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Aptamer based extraction followed by electrospray ionization-ion mobility spectrometry for analysis of tetracycline in biological fluids

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ABSTRACT

An extraction method based on aptamer sorbent followed by electrospray ionization-ion mobility spectrometry (ESI-IMS) has been developed for the analysis of tetracycline in human urine and plasma samples. The effect of extraction parameters on the extraction efficiency including washing (solvent type and volume) and elution (solvent type, volume and flow rate) were investigated. Under the optimized conditions, the linear dynamic ranges for tetracycline in urine and plasma samples were found to be 0.05–5.00 μg/mL and 0.10–5.00 μg/mL with detection limits of 0.019 and 0.037 μg/mL, respectively. The extraction efficiency was 86.5% for urine and it was 82.8% for plasma samples. The relative standard deviation was 5.9% and 6.3% for six replicate measurements of tetracycline at 1 and 2 μg/mL in urine and plasma samples, respectively.

1. Introduction

Sample preparation is an important step in the process of biological fluids analysis. It is applied for analyte enrichment and removes matrix or contaminants. During the last decade, various sample preparation methods have been developed such as liquid–liquid extraction (LLE), liquid phase microextraction (LPME), solid-phase extraction (SPE) and solid phase microextraction (SPME) [1]. The SPE is a well established method for sample clean-up and preconcentration for aqueous samples at trace levels. The main limitation associated with ordinary stationary phase SPE columns is low selectivity.

Recently, selective SPE methods have been developed to obtain an extract free from matrix interference in a single extraction step. The first strategy consists of using molecularly imprinted polymers (MIPs), which are sorbents possessing specific cavities complementary to the target analyte in size, shape, and position of the functional groups [2,3]. The second new selective approach involving antigen–antibody interactions called immunosorbents (ISs). The antibodies are used for their high affinity and specific molecular recognition but they are produced in animal bodies in response to proteins or other molecules recognized as foreign by their immune system. However, these biomacromolecules are very expensive. Recently, a third type of selective SPE sorbents called oligosorbents based on aptamers immobilized on a solid support has been developed [4,5]. Aptamers are oligonucleic acid or peptide molecules that bind to a specific target molecule with an affinity that can be comparable with those of antibodies. The process through which these molecules are isolated in vitro is called selective evolution of ligands by exponential enrichment (SELEX) [6]. Single stranded DNA aptamers that bind with high affinity and specificity to its ligand were identified by selection from an oligonucleotide library of 10^{15} molecules. The 3D structure of the oligonucleotide is highly influenced on the formation of the complex between an aptamer and its ligand. Aptamers, especially DNA aptamers, are easily and inexpensively synthesized and chemically modified. Furthermore, they are smaller in size, no limitation against any types of targets, reversible denaturation, and have high thermal stability [7]. The use of aptamers for SPE is in its infancy but aptamer affinity chromatography or DNA affinity chromatography in general has been already developed [8,9].

Aptamers have been applied in preconcentration, extraction, purification of targets on solid-phase extraction (SPE) column [10,11], and magnetic beads [12]. The immobilization of aptamers on beads were applied to purify proteins including thyroid transcription factor 1 (TTF1) [12], Thermus aquaticus DNA polymerase (Taqpolymerase) [13], and His-tagged proteins [14]. Aptamer columns are useful tools for extraction of proteins and peptides prior to analysis. A few methods have been developed for the extraction based on aptamers consisting of extraction and separation of a series of enantiomers using aptamer ligands (e.g., DNA aptamers, RNA aptamers, and bio-stable L-RNA aptamers) as chiral stationary phases on packed columns [15–21]. The main advantages of aptamer based extraction method are high selectivity and extraction recovery. Furthermore, aptamers are much cheaper than antibodies. In other words, antibodies are being replaced by

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aptamers for getting the same characteristics with much lower prices.

Antibiotics are an important group of pharmaceuticals that kill or slow down the growth of bacteria. Tetracycline is a broad-spectrum polyketide antibiotic, produced by the *Streptomyces* genus of Actinobacteria, indicated for using in veterinary medicine and treatment as well as prevention of microbial infections, such as respiratory tract infections, arthritis and severe acne [22].

