ELSEVIER

Journal of Chromatography B, 783 (2003) 297–301

JOURNAL OF CHROMATOGRAPHY B

www.elsevier.com/locate/chromb

Short communication

Simple liquid chromatographic method for the determination of cefotaxime in human and rat plasma

Sharon Sheue Nee Ling[a], Kah Hay Yuen[a,*], Susan A. Barker[b]

[a]*School of Pharmaceutical Sciences, University of Science Malaysia, 11800 Penang, Malaysia*
[b]*School of Pharmacy, Queen's University Belfast, Belfast BT9 7BL, UK*

Received 16 April 2002; received in revised form 19 August 2002; accepted 5 September 2002

Abstract

A high-performance liquid chromatographic method with ultraviolet (UV) detection was developed for measuring cefotaxime in rat and human plasma. The method used direct injection of the plasma supernatant after deproteinization with 70% perchloric acid. Degradation of cefotaxime in acidic medium was retarded by adding phosphate buffer before centrifuging the sample. The mobile phase was 0.05 *M* aqueous ammonium acetate–acetonitrile–tetrahydrofuran (87:11:2, v/v) adjusted to pH 5.5. Analysis was run at a flow-rate of 1.0 ml/min, and a detection wavelength of 254 nm was used. The method has a quantification limit of 0.20 μg/ml. The within- and between-day coefficients of variation and accuracy values were less than 8% and ±3%, respectively, while the recovery values were greater than 87% over the concentration range tested (0.20–50 μg/ml). The speed, sensitivity, specificity and reproducibility of this method make it particularly suitable for the routine determination of cefotaxime in human plasma. Moreover, only a relatively small sample plasma volume (100 μl) is required, allowing this method to be applied to samples taken from neonates.

Keywords: Cefotaxime

1. Introduction

Cefotaxime is a wide-spectrum, semisynthetic, third-generation cephalosporin antibiotic. Commercially available as the sodium salt, cefotaxime is administered intravenously. Various analytical methods, including microbiological assay and high-performance liquid chromatography (HPLC), have been developed for the determination of cefotaxime in biological samples [1–9]. The presence of cefotaxime's active metabolite has been shown to interfere with the determination of cefotaxime by microbiological assays [1]. In addition, the sensitivity of this technique limits quantification of the drug to concentrations >2 μg/ml [2].

Other reported methods are based on reversed-phase HPLC systems with UV detection. Signs et al. [3] and Scannes et al. [4] employed methods involving deproteinization with ice-cold methanol and incubation of the sample at −20 °C. In another method reported by Dell et al. [5], freeze-drying was used to prepare the samples, and the HPLC run time

*Corresponding author. Tel.: +60-4-657-7888; fax: +60-4-659-6517.
E-mail address: khyuen@usm.my (K.H. Yuen).

1570-0232/02/$ – see front matter
PII: S1570-0232(02)00657-8

was 20 min. Jehl et al. [6] employed a sample preparation method which needs approximately 40 min for the treatment of each sample. The sensitivity of most reported methods is in the range 0.2–1.0 μg/ml [1–8]. While the method reported by Brisson and Fourtillan [9] is sensitive to a limit of detection of 50 ng/ml, it involved back-extraction of the sample. An ion-pairing reagent was used in the method reported by Lecaillon et al. [2]. No information regarding variability is available in many studies [1–3,7,9]. As mentioned above, most of the reported methods involve elaborate sample preparation and were not sufficiently validated.

In this paper, we report a simple, sensitive and specific HPLC method for the determination of cefotaxime in human plasma using UV detection. The assay method was evaluated for accuracy, precision, recovery and linearity.

2. Experimental

2.1. Materials

Cefotaxime sodium was obtained from Orchid Chemicals (India). Anhydrous disodium hydrogen orthophosphate and acetonitrile were purchased from Fisher Scientific (Loughborough, Leics, UK). Ammonium acetate and glacial acetic acid were obtained from R&M marketing (Essex, UK). Perchloric acid (70%, w/w) was purchased from AJAX Chemicals (Sydney, Australia), while tetrahydrofuran was purchased from J.T. Baker (Phillipsburg, NJ, USA). All solvents and chemicals used were of AR or HPLC grade.

