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Research Article

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Dispersive Liquid - Liquid Microextraction for the Determination of Sulfonamide Residues in Egg Samples

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Abstract

A dispersive liquid-liquid microextraction combined with high-performance liquid chromatography-diode array detection was developed for the extraction and determination of 15 sulfonamide residues in the egg matrix. The effects of various parameters such as the type, volume, and composition of extraction solvent for sample treatment procedure, the type and volume of disperser solvent, centrifugation time, salting-out effect and solution pH were studied, and optimum conditions were established. Linearity was found in the range of 5.4 – 1 000 µg kg-1 with regression coefficients ranging from 0.9918 - 0.9987. The limit of detection (LOD) and limit of quantification (LOQ) values of the proposed method were in the range of 4.3 - 8.0 µg kg^{-1} and 12.9 - 24.0 µg kg^{-1}, respectively. Satisfactory intra-day and inter-day precision results in the range of 6.3 - 17.5% and 4.8 - 16.8%, respectively, were achieved. The accuracy of the method was acceptable with percentage recovery in the range of 73 - 108% and %RSD values in the range of 1.1 - 16.5%. The proposed method was applied in chicken egg samples obtained from supermarkets, and findings confirmed that the method is feasible to be used for extraction and determination sulfonamide residues in egg and related complex biological matrices.

Keywords: Dispersive liquid-liquid Microextraction (DLLME), Sulfonamides (SAs), Egg, DLLME-HPLC-DAD

Introduction

Many countries and organizations have established maximum residue limits (MRLs) for sulfonamides in foods of animal origin; for example, the European Union (EU) has established a maximum residue limit (MRL) of 100 µg kg-1 for total sulfonamides in foods of animal origin (EC regulation 37/2010). However, for eggs, where no residue limits have been set for sulfonamides there is “zero tolerance” limit, meaning that no residues should be permitted [1-3]. Therefore, monitoring of these compounds at a trace level is very important to comply with the above requirement, especially if the animal product is intended for human consumption.

Sulfonamides (structures ,Kow and pka values are shown in Table 1) have been detected in several matrices over the past years, including water [4], meat [5-12], milk, [13-17], egg [1, 18-23], infant formulas [24], honey [25, 26] and animal feed [27].Various analytical methods have been used to determine sulfonamide residues which include liquid chromatography with UV detection [17], diode array detection [9], fluorescence detection [10, 12], MS or MS/MS detection [1, 2, 6, 27, 28], and capillary zone electrophoresis (CZE) [5]. A number of sample preparation techniques have been used for extraction and clean-up of sulfonamides from various matrices, such as liquid-liquid extraction (LLE) [15], solid-phase extraction (SPE) [7, 15], supported liquid membrane (SLM) [16,18], molecularly imprinted polymer (MIP) extraction [11], pressurized liquid extraction (PLE) [6, 29], cloud point extraction (CPE) [17], QuEChERS [20, 27], and matrix solid-phase dispersion (MSPD) [12, 28]. However, current trends are focused on miniaturization of the sample preparation, extraction, and clean-up steps and on the enhancement of the environmental safety of these procedures [30].

Table 1: Linearity, LOD, and LOQ values obtained for 15 SAs

Compound	Regression equation(n= 6)	Correlation coefficient R^2	Linear range (µg kg-1)	LOD (µg kg^{-1}) (n =10)	LOQ (µg kg^{-1}) (n =10)
SGD	y = 0.003x + 0.030	0.9959	6.4 - 1000	4.3	12.9
SAM	y = 0.001x + 0.042	0.9918	16 - 1000	6.7	20.1
SAA	y = 0.003x + 0.010	0.9930	50 - 1000	6.4	19.2
SDZ	y = 0.010x + 0.189	0.9987	17 - 1000	7.2	21.6
STZ	y = 0.001x + 0.305	0.9967	16 - 1000	7.4	22.2
SPY	y = 009x + 0.068	0.9915	14 - 700	6.9	20.7
SMR	y = 0.075x + 0.404	0.9991	11 - 700	5.4	16.2
SMT	y = 0.005x + 0.283	0.9982	5.4 - 1000	4.5	13.5
SMM	y = 0.030x + 0.279	0.9974	10 - 1000	5.9	17.7
SCP	y = 0.034x + 0.588	0.9901	7 - 1000	4.7	14.1
SMX	y = 0.034x + 0.525	0.9960	8 -1000	5.1	15.3
SSO	y = 0.003x + 0.272	0.9976	18 - 1000	8.0	24.0
SBZ	y = 0.010x + 0.514	0.9939	14 - 1000	6.3	18.9
SQZ	y = 0.013x + 0.598	0.9952	13 - 500	6.7	20.1
SSA	y = 0.0004x + 0.004	0.9964	9 - 500	5.0	15.0

The DLLME procedure is an appropriate choice for the analysis of samples with a relatively simple matrix such as water. As a result, the DLLME procedure can be applied directly after simple sample preparation such as filtration, centrifugation, and pH adjustment [31]. Since the technique is not suitable for the direct extraction of compounds from solid samples, extensive sample pre-treatment such as homogenisation and extraction (in which analytes are released into a solvent) are required before being subjected to the DLLME procedure. Furthermore, the solvent used to extract the analyte from the sample matrix becomes a disperser solvent in the subsequent DLLME procedure. However, some challenges are encountered when extracting sulfonamides from egg samples; they exist at trace levels, and their extraction is hindered due to the complex nature of the egg matrix whereby some sulfonamides bind to the lipoprotein fraction of the egg. In general, the extent of analyte extraction from the solid sample is influenced by the solubility of the analytes, selectivity of the solvent, and matrix effects [32].The other challenge is that the method was initially developed for compounds which are neutral or non-polar. Polar compounds such as sulfonamides which have a wide polarity range (most polar to least polar) add to the complexity which means that extraction conditions must be optimized carefully to promote the neutral form of the analyte. Most DLLME applications reported in the literature are for environmental water samples [33, 34] with very few examples of complex biological matrices [18, 35-37]. Therefore, extending the application of the method to complex matrices such as eggs is of great significance for human health and safety as well as for meeting the standards and regulations when exporting such commodities.

Experimental

Standards and chemicals

Antibiotics standards included sulfaguanidine (SGD), sulfanilamide (SAM), sulfathiazole (STZ), sulfacetamides (SAA), sulfamethizole (SMT), sulfamethoxazole (SMX), sulfasalazine (SSA), sulfamonomethoxine (SMM), sulfaquinoxaline (SQX), sulfamerazine (SMR), sulfapyridine (SPY), sulfadiazine (SDZ), sulfabenzamide (SBZ), sulfachloropyridazine (SCP), and sulfisoxazole (SSO) were purchased from Sigma-Aldrich (Steinheim, Germany). All standards had purities higher than 98%. Methanol, acetone, and formic acid were obtained from Sigma-Aldrich (Steinheim, Germany). Acetonitrile (MeCN) was purchased from ROMIL Ltd. (Waterbeach, Cambridge, UK). Sodium hydroxide (NaOH) was supplied by Merck (Darmstadt, Germany). Trichloromethane, dichloromethane and 1,2-dichloroethane were purchased from Sigma-Aldrich, (Steinheim, Germany). All solvents were HPLC grade and reagents were analytical reagent (AR) grade. Ultrahigh purity (UHP) water (resistivity, 18.2 MΩ·cm at 25 °C) was generated using the Milli-Q® system (Millipore, Billerica, MA, USA).

Instrumentation

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

Preparation of sulfonamide standard solutions

Stock standard solutions (1 000 mg L-1) for each compound were prepared by dissolving 10 mg of accurately weighed standard of each compound in a mixture of methanol and ultrahigh purity water (1:1; v/v) in a 10 mL volumetric flask. Appropriate

dilution of these stock solutions with methanol- ultrahigh purity water (1:1; v/v) was used to prepare various concentrations of working solutions. All standard solutions were protected from light by covering the sample vials with aluminum foil and were kept at 4 ℃.

Chicken eggs sampling

Five cartons of half a dozen eggs of different brands were purchased from local supermarkets in Gauteng Province (South Africa). Twenty-five blank egg samples were collected from a non- commercial small-scale organic farmer. Thus, these eggs were from chickens which were not treated with antibiotics. To confirm this, the blank samples were initially screened for antibiotics prior to use in this study. Optimization studies were done using blank egg samples spiked with a mixture of 15 SAs at a concentration of 300 µg kg-1. For method validation, a mixture of 15 sulfonamides was spiked into the blank egg samples at a concentration range of 5 - 1 500 µg kg−1 in order to construct matrix-matched calibration curves.

Pre-treatment procedures for egg samples

In the sample pre-treatment procedure, the egg yolk and albumin from each of the brands were combined and blended using a food blender (Sunbeam®, Canada). Each blended sample consisted of a total of half a dozen eggs. For each blend, 5 g of sample was weighed into a 50 mL screw cap centrifuge tube and treated with 5 mL of organic solvent {(0.05% aqueous formic acid) in MeCN (15:85, v/v)}. The mixture was vortexed for 30 s to homogenize the mixture and to facilitate the extraction. The homogenized solution was centrifuged for 10 min at 4 000 rpm for complete phase separation and the resulting supernatant (acetonitrile extract from the egg sample) was filtered through a 0.45 µm nylon filter (Lenntech B.V., Rotterdamseweg, Delft, Netherlands) and a 1 mL aliquot of the MeCN extract was used for the subsequent DLLME procedure as a disperser solvent.

