ELSEVIER

Journal of Chromatography B, 686 (1996) 19–26

JOURNAL OF CHROMATOGRAPHY B: BIOMEDICAL APPLICATIONS

High-performance liquid chromatographic assay for meropenem in serum

H. Elkhaïli*, S. Niedergang, D. Pompei, L. Linger, D. Leveque, F. Jehl

Laboratory of Pharmacokinetics, Institute of Bacteriology, University Hospital Center, 3 Rue Koeberlé, 67000 Strasbourg, France

Abstract

High-performance liquid chromatographic procedures have been developed for the measurement of meropenem in serum. The separation was performed on an Ultrasphere XL-ODS analytical column (75×4.6 mm I.D.). The mobile phase consisted of 10.53 mmol/l ammonium acetate–acetonitrile (95:5, v/v) (pH 4). The UV detection was at 298 nm. The quantitation limit both in serum and water was 0.25 μg/ml. The method was validated in serum and aqueous solution over the concentration range 0.25–50 μg/ml. The extraction recovery from serum spiked with meropenem was 99.7±3.4%. The intra- and inter-assay coefficients of variation were below 6%. Stored at −80°C for three months at various concentrations in serum and in aqueous solution, meropenem did not reveal any appreciable degradation. After 24 h, it was also stable at 4°C in serum, aqueous solution and supernatant of extraction but not at room temperature. The stability of the drug was also confirmed in serum after repeated freezing–thawing cycles at −80°C on four consecutive days.

Keywords: Meropenem

1. Introduction

Meropenem, (4*R*,5*S*,6*S*)-3-[(3*S*,5*S*)-5-dimethylcarbamoylpyrrolidin-3-yl-thio]- 6 -[(1*R*)- 1 -hydroxyethyl]- 4 -methyl- 7 -oxo- 1 -azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, (ICI 194660, SM-7338), is a highly active, broad spectrum carbapenem antibiotic. It is effective in the treatment of a wide range of infections caused by many Gram-negative and Gram-positive organisms and possesses good activity against almost all clinically relevant aerobic, nutritionally fastidious and anaerobic bacterial species [1]. This compound is similar to imipenem in having a 6-α-hydroxyethyl group, but differs in a methyl group attached at C_1 and a dimethylcarbamoylpyrrolidinethio side chain attached at C_2 (Fig. 1) [2]. Of considerable importance is the fact that studies in animals and humans confirm its relative stability to hydrolysis by the proximal tubular brush border enzyme dehydropeptidase I (DHP-I), suggesting that a DHP-I enzyme inhibitor such as cilastatin is not necessary [1,3,4].

Meropenem has been determined in biological fluids by microbiological methods [5–7] or by high-performance liquid chromatography technique (HPLC) [3,8,9].

As further pharmacokinetic investigations are needed, we developed a rapid, sensitive and accurate

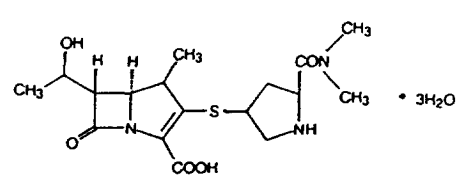

Fig. 1. Structure of meropenem.

*Corresponding author.

0378-4347/96/$15.00
PII S0378-4347(96)00205-8

HPLC method for the measurement of meropenem in biological fluids using UV detection.

2. Experimental

2.1. Reagents and chemicals

Meropenem was obtained as pure titrated powder from ICI (Alderly Park, UK). Stock solutions of 5 mg/ml were prepared in water and stored at −80°C. Acetonitrile, methylene chloride, ammonium acetate and acetic acid were all of analytical-reagent grade (Merck, Darmstadt, Germany). Water was obtained daily from a Milli-Ro-Milli-Q system (Millipore, Molsheim, France).

2.2. Chromatographic conditions

The isocratic liquid chromatograph consisted of a 126 solvent delivery module (Beckman, Fullerton, CA, USA), a Model 210 sample injection valve with a variable loop (Beckman) and a Model 166 variable-wavelength detector (Beckman). Chromatograms were processed by a Model 740 recording data processor (Millipore, Waters Division, Milford, MA, USA).

