Magnetic ionic liquids as extraction solvents in vacuum headspace single-drop microextraction

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A B S T R A C T

A vacuum headspace single-drop microextraction method based on the use of magnetic ionic liquids (vacuum MIL-HS-SDME) for the determination of short chain free fatty acids is described for the first time. The basis of the method involves the use of a rod magnet to aid in maintaining a small microdroplet of magnetic ionic liquid (MIL) during headspace single-drop microextraction (HS-SDME). The application favors reduced pressure conditions inside the sampling vial while maintaining the MIL droplet in the headspace. After extraction, the MIL microdroplet containing extracted FFAs is transferred to a headspace vial where static headspace desorption is performed, followed by gas chromatographic-mass spectrometry (GC-MS) analysis. A number of MILs were studied and the trihexyl(tetradecyl)phosphonium tris(hexafluoroacetylaceto)manganate(II) MIL was found to be the most suitable for the proposed method. A comparison with atmospheric pressure MIL-HS-SDME revealed that analytes reached equilibrium faster when reduced pressure conditions were applied and that an enhancement in the extraction efficiency of analytes under these vacuum conditions was observed at any extraction time. Under optimum conditions, the method requires only 20 µL of MIL placed at the end of a rod magnet and the evacuation of air using a modified extraction vial and a vacuum pump. Afterwards, 10 mL of sample containing 30% (w/v) of NaCl is injected in the vial and the vacuum MIL-HS-SDME is performed at 45 °C and 600 rpm for 60 min. The MIL microdroplet can easily be transferred to a 4.2 mL modified headspace vial for the headspace desorption and GC-MS analysis. The entire method is characterized by wide linearity ranges, low limits of detection for analytes (down to 14.5 µg L^{-1}), good reproducibility (with relative standard deviation lower than 13%), and relative recoveries ranging from 79.5% to 111%. The proposed vacuum MIL-HS-SDME was applied towards the analysis of two different milk samples with the majority of analytes being detected and quantified.

1. Introduction

With the introduction of Green Analytical Chemistry (GAC), a wide number of miniaturized strategies have been developed in environmental, biological, and food analysis [1,2]. The objective is primarily oriented towards the application of cheap, fast, and environmentally-safer procedures. Among liquid-phase microextraction (LPME) methods fulfilling the requirements of GAC, the single-drop microextraction (SDME) mode is popular since it is based on the exposure of a few microliters of the extraction solvent to the sample, typically with the aid of a micro-syringe [3]. The headspace mode (HS-SDME) is especially advantageous for the determination of volatile and semi-volatile compounds from samples of varying complexity.

Recently, the use of reduced pressure conditions has been reported in microextraction techniques that use the headspace (HS) as an intermediate phase between the sample and the extraction solvent [4–7]. Specifically, the strategy applied in headspace solid-phase microextraction (HS-SPME) has been beneficial for the determination

of volatile and semi-volatile compounds [4–7]. The reduced pressure conditions were achieved by evacuating air inside the extraction vial with the help of a vacuum pump. Often referred to as vacuum HS-SPME, an enhancement in the sensitivity, shorter sampling times, and lower extraction temperatures were reported [5–7]. However, in the case of HS-SDME, the vacuum option has not been studied due to the fact that the extraction solvents commonly used in HS-SDME detach or evaporate from the needle tip of the microsyringe during the air evacuation step.

Magnetic ionic liquids (MILs) are a subclass of ionic liquids (ILs) that possess a number of interesting properties of ILs while incorporating a paramagnetic component (in general, transition or rare-earth metal anions or metal complexes) [8]. MILs exhibit a strong response to external magnetic fields as well as low vapor pressure at room temperature, relatively high thermal stability, and tunable solvation properties [8]. These interesting properties make MILs suitable extraction solvents for all LPME modes. MILs have been mainly explored in dispersive liquid-liquid microextraction (DLLME) procedures [9–11] as magnetic separation avoids common centrifugation and filtration steps (often required in DLLME). This simplifies the entire method while minimizing sources of error.

The paramagnetic character of MILs allows for their exposure during HS-SDME with the aid of a rod magnet, thus avoiding the need of a micro-syringe to form and stabilize the MIL droplet [12]. In addition, larger volumes of the MIL extraction solvent can be loaded on the rod magnet compared to other types of solvents (including conventional ILs) when using micro-syringes [12]. Furthermore, the use of MILs in HS-SDME can facilitate working under reduced pressure conditions. In this case, air evacuation within the extraction vial can be achieved without compromising the stability of the MIL micro-droplet.

The theory for vacuum HS approaches predicts that reduced pressure conditions can be particularly beneficial for compounds with a low Henry's Law constant (K_H) value due to the mass transfer resistance of these analytes concentrated in the gas phase [4,7]. Short chain free fatty acids (FFAs) are a group of analytes possessing these characteristics [7] and are often present in milk and derivatives. Their presence is associated with health benefits, providing flavor and antimicrobial activity, and for being a direct cause of hydrolytic rancidity in milk and other dairy products [13]. In general, the determination of FFAs requires their derivatization *via* methyl esterification, following by gas chromatography-mass spectrometry (GC-MS) analysis [14]. However, this derivatization method consumes copious amounts of solvent and provides low resolution in GC and a high background in MS [14]. Furthermore, conventional methods for determining FFAs utilize liquid-liquid extraction (LLE) [15] or solid phase extraction (SPE) [16], that are not in concordance with GAC [1,2].

The aim the current study is to evaluate the suitability of MILs as extraction solvents in vacuum HS-SDME for the determination of a group of short-chained FFAs in milk samples. It is the first time that the vacuum mode can be combined with HS-SDME, and this is only possible due to the magnetic character of the MIL solvent, able to resist reduced-pressure conditions in the HS without falling. Furthermore, a new generation of MILs based on anions containing metal or rare earth complexes with hexafluoroacetilacetonate as ligands are used for the first time in microextraction. To sum up, the vacuum MIL-HS-SDME method does not require derivatization of the FFAs, what represent a further advantage over other reported methods [15]. A comparison of the extraction performance of the method with MIL-HS-SDME at atmospheric pressure is performed to highlight the inherent advantages of MILs under vacuum conditions. In addition, we study the use of a headspace desorption unit coupled to the GC-MS to facilitate the transfer of analytes from the MIL to the headspace. This is the first report on the use of a vacuum-assisted LPME method.

