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Molecularly imprinted solid phase extraction–pulsed elution–mass spectrometry for determination of cephalexin and α-aminocephalosporin antibiotics in human serum

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Abstract

A highly selective molecularly imprinted solid phase extraction (MISPE)–pulsed elution (PE) method coupled with electrospray mass spectrometry (MS) was developed for the rapid screening and determination of cephalexin in α-aminocephalosporin antibiotics. This method involved the solid phase extraction of cephalexin using a molecularly imprinted polymer micro-column, and pulsed elution with 1% trifluoroacetic acid in methanol, which contains sulindac as an internal standard for enhanced precision in MS detection. An LC/MS spectrometer was operated in the positive electrospray mode, and the selected-ion-recording (SIR) function was employed to detect the molecular ions of cephalexin, cefradine, cefadroxil and sulindac at *m/z* 348, 350, 363 and 357. Linearity was achieved in the cephalexin concentration range from 0.3 to 25 μg/ml (or 5–500 ng) (R^2 = 0.998). The detection limit was estimated at 0.04 μg/ml (or 0.8 ng) of cephalexin. Advantages of the newly developed MISPE–PE–MS, over the previously reported MISPE–DPE–FPE–UV, were evidenced in terms of detection limit, analysis time, solvent consumption, and simplicity of method development.

Keywords: Cephalexin; Aminocephalosporins; Molecularly imprinted polymer; Solid phase extraction; Pulsed elution; Mass spectrometry

1. Introduction

Cephalosporins are antibiotics used to treat a variety of infections in human. They have been grouped into three generations based primarily on their spectrum of antibacterial activities [1]. The first-generation cephalosporins have the highest activity against gram-positive bacteria, including most *Corynebacteria*, *Streptococci*, and *Staphylococci* (particularly *Staphylococcus aureus* and *Staphylococcus intermedius*). These cephalosporins also have activity against gram-negative bacteria, including some *Escherichia coli*, *Klebsiella pneumoniae*, *Actinobacte*, *Citrobacter*, *Enterobacter*, and indole-positive *Proteus* [2]. Cephalexin, 7-[(amino-phenylacetyl) amino]-3-methyl-8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid, belongs to the first generation of cephalosporins [3,4]. With the brand names of Ceporex (or Keflex) in the US, Novolexin in Canada, and many others outside North America, cephalexin is one of the top 20 drugs used in prescriptions worldwide. Tablets, capsules and liquid suspensions are mostly intended for oral administration. The current analytical methods reported for cephalexin determination are HPLC [5], HPTLC [6], electroanalytical techniques [7] and fluorometric analysis [8]. HPLC, although widely applied in many pharmaceutical industries, usually exhibits overlap with peaks from structural analogues in the chromatogram. The analysis normally requires longer than 7 min.

Molecular imprinting solid phase extraction (MISPE) has been intensively developed in the last 3 years. Basically it

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involves two steps: MIP polymerization and SPE (solid phase extraction). After a copolymerization between functional monomers (TFMAA) with the target analyte (as the imprinting molecule), in the presence of cross-linker (EGDMA), the functional groups are fixed in position inside the highly cross-linked polymeric structure. Subsequent removal of the imprinting molecule reveals the binding sites, which are complementary in size and shape to the analyte. The first application of MIP was used as stationary phases in affinity chromatography, specifically for enantioseparation of racemic mixtures of chiral compounds. This technology was further developed by Mullet et al. who successfully introduced differential pulsed elution (DPE) to eliminate the structural analogues [9]. In comparison with traditional stationary phase extraction materials, a unique property of MIPs can be highlighted on their lock-key relationship with the target molecule, and hence their selectivity is predetermined. The advantages of MISPE–PE include easy operation, rapid analysis and higher purity of extract. Solvent consumption is low, and the sample size can be as small as 20 μl [10].

A MISPE–DPE–final pulsed elution (MISPE–DPE–FPE) method was developed in our laboratory last year for screening cephalexin in α-aminocephalosporin antibiotics. In that 14% acetic acid in acetonitrile was used as DPE solvent to effectively eliminate interference by structurally similar analogues (cefradine, cefadroxil and ampicillin). Quantification of cephalexin was achieved by FPE with 1% trifluoroacetic acid (TFA) in methanol and direct UV detection at λ = 275 nm. However, one major concern arose from the fact that the measured UV signal was the sum of absorbance from the eluted cephalexin and the absorbance from 1% trifluoroacetic acid in methanol. The later created a very strong background signal, which significantly overlapped with the signal from cephalexin. The detection was partially optimized by shifting the detection wavelength up to 275 nm, which would lessen the background absorbance due to trifluoroacetic acid. This problem remained to be severe especially when trace amount of cephalexin was quantified. The introduction of DPE step also extended the total analysis time to 5 min.