Dispersive liquid-liquid microextraction procedure

The dispersive liquid-liquid microextraction procedure involved transferring 5.0 mL of UHP water, the pH of which was adjusted to 3.5 using 0.1 M HCl, into a 15.0 mL screw cap centrifuge tube. This was followed by the addition of a 1.0 mL aliquot of the egg sample extract (MeCN extract obtained from the sample pre-treatment procedure) and a rapid injection of 400 µL of dichloromethane (extraction solvent) into the mixture. It should be noted that the MeCN extract acted as a disperser solvent in this case and as a carrier for the sample. For complete dispersion and to facilitate the extraction, the mixture was subjected to vortex for 30 s and the resulting cloudy solution was centrifuged for 3 min at 4 000 rpm for complete phase separation. The dispersed fine particles of the extraction phase, which had settled at the bottom of the centrifuge tube were withdrawn by using a Hamilton 500 µL micro syringe and transferred into a 1.5 mL HPLC vial through a 400 µL insertion vial.The organic phase was dried in a gentle stream of nitrogen gas. Thereafter, the final residue was reconstituted with 100 µL of the mobile phase and subjected to the chromatographic analysis.

Chromatographic conditions for the separation of 15 sulfonamide compounds

Chromatographic conditions consisted of a binary mobile phase comprising of solvent A (0.1% formic acid at pH 2.73) and solvent B (acetonitrile) with a gradient elution of 10% B (0 - 1 min), which was gradually increased from 10% to 40% B for 1 - 4 min, and further increased from 40% to 60% B for 4 - 6 min. The mobile phase flow rate of 1.8 mL min−1 and an injection volume of 5 µL were used. Similarly, the column oven temperature of 40 °C and DAD detection wavelength of 265 nm were used.

Results and Discussion

Optimization of chromatographic conditions for the separation of 15 sulfonamide compounds

A chromatographic method for the separation of 15 sulfonamides was first developed and optimized. To investigate the condition for optimum peak shape and adequate resolution in the separation of target analytes, both ultrahigh purity (UHP) water and acidified ultrahigh purity water were evaluated as solvent A and organic solvents (acetonitrile and methanol) as solvent B. The best peak shape and satisfactory resolution of the target compounds were achieved using a binary mobile phase comprising solvent A (0.1% formic acid in UHP water at pH 2.73) and solvent B (acetonitrile). Both isocratic and gradient elution modes were investigated, and satisfactory peak shapes and resolution were achieved using a gradient elution program consisting mobile phase B (10%) for 0 - 1 min which was gradually (at 1% intervals) increased from 10% to 40% B for 1- 4 min and further increased from 40% to 60% B for 4 - 6 min. The effect of mobile phase flow rate was also evaluated in the range of 0.3 - 2 mL min-1 at 0.2 mL min-1 interval. Optimum resolutionand peak shape were obtained at a flow rate of 1.8 mL min-1 which was then selected as the optimum flow rate for the subsequent experiments. Furthermore, the column oven temperature was also optimized in the range of 25 - 45 ºC and best separation was observed at a temperature of 40 oC. The compounds were monitored using DAD at different wavelengths (260, 265, 270 and 280 nm) and at 265 nm all target analytes were detected with good sensitivity. Therefore, 265 nm was selected as the detection wavelength for the rest of the study. A chromatogram of 15 separated sulfonamide standards (100 µg L-1) is shown in (Supplemental, Figure S1), with baseline resolution of all 15 sulfonamides within 5.20 min, thus allowing for quantitation. Furthermore a chromatogram of blank egg sample spiked with mixture of SAs at concentration 100 µg L- shown in figure 1.

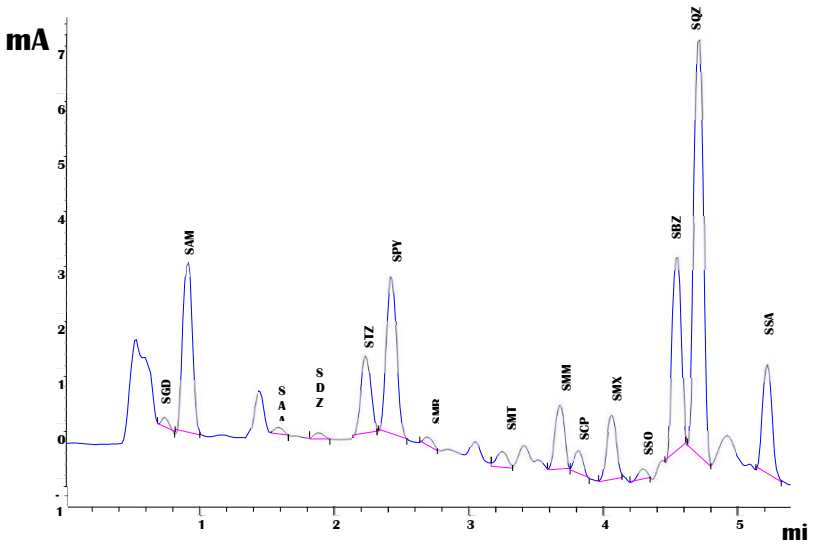


Figure 1: A chromatogram of blank egg sample spiked with mixture of SAs at concentration of 100 µg L-1.Chromatographic conditions: flow rate of 1.8 mL min−1; column temperature 40 °C; injection volume of 5 µL; wavelength of 265 nm. A binary mobile phase comprising of solvent A (0.1% FA in water) and 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).

Optimization of treatment procedure for egg samples

Relatively few applications have been devoted to the analysis of organic compounds in highly complex matrices, such as food and biological samples using DLLME [35-37]. In these types of matrices, analytes were pre-extracted from the sample matrix using appropriate extraction solvents and the extract was used as a disperser solvent for DLLME. One of the challenges is that the extract may not be compatible with the DLLME procedure due to the interaction of matrix components with the extraction solvent. Therefore, the extraction solvent should have a higher extraction capability for the analytes than the interferences and should be suitable to be used as a disperser solvent in DLLME.

To develop the sample pre-treatment procedure, the first step was to evaluate acetonitrile, methanol, and acetone as the organic solvent needed to effectively extract the target SAs from the egg matrix, and to determine the minimum possible volume of the selected organic solvent (in the current case MeCN) evaluated by varying its volume from 3 - 15 mL at 2 mL intervals. To investigate the optimum composition (acidification level) of the organic solvent (in the current case aqueous formic acid in acetonitrile), first the percentage of aqueous formic acid from 0.02 - 0.40% FA v/v and the proportions of the aqueous formic acid to organic solvent were evaluated by varying the proportions from 5/95 to 45/55 at 5 unit intervals.

Evaluation of organic solvent for the effective extraction of sulfonamide residues from egg samples

The type of organic solvent selected in a sample treatment procedure should efficiently dissolve the analytes from the bulk of the matrix. The selected extraction solvent should play the role of disperser solvent for the subsequent DLLME procedure [35-37]. Based on previous work in our laboratory, acetonitrile, methanol, and acetone were selected as potential extraction solvents in sample pre-treatment procedures. Their effect on the percentage recovery of the target compounds was investigated using 15.0 mL of each solvent as initial volume which was added to 5.0 g of the homogenized blank egg samples, previously fortified with a mixture of SAs at a concentration of 300 µg kg-1. Figure 2 shows that most analytes were reasonably extracted with all three solvents with the exception of a few analytes that had 30% or lower recoveries (SGD, SAA, SMR). Sulfaguanidine (SGD) could not be quantified in an acetone extract because it overlapped with an interfering compound. Due to interference challenges, acetone was not selected. When comparing MeCN to MeOH it was observed that the former gave good recoveries for most compounds. Furthermore, in several previous works [35, 37], MeCN was reported as being capable of denaturing the sample proteins, which results in a cleaner extract and a better release of polar residues bound to proteins. Therefore, acetonitrile was selected as the extraction solvent for further sample pre-treatment.