2. Experimental

2.1. Chemicals, reagents, materials and samples

The volatile free fatty acid (FFA) standard mix (certified reference material CRM46975) was purchased from Supelco (Bellefonte, PA, USA). The mix was a multi-component solution containing 10 mmol L^{-1} of propionic acid (C_3), iso-butyric acid (i-C_4), n-butyric acid (n-C_4), iso-valeric acid (i-C_5), n-valeric acid (n-C_5), iso-hexanoic acid (i-C_6), n-hexanoic acid (n-C_6), and n-heptanoic acid (n-C_7) dissolved in ultrapure water. The main physico-chemical properties of these analytes are detailed in Table S-1 of the Supplementary Material (SM). For extraction experiments, working solutions were prepared by dilution of the standard mix in ultrapure water containing 30% (w/v) of NaCl. Ultrapure water (18.2 MΩ cm) was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA) while NaCl (≥99.5%) was purchased from Fisher Scientific (FairLawn, NJ, USA). Optimization experiments were developed using a concentration of 0.02 mmol L^{-1} of FFAs (corresponding to 1.8 mg L^{-1} for C_3, 2.0 mg L^{-1} for i-C_4 and n-C_4, 2.4 mg L^{-1} for i-C_5 and n-C_5, 2.6 mg L^{-1} for i-C_6 and n-C_6, and 3.0 mg L^{-1} for n-C_7). Calibration curves of the entire vacuum MIL-HS-SDME-headspace desorption (HSD)-GC-MS method were developed with concentrations of FFAs ranging from 0.2 to 11 mg L^{-1}. Calibrations curves of the HSD-GC-MS method (without the preconcentration method) were obtained by dilution of the standard mix of FFAs in acetonitrile (Sigma-Aldrich, St Louis, MO, USA) with a concentration from 15 to 194 mg L^{-1}. All solutions were stored at −4 °C before use.
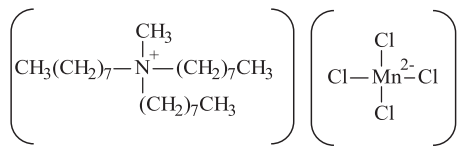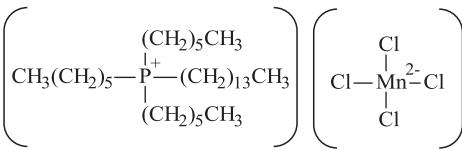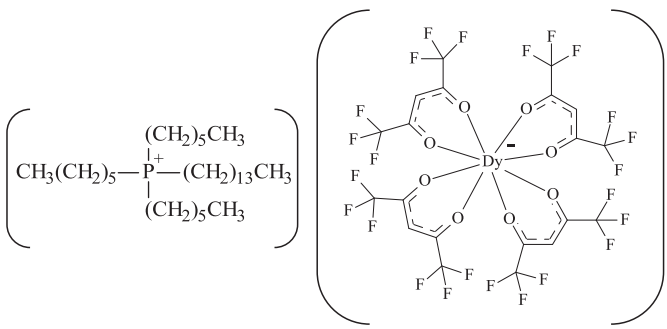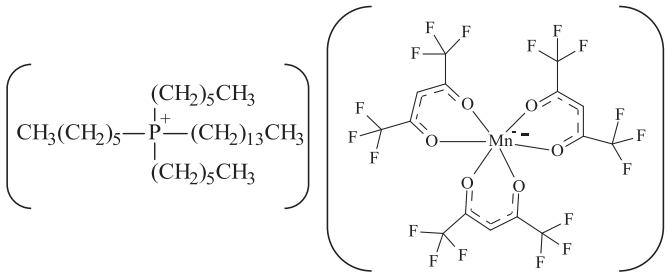
Four magnetic ionic liquids (MILs) were evaluated as extraction solvents, namely, trioctylmethylammonium tetrachloromanganate(II) ([aliquat$^+$]$_2$ [MnCl$_4$$^{2-}$]), trihexyl(tetradecyl)phosphonium tetrachloromanganate(II) ([P$_{6,6,6,14}$$^+$]$_2$ [MnCl$_4$$^{2-}$]), trihexyl(tetradecyl)phosphonium tetrakis(hexafluoroacetylaceto)dysprosate(III) ([P$_{6,6,6,14}$$^+$] [Dy(hfacac)$_4$$^-$]) and trihexyl(tetradecyl)phosphonium tris(hexafluoroacetylaceto) manganate(II) ([P$_{6,6,6,14}$$^+$] [Mn(hfacac)$_3$$^-$]). The MILs were synthesized and characterized according to recent studies [17–19]. Table 1 shows their structures and their main physicochemical properties. Fig. S-1 of the SM summarizes the characterization of the MILs.

Two different milk samples were purchased from a local supermarket (Ames, IA, USA). The samples included a low fat milk (with less than 1 g L^{-1} of total fat) and an organic reduced fat milk (with a total content of 19 g L^{-1} of fat). The total salt content of both samples was adjusted to 30% (w/v) of NaCl by dissolving the appropriate amount of NaCl in the sample.

2.2. Instrumentation

Analyses were carried out using a 7890B GC from Agilent Technologies (Santa Clara, CA, USA) equipped with a 5977A MS detector (single quadrupole). The GC-MS was coupled to an Agilent Technologies 7697A HS sampler unit for HSD of the analytes after extraction. The HSD of the analytes was achieved by working in the fill mode of the HS sampler (flow to pressure, 50 psi), and using a 150 °C, 165 °C and 175 °C as oven, loop and transfer line temperatures, respectively. The equilibration time was 10 min and the stirring rate 100 cycles min^{-1}. The GC separation was achieved using a MEGA-FFAP EXT crossbond capillary column (50 m L×0.20 mm I.D.×0.20 μm film thickness) purchased from Mega s.n.c (Legnano, MI, Italy). Helium was used as carrier gas at a flow rate of 1 mL min^{-1}. The inlet was maintained at 290 °C with a 5:1 split ratio. The temperature program consisted of the following: initially 100 °C during 2 min, then the temperature was increased at 5 °C min^{-1} up to 240 °C, and held for 3 min. The transfer line from the GC to the MS was kept at 250 °C. The MS was operated in electron ionization (EI) mode at 70 eV, using 230 °C and 150 °C as the source and quadrupole temperatures, respectively. Data was acquired in single ion monitoring (SIM) mode. The retention time, quantifier and qualifier ions of each FFA, together with the employed segment program are all shown in Table S-2 of the SM.

