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An Ultraperformance Liquid Chromatography Tandem−Mass Spectrometry Method for Determination of Multiclass Pharmaceuticals in Water Sample by Dispersive Liquid−Liquid Microextraction Combined with Ultrasound Assisted Reverse Extraction from Solidified Floating Organic Droplets

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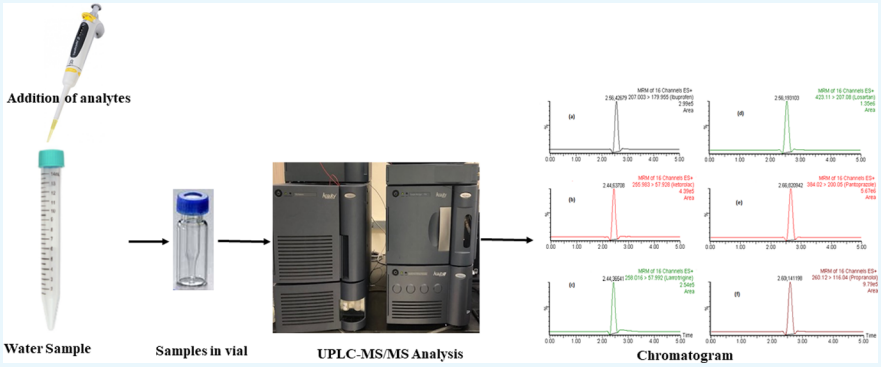

ABSTRACT: A novel, simple, and reliable ultraperformance liquid chromatography tandem-mass spectrometry (UPLC−MS/MS) assay based on dispersive liquid−liquid microextraction followed by ultrasound-assisted reverse extraction from solidified floating organic droplets was established for determination of multiclass pharmaceuticals in the water sample. Six commonly used drugs of various therapeutic classes: ibuprofen, ketorolac, lamotrigine, propranolol, pantoprazole, and losartan were extracted from water samples by using 50 μL 1-undecanol as extracting solvent and 400 μL acetonitrile as dispersive solvent. After collecting the floating organic droplets by cold centrifugation, an ultrasound-assisted back extraction procedure was performed to make the sample compatible for UPLC−MS/MS analysis. Acquity BEH C_{18} column (2.1 × 100; 1.7 μm) was used for separation of target analytes that were eluted by a gradient mobile phase composition of 15 mM ammonium acetate and acetonitrile at a flow rate of 0.25 mL/min. The sample ionization was performed by using electrospray ionization in positive mode, and multiple reaction monitoring was used for quantification of target analytes. After optimizing the assay conditions, all calibration curves were found to be linear with limit of detection and limit of quantification were ranged in between 0.06−0.15 and 0.16−0.41 ng/mL, respectively. The enrichment factor was found to be 172−192-fold and the relative recovery was ranged between 93.1 and 109.4% between target analytes. These satisfactory results confirmed that the proposed method is specific and reliable for application of trace analysis of target analytes in waste water samples.

INTRODUCTION

The consumptions of pharmaceutical and pharmaceutical care products are growing throughout the world due to increase in affordability and availability of generic medicine, high burden of diseases, and large population growth.[1] Therapeutic effects of these products are produced due to the presence of active pharmaceutical ingredient (API) as the main constituent in their formulations. The high consumption of APIs and their presence as pollutants in the environment has become a challenging issue in the 21st century. The amount of human consumed APIs reaching the environment depends on the consumption amount, excreted (via feces and urine), and finally discharged into the wastewater system.[2,3] Therefore, the influents of wastewater treatment plants (WWTPs) are being considered as the main source of drugs in the aquatic environment.[4] Since water is essential for healthy living, the

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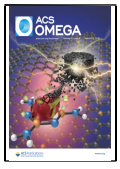

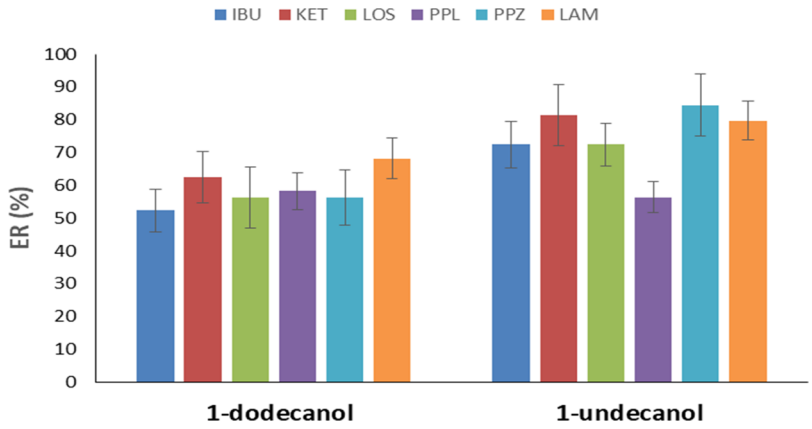

Figure 1. Effect of type of extraction solvent on the extraction recovery of target analytes by the DLLME-SFO procedure.

polluted form of water might lead to serious health and environmental issues.[5]

