Journal of Chromatography B, 895–896 (2012) 83–88

Contents lists available at SciVerse ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Development and validation of a rapid HPLC method for the determination of cefdinir in beagle dog plasma integrated with an automatic on-line solid-phase extraction following protein precipitation in the 96-well plate format

Ji Li[a,b,1], Li Wang[a,b,c,1], Zhao Chen[a,b], Rui Xie[a,b], You Li[a,b,d], Taijun Hang[c,**], Guorong Fan[a,b,*]

[a] Department of Pharmaceutical Analysis, School of Pharmacy, Second Military Medical University, No. 325 Guohe Road, Shanghai 200433, PR China
[b] Shanghai Key Laboratory for Pharmaceutical Metabolite Research, No. 325 Guohe Road, Shanghai 200433, PR China
[c] Department of Pharmaceutical Analysis, China Pharmaceutical University, Nanjing 210009, PR China
[d] College of Food Science & Technology, Shanghai Ocean University, No. 999 Hucheng Huan Road, Shanghai 201306, China

ARTICLE INFO

Article history:
Received 16 November 2011
Accepted 10 March 2012
Available online 20 March 2012

Keywords:
Cefdinir
On-line solid-phase extraction
HPLC
Pharmacokinetic study
96-Well protein precipitation

ABSTRACT

The high-performance liquid chromatography (HPLC) coupled with on-line solid phase extraction (SPE) and ultraviolet (UV) detection was developed for determining cefdinir in beagle dog plasma. After simple pretreatment for plasma with 6% perchloric acid, a volume of 100 μL upper layer of the plasma sample was injected into the self-made on-line SPE column. The analytes were retained on the trap column (Lichrospher C_{18}, 4.6 mm × 37 mm, 25 μm), and the biological matrix was washed out with the solvent (20 mM KH_2PO_4 adjusted pH 3.0) at flow rate of 2 mL/min. By rotation of the switching valve, the target analytes could be eluted from trap column to analytical column in the back-flush mode by the mobile phase (methanol–acetonitrile–20 mM KH_2PO_4 adjusted pH 3.0, 11.25:6.75:82, v/v/v) at flow rate of 1.5 mL/min, and then separated on the analytical column (Ultimate™ XB-C_{18}, 4.6 mm × 50 mm, 5 μm). The complete cycle of the on-line SPE preconcentration, purification and HPLC separation of the analytes was 4 min. The UV detection was performed at 286 nm. The calibration curves showed excellent linear relationship (R^2 = 0.9995) over the concentration range of 0.05–50 μg/mL. The optimized method showed good performance in terms of specificity, linearity, detection and quantification limits, precision and accuracy. This method was successfully applied to quantify cefdinir in beagle dog plasma to support the pre-clinical pharmacokinetic trial.

1. Introduction

Cefdinir (6R-[6α,7β(Z)]-7-[[(2-amino-4-thiazolyl)(hydroxyimino)acetyl]amino]-3-ethenyl-8-oxo-5-thia-1-azabicyclo[4.2.0] oct-2-ene-2-carboxylic acid) (shown in Fig. 1) first synthesized in 1988 [1], is an extended-spectrum, third-generation cephalosporin antibiotic for oral administration used in the treatment of mild-to-moderate bacterial infections. This compound, comparable in structure and in vitro activity to the oral agent cefixime, offers enhanced activity against methicillin-sensitive *Staphylococcus aureus* and *Staphylococcus epidermidis* as well as effective antimicrobial activity against strains of *Streptococcus* and *Neisseria* spp [2–9]. This can be attributed to its oximino side chain substituting the carboxymethoxyimino moiety (present in most orally active cephalosporins) at the 7-position, which sterically hinders the entry of the molecule into the active site of TEM-1β-lactamase [10]. Cefdinir is used for the treatment of community-acquired pneumonia, acute exacerbations of chronic bronchitis, acute maxillary sinusitis, pharyngotonsillitis, and uncomplicated skin and skin-structure infections in adults and adolescents; it is indicated for acute otitis media, uncomplicated skin and skin-structure infections, and pharyngotonsillitis in children [11].

Common separation methods had been reported for the determination of cefdinir in biological samples based on high performance liquid chromatography with UV [9,12–14] and tandem mass spectrometric detection [15] and microbiological assay [16]. Because of the hydrophilicity of cefdinir, protein precipitation (PP) is the major pretreatment method in the determination of cefdinir plasma sample by liquid chromatography. However, PP could not provide sufficient clean-up and sensitivity for the biological samples by LC-UV.

