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Research Article

Estimation of Some Useful Drugs in Water, Urine, and Medical Formulations Following Conventional and Modified Dispersive Microextraction Coupled with HPLC-UV

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Abstract: Dispersive Liquid-Liquid Microextraction (DLLME) coupled with high-performance liquid chromatography-ultraviolet spectroscopy was developed, as a fast and precise operation, for extractive recovery and estimation of two pharmaceuticals, viz. moxifloxacin and galantamine, from water, urine, and medical formulations. The process was investigated for extraction solvent (ES) and dispersive solvent (DS), as well as pH, temperature, and salt concentration. Extraction was found effective using methanol (CH_3OH) as the DS, employing 1, 1, 2, 2-tetrachloroethane ($C_2H_2Cl_4$) and chloroform ($CHCl_3$) as the ES for moxifloxacin and galantamine, respectively. The optimum pH was found to be 6.9 for moxifloxacin and 10.2 for galantamine. Temperature and salt were found to have some influence on the extraction efficiency of moxifloxacin but were insignificant for galantamine. An improvement of the operation in terms of extraction efficiency (ER%), preconcentration factor (PF), thermodynamic feasibility, and greenness was achieved during surfactant-aided DLLME (SDS-DLLME), where an anionic surfactant (sodium dodecyl sulphate (SDS)) was employed and no DS was required. Interestingly, the volume requirement for ES was found to be less compared to that in conventional DLLME, without compromising the performance. Moreover, quantitative recovery of both drugs was achieved using a single ES. Thus, mutual separation and simultaneous determination of moxifloxacin and galantamine may be designed. A two-phase separation with concomitant enrichment of the solute in the sediment phase occurred. The drugs in the sediment phase, on subsequent dilution with methanol, were determined using the high-performance liquid chromatography-ultraviolet (HPLC-UV) system. The negative free energy changes for the operation indicated that the process was thermodynamically feasible. The process was found to be effective for the spiked recovery of the studied drugs from real samples, viz., water, human urine, and commercial medical formulations.

***Keywords*:** Dispersive Liquid-Liquid Microextraction, surfactant aided Dispersive Liquid-Liquid Microextraction, water, urine, medicine

1. Introduction

Pharmaceuticals or drugs are the most useful compounds in the medical treatment of humans and animals. The commonly used drugs, such as antibiotics, analgesics, anti-inflammatory, are considered pseudo persistent pollutants due to their continual input and permanent presence in the environment.[1] The present report deals with recovery via

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extraction of moxifloxacin and galantamine using dispersive liquid-liquid microextraction. Moxifloxacin is used for the treatment of sinusitis, bronchitis, and urinary tract infection, and galantamine is mostly used for Alzheimer's disease. However, both of them show common side effects of nausea, vomiting, diarrhea, stomach pain, heartburn, loss of appetite, etc. Moreover, it is of alarm for a healthy human if exposed to sublethal doses of such drugs from non-point sources. Thus, assessment of the contamination level, via determination, of such drugs in environmental and biological samples becomes essential. In medical formulations, the estimation of drugs is important to assess either the activity or the presence of any spurious component.

In analytical chemistry sample preparation step before detection/determination becomes very crucial, considering the solute load and matrix complexity. Generally, sample preparation protocol consists of extraction via interference removal and solute preconcentration or enrichment. Liquid-Liquid Extraction (LLE)[2,3] and Solid-Phase Extraction (SPE) are widely used for certain drug analysis.[4-6] Recently, solid-phase microextraction,[7,8] cloud point extraction,[9,10] dispersive liquid-liquid microextraction, following HPLC and Gas Chromatograph (GC)[11,12] coupled with UV, fluorescence, mass spectroscopy or capillary electrophoresis,[13-21] etc., are gaining attention for detection and quantification of drugs. Among the various sample preparation techniques, Dispersive Liquid-Liquid Microextraction (DLLME) is a very attractive option, as it is simple, quick, efficient, less expensive due to less solvent volume requirement and compatibility with instrumental detection system.[22] The present report demonstrates Conventional DLLME (C-DLLME) and Surfactant aided DLLME (S-DLLME) for the recovery and preconcentration of moxifloxacin and galantamine in different water, urine, and medical formulations. The literature study reveals that there is only one preliminary study on DLLME of moxifloxacin while no report on DLLME of galantamine is available.

The process was investigated for different operational variables, such as nature and volume of extraction and dispersive solvent or surfactant (in case of S-DLLME), pH, equilibration time, temperature, and ionic strength. The interference study due to common anions was also made. The feasibility as well as the performance of C-DLLME, and S-DLLME was finally compared.

2. Material and methods

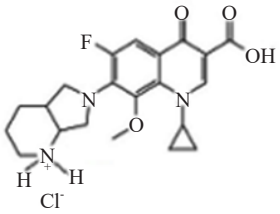
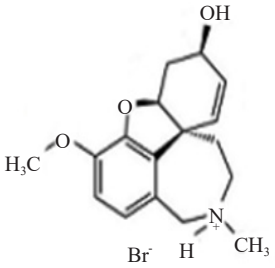
2.1 *Reagents and materials*

Standard samples of drugs viz. moxifloxacin and galantamine, investigated in the present study, were procured from Sigma-Aldrich, India. The structure and relevant physicochemical properties of the drugs were given in Table 1. Solvents such as 1,1,2,2-tetrachloroethane ($C_2H_2Cl_4$; 1.59 g/L, ES1), chloroform ($CHCl_3$; 1.49 g/L, ES2), carbon tetrachloride (CCl_4; 1.59 g/L, ES3), dichloromethane (CH_2Cl_2 ; 1.33 g/L, ES4), methanol (CH_3OH; 0.792 g/L, DS1), ethanol (C_2H_5OH (0.789 g/L, DS2)), acetonitrile CH_3CN (0.786 g/L, DS3) and acetone (C_3H_6O; 0.791 g/L, DS4) are of HPLC grade and were procured from Merck, India. Surfactants, such as anionic (Sodium Dodecyl Sulfate (SDS)), cationic (Cetyl Trimethyl Ammonium Bromide (CTAB)), non-ionic (polyoxyethylene 9.5 octylphenyl ether, TX-100), other solvents and reagents used are of Merck, Anal R grade. Hydrochloric acid and sodium hydroxide, procured from Merck, was used to adjust the pH of the samples and were diluted to 0.1 M before use. Sodium chloride (NaCl), potassium chloride (KCl), and sodium sulfate (Na_2SO_4) were procured from Merck, India. Milli-Q water, used in all the experiments, was produced on a Nano pure (Merck Ultrapure, India) water purification system.

2.2 *Apparatus*

Separation and determination of the drugs were carried out in an HPLC-UV system (Cecil; CE 4201). The spectral study was done using UV-Vis spectrophotometer (Shimadzu UV-2401PC) with the blank solution as the reference. A Systronics digital pH meter (Model no. 335) was used for pH measurement. The phase separation was achieved by a Rotofix centrifuge. A mixture of $(NH_4)H_2PO_4$, and CH_3CN (70:30), adjusted at pH 3 by ortho-H_3PO_4, was used as mobile phase maintaining a flow rate of 1 mL/min in HPLC. Sample solution (H_2O:CH_3CN (50:50)) was injected into HPLC by a micro-syringe (20 µL) following determination at the wavelength of 295 and 288 nm respectively for moxifloxacin and galantamine.

Table 1. Structures and some physicochemical properties of the target pharmaceuticals

Analyte	Chemical structure	Formula	Molecular weight	CAS number	pK_a
Moxifloxacin hydrochloride		$C_{21}H_{25}ClFN_3O_4$	437.9 g/mol	186826-86-8	pK_{a1} = 5.69 and pK_{a2} = 9.42
Galantamine hydrobromide		$C_{17}H_{22}BrNO_3$	368.3 g/mol	1953-04-4	8.2

2.3 *Preparation of standard and real samples*

A standard solution of 1000 µg/L of each drug was prepared in Milli-Q water and stored at 4 °C. Working solutions, of different concentrations, were prepared by appropriate dilution of the stock.

Water samples of different origins were suitably collected and stored at 4 °C. In the case of urine, an aliquot of the sample (50 mL) was taken from healthy male volunteers without any medication. The urine samples were treated with 2 M of KOH (5 mL) to precipitate carbamide, uric acid, glucose, calcium phosphate, etc.[23,24]

Two commercial medical formulations viz. moxifloxacin (as liquid) and galantamine (as tablet) were taken and working solutions were prepared using the desired volume of Milli-Q water. 1 mL of the liquid sample in the first case and one tablet in the second case were taken for sample preparation.

The spiked samples were prepared at concentration level up to 10 and 60 µg/L for moxifloxacin and galantamine respectively using 1000 µg/L standard solution and kept at a screw cap glass test tube with a conical bottom at room temperature for about 30 min and filtered through a 0.45 µm membrane prior to extraction.

2.4 *Extraction procedure*

In Conventional DLLME operation (C-DLLME), an aliquot of aqueous sample (5.0 mL) was placed in a 15 mL screw cap glass test tube with conical bottom. In a typical experiment, an optimum volume of DS (dispersive solvent; methanol) and ES (extraction solvent; $C_2H_2Cl_4$ for moxifloxacin and $CHCl_3$ for galantamine) was rapidly added into the sample solution, the pH of the solution was optimized and the mixture was gently shaken till the attainment of the equilibrium.

In the case of Surfactant aided DLLME (S-DLLME), suitable surfactant (SDS) was used instead of toxic DS. A cloudy solution was formed when the solute in the water sample was dispersed as fine droplets. The mixture was centrifuged for 4 min at 3000 rpm whereby the solute was enriched in the pellet phase at the bottom. The upper phase was withdrawn by a micropipette. The sediment phase was diluted to 6 mL using methanol and the solute was analyzed by HPLC.

