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Short communication

Simultaneous determination of cefotaxime and desacetylcefotaxime in human plasma and cerebrospinal fluid by high-performance liquid chromatography

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Abstract

A simple and sensitive HPLC method for the simultaneous determination of cefotaxime (I) and desacetylcefotaxime (II) in human plasma and cerebrospinal fluid (CSF) is described. The assay involves deproteinisation and subsequent separation on a reversed-phase HPLC column, with ultraviolet detection at 262 nm. Retention times were 6.8 and 2.2 min for cefotaxime and desacetylcefotaxime, respectively. Average recoveries for the analytes were 78% (I) and 88% (II) from both matrices. Linear responses were observed over a wide range (0.58–940 μg/ml for (I) in plasma, 0.80–55.8 μg/ml for (I) in CSF, 0.54–148 μg/ml for (II) in plasma and 0.50–36.0 μg/ml for (II) in CSF).

Keywords: Cefotaxime; Desacetylcefotaxime

1. Introduction

Cefotaxime sodium is a third-generation cephalosporin antibiotic used intravenously against a wide variety of bacteria. Its metabolite, desacetylcefotaxime, also has antimicrobial activity. Microbiological and high-performance liquid chromatographic (HPLC) methods have been employed to quantify the drug and its metabolite for pharmacokinetic studies. Microbiological techniques lack sensitivity and specificity and it has been seen that the active metabolite interferes with the determination of cefotaxime using these techniques [2,9,12,14,16]. HPLC analysis of cefotaxime (I) and desacetylcefotaxime (II) is also difficult, due to the polar nature of the analytes and their instability [9,19,20]. Various extraction and detection methods have been employed to assay cefotaxime and its metabolite in plasma, urine and CSF, but these methods were either not sensitive enough, or required large volumes of matrix [1–11,13–20]. A simple, rapid assay method for the simultaneous determination of cefotaxime and desacetylcefotaxime in plasma and CSF is described here, which requires small volumes of biological fluids, gives good separation and is sensitive and linear over a wide concentration range.

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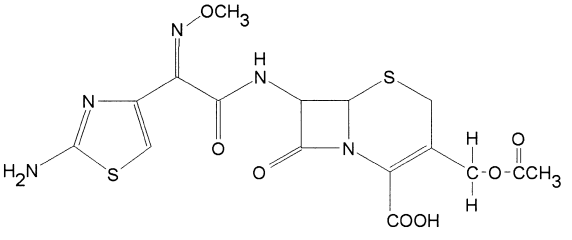
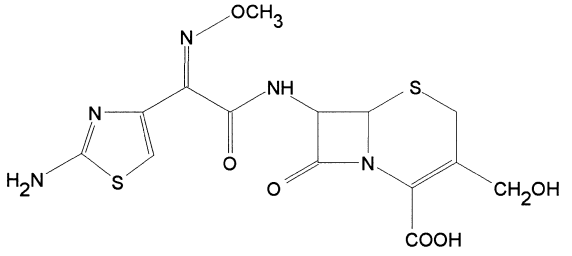
2. Experimental

2.1. Chemicals and reagents

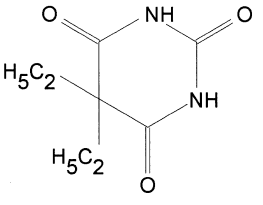
Cefotaxime (Fig. 1A), {3-[(acetyloxy)methyl]-7-[[(2-amino-4-thiazolyl)(methoxyimino)acetyl]amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid} and desacetylcefotaxime (Fig. 1B), {3-[(hydroxy)methyl]-7-[[(2-amino-4-thiazolyl)-(methoxyimino)acetyl]amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid} were obtained from Rousell Uclaf. Internal standard (5,5-diethyl-barbituric acid, Fig. 1C) was obtained from BDH (Ref. No. 798580).



(A) Cefotaxime



(B) Desacetylcefotaxime



(C) Diethyl barbituric acid (barbitone, internal, standard)

Fig. 1. Chemical structures of analytes. (A) Cefotaxime. (B) Desacetylcefotaxime. (C) Diethyl barbituric acid (barbitone, internal standard).

