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Talanta 64 (2004) 87–100
Talanta
www.elsevier.com/locate/talanta

Multiresidue determination of sulfonamides in a variety of biological matrices by supported liquid membrane with high pressure liquid chromatography-electrospray mass spectrometry detection

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Received 12 August 2003; received in revised form 13 February 2004; accepted 24 February 2004

Abstract

A high performance liquid chromatography (HPLC) coupled to a mass spectrometer (MS) was used for a simultaneous determination of 16 sulfonamide compounds spiked in water, urine, milk, and bovine liver and kidney tissues. Supported liquid membrane (SLM) made up of 5% tri-*n*-octylphosphine oxide (TOPO) dissolved in hexyl amine was used as a sample clean-up and/or enrichment technique. The sulfonamides mixture was made up of 5-sulfaminouracil, sulfaguanidine, sulfamethoxazole, sulfamerazine, sulfamethizole, sulfamethazine (sulfadimidine), sulfacetamide, sulfapyridine, sulfabenzamide, sulfamethoxypyridazine, sulfamonomethoxine, sulfadimethoxine sulfasalazine, sulfaquinoxaline, sulfadiazine, and sulfathiazole. Some of these compounds, such as, sulfaquinoxaline, sulfadiazine, sulfabenzamide, sulfathiazole and sulfapyridine failed to be trapped efficiently by the same liquid membrane (5% TOPO in hexylamine). The detection limits (DL) obtained were 1.8 ppb for sulfaguanidine and sulfamerazine and between 3.3 and 10 ppb in bovine liver and kidney tissues for the other sulfonamides that were successfully enriched with SLM; 2.1 ppb for sulfaguanidine and sulfamerazine and between 7.5 and 15 ppb in cow's urine, whereas the DL values in milk were 12.4 ppb for sulfaguanidine and sulfamerazine and between 16.8 and 24.3 for the other compounds that were successfully enriched by the membrane. Several factors affecting the extraction efficiency during SLM enrichment, such as donor pH, acceptor pH, enrichment time and the membrane solvent were studied.

Keywords: Sulfonamides; Biological matrices; Supported liquid membrane; Electrospray-mass spectrometry

1. Introduction

Sulfonamides are a group of antibacterial agents commonly used in veterinary practice to prevent infections in livestock, to treat diseases, and to promote growth [1,2]. The extensive use of sulfonamides in animal husbandry has been associated with the presence of sulfonamide residue in meat and meat products. In addition to the notable residues of these drugs in animal tissues, large amounts of these drug residue and their metabolites may also be egested in the faeces and urine. Eventually these compounds find their way in water and wastewater treatment locations and thereby become a health hazard to humans and also inhibit the growth of microorganisms involved in biological processes for wastewater treatment [2,3]. The presence of sulfonamide residue in food is of concern because some of the compounds are known to be carcinogenic [2,4] and they generally enhance the risk of developing antibiotic resistance [2,5], which makes the therapeutic use of similar medicine inefficient [5]. Recent evidence has implicated sulfamethazine as a possible thyroid carcinogenic agent [2,6]. Sulfonamide residue in food and animal tissues may be present in minute concentrations but may pose a health threat to consumers [2]. Therefore, monitoring of these compounds has attracted interest to the scientific communities.

Numerous methods have been employed to determine sulfonamide drugs such as TLC, GC, GC-MS, LC, etc. Thin layer chromatography (TLC) is prone to interferences and is inadequate for quantitative analysis [3,8]. Gas chromatography coupled to electron ionisation (EI) mass spectrometry (MS) is both sensitive and selective for the determination of sulfonamides but derivatisation of non-volatile and thermally labile sulfonamides is required prior to analysis [9,10].

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0039-9140/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2004.02.038

This increases the overall analysis time and it may lead to errors to the analytical technique. GC with atomic emission detection (AED) is one of the recent techniques applied for the determination of sulfonamide antibiotics [11]. Quantitative mass calculations of the sulfonamides are based on the proportion of the peak areas to the number of atoms in the compounds, C, S and N. Derivatisation is of course, required for the GC, and AED can only be used to identify already known species [11]. Capillary zone electrophoresis (CZE) coupled with nano-electrospray quasi-MS^3 has been applied successfully to the determination of sulfonamide in milk samples [12]. However, the separation suffers from interferences from salt and fat in milk, therefore a clean-up procedure was required prior to the analysis [12]. Liquid chromatography with fluorescence detection has also been reported to have a low limit of detection. However, the technique certainly requires derivatisation to improve the fluorescence properties for detection. Unfortunately, the resolution of this particular system was often poor and the detection was non-specific [13,14]. LC-MS using APCI [7,15], thermospray [16,17], thermospray tandem MS [18,19], in addition to UV [4,18,20] detection have been successfully applied to the determination of some of the sulfonamides in milk, chicken liver, swine muscle tissue, porcine muscle and swine wastewater. Packed column supercritical fluid chromatography (pSFC) using UV detection has recently been applied to the determination of eight sulfonamides and good resolution was achieved by coupling two columns in-line [2,4]. pSFC-MS using a moving belt and SFC-MS with EI source were useful for the determination of sulfonamides in kidney, biological matrices and an extract from *Claviceps purpurea* [21,22]. The moving belt interface has also been used with chromatography to analyze extracts of pig's kidneys for these drugs [23]. pSFC interfaced to FT-IR spectrometry has also been applied to determine eight sulfonamides [24,25] and satisfactory resolution was obtained with the exception of sulfamerazine, sulfadimethoxine and sulfapyridine, however FT-IR detection was found to be non-specific for these compounds. Capillary SFC has also been applied to separate these compounds but the technique failed to achieve complete separation of the test analytes [26].

Although, the determination of sulfonamides by LC-MS or LC-UV has been investigated [4,7,16–18,20], the analysis technique requires time for sample preparation and clean-up. From the environmental point of view, it is important to minimize the use of undesirable organic solvents in the determination of sulfonamides. The use of supported liquid membrane, as a sample clean-up technique that has been applied in this work, is such a technique.

2. Experimental

2.1. Standards and chemicals

All the 16 sulfonamides were obtained from Sigma (St. Louis, USA). Most standard stock solutions (1000 ppm) were prepared by dissolving 10 mg in 10 ml methanol. For those, which were not very soluble in the latter, such as sulfasalazine, 5-sulfaminouracil and sulfadiazine, a mixture of methanol-DMSO (1:1) was used. The working standard solutions were prepared from this stock by serial dilution. Ethyl acetate was from LAB-SCAN (Stillorgan, Dublin, Ireland). Stock solutions were stored in brown glass bottles and kept at 4 °C. All solvents used were of HPLC grade. Methanol and acetonitrile were purchased from BDH Laboratory (Poole, England). Ultra high purity (UHP) water was processed through a Millipore Quantum Ultrapure Ionex Gradient A10 purification system (Millipore, Molsheim, France). The aqueous solvents were filtered through cellulose nitrate membrane with 0.45 μm pore size and 47 mm diameter while the organic solvents were filtered through 0.45 μm organic membrane filter, type HVLP, Millipore (Dublin, Ireland). The structures and molecular weights of the sulfonamide compounds studied are presented in Table 1.