* Corresponding author at: School of Pharmacy, Second Military Medical University, No. 325 Guohe Road, Shanghai 200433, PR China.
Tel.: +86 21 8187 1260; fax: +86 21 8187 1260.
** Corresponding author at: Department of Pharmaceutical Analysis, China Pharmaceutical University, No. 24 Tongjiaxiang, Nanjing 210009, PR China.
Tel.: +86 25 8327 1090.
E-mail addresses: hangtj@cpu.edu.cn (T. Hang), guorfan@yahoo.com.cn (G. Fan).
[1] These authors contributed equally to this work.

1570-0232/$ – see front matter © 2012 Published by Elsevier B.V.
doi:10.1016/j.jchromb.2012.03.018

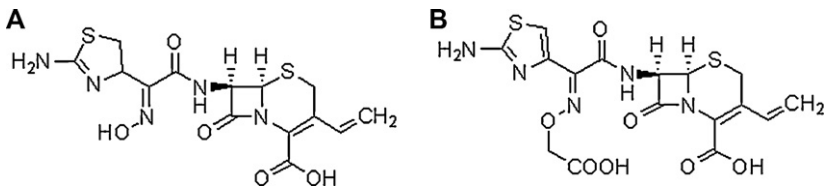

Fig. 1. Chemical structures of cefdinir (A) and the IS (B).

In developing an analytical method for pharmacokinetic studies, not only a suitable sensitivity needs to be achieved but also economy and labor-saving factors must be considered [17]. Although LC–MS/MS method can provide excellent sensitivity and selectivity, the apparatus is expensive, and the matrix effects are difficult to overcome especially when PP is used as sample pretreatment. Microbiological assay had poor reproducibility, accuracy and correlation. So a method meets high-throughput analysis together with economic, pragmatic, volant and convenient needs should be established in order to determine cefdinir in biological samples.

The preparation of biological samples for HPLC analysis, typically by liquid–liquid extraction (LLE) and solid phase extraction (SPE), can be labor-intensive and time-consuming [17,18]. A direct injection technique which is an important strategy can avoid complicate sample pre-treatment steps and increase the throughput of many bioanalytical methods. The on-line SPE procedure combined with HPLC analysis has been employed attributed to its simple sample preparation, short analysis time and low-cost in the 1980s [17–33]. However, up to date, there is no report on the determination of cefdinir using HPLC-UV coupled with on-line SPE.

This study presents an efficient on-line technique by coupling SPE with HPLC for determination of cefdinir in beagle dog plasma, which combined simplicity of self-made trap column with rapidity of short analytical column. The method was evaluated in terms of selectivity, sensitivity, linearity, accuracy, precision and stability in accordance to the recommendations published by the FDA [34], and when combined with 96-well format protein precipitation, it was successfully applied to the pharmacokinetic studies of cefdinir capsules in beagle dogs.

2. Experimental

2.1. Chemicals and reagents

Cefdinir capsules and cefdinir standard reference material (95.4% purity) were supplied by Guangzhou Baiyunshan Guanghua Parmceutical Co. Ltd. (Guangzhou, China). Cefixime (internal standard, IS, 99.0% purity) (shown in Fig. 1) was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Methanol and acetonitrile of HPLC grade were obtained from Caledon Laboratories Ltd. (Georgetown, Ont., Canada). Perchloric acid and phosphoric acid of HPLC grade were purchased from Tedia Company (Tedia Fairfield, OH, USA). Other chemicals were all of analytical grade. Deionized (18.2 MΩ/cm) water was obtained by means of a Milli-Q apparatus from Millipore (Bedford, MA, USA).

2.2. Apparatus

The HPLC system consisted of a Shimadzu system equipped with two LC-20 AD pumps, an LC-10 AD pump, an SIL-20 A autosampler, a CTO-20 AC column oven, an LV-306 R automatic high-pressure switching valve, an SPD-20 A UV–vis detector for the second column and a DGU-20 A3 degasser. Shimadzu LC-solution software was used for data acquisition and mathematical calculations. 96-Well plate refrigerated centrifuge (Model SC210A, Thermo Electron, USA) was also used. The Sirocco^{TM} 96-well plates were purchased from Waters Corporation (Milford, USA).

2.3. Liquid chromatographic conditions

Separation was performed on a 50 mm × 4.6 mm, 5 μm, Ultimate^{TM} XB-C_{18} column (Welch Materials, USA) with a 37 mm × 4.6 mm, 25 μm, Lichrospher C_{18} trap column (self-made). The trap column and analytical column temperature of 30 °C was maintained. The wavelength of the detection was at 286 nm. The loading solvent (Pump A) was 20 mM KH_2PO_4 buffer (pH 3.0) at the flow rate of 2.0 mL/min. The mobile phase (Pumps A and B) was methanol:acetonitrile:20 mM KH_2PO_4 (pH 3.0) (11.25:6.75:82; v/v/v) at the flow rate of 1.5 mL/min. The complete cycle time (extraction, elution, injection, analysis) was 4.0 min.

