ISSN : 0974-7419 Volume 16 Issue 5

Analytical CHEMISTRY
An Indian Journal

Full Paper

ACAIJ, 16(5) 2016 [183-191]

# Extraction of pazufloxacin mesilate using magnetic attapulgite clay nanoparticles prior to determination by fluorimetric detection

**Jinfeng Zheng, Hao Wu*, Nannan Gao, Shiwen Guo, Liming Du**
**Shanxi Normal University of Modern Arts and Sciences, Shanxi Linfen, 041004, (P.R.CHINA)**
**E-mail: fxcszx@163.com; lmd@dns.sxnu.edu.cn**

## ABSTRACT

In this paper, a kind of magnetic attapulgite nanocomposites was successfully synthesized and used as the adsorbent for the magnetic solid-phase extraction of pazufloxacin mesilate prior to ûuorimetric detection. Under optimized conditions, the linear range of pazufloxacin mesilate was 2–36 ng $mL^{-1}$, and the correlation coefficients of the calibration curves was 0.9998. The limits of detection of the method was 0.27 ng $mg^{-1}$. The results demonstrated that the proposed method was rapid, reliable, and efficient for the determination of PZFX in urine and plasma samples.

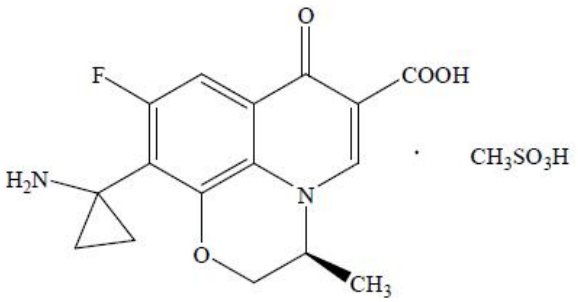

## KEYWORDS

Pazufloxacin mesilate;
Magnetic solid-phase extraction;
Fluorescence;
Magnetic attapulgite nanocomposites.

## INTRODUCTION

Pazufloxacin mesilate (PZFX, (S)-10-(1-aminocyclopropyl)-9-fluoro-3-Methyl-7-oxo-2,3-dihydro-7H-pyrid[1,2,3-de][1,4] benzoxazine-6-carboxylic acid monomethanesulfonate (Scheme 1), is one of the fourth generation quinolone antimicrobial agent with high antimicrobial activity in comparison with the similar quinolones[1]. PZFX has shown activity against gram-negative, gram-positive bacteria and anaerobic species, based on the inhibition of both DNA gyrase and topoisomerase IV[2,3]. It is currently widely used for the treatment of infections, such as respiratory organ infection, genitourinary organ infection, skin and parenchyma infection, and surgical infection[4]. Therefore, great attention has been devoted to the accurate and precise determination of PZFX in various biofluids including plasma and urine.

Many researchers have determined PZFX concentrations in serum, plasma or urine using high-performance liquid chromatography (HPLC)[5,6,7,8], fluorescence method[9,10] and chemiluminescence[11,12]. However, these methods have suffered several disadvantages, such as low sensitivity and poor anti interference ability make them impractical for complex samples. As a result, to establish a highly selective and sensitive method for the determination of PZFX in complex samples is necessary for academic research and practical application.




**Scheme 1 : Molecular structure of pazufloxacin mesylas (PZFX)**

Full Paper

Magnetic solid-phase extraction (MSPE), a promising technique for sample preparation, has received considerable attention in recent years. The magnetic sorbents can be uniformly dispersed into the sample solution by vortexing or shaking, which increasing the contact between analytes and the sorbents to ensure a fast mass transfer, then they can be readily isolated from sample matrix with an external magnet. MSPE is fast, simplicity, convenience and high throughput among sample preparations. MSPE has allowed the selective separation of organic compounds, including antibiotics, anti-inflammatory drugs, pesticides and phenolic compounds that are present in different matrices[13, 14].