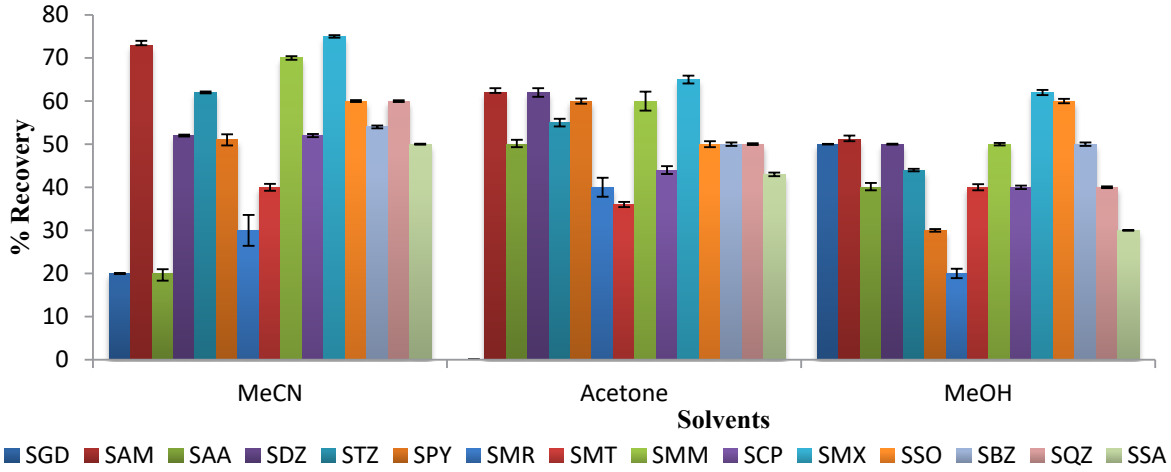


Figure 2: Evaluation of organic solvent for the extraction of SAs in blank egg sample spiked at 300 µg kg^{-1} concentration level, 15 mL each (acetonitrile, acetone, and methanol);n = 5.

Pre-treatment procedure for evaluation of the volume of extraction solvent used in egg samples

One of the objectives of modern analytical chemistry is miniaturization, i.e. reducing solvent use. Therefore, the minimum possible amount of organic solvent required to effectively extract the target SAs from the bulk of egg matrix was optimized. Thus, to evaluate the volume of extraction solvent used in the sample pre-treatment procedure, blank egg samples were fortified with a mixture of SAs at a concentration of 300 µg kg-1 with and compounds were extracted using different volumes (3, 5, 7, 10, and 15 mL) of MeCN. Figure 3 shows that the percentage recoveries of most target compounds were improved significantly (above 30%) with the exception of SSA which did not change at p = 0.05 significance level when the volume of MeCN was varied from 3.0 to 5.0 mL. The possible reason could be that 3.0 mL of MeCN was not sufficient to precipitate the proteins effectively and extract the target compounds. On the other hand, by increasing the volume of organic solvent from 5 to 7 mL, the extraction efficiency for three analytes (SAM, SMX and SSO) was improved, on the other hand, a significant decrease in the extraction efficiency was observed for another seven analytes (SGD,STZ, SPY, SMR, SBZ, SQZ, and SSA).However, no significant difference in the extraction efficiency was observed for the remaining five analytes (SAA, SMT, SMR, SCP and SMM) at p = 0.05 significance level. In general, it was noted that volumes higher than 5 mL did not significantly improve the percentage extraction recoveries of most analytes. These variations could possibly be due to the differences in analyte- matrix binding. Therefore, overall, no considerable changes were observed in percentage recoveries when the volume of MeCN was varied from 5 to 15 mL. In view of the environmental benefits of green chemistry and cost of the analysis, a volume of 5.0 mL MeCN was selected as optimum for the subsequent experiments since it gave reasonable percentage recoveries. A similar volume of MeCN has been used in the previous report for extraction of seven fluoroquinolones in chicken liver samples [35].

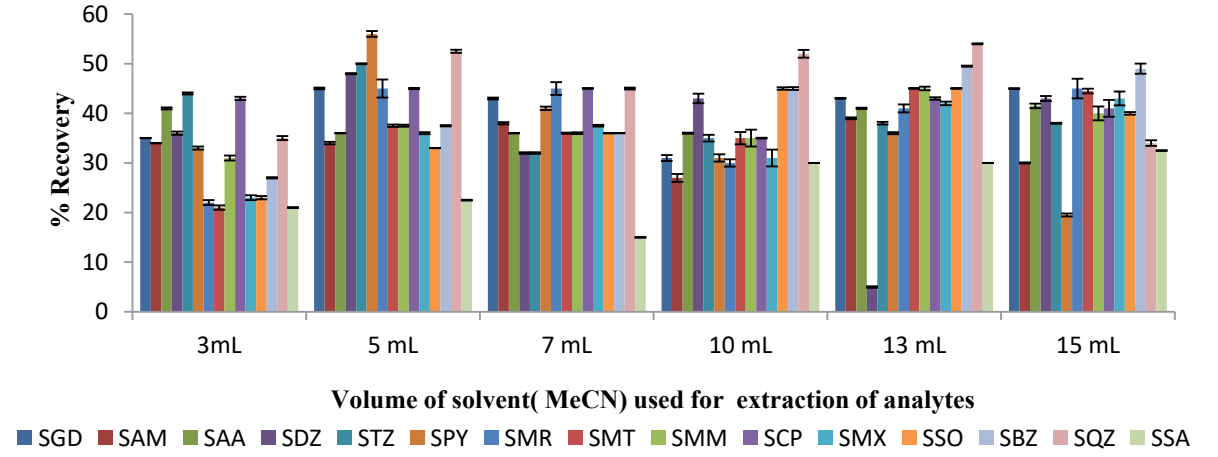


Figure 3: Evaluation of the volume of organic solvent used for isolation of target compounds; sample spiked at 300 µg kg-1 concentration level, MeCN (3 - 15 mL); n = 5.

Evaluation of acidic conditions that favor the extraction of sulfonamides from egg samples

Studies in the literature have shown that the extraction recoveries could be improved significantly by acidifying the extraction solvent for isolation of compounds from the bulk of the sample matrix [2, 35, 37]. This could be due to the fact that the acid promotes hydrolysis and unbinds the drugs which are bound to the lipoprotein fraction of the sample and also the acidic condition possibly keeps most of the sulfonamides in their neutral form.

In this study, the optimum amount of formic acid needed to acidify the extraction solvent was investigated by varying the

concentration of formic acid (FA) from 0.02 to 0.4% (v/v) in the aqueous component of the solvent system (aqueous FA to MeCN (25:75; v/v)). Figure 4 shows that maximum percentage recoveries for most target SAs were obtained when the percentage of formic acid was 0.05% (pH 3.0). Thus, 0.05% FA in the extraction solvent system was selected as optimum for the effective extraction of sulfonamide residues from the egg matrix.

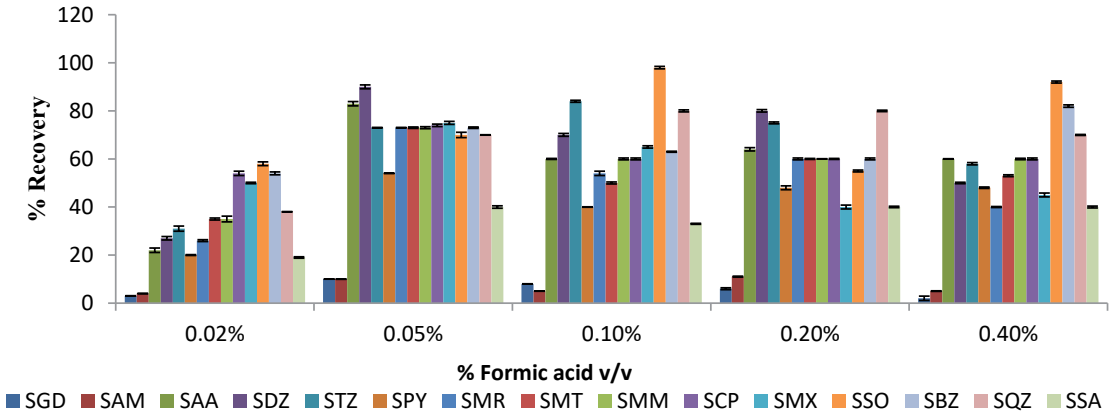


Figure 4: Evaluation of optimum acidic condition that favours the extraction of SAs in blank egg sample spiked at 300 µg kg-1 concentration level, 5 mL (25:75) (0.02, 0.05, 0.1, 0.2, and 0.4% FA; v/v)/MeCN; n = 5.

Evaluation of optimum aqueous/organic proportion for extraction of target compounds from egg samples

To evaluate the optimum composition of the extraction solvent (aqueous formic acid to acetonitrile) blank egg samples were fortified with a mixture of SAs at a concentration of 300 µg kg-1 and extracted with 5 mL of acidified extraction solvent. The proportions of the aqueous to organic solvent were varied from 5/95 to 45/55. Figure 5 demonstrates that the percentage recoveries obtained using aqueous FA to MeCN (15:85; v/v) were the best for most of the compounds except for SGD, SDZ and SSA which extracted better using 25/75, 35/65 and 45/55, respectively. Thus, the proportion of aqueous FA to MeCN in the ratio of 15:85 (v/v) was selected as optimum.