2.5 *Extraction recovery and preconcentration factor*

Extraction performance is commonly estimated by the extraction recovery (ER %) and Preconcentration Factor (PF). PF is defined as the ratio of the final analyte concentration in the sediment phase (C_{sed}) after DLLME to the initial concentration of analyte (C_0) in the sample solution, which is expressed as:

$$PF = \frac{C_{sed}}{C_0} \tag{1}$$

C_{sed} was calculated from a calibration graph obtained from a direct injection of the standard solutions of drugs, of different concentrations, to a suitable ES-DS (C-DLLME) and ES-surfactant (S-DLLME) combination. ER is the percentage of total analyte amount (n_0), which is extracted to the organic phase (n_{sed}). It can be related to the PF and the phase volume ratio (V_{sed}/V_0), and is expressed as:

$$ER\,\% = \frac{n_{sed}}{n_0} \times 100 = \frac{C_{sed}}{C_0} \times \frac{V_{sed}}{V_0} \times 100 = PF \times \frac{V_{sed}}{V_0} \times 100 \tag{2}$$

where V_{sed} and V_0 are the volumes of sediment and sample solution respectively.

2.6 *Method validation*

The reliability and suitability of the proposed method are assessed by the linearity, Limit of Detection (LOD), Limit of Quantification (LOQ), precision, and matrix effect. The regression coefficient (r^2) value, close to one, is used as the measure of linearity. LOD and LOQ are expressed as the ratio of signal (corresponding to analyte concentration) to noise (blank) equivalent to 3 and 10 respectively.

The precision in terms of inter-day and intra-day repeatability is expressed as the Relative Standard Deviation (RSD).

3. Results and discussion

Dispersive Liquid-Liquid Microextraction (DLLME) is developed as an extension of conventional Liquid-Liquid Extraction (LLE) for improved efficiency. It utilizes a pair of solvent systems viz. Extraction (ES) and Dispersive Solvent (DS) to extract solutes from the aqueous sample. The merit of DLLME is the requirement of the low volume of organic solvent, as well as the quick time of extraction. Further improvement in extraction recovery was achieved in surfactant (SDS) aided operation. The application of the proposed operation was validated for different samples of water, urine, and commercial medicine.

The efficiency of extraction is influenced by the operational variables such as nature and volume of both ES and DS or surfactant, pH of the sample solution, electrolyte concentration, and temperature. In order to maximize the efficiency optimization of variables constitutes the first step.

3.1 *Selection of extraction solvent in C-DLLME*

Extraction solvent plays a key role in governing the efficiency, and proper selection of ES, thus, becomes vital in Conventional DLLME (C-DLLME). The prime criteria for the selection of ES are that it must have a good affinity for the solute, lower solubility in water, be denser than water, and form an emulsion with DS. Moreover, low volatility and less toxicity of ES provide some added merit. In the present study, four different extraction solvents (500 µL), such as 1,1,2,2-tetrachloroethane ($C_2H_2Cl_4$; ES1), chloroform ($CHCl_3$; ES2), carbon tetrachloride (CCl_4; ES3), dichloromethane (CH_2Cl_2; ES4), in presence of methanol, as the DS (500 µL), was tested, at the initial solution pH of 4.6 for Moxifloxacin (Mox) and 6.2 for Galantamine (Gal). It was observed (Figure 1) that the extent of recovery was different

for different combinations of ES-DS. The ER % was found to follow an order: ES1 > ES2 > ES3 > ES4 for Mox and ES2 > ES4 > ES3 > ES1 for Gal. The highest recovery was obtained using $C_2H_2Cl_4$ (ES1) for Mox and $CHCl_3$ (ES2) for Gal. Therefore, the subsequent experiments were performed with the suitable ES-DS combination for the respective drug.

In Surfactant aided DLLME (S-DLLME) different surfactants (500 µL), such as anionic (Sodium Dodecyl Sulfate (SDS)), cationic (Cetyl Trimethyl Ammonium Bromide (CTAB)), and non-ionic (polyoxyethylene 9.5 octylphenyl ether, TX-100), were tested in place of methanol. It was found that the extraction efficiency, for each drug, was higher in presence of SDS (vide section 3.5).

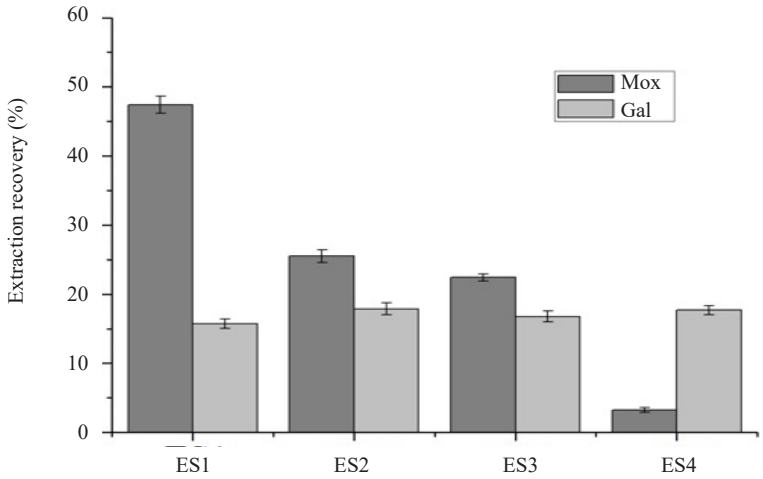


Figure 1. Selection of ES. Bar indicates standard deviations (n = 3)
Extraction condition: sample, 5 mL; DS1, 500 µL; ES, 500 µL; solution pH 4.6 for Mox, 6.2 for Gal; Time, 5 min

3.2 *Optimization of the extraction solvent volume*

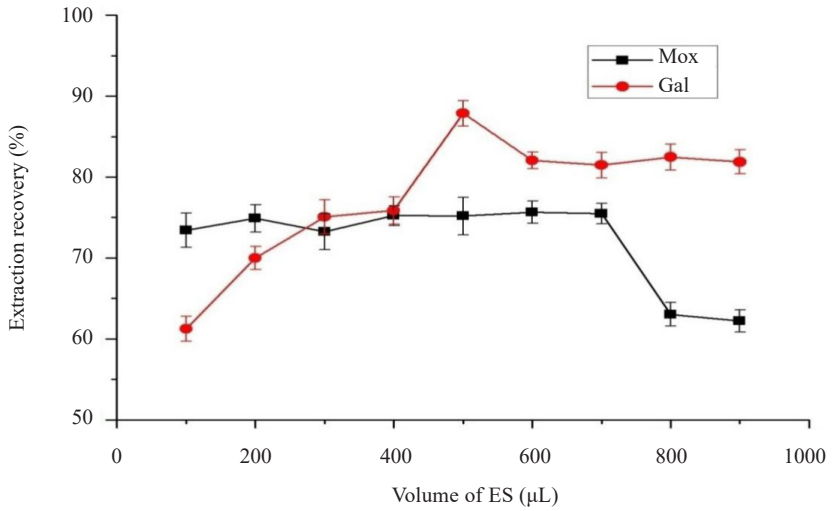


Figure 2. Optimization of ES volume; Bar indicates standard deviations (n = 3)
Extraction condition: same as that in Figure 1, except pH, optimized at 6.9 for Mox and 10.2 for Gal

In order to investigate the role of ES volume on extraction recovery, the volume of 1,1,2,2-tetrachloroethane (ES1)

for Mox and chloroform (ES2) for Gal was varied from 100 to 900 µL (Figure 2), in presence of 500 µL of methanol (DS1). The sample solution was maintained at the desired/optimized pH (6.9 for Mox and 10.2 for Gal), to maximize the solute recovery (vide section 3.7). It was found that extraction profiles were different for the studied solutes. The extraction recovery was found to increase smoothly for Mox with the increase of ES1volume from 100 to 700 µL and decrease sharply with further volume increase. On the other hand, the extraction recovery of Gal was found to increase rapidly up to the ES2 volume of 500 µL and decrease smoothly thereafter. Hence, subsequent studies were conducted with 1,1,2,2-tetrachloroethane (ES1) volume of 600 µL for Mox and with chloroform (ES2) volume of 500 µL for Gal.

3.3 *Selection of the dispersive solvent in C-DLLME*

Dispersive solvent has a definite role in assisting extraction, by increasing the surface area between the phases for solute mass transfer.[25] The dispersive solvent is selected such that it is miscible with both the aqueous (sample) and the organic solvent (ES), enhancing dispersion of ES as droplets into the sample solution. In the present case, methanol (DS1), ethanol (DS2), acetonitrile (DS3), and acetone (DS4) were tested as the dispersive solvent, in presence of the suitable extraction solvent for each drug (vide section 3.1) and at the initial solution pH of 4.6 for Mox and 6.2 for Gal. It was found that the extent of recovery follows an order: DS1 ≥ DS2 >> DS3 > DS4 for Mox and DS1 > DS2 > DS3 >> DS4 for Gal. Methanol gave the highest extraction recovery (Figure 3) and was chosen as the dispersing solvent for the subsequent studies.

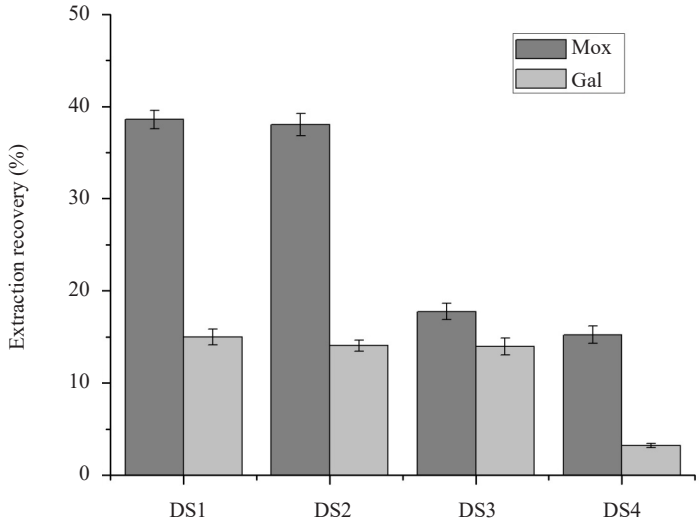


Figure 3. Selections of DS. Bar indicates standard deviations (n = 3)
Extraction condition: same as that in Figure 1, except ES1 600 µL for Mox, and ES2 500 µL for Gal

3.4 *Optimization of methanol volume*

Optimization of DS volume was carried out varying methanol volume from 100-900 µL, in presence of the suitable ES for each drug, maintaining the sample solution at the desired pH of 6.9 for Mox and 10.2 for Gal (vide section 3.7). The effect of DS volume on extraction recovery was shown in Figure 4. In the case of Mox, with increasing DS volume from 100 to 200 µL the percentage recovery was found to increase gradually and decrease thereafter. However, for Gal percentage recovery was found to increase up to 300 µL and gradual decrease thereafter. In presence of insufficient methanol, the extraction solvent could not disperse in water completely effecting poor extraction. Beyond the optimum volume of methanol, the decrease was due to the dilution effect. In the present case, 200 and 300 µL of methanol were found as the optimum volume of DS for Mox and Gal respectively.

