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Development and validation of SPE-LC-MS method for simultaneous determination of selected pharmaceuticals in hospital wastewater

Abstract

A fast-green liquid chromatography-mass spectrometry (HPLC-MS) method has been developed and validated for simultaneous determination of nine pharmaceuticals in hospital effluents in Al Khobar city, Kingdom of Saudi Arabia. The separation was performed on a short narrow bore column packed with superficially porous particles and selected ion monitoring (SIM) mode was used. Sample preparation and cleanup was executed using solid phase extraction (SPE) with Oasis hydrophilic-lipophilic balance (HLB) cartridges. The chromatographic parameters were adjusted to separate all the analytes in the shortest possible time. The method was validated according to ICH guidelines. The detection and quantification limits of the studied pharmaceuticals were ranged from 0.1 to 0.5µg L^{-1}, and 0.3 to 1.5µg L^{-1}, respectively. Among the studied analytes, levofloxacin and caffeine were detected at concentrations of 3µg L^{-1} and 60µg L^{-1} respectively.

Keywords: wastewater, fast analysis, hospital effluents, liquid chromatography, SPE

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Abbreviations: CECs, contaminants of emerging concern; EDTA, ethylene diamine tetra-acetic acid; LN, lincomycin hydrochloride; CF, caffeine; TM, trimethoprim; LV, levofloxacin; NF, norfolxacin; CP, ciprofloxacin; EF, enrofloxacin; SM, sulfamethazine; MT, metoprolol; ESI, electrospray ion source; SIM, selected ion monitoring; SPE, solid phase extraction

Introduction

Pharmaceuticals are considered as one of the major groups of contaminants of emerging concern (CECs). These are the contaminants that were unknown, unrecognized (not detectable) or not routinely monitored.[1] The continuous introduction of these contaminants into the environment can compensate their high transformation/removal rates, thus it is not necessary for these contaminants to persist in the environment to pose negative effects.[2,3] Due to their potential adverse effects on animals and humans at low levels of exposure they are receiving attention. They may pose toxic effects and alterations on the reproductive system of aquatic organisms[4–6] and the promotion of the development of resistant bacterial strains representing a health risk to humans and the environment. The prevalence of pharmaceuticals such as antibiotics, steroids and hormones and their metabolites in the environment has been an area of growing concern.[7–12]

The limited amount of knowledge regarding the levels of pharmaceutically active pollutants in the environment has precluded a good assessment of the effect of long-term exposures. Pharmaceuticals have been detected in a wide range of environmental samples, including surface water, drinking water, groundwater, sewage influent and effluent.[13,14] Hospital wastewater is one of the major sources of pharmaceuticals discharged into the environment.[15] In 1997, the U.S. Food and Drug Administration established that an environmental risk assessment should be conducted for new human use drugs if the average predicted environmental concentration of a drug is above 1µg L^{-1}. The EU has set this level at 0.01µg L^{-1}.

There is a lack of information about the prevalence and concentrations of these contaminants in Saudi waters. Limited studies have been conducted on these kinds of contaminants in Saudi Arabia. Qarni et al.[16] investigated 12 pharmaceuticals in the influent and effluent of hospital wastewater treatment plants in Riyadh and studied the efficiency of the wastewater treatment process.[16] Another study was conducted in the Western region and investigated the occurrence of emerging trace organic chemicals in wastewater effluents in this region.

Different chromatographic methods have been developed for the analysis of pharmaceuticals in the environment using liquid chromatography[12,17–20] and gas chromatography.[21] To the best of our knowledge no study reported the analysis and detection of pharmaceuticals as emerging contaminants in the Eastern province of Saudi Arabia. In the present study, a solid phase extraction coupled to LC-MS method was developed for the simultaneous determination of nine pharmaceuticals in the wastewater effluent of the King Fahd Hospital of the University, Al Khobar city, Saudi Arabia.

