Journal of Chromatography B, 879 (2011) 2423–2429

Contents lists available at ScienceDirect

Journal of Chromatography B

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

Simultaneous determination of cefdinir and cefixime in human plasma by RP-HPLC/UV detection method: Method development, optimization, validation, and its application to a pharmacokinetic study

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ARTICLE INFO

Article history:
Received 19 May 2011
Accepted 28 June 2011
Available online 6 July 2011

Keywords:
Cefixime
Cefdinir
Reversed-phase high performance liquid-chromatography
Optimization
Validation
Pharmacokinetic study

ABSTRACT

A novel isocratic reversed-phase high performance liquid-chromatography/ultraviolet detection method for simultaneous determination of cefdinir and cefixime in human plasma was developed and validated after optimization of various chromatographic conditions and other experimental parameters. Sample preparation based on a simple extraction procedure consisting of deproteination and extraction with 3 parts of 6% trichloroacetic acid aqueous solution followed by volume make up with the aqueous component of the mobile phase obtained best recoveries of the two analytes. Samples were separated on a Supelco Discovery HS C_{18} (150 mm × 4.6 mm, 5 μm) analytical column protected by a Perkin Elmer C_{18} (30 mm × 4.6 mm, 10 μm) guard cartridge. The mobile phase, methanol/acetonitrile (50/50, *v*/*v*):0.05% trifluoroacetic acid (19:81, *v*/*v*), operated at 50 °C column oven temperature was pumped at a flow rate of 2.0 mL min^{-1} and the column eluents were monitored at a wavelength of 285 nm. When Sample was injected into the Perkin Elmer high performance liquid-chromatography system through Rheodyne manual (or auto-sampler) injector equipped with 20 μL loop, separation was achieved within 4 min. The present method demonstrated acceptable values for selectivity, linearity within the expected concentration range (0.004–5.0 μg mL^{-1}; $r^2 > 0.999$ for both analytes), recovery (>95% for cefdinir and >96% for cefixime), precision (%RSD < 2.0 for cefdinir and <2.2 for cefixime), sensitivity (limit of detection: 1 ng mL^{-1} and lower limit of quantification: 4 ng mL^{-1} for both analytes), stability of solutions, and robustness. The method was efficiently applied to a pharmacokinetic study in healthy volunteers.

1. Introduction

Cefdinir and cefixime belong to oral broad spectrum 3rd generation cephalosporins, which are penicillin-like bactericidal agents that inhibit bacterial cell wall synthesis. Their oral bioavailability is attributed to a vinyl moiety at position 3 of the cephalosporin nucleus [1,2]. Cefdinir is highly effective against many gram positive and gram negative bacteria, and it is used to treat otitis media, soft tissue infections, and respiratory tract infections, including sinusitis, community-acquired pneumonia, and acute exacerbations of bronchitis [3–7]. On the other hand, cefixime has broad and potent activities against various pathogens especially gram negative organisms including *β-lactamase* producing strains, and its therapeutic uses include gonorrhea, tonsilitis, and pharyngitis [8,9].

To our knowledge, only three papers [10–12] have been reported so far on the liquid-chromatographic determination of cefdinir in biological samples. However, one of these methods [10] have utilized very expensive mass spectrometric detector, one involves tedious and expensive solid-phase extraction technique and it is not validated according to international guidelines [11], and the third one, which is not very much sensitive, hardly involves any sample pre-treatment technique [12]. Thus it is not suitable for selective extraction of cefdinir from plasma samples. On the other hand, several papers have been published on the liquid chromatographic determination of cefixime either alone [13–15] or in combination with other cephalosporins [16–20]. But no single paper has been reported so far that can simultaneously determine cefdinir and cefixime, which may be helpful in the analysis of these analytes in pharmaceuticals and pharmacokinetic and drug–drug interaction studies.

This study was thus designed to develop and validate a sensitive, precise, and accurate method for simultaneous determination of cefixime and cefdinir in human plasma using reversed-phase high performance liquid-chromatography (RP-HPLC) coupled with

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doi:10.1016/j.jchromb.2011.06.040

ultraviolet–visible (UV–Vis) detector. The proposed method was applied to a pharmacokinetic study in healthy human volunteers.

2. Experimental

2.1. Chemicals and reagents

Cefixime (cefixime trihydrate; purity ≥ 98%) and cefdinir (purity ≥ 95%) were purchased from Sigma–Aldrich® (via Analytical Measuring Systems, Karachi, Pakistan). HPLC-grade acetonitrile (ACN) and methanol (MeOH) and analytical-grade potassium dihydrogen phosphate (KH_2PO_4), sodium dihydrogen phosphate (NaH_2PO_4), 85% *o*-phosphoric acid (OPA), 85% formic acid (FA), trifluoroacetic acid (TFA; purity ≥ 98%), trichloroacetic acid (TCA; purity ≥ 99%), ethanol (EtOH), hydrochloric acid (HCl; purity 37%), sulfuric acid (H_2SO_4; purity 98%), and *n*-hexane were purchased from either Sigma–Aldrich®, Merck Chemicals (via Science Centre, Rawalpindi, Pakistan), or Scharlau (via Musaji Adam & Sons, Karachi, Pakistan). Ultra-pure water was prepared by a Millipore ultra-pure water system (Milford, USA). All these chemicals and reagents were used without further purification accept mobile phases, which were vacuum filtered through 0.45 μm pore size filters.