Chromatography was performed on a high-speed analytical column (75×4.6 mm I.D.) packed with 3 μm diameter particles (Ultrasphere XL-ODS, Beckman). The mobile phase consisted of 10.53 mmol/l ammonium acetate–acetonitrile (95:5, v/v), adjusted to pH 4 with glacial acetic acid. The flow-rate was set at 1.0 ml/min and the eluent was monitored at 298 nm. The range setting of the spectrophotometer depended on the concentration of meropenem measured.

2.3. Extraction procedure

2.3.1. Serum

As described previously [10,11], an aliquot of serum (0.5 ml) was mixed with an equal volume of acetonitrile in a 5-ml screw-capped glass tube on a Vortex mixer (Vortex, Cleveland, OH, USA). The tube was gently shaken by rotation for 10 min (20 rpm). The resulting mixture was centrifuged 10 min at 1000 *g*. The supernatant was transferred with a Pasteur pipette to another screw-capped glass tube, and 3.2 ml of methylene chloride were added. After shaking by rotation (20 rpm) for 10 min and centrifugation at 1000 *g* for 10 min, a 5-μl aliquot of the upper aqueous layer was injected into the column.

2.4. Recovery study

Five samples of serum were spiked with 0.5, 5 and 50 μg/ml of meropenem. They were assayed and the resulting peak areas were compared with peaks resulting from aqueous solutions at the same concentrations.

2.5. Accuracy and limit of quantitation

Quantification was based on peak areas measured by the integrator. The limit of quantitation was defined as the lowest antibiotic concentration which can be determined with confidence on a day-to-day basis. A standard curve was prepared for meropenem by spiking normal sera with increasing amount of antibiotic.

The linearity of the method was assessed from 0.1 to 50 μg/ml of antibiotic concentration. Each concentration was measured three times.

Accuracy was evaluated by calculating the mean percent differences between theoretical values and measured values.

2.6. Precision

Both within- and between-day reproducibilities were tested. Three concentrations of antibiotic were included in this study, the first high (50 μg/ml), the second middle (5 μg/ml) and the third low (0.5 μg/ml). Nine aliquots of each sample were tested on the same day and the resulting coefficient of variation (C.V.) indicated the within-day reproducibility. Aliquots of the same sample were tested once a day during nine days and the resulting C.V. indicated the between-day reproducibilities.

2.7. Specificity

Meropenem was assessed in the presence of most β-lactam antibiotics (e.g., clavulanic acid, amox-

ycillin, ampicillin, cefixime, cefotaxime, ceftazidime, cloxacillin, imipenem, cefalotin, mezlocillin, latamoxef, penicillin G, piperacillin, tazobactam, ticarcillin) as possible interfering compounds with this assay. Other antibiotics were included in this study (fusidic acid, ciprofloxacin, fosfomycin, vancomycin, itraconazole and hydroxyitraconazole, ofloxacin, pefloxacin, pristinamycin, rifampicin, roxithromycin, sulfamethoxazole, teicoplanin, tetracycline, trimethroprim, amikacin, gentamicin, tobramycin, netlimicin).

2.8. Stock solution stability

The working solution (5 μg/ml) of meropenem were repeatedly ($n=3$) injected into the chromatograph immediately after preparation (time 0) and at 1, 2, 3, 4 and 24 h after bench-top storage at room temperature and at 4°C. This injection protocol was repeated after 1, 3, 6, 8, 15, 30, 60 and 90 days storage of these solutions at −80°C.

2.9. Bench-top stability of meropenem after serum preparation

Quality control (QC) concentrations representing the low, middle to high end of the assay validation concentration range were analysed in triplicate (each time three aliquots of the sample were extracted and injected at the indicated times) at the following times: 0, 1, 2, 3, 4 and 24 h after bench-top storage at 4°C. QC concentrations tested were 0.5, 5 and 50 μg/ml. In fact this test indicates the stability of meropenem in the supernatant of extraction and is more representative of what really occurs in routine handling of samples or during pharmacokinetic studies.