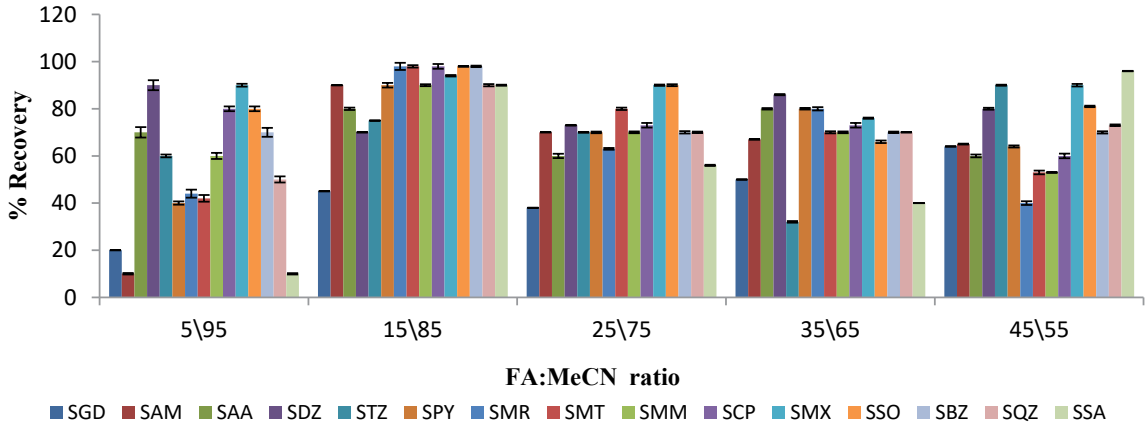


Figure 5: Evaluation of optimum formic acid to acetonitrile ratio for the extraction of SAs mixture in blank egg matrix spiked at 300 µg kg-1 concentration level, 5 mL acidified extraction solvent prepared by mixing of 0.05% FA with MeCN in the proportion of FA to MeCN ranging from 5:85 - 55:45, v/v, respectively; n = 5.

Optimization of the DLLME procedure for egg samples

In order to obtain high extraction efficiencies, the effects of parameters that affect the extraction and enrichment conditions such as type and volume of extraction solvent, the pH of solution, salting-out effect, and centrifugation time were evaluated and optimized. Initially, an appropriate extraction solvent for the DLLME procedure from three extraction solvents (dichloromethane, 1,2-dichloroethane, and trichloromethane) was investigated by injecting a mixture of disperser solvent (MeCN) and each of the extraction solvents. The volume of the extraction solvent and disperser solvent on the extraction efficiency were also evaluated by varying the volume of MeCN (extract) from 200 - 1 000 µL at 200 µL intervals and a disperser solvent from 0.5 - 2 mL at 0.5 mL intervals. The effect of pH on the extractability of SAs was also investigated by varying the pH of the aqueous solution from 2.5 to 6.5 at one pH unit interval. The effect of salt on the extraction efficiency for target compounds was evaluated by adding different amounts of NaCl from 2 to 10% (w/v)

Selection of extraction solvent for the DLLME procedure

As mentioned in section 3.2.4, acetonitrile (i.e. aqueous FA:MeCN; 15:85) was used to extract the target analytes from the egg matrix. Since DLLME requires a ternary solvent system, i.e. water, extraction solvent, and disperser solvent, the MeCN extract (as described above) was used as the disperser solvent [35, 37]. In selecting the extraction solvent for the DLLME pro-

cedure, the general requirements (already discussed in the previous sections) were considered. Based on these requirements and by taking into account the applications of the solvents in previously reported studies [1, 35, 37], in the present study, dichloromethane (DCM), 1,2-dichloroethane (DCE), and trichloromethane (TCM) were investigated and their effects on the extraction efficiency were evaluated. Figure 6 shows that the extraction efficiencies of most SAs appeared similar in both DCM and DCE. However, statistical t-test (at p= 0.05 level) confirmed that the extraction efficiencies of seven analytes (SGD, SAM, SDZ, SMT, SSO and SSA) were significantly better in the former than the latter solvent. For the rest of the compounds, it was noted that the observed difference in extraction efficiencies was not significant. Therefore, dichloromethane was selected as the extraction solvent for the subsequent experiments. Similarly, [1] also found DCM to be the best solvent for extracting SAs from egg samples using the conventional liquid extraction.

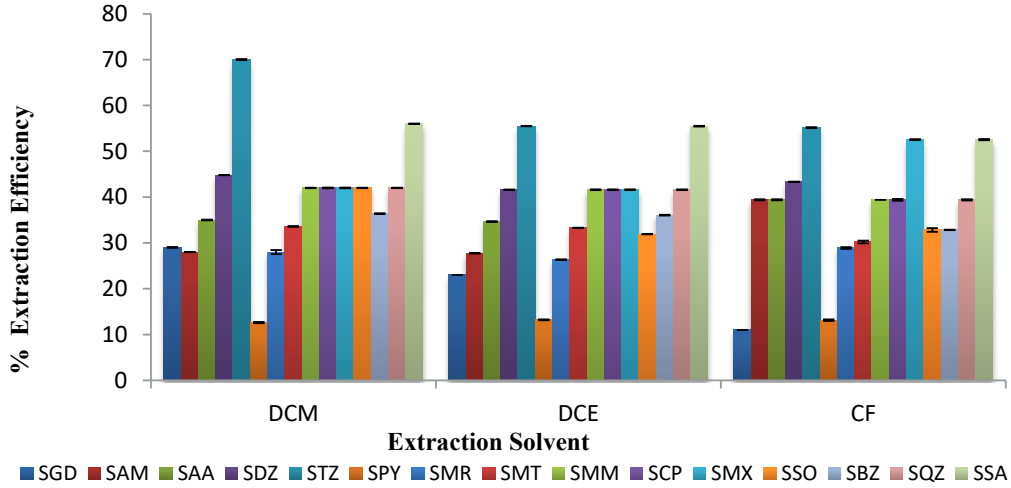


Figure 6: Effect of extraction solvents on extraction efficiencies for SAs in blank egg samples spiked at 300 µg kg-1 concentration level. DLLME conditions: water (5.0 mL), disperser solvent (1.0 mL of MeCN extract), extraction solvents (DCM, DCE and CF; 400 µL each); n = 5.c

Effect of sample pH

For DLLME, the partitioning of an analyte from an aqueous phase into a hydrophobic organic solvent is greater when a molecule is in its uncharged form. This could be achieved by controlling the pH of the solution. Sulfonamides are amphoteric due to the presence of both acidic and basic moieties in their structure. As a result, these compounds exist as charged compounds over a wide pH range. Thus, in this study, the effect of pH was investigated by varying the pH of the aqueous solution from 2.5 to 6.5 at 1 pH unit intervals using 0.10 M HCl. Figure 7 shows that the highest extraction efficiencies for most SAs were achieved at pH 3.5. It was observed that at this pH, the extraction efficiencies ranged from 10% (SPY) to 100% (SMX). Based on the experimental results, pH 3.5 was selected as the optimum pH for the subsequent experiments.

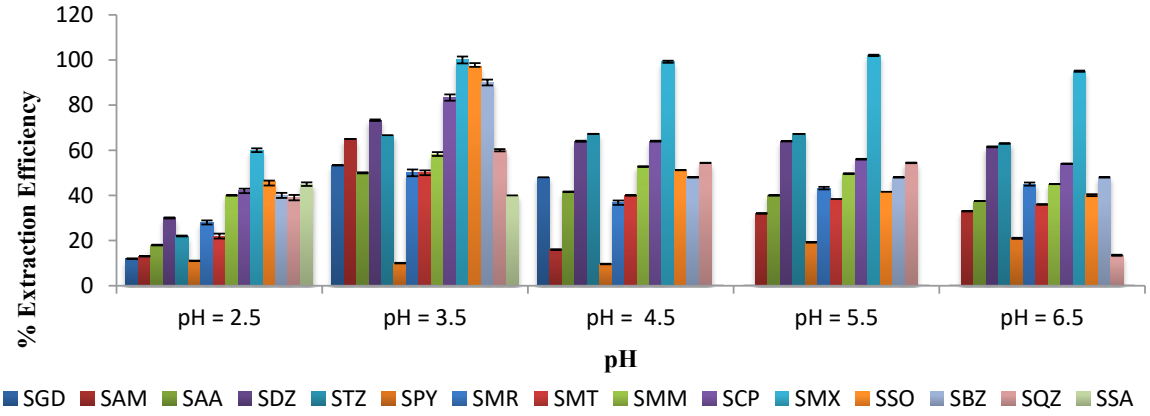


Figure 7: Effect of pH on the extraction efficiencies for SAs in blank egg samples spiked at 300 µg kg-1 concentration levels. DLLME conditions: water (5.0 mL, pH varying from 2.5 to 6.5); disperser solvent (1.0 mL, MeCN); extraction solvent (400 µL of dichloromethane); n = 5.

Effect of the volume of extraction solvent

The effect of the volume of extraction solvent on the extraction efficiency was evaluated by varying the volume of dichloromethane over the range of 200 - 1 000 µL, while the other experimental parameters were kept constant. Results in Figure 8 indicated that there was no considerable difference in the extraction efficiencies for most target compounds as the volume of the extraction solvent was increased from 400 - 800 µL. Only a few compounds, namely SSA, SAM, SMT, SSO and SCP (46, 20, 19, 6.4 and 6%, respectively) benefited from the increased volume. A further increase in the extraction volume to above 800 µL resulted in a lower extraction efficiency. This could be due to the formation of larger dichloromethane droplets and consequently an increase in the settled phase volume. Since the DLLME method promotes the use of smaller volumes where possible, 400 µL was then selected as the optimum volume of extraction solvent for the subsequent experiments.