Table 1
Structures and important physicochemical properties of the studied MILs.

MIL	Structure	MW (g mol^{-1})	Viscosity at 25 °C (cP)	µeff (µ$_B$)[a]
[aliquat$^+$]$_2$ [MnCl$_4$$^{2-}$]		1009	–[b]	–[b]
[P$_{6,6,6,14}$$^+$]$_2$ [MnCl$_4$$^{2-}$]		1103	75230[c]	–[b]
[P$_{6,6,6,14}$$^+$] [Dy(hfacac)$_4$$^-$]		1475	291.5[d]	5.8[d]
[P$_{6,6,6,14}$$^+$] [Mn(hfacac)$_3$$^-$]		1160	401.8[d]	9.7[d]

[a] Effective magnetic moment, in Bohr magnetons (µ$_B$).
[b] Non-reported data.
[c] Ref. [18].
[d] Ref. [19].

During extraction *via* vacuum MIL-HS-SDME, a Büchi Labortechnik AG type V-500 vacuum pump (Flawil, Switzerland) with a suction volume of 1.6 m^3 h^{-1} and a final vacuum of 10 mbar, and a Corning PC-420D magnetic stirring hotplate (Corning, NY, USA) were utilized.

Elemental analyses of MILs were obtained using a Perkin Elmer 2100 Series II CHN/S Analyzer (Waltham, MA, USA). Mass spectra of MILs were obtained using an Agilent 6230 TOF LC/MS (Santa Clara, CA, USA). A Renishaw Raman Spectrometer equipped with an Ar-ion laser operated at 488 nm and a charge coupled device detector was employed to record the Raman spectra.

2.3. Procedures

2.3.1. Cap design for vacuum MIL-HS-SDME and modification of the headspace vial

To perform the experiments in this study, a special cap was necessary for vacuum MIL-HS-SDME. The cap must permit the exposure of the magnet containing the MIL within the HS of the vial, but must also allow for the evacuation of the air within the vial and ensure a leak-tight seal. With these objectives, a polytetrafluoroethylene (PTFE)/silicone septum (Sigma-Aldrich) was pierced to introduce a NdFeB rod magnet (0.5 cm of diameter×5 cm of thick, B=0.66 T) from K & J Magnetics, Inc. (Pipersville, PA, USA). A pipette plastic bulb was coupled to a stainless steel screw cap (open-top, 8 mm center hole, Sigma-Aldrich). The pipette bulb with the cap and the rod magnet in the septum were assembled, as shown in Fig. 1 (Step 1). The modified cap was then attached to a 20 mL thread, clear glass vial (Sigma-Aldrich).

Once vacuum MIL-HS-SDME was completed, the MIL containing enriched analytes was subjected to HSD. A modified HSD vial was also developed following the adaptation proposed by Zhang et al. [20]. In this case, 10 mL HS sampling glass vials (Agilent Technologies) were filled with 12.5 g of glass beads (3 mm diameter). A glass insert containing a flat bottom was placed inside the vial and a crimped silver aluminum cap with a PTFE/silicone septum (Agilent Technologies) was used to seal the system. The modified vial contained a HS volume of 4.2 mL. A scheme of the device is represented in Fig. 1 (step 5).
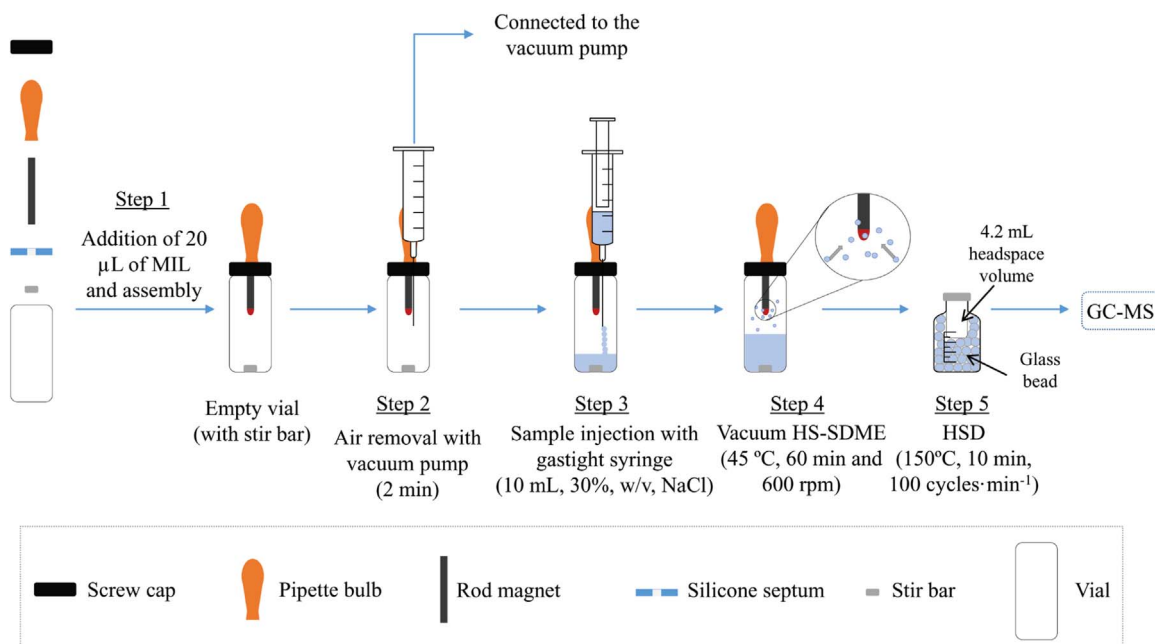
Fig. 1. Schematic of the vacuum MIL-HS-SDME procedure under optimum conditions.