2.10. Short and long term freezer stability of meropenem in serum

QC concentrations of 0.5, 5 and 50 μg/ml of meropenem in serum were analyzed in triplicate, and injected into the chromatograph immediately after preparation (time 0) and at 1, 2, 3, 4 and 24 h. These samples were stored at room temperature and at 4°C.

The injection protocol was repeated after 1, 3, 6, 8, 15, 30, 60 and 90 days storage of these solutions at −80°C.

3. Results

A representative chromatogram of serum containing meropenem is shown in Fig. 2. There was clear resolution of the compound of interest which had a retention time of 5.2 min. Extraction and chromatographic analysis of five separate blank serum samples confirmed that there were no endogenous peaks that coeluted with meropenem. A number of commonly administered antimicrobial agents were examined for possible interference with the HPLC method. None of the parent compounds coeluted with of meropenem. The lower limit of quantitation was 0.25 μg/ml. The linearity study was carried out with concentrations ranging from 0.25 to 50 μg/ml in serum. The regression analysis between peak areas and serum concentrations revealed that the method is linear ($r=1.0$). The equation of regression line is $y=4.832x-0.013$ where y=concentration and x= peak area. The extraction recovery from serum spiked with meropenem was 99.7±3.4% (Table 1). Precision values in aqueous solution were 4.3, 5.2, and 2.8% (relative standard deviation) as determined by injection ($n=9$) of 0.5, 5 and 50 μg/ml respectively (Table 2).

For serum, the intra-assay precision was characterized by coefficients of variation (C.V.) of 2.9% (0.5 μg/ml), 2.9% (5 μg/ml) and 3.2% (50 μg/ml) (Table 2). The values for inter-assay precision respectively were 5.0, 3.7 and 3.3%. Accuracy for targeted values were below 15% and 10% for low and high levels respectively (Table 3).

When stored at −80°C for three months in serum and in aqueous solution meropenem did not reveal any appreciable degradation, with all samples retaining more than 95% of their original concentrations values (Table 4). The working solution of meropenem was stable both at room temperature and 4°C (Fig. 3). Of interest is that after serum extraction, meropenem was stable at the various concentrations tested. The percentage of change compared to day 0 was −8.4% (0.5 μg/ml), −2.9% (5 μg/ml) and −0.9% (50 μg/ml) after 24 h. The stability of meropenem in serum was good at 4°C

