, and in the latter, the proportion of propylene glycol and parabens used as excipients was eliminated or reduced, respectively.

Materials and Methods

Vehicles. We studied 5 vehicles (Table 1). Vehicle 1 was simple syrup and the others were dilutions of it with other solutions in the proportion 50:50 (v/v). The simple syrup was prepared as a solution of 64% sucrose w/w in purified water, which is roughly equivalent to 85% w/v.[16] A concentrated solution of hydroxybenzoates was prepared by mixing 8 g of methyl p-hydroxybenzoate (methylparaben) and 2 g of propyl p-hydroxybenzoate (propylparaben) with propylene glycol qs 100 g.[12] The aqua conservans was made up of 1 g of this solution in purified water qs 100 mL.[11] In vehicle 4, the hydroxybenzoates were diluted at the same concentration as in the aqua conservans, without the addition of propylene glycol. Finally, vehicle 5 was prepared by using the lowest proportion of each hydroxybenzoate recommended for oral solutions and suspensions (0.015% methyl p-hydroxybenzoate and 0.01% propyl p-hydroxybenzoate),[15] without the addition of propylene glycol.

All excipients were prepared from pharmacopoeia-grade raw materials, provided by Acofarma (Madrid, Spain). The culture media was prepared from commercial media (Scharlab, Barcelona, Spain).

Efficacy of Antimicrobial Preservation. In this study the European Pharmacopoeia test of efficacy of antimicrobial preservation[17] was applied, which is stricter than the antimicrobial effectiveness test of United States Pharmacopoeia.[18] This test must show that the formulation provides adequate protection against adverse effects due to contamination or microbial growth during storage and use. The test consists of deliberate contamination of the preparation in the final container with a prescribed inoculum of suitable microorganisms, conservation of the inoculated preparation at a set temperature, withdrawing samples from the container at specified time intervals, and counting microorganisms in the samples taken. The preservative properties of the preparation are adequate if a significant decrease or no increase in the number of microorganisms occurs in the inoculated preparation after the prescribed times and temperatures. The acceptance criteria vary depending on the type of preparation (parenteral, ophthalmic, intrauterine, intramammary, otic, nasal, cutaneous, inhaled, oral, or rectal) and the degree of protection required.

The microorganisms used were *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Candida albicans*, *Aspergillus brasiliensis*, *Escherichia coli* (for oral administration vehicles), and *Zygosaccharomyces rouxii* (for oral administration vehicles with high sugar content), all of which were obtained from the Spanish Type Culture Collection in Valencia, Spain. The inoculum for each microorganism was prepared on the surface of soybean casein digest agar for bacteria, or Sabouraud-dextrose agar without the addition of antibiotics for fungi. Incubation is at 30°C to 35°C for 18 to 24 hours in the case of bacteria, at 20°C to 25°C for 48 hours

Table 2. Test Results for the 5 Vehicles Studied*,†,‡

Organism		*S aureus*		*P aeruginosa*		*C albicans*		*A brasiliensis*		*E coli*		*Z rouxii*	
Incubation time (days)		**14**	**28**	**14**	**28**	**14**	**28**	**14**	**28**	**14**	**28**	**14**	**28**
Sample No.	1	6.0	NI	5.0	NI	4.0	NI	3.0	−1.0	5.0	NI	4.0	NI
	2	0.2	−0.3	−1.1	0.3	−1.3	0.2	−0.6	−0.6	0.7	CM	−2.1	CM
	3	6.0	NI	5.0	NI	2.0	2.0	2.0	NI	5.0	NI	4.0	NI
	4	6.0	NI	5.0	NI	4.0	NI	2.0	NI	5.0	NI	4.0	NI
	5	6.0	NI	6.0	NI	4.0	NI	1.0	1.0	−1.0	2.0	4.0	NI

CM, growth of countless microorganisms; NI, no increase in number of viable microorganism as compared to the previous reading

* Positive numbers: log reduction in the inoculated dose of microorganisms after different incubation times (14 and 28 days).

† Negative numbers: increase in microorganism count after initial time of incubation.

‡ To meet the European Pharmacopoeia criteria for oral preparations, the antimicrobial activity of the vehicles studied must result in a 3 log reduction (equal or more) in the inoculated dose of bacteria after 14 days and NI as compared to the previous reading at 28 days. For fungi, these criteria change to 1 log reduction (equal or more) at 14 days and NI as compared to the previous reading at 28 days.

with *C albicans* and *Z rouxii*, and at 20°C to 25 °C for 1 week or until good sporulation is achieved with *A brasiliensis*. A minimum subculturing was sometimes required. A sterile liquid suspension containing sodium chloride 9 g /L was used to collect bacterial, *C albicans*, and *Z rouxii* cultures. For *A brasiliensis* the sterile liquid suspension must also contain 0.5 g/L polysorbate 80. Enough liquid should be used to reduce the suspended microbial count to about 10^8 organisms per milliliter. Subsequently, appropriate samples were removed from each suspension (0.1 mL from serial dilutions) and the number of colony-forming units per milliliter was determined in each suspension by plate count. This value was used to determine the inoculum and the reference values used in the assay. The suspensions should be used immediately.

The test then began with inoculation of the studied vehicles. Inoculate is done in the vehicles' final package with each of the test microorganisms in order to obtain an inoculum of 10^5 to 10^6 organisms per milliliter or per gram of preparation. The volume of the inoculum suspension did not exceed 1% of the volume of the product. It was thoroughly mixed to ensure homogeneous distribution. The inoculated product was maintained at 20°C to 25°C, and protected from light. At time zero and at suitable intervals, depending on the type of product (e.g., oral preparations at 14 and 28 days), a sample of each package (1 mL) is removed and the number of viable microorganisms determined by plate count. Results are the average of duplicate readings.

Results

To meet the European Pharmacopoeia criteria for oral preparations, the antimicrobial activity of a preservative must result in a 3 log reduction in the inoculated dose of bacteria after 14 days and no increase as compared to the previous reading at 28 days. For fungi, these criteria change to 1 log reduction at 14 days and no increase as compared to the previous reading at 28 days.

Table 2 shows the results of the assays. As can be seen, simple syrup (vehicle 1) was microbiologically stable during 15 days (a log reduction above 3 was detected at 14 days for every microorganism). *A brasiliensis* growth was detected at 28 days (a negative log reduction at 28 days). When this vehicle was diluted with purified water (vehicle 2), at each sampled time, the quality criteria were not met (log reduction was less than the desired one or an increase in growth was detected). Vehicle 3, simple syrup diluted with aqua conservans, met the quality criteria (log reduction was above 3 or 1 for bacteria or fungi, respectively, at 14 days and no increase at 28 days) owing to its containing propylene glycol and parabens in a final proportion of 0.04% and 0.01% w/w for methylparaben and propylparaben, respectively. Vehicle 4, aqua conservans without propylene glycol, met the microbiological quality criteria too. When vehicle 5 was assayed, in which aqua conservans was prepared without propylene glycol and lower proportions of parabens, bacterial growth (*E coli*) was detected at 14 days, and vehicle 5 did not meet quality criteria.

Discussion

Simple syrup (vehicle 1) was microbiologically stable during its declared validity period of 15 days[16] even though fungal growth was later detected. The quality criteria for vehicle 2 were not met since it lacked preservatives, except sucrose itself in too low a proportion (32% w/w) to prevent microbial contamination (<60% w/w).[3] The proportions of parabens in vehicle 3 (above or equal to the minimum proportion recommended, 0.015% and 0.01% for methylparaben and propylparaben, respectively[15]) prevent the growth of the inoculated microorganism. With the aim of eliminating the propylene glycol and reducing the parabens' proportions to the minimum, vehicles 4 and 5 were assayed. Vehicle 4, in which aqua conservans is prepared without propylene glycol to prevent its toxic action,

met the microbiological quality criteria. Therefore, the elimination of propylene glycol is possible when the aqua conservans is prepared with parabens above the lowest feasible level, in the final proportion (equal to vehicle 3). On the other hand, when vehicle 5 was assayed, prepared with aqua conservans without propylene glycol and the lower quantity of parabens recommended as preservatives for oral liquid formulations, bacterial growth was detected at 14 days. This minimum quantity of parabens in vehicle 5 is evidently insufficient to protect it from microbial contamination; indeed the final proportions (0.008% and 0.005% for methylparaben and propylparaben, respectively) in the diluted syrup were less than those recommended.

For all the above, when APIs are formulated with simple syrup diluted 50:50 v/v with aqua conservans (vehicle 3) or with aqua conservans without propylene glycol as solvent (vehicle 4), these vehicles are able to inhibit microbial growth with and without the use of propylene glycol, respectively, which is not recommended for pediatrics owing to its toxic effects. Thus, less toxic formulations can be used to administer APIs to this vulnerable group. But, if it is used, simple syrup diluted 50:50 v/v with purified water (vehicle 2) or dilute aqua conservans prepared with the lowest feasible proportion of hydroxybenzoates recommended to exert a preservative effect without propylene glycol (vehicle 5) is not able to inhibit growth if these vehicles become contaminated by microorganisms before or during API administration.

Taking into account that this study is made as the Pharmacopoeia test indicates, and the vehicles are tested without the API incorporation and are incubated not during the real administration of the doses, the microorganism contamination can be greater if adequate hygienic measures are not considered.

In conclusion, when diluted simple syrup is necessary to use in the formulation of an API in pediatrics, it is possible to use water with parabens at adequate proportions without being in excess to assure the effectiveness of its antimicrobial preservation and without propylene glycol used as solvent. Thus, if during the oral administration of the formulation, it is contaminated, the preservatives will be able to inhibit their growth.

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Disclosure The authors declare no conflicts or financial interest in any product or service mentioned in the manuscript, including grants, equipment, medications, employment, gifts, and honoraria. The authors had full access to all the data and take responsibility for the integrity and accuracy of the data analysis.

Acknowledgments This work was supported by a Research Project of the Fundación CajaCanarias in Spain (BIO27). The authors are very grateful to the following for their collaboration: Farmacia Feria, Instituto Tecnológico del Medicamento Individualizado (ITMI), and the Comisión Nacional de Unificación de Criterios en Formulación Pediátrica. The authors also wish to thank Jonatan Martín Rodríguez from the Organic Chemistry Department of La Laguna University for his advice on use of the McFarland Densitometer and standard set.

Accepted April 2, 2018

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AAPS PharmSciTech (2019) 20:208
DOI: 10.1208/s12249-019-1432-x

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Research Article

A High-Demanding Strategy to Ensure the Highest Quality Standards of Oral Liquid Individualized Medicines for Pediatric Use

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Received 6 March 2019; accepted 22 May 2019

Abstract. Individualized medicines for pediatrics are a useful alternative if there is no correct dosage marketed for this segment (easy to swallow, adequate volume and content, correct composition for pediatrics, good organoleptic properties, *etc.*). Its validation process must ensure quality testing: its content uniformity, physical (homogeneity after shaking), chemical, and microbiological stability. Some of these attributes are checked by the recommendations of European Pharmacopoeia (Ph. Eur.), International Conference of Harmonization (ICH), and National Formularies but others are not. The aim of this study is to develop a general high-demanding strategy to ensure the final quality of liquid dosage forms testing and developing standard operating processes (SOPs) for the elaboration of individualized oral liquid medicines for pediatric use. Furosemide was used as an example of the validation of an individualized liquid solution for pediatric use. Three SOPs were selected according to their composition and the recommendations of liquid dosage forms for pediatric use. Quality attributes according to National Formularies, Ph. Eur., and ICH were tested: pH, organoleptic properties, uniformity of mass of delivered dose from multidose containers, and chemical stability. In this study, a general high-demanding strategy was elaborated to validate oral liquid dosage forms, including validation of the analytical method used to test their quality. A second part focuses on the elaboration of liquid formulations for pediatrics with the highest standards of quality taking into account CQAs that were not contemplated by official compendial such as content uniformity and physical stability.

KEY WORDS: individualized medicines; pediatrics; quality; liquid dosage form.