Experimental

Materials and reagents

Ethylene diamine tetra-acetic acid disodium salt (EDTA), HPLC grade acetonitrile, methanol and ethanol were purchased from Sigma, Germany. Deionized water (18.2MΩ) was purified using Pure Lab Ultra water system (ELGA, High Wycombe, UK). All the studied analytes including lincomycin hydrochloride (LN), caffeine (CF), trimethoprim (TM), levofloxacin (LV), norfolxacin (NF), ciprofloxacin (CP), enrofloxacin (EF), sulfamethazine (SM), metoprolol (MT) and $^{13}C_3$ trimethoprim (internal standard) were purchased from Sigma, Germany. All standards purity was ≥98%. The chemical structures of the studied analytes are shown in Figure 1.

Instrumentation

LC-MS separation was performed using Prominence UFLC system (Shimadzu Corporation, Kyoto, Japan) consisting of SIL20AC autosampler, two LC-20AD pumps, DGU-20A degasing unit, a CTO-20A column oven, SPD-M20A photodiode array detector, CBM-20A

HPLC system controller and LCMS 2020 mass spectrometer. The mass analyzer was a single quadruple equipped with an electrospray ion source (ESI).

An Accucore C_{18} column, 30mm×2.1 inner diameter, 2.6µm particle size (Thermo Scientific, USA) was used for separation. The mobile phase composed of acetonitrile/water (15:85, v/v) at a flow rate of 0.6mL min^{-1}. The injection volume was 2µL. All analytes were eluted within 5 min. For maximum sensitivity selected ion monitoring (SIM) was used for analytes quantification with the ESI in the positive mode. The interface and the heat block temperatures were set at 350 and 200ºC, respectively. The desolvation line temperature was 250ºC. The nebulizing and drying gas flows were 1.5 and 10L min^{-1}, respectively. Ultra-pure nitrogen produced by Air-Tech nitrogen generator was used as the nebulizing and drying gas. The quantification ions of the studied analytes are listed in Table 1.

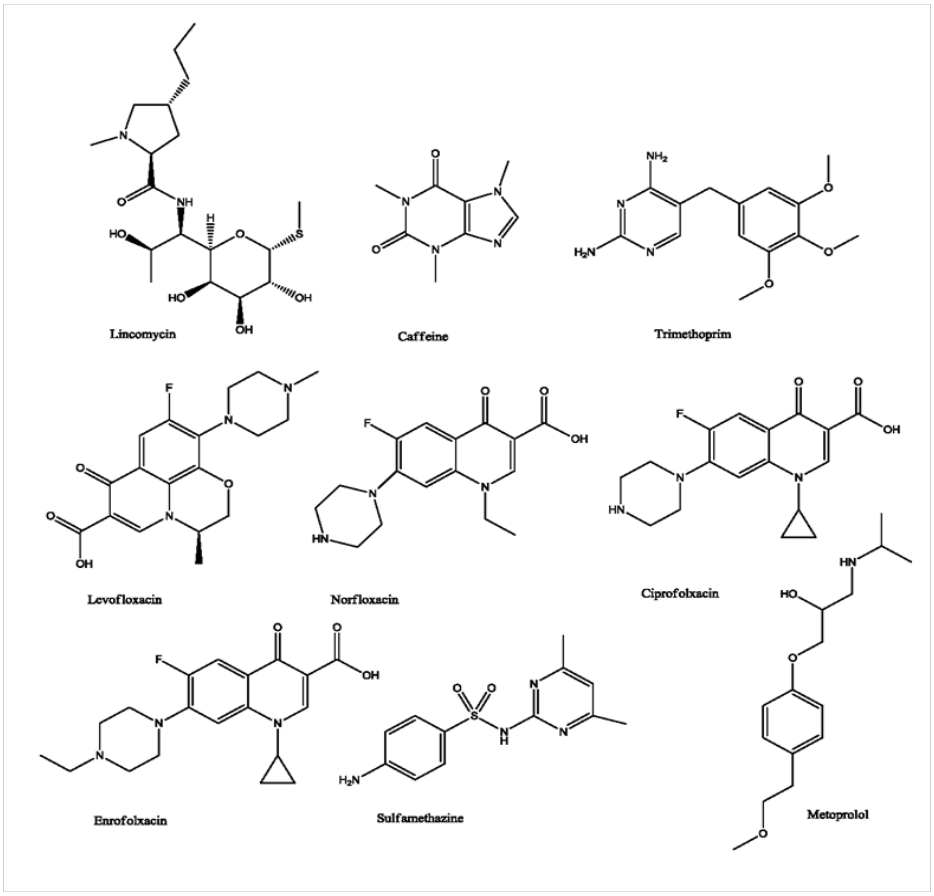

Figure 1 Structures of the studied analytes.