A few methods have been developed for the analysis of tetracycline in body fluids consisting of liquid chromatography with photometric detection [23], luminescence spectrometer [24], ultra-performance liquid chromatography coupled to quadrupole time of flight mass spectrometry (UPLC–QToF) [25], or to time of flight mass spectrometry (UPLC–ToF) [26]. Two sensors have also been reported for the determination of tetracycline in urine samples [27,28].

Ion mobility spectrometry (IMS) is another technique, which has been used for qualitative and quantitative analysis of a variety of compounds [29]. The IMS with electrospray as the ionization source has been used by Shu Li et al. for the analysis of antibiotics in liquid samples. "Qualitative and semi-quantitative capabilities of the ESI-IMS in analysis of antibiotics were demonstrated" [30]. In this work, a combination of solid-phase extraction using an aptamer-based sorbent and ESI-IMS has been developed for the determination of tetracycline in biological samples.

The secondary structure of ssDNA antitetracycline aptamer was predicted by Mfold program according to the free-energy minimization algorithm [31]. Antitetracycline aptamer composed of 76-mer size with central 40 nucleotides variable (19th–58rd nucleotides) flanked by 18 nucleotides each primer-binding constant region. The sequence and three-dimensional structure of aptamer play an important role in recognition and binding of the target molecule. This also implies on the specific groups present on the target molecule, such as tetracycline to which an aptamer binds [32]. The frequency of GTGG sequence in variable 40-nucleotide region of 76-mer aptamer appears to be significant for binding and recognition of antitetracycline aptamer to the target molecule (tetracycline) and it had high affinity for tetracycline (K_d = 63.6 nM) [31].

2. Experimental

2.1. Chemicals and reagents

Analytical standard of tetracycline (98%) was obtained from Sigma–Aldrich (Germany). Acetonitril (ACN), methanol (MeOH), acetic acid (HOAC), hydrochloric acid (HCl, 38%) and deionized water (HPLC grade) were purchased from Merck (Germany). Trizma hydrochloride, sodium azide, and CNBr-activated Sepharose (4B, 90 μm) were obtained from Sigma–Aldrich (Germany). Magnesium chloride, sodium chloride, sodium phosphate (Na_2HPO_4) and potassium dihydrogen phosphate (KH_2PO_4) were prepared from Merck (Germany). The 5′-amino-modified DNA oligonucleotides (sequence 5′-CGTACGGAATTCGCTAGCCCCCCGGCAGGCCACGGCTTGGGTTGGTC-CCACT GCGCGTGGATCCGAGCTCCACGTG-3′ for the aptamer and 5′-CATCGTAAGGTCGCTCCCGAGGCCCCGGACGGCACGGTTCG-GTTGACCCTCGCGTCAGGTGAGCCTCCTCGGTGCA-3′ for the scrambled oligonucleotide) with a C6 spacer arm were synthesized in Bioneer, South Korea. The sequence of the antitetracycline aptamer used in this work has been reported by Gu et al. [31]. A standard stock solution of tetracycline, 1000 μg/mL, was prepared in MeOH. The standard solution was protected against light and stored in the refrigerator. Working standard solutions of tetracycline were prepared daily by appropriate dilution of the stock solution using deionized water and 0.1 M acetic acid and were filtered through PTFE filters (0.22 μm particle size). The selection buffer contained 20 mM Trizma hydrochloride, 140 mM NaCl, 5 mM KCl, and 1 mM $MgCl_2$ at pH 7.4.

Table 1
Experimental parameters of the ESI-IMS.

Operation parameter	Setting
Electrospray voltage (kV)	11.4
Counter electrode voltage (kV)	9.0
Desolvation region length (cm)	4
Drift region length (cm)	11
Inner diameter of drift tube (cm)	4
Flow rate of sheath gas (mL min^{-1})	900
Flow rate of drift gas (mL min^{-1})	500
Sample flow rate (μL min^{-1})	6
Drift tube temperature (°C)	165