2.2. Preparation of standard solutions

Stock solutions of cefixime and cefdinir were prepared by dissolving weighed amounts of each in ACN:MeOH (50:50, *v*/*v*) and stored at −20 °C in amber glass vials.

2.2.1. Cefdinir analysis

For the calibration purpose, cefixime solution (to give a final concentration of 1.0 μg mL^{-1}) used as the internal standard (IS), to be added to all standard mixtures and plasma samples, was prepared by dilution of the corresponding stock solution with the mobile phase. Similarly, the cefdinir stock solution was also further diluted with mobile phase to give standard mixtures in the range of 0.004–5.0 μg mL^{-1} (eight concentration levels), each containing 1.0 μg mL^{-1} of the IS.

2.2.2. Cefixime analysis

Cefdinir solution (to give a final concentration of 1.0 μg mL^{-1}) used as the IS, to be added to all standard mixtures and plasma samples, was prepared by dilution of the corresponding stock solution with the mobile phase. While, stock solution of the cefixime was also further diluted with mobile phase to give standard mixtures in the range of 0.004–5.0 μg mL^{-1} (seven concentration levels), each containing 1.0 μg mL^{-1} of the IS.

Finally, standard solution containing 1.0 μg mL^{-1} each of cefdinir and cefixime (1:1 calibration mixture) was also prepared.

2.3. Sample preparation

Plasma was immediately separated from the blood samples, obtained from subjects in sodium heparin-vacutainer tubes, by centrifugation at 2500 × *g* for 10 min at 4 °C and stored at −20 °C until use. To extract cefixime and cefdinir from human plasma, 50 μL (1/4 parts) IS solution (to give a final concentration of 1.0 μg mL^{-1}) was mixed with 200 μL of the plasma before treating it with 600 μL (in single step as 1 × 600 or two steps as 2 × 300) of various protein denaturing and/or extraction solvents such as EtOH, ACN, MeOH, 6% TCA solution$_{(aqueous)}$, or their combinations by vigorous vertex-mixing for about 5 min. The samples were then centrifuged at 2500 × *g* for 10 min at 4 °C. The supernatants obtained were then collected in separate eppendorf tubes and diluted with the aqueous component of the mobile phase to the desired volume. Optionally, sample was then treated with 500 μL (2.5 parts) of *n*-hexane by vigorous mixing for about 2 min and centrifuged again at 2500 × *g* for 10 min at 4 °C to accomplish delipidation and removal of any late eluting hydrophobic impurities present. The upper organic layer was then discarded and the lower layer injected into the HPLC system.

2.4. Instruments

Chromatography was performed using HPLC system equipped with a pump, on-line vacuum degasser, auto-sampler, Peltier column oven, and UV–Vis detector (Perkin Elmer Series 200 HPLC system, Norwalk, USA). The chromatographic data was analyzed using Perkin Elmer Totalchrom chromatography workstation (version 6.3.1.) interfaced with the HPLC system through network chromatography interface (NCI) 900.

2.5. Chromatographic conditions and detection parameters

Analytes were separated using Supelco Discovery HS C_{18} (150 mm × 4.6 mm, 5 μm; Bellefonte, USA) analytical column protected by a Perkin Elmer RP_{18} (30 mm × 4.6 mm, 10 μm; Norwalk, USA) pre-column guard cartridge. Different isocratic mobile phases, consisting of MeOH, ACN, or ACN:MeOH (50:50, *v*/*v*) as the organic components and pH adjusted water with either 85% FA, 85% OPA, or TFA or 50 mM KH_2PO_4 buffer, pH adjusted with 85% OPA (pH 2.0–3.0) as the buffered aqueous components, pumped at various flow rates in the range of 1.0–2.5 mL min^{-1} were evaluated. Analyses were performed at different column oven temperatures in the range of 25–50 °C. The injection volume was kept 20 μL. The column eluents were monitored at various wavelengths in the UV range to select the most appropriate wavelength for simultaneous analysis of the two compounds.

To achieve the best separation and detection, the preliminary selected parameters were evaluated on the basis of one or more of the following factors: The %peak height ($\%H_{i,j}$), the %peak width at half height ($\%W_{i,j}^{1/2}$), the peak resolution ($R_{s;i,j}$), the asymmetry/tailing factor ($A_{i,j}$), and the retention time for individual compounds, *i* (cefdinir or cefixime) and individual tested values, *j* of the studied parameters, calculated using the following equations:

$$\%H_{i,j} = \left(\frac{H_{i,j}}{H_{i,j;\max}}\right) \times 100 \quad (1)$$

$$\%W_{i,j}^{1/2} = \left(\frac{W_{i,j}^{1/2}}{W_{i,j;\max}^{1/2}}\right) \times 100 \quad (2)$$

$$R_{s;i,j} = 1.18 \times \frac{t_{b;i,j} - t_{a;i,j}}{W_{a;i,j}^{1/2} + W_{b;i,j}^{1/2}} \quad (3)$$