2.2. Extraction of sulfonamides from kidney and liver tissues

Extraction from kidney and liver tissues were performed as reported by De Baere et al. [27], and also by Porter [28] with slight modifications. The samples (kidney and liver tissues) were acquired from the local abattoirs (slaughterhouses). Five grams of kidney and liver tissues were sliced, blended and then placed into a plastic centrifuge tubes. Some portions were spiked with a known amount of sulfonamide compounds and one portion from each of these biomatrices was not spiked and used as the control sample. Two milliliters of 0.1 M acetic acid in water was added to 10 ml ethyl acetate and the tissues were extracted using this acidified ethyl acetate. After the addition of acidified ethyl acetate, the samples were vortex-mixed for about 20 s. Three milliliters of acetone was added and then spanned at $3000 \times g$ for 10 min in a centrifuge. The organic phase was transferred into clean centrifuge tubes, and the same amount of organic phase was added to the remainder of the tissue sample in the centrifuge tubes and the extraction process was repeated. The organic phases collected were pooled and kept in the cold room for at least 1 h ready for the next steps of clean-up and/or enrichment.

2.3. Extraction of sulfonamides from milk spiked samples

The milk samples were filtered through a Whatman filter paper to remove solid particles. Then 10 ml of filtered milk was transferred into calibrated flasks, and spiked with a mixture of sulfonamide compounds to give the desired concentrations and diluted with MeOH to 10 ml. 0.1 M acetic acid in water was then added to the flasks and their contents were vortex-mixed for about 20 s. Three milliliters of acetone and 12 ml of ethyl acetate was then added and the flasks together with their contents were centrifuged for 10 min at $3000 \times g$

Table 1
Structures, molecular weights, pK_a values and CAS numbers for the sulfonamide compounds under this study

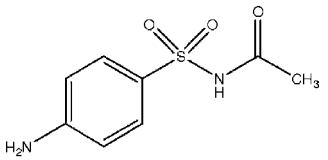
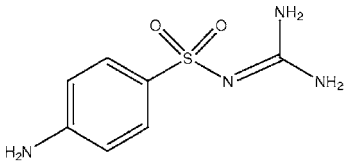
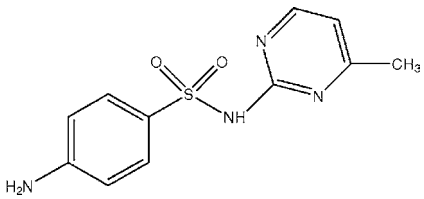
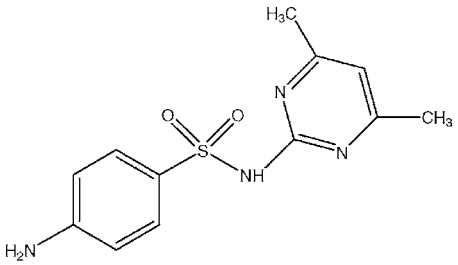
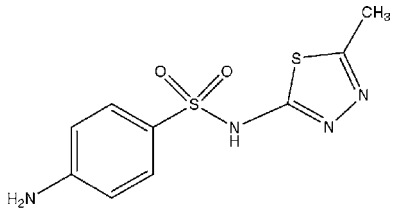
Sulfonamide structure, name and pK_a values of various functional groups in the structures	CAS number	Molecular weight
 1.3±0.1 5.6±0.5 Sulfacetamide	144–80–9	214.2
 0.5±0.1 0.4±0.1 3.3±0.4 (Guanidine-N) Sulfaguanidine	57–67–0	214.2
 1.6±0.1 6.9±0.5 0.4±0.1 (ring-N) Sulfamerazine	127–79–7	264.3
 0.4±0.1 7.0±0.5 2.8±0.2 (ring-N) Sulfamethazine	57–68–1	278.3
 1.2±0.1 7.0±0.5 Sulfamethizole	144–82–1	270.3

Table 1 (*Continued*)

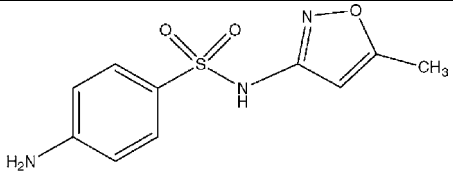
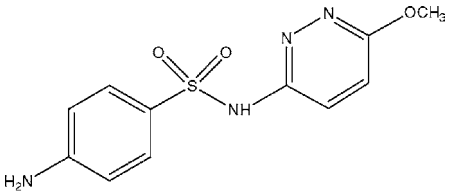
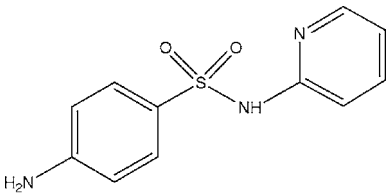
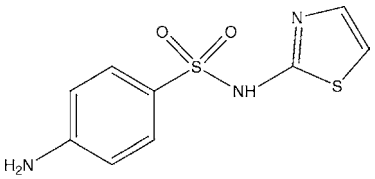
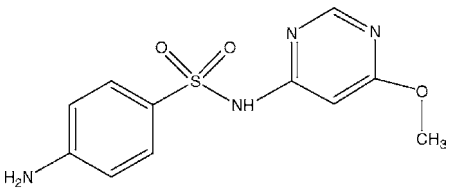
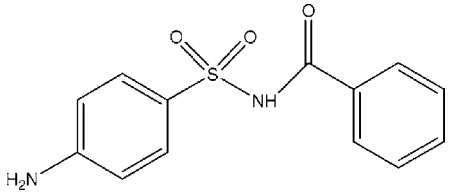
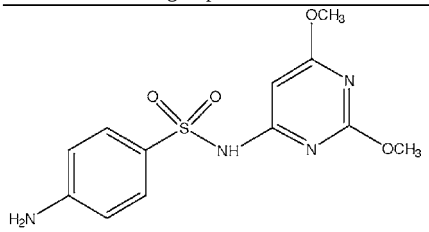
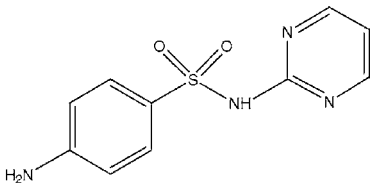
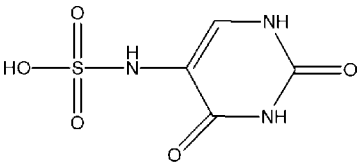
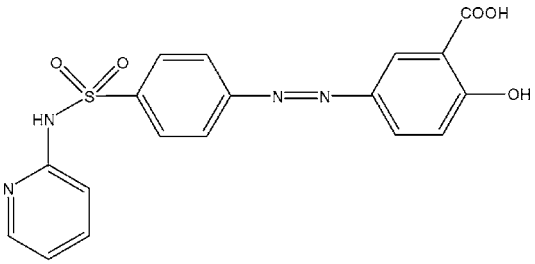
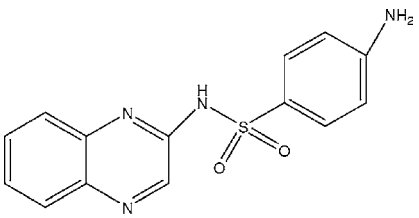
Sulfonamide structure, name and pK_a values of various functional groups in the structures	CAS number	Molecular weight
	723–46–6	253.3
1.4±0.1 7.7±0.5		
Sulfamethoxazole		
	80–35–3	280.3
0.8±0.1 7.3±0.5 2.2±0.5		
Sulfamethoxypyridazine		
	144–83–2	249.3
0.8±0.1 8.0±0.5 2.9±0.5		
Sulfapyridine		
	72–14–0	255.3
0.7±0.1 7.8±0.5 2.3±0.5		
Sulfathiazole		
	1220–83–3	280.3
0.8±0.1 6.7±0.5 2.9±0.4		
Sulfamonomethoxine		
	127–71–9	276.3
1.1±0.1 5.9±0.5		
Sulfabenzamide		

