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Determination of cefotaxime and desacetylcefotaxime in cerebrospinal fluid by solid-phase extraction and high-performance liquid chromatography

Jolanta J. Bafeltowska[a,*], Ewa Buszman[a], Krzysztof Mandat[b], Jadwiga Hawranek[b]

[a]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Medical University of Silesia, Jagiellońska 4, 41-200 Sosnowiec, Poland*

[b]*Division of Pediatric Surgery, Centre of Pediatrics and Rehabilitation, Truchana 7, 41-500 Chorzów, Poland*

Abstract

A high-performance liquid chromatographic procedure has been developed for the measurement of cefotaxime and desacetylcefotaxime in cerebrospinal fluid. Both compounds were isolated from cerebrospinal fluid samples using solid-phase extraction (SPE). LiChrolut RP-18 (200 mg; 3 ml) columns and a mixture of methanol–phosphate buffer pH 7 (1:1) were applied to elute cefotaxime and its desacetyl metabolite. The separation was performed on a LiChrospher 100 RP-18 (5 μm; 250×4 mm I.D.) column. The mobile phase consisted of 0.01 *M* acetate buffer pH 4.8–methanol (85:15), flow-rate was 1.5 ml/min. Cefotaxime and desacetylcefotaxime were detected at a wavelength of 254 nm by UV–Vis detector. The range of concentrations for method calibration and for analytical studies was 1.56–100 μg/ml. The quantitation limit in cerebrospinal fluid was 0.39 μg/ml for cefotaxime and 0.78 μg/ml for desacetylcefotaxime. The extraction recovery from cerebrospinal fluid spiked with cefotaxime and desacetylcefotaxime was 90.4–100.1% and 97.4–102.9%, respectively. The RSDs were below 10.7% for cefotaxime and 6.8% for desacetylcefotaxime. The developed SPE–HPLC method was applied for cefotaxime and desacetylcefotaxime determination in cerebrospinal fluid of children with hydrocephalus after intraventricular administration.

Keywords: Cefotaxime; Desacetylcefotaxime; Cephalosporins; Antibiotics

1. Introduction

Cefotaxime, a semisynthetic third-generation cephalosporin exhibits potent activity against many Gram-negative species, including *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Haemophilus influenzae* and *Neisseria gonorrhoeae*. It is stable to most bacterial β-lactamases.

In humans, cefotaxime is metabolized by esterases to the active metabolite desacetylcefotaxime and several non-active metabolites [1–6]. The desacetyl metabolite has about 10% of the activity of the parent against Enterobacteria, less against *Staphylococcus aureus* and *Bacillus fragilis*. The efficacy of antibiotic therapy is related to the length of time that drug levels are above the minimum inhibitory concentration (MIC) [1,7,8]. Pharmacokinetic studies

*Corresponding author. Tel.: +48-32-292-5541x400; fax: +48-32-266-7860.

E-mail address: jbafeltowska@slam.katowice.pl (J. Bafeltowska).

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can help to establish the optimal dosage regimens in specific populations.

A high-performance liquid chromatography (HPLC) method is the most common technique for cephalosporin determination in physiological fluids [3,5,9–11]. Several methods have been described for simultaneous quantification of cefotaxime and desacetylcefotaxime in plasma, serum and ocular aqueous humor, including HPLC [2–5,9,12–14] and microbiological [1] methods.

For cefotaxime and desacetylcefotaxime a HPLC method has been described by Kraemer et al. [2] based on solid-phase extraction (SPE) and reversed-phase HPLC with detection by ultraviolet absorbance at 254 nm. The SPE procedure offers advantages in terms of concentration of the analyte. In the method of simultaneous quantification of both compounds described by Dell et al. [4] the plasma or serum samples were mixed with chloroform–acetone to remove proteins and most lipid material. The aqueous phase was freeze-dried, reconstituted in mobile phase and chromatographed on a reversed-phase column using UV detection at 262 nm.