$$A_{i,j} = \frac{A5\%_{i,j} + B5\%_{i,j}}{2 \times A5\%_{i,j}} \quad (4)$$

where $H_{i,j}$ and $W_{i,j}^{1/2}$ were peak height and peak width at half height; *a* and *b* were the two adjacent peaks and *t* was their retention time; and *A*5% and *B*5% were the bandwidth of the front half and the tail half of the peak at 5% of the maximum peak height of the compound, *i* at tested value, *j* of the studied parameter and $H_{i,j;\max}$ and $W_{i,j;\max}^{1/2}$ were maximal values of $H_{i,j}$ and $W_{i,j}^{1/2}$ from whole range of tested values, *j* of the studied parameter.

The value "*j*" was selected as optimal when calculated $\%H_{i,j}$ achieved the highest value, $\%W_{i,j}^{1/2}$ the lowest value, $R_{s;i,j}$ at least the minimum acceptable value of 2 for the critical peak pair(s), and the $A_{i,j}$ the value closest to 1.

2.6. Method validation

The proposed analytical method was validated according to international guidelines with emphasis on selectivity, linearity within the expected concentration range, recovery, precision (repeatability and intermediate precision), sensitivity, stability of solutions, and robustness [21,22].

2.6.1. Selectivity

The selectivity of the method was verified by the separation of peaks in the chromatograms of the blank solvent, the blank plasma, the standard 1:1 calibration mixture, and the blank plasma sample spiked with the standard 1:1 calibration mixture.

2.6.2. Linearity

The linearity of the method was determined by plotting the response ratios (ratios of peak areas of analytes and IS) of the plasma samples, extracted, and analyzed after spiking with various standard mixtures, as a function of spiked concentrations of analytes and the slope (m), the intercept (b), and the correlation co-efficient (r^2) were determined from the regression analysis.

2.6.3. Recovery

To determine the %recovery, three nominal concentrations of the standard mixtures were spiked into plasma samples (200 μL; $n = 5$), extracted, and analyzed with triplicate injections. Response ratios of the spiked plasma samples were divided by the response ratios of the corresponding standard mixtures and multiplied by 100 to get %recovery.

2.6.4. Precision

The injection repeatability was determined by injecting the standard 1:1 calibration mixture at least 10 times. It was expressed by repeatability of peak areas and retention times of the analytes and determined as the mean ± standard deviation (SD) and the %residual standard deviation (%RSD) calculated from the data obtained.

Analysis repeatability was confirmed by analyzing five samples prepared individually from single plasma spiked with 1:1 calibration mixture. The result was expressed by repeatability of the recovered amount and determined as the mean ± SD and the %RSD calculated from the data obtained.

To determine the intermediate precision (intra- and inter-days reproducibility), the spiked samples prepared for the recovery studies were analyzed three times a day at 08:00, 15:00, and 22:00 h and for three successive days, respectively. The result was expressed as the reproducibility of the recovered amount and determined as the mean ± SD and the %RSD calculated from the data obtained.

2.6.5. Sensitivity

The sensitivity of the method was evaluated by determining the limit of detection (LOD) and lower limit of quantification (LLOQ) using the signal-to-noise ratio (S/N) approach at the values of 3 and 10, respectively.

2.6.6. Robustness

To determine the robustness of the developed method, effect of small deliberate variations in system parameters like the organic component of the mobile phase (±2%), the mobile phase flow rate (±0.2 mL min^{-1}), the column oven temperature (±5 °C), and the detection wavelength (±5 nm) was studied.

2.6.7. Stability

Short-term stability study of the analytes was evaluated in:

(a) Plasma samples stored over-night at room temperature (15–20 °C) after the first injection cycle and then re-injected on the next day;
(b) Plasma samples stored over-night in the refrigerator (at 4 °C) or in the freezer (at −20 °C), brought to room temperature, and injected within 1 h after thawing; and
(c) Standard stock solutions stored for 1 week at −20 °C, brought to room temperature, and injected within 1 h after thawing.

2.7. Application of the method to a pharmacokinetic study

The proposed method was applied to a pharmacokinetic study in healthy volunteers that was conducted as per the principles of the Declaration of Helsinki and its amendments. The protocol of the study was approved by the Ethical Committee of the Department of Pharmacy, University of Peshawar, Pakistan. A written informed consent was obtained from all participants before blood sampling.

Briefly, eight students of the Department of Pharmacy, University of Peshawar were recruited and blood samples were collected from them at various time intervals after administration of a single 400 mg cefixime peroral dose, processed, and analyzed as described above. The average concentration of the cefixime determined was plotted as a function of the sampling time subsequent to drug administration. This concentration versus time data was also subjected to pharmacokinetic software, PK-Solution to calculate various pharmacokinetic parameters including peak plasma concentration (C_{max}), time to reach peak plasma concentration (T_{max}), area under the concentration–time curve from zero to the last measurable plasma concentration point (AUC_{0-t}), elimination rate constant (K_{el}), terminal elimination half-life ($T_{1/2}$), mean residence time (MRT), and clearance (CL), etc.

