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Research Article

Hollow Fibre-Supported Liquid Membrane for the Determination of Sulfonamide Residues in Egg Samples

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In this study, a three-phase hollow fibre-supported liquid membrane (HF-SLM) technique incorporated with high-performance liquid chromatography (HPLC) coupled with diode array detection (DAD) was developed for the extraction, clean-up, and determination of fifteen sulfonamide residues in chicken egg samples. The residues were extracted from the 5 mL sample solution of pH 2.5 into a thin layer of organic phase (1-octanol with 10% TOPO) immobilised in hollow fibre pores and then back-extracted into approximately 6 μL of aqueous phase (pH 13) located in the lumen of the hollow fibre. After extraction, 6 μL of the acceptor phase was injected into an HPLC instrument for subsequent analysis. Under optimum conditions, the limit of detection (LOD) and limit of quantification (LOQ) values ranged from 0.8–7.9 $\mu g \cdot kg^{-1}$ and from 2.4–21.0 $\mu g \cdot kg^{-1}$, respectively, linearity in the range of 5 1 000 $\mu g \cdot kg^{-1}$, and intra- and inter-day precision (%RSD) values at three concentration levels (50, 100, and 500 $\mu g \cdot L^{-1}$) ranged from 6.2–15.7%, 7.3–15.0%, and 7.3–14.6%; and 6.4–17.4%, 4.3–16.2%, and 8.3–16.5%, respectively, were obtained. The accuracy of the method, expressed as percentage recovery, was in the range of 71.0–98.7%, with corresponding %RSD ($n = 6$) values ranging from 1.9–9.9% being obtained. The developed method provided enrichment factors in the range of 17.1 to 541.4. The applicability of the proposed method was also evaluated by analysing egg samples, which were randomly collected from local supermarkets located in Gauteng Province, South Africa. The results obtained revealed that the developed method has the potential to be used as an alternative method for the determination of sulfonamide residues in egg and related complex samples.

1. Introduction

Recent research trends involve miniaturisation of the traditional sample preparation techniques by greatly reducing the volume of extraction solvents from the millilitre (mL) to the microliter (μL) scale [1]. As a result, several miniaturised solvent-based sample preparation techniques are becoming widely applied in analytical research [1–9]. One is the hollow fibre-supported liquid membrane (HF-SLM) technique which was introduced by Pedersen-Bjergaard and Rasmussen in 1999 to overcome the drop instability problem encountered in single-drop liquid-phase microextraction (SD-LPME) technique [4]. Hollow fibre-supported liquid-phase microextraction is a mode of LPME that uses a porous polypropylene hollow fibre for immobilisation of organic solvent in its pores. Extractions with SLM have several advantages over the traditional liquid-liquid extraction (LLE). Some of these include the usage of very small volumes (μL) of organic solvents for the preparation of the membrane and the ability to extract large sample volumes (up to 1 L). The use of very small volumes of acceptor solution (5–25 μL) makes it possible to enrich the analytes substantially in a convenient

way without the need for solvent evaporation [3]. Furthermore, in the three-phase mode, analytes are extracted from the aqueous sample through a water-immiscible organic solvent immobilised in the pores of the hollow fibre into another aqueous phase present inside the lumen of the hollow fibre. The hollow fibre could serve as a barrier, and the extraction solvent (accepter phase) could not contact directly with the sample solution. Moreover, the hollow fibre could also act as a barrier and prevent interference caused by macromolecules or suspended solid particles in the sample solution. Thus, hollow fibre-supported liquid membrane microextraction is a more attractive and reliable sample preparation technique. In addition, the technique uses simple setups and materials which are inexpensive. The extraction efficiency of HF-SLM is governed by the partitioning of the analyte between the sample matrix and the immobilised solvent and further by the partition between the acceptor phase and the immobilised solvent [10–13]. Since the organic phase is protected by the hollow fibre, the stability is greatly improved, and higher stirring rates can be used to easily achieve equilibrium and reduce the extraction time. As a result, better extraction efficiency and sensitivity are achieved [12, 13]. The focus in most of the reported literature has mainly been on hydrophobic analytes because these analytes are easily extracted from aqueous sample solutions into the organic solvent. In addition, hydrophobic ionic analytes have large solubility differences in acidic and basic aqueous solutions and are extracted well into the aqueous acceptor phase. However, the extraction of hydrophilic analytes presents a challenge, due to the low solubility of polar (hydrophilic) analytes in organic solvents, and the small differences in their solubility in acidic and basic aqueous media. The extraction of hydrophilic analytes is commonly facilitated either by using a carrier or by modifiers [10] and further promoted by creating a pH gradient on both sides of the SLM [10, 11, 14]. The pH value in the sample solution should be appropriate to suppress analyte ionisation, while the pH in the acceptor solution should be adjusted to ensure total ionisation of the analytes. Extraction is further enhanced by strong agitation of the extraction system to reduce the stagnant boundary layer near the SLM and to induce convection in the sample. After extraction, the aqueous acceptor solution can be injected directly into the analysis system, e.g., high-performance liquid chromatography (HPLC), liquid chromatography mass spectrometry (LC-MS), or capillary electrophoresis (CE) [3, 4]. Furthermore, the addition of salt can also promote the extraction by decreasing the solubility of analytes in water and enhancing the partitioning of the analytes into the organic phase. The sensitivity of the method can be increased by decreasing the volume ratio of the acceptor to donor phase. However, the volume of the acceptor solution used for extraction must be adjusted depending on the coupled analytical technique. Thus, sample volumes in the 10–25 μL range are preferable, as volumes within this range can easily be injected into an HPLC instrument, so that the entire acceptor phase may be analysed, resulting in lower detection limits [1, 2].

This well-established SLM-based sample preparation technique has generally shown excellent performance to extract different types of residues from water and in a few aqueous extracts of food samples, mainly for the extraction of hydrophobic compounds or for a small number (less than eight) of polar compounds [1, 6, 10, 14]. However, the method is rarely applied for compounds having wider polarity ranges and complex matrices, with only a few applications being reported in the literature for biological samples, such as that reported by Tajabadi et al. on antibiotics in lamb and chicken (muscle and liver), fish, honey, and milk [15] and the application reported by Msagati and Nindi on 16 SAs in milk, liver, kidney, and urine [16]. Therefore, it is desirable to extend their application to complex matrices such as chicken eggs and for a large number of compounds having a wide range of polarities such as sulfonamides.

The use of membrane-based techniques for the determination of sulfonamides has been previously reported by Msagati and Nindi [16] using a porous polytetrafluoroethylene (PTFE) membrane (Millex®-FG Syringe Filter) which was impregnated with 5% (w/v) tri-n-octyl phosphine oxide (TOPO) dissolved in hexylamine. The method was developed and applied for the determination of 16 sulfonamide residues in water, urine, milk, bovine liver, kidney, and tissue samples. Tao et al. [17] used an ionic liquid 1-octyl-3-methylimidazolium hexafluorophosphate ([C8MIMI]) as a liquid membrane and 14% (w/v) TOPO as an additive in the three-phase HF-LPME configuration using a Q3/2 Accurel KM polypropylene hollow fibre for the determination of five sulfonamide residues in environmental water samples. Ramos Payan et al. [18] also used a similar hollow fibre for the determination of four sulfonamides and their main metabolites in environmental water samples. Shariati et al. [10] reported Aliquat 336 as a carrier for the determination of tetracycline in bovine milk, human plasma, and water samples. In all abovementioned cases, the technique showed excellent analytical performance (higher enrichment factors and excellent clean-up capability). Furthermore, several applications of hollow fibre techniques are reviewed by Bello-Lopez et al. [19]. However, to the best of our knowledge, the determination of SAs in chicken egg samples by HF-SLM coupled with HPLC-DAD has not been reported.

Thus, here we report the development and validation of a sample preparation method based on the three-phase HF-SLM technique, which is combined with high-performance liquid chromatography (HPLC) coupled with diode array detection (DAD) for the simultaneous determination of fifteen sulfonamide residues in egg samples.

2. Experimental

2.1. Standards and Chemicals. Table 1 shows the structures, K_{OW} values, and pKa values of the sulfonamides used in this study. Antibiotic standards included sulfaguanidine (SGD), sulfanilamide (SAM), sulfathiazole (STZ), sulfacetamide (SAA), sulfamethizole (SMT), sulfamethoxazole (SMX), sulfasalazine (SSA), sulfamonomethoxine (SMM), sulfaquinoxaline (SQX), sulfamerazine (SMR), sulfapyridine (SPY), sulfadiazine (SDZ), sulfabenzamide (SBZ),

Table 1: Structures, K_{OW}, and pKa values of studied sulfonamides.