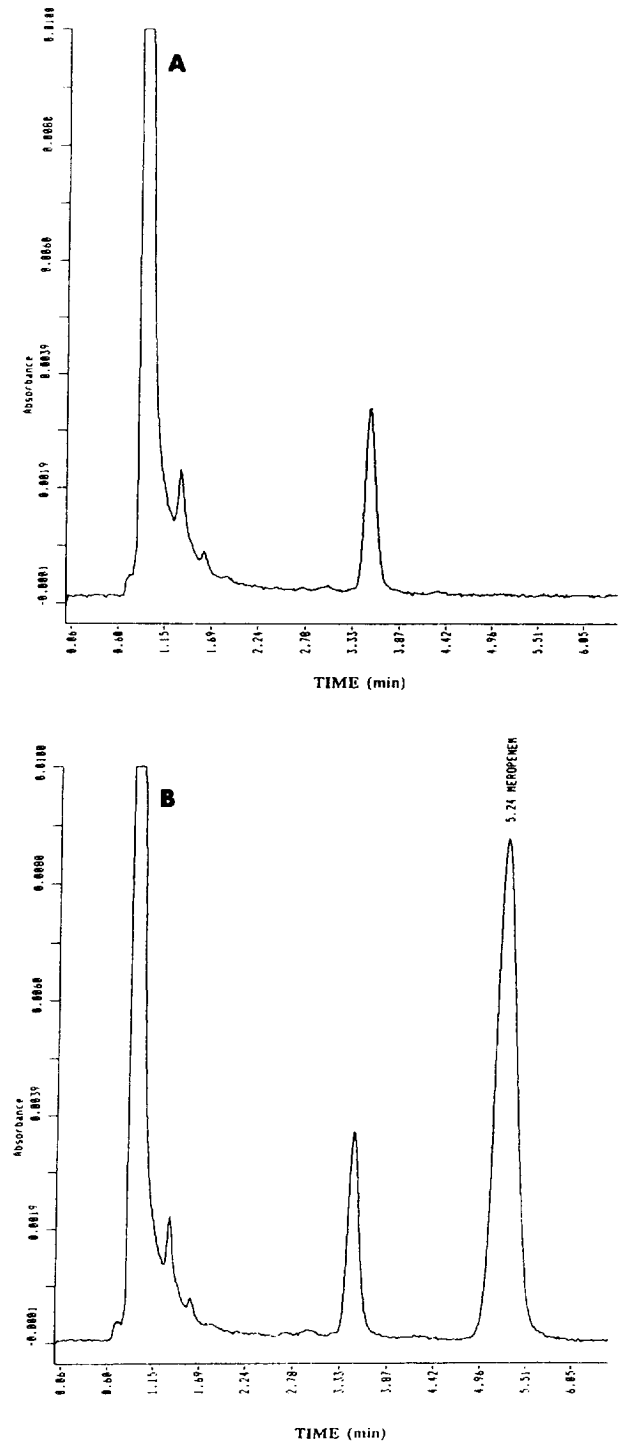

Fig. 2. Chromatograms: (A) meropenem-free normal serum; (B) serum spiked with 10 μg/ml meropenem. Detection wavelenght: 298 nm.

Table 1
Extraction recoveries

Concentration (μg/ml)	Recovery (n=5) (%)
0.5	95.8±3.7
5	101.7±1.1
50	101.7±3.2

(Fig. 4A), whereas an important degradation occurs after 24 h storage at room temperature (Fig. 4B). The values of meropenem stability in serum after repeated freezing–thawing cycles at −80°C on four consecutive days were C.V. of 13.7% (0.5 μg/ml), 2.7% (5 μg/ml) and 2.7% (50 μg/ml).

4. Discussion

HPLC proved to be particularly suitable for monitoring of carbapenems. Many methods have been proposed for meropenem assay, this drug generally being determined either by HPLC [3,8,9] or by microbiological assay [5,6]. The major drawback of microbiological assays is their lack of specificity when assaying samples from patients treated with several antibiotics or with an antimicrobial agent that produces active metabolites. They also suffer from poor reproducibility and accuracy and are somewhat time consuming and often result in delayed response (6–24 h) [11]. The microbiological methods often using *Escherichia coli* NIHJ as a test strain were sensitive. The lower limit of detection ranged from 0.06 to 0.1 mg/l [5–7].

For meropenem, an HPLC method has been described by Bax et al. [3] based on solid-phase extraction and reversed-phase chromatography with detection by ultraviolet absorbance at 296 nm. These procedures offer advantages in terms of concentration of the analyte (i.e. lower detection limit) and reduced background from endogenous components resulting in clean chromatograms. We describe here a simple extraction method. We commonly deproteinize samples with an equal volume of acetonitrile, the excess acetonitrile is then removed from the supernatant with methylene chloride, leaving concentration of the antibiotic in the supernatant. When compared with the Nilsson-Ehle et al. extraction method [9], this technique was easier to carry out. It enhances

Table 2
Precision of meropenem assay

Concentration (μg/ml)	Sample (n=9)	Intra-assay C.V. (%)	Inter-assay C.V. (%)
0.5	Serum	2.9	5.0
	Aqueous solution	4.3	
5	Serum	2.9	3.7
	Aqueous solution	5.2	
50	Serum	3.2	3.3
	Aqueous solution	2.8	