3. Results and discussion

3.1. Optimization of sample preparation

Of the various procedures/solvents tested for sample preparation, a simple procedure based on protein precipitation and extraction of plasma samples (200 μL) with three parts of 6% TCA aqueous solution (600 μL) followed by volume make to 1 mL with the aqueous component of the mobile phase obtained best recoveries of the two analytes.

The most fundamental problem regarding the analysis of these cephalosporins was their solubility, particularly of the cefdinir. As cefdinir is insoluble in water, MeOH, EtOH, acetone, and other frequently used organic solvents and cefixime in water, ether, and ethyl acetate, etc., we checked their solubility in acidified water (pH reduced with FA, OPA, TFA, TCA, HCl, and H_2SO_4, etc.), sodium phosphate buffer (50 mM NaH_2PO_4 buffer, pH 2.0–3.0 with 85% OPA), potassium phosphate buffer (50 mM KH_2PO_4 buffer, pH 2.0–3.0 with 85% OPA), and MeOH, ACN, or their mixtures. Results showed that cefixime was soluble in MeOH, water acidified with TCA, and ACN:MeOH (50:50, v/v) mixture. On the other hand, cefdinir was readily soluble in ACN:MeOH (50:50, v/v) and water acidified with TCA.

Various solvents were studied to optimize the extraction of the two analytes. As obvious from the result of the solubility study, acceptable recoveries were obtained only with 6% TCA and ACN:MeOH (50:50, v/v) mixture. However, peaks were broad and fronting in case of extraction with ACN:MeOH (50:50, v/v) or a mixture of ACN/MeOH (50/50, v/v):6% TCA (50:50, v/v). The reason might be the stronger solvent effect in case of extraction solvents containing MeOH and ACN. Comparative results of $\%H_{i,j}$, $\%W_{i,j}^{1/2}$, and $A_{i,j}$ of the two analytes with various extraction solvents are depicted in Fig. 1, showing that a simple extraction proce-

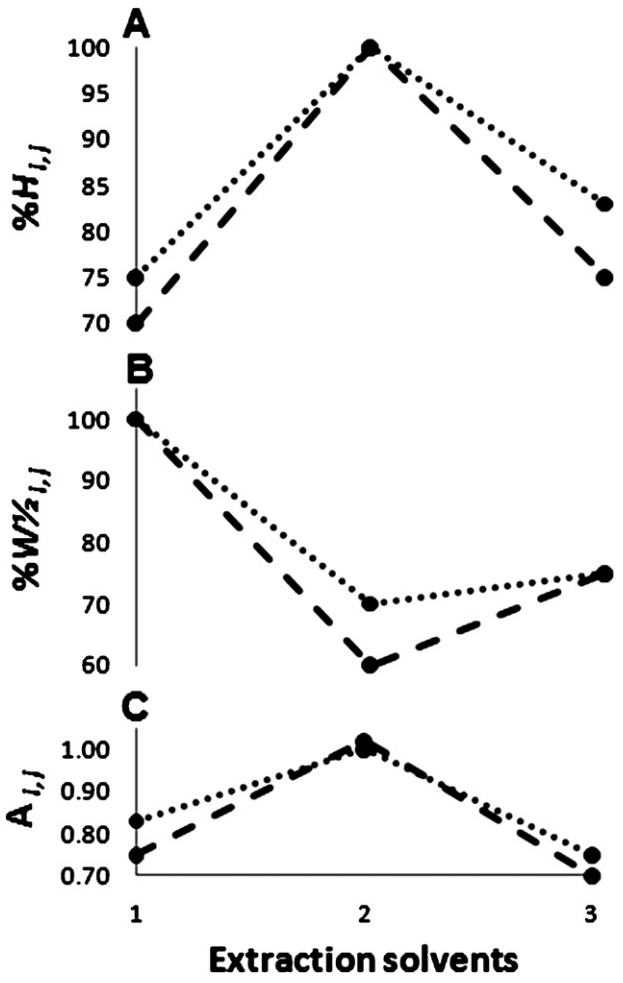

Fig. 1. Influence of extraction solvents, (1) ACN:MeOH (50:50, *v*/*v*), (2) 6% TCA, and (3) ACN/MeOH (50/50, *v*/*v*):6% TCA (50:50, *v*/*v*), on $\%H_{i,j}$ (A), $\%W^{1/2}_{i,j}$ (B), and $A_{i,j}$ (C) of cefdinir (dotted line) and cefixime (dashed line).

dure consisting of deproteination and extraction with 3 parts of 6% TCA solution$_{(aqueous)}$ followed by volume make up with the aqueous component of the mobile phase obtained maximum recoveries of both cefixime and cefdinir from human plasma samples.