Table 1 (*Continued*)

Sulfonamide structure, name and pK_a values of various functional groups in the structures	CAS number	Molecular weight
 0.7±0.1 6.5±0.5 2.4±0.5 Sulfadimethoxine	122–11–2	310.3
 1.6±0.1 6.8±0.5 0.35±0.2 Sulfadiazine	68–35–9	250.3
 11.5±0.5 (OH) 2.2±0.2 (amide) 5-Sulfaminouracil	5435–16–5	207.2
 1.9±0.2 (pyridine) 7.0±0.5 (amide) 2.9±0.1 (acid) 12.5±0.2 (phenol) Sulfasalazine	599–79–1	398.4
 -1.4±0.3 (q-N) 7.6±0.5 (amide) 1.2±0.1 (NH_2) Sulfaquinoxaline	59–40–5	300.2

NB: pK_a values were calculated using ACD pK_a DB program.

and then the organic phase was transferred into another centrifuge tube. The same amount of organic phase was again added to the remainder of the tissue samples, and the extraction process was repeated and then all organic fractions collected were pooled and kept in the cold room for at least 1 h before further purification and/or enrichment with SLM.

2.4. Extraction of sulfonamides from urine spiked samples

The urine sample was also obtained from the local abattoirs. Prior to analysis, urine matrix was buffered with 0.1 M acetic acid to a uniform pH of around 6. Then a known amount a sulfonamide mixture was spiked and then extracted using acidified ethyl acetate similarly to spiked milk samples.

2.5. Supported liquid membrane enrichment of sulfonamides

Supported liquid membrane (SLM) was used as a clean-up and/or enrichment pretreatment technique for sulfonamide samples prior to analysis with high performance liquid chromatography (HPLC). A mixture of 16 sulfonamides drugs were enriched using supported liquid membrane consisting of 5% tri-*n*-octyl phosphine oxide (TOPO) dissolved in hexylamine. The acceptor and the donor phases were both adjusted to their optimum pH values, i.e. 6 and 10, respectively. The SLM device consisted of two circular poly (vinylidene difluoride) (PVDF) blocks (diameter 120 mm, thickness 15 mm) with the grooves like an archimedes spiral (depth 0.25 mm, width 1.5 mm and length 250 cm giving a total volume of about 0.95 ml. Both sides of the holder were backed with aluminum blocks of 6 mm thickness, in which threads for the clamping screws were machined, to make the assembly stable. In addition, the donor channels of the PVDF blocks were equipped with an O-ring, outside the grooves for sealing the flow system. The liquid membrane support was by a porous PTFE membrane, type FG Millipore (Bedford, Ireland) with an average pore size of 0.2 μm, a total thickness of 175 μm of which 115 mm polyethylene backing, and a porosity of 70%. A Millipore filter, FG type with a pore size of 0.2 μm made of Teflon was impregnated with liquid membrane for 24 min turning its side every 12 min. The impregnated membrane was placed between the two PVDF blocks, with the rough side of the membrane facing the donor side and the whole construction was clamped together tightly and evenly with six screws. The two channels of the SLM unit were separated from each other by a liquid membrane forming the donor (feed) and acceptor (receiving) compartments. After installation of the impregnated membrane in the separator, both channels were flashed with ultra high purity (UHP) water to remove excess of the organic solvent from the surface of the membrane. The acceptor stagnant was flushed with the trapping buffer solution. Two peristaltic pumps Minipuls 3, Gilson, (Villiers-Le-Bel, France) were used to control the flow rates of the donor and acceptor phases independently. The tubes used for pumping solutions were acid-resistant (acid-flexible), Elkay Products, (Shrewsbury, MA, USA) with an i.d. of 1.2 mm for the donor and 0.60 mm for the acceptor. The various parts of the flow system were connected with 0.5 mm i.d. PTFE tubing and Alex screws fittings. The sample and buffer in the donor stream were emerging in a PTFE tee connection and then mixed in a coil (1.0 m × 0.5 mm i.d. coiled tubing) before entering the donor channel of the membrane device. The stagnant acceptor solution containing analytes were quantitatively bled into a 2 ml volumetric flask. The acceptor channel was then cleaned for 5 min first with UHP water and then with the acceptor buffer solution to clear the system of any remaining untrapped analytes before the next extraction.

2.6. High performance liquid chromatography-electrospray mass spectrometry

Samples were separated with a HP 1100 HPLC system consisting of a DAD detector, binary pump system, and thermostatted column compartment, coupled to ThermoQuest LCQ^{Deca} ion trap mass spectrometry (Finnigan, San Jose, USA) for detection of the separated sulfonamide drugs. A C_{18} Clipeus Higgins, 150 mm × 3.0 mm × 5 μm column was used to separate all compounds in a mixture by isocratic mobile phase. The separation was carried out at flow rates of 150 μl min^{-1} and was monitored at absorbance wavelength of 260 nm in addition to ES-MS. The mobile phase used was $A = 85\%$ (25 mM AcOH in water) and $B = 15\%$ (25 mM AcOH in MeOH) and the column temperature was set at 35 °C.

3. Results and discussion

3.1. Supported liquid membrane enrichment of a mixture of sulfonamides

Sulfonamides are known to possess amphoteric character due to the presence of amine groups in their structures, which because of their positions in the structure can either protonate or deprotonate depending on the pH of the environment. The polarity of sulfonamides also makes them soluble in polar organic solvents such as ethyl acetate. This solubility property can be exploited in the extraction of sulfonamides from a variety of biological matrices such as liver, kidney tissues, and milk and urine samples.

3.2. Optimization of the SLM system

For the efficient enrichment of sulfonamide compounds, factors that control the transfer of the analytes from the donor channel to the acceptor channel across the membrane and the entrapment of the analytes in the acceptor channel were optimized. For an efficient enrichment [29]; analytes in the sample solution need to be non-ionic or in an uncharged

Table 2
Extraction efficiency for 60 min extraction of 1 ppb sample of a mixture of sulfonamides in various solvents

Sulfonamide compounds	Di-*n*-hexyl ether	*n*-Undecane	Di-*n*-hexyl ether:*n*-undecane (1:1)	Di-*n*-hexyl ether:*n*-undecane (1:1) + 5% TOPO	Hexylamine + 5% TOPO
Sulfaguanidine	29	16	50	69	83
Sulfacetamide	–	–	–	–	50
Sulfamethoxazole	–	–	–	–	45
Sulfamerazine	–	–	–	–	69
Sulfamethizole	–	–	–	–	72
Sulfasalazine	–	–	–	–	61
5-Sulfaminouracil	–	–	–	–	56
Sulfamethazine	–	–	–	–	70
Sulfamethoxypyridazine	–	–	–	–	74
Sulfamonomethoxine	–	–	–	–	66
Sulfadimethoxine	–	–	–	–	56
Sulfapyridine	–	–	–	–	–
Sulfabenzamide	–	–	–	–	–
Sulfathiazole	–	–	–	–	–
Sulfadiazine	–	–	–	–	–
Sulfaquinoxaline	–	–	–	–	–

Donor pH 6.0; acceptor pH 10.0 and flow rate of 0.3 ml min^{-1}.

form before or diffuse across the membrane. The partition coefficient (K_p) of the analyte molecules between the organic solvent and the aqueous donor phase, have to be as large as possible for the target molecules. For the interfering compounds, it has to be low; and also an efficient trapping or conversion of analytes into the inactive form which in turn prevent back-diffusion into the donor channel, should take place from the stripping channel. Therefore, a number of parameters were optimized in order to achieve the objective of efficient trapping of the analyte.