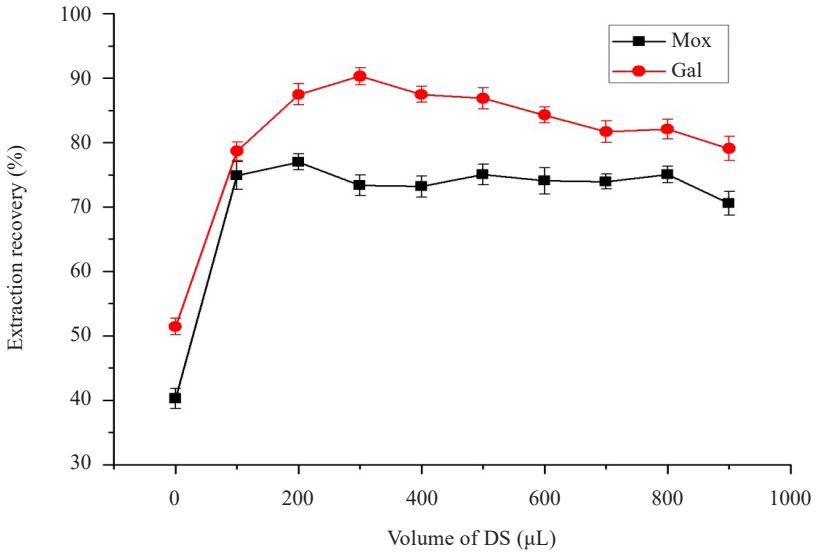


Figure 4. Optimization of the DS volume. Bar indicates standard deviations (n = 3)
Extraction condition: same as that in Figure 3, except pH optimized at 6.9 for Mox,10.2 for Gal

3.5 *Selection of surfactant for SAE (S-DLLME)*

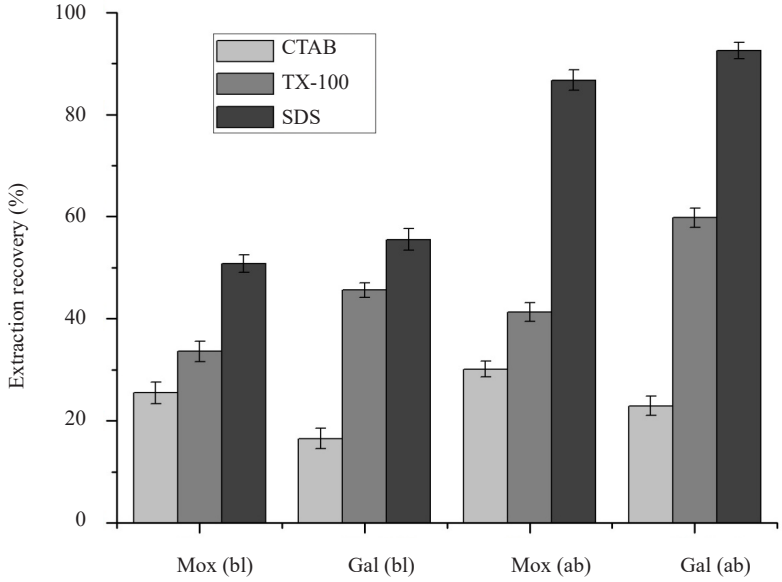


Figure 5. Selection of surfactant: bl = below CMC, ab = above CMC; Bar indicates standard deviations (n = 3)
Extraction condition: sample volume, 5 mL; SDS, 500 µL; ES, 100 µL, pH 4.6 for Mox, 6.2 for Gal; Time, 5min

C-DLLME, was though found efficient in terms of efficiency and Preconcentration Factor (PF), it utilizes some volume of DS. In order to improve the operation, in the context of safer route development, surfactant in place of DS was tested. All the three kinds of surfactants (500 µL), viz. cationic (Cetyl Trimethyl Ammonium Bromide (CTAB)), anionic (Sodium Dodecyl Sulfate (SDS)), and neutral (Triton X-100; TX-100), both below and above their Critical Micellar Concentrations (CMC), were employed, keeping the respective ES (100 µL, each) i.e., ES1 for Mox and ES2 for Gal, at the initial solution pH of 4.6 and 6.2 respectively. It was found that in the case of CTAB the extraction

recovery of both the drugs was less compared to the respective ES-DS combination, due to additional electrostatic repulsion between the cationic drugs and the cationic surfactant. In the case of TX-100 the extraction recovery of both the drugs was comparatively higher than in presence of CTAB, but still less compared to the respective ES-DS combination, at the optimized condition. The presence of non-ionic surfactant may impart micelle formation and solute dispersion. On the other hand, the extraction recovery of both the drugs was improved much in presence of SDS, due to electrostatic attraction between the cationic drugs and the anionic surfactant. The effect of SDS above CMC ($7.5.10^{-3}$ M) was much pronounced compared to that below CMC ($5.0.10^{-3}$ M). It may be assumed that SDS forms an effective micellar core to entrap the drug molecules resulting in improvement of both in ER % and Preconcentration Factor (PF) for each drug (Figure 5). Thus, SDS was selected, as the suitable surfactant, for S-DLLME operation.

3.6 *Optimization of SDS volume in S-DLLME*

In order to optimize the volume of SDS (> CMC), the study was carried out varying SDS from 100 to 900 µL, in the initial solution pH of 4.6 for Mox and 6.2 for Gal, employing 100 µL of the respective ES (Figure 6). The results indicated that maximum extraction recovery of 87.2 and 92.5% for Mox and Gal respectively occurred with 500 and 300 µL of SDS. The PF value was simultaneously enhanced for each drug. Interestingly, the volume requirement of the ES in S-DLLME was found less compared to that in C-DLLME. Moreover, the pH adjustment of the analyte solution was not required. Quantitative recovery was observed with a combination of ES1 (100 µL)-SDS ($7.5.10^{-3}$ M, 500 µL) for Mox (89%), and a combination of ES2 (100 µL)-SDS ($7.5.10^{-3}$ M, 300 µL) for Gal (93%) in S-DLLME, in contrast to the combination of ES1 (200 µL)-DS1 (200 µL) for Mox (77%) and ES2 (500 µL)-DS1 (300 µL) for Gal (90.3%) at the optimized pH in C-DLLME. Thus, more greenness in extraction was achieved with S-DLLME, due to the requirement of the lesser volume of ES and no DS.

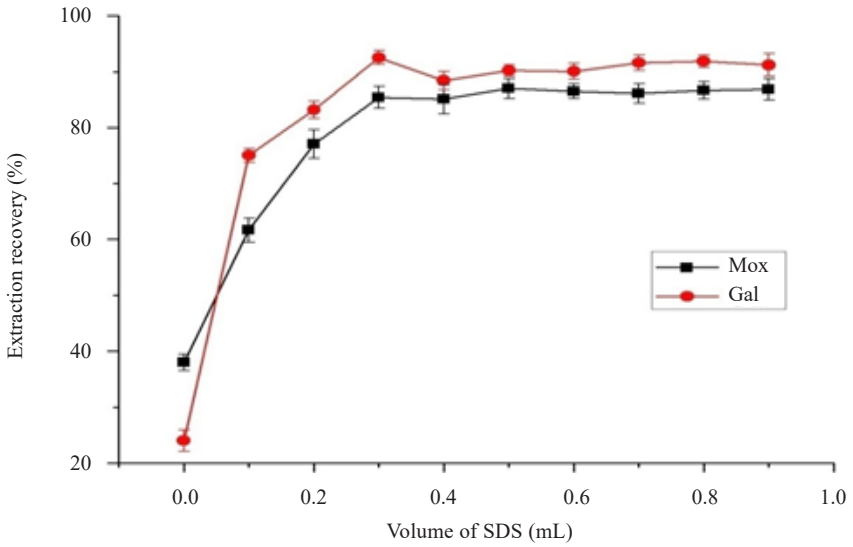


Figure 6. Optimization of SDS volume. Bar indicates standard deviations (n = 3)
Extraction condition: same as that in Figure 5

Further, the experiment was carried to investigate the possibility of mutual separation of the studied drugs using a single combination of binary solvent (ES-surfactant) mixture. Figure 7 illustrated that in presence of SDS either ES1 or ES2 may effectively recover (> 80%) both the drugs. However, the extent of recovery was found more with ES1-SDS (89.6% for Mox and 92.1% for Gal) than ES2-SDS (81.8% for Mox and 85.2% for Gal). The main advantage of SDS assisted extraction was that the requirement of ES volume was less compared to that in conventional DLLME. Thus, with a combination of ES1-SDS, about 90% of both drugs were extracted.

