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Research Article

Dispersive Liquid - Liquid Micro extraction Technique Coupled with High Performance Liquid Chromatography Diode Array Detector for the Determination of Sulfonamides in Water Samples

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Abstract:

A dispersive liquid-liquid microextraction combined with high-performance liquid chromatography with diode array detection (DLLME-HPLC-DAD) has been developed for the determination of 15 sulfonamides in water samples. The effects of experimental parameters on the performance of the method such as the type and volume of extractionand disperser solvents, pH of the solution, effect of salt, and centrifugation time were evaluated, and optimum conditions were established. Under these optimum conditions, linearity was found in the range of 2 - 1 000 µg L-1 with regression coefficients better than 0.9990. The limit of detection (LOD) and limit of quantification (LOQ) valueswere in the range of 0.6 - 7.8 µg L-1 and 1.5 - 8.3 µg L-1, respectively. Intra-day and inter-day precision ranged from 1.5 - 9.7% and from 3.1 - 9.9%, respectively. The accuracy of the method were also evaluated in samples from three different sources of water (river water, groundwater, and tap water) were found to range from 70.5 to 103.9% with %RSD values ranging from 0.5 to 9.8%, with the exception of sulfaguanidine in river water and groundwater where the percentage recovery ranged from 54.3 - 84.9% at the two higher concentration levels (100 and 400 µg L-1).Furthermore, the effect of sample matrix on the extraction of target compounds was evaluated using the relative recovery (%RR) of the samples from three different water sources relative to the ultrahigh purity (UHP) water sample spiked with target analytes, and found in the range of 70.3 - 106.1%.The proposed method was successfully applied to wastewater treatment plant samples and Sulfisoxazole (SSO) was detected in the influent samples in the range of 14.8 - 17.8 µg L-1 with corresponding %RSD, (n = 5) value of 1.6% to 1.9%.The results indicated that the proposed method is effective for the extraction and determination of the sulfonamides in water samples.

Key words: dispersive liquid-liquid microextraction (DLLME); sulfonamides (SAs); HPLC-DAD

Introduction

Currently, the use of antibiotics in human and veterinary medicine has become a common practice that has led to the gradual accumulation of antibiotics in the environment, which enter through sources of contamination such as wastewater, landfills, urban sites, as well as industrial and hospital effluents.(Manzetti and Ghisi 2014) Furthermore, due to the limited knowledge of the input, fate, and effects of most antibiotics in the environment, there is no regulation on the levels of these compounds in environmental matrices, including water systems, sediment, and soil (Kümmerer 2009)However, concerns about the occurrence, transport, and fate of veterinary drugs in the environment

have been ever increasing in recent years, and several initiatives have been launched to establish or strengthen the monitoring systems.(Seifrtová, Pena et al. 2008, Pan, Qiang et al. 2011, Iglesias, Nebot et al. 2013)

Sulfonamides may enter natural systems following different paths, via discharge from wastewater treatment plants (WWTPs), leakage from septic systems and agricultural waste storage facilities as well as the application of human and agricultural waste to land to supplement fertilisers.(García-Galán, Silvia Díaz-Cruz et al. 2008, Kümmerer 2009, Baran, Adamek et al. 2011) Sulfonamides are fairly soluble in water and their ionisation is highly dependent on the pH of the matrix (structures, K_{OW}. and pKa values are shown in Table S1, supplemental material). Thus, after disposal in soils they may enter surface runoff or be leached into the groundwater. Furthermore, these compounds may not be completely metabolised and a high proportion of them are possibly excreted directly in faeces (digested wastes) and urine. Therefore, both the parent compounds and their metabolites can be released directly to the environment.(Białk-Bielińska, Stolte et al. 2011) It is therefore important to develop simple, efficient, and eco-friendly analytical methods for the monitoring of these drug residues. Various methods have been developed for the determination of SA residues, such as gas chromatography-mass spectrometry (GC-MS),(Chiavarino, Crestoni et al. 1998) capillary electrophoresis with ultraviolet detection (CE-UV),(Wen, Li et al. 2011)and high-performance liquid chromatography (HPLC) with ultraviolet (UV),(Chitescu, Nicolau et al. 2011) fluorescence detection (FD),(Mor, Sahindokuyucu Kocasari et al. 2012, Arroyo-Manzanares, Gámiz-Gracia et al. 2014) MS or MS/MS,(Senta, Terzić et al. 2008, García-Galán, Díaz-Cruz et al. 2010, Tölgyesi, Berky et al. 2013) and electrochemical methods.(Fotouhi, Fatollahzadeh et al. 2012).

Since sulfonamides are found at very low (μg L^{-1}) levels and in most cases more than one type of compound (parent and metabolites) would be determined, efficient sample preparation (extraction, enrichment, and/or clean up) techniques are mandatory prior to instrumental analysis. Liquid-liquid extraction,(Koesukwiwat, Jayanta et al. 2007) solid-phase extraction,(García-Galán, Díaz-Cruz et al. 2010)solid-phase microextraction, (Lu, Chen et al. 2007) and pressurised liquid extraction(Yu, Tao et al. 2011) have thus been widely used as sample preparation methods for the extraction of sulfonamides from environmental and food matrices. Solid-phase microextraction (SPME) offers simpler steps, compatible with GC and LC separation techniques, and it is almost solvent-free, but it has inherent drawbacks in that the fibre can break easily, the fibre coating can be damaged, and the fibre assembly is relatively expensive.(Schmidt and Podmore 2015) On the other hand, pressurised liquid extraction (PLE) requires expensive instrumentation, and it is less selective.(Yu, Tao et al. 2011)

In recent years, liquid-phase microextraction (LPME) techniques have become widely applied techniques in analytical research areas,(Sarafraz-Yazdi and Amiri 2010, Asensio-Ramos, Ravelo-Pérez et al. 2011, Prosen 2014) due to their consumption of very small volumes (μL) of extraction solvent; one of the objectives of green chemistry is addressed by minimising the amount of solvent usage (miniaturisation) in the analytical process.

Among the above-mentioned recently developed sample preparation techniques, dispersive liquid-liquid microextraction (DLLME) is of particular interest due to the fact that the technique has several advantages, including simplicity of operation, rapidity, low cost, high recovery, and enrichment factors.(Rezaee, Assadi et al. 2006, Yan and Wang 2013). In DLLME, extraction is carried out between the sample and a cloud of fine extractant droplets formed when the mixture of extraction solvent, which is water immiscible, and the disperser solvent, which is miscible with both aqueous solvents and extraction solvents, is injected rapidly into an aqueous sample containing the analytes of interest.(Yan and Wang 2013). A very high contact area is generated between the aqueous phase and the extraction solvent; thus, analytes are rapidly extracted into the extraction phase.(Yan and Wang 2013). Therefore, analytes have to be in their neutral form for them to efficiently partition between the aqueous phase and the hydrophobic organic phase (extraction solvent). This explains why the technique was initially applied to neutral or non-polar organic compounds such as PAH which have larger octanol-water partition coefficient (K_{OW}) values. However, the application of DLLME for polar analytes poses a challenge, since they will rather remain in the aqueous phase than be extracted into the organic solvent. These analytes are soluble in water mainly in their ionised form; therefore, appropriate adjustment of sample pH is necessary to suppress ionisation of target analytes. Furthermore, by using an appropriate amount of salt to induce a "salting-out effect", the extractability of polar analytes can be improved as their solubility in the aqueous phase is reduced. Compounds with a wider range of polarity like sulfonamides present yet another dimension of complexity. As a result of such challenges, most of the reported DLLME methods often focused on the less polar group of sulfonamide compounds(Wen, Li et al. 2011, Xu, Su et al. 2011, Li, Li et al. 2016, Wang, Li et al. 2022) with a few exceptions occasionally being reported for some polar group of sulfonamides such as Herrera-Herrera et al.(Herrera-Herrera, Hernández-Borges et al. 2013) in 2013 reported for the determination of 11 sulfonamides together with 14fluoroquinolones using DLLME coupled with UHPLC-DAD. However, most of the reported methods require longer extraction times or use of advanced instrument. Furthermore, in some cases a higher volume of solvents and detection limits were reported. Therefore, it is desirable to develop a fast, cost effective and sensitive analytical method for simultaneous extraction and determination of wide number of sulfonamides with diverse polarity ranges.

Thus, the aim of the current study was to develop a fast, cost effective and sensitive DLLME method coupled with HPLC –DAD for the simultaneous extraction and determination of 15 sulfonamide compounds in diverse water samples. The effects of various parameters on the extraction efficiencies of target compounds such as the type and volume of extraction and disperser solvents, pH of the solution, effect of salt addition and centrifuge time for DLLME procedure were evaluated and optimum conditions were established. The performance of the developed method was validated and applied to wastewater samples. Results revealed that the proposed method is effective to be used for routine monitoring of sulfonamides and related drugs in diverse water samples

2. Methods and Materials

2.1. Standards and chemicals

Antibiotic standards included sulfaguanidine (SGD), sulfanilamides (SAM), sulfathiazole (STZ), sulfacetamides (SAA), sulfamethizole (SMT), sulfamethoxazole (SMX), sulfasalazine (SSA), sulfamonomethoxine (SMM), sulfaquinoxaline (SQX), sulfamerazine (SMR), sulfapyridine (SPY), sulfadiazine (SDZ), sulfabenzamide (SBZ), sulfachloropyridine (SCP), and sulfisoxazole (SSO), and were purchased from Sigma-Aldrich (Steinheim, Germany). All standards had purity 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, 1,2-dichloroethane and 1,1,2,2-tetrachloroethane were of HPLC grade and were purchased from Sigma-Aldrich (Steinheim, Germany). Ultrahigh purity (UHP) water (resistivity, 18.2 MΩ·cm at 25 °C) was generated using the Milli-Q® system (Millipore, Billerica, MA, USA).