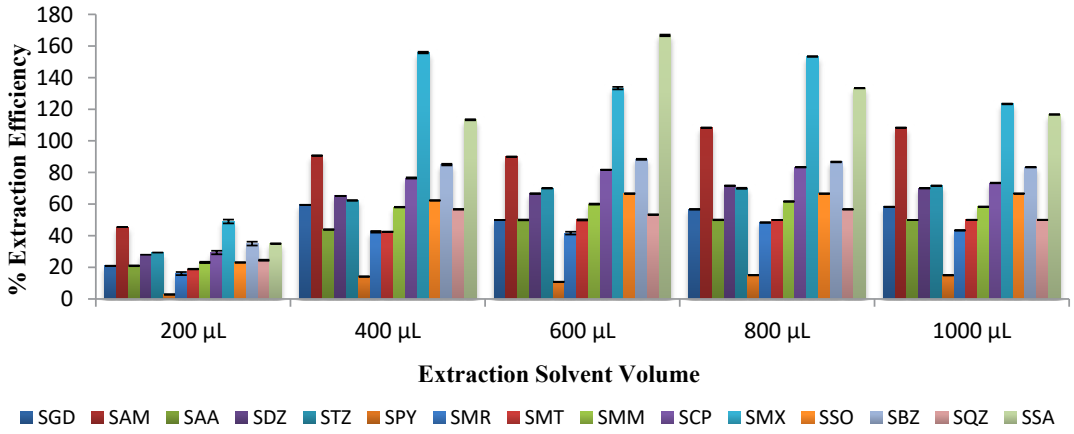


Figure 8: Effect of extraction solvent volume on the extraction efficiencies for SAs in blank egg samples spiked at 300 µg kg-1 concentration level. DLLME conditions: water (5.0 mL, pH 3.5); disperser solvent (1.0 mL, MeCN); extraction solvent (200 - 1000 µL, dichloromethane); n = 5.

Effect of disperser solvent volume

The influence of disperser solvent volumes on the extraction efficiency was investigated by varying the volume of MeCN extract (see above) from 0.5 - 2 mL. Experimental results in Figure 9 reveal that better extraction efficiencies were obtained when the volume of MeCN extract (i.e. disperser solvent in this case) was 1 mL. Lower disperser solvent volume usually results in a lower disperser to extraction solvent volume ratio, which consequently leads to a reduced number of droplets available for extraction. As a result, the transfer of the target analytes into the extraction solvent was insufficient as is evident from the lower extraction efficiencies. Furthermore, SPY which was poorly extracted at a volume of 0.5 mL MeCN showed a major improvement at a volume of 1 mL. On the other hand, at a disperser solvent volume of above 1.5 mL, a decrease in the extraction efficiencies for most target analytes was observed due to the increased solubility of the target analytes in the aqueous phase. Therefore, a disperser solvent volume (MeCN extract) of 1 mL was selected for the subsequent experiments as the optimum.

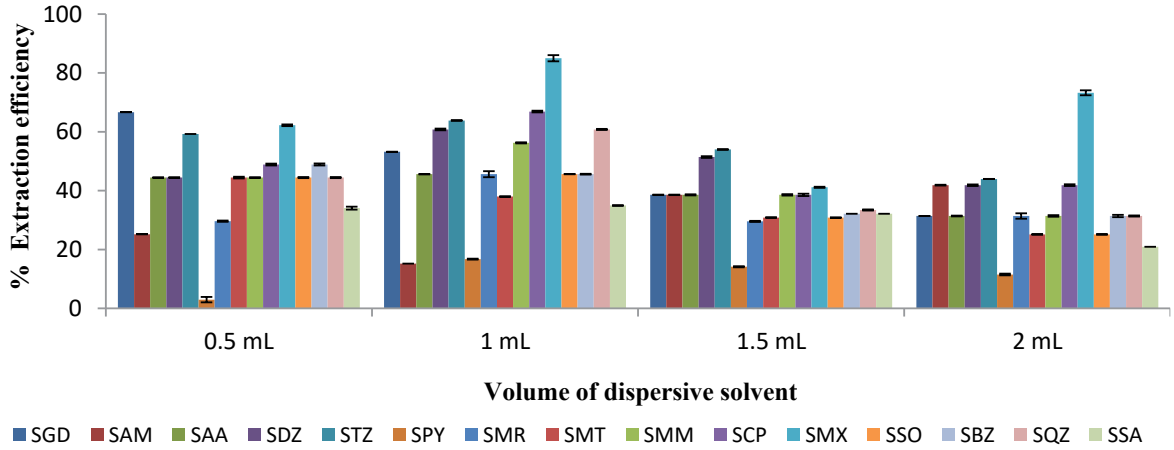


Figure 9: Effect of disperser solvent volume on the extraction efficiencies for SAs in blank egg samples spiked at 300 µg kg-1 concentration level. DLLME conditions: water (5.0 mL, pH, 3.5); extraction solvent (400 µL, dichloromethane); and disperser solvent (0.5 - 2 mL, MeCN extract); n = 5.

Effect of salt addition

In principle, addition of salt into the sample solution produces a salting-out effect, thus decreasing the solubility of target analytes into the aqueous sample solution and consequently promoting the transfer of analytes into the organic phase. Thus, the effect of salt on the extraction efficiency for target compounds was evaluated by adding different amounts of NaCl from 2 to 10% (w/v) keeping all the other experimental conditions constant. Figure 10 indicates that the addition of salt had no observed effect on the extraction efficiency for target compounds. Arroyo-Manzanares et al, 2014 observed similar results when they used NaCl (0% to 20% (w/v)) to improve the extraction efficiency for SAs using DLLME in milk samples. However, in some of the studies reported in the literature such as [35] for FQ in chicken liver, salt was not used in their DLLME procedure. On the other hand, Gure etal. 2014 found a decrease in recoveries upon the addition of salt in DLLME procedure for the determination of sulfonylurea herbicides (SUHs) in fruit juices due to the formation of a thick third phase in between the settled organic and aqueous phases that leads to a decrease in the volume of the settled organic phase. Similarly, Salami, etal. 2011 also found that the addition of NaCl to egg samples decreased the extraction yield of sulfonamides using microextraction by packed sorbent (MEPS) . Therefore, salt was not used in the subsequent procedures.

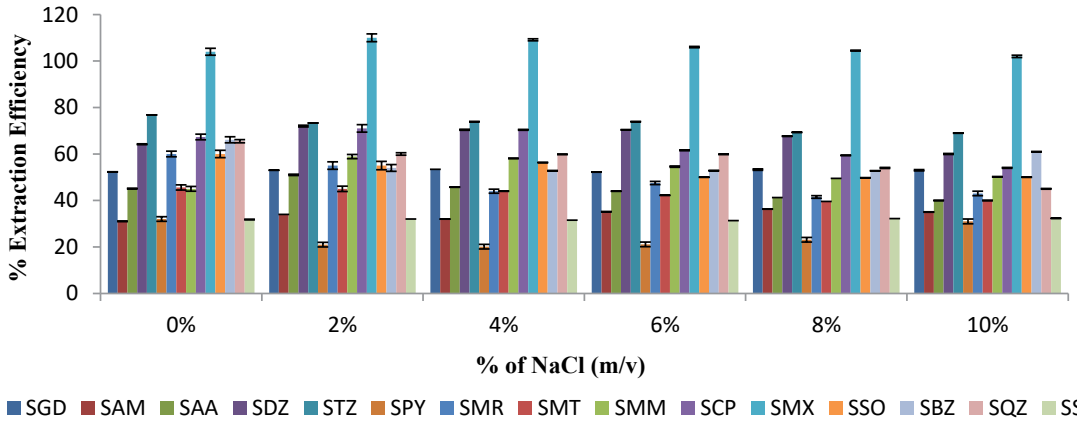


Figure 10: Effect of salt addition on the extraction efficiencies for SAs in blank egg samples spiked at 300 µg kg-1 concentration level. DLLME conditions: water (5.0 mL, pH, 3.5); extraction solvent (400 µL, dichloromethane); and disperser solvent (1 mL, MeCN); n=5.

Effect of centrifugation time

To evaluate the optimum time required for complete phase separation, centrifugation time was examined in the range of 3 - 10 min. Results shown in Figure 11 confirmed that 3 min was sufficient for a complete phase separation. Thus, a centrifugation time of 3 min was selected as the optimum.