2.4. Preparation of stock solutions, calibration samples and quality control (QC) samples

Both stock solutions of cefdinir and the IS were prepared in methanol at concentrations of 1 mg/mL. The cefdinir stock solution was diluted with 40% methanol to working solutions ranging from 0.5 to 500 μg/mL. A 5 μg/mL IS working solution was obtained by diluting the stock solution of IS with 40% methanol. All described solutions were protected from light, stored at 4 °C.

Calibration samples were obtained by diluting standard working solutions (10 μL) with drug-free dog control plasma (90 μL), to span a calibration standard range of 0.05–50 μg/mL (0.05, 0.1, 0.5, 1, 5, 10, 20 and 50 μg/mL). Quality control (QC) samples (0.1, 2, 40 μg/mL) were prepared in a similar way.

2.5. Sample preparation

Samples were prepared using protein precipitation in 96-well format plate. An eight-channel 100 μL pipetting tool (Eppendorf Research®, Eppendorf AG, Hamburg, Germany) was used for liquid transfer steps. 100 μL of subject plasma samples, standard curve samples and QC samples were added individually into a 96-well plate spiked by an eight-channel pipetting tool. Using an eight-channel 300 μL pipetting tool, 75 μL of 6% perchloric acid and 25 μL 5 μg/mL of IS were added to each sample (standards, QCs and subject samples) in order to precipitate the plasma protein. Plates were capped and mixed by vortex for 5 min and then subjected to centrifuge at 12,000 rpm for 10 min at 4 °C to remove any precipitated material. The supernatant was transferred to another 96-well plate, this 96-well plate was covered and sent to autosampler and 100 μL of the mixture was injected into the HPLC system.

2.6. On-line SPE procedure

The automated column switching HPLC system (Fig. 2) was operated according to the following procedure, where valve positions and switchover times are in parentheses: step 1 (valve 1; 0–0.2 min): a plasma sample was injected onto the trap column and the trap column was washed by loading solvent at a flow rate of 2 mL/min in order to remove endogenous interferences and enrich

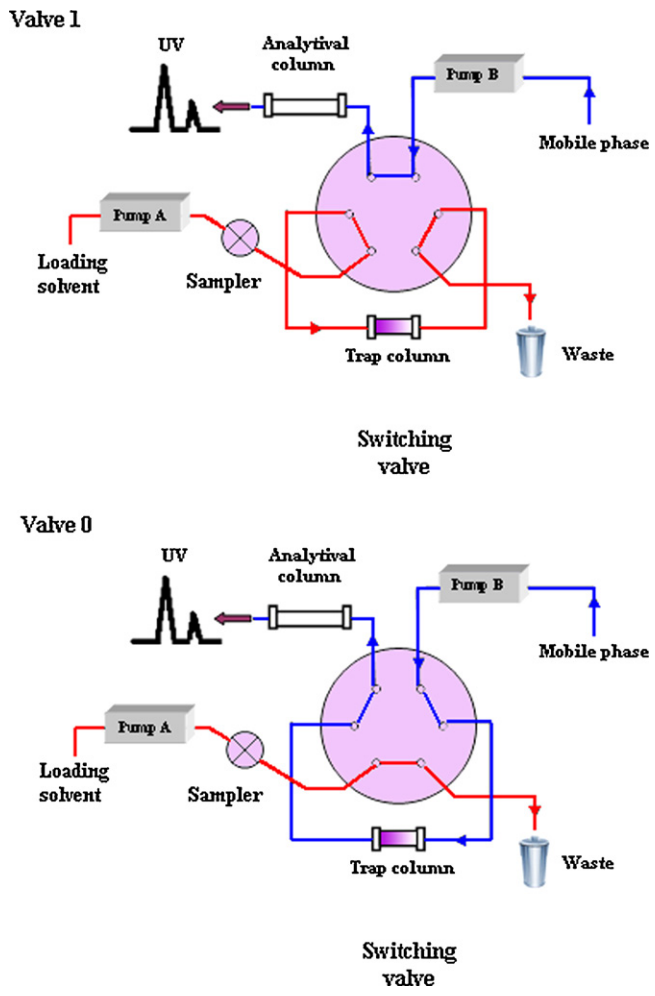

Fig. 2. Schematic diagram of the on-line SPE system using a six-port switching valve. Step 1: valve 1, step 2: valve 0, step 3: valve 1.

the interesting compounds. Data acquisition started in detector. Step 2 (valve 0; 0.2–2.5 min): the valve was switched from position 1 to 0 and the enriched compounds were eluted from trap column to analytical column in the back-flush mode for separating the analytes with the mobile phase. Step 3 (valve 1; 2.5–4 min): the valve was returned to the initial position, the trap column was equilibrated again with the loading solution for the next analysis and the analytical column was continuously eluted with the mobile phase until the end of this analytical procedure.