Attapulgite (ATP) is a hydrous layer-ribbon magnesium aluminum silicate with a fibrous structure and a theoretical formula $(Al_2Mg_2)Si_8O_{20}(OH)_2(OH_2)_4 \cdot 4H_2O$ that has a larger soecific surface area and unique nanoporous structure[15]. Recently, clay minerals have been widely used as low-cost, sustainable and effective adsorbents for the removal of various dyes[16, 17, 18, 19], heavy metal ions[20, 21, 22, 23] and organic compounds[24, 25, 26, 27] from wastewater. While, it is difficult to separate the attapulgite from aqueous solution when the particle size is on the nano-scale. Although traditional methods such as centrifugation and filtration can be applied to recover the attapulgite particles from aqueous solutions. The centrifugation method requires very high centrifugal speed and filtration method is prone to filter blockages, these methods cannot be applied in large scale in real work. It is necessary to find new method for the separation of attapulgite particles easily in large scale. Recently, the attapulgite/iron oxide magnetic composites as a kind of sorbent has aroused much interest[20, 28]. It not only enhances the extraction efficiency by increasing the contact between analytes and the sorbents, but also overcomes problems with conventional separation methods.

In this paper, we synthesized the magnetic attapulgite (MATP) nanocomposites by a solvent thermal method, and applied the magnetic nanocomposites to extract PZFX from pharmaceutical preparations and biological samples, followed by fluorimetric determination. The effects of various experimental parameters, such as type and volume of extraction solvent, amount of DBMNPs, extraction time, and pH were studied and optimized from the experimental results.

## EXPERIMENTAL

### Apparatus

Fluorescence signals were measured using a Cary Eclipse Fluorescence spectrophotometer (Agilent Technologies, USA) equipped with a xenon lamp and computer with Cary Eclipse 1.1 software. All measurements were performed in a 0.7 mL quartz cell maintained at 25.0 ± 0.5 °C, with 5.0 nm bandwidths for the emission and excitation monochromators. A pH meter (Model pHS-3C, Shanghai Tianda Apparatus Ltd., China) was used for pH adjustment. An ultrasonic cleaning Model KH 2200DV (Kunshan Hechuang Ultrasonic Instrument Co., Ltd., China) was used to assist adsorption and desorption.

### Chemicals and reagents

The standard drug sample of PZFX was purchased from the Chinese National Institute for the Control of Pharmaceutical and Biological Products. A 0.1 mg·mL$^{-1}$ stock standard solution was prepared by dissolving the standard drug sample in 5 mL of sodium hydroxide (0.01 M), and diluting to the mark in a 100 mL volumetric flask with double–distilled water. The standard working solution was prepared by diluting the stock standard solution with double-distilled water. Urine and plasma samples were obtained from several healthy volunteers. The mixture of 40 g/L SDS (ACROS ORCANICS) and 0.0006 mol/L aluminium ions was prepared by dissolving the reagents in double-distilled water. Britton–Robinson (BR, 0.04 mol·L$^{-1}$) buffer was used to control the pH. All standard solutions were stored at 4 °C, and brought to ambient temperature prior to use.

Iron (III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$) (Sigma–Aldrich, USA), ethylene glycol (EG; Sigma–Aldrich, USA), sodium acetate (NaAc, J&K Scientific Ltd., China), polyethylene glycol(PEG, Sigma–Aldrich, USA) and attapulgite clay (Jiuchuan Clay Technology Co., Jiangsu, China,) were used to pre-

pare the MATP. Milli-Q water (Millipore, Bedford, MA, USA) was used throughout the study. All solvents used were of analytical reagent grade.

### Synthesis of magnetic materials

### Preparation for the attapulgite clay

10 g attapulgite clay was stirred with 1 mol/L hydrochloric acid, and rinsed with distilled water repeatedly until there is no chloridion if inspected by 0.l mol/L silver nitrate solution at room temperature. After centrifugal separation, the sample was baked in 105 ºC for 2 h and smashed for stand-by.

### Manufacture of magnetic attapulgite clay

The MATP was synthesized by a modified reduction reaction of $FeCl_3$ based on a reported method[29]. In a typical procedure, 0.2 g pre-prepared attapulgite clay, 25 mL EG, 0.6 g $FeCl_3$, 1.0 g of polyethylene glycol and 1.2 g NaAc were measured and put into a 50 mL beaker. After stirred evenly, the mixture was sealed in a Teflon-lined stainless steel autoclave (50 mL capacity) and maintained at 190 ºC for 8 h. The obtained black magnetic attapulgite nanocomposite was collected by magnet and purified by magnetic separation method for three times by using absolute ethyl alcohol and distilled water respectively, and dried at vacuum at 60 ºC for 12 h.