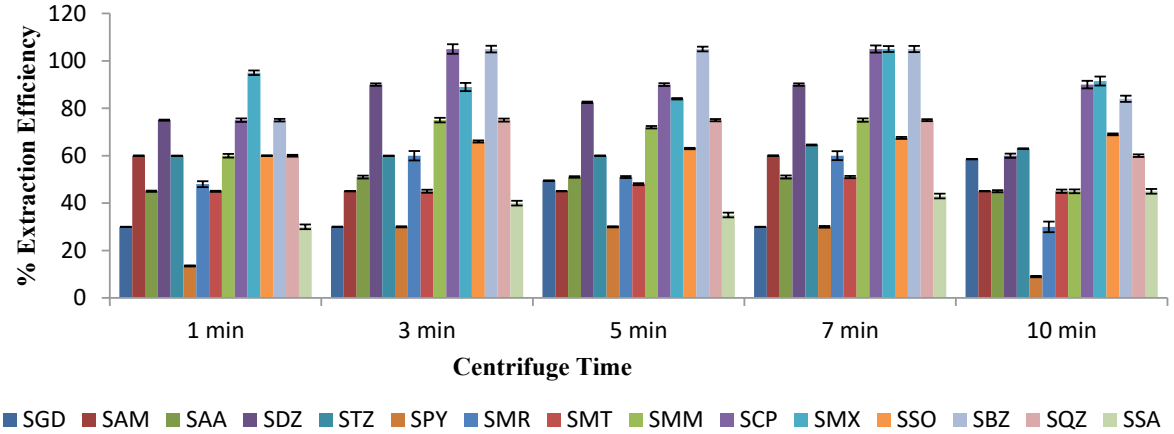


Figure 11: Effect of centrifugation time on the extraction efficiencies for SAs in blank egg samples spiked at 300 µg kg-1 concentration level. DLLME conditions: water volume (5.0 mL, pH 3.5; extraction solvent (400 µL, dichloromethane) and disperser solvent (1 mL, MeCN), centrifugation time (3 - 10 min); n = 5

DLLME method validation for egg samples

Under optimized 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 at concentrations ranging from 5 to 1 500 µg kg-1. Satisfactory linearity was obtained in the range of 5.4 - 1 000 µg kg-1 with coefficient of determinations ranging from 0.9918 - 0.9987 (Table1). The LOD and LOQ values were calculated based on 3 and 10 times standard deviation of blank egg extract with a minimum analyte concentration (4.0 µg kg-1), respectively. The LOD values were found in the range of 4.3 - 8.0 µg kg-1 while LOQ values were found to range between 12.9 and 24.0 µg kg-1. The intra-day and inter-day precision was evaluated by spiking blank egg samples at three concentration levels (50 µg kg- 1,100 µg kg-1, and 500 µg kg-1) of target analytes for three consecutive determinations in a single day and five determinations in five days, respectively. Intra-day precision expressed as relative standard deviation (%RSD) ranged from 6.3 - 16.4%, 9.2 - 17.5%, and 9.7 - 14.7%, while inter- day precision results at these three concentration levels were in the range of 9.3 - 14.5%, 6.4 - 16.8%, and 4.4 - 16.8%, respectively (shown in Table 2).

Table 2: Intra-day and inter-day precision result for 15 SAs in blank egg samples spiked at 50 µg kg^{-1}, 100 µg kg^{-1}, and 500 µg kg^{-1} levels

Compound	Intra-day (%RSD) (n = 6)			Inter-day (%RSD) (n = 6)		
	50 (µg kg^{-1})	100 (µg kg^{-1})	500 (µg kg^{-1})	50 (µg kg^{-1})	100 (µg kg^{-1})	500 (µg kg^{-1})
SGD	10.9	10.3	9.5	11.3	12.9	9.5
SAM	16.2	12.3	9.8	11.3	15.2	14.6
SAA	13.1	9.2	12.3	14.4	9.3	10.4
SDZ	15.3	13.2	13.8	14.3	10.4	6.9
STZ	15.8	12.4	15.1	14.3	8.2	7.3
SPY	6.3	10.7	9.7	14.5	11.5	4.8
SMR	14.4	11.8	9.8	14.0	9.2	5.5
SMT	11.8	15.4	13.7	13.0	10.4	15.3
SMM	15.4	10.6	10.1	12.7	15.8	10.2
SCP	14.9	15.9	9.3	13.7	11.8	16.8
SMX	16.4	14.7	10.8	11.5	7.8	12.6
SSO	15.6	17.5	14.7	12.1	13.5	4.4
SBZ	14.7	16.5	12.1	9.3	6.4	8.0
SQZ	12.5	14.8	9.7	12.8	14.0	11.0
SSA	9.9	13.0	14.1	13.7	16.8	7.2

The accuracy of the method was also evaluated by analyzing the recoveries of blank egg samples spiked at three concentration levels (50 µg kg-1, 100 µg kg-1, and 500 µg kg-1) and results obtained were in the range of 73.0-108.0% with %RSD values in the range of 1.1- 16.5% (Table 3). It was also noted that the %RSD values for SAM, SMT, and SSO were higher at the higher fortification level. The possible reason might be the type of analyte (some analytes bind more specifically to the lipoprotein fraction of the eggs) and the concentration-dependent matrix effect. Heller et al. 2002 also observed a similar effect in the extraction of most polar sulfonamides from the egg matrix using SPE-LC-MS. These experimental findings confirmed the feasibility of the proposed method for the determination of sulfonamide residues in real egg samples.

Table 3: Recoveries obtained for 15 SAs in blank egg sample spiked at 50 µg kg^{-1}, 100 µg kg^{-1}, and 500 µg kg^{-1} levels

Compound	Recovery (n = 6)					
	50 (µg kg-1)	%RSD	100 (µg kg-1)	%RSD	500 (µg kg-1)	% RSD
SGD	86.0	6.2	89.0	11.0	94.8	6.5
SAM	110.0	7.4	91.0	5.0	93.6	12.3
SAA	94.0	13.4	93.0	7.0	72.6	8.6
SDZ	80.0	4.8	90.0	12.1	78.2	2.1
STZ	86.0	5.7	95.0	9.7	93.2	6.0
SPY	98.0	4.6	96.0	2.1	94.0	3.8
SMR	80.0	8.6	90.0	3.1	78.0	8.1

SMT	92.0	9.10	90.0	4.6	88.6	12.6
SMM	94.0	6.9	94.0	3.2	88.0	7.9
SCP	92.0	4.9	96.0	4.3	73.3	7.7
SMX	108.0	3.6	95.0	3.7	79.2	7.2
SBZ	86.0	3.1	92.0	8.1	95.8	3.9
SSO	92.0	1.1	98.0	14.7	93.6	16.2
SQZ	86 .0	10.8	87.0	16.5	95.2	8.1
SSA	80.0	13.1	85.0	14.1	91.2	13.3

Table 4: Sulfonamide levels found in egg samples from supermarkets in Gauteng Province (South Africa) (n = 6)

Samples	SGD	SAM	SAA	SDZ	STZ	SPY	SMR	SMT	SMM	SCP	SMX	% RSD	SSO	SBZ	SQZ	SSA
A	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	17.8a	13.4	ND	ND	ND	ND
B	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	9.6a	8.8	ND	ND	ND	ND
C	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	17.8a	14.6	ND	ND	ND	ND
D	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	46.3a	11.4	ND	ND	ND	ND
E	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	11.6a	15.2	ND	ND	ND	ND

A, B, C D, and E are samples of different brands of eggs; a: µg kg-1; ND: not detected

Analysis of egg samples

The developed and validated method was applied to the determination of the target compounds in egg samples, which were collected from Gauteng Province (South Africa). Figures (supplemental S2 and S3) show chromatograms of a blank egg sample, and a real egg sample, respectively. In the case of the blank egg sample (supplemental Fig. S2), there are no interfering peaks in the retention times of the target compounds. On the other hand, as demonstrated in Figure 14, SMX was detected in the concentration range of 9.6 -46.3 µg kg-1 with the corresponding %RSD values in the range of 8.8 - 15.2% in all the analyzed real egg samples (Table 4). This indicates non-compliance in terms of the zero tolerance limit since the presence of such residues at any level is not permitted. However, these levels were much lower than the corresponding maximum residue limit of 100 µg kg−1 set for sulfonamides in other matrices, not including eggs. The other SAs that were not detected in the analyzed samples may not have been present at all or their concentrations were lower than the LOD of the developed method. Since the detection of sulfonamide residues in egg samples is a serious matter in terms of human health and international trade, more attention and work in this area is required to draw a valid conclusion. Thus, the experimental results confirmed that the proposed DLLME-HPLC- DAD method is feasible for quantitative analysis of sulfonamide residues in egg samples, and could be used for residue monitoring purposes.