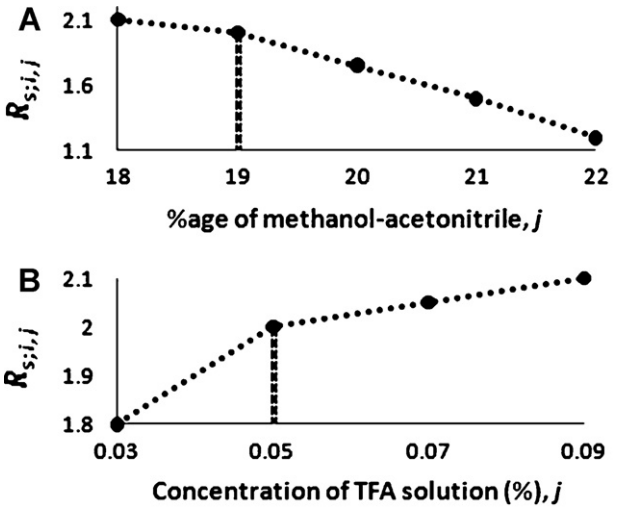

Fig. 2. Influence of (A) %age of ACN:MeOH (50:50, *v*/*v*) and (B) %age of TFA aqueous solution in the mobile phase on $R_{s;i,j}$ of cefdinir.

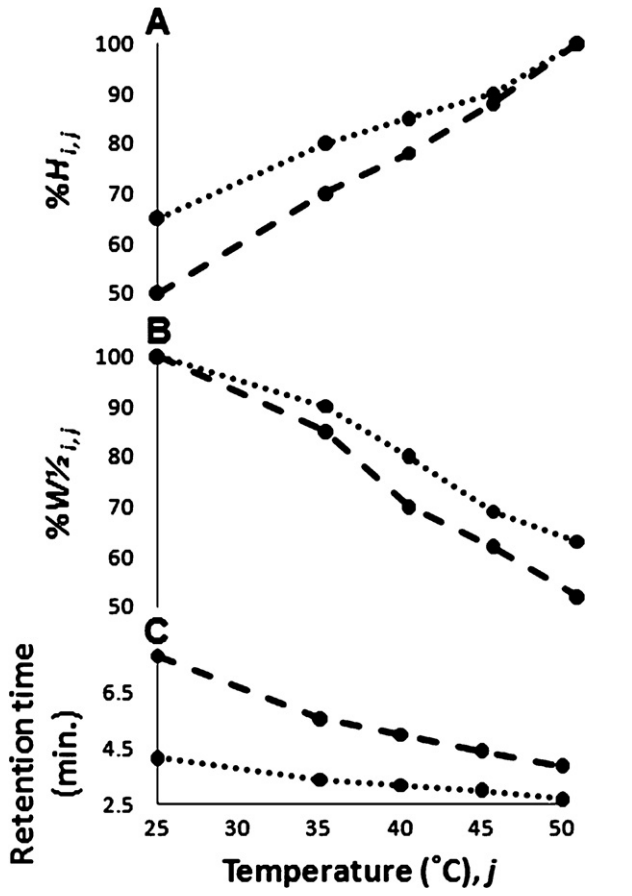

Fig. 3. Influence of temperature on (A) $\%H_{i,j}$, (B) $\%W^{1/2}_{i,j}$, and (C) retention time (min) of cefdinir (dotted line) and cefixime (dashed line).

These results were consistent with the previous results [10,16]. The solid-phase extraction procedure has also been reported for the extraction of the cefdinir from plasma samples [11]. Although, solid-phase extraction offers comparable recovery and clear chromatograms; however, it is a much more expensive procedure than liquid–liquid extraction and protein precipitation methods, so it was not utilized in this study.

3.2. Optimization of chromatographic conditions

Depending upon the nature of the analytes, a routinely used RP column, Supelco Discovery HS C_{18} (150 mm × 4.6 mm, 5 μm) was utilized for their separation.

Different isocratic mobile phases, consisting of MeOH, ACN, or ACN:MeOH (50:50, *v*/*v*) mixture as the organic and 50 mM KH_2PO_4 buffer, pH adjusted with 85% OPA (pH 2.0–3.0) or pH adjusted water with either 85% OPA, 85% FA, or TFA as the buffered aqueous components, were utilized.

Of the various organic components, ACN:MeOH (50:50, *v*/*v*) obtained better peak shapes of the two analytes as compared to MeOH or ACN alone. The reason might be the better solubility of the two analytes in ACN:MeOH (50:50, *v*/*v*) mixture. As depicted in Fig. 2A, 19% ACN:MeOH (50:50, *v*/*v*) in the mobile phase efficiently resolved the cefdinir peak from an unidentified peak in plasma samples.

Initially, 50 mM KH_2PO_4 buffer, pH adjusted with 85% OPA (pH 2.0–3.0) was used as the buffered aqueous component of the mobile phase but it was unable to resolve the cefdinir peak from an unidentified peak in plasma samples even in presence of less than 10% organic modifier in the mobile phases. The pH adjusted water (with

