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Determination of ceftazidime in plasma using high-performance liquid chromatography and electrochemical detection Application for individualizing dosage regimens in elderly patients

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Abstract

This study describes a sensitive HPLC–electrochemical detection method for the analysis of ceftazidime, a third-generation cephalosporin, in human plasma. The extraction procedure involved protein precipitation with 30% trichloroacetic acid. The separation was achieved on a reversed-phase column (250×4.6 mm I.D., 5 μm) packed with C_{18} Kromasil with isocratic elution and a mobile phase consisting of acetonitrile–25 m*M* KH_2PO_4–Na_2HPO_4 buffer, pH 7.4 (10:90, v/v). The proposed analytical method is selective, reproducible and reliable. The assay has a precision of 0.2–15.1% (C.V.) in the range of 5–200 μg ml^{-1}. (corresponding to 0.5 to 20 ng of ceftazidime injected onto the column), and is optimised for assaying 50 μl of plasma. The extraction recovery from plasma was approximately 100%. The method was highly specific for ceftazidime and there was no interference from either commonly administered drugs or endogenous compounds. This assay was used to measure ceftazidime in elderly patients for therapeutic drug monitoring.

Keywords: Ceftazidime

1. Introduction

Ceftazidime is a third generation cephalosporin often used in *Pseudomonas aeruginosa* infections [1–3]. Like other β-lactam antibiotics, the antimicrobial activity of ceftazidime is mainly due to inhibition of the biosynthesis of peptidoglycan which is a component of the bacterial cell wall [4].

Ceftazidime is excreted by the kidneys, almost exclusively by glomerular filtration and it is poorly bound to plasma proteins [5]. The serum half-life is prolonged after intravenous (i.v.) administration in patients that have diminished renal function (e.g. critically ill or elderly patients) [2,3,6]. In these populations an individualized therapy to optimize efficacy and minimize toxicity could be established.

A variety of methods have been published describing either the quantitation or the identification of

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ceftazidime in biological samples including liquid chromatography–mass spectrometry [7], a microbiological assay [8], or a fluorescence ELISA assay [9]. Nevertheless, the procedures most commonly employed in clinical studies are HPLC with UV detection [10–16]. Since most cephalosporins are electroactive, an alternative to UV detection might be electrochemical detection [17–19]. The majority of cephalosporins are electroreducible due to the reduction of the Δ^3 double bond of the cephem nucleus, whereas others can undergo an oxidation of the aminothiazole group [20,21]. Either reduction or oxidation properties of ceftazidime can lead to its quantification [17,21]. In a previous study, the authors described the potential to use amperometric detection in the oxidative mode using an aqueous solution, for the quantitation of ceftazidime [21].

In the present paper, a simple, sensitive and reliable assay for determination of ceftazidime in human plasma is described. The method is based on HPLC separation followed by electrochemical detection which, because of the sensitivity toward ceftazidime, used only 0.5–20 ng for injection (corresponding to a calibration range of 5–200 $\mu g\,ml^{-1}$) to the chromatographic apparatus. Thus, very low quantities of endogenous compounds were injected, giving a longer lifetime of the analytical columns.

2. Experimental

2.1. Drugs and chemicals

Ceftazidime pentahydrate was obtained from Sigma (St. Quentin Fallavier, France). HPLC-grade acetonitrile used in chromatography was purchased from Merck (Darmstadt, Germany). Trichloroacetic acid, sodium phosphate dibasic heptahydrate and potassium phosphate monobasic were acquired from Sigma. Blank plasma was obtained from the blood bank, and samples from patients in the Geriatric Department of the Hospital.

2.2. Preparation of standard solutions

Standard stock solution of ceftazidime (2.5 $mg\,ml^{-1}$, calculated as a base) were prepared in purified water. New solution were prepared weekly and aliquots were stored at −30°C until use. Standard curves were freshly prepared from plasma spiked with antibiotic concentrations in the range of 5–200 $\mu g\,ml^{-1}$.