2.2. Electrospray ionization-ion mobility spectrometry

The electrospray ionization-ion mobility spectrometer (ESI-IMS) used in this work was designed and constructed in our group at Isfahan University of Technology. In brief, the main parts of the instrument are: the IMS cell, the electrospray needle, two high voltage power supplies, a pulse generator, an analog to digital converter and a computer. The IMS cell was constructed from 16 aluminum rings, which were electrically isolated from each other by thin PTFE rings. The aluminum rings were connected together by a series of 5.6 MΩ resistors to form the electric field gradient. The cell consists of two sections: a desolvation and a drift region separated by a Bradbury–Nielsen ion gate that made of two series of parallel wires biased to a potential for blocking ion passage to the drift tube. The grid potential was removed for a short period of time by the pulse generator at a frequency of 25 Hz, allowing ion packets to enter into the cell. The electrospray needle (P/N 7768–01, Hamilton, Reno, NV, USA), which was fixed at one end of the cell, was inserted into a Teflon tube to eliminate the corona discharge problem. A six-port injection valve (Rheodyne, USA) having an external injection loop with the volume of 20-μL was used for the sample introduction. Nitrogen gas, after passing through a 13X molecular sieve (Fluka), was passed through the cell at 500 and 900 mL/min as the drift and desolvation gases, respectively. The high-speed A/D module (12-bit dynamic range) was used and the digital signal was averaged over a number of scans. The resulting ion mobility spectrum was then displayed on the monitor. The spectrometer was operated in the positive mode.

The electrospray solvent used in this work was methanol: water 95:4 with 1 mL of 0.1 M acetic acid. All mobility data were collected by data acquisition software and each IMS spectrum being the average of 50 individual spectra. Table 1 summarizes the operating conditions.

2.3. Extraction procedures

2.3.1. Sorbent preparation

The sorbent preparation was carried out based on the work of Pichon et al. who used an aptamer-based sorbent for the analysis of cocaine in human plasma [22]. The 1000 μg/mL Antitetracycline aptamers were made in (200 mM Na_2HPO_4 and 5 mM $MgCl_2$, pH 8) and then renatured by heating at 75 °C for 5 min and let for 40 min at room temperature. Dry CNBr-activated sepharose (35 mg) was swollen and washed six times with 1 mL of 1 mM HCl. Aptamer solution (150 μL) was mixed with the gel at room temperature overnight. The sorbent was then packed between two frits into a 1 mL SPE cartridge and washed with 3 mL of 200 mM Na_2HPO_4 (pH 8). Remaining active groups of the support were

blocked by a 0.1 M Trizma solution (pH 8) for 2 h at room temperature. The gel was then washed alternately three times with 2 mL of an aqueous saline buffer (0.1 M acetate + 0.5 M NaCl, pH 4) and 2 mL of a Trizma buffer (0.1 M + 0.5 M NaCl, pH 8) to remove noncovalently bound aptamers. To evaluate nonspecific interactions between tetracycline and the sepharose-based sorbent, a blank sorbent was prepared following the same procedure but without aptamers. Various fractions of the selection buffer at 4 °C were then percolated through the oligosorbent to study the strength of the interactions between tetracycline and immobilized aptamers. To estimate the risk of nonspecific interactions between tetracycline and single-stranded DNA, the same immobilization procedure was followed with a scrambled aptamer. Scrambled aptamer is an aptamer with the same base composition but in a random order, immobilized to check for possible nonspecific interactions between an oligonucleotide and tetracycline. The scrambled have no affinity for tetracycline. Above steps were repeated for scrambled aptamer.

The cartridge was pre-conditioned with 5 mL of MeOH followed by 5 mL of deionized water. Then, 5 mL of selection buffer that consisted of 5% (v/v) MeOH was used for conditioning the cartridge. The percolation samples consisted of the selection buffer were spiked with the appropriate volumes of tetracycline and passed through the column. The optimized extraction procedure consisted of the percolation of 200 μL of the selection buffer at 4 °C containing variable amounts of tetracycline followed by a washing step with 300 μL of the selection buffer at 4 °C. The analytes retained on the SPE cartridge were eluted by 300 μL of a water/methanol solution (20/80, v/v) at ambient temperature. 20 μL of the elution fraction was injected into the IMS.

2.4. Real samples

2.4.1. Plasma and urine samples

Drug-free human plasma sample was obtained from the Isfahan University of Technology Clinic Center and kept frozen at −18 °C until analysis. Urine sample was collected from a healthy volunteered woman who had not taken any medication. Prior to use, the plasma sample was allowed to thaw at room temperature. Plasma proteins were precipitated with 0.05 M $HClO_4$ solution with the same sample volume. The plasma and urine samples were diluted 3 times before the extraction to reduce the matrix effects. Both plasma and urine samples were filtered through a 0.45 and 0.22 μm syringe filters (PTFE, Bioneer) before introducing into the sorbents.