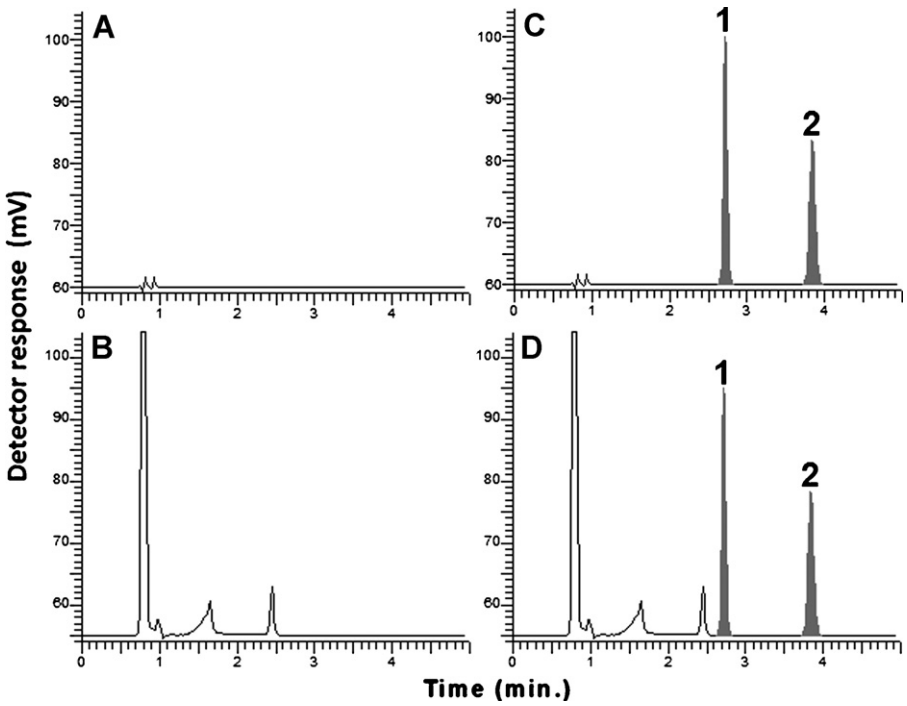

Fig. 4. Representative RP-HPLC chromatograms of different samples analyzed under optimum conditions *Chromatograms:* (A) the blank mobile phase; (B) the blank plasma sample; (C) the standard 1:1 mixture; and (D) the blank plasma sample spiked with standard 1:1 mixture. *Peaks:* 1. cefdinir (2.71 min) and 2. cefixime (3.88 min).

85% OPA and 85% FA, pH 2.0–3.0) was also tried as the acidified aqueous component of the mobile phase but the same problem persisted. Moreover, the retention time of the cefixime was comparatively longer with mobile phases containing FA. Finally, these problems were solved by using pH adjusted water with TFA as the aqueous component of the mobile phase. As shown in Fig. 2B, of the different concentrations of TFA aqueous solution used, 0.05% TFA solution was optimal for the separation of the two analytes.

The mobile phase was pumped at various flow rates in the range of 1.0–2.5 mL min^{-1} but the selected flow rate of 2.0 mL min^{-1} was able to efficiently resolve the two analytical peaks in less than 4 min without building too much backpressure on the column.

Separation of the analytes was performed at various column oven temperatures in the range of 25–50 °C. Peak shapes and heights were improved and retention times were decreased with increasing temperature without affecting peak areas and resolution. So depending upon these parameters, 50 °C was selected to be the optimum temperature for the separation of these two analytes (Fig. 3).

In short, of the variety of chromatographic conditions studied, best results were obtained with the following combination of parameters: Stationary phase, Supelco Discovery HS C_{18} (150 mm × 4.6 mm, 5 μm; Bellefonte, USA); Mobile phase, ACN/MeOH (50/50, *v*/*v*):0.05% TFA $solution_{(aqueous)}$ (19:81, *v*/*v*) in isocratic mode; Flow rate, 2.0 mL min^{-1}; Column oven temperature, 50 °C; and Injection volume, 20 μL. Fig. 4 shows representative chromatograms, at optimum chromatographic conditions, of the blank mobile phase, blank plasma sample, standard 1:1 calibration mixture, and the plasma sample spiked with the standard 1:1 calibration mixture. Under the specified conditions, the mean retention times were 2.71 and 3.88 min for cefdinir and cefixime, respectively.

3.3. Optimization of detection wavelength

The column eluents were monitored at various wavelengths in the UV range. The maximum response of the two analytes was observed in the range of 275–285 nm. However, the response of the extraneous peaks was minimal at 285 nm. So 285 nm was selected to be the optimum wavelength for simultaneous determination of cefdinir and cefixime.

3.4. Method validation

3.4.1. Selectivity

The method was selective for simultaneous determination of cefixime and cefdinir as both the target peaks were well resolved from each other, from other peaks of extraneous and endogenous substances in spiked plasma samples, and from the peak of the sample solvent as depicted in the RP-HPLC chromatogram of preparations: the blank solvent; the blank plasma sample; the standard 1:1 calibration mixture; and the plasma sample spiked with the standard 1:1 calibration mixture (Fig. 4).

Table 1
Calibration range, linearity, and sensitivity of the proposed method for simultaneous determination of cefdinir and cefixime.

