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Highly Sensitive Fluorescence Methods for the Determination of Alfuzosin, Doxazosin, Terazosin and Prazosin in Pharmaceutical Formulations, Plasma and Urine

Xiaozhen GUO, Hao WU,† Shiwen GUO, Yating SHI, Juanli DU, Panpan ZHU, and Liming DU†

School of Chemistry and Material Science, Shanxi Normal University, Shanxi Linfen, 041004, P. R. China

Polymeric ionic liquid-coated magnetic nanoparticles have been successfully prepared as adsorbents for the magnetic solid-phase extraction of four drugs, namely alfuzosin, doxazosin, terazosin and prazosin, from pharmaceutical preparations, urine samples and plasma samples. The four drugs were detected by fluorescence spectrophotometer. Several extraction parameters, including the pH of the solution; the type, ratio and volume of the desorbing reagent; the amount of adsorbent; the time of the extraction and desorption processes; and the addition of NaCl, were investigated and optimized. Linear responses were determined for the four drugs in the concentration range of 0.5 – 45 ng mL^{-1}. The limit of detection values for alfuzosin, doxazosin, terazosin and prazosin, which were defined as three times the standard deviation of a blank sample, were determined to be 0.035, 0.034, 0.027 and 0.028 ng mL^{-1} (n = 11), respectively. Furthermore, this new method gave preconcentration factors of 114.5, 111.3, 111.1 and 108.5 for these four drugs.

Keywords Magnetic solid phase extraction, α1-adrenoreceptor blocker, fluorescence, Fe_3O_4@PIL

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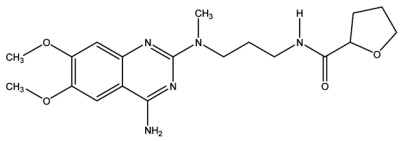
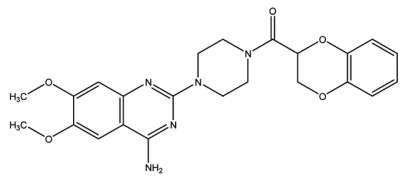
Introduction

Benign prostatic hyperplasia (BPH) is a common disorder of the male urogenital tract and is the main cause of lower urinary tract symptoms (LUTS) in older men. BPH affects 50 – 90% of the male population aged from 50 to 85 years.[1] Compared with surgery, pharmacological intervention can result in significant improvements in the symptoms of BPH with a reduced risk of serious side effects. Selective α1-adrenoreceptor blockers, which are the main class of pharmacological drugs used for the treatment of BPH, can be widely distributed in the prostatic tissues. Drugs belonging to this class can therefore be used to inhibit the sympathetic stimulation of the prostatic smooth muscles and relieve urinary obstruction.[1] Alfuzosin (ALF), doxazosin (DOX), terazosin (TER) and prazosin (PRA) are well-known inhibitors of the α1-adrenoreceptor.[1,2] The structures of the four drugs are shown in Scheme 1. It is noteworthy that all four of these compounds are based on a similar 4-amino-6,7-dimethoxyquinazoline core.

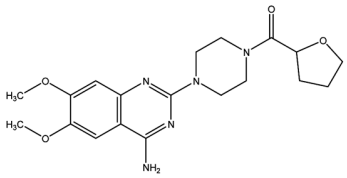
The development of sensitive analytical methods for the detection and evaluation of these drugs in human samples is highly desired because of their strong potency. A variety of different analytical methods have been reported for the determination of ALF, DOX, TER and PRA in pharmaceutical formulations and biological samples, including spectrophotometry,[3–6] fluorometry,[7,8] voltammetry,[9–11] high-performance liquid chromatography (HPLC)[12–19] and high-performance thin-layer chromatography[20,21] methods. Among these methods,



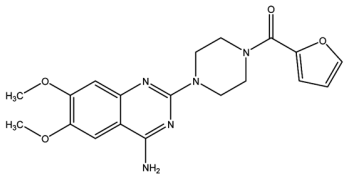
a) ALF



b) DOX



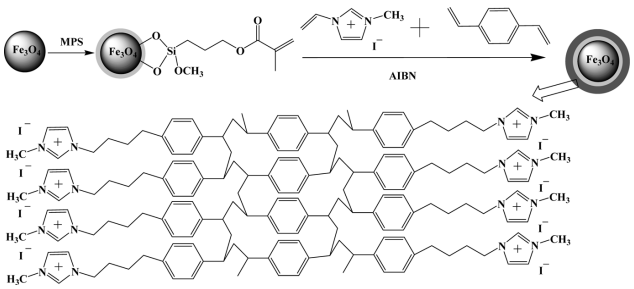
c) TER



d) PRA

Scheme 1 The structures of the drugs.