The recent advance of electrospray MS methods for chemical analysis, structural identification and databases allows increased amounts of information to be generated in shorter periods of time. As researchers embrace different approaches for the collection of information on pharmaceutical properties, MISPE–MS emerges as an advantageous technique for a variety of screening applications. Recently, some groups reported the successful use of MIPs as stationary phase in LC–MS system. Koeber et al. reported a highly selective sample cleanup procedure based on the use of MIPs as on-line separation materials [11]. Xie et al. also reported the successful development of a coupled liquid chromatography and mass spectrometry (LC–MS) system that combined a MIP column and a MS detector for affinitive separation and on-line identification of antitumor components [12]. However, their investigation mainly focused on the affinitive chromatography instead of selective solid phase extraction for quantitative analysis. In our present research, an on-line molecularly imprinted solid phase extraction–pulsed elution (MISPE–PE) method was coupled with a quadrupole MS detector to replace the UV detector. For enhanced precision in cephalexin determination, sulindac was used as an internal standard in the PE solvent. A simple MISPE–PE–MS method was developed for on-line extraction and determination of cephalexin in α-aminocephalosporin antibiotics. A comparison between the newly developed MISPE–PE–MS and the conventional MISPE–DPE–FPE–UV method was made, in terms of analysis time, solvent consumption, and detection limit.

2. Experimental

2.1. Chemicals

Cephalexin (CFL), cefradine (CFR), cefadroxil (CFD) and sulindac were purchased from Sigma (St. Louis, MO, USA). Isoproturon was purchased from Chemical Service (West Chester, PA, USA). Trifluoroacetic acid (TFA), spectrophotometric grade, was purchased from Aldrich (Milwaukee, WI, USA). Methanol and chloroform, HPLC grade, were purchased from Caledon (Georgetown, ON, Canada). As cephalexin was available only as a hydrate ($C_{16}H_{17}N_3O_4S{\cdot}H_2O$) that is not soluble in $CHCl_3$, the white powder was first dissolved in CH_3OH before dilution with $CHCl_3$ to make up standard solutions.

2.2. Synthesis of cephalexin MIP

A molecularly imprinted polymer was prepared using cephalexin as the template molecule and 2-(trifluoromethyl) acrylic acid (TFMAA) as the functional monomer, in accordance with Guo and He's method [13]. To 5 ml of acetonitrile, 1 mmol of cephalexin and 4 mmol of TFMAA were added. After the cephalexin was dissolved completely, 20 mmol of the cross-linking agent ethylene glycol dimethacrylate (EGDMA) and 40 mg of the initiator 2,2′-azobisisobutyronitrile (AIBN) were added. The mixture was poured into a glass ampoule, degassed with sonication, and then bubbled with nitrogen for 5 min. Degassing and bubbling were repeated three times. The ampoule was sealed under vacuum and placed into a thermostatic bath at 60 °C for 24 h. After the ampoule was crushed, the bulk MIP was ground to obtain a suitable size range of cephalexin MIP particles that passed through an 80 μm sieve but not a 20 μm sieve. The resulting particles were washed with methanol and 20% aqueous acetic acid solution until cephalexin could no longer be detected with spectrophotometry at 240 nm and electrospray ionization quadrupole mass spectrometry at *m/z* 348. The particles were then washed with distilled water and dried to constant weight under vacuum at 60 °C.

2.3. CFL MIP micro-column

A slurry of these particles (50 mg) in methanol was manually injected from a syringe through a plastic connection tubing to a stainless steel column (50 mm length × 0.8 mm i.d.) immersed in an ultrasonic water bath. After the column was fully packed over 90 min, sonication was continued for 30 min to attain uniform packing. A zero-volume union was put on each end of the packed column before acetonitrile was pumped through for 2–3 h to achieve tight packing. The micro-column was washed with 1% TFA in methanol to remove all imprint molecules and yield binding sites inside the MIP particles. Approximately 40 mg of MIP particles was contained in the micro-column.

2.4. Molecularly imprinted solid phase extraction–pulsed elution

2.4.1. Instrumentation

An Eldex 9600 HPLC pump (San Carlos, CA, USA) or CC-30S micrometer pump was used as solvent delivery system. A Rheodyne 7125 or Cheminert VIGI C2XL extended life injector valve (Valco Instruments, Houston, TX, USA) equipped with a 20 μl sample loop was used for sample injection and PE. The breakthrough and elution of analytes were monitored by a Gilson 110 (Middleton, WI, USA) or Bischoff Lambda 1010 (Leonberg, Germany) UV detector at a wavelength of 240–275 nm. The breakthrough and PE peak areas were recorded and integrated by a Dionex 4270 integrator (Sunnyvale, CA, USA).