2.2. Instrumentation

The HPLC system consisted of mostly Jasco (Tokyo, Japan) components, including a PU-980 pump, a UV-975 UV–Vis detector and an 807-IT integrator. A Rheodyne 7725 (Cotati, CA, USA) sample injector was fitted with a 20 μl sample loop. A Zorbax SB-C_{18} (Palo Alto, CA, USA) column (5 μm, 250×4.6 mm I.D.), fitted with a refillable guard column (Upchurch Scientific, Oak Harbour, WA, USA) packed with Perisorb RP-18 (30–40 μm, pellicular) powder (Upchurch), was used for chromatographic separation. The mobile phase consisted of 0.05 *M* aqueous ammonium acetate–acetonitrile–tetrahydrofuran (87:11:2, v/v) adjusted to pH 5.5 using glacial acetic acid, which was delivered at a flow-rate of 1.0 ml/min. The detection wavelength was set at 254 nm with a sensitivity range of 0.005 a.u.f.s. and the samples were quantified using peak height.

2.3. Sample preparation

Plasma (100 μl) was pipetted into an Eppendorf microcentrifuge tube and deproteinized by adding 10 μl 70% w/w perchloric acid. The mixture was vortex-mixed for 30 s (Stuart Scientific, UK). A volume of 100 μl of 0.5 *M* aqueous disodium hydrogen orthophosphate was immediately added to the sample, which was vortex-mixed for a further 5 s. The sample was then centrifuged (Eppendorf, Hamburg, Germany) at 12 800 *g* for 10 min. A 20 μl aliquot of the supernatant was then injected onto the column. For samples with concentrations >50 μg/ml, the supernatant was diluted with mobile phase prior to analysis.

2.4. Assay qualification

Calibration curves were constructed by spiking blank human plasma with a known amount of cefotaxime. A 250 μl aliquot of 1 mg/ml cefotaxime in distilled water was measured and made up to 5 ml volume with blank human plasma to give a final concentration of 50 μg/ml. Subsequent concentrations were prepared by serial dilution of this spiked concentration with blank plasma. These plasma standards were also used to determine the within-day accuracy and precision. For this, six replicates of each concentration were determined in a single day. For between-day evaluation, analysis was carried out on a single sample of each concentration daily for 6 days with a calibration curve constructed on each day of analysis. The accuracy was expressed as percent difference from the spiked concentration, whereas the precision was the coefficient of variation of the replicates. In addition, the absolute recovery (n=6) of the method was estimated by comparison with solutions of the drug at corresponding concentrations.