The sample analysis in waste water is challenging due to its complex nature by the presence of suspended and dissolved organic and inorganic chemicals. Moreover, the presence of trace levels of APIs in waste water samples, its extraction and pre-concentration are also a crucial step to improve the sensitivity as well as selectivity of analytical methods.[6] As a consequence, highly sensitive and specific analytical techniques are required for determination of APIs at trace levels in waste water samples.[7] Therefore, expensive and complex solid phase extraction (SPE) method is the most commonly employed to concentrate the analytes present in wastewater samples rather than the economical and simple traditional liquid−liquid extraction (LLE).[8] In recent decades, dispersive liquid−liquid microextraction (DLLME) has been emerged as a modern sample pretreatment method, which garnered considerable attention from researchers for the trace determination of analytes due to its simple, inexpensive, and environment friendly approach and is advantageous due to short sample preparation time and high enrichment factor [EF].[9] In DLLME procedure, usually, extractive solvents are mixed with dispersive solvents and then forcibly injected into sample matrices, which leads to the dispersion of extraction solvent in the form of small fine droplets throughout the matrices and samples that become cloudy is manually separated after the centrifugation process. DLLME-coupled with solidification of floating organic droplets (DLLME-SFO) that is first developed by Leong and Huang is an advance form of liquid phase microextraction technique.[10] Unlike DLLME technique, a less toxic water insoluble extraction solvent (e.g., 1-undecanol, 1-dodecanol) in a trace amount is usually used in the DLLME-SFO procedure, which is considered as more environment friendly approach for a sample extraction procedure. After dispersion of the extracting solvent with the help of the dispersive solvent, the samples become cloudy and the analyte is transferred to the extraction solvents, which floats on the top of the extraction tube after centrifugation due to its low density. After centrifugation, the organic droplets solidified by immediately transferring the extraction tube into an ice bath and then collected carefully to a suitable vial, which further become melted and liquefied at ambient temperature and then it was finally injected for analysis. As a result, it has attracted much attention in the environmental analysis community and was used to detect various organic compounds in environmental water samples.[11] In spite of its advantages, DLLME-SFO procedure is more commonly coupled with traditional high-performance liquid chromatographic (HPLC) technique for determination of analytes and has limited application with liquid chromatography tandem-mass spectrometry (LC−MS/MS)-based analysis.[11−13] However, LC−MS/MS or ultra-performance liquid chromatography tandem-mass spectrometry UPLC−MS/MS is advance, highly sensitive, specific, and one of the most preferred techniques for quantitative analysis. It has been assumed that the final liquefied organic droplets phase of the DLLME-SFO procedure are highly viscous in nature (due to extractive solvents e.g., undecanol and dodecanol) and have compatibility issue with the mass spectrometric-based detection system, which limit its application in HPLC−MS/MS analysis. To overcome the incompatibility challenge, Canales et al. modified the DLLME-SFO procedure by the addition of ultrasound-assisted back extraction (UABE) of the analytes into mass spectrometer friendly and compatible aqueous organic solvents (acetonitrile, methanol, or mobile phase) before the analysis of some toxic heterocyclic aromatic amines in natural water samples.[5] After application of the UABE procedure, the MS compatible organic solvents facilitate the injection of the DLLME-SFO extracted compound directly to the MS/MS system. Recently, we have modified and simplified the DLLME-SFO-UABE procedure for the quantitative analysis of suvorexant in human urine matrices.[14] However, to the best of our knowledge, DLLME-SFO-UABE has not yet been applied in the analysis of pharmaceutical analytes in water samples. Therefore, a novel DLLME-SFO-UABE procedure coupled to the UPLC−MS/MS method was developed for the quantitative determination of six most commonly used pharmaceuticals of various therapeutic classes; ibuprofen (IBU) and ketorolac (KET) used as analgesic, lamotrigine (LAM) used as an anticonvulsant, propranolol (PPL) used as a β-blocker, losartan (LSR) used as an antihypertensive agent, and pantoprazole (PPZ) used as a proton pump inhibitor in water samples. Since the expected levels of pharmaceuticals in water samples depend upon their amount of consumption during treatment of disease. Most of the above selected pharmaceuticals are the choice of drug for their respective classes, and are also being used for long term treatment in chronic diseases. Therefore, the frequency of administering is high, which increases the possibility of their occurrence in waste water samples. Compared to a previously reported method for the above target analytes in water samples, the extraction procedure of this assay is more environment friendly, simple, and