† To whom correspondence should be addressed.
E-mail: fxcszx@163.com; lmd2004@163.com



Scheme 2 The structure of the polymeric material coating the Fe_3O_4 NP.

fluorometry is considered to provide a high level of sensitivity, as well as being one of the most convenient of the different analytical techniques for pharmaceutical analysis. However, fluorometry cannot be used for the direct detection of drugs in biological samples without first subjecting the sample to a pretreatment process.

Magnetic solid-phase extraction (MSPE) has recently emerged as a promising technique for the preparation of analytical sample preparation, and has attracted considerable interest from analytical scientists working in a variety of different fields.[22-24] MSPE represents a new form of extraction based on the use of magnetic or magnetizable adsorbents, which can be readily isolated from a sample matrix with an external magnet. Furthermore, the adsorbents used in MSPE can be uniformly dispersed in a sample solution by sonication, vortex mixing or simple shaking, which makes the contact area between the adsorbents and the analytes large enough to allow for a rapid mass transfer. Compared with other methods, MSPE can be used to achieve higher levels of extraction efficiency, as well as greater enrichment factors.

The type of adsorbent used in an MSPE process can have a direct impact on the extraction efficiency. The choice of adsorbent is therefore critical to the successful practical application of this technique. Iron oxide (Fe_3O_4) nanoparticles are magnetic and could therefore be used as an adsorbent for the MSPE of drug compounds from biological samples. However, when it is used as the adsorbent of MSPE, its surface is often coated with different specific organic reagents because naked Fe_3O_4 is easy to aggregate, and not selective and not suitable for the samples with complicated matrix.[25]

Based on their unique physicochemical properties, ionic liquids (ILs) have been successfully applied to several different areas of analytical chemistry as extraction solvents, especially for the extraction and preparation of biological samples.[26,27] The use of IL-coated Fe_3O_4 magnetic nanoparticles (MNPs) as adsorbents would combine the unique properties of ILs with the advantages of magnetic materials. However, the results of previous research in this area have shown that ILs can be physically absorbed onto the surfaces of MNPs and can therefore appear in the final extract, leading to contamination and interference issues. The development of ILs that are covalently bound to some form of support material could therefore provide higher levels of stability and minimize the IL losses generally incurred during the extraction and elution stages of existing MSPE processes.[28,29]

In this study, we have synthesized polymeric IL-coated Fe_3O_4 (MNPs) as adsorbent for the MSPE process. The structure of the material is presented in Scheme 2. Furthermore, this material allowed for the efficient extraction of ALF, DOX, TER and PRA from pharmaceutical preparations and biological samples, with the extracted materials being determined by fluorimetric analysis. To the best of our knowledge, this study represents the first reported account of the use of an extraction technique of this type for the fluorometric determination of ALF, DOX TER and PRA. The effects of several extraction parameters, including the type and volume of eluent, amount of adsorbent, extraction time and pH, have also been studied and optimized.

Experimental

Apparatus

Fluorescence signals were measured on a Cary Eclipse fluorescence spectrophotometer (Agilent Technologies, Palo Alto, California, USA) equipped with a xenon lamp. The analytical results were evaluated using Ver. 1.1 of the Cary Eclipse software. All of the fluorescence measurements were performed in a 0.7-mL quartz cell, which was maintained at 25.0 ± 0.5°C. A bandwidth of 5.0 nm was used for the emission and excitation monochromators. An ultrasonic cleaning system (Model KH 2200DV, Kunshan Hechuang Ultrasonic Instrument Co., Ltd., Jiangsu, China) was used to assist in the adsorption and desorption of the samples. A pH meter (Model pHS-3C, Shanghai Tianda Apparatus Ltd., Shanghai, China) was used to measure the pH values of the different solutions for the pH adjustment experiments.