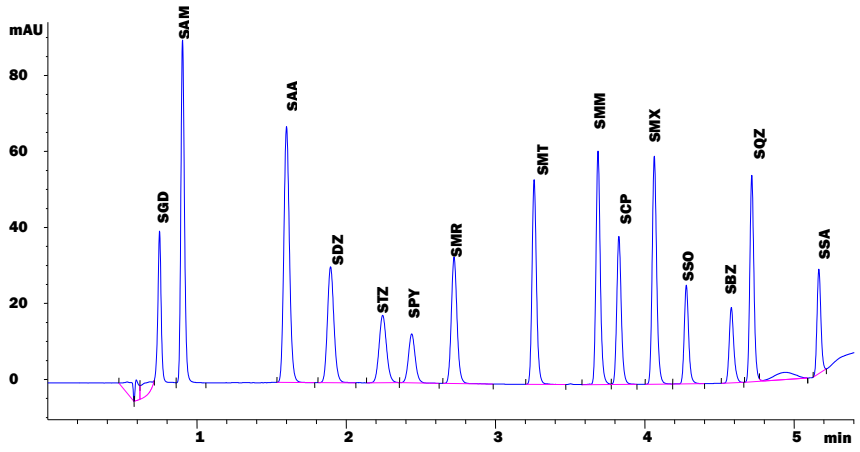


Figure S1: A chromatogram of 15 separated sulfonamide standards (100 µg L-1) at 265 nm. Chromatographic conditions: flow rate of 1.8 mL min−1, column temperature 40 °C, injection volume of 5 µL, wavelength of 265 nm. A binary mobile phase comprising of solvent A (0.1% FA water) and solvent B (acetonitrile)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

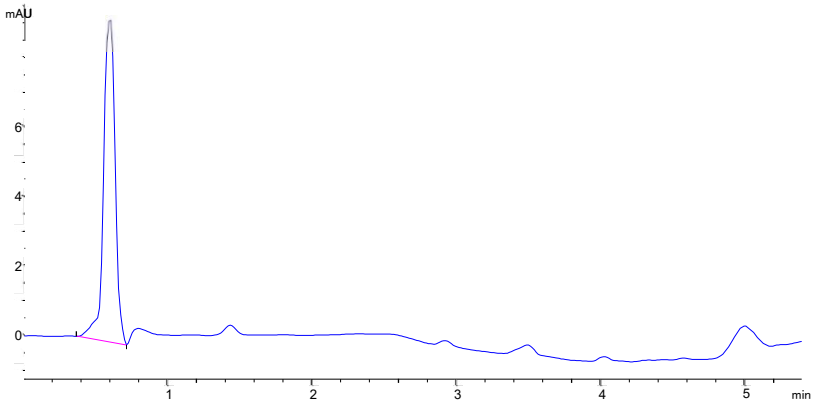


Figure S2: A chromatogram of blank egg sample. Chromatographic conditions: flow rate of 1.8 mL min−1; column temperature 40 °C; injection volume of 5 µL; wavelength of 265 nm. A binary mobile phase comprising of solvent A (0.1% FA in water) and solvent B (acetonitrile) with a gradient elution program of 10% B (0 - 1 min), 40% (1 - 4 min) and 60% B (4 - 6 min).

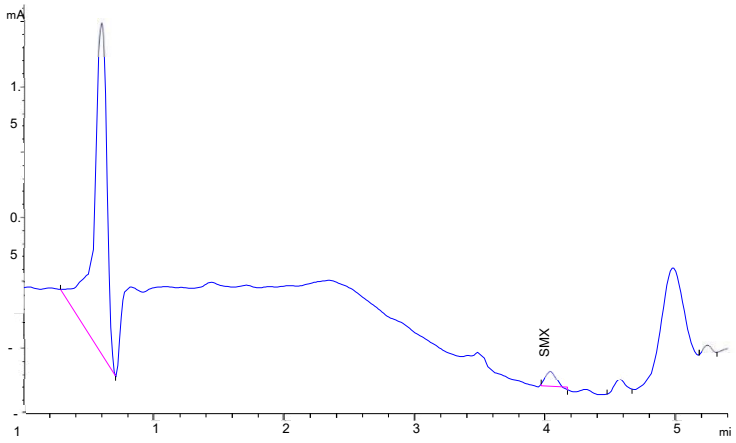
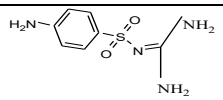
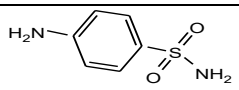
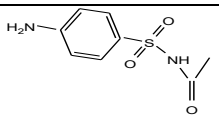
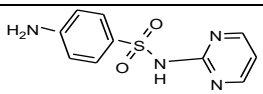
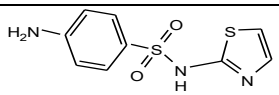
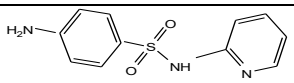
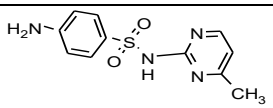
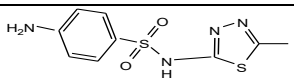
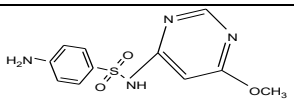
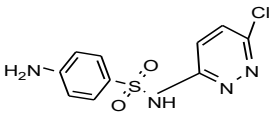
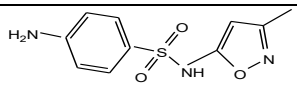
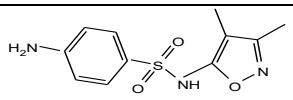
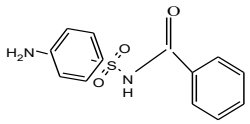
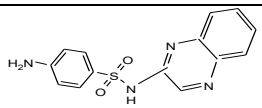
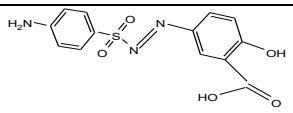


Figure S3: A chromatogram of real egg sample. Chromatographic conditions: flow rate of 1.8 mL min−1; column temperature 40 °C; injection volume of 5 µL; wavelength of 265 nm. A binary mobile phase comprising of solvent A (0.1% FA in water) and solvent B (acetonitrile) with a gradient elution program of 10% B (0 – 1 min), 40% B (1 - 4 min) and 60% B (4 - 6 min).

Table S1

Compounds	Structures	CAS Number	K_{OW}	pKa Values (pKa_1, pKa_2, pKa_3)	Reference
SGD		57-67-0	-1.07	0.5± 0.1; 0.4 ± 0.1; 3.3 ± 0.4	(Msagati and Nindi 2004)
SAM		63-74 - 1	-0.67	2.4; 10.4	(Dub etal 2011)
SAA		144 - 80-9	0.11	1.3 ± 0.1; 5.6 ± 0.5	(Msagati and Nindi 2004)
SDZ		68-35-9	0.81	1.6 ± 0.1;6.8 ± 0.50; 0.35 ± 0.2	(Msagati and Nindi 2004)
STZ		72-14 - 0	0.72	0.7 ± 0.1;7.8 ±0.5;2.3±0.5	(Msagati and Nindi 2004)
SPY		444-83-2	1.07	0.8±0.1;8.0±0.5, 2.90 ±0.5	(Msagati and Nindi 2004)
SMR		127- 97- 7	0.14	1.6 ± 0.1, 6.9 ± 0.5, 0.4 ± 0.1	(Msagati and Nindi 2004)
SMT		144 - 82-1	0.53	1.2± 0.1;7.0 ± 0.5	(Msagati and Nindi 2004)
SMM		1220 - 83-3	0.89	0.8 ± 0.1, 6.7 ± 0.5, 2.9 ± 0.4	(Msagati and Nindi 2004)
SCP		80-32-0	2.04	1.80, 5.70	(Msagati and Nindi 2004)
SMX		723- 46 - 6	0.89	1.4 ± 0.1, 7.7± 0.5	(Msagati and Nindi 2004)

SSO		127-69-5	1.01	1.50, 5.00	(Abdallah etal 2014)
SBZ		127 - 71 - 9	-	1.1±0.1; 5.90±0.5	Msagati and Nindi 2004)
SQZ		59 - 40 - 5	1.69	-1.4 ± 0.3 ,1.2 ± 0.1, 7.6 ± 0.3	(Msagati and Nindi 2004)
SSA		599 - 79 -1	3.8	1.9 ± 02; 2.9 ± 0.1; 1.2 ± 0.2; 7± 0.5	(Msagati and Nindi 2004)

Comparison of the proposed DLLME method for egg samples with other similar reported methods

The developed method was compared with the other reported methods such as pressurized liquid extraction followed by high-performance liquid chromatography coupled to tandem mass spectrometry (PLE-HPLC-MS/MS), pressurized liquid extraction coupled to liquid chromatography-tandem mass spectrometry (PLE-LC-MS/MS), liquid chromatography-mass spectrometry (LC-MS), solid-phase extraction coupled with ultra-high performance liquid chromatography-tandem mass spectrometry (SPE-UHPLC-MS/MS), matrix solid-phase dispersion and ultra-high performance liquid chromatography-tandem mass spectrometry (MSPD- UHPLC-MS/MS), QuEChERS-UHPLC-MS/MS, liquid-liquid extraction (LLE) coupled to UHPLC-MS/MS, molecularly imprinted polymer cartridges coupled to high-performance liquid chromatography with UV detection (MIP-HPLC-UV), DLLME-HPLC-DAD, –matrix solid-phase dispersion followed by HPLC with fluorescence detection (FLD) (MSPD-HPLC-FLD), modified QuEChERS-HPLC-FLD, Aqueous Two-Phase System ATPS Extraction HPLC-UV, Graphene-Functionalized Melamine Sponges Gmes Microextraction HPLC-DAD, Deep Eutectic Solvent Dispersive Liquid Microextraction DES-DLLME-HPLC-DAD, ionic liquids molecularly imprinted polymer solid phase extraction coupled with ultra-high performance liquid chromatography-tandem mass spectrometry IL-MIP-SPE-HPLC-Ms/Ms, and Pressure-Assisted Electrokinetic Injection Coupled With Capillary Zone Electrophoresis PAEKI-CZE-DAD methods, based on amount (volume) of solvent usage, linearity, recovery, extraction time and LODs. The data given in Table 5 confirmed that the developed method has a wider linear range, requires smaller volumes of organic solvents, and has a shorter extraction time than most of the reported methods. Most of the methods shown in the table 5, need additional technique for cleanup, which is more time consuming. Furthermore, a larger number of compounds with wide polarity ranges were analyzed in the proposed method. The other benefit of the proposed method is that the procedures are simple and inexpensive materials are used which could be accessible in any research laboratory. However, the recovery and LOD values were comparable.