### Process of magnetic solid-phase extraction

Typically, all PZFX samples were prepared to preselected concentrations; pH was adjusted with a BR solution in a 50 mL colorimetric tube. Subsequently, 2 mg of MATP were added. After the magnetic materials was uniformly dispersed by ultrasonic, the sample was extracted for 15 min, and then put a bar magnet close to the bottom of tube to separate MATP from solution. The supernate was abandoned and the MATP used to adsorb PZFX were dried by small flow rate of nitrogen and desorbed by 1 mL of mixed solvents of $AlCl_3$ and SDS, ultrasound for another 10 min.. The magnet was again placed the bottom of tube, and the supernatant was transferred into quartz cells, and in the end analyzed by fluorescence spectrophotometer.

## RESULT AND DISCUSSION

### Optimization of magnetic solid-phase extraction conditions

In order to get the best analytical results of MATP for PZFX, 0.1 mL 0.01 g/L pazufloxacin mesilate was chosen as the target object to investigate the influence of buffer system, the pH value, absorbent dosage, extraction time, extraction temperature, and desorption conditions (desorption solvent type and concentration, desorption solvent volume and desorption time).

### Influence of buffer system

The influence of four kinds of commonly used buffer system with BR buffer solution, sodium acetate buffer solution (SC), phosphate–potassium dihydrogen phosphate buffer solution (PPDP), phthalate two potassium formate buffer system (PTPF) (pH=4) on the fluorescence intensity after extrac-

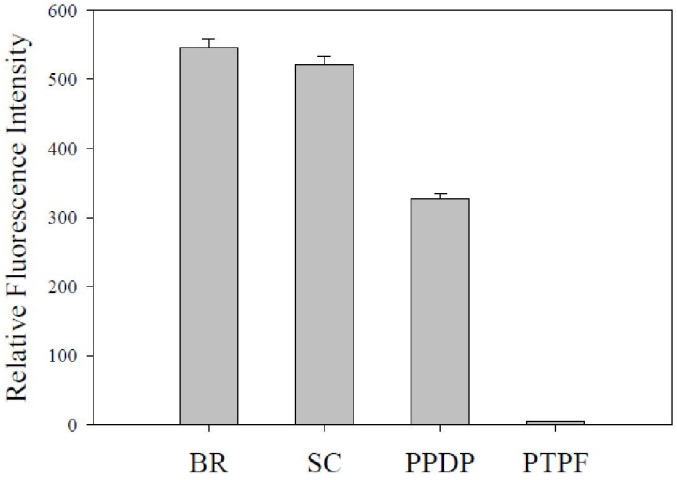



**Figure 1 : The influence of different buffer system**

tion was investigated respectively. Figure 1 shows the experimental results. Results show that almost no fluorescence intensity can be observed for the potassium acid phthalate buffer system after extraction, while the highest fluorescence intensity is observed in BR buffer solution. In the following experiment, the BR buffer solution is defined as the buffer system.

**Influence of the pH value of the solution**

The pH value of the solution is an important parameter in the research of separation-enrichment due to the great influence on the form and the distribution in the ionic liquid of the analyte. In order to obtain an ideal enrichment efficiency, the influence of pH value from 2.0 to 12.0 on the extraction efficiency of PZFX was investigated. The pH value can be adjusted by add BR buffer solution with different pH value. As shown in the following Figure 2, the highest extraction efficiency was obtained at pH 4.0. When the pH value is higher or lower than this, the extraction efficiency will decreases. Therefore, the pH value was selected as 4.0 in the following experiment.

**The amount of absorbent**

It is obvious that the amount of MATP has great influence on the extraction efficiency of PZFX. In the range of 1-8 mg, with the increasing of MATP, the fluorescence intensity also increase, and reach the maximum value at 2 mg (Figure 3). When the amount increased over 2 mg, more than 1 mL eluting solvent must be added to ensure all PZFX can be desorbed, and it might dilute the analytes in solvent correspondingly. Therefore, the amount of MATP was chosen as 2 mg.

**Optimization of the extraction time**

The optimization of extraction time was to get the best extraction efficiency and the shortest extraction time. Extraction time from 5 min to 40 min range with the interval 5 min was considered. The results are showed in the following Figure 4 it is clearly seen that, on the premise of the high fluorescence intensity, 10 min can be regarded as the best extraction time.