2.4.2. % Binding evaluations of CFL, CFR, CFD and AMP

The % binding of CFL on the MIP micro-column was investigated by single loading injections (20 μl) of CFL standard solution (20 μg/ml), first bypassing the micro-column and then through the CFL MIP micro-column. $CHCl_3$ was used as the mobile phase at a flow rate of 0.5 ml/min. The flow injection analysis (FIA) and breakthrough peaks, respectively, were recorded. The % bindings of CFR, CFD and AMP were investigated following the same procedure.

2.4.3. Binding capacity evaluation of CFL, CFR, CFD and AMP

The binding capacity of this CFL MIP micro-column was investigated by performing multiple loading injections of a 20 μg/ml CFL standard solution (prepared in $CHCl_3$/CH_3OH, 80:1 (v/v)). Binding saturation was finally observed when the breakthrough peak area of each loading injection became identical to the FIA peak area. The binding capacities for CFR, CFD and AMP were investigated following the same procedure.

2.5. MISPE–PE–MS

2.5.1. Instrumentation

MISPE of CFL was performed on the CFL MIP micro-column, with chloroform as the mobile phase. A Shimadzu LC-610 pump equipped with a Shimadzu SCL-6B system controller was used to deliver chloroform at a flow rate of 0.05 ml/min. A Rheodyne 7125 switching valve (Cotati, CA, USA) equipped with a 20 μl sample loop was used for sample injection and PE. 1% TFA in methanol, containing sulindac as the internal standard (20 μg/ml), was used as the PE solvent for quantitative determination of the bound CFL. A fused silica capillary (50 cm length and 75 μm i.d.) was used to connect the MIP micro-column with the mass spectrometer. It was estimated that the delay time (between sample injection and MS detection) was 3–4 min. A Quattro triple quadrupole mass spectrometer (Micromass®) employing positive ion electrospray ionization (ESI) was used to monitor the PE eluate. Data were processed under the control of a Micromass Professional Station, Masslynx version 3.5. Selected ion recording (SIR) was employed as the MS data collection mode. For each run, the MS detector was set at the following *m/z* values: CFL (348), CFR (350), CFD (363) and sulindac (357).

2.5.2. Ionization of CFL, CFR and CFD

Ionization of cephalexin, cefradine and cefadroxil during pulsed elution was investigated in the presence of 1% trifluoroacetic acid + CH_3OH. Cephalexin, cefradine and cefadroxil prepared with 1% TFA in CH_3OH, in concentrations from 20 to 300 μg/ml, were injected directly to the mass spectrometer, bypassing the MIP micro-column. The MS peaks of cephalexin, cefradine and cefadroxil were observed at *m/z* 348, 350 and 363, respectively.

MISPE–PE–MS was performed by single loading injections (20 μl) of a mixture containing CFL (20 μg/ml), CFR (20 μg/ml) and CFD (20 μg/ml) onto the CFL MIP micro-column. Sulindac was chosen as the internal standard. 1% TFA + CH_3OH, containing 20 μg/ml of sulindac, was used as PE solvent for MISPE–PE–MS. Mass spectra of these PE results were recorded.

2.5.3. Investigation of ionization competition

Ionization competition between CFL, CFR and CFD was investigated by loading a 20 μl aliquot of CFL standard solution (20 μg/ml) onto the MIP micro-column. $CHCl_3$ was used as the mobile phase at a constant flow rate of 0.05 ml/min. PE was performed with 1% TFA + 20 μg/ml sulindac + CH_3OH, containing CFR and CFD at varying concentrations in the range from 4 to 75 μg/ml, individually. Mass spectra of these PE results were recorded.

2.5.4. Serum analysis by MISPE–PE–MS

Human serum (Sigma Immuno Chemicals, S5143) was spiked with CFL, followed by treatment with an octadecyl C18 SPE cartridge (T. Baker, 7020-03). The extracted CFL

Table 1
Summary of % binding and binding capacity of CFL MIP micro-column for cephalosporin antibiotics prepared in $CHCl_3$ (with $CHCl_3$ as mobile phase, at flow rate of 0.5 ml/min)

Cephalosporin	Concentration (μg/ml)	% Binding	Binding capacity (μg)
CFL	23.2–56.8	92 ± 3 (n = 16)	7.3
CFD	20.0	79 ± 2 (n = 10)	3.8
CFR	20.8	72 ± 6 (n = 6)	1.7

was eluted with 3 ml of methanol. The eluent was collected and diluted with chloroform to a final CFL concentration in the range from 0.1 to 50 μg/ml. A standard calibration curve was constructed by performing single loading injections for MISPE, followed by multiple PEs with 1% TFA + 20 μg/ml sulindac + CH_3OH for electrospray MS analysis.

Human serum was spiked to contain CFL (13.5–25 μg/ml), CFR (60.5 μg/ml) and CFD (31.6 μg/ml). MISPE–PE–MS analysis was performed as described above.