2.3.2. Vacuum MIL-HS-SDME and MIL-HS-SDME procedures

The vacuum MIL-HS-SDME procedure is summarized in Fig. 1. The modified cap was assembled and a certain volume of MIL was placed on the end of the rod magnet. The cap was then screwed onto a 20 mL empty vial containing a magnetic PTFE stir bar (15 mm×4.5 mm; Sigma-Aldrich) (step 1 of Fig. 1). The reduced pressure conditions inside the vial were achieved using a vacuum pump. The tubing of the vacuum pump was connected to the barrel of a 5 mL plastic medical syringe equipped with a detachable 22 gauge metallic needle possessing a beveled tip (Sigma-Aldrich). The needle was inserted through the pipette bulb and the septum of the cap and the air was evacuated for 2 min (Fig. 1, step 2). Then, 10 mL of aqueous sample containing analytes and 30% (w/v) of NaCl was injected to the device through the same hole using a 10 mL Hamilton® gastight syringe (Sigma-Aldrich) (Fig. 1, step 3) and the vial was immediately placed on the magnetic stir plate. Vacuum MIL-HS-SDME extraction was carried out by exposing the MIL to the HS of the vial and under control of the extraction time, temperature and agitation speed. The system was then opened, and the vial was allowed to equilibrate to atmospheric pressure. A microdroplet of MIL containing extracted analytes was transferred to a HS vial of 10 or 4.2 mL, depending on the experiment, for HSD and GC-MS analysis (Fig. 1, step 5). Each experiment was repeated in triplicate (n=3).

Under optimum conditions, 20 µL of $[P_{6,6,6,14}^+]$ $[Mn(hfacac)_3^-]$ MIL were placed on the rod magnet, and vacuum MIL-HS-SDME was performed at 45 °C for 60 min using an agitation speed of 600 rpm.

For MIL-HS-SDME (at atmospheric pressure), extractions were performed using 20 µL of $[P_{6,6,6,14}^+]$ $[Mn(hfacac)_3^-]$ MIL using the modified device of vacuum MIL-HS-SDME to mimic the same HS of both methodologies. A similar procedure to that used for vacuum MIL-HS-SDME was performed, but without the air evacuation step (Fig. 1, step 2). Experiments were repeated in triplicate (n=3).

2.3.3. Quality assurance and quality control procedures

The quality assurance and quality control of the developed method was evaluated by the determination of the analytical performance of the entire method, the reproducibility, the extraction efficiency and the relative recovery.

The calibration curves of each FFA using the entire method of vacuum MIL-HS-SDME-HSD-GC-MS were obtained by using external calibration.

The limits of detection (LODs) and limits of quantification (LOQs) were estimated using the signal to noise ratio method. This methodology is based on the measuring of the chromatographic noise corresponding to blank samples which have been subjected to the entire method. The LODs and the LOQs were calculated as three or ten time the signal to noise ratio, respectively.

The reproducibility was estimated as the relative standard deviation (RSD). The extraction performance was determined by the enrichment factors (E_F). The E_F values were calculated as the ratio between the predicted concentration obtained using HSD-GC-MS calibration curves (without the vacuum MIL-HS-SDME step) and the spiked concentration of each FFA. Table S-3 of the SM includes the analytical performance of the HSD-GC-MS calibration curves. 20 µL of the FFA standard solutions in acetonitrile were placed in the modified HSD vials and subjected to the HSD-GC-MS analysis to obtain these calibration curves. The relative recovery (RR) was calculated as the ratio of the predicted concentration obtained using the calibration curves of the entire method and the spiked concentration of each FFA.

The total uncertainty of the analytical results was estimated following the *bottom-up* approach adopted by the International Organization for Standardization (ISO) [21]. The *bottom-up* approach estimates the total uncertainty by the identification and combination of all uncertainty sources associated with the analytical results [22,23]. In this study, the most important contribution to the uncertainty is associated with the calibration. Thus, the total uncertainty was estimated as the calibration uncertainty and considering a coverage factor (*k*) of 2.

3. Results and discussion

3.1. Selection of the MIL

In this study, direct GC-MS injection of the MIL containing FFAs after vacuum MIL-HS-SDME was not possible due to the incompatibility of IL solvents with GC. Therefore, we proposed the combination of the extraction method with HSD-GC-MS. The HSD step was carried out using a HS sampler unit to ensure the volatilization of analytes from the MIL (without volatilization of the MIL) followed by their transfer to GC-MS without loss of sensitivity.

The criteria for MIL selection were based on both their compat-

ibility with the vacuum MIL-HS-SDME procedure as well as with the subsequent HSD-GC-MS analysis. The nature of the MIL should also be suitable for the extraction of the target analytes. A hydrophobic MIL possessing a relatively high viscosity is needed to ensure that the MIL microdroplet does not fall from the rod magnet during the air evacuation step (Fig. 1, step 2). High thermal stability of the MIL is also mandatory to desorb analytes from the solvent during the HSD step but to avoid volatilization and/or degradation of the MIL. This last requirement is especially important because degradation products from the MIL may generate peaks within the GC-MS chromatogram that can interfere in the determination of the analytes. Several different MILs were selected as possible extraction solvents, including [aliquat$^+$]$_2$ [MnCl$_4$$^{2-}$], [P$_{6,6,6,14}$$^+$]$_2$ [MnCl$_4$$^{2-}$], [P$_{6,6,6,14}$$^+$] [Dy(hfacac)$_4$$^-$] and [P$_{6,6,6,14}$$^+$] [Mn(hfacac)$_3$$^-$]. 20 mg of each MIL were placed in 10 mL HS vials and HSD-GC-MS experiments were performed using the SCAN mode. Fig. 2 shows representative HSD-GC-MS chromatograms obtained after these experiments. The observed chromatographic peaks were due to the degradation products of the MIL and were generated by applying extreme conditions of temperature and pressure in the HSD (10 min of equilibration time, 200 °C, 215 °C and 225 °C as oven, loop and transfer line temperatures, respectively, and agitation at 36 cycles min^{-1}). Thus, it can be assumed that each chromatogram represents the background of the MIL in the GC-MS under these conditions. From Fig. 2, higher backgrounds were observed using MILs containing [MnCl$_4$]$^{2-}$ anions, with [aliquat$^+$]$_2$ [MnCl$_4$$^{2-}$] possessing the highest background.

The use of [P$_{6,6,6,14}$$^+$] [Dy(hfacac)$_4$$^-$] and [P$_{6,6,6,14}$$^+$] [Mn(hfacac)$_3$$^-$] in HSD-GC-MS resulted in drastically reduced backgrounds (Fig. 2(C) and (D)). From these two MILs, the [P$_{6,6,6,14}$$^+$] [Mn(hfacac)$_3$$^-$] MIL possesses a relatively high viscosity (see Table 1) making it more suitable for vacuum MIL-HS-SDME; therefore, it was selected for subsequent method development.

