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Short communication

Measurement of cefuroxime in human bronchoalveolar lavage fluid by high-performance liquid chromatography after solid-phase extraction

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Abstract

A sensitive and selective method for the determination of cefuroxime in bronchoalveolar lavage (BAL) fluid using high-performance liquid chromatography (HPLC) with UV detection at 280 nm after solid-phase extraction with C_{18} cartridges was developed. A Waters symmetry C_{18} column was used and the mobile phase was acetonitrile–0.05 *M* ammonium phosphate buffer (pH 3.2) (15:85, v/v). The method enabled the determination of cefuroxime at concentrations below 100 ng/ml, with a linear calibration curve at concentrations of 5–100 ng/ml for 400 μl of BAL. The intra- and inter-assay coefficient of variations for 10, 40 and 80 ng/ml were between 5.3 and 8.9%. Analytical recoveries were between 92.7 and 106.2%. The detection limit was 1 ng/ml at a signal-to-noise ratio of 3:1 using 400 μl of BAL. The method was successfully used for the analysis of BAL fluid from patients after oral administration of 500 mg cefuroxime axetil twice daily.

Keywords: Cefuroxime

1. Introduction

Cefuroxime sodium is a parenteral cephalosporin, categorized as a second generation agent [1]. Cefuroxime axetil (Zinnat®) is an ester prodrug of cefuroxime {(6*R*, 7*R*)-3-carbamoyloxymethyl-7-[2-(2-furyl)-2-(methoxyimino)acetamido]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid}, which is rendered more lipophilic by esterification of the C-4 carboxyl group of the molecule by the racemic 1-acetoxyethyl bromide, thus enhancing absorption. The absorbed ester is hydrolysed in the intestinal mucosa and in the portal circulation [2]. Products of the de-esterification are active cefuroxime, acetaldehyde and acetic acid. Cefuroxime axetil itself is not detected in the systemic circulation.

The penetration of cefuroxime into the bronchial and pulmonary secretions after administration of cefuroxime axetil was studied in nine patients.

For the determination of cefuroxime in plasma, usually protein precipitation procedures with organic solvents [3,4] or with a solution of acid in water [4–6] or with guanidinium chloride [7] are used for sample pretreatment. These techniques, however, suffer from sample dilution; their sensitivity (≥400 ng/ml) is related to the volume of sample injected. A

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two-step extraction procedure [8,9] has a detection limit of 50 ng/ml. A column-switching technique was used as on-line sample clean up [10] with a detection limit of 500 ng/ml. None of these methods are suitable for low concentrations of cefuroxime (<50 ng/ml).

To improve the detection limit we developed a sensitive HPLC method with solid-phase extraction for the specific determination of cefuroxime in BAL fluid.

2. Experimental

2.1. Materials

Cefuroxime sodium and cephaloridine hydrate were kindly supplied by Glaxo (Paris, France). Methyl *tert.*-butyl ether (analytical grade), methanol (HPLC grade) and acetonitrile (low UV) were from Lab Scan (Dublin, Ireland). All other reagents were analytical grade from Merck (Darmstadt, Germany).

Isolute C_{18} cartridges, non-end capped (100 mg, 1 ml) were purchased from IST (MID Glamourgan, UK). Pure water, 18 MΩ, was obtained using a Gelman Water-I-Laboratory system (Gelman Science, Ann Arbor, MI, USA).

2.2. Preparation of standards

Stock solutions (1 mg/ml) of cefuroxime and cephaloridine were made in 0.02 *M* sodium phosphate buffer (pH 6.0) and stored at −70°C. Working solutions were prepared weekly by suitable dilution.

2.3. Apparatus

The HPLC system consisted of a Varian 9010 solvent pump (Varian Associates, Walnut Creek, CA, USA), a Merck Hitachi AS 2000 autosampler (Merck, Darmstadt, Germany) and a HP 3394 integrator (Hewlett-Packard, Palo Alto, CA, USA).

The Perkin-Elmer LC 235 diode array detector (Norwalk, CT, USA) was set at 280 nm. Samples were processed with a Vac Master 20 apparatus (IST, MID Glamorgan, UK).