3.3. Selection of the membrane solvent

Organic solvent is one of the important parameters that can influence not only efficiency but also selectivity of enrichment of the liquid membrane. The extent of analyte extraction from the sample matrix can be expressed as the extraction efficiency, e.g. %*E*, and is defined as the fraction of the analyte extracted into the acceptor phase to the total amount of the analytes in the sample [29,30], thus:

$$E = \frac{C_a V_i}{(C_d V_s)}$$

where C_a is the concentration of the analyte in the acceptor measured after extraction as a peak area, related to a calibration graph, V_i the volume of the injection loop, C_d the concentration of the analyte in the sample before extraction and V_s is the volume of the sample loop [29].

Therefore, there is a need to investigate the best membrane that can extract analytes from the sample matrix efficiently. Several membrane solvents were investigated for possible use to enrich sulfonamides compounds. These include, (i) di-*n*-hexylether (ii) *n*-undecane (iii) di-*n*-hexylether + *n*-undecane (1:1), (iv) 5% (TOPO in di-*n*-hexylether + *n*-undecane (1:1); (v) Hexylamine and (vi) 5% (TOPO in hexylamine. A summary of the extraction efficiency obtained from each membrane as shown in Table 2 shows a summary of 5% TOPO in hexylamine.

The results from Table 2, show that 5% TOPO in hexylamine was the best supported liquid membrane with respect to enriching compounds of interest. Many sulfonamide compounds enriched using this membrane with the exception of five compounds, that included; sulfapyridine, sulfathiazole, sulfabenzamide, sulfadiazine and sulfaquinoxaline. It is not very obvious why these compounds failed to extract using this membrane. The pK_a values of the sulfonamides of interest ranged from 5.9 to 8.0. However, most compounds that trapped have pK_a values that are lower than the least pK_a value of the compounds that failed to trap. In addition, all these compounds, which failed to trap have an unsubstituted side chain ring (the ring attached to the sulfanilamide group in the sulfonamide structure) in their structures. The substitution is believed to play an important role in forming bonding with the TOPO [30]. TOPO has been reported to be an efficient chemical extractant due to the presence of electron pairs on the oxygen atom, that offer the possibility of forming hydrogen-bond complexes of various composition [30]. This process is probably responsible for providing selectivity to these groups of compounds. It is also possible that the structural similarity of hexylamine to sulfonamide compounds, in terms of the presence of amine groups might have contributed to the success of this membrane in comparison to the other liquid membranes attempted.

3.4. Optimization of the donor stream pH

The critical step in the method development for the determination of sulfonamide residues is the clean-up procedure, especially since sulfonamides have amphoteric properties [27]. Therefore, pH control is one of the important parame-

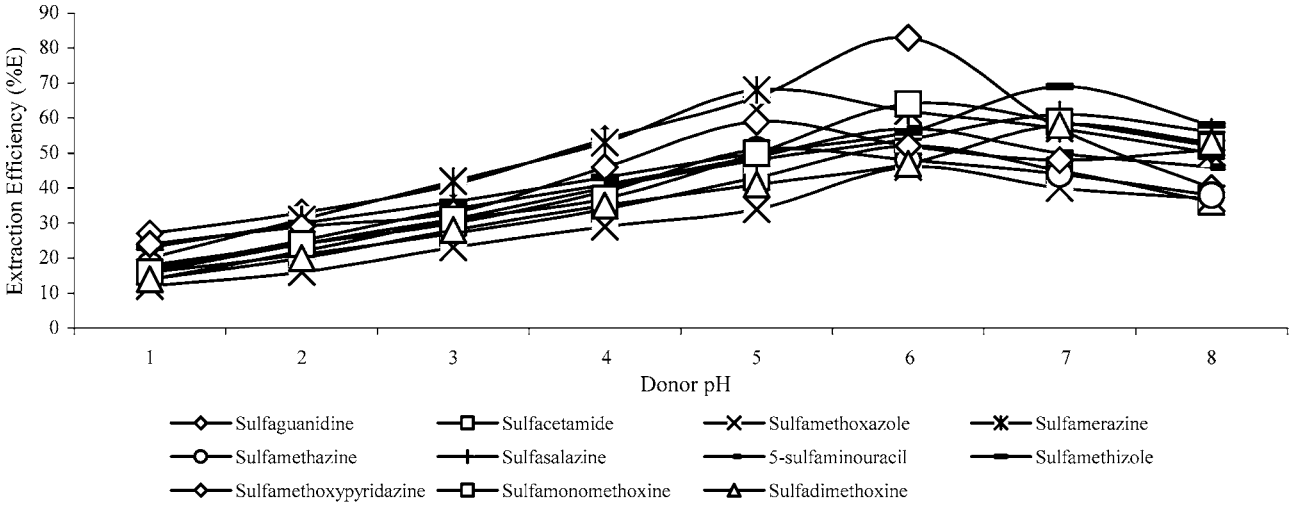


Fig. 1. Donor pH Optimization for sulfonamides; membrane, hexylamine + 5% TOPO, acceptor pH = 10, extraction time = 60 min, flow rate = 0.3 ml min^{-1}.

ters to control. Since the analyte need to be in neutral form for sulfonamides to be trapped by the liquid membrane, pH of the donor channel had to be optimized to establish the conditions for neutrality of compounds during enrichment process. The optimum pH was found to be between pH 5.0–7 (Fig. 1).

The amphoteric sulfonamides would therefore form positive, neutral as well as negative ions depending on the pH of the environment. The pH optimization in the process of clean-up and enrichment with SLM was therefore crucial for both feed (donor) channel and the acceptor (stripping) channel. The amphoteric nature of sulfonamides makes them behave both as acids and abases depending on the pH of the environment. At high pH, the amine group, which is not directly attached to the ring, loses its proton to create a negative charge on the molecule. At low pH values, the amine group, which is attached directly to the ring, is protonated and therefore a positive charge to the molecule is formed. It is evident therefore that in between these two extremes there will be a range for neutral uncharged species. A theoretical prediction for the best and optimal pH has been reported to be at two units below the pK_a lowest acidic value. In present work however, this prediction is applicable in this situation [29] as shown in Table 1.

3.5. Optimization of the acceptor stream pH

The pH of the stagnant acceptor phase also played an important role in enhancing the extraction efficiency of the target analyte. Theoretically, it has been reported that, for nearly complete trapping for the analytes, the pH on the acceptor side should be at least 3.3 pH units higher than the pK_a of the analyte of interest [29]. Fig. 2 show that, the majority of the compounds have their optimal acceptor pH at around 10. Table 1 shows the pK_a values compounds of study. The majority of the compounds studied fall between 5 and 8.