INTRODUCTION

In the development of individualized medicines for pediatric use, a good validation strategy is essential to ensure a quality target product profile (QTTP) during the stability period of the formulation. This kind of medicine can be defined as non-sterile liquid preparation produced by licensed hospitals and community pharmacies when appropriate medicine for children is unavailable.

The identification of critical quality attributes (CQAs) is important in order to produce high-quality oral liquid formulations. In the USA, 10 compounded drugs out of 29 failed quality testing when checked by regulatory agencies (1). This could mean that a strategy is required that focuses on the evaluation of the quality of individualized liquid medicines prepared by community pharmacies or hospitals, as those published for medicine manufacture by pharmaceutical industries may not be suitable for this kind of formulations.

In individualized liquid medicines for oral use, one of the most important CQA is content uniformity; each dose must label the amount of active pharmaceutical ingredient (API). This is especially important in the case of suspensions, where the API might not be homogenously distributed in the formulation. According to the European Pharmacopoeia (Ph. Eur.), these individualized liquid medicines stored in multidose containers are only required to comply with the test for uniformity of mass delivered from multidose containers (2–4). This test evaluates the uniformity of the weight of each dose, assumes a homogeneous distribution of the API in the whole formulation (5). In 2017, Schlatter *et al.* published an article where the uniformity of doses of a suspension was not tested following Ph. Eur. recommendations. Therefore, content uniformity cannot be ensured (6).

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Published online: 03 June 2019

Physical stability is another important attribute. During storage, there must be no crystallization, sedimentation, or other physical processes that could affect the stability (quality) of the formulation. In addition, if some of these processes take place during storage, it must be assured that, after shaking the individualized medicine, a homogenous formulation is obtained. Although this is important, it becomes even more so when the formulations are stored at 5°C as they are not usually tested (6–10).

Chemical stability is another point that must be checked as a CQA in individualized medicines for pediatrics. The medium used to dissolve API, the excipients, or the pH are elements which can affect stability and so must be tested. Last but not least, microbiological stability is as significant as the other points. The growth of microorganisms produces changes in the pH that could produce a reduction in the stability of the molecule apart from producing health problems in the patient. However, there are some articles without microbial stability testing, even when there were no preservatives in the composition (8,11,12). Moreover, in some cases, the pH variation was not followed during the stability test despite the importance of this parameter as an indicator of the API stability or microbial contamination (13,14).

In addition, a stability test must be carried out following the International Conference of Harmonization (ICH) guidelines. Some National Formularies recommend checking their organoleptic properties and pH (2,3). All these tests would cover two of the five CQAs proposed.

As pointed out, most authors validate each formulation taking into account different CQAs. That is the reason why a high-demanding strategy is needed in order to ensure the quality of liquid dosage and unify criteria.

The physicochemical properties of the API (solubility, particle size, class in the Biopharmaceutics Classification System (BCS), *etc.*) will have an enormous influence on these CQAs. Solubility, particle size, and dose will affect the content uniformity of the formulation because they determine if a certain formulation will be a solution or a suspension. The antimicrobial activity of the API or if it is photosensitive will also have an influence in the stability of the formulation.

The excipients used in individualized medicine will influence the quality, so it is another important point during its design. A very common way to prepare these formulations is to manipulate or compound the authorized and marketed tablets. This means that the API and excipients used will be transferred. These might not be suitable for children (15), may not be soluble in water or even interfere with the distribution of the API (16). In this sense, it is suggested to start from the API and add the least number of excipients and in the lowest proportion to produce their effect in the formulation (17,18).

Certainly, the elaboration of a good standard operating procedure (SOP) is essential to ensure the quality of the final formulation. The SOP must include information about packaging. The guideline regarding packaging for pharmaceutical products published by WHO in 2002 must be followed (19). Quality packaging selection is essential to ensure protection of the API in the formulation (light, moisture, oxygen). In addition, the compatibility of the packaging with the API is very important: interaction between container and substances, release of chemicals from packaging materials, absorption or adsorption of substance by packaging materials, degradation of packaging materials, *etc.*

A class IV drug, furosemide, was used as a case of the validation of a liquid individualized medicine for pediatric use (20,21). Furosemide is a loop diuretic indicated for the treatment of cardiac and renal edema in pediatrics; it blocks the co-transport system $Na^+K^+2Cl^-$ which is placed on the ascending limb of the loop of Henle. The diuretic action is the result of the inhibition of the reabsorption of sodium chloride in this segment of the loop of Henle (22,23).

It is marketed in the USA and France in tablet form and as an oral solution (24,25). In other countries such as Belgium, Spain, Norway, or Sweden, it is only available in tablet form so an oral formulation for pediatric use is required in community pharmacies and hospitals (26–29).

The oral dose for a newborn child is 1–4 mg/kg body weight each 12–24 h and 1–2 mg/kg body weight each 6–12 h in nurslings and older children (22).

The solubility of furosemide is pH dependent; its maximum solubility is to be reached at pH greater than 8, 21.9 mg/ml at 30°C (21). In addition, bioavailability is very low, near to 20–60%, due to its low permeability (30).

Although there are SOPs in the literature for the elaboration of oral formulations of furosemide (some of them using already commercial dosage forms), there is a deficiency of published data related to dose homogeneity, stability, and in general, about the steps to ensure its quality (31–37).

The aim of this work is to develop a high-demanding strategy, based on the QTTP and CQAs, to be used during the validation process of liquid dosage forms, solutions, or suspensions, and thus ensure its quality.

This global strategy has been developed based on the data obtained during the validation of furosemide and from previous studies with different APIs (dexamethasone, flecainide, ursodeoxycholic acid, carbamazepine, and acetazolamide) (12,38–40). These have different physicochemical characteristics (solubility, permeability, dose, particle size…) so a general strategy can be reached.

The selections of the APIs took into account several aspects. One of these aspects was the most commonly demanded APIs in hospitals and community pharmacies to treat ailments in pediatrics and did not have a commercial formulation. Another aspect was the problems that arose during the elaboration of several formulations using previously proposed SOPs. And finally, the excipients used in these formulations must be adequate for pediatric use and have sufficient data regarding safety.

MATERIALS AND METHODS

Furosemide was pharmacopeia grade and was provided by Acofarma (Barcelona, Spain). Purified water was obtained from a water purification system (Puranity TU 12, VWR, USA). Methanol was analytical grade (Sigma-Aldrich, Madrid, Spain).

Formulations obtained in the literature with a high number of excipients or not accepted for pediatric used were discard. Three different formulations of furosemide (2 mg/ml), contained in multidose containers, were chosen to evaluate its quality. F1 is the most used formulation until 2017 (37), F2 is the formulation proposed by ISPHC (International Society of

Pharmaceutical Compounding) (35), and F3 is the formulation published in 2018 in the Spanish National Formulary (34), see Table I.

The excipients of the different formulations were prepared as follows:

– Sörensen buffer was prepared as a solution of 19.2% of KH_2PO_4 *v/v* and 80.8% of Na_2HPO_4 *v/v* in purified water.
– The aqua conservans without propylene glycol (ACWP) was prepared by mixing 0.8 g of methyl p-hydroxybenzoate (methylparaben) and 0.2 g of propyl p-hydroxybenzoate (propylparaben) in 1 l of purified water at 50°C.
– The simple syrup with ACWP was prepared as a solution of 64% sucrose *w/w* and 36% *w/w* of ACWP at constant shaking until obtaining a homogeneous solution which was then filtered.

F1 was elaborated according to the following standard operating procedure (SOP):

– 200 mg of furosemide was weighted.
– Measure 70 ml of Sörensen buffer in a 100-ml graduated cylinder and transferred it to a 250-ml beaker.
– Furosemide was transferred to the beaker with constant magnetic stirring.
– Maintain under magnetic stirring at medium power until getting a solution.
– The solution is then transferred to a 100-ml graduated cylinder and complete with simple syrup with ACWP.
– Finally, it is packaged in a 125-ml amber bottle with dispenser closure.

F2 was elaborated according to the following SOP:

– 200 mg of furosemide, 6.96 g of $Na_2HPO_4 \cdot 2 H_2O$, and 63 mg of monohydrated citric acid were weighted.
– 80 ml of ACWP was measured in a 100-ml graduated cylinder and transferred to a 250-ml beaker.
– $Na_2HPO_4 \cdot 2 H_2O$ and the monohydrated citric acid were transferred to the beaker with constant magnetic stirring. Maintain magnetic stirring at medium power until obtaining a solution.
– Furosemide was transferred to the beaker with constant magnetic stirring.
– Maintain under magnetic stirring at medium power until obtaining a solution.
– The solution is then transferred to a 100-ml graduated cylinder and completed with ACWP.
– Finally, it is packaged in a 125-ml amber bottle with dispenser closure.

F3 was elaborated according to the following SOP:

– 200 mg of furosemide, and 1.5 g of $Na_2HPO_4 \cdot 12 H_2O$ were weighted.
– 80 ml of ACWP was measured in a 100-ml graduated cylinder and transferred to a 250-ml beaker.
– $Na_2HPO_4 \cdot 12 H_2O$ was transferred to the beaker with constant magnetic stirring. Maintain under magnetic stirring at medium power until obtaining a solution.
– Furosemide was transferred to the beaker with constant magnetic stirring.
– Maintain under magnetic stirring at medium power until obtaining a solution.
– The solution is then transferred to a 100-ml graduated cylinder and complete with ACWP.
– Finally, it is packaged in a 125-ml amber bottle with dispenser closure.

As mentioned, all formulations were packaged in amber bottles (Envases Farmacéuticos SIREP, Spain) taking into account the effect of light on the stability of furosemide. These bottles are made of polyethylene terephthalate (PET), and comply with the Ph. Eur. requirements and seemingly do not interact with any substance in the composition. In addition, a dispenser closure was added in order to make dose measurement easy with the use of a plastic syringe.

Furosemide was analyzed applying an adapted high-performance liquid chromatography (HPLC) method to ultra-high-performance liquid chromatography (UHPLC) in an Acquity UPLC® H-Class System with a X-Select® C18 reversed phase column 2.5 μm XP (2.1 × 75 mm) (Waters, Milford, MA, USA) (21,41). The data acquisition software was Astra 6.0.1. (Chromatographic Manager, Waters Corporation). The mobile phase was ammonium phosphate buffer 0.01 M:methanol (57:43, *v/v*), at a flow rate of 0.4 ml/min. The

Table I. Composition and Characterization of 2 mg/ml Furosemide Solutions

Formulation	F1	F2	F3
Furosemide (mg)	200	200	200
Na_2HPO_4 2 H_2O (g)	–	6.96	–
Na_2HPO_4 12 H_2O (g)	–	–	1.50
Sörensen buffer, pH 7.4 (ml) (19.2% of KH_2PO_4 *v/v* and 80.8% of Na_2HPO_4 *v/v* in purified water)	70	–	–
Monohydrate citric acid (mg)	–	63	–
Diluent (qs 100 ml)	Symple syrup with ACWP	ACWP	ACWP
pH	6.92 ± 0.01	7.90 ± 0.01	7.87 ± 0.01

ACWP, aqua conservans without propylene glycol; *qs*, amount which is enough to complete 100 ml. pH is expressed as mean value ± SD. *SD*, standard deviation

UV detection was at 273 nm. The injection volume was 10 µl. All chemicals and reagents were UPLC grade. All samples and solvents were filtered with 0.2 µm pore-size filters (Millipore, Billerica, MA).

The validation of the analytical method was done following the ICH guideline for this purpose (42). The variance analysis (ANOVA) of the linear regression confirmed the linearity of the method, through rejection of the null hypothesis of deviation from linearity for a significance level of 0.05 ($\alpha = 0.05$). Eight standard solutions were prepared weighting 10 mg of furosemide and adding 10 ml of a solution to promote the solubility of the API, diluting solution. This solution was made of 50% *v/v* of acetonitrile, 2.2% *v/v* of acetic acid, and purified water in quantity sufficient to 100 ml (42). This first standard solution was diluted with mobile phase to a concentration interval 6–20 µg/ml. The variance analysis (ANOVA) was carried out to confirm the linearity of the method.