2.7. Pharmacokinetic study in beagle dogs

Six healthy beagle dogs (9.0–10.0 kg) were purchased from the Experimental Animal Center of Second Military Medical University. Animal welfare and experimental procedures were strictly in accordance with the guide for the care and use of laboratory animals and the related ethical regulations of Second Military Medical University. After being fasted overnight, each dog ($n = 6$) was administrated with 100 mg of cefdinir capsule. Animal had access to water and food 4 h after drug administration. About 0.5 mL of blood samples were collected into heparinized tubes before administration (0 h) and post-dose at 0.5, 1, 1.5, 2, 2.5, 3, 5, 8, 10, 12, 24 and 36 h. The blood samples were separated by centrifugation at 3000 rpm for 10 min and were stored at −20 °C until analysis.

3. Results and discussion

3.1. HPLC-UV conditions

3.1.1. Selection of analytical column

There were two reasons to choose a short column as an analytical column. Firstly, short column could get rapid separation and high-though put. Secondly, due to the high speed (1.5 mL/min) and series connection of the trap column and analytical column, a short column could get a suitable pressure.

In our early study, several kinds of columns were compared, such as Ultimate™ XB-C_{18} column (4.6 mm × 50 mm, 5 μm), Diamonsil C_{18} column (4.6 mm × 50 mm, 5 μm), Lichrospher C_{18} column (4.6 mm × 50 mm, 5 μm), Agilent ZORBAX SB-C_{18} column (4.6 mm × 50 mm, 3.5 μm) and Waters Symmetry C_{18} column (4.6 mm × 50 mm, 5 μm). Tailing of peaks occurred when Diamonsil C_{18} column (4.6 mm × 50 mm, 5 μm) was used. Lichrospher C_{18} column (4.6 mm × 50 mm, 5 μm) could get better peak shape, but the analytes could not be separated from endogenous interference due to the short retention time of the analytes. Although Agilent ZORBAX SB-C_{18} column (4.6 mm × 50 mm, 3.5 μm) and Waters Symmetry C_{18} column (4.6 mm × 50 mm, 5 μm) had no shortages mentioned above, when either of them was connected with the trap column in series, the pressure was too high (≥2000 psi) to keep the system stable. For its acceptable performance and back pressure (≤1500 psi), an Ultimate™ XB-C_{18} column (4.6 mm × 50 mm, 5 μm) was selected.

3.1.2. Composition of mobile phase

It is important to optimize the composition of mobile phase to achieve good resolution, symmetric peak shapes and short running time for both analyte and IS different mobile phase combinations of phosphate buffer, methanol and acetonitrile and their percentage were considered. As shown in Fig. 3, cefdinir was eluted between two interferences. When methanol or acetonitrile alone was used as organic phase, cefdinir could not be separated from them. It was found that when the organic phase contained methanol and acetonitrile (5:3; v/v), analyte, IS and endogenous interference could obtain satisfactory separation. In addition, as the concentration of organic phase increased, the retention time of the analytes was shorter and the resolution was worse. When the organic phase concentration decreased, the analytical time was longer. In this work, using the mixture (organic solvent:water, 18:82; v/v) as mobile phase could get better resolution and running time. Therefore, methanol–acetonitrile–20 mM KH_2PO_4 (pH 3.0) (11.25:6.75:82; v/v/v) was selected as mobile phase.