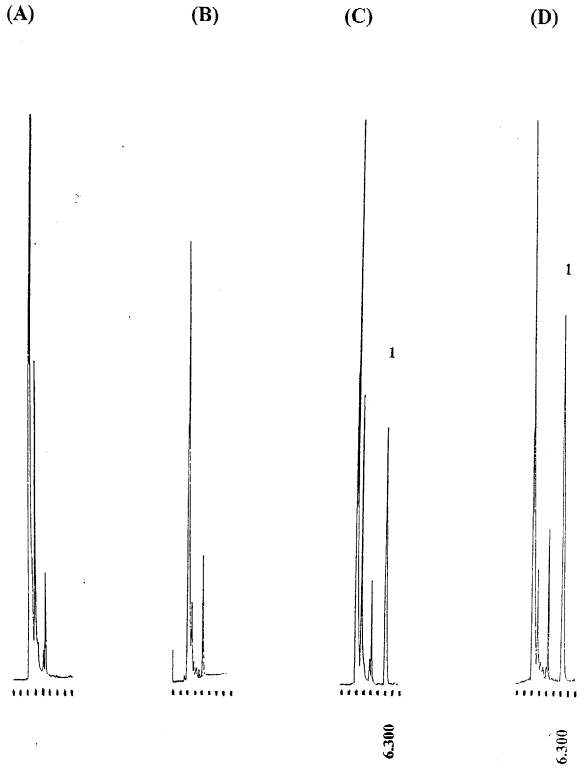

Fig. 1. Chromatograms of cefotaxime in (A) blank human plasma, (B) blank rat plasma, (C) human plasma spiked with 25 μg/ml cefotaxime, and (D) rat plasma containing 37.1 μg/ml cefotaxime, obtained 40 min after intravenous administration of 5.0 mg cefotaxime (*y*-axis, attenuation 64; *x*-axis, chart speed 2.0 mm/min). Peak: 1=cefotaxime.

3. Results

Chromatograms obtained for blank human and rat plasma as well as blank human plasma spiked with 25 μg/ml cefotaxime are shown in Fig. 1A–C, while Fig. 1D shows a chromatogram of a plasma sample obtained from a rat 40 min after intravenous administration of 5.0 mg cefotaxime. The cefotaxime peak, which has a retention time of approximately 6.3 min, was well resolved and free of interference from endogenous compounds in both human and rat plasma. At least four batches of both human and rat plasma were tested. Thus, blank human plasma was used in the preparation of the calibration curves because it was readily available. The drug was found to be stable in both human and rat plasma for at least 12 h at room temperature with no significant change in plasma concentration. The total run time for each sample was 7.5 min.

The standard calibration curves ($n=6$) were linear over the concentrations used, with a mean correlation coefficient of $0.9999 \pm 1.3 \cdot 10^{-4}$ (C.V. $1.3 \cdot 10^{-2}$%), a mean slope of $1.1 \cdot 10^{-3} \pm 9.0 \cdot 10^{-5}$ (C.V. 8.5%) and a mean intercept of $4.7 \cdot 10^{-2} \pm 1.8 \cdot 10^{-2}$ (C.V. 38.5%). Note that equal weighting was given to all points in the regression analysis. The absolute recovery, and within- and between-day accuracy and precision values of the assay method are presented in Table 1. The coefficient of variation (C.V.) values for both within-day and between-day were all <8%, whereas

Table 1
Absolute recovery and within- and between-day precision and accuracy ($n=6$)

Conc. (μg/ml)	Recovery (%)		Within-day		Between-day	
	Mean	C.V.	Precision (C.V. %)	Accuracy (% error)	Precision (C.V. %)	Accuracy (% error)
0.20	102.7	6.3	7.7	−1.5	5.4	2.6
0.39	89.6	9.9	5.2	−2.3	4.3	0.2
0.78	87.8	6.5	6.2	1.5	3.4	1.3
1.56	94.5	5.2	6.1	2.2	1.9	0.4
3.13	88.8	5.9	5.9	1.2	1.9	0.5
6.25	90.1	6.5	6.7	2.3	4.2	−1.2
12.5	95.1	6.2	7.3	−0.7	3.3	1.6
25.0	92.2	4.3	4.9	1.6	5.1	1.8
50.0	91.4	5.8	5.4	2.5	2.8	1.1

the percentage error was always <3%. For eight of the nine concentrations determined, the between-day C.V. values were smaller than the within-day values, suggesting that the precision was not compromised for between-day analyses.

The limit of quantification was approximately 0.20 μg/ml, the lowest concentration used in the construction of the calibration curves. At this concentration, both the between- and within-day accuracy and precision were within ±8%. This value is comparable to that reported by Jehl et al. [6], even though a smaller supernatant volume was injected in the study reported here. Furthermore, the present method was found to be more sensitive than previously reported using most other methods [1–5,7,8].