Table 1 m/z and retention times of the studied analytes

Analyte	m/z (+)	Retention time (min)
Ciprofloxacin	332.20	3.1
Enrofloxacin	360.15	4.1
Lincomycin	407.15	1.8
Norfloxacin	320.15	2.9
Trimethoprim	291.35	2.5
Caffeine	195.15	2.4
Levofloxacin	362.10	2.8
Metoprolol	268.20	5.1
Sulfamethazine	279.10	4.4

Standard solutions and calibration

Stock standard solutions (1000mg L^{-1}) were prepared separately in methanol and stored in dark at –20ºC. Appropriate dilutions of the stock solution in the mobile phase were performed producing the working standard solutions in the range of 10–6000µg L^{-1}. Each standard was injected in triplicate. The calibration curve was obtained by plotting the concentration versus the ratio of the peak area of the analyte to the peak area of the internal standard.

Water sample collection and preparation

Samples of wastewater effluents were collected from King Fahd Hospital of the University, Al Khobar city, Saudi Arabia in amber glass bottles with Teflon™ lined caps and kept in ice box and then transferred to the laboratory to be analyzed. Samples were filtered using cellulose acetate filters (0.45 µm). Two grams of EDTA were added to 500mL of the wastewater sample and rolled on a roller for 20 min for complete dissolution and was extracted within 48 hours of sample collection. 50ng of the internal standard ($^{13}C_3$ trimethoprim) was then added prior to sample extraction.

Solid phase extraction (SPE) was employed for sample clean-up and preconcentration. Oasis HLB cartridges (60mg, 3mL), purchased from Waters Corporation, USA were used. The cartridges were first conditioned with 5mL methanol and then 5mL ultrapure water. 500mL wastewater samples were then extracted through the cartridges at a flow rate of 3mL min^{-1}. After the extraction of the whole sample,

the cartridges were rinsed with 10mL ultrapure water then with 5% methanol in water and the cartridges were then dried under vacuum. Finally, elution was performed with 5mL methanol. The extract was then filtered through 0.2µm syringe filters and 1mL aliquot was quantitatively transferred into 1.5mL HPLC vial and evaporated to dryness under a gentle stream of nitrogen. The residue was then reconstituted in 100µL of the mobile phase and the concentrated extract was transferred into a vial insert, capped and submitted for LC-MS analysis.

Results and discussion

Optimization of SPE method

Before separating the studied analytes, samples should be pre-treated to remove the sample matrices and eliminate the interfering components. Moreover, this step is necessary to isolate and enrich the analytes before instrumental determination.

In the proposed method, Oasis HLB cartridges were selected as they provide higher extraction capacity than C_{18} sorbents and also can be used without pH adjustment which could simplify sample handling.[22]

Several trials were made to enhance the recovery; the first trial was based on extracting the analytes without adding EDTA, however low recoveries for levofloxacin, norfloxacin, ciprofloxacin and enrofloxacin were observed (ranged from 5.4% to 14.1%). This was mainly attributed to their chemical structures as they can form chelates with metal cations that are soluble in water or glassware,[23] therefore EDTA was added to improve their extraction efficiencies. Significant enhancement of the recoveries of the quinolones antibiotics (86–107%) was obtained as shown in Figure 2.