2.4.2. Drug administration

The proposed method was applied to analyze plasma and urine samples of a healthy 23 years old volunteered woman before any drug taking. Then, a 500 mg tetracycline soft gel capsule was administrated to the volunteer and her blood and urine samples were collected in different times. The samples were analyzed after sampling according to the procedure described above.

3. Results and discussion

3.1. Ion mobility spectra

The ion mobility spectrum of tetracycline is shown in Fig. 1. The reduced mobility value of tetracycline ion is 1.20 ± 0.02 cm^2/Vs based on the reduced mobility of nicotinamide (1.85 cm^2/Vs) [33].

3.2. Optimization of extraction condition

Fig. 2 shows parameters needed to be optimized for the extraction method. Factors affecting the extraction efficiency such as choice of washing and elution solvent, washing and elution volume, concentration of MeOH in elution solvent and elution flow rate

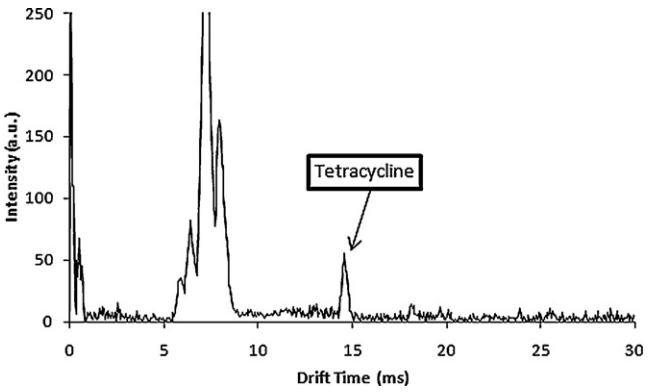

Fig. 1. The ion mobility spectrum of tetracycline in MeOH.

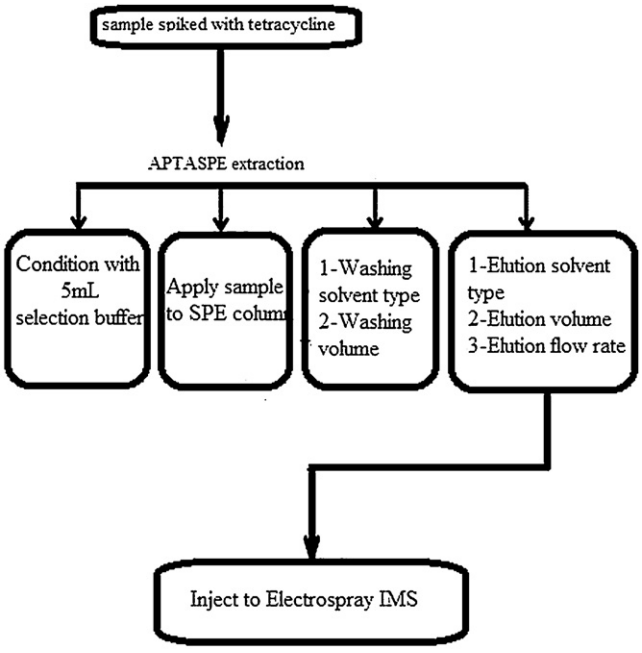

Fig. 2. Parameters needed to be optimized for the extraction.

were studied and optimized. All experiments were performed in triplicate. After the samples had been passed through the aptamer column, they were washed by 300 μL of selection buffer:MeOH, 95:5. The results of washing solution with different volume percent of methanol are listed in Table 2. Fig. 3 also shows that the optimized washing volume is 300 μL. The properties of selected elution solvent must be compatible with ESI-IMS. Based on the above consideration, MeOH/water and ACN/water were considered as the elution solvent. The extraction results in Table 3 show that MeOH could provide the higher extraction efficiency (about 2 times) than that ACN for the target analyte. Therefore, MeOH was

Table 2
Effect of MeOH percent on intensity and extraction recovery of washed tetracycline (1 μg/mL).

MeOH in selection buffer (%)	Intensity of washed sample (a.u.)	Relative recovery (%)
0	1094 (5.8)[a]	67.1
1	860 (6.4)	52.8
5	358 (7.0)	22.0
10	550 (5.9)	33.7
20	617 (5.3)	37.8

[a] Relative standard deviation (n = 3).

Table 3
Effect of elution solvent type on intensity and extraction recovery (1 μg/mL).

