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Liquid chromatographic determination of the plasma concentrations of cefotaxime and desacetylcefotaxime in plasma of critically ill patients

M.T. Rosseel[a,*], K.H. Vandewoude[b]

[a] *Faculty of Medicine and Health Sciences, Heymans Institute of Pharmacology, Ghent University, De Pintelaan 185, B-9000 Ghent, Belgium*
[b] *Department of Intensive Care, Ghent University Hospital, De Pintelaan 185, B-9000 Ghent, Belgium*

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Abstract

A method for the simultaneous determination of cefotaxime (CTX) and desacetylcefotaxime (DES) in plasma was developed, using acetonitrile protein precipitation and high-performance liquid chromatography (HPLC) with UV-detection at 285 nm. Desacetylcefotaxime was also analysed after conversion in highly acidic medium to its lactone form (DES-lactone). The chromatographic separation was performed on a C18 Aqua column. The lower limit of quantitation was 1 μg/ml for CTX and 0.5 μg/ml for DES and DES-lactone, using 25 μl of plasma samples. The linearity of the calibration curves was satisfactory as indicated by correlation coefficients of ≥0.990. The within-day and between-day precisions were <12% (n = 18) for the two products and the accuracy was between 88 and 101%. The developed HPLC method was applied for CTX and DES determination in plasma samples of critically ill patients after continuous intravenous infusion of CTX.

Keywords: Cefotaxime; Desacetylcefotaxime; HPLC

1. Introduction

Cefotaxime (CTX), a semisynthetic third-generation cephalosporin, exhibits potent activity against many Gram-negative and Gram-positive species.

In humans, CTX is metabolized by esterases to its active metabolite desacetylcefotaxime (DES) and several non-active metabolites [1].

For betalactams, the important determinant of their efficacy is the time the plasma concentration is above the minimal inhibitory concentration (MIC) for the presumed pathogen [2]. Previous data for different betalactams suggest that pharmacodynamic parameters are improved by continuous intravenous infusion. Cefotaxime is well established as an effective and well-tolerated drug for parenteral treatment of moderate and severe pneumonia in hospitalised patients.

Describing the pharmacokinetic profile of CTX in critically ill patients after continuous intravenous infusion is of interest and can help to establish the optimal dosage required in order to optimize antibacterial effect.

Several HPLC methods have been described for simultaneous quantification of CTX and DES in plasma. Some use solid-phase extraction (SPE) [3,4] and reversed-phase HPLC, others protein precipitation [5–7] or protein precipitation followed by solvent extraction as purification step [8–11]. These methods were either not sensitive enough or required large volumes of matrix [3–6,8–11]. Only the selectivity towards endogenous plasma material is tested [3–11]. Capillary zone electrophoresis and micellar electrokinetic capillary chromatography (MECC) with direct injection of plasma for determination of CTX and DES have also been described [12].

The aim of the study was to develop a HPLC method for the quantification of CTX and DES in human plasma samples of critically ill patients after continuous intravenous infusion of CTX. A C18 Aqua column retaining both polar

* Corresponding author. Tel.: +32 9 2403359; fax: +32 9 2404988.
E-mail address: marietherese.rosseel@ugent.be (M.T. Rosseel).

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and non-polar compounds significantly longer than a conventional end-capped C18 column was used.

Selectivity towards co-administered drugs was tested. As plasma components and (or) co-medication can interfere with the very polar DES [10], DES was also analysed after conversion to its more lipophilic lactone form.

2. Experimental

2.1. Chemicals and reagents

Cefotaxime sodium salt, DES sodium salt and DES-lactone were obtained from Hœchst Marion Roussel (Frankfurt, Germany). The internal standard cefuroxime was obtained from Glaxo (Greenford, UK). Ammoniumdihydrogen phosphate, phosphoric acid, triethylamine (TEA) and tris(hydroxymethyl)aminomethane (TRIS) pro analysis were from Merck (Darmstadt, Germany). Acetonitrile far UV was from Acros Organics (Geel, Belgium) and 60% perchloric acid was from UCB (Leuven, Belgium). Pure water was obtained with a reverse osmosis system from Labo-eco (Aartselaar, Belgium) and further purified with a Simplicity 185, ultra pure water system from Millipore (Bedford, MA, USA).