2.3. Sample preparation

Ceftazidime determination was based on a precipitation of plasma proteins with 30% trichloroacetic acid (TCA). To 50 μl plasma sample was added an equal volume of TCA and the mixture was mixed for 30 s. Then, 300 μl of 500 m*M* phosphate buffer (pH 7.4) and 600 μl of mobile phase were added, the mixture was mixed for 10 s and centrifuged at 3500 *g* for 5 min in a conical tube. Finally, 100 μl of the supernatant was mixed with 900 μl of mobile phase, 20 μl of which was analysed by HPLC.

2.4. Instrumentation and chromatographic conditions

The assay instrumentation included a constant-flow pump Model LC-10AD (Shimadzu, Japan), an ESA Coulochem II (Bedford, USA) electrochemical detector coupled with dual electrode analytical cell Model 5010 (ESA) and a guard cell Model 5020 (ESA) placed between the pump and the injector. Applied electrode potentials were set at +1 V for the guard cell and +400 mV and +650 mV for detectors 1 and 2, respectively. The peak areas, produced by oxidation at detector 2, were integrated using a Shimadzu Model C-R6A chromatopac integrator. Samples were injected into a Rheodyne Model 7125 valve fitted with a 20-μl loop.

Chromatography was carried out on a reversed-phase column (250×4.6 mm I.D., 5 μm) packed with C_{18} Kromasil (Interchim, France) preceded by a guard column filled with C_{18} Kromasil (10×4.0 mm I.D., 5 μm). The mobile phase consisted of acetonitrile–25 m*M* KH_2PO_4–Na_2HPO_4 buffer, pH 7.4 (10:90, v/v). The mobile phase components were passed through a 0.22 μm filter (Whatman, UK) and degassed for 10 min before use. Elution was isocratic at a flow-rate of 0.9 $ml\,min^{-1}$. Chromatographic separations were achieved at ambient column temperature.

2.5. Evaluation of potential interfering substances

A number of antimicrobial drugs used in combination with ceftazidime against *Pseudomonas aeruginosa* or usually employed in antibiotic therapy were examined for possible interference with the HPLC assay (amikacin, gentamicin, tobramycin, netilmicin, ticarcillin, imipenem, piperacillin, cefsulodin, ciprofloxacin, ofloxacin, pefloxacin, spiramycin and erythromycin). This evaluation included some other common drugs such as: acetaminophen, salicylate, amitriptyline, desipramine, imipramine, caffeine, digoxin, carbamazepin, phenobarbital, phenytoin, cimetidine and ranitidine. All drugs were tested at plasma concentration ranges commonly observed after normal dose regimens. The chromatographic specificity was also tested by injecting blank plasma samples from healthy human subjects.

2.6. Validation

The method was validated as to accuracy and precision (intra- and inter-day), linearity, limit of quantitation (LOQ) and limit of detection (LOD) and recovery [22]. The concentrations of ceftazidime in the analytical standards were 5, 10, 30, 75, 130 and 200 $\mu g\ ml^{-1}$.

Intra-day repeatability of the assay was assessed by performing replicate analyses of spiked samples at high, middle, and low concentrations in plasma against a calibration curve on the same day. Inter-day repeatability was assessed for calibration curves with the same standards on different days.

Intra-assay reproducibility was determined for calibration curves prepared the same day in replicate ($n=5$) using the same stock solutions. Inter-assay reproducibility was determined for calibration curves prepared on different days using different stock solutions ($n=10$).

LOD was determined as the lowest concentration for which the peak area had a signal-to-noise ratio of 3:1. LOQ was determined as the lowest concentration for which the precision in measuring the peak-area response was lower than 20%.