3.6. Optimization of extraction time

The effect of time in the enrichment of sulfonamide compounds was investigated. Fig. 3 shows that, the extraction efficiency is dependent on time. The maximum extraction efficiency was obtained at around 60 min and after that the efficiency slowed down. This may be attributed from the fact that, the efficiency of the extraction of the SLM system depends on the pH of the acceptor channel. As the analyte continue to be trapped, the pH of the acceptor may shift from the set pH and this may cause the analyte molecule to back extract.

3.7. Extraction efficiency with variation in analyte concentrations in the donor stream

To study the trend and behaviours on the extraction efficiency when the concentration in the donor stream is varied, samples with concentrations ranging between 5 ppm to 1 ppb of the sulfonamide compounds were prepared and enriched in the same membrane. From the results, (not shown), it was observed that, as the concentration of the analytes decreases in the donor stream, the efficiency of the membrane increased. This trend may be attributed by the fact that, at low concentrations, the acceptor solutions are not saturated easily with analyte and hence high efficiency. On the contrary, at high concentrations, the acceptor solution is saturated with ionized analyte quickly, which results in some back extractions of the analyte molecules, which are not efficiently changed into inactive form by the acceptor, and this would lower the pre-concentration factor.

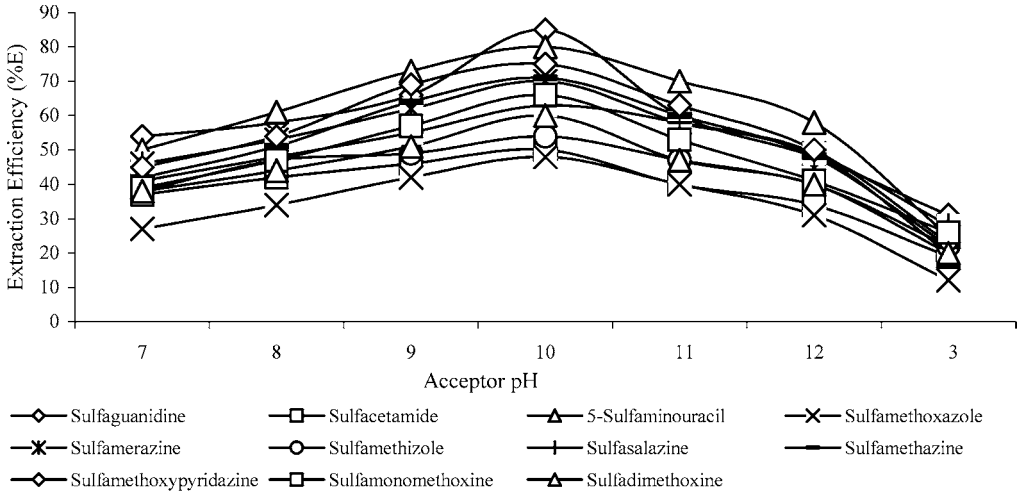


Fig. 2. Acceptor pH Optimization for sulfonamides: membrane, hexylamine + 5% TOPO, donor pH, 6.0, extraction time, 60 min, flow rate, 0.3 ml min^{-1}.

3.8. Chromatographic elution of sulfonamides

The retention and selectivity factors are highly affected by the pH of the mobile phase. Taking into consideration of the amphoteric character of sulfonamides, the eluent (mobile phase) has to be carefully adjusted to an appropriate pH. The pH range was varied from pH values of 2.0–8.0 in an attempt to achieve, the optimum pH for the separation of sulfonamides. It was established that, a pH value around 5.0–6.5 resulted in better resolution and this pH value was used for successful used for the separation of the sulfonamide.

3.9. LC-ES-MS of a mixture of sulfonamides after SLM sample clean up and enrichment

The compounds were separated on a C_{18} reversed phase microbore column and detected with the electrospray ion trap mass spectrometer. Acetic acid was incorporated in the mobile phase to assist the formation of the charged droplets and hence produce protonated molecular ions. The results as displayed in Fig. 4a and b show a successful separation, with Fig. 5a and d showing results after SLM treatment from different one of the biological matrices. Selected ion monitoring provided a powerful selective tool for identification of the separated compounds.

3.10. Comparison of extraction from different biological matrices

The extraction of sulfonamide residues was performed from edible products, that is meat (liver and kidney tissues), milk and from by-products, which in this case was urine. The summary results of the results are shown in Table 4.

3.11. Extraction from spiked milk

Drug residues can be bound to proteins in milk. Since milk is consumed, there is every reason to monitor the presence of drug residues to establish whether the product is fit for

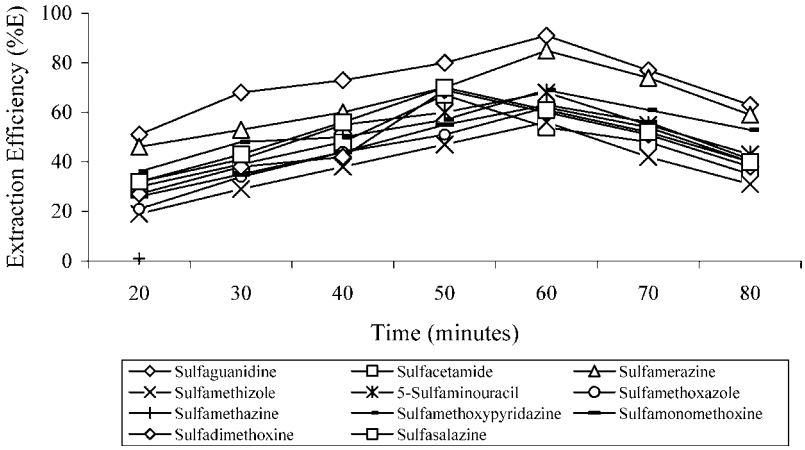


Fig. 3. Extraction time optimization-SLM for sulfonamides; acceptor channel optimization-SLM for Sulfonamides: membrane, hexylamine + 5% TOPO, donor pH, 6.0, acceptor pH, 10, flow rate, 0.3 ml min^{-1}.