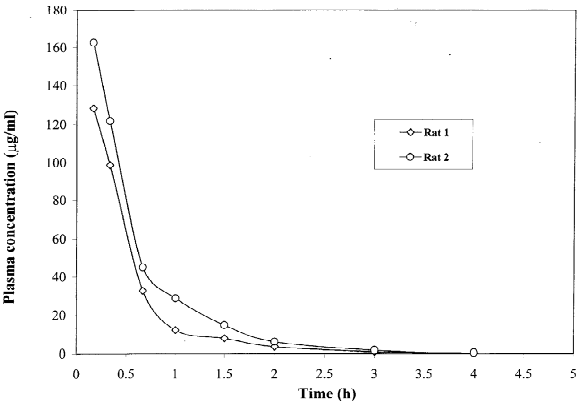

Fig. 2. Plasma cefotaxime concentration versus time profiles for two rats following intravenous administration of 14.3 mg/kg cefotaxime.

4. Discussion

During assay development, various deproteinizing agents, such as acetonitrile, methanol, tetrahydrofuran, phosphoric acid and perchloric acid, were investigated. Organic solvents produced a more turbid sample and perchloric acid was found to provide the cleanest chromatogram. However, stability studies have shown that hydrolysis of cefotaxime takes place in strong acid. Cefotaxime solutions exhibit maximum stability in the range pH 4.3–6.5 [10,11]. Therefore, phosphate buffer was added to increase the pH of the sample to approximately 5 immediately after perchloric acid treatment. Degradation of cefotaxime was retarded by the addition of this buffer, resulting in an average recovery of 92.5%. The supernatants were stable for at least 5 h at ambient temperature (24–26 °C) with less than 5% degradation. Thus it is suggested that the samples should be freshly treated prior to analysis (or should be analyzed within 5 h after treatment).

The mobile phase used was adapted from Jehl et al. [6]. The concentration of buffer as well as the pH of the mobile phase was found to be critical for optimizing the sensitivity. By increasing the concentration of ammonium acetate buffer from 20 to 50 m*M*, the sensitivity was found to increase by approximately 16.4%, while pH 5.5 resulted in better sensitivity compared with pH 5.0. Tetrahydrofuran was added to separate an endogenous compound eluting near the retention time of cefotaxime. Less or more than 2% of tetrahydrofuran resulted in poorer separation. Since an extraction procedure was not involved in this method and good reproducibility was obtained, an internal standard to overcome sample-to-sample variation was found to be unnecessary.

The cleaner samples and smaller injection volumes employed in the present study suggest that a longer column life can be achieved. Moreover, the sample preparation involved simple procedures and the total run time for each sample was only 7.5 min.

The present method was used to analyze plasma samples from two rats after intravenous administration of 14.3 mg/kg cefotaxime solution. Fig. 2 shows the plasma concentration versus time profiles obtained.

5. Conclusion

In summary, the HPLC method described here is simple, sensitive, reproducible and requires only a small sample volume, and is suitable for the determination of plasma cefotaxime in routine measurements for pharmacokinetic/bioavailability studies.

References

[1] T. Bergen, R. Solberg, Chemotherapy 27 (1981) 155.
[2] J.B. Lecaillon, M.C. Rouan, C. Souppart, N. Febvre, F. Juge, J. Chromatogr. 228 (1982) 257.
[3] S.A. Signs, T.M. File, J.S. Tan, Antimicrob. Agents Chemother. (1984) 652.
[4] T. Scanes, A.F. Hundt, K.J. Swart, H.K.L. Hundt, J. Chromatogr. B 750 (2001) 171.
[5] D. Dell, J. Chamberlain, F. Coppin, J. Chromatogr. 226 (1981) 431.
[6] F. Jehl, P. Birckel, H. Monteil, J. Chromatogr. 413 (1987) 109.
[7] R.L. Yost, H. Derendorf, J. Chromatogr. 341 (1985) 131.
[8] K.B. Patel, D.P. Nicolau, C.H. Nightingale, R. Quintiliani, Antimocrob. Agents Chemother. (1996) 2805.
[9] A.M. Brisson, J.B. Fourtillan, J. Chromatogr. 223 (1981) 393.
[10] V.D. Gupta, J. Pharm. Sci. 73 (1984) 565.
[11] S.M. Berge, N.L. Henderson, M.J. Frank, J. Pharm. Sci. 72 (1983) 59.