Chemicals and reagents

Standard drug samples of DOX, ALF, TER and PRA were purchased from the Chinese National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Stock solutions of the different samples were prepared at a concentration of 0.1 mg mL^{-1} by dissolving the appropriate amount of each standard drug in 5 mL of acetic acid, and then diluting the resulting solution to 50 mL in a volumetric flask with double-distilled water. Standard working solutions were prepared by diluting the corresponding standard stock solutions with double-distilled water. Urine and plasma samples were obtained from several healthy volunteers. Britton–Robinson (BR, 0.04 mol L^{-1}) buffer was used to control the pH. All of the standard solutions were stored at 4°C prior to being used and brought to ambient temperature before being analyzed.

Iron(III) chloride hexahydrate ($FeCl_3{\cdot}6H_2O$; Sigma-Aldrich, St. Louis, Missouri, USA), ethylene glycol (EG; Sigma-Aldrich), 1,2-ethylenediamine (EDA; J&K Scientific Ltd., Jiangsu, China)) and sodium acetate (NaAc; J&K Scientific Ltd.) were used to prepare Fe_3O_4 (MNPs). Ammonia (Haohua Chemical Reagent Co., Ltd., Luoyang, China), 3-(trimethoxysilyl)propyl methacrylate (MPS; Tokyo Chemical Industry; Tokyo, Japan), iodide-vinyl-3-methylimidazolium ([VMIM][I]; Chengjie Chemical Co., Ltd., Shanghai, China), divinylbenzene (DVB; Tokyo Chemical Industry) and 2,2′-azoisobutyronitrile (AIBN; Tokyo Chemical Industry) were purchased for the synthesis of the novel adsorbent.

General procedure

The polymeric IL-coated Fe_3O_4 particles were synthesized according to a procedure described in the literature.[30] The procedure used for the MSPE was as follows: 1 mL of a 2 μg mL^{-1} sample was added to a 50-mL tube, followed by 10 mg of the adsorbent. The tube was then sealed and sonicated for 2 min. The magnetic adsorbent was then rapidly separated from the solution using an external magnet, and the supernatant was directly decanted into a separate vial. The magnet was then

removed and the adsorbed target compounds were eluted from the adsorbent with acetone (0.3 mL) following 2 min of vigorous ultrasonic irradiation. The magnet was then reapplied to the tube and the supernatant was transferred to a quartz cell. The fluorescent intensity of the sample in the cell was then measured for the determination of DOX, ALF, TER or PRA. Fluorescence intensity calibration curves were constructed for known concentrations of the different test drug samples.

Sample pretreatment

Ten tablets of each drug were weighed and carefully powdered. An amount of powder equivalent to the average weight of each tablet was accurately weighed into a 50-mL volumetric flask and then dissolved in 5 mL of acetic acid. The resulting solution was subjected to ultrasonic irradiation for about 30 min before being diluted to 50 mL with double-distilled water. The solution was then filtered with the first 15 mL of the filtrate being discarded. An aliquot (10 mL) of the remaining filtrate was transferred to a 100-mL volumetric flask and diluted to 100 mL with a BR buffer solution.

Plasma samples (2.0 mL) were spiked with 10 μL of each drug solution, and the resulting mixtures were deproteinized with 8 mL of acetonitrile, followed by centrifugation at 4000*g* for 10 min. Five milliliter samples of the clear supernatants were then collected and diluted to 50 mL with a BR buffer solution, and the resulting solutions were subjected to the general analytical procedure.

Ten milliliter samples of fresh human urine were injected into individual centrifuge tubes, and spiked with 10 μL solutions containing different concentrations of the four different drugs. The resulting mixtures were then centrifuged at 4000*g* for 5 min. The clear supernatants (5.0 mL) were placed into 50 mL glass tubes and diluted to 50 mL with a BR buffer solution, before being subjected to the general analytical procedure.

Results and Discussion

Effect of the sample pH

The pH of the sample solution can have a dramatic affect on the extraction efficiency of an adsorbent because different analytes can exist in different forms at the different working pH values. To obtain the desired preconcentration efficiencies, we studied pH values in the range of 2.0 – 12.0 by adjusting the pH of the sample solution *via* the addition of a BR buffer solution. The results shown in Fig. 1 revealed that the maximum fluorescence intensities were obtained at pH values of 10.0, 8.0, 8.0 and 11.0 for ALF, DOX, TER and PRA, respectively. The pK_a values of ALF, DOX, TER and PRA are different from each other, and the pK_a values of ALF, DOX, TER and PRA are 8.13, 6.94, 7.10 and 6.50, which is lower than the solution pH. Under this condition, they all exist in an anion state and bear negative charges, and magnetic material bears positive charges. As a result, the drugs can be adsorbed on the sorbent *via* electrostatic interactions, allowing for higher extaction efficiency. At the same time, according to the literature,[31] due to the difference in the structure of the drugs, van der waals forces, the π–π bonds and hydrophobic interactions would also have an effect on extaction efficiency. Thus, there exists a difference in optimum pH value. A variety of different buffers (borate, phosphate and BR) were investigated in terms of their effect on the pH and the results showed that BR was the optimum buffer system. In the process of adsorption, the solution pH is higher than the pK_a values of the analytes. Under this condition, they all exist in anion state and bear negative charges, and magnetic material