In addition, an *F* test was carried out to evaluate possible differences, apart from the ones produced by experimental error, between the results of the analysis of the standard solutions and days. A significance level of 0.05 was used ($\alpha = 0.05$).

The method precision (as repeatability) was determined by a sixfold analysis of the same sample. System accuracy was expressed as percentage recovery by assay of a known added amount of drug ($n = 9$). The detection and quantitation limits, based on the standard deviation of the response and slope, were also checked. Robustness was also tested to establish the effect of operational parameters on the analysis results. The flow rate (0.4 ± 0.5 ml/min), injection volume (10 ± 0.3 µl), mobile phase composition ($57 \pm 5/43 \pm 5$), and column performance over time were determined to confirm the method's robustness. To calibrate the UPLC system and monitor its performance, we analyzed a furosemide solution sample daily as standard.

A 20 µg/ml solution of furosemide was stored at 80°C (Heraeus UT 6060, Spain) to test the ability of the method to follow the degradation of the API and check if it was able to detect and quantify any product degradation. This solution was analyzed at 1, 2, 24, and 48 h.

To determine the capability of the method to extract the API of every formulation studied, ten samples of 5 ml each were prepared. Two milligrams of furosemide and all the excipients of each formulation were weighed in the right amount. After homogenization of the samples, they were first mixed with the diluting solution to improve the solubility of the API and then diluted to a concentration of 14 µg/ml.

An exam of the organoleptic properties, pH, and a verification of the volume/mass must be done according to the Spanish National Formulary, either it is a suspension or solution (43,44). The pH of each formulation was measured in a Crison GLP 21 pHMeter. A 3-ml sample was taken from each solution at 0, 15, 30, and 60 days. The measurement was done in duplicate at 25°C. A *t* test was done to evaluate the significance between pH value and time with a significance level of 0.05 ($\alpha = 0.05$), data not shown.

The European Pharmacopoeia (Ph. Eur.) simply recommends uniformity test of mass of delivered doses from multidose containers for this kind of individualized medicines: "Weight individually 20 doses taken at random from one or more containers with the measuring device provided and determine the individual and average masses. Not more than 2 of the individual masses deviate from the average mass by more than 10 per cent and none deviates by more than 20 per cent." (4). Those 20 doses were taken out with a 5-ml syringe (BD Discardit™ II) for oral use after the formulations were manually shaken, 10 times inverted 180°, before taking out a new dose (45). This device allows accurate dose measurement and controlled administration to the buccal cavity for all ages (15).

Chemical stability of the API was tested following the respective guidelines of the ICH. This guideline establishes a limit of ± 5% of the declared value (DV) (46). All formulations were placed and duplicated under three different conditions: 5 ± 0.1°C/10 ± 5 relative humidity (RH) (Fridgestove P-selecta Welidow type, Spain), 25 ± 0.5°C/45 ± 5 RH (Memmert ULP500, Spain), 40 ± 0.1°C/20 ± 5 RH (Heraeus UT6060, Spain). Five-milliliter samples were taken, with a 5-ml syringe (BD Discardit™ II) each couple of days and their content was measured and expressed as percentages of the DV. Samples were first mixed with the solution to improve the solubility of the API and then diluted to a concentration of 14 µg/ml.

Powder XRD spectra were acquired from X'Pert PRO X-ray diffractometer (PANalytical, Madrid, Spain) to determine the structure of the crystals formed in the formulations during storage. CuKα radiation ($\lambda = 1.5406$ Å) was employed, and 2θ data were collected from 5.00° to 100° with a scanning rate of 0.03 s^{-1}. Crystalline phases were identified by comparing the experimental diffraction patterns with a furosemide pure pattern and using the Joint Committee on Powder Diffraction Standards (JCPDS).

The variation of pH was determined due to the influence of the pH with attributes like stability or solubility (47).

Finally, all the data enables the establishment of a stability period. This may be understood as the period where individualized medicine meets the CQAs.

The results obtained from the validation of formulations made of furosemide were used with previously published data of different APIs: flecainide, acetazolamide, dexamethasone, and carbamazepine to elaborate the most general strategy possible (38–40).

RESULTS AND DISCUSSION

The ANOVA of the linear regression confirmed the linearity of the method, through rejection of the null hypothesis of deviation from linearity for a significance level of 0.05 ($\alpha = 0.05$). The coefficient of variation of the method was 4.34%. The equation of the regression line was Area ($\mu V \cdot sec^{-1}$) = 80,135 · C (µg/ml); $r^2 = 0.99$. It is precise (0.7%), accurate (99.29%), and has a detection and quantification limits of 1.78 and 5.41 µg/ml, respectively.

The *F* test showed no difference between the results of the analysis of standard solutions and days. The regression line could then be used to obtain the concentration of API in each sample. However, a pure pattern was always used in order to ensure optimal performance of the system.

Figure 1 shows the chromatogram obtained by the UHPLC method for furosemide as pure pattern and also how this peak changes over time when it is stored for 48 h at 80°C. Moreover, at 0.6 min, another peak may be observed

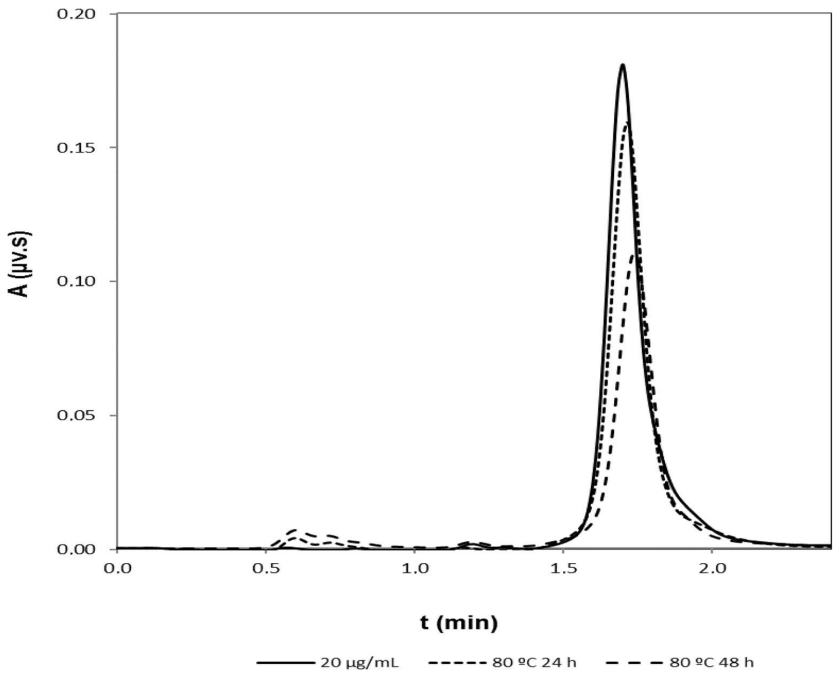

Fig. 1. Chromatogram obtained by UHPLC for furosemide

with an area increase; this may be due to a degradation product of the furosemide.

Developing a good analytical method is essential to ensure that it is efficient to analyze all the CQAs of the formulation. Table II shows a summary of the main properties of different analytical methods used to study different APIs. The method must be precise and accurate. In addition, it should follow the degradation of the API and quantify degradation products, as this might be dangerous above a certain limit (48).

As may be seen, all the APIs were analyzed over a short period of time when the UHPLC system was used. All methods showed values of precision and accuracy within the limits and they were all able to detect the degradation of the API, fundamental when testing their chemical stability.

The average extraction yield of the furosemide for F1 is 103 ± 3.0% and 100 ± 1.8% for F2. In the case of F3, it was not carried out because its composition is very similar to F2. Table III shows a summary of the extraction yield for the different API studied.

It is fundamental to check that the excipients of the formulation do not interfere with the analysis of the API and that it is possible to extract the right amount from a complex matrix (simple syrup, glycerol, cellulose, *etc.*). In this case, for all APIs studied, correct extraction was possible for each method.

According to the BCS, furosemide is a class IV due to its low solubility and low permeability. Nevertheless, the amount of API required to develop a formulation of 2 mg/ml enables the dose to dissolve in our formulation. Furosemide is a good example which proves that the BCS needs to be adapted for pediatrics. BCS is focused on adults because in this classification, a high solubility drug means that the highest dose permitted (for adults) is dissolved in 250 ml of purified water. However, in pediatrics, this dose is much lower and permeability is also different. For that reason, a pediatric-BCS could be useful in order to select the appropriate amount and number of excipients during the design of individualized medicines for pediatric use (49,50).

Table II. Summary of the Main Characteristics of Different Analytical Methods Used to Validate Oral Liquid Individualized Medicines

API	System	Analysis time (min)	Precision (%, < 1%)	Accuracy (%, 97–103%)	Detect degradation/ products
Furosemide	UHPLC	2.70	0.70	99.3	Yes/no
Dexamethasone (38)	UHPLC	1.70	0.48	98.7	Yes/yes
Flecainide (39)	UHPLC	0.80	0.21	99.1	Yes/no
Ursodeoxycholic acid (12)	HPLC	8.00	0.93	102	Yes/no
Acetazolamide (40)	UHPLC	2.50	0.71	102	Yes/no

Table III. Extraction Yields (%) for the API Studied

API	Formulation	Extraction yield (%)
Furosemide	F1	103 ± 3.0 (n = 10)
	F2	100 ± 1.8 (n = 10)
Dexamethasone (38)	F1	103 ± 4.3 (n = 5)
	F2	100 ± 3.4 (n = 5)
Flecainide (39)	F1	111 ± 10.2 (n = 20)
Ursodeoxycholic acid (12)	F1	95.1 ± 0.06 (n = 20)
Acetazolamide (40)	F1	105 ± 6.5 (n = 6)

All solutions showed a pH near 7 and a transparent aspect when they were elaborated. They were odorless and insipid. Table IV shows the mass uniformity test performed for the three formulations. All formulations met the Ph. Eur. test for mass uniformity of multidose containers.

In the case of furosemide, the API is dissolved in the entire formulation; it is a solution. However, in case of suspension where it is not completely dissolved in the medium, this test is insufficient to ensure homogeneity of the dose. For example, in the case of validation of an individualized medicine of acetazolamide, all formulations tested met the mass uniformity test. However, once these doses were analyzed, a lack of homogeneity between doses was detected. Table V shows the content uniformity data for APIs studied, obtained from already published articles, which where formulated as suspensions.

Table IV. Mass Uniformity Test (Ph. Eur.) for F1, F2, and F3

Dose	F1, mg		F2, mg		F3, mg	
1	5.46		5.22		5.09	
2	5.48		5.25		5.12	
3	5.50		5.28		5.17	
4	5.50		5.23		5.17	
5	5.48		5.25		5.11	
6	5.51		5.24		5.12	
7	5.51		5.24		5.11	
8	5.50		5.24		5.12	
9	5.51		5.25		5.12	
10	5.49		5.25		5.20	
11	5.50		5.25		5.16	
12	5.51		5.27		5.12	
13	5.48		5.23		5.14	
14	5.50		5.27		5.15	
15	5.48		5.26		5.13	
16	5.50		5.28		5.16	
17	5.51		5.27		5.14	
18	5.50		5.24		5.14	
19	5.49		5.27		5.18	
20	5.48		5.22		5.13	
Average	5.49		5.25		5.14	
	LL	UL	LL	UL	LL	UL
10%	4.94	6.04	4.73	5.78	4.62	5.65
20%	4.40	6.59	4.20	6.30	4.11	6.17

LL, lower limit; *UL*, upper limit

For this reason, a content uniformity test is suggested in the validation of suspensions; to ensure that in each dose is the right amount of API. Mass uniformity test of multidose containers could be adapted to this task and individual content of 20 doses and its average could be measured. As limits: no more than 2 of the individual content deviates from the average content by more than 10% and none deviate by more than 20%. Relative standard deviation (RSD) could be calculated to translate this deviation, in more than 10 or 20%, into a numerical meaning. This test was done in previously published articles, during the validation of different individualized medicines for pediatric use. A total of 7 formulations out of eleven were discarded due to the absence of content uniformity. It must be highlighted that all these formulations met the test of mass uniformity recommended by the Ph. Eur. (12,38–40).