2.4. HPLC conditions

A Waters Symmetry C_{18}, 5 μm, 100 Å, analytical column (150×3.9 mm I.D.) with a Symmetry C_{18} Sentry guard column, 5 μm (20×3.9 mm I.D.) (Millipore, Milford, MA, USA) was used. A mobile phase consisting of acetonitrile–0.05 *M* ammonium phosphate buffer (pH 3.2) (15:85, v/v) (Glaxo Wellcome, laboratory manual) was pumped at a flow-rate of 0.8 ml/min. All operations were carried out at room temperature.

2.5. Assay application

Nine patients (mean age 57.9 years, mean weight 65.3 kg) received 500 mg cefuroxime axetil orally twice daily for 3 days and 500 mg during breakfast at the day of bronchoscopy. No other cephalosporins were co-administered to the patients. BAL fluid was obtained after fiberbronchoscopy. The BAL fluid was centrifuged (10 min, 1150 *g*, 4°C) and the supernatant was stored at −70°C.

2.6. BAL extraction procedure

BAL supernatant samples (400 μl or 200 μl) were diluted with 1 ml of 0.05 *M* citrate buffer (pH 6.0). Thirty μl (30 ng) of the internal standard cephaloridine in 0.02 *M* phosphate buffer (pH 6.0) were added.

The C_{18} cartridges were wetted with 2 ml of acetonitrile, 1 ml of water and 1 ml of 0.05 *M* citrate buffer (pH 6.0). The diluted BAL samples were loaded immediately on the wet columns and allowed to filter without vacuum. The columns were then successively washed with 1 ml of 0.05 *M* citrate buffer (pH 6.0), with 0.5 ml of water, twice with 0.4 ml of methyl *tert.*-butyl ether and three times with 1 ml of acetonitrile. Between each washing step, the columns were dried for at least 1 min under full vacuum. Cefuroxime and the internal standard were eluted with 1 ml of methanol. The eluates were evaporated under nitrogen at 40°C. The residues

were dissolved in 55 μl of the mobile phase and about 40 μl was injected on the analytical column.

2.7. Quantification

For cefuroxime quantification, calibration graphs were constructed by 1/concentration squared weighed linear regression analysis of the peak-height ratios cefuroxime /internal standard versus the cefuroxime concentrations.

3. Results and discussion

The extraction method provides a reliable sample clean-up for chromatography. Retention and extraction of cefuroxime and the internal standard are based on secondary silanol interactions of the products with the polar silica surface (residual silanols) of the non-end capped C_{18} cartridges.

Absolute recovery of cefuroxime obtained from the slope of the calibration curves with and without extraction was 80.4% and absolute recovery of 75 ng/ml of cephaloridine using 400 μl BAL fluid was 60.0%±2.3% S.D. (n=3). Retention times using the chromatographic method described were for cefuroxime 9.17 min and for cephaloridine 6.25 min (Fig. 1). No interfering peaks are present in the blank BAL fluid of a patient. Other antibiotics, such as vancomycin, amoxicillin/clavulanic acid, co-administered to some of the patients, do not interfere with either cefuroxime or the internal standard on the chromatogram.

Fig. 2 shows a chromatogram of a BAL sample of a patient after cefuroxime axetil treatment, extracted and assayed by this method. Peak identity was confirmed by co-injection of cefuroxime standard with the BAL sample.

Data on the intra- and inter-assay precision and accuracy of quality control samples are shown in Table 1. The relative standard deviations and the deviations from the nominal value are acceptable (<15%).

The calibration graphs were linear in the concentration range evaluated (5 to 100 ng/ml BAL fluid). A typical calibration curve is y=0.0312x+

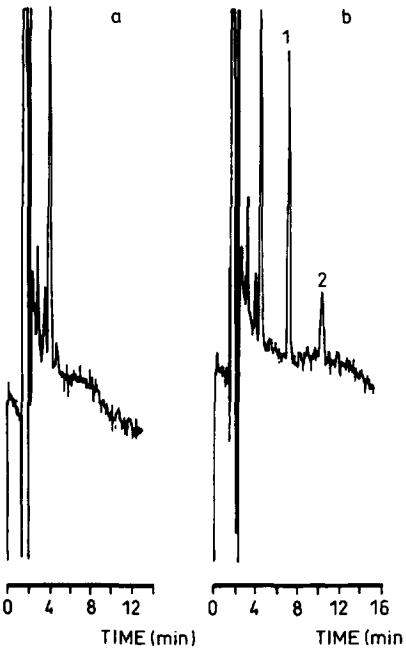

Fig. 1. Representative HPLC chromatograms of extracts of 200 μl BAL fluid of a patient without cefuroxime treatment. (a) Blank BAL fluid, (b) BAL fluid spiked with 20 ng/ml cefuroxime (2) and cephaloridine (internal standard) (1). Column, Symmetry C_{18}; mobile phase, acetonitrile–0.05 M ammonium phosphate buffer (pH 3.2) (15:85, v/v); flow-rate, 0.8 ml/min; detection wavelength, 280 nm.