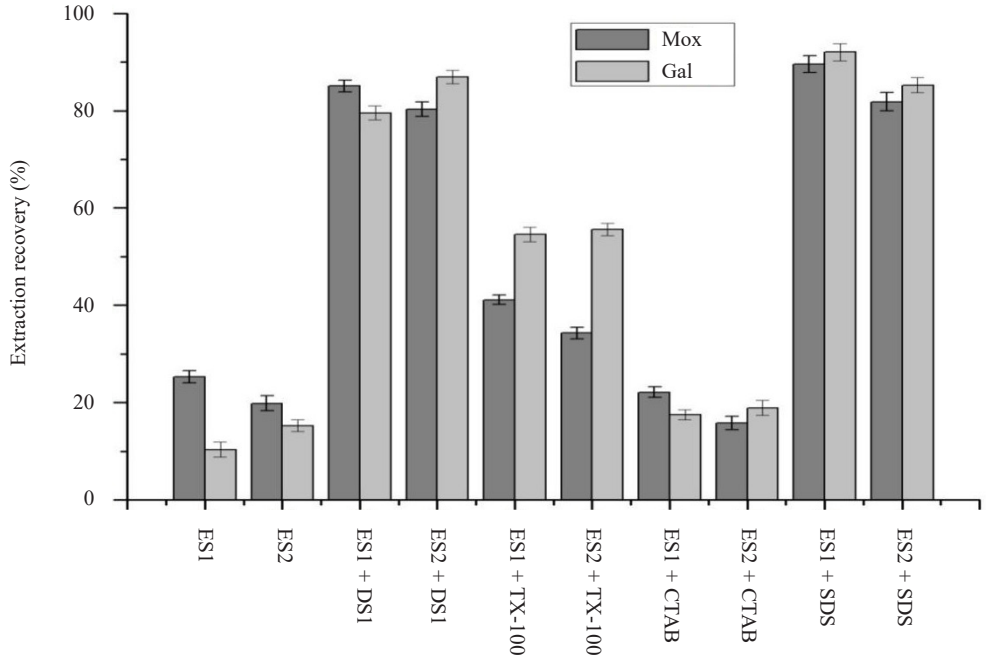


Figure 7. Extraction performances in different solvent systems. Bar indicates standard deviations (n = 3)

3.7 *Effect of pH*

pH-dependent solubility and stability of the drug truly govern the transfer of target analyte from aqueous to organic phase in liquid-phase microextraction. Due to the acid-base property of the drug, ionization occurs with the change of solution pH and hence pH has a direct influence on the extraction. The unionized species is more likely to be extracted into the extraction solvent than the ionized form.[26] It was found that for both the drug's extraction increased with the increase of pH. The highest extraction was found to occur at pH 6.9 and 10.2 for Mox and Gal respectively, in conventional DLLME (Figure 8). The pH-dependent extraction behavior may be assessed from the dissociation constant (pK_a) of the drugs. Moxifloxacin contains one basic amine group ($-NH_2$) and one acidic group (-COOH) that corresponds to two pK_a (pK_{a1} and pK_{a2}) values. Moxifloxacin tends to exist as the cation at pH below pK_{a1} of 5.69. An increase of pH above this would increase the portion of Mox to exist as the neutral molecule. However, the further increase of pH above pK_{a2} of 9.42 would change the neutral species to the anionic form. So, Mox is expected to be extracted more efficiently between pH 5.69 to 9.42.[27] Similarly, the greater extraction efficiency of Gal at a pH of 10.2 may be explained by its pK_a value (8.2). It is expected that Gal exists as neutral species and is extracted above a pH value of its pK_a.

The extraction recovery was found to be higher at the initial solution pH of 4.6 for Mox (86.7%) and at the initial solution pH of 6.2 for Gal (92.5%) in S-DLLME employing SDS concentration higher than CMC (Figure 5), compared to those in C-DLLME at optimized pH condition of 6.9 for Mox and 10.2 for Gal (77% for Mox and 90.3% for Gal, Figure 4). However, below CMC the extent of extraction was found to be less (50.8% for Mox and 55.5% for Gal, Figure 5), compared to those of C-DLLME (Figure 4). Therefore, the pH-dependent extraction behavior of the drugs, using 800 µL of SDS below CMC ($5.0.10^{-3}$ M), was further investigated (Figure 9).

Moxifloxacin extraction showed an almost linear and gradual increase up to a pH increase of 6.5 with a slight gradual decrease beyond it. The highest recovery of 88.6% was observed between pH of 6.5 to 7.3. The extraction behavior of galantamine with the change of solution pH showed a different trend. While 94% recovery occurred at pH 9.0 almost 80% recovery was observed at pH 7.6. The change in the extraction behavior of the drugs for S-DLLME compared to that of the C-DLLME may be due to the acid/base interaction of SDS influencing the micellar core structure formation.[28] In S-DLLME, below CMC of SDS, at least 80% recovery of the drugs at neutral solution pH condition was observed particularly for real sample analysis.

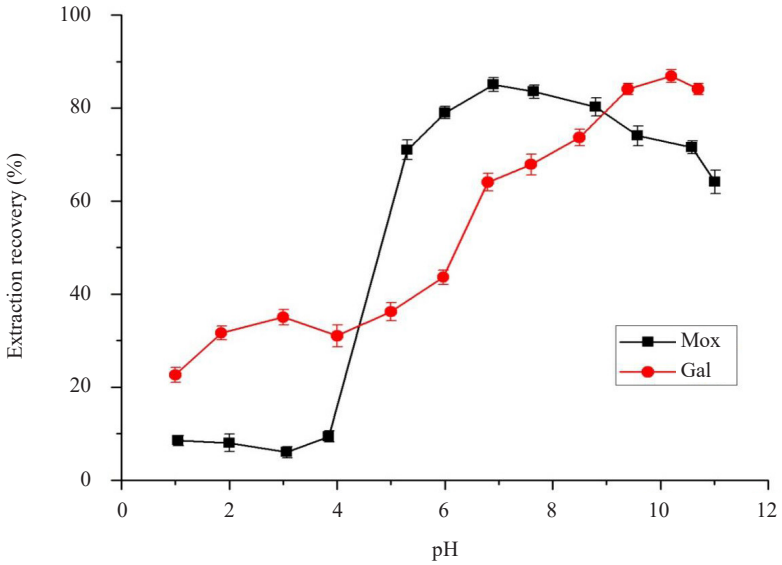


Figure 8. Effect of pH on C-DLLME. Bar indicates standard deviations (n = 3)
Extraction condition: sample, 5 mL; DS1, 200 µL for Mox and 300 µL for Gal; ES1, 600 µL for Mox and ES2, 500 µL Gal; Time, 5 min

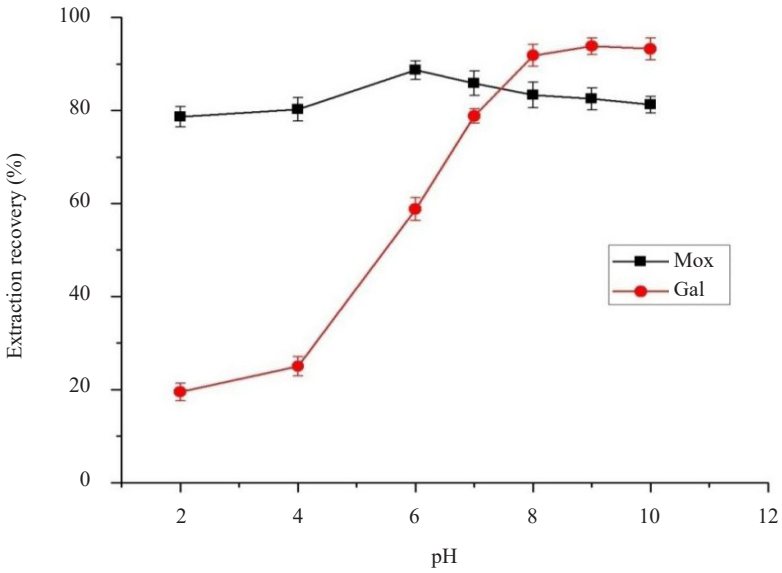


Figure 9. Effect of pH on S-DLLME. Bar indicates standard deviations (n = 3)
Extraction condition: same as that in Figure 5, except SDS (< CMC, 800 µL)

3.8 *Effect of extraction time*

During DLLME, when ES is dispersed into the aqueous phase it transforms into fine droplets and phase separation is done by shaking and centrifugation. Such mechanical operation assists to reach the equilibrium via mass transfer that constitutes the limiting step of extraction. The fine droplets could extract analyte rapidly owing to shorter diffusion distance and large specific surface area.[29] The extraction profile in conventional and SDS aided DLLME was shown in Figure 10 and Figure 11 for Mox and Gal respectively. The equilibrium was reached essentially within 4 min for both the drugs in both C-DLLME and S-DLLME (SDS > CMC). However, the ER % of the drugs was found to be higher in the S-DLLME than in the C-DLLME.

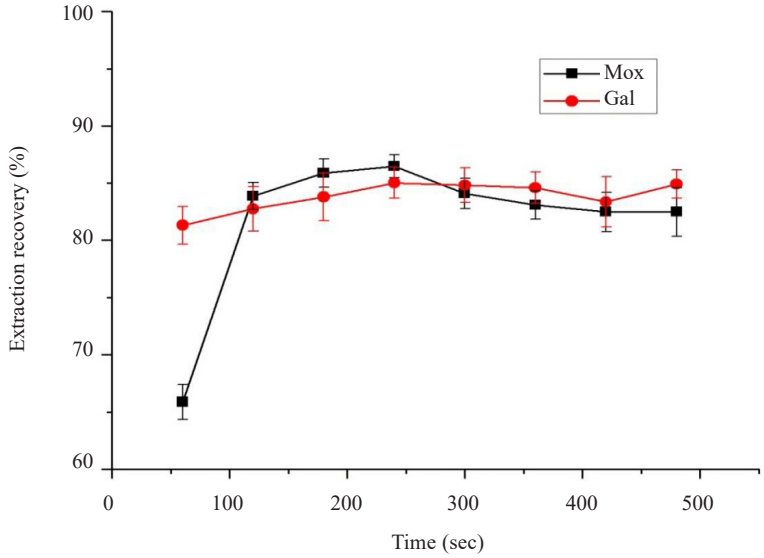


Figure 10. Effect of extraction time on C-DLLME. Bar indicates standard deviations (n = 3)
Extraction condition: same as that in Figure 8, except pH, 6.9 for Mox and 10.2 for Gal