3.2. Sample preparation

The extraction of plasma samples was optimized in our preliminary studies by comparing liquid–liquid extraction, solid-phase extraction and protein precipitation. Neither protein precipitation nor liquid–liquid extraction is suitable for cefdinir extraction from plasma because of lower sensitivity and more interference of endogenous components in the former and tediousness, solvent- and time-consuming in the latter. Although off-line solid-phase extraction can get better sensitivity and introduce less interference, it is time consuming and wasteful. Given the shortcomings mentioned above, the SPE coupled with HPLC method was used to determine cefdinir in dog plasma, which could achieve rapid and high-throughput analysis. In this study, to extend the lifetime of the trap column and avoid the time consuming process which was used to clean the trap column after every injection, samples were simply purified by protein precipitation before injecting onto the trap column. In addition, several protein precipitation reagent including 6% perchloric acid, 10% perchloric acid and acetonitrile were

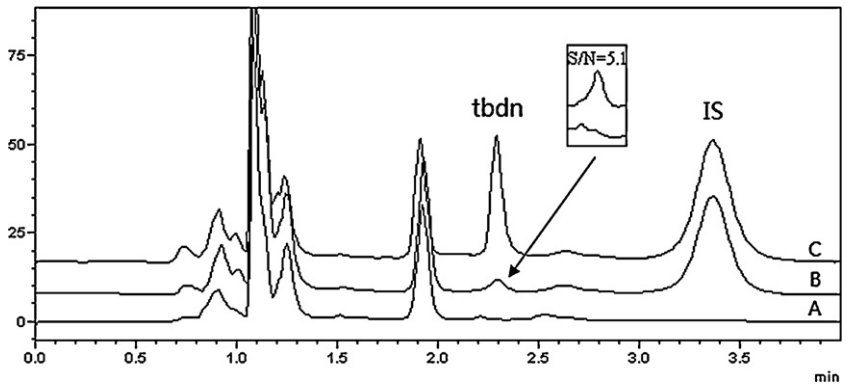

Fig. 3. Representative HPLC chromatograms of the method: (A) blank plasma; (B) blank plasma sample spiked with cefdinir at LLOQ and 5 μg/mL IS; (C) test plasma sample obtained at 1.0 h after the oral dose of 100 mg cefdinir capsules to a beagle dog.

investigated. Comparing to acetonitrile, the analyte peak shape and response were better when perchloric acid (6% or 10%) was used as protein precipitation reagent. In addition, there was no different efficiency between 6% perchloric acid and 10% perchloric acid. Therefore, 6% perchloric acid was selected. In order to increase sample throughput and reduce residual precipitated protein, the new Sirocco™ 96-well plates from Waters Corporation were used, resulting in a shorter sample preparation time and a longer trap column lifetime.

3.3. On-line SPE procedure development

3.3.1. Selection of trap column

In our experiment, it is necessary to select the suitable trap column which could maintain low pressure and good reproducibility under high speed of the loading solution (2 mL/min) and eluent (1.5 mL/min). Huang et al. [23] and Chang et al. [33] used Strata-X column (20 mm × 2.0 mm, 25 μm, Phenomenex Inc., Torrance, CA, USA) and Oasis HLB cartridge column (1 mm × 50 mm, 25 μm, Waters, Milford, USA), two of the common commercial on-line SPE columns, respectively. However, the former one was inappropriate in our experiment, because the small inner diameter and high speed of the loading solution and eluent induced higher pressure and the tailing peaks of cefdinir and IS were observed. In addition, it is designed to analyze neutral and aromatics. Although the latter one can be replaced by a 4.6 mm × 20 mm Oasis HLB cartridge column, which had no problem mentioned above, it was more expensive than the Lichrospher C_{18} column (4.6 mm × 37 mm, 25 μm) packed in our laboratory. This self-made column had many advantages: it permitted large injection volume (≥100 μL), so that the analytes could not only be purified but also be preconcentrated; it permitted high speed of the loading solvent which was more effective to wash the endogenous compounds and reduce the analytical time; and it was cheap and durable. Furthermore, most of endogenous interference was removed by using trap column, which enhanced the sensitivity of the analyte (LLOQ from 20 dropped to 0.05 μg/mL).

3.3.2. Selection of loading solvent

At the beginning of the loading solvent optimization, pure water was selected, but almost no cefdinir or IS peaks was observed in chromatogram, which indicated that the analytes were ionized in water and were washed together with the endogenous components. Therefore, an acid solution should be used as loading solution to keep the analytes as molecular state that could be retained on the SPE column. And we selected phosphate buffer as loading solution. In order to optimize the purification and concentration of analytes in the trap column, phosphate buffer at different pH (2.5, 3.0, 3.5 and 4.0) was tested. The response of the analytes was lower at pH 3.5 and 4.0 than the other two. And there was no significant difference between pH 2.5 and 3. So we chose a phosphate buffer with a pH of 3.0.