In addition, uniformity of dosage units test could be used to calculate content uniformity. Individual content of 10 doses could be used to calculate the acceptance value of each formulation. This would be more precise than the first test and could detect formulations with individual values within the limits ± 10% but with an AV higher than 15 (AV limit for 10 doses), see Table V.

Generally speaking, when mass uniformity limits are used to check content uniformity, if a formulation does not meet this test, it will not meet the test for uniformity of dosage units (flecainide F1). However, in the case of ursodeoxycholic acid F1, there is no individual value which deviates by ± 10% of the average content but its AV value is greater than 15. So, as explained before, the determination of AV is stricter than knowing how many individual values deviate ± 10/20% of average content.

Another important point in the validation of liquid formulation is the physical stability of suspensions. Understanding the behavior of the formulation is essential to obtain a homogeneous suspension once it is shaken after several hours of standing. For this reason, rheological studies should be carried out during the validation of suspension. Differentiating between a Newtonian and a non-Newtonian fluid is basic to know how a liquid individualized medicine should be shaken. In the first type, viscosity is independent of shear rate, which means that it does not matter how much a formulation is shaken; its viscosity will not change (dexamethasone and acetazolamide (12,38–40).. In the case of the non-Newtonian fluid, viscosity changes with shear rate, if the formulation is shaken vigorously, its viscosity decreases and would be easier to re-suspend the API and get a homogeneous formulation after standing, for example, ursodeoxycholic acid and acetazolamide (12,40).

Obtaining a homogeneous suspension after standing can be checked, as done in the case of dexamethasone, ursodeoxycholic acid, or acetazolamide. Each formulation was placed into a 100-ml graduated cylinder and deposited in a 5 ± 0.1°C (Fridge-stove P-selecta Welidow type, Spain) for 30 days. Doses (5 ml) were taken from Z1 (top of the formulation) and Z3 (bottom of the formulation) after shaking (10 times inverted 180°) and left to stand for several minutes. Then, their content, expressed as % DV, was studied in order to see how homogenous the suspension was.

Table V. Content Uniformity Test and Dmax/*t* value

Formulation		Content uniformity test				Dmax/*t*
		± 10%	± 20%	RSD	AV	
Dexamethasone (38)	F1	0	0	5.24	7.62	0.02
	F2	0	0	1.68	4.63	0.06
Flecainide (39)	F1	8	4	17.84	54.80	ND
	F3	0	0	2.20	4.35	ND
Ursodeoxycholic acid (12)	*F1*	*0*	*0*	*7.32*	*28.82*	*5.93*
	F2-H3	1	19	65.35	132.81	2.20
Acetazolamide (40)	F1	4	3	12.42	22.64	0.11
	F3	7	2	12.6	30.21	1.63
Carbamazepine^{MTP}	F1A	0	0	3.45	8.68	0.30
	F3A	1	0	4.10	9.45	0.42

ND, not determined; *MTP*, manuscript to be prepared. Italic data: none individual value greater than ± 10% of the average content but with AV > 15

Maximum difference between Z1 and Z3, divided by the resting time (Dmax/*t*), was used as an indicator of homogeneity of a suspension and to know how fast the sedimentation process takes place. Table V shows Dmax/*t* values for all the formulations studied by our group. Although it is not possible to establish a solid correlation between Dmax/*t* and AV, it can be seen that when Dmax/*t* has values below 1, AV is less than 15 (below the pharmacopeia limit for 10 samples). Formulation acetazolamide F1 is the only one which, having values for Dmax/*t* < 1, shows AV > 15. In this case, another 20 doses should be analyzed to confirm this AV value. More data are needed to confirm this limit value of 1 for Dmax/*t* to meet content uniformity and confirm homogeneous of suspensions after shaking.

Table VI. Evolution of the Declared Value (% DV) of Furosemide in Time for F1, F2, and F3 at Different Conditions

	Time (days)		% DV			
			0	15	30	60
F1	5°C	Average	100.0	ND	94.31	101.1
		SD	0.00	ND	20.76	0.38
	25°C	Average	100.0	ND	94.25	98.04
		SD	0.00	ND	29.66	2.53
	40°C	Average	100.0	ND	107.9	107.0
		SD	0.00	ND	11.74	1.03
F2	5°C	Average	100.0	ND	119.0	106.1
		SD	0.00	ND	2.72	0.62
	25°C	Average	100.0	ND	107.4	102.3
		SD	0.00	ND	4.32	1.71
	40°C	Average	100.0	ND	105.6	99.14
		SD	0.00	ND	0.26	0.52
F3	5°C	Average	100.0	104.0	98.93	ND
		SD	0.00	0.99	2.90	ND
	25°C	Average	100.0	104.5	98.68	ND
		SD	0.00	1.91	0.31	ND
	40°C	Average	100.0	105.9	98.94	ND
		SD	0.00	0.26	3.14	ND

ND, not determined; *SD*, standard deviation

A chemical stability test, following the ICH guideline, should be carried out during the validation of a liquid formulation either solution or suspension. Table VI shows the stability of furosemide in different formulations. At 30 and 60 days of storage at 5°C and possibly because of the mayor concentration of salts and the temperature, F2 showed the formation of crystals. These crystals were studied by X-ray diffraction and there was no furosemide in its composition. Table VII shows the composition of crystals found in F2. Hence, F2 does not fulfill quality standards due to the formation of crystals.

F1 and F2 are chemically stable for 60 days at 5°C and 25°C, respectively. In the case of F3, the results obtained agreed with stability period established by Spanish National Formulary, stable for 30 days at 25°C (34).

In Table VIII, the pH changes over time are shown. In all formulations and storage conditions, there is a decrease in pH value except for F1 at 25°C. However, there is no statistical difference between pH and time for all storage conditions ($p > 0.05$). pH variation is an important tool which could indicate degradation of an API or microbial contamination. The pH results coincide with the chemical stability test and no degradation was detected in the API. pH is important in APIs, like furosemide or dexamethasone, where pH has an influence in their solubility and stability (38,47).

Due to the fact that there were preservatives in these formulations and the concentration was within the recommended range established by European Medicine Agency (EMA), there was no need for a microbiological study. However, the Ph. Eur. recommends doing the microbiological examination of non-sterile products in the case of APIs which does not have antimicrobial activity or when there is no preservative in their formulation (51).

In the case of validated individualized medicines, if the formulation does not contain preservatives in its composition, we elaborate, at the pharmacy compounding laboratory, the formulation following the SOP proposed. Then, samples were analyzed at 0, 15, and 30 days of storage at room temperature (25°C), simulating the conditions under which daily doses are removed. The microbial count was considered to be the average number of colony forming units (cfu) found in the appropriate medium by plate count method. Liquid oral

Table VII. Composition of Crystals Found in F2

Semi quantification [%, *w*/*w*]	Chemical formula	Matched lines	Mineral name	Common name
88	HNa2(PO4) (H2O)2	133	Dorfmanite, syn	Sodium hydrogen phosphate dihydrate
4	C6H8O7	12		
8	HNa2(PO4)	75	Nahpoite, syn	Disodium hydrogen phosphate(V)

formulations meet microbial requirements if the total aerobic microbial count was less than 10^2 cfu/ml, the total combined yeast/mold count less than 10^1 cfu/ml, and the absence of *Escherichia coli* was confirmed (3,51–53).

In the case of previously published formulations made of flecainide or dexamethasone due to their microbiological stability, their stability period was lower than their chemical stability (38,39).

Recently, Zahalka *et al.* examined the stability of an oral formulation of furosemide which had a similar composition to F3, with a good stability period (54). Saccharin was included in their formulation to improve palatability; a color change and a pH decrease were detected when sucrose was used. In F1, a pH decrease was detected but did not affect the chemical stability when stored at 5°C. In addition, methylparaben was included to ensure antimicrobial preservation but, according to the EMA, there is not sufficient clinical evidence regarding the effect of methylparaben and propylparaben as preservatives in children. Due to the importance of preventing microbial contamination in children, a concentration range has been agreed on for both preservatives to ensure good antimicrobial activity and safety (55).

Therefore, as the case of our formulation, using methylparaben and propylparaben ensured that there would be no microbial contamination and thus safe for children.

According to everything exposed above, a high-demanding strategy to validate liquid individualized medicines has been elaborated to ensure QTTP and control CQAs, see Fig. 2.

Table VIII. pH Evolution at Different Storage Conditions for F1, F2, and F3

	pH				
	Time (days)	0	15	30	60
F1	5°C	6.92	ND	6.90	6.40
	25°C	6.92	ND	5.80	6.80
	40°C	6.92	ND	6.90	6.30
F2	5°C	7.90	ND	ND	7.50
	25°C	7.90	ND	ND	7.80
	40°C	7.90	ND	ND	7.60
F3	5°C	7.87	7.61	6.98	ND
	25°C	7.87	7.54	7.13	ND
	40°C	7.87	7.70	7.09	ND

This strategy can be divided in two different parts, the first is to discern the suitability of the method to validate liquid formulations. The analytical method used needs to comply with some tests according to ICH guideline: precision, accuracy, detections, and quantifications limits; it should be able to detect and quantify degradation products (especially if it could produce health problems above a certain limit). Moreover, the capability of the method to extract the right amount of API from the formulation must be checked too.

The second part concerns the validation of liquid formulations, which should start with its organoleptic properties and pH. If a SOP is available, organoleptic properties and pH should agree with it. If it is not, this must be taken into account in order to establish the stability period. Then, once mass uniformity of the liquid formulation has been checked, a distinction between suspension and dissolution must be carried out. In the first case, content uniformity and physical stability must be ensured. Finally, a chemical and if needed, a microbiological stability test should be done. If the results of any these tests do not meet the requirement standards of quality for any formulation, this should be re-designed in order to improve the quality.

CONCLUSIONS

A general strategy to validate the final quality of oral liquid individualized medicine (solutions or suspensions) was developed. The proposed strategy is more restrictive than the requirements of the actual compendial for this type of formulations. It was created based on previous data from liquid preparations of dexamethasone, acetazolamide, carbamazepine, ursodeoxycholic acid, flecainide, and more recently, furosemide.

This strategy included two different actions: analytical method validation and liquid formulation validation. In order to perform a correct quantification of the API, the method must be lineal, precise, accurate, and be capable of detecting degradation. Moreover, the method must ensure that there are no interferences between the analysis of the API and the excipients of the formulation.