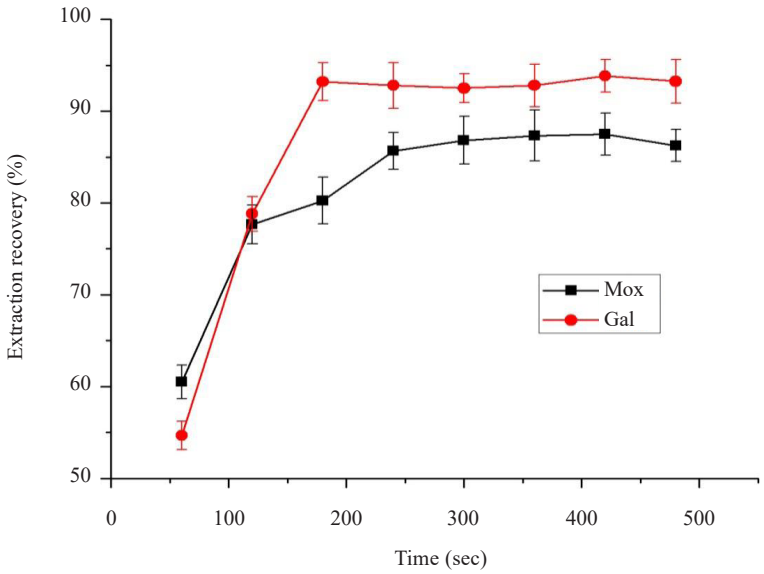


Figure 11. Effect of extraction time on S-DLLME. Bar indicates standard deviations (n = 3)
Extraction condition: same as that in Figure 9, except SDS (> CMC, 100 µL)

3.9 *Effect of temperature*

The temperature may govern the extraction either by modifying the mass transfer rate of the analyte and/or changing the partition equilibrium between the phases. In order to examine the effect of temperature on DLLME, both C-DLLME and S-DLLME aided the experiments were carried out without optimizing the volume of ES, DS, or the surfactant and the solution pH, varying the temperature from 10 to 70 °C. The results indicated that in the case of C-DLLME there was no significant effect for Gal. A maximum recovery of 44.3% was observed at around 40 °C for Mox (Figure 12), although, less than that at the optimized pH condition. However, S-DLLME remained unaffected by the change of operational temperature. A maximum recovery of 87 and 93% for Mox and Gal respectively was observed at the room temperature and optimized ES1-SDS combination.

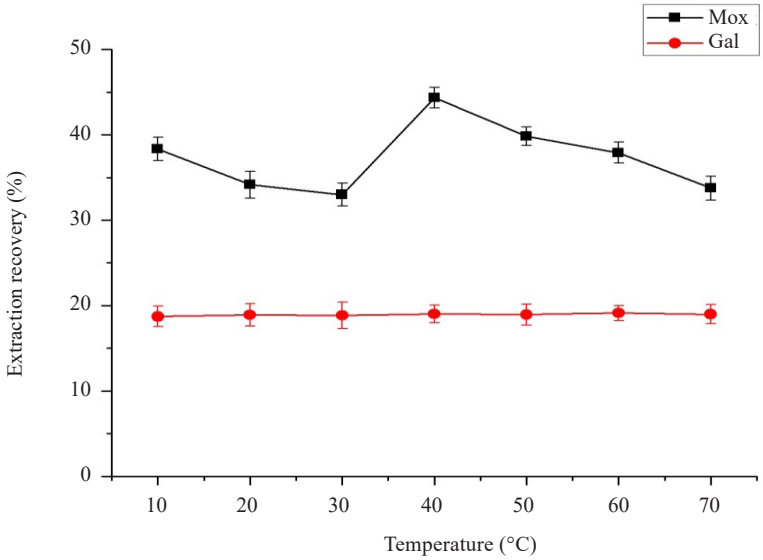


Figure 12. Effect of temperature. Bar indicates standard deviations (n = 3)

3.10 *Effect of salt*

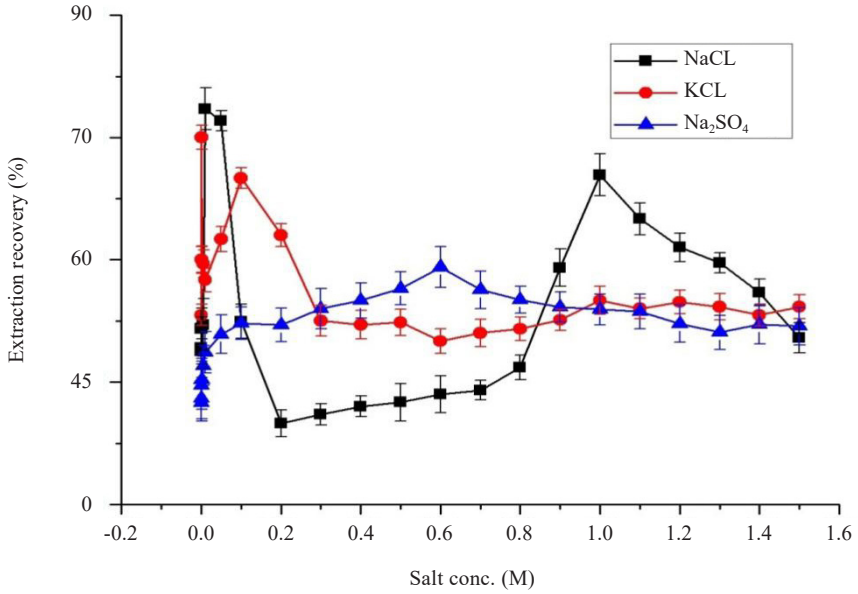


Figure 13. Effect of salt for Mox. Bar indicates standard deviations (n = 3)

It is found that the addition of salt increases the DLLME performance similarly as in the conventional liquid-liquid extraction. When the salt is added, the transfer of analyte into the organic phase increased due to the lesser availability of free water in the aqueous phase.[30] Initially, NaCl and KCl (0-1.5 M) were added to the sample solution, and recovery of Gal remained essentially unaltered but a significant effect was observed on the recovery of Mox. The experiment was carried out for the experiment condition of Figure 1 for C-DLLME without optimizing the volume of ES, DS, and the pH. Higher recovery of Mox (79% and 70% respectively) at two concentration regions viz. low [0.03-0.08 (M)] and high [1.05-1.15 (M)] was found (Figure 13). The increased recovery at the low NaCl concentration region may be

due to the common ion (Cl^-) effect as moxifloxacin hydrochloride was taken as the analyte. Again, in the high NaCl concentration region, the salting-out effect may become pronounced. A similar effect on Mox recovery was observed with KCl (0-1.5 M) addition. The extent of extraction was found to be 76% at lower KCl concentration (0.05 M) and 70% at higher KCl concentration of (1.0 M). However, observed high recovery (60%) in presence of Na_2SO_4 (0.7 M) may be due to the salting-out effect (Figure 13). A similar study of the salt addition on S-DLLME, reflected insignificant effects on ER % and PF for both the drugs. This is probably because of the high enrichment influence of the micellar core of SDS for the analytes.

3.11 *Thermodynamic feasibility*

The feasibility of any chemical interaction is governed by Gibbs standard free energy change (ΔG^0). At any point, a negative ΔG^0 indicates the feasible pathway. In the present case of extraction free energy change was calculated from the Gibbs-Helmholtz equation as:

$$\Delta G^0 = -RT \ln K_c \tag{3}$$

where R is the universal gas constant (J/mol/K), T is the absolute temperature (K) and K_c is the equilibrium constant, which can be calculated from the ratio of equilibrium analyte concentration in sediment phase to that of the aqueous phase.

ΔG^0 values are found to be -8.33 and -6.30 kJ/mole for Mox and Gal respectively for C-DLLME. The negative values indicate that extraction is thermodynamically feasible. In the case of S-DLLME $-\Delta G^0$ value increases for both the drugs. In the case of Mox, $-\Delta G^0$ was found to increase from 8.33 to 9.36 kJ/mole and 6.30 to 9.37 kJ/mole for Gal. The higher $-\Delta G^0$ value compared to C-DLLME indicates a more favorable extraction in S-DLLME for the drugs.

3.12 *Quantitative analysis*

The Calibration curve for each drug was found linear under the optimized condition. The validity testing was done using the parameters viz. the correlation of linear determination (r^2), the Limit of Detection (LOD), and the Limit of Quantification (LOQ) (Table 2). The values were found almost similar for C-DLLME, and S-DLLME. Svinyarov et al.[31] and Lopez et al.[32] reported the LOD for Gal of 0.5 and 0.6 µg mL^{-1} respectively during solid-phase extraction. In the case of Mox, Herrera-Herrera et al.[33] found the LOD values of 3.47, 1.3, and 1.07 µg L^{-1} for water, mineral water, and runoff water respectively after DLLME. Szultka et al.[34] using HPLC-UV and LC-MS/MS after SPE reported the LOD values of 0.041 and 0.139 µg mL^{-1} respectively for Mox. The present study shows improved LOD compared to the reported methods. The r^2 values are found to be >0.995 in all the studied samples except for urine. A somewhat lower value of r^2 for urine may be due to the matrix composition. The matrix effect has little influence on the samples of water and drug.

The optimum extraction condition for Mox was found to be, ES1 (600 µL)-DS1 (200 µL), with 4 min of extraction time at the optimized pH of 6.9 in C-DLLME, and ES1 (100 µL)-SDS ($7.5.10^{-3}$ M, 500 µL), with 4 min of extraction at initial solution pH of 4.6 in S-DLLME. In both cases, recoveries were found greater than 85% and the LODs ranged from 0.9 to 1.0 µg/L. On the other hand, the optimum extraction condition for Gal was found to be, ES2 (500 µL)-DS1 (300 µL) with 4 min of extraction time at the optimized pH of 10.2 in C-DLLME, and ES1 (100 µL)-SDS ($7.5.10^{-3}$ M, 300 µL) with 4 min of extraction time at initial solution pH of 6.2 in S-DLLME. In both cases, recoveries greater than 90% were obtained and LODs ranged from 4.25 to 5.0 µg/L.