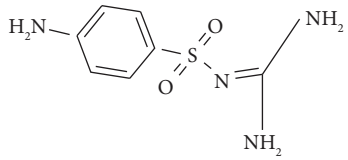
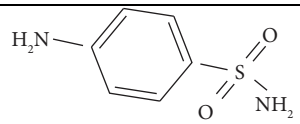
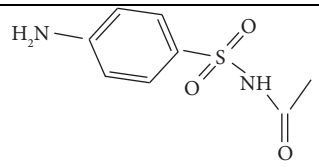
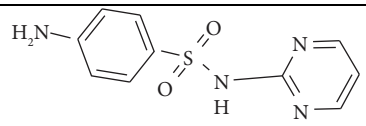
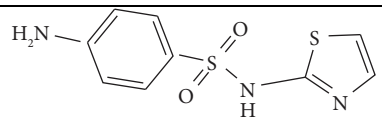
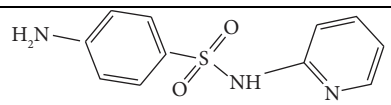
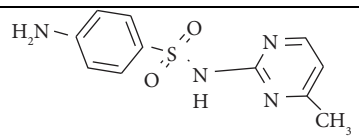
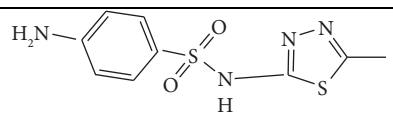
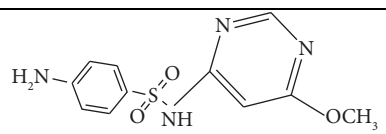
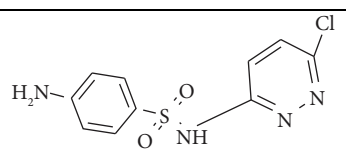
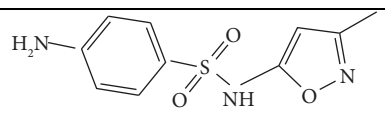
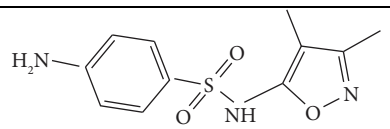
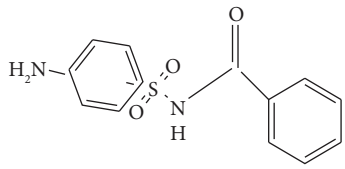
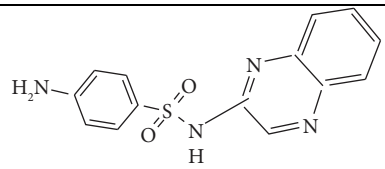
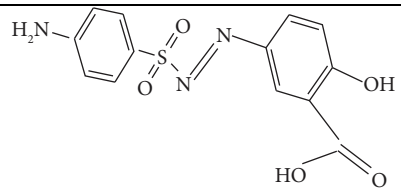
Compound	Structures	CAS no.	K_{OW}	pKa values	Reference
SGD		57-67-0	−1.07	0.5 ± 0.1; 0.4 ± 0.1; 3.3 ± 0.4	[16]
SAM		63-74-1	−0.67	2.4; 10.4	[20]
SAA		144-80-9	0.11	1.3 ± 0.1; 5.6 ± 0.5	[16]
SDZ		68-35-9	0.81	1.6 ± 0.1; 6.8 ± 0.50; 0.35 ± 0.2	[16]
STZ		72-14-0	0.72	0.7 ± 0.1; 7.8 ± 0.5; 2.3 ± 0.5	[16]
SPY		444-83-2	1.07	0.8 ± 0.1; 8.0 ± 0.5; 2.90 ± 0.5	[16]
SMR		127-97-7	0.14	1.6 ± 0.1; 6.9 ± 0.5; 0.4 ± 0.1	[16]
SMT		144-82-1	0.53	1.2 ± 0.1; 7.0 ± 0.5	[16]
SMM		1220-83-3	0.89	0.8 ± 0.1; 6.7 ± 0.5; 2.9 ± 0.4	[16]
SCP		80-32-0	2.04	1.80; 5.70	[16]
SMX		723-46-6	0.89	1.4 ± 0.1; 7.7 ± 0.5	[16]
SSO		127-69-5	1.01	1.50; 5.00	[21]

Table 1: Continued.

Compound	Structures	CAS no.	K_{OW}	pKa values	Reference
SBZ		127-71-9 -	—	1.1 ± 0.1; 5.90 ± 0.5	[16]
SQZ		59-40-5	1.69	−1.4 ± 0.3; 1.2 ± 0.1; 7.6 ± 0.3	[16]
SSA		599-79-1	3.8	1.9 ± 02; 2.9 ± 0.1; 1.2 ± 0.2; 7 ± 0.5	[16]

sulfachloropyridazine (SCP), and sulfisoxazole (SSO); all were purchased from Sigma-Aldrich (Steinheim, Germany). All chemicals and reagents used in this study were of analytical grade, the solvents were of HPLC grade, and the standards had purities higher than 98%. Methanol, acetone, formic acid, di-n-hexyl ether, n-undecane, tri-n-octyl phosphine oxide (TOPO), 1-octanol, and triethylamine were obtained from Sigma-Aldrich (Steinheim, Germany). Acetonitrile (MeCN) was purchased from ROMIL Ltd (Waterbeach, Cambridge, UK), while 1-undecanol and sodium hydroxide were supplied by Merck (Darmstadt, Germany). Ultrapure water (UHP) (resistivity, 18.2 MΩ cm at 25°C) was generated using a Milli-Q® System (Millipore, Billerica, MA, USA).

Hollow fibre PPQ 3/2 Accurel KM polypropylene (600 μm inner diameter, 200 μm wall thickness, and 0.2 μm average pore size) was purchased from Membrana GmbH (Wuppertal, Germany).

2.2. Instrumentation. An Agilent 1260 series high-performance liquid chromatography (HPLC) system (Agilent Technologies, Waldbronn, Germany) was used for the chromatographic separations. The HPLC consisted of a binary pump, vacuum degasser, thermostatic column compartment, auto-sampler, and diode array detector (DAD). Data acquisition was achieved using the Agilent Chem Station (version 1.9.0) software. Chromatographic separations were carried out using a ZORBAX Eclipse Plus C18 column (100 mm × 4.6 mm, 3.5 μm) from Agilent Technologies, Inc. (Santa Clara, California, USA). A vortex mixer obtained from VELP Scientifica (Usmate Velate (MB), Italy) and a centrifuge from Thermo Electron Corporation (Massachusetts, USA) were used for sample preparation. Nitrogen gas was used for drying the samples.

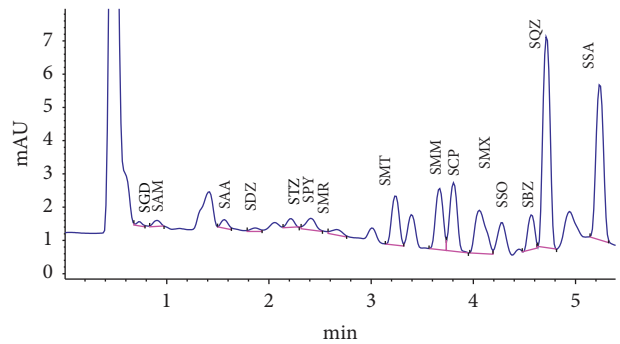


Figure 1: Chromatogram of blank egg samples spiked with 100 μg kg^{-1} SAs mixture. Chromatographic conditions: flow rate of 1.8 mL·min^{-1}, column temperature of 40°C, injection volume of 5 μL, and wavelength of 265 nm. A binary mobile phase comprising solvent (a) (0.1% FA water) and solvent B (acetonitrile) with a gradient elution mode of 10% B (0-1 min), 40% (b) (1–4 min), and 60% B (4–6 min).

2.3. Sampling of Chicken Eggs. Cartons of half a dozen eggs of different brands were purchased from local supermarkets in Gauteng Province (South Africa). Other 25 blank egg samples were collected from noncommercial small-scale organic farmers. Thus, these eggs were from chickens which were not treated with antibiotics. To confirm this, blank samples were initially screened for antibiotics prior to use in this study. Optimisation studies were done using blank egg samples spiked with 300 μg·kg^{-1} of the mixture of 15 SAs. For method validation, a mixture of 15 sulfonamides was spiked into the blank egg samples at a concentration range of 5–1000 μg·kg^{-1} in order to construct matrix-matched calibration curves.