3. Results and discussion

3.1. Molecular recognition on CFL MIP micro-column

Previous studies regarding molecular recognition of MIPs mainly focused on the investigation of static batch binding. Guo and He in 2000 reported their investigation of CFL batch binding in aqueous solution. Their experiment was performed by immersing the sized and washed CFL MIP particles (20 mg) into 10 ml of known concentration of the selected cephalosporins in water at 25 °C for 16 h. Technically speaking, however, their static binding investigation could not give sufficient support to the application of this MIP for the on-line MISPE due to a lack of knowledge on the dynamic binding performance under flowing conditions.

% Binding was used as a very straightforward criterion for evaluating the molecular recognition ability of CFL MIP particles for CFL and its structural analogues. For investigation of % binding, FIA peak area was obtained by injecting the sample solution bypassing the MIP micro-column, while the breakthrough peak area was achieved by injecting the sample solution through the MIP micro-column. The % binding was calculated as the quotient

$$\%\,\text{binding} = \frac{\text{FIA peak area} - \text{breakthrough peak area}}{\text{FIA peak area}} \times 100\%$$

When $CHCl_3$ was used as the sample solvent and mobile phase for MISPE, 90–95% binding of CFL could be achieved on this CFL MIP micro-column, as shown in Table 1. By comparison, CFR and CFD had only 68–76% and 78–80% binding under the dynamic conditions.

The binding capacity of the MIP micro-column was evaluated in this work by multiple 20 μl loading injections of a 24.7 μg/ml CFL standard solution, with $CHCl_3$ as the mobile phase, at a flow rate of 0.5 ml/min. Each injection would load 0.50 μg of CFL onto the micro-column. Saturation of the MIP recognition sites was reached after approximately 57 loading injections. Based on all the break-through peak areas before the micro-column saturation, a binding capacity of total mass of 7.3 μg was determined for the CFL bound to the ~40 mg of MIP particles. However, this CFL MIP micro-column did not show high binding capacity for the structural analogues studied. After approximately 10 loading injections of 20.8 μg/ml of CFR standard solution, a total binding capacity of 1.7 μg of CFR was achieved on this CFL MIP micro-column. By comparison, CFD could achieve a binding capacity of approximately 3.8 μg of CFD on this CFL MIP micro-column, suggesting that CFD could form stronger hydrogen bonding with the recognition sites than CFR. The present % binding and capacity results should be interpreted as dependent on the binding strength (thermodynamics) and rate (kinetics). The high % binding and capacity results for CFL can potentially be utilized to develop a highly selective MISPE–PE method for the accurate determination of CFL.

3.2. Pulsed elution (PE)

Although CFL achieved 90–95% binding on CFL MIP particles, the other α-aminocephalosporin compounds, CFR and CFD, also achieved 68–76 and 78–80% binding, respectively. Due to a lack of absolute specificity afforded by the CFL MIP, differential pulsed elution (DPE) with an intermediate solvent (14% CH_3COOH + CH_3CN) to wash CFR and CFD out of the micro-column was deemed necessary [14]. However, the involvement of a DPE step would not only require additional labor-intensive method development, but also extends the analysis time and causes result variations. Besides, the sensitivity of the method was significantly affected by the background interference attributed to TFA in PE when coupled with a UV detector. A MS detector was hence employed to replace the UV detector, for achieving mass spectral resolution of CFL from CFR and CFD. MS would afford higher sensitivity and freedom from spectral interference caused by the PE solvent.

3.3. MISPE–PE–MS

3.3.1. MS behavior of α-aminocephalosporin antibiotics

As shown in Fig. 1, the α-aminocephalosporin antibiotics contain both amino and carboxylic groups in each molecular structure. Theoretically they will favor both positive and negative electrospray. However, negative electrospray was found to be less efficient. Firstly, $CHCl_3$ was used as the mobile phase in which dissociation of a proton from the carboxylic group became less possible. The second consideration was about the PE solvent containing TFA, which might promote the protonation of α-amino groups. By associating 1 proton from TFA, the α-aminocephalosporin molecule would now be detectable as a positive ion. Therefore, the choice of a