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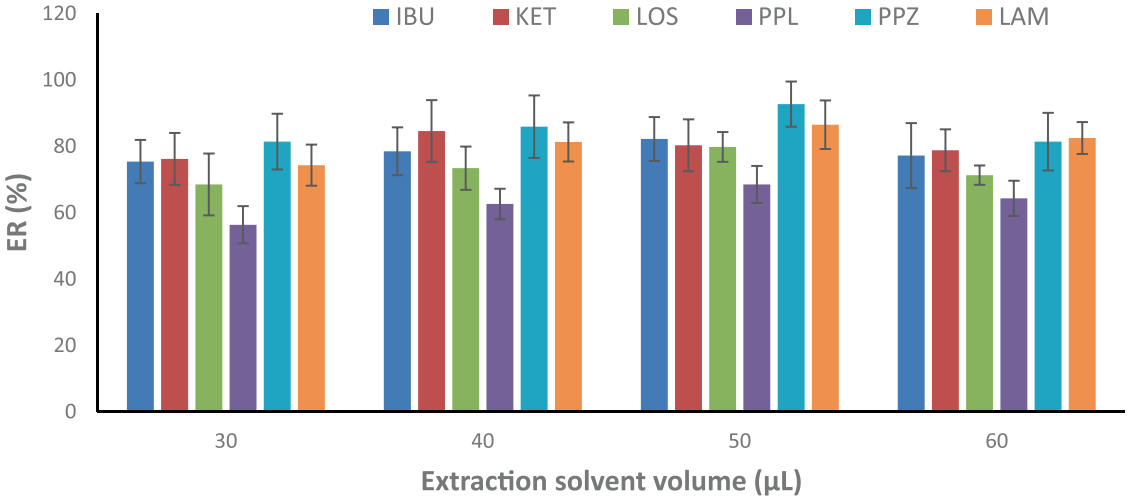

Figure 2. Effect of volume of 1-undecanol (extraction solvent) on the extraction recovery of target analytes by the DLLME-SFO procedure.

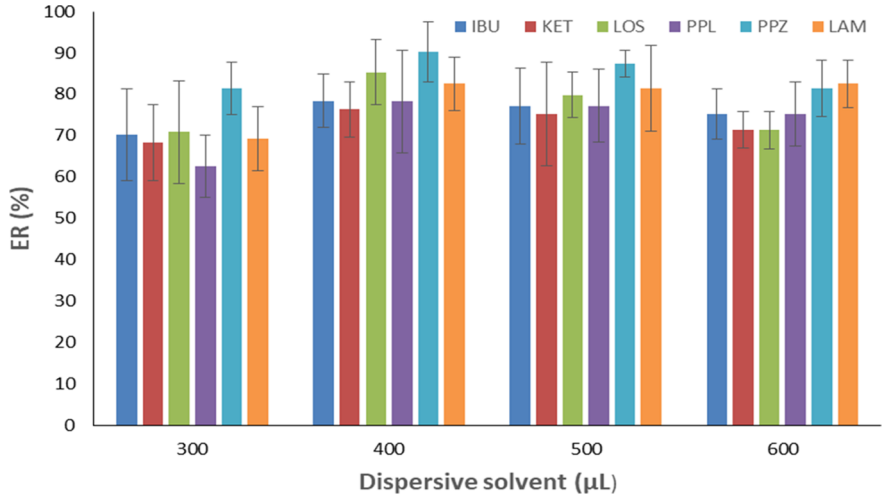

Figure 3. Effect of volume of acetonitrile (dispersive solvent) on the extraction recovery of target analytes by the DLLME-SFO procedure.

economical with satisfactory EF and extraction recovery (% ER).

RESULTS AND DISCUSSION

The EF and ER of analytes from the water samples by the proposed DLLME-SFO-UABE procedure were initially guided by mass transfer from water samples to extraction solvent followed by back extraction (BE) steps to BE solvent. Therefore, the influence of parameters that affects these two steps was carefully studied and their experimental conditions were optimized to achieve extraction efficiency of the target analytes in a shortest possible time frame.

Evaluation of DLLME-SFO Conditions. *Optimization of Extraction Solvent.* The selection of an appropriate extracting solvent is a crucial step in the DLLME-SFO procedure. To maximize the extraction efficiency of target analytes, an ideal extracting agent should be low volatile in nature, density should be lower than water so that it can appear on top, low solubility in water for high partitioning effects, and can easily be solidified at low temperature. Keeping in mind of the abovementioned requirements and based on the previous literature, 1-undecanol and 1-dodecanol were evaluated as extraction solvents in this experiment.[5,14] The % ER was evaluated by using 40 μL of each extractant containing 400 μL of acetonitrile in triplicates (n = 3). Herein,1-undecanol was considered as the extractive solvent as % ER of all analytes was comparatively higher with it except PPL, which showed a % ER slightly higher with 1-dodecanol (Figure 1). Although the melting point of 1-dodecanol is higher than 1-undecanol, but the solid droplets produced with 1-dodecanol was not in proper shape during collection from cooling centrifuge tube, which make it difficult to transfer from sample tubes. Comparatively, the droplet shape produced by 1-undecanol was more suitable and convenient.

According to the previous literature, the volume of extraction solvent has great impact on the extraction of analytes from samples in the DLLME-SFO procedure.[15] Extraction of analytes in the DLLME procedure occurs by the dispersion of fine droplets of extraction solvent in an aqueous sample. The dispersion of fine droplets depends upon the optimal ratio of the extracting agent and dispersive agents. During optimization of this parameter, the extracting solvent volume (1-undecanol) was increased (30, 40, 50, and 60 μL) while keeping the dispersive agent volume constant (400 μL) in triplicates (n = 3) to select a suitable volume for further experiments. The % ER of the analytes was increased by increasing the volume of the extracting agent from 30 to 50 μL; however, it was further decreased at 60 μL volume. The 50 μL

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volume of 1-dodecanol produced maximum recovery for all analytes except KET and therefore was considered for the proposed assays. Overall, the average % ER was 81.6 ± 8.1% with 50 μL of 1-undecanol (Figure 2). Also, the increase in volume (60 μL) caused an increase in the floating phase volume, and as a consequence, the concentration of the analytes in this phase slightly decreased.