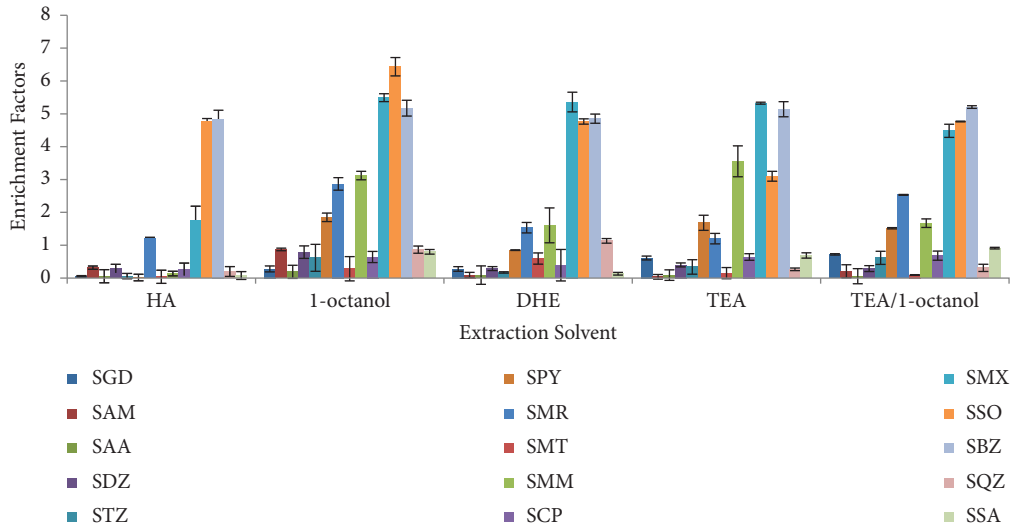


FIGURE 2: Evaluation of the organic solvent. Extraction conditions: 300 $\mu g \cdot kg^{-1}$ SAs mixture spiked in blank egg samples; hollow fibre length (4 cm); 5 mL of aqueous sample solution (pH 4); 6 μL of aqueous acceptor phase (pH 12); HA, 1-octanol, DHE, TEA, and a mixture of TEA and 1-octanol (50 : 50; v/v) as an SLM; stirring rate: 400 rpm; extraction time: 60 min; and $n = 5$.

2.4. Preparation of Sulfonamide Standard Solutions. Stock standard solutions (1000 $mg \cdot L^{-1}$) for each compound were prepared by accurately weighing out 10 mg of each standard in a 10 mL volumetric flask and dissolving the mixture to the mark with MeOH-UHP water (1 : 1; v/v). The working standards were prepared by diluting appropriate volumes of these stock solutions with MeOH-UHP water (1 : 1 v/v). All standard solutions were protected from light by covering the volumetric flasks with aluminium foil and kept at 4°C until use.

2.5. Pretreatment Procedure for Analysis of Egg Samples. Both the yolk and albumin of the six eggs in each carton were combined and blended using a food blender (Sunbeam®, Canada). From each blend (treated separately), 5 g was weighed into a 50 mL screw cap centrifuge tube and treated with 5 mL of organic solvent {(0.05% aqueous formic acid): MeCN (15 : 85, v/v)}. The mixture was vortexed for 30 s for homogenisation to facilitate extraction. The homogenised solution was centrifuged for 10 min at 4000 rpm, the resultant supernatant (acetonitrile extract) was filtered through a 0.45 μm nylon filter, and then a 1 mL aliquot was dried under a stream of nitrogen gas. The residue was reconstituted using 5 mL of donor aqueous phase (pH 2.5) and used as the sample solution for the HF-SLM procedure. To facilitate the efficient extraction of these hydrophilic SAs into the hydrophobic organic membrane liquid, the pH of the donor phase should be adjusted to convert them into their neutral form. Accordingly, the pH of the donor phase was adjusted to pH 2.5 using a 0.1 M HCl solution, and the pH of the acceptor phase was adjusted to pH 13 with a 0.1 M NaOH solution.

2.6. Hollow Fibre-Supported Liquid Membrane (HF-SLM) Microextraction Procedure. For the extraction procedure, the porous HF was cut into 4 cm long pieces and then one end of each piece was sealed using heat. The heat-sealed fibres were cleaned with acetone in an ultrasonic bath and were air-dried for 15 min. The unsealed end of the fibre was connected to a needle of a Hamilton® 100 μL HPLC syringe (Hamilton Company, Reno, Nevada, USA) that was filled with basic (pH 13) acceptor solution for filling the lumen of the hollow fibre (HF) (16–20 μL). Thereafter, the membrane, still attached to the syringe, was dipped into 10% TOPO in 1-octanol for a few seconds (15–20 s) for the HF pores to become impregnated with the solvent. Any surplus organic solvent on the HF surface was removed by immersing the tube in ultrapure water and shaking it carefully for 30 s. The HF-SLM was then immersed in a 5 mL sample solution as described above, and the solution was stirred using a magnetic stirrer at a stirring speed of 600 rpm for 90 min. The fibre still attached to the syringe was then removed from the sample solution. The tip of the sealed end was carefully cut off, thus allowing the acceptor phase (16–20 μL) containing the extracted analyte to be collected into a 400 μL HPLC insertion vial which was then placed in a 5 mL HPLC vial. The extracted sample was then analysed using HPLC.

2.7. Chromatographic Conditions for the Separation of 15 Sulfonamide Compounds. The chromatographic separation method used consisted of a binary mobile phase comprising of solvent A (0.1% FA in water) and solvent B (acetonitrile). The separation was performed using a gradient elution mode of 10% B (0-1 min), gradually increased from 10%–40% B (from 1–4 min), and further increased from 40%–60% B (from 4–6 min). The mobile phase flow rate of 1.8 $mL \cdot min^{-1}$ and an injection volume of 5 μL were used. Similarly, the column oven temperature was maintained at 40°C. All target compounds were detected using a wavelength of 265 nm (Supplemental Figure S1). A chromatogram of blank egg samples spiked with 100 $\mu g \cdot kg^{-1}$ SAs mixture is shown in Figure 1.

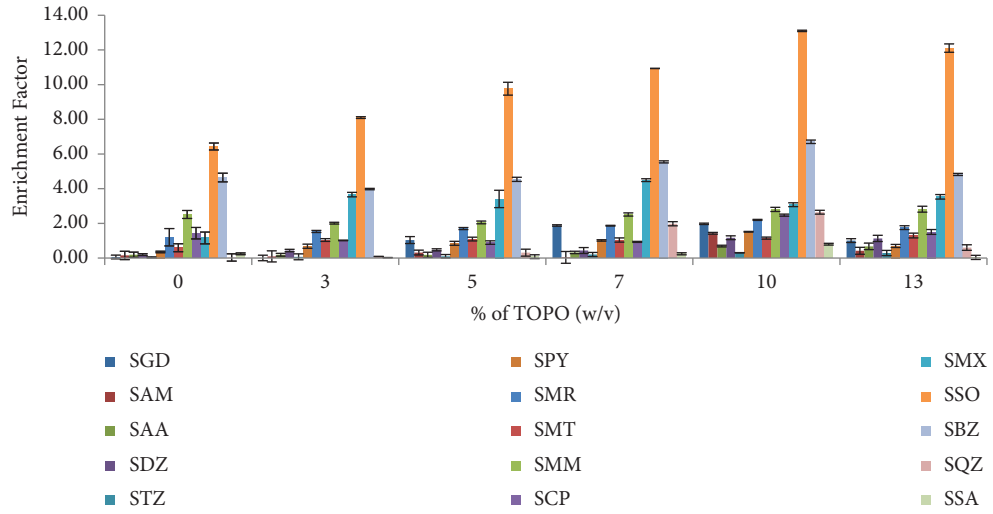


Figure 3: Evaluation of modifier. Extraction conditions: 300 $\mu g \cdot kg^{-1}$ SAs mixture spiked in blank egg samples; hollow fibre length (4 cm); 5 mL of aqueous sample solution (pH 4); 6 μL of aqueous acceptor phase (pH 12); 0–13% TOPO in 1-octanol as an SLM; stirring rate: 400 rpm; extraction time: 60 min; and $n = 5$.

3. Results and Discussion

3.1. Optimisation of Hollow Fibre-Supported Liquid Membrane (HF-SLM) Procedure for Egg Samples. To obtain the best analytical performance, the influence of different experimental parameters, including the extraction solvent, sample pH, length of hollow fibre (acceptor phase volume), sample volume, the salting-out effect, stirring rate, and extraction time on the performance of the method, was optimised via one parameter at a time.