2.2. Sampling of tap water, groundwater, river water and wastewaters from wastewater treatment plant (influents and effluent)

For the purposes of validating the proposed method, four different types of water samples were collected using 2.5 L sampling bottles: Tap water I sample from Pretoria, South Africa; tap water II sample from Florida, South Africa; river water sample from Suikerbosrand River (Gauteng, South Africa); and groundwater sample from Florida, South Africa. Wastewater treatment plant samples (influent and effluent) were collected from the *Daspoort* Wastewater Treatment Plant (WWTP) located at latitude 25.7345^0 south and longitude 28.1781^0 East coordinates in Pretoria, Gauteng Province, South Africa. These samples were collected from three different locations: -i) before entry into the treatment plant (Influent I); ii) before entry into the treatment plant from a different location (influent (II); and iii) after treatment at the outlet (effluent). The grab sampling method was used, and the samples were collected into amber glass bottles, which had previously been acid-washed and rinsed with UHP water and then flushed with wastewater before collection. Each sample was collected in triplicate into 2.5 L bottles. The water samples were filtered through Whatman filter paper and 0.45 µm nylon filters, respectively, and then stored in amber glass bottles in a refrigerator at 4 °C until analysis.

2.3. Instrumentation

An Agilent 1260 series high-performance liquid chromatographic system (Agilent Technologies, Waldbronn, Germany) was used for all separations. The HPLC consisted of a binary pump, vacuum degasser, thermostatted column compartment, auto-sampler, 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), centrifuge (Thermo Electron Corporation, Massachusetts, USA) and a micro balance (Mettler Toledo XP6U) were used for sample preparation. A Hamilton® 500 µL syringe (Hamilton Bonaduz AG, Switzerland) was used for withdrawing the settled phase in the DLLME procedure and nitrogen gas was used for drying the samples.

2.4. Preparation of sulfonamide standard solutions

Stock standard solutions (1 000 mg L^{-1}) for each compound were prepared by dissolving10 mg of accurately weighed standard of each compound in 10 mL of a mixture of methanol and ultrahigh purity water (1:1; v/v). 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 aluminium foil and were kept at 4 °C until use.

2.5. Optimisation of chromatographic conditions for the separation of 15 sulfonamide compounds

A chromatographic method for the separation of 15 sulfonamides was first developed and optimised. To investigate the conditions for optimumpeak shape and adequate resolution in the separation of target analytes, both ultrahigh purity (UHP) water and acidified ultrahigh purity water were evaluated as a solvent A and organic solvents (acetonitrile and methanol) as a solvent B. 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. Both isocratic and gradient elution modes were investigated Furthermore, the column oven temperature was also optimised in the range of 25 - 45 ºC and compounds were monitored using DAD at different wavelengths (260, 265, 270 and 280 nm).

2.6. Optimisation of DLLME conditions

In DLLME, the factors that can affect the efficiency of extraction include type and volume of extraction and disperser solvent, the pH of the solution, salting-out effect and the extraction time. A disperser solvent must disperse the extraction solvent (form fine droplets) into the aqueous solution while the volume of extraction and disperser solvents would have a significant effect on the enrichment factor (EF). Therefore, it is important to optimise all the factors in order to obtain good performance.

2.6.1. Evaluation of extraction solvent

In DLLME it is very important to select an appropriate extraction solvent that is capable of extracting the target compound, has a low solubility in water, good chromatographic behaviour (i.e. should not absorb at the wavelength of target analytes), has a higher density than water, and is able to form two phases in the presence of a disperser solvent.(Rezaee, Assadi et al. 2006, Schmidt and Podmore 2015). Based on the above criteria, dichloromethane, CH_2Cl_2 (1.32 g mL^{-1}), trichloromethane, $CHCl_3$ (1.47 g mL^{-1}), 1,2-dichloroethane, $C_2H_4Cl_2$ (1.25 g mL^{-1}), and 1,1,2,2-tetrachloroethane, $C_2H_2Cl_4$ (1.59 g mL^{-1}) were selected as potential solvents for this study. The extraction solvents wereevaluated at a volume of 600 µL and using 1 mL of MeCN as the dispersersolvent; these are conditions adapted from previous reports in the literature.(Rezaee, Assadi et al. 2006, Wen, Li et al. 2011, Xu, Su et al. 2011, Yan and Wang 2013, Li, Li et al. 2016).

2.62. Effect of the volume of extraction solvent

The volume of the extraction solvent has an impact on the enrichment factors. If very low volumes are used, the extraction solvent may not be distributed well in the aqueous phase resulting in low enrichment factors. On the other hand, high volumes of extraction solvent may lower the optimum ratio of the disperser solvent to the extraction solvent volume. This consequently reduces the amount of droplets formed which are necessary for the extraction process thereby lowering the enrichment factors.(Saraji, Khalili Boroujeni et al. 2011) Therefore, the effect of extraction solvent volume on the enrichment factor was investigated over a range of 200 - 1 000 µL, while all other experimental parameters were maintained constant.

2.6.3. Evaluation of disperser solvent

Evaluation of an appropriate disperser solvent is important since it can affect the enrichment factors of target analytes. Disperser solvents must fulfil the condition of being miscible with both water and the extraction solvents.(Rezaee, Assadi et al. 2006)

In this study, methanol, acetonitrile, ethanol and acetone were selected as potential disperser solvents and their effect on the enrichment factor was evaluated using 1 mL of each solvent and 600 µL of dichloromethane as the extraction solvent.

2.6.4. Effect of the volume of disperser solvent

To determine the optimum volume of the disperser solvent, various volumes (200 - 1 200 µL) of acetonitrile containing a fixed volume of dichloromethane (400 µL) were evaluated.

2.6.5. Effect of the solution pH

In principle, DLLME is most appropriate for extracting neutral or non-charged compounds from aqueous to organic phase, thus in a way, making it a limited technique. Therefore, pH control becomes essential to extend is applicability to ionic and polar compounds such as sulfonamides. Theoretically, SAs are positively charged in acidic medium at two pH units below their acidic pKa values, neutral at pH between pKa_1 and pKa2, and negatively charged at 3.3 pH units above their basic pKa_2 values. The pKa_1 values of target compounds are in the range of 0.9 - 2.5

and their pKa_2 values are in the range of 5.3 - 11.3. One of the challenges when dealing with analytes of diverse polarity is to find a suitable pH in which the majority of target analytes can be extracted in their neutral form. Therefore, the selected pH must be adequate for the extraction of all analytes. Thus, the effect of the solution pH was evaluated by varying it in the range of 2.5 - 7.5 using HCl (0.1 M) and/or NaOH (0.1 M) and keeping the other conditions constant.

2.6.6 Effect of salt addition

The addition of salt into the sample solution decreases the solubility of the analytes, thus resulting in the transfer of analytes into the organic phase. Therefore, the effect of salt on the enrichment factors of target compounds was tested by adding NaCl in the range of 0 - 8 % (w/v), while keeping all the other experimental conditions constant(Xu, Su et al. 2011).

2.6.7 Effect of centrifugation time

Adequate centrifugation time is important for the formation of phase separation. A very long centrifugation should be avoided because a centrifuge generates heat as it rotates, which can destabilise phase separation. Therefore, in this study, the effect of centrifugation time was evaluated in the range of 1 - 10 min.

2.7. Dispersive liquid-liquid microextraction procedure for water samples

Into 15 mL screw cap centrifuge tubes, 5 mL of ultrahigh purity (UHP) water, with the pH previously adjusted to 3.5 using 0.1 M HCl that contained 0.10 g (2%; w/v) NaCl, was transferred, and spiked with a mixture of SAs at a concentration of 100 µg L^{-1}. Thereafter, the mixture of extractant and disperser (400 µL and 600 µL, respectively) was rapidly injected into the aqueous solution containing the sample using an aseptic disposable syringe. The mixture was then vortexed for 30 s to ensure complete dispersion and to facilitate the extraction. The dispersed fine particles of the extraction phase, which had settled at the bottom of a screw cap centrifuge tube, were transferred to a 1.5 mL HPLC vial (Agilent, Germany) through a 400-µL vial insert using a Hamilton 500-µL microsyringe and dried under a gentle stream of nitrogen gas. Thereafter, the remaining residue was reconstituted with 100 µL of the mobile phase and subjected to chromatographic analysis.

2.8. Chromatographic conditions for the separation of 15 sulfonamide compounds

The chromatographic conditions for the separation of 15 SAs were as follows: 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. The column oven temperature of 40 °C and DAD monitoring wavelength of 265 nm was used throughout the analysis.