The recovery of ceftazidime from the plasma was determined by comparing the slopes from the ex-

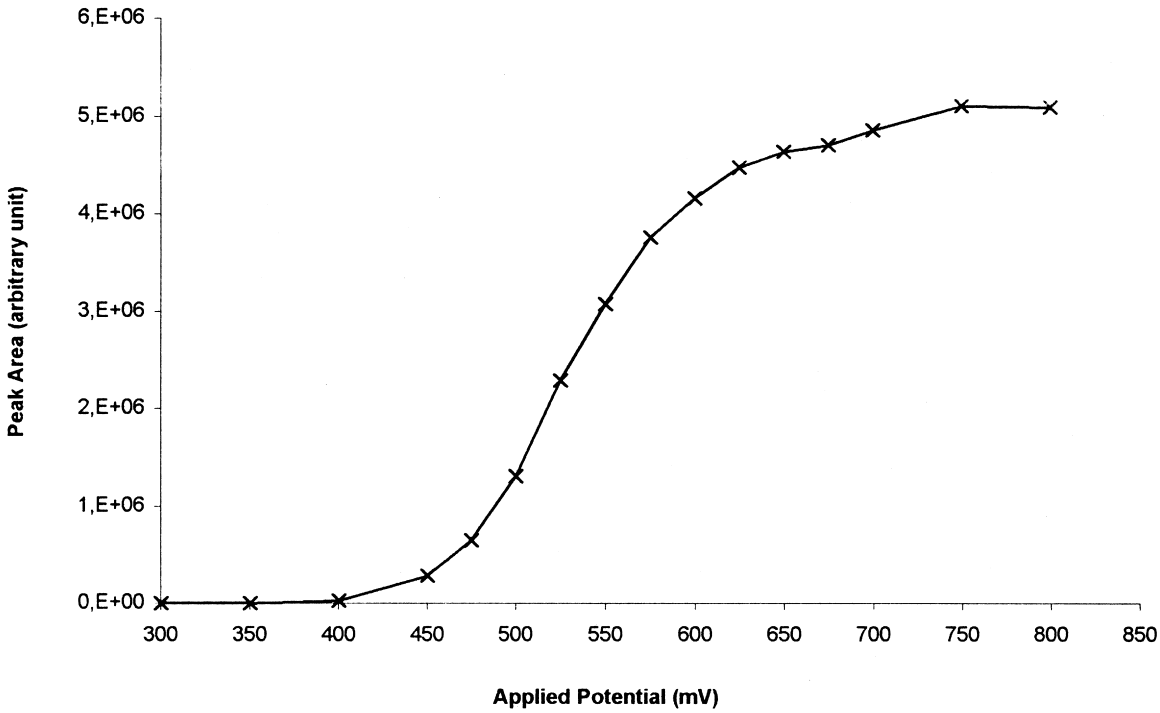


Fig. 1. Hydrodynamic voltammogram of ceftazidime, the details of HPLC conditions are described in Section 2.

tracted plasma samples with those obtained from unextracted aqueous standards of the same concentrations. The percent recovery was obtained from the mean of four replications.

An external standard method was used in the calibration and evaluation of the unknown samples.

3. Results and discussion

3.1. Electrochemical detection

The presence of an aminothiazole substituent in ceftazidime permits detection by amperometric detection in the oxidative mode. The hydrodynamic voltammogram for ceftazidime under the chromatographic conditions described above is shown in Fig. 1 (average of duplicate assays). The working potential to the second electrode was set at +650 mV although the sigmoidal curve plateau was obtained at a higher voltage. Indeed, using applied potential above +675 mV, the background current and range of interferences were greatly increased. A +400 mV potential applied to the first electrode eliminated some interferences due to other drugs or endogenous compounds.

3.2. Linearity

The linearity of this assay was statistically confirmed. For each calibration, the slope was different from zero and the intercept was not different from zero using Student's *t*-test ($\alpha=0.05$). Calculated residuals are normally distributed (data not shown) and have equal variances. Homoscedasticity was statistically confirmed using the Hartley test. Consequently, unweighted least squares linear regression of the peak-area as a function of the theoretical concentrations was applied to each standard curve. The correlation coefficient (r) of the linear regression, from inter-day reproducibility, was 0.9998 ± 10^{-4} (mean±S.D.).