2.2. Subjects

Blood samples were obtained from five critically ill patients (aged 28–70 years) with severe community acquired pneumonia or early onset nosocomial ventilator associated pneumonia.

A loading dose of 2 g of CTX was given intravenously over 30 min, followed by 4 g of CTX in continuous infusion over 24 h, divided in two portions of 2 g in 50 ml dextrose 5%. Concomitant antibiotic therapy was allowed with the exception of other cephalosporins. All blood samples were drawn from an arterial line, and sampled in heparinized tubes with venosafe VF-106Sahl Autosep 6 ml gel + LiHep. Blood sampling was done before and immediately after the loading dose, every 4 h during the 1st day and subsequently every 12 h until day 4 of treatment. The plasma samples were obtained by direct centrifugation of the blood samples for 10 min at 2000 × *g* at 4 °C, and stored at −20 ° C until analysis, at least within 4 weeks. Approximately, 34 different drugs (mainly analgetics, antihypertensives, diuretics, anti-epileptics, antibiotics, antidepressants, . . .) were administered to these patients.

2.3. Apparatus and chromatographic conditions

The HPLC system consisted of a Varian 9010 solvent delivery system (Varian Associates, Walnut Creek, CA, USA), an automatic injector Merck–Hitachi AS 2000A, with a 100 μl loop (Merck), a Waters UV detector 2487 (Waters, Milford, MA, USA) and a HP integrator (Hewlett-Packard, Avondale, PA, USA). The compounds were separated on a C18 Aqua column (150 mm × 4.6 mm, 5 μm) with a guard column (4.0 mm × 3.0 mm) packed with reversed-phase C18 material (Phenomenex, Torrance, CA, USA). The mobile phase consisted of a mixture of 50 mM ammonium dihydrogen phosphate buffer (pH 3.0)–acetonitrile–triethylamine (87.8:12.0:0.2, v/v/v) adjusted to pH 3.0 with 85% phosphoric acid at a flow rate of 1 ml/min. UV detection was performed at 285 nm.

2.4. Analytical procedure

2.4.1. Two different methods were used

For the assay of CTX and DES: to a 25 μl volume of plasma in 1.5 ml Eppendorf tube, 20 μl of an aqueous cefuroxime solution (10 μg/ml) as internal standard (IS), and 50 μl of acetonitrile were added (plasma samples not within the range of the calibration curves were diluted with blank plasma and analysed twice). The plasma was vortex mixed for 15 s. Subsequently, the tubes were centrifuged at 11,600 × *g* for 5 min in an Eppendorf 5415C centrifuge (Eppendorf, Hamburg, Germany). The supernatant was transferred into a clean 5 ml glass tube and was evaporated to dryness under a gentle stream of nitrogen at room temperature. The residue was dissolved in 125 μl of 50 mM ammonium dihydrogen phosphate buffer (pH 3.0)–acetonitrile (88:12, v/v). A volume of 100 μl was injected onto the HPLC column.

For the assay of DES in its lactone form: a 25 μl volume of plasma was diluted with 75 μl of NaCl 0.9% in a 1.5 ml Eppendorf tube and 200 ng cefuroxime as IS and 20 μl of 20% perchloric acid were added. The solution was vortex mixed for 15 s. Subsequently, the tubes were centrifuged at 11,600 × *g* for 5 min and were kept at room temperature for 1 h until the formation of the lactone form was complete; again the solution was centrifuged. 125 μl of the supernatant was added to 50 μl of 50 mM ammonium dihydrogen phosphate buffer (pH 3.0) and 28 μl of 2 M TRIS in a 1.5 ml Eppendorf tube. The solution was vortex mixed for 15 s. A volume of 100 μl was injected onto the HPLC column.