3.1.1. Evaluation of the Organic Solvent Used as a Liquid Membrane in HF-SLM Method. The nature of the organic solvent used as a liquid membrane plays an important role in HF-SLM. The solvent should be impregnated in the pores of the fibre; thus, the polarity of the organic solvent should be very close to the polarity of the polypropylene fibre. It should have a low solubility in water to prevent dissolution into the aqueous phase, and a low volatility, which will restrict solvent evaporation during extraction. Furthermore, it is important that an organic solvent must dissolve the target analytes and have a low viscosity to increase mass transfer through the membrane. On the basis of these considerations and by taking into account the previously reported literature [10, 16], 1-octanol (1-Oct), hexylamine (HA), dihexyl ether (DHE), 1-undecanol, undecane, and triethylamine (TEA) were evaluated as potential candidates for the organic membrane solvent. Higher enrichment factors were obtained for most sulfonamides when 1-Oct, DHE, and a mixture of TEA/1-Oct (1 : 1 v/v) were used (shown in Figure 2). A significant improvement was observed in the enrichment factors of six (SGD, SPY, SMR, SMX, SSO, and SQZ) and three (SMR, SSO, and SQZ) analytes (at $p = 0.05$ significance level) in 1-Oct, than in HA and TEA, respectively. However, the enrichment factors (EFs) of all studied analytes obtained using 1-Oct, DHE, and TEA/1-Oct were not significantly different at $p = 0.05$ level. Therefore, to select the best performing solvent, the enrichment factors of each analyte obtained using each solvent (1-Oct, DHE, and a mixture of TEA/1-Oct) were directly compared. The enrichment factors for ten analytes were higher in 1-Oct than in DHE, and similarly, the enrichment factors for eleven analytes were better in 1-Oct than in TEA/1-Oct. Therefore, 1-octanol was selected as the extraction solvent for the subsequent experiments. However, most of the hydrophilic compounds (SGD, SAA, and SMT) were not sufficiently enriched even with 1-octanol. This could be attributed to their lower K_{OW} values. Therefore, their extraction could be improved by using a modifier or a carrier. On the other hand, some of the relatively nonpolar SAs (SMX, SSO, and SBZ) were extracted well. This could be due to the nonpolar nature of the analytes which enhances their extraction in nonpolar organic solvents.

3.1.2. Evaluation of Modifiers for the HF-SLM Method. Most sulfonamides are hydrophilic drugs ($K_{OW} < 1$) with high solubility in aqueous media. Their direct extraction by passive diffusion from the donor phase through the organic membrane immobilised in the fibre pores and further into the acceptor phase would be very difficult. Tri-n-octyl phosphine oxide (TOPO) is one of the commonly used additives (carriers) to facilitate or enhance such transfer. In general, TOPO is dissolved in organic solvents in liquid membranes, and it serves as an efficient extractant for polar analytes such as sulfonamides because of its ability to form a hydrogen bond complex with SAs [16]. Thus, in the current study, the effect of the addition of TOPO in 1-octanol was evaluated by varying its composition from 0 to 13% (w/v). There are two possible reasons for the known capability of TOPO to act as a carrier: (i) it increases the polarity of the organic phase due to the presence of polar bonds, and (ii) it forms complexes with unprotonated (neutral) analytes

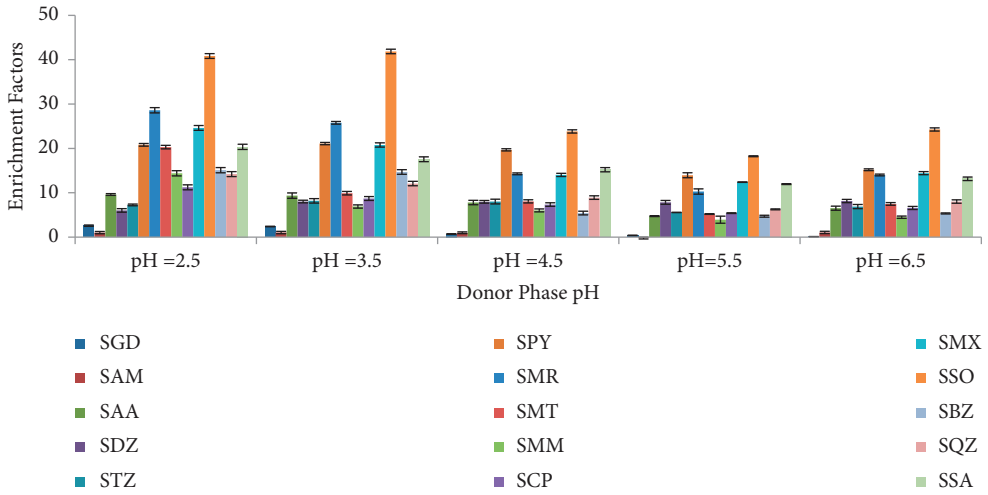


FIGURE 4: Evaluation of donor phase pH. Extraction conditions: 300 $\mu g \cdot kg^{-1}$ SAs mixture spiked in blank egg samples; hollow fibre length (4 cm); 5 mL of aqueous sample solution (pH 2.5–6.5); 6 μL of aqueous acceptor phase (pH 12); 10% TOPO in 1-octanol as an SLM; stirring rate: 400 rpm; extraction time: 60 min; and $n = 5$.

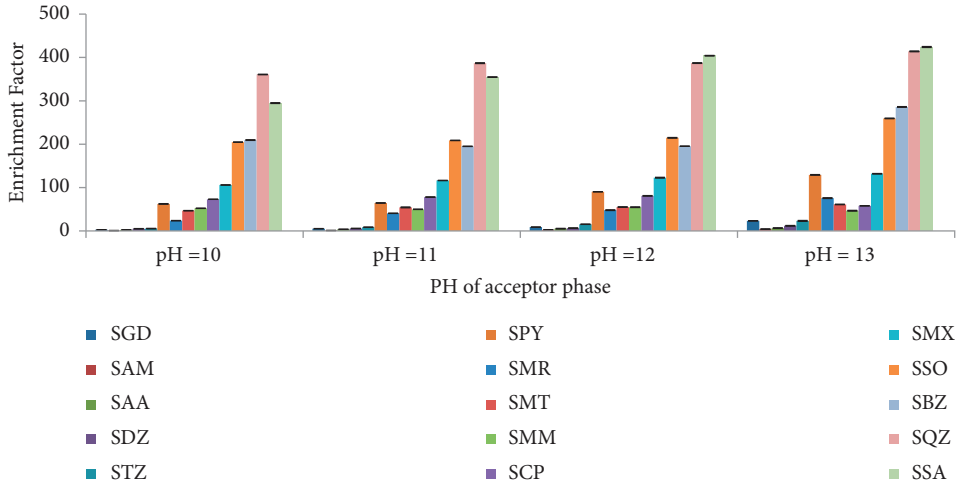


FIGURE 5: Evaluation of acceptor phase pH. Extraction conditions: 300 $\mu g \cdot kg^{-1}$ SAs mixtures spiked in blank egg samples; hollow fibre length (4 cm); 5 mL of aqueous sample solution (pH 2.5); 6 μL of aqueous acceptor phase (pH 10–13); 10% TOPO in 1-octanol as an SLM; stirring rate: 400 rpm; extraction time: 60 min; and $n = 5$.

through H-bonding, i.e., the acidic hydrogen of non-protonated analytes and phosphoryl oxygen atom of TOPO [16]. Figure 3 demonstrates that the enrichment factors (EFs) of all sulfonamides increased with increasing TOPO content and reached a maximum at 10% (w/v) TOPO in 1-octanol. A further increase in the TOPO content resulted in lower enrichment factors. The viscosity of the organic solvent at a higher concentration of TOPO increased, and the flux of drugs from the donor phase to the acceptor phase was reduced, thus resulting in lower enrichment factors. Likewise, Tao et al. found that the enrichment factors of SAs doubled when incorporating 14% TOPO in $[C_8MIM][PF_6]$, compared to those when using the ionic liquid alone [17]. Moreover, Shariati et al. also found that the extraction of highly hydrophilic compounds such as tetracycline was improved significantly when using 1-Oct containing 10% (w/v) Aliquat-336 as a carrier [10]. Therefore, 10% (w/v) TOPO in 1-Oct was selected as the optimum for the subsequent experiments.