3.2. HSD-GC-MS analysis

After selecting the most suitable MIL, separation and detection of the studied FFAs was optimized using HSD-GC-MS. The HSD unit is an extra module of the GC-MS. It permits to volatilize analytes present in the desorption vial by heating, pressurization and agitation. The HSD unit operates in fill mode, meaning that all desorbed analytes are transferred to the GC injector. Section 2.2 details the optimum GC-MS conditions. The key aspect to developing the HSD-GC-MS methodology was optimization of the parameters for the HSD step. The oven temperature of the HSD did not exceed the boiling point of the analytes (206 °C, corresponding with the boiling point of n-C$_6$), while the loop and transfer line temperatures were always 15 °C and 25 °C higher than the oven temperature. In this particular application, the maximum signal for the analytes was reached at an oven temperature of 150 °C (with the loop and transfer line temperature at 165 °C and 175 °C, respectively). These mild temperature conditions aided in generating a much lower background of the [P$_{6,6,6,14}$$^+$] [Mn(hfacac)$_3$$^-$] MIL. Regarding the equilibration time during the HSD, equilibration times longer than 10 min did not significantly improve the sensitivity of the analytes, while an increase in the stirring rate up to 100 cycles min^{-1} provided high sensitivity of analytes. As a result, the typical chromatograms of FFAs obtained after vacuum MIL-HS-SDME-HSD-GC-MS (SIM mode) are those represented in Fig. 3 (in red). In Fig. 3, a chromatogram from the extraction of a blank (only ultrapure water, no analytes) is overloaded (in black). No interferences from the blank were observed. The total time of the HSD step was ~15 min.

3.3. Influence of the extraction time in vacuum MIL-HS-SDME and MIL-HS-SDME

The first objective of this study was to determine the enhancement in analyte extraction efficiency when reduced pressure conditions are combined with MIL-HS-SDME. The influence of the extraction time was studied in the range between 20 and 100 min using both vacuum MIL-HS-SDME and MIL-HS-SDME (with no vacuum). Previous studies reported the positive effect from the addition of salt for the extraction of FFAs by similar approaches that used the HS as an intermediate phase [7,24,25]. Thus, a 30% NaCl (w/v) content was set to favor the transfer of the analytes to the HS. The sample volume was fixed at 10 mL to ensure adequate preconcentration and the spiked level of analytes was 0.02 mmol L^{-1}. 20 μL of the [P$_{6,6,6,14}$$^+$] [Mn(hfacac)$_3$$^-$] MIL was added to the rod magnet and the extraction time was kept at 35 °C with a stirring speed of 400 rpm in all experiments. The subsequent HSD was performed using 10 mL HS vials and the conditions described in Section 2.2.

(A) [aliquat$^+$]$_2$ [MnCl$_4$$^{2-}$]

(B) [P$_{6,6,6,14}$$^+$]$_2$ [MnCl$_4$$^{2-}$]

(C) [P$_{6,6,6,14}$$^+$] [Dy(hfacac)$_4$$^-$]

(D) [P$_{6,6,6,14}$$^+$] [Mn(hfacac)$_3$$^-$]
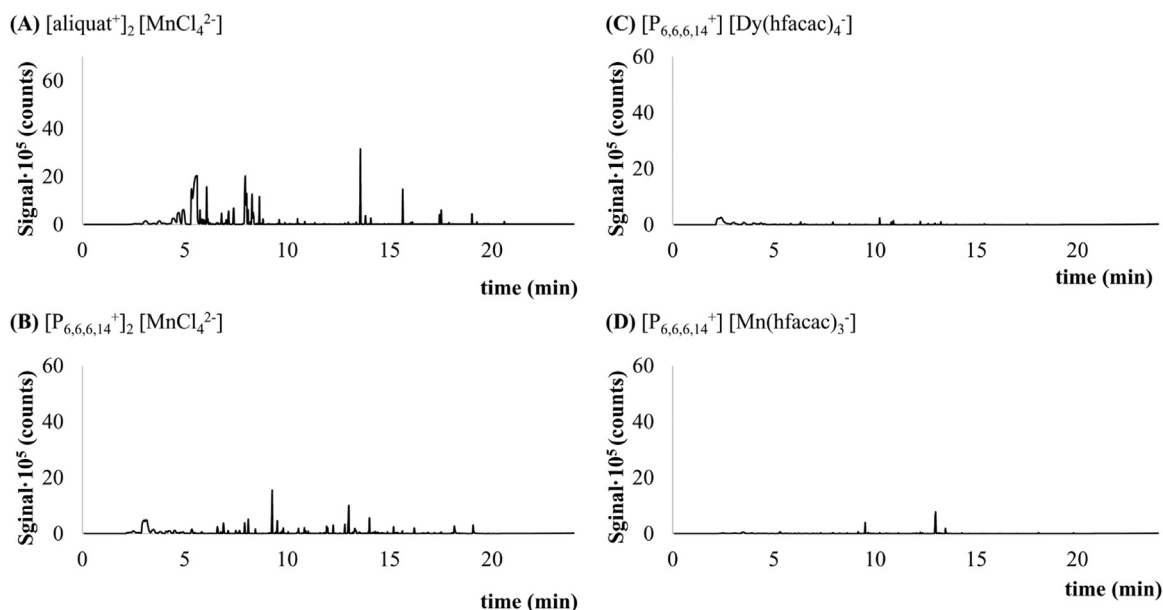
Fig. 2. Representative chromatograms showing the background for different MILs in the HSD-GC-MS method. Experimental conditions: 20 mg of MIL in a 10 mL HS vial, HSD (10 min, 200 °C oven, 21 5 °C loop and 225 °C transfer line, agitation at 36 cycles·min^{-1}), inlet (220 °C), separation in a HP-5 ms column (30 m L×0.250 mm I.D.×0.25 μm of film thickness, Agilent Technologies), oven GC program (initial: 80 °C; 10 °C min^{-1} up to 320 °C), MS detection (EI, SCAN mode, m/z range: 50–600).