**Optimization of extraction temperature**

For all the analytical methods of separation and enrichment, we always want to finish the extraction in the most close to room temperature conditions, and at the same time, achieve the complete extraction. From the experimental results the fluorescence intensity did not change significantly in the temperature range of 20–80 ºC. So, in order to simplify the experiment, the extraction was conducted at room temperature.

**Influence of desorption solvent type**

It is necessary to use solvent to elute the analyte

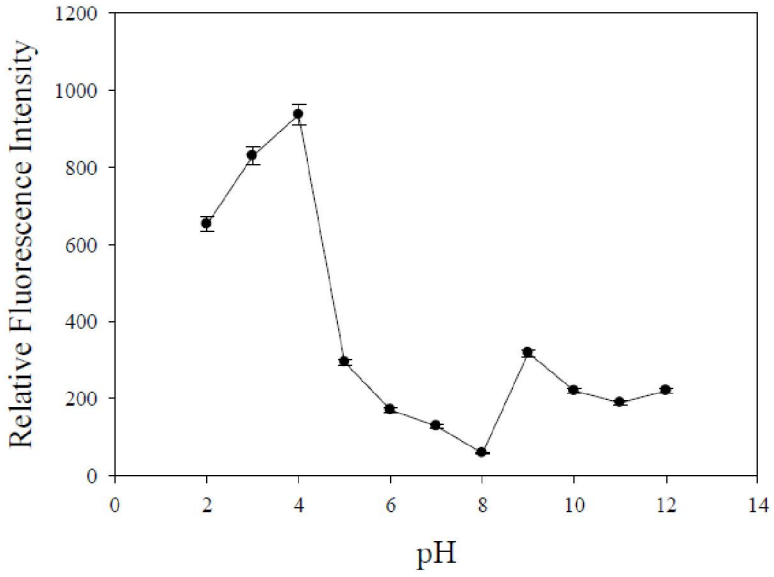



**Figure 2 : The influence of the pH value of the solution**

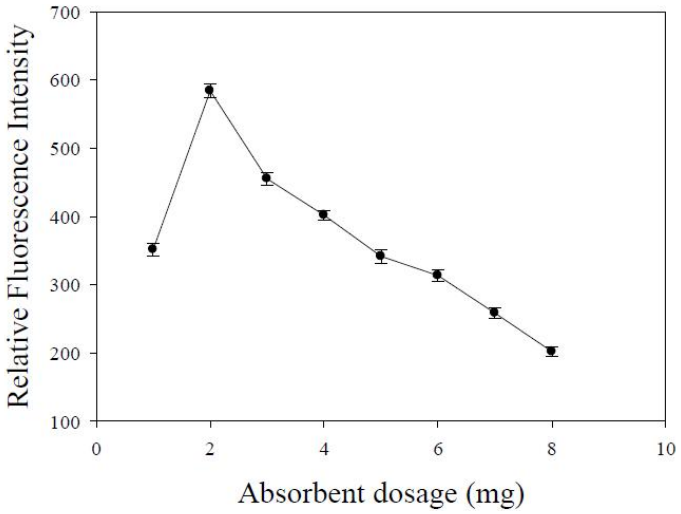



**Figure 3 : The influence of the amount of absorbent**

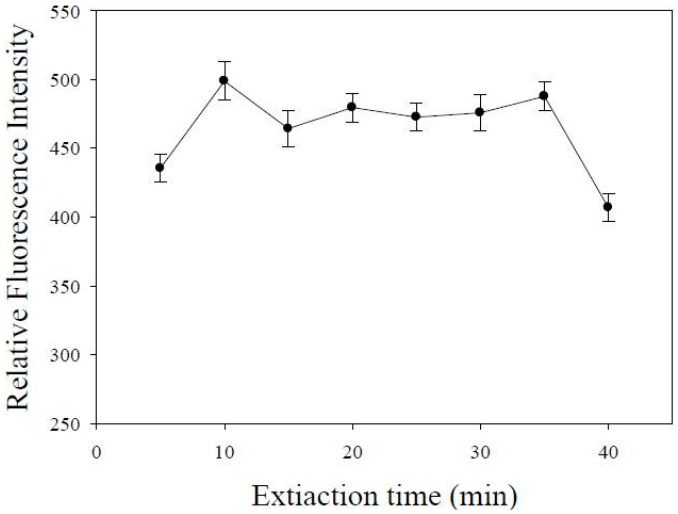



**Figure 4 : The influence of extraction time**

from MATP particles before fluorimetric determination, so the suitable solvent is indispensable which can elute the adsorbed analytes as much as possible. Five types of eluting solvent with $Ca^{2+}$, $Fe^{3+}$, $Mn^{2+}$, $Co^{2+}$, and $Al^{3+}$ were selected. As a result (Figure 5), the eluting solvent with aluminum ions showed the highest fluorescence intensity. Therefore, it is optimum to choose the aluminum ions as the appropriate eluting solvent in the following work. On the other hand, SDS can improve the desorption efficiency. So the mix of $Al^{3+}$and SDS was selected as the desorption solvent.