3.3.3. The optimization of switching time

Switching time included two sections (transferring time and resetting time). A suitable transferring time could obtain high recovery of the analyte and IS and limit the transfer of unwanted interfering compounds from the trap column to the analytical column. Different transferring times (0.1, 0.2 and 0.3 min) were studied, and it was found that 0.2 min had highest response and least endogenous interferences in chromatogram. Furthermore, the resetting time (2.0, 2.5 and 3.0 min) was also investigated. The response of cefdinir and IS decreased 25% when resetting time changed from 2.5 min to 2.0 min. However, there was no different response when the resetting time was 2.5 or 3.0 min, which indicated that 2.5 min was sufficient to transfer the analytes from trap column to analytical column. And because the analyzing time was 4.0 min, the resting 1.5 min, was enough to recover the condition of trap column. Therefore, the ideal resetting time was 2.5 min for the assay.

3.4. Method validation

In the present study, on-line SPE with HPLC-UV method was considered to be a preferred technique due to its sensitivity, speed and selectivity. And a full validation was performed in accordance to the recommendations published by FDA [34].

3.4.1. Specificity

Specificity is described as the ability of a method to discriminate the analyte from all potentially interfering substance. Specificity of the method was investigated by blank plasma detection, peak purity and spiking experiments with pure standard compounds. To test the specificity, six blank beagle dog plasma samples and the corresponding spiked plasma samples were compared. As shown in Fig. 3, there was no significant interference from endogenous substances observed at the retention times of the analytes. Typical retention times for cefdinir and cefixime were 2.3 and 3.4 min, respectively.

3.4.2. Sensitivity and linearity

The LLOQs for cefdinir were 0.05 μg/mL in plasma with a precision of 6.83%, which was sufficient for preclinical pharmacokinetic studies.

The linearity of an analytical procedure is its ability to obtain test results which are directly proportional to the concentration of analyte in the sample. A weighted ($1/x$) linear regression was used

Table 1
Accuracy and precision for cefdinir in spiked plasma samples ($n = 5$).

QC sample	Nominal concentration (μg/mL)	Mean measured concentration (μg/mL)	Accuracy (%)	Precision (RSD %)
Within-run accuracy and precision run I ($n = 5$)				
LQC	0.1	0.109	109.00	2.79
MQC	2	2.038	101.90	2.45
HQC	40	43.454	108.64	2.31
Within-run accuracy and precision run II ($n = 5$)				
LQC	0.1	0.103	103.00	9.33
MQC	2	1.886	94.30	1.50
HQC	40	41.145	102.86	2.74
Within-run accuracy and precision run III ($n = 5$)				
LQC	0.1	0.100	100.00	5.62
MQC	2	2.000	100.00	0.90
HQC	40	41.095	102.74	1.34
Between-run accuracy and precision ($n = 15$)				
LQC	0.1	0.104	104.00	7.04
MQC	2	1.975	98.75	3.75
HQC	40	41.898	104.74	3.40

Table 2
Recoveries of cefdinir and I.S. in spiked plasma samples ($n = 5$).

Compound	Concentration (μg/mL)	Recovery (%) (mean ± SD)	RSD (%)
Cefdinir	0.1	73.59 ± 5.32	7.23
	2	75.20 ± 4.18	5.56
	40	76.67 ± 3.80	4.96
I.S.	5	74.97 ± 5.22	6.96

to perform standard calibrations. The mean calibration equations were $y = 0.5851x + 0.02236$ ($R^2 = 0.9995$), where y represented the peak area ratios of the analyte to the IS and x represented the plasma concentration of analyte in μg/mL. Calibration curves were linear in the range 0.05–50 μg/mL.

3.4.3. Accuracy, precision and extraction recovery

Five replicate samples at each QC concentrations were analyzed in three separate runs. Accuracy was determined by calculating the ratios of the predicted concentrations to the spiked values and with the precision expressed as RSD. The results in Table 1 show that the within- and between-run relative standard deviations at three QC levels were all below 9.33%. It was shown that the accuracy was from 94.30% to 109.00%. The extraction recovery of analytes in our experiment was the response of the analytes added to and extracted from plasma, compared to that of the true concentration of the pure authentic standard. And it (Table 2) was found to be 73.59 ± 5.32%, 75.20 ± 4.18% and 76.67 ± 3.80% at the concentration of 0.1, 2 and 40 μg/mL, respectively. The extraction recovery of IS was 74.97 ± 5.22%.

3.4.4. Stability

Analyte stability determinations comprised short-term temperature stability, long-term stability, autosampler stability and freeze–thaw cycles stability, which were evaluated by analyzing three QC levels in triple. The QC samples were analyzed after storage at room temperature for 2 h, at −20 °C for 20 days, in the autosampler at room temperature for 8 h after sample preparation and after three freeze–thaw cycles, which consisted of storage at −20 °C for a minimum of 12 h followed by thawing at room temperature. Cefdinir primary stock solution (1 mg/mL in methanol) was stable for at least 20 days (data not shown) at −20 °C. The stability study results of cefdinir under various conditions were summarized in Table 3. It was demonstrated that cefdinir was stable under various conditions.