Parameters	Analytes	
	Cefdinir	*Cefixime*
Calibration range (μg mL^{-1})	0.004–5.0	0.004–5.0
Linearity		
Standard mixtures		
Regression equation	$y = 0.9760x + 0.0422$	$y = 0.9270x - 0.0469$
Correlation co-efficient, r2	0.9998	0.9994
Spiked plasma samples		
Regression equation	$y = 0.9760x + 0.0422$	$y = 0.9270x - 0.0469$
Correlation co-efficient, r2	0.9998	0.9998
Sensitivity		
Limit of detection, LOD		
ng mL^{-1}	1	1
On column (pg)	20	20
Lower limit of quantification, LLOQ		
ng mL^{-1}	4	4
On column (pg)	80	80

y is the response ratio and *x* is the concentration.

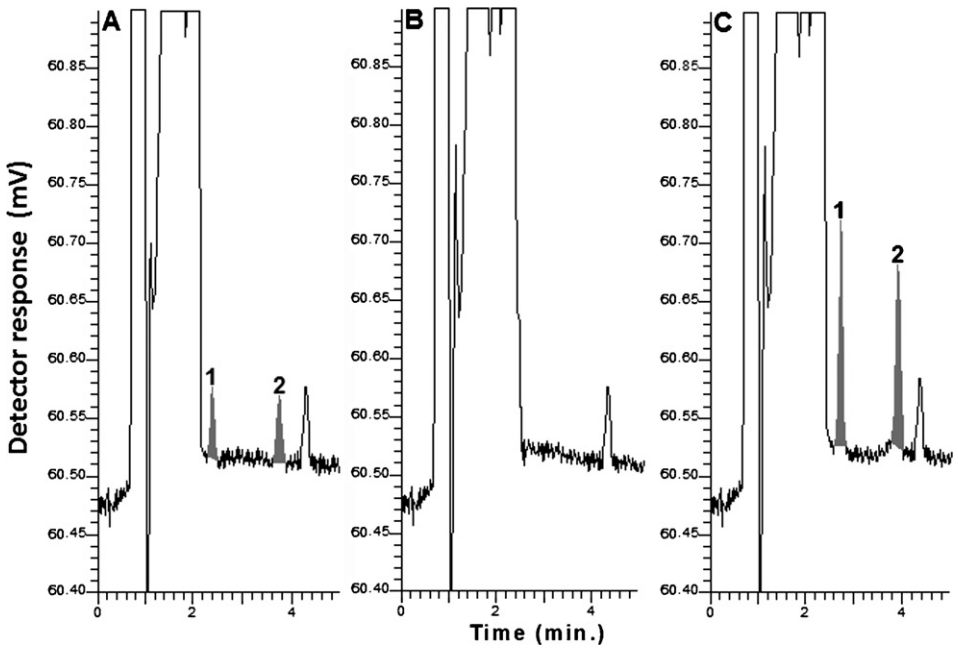

Fig. 5. RP-HPLC chromatograms showing peaks of (1) cefdinir and (2) cefixime at the level of LOD (A) and LLOQ (C), where B is the blank plasma sample.

3.4.2. Linearity

The proposed method was linear within the studied concentration range of 0.004–5.0 μg mL^{-1} not only in standard mixtures but also in spiked plasma samples. Regression equations describing the calibration curves for standard mixtures and spiked plasma samples and results of correlation co-efficient (r^2) of the two analytes are summarized in Table 1.

3.4.3. Sensitivity

The LODs and LLOQs for the two analytes are also given in Table 1, showing that the proposed method was more sensitive than the previously reported methods for determination of cefdinir in biological samples [10–12] and that it can used to determine the concentration of cefdinir and cefixime in the plasma even after 36 h of single 300 and 400 mg oral dose, respectively. Chromatograms

Table 2
Recovery and precision of the proposed method for simultaneous determination of cefdinir and cefixime.

Parameters	Analytes	
	Cefdinir Mean ± SD; %RSD	*Cefixime*
Recovery		
Spiked concentration level 1[a]	[d]98.95 ± 2.71; 2.74	[d]97.67 ± 2.25; 2.31
Spiked concentration level 2[a]	[d]95.73 ± 1.14; 1.19	[d]96.40 ± 1.51; 1.57
Spiked concentration level 3[a]	[d]98.67 ± 1.04; 1.05	[d]98.17 ± 1.04; 1.06
Precision		
Repeatability		
Injection repeatability		
Standard mixture[b]	[e]87,606 ± 981; 1.12	[e]85,012 ± 1156; 1.36
Standard mixture[b]	[f]2.71 ± 0.03; 0.98	[f]3.88 ± 0.03; 0.73
Analysis repeatability		
Standard mixture[a]	[g]998.42 ± 18.22; 1.82	[g]982.60 ± 12.40; 1.26
Intermediate precision		
Intra-day reproducibility		
Spiked concentration level 1[c]	[g]4.625 ± 0.075; 1.622	[g]4.658 ± 0.088; 1.885
Spiked concentration level 2[c]	[g] 484.667 ± 7.506; 1.549	[g] 484.333 ± 5.033; 1.039
Spiked concentration level 3[c]	[g] 2425 ± 25; 1.0	[g]2435 ± 21.4; 0.9
Inter-days reproducibility		
Spiked concentration level 1[c]	[g]4.608 ± 0.088; 1.905	[g]4.650 ± 0.100; 2.151
Spiked concentration level 2[c]	[g]483.333 ± 8.083; 1.672	[g] 481.333 ± 7.506; 1.155
Spiked concentration level 3[c]	[g]2422 ± 27.5; 1.1	[g]2428 ± 27.5; 1.133

Spiked concentration level 1 = 0.005 μg mL^{-1}; spiked concentration level 2 = 0.5 μg mL^{-1}; spiked concentration level 3 = 2.5 μg mL^{-1}; and standard mixture = 1.0 μg mL^{-1}.