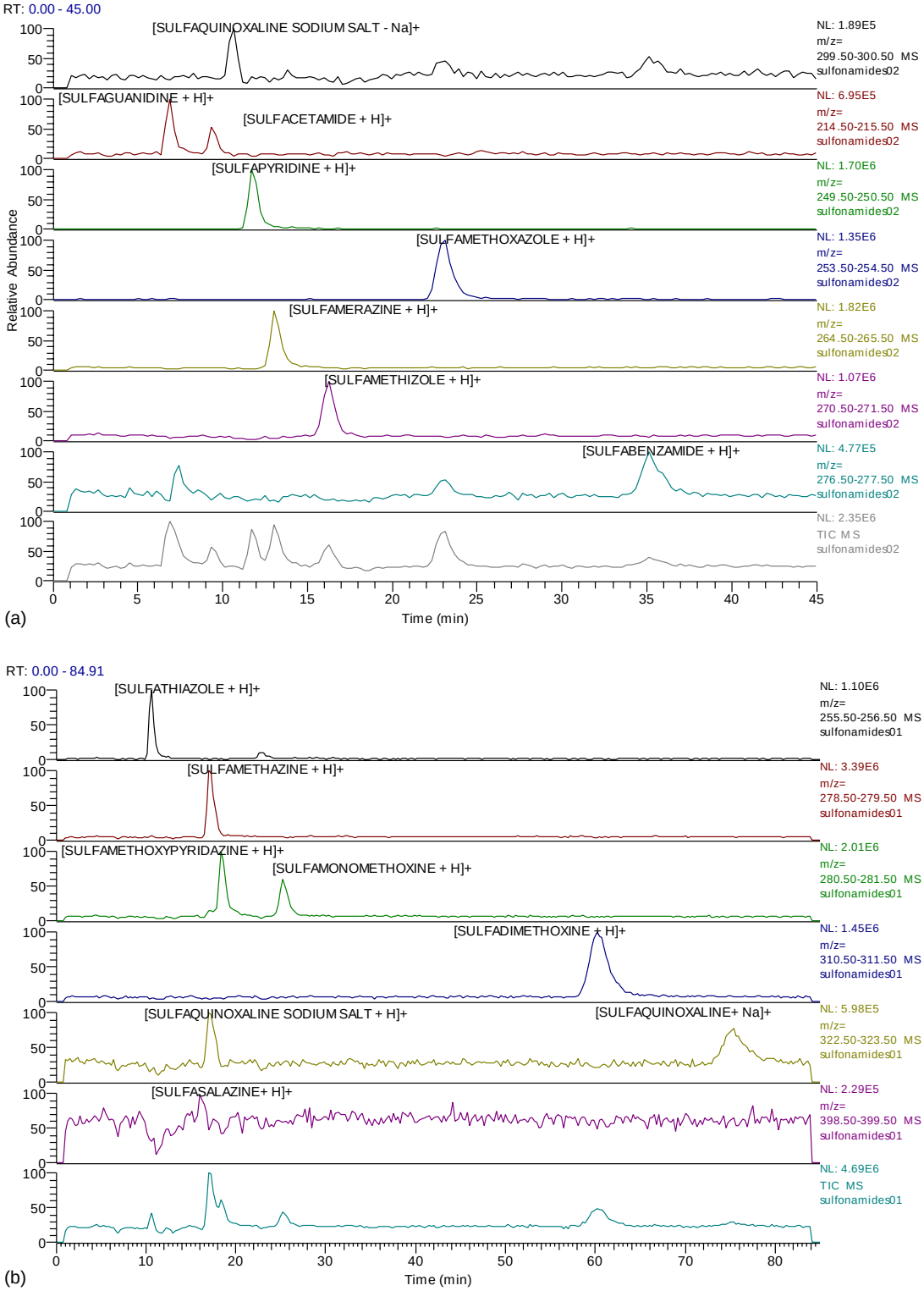
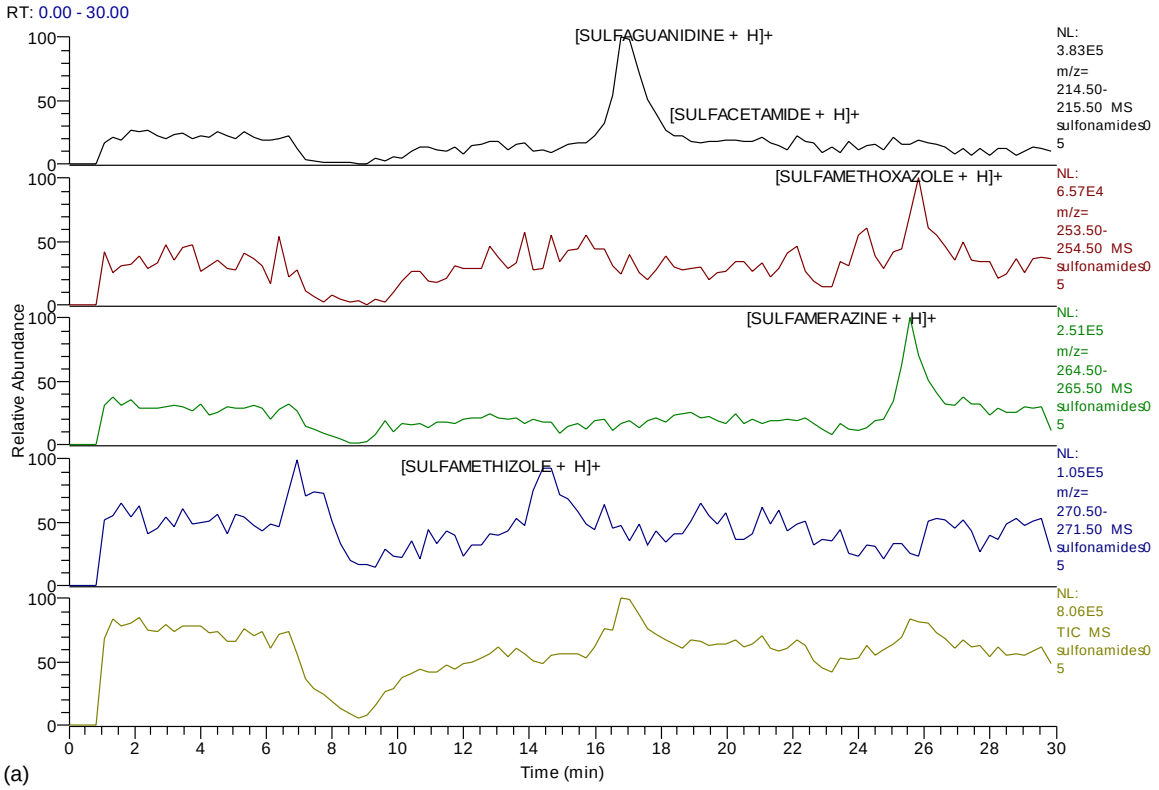


Fig. 4. (a) LC-ES-SIM-MS of sulfonamides spiked in water; concentration = 1 ppm. (b) LC-ES-SIM-MS of sulfonamides spiked in water; concentration = 1 ppm.

consumption or not. However, relatively lower efficiencies were registered in milk-spiked samples as compared with the other matrices. This may be attributed to the presence of non-homogeneous milk-plasma, which contains a colloidal solution of globular proteins, a dispersion of lipoproteins and a dispersion of casein micelles [31]. Milk is considered an emulsion of fat droplets in a complex aqueous milk plasma [31], consisting of a mixture of water, proteins, lipids, enzymes, minerals, phosphatides and other compounds [31]. Therefore, because of these physicochemically occurring