2.5. Calibration curves and validation of the method

Stock solutions of CTX (1 mg/ml) and of DES (1 mg/ml) were prepared in water, dilutions were made in water. Calibration curves were prepared in blank human plasma, aliquoted and stored at −20 °C. Cefotaxime in plasma, kept at −20 °C, is stable for at least four weeks.

The calibration curves consisted of seven points ranging from 0.5 to 25 μg/ml of DES and from 1 to 50 μg/ml of CTX. Quality control (QC) samples at low (1 μg/ml DES, 2 μg/ml CTX), medium (10 μg/ml DES, 20 μg/ml CTX), and high (20 μg/ml DES, 40 μg/ml CTX) concentrations were prepared by spiking blank human plasma with DES-diluted and CTX-diluted stock solution (other than the curve stock solutions). These samples were aliquoted and stored at −20 °C until use. The calibration curve equations were estimated for the concentration range used by linear least-squares regression model, using a weighing factor $1/\text{conc}^2$. Selectivity of

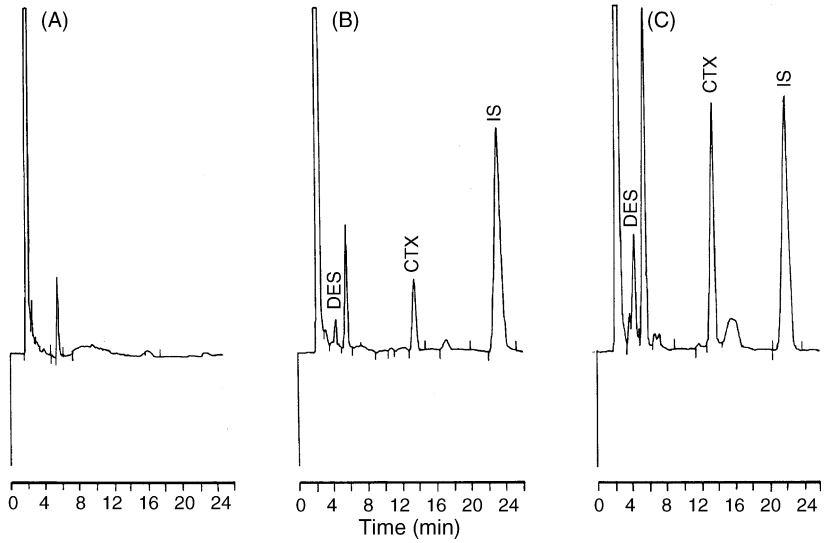

Fig. 1. Chromatograms of (A) blank patient plasma, (B) blank patient plasma spiked with 0.75 μg/ml DES and 2.5 μg/ml CTX and (C) patient D plasma sample containing 3.4 μg/ml DES and 9.0 μg/ml CTX 96 h after continuous CTX infusion. No detectable plasma concentrations of DES-lactone are present in the patient plasma sample after continuous CTX infusion. The internal standard (IS) cefuroxime is also present.

the assay in respect to endogenous plasma matrix substances and to co-administered drugs, was determined by analysis of blank plasma from 10 different critically ill patients taking 44 different drugs. Accuracy, between-day and within-day precision of the method, were determined by assay, six replicates of each of the three quality control samples on 3 different days. The accuracy was calculated at each test concentration and was obtained by dividing the mean measured concentration, obtained from all quality control samples on the 3 days, by the nominal concentration and multiplying with 100%. The within-day and between-day precisions were obtained by analysis of variance (ANOVA) for each test concentration. Accuracy and precision are expressed as analytical recovery and relative standard deviation (R.S.D.), respectively. The limit of quantification (LOQ) is defined as the lowest concentration of the calibration curve, six times analysed, for which the R.S.D. is less than 20% and the accuracy is between 80 and 120%.