Table 5: Comparison of the proposed DLLME method for determination of sulfonamide residues in egg samples with other similar methods reported in the literature

Method	Matrices	Compounds	Volume and type of solvents	Analysis time	Linearity (µg kg-1)	Recovery (%)	LOD (µg kg^{-1})	Reference
SPE HPLC-DAD	8SA	liver and muscle	20 mL ACN and 5 g anhydrous sodium sulfate.	> 40 min	0.1-100	7.5 and 16.2 µg kg−1,	70-108	Moga, etal 2021
ATPS extraction HPLC-UV	2SAsc	milk, egg and water	20mLof trichloroaceticacid(10%)	50 min	100-9000	2.92–3.64 (milk),2.90–3.49 (egg)	97.14–99.52 (milk),96.90–99.30 (egg)	Lu etal 2016

GMeS microextraction HPLC-DA	8 SAs	milk, egg and water	8.7 mL of DDW, 0.3 mL trichloroaceticacid aqueous solution (15% w/v) and-sodium chloride (6% w/v).,	30 min		.31–0.91 (milk), 0.96–1.32 (egg	90–105 (milk), 90–108 (egg)	Chatzimitakos etal 2017
SPE-LC-MS/MS	Egg	8As 4TC 5Qs 4SAs 4 Macs	10 mL of MeCN 1 mL of 0.5 M citric acid 0.5 mLof 0.1 M Na2EDTA	3 - 4 h	5-100	54-76 (SAs)	NR	(Frenich et al. 2010)
SPE-UHPLC-MS/MS	Egg	8As 4TC 5Qs 4SAs 4Macs	10 mL of 1% CH3COOH in MeCN	< 1 h	5-100	56-76 (SAs)	NR	(Frenich et al. 2010)
MSPD- UHPLC-MS/MS	Egg	8As 4TC 5Qs 4SAs 4Macs	3 mL MeOH 3mL MeCN 3mL CH3COOH solution in MeOH	3 - 4 hrs	5-100	62-89(SAs)		(Frenich et al. 2010)
Solvent Extraction with UHPLC-MS/MS	Egg	8As 4TC 5Qs 4SAs 4Macs	3mL MeOH 3mLMeCN 3mL NH3solution in MeOH	3 - 4 hrs	3-4 hrs	73-89 (SAs)	6.1 -114.2(decision limit for SAs	(Frenich et al. 2010)
MIP- HPLC-UV	Chicken Meat	4SAs	30mL CH2Cl2 10mL tolune 0.5 mL MeOH	> 1hr	0.5 - 150	93-105	0.1-0.5	(Karimi and Aboufazeli 2014
Modified QuEChERS-HPLC-FD	Chicken meat and egg	8SAs	MeCN	> 10 min	21.0-1000.0(muscle) 13.6-1000(egg)	65.9-88.1	5.8-19.9 (muscle) 13.6-1000,0 (egg)	(Huertas-Pérez et al. 2016)
DLLME-HPLC-DAD	Chicken Liver	7 FQs	5 mL of 25 mM H3PO4:MeCN (30:70) 200 µL CHCl3 1mL MeCN	<20 min	30.0 – 500.0	83.0 - 102,0	5.0 - 190	(Moema et al. 2012)
SPE-HPLC-FLD	5SAs	pig and poultry manure and digestate	Et0Ac/CAN-MECN/ MeOH(50/25/25) (V/V/V)	NR		13.53-23.30	77.00 -121.16	Osinski, 2022
DES-DLLME-HPLC-DAD	2SAs		v 2g of DES (tetrabutylammonium bromide, malonic acid and hexanoic acid (1:1:1, mol/mol))	30min		3(sulfamethoxazole) and 7(sulfamethazine)		Shishov etal 2020
IL-MIP-SPE-HPLC-ms/ms	21SAs	Egg	10µL IS aand 10 mL phosphate buffer	> 12 min	0.5-200	0.1-1.5	84.3-105.8	Suo etal 2022

DLLME-HPLC-DAD and DMSPE-HPLC-DAD	Swine Muscle	7 Qs	300 µL CH2Cl2 1.5 mL MeCN	< 20 min	30.0– 300.0	93 - 104.7 95.5 - 111.0	5.6 - 23.8	(Tsai et al. 2009)
PAEKI-CZE-DAD	6SAs	milk, pork and egg	20mM NaH^2PO4	NR	0.01-10µgmL-1	0.0018–0.0163 µg/mL,(milk) 0.0083–0.0638 µg/mL(pork) and 0.0052–0.0478 µg/(egg)	89-113	Yang, etal 2020
PLE -HPLC-MS/MS and PLE - LC-MS/MS	Muscles, livers and kidneys (swine, bovin and chicken)	18 SAs	MeCN 3 mL MeOH	> 5 min	NR	71.1 to 118.3	3.0	(Yu et al. 2011)
MSPD-HPLC-FL	Chicken Liver	7 SAs	8mL acetone 1 mL hexane	.> 30 mins	5 - 1000	> 84.6	NR	(Zhang et al. 2012
DLLME-HPLC-DAD	Egg	15 SAs	5mL0.05% FA: MeCN (15:85) 400 µL CH2Cl2 1mL MeCN	< 15 min	5.4 - 1000.0	73 - 108	4.3 – 8.0	Present study

MIP: molecularly imprinted polymer; DMSPE: dispersive micro-solid-phase extraction; MSPD: matrix solid-phase dispersion; FQs: fluoroquinolones; Qs: quinolones; Ant: anthelmintic; TC: tetracycline; Mac: macrolide; Na2EDTA: disodium ethylene diamine tetra acetic acid; ATPS aqueous two-phase system extraction; Gmes; graphene-functionalized melamine sponge microextraction; DES-DLLME-HPLC-DAD; deep eutectic solvent dispersive liquid liquid microextraction; IL-MIP-SPE-HPLC-Ms/Ms ionic liquids molecularly imprinted polymer solid phase extraction, and PAEKI-CZE-DAD; pressure-assisted electro kinetic injection coupled with capillary zone electrophoresis; NR: not reported

Conclusions

In the current study, dispersive liquid-liquid microextraction combined with high-performance liquid chromatography-diode array detection has been developed, validated and applied for the extraction, clean-up and quantitative determination of 15 sulfonamide residues in egg samples. The effects of various parameters were evaluated, and optimum conditions were established. Under optimum conditions, linearity was found in the concentration range of 5.4 - 1 000 µg kg-1 with regression coefficient of 0.9918 - 0.9987. Reasonable limit of detection (LOD) and limit of quantification (LOQ) values were achieved in the range of 4.3 - 8.0 µg kg-1 and 12.9 -24.0 µg kg- 1, respectively. Satisfactory intra-day and inter-day precision results in the range of 6.3 - 17.5% and 4.8 - 16.8%, respectively, were achieved.

The accuracy (percentage recovery) of the method was acceptable in the range of 73 - 108% with %RSD values of 1.1 to 16.5%. Based on these findings, the developed method was applied to real samples, which were obtained from supermarkets. Sulfamethoxazole (SMX) was detected in all analyzed samples in the range of 9.56 - 46.3 µg kg-1 with the corresponding %RSD values ranging from 8.8 to 15.2% indicating non-compliance as sulfonamides are prohibited in laying hens [3]. Since there is a small peak present at the same retention time as #11 in the blank egg sample, additional confirmation of the presence of SMX (by MS) would be recommended for future work. However, these findings have indicated the need for comprehensive analysis to be considered in order to draw a valid conclusion about the presence of SA residues in the various brands of eggs available in the retail market.

Compared with other reported methods, the developed method has several advantages, such as wide linear range, consumption of a small volume of solvents, shorter analysis time, and the capability to analyze larger numbers of analytes with a wide range of polarities. Thus, it has been concluded that the proposed method is feasible and can be used as an alternative method for the analysis of sulfonamide residues in egg and related complex biological matrices [38-47].

Data availability

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

Conflict of interests

All authors declare that they have no conflict of interest

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