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On-line solid-phase extraction of ceftazidime in serum and determination by high-performance liquid chromatography

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Abstract

A column-switching high-performance liquid chromatographic assay is described for the determination of ceftazidime (a third-generation cephalosporin) in human serum. The method does not require prior sample pretreatment. Serum is directly injected in a first chromatographic column for sample clean-up and extraction. Thereafter, using an on-line column-switching system, the drug is quantitatively transferred and separated on a second, analytical column followed by determination using ultraviolet absorption at 258 nm. The technique allows direct, rapid, precise, and simple determination of ceftazidime in serum over the range of 1–250 μg/ml using 12.5 μl of serum. This method was applied to study the pharmacokinetics of the drug in patients undergoing vascular surgery.

1. Introduction

Several assays are currently in use for determining ceftazidime [1] concentration in biological fluids, including bioassays and liquid chromatographic methods [2–7]. High-performance liquid chromatography (HPLC) is one of the most extensively and frequently used analytical techniques for determining the concentration of drugs in body fluids. The method usually consists in the isolation of the drug from interfering serum concomitants using liquid–liquid or solid-phase extraction prior to the HPLC determination. The liquid–liquid extraction procedure concentrates the drug and therefore improves the detection limit, but involves several separate steps, which not only make the method tedious and time-consuming but also increase the potential of introducing a bias in the results. A method involving direct injection of serum samples into the HPLC system can simplify the assay and decrease the chance for sample contamination. The on-line solid-phase extraction of drugs in serum is gaining widespread application [8]. In column switching, a fraction of the effluent from a primary column is selectively transferred to a secondary column. In this method the primary column produces an on-line sample clean-up. Using a column-switching approach, a rapid, direct, accurate, and precise on-line solid-phase method has been developed for the determination of ceftazidime in serum. No sample pretreatment is necessary. The method has demonstrated a 96% recovery, and a repeatability and

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reproducibility better than 5% using 12.5 μl of serum.

2. Experimental

2.1. Reagents and chemicals

All chemicals were reagent or analytical grade. Acetonitrile was purchased from E. Merck (Darmstadt, Germany). Sodium dihydrogen orthophosphate was supplied by Carlo Erba Farmitalia (Milan, Italy). Ceftazidime was from Glaxo. Analytical grade, filtered water was obtained daily from an Elgastat UHQ PS apparatus (ELGA, High Wycombe, Bucks, UK). Drug-free serum for preparation of standard solutions was obtained from a single human donor.

2.2. Apparatus

The HPLC system consisted of a Varian (Walnut Creek, CA, USA) Model Vista 5500 HPLC pump, a Spectra Physics Model SP-8000B solvent-delivery system, a Model 9060 Polychrom Diode Array ultraviolet detector, and a Model SP-4290 integrator. The injector was a Rheodyne Model 7125 manual injection valve equipped with a 50-μl sample loop. The analytical column was a HP ODS analytical column (15 cm × 0.4 cm I.D., particle size 5 μm) from Hewlett-Packard (Palo Alto, CA, USA). The extraction column was 5 cm × 0.4 cm I.D., dry-filled with a C_8 40-μm silica. The coupled-column system was operated by two pneumatic, six-port, automated switching valves (Valco, Schenkon, Switzerland), controlled by the HPLC system. The block diagram of the HPLC system is shown in Fig. 1.

2.3. Procedure

An aliquot (50 μl) of the biological material (serum + 10 m*M* aqueous sodium dihydrogen orthophosphate, 1:3, v/v) was injected, without any sample preparation directly onto the extraction column. It was brought onto the column by mobile phase 1 [10 m*M* aqueous sodium dihydrogen orthophosphate–acetonitrile (96:4, v/v), pH 5.0], delivered by pump 2, while pump 1 delivered the same mobile phase 1 to the analytical column, with the switching valves at the initial position (Fig. 1A). The mobile phase 1, which passed through the extraction column