3.3. Specificity, selectivity and interference

As shown in Fig. 2, ceftazidime was well resolved and no interference from endogenous plasma components or any of the tested drugs was observed.

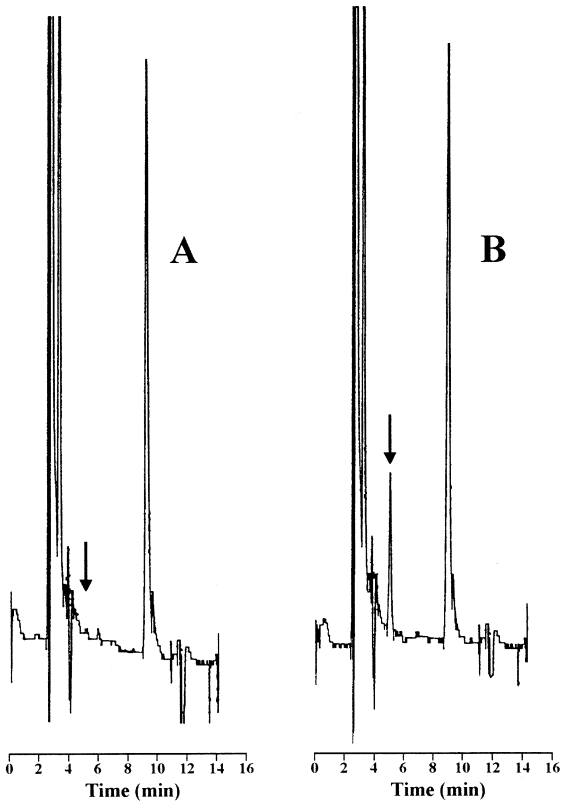


Fig. 2. Typical chromatograms of blank plasma (A) and of plasma spiked with 5 μg ml^{-1} of ceftazidime (B). Retention time for ceftazidime was 5.2 min.

3.4. Recovery, LOQ and LOD

The extraction recovery of ceftazidime from 50 μl of plasma was 100.9±4.9% (mean±S.D.). This result was equal to or even better than those obtained with methanolic precipitation [1,10] or those with on-line solid-phase extraction [16]. The LOD was 0.75 μg ml^{-1} and the LOQ was 3 μg ml^{-1}.

3.5. Precision and accuracy

The results for intra- and inter-day precision and accuracy are presented in Table 1. The C.V. for intra- and inter-day reproducibilities ranged from 0.44 to 6.00%, except for the lowest concentration (5 μg ml^{-1}) for which the C.V. was 15.1% for inter-day reproducibilities (Table 2).

Table 1
Intra- and inter-day precision and accuracy of ceftazidime plasma analysis

Spiked concentration ($\mu g\ ml^{-1}$)	n	Measured concentration[a] ($\mu g\ ml^{-1}$)	C.V. (%)	Mean recovery (%)	Relative error (%)
Intra-day precision					
7.5	7	7.53±0.29	3.8	100.46	0.5
40	7	39.95±0.80	2.0	99.88	0.1
125	7	124.80±0.87	0.7	99.84	0.2
Inter-day precision					
5	4	4.96±0.31	6.1	99.2	0.8
10	4	9.41±0.14	1.5	94.13	5.9
30	4	30.98±0.77	2.5	103.28	3.3
75	4	73.45±1.32	1.8	97.93	2.1
130	4	132.09±1.37	1.0	101.61	1.6
200	4	199.11±0.96	0.5	99.55	0.5

[a] Means±S.D.