Optimization of Dispersive Solvent. An ideal dispersive solvent should be miscible with both aqueous and organic phase (extracting solvent) and the properties to disperse the extraction solvent in sample solution in form of fine droplets, which results as cloudy solution. In the cloudy solution, the surface contact between the aqueous and organic phase is enhanced, which facilitates for achieving the equilibrium very rapidly between aqueous phase and extraction solvent, resulting in higher extraction efficiency of the targets analytes. Therefore, the selection of the type and volume of the dispersive solvent is a crucial step in the DLLME-SFO procedure. For finding a suitable dispersive agent, acetonitrile, ethanol, and methanol were tried by using 500 μL of each solvent containing 50 μL of 1-undecanol in triplicate (n = 3). Among them, acetonitrile produced very fine droplets in cloudy state, which gives the maximum extraction efficiency and therefore was selected as the dispersive solvent for further experiments (Supplemental Figure S1).

In addition, the volume acetonitrile (dispersive solvent) was further optimized by using its different volumes (300, 400, 500, and 600 μL) containing 50 μL of 1-undecanol in triplicates (n = 3). It was observed that the variation in volume of dispersant also changed the volume of the solid floated phase and therefore affects the extraction efficiency. As illustrated in Figure 3, by increasing the volume of the dispersive agent from 400 to 600 μL, the mean % ER was decreased, although it was lowest with 300 μL volume, which might be due to the cloudy state that was not formed satisfactorily with 300 μL. Therefore 400 μL of acetonitrile (8:1, v/v, dispersive:extractive solvent ratio) was selected for further study.

Optimization of UABE Conditions. Due to highly viscous nature of melted organic droplets, it is not suitable for a direct injection in UPLC−MS/MS. In some previous studies, sample dilution was used to decrease the viscosity of the extracted sample. However, the extractive reagent would be still present and it may also result to compromise the sensitivity of the method. Therefore, in this study, an additional UABE step was introduced to overcome this issue. In UABE procedure, the analytes were used to back extract from floating organic droplets in those aqueous organic solvents, which are compatible to the mass spectrometric analysis. This strategy overcomes the compatibility issue by avoiding direct injection of melted organic droplets in the UPLC−MS/MS system. During optimization of the BE experimental procedure, BE solvent type and its volume, ultrasonic bath temperature, and time required for BE of analytes were evaluated.

Effect of Type and Volume of BE Solvent. During BE solvent selection, its compatibility with chromatographic separation and extraction capability for the compound of interest was considered. Considering this, pure acetonitrile, acetonitrile with 0.1% formic acid, and acetonitrile with 0.1% 15 mM ammonium acetate (mobile phase) were evaluated. Among them, the BE efficiency was higher with acetonitrile with 0.1% 15 mM ammonium acetate (mobile phase) and was chosen as the BE solvent (Supplemental Figure S2). Further different volumes of optimized BE solvent (100, 150, 200, 250, and 300 μL) were evaluated. It was observed that the phase separation did not happen with 100 μL of BE solvent; however, the BE efficiency was highest with 150 μL of BE solvent and was selected as BE solvent in this study (Supplemental Figure S3).

Effect of Ultrasonic Temperature and Time on BE Efficiency. Ultrasonic irradiation enhanced the dispersion process by increasing the surface contact, and therefore facilitate the mass transfer between two phases. Therefore, the BE efficiency of analytes by keeping them in ultrasonic water between different temperature and time were evaluated. Since the temperature affects the analytes partition constants, therefore, BE efficiency was evaluated after keeping the samples in ultrasonic water bath at different temperatures (20, 30, 40, and 50 °C). It was observed that the BE efficiency increases after increasing the temperature of ultrasonic water bath and was highest with 50 °C (Supplemental Figure 4S). However, herein, it was fixed at 40 °C to avoid solvent evaporation. Furthermore, to provide optimum ultrasonic assisted quantitative contact mixing between melted SFO and BE solvent, exposure of different ultrasonication time (15, 25, 35, and 45 min) on BE efficiency was evaluated. Finally, the ultrasonication time was fixed to 25 min, as BE efficiency was the highest with a temperature of 40 °C (Supplemental Figure 5S).

Optimization of Mass Spectrometry and Chromatographic Conditions. Initially, mass spectrometry tuning was performed by continuous infusing of 400 ng/mL solution of analytes (IBU, PPL, PPZ, LSR, and LAM and KET) at a flow rate of 15 μL/min by combining flow with the mobile phase solution. All compounds were found to be sensitive in ESI positive mode and produced stable precursor ions according to their respective molecular weight. Furthermore, their precursor ions produced different expected product (daughter) ions after fragmentation. The precursor to product ions, which produced maximum ionic responses, was selected for MRM monitoring. Furthermore, the cone voltage and collision energy was optimized manually for each analyte to achieve highest responses.