Relative recovery (%)	Intensity (a.u.)	Solvent type (ACN:H_2O)	Relative recovery (%)	Intensity (a.u.)	Solvent type (MeOH:H_2O)
26.5	432 (3.9)[a]	ACN	53.1	869 (4.8)	MeOH
36.4	593 (4.6)	95:5	66.0	1075 (5.9)	95:5
43.6	711 (4.9)	90:10	72.9	1189 (4.6)	90:10
51.8	845 (3.7)	80:20	91.7	1495 (4.9)	80:20
54.6	890 (4.5)	70:30	64.8	1056 (4.5)	70:30

[a] Relative standard deviation ($n = 3$).

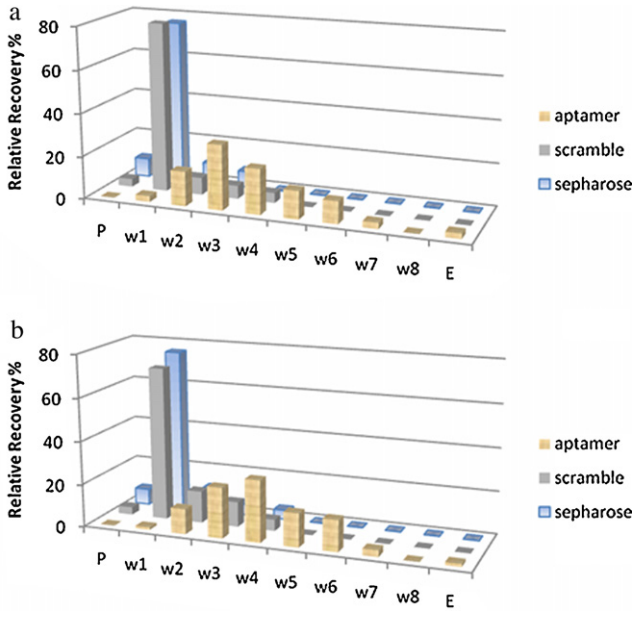

Fig. 3. Comparison of the retention of tetracycline on the oligosorbent, scrambled and on the sepharose sorbent for **(a)** plasma and **(b)** urine samples when selection buffer was passed through the sorbent. Percolation: 200 μL of selection buffer spiked with 1 μg/mL tetracycline. Washing with the selection buffer containing 5% (v/v) MeOH:W1 = 200 μL and W2-W8 = 100 μL.

selected for further experiments and methanol:water in the ratio of 8:2 was selected as the elution solvent. The effect of elution volume (ranging from 50 μL to 500 μL) on the extraction efficiency was also investigated. The extraction efficiency of the analyte was decreased by increasing the elution volume and results in Table 4 shows that the optimized elution volume is 300 μL.

The relative recovery for tetracycline extraction was increased by increasing the elution flow rate; hence, direct injection of the samples was selected.

3.3. Validation

To determine the applicability of the proposed method in analyzing real samples and investigate the effect of matrix on the determination of tetracycline, the optimized conditions were used for the analysis of tetracycline in plasma and urine samples. Plasma samples spiked with analyte at the concentration level of 2 μg/mL. Then proteins were precipitated by adding 0.05 M $HClO_4$ with the same sample volume. The relative recovery (RR) of the analyte was obtained from the following equation: $RR\ (\%) = (A1 - A2)/A3 \times 100$ where A1, A2 and A3 are peak areas of the analyte in spiked sample extract, unspiked sample extract and spiked deionized water extract, respectively. The RR value was obtained 71.3% (RSD = 6.1%). In order to reduce matrix effect and to increase RR, plasma sample was diluted 3 times with the selection buffer. The dilution enhanced the relative recovery to 82.8% (RSD = 6.3%). Analysis of urine sample was performed with 3 times dilution. The relative recovery for the spiked urine sample at 1 μg/mL concentration level was 86.5% (RSD = 5.9%). According to the USA FDA [34] "a calibration curve should be prepared in the same biological matrix as the samples in the intended study by spiking the matrix with known concentrations of the analyte". Therefore, the calibration curves of tetracycline in plasma and urine samples were carried out and the results are listed in Table 5. This table shows the standard deviation of the intercept, slope and coefficient of determination (R^2) values.

According to the results, the linear dynamic ranges are about 2 orders of magnitude and the correlation coefficient were above 0.990 for the compound in both urine and plasma samples.