Fabre and Kok [5] determined cefotaxime and its deacetylated metabolite in biological samples by liquid chromatography with indirect electrochemical detection. Castaneda Penalvo et al. [6] described capillary zone electrophoresis and micellar electrokinetic capillary chromatography (MECC) with direct injection of plasma for determination of cefotaxime and its metabolite. They suggest that MECC is an attractive alternative to HPLC in therapeutic drug monitoring.

We propose a simplified SPE procedure of cerebrospinal fluid with methanol–phosphate buffer pH 7.0 (1:1) to elute cefotaxime and desacetylcefotaxime, separation on a reversed-phase column and detection at 254 nm.

The aim of this study was to develop the method of simultaneous cefotaxime and desacetylcefotaxime determination in cerebrospinal fluid to conduct pharmacokinetic studies during central nervous system infection treatment in children with hydrocephalus.

2. Experimental

2.1. Chemicals

Cefotaxime sodium salt was obtained from European Pharmacopoeia Conseil de l'Europe (Strasbourg, France). Desacetylcefotaxime was kindly donated by the Institute of Biotechnology and Antibiotics (Warsaw, Poland). The other chemicals: methanol (HPLC grade) and phosphate buffer pH 7.0 were obtained from Merck (Darmstadt, Germany), sodium acetate and 0.1 *M* acetic acid in the highest available purity were from Chemical Reagent (Gliwice, Poland), C_{18} SPE columns Bakerbond spe Octadecyl (200 mg, 3 ml) were from J.T. Baker (Deventer, The Netherlands).

2.2. Chromatographic conditions

The isocratic liquid chromatographic system (Merck–Hitachi, Darmstadt, Germany) consisted of a pump (Model D-7100) connected via a reversed-phase LiChrospher 100 RP-18 column (5 μm, 250× 4 mm I.D., Merck) to a UV–Vis detector (Hewlett-Packard, Model 1050, Waldbronn, Germany) with a fixed wavelength of 254 nm. The injector was a Rheodyne (Model 7125) manual injection valve, fitted with a 20-μl sample loop (Cotati, CA, USA). Chromatograms were processed by a HPLC-System-Manager HSM D-7000 (Merck–Hitachi). The column was kept in methanol (100%) when not in use for more than 2 days. The mobile phase, 0.01 *M* acetate buffer pH 4.8–methanol (85:15), was freshly prepared and degassed by 10 min sonification in an ultrasonic bath (Ultron, Model U-501, Olsztyn, Poland). The flow-rate was 1.5 ml/min at room temperature (23 °C) (column oven Model L-7360, Merck).

2.3. Preparation of standard solutions and standard samples

Stock solutions of cefotaxime and desacetylcefotaxime (1 mg/ml) were prepared in phosphate buffer pH 7.0. Standard solutions containing the analytes in the concentration range 15.6–500 μg/ml were obtained by dilution with the same buffer.

For calibration curves, as well as for precision and accuracy evaluation, spiked control samples containing cerebrospinal fluid and cefotaxime or desacetylcefotaxime in concentrations 1.56, 3.12, 12.5, 50 and 100 μg/ml were prepared.

2.4. Cerebrospinal fluid samples and clinical application

To develop a method of determination of cefotaxime and desacetylcefotaxime in cerebrospinal fluid, blank cerebrospinal fluid was used. All cerebrospinal fluid specimens were collected and frozen at −20 °C until assayed.

From March 2000 to September 2001 cefotaxime and desacetylcefotaxime were monitored in two children with hydrocephalus for therapeutic purposes in shunt infections. The children were from 91 to 463 days old during the antibiotic therapy. Cefotaxime was administered intraventricularly in doses 50–10 mg/day, depending on cefotaxime and desacetylcefotaxime concentrations in cerebrospinal fluid during therapeutic drug monitoring.