3.5. Application of the assay

The method described above has successfully been applied to analyze plasma samples obtained from 6 healthy beagle dogs which received single doses of 100 mg cefdinir capsules. The mean plasma concentration–time profiles for cefdinir after oral administration of 100 mg cefdinir capsules was presented in Fig. 4. Cefdinir could be detected at all time points over the duration. Pharmacokinetic parameters were determined by non-compartment analysis method. The elimination half-life ($t_{1/2}$) was 4.04 ± 0.18 h.

Table 3
Stability results of cefdinir in spiked plasma samples ($n = 3$).

Sample condition	Nominal concentration (μg/mL)	Mean determined concentration (μg/mL)	RSD (%)	Accuracy (%)
Short-term stability (2 h at room temperature)	0.1	0.108	5.75	108.00
	2	1.923	0.34	96.15
	40	39.198	6.61	98.00
long-term stability (20 days at −20°C)	0.1	0.103	2.01	103.00
	2	2.041	1.44	102.05
	40	40.455	0.94	101.14
Autosampler stability (8 h at room temperature)	0.1	0.097	6.20	97.00
	2	1.943	1.03	97.15
	40	42.554	2.00	106.38
Freeze–thaw stability (3 cycles)	0.1	0.099	2.36	99.00
	2	1.987	4.65	99.35
	40	39.978	1.01	99.94

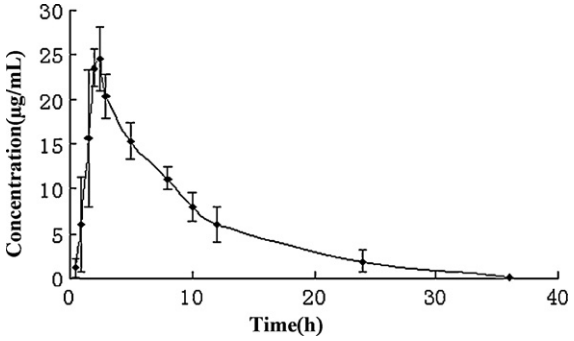

Fig. 4. Mean drug plasma concentration–time curves of cefdinir after single oral dose of 100 mg.

The AUC_{0-24} and $AUC_{0-\infty}$ values were 207.25 ± 33.85 h μg/mL and 207.74 ± 33.92 h μg/mL, respectively.

4. Conclusion

A rapid, sensitive and specific on line SPE-HPLC method has been developed for the determination of cefdinir in beagle dog plasma. The adequate selectivity, sensitivity, precision, accuracy and economy make it suitable for high-throughput pharmacokinetic study. In addition, in our experiment, on line SPE combined with simple sample pretreatment, 96-well protein precipitation, could provide sufficient clean-up of the biological samples prior to analysis and shorten sample preparation time. Finally, the method has been successfully applied to the pharmacokinetic study of cefdinir in beagle dogs and is also potentially helpful for the determination of other antimicrobial agents.

Acknowledgments

This work was supported by Platform on Research of Metabolism Technology of Traditional Chinese Medicine founded by Science & Technology Department of Shanghai, PR China (04DZ19216) and the National Science & Technology Major Special Project for "Major New Drugs Innovation and Development" of China (2009ZX09301-011).