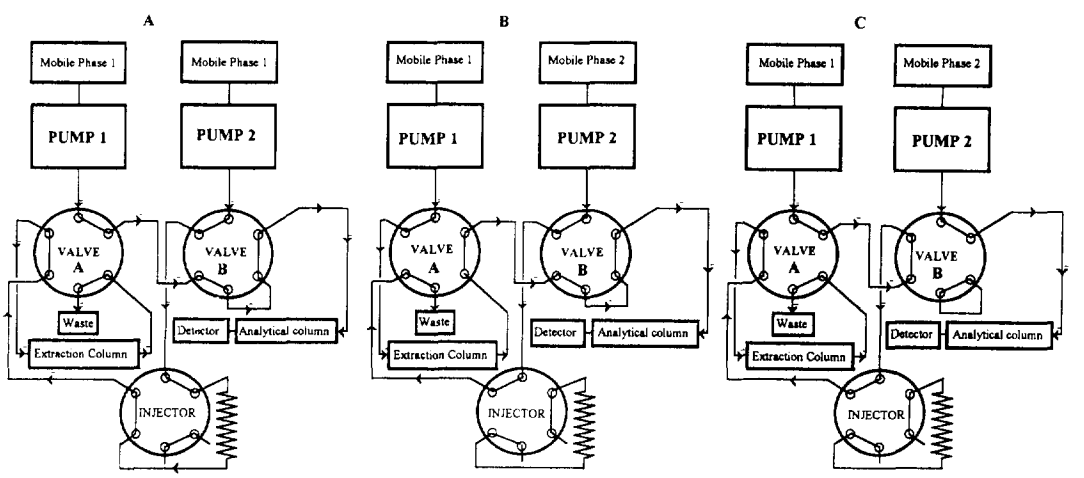

Fig. 1. Scheme of column switching. (A) Injection and extraction of sample (mobile phase 1 from pump 2 to extract ceftazidime and from pump 1 to prepare analytical column). (B) Elution of the analyte-containing fraction from the extraction column into the analytical column, with mobile phase 1 from pump 1. (C) Separation of the drug with mobile phase 2 from pump 2 and conditioning of extraction column with mobile phase 1 from pump 1.

and was directed to waste, removed the bulk of the serum matrix while quantitatively retaining ceftazidime on the stationary phase. After 0.9 min the analyte fraction in the pre-column was eluted to the analytical column, by switching the A valve, with mobile phase 1 from pump 1, and the mobile phase in pump 2 changed from mobile phase 1 to mobile phase 2 [10 m*M* aqueous sodium dihydrogen orthophosphate–acetonitrile (92:2, v/v) pH 5.0] (Fig. 1B). After 3.5 min, valve B was switched and mobile phase 2 from pump 2 passed through the analytical column, where ceftazidime was separated and detected by UV, while the switching of valve A allowed pump 1 to maintain mobile phase 1 to flow through the extraction column to prepare it for next sample (Fig. 1C). The flow-rates in both columns were the same (1 ml/min). The pre-column was backflushed each day with acetonitrile–water (50:50, v/v) for 20 min.

2.4. Chromatographic conditions

All analyses were performed at room temperature. Sample aliquots of 50 μl were injected into the chromatograph. Column elution was carried out with a flow-rate of 1 ml/min and a pressure of 150 atm. The effluent from the analytical column was monitored by UV at a wavelength of 258 nm. The cycle time of one analysis was 12 min.

2.5. Quantification

Standards for the calibration curve were made by spiking control serum at concentrations of 1, 5, 10, 25, 50, 100, 250 μg/ml. Each spiked serum standard was injected eight times. The calibration data of peak area against the concentration of the drug were fitted to a linear, unweighted, model. The resultant linear regression curves were used to calculate the drug concentrations in the samples. Based on a 50-μl sample volume (serum–10 m*M* aqueous sodium dihydrogen orthophosphate, 1:3, v/v), the detection limit was of the order of 0.5 μg/ml.