## Optimization of the desorption solvent concentration

The concentration of SDS, which is a part of desorption solvent and an important factor for analytical condition, influence the desorption efficiency directly. The influence of SDS was investigated in the 12 g/L–40 g/L range on the desorption efficiency, and found the best desorption efficiency occurred in 40 g/L under the same extraction conditions (Figure 6). Besides, we also found that when the concentration of SDS exceeds this value, the SDS cannot be dissolved. For the influence of aluminium ions in the 0.0002–0.0016 mol/L range was also studied. Therefore, for the overall consideration of sensitivity and linear range, the optimum value for the concentration for SDS and aluminium ions are 40 g/L and 0.0006 mol /L respectively.

## Optimization of desorption solvent volume

In order to decrease the dosage of desorption solvent and analysis the target object to the max ex-

Full Paper

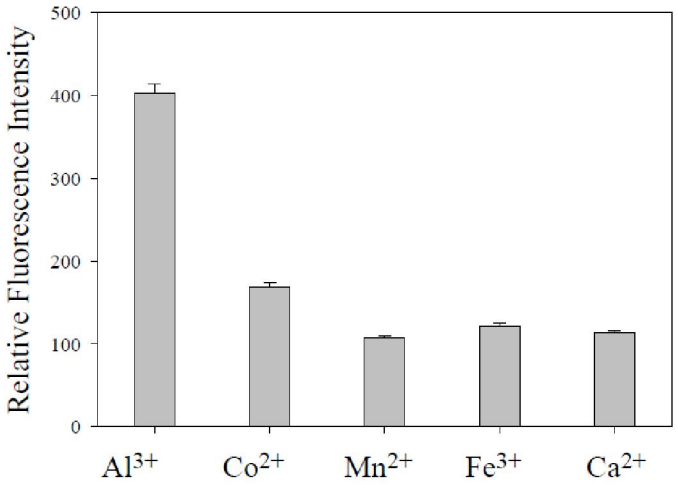



**Figure 5 : The influence of desorption solvent**

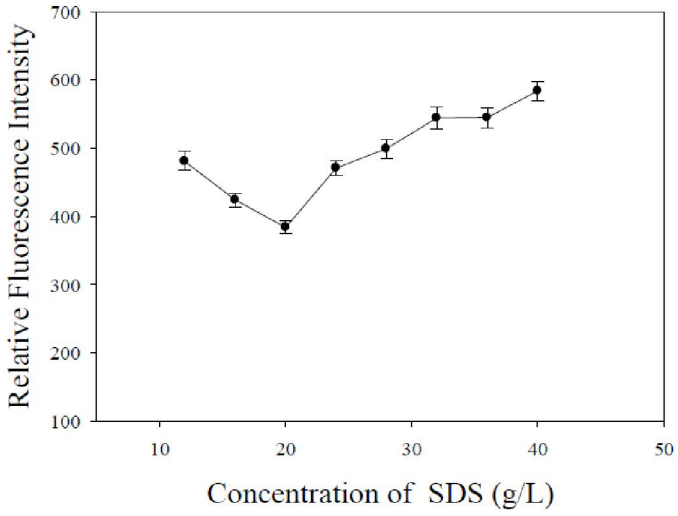



**Figure 6 : The influence of concentration of SDS**

tent, the influence of the mixed solution of 1 mL 0.0004 mol/L $AlCl_3$ and 20 g/L SDS with six different volume was investigated. As can be seen form the following Figure 7, the intensity of fluorescence decrease with the increase of the volume of analytical agent. Therefore, the volume of the mixed solution can be chosen as 1 mL.