Methanol (Burdick and Jackson Chrompure) and acetonitrile (Baxter) were used without further purification. Sodium acetate (analytical reagent) was obtained from Fluka and *o*-phosphoric acid (89%) and acetic acid (Pro Analysi) were obtained from Merck. Water was purified by RO 20SA (Millipore) reverse osmosis system and Milli-Q® (Millipore) polishing system.

2.2. Preparation of plasma calibration standards

Concentrated stock solutions of cefotaxime and desacetylcefotaxime were prepared separately at concentrations of 14324 μg/ml and 2955 μg/ml, respectively in water for the preparation of plasma calibration standards. Calibration solutions of cefotaxime and desacetylcefotaxime are reported to be stable in water at 5°C for at least 14 days [17], and at least 12 months at −70°C [2].

A stock of pooled human drug-free plasma obtained from healthy volunteers was spiked from the abovementioned solutions to attain concentrations of 940 μg/ml cefotaxime and 148 μg/ml desacetylcefotaxime. This plasma pool was diluted serially with drug-free plasma to give 12 plasma calibration standard concentrations down to 0.500 μg/ml and 0.402 μg/ml, respectively.

Cefotaxime is reported to be stable for at least three months in plasma when stored at −20°C [17] and at least 12 months when stored at −70°C [2,14].

2.3. Preparation of CSF calibration standards

Concentrated stock solutions of cefotaxime and desacetylcefotaxime were prepared separately at concentrations of 1173 μg/ml and 118 μg/ml, respectively in water for CSF calibration standards.

A stock of simulated CSF (10% solution of normal plasma in water) was spiked from the abovementioned solutions to attain concentrations of 55.8 μg/ml cefotaxime and 36.0 μg/ml desacetylcefotaxime. This pool was diluted serially with drug-free ''CSF'' to give 10 CSF calibration standard concentrations down to 0.403 μg/ml and 0.11 μg/ml, respectively. All biological standards and samples were stored at −70°C until assayed.

The internal standard solution consisted of 110 mg barbitone dissolved in 7 g of a mixture of 0.1 *M*

sodium acetate–methanol (3:7). The pH of this mixture was adjusted to 5.2 with acetic acid.

2.4. Apparatus and chromatographic conditions

Assays were carried out on a modular chromatograph. Samples were injected with a Hewlett-Packard series 1050 autosampler injector (Hewlett-Packard, Palo Alto, CA). A Shimadzu Model SPD-6A UV detector (Shimadzu, Kyoto, Japan) was used, monitoring at wavelength 262 nm.

The detector was interfaced by a Hewlett-Packard model 35900E A/D convertor (Hewlett-Packard, Palo Alto, CA) to a computer workstation running Hewlett-Packard HPLC^{2D} ChemStation version 2.05 software. Calibration curves were constructed using peak height ratios (analyte:internal standard) of the compounds.

Separations were performed on a Phase-Sep, Spherisorb ODS2, C_{18}, 5 μ, 4.6×150 mm stainless steel column protected by an Upchurch precolumn dry-filled with Perisorb® RP18 (30–40 μ) packing.

The compounds were eluted with a mobile phase of 0.007 *M* H_3PO_4–acetonitrile (85:15). The chromatography was performed isocratically at a flow-rate of 1.3 ml/min at room temperature.

2.5. Extraction procedure

Step 1: Plasma and CSF samples were thawed at room temperature. *Step 2:* Internal standard solution (100 μl) was added to 100 μl of CSF or plasma, in a 1.5 ml microfuge tube. *Step 3:* The samples were vortexed for 30 s and left on ice for 10 min, due to the heat lability of the analytes [11,14,19]. *Step 4:* The mixture was centrifuged for 10 min at 2000 *G*. *Step 5:* The samples were diluted by adding 20 μl of supernatant to 80 μl distilled water and mixed. A 20 μl aliquot was injected onto the HPLC column.