[a] $n = 5$.
[b] $n = 10$.
[c] $n = 3$ (where n is the number of samples).
[d] Recovery (%).
[e] Peak area.
[f] Retention time (min).
[g] Quantity recovered (ng mL^{-1}).

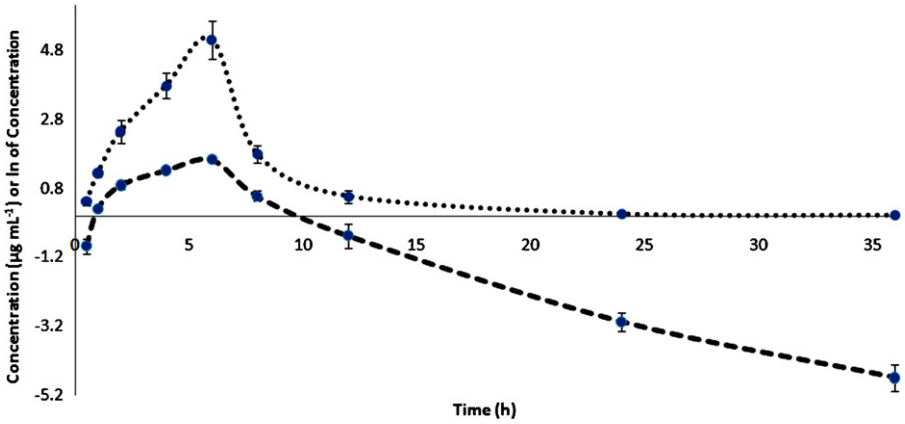

Fig. 6. Plasma drug concentrations–time curves for cefixime 400 mg capsule given as single oral dose in healthy Pakistani male volunteers Dotted line represents standard and dashed line represents semi-logarithmic (ln) plots; Each point is a mean of eight volunteers; and Error bars represent SD.

Table 3
Non-compartmental pharmacokinetic parameters for cefixime 400 mg given as single oral dose in healthy Pakistani male volunteers.

Pharmacokinetic parameters	Values (mean ± SD)
Time to peak concentration (T_{max}) [h]	6 ± 0
Peak concentration (C_{max}) [µg mL^{-1}]	5.1 ± 0.5
Area under concentration–time curve (AUC_{0-36}) [µg h mL^{-1}]	33.0 ± 4.0
$AUMC_{0-\infty}$ [µg h^2 mL^{-1}]	214.9 ± 32.0
Mean residence time (MRT) [h]	6.5 ± 0.3
Elimination rate constant (K_{el}) [h^{-1}]	0.14 ± 0.42
Half-life ($T_{1/2}$) [h]	5.13 ± 1.23
Clearance (CL) [mL h^{-1}]	12,119 ± 1521

showing peaks of cefixime and cefdinir at the level of LOD and LLOQ are given in Fig. 5.

3.4.4. Recovery

Results of the recovery studies with the selected extraction procedure are summarized in Table 2, showing that the recovery of the two analytes was more than 95% at all the three nominal concentration levels for plasma samples.

3.4.5. Precision

Results of the repeatability (injection and analysis) and intermediate precision (intra- and inter-days reproducibility) are also summarized in Table 2, showing complete agreement among the repeated injections (both retention times and peak areas), repeated analyses, and intra- and inter-days studies.

3.4.6. Robustness

Results of slight changes in various system parameters like the organic component of the mobile phase, the mobile phase flow rate, the column oven temperature, and the detection wavelength indicated that the method was robust as its performance was negligibly affected by minor changes in these parameters.

3.4.7. Stability

Short-term stability study indicated that spiked plasma samples remained stable for at least 24 h even at room temperature (30 °C). On the other hand, standard solutions of cefixime and cefdinir remained stable for at least 1 week when kept frozen.

3.5. Applicability of the method

This method was a part of an extensive "pharmacokinetics and pharmacokinetic drug–drug interactions studies in healthy human volunteers". Initially, the method was applied for studying the pharmacokinetics of cefixime in healthy adult volunteers, results of which are depicted in Table 3 and Fig. 6. It could be conveniently applied to pharmacokinetic studies of cefdinir, and for the analyses of the two analytes in various pharmaceutical dosage forms as well.

4. Conclusion

This study was aimed to develop a simple and reliable isocratic RP-HPLC/UV detection method for simultaneous determination of cefdinir and cefixime in human plasma. For this purpose, the effect of various chromatographic conditions and other experimental parameters on the analysis of these analytes was studied, which resulted in a method that was superior to the previously reported methods for determination of these analytes particularly cefdinir with respect to economy, simplicity, sensitivity, and throughput. The proposed method could be efficiently applied for the analysis of these analytes in pharmaceuticals and in various biological matrices in the context of clinical research.

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