Once the composition of the formulation has been proposed and preliminary test has been done (organoleptic properties, pH, and mass uniformity), depending on the characteristics of the API, two different pathways can be followed. In the case of suspensions, its content uniformity and physical stability (viscosity, rheological properties, resuspendibility, *etc.*) must be studied. Subsequently, a chemical

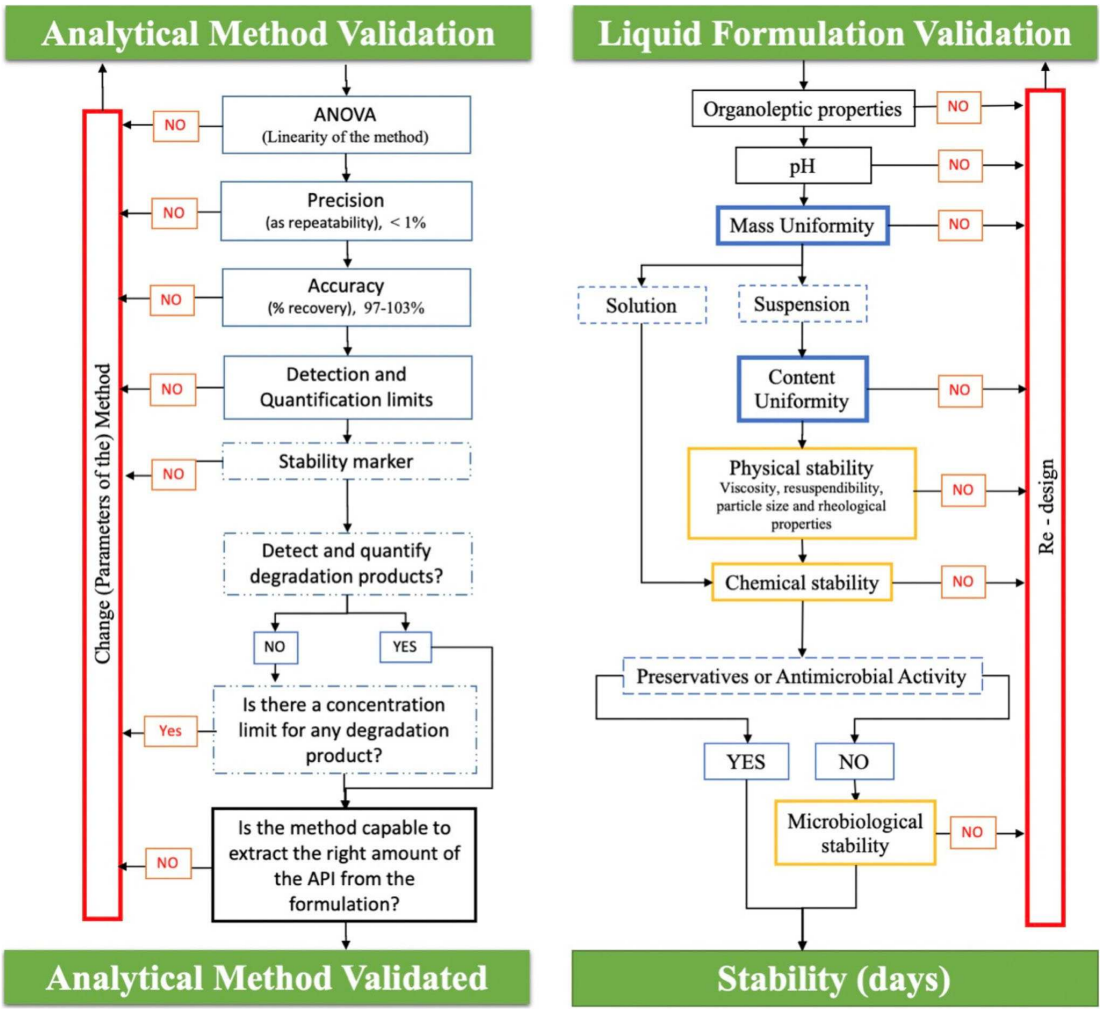

Fig. 2. A general high-demand strategy to validate liquid oral formulation for pediatric use

stability test based on the ICH guideline needs to be carried out. If the API studied does not have antimicrobial activity or there are no preservatives in its composition, a microbiological stability test has to be carried out following the compendial recommendations.

If the obtained data coincides with additional requirements proposed at this strategy, a stability period can be proposed and the medicine can be used.

ACKNOWLEDGMENTS

This work was supported by a Research Project of Fundación CajaCanarias in Spain (BIO27). The authors are very grateful to the following for their collaboration: Instituto Tecnológico del Medicamento Individualizado (ITMI) and Comisión Nacional de Unificación de Criterios en Formulación Pediátrica. The authors also to thank Cajasiete and Universidad de La Laguna for their contribution with a predoctoral contract.

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PUBLISHER'S NOTE

DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY
https://doi.org/10.1080/03639045.2020.1717516

Taylor & Francis
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RESEARCH ARTICLE

Design and optimization of a child-friendly dispersible tablet containing isoniazid, pyrazinamide, and rifampicin for treating tuberculosis in pediatrics

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ABSTRACT

Objective: Develop a child-friendly Fixed Dose Combination (FDC) water-dispersible tablet for Tuberculosis (TB) treatment, with 50, 150, and 75 mg of isoniazid, pyrazinamide and rifampicin respectively. This new formulation must contain the lowest number of excipients accepted for pediatrics and fulfill all the pharmacopeia requirements.

Significance: At present, there is no adequate market dosage form available for children. There is, however, one in a prequalification phase by the World Health Organization but its composition contains excipients which may not be suitable for pediatrics. Therefore, this new formulation would cover this therapeutic gap.

Methods: A factorial design, based on three quantitative factors (compression force and concentration of AcDiSol® and Explosol®) at three levels each, was performed to elucidate their influence over disintegration time and friability. In addition, the influence of the press speed on disintegration time, friability, tensile strength, fineness of dispersion and content uniformity over the target tablet was tested. A stability test was done following ICH guideline for accelerated conditions.

Results: Tablets developed with 9% w/w of Explosol® and a compression force of 16 kN disintegrated in less than 3 min and showed a friability below 1% when 15-mm punches were used. The tableting process could be done up to 25 and 50 cycles/minute ensuring good quality attributes when 15 and 12-mm punches were used, respectively. All APIs remained inside the limit of ± 5% of drug content till 6 months of storage.

Conclusion: A high-quality child-friendly FDC water-dispersible tablet was developed improving the treatment of TB in pediatric.

ARTICLE HISTORY
Received 4 October 2019
Revised 7 January 2020
Accepted 13 January 2020

KEYWORDS
Tuberculosis; children; pediatric; dispersible tablet; treatment; direct compression

Introduction

Tuberculosis (TB) caused the death of 233,000 children in 2017 and one of the main reasons of mortality was the lack of child-friendly formulations for its treatment [1]. Since 2015 The World Health Organization (WHO), the National Institutes of Health (NIH) and The European Medicines Agency (EMA) have been publishing articles regarding the need for efficient studies for global health and formulations focused on pediatrics for treating TB: list of pediatrics needs [2–6].

TB is an infectious disease produced by *Mycobacterium tuberculosis*. The first-line treatment is based on the combination of three active pharmaceutical ingredients (APIs): isoniazid (INH), pyrazinamide (PZA) and rifampicin (RFP). In 2014, WHO increased its daily doses to 10 mg/kg of INH, 35 mg/kg of PZA, and 15 mg/kg of RFP based on previous experience, the increase of resistance, and dose inefficiency. Thus, according to the WHO, the dose of API per tablet should be 50, 150, and 75 mg of INH, PZA, and RFP respectively [7]. However, according to Piñeiro et al., these doses may not be suitable for all ages and may produce cases of under or overdoses [8].

As there is scientific evidence proving the benefits to the patient's health when a fixed-dose combination (FDC) dosage form is used, this becomes the main aim to improve TB treatment in pediatrics [9–11]. The best option seems to be the development of orodispersible tablets, which disintegrates inside the mouth. However, this is not possible due to the high doses of the different APIs required to treat TB. The development of an orodispersible tablet with such doses means a larger tablet and the increase of the possibilities of choking and chewing. Therefore, an interesting alternative could be the development of water-dispersible tablets. In 2018 TB alliance presented a FDC dispersible tablet which has been prequalified by the WHO. This new formulation is made with the recent recommended doses of APIs, but contains excipients such as povidones, aspartame and flavors which may not be suitable for pediatrics, as EMA and other institutions recommend [12–14].

The aim of this study is to develop a child-friendly FDC dispersible tablet for TB treatment with 50, 150, and 75 mg of INH, PZA, and RFP, respectively, using direct compression. This new tablet must be made with the lowest number of excipients and in the lowest percentages. All of them must be accepted for pediatrics following EMA guidelines regarding drug formulation. In addition, such tablets have to be suitable for different ages and body weights [4]. Furthermore, it must comply with Ph. Eur. quality attributes for dispersible tablets (disintegration time, friability,

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content uniformity, fineness of dispersion, and effectiveness of the score lines in the case of 15-mm tablets) [15].

Materials and methods

Materials

INH (Acofarma®), PZA (Sygma-Aldrich®), and RFP (Fagron®) have been used as the API to develop a FDC Tablet for TB treatment. The following excipients were used: AcDiSol® (Croscarmellose Sodium, FMC Corp., Philadelphia, PA), Avicel® PH102, (Microcrystalline Cellulose, FMC Corp., Philadelphia, PA), Explosol® (Sodium Starch Glycolate, Blanver, Taboão da Serra, Spain), CompactCel® (Isomalt, sucralose, betadex, carboxymethylcellulose sodium, Biogrund GmbH, Hünstetten, Germany), Luzenac® (talc, Imerys Talc, Paris, France) and CabOSil® (fumed silica, Cabot CorporaFon, Boston, MA). Purified water was obtained from a water purification system (Puranity TU 12, VWR, Radnor, PA).

UHPLC analysis

All APIs were analyzed by reversed phase Ultra High-Performance Liquid Chromatography (UHPLC) in an Acquity UHPLC® H-Class System (Waters Corporation, Milford, MA) using Astra 6.0.1 as acquisition software (Chromatographic Manager, Waters Corporation, Milford, MA).

INH and PZA were analyzed with a method based on an UHPLC gradient method [16] and RFP was analyzed using a method adapted from High Performance Liquid Chromatography (HPLC) [17]. For both methods, the chromatographic conditions were: XSelect™ CSH™ C18 (75 mm × 2.1 mm id, 2.5 μm) reserved phased column; Acetonitrile:Phosphate Buffer pH 3.7 as mobile phase in proportion of 2:98 (v/v) for INH and PZA, and 38:62 (v/v) for RFP; flow rate of 0.5 ml/min; 242 nm of wavelength. All chemicals and reagents were of analytical grade. All samples and solvents were filtered with 0.2 μm pore-size filters (Millipore, Billerica, MA) before proceeding with chromatographic analysis.

The validation of the analytical method was done according to the ICH guideline using standard solutions with concentrations from 10.0 to 27.0 μg/ml for INH, PZA and RFP. [18]. The variance analysis (ANOVA) was carried out to confirm the linearity of the method.

The method precision (as repeatability) was determined by a six-fold analysis of the same sample. System accuracy was expressed as percentage recovery by assay of a known added amount of drugs ($n = 9$). The detection and quantitation limits, based on the standard deviation of the response and slope, were also checked for each API. Robustness was also tested to establish the effect of operational parameters on the analysis results. To calibrate the UHPLC system and monitor its performance, a solution sample containing all APIs was analyzed daily as standard.

A solution of INH, PZA and RFP with a pH of 7 was stored at 50 °C (Heaeus UT 6060, Spain) during 72 h in order to observe the capability of the method concerning degradation and to detect/quantify degradation products.

In addition, the method must be capable of analyzing the content and obtain the declared amount of APIs in each tablet from a complex matrix (non-soluble excipients mainly). For this reason, each ingredient of one tablet was weighed, dissolved in 50 ml of methanol and diluted with water up to 250 ml. Then, it was filtered using 110 mm filter paper (Albet LabScience, Spain) and diluted to UHPLC analysis. This procedure was repeated 10 times and the average amount was calculated and expressed as labeled content.

Optimization of blending process

APIs and excipients were weighted and blended in a V-Type Blender (FTLMV-0,5, FILTRA® VIBRACIÓN, Barcelona, Spain) with a mixing power of 0.12 kW for 5, 10 and 15 min. At each time, the powder mix was placed in a rectangular container which was divided in 5 different zones and a sample of 200 mg was taken. Finally, its content in API was determined as described above.

Process Capability index (CpK in Equation (1)) was used to know if the mixing process satisfied quality specification in terms of content uniformity:

$$CpK = \min\left(\frac{USL-\mu}{3\sigma}, \frac{\mu-LSL}{3\sigma}\right) \quad (1)$$

where μ and σ are the average and the standard deviation respectively, and USL/LSL are upper and lower specification limits using ±15% as limits for the theoretical content that should be in these samples.

Flow properties of the powder mix were evaluated according to Ph. Eur. tests: angle of repose (Granulate Tester GTB, Erweka, Germany), Carr's Index, and Hausner's Ratio (Tapped Density Tester SVM 223, Erweka, Germany). Other flow properties such as: flow rate, volume flow rate, mass flow rate and flow angle were tested using a 100 ml steel hopper and a 15 mm cylindrical nozzle [19,20].

Preparation of the tablets

Tablets were obtained by direct compression of the powder mix in an instrumented eccentric tablet machine XP1, Research Tablet Press (Korsch, Germany) using 15-mm flat-faced bisect punches (FFBP) and 12-mm flat-faced with beveled edge (FFBE). Tablets were produced with different compressions forces and press speed. Compression force and press speed were controlled by PharmaReseach® (Korsch, Germany).