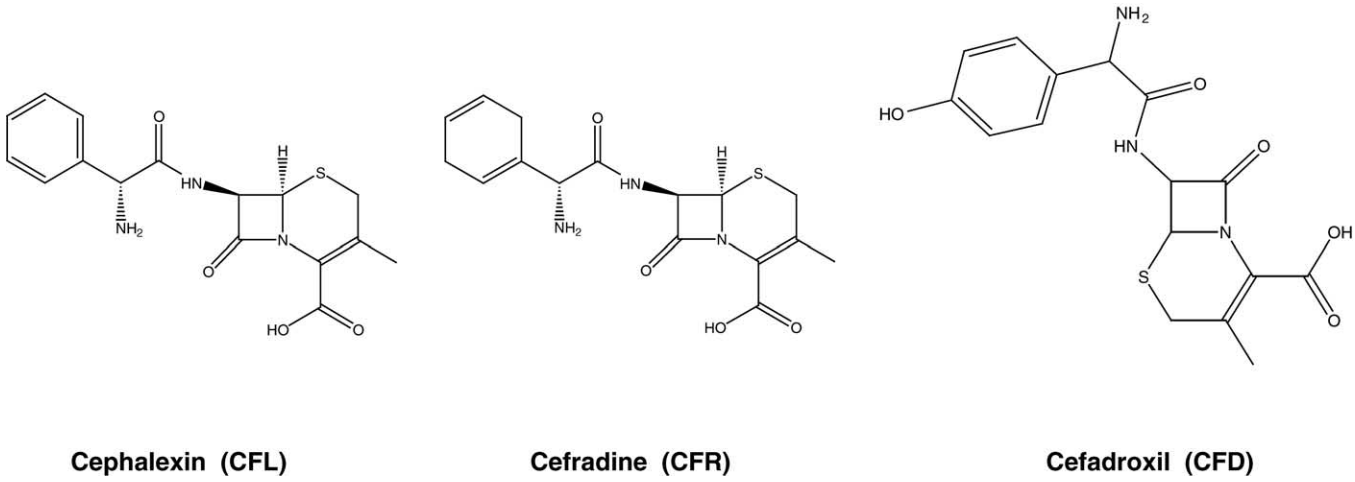

Fig. 1. α-Aminocephalosporin antibiotics investigated in this work.

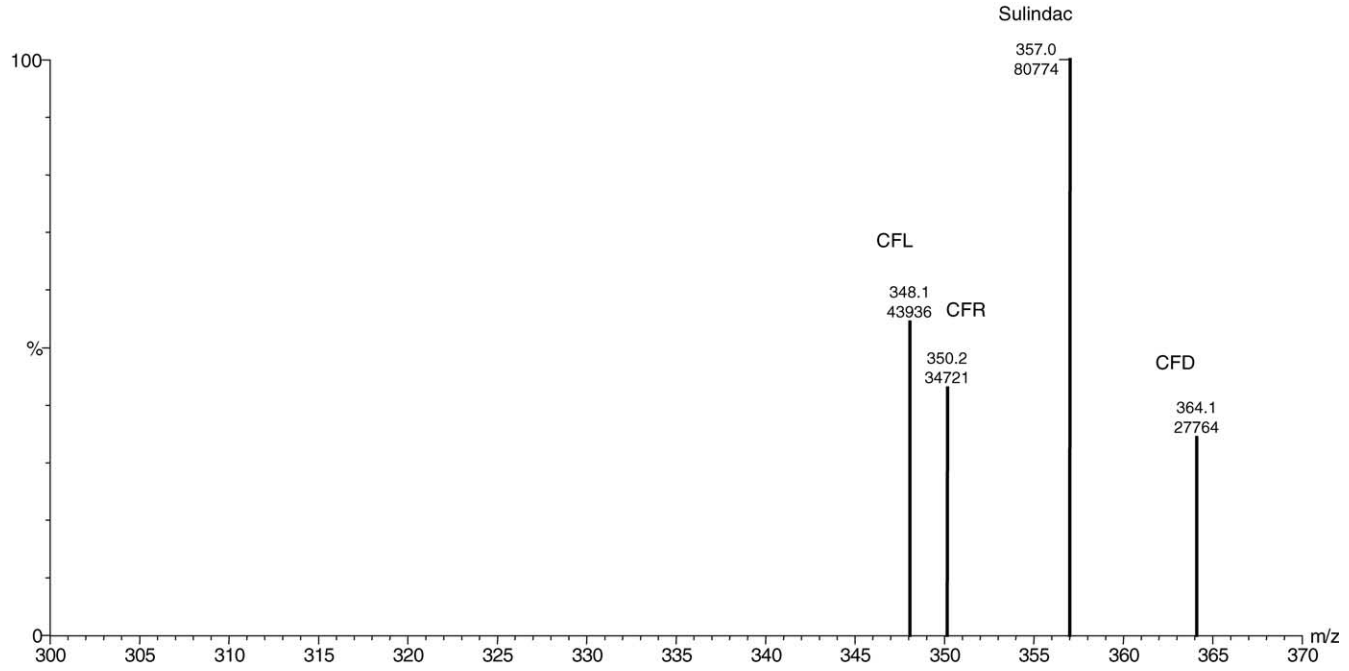

Fig. 2. Selected ion recording (SIR) mass spectrum of MISPE–PE for CFL, CFR and CFD (with 1% TFA + CH_3OH as PE solvent, containing 20 μg/ml of sulindac as internal standard).

positive electrospray mode was more appropriate. MISPE was performed with a standard mixture containing 20 μg/ml each of CFL, CFR and CFD. It was followed by PE with 1% TFA + CH_3OH, which afforded a PE efficiency of 99 (±1.8)% [14]. Electrospray MS analysis of the PE eluate exhibited peaks for CFL, CFR and CFD at *m/z* 348, 350 and 364, respectively.