To achieve suitable separation of target analytes, the chromatographic condition was optimized by using different mobile phase conditions (isocratic/gradient, composition, and flow rate) and different sizes of columns. The gradient mode of flow was preferred to avoid early elution of analytes. Due to the presence of multianalytes, Acquity BEH C_{18} column of 2.1× 100 mm size was selected. Different compositions of ammonium acetate buffer (5−20 mM) with methanol and acetonitrile as organic modifiers were tried at different flow rates. Although baseline separation was achieved using methanol as organic modifier, but the column pressure greatly increased due to higher ratio of buffer in initial conditions. Therefore, acetonitrile was chosen as the organic modifiers. In addition, it was found that the higher flow rate resulted in lower resolution and shorter analysis time. For the purpose of achieving reasonable resolution within the shortest running time and good MS performance, the flow rate was set at 0.25 mL/min. Finally, 15 mM ammonium acetate with acetonitrile as an organic modifier at a flow rate of 0.25 mL/min was fixed as a mobile phase with gradient elution. All analytes were separated between 2 and 3 min; however, the total run time was fixed to 5 min to reach the initial conditions.

Method Validation. The developed DLLME-SFO-UABE coupled to UPLC−MS/MS method was validated for its

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ACS Omega 2021, 6, 7524−7532

Table 1. Optimized MS/MS Parameters of Target Analytes Used for Determination in this Study

			quantification		
compound	precursor ion (m/z)	cone voltage (V)	product ion (m/z)	collision energy	dwell time (sec)
ibuprofen	207.0	58	179.9	22	0.015
ketorolac	256.0	46	57.9	32	0.015
lamotrigine	258.0	46	58.0	22	0.015
propranolol	260.1	30	116.0	18	0.015
pantoprazole	384.0	20	200.1	14	0.015
losartan	423.1	22	207.1	20	0.015

selectivity, linearity, limit of detection (LOD), limit of quantification (LOQ), EF, ER, and precision (% RSD). All calibration curves (CCs), which were prepared by spiking the target analytes in deionized water samples were in the range of 0.41−100 ng/mL for IBU and LAM, 0.16−40 ng/mL for LSR and PPL, 0.33−100 ng/mL for KET, and 0.21−50 ng/mL for PPZ. The CCs were found to be linear with a correlation coefficient (r^2) of ≥0.995. The precision, which is measured in terms of relative standard deviation (% RSD), was found to be ≤15% at the all concentration levels of CCs. The method was found to be selective and specific during screening of blank matrixes. The LOD and LOQ were determined according to IUPAC recommendation with a signal-to-noise ratio (S/N) of 3.3 and 10.0, respectively. The LOD and LOQ values of analytes were found in the range of 0.06−0.15 and 0.16−0.41 ng/mL, respectively. The EF values, calculated according to the proposed eq 1, were in the range of 172−192-folds, while the % ER values were in the range of 84.6 to 94.6% with a % RSD of 0.82 to 7.11% between all analytes. The matrix effects (% ME), evaluated by the pre-column infusion method, did not produce any significant ion suppression or enhancement effects calculated by proposed eq 4. The slight ion enhancement effects were observed for all analytes except PPL (ion suppression effects) and were within the acceptable limit of ±15%. These results confirmed that the matrix under analysis did not significantly have impact on the analytical performance of the studied compounds after applying the proposed extraction procedure.

The linearity range, correlation coefficient, LOD, LOQ, EF, and % ER of targeted analytes are summarized in Table 2.

Application in Real Samples. To evaluate the reliability and applicability of the proposed analytical method to real samples, the optimized assay was applied for the determination of the target analytes in the raw waste water, which was obtained from influents of WWTP of "King Saud University, Riyadh Saudi Arabia." Initial analysis confirmed that no target analytes were found in the collected samples, indicating that either the analytes were below the LOD for this assay or the samples were free of these drugs. Therefore, these samples were spiked with different concentrations (1.5, 5, 15, and 40 ng/mL) of QC samples and were extracted by the proposed DLLME-SFO procedure. After UPLC−MS/MS analysis, the percentage relative recoveries were measured as mentioned in eq 3, by comparing the response of analytes in blank samples spiked before the procedure with the response of the corresponding samples spiked after the application of the proposed procedure. According to Table 3, the recoveries of the analytes were varied between 93.1 and 109.4% with the % RSD of 0.99 to 7.81%. These results indicate that the proposed novel method is suitable for trace determination of these multiclass pharmaceuticals in water samples. Typical MRM chromatograms of IBU, KET, LSR, LAM, PPZ, and PPL after administration of 15 ng/mL in spiked waste water samples are shown in Figure 4.