3. Results and Discussion

For chromatic separation of sulfonamide compounds, optimum resolution and peak shape were obtained at a flow rate of 1.8 mL min^{-1}. 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). Satisfactory peak shapes and resolution were achieved using a gradient elution program consisting of 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. Furthermore, best separation was observed at a temperature of 40 °C and at a detection wavelength of 265 nm, all target analytes were detected with good sensitivity. Therefore, 265 nm was selected as the detection wavelength. The chromatogram obtained after optimising all chromatographic parameters is shown in **Figure 1**, with baseline resolution of all 15 Sulfonamides within 5.20 min, thus allowing for quantitation.

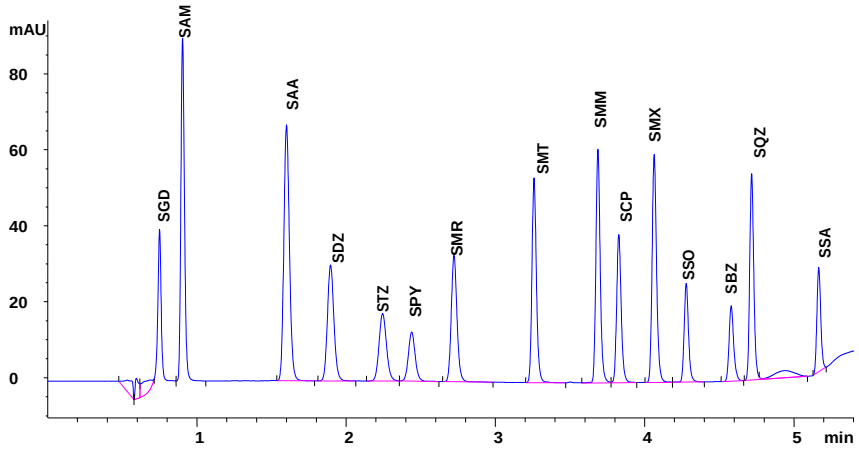

Figure 1: A chromatogram of 15 separated sulfonamide standards (100 µg L-1) at 265 nm. Chromatographic conditions: flow rate of 1.8 mL min−1, column temperature 40 °C, injection volume of 5 µL, wavelength of 265 nm. A binary mobile phase comprising of solvent A (0.1% FA water) and solvent B (acetonitrile)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

On the other hand, experimental results for optimization of extraction solvent type shown in **Figure 2** revealed that the enrichment factors of most target analytes obtained using dichloromethane (DCM) and 1,2-dichloroethane (DCE) were not significantly different at $p = 0.05$ significance level (t-test).

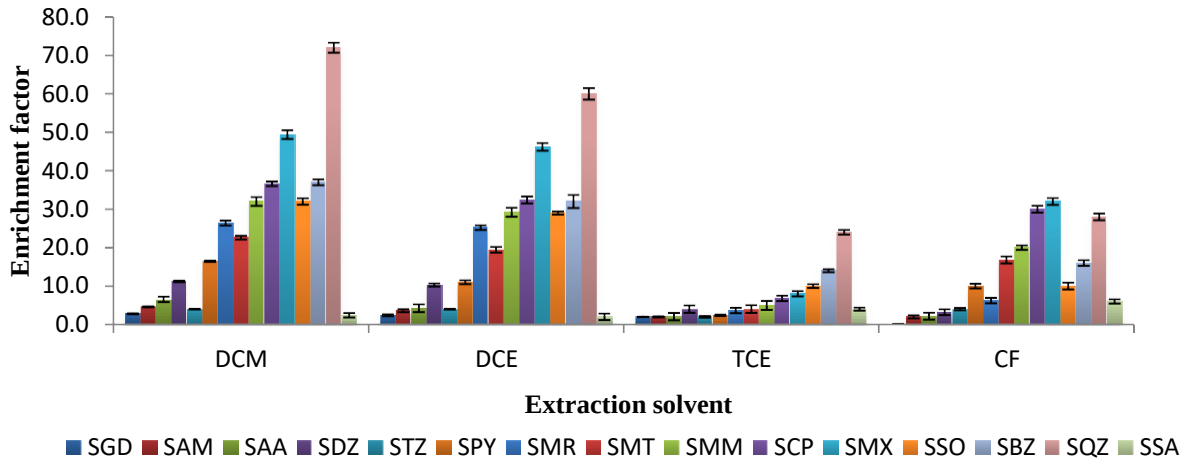

Figure 2: The effect of extraction solvent type on the enrichment factors of analytes. Extraction conditions: sample volume, 5.0 mL; disperser solvent type and volume; acetonitrile, 1.0 mL; extraction solvents (dichloromethane, dichloroethane, trichloroethane and chloroform) and volume 600 µL; spiked concentration, 100 µg L-1; n = 5.

However, significant differences (at p = 0.05) in enrichment factors for SPY and SQZ were observed using DCM. Thus, based on the experimental results, dichloromethane (DCM) was selected as an extracting solvent for the subsequent experiments. A similar trend was observed for the extraction of polar analytes (i.e., fluoroquinolones) in previous reports in the literature.[38] In the case of dispersive solvent type selection, results shown in **Figure 3** confirmed that the highest enrichment factors, in the range of 1.6 for SGD (more polar) to 32.0 for SQZ (less polar) were obtained when acetonitrile was used as a disperser solvent.

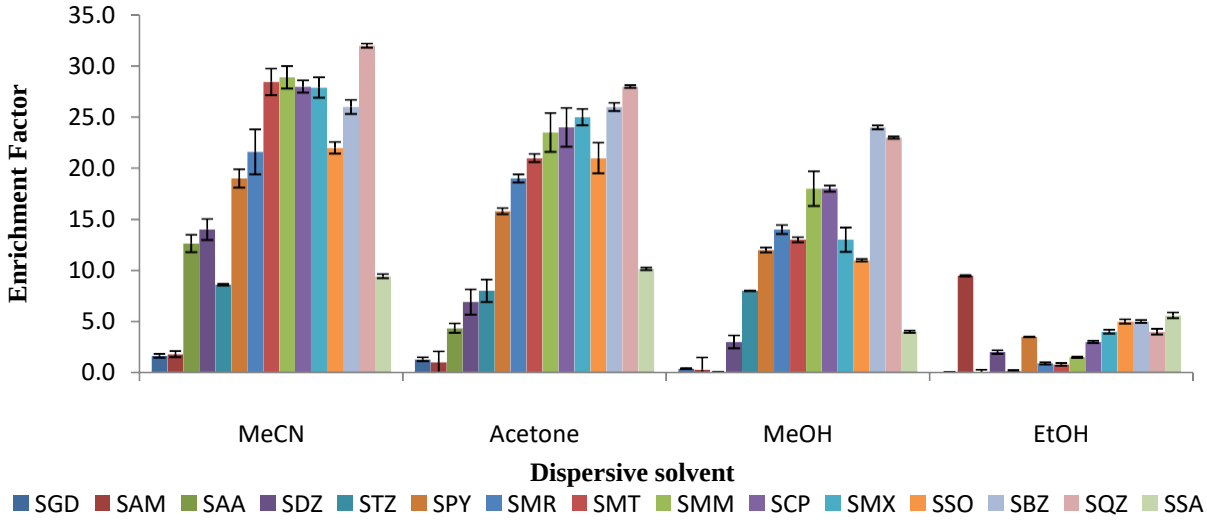

Figure 3: The effect of disperser solvent type on the enrichment factor of analytes. Extraction conditions: sample volume, 5.0 mL; types of disperser solvents (acetonitrile, acetone, methanol and ethanol) volume, 1.0 mL; extraction solvent and volume, (dichloromethane, 600 µL); spiked concentration, 100 µg L-1; n = 5

Acetone and methanol gave enrichment factors in the range of 1.3 (SGD) to 28.0 (SQZ) and 0.4 (SGD) to 24.0 (SBZ), respectively. The lowest enrichment factors for most analytes (0 to 9.6) were observed when ethanol was used as the disperser solvent. The miscibility of DCM and MeCN which resulted in the formation of distinct fine droplets (i.e.confirmation of improved large surface area) could explain why it performed better than the other solvents. On the other hand, ethanol could not disperse the extraction solvent well as confirmed by the poor formation of the cloudy solution. Thus, based on experimental results, acetonitrile was selected as the disperser solvent for the subsequent experiments.

Experimental results obtained for evaluation of the volume of extraction and dispersive solvents shown in **Figure 4** and **Figure 5** respectively revealed that the enrichment factors of all analytes increased as the volume of the extraction solvent increased from 200 - 400 µL.