3.1.3. Evaluation of Donor Phase pH. The pH of the donor and acceptor solutions should be carefully selected based on the nature of the target analyte. Sulfonamides are known as amphoteric molecules with both weakly acidic and basic properties. Thus, for the effective transfer of the target analytes into the organic phase, the pH of the donor solution should be adjusted in such a way that the analyte of interest is neutral or in the uncharged form. Therefore, the solution pH should be adjusted to a pH that suppresses the ionisation of the analyte. For this reason, the donor phase pH was evaluated in the pH range of 2.5–6.5. Figure 4 shows that higher enrichment factors for most analytes were observed at pH 2.5 and 3.5, probably because most of these analytes

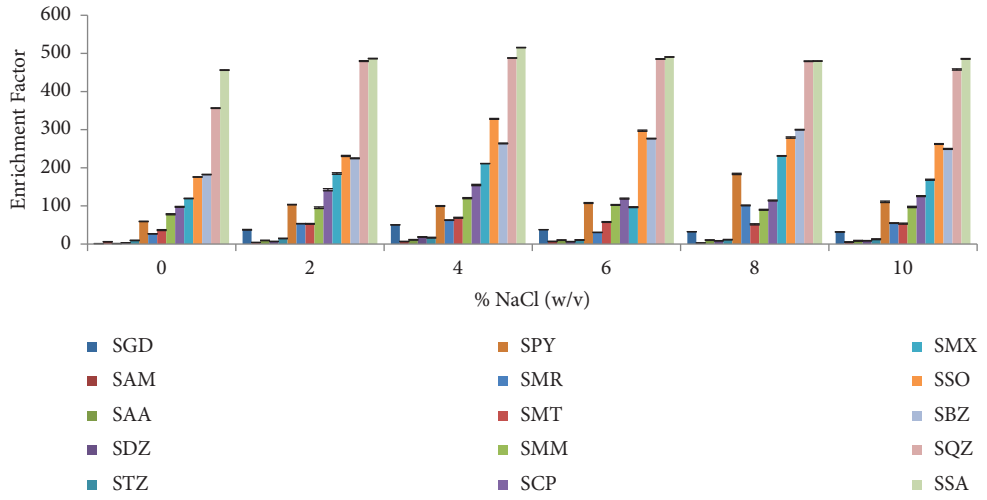


Figure 6: Effect of salt addition. Extraction conditions: 300 μg·kg^{-1} SAs mixture spiked in blank egg samples; hollow fibre length (4 cm); 5 mL of aqueous sample solution (pH 2.5); 6 μL of aqueous acceptor phase (pH 13); 10% TOPO in 1-octanol as an SLM; NaCl (0–10%); stirring rate: 400 rpm; extraction time: 60 min; and $n = 5$.

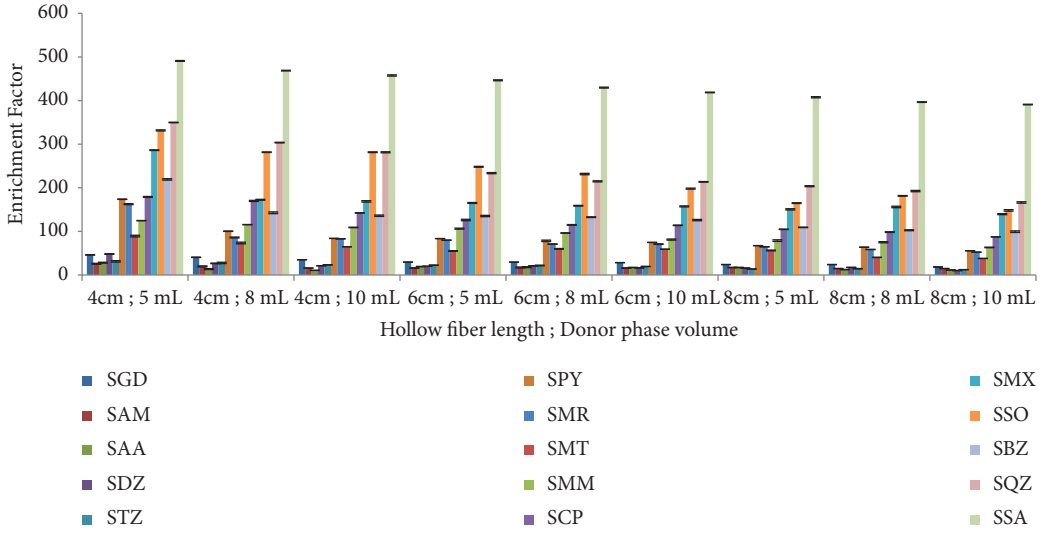


Figure 7: Length of hollow fibre (acceptor volume) and volume of sample solution. Extraction conditions: 300 μg·kg^{-1} SAs mixture spiked in blank egg samples; hollow fibre length (4–8 cm); volume of aqueous sample solution (pH 2.5) from 5–10 mL; 6–12 μL of aqueous acceptor phase at pH 13; 10% TOPO in 1-octanol as an SLM; NaCl (4%); stirring rate: 400 rpm; extraction time: 60 min; and $n = 5$.

were predominantly neutral under these conditions. The enrichment factors obtained for five of the target analytes (SSA, SMX, SMM, SMT, and SMR) were significantly higher at pH 2.5 (at $p = 0.05$ significance level); only SDZ extracted favourably at pH 3.5, while the rest of the analytes did not show any difference between the two conditions. Therefore, based on the current experimental results, pH 2.5 was selected as the optimum donor phase pH for subsequent experiments.

3.1.4. Evaluation of Acceptor Phase pH. The acceptor phase should induce ionisation of target compounds to prevent back-extraction into the organic phase. For this reason, the acceptor phase pH was evaluated in the range of 10–13. Figure 5 shows that the enrichment factors obtained for most of the target analytes were significantly higher (at $p = 0.05$ level) at pH 13 except for SCP and SMM which had significantly higher values at pH 12. Therefore, pH 13 was selected as the optimum pH for the acceptor solution in the subsequent experiments. Tao et al. also reported that pH 13 was appropriate to sufficiently trap five sulfonamide compounds in the acceptor phase [17].

3.1.5. Effect of Salt Addition. The salting-out effect is widely used to increase the extraction of polar target compounds by decreasing their solubility in the aqueous phase or enhancing their partitioning into the organic phase [22]. In this work, the effects of different concentrations of NaCl in the

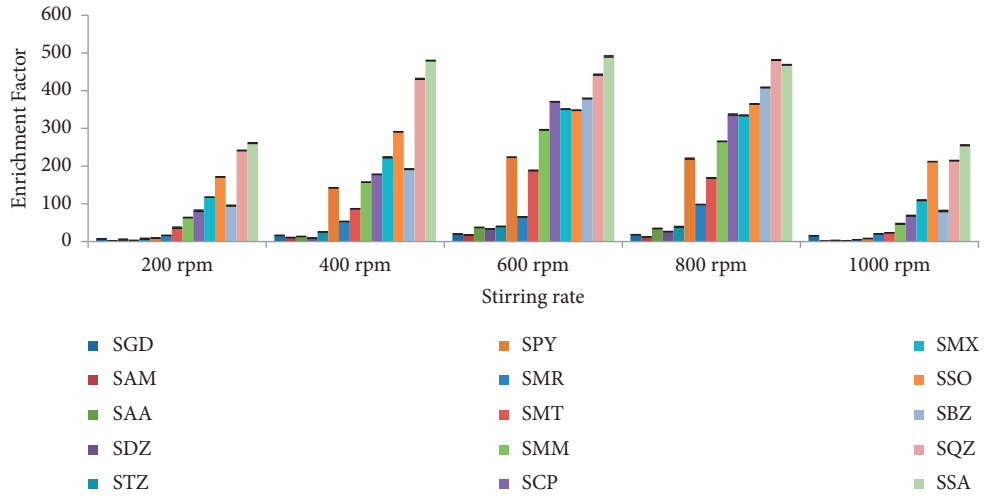


Figure 8: Effect of the stirring rate. Extraction conditions: 300 $\mu g \cdot kg^{-1}$ SAs mixtures spiked in blank egg samples; hollow fibre length (4 cm); 5 mL of aqueous sample solution (pH 2.5); 6 μL of aqueous acceptor phase at pH 13; 10% TOPO in 1-octanol as an SLM; NaCl (4%, w/v); stirring rate: 200–1000 rpm; extraction time: 60 min; and $n = 5$.

