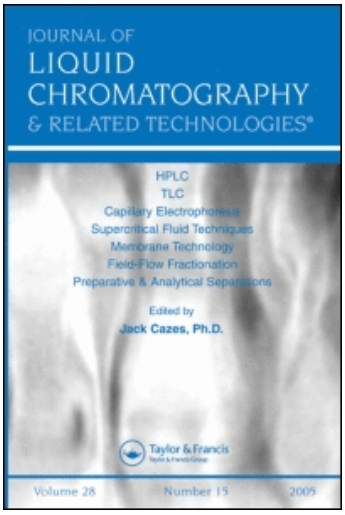

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RAPID AND SENSITIVE DETERMINATION OF MEROPENEM IN RAT PLASMA BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

A sensitive and rapid high-performance liquid chromatographic method using UV detection, has been developed for the analysis of meropenem in plasma. This involved a single liquid-solid extraction on Isolute C_{18}-EC column in the presence of an internal standard (ceftazidime). Analysis was performed by isocratic elution with a mobile phase consisting of 0.01 M acetate buffer pH 4.8 containing 15% (v/v) methanol, with UV detection at 296 nm. The limit of quantitation of the assay was 0.125 mg/L. The method was applied to a pharmacokinetic study in rats receiving 100 mg/kg by IV route with a good accuracy (94-101%) and precision (less than 13%).

INTRODUCTION

Meropenem, (1R, 5S, 6S)-2-[(3S, 5S)-5-dimethylamino-carbonylpyrrolidin-3-ylthio]-6-[(R)-1-hydroxyethyl]-1-methylcarbapenem-2-em-3-carboxylic acid, is a new carbapenem antibiotic with a broad spectrum of *in vitro* activity against both gram-positive and gram-negative bacteria including anaerobes such as *Bacteroïdes fragilis,* and *Pseudomonas aeruginosa.*[1,2] Meropenem is as stable against renal metabolic degradation as imipenem combined with a dehydropeptidase I inhibitor such as cilastatin.[3]

Carbapenems have been proposed to treat meningitis. Interestingly, meropenem is less potent than imipenem in inducing central nervous system (CNS) toxicity.[4] In order to investigate its CNS diffusion in small laboratory animals, a sensitive analytical method using low plasma volumes (0.1 mL) is required.

Two microbiological assays[5,6] have been reported for meropenem, but these procedures suffer from a lack of selectivity and are time-consuming. Several HPLC methods have been proposed[7-11] but rarely accuracy and precision were indicated. One of these involved protein precipitation only[8] and another one, protein precipitation followed by a washing solvent step[9] which is time-consuming. Two other methods described solid-phase extraction (SPE)[7,10] but without indication of the limit of quantitation.

A recent method using β-hydroxytheophylline as internal standard (IS) involved a step of drug extraction by single filtration of plasma.[11] Only one paper[9] gave indications about the stability of this carbapenem in different temperature conditions. We now describe a simple, accurate, and precise HPLC method which employs SPE for assaying meropenem in rat plasma at the detection wavelength of 296 nm.

EXPERIMENTAL

Chemicals

Meropenem was kindly supplied by Zeneca-Pharma (Cergy, France) and ceftazidime (internal standard) by Glaxo-Wellcome Laboratories (Paris, France). HPLC grade methanol and ethylene glycol were obtained from Carlo-Erba (Milan, Italy). Analytical grade acetic acid and sodium acetate were purchased from Labosi (Paris, France) and Prolabo (Paris, France), respectively and used for the preparation of an aqueous acetate buffer (0.01 M, pH 4.8). N-[2-hydroxyethyl] piperazine-N'-[2-ethanesulfonic acid] = HEPES was obtained from Sigma (Paris, France).

The extraction columns (Isolute C_{18} EC, 100 mg/1 mL) were from Touzart Matignon (Paris, France). HPLC-grade water was obtained before use, through a Milli Q- plus water purification system (Millipore Corp., USA).

Chromatography

The HPLC system consisted of a model L 6000 Merck-Hitachi pump and a model 717 plus Waters autosampler with models 484 Waters UV absorbance detector and 746 integrator-recorder. The guard column (20 x 3.9 mm i.d), which goes between the pump and the injector, was tap-filled with LiChroprep RP-18 (25-40 µm, Merck, Darmstadt, Germany). The precolumn LiChroCART 4-4 LiChrospher 100 RP-18 (5µm) was purchased from Merck (Darmstadt, Germany), and was changed after the injection of 300 samples. The analytical column (250 x 4.6 mm i.d) was packed with Kromasil C_{18}, 5 µm particle size (AIT, France) and the mobile phase consisted of 0.01 M aqueous acetate buffer pH 4.8 containing 15 % (v/v) methanol. Prior to use, the mobile phase was filtered through a HVLP 04700 Durapore membrane (Millipore Corp., USA). This was carried through the column at 1.0 mL/min and the detection wavelength was set at 296 nm. All separations were carried out at room temperature.

Standard Solutions

The stock solution (640 mg/L) of meropenem was prepared weekly in HPLC-grade water, and stored at -80°C. The stock solution (1000 mg/L) of ceftazidime was weekly prepared in HPLC-grade water and stored in similar conditions.