3. Results and discussion

3.1. Extraction

Ultrafiltration as well as many extraction techniques and protein precipitation with various solvents were investigated, as the analytes in question are very polar and thus difficult to extract. The optimum pH range of stability is 4.3 to 6.5 [20] and acidic protein precipitating reagents cause significant degradation of cefotaxime to desacetylcefotaxime and to a lactone [18,20]. The final protein precipitation procedure was based on that published by Signs et al. [19]. It was necessary to dilute the supernatant before injection.

3.2. Method validation

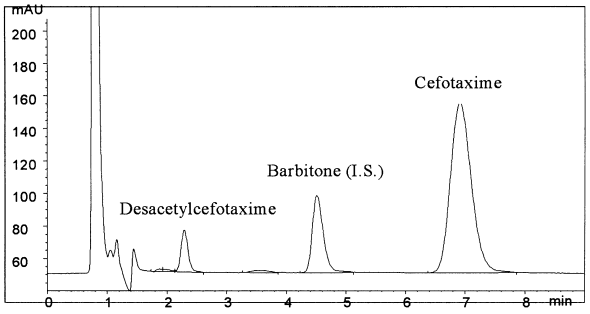
Calibration curves for cefotaxime and desacetylcefotaxime in CSF were generated by linear (weighted $1/\text{conc.}^2$) regression of peak height ratios against their respective concentrations, while cefotaxime and desacetylcefotaxime in plasma were calibrated using a Wagner (log quadratic) regression of peak height ratios against concentration, over the calibration ranges (0.500–940 μg/ml for cefotaxime in plasma, 0.403–55.8 μg/ml for cefotaxime in CSF, 0.402–148 μg/ml for desacetylcefotaxime in plasma, and 0.11–36.0 μg/ml for desacetylcefotaxime in CSF).

3.3. Chromatography

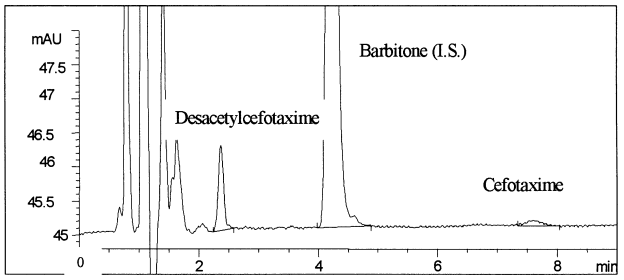
Good chromatographic results were obtained with the extracts of both matrices. Typical chromatograms obtained from blank plasma and plasma containing cefotaxime and desacetylcefotaxime in plasma and CSF are shown in Fig. 2. Under the chromatographic conditions described, cefotaxime and desacetylcefotaxime had retention times of 6.8 and 2.2 min, respectively, with no interfering co-elution of endogenous substances. Fig. 2A shows a subject plasma sample taken immediately post infusion, Fig. 2B shows a CSF subject sample taken 6 h post infusion and Fig. 2C shows a blank control study plasma sample.

3.4. Recovery

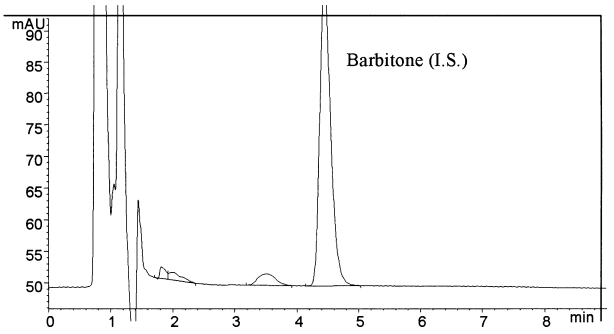
Extraction recovery of cefotaxime and desacetylcefotaxime from plasma and CSF was assessed in triplicate by calculating the ratio (analyte:internal standard) of the peak heights in extracts and compar-



A (Cefotaxime / desacetylcefotaxime in patient plasma (383 / 29.3 μg/ml)



B (Cefotaxime / desacetylcefotaxime in patient CSF (BLQ / 1.64 μg/ml)



C (Blank control study plasma sample of patient)

Fig. 2. Chromatograms of study samples (A and B) and a blank plasma sample extract (C). (A) Cefotaxime/desacetylcefotaxime in patient plasma (383/29.3 μg/ml). (B) Cefotaxime/desacetylcefotaxime in patient CSF (BLQ/1.64 μg/ml). (C) Blank control study plasma sample of patient.

ing them with the same ratio in pure solutions. Extraction recovery of internal standard was determined using internal standard:analyte ratio. The mean extraction yields were 78%, 88% and 74% for cefotaxime, desacetylcefotaxime and barbitone, respectively.