Comparison with the Literature. The characteristics of the present assay were compared with previously reported methods of these target analytes. As evident in Table 4, the proposed assay offers high CCs ranges and comparable % ER for target analytes in comparison with other reported methods.[16−19] Although the calculated LODs for most of the analytes are slightly higher than the previously reported work, but this assay offers simple and economical extraction procedure without drying and reconstitution steps in comparison to previously reported SPE methods. A large volume of organic solvents consumes during conditioning,

Table 2. Linearity, Coefficient of Determination, LOD, LOQ, EF, and ER (%) of Target Analytes Using the Proposed DLLME-SFO-UABE Method[a]

					EF		ER (%)	
compounds	LR (ng/mL)	r^2	LOD (ng/mL)	LOQ (ng/mL)	mean	RSD (%)	mean	RSD (%)
ibuprofen	0.41−100	0.996	0.15	0.41	183	2.82	88.7	7.11
ketorolac	0.33−80	0.997	0.12	0.33	189	3.20	86.3	4.84
lamotrigine	0.41−100	0.995	0.15	0.41	177	2.07	89.9	0.82
losartan	0.16−40	0.995	0.06	0. 16	180	6.71	93.3	1.12
pantoprazole	0.21−50	0.996	0.08	0.21	192	3.85	84.6	2.21
propranolol	0.16−40	0.997	0.06	0. 16	172	8.75	94.6	1.71

[a]LR = Linearity range, r^2 = coefficient of determination, LOD = limit of detection, LOQ = limit of quantification, EF = enrichment factor, ER = extraction recovery, and RSD = relative standard deviation (n = 3).

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ACS Omega 2021, 6, 7524−7532

Table 3. Recovery Study of the Target Analytes Spiked in Waste Water by Applying the Proposed Assay Procedure[a]

compounds	concentration added (ng/mL)	concentration determined (ng/mL)	RR (%)	RSD, (%)
ibuprofen	1.5	1.54	102.0	0.99
	5.0	4.86	97.2	1.63
	15	15.2	101.1	2.31
	40	40.8	101.9	2.22
ketorolac	1.5	1.53	102.2	2.09
	5.0	5.25	104.9	6.98
	15	16.0	106.6	5.12
	40	40.2	100.5	2.12
lamotrigine	1.5	1.48	99.1	1.40
	5.0	4.66	93.1	7.81
	15	15.8	105.3	2.53
	40	38.3	101.7	5.67
losartan	1.5	1.57	104.6	1.27
	5.0	5.47	109.4	8.67
	15	15.4	102.7	7.31
	40	40.8	102.0	5.08
pantoprazole	1.5	1.58	105.5	3.17
	5.0	5.22	104.4	2.21
	15	15.3	102.2	2.71
	40	40.4	101.0	2.37
propranolol	1.5	1.51	100.7	3.03
	5.0	4.69	93.8	3.90
	15	14.5	96.6	6.24
	40	39.7	99.2	4.88

[a]RR = Relative recovery and RSD = relative standard deviation (n = 3)

washing, and elution of analytes from cartridge in SPE procedure. Meanwhile, herein, approx. 0.6 mL of organic solvents were consumed for the proposed extraction method. According to these results, the proposed work represents more environment friendly, satisfactory extraction efficiency with simple and economical extraction procedure for the target compounds.

CONCLUSIONS

In this study, a novel DLLME-SFO-UABE-based sample extraction coupled to UPLC−MS/MS analysis was employed for determination of six most commonly used pharmaceutical analytes in waste water samples. This assay offers high enrichment factor (172−192) and relative recovery (93.1 to 109.4%) with acceptable sensitivity. Compared to previously reported assay of these target analytes by the expensive and complex SPE method, this assay is more simple and economical in nature. Moreover, the method can be considered as environment friendly due to consumption of low volume and less toxic solvents in sample preparation. These satisfactory results confirmed that the assay has strong potential for application of trace analysis of target analytes in waste water samples.

EXPERIMENTAL SECTION

Chemicals and Reagents. IBU, PPL, PPZ, and KET were obtained from "Sigma-Aldrich St. Louis, MO, USA" whereas LSR and LAM were obtained from Amriya Pharmaceutical Industries (Cairo, Egypt) and Delta Pharma (Cairo Egypt), respectively. Ammonium acetate and formic acid were obtained from "Qualikems Fine Chemical Private. Ltd. Vadodara, India" and "Loba Private. Ltd. Mumbai, India", respectively. Acetate buffer (pH 4.6) and 1-undecanol (99% purity) were purchased from "Sigma Aldrich, St. Louis, MO, USA." 1-dodecanol (98% purity) was from "Acros Organic, New Jersey, USA." Methanol and acetonitrile were purchased from "Fisher Scientific Limited, Leicestershire UK." All APIs used in this work were of highest purity available (≥98%), whereas the reagents and organic solvents were of analytical grade and HPLC grade, respectively. High standard and ultrapure (Milli-QR grade) water (0.22 μm, pore size) was used for working standard samples preparation.

Waste Water Sample. The raw waste water was obtained in polyethylene bottles from influents of WWTP of "King Saud University, Riyadh Saudi Arabia" and was used without further dilution. The waste water samples were centrifuged at 4000 rpm for 4 min before spiking to the working solution of target analytes to remove any suspended particulate and were applied for DLLME-SFO-UABE procedure.

Preparation of Stock Solution, Calibration Standard (CS), and Quality Control (QC) Samples. The stock solutions of analytes (IBU, PPL, PPZ, KET, LSR, and LAM) were prepared by accurately weighing their reference standards in methanol to achieve 1 mg/mL concentration. The working solutions for CS and QC samples were prepared by further dilution of stock solutions with 50% acetonitrile and were stored for 15 days only in a pharmaceutical refrigerator maintained at 4 °C. Separate fresh solutions were prepared every 15 days and when required during analysis.