Experimental design

The variables selected for the experimental design of dispersible tablets were the levels of excipients with function as disintegrate (AcDiSol® and Explosol®) and compression forces (kN). These were chosen to evaluate their influence on disintegration time and friability on 15-mm tablets. For this purpose, a factorial design based on 3 quantitative factors (compression force and concentration of AcDiSol® and Explosol®) at three different levels each was used. Table 1 shows the coded levels and values of the design variables. Therefore, a 3^3-factorial design was performed with 27 different combinations of variables and replicating the center point three times, which meant the elaboration of 30 batches. Sodium starch glycolate shows better properties than croscarmellose sodium according to the literature [21–25]. For this reason, percentages from 2 to 9% w/w of Explosol® where used and 0 to

Table 1. Coded levels and values of design variables to the development of dispersible tablets.

Factor	−1	0	+1
% AcDiSol® (A, w/w)	0.00	2.50	5.00
% Explosol® (B, w/w)	2.00	6.00	9.00
Compression force, kN (C)	11.0	14.0	16.0

Table 2. Composition in mg and % (w/w) of formulations 1–9, each of which was compressed to the three compressions forces, to develop the dispersible tablets following the experimental design.

	Formulation								
Ingredient (mg)	1	2	3	4	5	6	7	8	9
Isoniazid (mg)	50	50	50	50	50	50	50	50	50
Pyrazinamide (mg)	150	150	150	150	150	150	150	150	150
Rifampicine (mg)	75	75	75	75	75	75	75	75	75
AcDiSol® (%)	–	–	–	2.5	2.5	2.5	5	5	5
Avicel® (%)	57	53	50	54	50	47	52	48	45
Explosol® (%)	2	6	9	2	6	9	2	6	9
CompactCel® (%)	7	7	7	7	7	7	7	7	7
CabOSil® (%)	1	1	1	1	1	1	1	1	1
Luzenac® (%)	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Total (mg)	900	900	900	900	900	900	900	900	900

5% w/w of AcDiSol® in order to verify if the second one improves disintegration time or friability.

Table 2 shows the final composition of formulations 1 to 9, each of which was compressed at three compression forces to develop the dispersible tablets.

A statistical approach is used to fit a model using Design-Expert 9.0.3 (Stat-Ease Inc., Minneapolis, MN). Logarithmic values for disintegration time and inverse of square root for friability were used to improve the quality of the model. The *p* value was used in each case to know which terms were significant for each response and *R*-squared (R^2), adjusted *R*-squared (R^2_{adj}) and predicted *R*-squared (Q^2) were used to measure the goodness of the model [26]. All tests were performed at 5% level of significance ($\alpha = 0.05$). The complete model equation is as follows:

$$y = \beta_0 + \beta_A X_A + \beta_B X_B + \beta_C X_C + \beta_{AB} X_A X_B + \beta_{AC} X_A X_C + \beta_{BC} X_B X_C + \varepsilon \quad (2)$$

where *A* is the AcDiSol® (%, w/w), B is the Explosol® (%, w/w), and C is the compression force (kN).

Optimization and characterization of the dispersible tablet

When a formulation complied with the requirements of dispersible tablets in terms of friability and disintegration time the influence of press speed is tested tableting at 10, 25, and 50 cycles/min. Tablets are then characterized testing disintegration time, friability, tensile strength, content uniformity, fineness of dispersion, and effectiveness of score lines as critical quality attributes (CQAs).

Disintegration time: Disintegration time of 6 tablets was determined using a disintegration tester (Disintegrator Tester ZTx20, Erweka, Germany) following the Ph. Eur. recommendations [27]. The time that all the tablets disintegrated was used or accepted for the study.

Friability: This was carried out using a friability test (Tablet Friability/Abrasion Tester TAR Series, Erweka, Germany) following the Ph. Eur. guideline [28].

Tensile strength: This was measured for each batch (Hardness Tester TBH 125 Series, Erweka, Germany) following the recommendations given by Ph. Eur. and USP, Equation (3) [29,30]:

$$TS = \frac{2 \cdot p}{\pi \cdot d \cdot l} \quad (3)$$

where *p*, *d*, and *l* are tablet breaking force, tablet diameter, and tablet thickness, respectively.

Content uniformity: This was tested according to the uniformity of dosage units test by Ph. Eur. [31]. The content of 10 dispersible tablets for each batch was analyzed using a UHPLC system and their acceptance value was calculated.

Fineness of dispersion: Two dispersible tablets dissolved in 100 ml of purified water must pass through a sieve with 710 µm of nominal mesh aperture [15].

Effectiveness of score lines: As 15-mm tablets have score lines, suitability must be tested in terms of mass uniformity. First, 30 tablets were chosen randomly and broken by hand. One half was used for the test and the other half were rejected. 30 parts were weighed, and the average mass was calculated.

Tableting properties

Critical process parameters, such as compression force and press speed, were controlled and signals were imported from Extended Data Analysis® (EDA) (Korsch, Germany) and analyzed using a macro for MS Excel (Microsoft Corporation). Compression process were controlled using a control chart of compression forces and establishing stop reasons when the compression force was greater than 3% of target force.

The *K* value was obtained from the slope of straight-line interval of the Heckel plot using the data from the space between the upper and lower punch and matrix diameter to calculate the relative density of the material (D) according to Equation (4) [32,33]:

$$\ln\left(\frac{1}{1-D}\right) = K \cdot F + A \quad (4)$$

where *F* is the compression force and A is a constant.

Mean yield pressure (Py) and strain-rate sensitivity (SRS) were calculated using K following Equations (5) and (6):

$$Py = \frac{1}{K} \quad (5)$$

$$SRS = \frac{Py1 - Py2}{Py1}.100 \quad (6)$$

where Py1 and Py2 are the yield pressure at low (10 strokes/min) and high speed (50 strokes/min), respectively.

Plasticity, Equation (7), were estimated from the force–displacement compression profile using the average energy consumption within the different compaction phases: W1 (friction work), W2 (network) and W3 (elastic work) [32,34–36]:

$$PL = \left(\frac{W2}{W2 + W3}\right).100 \quad (7)$$

Stability test

A stability test was done placing 15-mm tablets under accelerated conditions (40 ± 2 °C/75% ± 5 Relative Humidity (RH)) following ICH guideline: stability testing of new drugs substance and products (Q1A(R2)). The content of INH, PZA and RFP was measured during 6 months of storage and express as % of declared value [37].

Results and discussion

The ANOVA of the linear regression confirmed the linearity of the analytical method to all the API studied through rejection of the null hypothesis of deviation from linearity for a significance level of 0.05. Characteristics of the method for each API are shown in Table 3.

The average extraction yield of each API from the tablets is 103 ± 2.07% for INH, 98.4 ± 1.95% for PZA, and 98.33 ± 0.95% for

Table 3. Characteristic of the method used to the analysis by UHPLC of each API.

API	INH	PZA	RFP
Calibration curve	A = 31,925.3·C	A = 35,181·C	A = −150,190 + 49,378·C
R^2	0.99	0.99	0.98
CV (%)	3.11	3.32	5.89
Precision (%, <1%)	0.28	0.16	0.23
Accuracy (%, 97-103%)	98.0	97.7	97.7
Detection limit (μg/ml)	1.70	1.74	3.10
Quantification limit (μg/ml)	5.16	5.28	9.40

A: peak area (μV·sec); C: concentration (μg/ml); CV: coefficient of variation.

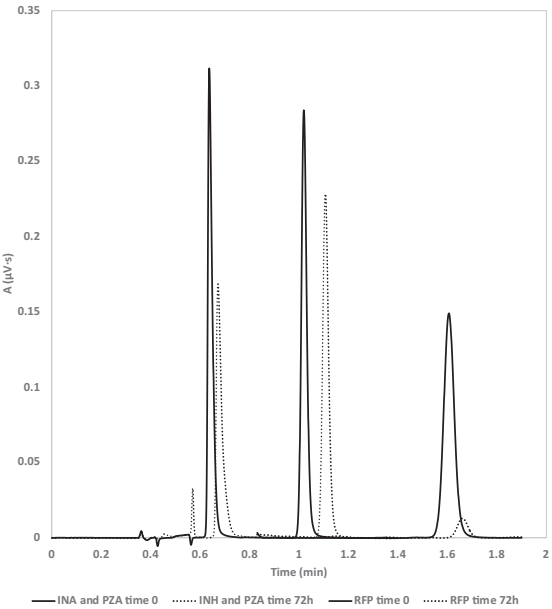

Figure 1. INH (0.6 min), PZA (1 min), and RFP (1.6 min) as pure patter chromatographic peaks (continuous bold line). Discontinuous line represents the decrease of signals for each API after 72 h of storage at 50 °C in a medium with a pH of 7.

RFP. In this case the average extraction is correct as it is near to 100%.

Figure 1 shows the chromatogram for each API obtained by the UHPLC method as pure patterns and also how these peaks change over time under 50 °C of storage.

The selection of excipients was carried out taking into account the complexity of our ideal formulation. All excipients need to be suitable for direct compression and provide good flow properties to ensure API's content. A taste-masking excipient is needed as INH has a bitter taste and they have to be accepted for pediatrics. In addition, the tablets must disintegrate in less than 3 min and have a friability below 1% [28,38].

The first selection of excipients was done taking into account the most common excipients used in published papers related to the development of dispersible tablets: croscarmellose sodium, sodium starch glycolate, crospovidone, microcrystalline cellulose, magnesium stearate, and talc [39–43]. Therefore, we selected the excipients according to their function (lubricant, (super)disintegrant, glidant, etc.), physical characteristics (water-solubility, particle size and shape) and safety.

According to the literature, it is described the incompatibility of the three APIs with lactose and some colorants in the case of INH. Incompatibilities with other excipients were not found. In addition, the excipients finally used in this child-friendly formulation were of the same nature as those used in commercially available oral dosage forms for treating TB in adults. Therefore, the compatibility of the excipients with the APIs it is ensure [44–48]

All of these excipients are generally recognized as safe (GRAS). However, due to the number of tablets which have to be taken in order to treat TB, some excipients were preferred instead of others. Crospovidone was not included in the formulation due to the lack of data in terms of acceptable daily intake and safety in children. In addition, as a lubricant, talc was preferred instead of magnesium stearate because of its laxative effect and mucosal irritation when large quantities are taken [23].

Previous test of powder flow, mixing time to obtain a homogenous powder and tableting process were done to find the right number and percentage of each excipient.

Our objective was to obtain dispersible tablets with a disintegration time below 3 min, according to WHO requirements. Hence, a high disintegration force with a low amount of excipient is required and therefore, superdisintegrants were preferred.

Explotab® and AcDiSol® were selected as theses excipients have a high disintegration force at low concentrations and physical properties useful to develop these tablets. The disintegration force of Explotab® does not seem to be affected by concentration of lubricant or compression force. AcDiSol® also has a good disintegration force and imparts exceptional long-term dissolution stability in comparison to other superdisintegrants. However, at high concentrations of excipient, tablets could become soft when stored with an elevated relative humidity [21–25].

The relationship between concentrations of excipient and disintegration time and friability are very important and therefore studied carefully.

During the first trails, adherence of powder mix to the surface of punches was noticed which made the tableting process difficult. To reduce such adherence, talc (Luzenac®) was increased from 1 to 2.5% w/w improving the situation.

CompactCel® was added to the formulation in order to mask the bitter taste of INH; one of the problems of poor adherence to treatment [49]. This complex excipient was chosen instead of other excipients due to the composition (isomalt, sucralose, betadex, carboxymethylcellulose sodium), and also because of the superior performance in terms of disintegration time and friability. It was added at 7% w/w because, along with microcrystalline cellulose (Avicel®), reduced powder adherence to punch surfaces [50].

The flow properties according to Carr's Index, Hausner's ratio, and flow angle were very poor when no glidant was used. Although the incorporation of 1% w/w CabOSil® did not improve the value of these parameters it produced a relevant improvement in flow rate, from 95.8 to 28.8 s/100g [19,20,23].