2.6. Comparison of desacetylcefotaxime concentrations analysed as desacetylcefotaxime and as desacetylcefotaxime-lactone

Critically ill patient plasma concentrations of DES after continuous intravenous infusion of CTX, determined using the acetonitrile protein precipitation method, were compared

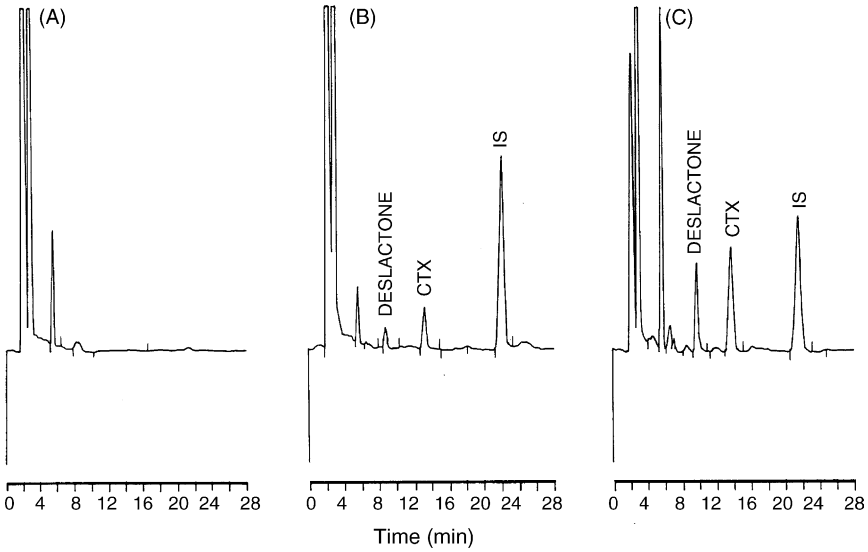

Fig. 2. Chromatograms of (A) blank patient plasma, (B) blank patient plasma spiked with 0.75 μg/ml DES (converted to DES-lactone during the analysis) and 2.5 μg/ml CTX and (C) patient D plasma sample containing 4.2 μg/ml DES-lactone (DES is converted to DES-lactone) and 8.9 μg/ml CTX 96 h after continuous CTX infusion. The internal standard (IS) cefuroxime is also present.

with DES-lactone concentrations, obtained after conversion of DES to its lactone form, by orthogonal distance regression [13].

The slope and intercept obtained were examined by a *t*-test to determine whether they were significantly different from 1 and 0, respectively. Ninety-five percent confidence limits were calculated to check whether the ideal slope and intercept fell within these limits. The confidence limits were calculated as follows: [slope or intercept] ± [standard deviation × t (α = 0.05, n = 2 d.f.)] [14].

3. Results and discussion

3.1. Chromatography

Fig. 1 shows chromatograms of blank patient plasma, blank patient plasma spiked with 0.75 μg/ml DES and 2.5 μg/ml CTX, and plasma obtained from a patient after continuous CTX intravenous infusion. The retention times of DES, CTX and cefuroxime (the internal standard) were 3.97, 13.21 and 21.37 min, respectively, under the chromatographic conditions described. Fig. 2 shows chromatograms of blank patient plasma, blank patient plasma spiked with 0.75 μg/ml DES (converted to DES-lactone in highly acidic medium) and 2.5 μg/ml CTX and plasma obtained from a patient after continuous CTX infusion (DES is converted to DES-lactone). The retention times of DES-lactone, CTX and cefuroxime (the internal standard) were 9.36, 13.37 and 21.21 min, respectively, under the chromatographic conditions described. No peaks from endogenous or co-medication compounds are present.

3.2. Validation

The regression analysis between the peak height ratios (product to internal standard) against their respective concentrations revealed that the calibration curves were linear in their respective ranges.

The mean ± S.D. regression lines are y = 0.114 (±0.009) x + 0.012 (±0.007) (r = 0.998), (n = 5), y = 0.151 (±0.035) x + 0.015 (±0.017) (r = 0.994), (n = 5) and y = 0.118 (±0.015) x + 0.053 (±0.067) (r = 0.990), (n = 5) for CTX, DES and DES-lactone, respectively.