References

[1] Y. Inamoto, T. Chiba, T. Kamimura, T. Takaya, J. Antibiot. 41 (1988) 828.
[2] B.M. Briggs, R.N. Jones, M.E. Erwin, M.S. Barrett, D.M. Johnson, Diagn. Microbiol. Infect. Dis. 14 (1991) 425.
[3] E.H. Gerlach, R.N. Jones, S.D. Allen, F.P. Koontz, P.R. Murray, M.A. Pfaller, J.A. Washington, M.E. Erwin, Diagn. Microbiol. Infect. Dis. 15 (1992) 537.
[4] Y. Inamoto, T. Chiba, T. Kamimura, T. Tayaka, J. Antibiot. 41 (1988) 828.
[5] Y. Mine, T. Kamimura, Y. Watanabe, S. Tawara, Y. Matsumoto, F. Shibayama, H. Kikuchi, T. Takaya, J. Antibiot. 41 (1988) 1873.
[6] H.C. Neu, G. Saha, N.X. Chin, Antimicrob. Agents Chemother. 33 (1989) 1795.
[7] S.R. Scriver, B.M. Willey, D.E. Low, A.E. Simor, Eur. J. Clin. Microbiol. Infect. Dis. 11 (1992) 646.
[8] R. Wise, J.M. Andrews, D. Thornber, J. Antimicrob. Chemother. 28 (1991) 239.
[9] M. Richer, S. Allard, L. Manseau, F. Vallee, R. Pak, M. Lebel, Antimicrob. Agents Chemother. 39 (1995) 1082.
[10] K. Sakane, Y. Inamoto, T. Takaya, Jpn. J. Antibiot. 45 (1992) 909.
[11] D.R. Guay, Clin. Ther. 24 (2002) 473.
[12] C.S. Lepsy, R.J. Guttendorf, A.R. Kugler, D.E. Smith, Antimicrob. Agents Chemother. 47 (2003) 689.
[13] Y. Okamoto, K. Itoh, Y. Namiki, J. Matsushita, M. Fujioka, T. Yasuda, J. Pharm. Biomed. Anal. 14 (1996) 739.
[14] A. Hishida, K. Ohishi, S. Nagashima, M. Kanamaru, M. Obara, A. Kitada, Antimicrob. Agents Chemother. 42 (1998) 1718.
[15] Z.J. Chen, J. Zhang, J.C. Yu, G.Y. Cao, X.J. Wu, Y.G. Shi, J. Chromatogr. B 834 (2006) 163.
[16] P.J. Cook, J.M. Andrews, R. Wise, D. Honeybourne, J. Antimicrob. Chemother. 37 (1996) 331.
[17] L. Tasso, T.D. Costa, J. Pharm. Biomed. Anal. 44 (2007) 205.
[18] L. Chen, A. Yu, X. Zhuang, K. Zhang, X. Wang, L. Ding, H. Zhang, Talanta 74 (2007) 146.
[19] R. Xie, J. Wen, H. Wei, G.R. Fan, D.B. Zhang, J. Pharm. Biomed. Anal. 52 (2010) 114.
[20] S.O. Choi, S.Y. Um, S.H. Jung, S.J. Jung, J.I. Kim, H.J. Lee, S.Y. Chung, J. Chromatogr. B 830 (2006) 301.
[21] E. Rozet, R. Morello, F. Lecomte, G.B. Martin, P. Chiap, J. Crommen, K.S. Boos, Ph. Hubert, J. Chromatogr. B 844 (2006) 251.
[22] C.H.P Bruins, C.M Jeronimus-Stratingh, K. Ensing, W.D van Dongen, G.J. de Jong, J. Chromatogr. A 863 (1999) 115.
[23] W.H. Huang, J. Yang, J. Zhao, C.Z. Wang, C.S. Yuan, S.P. Li, J. Pharm. Biomed. Anal. 53 (2010) 906.
[24] D. Teshima, N. Kitagawa, K. Otsubo, K. Makino, Y. Itoh, R. Oishi, J. Chromatogr. B 780 (2002) 21.
[25] J. Huclová, D. Šatínský, T. Maia, R. Karlíček, P. Solich, A.N. Araújo, J. Chromatogr. A 1087 (2005) 245.
[26] Y. Li, J.P. Gao, X. Xu, L.X. Dai, J. Chromatogr. B 838 (2006) 50.
[27] Q.Q. Wang, X.S. Li, S.J. Dai, L. Ou, X. Sun, B.Z. Zhu, F. Chen, M.M. Shang, H.F. Song, J. Chromatogr. B 863 (2008) 55.
[28] C. Estrela Rde, M.C. Salvadori, G. Suarez-Kurtz, Rapid Commun. Mass Spectrom. 18 (2004) 1147.
[29] M. Kenk, M. Greene, M. Lortie, R.A. deKemp, R.S. Beanlands, J.N. DaSilva, Nucl. Med. Biol. 35 (2008) 515.
[30] S. Calderoli, E. Colombo, E. Frigerio, C.A. James, M. Sibum, J. Pharm. Biomed. Anal. 32 (2003) 601.
[31] Y.C. Barrett, B. Akinsanya, S.Y. Chang, O. Vesterqvist, J. Chromatogr. B 821 (2005) 159.
[32] Y. Alnouti, K. Srinivasan, D. Waddell, H. Bi, O. Kavetskaia, A.I. Gusev, J. Chromatogr. A 1080 (2005) 99.
[33] Y.W. Chang, H.T. Yao, S.H. Hsieh, T.J. Lu, T.K. Yeh, J. Chromatogr. B 857 (2007) 164.
[34] Guidance for Industry, Bioanalytical Method Validation, US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Veterinary Medicine (CVM), May 2001, Available from: http://www.fda.gov/cder/guidance/4252.