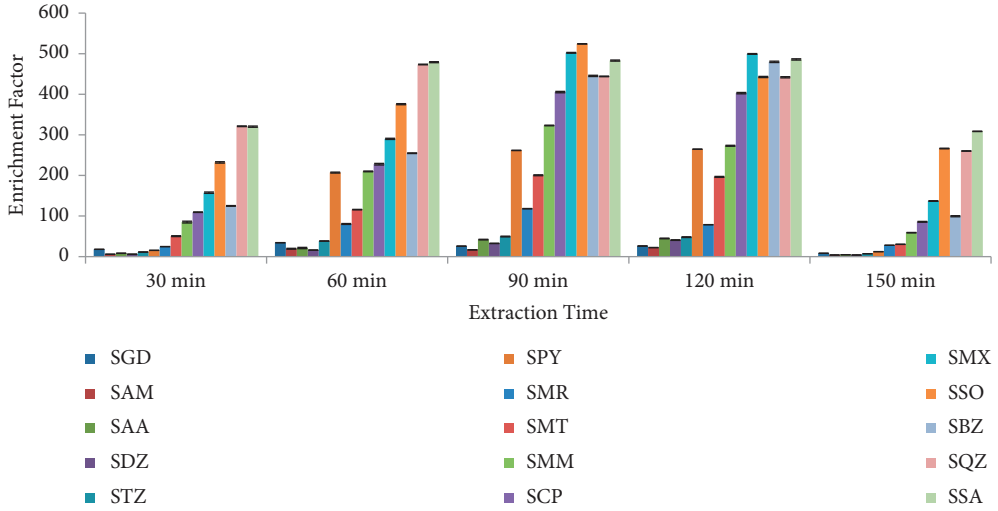


Figure 9: Effect of the extraction time. Extraction conditions: 300 $\mu g \cdot kg^{-1}$ SAs mixture spiked in blank egg samples; hollow fibre length (4 cm); 5 mL of aqueous sample solution (pH 2.5); 6 μL of aqueous acceptor phase at pH 13; 1-octanol in (10% TOPO) as an SLM; NaCl (4%); stirring rate: 600 rpm; extraction time: 30–150 min; and $n = 5$.

range of 0–10% (w/v) were evaluated. Results in Figure 6 show that the enrichment factors increased with increase in the concentration of NaCl from 0 to 4% (w/v) and remained constant from 4%–6%. A further increase in the concentration of NaCl resulted in a decrease in the enrichment factors due to the increased viscosity of the aqueous solution that could make the mass transfer difficult. The mass transfer is possibly affected by the electrostatic interaction between the analytes and the salt molecules. Therefore, 4% NaCl was selected as the optimum concentration of the salt for the subsequent experiments.

3.1.6. Length of Hollow Fibre (Acceptor Volume) and Volume of Sample Solution. Fibre length is an important factor as it provides the surface area to promote the transport of analytes to the extraction solvent and subsequently improving enrichment factors. It is clear that EFs are closely related to the volume ratio of donor to acceptor phase. To some extent, the preconcentration factor is improved upon increasing the phase ratio between the sample volume and the acceptor phase volume. In the present study, the effects of sample volume and the acceptor phase volume on the enrichment factors of target analytes were examined by varying the sample volume in the range of 5–10 mL and the length of hollow fibre (acceptor volume) in the range of 4–8 cm. Figure 7 shows that the highest enrichment factors (25.8–490.8) were obtained when the volume of the sample was 5 mL and the corresponding hollow fibre length was 4 cm. When hollow fibre lengths of 6 and 8 cm (with the corresponding acceptor phase volume of 6–10 μL, respectively) were used, it was mechanically difficult to perform the

Table 2: Linearity, LOD, and LOQ values obtained for 15 SAs.

Compound	Regression equation ($n = 6$)	Correlation coefficient R^2	Linear range ($\mu g\ kg^{-1}$)	LOD ($\mu g\ kg^{-1}$) ($n = 10$)	LOQ ($\mu g\ kg^{-1}$) ($n = 10$)
SGD	$y = 0.001x + 0.070$	0.9974	9–700	4.0	12.0
SAM	$y = 0.008x + 0.254$	0.9982	7–1000	0.8	7.0
SAA	$y = 0.003x + 0.012$	0.9993	7–1000	4.9	14.7
SDZ	$y = 0.001x + 0.067$	0.9987	7–1000	3.1	9.3
STZ	$y = 0.004x + 0.285$	0.9987	7–700	1.9	5.7
SPY	$y = 0.003x + 0.194$	0.9991	7–700	2.9	8.7
SMR	$y = 0.001x + 0.092$	0.9978	7–1000	3.1	9.3
SMT	$y = 0.003x + 0.074$	0.9981	7–1000	2.8	8.4
SMM	$y = 0.001x + 0.110$	0.9983	8–700	3.9	11.7
SCP	$y = 0.004x + 0.182$	0.9962	5–1000	2.2	6.6
SMX	$y = 0.018x + 0.564$	0.9969	5–700	2.1	6.3
SSO	$y = 0.002x + 0.341$	0.9975	8–700	3.0	9.0
SBZ	$y = 0.002x + 0.032$	0.9986	6–1000	2.7	8.1
SQZ	$y = 0.016x + 0.079$	0.9985	5–1000	0.8	5.0
SSA	$y = 0.016x + 0.020$	0.9984	9–1000	7.0	21.0

Table 3: Intra-day and inter-day precision results for 15 SAs in blank egg samples spiked at 50 $\mu g \cdot kg^{-1}$, 100 $\mu g \cdot kg^{-1}$, and 500 $\mu g \cdot kg^{-1}$ levels.

Compound	Intra-day (%RSD) ($n = 6$)			Inter-day (%RSD) ($n = 6$)		
	50 ($\mu g\ kg^{-1}$)	100 ($\mu g\ kg^{-1}$)	500 ($\mu g\ kg^{-1}$)	50 ($\mu g\ kg^{-1}$)	100 ($\mu g\ kg^{-1}$)	500 ($\mu g\ kg^{-1}$)
SGD	15.7	14.4	12.0	17.4	15.7	14.7
SAM	14.5	11.1	13.0	15.1	13.8	14.8
SAA	15.2	11.4	14.6	14.7	16.2	14.6
SDZ	17.0	12.9	13.9	9.6	12.0	14.8
STZ	6.4	7.3	9.2	13.1	13.6	11.7
SPY	12.0	14.3	9.2	15.7	15.9	13.3
SMR	8.1	10.8	11.9	16.4	11.3	16.5
SMT	9.7	11.8	8.6	9.2	14.6	11.2
SMM	12.3	14.2	7.9	15.3	15.2	15.7
SCP	8.6	8.6	9.6	9.0	10.3	8.3
SMX	6.9	15.0	9.9	7.5	11.6	11.5
SSO	7.1	10.2	10.8	7.7	10.8	11.1
SBZ	13.2	11.9	8.1	14.0	11.2	14.2
SQZ	9.4	7.9	7.3	9.0	10.9	11.8
SSA	6.2	8.7	12.4	6.9	4.3	9.2

extraction because the agitation made side of the fibre connected to the needle tip unstable (detached). For the case of 4 cm hollow fibre length with 8 mL and 10 mL sample volumes, lower enrichment factors in the range of 13.5–468.6 and 10.6–457.5, respectively, were observed due to a reduction in the efficiency of convection. Thus, a hollow fibre length of 4 cm and a sample volume of 5 mL were selected as the optimum conditions for fibre length and sample volume in the subsequent steps.

3.2. Effect of the Stirring Rate. Stirring the sample solution can increase the mass transfer and diffusion of the analyte from the donor phase into the acceptor phase, therefore reducing the time required to achieve thermodynamic equilibrium. On the other hand, a high stirring speed may cause the instability of organic solvent in hollow fibre pores and generate air bubbles around the fibre, which can promote solvent evaporation. Thus, the stirring speed was evaluated in the range of 200 to 1000 rpm. The results shown in Figure 8 revealed that the highest enrichment factors were obtained at a stirring rate of 600 rpm. A decrease in the extraction efficiency was observed at higher stirring rates because air bubbles were generated, which resulted in a loss of solvent, thereby lowering the enrichment factors. Therefore, 600 rpm was selected as the optimum stirring speed for subsequent experiments.

3.3. Effect of the Extraction Time. To study the effect of extraction time on the enrichment factors of target analytes, different extraction times in the range of 30–150 min at 30 min intervals were evaluated. Figure 9 shows that the enrichment factors increased with an increase in the extraction time up to 90 min. However, a further increase in the extraction time to above 90 min resulted in low enrichment factors. This may be attributed to the destabilisation of the liquid membrane at a higher stirring rate for a longer period. Therefore, 90 min was selected as the optimal extraction time for the subsequent experiments.

Table 4: Recovery results obtained for 15 SAs in blank egg sample spiked at 50 $\mu g \cdot kg^{-1}$, 100 $\mu g \cdot kg^{-1}$, and 500 $\mu g \cdot kg^{-1}$ levels.