0.0346 (r=0.9987). The BAL cefuroxime concentrations after oral adminstration of 500 mg cefuroxime twice daily were between 5.5 and 101 ng/ml and these values are in the linear range of the assay. Cefuroxime was detected to at least 1 ng/ ml, using 400 μl BAL, with a signal-to-noise ratio of 3:1. The lower limit of quantitation (LOQ) for cefuroxime is 5 ng/ml, using 400 μl BAL fluid. At the LOQ, the precision and the deviation of the nominal value are below 20%.

The method used has the required sensitivity, accuracy and reproducibility to analyse cefuroxime in BAL fluid. Previously, Baldwin et al. [11] determined cefuroxime in BAL fluid by a microbiological plate assay; the lower limit of sensitivity was 50 ng/ml; in some of their patients, the concentrations were below the lower limit of detection.

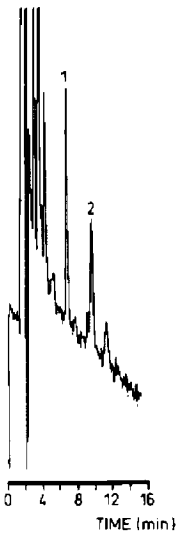

Fig. 2. Representative HPLC chromatogram of 200 μl BAL fluid of a patient receiving twice daily 500 mg cefuroxime axetil orally. Peaks: (2) cefuroxime, 54 ng/ml (calculated); (1) cephaloridine, internal standard. Column, Symmetry C_{18}; mobile phase, acetonitrile–0.05 *M* ammonium phosphate buffer (pH 3.2) (15:85, v/v); flow-rate, 0.8 ml/min; detection wavelength, 280 nm.

Table 1
Intra- and inter-assay precision and accuracy for cefuroxime analysis in BAL fluid

Nominal concentration (ng/ml)[a]	Concentration found (mean, %)	Coefficient of variation (%)
Intra-assay[b]		
10	99.7	6.6
40	92.7	5.3
80	106.2	8.9
Inter-assay[c]		
10	101.2	8.1
40	97.3	6.6
80	103.2	7.1

[a] 400 μl BAL fluid was processed.
[b] Six replicates of each sample were analysed.
[c] Replicates were analysed on three consecutive days.

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References

[1] C.H. O'Callaghan, R.B. Sykes, A. Griffiths and J.E. Thornton, Antimicrob. Agents Chem., 9 (1976) 511.
[2] S.M. Harding, P.E.O. Williams and J. Ayrton, Antimicrob. Agents Chem., 25 (1984) 78.
[3] C.A. Sanders and E.S. Moore. Clin. Chem., 32 (1986) 2109.
[4] J.B. Lecaillon, M.C. Rouan, C. Souppart, N. Febvre and F. Juge, J. Chromatogr., 228 (1982) 257.
[5] Y.A. Hekster, A.M. Baars, T.B. Vree, B. Van Klingeren and A. Rutgers, J. Antimicrob. Chemother., 23 (1980) 65.
[6] M.J. Koot, F.N. IJdenberg, R.M. Stuurman, J. Poell, L.J. Bras, J.J. Langemeijer and L. Lie-A-Huen, Pharm.-Weekbl.-Sci. Ed., 14 (1992) 360.
[7] D.A. Leigh, J. Antimicrob. Chemother., 23 (1989) 877.
[8] A.M. Brisson and J.B. Fourtillan, J. Chromatogr., 223 (1981) 393.
[9] V. Le Gros, R. Farinotti, N. Brion, J.F. Regnard, J. Azorin, A.M. Parrot and D. Chiche, Path. Biol., 40 (1992) 545.
[10] Y.J. Lee and H.S. Lee, Chromatographia, 30 (1990) 80.
[11] D.R. Baldwin, J.M. Andrews, R. Wise and D. Honeybourne, J. Antimicrob. Chemother., 30 (1992) 377.