When 50% w/w of Avicel® was added, no punch surfaces adherence was observed, regardless of type (FFBP or FFBE), and disintegration time and friability were near to the

Table 4. Evolution of CpK over time for each API.

Cpk			
Time (min)	5	10	15
INH	0.89	0.61	3.79
PZA	3.05	2.63	2.14
RFP	3.18	1.98	2.53

recommendations established by EMA and WHO for dispersible tablets: 2.33 min and 0.87%. Moreover, the use of this concentration of Avicel® reduced the blending process from 20 to 15 min.

Therefore, taking into account the results of the previous test, we adjusted the excipients and their concentrations as follows: 2.5% w/w Luzenac®, 1% w/w CabOSil®, 7% w/w CompactCel®, and 50% w/w of Avicel®.

Cpk value could be used to classify production process, according to USP: "exceeding 1.33 shows that the process is adequate to meet specifications" [51].

To establish an optimum mixing time, Cpks values were estimated. Table 4 shows the evolution over time for each API. As can be seen, at 15 min, the blending process is under control (CpK >1.33). INH is the only one that required more time to reach this CpK value, due to the lower proportion in the mixture. The other APIs showed CpK >1.33 after 5 min of mixture.

According to Hausner's ratio and Carr's Index, the flow properties of the powder can be classified as acceptable, which agrees with angle of repose (39.3, fair). Mass flow rate, volume flow rate, flow rate, and flow angle were the following: 4.59 ± 0.99 g/s, 10.1 ± 0.40 s/100 ml, 20.0 ± 0.87 s/100g and $78.2 \pm 1.72°$, respectively.

As already stated, our aim was to evaluate the influence of the concentration of excipients (AcDiSol® and Explosol®) and compression force on the disintegration time and friability of 15-mm water-dispersible tablets.

The results obtained with the different batches of tablets produced according to the experimental design are shown in Table 5.

Using a regression analysis, the relation between the studied factors (excipients and compression force) with the changes produced in tablet properties (disintegration time and friability) was studied. The statistical parameters to evaluate the goodness of the model are shown in Table 6.

Values for R^2, R^2_{adj}, and Q^2 are greater than 0.5, and their difference is not less than 0.3. Therefore, the indicators suggest a high quality of the model for fitting and predicting the effects on disintegration time and friability [26]. This lack of fitting in both responses was not significant.

Once the non-statistically significant terms were removed, the model equation for each response was

$$\text{Log (disintegration time)} = 2.11 - 0.04 \cdot \text{B} + 0.11 \cdot \text{C} - 0.09 \cdot \text{AC} \quad (8)$$

$$\frac{1}{\sqrt{Friability}} = 0.99 - 0.06 \cdot B + 0.13 \cdot \text{C} - 0.05 \cdot AB \quad (9)$$

where A is the AcDiSol® (%, w/w), B is the Explosol® (%, w/w), and C is the compression force (kN).

As may be seen from the equations, the concentration of A does not have any statistically significant influence over disintegration time (p value: 0.38) or friability (p value: 0.37). The concentration of B has a negative influence over disintegration and a positive one over friability, mainly because of its properties as a superdisintegrant (p value: 0.0109 and <0.0001, respectively) [23]. C, as expected, increase disintegration time and reduce friability of the tablet (p value <0.0001 for both responses).

Table 5. Experimental results: disintegration time and friability obtained with different batches of tablets according to the experimental design.

	Factors			Responses	
Batch no.	A (%, w/w)	B (%, w/w)	C (kN)	Disintegration time (s)	Friability (%)
1	0.0	2.0	11	69	1.44
2	0.0	2.0	14	145	0.95
3	0.0	2.0	16	270	0.82
4	0.0	6.0	11	80	1.36
5	0.0	6.0	14	141	1.02
6	0.0	6.0	16	195	0.83
7	0.0	9.0	11	100	1.43
8	0.0	9.0	14	132	0.97
9	0.0	9.0	16	170	0.74
10	2.5	2.0	11	124	1.09
11	2.5	2.0	14	128	0.79
12	2.5	2.0	16	140	0.75
13	2.5	6.0	11	86	1.31
14	2.5	6.0	14	120	0.91
15	2.5	6.0	16	146	0.84
16	2.5	9.0	11	85	1.79
17	2.5	9.0	14	107	1.17
18	2.5	9.0	16	130	0.90
19	5.0	2.0	11	145	1.05
20	5.0	2.0	14	155	0.78
21	5.0	2.0	16	175	0.64
22	5.0	6.0	11	124	1.53
23	5.0	6.0	14	132	1.07
24	5.0	6.0	16	141	0.88
25	5.0	9.0	11	113	1.66
26	5.0	9.0	14	121	1.10
27	5.0	9.0	16	135	0.84
28	2.5	6.0	14	128	1.02
29	2.5	6.0	14	142	0.91
30	2.5	6.0	14	139	0.93

A: AcDiSol® (%, w/w); B: Explosol® (%, w/w); C: compression force (kN).

Table 6. Quality of the experimental design using regression analysis.

	Disintegration time (min)	Friability (%)
Model (p value)	<0.0001	<0.0001
R-squared (R^2)	0.81	0.94
Adjusted R-squared (R^2_{adj})	0.76	0.92
Predicted R-squared (Q^2)	0.57	0.88
Lack of Fit (p value)	0.19	0.36

There are two interactions which are statistically significant (p value <0.0001) and both showed a negative effect over their response: AC in the case of disintegration time and AB for friability. Such negative effect means that the effect of one parameter is lower when the value of the other is high.

Figure 2 shows the 3D response surface for the predicting model. In black, the highest desirability, the conditions where the minimum disintegration time and friability is obtained using the lowest number of excipients. Therefore, the tablets that meet these conditions are those corresponding to formulation 3 (Table 2) produced without AcDiSol® with 9% w/w of Explosol® and a compression force of 16 kN (batch number 9 in Table 5).

This batch was also compressed using the 12-mm FFBE punches with the same compression pressure (9 kN/cm^2). As can be seen, in Table 7, when the 12-mm punches were used, the weight of the tablets was reduced by 50% and they meet disintegration time (<3 min) and friability test (<1% less of initial weight).

Since the previous tableting process was done at 10 cycles/min press speed, the influence of this on CQAs using 15- and 12-mm punches at a compression force of 16 and 10 kN, respectively, was tested.

Table 8 shows how quality attributes changes with press speed for both punches. Due to the improved strength transmission at slower press speed, at 10 cycles/min tensile strength showed the highest value whereas at 50 cycles/min showed the lowest: this will have an influence on friability and disintegration time. At the slowest press speed, as the tensile strength increases friability decreases and so will require a longer period to disintegrate. The 12-mm tablets showed a 6 times lower friability than the larger ones. This could be explained by the best strength transmission when a flat face is used compared to when score lines are present. Furthermore, due to the beveled edge in these tablets, the possibility of chipping during the friability test is reduced [52,53].

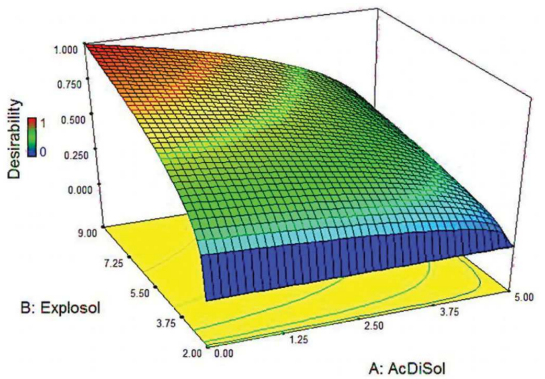

Figure 2. Response 3D-surface for factors A and B when C is 16 kN.

Table 7. Comparison of tablet properties using the same compression pressure and composition but different punches.

Formulation 3 (batch no. 9)		
Punches	12-mm FFBE	15-mm FFBP
Compression pressure (kN/cm^2)	9.00	
Compression force (kN)	10.0	16.0
Tablet weight (mg)	450	900
Disintegration time (s)	150	170
Friability (%)	0.09	0.74

Acceptance value (VA) was always below 15, regardless of press speed or the type of punches used.

In the case of 15-mm tablets, the tableting process could be done up to 25 cycles/minute ensuring good quality attributes since at 50 cycles/minute friability is greater than 1%. The highest press speed could be used for the 12-mm tablets since it showed good quality attributes at this speed. In this sense, this could be an alternative, in terms of industrial development, due to the improved friability in comparison with 15-mm tablets. However, as these tablets have 50% of the required daily dose, two tablets would need to be taken instead of one.

Finally, effectiveness of score lines: 15-mm tablets produced at 25 cycles/minute fulfilled this test since none of the 30 half tablets deviate in more than ± 15% of the average mass, which means that they could be split correctly. Moreover, the subdivision of these tablets could be useful to improve the dose scheme.

Compaction data obtained from an instrumented tableting machine enable rationale scientific designing of a tablet formulation with the desired quality attributes. Additionally, the parameters derived from the Heckel plot like mean yield pressure and SRS or those obtained from compression curves, like plasticity, give us information which is important for production efficiency and the final tablet quality [33,54,55].

The material had a plasticity of 92.0 ± 0.20 ($n = 26$) and it is independent of matrix diameter and press speed. Mean yield pressure is not influenced by press speed but depends on the diameter of the matrix: 12-mm (3.59 ± 0.68 kN) and 15-mm (81.0 ± 1.75 kN), $n = 5$.

The SRS value could be useful in order to catalog our product according to Robert and Rowe classification which goes from very soft to a moderately hard/brittle material [36]. Taking into account the low values obtained for SRS, 3.5 and 21.8 for 15-mm and 12-mm respectively, the material seems not to be affected by press speed.

In the literature, it is well describe the instability of RFP when it is in combination with INH in solution. However, there is not data about the stability of these APIs at pediatric doses in solid state. Singh and Mohan in 2003 described a reduction up to 7% of RFP content on a four-drug FDC available in the market for adults [56]. Moreover, this solid dosage form included Ethambutol

Table 8. Variation of CQAs according to press speed for 12 and 15-mm punches.

Punches	15-mm FFBE			12-mm FFBP		
Cycles/minute	10	25	50	10	25	50
Mass variation						
Average ± SD	0.92 ± 0.004	0.90 ± 0.004	0.89 ± 0.012	0.45 ± 0.002	0.44 ± 0.003	0.41 ± 0.002
RSD	0.43	0.44	1.32	0.53	0.57	0.53
Friability (%)	0.85	0.87	1.01	0.09	0.14	0.17
Disintegration time (sec)	160	155	132	150	136	125
Tensile strength (N/cm^2)						
Average ± SD	171 ± 9.82	165 ± 9.39	159 ± 3.64	165 ± 5.61	151 ± 4.10	145 ± 5.69
RSD	5.73	5.68	2.29	3.39	2.71	3.93
Content uniformity						
INH						
DV, %	102 ± 2.64	97.2 ± 3.21	101 ± 3.06	102 ± 3.37	99.1 ± 4.41	98.6 ± 11.1
AV	6.74	9.05	7.35	8.10	10.59	11.09
PZA						
DV, %	100 ± 0.87	99.1 ± 1.62	97.7 ± 1.45	93.7 ± 1.10	92.9 ± 1.02	98.5 ± 8.11
AV	2.08	3.88	4.27	7.46	7.99	8.12
RFP						
DV, %	100 ± 1.86	99.5 ± 2.35	99.6 ± 1.33	92.75 ± 1.17	90.4 ± 2.63	98.5 ± 10.6
AV	3.99	5.63	3.19	8.56	14.40	10.60
Fineness of dispersion	Ok	Ok	Ok	Ok	Ok	Ok

SD: standard deviation; RSD: relative standard deviation; DV: declared value; AV: acceptance value.