3.5. Sensitivity

Compared to previously published methods [4–7,10,15], sensitivity was improved. The lower limits of quantification were determined as 0.58 μg/ml for cefotaxime in plasma, 0.800 μg/ml for cefotaxime in

CSF, 0.540 μg/ml for desacetylcefotaxime in plasma and 0.500 μg/ml for desacetylcefotaxime in CSF.

The LLOQ is defined as that concentration of the analyte which can still be determined with acceptable precision (C.V. %<20) and accuracy (bias<20%) for the purposes of the particular application.

3.6. Inter- and intra-day accuracy and precision

Within a single batch, the method was found to be both accurate and precise (Table 1). Quality controls were included in every batch of samples assayed. Since the number of study samples in this case was so low and since the assays of these samples were performed over a period of about 8 months, a limited re-instatement validation was performed on three occasions with a freshly prepared set of calibration standards and quality controls. These new standards and controls were prepared as closely as possible to the original validated set. As the concentrations of the prepared controls were not exactly the same concentrations on all these occasions, the measured concentrations on each occasion were correlated against the original validated nominal concentrations. The mean r^2 values were 0.9966 and 0.9947 for cefotaxime in plasma and CSF, respectively and 0.9962 and 0.9975 for desacetylcefotaxime in plasma and CSF, respectively. These correlation coefficients indicate that the found concentrations of subsequent sets of quality controls were accurate. The inter-day reproducibility of the calibration curve slope of the linear (weighted $1/\text{conc.}^2$) regression in the case of CSF is presented in Table 2 while in the case of the plasma samples, where a log-quadratic ($\ln(y) =$

Table 2
Coefficients of variation for calibration curve slopes for all study batches over a period of 8 months

Analyte	Plasma (*b* parameter) ($n=4$)	CSF ($n=3$)
Cefotaxime	5.5	8.0
Desacetylcefotaxime	21.2	12.8

Table 1
Intra-day accuracy and precision

	Code	QC A	QC B	QC C	QC D	QC E	QC F	QC G	QC H	QC I	QC J
Cefotaxime in plasma	Nominal (μg/ml)	N/A	0.600	2.39	4.07	14.5	35.0	85.3	194	388	733
	Mean ($n=5$)		0.602	2.46	4.15	15.0	33.7	86.5	200	405	783
	% Nom		100.0	102.8	101.9	104.1	96.3	101.4	103.5	104.6	106.8
	C.V. (%)		10.2	6.0	14.7	9.2	6.8	9.6	6.4	8.9	7.7
Cefotaxime in CSF	Nominal (μg/ml)	N/A	0.630	1.25	N/A	2.70	4.06	6.06	12.1	24.2	43.7
	Mean ($n=5$)		0.604	1.23		2.53	3.90	6.14	12.4	25.9	45.8
	% Nom		95.9	98.1		93.8	96.1	101.3	101.9	106.9	104.8
	C.V. (%)		4.4	6.6		11.5	8.5	0.8	2.2	0.9	0.6
Desacetyl-cefotaxime in plasma	Nominal (μg/ml)	0.170	0.320	0.600	1.64	3.27	6.07	8.90	24.7	49.5	89.0
	Mean ($n=5$)	0.198	0.331	0.648	1.50	3.25	6.13	9.05	25.43	49.9	89.8
	% Nom	119.4	103.6	107.8	91.8	99.5	101.0	101.7	102.8	100.9	100.9
	C.V. (%)	10.8	11.1	4.6	10.1	4.0	5.2	3.2	3.2	2.5	3.1
Desacetyl-cefotaxime in CSF	Nominal (μg/ml)	N/A	0.360	0.720	N/A	1.55	2.33	3.48	6.96	13.9	25.1
	Mean ($n=5$)		0.339	0.692		1.54	2.28	3.52	7.22	14.9	26.4
	% Nom		93.8	96.1		99.5	97.6	101.1	103.7	107.1	105.4
	C.V. (%)		2.5	4.8		1.2	6.5	0.2	1.1	2.2	1.7

