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 (INH), pyrazinamide (PZA) and rifampicin (RFP) respectively. This new formulation must contain the lowest possible number of excipients, all accepted for pediatrics, and fulfill all the pharmacopoeia requirements for this type of tablet (friability, disintegration time, fineness of dispersion and content uniformity).

Significance: TB is an infectious disease which caused the death of 233,000 children in 2017. 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 some excipients which may not be suitable for pediatrics. Therefore, this new formulation would cover this therapeutic gap.

Methods: A factorial design, based on 3 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: A water-dispersible tablet was developed according to international recommendations in terms of excipients for pediatrics and meeting Ph. Eur. requirements. In addition, its production has been optimized to be elaborated at maximum eccentric press speed but maintaining quality requirements.

Conclusion: A high-quality child-friendly FDC water-dispersible tablet was developed improving the treatment of TB in pediatric.

Keywords: Tuberculosis; children; pediatric; dispersible tablet; treatment; direct compression.

1. 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 not 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, content uniformity, fineness of dispersion and effectiveness of the score lines in the case of 15-mm tablets) [15].

2. Materials and Methods.

2.1. Materials

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).

2.2. UHPLC analysis.

All APIs were analysed 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).

INH and PZA were analysed with a method based on an UHPLC gradient method [16] and RFP was analysed using a method adapted from High Performance Liquid Chromatography (HPLC) [17]. For both methods, the chromatographic conditions were: XSelect™ CSH™ C18 (75 mm x 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 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 sixfold 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 50ºC (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.

2.3. Optimization of blending process.

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 [19,20].

2.4. 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).

2.5. 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 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 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 2-9% 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.

Table 2 shows the final composition of formulation 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, 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^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:

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

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

2.6. 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/minute. 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 were 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.

2.7. 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.

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 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 (net work) and W3 (elastic work). [32,34-36].

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

2.8. Stability test.

A stability test was done placing 15-mm tablets under accelerated conditions (40 ± 2 ºC/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].

3. 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 are: 103 ± 2.07% for INH, 98.4 ± 1.95% for PZA and 98.33 ± 0.95% for 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 50ºC 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 minutes 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.

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 minutes, 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 [44]. 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 [45].

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 28.8s/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 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.

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 show that the process is adequate to meet specifications [46].

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 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.

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/100g and 78.2 ± 1.72º 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) were studied. The statistical parameters to evaluate the goodness of the model is 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 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) [23]. 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 2 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 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/cm2). 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. 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 [47,48]. 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 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 [33,49,50].

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 [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 upto 7% of RFP content on a four-drug FDC available in the market for adults [51]. Moreover, this solid dosage form included Ethambutol in its composition which is able to caption moisture making FDC more instable [52].

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 are currently ongoing in the laboratory to study the influence of light or moisture.

4. 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.

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Tables:

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 1. Coded levels and values of design variables to the development of dispersible tablets.

	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

Table 2. Composition in mg and % (w/w) of formulations 1 to 9, each of which was compressed to the three compressions forces, to develop the dispersible tablets following the experimental design.

API	INH	PZA	RFP
Calibration curve	A = 31925.3·C	A = 35181·C	A = - 150190 + 49378·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

Table 3. Characteristic of the method used to the analysis by UHPLC of each API. A: Peak Area (µV·sec). C: Concentration (µg/ml). CV: coefficient of variation.

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 4. Evolution of CpK over time for each API.

	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. 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)

	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 6. Quality of the experimental design using regression analysis.

Formulation 3 (Batch Nº9)		
Punches	**12-mm FFBE**	**15-mm FFBP**
Compression Pressure (kN/cm2)	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 7. Comparation of tablet properties using the same compression pressure and composition but different 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/cm2)	**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

Table 8. Variation of CQAs according to press speed for 12 and 15-mm punches. SD: standard deviation; RSD: relative standard deviation; DV: declared value; AV: acceptance value.

Figures:

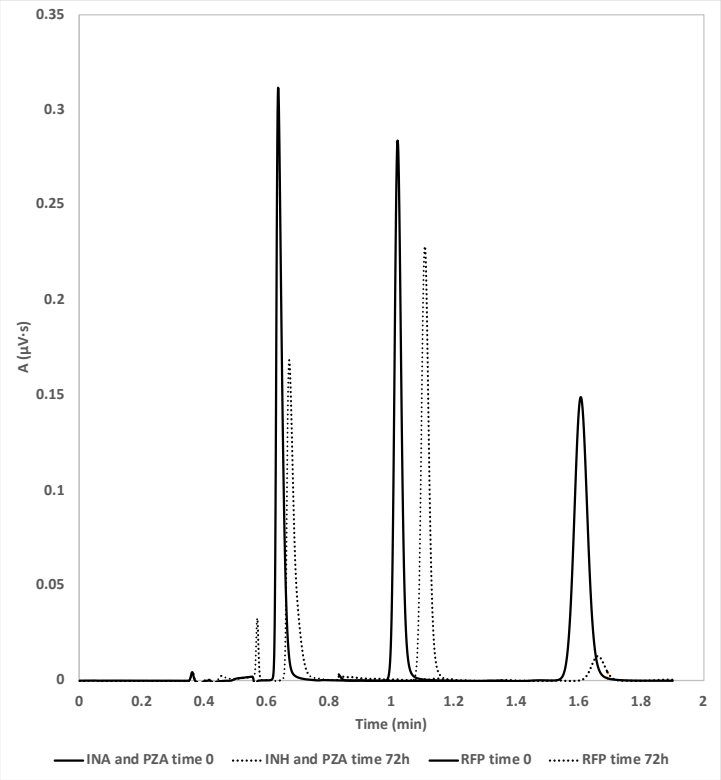

Figure 1. INH (0.6 min), PZA (1 min) and RFP (1.6 min) as pure patter chromatographic peaks (continuous bold line). Discontinuous line represents the decrease of signals for each API after 72h of storage at 50ºC in a medium with a pH of 7.

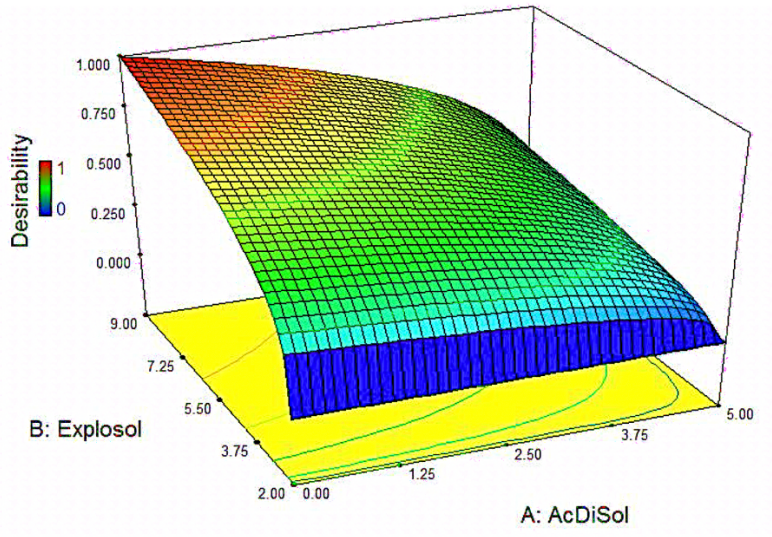

Figure 2. Response 3D-surface for factors A and B when C is 16 kN.

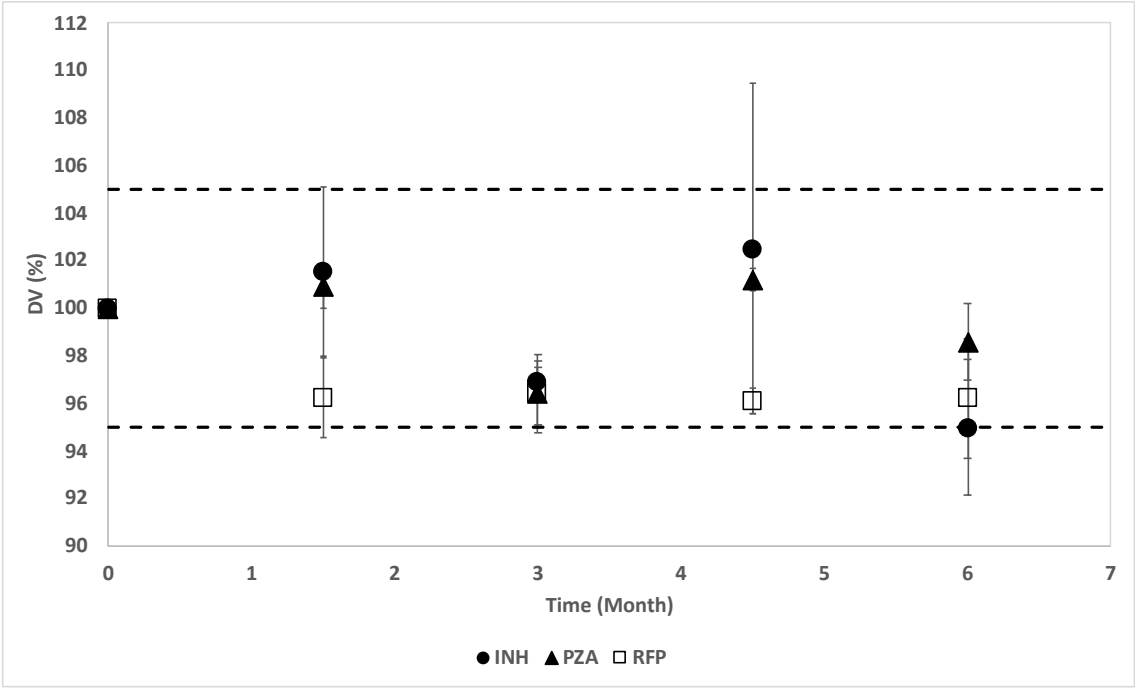

Figure 3. Stability of INH, PZA and RFP in 15 mm tablet under accelerated conditions. DV: Declared value. n=3.