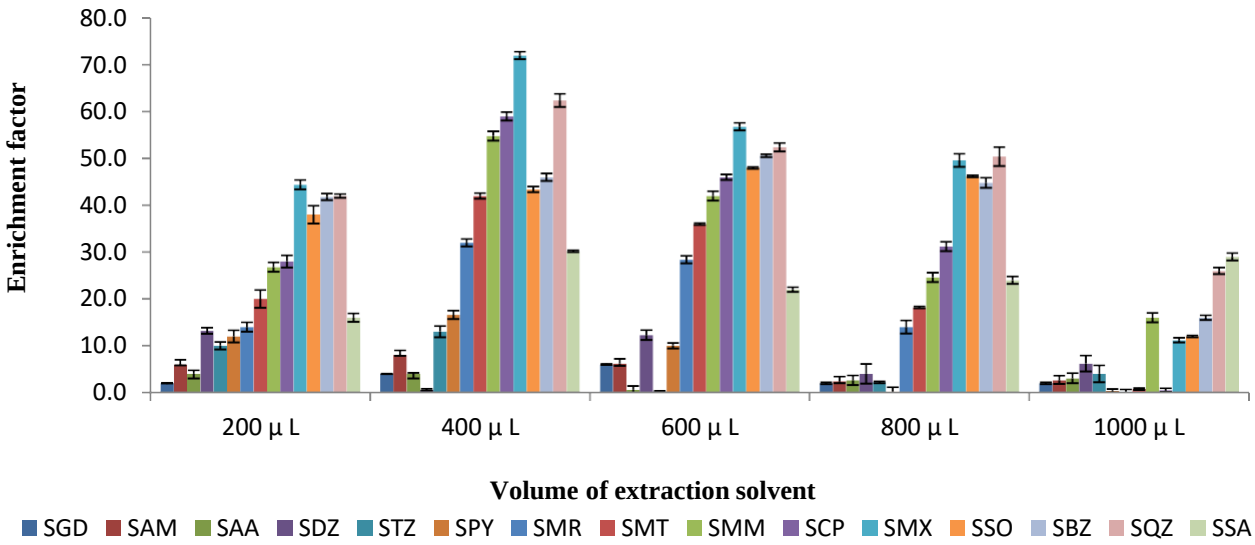

Figure 4. Effect of volume of extraction solvent on the enrichment factor of analytes. Extraction conditions: sample volume, 5.0 mL; disperser solvent type and volume, acetonitrile, 1.0 mL; extraction solvent and volume (dichloromethane, 200-1 000 µL); spiked concentration, 100 µg L-1; n = 5.

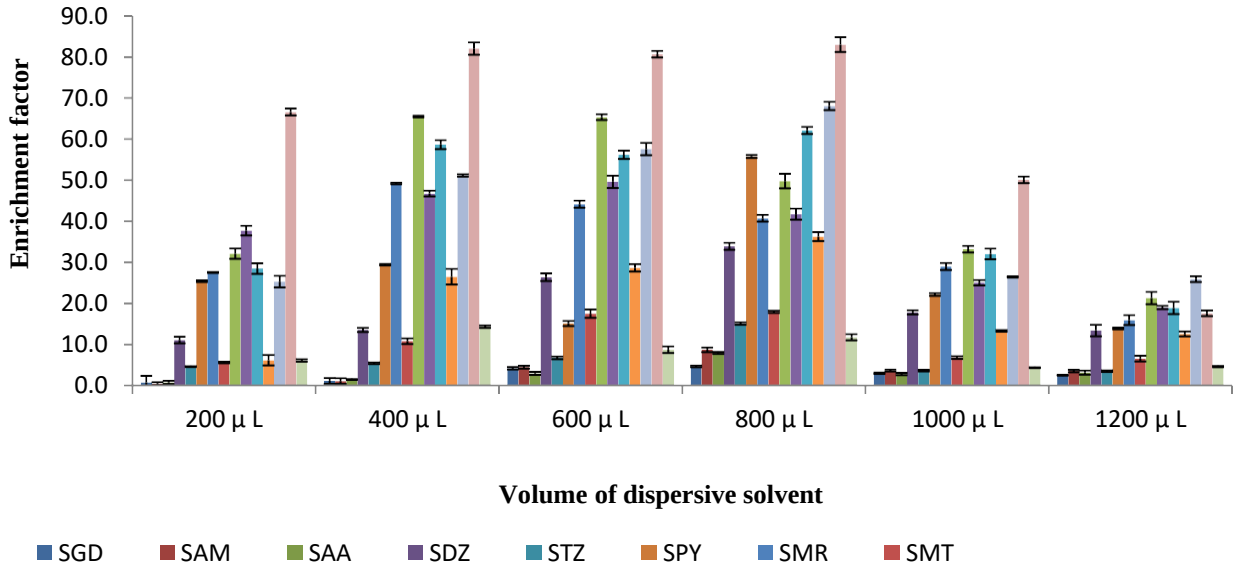

Figure 5: Effect of volume of disperser solvent on the enrichment factor of analytes. Extraction conditions: water sample volume, 5.0 mL; disperser solvent and volume, acetonitrile, (200–1 200 µL); extraction solvent and volume; dichloromethane, 400 µL; spiked concentration, 100 µg L-1; n = 5.

However, the enrichment factors gradually decreased after 400 µL probably due to the decrease in the volume ratio of the disperser to the extraction solvent. Therefore, 400 µL was selected as the optimum volume of dichloromethane for the subsequent experiments. Similarly, it was observed that at a lower volume of acetonitrile (i.e., below 200 µL) no cloudy solution was formed. The most probable reason could be that at low disperser solvent volume, the extraction solvent could not be dispersed well in aqueous solution. However, between 200 µL and 800 µL, the enrichment factors increased and then decreased at higher volumes of acetonitrile. The lower values of enrichment factors at higher disperser solvent volumes could be due to the solubility of the target compounds into the aqueous phase as the disperser solvent partitions more into the aqueous phase.[39-41] Furthermore, as shown in **Figure 5**, there were no obvious differences in the enrichment factor values obtained at 600 µL and 800 µL. Therefore, in view of the environmental benefits of green chemistry, the lower volume of 600 µL was selected.

On the other hand, results shown in **Figure 6** confirmed that the enrichment factors obtained between pH 2.5 and 4.5 for most polar analytes (SGD, SAM, SAA) were very low (1.8 - 9.82) which indicate that these analytes were not extracted well.

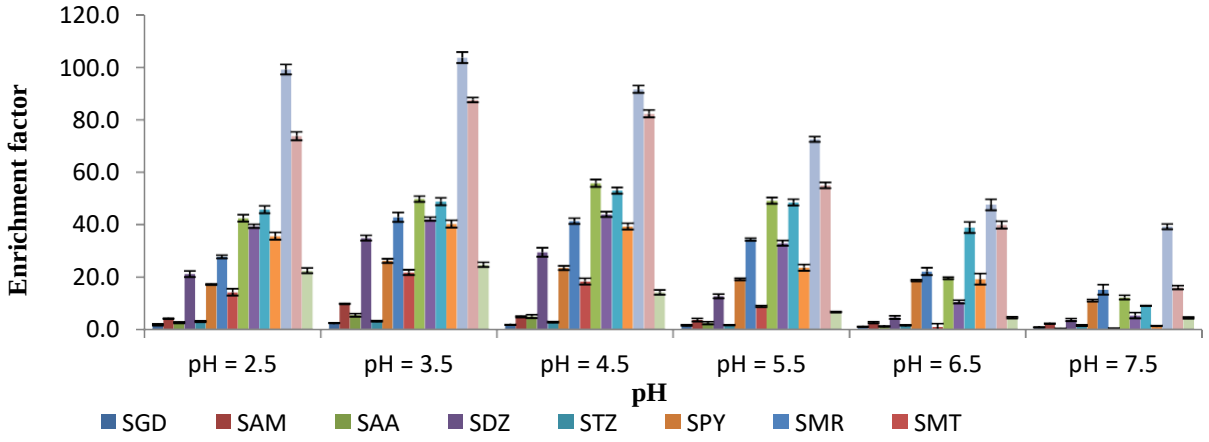

Figure 6: Effect of the sample pH on the enrichment factor of sulfonamides. Extraction conditions: sample volume, 5 mL whose pH adjusted from 2.5 to 7.5; spiked concentration, 100 µg L-1; extraction solvent type and volume, 400 µL of dichloromethane; disperser solvent type and volume, 600 µL of acetonitrile; n = 5.

The poor enrichment factors observed for SGD, SAM, and SAA are due to their very low K_{OW} values (i.e., -1.07 to 0.11), making it difficult to extract them using relatively less polar organic solvents. The low enrichment factors ranging from 2.80 to 3.22 exhibited by STZ may be attributed to the three pKa values (0.7 ± 0.1, 7.8 ± 0.5, and 2.3 ± 0.5) which makes it difficult to establish a specific pH where the analyte exists as neutral. However, slightly improved EF values were obtained at pH 3.5 for all four analytes. All relatively less polar analytes were extracted well from pH 2.5 to 4.5 due to their relatively high K_{OW} values, with the exception of SSA. While it is the least polar (K_{OW} = 3.8) it would have been expected to perform better. However, the relatively lowerenrichment factors (14.2 - 27.95) may be due to its four pKa values (1.9 ± 02; 2.9 ± 0.1; 1.2 ± 0.2; 7 ± 0.5), thus again posing a challenge to obtain a suitable pH. Based on the experimental results, pH 3.5 was selected as optimum pH for the subsequent experiments.

Results for the effect of adding salt on the extraction efficiency of target compounds shown in **Figure 7** indicate that enrichment factors for all target analytes showed some improvement when 0.1 g (2%, w/v) of NaCl was added than when the extraction was done without the addition of salt.