Fig. 3. Chromatograms obtained after vacuum MIL-HS-SDME-HSD-GC-MS (optimum conditions) using an aqueous solution containing FFAs at 5 mg L^{-1} (in re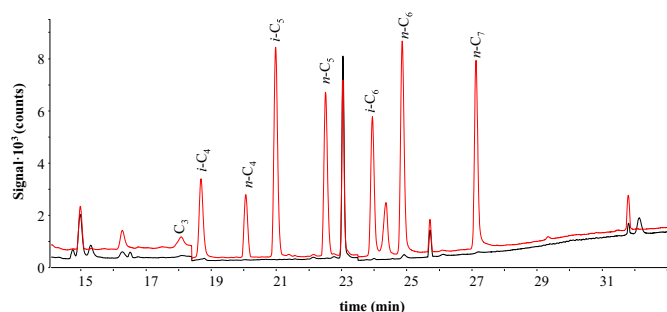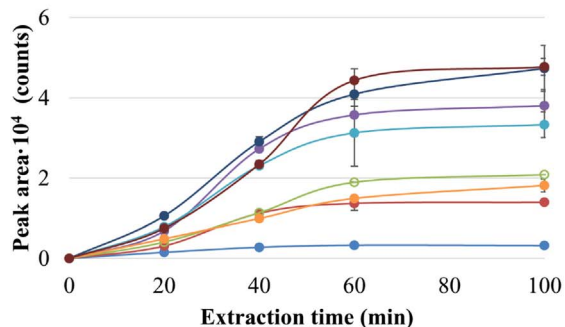d), and an aqueous solution without FFAs (in black). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

The extraction time profiles obtained using vacuum MIL-HS-SDME and atmospheric pressure MIL-HS-SDME are represented in Fig. 4. When MIL-HS-SDME experiments were performed without vacuum, analytes did not achieve equilibration in the range of times studied. On the contrary, the majority of analytes achieved equilibrium at 60 min using vacuum MIL-HS-SDME. Furthermore, an enhancement in the extraction efficiency was observed at any extraction time when reduced pressure conditions were applied. This enhancement was more drastic for less volatile compounds, especially for i-C$_6$, for which vacuum MIL-HS-SDME provided peak areas up to 14 times higher than MIL-HS-SDME (see Table S-4 of the SM). For more volatile compounds (from C$_3$ to i-C$_5$, with the exception of n-C$_4$), the highest ratios of peak area for vacuum MIL-HS-SDME *versus* MIL-HS-SDME were achieved at 40 min, while 60 min was required to observe the highest differences between both techniques in the case of less volatile FFAs and n-C$_4$ (Table S-4 of the SM). This improvement in the extraction efficiency using reduced pressure conditions is in agreement with results reported for vacuum HS-SPME for the same compounds [7].

Based on these results, vacuum MIL-HS-SDME was selected as the optimum HS-SDME procedure for the determination of FFAs using an optimal extraction time of 60 min.

3.4. Optimization of other parameters influencing vacuum MIL-HS-SDME

After demonstrating the superior extraction efficiency of vacuum MIL-HS-SDME, other parameters that influence the extraction performance were optimized, including the MIL volume, the extraction temperature, and the stirring rate. The remaining parameters were fixed for the same reasons explained in Sections 3.2 and 3.3 ([P$_{6,6,6,14}^{+}$] [Mn(hfacac)$_3^{-}$] as MIL, 10 mL of sample containing 30% (w/v) of NaCl, a spiked level of 0.022 mmol L^{-1} and subsequent HSD using 10 mL HS vials). Experiments were performed in triplicate (n=3).

3.4.1. Influence of MIL volume

Drop stability during SDME is an essential condition for successful use of the technique [26]. When conventional SDME is performed by exposure of the extraction solvent using a micro-syringe, the stability of the microdroplet hanging from the needle tip is limited. In addition, agitation of the sample can cause perturbations in the microdroplet resulting in its detachment from the needle tip. The marriage of MILs to the rod magnet support allows for larger extraction solvent volumes to be exposed. Thus, the extraction volume in conventional SDME is on the order of 1–3 µL while MIL-HS-SDME permits larger volumes to be employed without sacrificing microdroplet stability.

In vacuum MIL-HS-SDME, the influence of the MIL volume was studied in the range between 10 and 30 µL. Fig. S-2 of the SM shows the obtained extraction efficiencies of all FFAs expressed as chromatographic peak areas. No significant differences in reproducibility were found within all ranges of volumes studied, showing that the MIL microdroplet is stable at the studied extraction conditions. However, volumes higher than 30 µL detached from the needle tip during the air evacuation step. Fig. S-2 of the SM also reveals that the extraction efficiencies of all FFAs, with the exception of C$_3$, initially increased when the MIL volume was increased from 10 µL to 20 µL and then decreased at MIL volumes higher than 20 µL, probably because diffusion is slower when higher microdroplet volumes are employed. This behavior was not observed for the highly volatile compound C$_3$, for which the extraction efficiency reached a maximum using 30 µL of MIL. However, a MIL volume of 20 µL was selected as the optimum value.
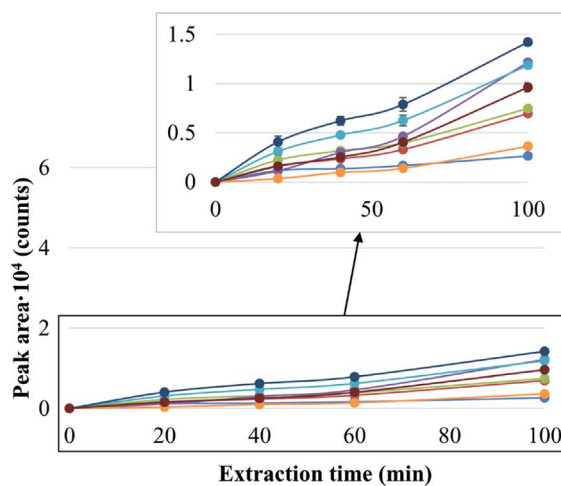
(A) Vacuum MIL-HS-SDME

(B) MIL-HS-SDME

Fig. 4. Comparison of the extraction-time profiles obtained after MIL-HS-SDME performed under reduced pressure conditions – vacuum MIL-HS-SDME **(A)** – or atmospheric pressure conditions –MIL-HS-SDME **(B)** –. Experimental conditions (n=3): 20 µL of [P$_{6,6,6,14}^{+}$] [Mn(hfacac)$_3^{-}$], 10 mL ultrapure water containing 30% (w/v) NaCl and 0.02 mmol L^{-1} of FFAs, 35 °C extraction temperature, 400 rpm stirring speed, followed by HSD in 10 mL headspace vials, and GC-MS.