3. Results and discussion

The HPLC method described overcomes the problems of complicated procedures of the previously reported methods [2–6]. No pretreatment and no internal standards are required. The method allows the direct injection of serum by use of the precolumn-switching technique, and high sensitivity can be achieved in a relatively short analytical time. A number of combinations of mobile phase 1 were investigated with different organic modifiers (methanol, acetonitrile) at various concentrations (3–15%, v/v). The final composition was adjusted to achieve a good clean-up of the sample, a short retention time on the precolumn, and a long retention time on the analytical column. Acetonitrile was preferred because it gave the same retention time as methanol at a lower concentration, minimizing baseline perturbations resulting from column switching. The connection time of the precolumn to the analytical column was optimized by stepwise reduction until the peak area of ceftazidime started to decrease. An optimum, yet safe, connection time was found to be 2.6 min. A longer connection time did not affect significantly the resolution and the sensitivity: in fact it increased only the retention time of ceftazidime, because mobile phase 1 did not elute the analyte in the second column. The separation and sensitivity of the chromatographic system may be improved if the analyte is reconcentrated at the top of the secondary column to reduce band broadening. Therefore a solvent of low elution strength was used for the primary column and a less retentive packing material. The sensitivity of the proposed method can be further improved by increasing the sample volume, and it can therefore be used to analyse samples containing low amounts of ceftazidime with acceptable precision and recovery.

Fig. 2 shows chromatograms obtained after injecting both a blank and a spiked serum. The absence of interferences demonstrates the clean-up efficiency. Ceftazidime was found to have a retention time of 8.9 min and was well separated from the other detectable components in human serum at the selected wavelength.

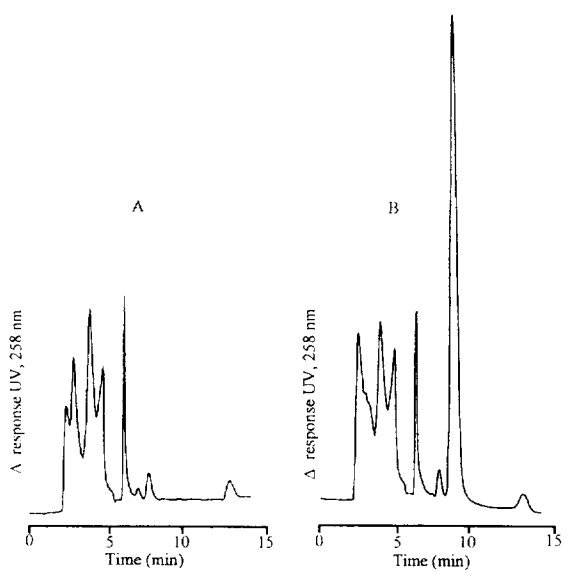

Fig. 2. Chromatograms of (A) drug-free normal serum and (B) serum spiked with 20 μg/ml ceftazidime

3.1. Linearity, precision, accuracy and recovery

Blank serum spiked with 1, 5, 10, 25, 50, 100, 250 μg/ml of ceftazidime was analysed on several days using the proposed method. The regression line obtained obeyed the equation $y = (12.21 \pm 0.13)x + (-24.07 \pm 10.59)$, the correlation coefficient being $r_2 = 0.998$. On average, the slope of the calibration graph obtained on six different days did not vary by more than 2%, demonstrating a good stability of the measuring system. The accuracy was +12% for the lower limit of quantitation and +1.3% for 250 μg/ml. Values for precision (coefficient of variation, C.V.) at these concentrations were 4.3 and 2.8%, respectively. The results are shown in Table 1. Recovery was determined by the ratio of the peak area resulting from spiked serum standards to the peak area resulting from an aqueous solution at the same drug concentration injected directly onto the analytical column. The recovery of ceftazidime was found to be 96% in serum samples.

3.2. Application

The procedure described above was employed in a pharmacokinetic study of five patients undergoing vascular surgery, after administration of ceftazidime for antimicrobial prophylaxis.