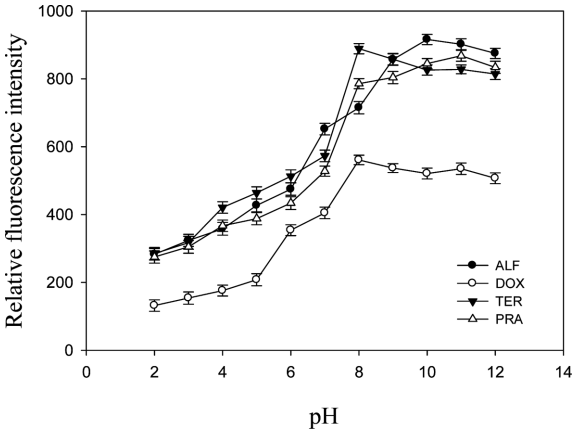


Fig. 1 The effect of the pH value of the solution. Extraction conditions: sample solution, 50 mL; Fe_3O_4@PIL, 8 mg; extraction time, 10 min for TER and PRA, 15 min for ALF and DOX; desorption time, 2 min for DOX and PRA, 3 min for ALF, and 5 min for TER; desorption solvent, 0.3 mL of acetone and 0.1 mL of BR buffer solution (pH 2.0).

bears positive charges. As a result, the drugs can be adsorbed on the sorbent *via* electrostatic interactions, making the higher extaction efficiency. The amount of BR used in the extraction process was also evaluated, and the results revealed that variations in the concentration of BR had very little impact on the extraction efficiency. Based on these results, a BR buffer solution (pH 10.0, 8.0 and 11.0) was used to maintain the pH throughout the experiment.

Amount of magnetic material

To determine the optimum amount of the adsorbent for the MSPE process, we investigated different amounts of adsorbent in the range of 2 – 10 mg. The results of these experiments showed that increasing the amount of adsorbent led to an increase in the fluorescence intensity until a maximum value was reached at 8 mg. Increasing the amount of the adsorbent beyond 8 mg made it increasingly difficult to achieve the desorption of the drug molecules from the adsorbent, which led to a slight decrease in the fluorescence intensity. Based on these results, the optimum amount of adsorbent was determined to be 8 mg and this amount was used in the subsequent experiments.

Effect of the extraction time

The extraction time profiles were studied by varying the adsorption time between 5 and 30 min. The results of these experiments showed that the fluorescence intensities of ALF and DOX reached their maximum values at 15 min, whilst those of TER and PRA reached their maximum values at 10 min (Fig. S1, Supporting Information) . Due to the difference in the adsorption mechanism, the difference in the optimum extraction time would occur. Based on these results, we selected an extraction time of 10 min for TER and PRA in the subsequent experiments, and an extraction time of 15 min for ALF and DOX.

Effect of the type, proportion and volume of the desorbing solution

Three parameters need to be optimized for a good desorption process, including the type, proportion and volume of the desorbing solution. In this study, we investigated a variety of conventional solvents (with the addition of a BR buffer solution,

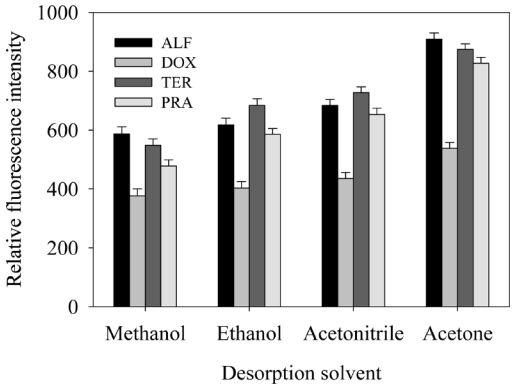


Fig. 2 The effect of the desorption solvent. Extraction conditions: sample solution, 50 mL; Fe_3O_4@PIL, 8 mg; extraction time, 10 min for TER and PRA, 15 min for ALF and DOX; desorption time, 2 min for DOX and PRA, 3 min for ALF, and 5 min for TER.