The acceptability of the process was indicated by precision and accuracy. The precision and accuracy of the method were assessed under the optimized conditions for both intra and inter-days. While the precision expresses the closeness of the successive measurements of an analyte, the accuracy estimates the deviation of mean result from the individual concentration. The Quality Control (QC) samples were prepared at low, medium, and high concentrations of 2.5, 5, and 10 µg/L for Mox and 10, 30, and 50 µg/L for Gal and analyzed on the same day (intra-day assay) and three different days (inter-day assay). Calculations were based on three replicates (intra-day and inter-day) of QC samples. The obtained results were expressed as the Relative Standard Deviation (RSD) and the Relative Error (RE).

The obtained results were presented in Table 3 and Table 4 for Mox and Gal respectively. The relative error was found to be in between 7.6 and 39.8% for C-DLLME and 8.0 and 15.2% for S-DLLME in the case of Mox; and the case of Gal in between 9.1 and 20.7 % for C-DLLME and 2.9 and 16.6% for S-DLLME. In C-DLLME, RSD was found to be range from 0.80-1.51 for Mox and from 1.21-1.59 for Gal. In S-DLLME, RSD was found to be range from 0.48-1.39 for Mox and from 0.58-1.14 for Gal (Table 3, 4). Thus, S-DLLME was found to be more precise and accurate than C-DLLME. The results demonstrated that the values were within the acceptable range recommended by the Food and Drug Administration (FDA).

Table 2. Analytical performance of C-DLLME and S-DLLME

Parameter	Sample	C-DLLME		S-DLLME	
		Moxifloxacin	Galantamine	Moxifloxacin	Galantamine
Linear equation of the calibration; r^2	Mineral water	y = 0.852x + 0.073; 0.998	y = 0.863x + 0.126; 0.999	y = 0.873x + 0.150; 0.999	y = 0.906x + 0.093; 0.999
	Tap water	y = 0.818x + 0.134; 0.995	y = 0.880x + 0.464; 0.998	y = 0.832x + 0.302; 0.996	y = 0.879x – 0.026; 0.993
	River water	y = 0.805x + 0.200; 0.997	y = 0.841x – 1.086; 0.995	y = 0.829x + 0.286; 0.991	y = 0.896x – 0.497; 0.995
	Rain water	y = 0.820x + 0.184; 0.996	y = 0.868x – 0.254; 0.997	y = 0.819x + 0.308; 0.990	y = 0.862x – 0.913; 0.997
	Urine	y = 0.626x + 0.112; 0.977	y = 0.899x – 0.578; 0.981	y = 0.711x + 0.817; 0.974	y = 1.024x – 2.419; 0.978
	Medicine 1	y = 0.834x + 0.046; 0.995	-	y = 0.810x + 0.347; 0.991	-
	Medicine 2	-	y = 0.871x – 0.024; 0.995	-	y = 0.896x – 0.390; 0.997
LOD (µg/L)	Mineral water	1.00	5.00	0.90	4.25
	Tap water	1.25	4.25	1.00	4.00
	River water	1.42	5.50	1.25	4.75
	Rain water	1.30	5.00	1.30	4.50
	Urine	2.01	4.00	1.75	4.00
	Medicine 1	0.95	-	0.90	-
	Medicine 2	-	5.25	-	4.50
LOQ (µg/L)	Mineral water	2.50	10.00	2.50	8.00
	Tap water	2.75	9.50	2.50	8.50
	River water	2.50	10.50	2.25	9.00
	Rain water	2.40	9.00	2.50	9.00
	Urine	2.75	9.00	2.35	8.50
	Medicine 1	2.10	-	2.00	-
	Medicine 2	-	8.50	-	8.50

y: detector response (chromatographic peak area), x: concentration

Table 3. Analytical precision and accuracy of C-DLLME and S-DLLME for Mox

Sample	Added (µg/L)	C-DLLME Found mean ± SD; RSD %; RE %		S-DLLME Found mean ± SD; RSD %; RE %	
		Inter day	Intra day	Inter day	Intra day
Mineral water	2.5	2.10 ± 0.02; 1.25; 16.0	2.12 ± 0.02; 1.14; 15.2	2.21 ± 1.25; 0.93; 11.6	2.22 ± 1.1; 0.97; 11.2
	5.0	4.40 ± 0.06; 1.44; 12.0	4.30 ± 0.05; 1.34; 14.0	4.52 ± 1.38; 0.61; 9.6	4.43 ± 1.24; 0.82; 11.4
	10.0	8.52 ± 0.11; 1.29; 14.8	8.45 ± 0.10; 1.23; 15.5	8.87 ± 1.36; 1.05; 11.3	8.89 ± 1.35; 0.93; 11.1
Tap water	2.5	2.02 ± 0.02; 1.24; 19.2	2.09 ± 0.02; 1.20; 16.4	2.15 ± 1.34; 0.48; 14.0	2.16 ± 1.26; 0.83; 13.6
	5.0	4.27 ± 0.06; 1.51; 14.6	4.37 ± 0.06; 1.44; 12.6	4.35 ± 1.42; 1.07; 13.0	4.52 ± 1.23; 0.61; 9.6
	10.0	8.22 ± 0.12; 1.42; 17.8	8.31 ± 0.11; 1.56; 16.8	8.67 ± 1.35; 1.03; 13.3	8.65 ± 1.25; 0.93; 13.5
River water	2.5	2.03 ± 1.34; 0.99; 18.8	2.04 ± 1.25; 0.98; 18.4	2.12 ± 1.20; 0.78; 15.2	2.20 ± 1.25; 0.80; 12.0
	5.0	4.62 ± 1.28; 0.93; 7.6	4.06 ± 1.45; 0.98; 18.8	4.38 ± 1.29; 1.03; 12.4	4.39 ± 1.32; 0.94; 12.2
	10.0	8.12 ± 1.42; 1.02; 18.8	8.13 ± 1.56; 1.18; 18.7	8.64 ± 1.44; 1.11; 13.6	8.78 ± 1.45; 0.98; 12.2
Rain water	2.5	2.05 ± 1.20; 0.81; 18.0	2.20 ± 1.35; 0.98; 12.0	2.08 ± 1.26; 0.83; 16.8	2.24 ± 1.29; 1.02; 10.4
	5.0	4.22 ± 1.34; 1.02; 15.6	4.28 ± 1.25; 0.93; 14.4	4.35 ± 1.35; 1.07; 13.0	4.48 ± 1.45; 1.39; 10.4
	10.0	8.54 ± 1.33; 0.89; 14.6	8.54 ± 1.38; 0.97; 14.6	8.62 ± 1.53; 1.09; 13.8	8.95 ± 1.56; 1.17; 10.5
Urine	2.5	1.65 ± 1.27; 0.80; 34.0	1.72 ± 1.26; 0.96; 31.2	2.25 ± 1.34; 1.02; 10.0	2.37 ± 1.28; 0.53; 5.2
	5.0	3.01 ± 1.36; 0.99; 39.8	3.10 ± 1.24; 0.96; 38.0	4.54 ± 1.45; 1.09; 9.2	4.60 ± 1.41; 0.98; 8.0
	10.0	6.50 ± 1.39; 0.92; 35.0	6.60 ± 1.33; 0.60; 34.0	7.82 ± 1.42; 1.10; 21.8	7.81 ± 1.35; 0.93; 21.9
Medicine 1	2.5	2.01 ± 1.25; 0.99; 19.6	2.07 ± 1.35; 1.04; 17.2	2.15 ± 1.34; 0.48; 14.0	2.20 ± 1.26; 0.80; 12.0
	5.0	4.35 ± 1.34; 1.07; 13.0	4.37 ± 1.41; 1.06; 12.6	4.24 ± 1.25; 0.95; 15.2	4.41 ± 1.56; 1.19; 11.8
	10.0	8.10 ± 1.51; 1.07; 19.0	8.40 ± 1.62; 1.23; 16.0	8.67 ± 1.48; 1.15; 13.3	8.75 ± 1.60; 1.12; 12.5