## Influence of desorption time

The influence of desorption time on the analytical efficiency can be observed in the following Figure 8. It can be seen that the fluorescence intensity reaches the max value at 7 min and extending the analytical time on the basis of 7 min did not play a good effect. Besides, when the analytical time is 3 min, a high analytical efficiency also can be achieved. Therefore, in order to balance the analytical efficiency and the analytical time, 3 min is best.

## The excitation and emission spectra

The excitation and emission spectra of PZFX after extraction are investigated. The excitation and emission wavelength locate at 248 nm and 389 nm, respectively. At the same time, the excitation and emission spectra of sample with only buffered solution were determined after MATP extraction. It can conclude that the buffered solution has no influence on the measured spectra. Therefore, we use the mentioned wavelengths as the excitation and emission spectra for PZFX.

## Analytical performance

Under the optimized conditions, a series of standard solution containing PZFX with an different concentration levels were prepared to establish the cali-

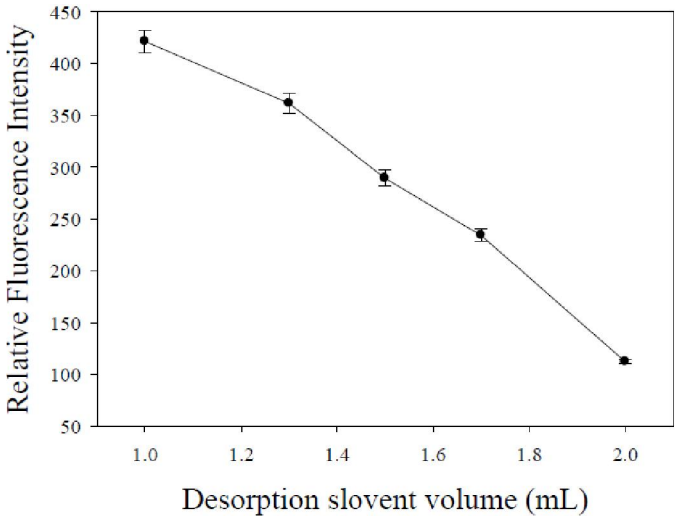



**Figure 7 : The influence of desorption solvent volume**

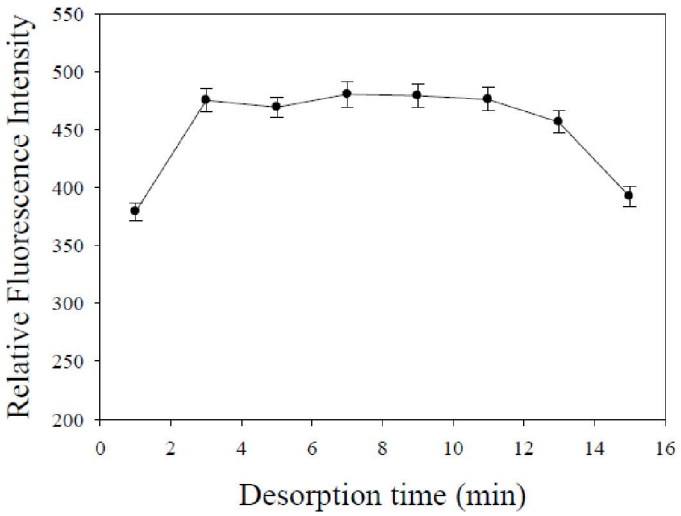



**Figure 8 : The influence of desorption time**

bration curve, and satisfactory correlation coefficient of 0.9997 was obtained in the concentration range of 2-36 ng $mL^{-1}$, which indicated a good linear. According to the IUPAC method, the lower detection limit of this method are calculated by LOD=$KS_0/S$, where $K$ is 3, $S_0$ is the standard deviation of blank sample, and $S$ is the slope of the standard curve. The relative standard deviations (RSD) of this method was 2.32%. Comparing the proposed method with others(listed in TABLE 1), it can easily seen that the limit of detection is lower.