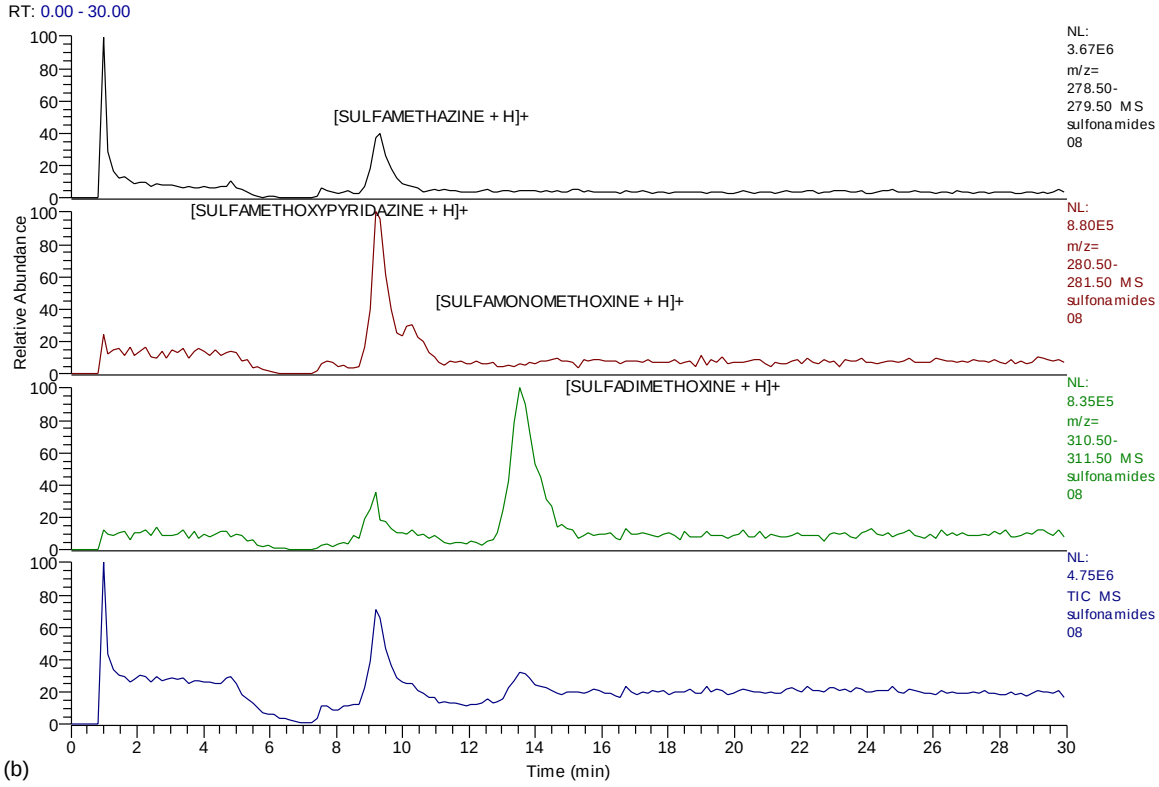
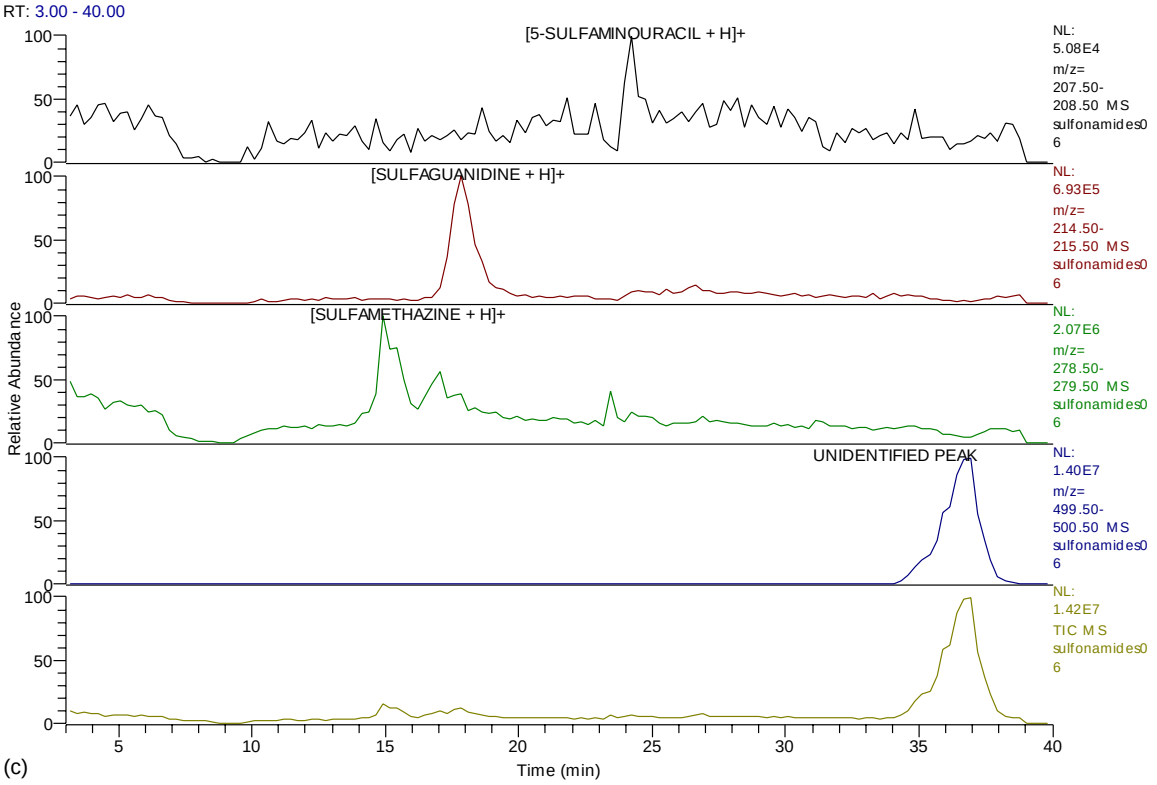


Fig. 5. (a) LC-ES-SIM-MS of sulfonamide drugs in spiked urine after SLM enrichment: concentration = 0.01 ppm. (b) LC-ES-SIM-MS of sulfonamide drugs in spiked urine after SLM enrichment: concentration = 0.01 ppm. (c) LC-ES-SIM-MS of sulfonamide drugs in spiked urine after SLM enrichment: concentration = 0.01 ppm. (d) LC-ES-SIM-MS of sulfonamide drugs in spiked urine after SLM enrichment: concentration = 0.01 ppm.