Mass Spectrometry Instrumentation and Chromatographic Conditions. The UPLC system coupled to a triple quadrupole mass detector (MS/MS) (Waters Corp., Milford, MA, USA) was used in this study. Acquity BEH C_{18} column (2.1 × 100; 1.7 μm) was used for separation of target analytes having column oven temperature maintained at 38 °C. The gradient mobile phase comprising of (A) acetonitrile and (B) 15 mM ammonium acetate buffer were eluted at a flow rate of 0.25 mL/min programmed as follows: 0−0.5 min, from A−B (10:90, v/v) to A−B (90:10, v/v); 0.5−1 min, from A−B (90:10, v/v) to A−B (10:90, v/v) and remained to return to initial condition till 5 min prior to next injection. The temperature of auto-sampler was maintained at 12 °C and the volume of sample injection was fixed to 5 μL.

The analytes ionization was performed in an electrospray ionization (ESI) probe operated in positive-ion mode. The multiple reaction monitoring (MRM) mode was used for identification and quantification of target analytes. The optimized mass spectrometer parameters were as follows: source temperature, 150 °C; desolvation temperature, 350 °C; capillary voltage, 1.58 kV; extractor voltage, 3.0 kV; and the cone and desolvation gas flow rates were 50 and 650 L/h, respectively. Auto-generated ultrapure nitrogen was used as cone gas and argon was used as collision gas. To choose the analytes fragmentation patterns in MRM mode, direct infusions (via syringe pump) into the MS/MS of all analytes at 400 ng/mL concentrations were performed and the

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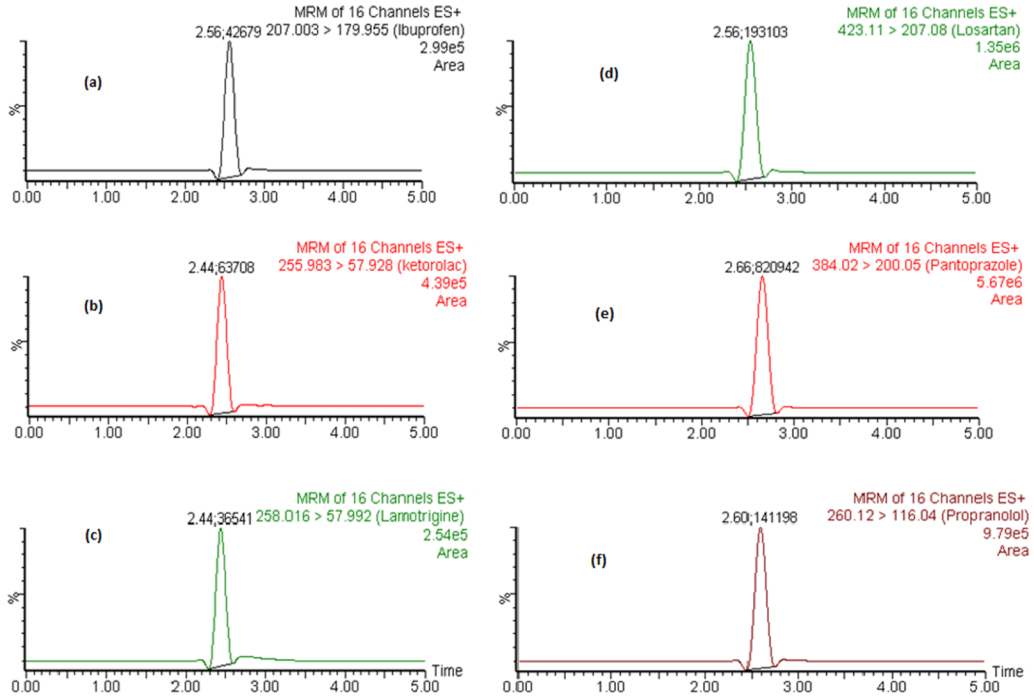

Figure 4. Representative MRM chromatograms of (a) ibuprofen, (b) ketorolac, (c) lamotrigine, (d) losartan, (e) pantoprazole, and (f) propranolol in spiked waste water samples.

Table 4. Comparison between the Proposed Method with Previously Reported Methods for Determination of Target Analytes in Water Samples[a]

analytical method	analytes	linearity range (ng/mL)	LOD (ng/mL)	% ER	extraction method	extraction cost	reference
SPE-LC-MS/MS	ketorolac	0.2−59.5	0.03	76.3	SPE	high	16
	ibuprofen	1.5−151	0.03	111.7			
	propranolol	0.2−6.6	0.08	87.5			
SPE-DLLME-LC-MS/MS	pantoprazole	0.1−15	0.03	91	SPE-DLLME	high	17
SPE-UPLC-MS/MS	losartan	2.5−500	0.80	103	SPE	high	18
SPE-LC-QTOF-MS	lamotrigine		0.01		SPE		19
DLLME-SFO-UABE-UPLC-MS/MS	ibuprofen	0.41−100	0.15	88.7	DLLME-SFO-UABE	low	this method
	ketorolac	0.33−80	0.12	86.3			
	propranolol	0.16−40	0.06	94.6			
	pantoprazole	0.21−50	0.08	84.6			
	losartan	0.16−40	0.06	93.3			
	lamotrigine	0.41−100	0.15	89.9			