Table 4. Analytical precision and accuracy of C-DLLME and S-DLLME for Gal

Sample code	Added (µg/L)	C-DLLME Found:mean ± SD; RSD %; RE %		S-DLLME Found:mean ± SD; RSD %; RE %	
		Inter day	Intra day	Inter day	Intra day
Mineral water	10.0	8.67 ± 1.42; 1.03; 13.3	8.78 ± 1.35; 0.98; 12.2	9.23 ± 1.34; 0.93; 7.7	9.25 ± 1.25; 0.97; 7.5
	30.0	26.26 ± 1.52; 1.00; 12.4	26.35 ± 1.54; 1.12; 12.1	27.72 ± 1.48; 1.15; 7.6	27.80 ± 1.46; 1.07; 7.3
	50.0	44.12 ± 1.55; 1.10; 11.7	44.23 ± 1.46; 1.10; 11.5	45.84 ± 1.45; 1.09; 8.3	45.92 ± 1.54; 1.18; 8.1
Tap water	10.0	8.82 ± 1.21; 0.86; 11.8	8.93 ± 1.22; 0.95; 10.7	9.10 ± 1.26; 0.84; 9.0	9.15 ± 1.23; 0.94; 8.5
	30.0	26.72 ± 1.63; 1.25; 10.9	26.65 ± 1.23; 0.91; 11.1	27.35 ± 1.42; 1.09; 8.8	27.45 ± 1.49; 1.06; 8.5
	50.0	45.43 ± 1.44; 1.07; 11.1	45.65 ± 1.32; 1.01; 10.7	44.75 ± 1.55; 1.16; 10.5	44.90 ± 1.48; 1.14; 10.2
River water	10.0	8.03 ± 1.23; 0.83; 19.7	8.24 ± 1.24; 0.44; 17.6	9.10 ± 1.36; 0.84; 9.0	9.12 ± 1.35; 1.05; 8.8
	30.0	24.10 ± 1.35; 1.02; 19.6	24.22 ± 1.40; 1.10; 19.2	27.15 ± 1.42; 1.05; 9.5	27.42 ± 1.41; 1.06; 8.6
	50.0	39.65 ± 1.25; 0.92; 20.7	40.12 ± 1.34; 0.96; 19.7	44.6 ± 1.58; 1.20; 10.8	44.90 ± 1.51; 1.14; 10.2
Rain water	10.0	8.75 ± 1.38; 1.04; 12.5	8.96 ± 1.25; 0.93; 10.4	9.01 ± 1.32; 0.99; 9.9	9.17 ± 1.25; 0.98; 8.3
	30.0	26.16 ± 1.25; 0.96; 12.8	26.27 ± 1.24; 0.93; 12.4	27.10 ± 1.25; 0.95; 9.6	27.29 ± 1.46; 1.12; 9.0
	50.0	43.54±1.41; 1.02; 12.9	43.76±1.32; 0.99; 12.4	43.95±1.48; 1.02; 12.1	44.21 ± 1.38; 1.04; 11.5
Urine	10.0	9.09 ± 1.37; 0.95; 9.1	9.11 ± 1.27; 0.87; 8.9	9.25 ± 1.22; 0.97; 7.5	9.31 ± 1.35; 1.00; 6.9
	30.0	26.57 ± 1.51; 1.09; 11.4	26.80 ± 1.48; 1.13; 10.6	24.85 ± 1.26; 0.87; 17.1	25.00 ± 1.44; 1.10; 16.6
	50.0	44.96 ± 1.59; 1.22; 10.0	45.21 ± 1.59; 1.06; 9.5	48.28 ± 1.58; 1.11; 3.4	48.51 ± 1.43; 1.09; 2.9
Medicine 2	10.0	8.96 ± 1.33; 0.89; 10.4	9.01 ± 1.30; 0.99; 9.9	9.02 ± 1.34; 0.88; 9.8	9.08 ± 1.42; 0.95; 9.2
	30.0	26.90 ± 1.23; 0.87; 10.3	26.92 ± 1.34; 0.91; 10.2	27.15 ± 1.39; 0.58; 9.5	27.22 ± 1.53; 1.18; 9.2
	50.0	44.82 ± 1.56; 1.22; 10.3	45.01 ± 1.52; 1.09; 9.9	44.20 ± 1.42; 1.06; 11.6	44.66 ± 1.56; 1.07; 10.6

3.13 *Analysis of real samples*

Table 5. Relative performance of C-DLLME and S-DLLME in real samples

Sample		Extraction recovery (ER %), Preconcentration factor (PF)			
Type	Composition	Mox		Gal	
		C-DLLME	S-DLLME	C-DLLME	S-DLLME
Tap water	pH: 6.71; EC: 625; Eh: 21.3; TDS: 400; F^-: 0.86; Cl^-: 251.22; NO_3^-: 24.32; SO_4^{2-}: 3.04; TH: 92.50	83.05, 24.08	51.15, 51.15	89.23, 9.81	91.16, 53.78
Rain water	pH: 6.49; EC: 20.03; Eh: 28.7; TDS: 12.82; F^-: 0.072; Cl^-: 0.1; NO_3^-: 0.097; SO_4^{2-}: 0.241; Na^+: 0.097; K^+: 0.027; Ca^{2+}: 0.317; Mg^{2+}: 0.070; HCO_3^-: 0.06	85.30, 24.73	24.73, 53.10	87.56, 9.63	93.66, 55.25
River water	pH: 6.78; EC: 608; Eh: 25.0; TDS: 389.12; DO: 7.01; TH: 102.4; PO_4^{3-}: 0.098; NO_3^-: 0.032	81.20, 23.54	86.40, 50.97	80.34, 8.84	89.20, 52.62
Urine	pH: 6.57; EC: 19.14; Eh: 29.6; TDS: 12.24; Urea: 9.3; Cl^-: 1.87; Na^+: 1.17; K^+: 0.750; Creatinine: 0.670; Inorganic sulfur: 0.163	62.32, 18.07	78.20, 46.13	70.90, 9.99	96.20, 56.75
Medicine 1	Nature: Liquid for intravenous administration; Each 100 mL contains: Moxifloxacin: 400 mg, Mannitol: 5 g	82.37, 23.88	87.51, 51.62		
Medicine 2	Nature: Solid as tablet; Galantamine: 4 mg Titanium dioxide (color)			89.61, 9.85	90.25, 53.24

All except pH, EC (µS/m), Eh (mV) in mg/L

Table 6. Comparison with reported methods

Method	Sample origin	Vol. of ES (µL)	ER %	PF	Reference
	Galantamine				
Ionic liquid-supported solid-liquid extraction using HPLC-UV	Plant	12 #	97.86-100.86	-	[31]
Solid-phase extraction and reversed-phase high performance liquid chromatography diode array detector	Plant	-	91.3	-	[32]
High-performance liquid chromatographic method with UV photodiode-array, fluorescence and mass spectrometric detection	Bio	-	81.0	-	[35]
DLLME coupled with HPLC-UV Conventional/SDS assisted	Water, urine and medical formulations	500/100	86.88/92.00	10.94/46.92	Present report
	Moxifloxacin				
Dispersive liquid-liquid microextraction combined with ultra-high performance liquid chromatography	Mineral, runoff water	658	94.00, 111.00	-	[33]
Solid phase microextraction fibers and liquid-chromatography-tandem mass spectrometry	Whole blood	-	99.29	-	[34]
Solid phase extraction combined with liquid chromatography	Dilatation and curettage material	-	92.70	-	[36]
DLLME coupled with HPLC-UV Conventional/SDS assisted	Water, urine and medical formulations	200/100	85.03/89.56	26.35/45.39	Present report

mL

Tap water was collected from Kalyani University tap, rainwater was collected in the rainy season and river water from the Hooghly river (22049' N, 88020' E; West Bengal, India). Urine samples used were collected from healthy young male adults without any medication. Initially, samples were found free from the contamination of the drugs. The samples were spiked with the desired level of standard drug solution prior to extraction. Table 5 indicates the efficiency of C-DLLME and S-DLLME in real samples in terms of ER % and PF. The data reveals that both ER % and PF increase in S-DLLME compared to C-DLLME. Although both C-DLLME and S-DLLME are effective, considering the low volume of ES, without any requirement of DS, higher ER % and PF as well as greater thermodynamic feasibility the S-DLLME is highly recommended for the extraction of pharmaceuticals from water and bio-samples. Finally, the present method is compared with the reported methods (Table 6).

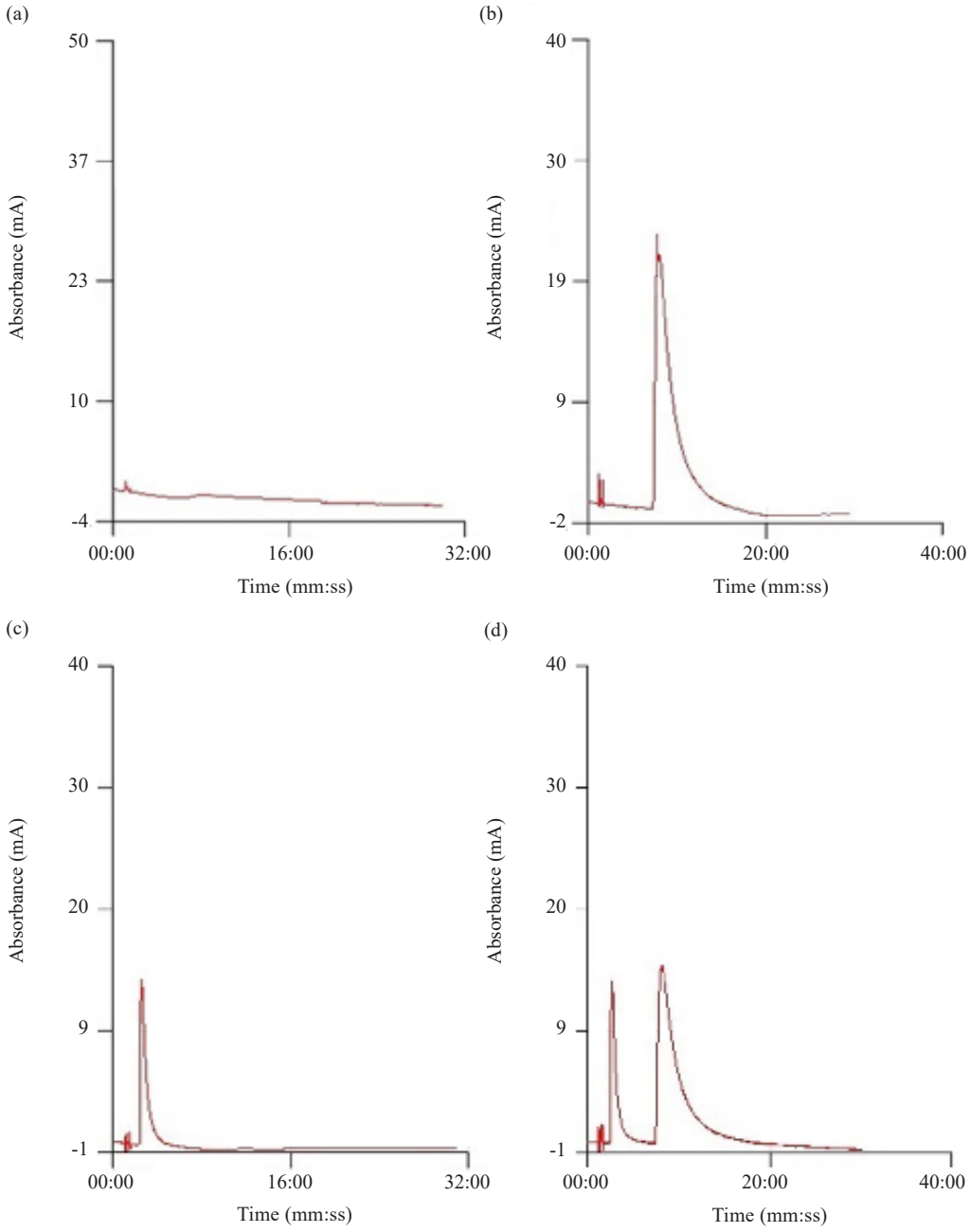


Figure 14. Chromatogram of (a) M-phase, (b) Mox-M-phase, (c) Gal-M-phase, (d) Mox-Gal-M-phase
Experimental condition: M-phase, $(NH_4)H_2PO_4$:CH_3CN: 70:30, adjusted at pH 3 by ortho-H_3PO_4;
Flow rate, 1 mL/min; spiked samples concentration, Mox: 2.5 µg/L and Gal: 10 µg/L

Further, the HPLC analyses of the samples were performed to identify and quantify each drug in the samples. The chromatograms of some representative samples were presented in Figure 14-16 (Figure 14, blank; and spiked: Figure 15, tap water; Figure 16: urine). The peak retention time parameter was matched with the standard, to identify the respective drug. Some additional peaks in tap water and urine sample indicated the matrix effect. The quantification of the peak area indicated the drug concentration in the sample.