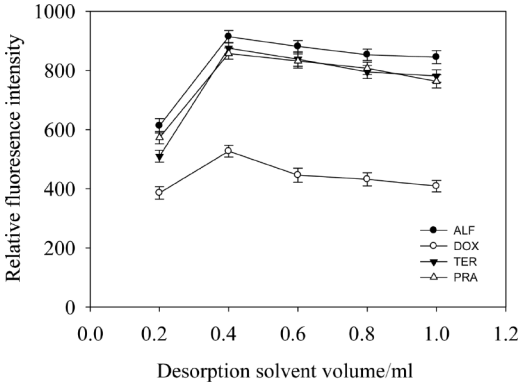
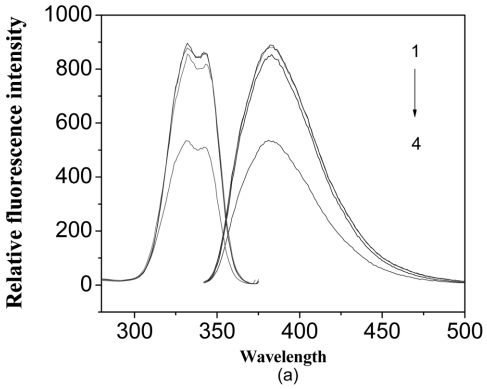


Fig. 3 The effect of the desorption solvent volume. Extraction conditions: sample solution, 50 mL; Fe_3O_4@PIL, 8 mg; extraction time, 10 min for TER and PRA, 15 min for ALF and DOX; desorption time, 2 min for DOX and PRA, 3 min for ALF, and 5 min for TER; desorption solvent, acetone and BR buffer solution (pH 2.0).



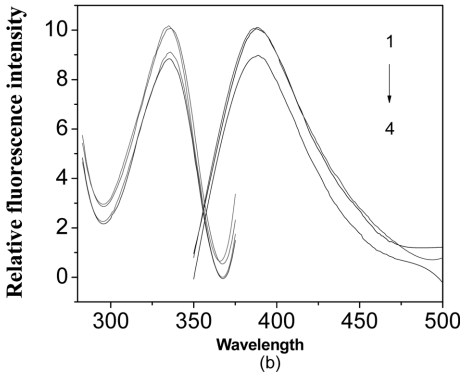


Fig. 4 Excitation and emission spectra. (a) Excitation and emission spectra of drugs that have been desorbed: (1) ALF, (2) TER, (3) PRA and (4) DOX. (b) Excitation and emission spectra of drugs before extraction: (1) ALF, (2) TER, (3) PRA and (4) DOX.

pH 2.0), including methanol, ethanol, acetonitrile and acetone, to determine the optimum desorption solvent (Fig. 2). The results of these experiments indicated that acetone (with BR buffer solution, pH 2.0) was the optimum desorption solvent for all four of the drug samples, due to the fact that the solubility of the drugs in acetone is higher than the rest of the solvents. To find the best ratio of acetone to BR buffer for the desorbing solution, we evaluated the effects of a variety of different mixed solutions of acetone and BR buffer solution (*i.e.*, 1:3, 2:2 and 3:1, v/v). The results of these experiments showed that the use of a higher proportion of acetone led to higher fluorescence intensity (Fig. S2, Supporting Information). Based on these results, a mixture consisting of 0.3 mL of acetone and 0.1 mL of BR buffer solution (pH 2.0) was used as the eluent. Subsequent experiments revealed that 0.4 mL was the optimum volume for desorbing the drugs from the adsorbent (Fig. 3). Given that an increase in the elution volume led to a decrease in the intensity of the analytical signals, we selected 0.4 mL as the optimum eluent volume for the whole experiment.

Effect of desorption time

The time allowed for the ultrasonic irradiation of the adsorbent for the elution of the analytes was also optimized to minimize the time required for the processing of the samples. A variety of different times were therefore investigated for the desorption process (*i.e.*, 1, 2, 3, 5, 7 and 9 min) (Fig. S3, Supporting Information), and the results revealed that the fluorescence intensities of DOX and PRA reached their maximum values at 2 min, whilst those of ALF and TER reached their maximum values at 3 and 5 min, respectively.