## Interference

An evaluation of the interference of MATP extraction on the PZFX and the selectivity of the method was conducted by investigating the coexisting materials with PZFX from the biological samples. The tolerance limit was defined as the concentration of the added interfering substance that cause less than ±5% relative error in the determination of the drug. Aliquots of aqueous solutions containing 20 ng·$mL^{-1}$ PZFX and certain amounts of other chemical species were obtained, and the proposed procedure was followed to study the selective separation and determination of the drug. The results show that 1000-fold $Na^+$, $K^+$, $Cu^{2+}$, $Ca^{2+}$, $Mn^{2+}$, $Fe^{2+}$, $Sn^{2+}$, $Al^{3+}$, $SO_4^{2-}$, $PO_4^{3-}$, $Cl^-$, $SiO_3^{2-}$, $NO_3^-$, $C_2O_4^{2-}$, $NO_2^-$, $CH_3COO^-$, $ClO_3^-$, $F^-$, $Br^-$, $H_2PO_4^-$, glucose, a-lactose, L-glutamic acid, urea, and citric acid, and 500-fold $CO_3^{2-}$, $Cr_2O_7^-$, $I^-$, $Zn^{2+}$, $Fe^{3+}$, and $MnO_4^-$ did not interfere with the determination, indicating the high selectivity of the proposed method.

## Analysis and applications

Full Paper

**TABLE 1 : Comparison with other proposed methods**

| Methods | Linear range (ng mL$^{-1}$) | Correlation coefficient | Limit of detection (ng mL$^{-1}$) | RSD (%) (n=11,*c*=100 ng mL$^{-1}$) |
|---|---|---|---|---|
| CE-ECL | 20-10,000 | 0.9968 | 4.0 | 4.28 |
| HPLC-FL | 20-5,000 | 0.9969 | 10 | 5.49 |
| MISPE-CL | 2.5-250 | 0.9991 | 0.7 | 3.7 |
| MIPs-CA | 5-5,000 | 0.9901 | 1.8 | 5.3 |
| This work | 2-36 | 0.9997 | 0.27 | 2.32 |

**CE-ECL: capillary electrophoresis with electrochemiluminescence detection; HPLC-FL: high-performance liquid chromatography (HPLC) method with fluorescence detection; MISPE-CL: molecularly imprinted polymer solid-phase extraction (MISPE) with flow-injection chemiluminescence; MIPs-CA: molecularly imprinted polymers technology and capacitive transducer technology.**

**TABLE 2 : Fluorimetric determination of the drug in spiked urine and spiked 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] |
| PZFX | 5 | 4.84 | 96.8±2.12 | 5 | 5.11 | 102.2±2.21 |
| | 10 | 9.79 | 97.9±2.37 | 10 | 19.01 | 95.1±2.35 |
| | 20 | 19.78 | 98.9±2.23 | 20 | 19.75 | 98.76±1.92 |

[a] **Average of five determination**

### The measurement with the additional standard urine samples

2 mL fresh urine from a healthy volunteer (no any drugs for nearly one week) was mixed the standard PZFX with 0.1 mL by centrifuging for 10 min in a centrifuge tube. 1 mL supernatant liquid of the sample was taken to measure the concentration of drugs. The process repeated for three times to ensure the accuracy. The results is listed in TABLE 2. It can be seen that the average recovery is in the range of 96.88 % to 98.9 %.

### The measurement with the additional standard plasma samples

Similarly, 1.0 ml plasma was mixed uniformly with the 0.1 mL standard solution by volution for 3 min. After that, 9 mL acetonitrile was mixed to wipe out the protein in the plasma. Take 5 mL supernatant liquid after the solution was centrifuged for 10 min at a speed of 3500 rpm to measure the concentration of the drug for five times. The average recovery among 95.1%–102.2%. The result is listed in TABLE 2.

## CONCLUSIONS

In conclusion, the MATP was successfully fabricated with high quality which was used to magnetic solid-phase extraction experiment. Combined with the fluorescence spectrophotometer, a method of enrichment PZFX was built in urine and plasma. It provides a simple, fast method with no organic solvent, high sensitivity, and repeatability. The additional experiments of urine and plasma verify a good accuracy and precision for the PZFX in the biological samples by MATP extraction method.

## ACKNOWLEDGEMENT

Financial Supporting by the Shanxi Province Natural Science Foundation for Youths (No. 2014021018-3) is gratefully acknowledged.

## REFERENCES

**[1]** M.Takei, H.Fukuda, R.Kishii, M.Hosaka; Antimicrob.Agents Chemother., **45**, 3544 **(2001)**.

**[2]** M.Nagasawa, S.Nakamura, M.Miyazaki, Y.Nojima, H.Hayakawa, Y.Kawamura; Jpn.J.antibiot., **55**, 259 **(2002)**.

**[3]** N.Nomura, J.Mitsuyama, Y.Furuta, H.Yamada, M.Nakata, T.Fukuda, M.Takahata, S.Minami; Jpn.J.antibiot., **55**, 412 **(2002)**.