Compound	Recovery ($n = 6$)					
	50 ($\mu g \cdot kg^{-1}$)	%RSD	100 ($\mu g \cdot kg^{-1}$)	%RSD	500 ($\mu g \cdot kg^{-1}$)	%RSD
SGD	96.0	9.3	88.0	8.3	90.8	8.3
SAM	96.5	8.6	74.0	9.9	97.2	6.1
SAA	71.3	9.5	88.1	9.5	87.5	5.4
SDZ	86.0	9.1	71.0	7.9	75.1	6.5
STZ	95.5	9.1	98.4	6.1	71.2	4.4
SPY	98.7	8.6	94.0	5.3	96.1	3.4
SMR	93.6	5.4	86.0	4.7	86.0	4.8
SMT	88.0	4.3	84.0	8.0	81.9	8.1
SMM	88.0	9.8	94.4	3.0	97.2	1.9
SCP	90.8	5.5	95.2	7.3	78.9	4.2
SMX	80.1	4.6	89.4	9.2	76.7	3.1
SBZ	71.0	2.0	81.5	7.1	96.5	2.5
SSO	89.0	4.4	94.3	8.5	75.6	2.4
SQZ	92.0	9.5	96.6	5.4	85.0	6.8
SSA	85.3	9.9	77.3	9.2	88.5	6.8

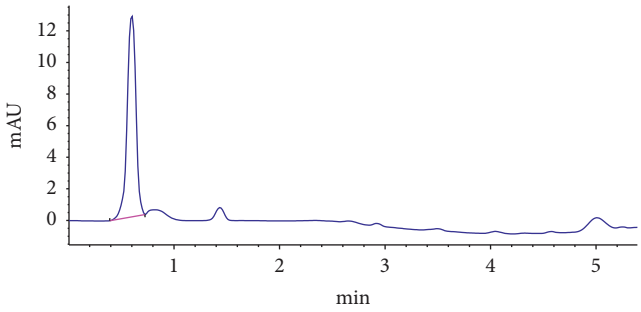


Figure 10: Chromatogram of a blank egg sample. Chromatographic conditions: flow rate of 1.8 mL min^{-1}, column temperature of 40°C, injection volume of 5 μL, and wavelength of 265 nm. A binary mobile phase comprising solvent (a) (0.1% FA water) and solvent (b) (acetonitrile) with a gradient elution mode of 10% B (0-1 min), 40% B (1–4 min), and 60% B (4–6 min).

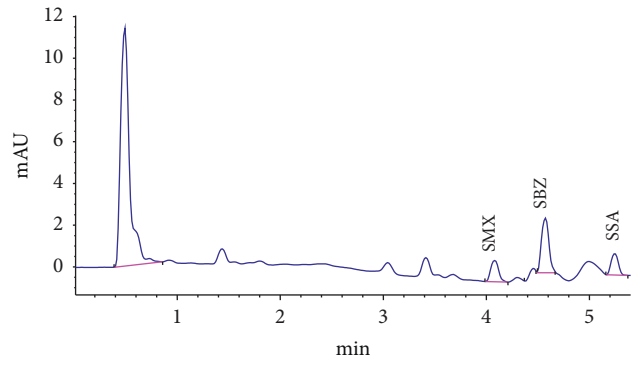


Figure 11: Chromatogram of a real egg sample. Chromatographic conditions: flow rate of 1.8 mL·min^{-1}, column temperature of 40°C, injection volume of 5 μL, and wavelength of 265 nm. (a) Binary mobile phase comprising solvent A (0.1% FA water) and solvent (b) (acetonitrile) with a gradient elution mode of 10% B (0-1 min), 40% B (1–4 min), and 60% B (4–6 min).

Under the optimised conditions, the enrichment factors for most polar analytes (SGD, SAM, SAA, SDZ, and STZ) were found to range from 16.74–49.51, while the EFs of polar analytes were in the range of 118.08–524.30. The most probable reason for the lower enrichment factors for most of the polar analytes is due to their hydrophilic nature which makes them difficult to extract in a nonpolar solvent. Furthermore, it was a challenge to establish an appropriate pH value in which compounds such as SGD, SDZ, and STZ with three pKa values existed as neutral compounds. However, in the current study, compounds such as SDZ, STZ, SPY, SQZ, and SBZ were well separated and extracted in 10% TOPO in 1-octanol with EFs in the range of 32.63–445.30 which failed to be extracted in 5% TOPO in hexylamine, as reported by Msagati and Nindi [16]. Therefore, the proposed method is attractive and would be an excellent alternative method for monitoring SAs in complex matrices.

4. HF-SLM Method Validation for Egg Samples

Under the optimised conditions, the performance of the proposed method was evaluated by investigating parameters such as linearity and range, limit of detection (LOD), limit of quantification (LOQ), precision (intra-day and inter-day), and accuracy (percentage recovery). Linearity was evaluated by plotting a calibration curve using matrix-matched calibration standards prepared by spiking blank egg samples with sulfonamide mixture at concentrations in the range of 2 $\mu g \cdot kg^{-1}$ to 1000 $\mu g \cdot kg^{-1}$ (seven concentration levels with six replicates per level). Satisfactory linearity was obtained in the range of 5–1000 $\mu g \cdot kg^{-1}$ with coefficient of determination in the range of 0.9962–0.9993 (Table 2). The LOD and LOQ values were calculated based on 3 and 10 times the standard deviation of blank egg extract with a minimum analyte concentration of 0.7–1.0 $\mu g \cdot kg^{-1}$, respectively. The LOD values were in the range of 0.8–7.9 $\mu g \cdot kg^{-1}$, while LOQ values ranged from 2.4–21.0 $\mu g \cdot kg^{-1}$ for ten replicates. The intra-day and inter-day precisions were investigated by spiking the blank egg samples at three concentration levels (50 $\mu g \cdot kg^{-1}$, 100 $\mu g \cdot kg^{-1}$, and 500 $\mu g \cdot kg^{-1}$) with six replicates ($n = 6$). For the intra-day precision, the analysis was done three times (i.e., at 8-hour intervals) within a single day,

Table 5: Sulfonamide levels found in egg samples from supermarkets located in Gauteng Province (South Africa) ($n = 6$).

Sample	SGD	SAM	SAA	SDZ	STZ	SPY	SMR	SMT	SMM	SCP	SMX	%RSD	SSO	SBZ	%RSD	SQZ	SSA	%RSD
F	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	19.2[a]	7.4	ND	59.0[a]	7.3	ND	50.0[a]	9.2
G	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	—	ND	ND	—	ND	ND	—
H	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	16.4[a]	11.8	ND	56.0[a]	10.6	ND	33.0[a]	9.7
I	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	—	ND	ND	—	ND	ND	—
J	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	68.0[a]	9.6	ND	61.5[a]	12	ND	61.2[a]	12.6

F, G, H, I, and J are new batches of egg samples collected from five brands (supplier): a: $\mu g \cdot kg^{-1}$; ND: not detected.

Table 6: Comparison of the proposed HF-SLM method for egg samples with other HF-LPME methods reported in the literature.

Methods	Matrices	Compounds	Linear range	Recovery (%)	LOD	Ref
HF-LPME-UV	Bovine milk, human plasma, and water	3 TC	0.5-1000 $\mu g \cdot L^{-1}$	Not reported	0.5-1.0 $\mu g \cdot L^{-1}$	[10]
HF-SLM-MS	Urine, milk, and bovine liver	16 SAs	NR	>90%	3.3-10.0 (liver) $\mu g \cdot kg^{-1}$, 2.5-15.0 (urine) $\mu g \cdot L^{-1}$, 12.4-24.3 (milk) $\mu g \cdot L^{-1}$	[16]
HF-LPME-UV	Environmental water	5 SAs	1-2000 $ng \cdot L^{-1}$	82.2–103.2	0.1-0.4 $\mu g \cdot L^{-1}$	[19]
HF-LPME-GC	Sausage, corned meat, fresh meat, and salted eggs	2 carcinogenic nitrosamines	5000-25000 $\mu g \cdot kg^{-1}$	100.0 and 1010	330.0 and 20.0 $\mu g \cdot L^{-1}$	[25]
HF-LPME-DAD/FL	Bovine urine and water	8 FQ	0.02-1 000 $\mu g \cdot L^{-1}$	76.0–100.5	0.0003-0.016 $\mu g \cdot L^{-1}$	[26]
VA–HF–LPME-DAD	Eggs	2 carotenoids	50-1000 $\mu g \cdot kg^{-1}$	90.8–101.8	0.038 and 0.045 $\mu g \cdot kg^{-1}$	[27]
HF-LPME-DAD/FLD	Human urine	3 nonsteroidal anti-inflammatory drugs	41-10000 $\mu g \cdot L^{-1}$	82.3–99.0	12.53-40.00 $\mu g \cdot kg^{-1}$	[28]
SPE-LC-MS/MS	Eggs	10 SAs	NR	99.8–100.4	<20.5 (CC $\propto$)	[29]
SPE-UHPLC-MS/MS	Eggs	8 As, 4 TC, 5 Qs, 4 SAs, 4 Macs	5-100 $\mu g \cdot kg^{-1}$	54.0-76.0 (SAs)	NR	[30]
QuEChERS- UHPLC-MS/MS	Eggs	8 As, 4 TC, 5 Qs, 4 SAs, 4 Macs	5-100 $\mu g \cdot kg^{-1}$	56.0-76.0 (SAs)	NR	[30]
MSPD- UHPLC-MS/MS	Eggs	8 As, 4 TC, 5 Qs, 4 SAs, 4 Macs	5-100 $\mu g \cdot kg^{-1}$	62.0-89.0 (SAs)	NR	[30]
SE-UHPLC-MS/MS	Eggs	8 As, 4 TC, 5 Qs, 4 SAs, 4 Macs	5-100 $\mu g \cdot kg^{-1}$	73.0-89.0 (SAs)	6.1-114.2 (CC α) SA	[31]
LLE-SPE-HPLC-MS/MS	Eggs	10 SAs	NR	91.0–146.0	7.4–26.0 (CC α)	[31]
HF-SLM-DAD	Eggs	15 SAs	5-1000 $\mu g \cdot kg^{-1}$	71.0–98.7	0.8-7.9 $\mu g \cdot kg^{-1}$	Proposed method