To estimate the risk of nonspecific interactions between tetracycline and the activated sepharose or scrambled aptamer, the same experiments were carried out in parallel on a sepharose sorbent and scrambled sorbent, followed the same procedure. The recovery yields of tetracycline in the percolated and washing fractions in plasma and urine samples are shown in Fig. 3(a) and (b) respectively. After the sample percolation and the first two washing fractions, tetracycline was eluted from the sepharose and scrambled sorbents, whereas 87% and 81% of the tetracycline was still retained on the aptamer sorbent in urine and plasma samples, respectively. According to the results, a 300 μL washing solution with 5% MeOH should break low nonspecific interactions between the sorbent and drug without affecting the retention of tetracycline by aptamer.

Results of the extraction for the different sorbents are shown in Fig. 4(a) and (b). 7% of tetracycline was recovered in the elution fraction for both control supports, indicating that few nonspecific interactions were developed during this extraction procedure.

Table 4
Effect of elution volume on intensity and extraction recovery (1 μg/mL).

Elution volume (μL)	Intensity (a.u.)	Relative recovery (%)
50	879 (5.0)[a]	54.0
100	992 (5.7)	60.9
150	1098 (4.9)	67.4
200	1097 (5.5)	67.3
250	1236 (6.3)	77.2
300	1490 (6.4)	89.4
350	1389 (5.9)	85.2
400	1187 (5.6)	72.8
500	1070 (6.6)	65.6

[a] Relative standard deviation ($n = 3$).

Table 5
Analytical parameters for spiked solutions of tetracycline in plasma and urine matrix.

	Urine	Plasma
Intercept	0.509 ± 0.107[a]	0.721 ± 0.137
Slope	0.658 ± 0.023	0.672 ± 0.029
Regression coefficient (r^2)	0.9852	0.9834
LOD (μg/mL)	0.019	0.037
Lower LOQ (μg/mL)	0.05 (80.0)[b]	0.10 (83.1)
Upper LOQ (μg/mL)	5.00 (88.6)	5.00 (86.4)

[a] Standard deviation ($n = 3$).
[b] Relative recovery (%).

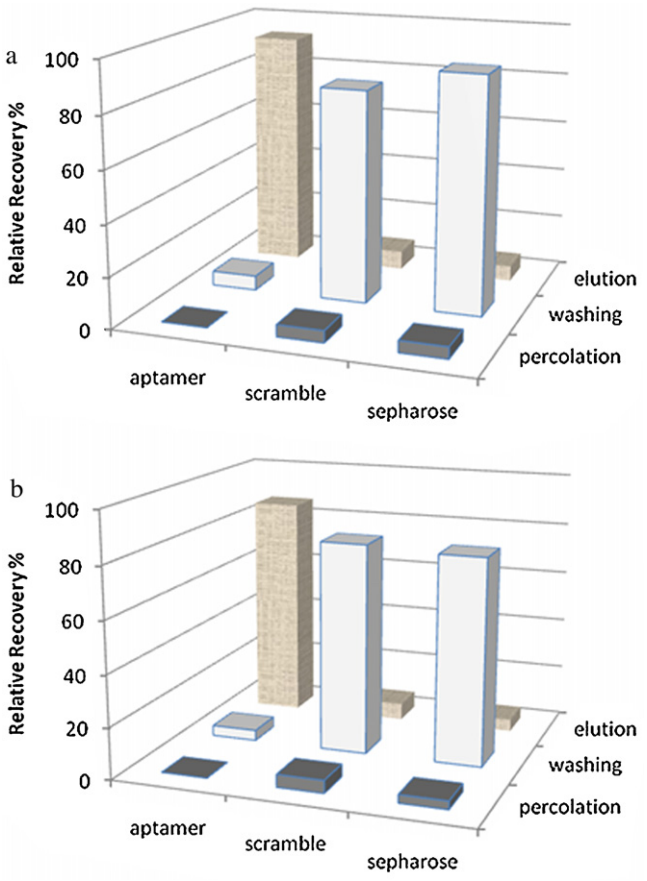

Fig. 4. Extraction profile of tetracycline from (a) plasma and (b) mediums on the oligosorbent and both control sorbents.