Table 2
Analytical performance of the vacuum MIL-HS-SDME-HSD-GC-MS method (n=9 calibration levels).

Analyte	Working range (mg L^{-1})	(Slope ± SD[a])·10^{-4}	R[b]	$S_{y/x}$[c]·10^{-4}	LOD[d] (µg L^{-1})	LOQ[e] (µg L^{-1})
C$_3$	0.8–7.6	0.43 ± 0.03	0.990	0.17	216	721
i-C$_4$	0.2–8.7	2.8 ± 0.1	0.996	0.78	70.1	234
n-C$_4$	0.8–8.7	2.5 ± 0.1	0.991	1.0	47.3	158
i-C$_5$	0.3–10	7.7 ± 0.3	0.996	2.5	14.5	48.4
n-C$_5$	0.3–5.2	4.9 ± 0.2	0.996	0.82	55.3	184
i-C$_6$	0.3–11	3.5 ± 0.1	0.997	1.1	70.3	234
n-C$_6$	0.1–11	7.9 ± 0.2	0.996	2.8	21.3	71.0
n-C$_7$	0.1–13	6.5 ± 0.3	0.995	3.1	17.5	58.2

[a] Standard deviation of the slope.
[b] Correlation coefficient.
[c] Standard deviation of the residuals (or error of the estimate).
[d] Limit of detection, calculated as 3 times the signal-to-noise ratio.
[e] Limit of quantification, calculated as 10 times the signal-to-noise ratio.

3.4.2. Influence of the extraction temperature

Extraction temperature is another important parameter in HS-SDME [27]. The influence of this variable was studied for vacuum MIL-HS-SDME in the range between 25 and 55 °C. When the temperature was increased at values higher than 55 °C, the MIL microdroplet became unstable due to undesirable water microdroplets that deposited around the MIL.

The results obtained are represented in Fig. S-3 of the SM. For most volatile analytes (from C$_3$ to n-C$_5$), the extraction efficiency increased with increasing temperature up to an optimum value, where the extraction efficiency was then observed to decrease. The optimal temperatures were 35 °C for n-C$_4$ and 45 °C for the rest of the FFAs. The initial increment is likely due to an increase in the sample temperature which favors mass transfer of the analyte to the HS as long as the vapor pressure of the analytes decrease. However, higher temperatures may coincide with a more pronounced decrease in the vapor pressure resulting in analytes that reside in the gas phase rather than in the MIL microdroplet prompting a decrease in the amount of analyte extracted.

A final decrease in the extraction efficiency at higher extraction temperatures was not observed for the less volatile analytes (i-C$_6$, n-C$_6$ and n-C$_7$). Thereby, i-C$_6$ did not achieve equilibration in the temperature range studied and the extraction efficiency increased with higher temperature. Meanwhile, n-C$_6$ and n-C$_7$ achieved equilibrium at ~40 and 45 °C, respectively, and the extraction efficiency was kept constant at higher extraction temperatures. Therefore, 45 °C was selected as the optimum (and mild) extraction temperature for vacuum MIL-HS-SDME.

3.4.3. Influence of stirring rate

The mass transfer of analytes to the headspace in HS-SDME can be accelerated by the application of constant stirring [28,29]. The effect of stirring rate in vacuum MIL-HS-SDME was investigated by performing experiments between 200 and 600 rpm. The obtained results are shown in Fig. S-4 of the SM. The results indicate that microdroplet stability was independent of the studied stirring rate. However, stirring rates higher than 600 rpm caused detachment of the MIL microdroplet. Based on these results, an optimum stir rate of 600 rpm was selected.

3.4.4. Ensuring a higher preconcentration in the entire method

After optimization of vacuum MIL-HS-SDME method, another parameter to consider is the phase ratio in the subsequent step of HSD step [20]. The HSD phase ratio is defined by the ratio between the volume of the gas phase (or HS volume) and the initial volume (or MIL microdroplet volume). An improvement in the preconcentration of the entire method should result by the reduction of the HSD phase ratio. Two strategies can be applied to achieve this goal: (1) increase the MIL microdroplet volume or (2) decrease the HS volume.

Increasing the MIL microdroplet volume is not possible because, as

discussed in Section 3.4.1, this parameter also has an important influence in vacuum MIL-HS-SDME with 20 µL being the optimum MIL volume.

Thus, the effect of reducing the HS volume was investigated. Experiments were performed using optimum conditions for vacuum MIL-HS-SDME by varying the HS vial size during the HSD step. The smallest commercially available HS vials with a volume of 10 mL and a modified HS vial with a HS volume of 4.2 mL (see Section 2.3.2) were examined. Therefore, the HSD phase ratio was reduced from 500 (10/0.020) to 210 (4.2/0.02), respectively. As theory predicted, the reduction of the HS volume in the HSD (from 10 to 4.2 mL) ensured a higher overall method preconcentration. The extraction efficiency of FFAs increased from 39% to 62% using the modified 4.2 mL HS vials (see Table S-5 of the SM). Similar behavior was previously reported for the extraction of polychlorinated biphenyls (PCBs) using *in situ* IL-DLLME coupled to HSD-GC-electron capture detector (ECD) [20]. The response of PCBs was increased from 20% to 40% by employing similar 4.2 mL HS vials during the HDS step [20]. Based on this result, 4.2 mL was selected as optimum HS volume in the HSD step.

3.5. Analytical performance

Evaluation of the analytical performance for the vacuum MIL-HS-SDME-HSD-GC-MS method was investigated in terms of the linearity ranges, correlation coefficients (R), sensitivities, limit of detections (LODs), and limit of quantifications (LOQs). The procedure for the determination of these parameters was described in Section 2.3.3. Table 2 summarizes the obtained results for the studied FFAs.

The calibration curves produced wide linearity ranges, from 0.1 to 13 mg L^{-1}, and R ranged between 0.990 and 0.997. The sensitivity of the method, expressed as the slopes of the calibration curve, was between $(0.43 ± 0.03)·10^{-4}$ for C$_3$ and $(7.9 ± 0.2)·10^{-4}$ for n-C$_6$.

The LODs and LOQs were estimated and verified as described in Section 2.3.3. Low LODs were obtained, ranging between 14.5 µg L^{-1} for i-C$_5$ and 216 µg L^{-1} for C$_3$.