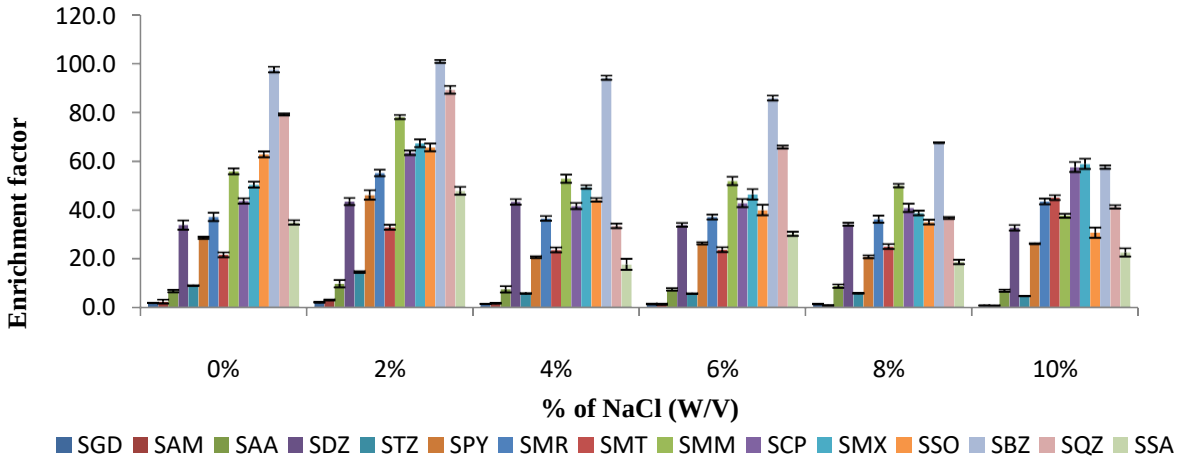

Figure 7: Effect of salt addition. Extraction conditions: sample volume, 5 mL; spiked concentration, 100 µg L-1 at pH, 3.5; extraction solvent type and volume, 400 µL of dichloromethane; disperser solvent type and volume, 600 µL of acetonitrile; n =5.

The t-test was used to verify the significance of adding salt. The statistical test at (p = 0.05 level) confirmed that the enrichment factors obtained for ten analytes (SDZ, STZ, SPY, SMR, SMT, SMM, SCP, SMX, SQZ and SSA) using 2% NaCl were significantly better than those obtained at 0% NaCl. There was, however, no significant gain (at p = 0.05 level) in the enrichment factors of the other five analytes (SGD, SAM, SAA, SSO, and SBZ). For extremely polar compounds (SGD, SAM, and SAA) with their highly hydrophilic nature (very low K_{OW} values), the addition of salt may not overcome their affinity for water, hence explaining their poor extractability. However, the latter two analytes were relatively less polar, and a salting-out effect may not have such a significant effect on their extraction. It was noted that the enrichment factors declined after addition of 0.2 g (4%, w/v) NaCl. The lower values of enrichment factors observed at higher salt concentrations could be attributed to the increased viscosity of the sample solution, which reduced the mass transfer process of target analytes from the aqueous phase to the organic phase. Thus, 0.1 g (2%, w/v) NaCl was selected as the optimum quantity of salt for the subsequent experiments. Xu et al. also found that 3% NaCl was sufficient to induce a salting-out effect in the extraction of sulfonamides from various matrices. Experimental results shown in **Figure 8** revealed that the enrichment factors of target compounds increased when the centrifugation time was increased to 3 min and then remained constant up to 5 min. However, at longer centrifugation times, a gradual decrease in EFs was observed, due to the dissolution of the settled phase. Thus, 3 min was selected as the optimum centrifugation time for complete phase separation.

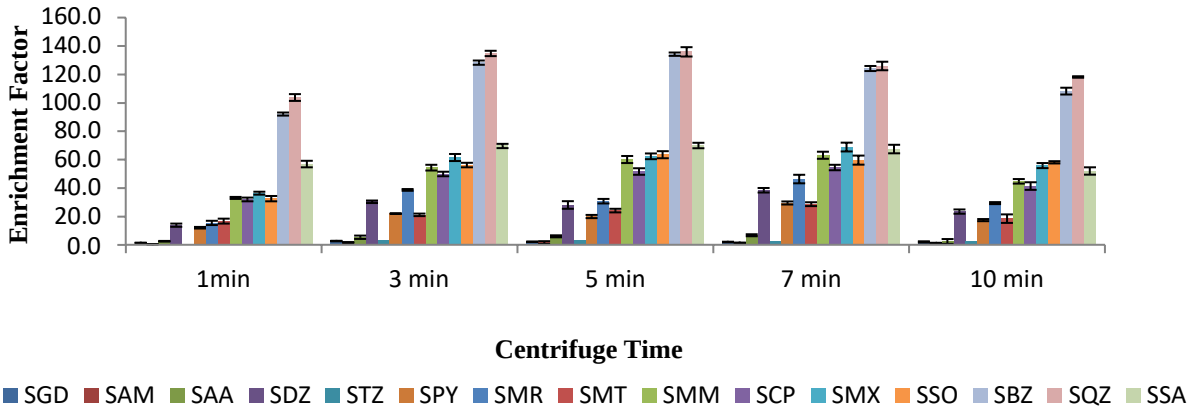

Figure 8: Effect of centrifugation time. Extraction conditions: sample volume, 5 mL; spiked concentration, 100 µg L-1 at pH, 3.5; 2% NaCl (w/v); extraction solvent type and volume, 400 µL of dichloromethane; disperser solvent type and volume, 600 µL of acetonitrile; n = 5; and centrifuge speed: 4 000 rpm.

Under the optimum conditions, the enrichment factors (EFs) for most of the polar analytes (SGD, SAM, SAA, and STZ) still remained low (1.6 - 5.5) indicating that these conditions were not favourable for their efficient extraction. This could be due to their very low K_{OW} values (SGD, SAM, SAA) and probably for STZ due to its three pKa values which makes it difficult to find a pH at which these analytes remain uncharged (neutral). Msagati and Nindi(Msagati and Nindi 2004) also reported similar challenges when extracting STZ, SBZ, and SDZ, although acceptable EFs for the latter two compounds were recorded in our work (30.3 - 128.3) shown in **Figure 8**.

4. Method validation

Under optimised conditions, the developed method was validated using parameters such as linearity, limit of detection (LOD), limit of quantification (LOQ) and precision (intra-day and inter-day). Linearity was evaluated using calibration standards prepared by spiking ultrahigh purity (UHP) water at a concentration range of 0.5 - 2 000 µg L^{-1} and a satisfactory linearity was obtained from 2 - 1 000 µg L^{-1} with regression coefficients better than 0.9990. The LOD and LOQ values were calculated based on 3- and 10-times standard deviation of UHP water spiked with a minimum analyte concentration (0.3 - 0.5µg L^{-1}) respectively. The LOD values ranged from 0.6 µg L^{-1} - 7.8 µg L^{-1} while LOQ values were obtained in the range of 1.8 - 23.4 µg L^{-1} (**Table 1**).

Compound	Regression equation (n = 6)	R^2	Linear Range (µg L^{-1})	LOD (µg L^{-1})	LOQ (µg L^{-1})
SGD	y = 0.008x + 0.045	0.9991	8 - 1 000	2.7	8.1
SAM	y =0.018x + 0.017	0.9998	9 - 1 000	6.4	19.2
SAA	y = 0.057x + 0.125	0.9999	3 - 1 000	1.0	3.0
SDZ	y = 0.231x + 0.198	0.9996	4 - 1 000	2.2	6.6
STZ	y = 0.015x + 0.110	0.9999	5 - 1 000	1.8	5.4
SPY	y = 0.054x + 0.142	0.9999	3 - 1 000	1.2	3.6
SMR	y = 0.341x - 2.631	0.9997	8 - 1 000	7.8	23.4
SMT	y = 0.133x + 0.225	0.9998	2 - 1 000	0.6	1.8
SMM	y = 0.523x + 1.118	0.9992	3 - 1 000	0.9	2.7
SCP	y= 0.273x - 0.232	0.9991	3 - 1 000	1.3	3.9
SMX	y = 0.4034x - 0.713	0.9998	2 - 1 000	1.8	5.4
SSO	y = 0.296x + 0.388	0.9999	4 - 1 000	1.7	5.1
SBZ	y = 0.310x + 0.323	0.9996	5 - 1 000	2.0	6.0
SQZ	y = 0.508x + 0.101	0.9999	2 - 1000	0.6	1.8
SSA	y = 0.051x + 0.457	0.9999	4 - 1000	1.8	5.4

Table 1: Quantitative result of linearity, and LOD and LOQ values obtained for 15 SAs

Inter-day and intra-day precision was investigated by spiking UHP water samples with a mixture of target analytes at three concentration levels (40 µg L^{-1}, 100 µg L^{-1}, and 400 µg L^{-1}) for three consecutive determinations in a single day and five determinations in five days, respectively. Intra-day %RSD values at concentration levels of 40 µg L^{-1}, 100 µg L^{-1}, and 400 µg L^{-1} were in the range 4.1 - 9.5%, 3.6 - 9.7%, and 1.5 - 9.3%, respectively, while for inter-day precision at the same three concentration levels, the ranges were 3.4 - 9.9%, 5.0 - 9.6%, and 3.1 - 9.9%, respectively (**Table 2**).