Table 2
Intra- and inter-day reproducibilities of the HPLC assay of ceftazidime

Spiked concentration ($\mu g\ ml^{-1}$)	Intra-assay reproducibility ($n=5$)		Inter-assay reproducibility ($n=10$)	
	Measured concentration[a] ($\mu g\ ml^{-1}$)	C.V. (%)	Measured concentration[a] ($\mu g\ ml^{-1}$)	C.V. (%)
5	5.48±0.37	6.8	5.09±0.77	15.1
10	9.75±0.26	2.6	9.95±0.56	5.6
30	29.77±1.02	3.4	30.70±1.03	3.3
75	73.38±1.78	2.4	73.83±2.08	2.8
130	132.82±1.79	1.4	130.26±1.48	1.1
200	198.81±0.88	0.4	200.17±0.79	0.4

[a] Means±S.D.

3.6. Accuracy in extended concentration range

When ceftazidime is administered in an i.v. bolus for pharmacokinetics studies, antibiotic plasma concentrations can achieve values greater than 200 $\mu g\ ml^{-1}$ and lower than 5 $\mu g\ ml^{-1}$ 8 h after injection [1]. Because of this, accuracy (intra-day repeatability) in the determination of ceftazidime outside the calibration concentration range was assessed. Sample volume was the same as for the calibration curve (50 μl), but the volume of the supernatant that was removed after centrifugation was 100 μl (dilution factor, D.F.=1), 50 μl (D.F.=2) or 200 μl (D.F.=0.5), to which 900, 950 or 800 μl of mobile phase were added, respectively. The results, presented in Table 3, were satisfactory.

Table 3
Ceftazidime quantitation in extended concentration range

Spiked concentration ($\mu g\ ml^{-1}$)	D.F.	Measured concentration ($\mu g\ ml^{-1}$)	C.V. (%)
2.5	0.5	4.91±0.12	2.5
300	1	311.76±4.14	1.3
300	2	154.76±1.73	1.1
400	1	400.10±2.56	0.6
400	2	203.83±1.54	0.8

[a] Means±S.D.
D.F.=dilution factor; data corresponding at three determinations.

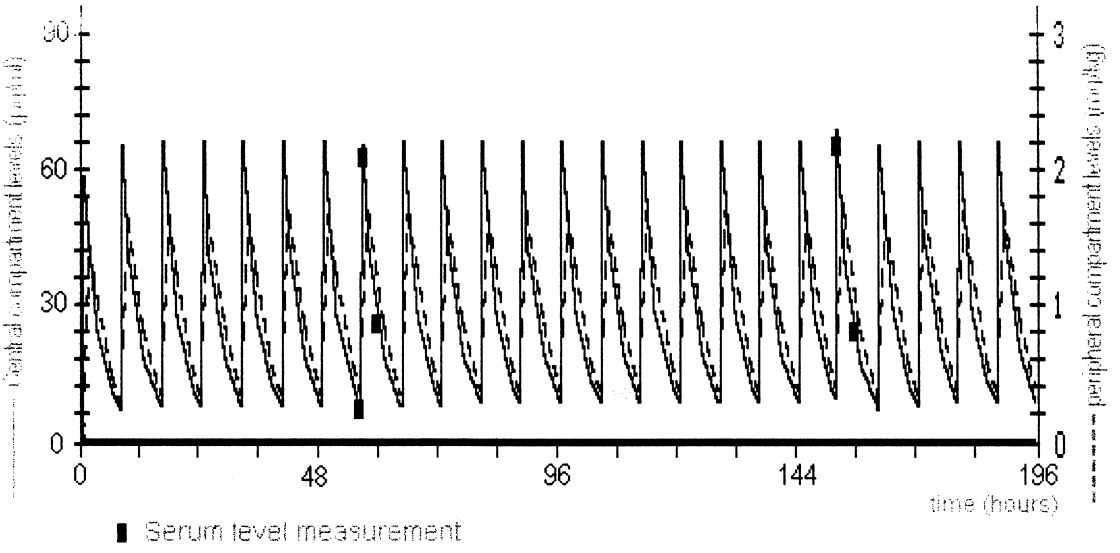
3.7. Stability

Stability was not studied for this assay since several authors have thoroughly described the degradation of ceftazidime under various conditions (pH, temperature, medium,..) [1,14,15,23]. In the present study, it was observed as previously described, that ceftazidime in plasma was unstable at 4°C, but was

stable for at least 5 days when stored at −20°C [1,23]. However, the stability of ceftazidime was better in the mobile phase once the extraction procedure was carried out.