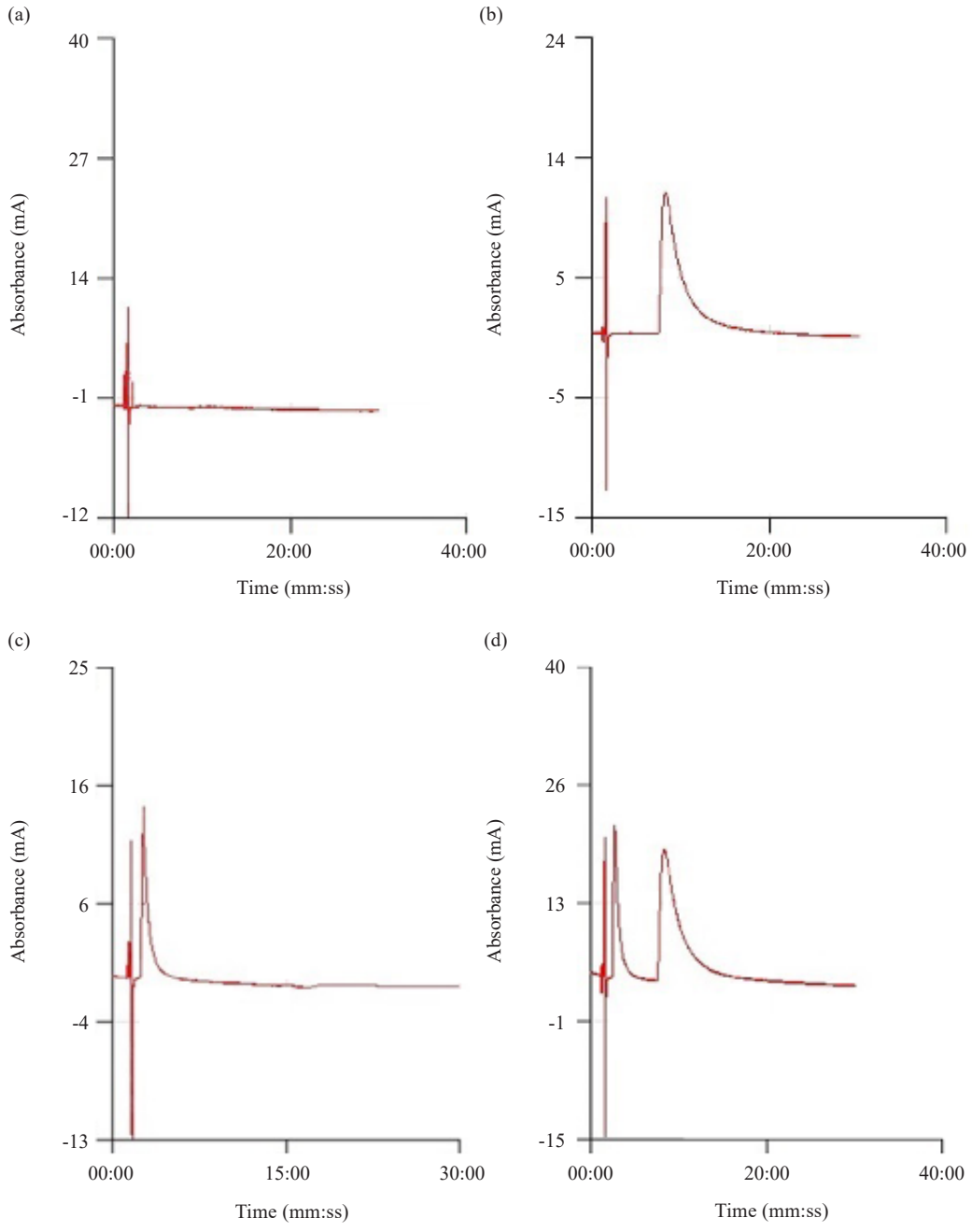


Figure 15. Chromatogram of (a) tap water, (b) Mox-tap water, (c) Gal-tap water, (d) Mox-Gal-tap water
Experimental condition: same as that in Figure 14

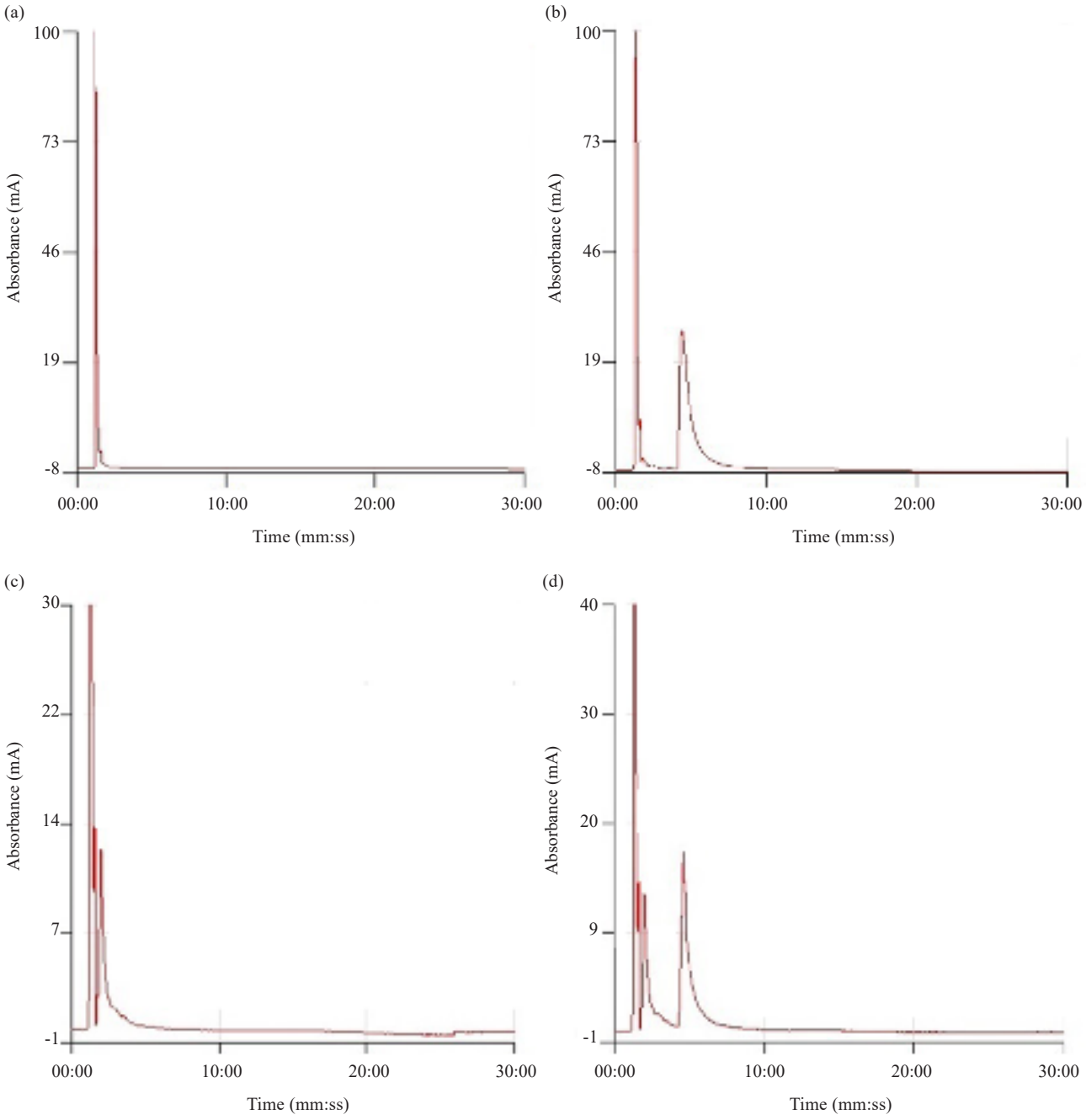


Figure 16. Chromatogram of (a) urine, (b) Mox-urine, (c) Gal-urine, (d) Mox-Gal-urine
Experimental condition: same as that in Figure 14

4. Conclusions

Extractive recovery prior to determination of pharmaceuticals, viz. moxifloxacin and galantamine, was developed using C-DLLME coupled with HPLC-UV. The optimized condition was found to be ES1-DS1 at pH 6.9 and 5 min for Mox, and ES2-DS1 at pH 10.2 and 5 min for Gal. The method is simple, sensitive, rapid, cost-effective, the linear range of determination, thermodynamically feasible. An improved operation in terms of efficiency, preconcentration factor, spontaneity, and greenness is achieved in S-DLLME using ES1 and SDS (> CMC). The low volume requirement of ES, no use DS, and no need for pH adjustment make the process more promising. Similar extraction of the drugs can

be achieved with SDS (< CMC), but with some higher volume. Temperature plays an insignificant role in improving extraction efficiency. The addition of some common ion to the sample solution was found to somewhat enhance the extraction efficiency of Mox, although much less than that of C-DLLME at the optimized condition and S-DLLME. The method can be applied for the analysis of different water and urine samples as well as in medical formulations. The method may be used for assessing the active component as well as to detect spurious medicines.

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Conflict of interest

The authors declare that there is no conflict of interest.

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