The cerebrospinal fluid samples were taken from patients from 10 to 28 h after intraventricular administration of antibiotic. The samples of cerebrospinal fluid were taken from children by anterior fontanelle, external drainage, puncture of the shunt reservoir or in the course of the operation.

2.5. Solid phase extraction of cerebrospinal fluid samples

C_{18} SPE columns were conditioned with 2×1 ml of methanol and 2×1 ml of water (do not allow column to run dry) in a J.T. Baker vacuum manifold (12 columns). One ml of cerebrospinal fluid samples were applied to the preconditioned extraction columns and then they were washed with 2×1 ml of water. Application of vacuum (400 mbar) for 10 min, and centrifugation for 15 min at 3000 *g* removed traces of humidity prior to elution of absorbed compounds with a 400-μl mixture of methanol–phosphate buffer pH 7.0 (1:1). The eluates were collected in 2.0-ml Eppendorf tubes and 20-μl aliquots were injected onto the HPLC column.

3. Results

Representative chromatograms of drug-free cerebrospinal fluid and cerebrospinal fluid sample containing 12.5 μg/ml cefotaxime and 12.5 μg/ml desacetylcefotaxime are shown in Fig. 1. The retention times of desacetylcefotaxime and cefotaxime are in the order 3.3 and 13.8 min, respectively. Extraction and chromatographic analysis of six separate blank cerebrospinal fluid samples confirmed that there were no endogenous peaks that coeluted with both analyzed compounds.

In this study an internal standard was not used. According to Pehourcq and Jarry [3], the use of an internal standard is a controversial point, since one can never be sure that, for a given sample, the extraction recoveries of the internal standard and the drug will be identical. Moreover, an internal standard may interfere with endogenous cerebrospinal fluid compounds which absorb UV light at the same wavelength.

The specificity of the HPLC method was examined by determination of samples containing

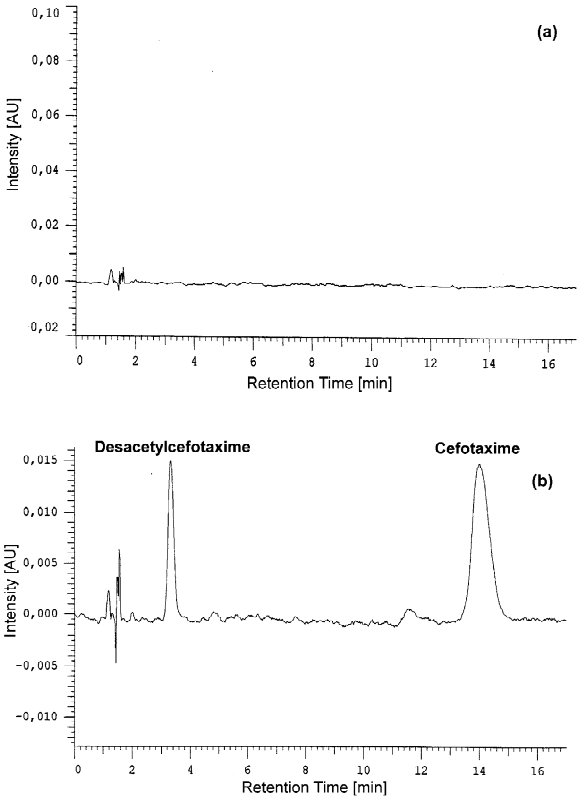

Fig. 1. Chromatograms of blank cerebrospinal fluid (a), and cerebrospinal fluid containing 12.5 μg/ml of cefotaxime and 12.5 μg/ml of desacetylcefotaxime (b).

cefepime, ceftriaxone, ceftazidime, cefuroxime, cefradine, piperacillin, imipenem, meropenem, vancomycin, netilmicin and fluconazole added in a quantity of 100 μg/ml to drug-free cerebrospinal fluid samples. On chromatograms, interfered peaks were not found.