Effect of NaCl

Salting out effects have been well established in previous studies through the addition of different salts (mostly NaCl and Na_2SO_4) to the samples.[32] In this study, we conducted a series of experiments involving the addition of different amounts of NaCl (*i.e.*, 0, 0.5, 1, 1.5, 2 g) to the drug solutions to evaluate the effect on the extraction efficiency (Fig. S4, Supporting Information). The results showed that the addition of salt had no discernible impact on the extraction efficiency. Therefore, no salt was added to the subsequent optimization experiments.

Excitation and emission spectra

The excitation and emission spectra of the four drugs evaluated in the current study were recorded after the extraction process (Fig. 4). The spectra clearly showed that the excitation and emission peaks of the four different drugs occurred at 332 and 383 nm. Given that the reagent blanks had no effect on the determination of the drugs, these wavelengths were selected as the optimal excitation and emission conditions.

Table 1 Analytical parameters for ALF, DOX, TER and PRA (n = 11)

Analyte	Linear regression equation/ng mL^{-1}	Linear range/ ng mL^{-1}	Correlation coefficient	LOD/ ng mL^{-1}	RSD, %	
					Intraday	Interday
Alfuzosin	y = 20.453+20.294c	0.5 - 45	0.9993	0.035	2.31	2.34
Doxazosin	y = 10.397+13.181c	0.5 - 45	0.9996	0.034	3.27	3.19
Terazosin	y = 20.493+18.258c	0.5 - 45	0.9997	0.027	2.82	2.73
Prazosin	y = 3.9239+19.721c	0.5 - 45	0.9997	0.028	3.07	3.16

Table 2 Comparison with other proposed methods

Method	Linear range/ng mL^{-1}				LOD/ng mL^{-1}				Reference
	Alfuzosin	Doxazosin	Terazosin	Prazosin	Alfuzosin	Doxazosin	Terazosin	Prazosin	
HPLC-DAD	250 - 11000	—	—	—	50	—	—	—	12
HPLC-PDA	—	2000 - 500000	2000 - 500000	2000 - 500000		109	65	33	13
Fluorescence	—	—	1 - 7000	—	—	—	0.304	—	8
UV-spectrophotometry	—	450 - 6772	—	—	—	220	—	—	3
UV-spectrophotometry	1000 - 10000	—	—	—	30	—	—	—	4
HPLC-fluorescence	—	1 - 25	—	—	—	0.5	—	—	16
HPLC-ESI-MS\MS		0.2 - 50	—	—		0.05	—	—	19
Fluorescence	5 - 300	5 - 300	—	—	0.16	0.21	—	—	7
This work	0.5 - 45	0.5 - 45	0.5 - 45	0.5 - 45	0.035	0.034	0.027	0.028	

Table 3 Determination of the drugs in tablets

Tablet	The proposed method			The reference methods	
	Found (mg/grain)	Equivalent nominal content (%) ± S.D.[a]	Recovery, %	Found (mg/grain)	Equivalent nominal content (%) ± S.D.[a]
Alfuzosin	2.51 ± 0.0041	100.4 ± 1.67 (t, 0.33; F, 1.15)	100.4 ± 2.18	2.51 ± 0.0045	100.5 ± 1.80
Doxazosin	3.90 ± 0.0047	97.5 ± 1.16 (t, 0.96; F, 1.12)	97.7 ± 0.82	4.02 ± 0.0048	100.5 ± 1.20
Terazosin	2.01 ± 0.0042	100.5 ± 2.03 (t, 0.97; F, 2.02)	100.3 ± 2.05	1.96 ± 0.0110	100.1 ± 2.45
Prazosin	0.99 ± 0.0046	100.5 ± 2.03 (t, 0.96; F, 1.22)	0.99 ± 1.37	1.01 ± 0.0035	100.1 ± 1.67

The tabulated values of t and F at the 95% confidence limit are t = 2.57 and F = 6.26. Average of six determinations.

Analytical Performance

The analytical performance of our newly developed method was evaluated under the optimum conditions, and the results are summarized in Table 1. The results revealed that the calibration curves of the drugs gave correlation coefficients in the range of 0.9993 to 0.9997 for concentrations of 0.5 - 45 ng mL^{-1}. According to the IUPAC definition, the lower limit of detection (LOD, 3σ) values of our newly developed method, which were defined as three times the standard deviation of the blank signal intensity for 11 determinations, were 0.035, 0.034, 0.027 and 0.028 ng mL^{-1} for ALF, DOX, TER and PRA, respectively. Furthermore, a brief comparison of different analytical methods for the determination of ALF, DOX, TER and PRA is listed in Table 2.