Table 3
Accuracy of meropenem assay

Theoretical value (μg/ml)	Aqueous solution		Serum	
	Measured value (μg/ml)	% difference from theoretical value	Measured value (μg/ml)	% difference from theoretical value
0.1	0.098	2	0.122	22.0
0.25	0.260	4	0.281	12.4
0.5	0.504	0.8	0.513	2.6
1	0.988	1.2	0.948	5.2
5	5.069	1.4	5.027	0.5
25	24.876	0.5	24.920	0.3
50	50.055	0.1	50.038	0.1

Table 4
Long term stability of meropenem at −80°C both in serum and working solution

Concentration (μg/ml)	Storage period days	% change compared to day 0, time 0	
		Working solution	Serum
0.5	1		+3.7
	3		−1.9
	6		+2.8
	8		+8.4
	15		+3.7
	30		+0.9
	60		+0.9
	90		−0.9
5	1	+1.7	+0.9
	3	0	0
	6	+7.8	+1.8
	8	−2.6	+13.0
	15	−3.4	+5.5
	30	−4.3	+0.9
	60	−2.6	+5.5
	90	−0.9	−0.9
50	1		−1.9
	3		+6.7
	6		+10.5
	8		+13.3
	15		+4.8
	30		+1.9
	60		+9.5
	90		0

reproducibility and concentrates antibiotic by reducing the final volume of the aqueous phase [13]. For the mobile phase the use of acetonitrile is preferable to methanol which precipitates phosphate anions and damages columns rapidly.

The good reproducibility of the assays in serum in

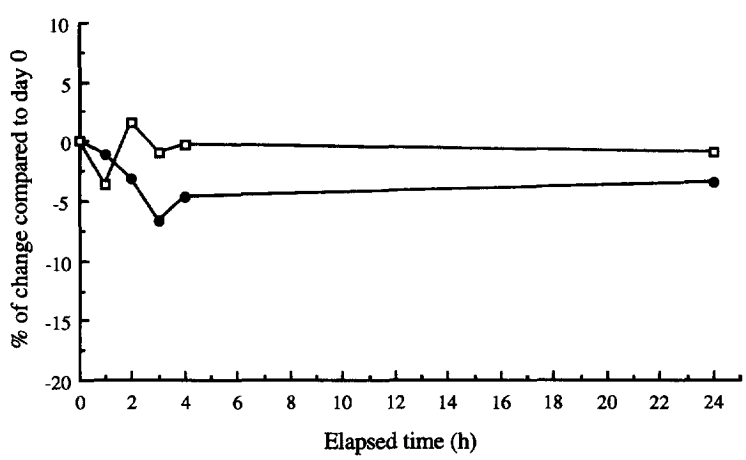

Fig. 3. Assay of stability of meropenem (5 μg/ml) at room temperature (□) and 4°C storage in working solution (●).