The linearity study was carried out with cefotaxime and desacetylcefotaxime concentrations in cerebrospinal fluid ranging from 1.56 to 100 μg/ml. The regression analysis between peak areas and cerebrospinal fluid concentrations of each compound revealed that the standard curves are linear: desacetylcefotaxime $y = 5.637 \times 10^{-5}x$ ($r = 0.9921$), cefotaxime $y = 3.843 \times 10^{-5}x$ ($r = 0.9984$). The quantitation limits were 0.78 μg/ml for desacetylcefotaxime and 0.39 μg/ml for cefotaxime. The extraction recovery from cerebrospinal fluid spiked with cefotaxime and desacetylcefotaxime was respectively: 90.4–100.1% and 97.4–102.9%.

The precision of the method was characterized by RSDs of 1.4–10.7% (1.56–100 μg/ml) for cefotaxime and of 3.7–6.8% (1.56–100 μg/ml) for desacetylcefotaxime (Table 1).

The method of determination of cefotaxime and desacetylcefotaxime in cerebrospinal fluid by the SPE–HPLC technique was applied to determine the levels of both compounds in cerebrospinal fluid samples in two children with hydrocephalus after intraventricular administration of cefotaxime. A typical chromatogram of a processed cerebrospinal fluid sample of a patient is shown in Fig. 2.

Table 1
Precision and accuracy of cefotaxime and desacetylcefotaxime assay ($n = 5$)

Substance	Concentration (μg/ml)			RSD (%)	Recovery (%)
	Added	Found			
		Mean	SD		
Cefotaxime	1.56	1.56	0.167	10.7	100.0
	3.12	2.86	0.130	4.6	90.4
	12.50	11.96	0.365	3.0	95.7
	50.00	49.58	0.709	1.4	99.2
	100.00	100.14	5.051	5.0	100.1
Desacetyl-cefotaxime	1.56	1.56	0.106	6.8	100.3
	3.12	3.05	0.112	3.7	97.6
	12.50	12.18	0.626	5.1	97.4
	50.00	51.46	1.903	3.7	102.9
	100.00	102.22	4.644	4.5	102.2

SD, standard deviation; RSD, relative standard deviation.

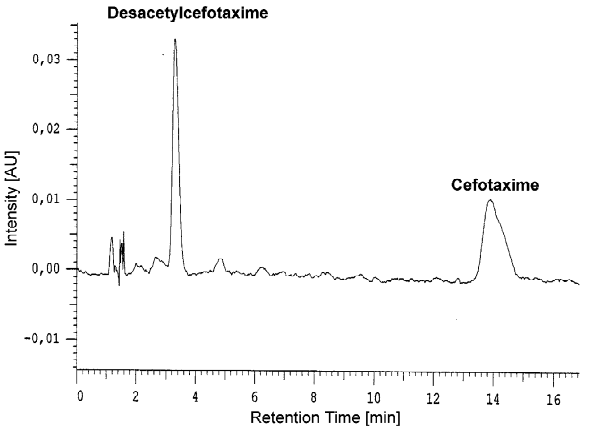

Fig. 2. Chromatogram of cerebrospinal fluid from a patient receiving intraventricularly 15 mg of cefotaxime 24 h before taking a sample.

The cefotaxime and its desacetyl metabolite concentrations determined at steady state after intraventricular treatment were higher (from 17.7 to 112.8 μg/ml for cefotaxime and from 1.4 to 17.2 μg/ml for desacetylcefotaxime) than the therapeutic recommended range (<20 μg/ml for cefotaxime) [1,2,7]. The values of ventricular cerebrospinal fluid concentrations of cefotaxime and desacetylcefotaxime after intraventricular cefotaxime administration are shown in Fig. 3.