NR: not reported; TC: tetracycline; FQ: fluoroquinolones; Mac: macrolides, and As: anthelmintics.

while for the inter-day precision the analysis was done once per day over five days. Intra-day %RSD values ranged from 6.2–15.7%, 7.3–15,0%, and 7.3–14.6% at concentration levels of 50 $\mu g \cdot kg^{-1}$, 100 $\mu g \cdot kg^{-1}$, and 500 $\mu g \cdot kg^{-1}$, respectively, while the inter-day %RSD values at the same concentration levels were in the range of 6.9–17.4%, 4.3–16.2%, and 8.3–16.5%, respectively (Table 3).

The accuracy of the method was also evaluated by analysing the recovery of blank egg samples spiked at three concentration levels (50 $\mu g \cdot kg^{-1}$, 100 $\mu g \cdot kg^{-1}$, and 500 $\mu g \cdot kg^{-1}$) in six replicates. The recoveries obtained at these concentration levels were in the range of 71.0–98.7%, 71.0–98.4%, and 71.2–97.2% with the corresponding %RSD values in the range of 2.0–9.9, 3.0–9.9, and 1.9–8.3%, respectively (Table 4). The validation results indicate that the HF-SLM method has adequate sensitivity, precision, and accuracy for it to be used for the determination of SAs in real egg samples.

5. Analysis of Egg Samples

The optimised and validated HF-SLM method was applied for the determination of sulfonamide residues in egg samples collected from supermarkets in Gauteng Province (South Africa). Figures 10 and 11 show the chromatograms of a blank egg sample and a real egg sample, respectively. As illustrated in Figure 10, no interfering peaks were observed at the target compound retention times in the blank egg sample. The few unknown peaks observed did not correspond to the analytes under study. On the other hand, results shown in Table 5 revealed that SMX, SBZ, and SSA were detected at concentrations in the range of 16.4–68.0 $\mu g \cdot kg^{-1}$, 56.0–61.50 $\mu g \cdot kg^{-1}$, and 33.0–61.2 $\mu g \cdot kg^{-1}$, respectively, with the corresponding %RSD values of 7.4–9.6%, 7.3–10.6%, and 9.2–12.6% in real egg samples. Sulfonamide residues were recorded in three of the five brands of egg samples from different suppliers, indicating noncompliance with the legislation, since these compounds should not be present in chicken eggs. In South Africa, sulfonamides are regulated based on the international values stated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) for the total residues of all substances within the sulfonamide groups that should not be exceeded 0.1 $\mu g \cdot kg^{-1}$ for various foodstuffs of animal origin but not for chicken eggs [23]. The fact that other SAs were not detected in the analysed samples may signify that there were no residues, or that their

concentrations were lower than the LOD values of the developed method. Some previous studies have also reported that large amounts of antibiotics in food-producing animals are being used in South Africa, including those that have been banned for use in other countries [24]. It is therefore important to have focused residue monitoring protocols in place for food-producing animals and foods of animal origin to protect human health and to facilitate international trade of these commodities. Since any amount of sulfonamide residue in egg samples indicates noncompliance with legislation, the method could be extended to HF-SLM-LC-MSMS to further improve the sensitivity.

6. Comparision of the Proposed Method with Other Reported Methods

The developed method was compared with the other reported methods such as supported liquid membrane combined with mass spectrometry (SLM-MS), hollow fibre-based liquid-phase microextraction combined with diode array detection (HF-LPME-DAD), and vortex-assisted HF-LPME-DAD/FL, HF-LPME-GC, HF-LPME-UV, SPE-LC-MS/MS, QuEChERS-UHPLC-MS/MS, MSPD-UHPLC-MS/MS, and LLE-UHPLC-MS/MS, based on linearity, percentage recovery, and LOD values. As shown in Table 6, comparable linearity ranges, and reasonable LOD values and percentage recovery were obtained using the developed method. Therefore, the proposed method is an excellent alternative method for the determination of sulfonamide residues in egg and other complex matrices.

7. Conclusions

In the present study, a simple, efficient, and environmentally friendly sample preparation technique based on three-phase hollow fibre-supported liquid membrane (HF-SLM) was successfully developed and applied to the determination of sulfonamide residues in real egg samples collected from various supermarkets. Under optimum conditions, LOD and LOQ values ranged from 0.8–7.9 $\mu g \cdot kg^{-1}$ and from 2.4 to 21.0 $\mu g \cdot kg^{-1}$, respectively. Linearity was found in the range of 5–1 000 $\mu g \cdot kg^{-1}$, and reasonable intra- and inter-day precision (%RSD) values in the range of 6.2–17.4, 4.3–16.2, and 7.3–16.5, respectively, were obtained. The accuracy of the developed method, expressed as percentage recovery, was in the range of 71.0–98.7%, with corresponding %RSD values ranging from 1.9–9.9%. The developed method was found to have reasonable enrichment factors which ranged between 17.1 and 524.3. When applied to the real egg samples, 60% of the brands were found to be noncompliant as they contained SMX, SBZ, and SSA at concentration ranges of 16.4–68.0 $\mu g \cdot kg^{-1}$, 56.0–61.5 $\mu g \cdot kg^{-1}$, and 33.0–61.2 $\mu g \cdot kg^{-1}$, respectively. The proposed method compares with other similar reported methods, and we found that most of the reported HF-SLM-based methods for sulfonamides often focused on the less polar group as well as small number of sulfonamide compounds. However, there are a number of sulfonamide compounds that are widely applied or used in both humans and animals which may have a potential to cause health effect. In the current study, very large number of sulfonamides are extracted and determined in a single procedure. Compared to similar method reported by Msagati and Nindi in 2014,compounds such as SDZ, STZ, SPY, SQZ, and SBZ which failed to be extracted using 5% TOPO in hexamine were well separated and extracted in the current work using 10% TOPO in 1-octanol with EFs in the range of 32.63–445.30. Furthermore, in almost all reported similar methods for egg samples complex HF-SLM setup and expensive instruments were used. However, in the current method we developed simplified, cost-effective (which can be implemented in any laboratories where resources are limited), and sensitive method that is able to simultaneously extract and determine the available and widely used sulfonamide compounds with wider polarity ranges (from relatively most polar to less polar one) in a single simplified procedure. It can be concluded that the developed HF-SLM method is suitable to be used as an alternative method for the determination of sulfonamide residues in egg and related complex samples.

Data Availability

The data that support the findings of this study are available upon request from the authors.

Conflicts of Interest

All the authors declare that they have no conflicts of interest.

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Supplementary Materials

Figure S1 A chromatogram of 15 separated sulfonamide standards (100 $\mu g\ L^{-1}$) at 265 nm. Chromatographic conditions: flow rate of 1.8 mL min^{-1}, column temperature 40°C, injection volume of 5 μL, and wavelength of 265 nm. A binary mobile phase comprising solvent A (0.1% FA water) and solvent B (acetonitrile) with a gradient elution program of 10% B (0–1 min), 40% B (1–4 min), and 60% B (4–6 min). (*Supplementary Materials*)

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