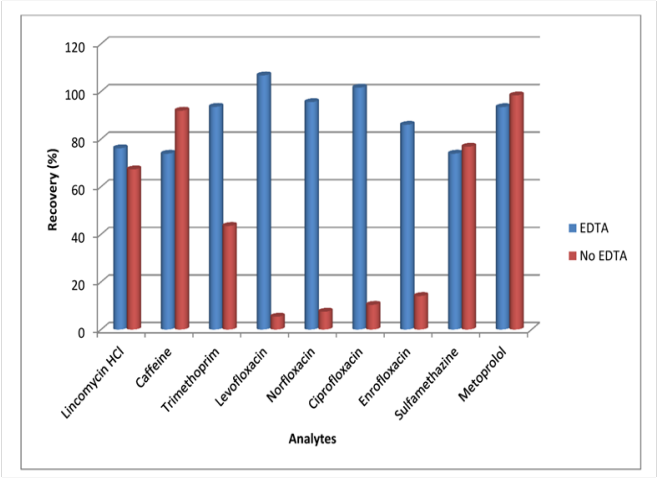

Figure 2 Effect of EDAT addition on the recovery of the studied analytes on Oasis HLB cartridges.

Optimization of the proposed HPLC method

The developed method was designed in a way that all the analytes could be separated in the shortest possible time with minimum solvents consumption. In this context, a short (3cm) narrow bore (2.1mm) column packed with superficially porous particles was selected. These particles could provide fast and efficient separation compared to traditional LC columns.[24] The volume fraction of acetonitrile in the mobile phase has the most significant impact on the separation of the studied analytes. A flow rate of 0.6mL min^{-1} was used because it offered minimum waste generation (3mL/min per run) and short analysis time (5.1 min).

By using 15% acetonitrile, all analytes were eluted in 5 min. However, when 20% acetonitrile was used, coelution between many peaks was observed. Good separation, peak symmetry, resolution and selectivity for the studied analytes were obtained upon using acetonitrile: water in (15:85, v/v) and a flow rate of 0.6mL min^{-1}. Figure 3 shows the separation of the studied analytes.

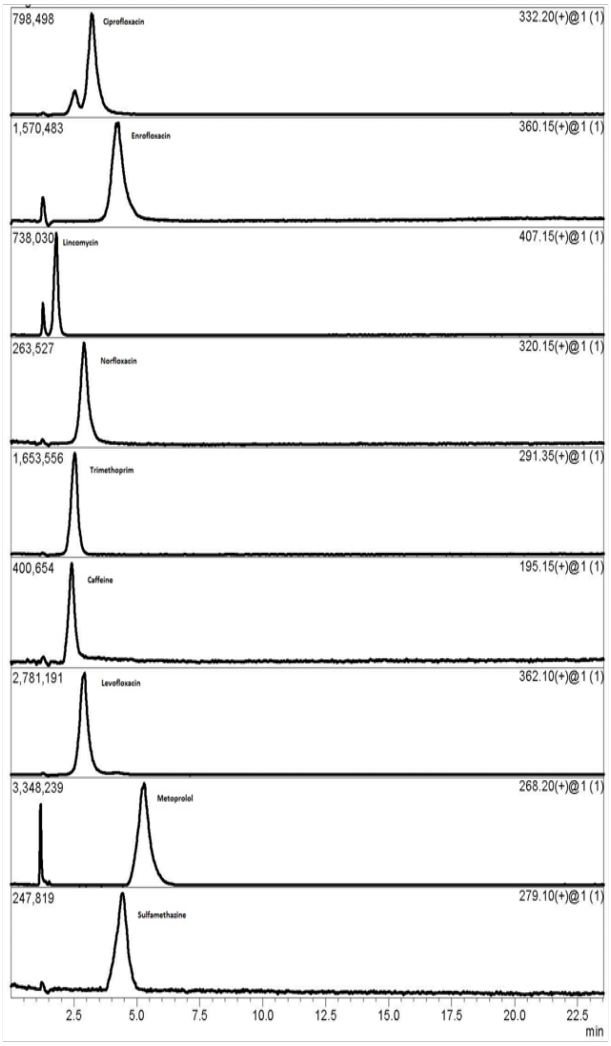

Figure 3 The separation of the studied analytes using SIM mode.

Validation of SPE-LC method

The validation of the method was performed according to ICH guidelines. Linearity, precision, detection and quantification limits and accuracy were studied.

The linearity of the proposed method was assessed for each analyte using seven concentrations in the calibration range of each standard. The analysis was performed in triplicate as described in the experimental section. The high correlation coefficient values (R^2 ≥0.99) reflected the good linearity of the calibration curves.