4. Discussion

HPLC is an analytical approach for quantitating β-lactam antibiotics in different biological fluids [2–4,9,12,13]. In this study cefotaxime and its active metabolite desacetylcefotaxime were determined simultaneously in the cerebrospinal samples by the use of SPE–HPLC. Due to HPLC specificity and sensitivity, this method is more suitable than microbiological assays [3]. Microbiological determinations of cefotaxime are not selective, since its metabolite desacetylcefotaxime interferes with the assay techniques, and thus are not appropriate for

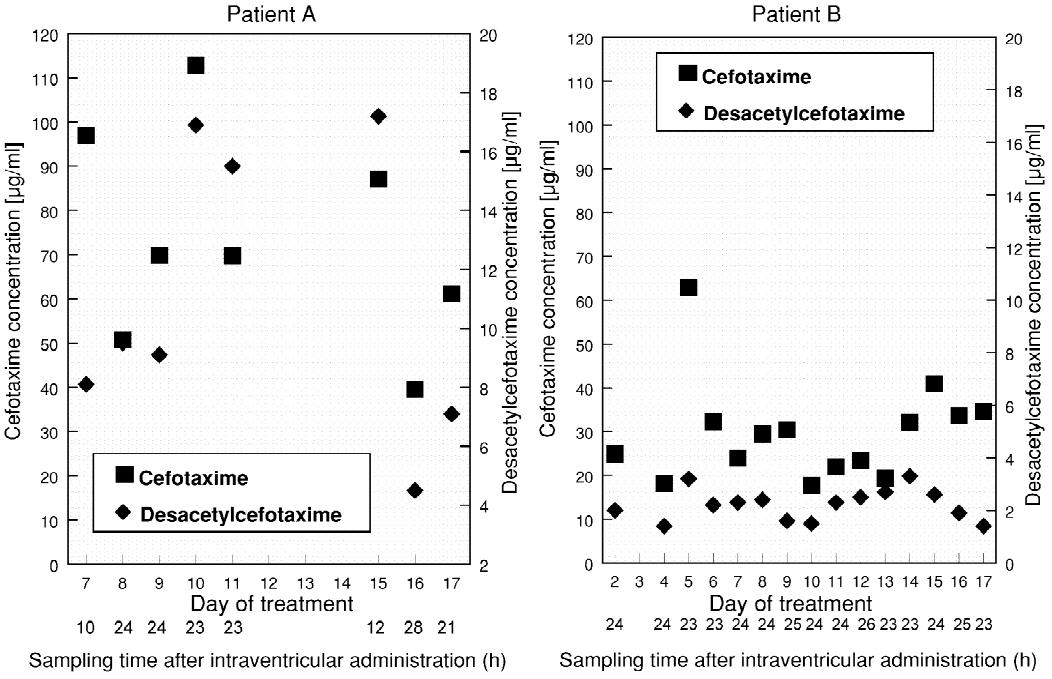

Fig. 3. Patients' ventricular cerebrospinal fluid concentrations of cefotaxime and desacetylcefotaxime after intraventricular cefotaxime administration.

pharmacokinetic studies [9]. Moreover, microbiological methods are very time-consuming.

The HPLC method for cefotaxime and desacetylcefotaxime determination described here represents a rapid, sensitive, efficient and analytical approach for quantitating both compounds in cerebrospinal fluid. The analytical characteristics of the proposed method are satisfactory for pharmacokinetic and clinical laboratory investigations.

Cefotaxime or ceftriaxone are usually used in the therapy of meningitis. These agents are active against ampicillin-resistant *Haemophilus influenzae* and most strains of penicillin-resistant *Streptococcus pneumoniae* [5,8,11,15].

The developed method has been applied to the analyses of cerebrospinal fluid cefotaxime and desacetylcefotaxime concentrations in children with hydrocephalus after intraventricular administration of cefotaxime. The cefotaxime and desacetylcefotaxime levels determined at steady state were much higher than the therapeutic recommended concentrations. The results of pharmacokinetic studies will be reported elsewhere. The method of cefotaxime and desacetylcefotaxime determination in cerebrospinal fluid by the use of SPE–HPLC technique is specific, precise and allows to conduct pharmacokinetic studies.

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