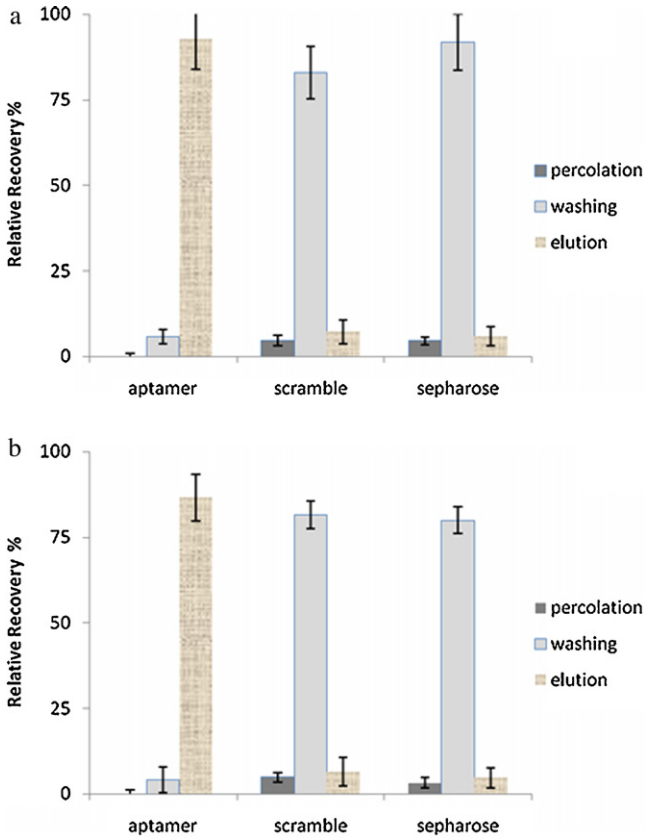

Fig. 5. Repeatability of the extraction protocol on the oligosorbent, scrambled and on the sepharose sorbent in (a) plasma and (b) urine samples.

Repeatability of the extraction procedure is shown in Fig. 5(a) and (b) for plasma and urine samples, respectively.

The extraction recoveries of 86.5% and 82.8% were obtained for the aptamer sorbent, with RSD values lower than 6.3% and 5.9% ($n = 3$), whereas the extraction recoveries were found to be $4.9 \pm 1.2\%$ and $3.5 \pm 1\%$ for the sepharose sorbent in plasma and urine samples, respectively.

3.4. Analysis of urine and plasma sample

Quantification of tetracycline in the samples was performed using single standard addition method. Urine and plasma of a 23-year-old volunteered woman who received oral administration of 500 mg tetracycline, was chosen as the real samples. Urine samples were diluted 3 times while plasma samples were diluted 3 times after protein precipitation with the same volume of $HClO_4$ 5% (v/v). The concentrations of tetracycline found in plasma and urine samples are presented in Table 6. The spectra obtained by ESI-IMS for blood (6 h after drug-taking) and urine (4 h after drug-taking) are shown in Fig. 6(a)–(d). The objective of this study was to show the capability of the proposed method to clean up the sample. The ion mobility spectra of the urine and plasma samples of the volunteered woman before drug taking (blank sample) did not show any spectral interference at the tetracycline ion peak. Whereas, the ion mobility spectra of the urine and plasma samples of the volunteer after drug taking show the ion peak of the tetracycline at its position.