3.3.2. Internal standards

Previous MISPE–DPE–FPE using UV detection for serum analysis was found to be unsatisfactory, in terms of sensitivity, due to background interference caused by TFA in the PE solvent [15]. Electrospray MS detection may permit a selective detection of CFL with total elimination of the background interference arising from the TFA. For quantitative analysis, precision could be enhanced by adopting the internal standard method. Conventionally there are two different ways for introducing an internal standard to the analysis procedure [16]. The surrogate introduction method involves addition of the internal standard prior to any procedures (including extraction and purification). This method compensates for signal loss attributed to the sample preparation procedure. In contrast, the volumetric introduction method involves the addition of an internal standard prior to instrumental analysis, particularly to address errors. However, for either method to be effective

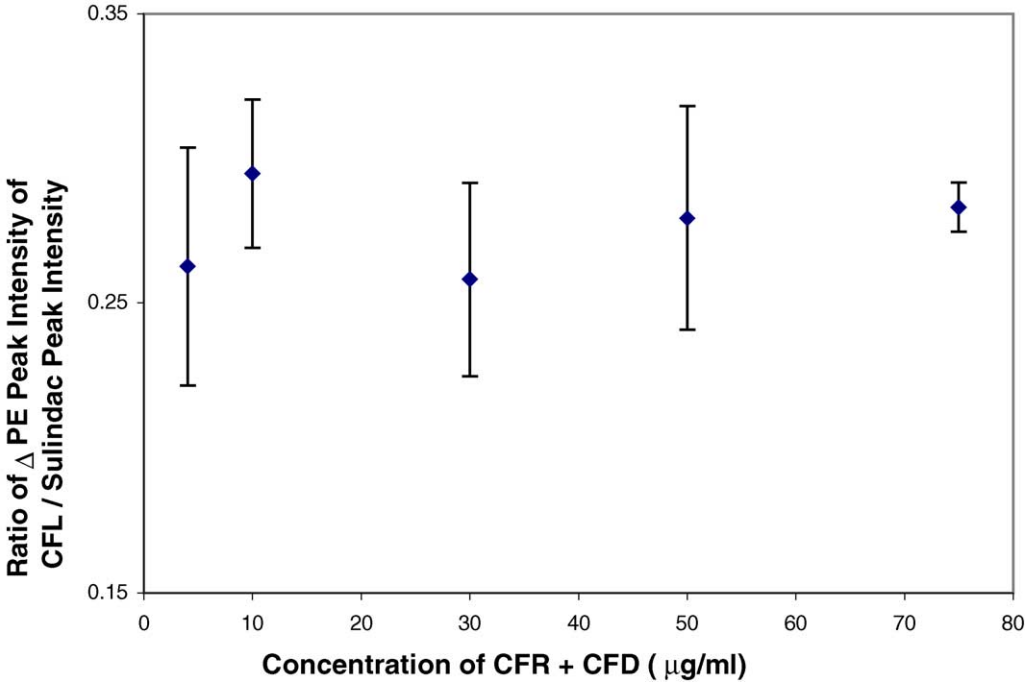

Fig. 3. Investigation of ionization competition between CFL and structural analogues (CFD and CFR) of varying concentrations.

the analytes and the internal standard must be introduced to the MS detector simultaneously. This can hardly be realized in the MISPE–PE procedure because the internal standard would not be extracted by the MIP micro-column.

By mixing the internal standard with the PE solvent, the extracted analytes and internal standard (sulindac) would be eluted and introduced to the MS detector simultaneously. Application of sulindac as an internal standard had previously been reported in LC–MS determination of celecoxib in plasma [17]. Sulindac proved to be a good candidate for LC–MS analysis, as it could be detected and quantified at considerably low concentrations at *m/z* 357. Our investigations also found that sulindac was very stable in the PE solvent. The selected ion recording (SIR) mode was employed for effectively improving the detection limit. Four major *m/z* channels were set in the SIR mode: 348 (CFL), 350 (CFR), 357 (sulindac) and 364 (CFD), for monitoring the PE of these α-aminocephalosporins simultaneously (Fig. 2).

3.3.3. Ionization competition between CFL and structural analogues

One major concern was about the ionization competition between CFL and its structural analogues, which would affect the accurate MISPE–PE–MS quantification of CFL in the presence of CFR and CFD. This was investigated by performing MISPE with 20 μg/ml CFL standard solution ($CHCl_3$). Afterwards, PE was performed by injecting 1% TFA + 20 μg/ml sulindac + CH_3OH containing CFR and CFD at concentrations ranging from 4 to 75 μg/ml, individually. As can be observed in Fig. 3, a fairly constant ratio value of ΔPE peak area intensities of CFL to sulindac was obtained, independent of CFR and CFD concentrations. These results demonstrated that there was no significant ionization competition from CFR and CFD.