Interferences

Aliquots of aqueous solutions containing 40 ng mL^{-1} each of the four drugs and certain amounts of other chemical species were obtained, and the proposed procedure was followed to study the selective separation and determination of the four drugs from the pharmaceutical formulations and biological samples with various chemical species. The tolerance limit was defined as the concentration of the added interfering substance that causes less than ±5% relative error in the determination of the four drugs. The results indicate that 1000-fold Na^+, K^+, Ca^{2+}, Mg^{2+}, Zn^{2+}, Fe^{2+}, Fe^{3+}, Sn^{2+}, Mn^{2+}, Cu^{2+}, Al^{3+}, PO_4^{3-}, SiO_3^{2-}, NO_3^-, Cl^-, SO_4^{2-}, CO_3^{2-}, F^-, Br^-, ClO_3^-, NO_2^-, CH_3COO^-, $H_2PO_4^-$, glucose, L-glutamic acid, aspartate, asparagine, serine, glycine, histidine, threonine, citrulline, alanine, arginine and urea, 500-fold CrO_4^{2-}, $Cr_2O_7^-$, Zn^{2+} and 50-fold Fe^{2+}, ClO_3^- and MnO_4^{2-} did not interfere with the determination, indicating the high selectivity of the proposed method. Based on our experimental results, the developed method exhibits excellent selectivity for drugs adsorption and is suitable for the analysis of samples with a complicated matrix.

Analysis of pharmaceutical formulation

To further assess the applicability of our newly developed method, we analyzed commercially available tablets containing ALF, DOX, TER and PRA as previously described. A standard addition method was applied to confirm the proposed method, which involved the addition of the four different drugs to the previously analyzed tablets. The data shown in Table 3 revealed that the recovery of the spiked samples was satisfactory and demonstrated the capability of this new method for the determination of the four different drugs of interest.

Analysis of spiked human urine and plasma

This newly developed method was also applied to analyze the drugs in spiked human urine and plasma samples, and the results

Table 4 Fluorometric determination of the drugs in spiked urine and plasma (n = 5)

Sample	Spiked urine			Spiked plasma		
	Amount added/ ng mL^{-1}	Amount found/ ng mL^{-1}	Recovery, % ± S.D.[a]	Amount added/ ng mL^{-1}	Amount found/ ng mL^{-1}	Recovery, % ± S.D.[a]
Alfuzosin	1	0.99	99.72 ± 2.41	1	1.05	105.62 ± 1.09
	5	4.82	96.48 ± 1.71	5	5.12	102.43 ± 2.01
Doxazosin	1	0.99	99.34 ± 1.40	1	1.01	101.73 ± 2.27
	5	5.06	101.2 ± 2.02	5	4.99	99.85 ± 2.62
Terazosin	1	1.02	102.7 ± 1.24	1	0.96	96.75 ± 1.82
	5	4.93	98.78 ± 2.73	5	4.98	99.63 ± 2.48
Prazosin	1	0.99	99.14 ± 2.85	1	0.96	96.75 ± 1.31
	5	4.98	99.65 ± 2.73	5	5.13	102.62 ± 1.94

are shown in Table 4. The accuracy of the method was evaluated by investigating the recovery of the studied drugs at concentrations of 1 and 5 ng mL^{-1}. The resulting mean values for the recoveries ranged from 96.75 to 105.62% and 96.48 to 102.71% for the human urine and plasma, respectively, indicating good accuracy and precision for both samples.

Conclusion

In this study, we have successfully synthesized a polymeric IL-coated Fe_3O_4 MNP material using simple methods. The material was subsequently evaluated as a recyclable adsorbent for the extraction of ALF, DOX, TER and PRA in pharmaceutical preparations and biological samples, followed by fluorometric determination. Compared with existing methods, our newly developed method for the determination of these drugs provided high sensitivity, low LOD values and good recovery, and was also found to be suitable for the determination of different analytes in real samples with complex matrices.

Acknowledgements

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Supporting Information

Fig. S1, The effect of extraction time; Fig. S2, the effect of desorption solvent ratio; Fig. S3, the effect of desorption time; Fig. S4, the effect of NaCl. This material is available free of charge on the Web at http://www.jsac.or.jp/analsci/.

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