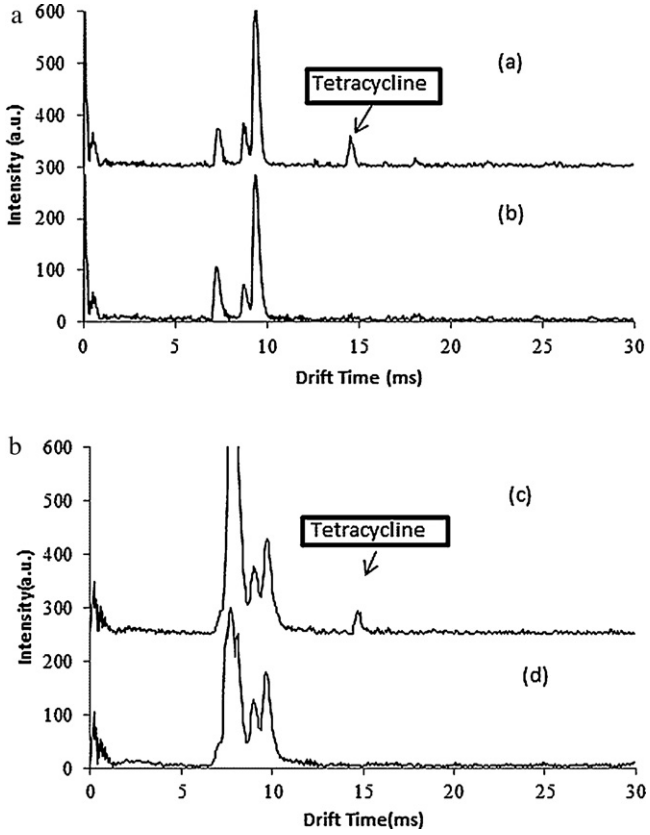

Fig. 6. (a) Extracted from plasma sample after 6 h from drug-taking, (b) extracted plasma sample before drug-taking (blank), (c) extracted from urine sample after 6 h from drug-taking and (d) extracted urine sample before drug-taking (blank).

Table 6
Concentration of tetracycline found in plasma and urine samples obtained after oral administration of 500 mg tetracycline.

Sampling time after drug-taking (h)	Urine			Plasma		
	Tetracycline added (μg/mL)	Tetracycline found (μg/mL)	Relative recovery (%)	Tetracycline added (μg/mL)	Tetracycline found (μg/mL)	Relative recovery (%)
2	–	3.00 (7.3)[a]	–	–	0.10 (6.5)	–
	1.00	3.80 (6.8)	88	1.00	0.98 (5.1)	82
4	–	3.61 (6.7)	–	–	0.32 (9.1)	–
	1.00	4.43 (5.8)	91	1.00	1.21 (7.3)	89
6	–	5.08 (6.9)	–	–	0.52 (7.0)	–
	1.00	5.75 (5.2)	84	1.00	1.33 (5.2)	77
8	–	2.12 (6.0)	–	–	0.29 (6.8)	–
	1.00	3.24 (6.3)	86	1.00	1.18 (4.7)	82

[a] Relative standard deviation ($n = 3$).

Table 7
Comparison of analytical performance data of the proposed method and other techniques in the determination of tetracycline in urine and plasma samples.

Extraction method	Detection system	Sample	LDR (ng/mL)	LOQ (ng/mL)	RSD (%)[a]	Recovery (%)	Reference
SPE-UPLC	QToF-MS	Urine	0.5–10	0.455	6.9	97.9 ± 6.8	[24]
Direct dilution	UPLC-ToF	Urine	NR[b]	NR	NR	175–223	[25]
–	Luminescence	Urine	1000–5000	0.075	<6	96.4	[23]
–		Plasma	1000–5000	800	<6	101	[23]
Ion pair RP-HPLC	UV	Urine	$(2–20) \times 10^4$	NR	3	–	[22]
		Plasma	$200–2 \times 10^4$	NR	10	–	[22]
–	Fluorosensor	Urine	4–356	0.4	1.1	–	[26]
–	Optical fiber	Urine	89–89,000	89	0.55	–	[27]
Aptamer SPE	ESI-IMS	Urine	50–5000	50	7	86.55	This method
		Plasma	100–5000	100	6.3	82.84	This method

[a] Relative standard deviation ($n = 3$).
[b] Not reported.

3.5. Comparison of the present method with other methods

The combination of antitetracycline aptamer-based sorbent with ESI-IMS was successfully applied for the analysis of tetracycline from human plasma and urine samples. Table 7 summarizes the detection limits and linear response ranges obtained for tetracycline by this study and previously reported methods. The results show that the dynamic range of the proposed method is better than or comparable with previously reported methods. The results of Table 7 also show that the limit of detection of the proposed method is lower than luminescence [23] and comparable with the others. The detection limit of UHPLC-MS/MS is lower than that the proposed method, however, the hyphenated MS is a sophisticated method relative to the proposed method. The IMS is also generally considered as a simple and fast technique [29].

4. Conclusion

A selective separation of an analyte from the complex biological fluids is an important step in chemical analysis. Aptamer based sorbents are one of the best candidates for separation of an analyte from a complex matrix. Aptamers have excellent selectivity and recovery factor and they are much cheaper than antibodies. In the present study, the combination of using aptamer as a selective separation technique with ESI-IMS has been introduced. This combination provided a selective, simple, fast and dervitazation free method for analysis of tetracycline in human plasma and urine samples. In addition, the proposed method has a compatible detection limit with a wide dynamic range relative to the reported methods.

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