Only one of the blank plasma samples collected from 10 different critically ill patients had an interfering peak with DES on the chromatogram. As it was the only patient taking ciprofloxacine, the interfering peak was probably a metabolite of ciprofloxacine. The R.S.D. obtained in the study of within-day and between-day precision was less than 9% for CTX, less than 12% for DES and less than 11% for DES-lactone. The accuracy was between 97 and 101% for CTX, between 95 and 100% for DES and between 88 and 99% for DES-lactone (Tables 1 and 2).

The LOQ was 1 μg/ml for CTX, using 25 μl of plasma with R.S.D. of 3.4% and an accuracy of 98%. The LOQ was 0.5 μg/ml for DES, using 25 μl of plasma with R.S.D. of 1.9% and an accuracy of 90%.

The precision and accuracy data demonstrate that the method is acceptable for CTX and for DES determined in both forms. For the diluted samples, analysed twice, the deviation from each other did not exceed 15%. The QC samples for CTX and DES, two for each concentration, did not deviate

Table 1
Precision (R.S.D.) and accuracy (analytical recovery) of the HPLC analysis of cefotaxime in human plasma

Concentration (μg/ml)	Accuracy (%)	Within-day precision (%) (n = 18, d.f. = 15)	Between-day precision (%) (n = 3, d.f. = 2)
2	100.9	2.6	3.5
20	99.7	3.6	8.6
40	97.3	3.3	

Table 2
Precision (R.S.D.) and accuracy (analytical recovery) of the HPLC analysis of desacetylcefotaxime in human plasma

Concentration (μg/ml)	Accuracy (%)	Within-day precision (%) (n = 18, d.f. = 15)	Between-day precision (%) (n = 3, d.f. = 2)
Desacetylcefotaxime			
1	99.3	4.2	4.7
10	98.5	3.7	11.3
20	95.6	3.8	2.2
Desacetylcefotaxime-lactone			
1	88.6	10.9	3.8
10	94.0	4.5	4.1
20	98.2	3.1	8.7

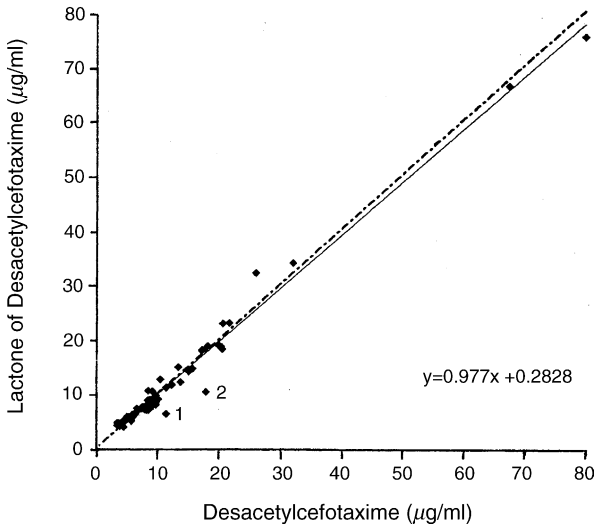

Fig. 3. Correlation between DES plasma concentrations from five patients after continuous i.v. infusion of CTX (65 samples) determined as DES and as DES-lactone. (—) Line fitted by orthogonal distance regression. (----) Line of equality. Two plasma samples (1 and 2) from patient B, with co-administration of metronidazole to the patient 84 and 96 h after starting the continuous infusion of CTX, have metronidazole interfering with DES on the chromatogram.

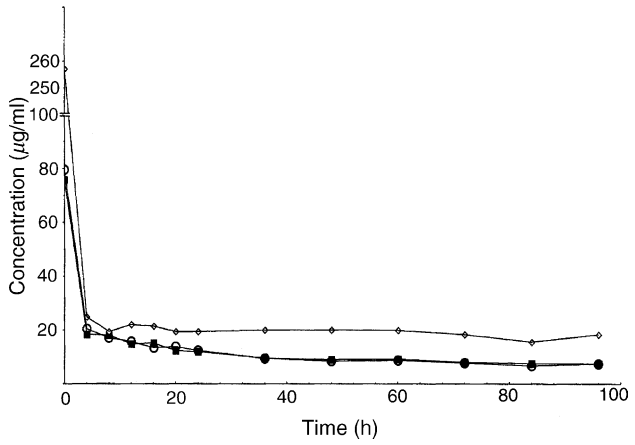

Fig. 4. The plasma concentration–time profiles of CTX (◊) and DES (◯) in patient C after a loading dose of 2 g of CTX over 30 min followed by a continuous i.v. infusion of CTX (4 g over 24 h). The plasma concentration–time curve of DES-lactone (■) (DES is converted to DES-lactone during the analysis) coincides with the curve of DES.