Compound	Intra-day (%RSD) (n = 6)			Inter-day (%RSD) (n = 6)		
	40 (µg L^{-1})	100 (µg L^{-1})	400 (µg L^{-1})	40 (µg L^{-1})	100 (µg L^{-1})	400 (µg L^{-1})

SGD	9.7	8.1	8.9	9.1	9.6	5.9
SAM	8.4	9.6	7.9	6.3	8.0	9.0
SAA	9.2	9.7	8.3	5.0	8.0	9.9
SDZ	8.7	8.8	3.2	8.0	7.5	6.4
STZ	7.0	9.5	9.3	9.7	8.5	9.5
SPY	9.0	9.4	7.8	5.8	6.8	9.2
SMR	9.1	8.3	2.4	9.5	8.9	5.2
SMT	9.2	8.9	6.9	3.4	8.3	3.8
SMM	5.8	4.1	3.2	7.2	6.9	3.1
SCP	9.5	7.7	7.9	8.6	8.8	7.0
SMX	8.5	4.2	4.6	9.7	5.0	6.1
SSO	9.0	6.9	7.9	9.6	8.0	7.9
SBZ	9.3	8.8	3.2	9.0	6.6	9.0
SQZ	5.7	3.6	1.5	9.9	9.8	6.0
SSA	4.1	5.0	8.3	7.2	9.4	4.7

Table 2. Intra-day and inter-day precision results (%RSD) for water samples spiked at 40 µg L^{-1}, 100 µgL^{-1}, and 400 µg L^{-1} levels of 15 SAs

The accuracy of the method was evaluated by spiking the same levels of sulfonamide standard mixtures in three different types of water samples (river water, groundwater, and tap water) and the percentage recoveries were calculated. The results shown in **Table 3** revealed that satisfactory recoveries, ranging from 70.5 to 103.9% with %RSD values ranging from 0.5 to 9.8%, were obtained, with the exception of SGD in river water and groundwater where recoveries in the range of 54.3 - 84.9% at the two higher concentration levels (100 µg L^{-1} and 400 µg L^{-1}) were obtained. This lower recovery of SGD may be attributed to its very low K_{OW} value (-1.07).

Compounds	Recovery																					
	River water						Ground water					Tap water I (Pretoria)						Tap water II (Florida)				
	40 (µg L^{-1})		100 (µg L^{-1})		400 (µg L^{-1})		40 (µg L^{-1})		100 (µg L^{-1})		400 (µg L^{-1})	40 (µg L^{-1})		100 (µg L^{-1})		400 (µg L^{-1})		40 (µg L^{-1})		100 (µg L^{-1})		400 (µg L^{-1})	
	% RSD	%R	% RSD	%R	% RSD	%R	% RSD	%R	% RSD	%R	% RSD	% RSD	%R	% RSD	%R	% RSD	%R	% RSD	%R	% RSD	%R	% RSD	% RSD
SGD	76.0	7.4	63.1	10.0	56.4	5.7	84.9	3.3	73.5	8.2	53.8	78.6	8.2	66.3	7.3	65.3	9.2	79.7	5.6	64.4	8.7	69.4	3.6
SAM	84.1	5.0	95.4	5.3	72.2	3.2	76.4	9.7	98.8	8.3	71.8	81.7	9.8	71.1	1.8	74.8	6.7	81.7	9.8	98.1	7.4	71.0	1.8
SAA	70.5	7.0	72.1	4.9	79.1	4.1	80.8	4.2	77.9	6.7	75.3	72.0	5.8	75.0	8.7	83.5	7.1	80.8	5.3	80.8	9.2	74.7	5.7
SDZ	99.5	1.9	97.9	6.3	75.6	2.3	102.4	6.2	100.0	5.5	77.1	99.4	3.5	99.2	3.8	93.3	3.3	98.5	2.0	96.9	7.6	93.4	2.5
STZ	94.7	4.2	97.6	5.9	99.6	1.6	91.7	7.4	80.4	8.9	91.7	87.2	6.4	93.8	8.8	88.4	5.5	87.2	8.2	78.8	8.6	103.2	2.8
SPY	99.9	4.8	86.3	8.6	84.1	4.3	96.0	7.4	98.3	7.4	78.6	93.3	9.6	99.5	6.2	86.9	3.6	88.3	2.7	79.2	8.3	82.2	8.4
SMR	81.4	2.1	74.1	7.0	73.0	2.1	83.3	3.5	101.1	3.7	79.1	90.5	1.9	97.6	4.0	93.5	2.5	86.1	4.7	72.9	4.7	82.4	1.6
SMT	98.5	4.0	93.0	9.4	99.8	5.6	98.7	7.8	97.1	7.6	101.5	92.3	5.1	96.9	7.5	85.2	3.4	90.7	2.1	74.7	7.9	100.3	3.8
SMM	83.6	8.0	96.3	3.9	88.1	1.0	80.9	4.9	89.0	5.6	95.7	94.2	8.0	99.6	3.2	73.5	1.8	101.1	9.6	71.6	4.2	96.7	0.6
SCP	98.3	1.3	92.2	3.7	101.3	2.0	101.2	2.8	94.5	6.9	99.1	99.7	2.8	83.1	3.5	98.6	1.7	103.6	3.8	70.8	4.0	103.9	0.9
SMX	80.4	3.5	86.6	3.8	91.8	2.3	86.9	5.1	80.9	4.8	88.6	87.5	7.9	91.4	4.5	91.1	1.3	99.4	5.4	77.0	3.8	103.7	1.2
SSO	99.3	5.6	100.5	2.5	79.8	4.1	101.6	4.0	101.6	4.8	81.8	97.9	1.4	97.0	7.4	86.9	1.0	89.0	3.3	92.2	5.8	71.9	2.9
SBZ	95.9	7.7	99.8	4.3	101.4	2.0	93.8	5.4	95.1	9.5	100.7	99.1	5.8	85.0	6.8	101.4	2.0	101.4	6.1	94.3	5.2	101.1	1.5
SQZ	96.8	7.5	86.7	4.9	101.4	1.1	100.6	3.0	90.2	8.0	102.1	99.3	4.0	99.4	1.8	102.6	0.9	95.1	6.5	99.3	1.8	76.8	0.9
SSA	98.8	8.1	87.7	7.2	88.5	3.4	96.3	6.9	101.1	4.2	94.0	93.9	9.6	91.0	5.8	97.9	6.5	92.8	4.5	81.2	4.8	101.7	6.2

Table 3: The recovery (%R) result obtained by comparing the ratio of concentration of each analyte found in spiked river water, groundwater samples. tap water I (Pretoria, South Africa) and tap water II (Florida, South Africa) at 40 µg L-1,100 µg L-1, and 400 µg L-1 levels of a mixture of SAs with the spiked concentration and results were multiplied by 100.

Furthermore, in order to study the effect of sample matrix on the extraction process, the relative recovery (RR), which is defined as the peak area ratio of the natural water sample to the UHP water sample spiked with analytes at the same concentration levels (40 µg L^{-1}, 100 µg L^{-1}, and 400 µg L^{-1}) were calculated and found in the range of 70.3 - 106.1%, 72.5 - 105.4%, and 72.6 - 104.9%, respectively (Table S2, Supplemental material). The recovery results were in agreement with those previously reported in the literature on the same sample and matrix.[34] The result demonstrated that the matrices of the real water samples do not have significant effects on the proposed DLLME method

for the extraction of target analytes from real water samples. The selectivity of the proposed method was also evaluated by comparing the chromatograms of the blank (unspiked) water samples with the corresponding spiked water samples. Results shown in **Figures S1 – S5** (supplemental material) confirmed that no interferences were observed at the retention times of the target analytes.

5. Application of the proposed method to Wastewater samples from Wastewater Treatment Plant (WWTP)

To demonstrate the applicability of the proposed DLLME method, influent and effluent samples of the WWTP were collected, i.e., influent I, influent II, and effluent samples; these samples were analysed to assess the presence and levels of sulfonamides. A parallel group of the same samples was also analysed after spiking with 100 µg L^{-1} of target compounds. As shown in **Table 4**, SSO was detected in the influent I sample at an average concentration of 17.8 µg L^{-1} with corresponding %RSD, (n = 5) value of 1.6% and in the influent II sample at 14.8 µg L^{-1} with corresponding %RSD (n = 5) value of 1.9%.