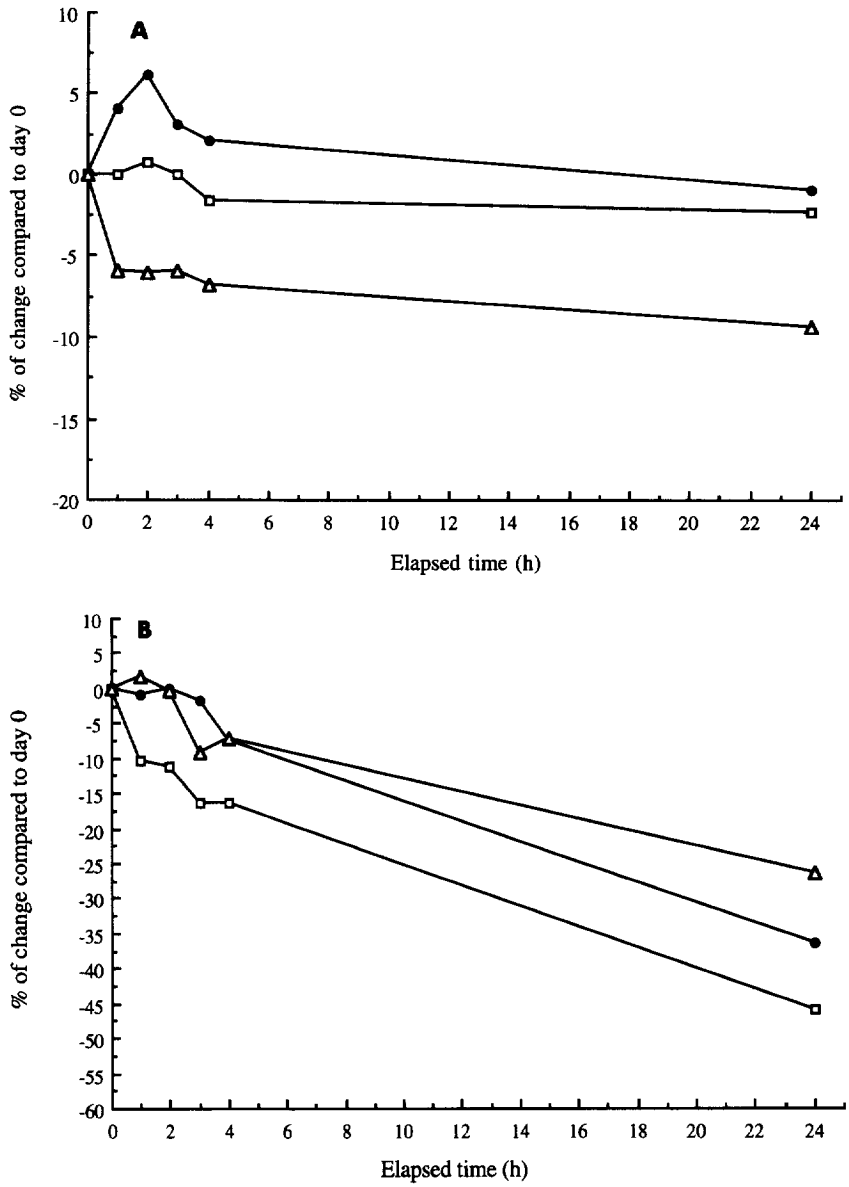

Fig. 4. Assay of stability of meropenem in serum: storage at 4°C (A); storage at room temperature (B). Concentrations tested were 0.5 (●), 5 (□) and 50 μg/ml (△).

conjunction with the proven linearity indicate that an internal standard to overcome sample to sample variation is not necessary. In our study neither serum sample dilution nor adsorption on to preconditioned extraction columns is needed. The limit of quantitation is lower than that determined by Nilsson-Ehle et al. [9] (0.4 μg/l) and Burman et al. (0.5 μg/ml) [12], but fourfold higher than that determined by Bax et al. (0.06 μg/ml) [3]. The assay stability of meropenem is poorly documented [14]. In this work, the stability of meropenem is well documented. The stability of meropenem at concentrations tested in working solution and supernatant of extraction was good at 24 h. In contrast, more than 40% of product

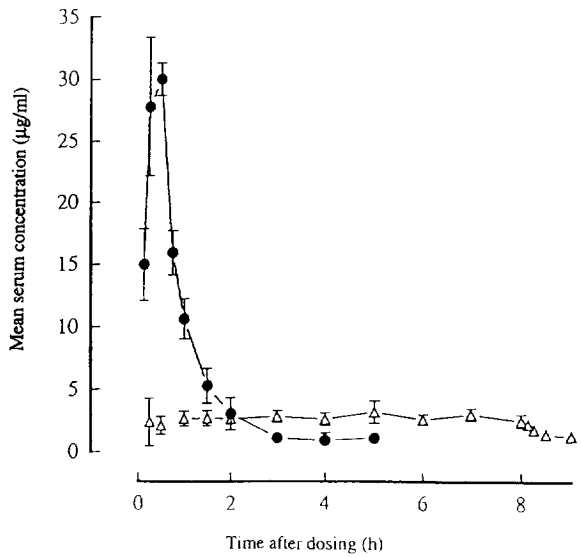

Fig. 5. Mean (±S.E.M) serum concentrations–time profiles following administration of 250 mg meropenem to micropig by 30-min infusion (●) or 250 mg meropenem by continuous infusion (△).

was lost when serum was allowed to stand at room temperature for 24 h after preparation. Meropenem could be stored at −80°C for at least three months both in aqueous solutions and serum. The stability of the drug was also confirmed in serum after repeated freezing–thawing cycles at −80°C on four consecutive days. In our laboratory this newly developed and validated HPLC technique allows us to determine the pharmacokinetic profile of meropenem with Yucatan micropig used as an experimental model (Fig. 5). The pharmacokinetic parameters of meropenem in micropig were similar to those in man (Table 5).