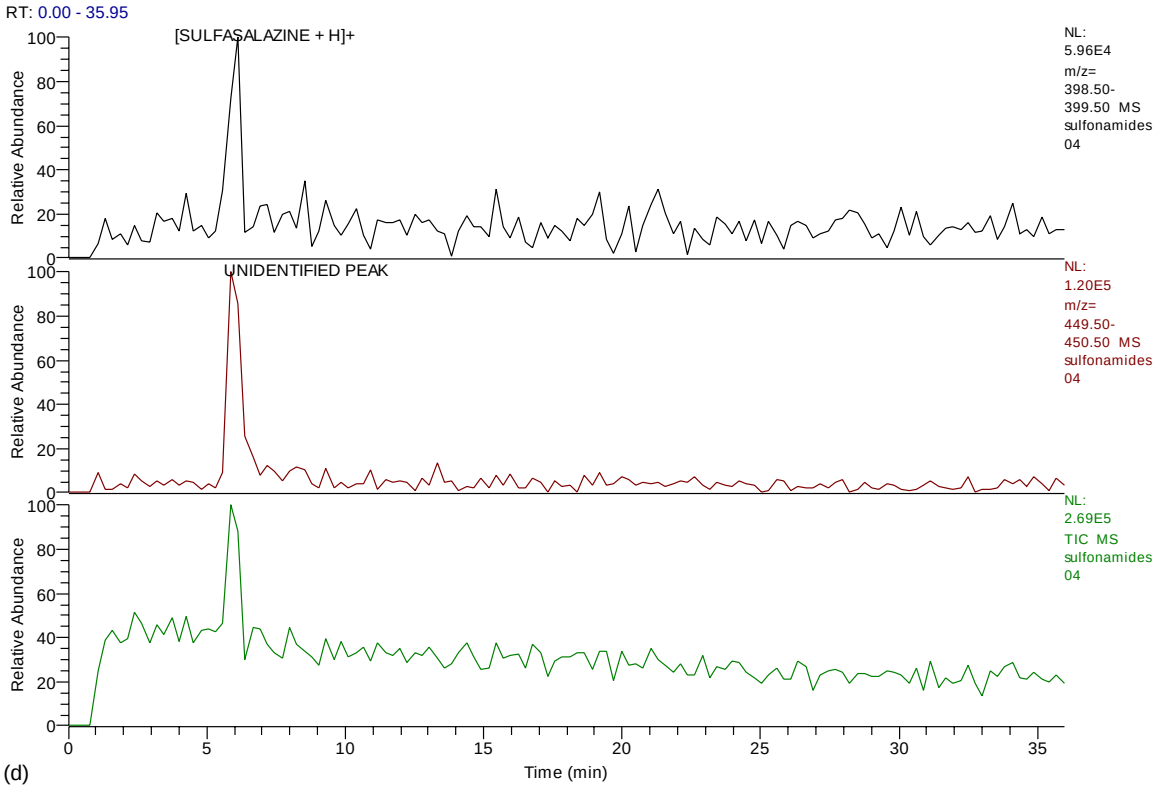


Fig. 5. (*Continued*).

Table 3
Results obtained when sulfaguanidine concentrations in the donor stream was varied

Sulfonamide compound	Concentration 0.01 ppm				
	Water	Urine	Milk	Liver	Kidney
Sulfadimethoxine	57 (2.1)	45 (2.4)	41 (4.3)	51 (3.2)	53 (3.0)
Sulfaguanidine	93 (1.9)	86 (3.0)	77 (6.2)	88 (3.5)	90 (2.7)
Sulfamerazine	70 (2.4)	63 (3.5)	60 (5.9)	65 (3.4)	67 (2.7)
Sulfamethizole	58 (3.7)	49 (3.3)	45 (5.7)	54 (2.8)	56 (2.1)
Sulfamethoxazole	47 (5.1)	37 (4.8)	34 (6.1)	42 (3.9)	44 (3.2)
Sulfamethoxypyridazine	68 (2.8)	62 (3.4)	58 (5.8)	64 (4.2)	66 (3.6)
Sulfacetamide	69 (1.9)	64 (3.0)	60 (5.8)	64 (4.2)	69 (5.7)
Sulfamethazine	58 (4.5)	48 (4.5)	47 (7.5)	52 (4.3)	55 (3.8)
Sulfamonomethoxine	66 (3.3)	58 (2.8)	55 (6.8)	61 (5.2)	64 (4.3)
5-Sulfaminouracil	73 (3.9)	64 (5.9)	62 (6.2)	69 (4.1)	71 (3.9)
Sulfasalazine	75 (3.5)	69 (4.8)	65 (7.2)	71 (3.7)	73 (4.1)

NB: values in parantheses are % relative standard deviations for $n = 5$. The donor and acceptor pH's remained stagnant.

different phases in milk, drugs may be unevenly distributed and this may cause the residues to predominantly remain in one phase even after acidification or decreaming [31].

3.12. Extraction from urine

Urine on the other hand, contains neither lipids nor proteins as milk does. This eliminates the problem of producing emulsions and foams during the extraction with organic solvents. However, both conjugated and non-conjugated drugs residues are known to be excreted together with urine [31]. Hence, hydrolysis may be a necessary step to release the conjugated drugs. The addition of acetic acid not only keeps sulfonamide drugs at the right pH which ensures that they in uncharged form but also enhances acid hydrolysis to set free the conjugated drugs.

3.13. Extraction from the kidney and liver tissues

In most cases, liver and kidney tissues are regarded as target organs to find drug residues. The presence of very active anabolic enzyme systems such as cytochrome P450 complex and reductase activity in liver leads to the post-mortem in the drug metabolism, which may eventually result into the inactivation of the drugs [31]. The results from Table 3 show slightly higher recovery from kidney tissue as compared to liver tissue probably from the fact that traces of such enzymes might have been active in liver tissue and hence relatively suppressed the detection in liver spiked samples.

3.14. Detection limit (DL)

The detection limits in the determination of sulfonamide compounds was determined as the three times the standard deviation of the blank. The DL obtained from kidney and liver tissue extraction was 1.8 ppb for sulfaguanidine and sulfamerazine and between 3.3 and 10 ppb for the rest. For urine was 2.1 ppb for sulfaguanidine and sulfamerazine and between 7.5 and 15 ppb for the rest while from milk spiked samples it was 12.4 ppb for sulfaguanidine and sulfamerazine and between 16.8 and 24.3 for the rest.

3.15. Method precision and accuracy

Bovine urine matrix was spiked with a mixture of sulfonamides of known concentrations ranging from 10 ppb to

Table 4
Comparison of extraction efficiencies from different matrices

Sulfonamide compound	% E for Concentration 0.01 ppm				
	Water	Urine	Milk	Liver	Kidney
Sulfadimethoxine	57 (2.1)	45 (2.4)	41 (4.3)	51 (3.2)	53 (3.0)
Sulfaguanidine	93 (1.9)	86 (3.0)	77 (6.2)	88 (3.5)	90 (2.7)
Sulfamerazine	70 (2.4)	63 (3.5)	60 (5.9)	65 (3.4)	67 (2.7)
Sulfamethizole	58 (3.7)	49 (3.3)	45 (5.7)	54 (2.8)	56 (2.1)
Sulfamethoxazole	47 (5.1)	37 (4.8)	34 (6.1)	42 (3.9)	44 (3.2)
Sulfamethoxypyridazine	68 (2.8)	62 (3.4)	58 (5.8)	64 (4.2)	66 (3.6)
Sulfacetamide	69 (1.9)	64 (3.0)	60 (5.8)	64 (4.2)	69 (5.7)
Sulfamethazine	58 (4.5)	48 (4.5)	47 (7.5)	52 (4.3)	55 (3.8)
Sulfamonomethoxine	66 (3.3)	58 (2.8)	55 (6.8)	61 (5.2)	64 (4.3)
5-Sulfaminouracil	73 (3.9)	64 (5.9)	62 (6.2)	69 (4.1)	71 (3.9)
Sulfasalazine	75 (3.5)	69 (4.8)	65 (7.2)	71 (3.7)	73 (4.1)

NB: values in parantheses are % relative standard deviations for $n = 5$.

1 ppm. Three sets of samples were prepared and from each five replicates were analyzed by LC-ES-MS after enrichment with SLM. Accuracy was determined by calculating the percentage ratio of the amount recovered to that spiked while the relative standard deviation values gave the measure of the method precision. The results for accuracy was as high above 90% while the standard deviation values range from 2.4 to 7.9

4. Conclusions

The sulfonamide compounds of interest in this work have been separated and detected by LC-ES-MS and the enrichment and/or clean-up method was by SLM technique. Eleven out of 16 sulfonamide compounds were successfully enriched using 5% TOPO in hexylamine liquid membrane. The high extraction efficiencies of this membrane are probably due to the fact that, it combined both qualities of hexylamine and the TOPO. The use of selected ion monitoring provided additional selectivity and sensitivity in the determination of these compounds.

Acknowledgements

This work was financially supported by UB-Research and Publication. Private financial assistance in support of TAM by JTMN was greatly appreciated. Prof. J.Å. Jönsson of the University of Lund for the calculation of pK_a values.

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