more than 15% from the nominal value during the five runs of patient plasma samples performed. These QCs stored at −20 °C were used for a period of one month and found to be stable [7]. The C18 Aqua column can be used without problems for at least 1 year.

3.3. Comparison of the two methods used to determine both forms of desacetylcefotaxime

A comparison of the DES plasma concentrations determined in paired samples of five patients, using the protein precipitation method and the assay after conversion of DES to its lactone form is illustrated in Fig. 3.

The DES concentrations were placed on the x-scale as this is the reference method. The slope and intercept of the orthogonal distance regression line with their confidence limits (α = 0.05) were 0.9774 ± 0.0351 and 0.2828 ± 0.6089 μg/ml, respectively (n = 65). The confidence intervals encompassed the ideal slope of unity and the ideal intercept of zero, indicating that the two methods are comparable to each other. The agreement between DES patient plasma concentrations determined by both methods is good.

Fig. 4 shows the plasma concentration–time curve of CTX and DES in a critically ill patient after continuous intravenous infusion of CTX. The plasma concentration–time profiles of DES, determined with both methods, were superimposable.

4. Conclusions

A sensitive, selective and validated analytical method for the determination of CTX and DES in plasma of critically ill patients was established by HPLC.

Selectivity in respect to co-administered drugs for the polar DES is enhanced by determination of DES also in its more lipophilic lactone form.

This method proved to be suitable for clinical pharmacokinetic studies of CTX. The results of the pharmacokinetic studies will be reported elsewhere.

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References

[1] K.B. Patel, D.P. Nicolau, C.H. Nightingale, R. Quintiliani, Diagn. Microbiol. Infect. Dis. 22 (1995) 49.
[2] J.D. Turnidge, Diagn. Microbiol. Infect. Dis. 22 (1995) 57.
[3] H.J. Kraemer, R. Gehrke, A. Breithaupt, H. Breithaupt, J. Chromatogr. B 700 (1997) 14.
[4] J.J. Bafeltowska, E. Buszman, K. Mandat, J. Hawranek, J. Chromatogr. A 976 (2002) 249.
[5] L. Hary, M. Andrejak, J. Chromatogr. 419 (1987) 396.
[6] L. Hakim, D.W.A. Bourne, E.J. Triggs, J. Chromatogr. 424 (1988) 111.
[7] T. Scanes, A.F. Hundt, K.J. Swart, H.K. Hundt, J. Chromatogr. B 750 (2001) 171.
[8] D. Dell, J. Chamberlain, F. Coppin, J. Chromatogr. 226 (1981) 431.
[9] R.L. Yost, H. Derendorf, J. Chromatogr. 341 (1985) 131.
[10] J.B. Lecaillon, M.C. Rouan, C. Souppart, N. Febvre, F. Juge, J. Chromatogr. 228 (1982) 257.
[11] F. Demotes-Mainard, G. Vincon, J.L. Bouchet, C. Jarry, H. Albin, Ann. Biol. Clin. 42 (1984) 301.
[12] G. Castaneda Penalvo, M'. Kelly, H. Mailloïs, H. Fabre, Anal. Chem. 69 (1997) 1364.
[13] D.L. Massart, B.G.M. Vandeginste, L. Buydens, S. de Jong, P. Lewi, J. Smeyers-Verbeke, Handbook of Chemometrics and Qualimetrics: Part A, Elsevier, Amsterdam, 1997.
[14] C. Hartmann, J. Smeyers-Verbeke, W. Penninckx, D.L. Massart, Anal. Chim. Acta 338 (1997) 19.