**[4]** X.Yang, X.You; Med.Antibiot.Overseas (in Chinese), **24**, 80 **(2003)**.

**[5]** S.Watabe, Y.Yokoyama, K.Nakazawa, K.Shinozaki, R.Hiraoka, K.Takeshita, Y.Suzuki; J.Chromatogr.B, **878**, 1555 **(2010)**.

**[6]** J.Sousa, G.Alves, G.Campos, A.Fortuna; A.J.Chromatogr.B, **930**, 104 **(2013)**.

**[7]** P.B.Phapale, H.W.Lee, S.D.Kim, M.S.Lim, D.D.Kale, J.M.Lee, J.H.Park, S.O.Moon, Y.R.Yoon; Chromatographia, **71**, 101 **(2010)**.

**[8]** Q.Li, R.Wang, F.Pei; Asian J.Drug Metab Pharmacokinetics, **4**, 289 **(2004)**.

**[9]** X.Cao, L.Lian, H.Li, Y.Wu, D.Lou; Anal.Sci., **30**, 817 **(2014)**.

**[10]** C.Shilv, M.Hongwu, Z.Huichun, F.Ruiqing, J.Linpei; Anal.Sci., 20, **(2004)**.

**[11]** X.L.Wang, S.L.Chen, H.C.Zhao, L.P.Jin, X.Li; Anal.Lett., **38**, 971 **(2005)**.

**[12]** C.Yang, Z.Zhang, S.Chen, F.Yang; Microchim.Acta., **159**, 299 **(2007)**.

**[13]** K.Aguilar-Arteaga, J.Rodriguez, E.Barrado; Anal.Chim.Acta., **674**, 157 **(2010)**.

**[14]** C.A.Ibarra, X.Feng, V.K.Schoft, T.F.Hsieh, R.Uzawa, J.A.Rodrigues, A.Zemach, N.Chumak, A.Machlicova, T.Nishimura; Sci., **337**, 1360 **(2012)**.

**[15]** V.Drits, G.Sokolova; Am.Inst.Phy., **(1971)**.

**[16]** Y.Liu, W.Wang, A.Wang; Powder Technol., **225**, 124 **(2012)**.

**[17]** R.Giustetto, O.Wahyudi; Microporous Mesoporous Mater., **142**, 221 **(2011)**.

**[18]** H.Chen, J.Zhao, A.Zhong, Y.Jin; Chem.Eng.J., **174**, 143 **(2011)**.

**[19]** Z.Zhang, W.Wang, A.Wang; J.Environ.Sci., **(2015)**.

**[20]** L.Chen, J.Xu, J.Hu; J.Radioanal.Nucl.Chem., **297**, 97 **(2012)**.

**[21]** J.Potgieter, S.Potgieter-Vermaak, P.Kalibantonga; Miner.Eng., **19**, 463 **(2006)**.

**[22]** Y.Chang, H.Liu, F.Zha, H.Chen, X.Ren, Z.Lei; Chem.Eng.J., **167**, 183 **(2011)**.

**[23]** H.Chen, Y.Zhao, A.Wang; J.Hazard.Mater., **149**, 346 **(2007)**.

**[24]** X.Guo, Y.Yao, G.Yin, Y.Kang, Y.Luo, L.Zhuo; Appl.Clay Sci., **40**, 20 **(2008)**.

**[25]** A.Xue, S.Zhou, Y.Zhao, X.Lu, P.Han; Appl.Clay Sci., **48**, 638 **(2010)**.

**[26]** N.Frini-Srasra, E.Srasra; Surf.Eng.Appl.Electrochem., **45**, 306 **(2009)**.

**[27]** A.Al-Futaisi, A.Jamrah, R.Al-Hanai; Desalination, **214**, 327 **(2007)**.

**[28]** Q.H.Fan, P.Li, Y.F.Chen, W.S.Wu; J.Hazard.Mater., **192**, 1851 **(2011)**.

**[29]** B.Mu, Q.Wang, A.Wang; J.Mater.Chem.A, **1**, 7083 **(2013)**.