Compound	Influent I				Influent II				Effluent			
	C_o	C_s	%R	%RSD	C_o	C_s	%R	%RSD	C_o	C_S	%R	%RSD
SGD	**ND**	**75.9**	**75.9**	**5.5**	**ND**	**70.9**	**70.9**	**5.2**	**ND**	**83.1**	**83.1**	**3.8**
SAM	**ND**	**93.3**	**93.3**	**2.9**	**ND**	**96.8**	**96.8**	**5.6**	**ND**	**96.8**	**96.8**	**5.8**
SAA	**ND**	**97.8**	**97.8**	**4.3**	**ND**	**97.8**	**97.8**	**3.2**	**ND**	**81.1**	**81.1**	**4.4**
SDZ	**ND**	**98.5**	**98.5**	**3.4**	**ND**	**73.9**	**73.9**	**0.7**	**ND**	**81.1**	**81.1**	**1.8**
STZ	**ND**	**96.0**	**96.0**	**3.7**	**ND**	**93.8**	**93.8**	**5.9**	**ND**	**98.7**	**98.7**	**1.3**
SPY	**ND**	**94.1**	**94.1**	**6.3**	**ND**	**98.8**	**98.8**	**2.7**	**ND**	**92.3**	**92.3**	**1.9**
SMR	**ND**	**83.3**	**83.3**	**1.1**	**ND**	**82.4**	**82.4**	**3.9**	**ND**	**80.7**	**80.7**	**3.4**
SMT	**ND**	**91.2**	**91.2**	**1.9**	**ND**	**92.9**	**92.9**	**3.5**	**ND**	**94.2**	**94.2**	**4.1**
SMM	**ND**	**90.1**	**90.1**	**0.7**	**ND**	**86.0**	**86.0**	**1.8**	**ND**	**82.2**	**82.2**	**2.0**
SCP	**ND**	**98.9**	**98.9**	**1.3**	**ND**	**99.6**	**99.6**	**0.9**	**ND**	**101.7**	**101.7**	**1.6**
SMX	**ND**	**99.1**	**99.1**	**1.9**	**ND**	**99.1**	**99.1**	**0.2**	**ND**	**100.3**	**100.3**	**2.7**
SSO	**17.8**	**105.4**	**87.6**	**1.6**	**14.8**	**107.3**	**92.5**	**1.9**	**ND**	**93.0**	**93.0**	**4.8**
SBZ	**ND**	**102.2**	**102.2**	**2.6**	**ND**	**99.9**	**99.9**	**1.5**	**ND**	**101.5**	**101.5**	**2.9**
SQZ	**ND**	**80.5**	**80.5**	**2.0**	**ND**	**81.3**	**81.3**	**4.4**	**ND**	**107.7**	**107.7**	**2.0**
SSA	**ND**	**80.7**	**80.7**	**1.1**	**ND**	**70.5**	**70.5**	**4.3**	**ND**	**85.4**	**85.4**	**2.8**

Table 4: Sulfonamide levels found in wastewater samples from wastewater treatment plant (WWTP), Pretoria, South Africa and spike recovery (%R, %RSD, n = 5 and two injections for each sample)

The chromatograms of SSO in influent I and II samples are shown in **Figures S6 and S7**, respectively (supplemental material). Sulfisoxazole was also detected in the influent of WWTPs in the USA in the range of 3.2 to 22.1 ng L^{-1},(Spongberg and Witter 2008)which was lower than that detected in the current work. It has also been detected in WWTP effluents in Canada (19 - 34 µg L^{-1}),(Rodríguez-Cabo, Rodríguez et al. 2011) in the USA (3.2 to 11.9 ng L^{-1}),(Spongberg and Witter 2008) and in Japan (0.13 -1.6 ng L^{-1})(Díaz-Cruz, García-Galán et al. 2008) One of the possible reasons for the presence of sulfisoxazole is its wide application in human medicine on its own or together with erythromycin for urinary tract infections.(Chung 2008) In addition, the compound is also excreted mainly in urine in an uncharged form. On the other hand, none of the target compounds were detected in the effluent samples.

Sulfonamides have been detected in WWTPs in Europe, America, Asia, Australia,(Zhang and Li 2011) and in Africa.(Faleye, Adegoke et al. 2018) Among these, sulfamethoxazole was the most frequently detected, followed by sulfamethazine, sulfapyridine, and sulfadiazine.(Faleye, Adegoke et al. 2018, Zhang, Bai et al. 2023). The highest reported concentration of sulfamethoxazole in WWTP influent was 5 597 ng L^{-1} and in WWTP effluent it was 6 000 ng L^{-1} in Europe, America, Asia and Australia,(Díaz-Cruz, García-Galán et al. 2008). United nation reported minimum and maximum concentrations for Africa were in the range of 0.1 - 29.0 µg L^{-1}respectively(Faleye, Adegoke et al. 2018). Sulfamethoxazole has been detected wastewater effluents ranging between 0.15 and 10 µg L^{-1} in African wastewater effluents.(Ncube, Nuapia et al. 2021) Considering South Africa, the average concentration of sulfamethoxazole in surface water was reported as 7.3 µg L^{-1}(Faleye, Adegoke et al. 2018) . Due to the combination of trimethoprim and sulfamethoxazole used for the treatment of *Pneumocystis pneumonia* in people with HIV/AIDS, sulfamethoxazole is widely used and hence the most commonly detected sulfonamide in surface water systems in South Africa. Neube etal (Ncube, Nuapia et al. 2021) also reported the presence of sulfonamides (sulfamethiozole, sulfamethazine and sulfamethoxaxole) in their study for the presence of antibiotics in a South African stream.

6. Comparison of the proposed method with other reported DLLME methods for sulfonamide compounds

The proposed method was also compared with the other reported DLLME methods based on the number of analysed analytes, volume of solvent consumption, extraction time, linearity range, recovery values, and LOD values. The results shown in **Table 5** confirmed that the proposed method has a wider linear range, short extraction time (less than 5 min), a lower consumption of organic solvents than the methods reported in the literature,(Wen, Li et al. 2011, Li, Li et al. 2016) a larger number of analytes, and a wide range of K_{OW} values (-1.07 - 3.8). However, the percentage recovery and LOD values were similar to those reported by Wen et al.(Wen, Li et al. 2011) and Wang et al (Wang, Li et al. 2022) and higher than the values reported by Xu et al.(Xu, Su et al. 2011) Therefore, the results indicated that the proposed method is feasible to be used as an alternative method for the determination of Sulfonamides in water samples.

Method	Matrix	Analyzed no Sulfonamides	Solvent types and amount (extraction, disperser)	Extraction time	Linearity (µg L-1)	Recovery (%)	LOD (µg L-1)	Reference
DLLME - CE-DAD	Lake, pond and tap water	5	5 mL of chlorobenzene, 800 µL of DMSO	40 - 45 min	20 - 570	53.6 - 94.0	0.02 - 0.57	[32]
IL-based MADLLME-HPLC – FD	Water, honey milk and plasma	6	100 µL of [C6MIM][PF6], 0.75 mL of MeOH	15 min	0.1 - 5	95.0 - 107.7	0.011 - 0.018 (river water)	[33]
LDS-SD-DLLME - DME	Environmental water	4	200 µL of octanol 750 µL of MeOH 600 µL of MeCN	17 min	1.0 - 500 to 10 - 500	NR	0.22-1.92	[34]
UA-DLLME-HPLC-DAD	Environmental water and seafood	7	500 µL $C_2H_2Cl_4$ 900µL MeCN	More than 13min	5 - 5000	80.0 – 116.0%	0.7–7.8	[35]
DLLME - UHPLC - DAD	Mineral and runoff water	11 and 14 Qs	685 µL of $CHCl_3$, 1 250 µL of MeCN	15 min	5 - 1000	82.0 - 115.0%	0.8 - 32.1	[36]
(UA-DLLME) - UPLC-MS/MS	water	13	-	More than 10 min	-	80.3% - 101.8	0.6 ng/L and 2.4 ng/L	[37]
DLLME - HPLC -DAD	Tap water, river water and wastewater	15	400 µL of CH2Cl2 600 µL of MeCN	5 min	2 - 1000	70.8 - 103.9	0.6 - 7.8	Current work

Table 5: Comparison of the proposed DLLME method for water samples with other reported similar methods

7. Conclusions

In this study, dispersive liquid-liquid microextraction combined with high performance liquid chromatography-diode array detection has been evaluated and successfully developed for the simultaneous extraction and determination of 15 sulfonamides in real water samples. The effects of various parameters on the extraction efficiency were evaluated, and optimum conditions were established. Under optimised conditions, linearity was found in the range of 2 - 1 000 µg L^{-1} with regression coefficients better than 0.9990. The limit of detection (LOD) and limit of quantification (LOQ) values were in the range of 0.6 - 7.8 µg L^{-1} and 1.8 - 23.4 µg L^{-1} respectively. Intra-day (n = 6) and inter-day (n = 6) precision expressed as %RSD were in the range of 1.5 - 9.7% and 3.1 - 9.9%, respectively.

The accuracy of the method was also evaluated in samples from three different sources of water (river water, groundwater, and tap water) and found to range from 70.5 to 103.9% with %RSD values ranging from 0.5 to 9.8% for 14 target analytes, with the exception of SGD, where recoveries in the range of 53.8 - 84.9% were obtained in river water and groundwater, with the corresponding %RSD values in the range of 0.5 - 9.5%.

Satisfactory relative recoveries (RR) in the range of 70.3 - 106.1% obtained provided evidence that the proposed DLLME method was not affected by the matrix effects. Therefore, the proposed method was applied for the determination of sulfonamides in wastewater treatment plant samples. Both SMX and SSO were detected; however, SMX was not quantifiable and SSO was in the range of 14.8 -17.8 µg L^{-1} in WWTP influents with %RSD values in the range of 1.6 - 1.9%. In comparison with other reported methods, the proposed DLLME method is adequate to be used as an alternative method for the determination of sulfonamides in water samples.