3.4. Serum analysis

3.4.1. Linearity of standard calibration curve

The determination of CFL in human serum was carried out by the volumetric introduction method (using sulindac as the internal standard). The standard calibration curve of MISPE–PE–MS for CFL serum sample analysis was constructed on the same day as for serum sample analysis. A good linear range (R^2 = 0.9968) was confirmed within the concentration range from 0.2 to 25 μg/ml (or 5–500 ng of CFL), which covers the therapeutic plasma levels of 5–25 μg/ml [18].

3.4.2. Accuracy and recovery of the method

After extraction with the C18 SPE cartridge, human serum samples containing CFL in the concentration range of 13.5–25 μg/ml, each spiked with CFR at a concentration of 60.5 μg/ml and CFD at a concentration of 31.6 μg/ml, were analyzed using electrospray MS detector. An electrospray MS chromatogram was displayed in Fig. 4. After MISPE, PE was performed three times using 1% TFA + CH_3OH, containing 20 μg/ml of sulindac (internal standard). Selected ion recording mode (SIR) allowed a separate detection of cephalosporins and sulindac at *m/z* 348 (CFL), 350 (CFR), 357 (sulindac) and 364 (CFD), in each PE step. The CFL concentration was determined from a standard calibration curve, and a 93 ± 1% recovery of CFL was achieved. This confirmed the suitability of MISPE–PE–MS for the determination of