The precision of the developed method was evaluated by analyzing three replicates of the samples at the same day (within-day precision) and on three consecutive days (between-day precision) at two concentration levels (100µg L^{-1} and 1000µg L^{-1}). The RSDs were ranged from 1.7% to 10% and 3.4% to 10.2% for within-day and between-day precision, respectively. The obtained results reflected the high precision of the developed method (Table 2).

Table 2 Intra-day and inter-day precision of the studied analytes

Analyte	Intra-day precision (RSD[a] %)		Inter-day precision (RSD %)	
	Low conc.	High conc.	Low conc.	High conc.
Lincomycin HCl	6.4	7.2	6.8	7.2
Caffeine	7.9	6.8	7.6	7.5
Trimethoprim	1.7	3.5	3.4	4.5
Levofloxacin	6.1	6.7	6.4	6.8
Norfloxacin	6.3	7.6	5.9	6.2
Ciprofloxacin	4.6	5.3	5.7	5.8
Enrofloxacin	10	9.7	9.3	10.2
Sulfamethazine	2.11	3.5	4.2	3.6
Metoprolol	4.7	3.9	4.1	5.3

[a]RSD, relative standard deviation of 3 determinations (%).

Table 3 Figures of merit of the developed method for the studied analytes

Analytes	Range (µg L^{-1})	r^2	% Recovery±SD[a]		LOD[b] (µg L^{-1})	LOQ[c] (µg L^{-1})
			Low Conc.	High Conc.		
Lincomycin HCl	10–1000	0.9996	76.2±4.9	76.6±5.5	0.1	0.3
Caffeine	20–2000	0.9999	74.6±5.9	75.2±5.1	0.2	0.6
Trimethoprim	20–2000	0.9983	93.2±1.6	92.7±3.2	0.3	0.9
Levofloxacin	60–6000	0.998	106.7±6.5	105.9±7.1	0.4	1.2
Norfloxacin	40–4000	0.9979	95.1±6.0	94.7±7.2	0.4	1.2
Ciprofloxacin	50–5000	0.9995	102.3±4.7	102.8±5.4	0.3	0.9
Enrofloxacin	60–6000	0.9956	86.7±8.7	86.9±8.4	0.4	1.2
Sulfamethazine	10–1000	0.9975	74.9±1.6	75.3±2.6	0.1	0.3
Metoprolol	50–5000	0.9975	93.4±4.4	93.7±3.7	0.5	1.5

[a]Standard deviation.
[b]Limit of detection.
[c]Limit of quantification.

Detection limits (LOD) and quantification limits (LOQ) were determined according to International Conference on Harmonization (ICH) recommendations[25] using signal to noise ratio approach. LODs and LOQs were calculated as the lowest concentrations producing signal-to noise ratio values of three, and ten, respectively (Table 3). The recovery is determined by using standard addition method at two concentration levels. The mean percentage recoveries and their standard deviations were calculated. The recoveries were within the range of 74.6 to 106.7%.

Analysis of the studied analytes

The developed SPE-HPLC-MS method was successfully applied to the analysis of different pharmaceuticals in real wastewater samples. After extraction, samples were analyzed to demonstrate the method applicability. Levofloxacin and caffeine could be quantified at 3µg L^{-1} and 60µg L^{-1}, respectively while other analytes were below their LODs.

This high load of caffeine in the effluent wastewater could be due to the direct disposal of unconsumed caffeine-containing products.[26] This result is in agreement with other published studies e.g.[27,28] as the levels of caffeine in the range of 0.21–398µg L^{-1} have been reported to be found in wastewater effluents.

Conclusion

High speed SPE-LCMS method has been developed and optimized for the determination of lincomycin, caffeine, trimethoprim, levofloxacin, norfolxacin, ciprofloxacin, enrofloxacin, sulfamethazine and metoprolol in hospital effluents using a short column packed with superficially porous particles operated in SIM mode. The developed method was validated according to ICH guidelines. The detection limits were in the range of 0.1−0.5µg L^{-1}. All the studied pharmaceuticals were eluted in 5 min with minimum solvent consumption. The developed method could be used to investigate the efficiency of wastewater treatment plants in eliminating pharmaceuticals.

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Conflict of interest

The author declares that there is no conflict of interest.

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