[a]SPE-LC-MS/MS: Solid phase extraction-liquid chromatography tandem mass spectrometry; DLLME-LC-MS/MS: dispersive liquid−liquid microextraction-liquid chromatography tandem mass spectrometry; SPE-UPLC-MS/MS: solid phase extraction-ultraperformance liquid chromatography tandem mass spectrometry; SPE-LC-QTOF-MS: solid phase extraction-liquid chromatography quadrupole time of flight mass spectrometry; and DLLME-SFO-UABE-UPLC-MS/MS: dispersive liquid−liquid microextraction-solidified floating organic droplets-ultrasound assisted back extraction-ultraperformance-liquid chromatography tandem mass spectrometry.

fragmentation patterns of target analytes were recorded. The optimized compound specific parameters for all analytes are depicted in Table 1. The UPLC−MS/MS system was operated by using the "MassLynx software (version 4.1)" and the data were processed using the "Target LynxTM program."

DLLME-SFO-UABE Procedure for Sample Extraction. For extraction of the analytes from water samples, first, 12 mL of deionized water was transferred into a 15 mL capacity of tube. Then, 60 μL of working standard solution of CS or QC was spiked into each sample except blank. Samples were vortexed for few seconds to ensure appropriate mixing followed by the addition of 1 mL of solution of cold acetate buffer (pH = 4.6). Then, 400 μL solution of acetonitrile containing 50 μL of 1-undecanol was injected to each tube, which worked as a dispersive and extractive agent, respectively. The samples now become a cloudy mixture due to dispersion of 1-undecanol in the form of fine droplets throughout the samples. Consequently, the samples were gently vortex-mixed for approx. 1 min for mass transfer of the target analytes from sample matrix to fine droplets of 1-undecanol. Then, the cloudy samples were centrifuged in a cold centrifuge machine maintained at 10,500 rpm, at 3 °C maintained for 10 min. The fine droplets were solidified in the form of disc-shaped pellets on the surface of the tube. These pellets were carefully collected into Eppendorf tubes (1.5 mL) with the help of a spatula, which was instantaneously melted in the form of

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ACS Omega 2021, 6, 7524−7532

viscous liquid. Then, the samples were diluted with 150 μL of acetonitrile, vortex-mixed, and all samples were placed to ultrasonic bath for 25 min (maintained at 40 °C) for back extraction of target analytes. After back extraction into acetonitrile, the final samples were centrifuged for 4 min at 4500 rpm, and at this time, the 1-undecanol solvents were solidified at the bottom of the tube. After that, 120 μL of supernatant samples was transferred to a glass insert tube ready for 5 μL to inject for UPLC−MS/MS analysis.

Evaluation of EF, ER, and Relative Recovery. The EF and % ER for the analytes were used as the parameters to evaluate the extraction efficiency during optimization of different experimental parameters.

The EF was defined as the ratio of the final target analytes concentration in the acceptor solution i.e., floated phase (C_f) to the initial target analytes concentration in the aqueous sample solution (C_0):

$$EF = C_f/C_0 \quad (1)$$

In this study, the EF values of the compounds were calculated by considering the analytes concentration in the floating phase since this extraction procedure may have evaluated in different separation/detection systems and not only to LC−MS/MS for which, as mentioned. A further back step procedure (reverse extraction) was required here only to overcome the compatibility issue with MS/MS detection.[5]

The % ER was expressed as the total percentage amount of target analytes extracted into the floated phase by the proposed extraction procedure:

$$\%ER = EF \times (V_f/V_0) \times 100 \quad (2)$$

where V_f and V_0 are the volume of the floating phase and the sample solution, respectively.

The % *RR* was defined as the % amount of analyte recovered from the matrix (real samples) with reference to the extracted standard (standard spiked into the same matrix).

$$\mathrm{RR}(\%) = \frac{C_{found} - C_{real}}{C_{added}} \times 100 \quad (3)$$

where C_{found} is the concentration of analyte after adding a known amount of working standard to the real sample, C_{real} is the analyte concentration in real samples, and C_{added} represents to the concentration of a known amount of working standard that was spiked into the real samples.

Evaluation of Matrix Effects. Matrix effects (ME) in the form of ion suppression/enhancement is one of the drawback of ESI-MS/MS analysis. It can be measured either by the quantitative (post-column infusion) or qualitative (pre-column infusion) method.

Herein, the ME was measured by quantitative method in which the signal response of analytes in the sample matrix was compared with the signal response in the pure solvent. The percentage difference (% ME) between the signal response in spiked and solvent samples was used as an indicator of matrix effects.

$$\mathrm{ME}(\%) = \frac{\text{signal response in spiked samples}}{\text{signal response in solvent samples}} \times 100 \quad (4)$$

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.0c06047.

Effect of type of dispersive solvent on the extraction recovery of target analytes by DLLME-SFO procedure, effect of type and volume of UABE agent on the extraction recovery of target analytes in UABE procedure, effect of ultrasonic temperature and time on the extraction recovery of analytes in UABE procedure (PDF)

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Notes

The authors declare no competing financial interest.

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ACS Omega 2021, 6, 7524−7532