3.8. Clinical application

Therapeutic drug monitoring (TDM) of ceftazidime was carried out in one infected 85-year-old patient with creatinine clearance estimated as 38 ml/min/1.73 m^2 [24]. Ceftazidime was given by intermittent i.v. infusion over 30 min. A total of five measured serum ceftazidime concentrations were collected during therapy. At dose number 8, a first set of three blood samples were taken, 5 min before starting of infusion, 5 min and 3 h after the end of infusion. At dose number 20, an additional set of two blood samples were taken, 5 min and 3 h after the end of infusion. Plasma samples were analysed by the chromatographic assay describe above. Bayesian adaptative control of ceftazidime was computed with USC*PACK PC clinical programs [25] using a two-compartment model with intravenous administration (Fig. 3). The free fraction value was 0.83. The error pattern of the chromatographic ceftazidime assay was determined, and its polynomial equation was found to be: $S.D.=0.3974+0.0268\times C-0.0001\times C^2$, where S.D. is in $\mu g\,ml^{-1}$ and C is the measured concentration in $\mu g\,ml^{-1}$. Individual pharmacokinetic parameters were found as followed, V_c (the apparent volume of distribution in the central compartment)=0.17 $l\,kg^{-1}$, K_{cp} (the rate constant from the central to the peripheral compartment)= 0.31 h^{-1}, K_{pc} (the rate constant in the reverse direction)=1.35 h^{-1}, K_{slope} (the increment of the elimination rate for each unit of creatinine



LEVEL #	TIME (HH:MM)	SERUM LEVEL (MCG/ML)	SERUM LEVEL SD (MCG/ML)	MODEL ESTIMATE (MCG/ML)	DIFFERENCE (MCG/ML)
1	55:46	8.11	0.608	8.61	0.5049
2	56:27	63.58	1.697	64.01	0.4337
3	59:06	26.48	1.037	27.46	0.9825
4	152:01	65.68	1.726	65.77	0.0892
5	155:31	25.12	1.008	23.19	-1.9324

The final minimized value of the Bayesian objective function = 9.2451

Fig. 3. Ceftazidime concentration versus time curve following i.v. perfusion every 8 h; differences between estimated and measured values of ceftazidime concentration.

clearance)=0.0068 h^{-1}, K_{int} (the nonrenal component of the elimination rate)=0.057 h^{-1}. Thus the overall $K_{el}=K_{int}+K_{slope}\times C_{cr}$ where C_{cr} is the creatinine clearance in ml $min^{-1}\times 1.73$ m^2. The elimination rate of the drug (K_{el}), the plasma distribution half-life ($t_{1/2\alpha}$), the apparent elimination half-life ($t_{1/2\beta}$) and the total body clearance of ceftazidime were respectively estimated as 0.317 h^{-1}, 0.402 h, 2.799 h and 63.5 ml min^{-1}. The values of these parameters are in agreement with previous pharmacokinetic studies in elderly patients [26,27].

4. Conclusion

Ceftazidime is frequently overdosed in the elderly because renal function is not considered. In this way, ceftazidime dose-adjustment in the elderly, based on population pharmacokinetic parameters, can lead to a better antibacterial efficacy and also to cost savings.

The analytical characteristics of this HPLC assay for the quantitation of ceftazidime in plasma appear useful in clinical pharmacokinetic because of the small sample volume necessary, the simplicity of sample preparation, the short run time and lack of interference.

Electrochemical detection for determination of ceftazidime in human plasma constituted a powerful technique, superior to UV detection with regard to sensitivity, linearity and precision [21].

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