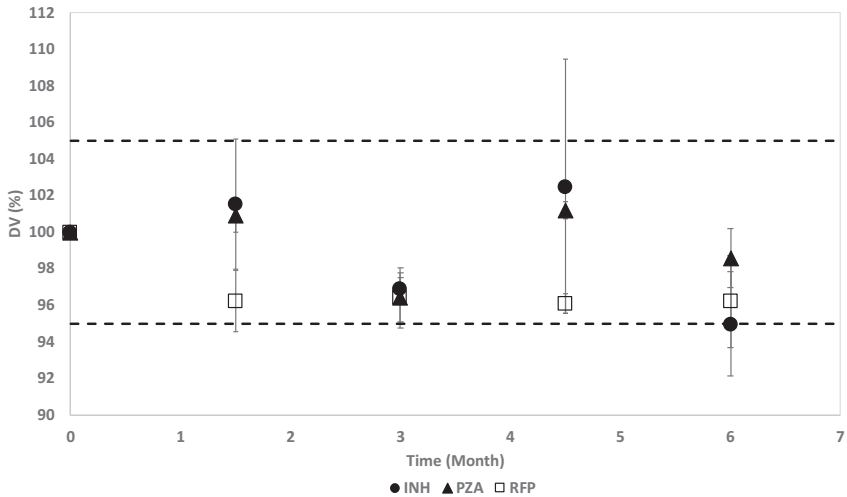

Figure 3. Stability of INH, PZA and RFP in 15 mm tablet under accelerated conditions. DV: declared value. $n = 3$.

in its composition which is able to caption moisture making FDC more instable [57].

Figure 3 shows the variation of drug content of 15-mm tablets storage at accelerated conditions for 6 months express as % of declared value. In our formulation all APIs remained inside the limit of ± 5% of drug content till 6 months of storage. More stability studied is currently ongoing in the laboratory to study the influence of light or moisture.

Conclusions

According to the results obtained, a high-quality child-friendly water-dispersible tablet containing INH, PZA and RFP for TB treatment has been developed in a design space using the lowest number of excipients and in the lowest proportion; all of them accepted by pediatrics (as EMA recommends). This new dosage form meets compendial requirements in terms of friability, disintegration time, and content uniformity and could be a vial alternative for treating tuberculosis in pediatrics.

Acknowledgments

The authors thank CajaSiete and Universidad de La Laguna for their contribution with a predoctoral contract and Biogrund GmbH, IMCD Iberia, Acofarma, and DFE Pharma for the selfless donation of raw materials.

Disclosure statement

No authors have any financial/conflicting interests to disclose.

Funding

This work was supported by a research project [ProID2017010094] co-funded by Gobierno de Canarias (Spain) and FEDER.

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Boletín nº 48 • Septiembre y Octubre 2016

fórmula+ acofar

Análisis de la homogeneidad de las dosis en fórmulas magistrales líquidas orales de uso pediátrico para el aseguramiento de su calidad.

Introducción

Cuando se elabora una Fórmula Magistral (FM) el objetivo final debe ser asegurar que el paciente reciba durante todo el tratamiento la cantidad necesaria de principio activo (API) en cada dosis administrada. En ocasiones, especialmente en el caso de las suspensiones, existe el riesgo de no poder cumplir con dicho objetivo o al menos, de no tener la seguridad de cumplirlo porque no disponer de un control de calidad apropiado para el producto acabado.

En formulación magistral, al no elaborar lotes, no podemos aplicar los ensayos oficinales que nos permiten controlar la calidad del producto final. En el caso de las FM líquidas orales, los ensayos sobre el producto acabado no van más allá de la inspección visual de sus características físicas o de la estabilidad documentada del API. En casos como el de las suspensiones con bajas dosis, sería conveniente disponer de datos relacionados con la homogeneidad del API en la FM y de toda la información necesaria que permita decidir si el PNT utilizado ofrece las garantías necesarias para asegurar la calidad la FM.

El objetivo del estudio se centró en analizar la utilidad de los ensayos de uniformidad de masa de las dosis obtenidas de envases multidosis y el ensayo de uniformidad de contenido de preparaciones unidosis de la Farmacopea Española (RFE), adaptados a FM líquidas orales pediátricas, para comprobar la homogeneidad del API en las dosis. Para ello se elaboraron diferentes FM de ranitidina, flecainida y acetazolamida que constituían sistemas dispersos homogéneos y heterogéneos.

Material y métodos

Los APIs y excipientes fueron suministrados por Acofarma (España): flecainida acetato (F), sacarosa, glicerol (G), acetazolamida (A), metilcelulosa (MC)1000, hidroxipropilmetilcelulosa (HPMC) 4500, ranitidina clorhidrato (R), jarabe simple (JS), excipiente Acofar (JEA), jarabe sin azúcar (JSA), excipiente Acofar jarabe (EAJ), esencia fresa oral (Ef) y agua purificada (AP).

Fórmulas magistrales

- **Ranitidina, Clorhidrato 5 mg/mL.** Con ranitidina se elaboraron tres formulaciones (Tabla 1) con diferentes bases comercializadas por Acofarma. Al ser disoluciones estas formulaciones nos sirvieron para comprobar la variabilidad de referencia.

Composición	R1	R2	R3
R (g)	0,5	0,5	0,5
Ef	(csp saborizar)		
AP (mL)	10		
JEA (csp, mL)	100	100	
EAJ (csp, mL)			
JSA (csp, mL)			100

Tabla 1.- Composición de las formulaciones de ranitidina.

- **Flecainida, Acetato 20 mg/mL.** La flecainida también se formuló en disolución, existen variables en el proceso de elaboración que si no se controlan podrían incidir en la insolubilización parcial del API y en una dispersión no homogénea. Se elaboraron cuatro fórmulas (Tabla 2).

Composición	F1	F2	F3	F4
F (g)	2	2	2	2
G (mL)		10		25
AP (mL)			50[1]	25
JS (csp, mL)	100	100	100	100

Tabla 2.- Composición de las formulaciones de flecainida.

- **Acetazolamida (A) 20 mg/mL.** La acetazolamida se formuló en suspensión. Sus propiedades físico-químicas dificultan la obtención de suspensiones con una dispersión homogénea del API. Se elaboraron tres fórmulas (Tabla 3).

Composición	A1	A2	A3
A (g)	2	2	2
G (mL)		20	20
Citrato (pH 4.00) (mL)		30	35
Cítrico concentrado (csp pH 4,0)	0,1		
MC 1000 1%	50		

Composición	A1	A2	A3
HPMC 4500 2%		20	15
JS (csp, mL)	100	100	100

Tabla 3.- Composición de las formulaciones de acetazolamida.

Ensayos de uniformidad de masa y contenido de la RFE

Uniformidad de masa (UM) de las dosis obtenidas de envases multidosis[2]. Este ensayo se aplicó a todas las formulaciones elaboradas, las dosis de 5 mL se tomaron aleatoriamente utilizando una jeringa dosificadora previa agitación del envase invirtiéndolo 10 veces.

Uniformidad de contenido (UC)[3]. Este ensayo, que no está indicado para preparaciones multidosis, lo adaptamos a las FM elaboradas por no ser tan restrictivo como el ensayo de uniformidad de las preparaciones unidosis. Lo modificamos utilizando la conversión de las cantidades de principio activo determinadas para cada dosis a % del valor declarado (dosis que queremos administrar). Para evitar repeticiones de los ensayos se determinaron contenidos de 20 dosis en vez de 10, como determina el ensayo original, salvo en el caso de las formulaciones de referencia de ranitidina que se realizaron las 10 determinaciones. Con esta adaptación se pretende incrementar la representatividad del muestreo del ensayo e incorporar al criterio los límites del ensayo de masa (10 y 20%).

Métodos analíticos

Los APIs se analizaron por Cromatografía Líquida de Ultra Alto Rendimiento. La viscosidad de las formulaciones se determinó con un viscosímetro Brookfield®.

1. Se calentó a 37ºC para facilitar la disolución completa de la flecainida.

2. RFE, 2.9.27. Uniformidad de masa de las dosis obtenidas de envases multidosis (01/2008, 20927), 2012, in: Ministerio de Sanidad y Consumo, Agencia Española de Medicamentos y Productos Sanitarios, eds. Real Farmacopea Española 5ª edición, Madrid..

3. RFE, 2.9.6. Uniformidad de contenido de las preparaciones unidosis (01/2008, 20906), 2012, en: Ministerio de Sanidad y Consumo, Agencia Española de Medicamentos y Productos Sanitarios, eds. Real Farmacopea Española 5ª edición, Madrid..

Resultados y discusión

La ranitidina se seleccionó para establecer la variabilidad de referencia de las dosis extraidas desde disoluciones elaboradas con diferentes bases de Acofarma.

Para el ensayo de UM, se determinaron los pesos de 20 dosis extraídas aleatoriamente. El valor medio de las masas fue de 6,39±0,08 g y ninguna unidad sobrepasó el límite de ±10% de la masa media. La desviación estándar relativa (DSR) fue muy baja (1,31) a pesar de usar dosis extraídas de 2 formulaciones diferentes, el ensayo se cumple. Las tres formulaciones cumplieron el ensayo de UC. Las DSR oscilaron entre un 4,0 y 6,9, por lo que se puede afirmar que las disoluciones de ranitidina presentan una distribución homogénea de las dosis con una variabilidad inferior al 7%.

La flecainida tiene una solubilidad acuosa elevada. Las FMs que más utilizadas tienen una composición similar a las suspensiones que se elaboran partiendo de la forma farmacéutica, por lo que su composición es típica de suspensiones (F1). Como no es posible solubilizar por completo la flecainida en el JS y en el viscosizante, se obtiene es una disolución opalescente. En F2 y F4, a pesar del glicerol, se observan distintos grados de opalescencia por insolubilización parcial de la flecainida. La solubilidad de la flecainida en agua es de 48,4 mg/mL a 37ºC, para solubilizarla hay que partir de 50 mL de agua y calentar con agitación, añadiendo luego el JS. Así se elaboró la F3, única que constituía una disolución transparente. Todas las formulaciones de flecainida cumplen con el ensayo de UM, con DSRs bajas, entre 0,31 y 0,77%. En el ensayo de UC, aplicamos el criterio adaptado de la RFE. Solo la F3 lo cumplió siendo la que menor variabilidad presentó (DSR de 4,5). La solubilización de la flecainida es el factor crítico que permite obtener FM con dosis homogéneas.

La solubilidad acuosa de la acetazolamida es muy baja, la concentración en suspensiones pediátricas es de 20 mg/mL. Su pH de máxima estabilidad está entre 4 y 4,5. La baja proporción de API y su baja solubilidad dificulta la obtención de FM con el API disperso homogeneamente. La A1 es la FM más utilizada en la práctica. A las restantes se les incorporó glicerol y se ajustó el pH al de máxima estabilidad (4-4,5). Las FM A3 y A4 incorporan dos niveles de HPMC 4500 que permiten modificar la viscosidad a valores superiores e inferiores a los obtenidos con MC. Las viscosidades a 25ºC fueron de 55, 89 y 34,5 mPa.s para A1, A3 y A4 respectivamente.

Las tres formulaciones cumplen el ensayo de UM. La DSR osciló entre 2,01 y 4,09. Ninguna de las FM cumple con el ensayo de UC. La DSR superó en todas las formulaciones el 10% y no es posible asegurar la homogeneidad de las dosis a pesar de los cambios en la composición.

Conclusiones

El ensayo de UM de la RFE no da información relevante sobre la homogeneidad de las dosis independientemente de la proporción en API de las suspensiones. El ensayo de UC de la RFE con las modificaciones propuestas, es una alternativa útil para la determinación de la uniformidad de las dosis en suspensiones. Las formulaciones que no cumplieron con el ensayo de UC tampoco cumplieron con el ensayo adaptado propuesto.

Valores de la DSR superiores al 2% en el ensayo de UM pueden ser indicativos de no homogeneidad en las dosis.

Las suspensiones de acetazolamida elaboradas no permiten dosificar de forma homogénea el API.

Por todo lo anterior proponemos, para comprobar la homogeneidad de las dosis en suspensiones con bajo contenido en API, el ensayo de UM para preparaciones multidosis de la RFE, sustituyendo la determinación de las masas de las dosis por la determinación de sus contenidos en API expresados como % del valor declarado.

En caso de que la FM no satisficiera el ensayo, su PNT debería revisarse analizando los procesos críticos para la homogeneidad de las dosis.

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