Serum levels of ceftazidime from a patient after i.v. administration of a 2-g dose are shown in Fig. 3. The regression analysis on individual data was achieved by assuming a two-compartment model. Table 2 summarizes the main pharmacokinetic parameters (means ± S.D.) of the patients: terminal half-life ($t_{1/2\beta}$), area under the serum concentration–time curve (AUC), volume of distribution of the first compartment (V_1), volume of distribution at steady state (V_{ss}) and serum clearance (Cl_s). The comparison between our estimates of the pharmacokinetic parameters and those reported in Ref. [1] shows that elimination half-life in our patients increases and total serum clearance decreases while the

Table 1
Precision, accuracy and linearity for ceftazidime (spiked serum)

Nominal (μg/ml)	Actual value (mean ± S.D., $n = 8$) (μg/ml)	Precision (%)	Accuracy (%)
1	1.12 ± 0.04	4.3	+12.0
5	5.32 ± 0.20	3.8	+6.4
10	9.86 ± 0.45	4.6	−1.4
25	24.06 ± 1.07	4.4	−3.8
50	49.30 ± 1.73	3.5	−1.4
100	98.27 ± 4.34	4.4	−1.8
250	253.34 ± 7.34	2.8	+1.3

Sample standard regression line $y = (12.21 \pm 0.13)x + (-24.07 \pm 10.59)$.

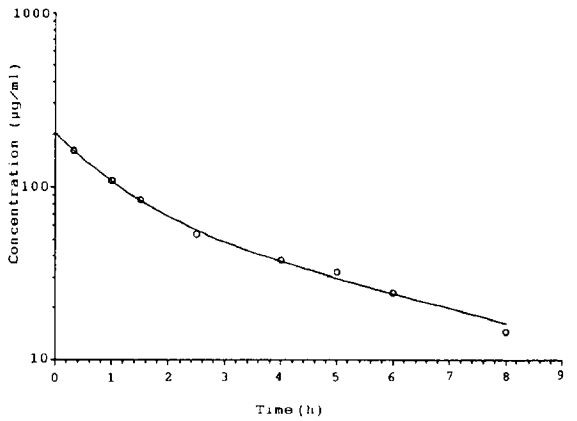

Fig. 3. Serum concentration–time curve of ceftazidime from a patient after 2-g i.v. administration.

difference between the distribution volumes at steady state is not statistically significant. The kinetic differences may be explained taking into account that several factors during vascular surgery may influence the pharmacokinetic profile of drugs.

4. Conclusions

The column-switching HPLC method described has advantages over conventional HPLC in that it allows the on-line determination of ceftazidime without sample pretreatment. The system has the advantages of speed of analysis, high recovery, and it is relatively inexpensive.

The method is sufficiently sensitive to analyse ceftazidime in small sample volumes (e.g. from pediatric patients). The assay is suitable for human pharmacokinetic studies and clinical purposes. The analysis of concentrations in patients after a 2-g i.v. dose are reported to document the utility of this method in clinical pharmacokinetic studies.

Table 2
Pharmacokinetic parameters after intravenous administration of 2 g ceftazidime (means ± S.D.)

$t_{1/2\beta}$ (h)	AUC (mg h/l)	Cl_s (ml/min)	V_1 (l)	V_{ss} (l)
3.65 ± 0.624	519.62 ± 97.34	64.15 ± 12.44	9.86 ± 1.42	16.30 ± 2.74

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References

[1] D.M. Richards and R.N. Brogen, Drugs, 29 (1985) 105.
[2] Y.W.F. Lam, M.H. Duroux, T.G. Gambertoglio, S.L. Barriere and B.T. Guglielmo, Antimicrob. Agents Chemother., 32 (1988) 298.
[3] M.L. van Ogtrop, H. Mattie, H.F.L. Guiot, E. Van Strijen, A.M. Hazekamp-Van Dokkum and R. Van Furth, Antimicrob. Agents Chemother., 34 (1990) 1932.
[4] C.M. Myers and T.L. Blumer, Antimicrob. Agents Chemother., 24 (1983) 343.
[5] J.S. Leeder, M. Spino, A.M. Tesoro and S.M. MacLeod, Antimicrob. Agents Chemother., 24 (1983) 720.
[6] R.L.Yost and H. Derendorf, J. Chromatogr., 341 (1985) 131.
[7] F. Jenl, P. Brickel and H. Monteil, J. Chromatogr., 413 (1987) 109.
[8] K.A. Ramsteiner, J. Chromatogr., 456 (1988) 3.