Data availability

The raw data required to produce these findings can be obtained from authors with reasonable request

Conflict of interests

All the authors declare that they have no conflict of interest

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References

1. Arroyo-Manzanares, N., et al. (2014). "Alternative sample treatments for the determination of sulfonamides in milk by HPLC with fluorescence detection." Food Chemistry 143: 459-464.
2. Asensio-Ramos, M., et al. (2011). "Liquid phase microextraction applications in food analysis." Journal of Chromatography A 1218(42): 7415-7437.
3. Baran, W., et al. (2011). "Effects of the presence of sulfonamides in the environment and their influence on human health." Journal of Hazardous Materials 196: 1-15.
4. Białk-Bielińska, A., et al. (2011). "Ecotoxicity evaluation of selected sulfonamides." Chemosphere 85(6): 928-933.
5. Chiavarino, B., et al. (1998). "Determination of sulfonamide antibiotics by gas chromatography coupled with atomic emission detection." Journal of Chromatography B: Biomedical Sciences and Applications 706(2): 269-277.

6. Chitescu, C. L., et al. (2011). "Simultaneous analysis of four sulfonamides in chicken muscle tissue by HPLC." Food Additives & Contaminants: Part A 28(8): 1013-1020.
7. Chung, J. K. (2008). "The research of the antibiotics reduction in aquarium and the environmental influence of parasiticide." Korea: Ministry of Food, Agriculture, Forestry and Fisheries & Pukyong National University Press.
8. Díaz-Cruz, M. S., et al. (2008). "Highly sensitive simultaneous determination of sulfonamide antibiotics and one metabolite in environmental waters by liquid chromatography-quadrupole linear ion trap-mass spectrometry." Journal of Chromatography A 1193(1-2): 50-59.
9. Faleye, A. C., et al. (2018). "Antibiotic residue in the aquatic environment: status in Africa." Open Chemistry 16(1): 890-903.
10. Fotouhi, L., et al. (2012). "Electrochemical behavior and voltammetric determination of sulfaguanidine at a glassy carbon electrode modified with a multi-walled carbon nanotube." Int. J. Electrochem. Sci 7: 3919-3928.
11. García-Galán, M. J., et al. (2010). "Determination of 19 sulfonamides in environmental water samples by automated on-line solid-phase extraction-liquid chromatography-tandem mass spectrometry (SPE-LC-MS/MS)." Talanta 81(1-2): 355-366.
12. García-Galán, M. J., et al. (2010). "Determination of 19 sulfonamides in environmental water samples by automated on-line solid-phase extraction-liquid chromatography–tandem mass spectrometry (SPE-LC–MS/MS)." Talanta 81(1-2): 355-366.
13. García-Galán, M. J., et al. (2008). "Identification and determination of metabolites and degradation products of sulfonamide antibiotics." TrAC - Trends in Analytical Chemistry 27(11): 1008-1022.
14. Herrera-Herrera, A. V., et al. (2013). "Dispersive liquid-liquid microextraction combined with ultra-high performance liquid chromatography for the simultaneous determination of 25 sulfonamide and quinolone antibiotics in water samples." Journal of Pharmaceutical and Biomedical Analysis 75: 130-137.
15. Iglesias, A., et al. (2013). "Determination of the Presence of Three Antimicrobials in Surface Water Collected from Urban and Rural Areas." Antibiotics 2(1): 46-57.
16. Koesukwiwat, U., et al. (2007). "Solid-phase extraction for multiresidue determination of sulfonamides, tetracyclines, and pyrimethamine in Bovine's milk." Journal of Chromatography A 1149(1): 102-111.
17. Kümmerer, K. (2009). "Antibiotics in the aquatic environment - A review - Part I." Chemosphere 75(4): 417-434.
18. Li, X., et al. (2016). "Dispersive liquid–liquid microextraction coupled with single-drop microextraction for the fast determination of sulfonamides in environmental water samples by high performance liquid chromatography-ultraviolet detection." Analytical Methods 8(3): 517-525.
19. Lu, K. H., et al. (2007). "Trace determination of sulfonamides residues in meat with a combination of solid-phase microextraction and liquid chromatography-mass spectrometry." Talanta 72(3): 1082-1087.
20. Manzetti, S. and R. Ghisi (2014). "The environmental release and fate of antibiotics." Marine Pollution Bulletin 79(1-2): 7-15.
21. Mor, F., et al. (2012). "Determination of sulphonamide residues in cattle meats by the Charm-II system and validation with high performance liquid chromatography with fluorescence detection." Food Chemistry 134(3): 1645-1649.
22. Msagati, T. A. M. and M. M. Nindi (2004). "Multiresidue determination of sulfonamides in a variety of biological matrices by supported liquid membrane with high pressure liquid chromatography-electrospray mass spectrometry detection." Talanta 64(1): 87-100.
23. Ncube, S., et al. (2021). "Trace detection and quantitation of antibiotics in a South African stream receiving wastewater effluents and municipal dumpsite leachates." Frontiers in Environmental Science: 365.
24. Pan, X., et al. (2011). "Simultaneous determination of three classes of antibiotics in the suspended solids of swine wastewater by ultrasonic extraction, solid-phase extraction and liquid chromatography-mass spectrometry." Journal of Environmental Sciences 23(10): 1729-1737.
25. Prosen, H. (2014). "Applications of liquid-phase microextraction in the sample preparation of environmental solid samples." Molecules 19(5): 6776-6808.
26. Rezaee, M., et al. (2006). "Determination of organic compounds in water using dispersive liquid–liquid microextraction." Journal of Chromatography A 1116(1-2): 1-9.
27. Rodríguez-Cabo, T., et al. (2011). "Dispersive liquid–liquid microextraction using non-chlorinated, lighter than water solvents for gas chromatography–mass spectrometry determination of fungicides in wine." Journal of Chromatography A 1218(38): 6603-6611.
28. Sarafraz-Yazdi, A. and A. Amiri (2010). "Liquid-phase microextraction." TrAC Trends in Analytical Chemistry 29(1): 1-14.
29. Saraji, M., et al. (2011). "Comparison of dispersive liquid–liquid microextraction and hollow fiber liquid–liquid–liquid microextraction for the determination of fentanyl, alfentanil, and sufentanil in water and biological fluids by high-performance liquid chromatography." Analytical and Bioanalytical Chemistry 400(7): 2149-2158.
30. Schmidt, K. and I. D. Podmore (2015). "Solid phase microextraction (SPME) method development in analysis of volatile organic compounds (VOCS) as potential biomarkers of cancer." Journal of Molecular Biomarker & Diagnosis 6(6).
31. Seifrtová, M., et al. (2008). "Determination of fluoroquinolone antibiotics in hospital and municipal wastewaters in Coimbra by liquid chromatography with a monolithic column and fluorescence detection." Analytical and Bioanalytical Chemistry 391(3): 799-805.
32. Senta, I., et al. (2008). "Simultaneous determination of sulfonamides, fluoroquinolones, macrolides and trimethoprim in wastewater and river water by LC-tandem-MS." Chromatographia 68(9): 747-758.
33. Spongberg, A. L. and J. D. Witter (2008). "Pharmaceutical compounds in the wastewater process stream in Northwest Ohio." Science of The Total Environment 397(1-3): 148-157.
34. Tölgyesi, Á., et al. (2013). "Analysis of sulfonamide residues in real honey samples using liquid chromatography with fluorescence and tandem mass spectrometry detection." Journal of Liquid Chromatography & Related Technologies 36(8): 1105-1125.
35. Wang, Y., et al. (2022). "Simultaneous Determination of Sulfonamides Antibiotics in Environmental Water and Seafood Samples Using Ultrasonic-Assisted Dispersive Liquid-Liquid Microextraction Coupled with High Performance Liquid Chromatography." Molecules 27(7): 2160.
36. Wen, Y., et al. (2011). "Dispersive liquid–liquid microextraction coupled with capillary electrophoresis for simultaneous determination of sulfonamides with the aid of

experimental design." ELECTROPHORESIS 32(16): 2131-2138.
37. Xu, X., et al. (2011). "Ionic liquid-based microwave-assisted dispersive liquid–liquid microextraction and derivatization of sulfonamides in river water, honey, milk, and animal plasma." Analytica Chimica Acta 707(1-2): 92-99.
38. Yan, H. and H. Wang (2013). "Recent development and applications of dispersive liquid-liquid microextraction." Journal of Chromatography A 1295: 1-15.
39. Yan, H. and H. Wang (2013). "Recent development and applications of dispersive liquid–liquid microextraction." Journal of Chromatography A 1295: 1-15.
40. Yu, H., et al. (2011). "Development of a high-performance liquid chromatography method and a liquid chromatography–tandem mass spectrometry method with the pressurized liquid extraction for the quantification and confirmation of sulfonamides in the foods of animal origin." Journal of Chromatography B 879(25): 2653-2662.
41. Zhang, L., et al. (2023). "Occurrence, bioaccumulation and ecological risks of antibiotics in the water-plant-sediment systems in different functional areas of the largest shallow lake in North China: Impacts of river input and historical agricultural activities." Science of The Total Environment 857.
42. Zhang, T. and B. Li (2011). "Occurrence, transformation, and fate of antibiotics in municipal wastewater treatment plants." Critical reviews in environmental science and technology 41(11): 951-998.

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