The reproducibility and extraction performance of the method were also evaluated and the results are shown in Table 3. The reproducibility was estimated as RSD of intra-day experiments, and at two different spiked levels of FFAs (n=3). For the low spiked level 1, acceptable RDS values were reported and ranged from 2.5% for C$_3$ to 13% for n-C$_4$.

The extraction performance of the vacuum MIL-HS-SDME method was studied by determining the E$_F$ values at two spiked levels of FFAs (Table 3), as described in Section 2.3.3. For the spiked level 1, the obtained E$_F$ values ranged between 15 and 31.

The accuracy of the method was evaluated using the RR and using the calibration curves reported in Table 2. Results are presented in Table 3 for the two spiked levels. RR values between 79.5% and 94.4% were achieved for the spiked level 1.

The developed method was also compared with other reported

Table 3

Analytical performance of the vacuum MIL-HS-SDME-HSD-GC-MS method in terms of extraction efficiency and reproducibility.

Analyte	Spiked level 1				Spiked level 2			
	Spiked level (mg L^{-1})	RSD[a] (%)	RR[b] (%)	E$_F$[c]	Spiked level (mg L^{-1})	RSD[a] (%)	RR[b] (%)	E$_F$[c]
C_3	0.95	2.5	94.4	15	2.7	4.4	109	7.6
i-C_4	1.1	7.7	79.5	17	3.1	4.9	97.5	13
n-C_4	1.1	13	86.3	15	3.1	5.9	100	11
i-C_5	1.3	6.5	88.6	18	3.6	7.4	97.4	20
n-C_5	1.3	3.3	89.8	18	3.6	9.1	111	18
i-C_6	1.4	5.6	93.7	24	4.0	11	104	33
n-C_6	1.4	6.3	87.1	26	4.0	6.9	106	34
n-C_7	1.6	8.5	93.9	31	4.6	6.8	107	37

[a] Relative standard deviation (n=4).
[b] Relative recovery.
[c] Enrichment factor.

methods for the determination of FFAs (Tables S-6 and S-7). Thus, if the proposed method is compared with conventional methods that utilize GC-MS [15], it can be highlighted that the proposed method does not require any derivatization of the analytes. Furthermore, the proposed method is solvent-free, contributing to a safer methodology [1]. On the other hand, the proposed vacuum MIL-HS-SDME represents an alternative to other HS-SPME methods such as multiple headspace (MHS)-SPME [24] and vacuum HS-SPME [7]. Compared to MHS-SPME, this technique is easier and requires shorter sampling time as long as MHS-SPME implies four successive HS-SPME extractions of the same extraction vial [24]. In addition, although shorter sampling times are required for vacuum HS-SPME, the possibility of performing parallel extractions in vacuum MIL-HS-SDME demonstrates its higher throughput. Thus, the proposed methodology can be included in the GAC [1,2].

3.6. Analysis of milk samples

The method was applied for the analysis of a low fat milk (with less than 1 g L^{-1} of total fat) and an organic fat reduced milk (with a total content of 19 g L^{-1} of fat). The primary component found in milk lipids is triacylglycerides, compounds consisting of a glycerol molecule linked to three fatty acid chains that yield a triester [13]. FFAs are formed in milk by the enzymatic breakdown of the triacylglycerides [13]. Thus, milk with different fat contents are suspected to contain different amounts of FFAs. Table 4 shows the estimated value of FFAs for both samples. In the case of the low fat milk, i-C_4 was not detected while C_3 was detected but not quantified. The remaining FFAs were quantified, with concentrations between 0.6 ± 0.3 mg L^{-1} and 0.7 ± 0.3 mg L^{-1}. When the organic fat reduced milk was analyzed, i-C_5 could not be detected. For the rest of the FFAs, the estimated concentration was higher with respect to the low fat milk, and the concentration of FFA ranged from 0.7 ± 0.2 mg L^{-1} to 5.5 ± 0.3 mg L^{-1}. The FFA values obtained are in accordance with the fat content reported for milk manufacturers, with low fat milk having the lowest amount. Similar amounts of short chain FFAs were detected with other reported procedures [7,15], with main features summarized in Tables S-6 and S-7 of the SM. The current method shows similar analytical performance but minimizes the extraction time while requiring low extractant (MIL) volumes and no derivatization of FFAs.

4. Conclusions

It has been successfully demonstrated the possibility of using vacuum HS-SDME for first time. The use of relatively large amounts of MILs suspended onto a rod magnet in the HS of a sample (i) while permitting the use of reduced pressure conditions (ii) are benefits derived from the magnetic characters of these solvents.

The proposed vacuum MIL-HS-SDME method provides a powerful

Table 4

Analysis of milk samples using the vacuum MIL-HS-SDME-HSD-GC-MS method under optimum conditions.

Analyte	Concentration found ± U[a] (mg L^{-1})	
	Low fat milk	Organic fat reduced milk
C_3	> LOD, < LOQ[b]	5.5 ± 0.3
i-C_4	n.d.[c]	0.7 ± 0.2
n-C_4	0.6 ± 0.3	4.4 ± 0.3
i-C_5	0.7 ± 0.3	n.d.[c]
n-C_5	0.6 ± 0.1	1.6 ± 0.1
i-C_6	0.7 ± 0.2	0.8 ± 0.2
n-C_6	0.6 ± 0.3	1.6 ± 0.3
n-C_7	0.6 ± 0.4	1.1 ± 0.4

[a] Calibration uncertainty.
[b] Detected but non-quantified.
[c] Non-detected.

approach for the determination of a group of short chain FFAs (from C_3 to n-C_7), responsible for the aroma of milk and other dairy products.

The advantages exploited by reduced pressure conditions are demonstrated using the [P$_{6,6,6,14}^+$] [Mn(hfacac)$_3^-$] MIL as extraction solvent. With the proposed method, analytes reach equilibrium faster than regular atmospheric pressure MIL-HS-SDME (thus supporting the need of a vacuum approach) and, in addition, an enhancement in the extraction efficiency for all analytes was demonstrated when vacuum MIL-HS-SDME was applied at any extraction time.

As additional advantages, the method does not require the derivatization of the FFAs to their methyl ester analogues and, combined with HSD, analytes are determined in an automated approach using GC-MS without any interferences coming from the MIL solvent. After proper optimization and validation of the entire method, a comparison with other reported methods for FFAs determination revealed similar throughput and sensitivity. Furthermore, the applicability of the method is demonstrated with the analysis of milks containing different fat content.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.talanta.2017.05.021.

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