$a(\ln(x)^2) + b\ \ln(x) + c$) regression was used, the RSD of the *b* parameter is reported. Considering the long intervals between batches assayed and the fact that the assay method had to be re-instated on each occasion, this represents quite a high degree of ruggedness.

3.7. Specificity

Interference from endogenous substances did not occur from drug-free plasma (Fig. 2C). All peaks of interest were well separated.

3.8. Application

The described method can be used to assay large numbers of samples in a relatively short time. The small volumes of biological fluids required for the extraction allowed this method to be applied to samples taken from neonates and children.

3.9. Conclusion

This method is an effective method of rapidly and simultaneously quantifying cefotaxime and desacetylcefotaxime in human plasma and CSF.

References

[1] T. Annesley, K. Wilkerson, K. Matz, D. Giacherio, Clin. Chem. 30 (6) (1984) 908–910.
[2] T. Bergen, R. Solberg, Chemotherapy 27 (1981) 155–165.
[3] M.-D. Blanchin, M.-L. Rondot-Dudragne, H. Fabre, B. Mandrou, Analyst 113 (1988) 899–902.
[4] A.M. Brisson, J.B. Fourtillan, J. Chromatogr. 223 (1981) 393–399.
[5] C.Y. Chan, K. Chan, G.L. French, J. Antimicrob. Chemother. 18 (1986) 537–545.
[6] D. Dell, J. Chamberlain, F. Coppin, J. Chromatogr. 226 (1981) 431–440.
[7] F.M. Demotes-Mainard, G.A. Vinçon, C.H. Jarry, H.C. Albin, J. Chromatogr. 336 (1984) 438–445.
[8] H. Fabre, G. Castaneda Penalvo, J. Liq. Chromatogr. 18 (18-19) (1995) 3877–3887.
[9] H. Fabre, W.Th. Kok, Anal. Chem. 60 (1988) 136–141.
[10] H. Fabre, M.-D. Blanchin, U. Tjaden, Analyst 111 (1986) 1281–1284.
[11] C.E. Fasching, L.R. Peterson, Antimicrob. Agents Chemother. (1982) 628–633.
[12] L. Hakim, D.W.A. Bourne, E.J. Triggs, J. Chromatogr. (Biomed. Appl.) 424 (1988) 111–117.
[13] E.M. Hammond, M. Legge, A.B. MacLean, Med. Lab. Sci. 41 (1984) 299–301.
[14] F. Kees, E. Strehl, K. Seeger, G. Seidel, P. Dominiak, H. Grobecker, Arzneimittel-Forschung/Drug Res. 31(I) (2) (1981) 362–365.
[15] R. Krausse, U. Ullmann, G. Brandstetter, P. Kratochvil, Arzneimittel-Forschung/Drug Res. 34(II) (12) (1984) 1787–1791.
[16] M. LeBel, J.F. Ericson, D.H. Pitkin, J. Liq. Chromatogr. 7 (5) (1984) 961–968.
[17] J.B. Lecaillon, M.C. Rouan, C. Souppart, N. Febvre, F. Juge, J. Chromatogr. 228 (1982) 257–267.
[18] C.M. Paap, M.C. Nahata, J. Liq. Chromatogr. 12 (12) (1989) 2385–2395.
[19] S.A. Signs, T.M. File, J.S. Tan, Antimicrob. Agents Chemother. (1984) 652–655.
[20] R.L. Yost, H. Derendorf, J. Chromatogr. 341 (1985) 131–138.