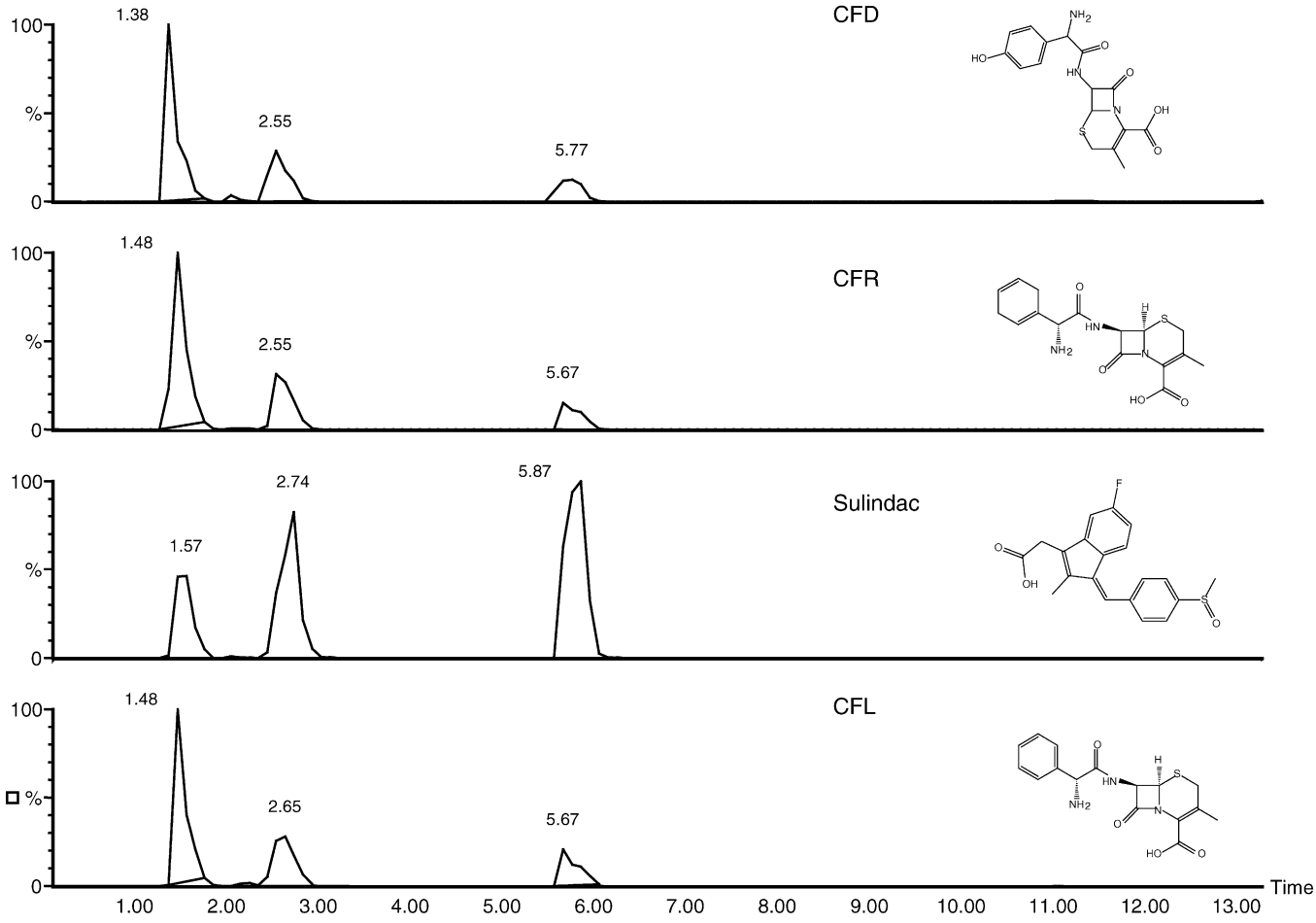

Fig. 4. MISPE–PE–MS for the determination of CFL and structural analogues (CFD and CFR) in a serum sample. Each figure corresponds to the chromatogram of the target *m/z* for each compound from a single experiment involving three pulsed elution steps.

Table 2
Comparison of MISPE–PE–MS with MISPE–PE–UV

Method for serum analysis	Linear range mass (ng) concentration (μg/ml)	Regression coefficient (R^2)	LOD (ng) (μg/ml)	LOQ (ng) (μg/ml)	Solvent consumption (ml/min)	Analysis time (min)	Percentage recovery
MISPE–DPE–FPE–UV							
$CHCl_3$ as mobile phase, 14% CH_3COOH + CH_3CN as DPE solvent, and 1% TFA + CH_3OH as FPE solvent	20–530, 0.8–27	0.9884	5, 0.3	17, 0.9	0.5	5–6	105 ± 2
MISPE–PE–MS							
$CHCl_3$ as mobile phase, and 1% TFA + 20 μg/ml sulindac + CH_3OH as PE solvent containing internal standard	5–500, 0.3–25	0.9968	0.8, 0.04	2.6, 0.1	0.05	3–4	93 ± 1

CFL in serum samples that might contain CFR and CFD (Table 2).

3.4.3. Limits of detection and quantification

The LOD and LOQ for CFL in serum were determined by analyzing serum samples spiked with CFL at relatively low concentrations of CFL (0.25–25 μg/ml) (or 5–500 ng of CFL) using the developed MISPE–PE–MS method. The achieved LOD for CFL (expressed as 3 × standard deviation of the blank) in serum was 0.04 μg/ml (or 0.8 ng of CFL). The achieved LOQ for CFL in serum was 0.13 μg/ml (or 2.6 ng of CFL) (expressed as 10 × standard deviation of the blank) (Table 2).

3.4.4. MISPE–PE–MS analysis time

When coupled with the UV detector, MISPE must employ a DPE step to eliminate the co-extracted structural analogues, before the FPE step for CFL quantification. The DPE step, although proves to be successful in eliminating structural analogues, increases the total analysis time. Under the mobile phase flow rate of 0.5 ml/min, a single MISPE–DPE–FPE–UV analysis took 5–6 min for each run. The MS spectrometer easily distinguished CFL, CFR and CFD simultaneously, without any spectral overlap. Thus, the DPE step could be eliminated. Method development with any MIP micro-column for PE–MS analysis became straightforward and simple. The analysis time of each MISPE–PE–MS, at a mobile phase flow rate of 0.05 ml/min, was as short as 3–4 min (Table 2).

4. Conclusion

The molecular recognition properties of cephalexin-MIP particles were evaluated in the present work. The binding results confirmed a high selectivity for cephalexin. A micro-column packed with these particles was used for the fast, quantitative extraction of cephalexin from a single injection of sample solution. The micro-column proved to be stable and robust, with an unbeatable lifetime in chloroform up to thousands of MISPE analyses, which is promising for high-throughput screening. However, selectivity toward α-aminocephalosporins turns out to be a challenge, due to the fact that the recognition sites could not 100% differentiate between structural analogues. Removal of cefradine and cefadroxil would require an intermediate DPE step. However, the involvement of DPE step extends the analysis time, and cause variations of result.

In the specific determination of cephalexin and other α-aminocephalosporins found in combinatorial drug libraries, the use of MS detection was ideal. Each α-aminocephalosporin has a characteristic molecular mass for unequivocal peak identification in the mass spectrum during PE. By applying the internal standard method, with sulindac mixed with the PE solvent, precise quantification of eluted CFL in serum was achieved. The total analysis time of each MISPE–PE–MS run was as short as 3 min. The achieved LOD and LOQ for human serum sample analysis were as low as 0.04 μg/ml (or 0.8 ng) and 0.13 μg/ml (or 2.6 ng) of CFL, respectively. The linear dynamic range from 0.3 to 25 μg/ml (or 5–500 ng) of CFL, which fully covers the therapeutic plasma cephalexin level.

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