Table 5
Pharmacokinetic parameters of meropenem in plasma, after intravenous administration to micropig and man[a]

Parameter	Micropig	Man
C_{max} (μg/ml)	29.9 ±1.3	24.8 ±1.4
AUC (μg h/ml)	27.4 ±2.4	27.2 ±2.2
$T_{1/2}$ (h)	0.78±0.3	0.83±0.02
Cl_p (ml/min/kg)	4.6 ±1.0	4.0

[a] C_{max}, maximum concentration of meropenem in serum; AUC, area under concentration–time curve for an 8-h dosing period; $T_{1/2}$, half-life; Cl_p, total body clearance.

The HPLC method for meropenem described here represents a rapid, sensitive, efficient and precise analytical approach for quantitating this drug in serum. The analytical characteristics of the proposed method and the minimum sample handling are satisfactory for pharmacokinetic and clinical laboratory investigations.

Acknowledgments

We wish to thank Mrs M. Hoehn for her excellent secretarial work.

References

[1] J.R. Edwards, P.J. Turner, C. Wannop, E.S. Withnell, A.J. Grindey and K. Nairn, Antimicrob. Agents Chemother., 33 (1989) 215.
[2] M. Sunagawa, M. Matsumura, H. Inoue, N. Fukasawa and M. Kato, in Proceedings and Abstracts of the Twenty-Seventh Interscience Conference on Antimicrobial Agents and Chemotherapy, New York, American Society for Microbiology, Washington, 1987, Abstract 752, p. 228.
[3] R.P. Bax, W. Bastain, A. Featherstone, D.M. Wilkinson, M. Hutchison and S.J. Haworth, J. Antimicrob. Chemother., 24 (Suppl. A) (1989) 311.
[4] R.N. Jones, K.E. Aldridge, S.D. Allen, A.L. Barry, P.C. Fuchs, E.H. Gerlach and M.A. Pfaller, Antimicrob. Agents Chemother., 33 (1989) 562.
[5] R. Wise, M. Logan, M. Cooper, J.P. Ashby and J.M. Andrews, Antimicrob. Agents Chemother., 34 (1990) 1515.
[6] A. Leroy, J.P. Fillastre, F. Borsa-Lebas, I. Etienne and G. Humbert, Antimicrob. Agents Chemother., 36 (1992) 2794.
[7] M. Chimata, M. Nagase, Y. Suzuki, M. Shimomura and S. Kakuta, Antimicrob. Agents Chemother., 37 (2993) 229.
[8] M.P. Harrison, S.R. Moss, A. Featherstone, A.G. Fowkes, A.M. Sanders and D.E. Case, J. Antimicrob. Chemother., 24 (Suppl. A) (1989) 265.
[9] I. Nilsson-Ehle, M. Hutchison, S.J. Haworth and S.R. Norrby, Eur. J. Clin. Microbiol. Infect. Dis., 10 (1991) 85.
[10] F. Jehl, C. Gallion and H. Monteil, J. Chromatogr., 531 (1990) 509.
[11] F. Jehl, P. Birckel and H. Monteil, J. Chromatogr., 413 (1987) 109.
[12] L. Burman, I. Nilsson-Ehle, M. Hutchison, S.J. Haworth and S.R. Norrby, J. Antimicrob. Chemother., 27 (1991) 219.
[13] J.T. Rudrik and R.E. Bowdon, J. Liq. Chromatogr., 4 (1981) 1525.
[14] ICI Pharmaceuticals, Laboratory Data, 1990.