The working solutions were prepared by dissolving the stock solution in HPLC-grade water, at final concentrations of 640-320-160-80-40-20-10-5-2.5-1 mg/L for meropenem and 800 or 50 mg/L for IS, respectively. Two calibration standards: (0-0.125-0.25-0.5-1.0-2.0 mg/L and (4.0-8.0-16.0-32.0-64.0 mg/L) were made daily from pooled rat plasma. These were prepared according to the sample preparation procedure described below.

Sample Preparation

The SPE column (Isolute C_{18} EC) was conditioned by elution with 3 x 1 mL of methanol then 3 x 1 mL of 0.01 M pH 4.8 acetate buffer. Plasma sample (0.1 mL) supplemented by 0.9 mL of 0.01 M pH 4.8 acetate buffer containing IS (4 mg/L or 0.250 mg/L, final concentrations), was loaded onto the conditioned column.

Table 1

Within-Day Variability and Accuracy in Measured Meropenem Concentrations in Plasma

Concentration Spiked (mg/L)	Concentration Found (mg/L) mean ± SD	Accuracy (%)	CV (%)
0.125 (n=8)	0.136 ± 0.014	108.9	10.0
2.0 (n=8)	1.97 ± 0.12	98.3	6.0
4.0 (n=8)	4.08 ± 0.14	102.0	3.3
64.0 (n=8)	65.23 ± 2.64	101.9	4.1

This fraction was washed with 3 x 0.15 mL of 0.01 M pH 4.8 acetate buffer. Both meropenem and IS were recovered with 2 x 0.1 mL of a mixture: 0.01 M pH 4.8 acetate buffer/methanol (50:50, v/v), and an aliquot of 20 µL was injected onto the chromatographic system from a refrigerated autosampler.

RESULTS AND DISCUSSION

Analytical Conditions

Solid-phase extraction (SPE) provides fast and efficient sample preparation, it reduces sample handling and eliminates the risk of emulsification.[13,14] The use of ceftazidime as IS provides a good reproducibility (Table 1), whereas in our experience the β-hydroxytheophylline showed a non-linear calibration curve. Two UV detection peaks are available for the analytical determination of meropenem in biological fluids.

Two groups of co-workers[7,12] have selected a wavelength below 230 nm for UV detection, which gives a good sensitivity but in contrast a poor specificity. Others[8-11] selected a wavelength at 296 nm as we did, which optimizes the specificity of the assay (Figure 1).

Linearity

Standard curves were constructed by plotting the peak-area ratio of meropenem to the internal standard versus the concentration of meropenem.

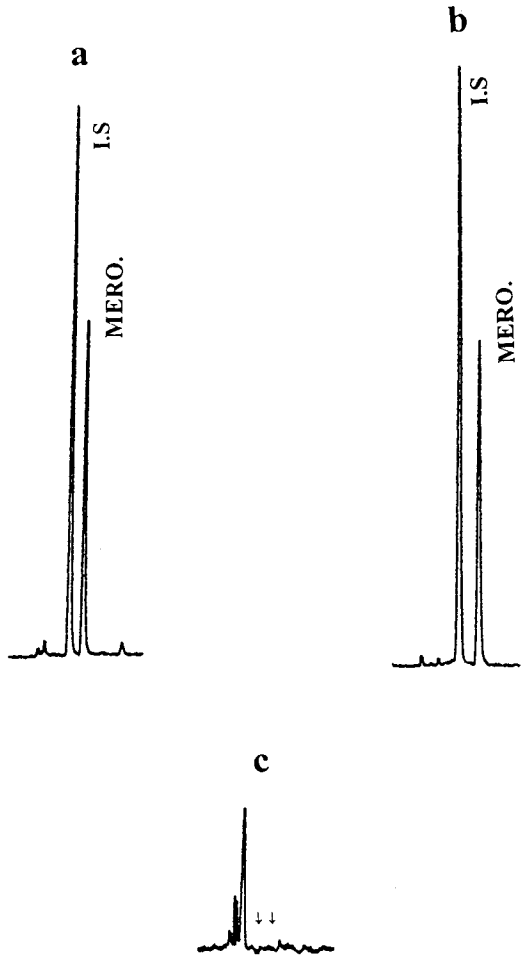

Figure 1. Chromatograms obtained from extract of (a) plasma spiked with meropenem (MERO., 16 mg/L) [RT = 7.3 min.] and internal standard (I.S) [RT = 5.8 min.]. Attenuation =32 mV, (b) plasma sample obtained at the 15th min. from a rat in a pharmacokinetic study (MERO., 13.3 mg/L). Attenuation =32 mV. (c) rat blank plasma. Attenuation =8 mV.

The calibration curves in plasma were linear over the following ranges 0.125-2.0 mg/L and 4.0-64.0 mg/L respectively, and characterized by the equation: y =1.152x - 0.0014, (r =0.9995, n =6) and y =0.0837x - 0.108, (r =0.9992, n =6) for the low and high ranges, respectively.

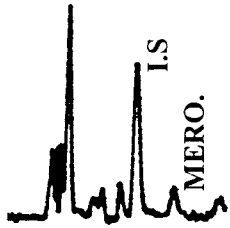

Figure 2. Chromatogram obtained from an extract of blank plasma spiked at the limit of quantitation : 0.125 mg/L of MERO. Attenuation =8 mV.

Recovery

The absolute recovery of the SPE was determined by comparing the peak areas of meropenem obtained from freshly prepared spiked plasma sample extracts to those obtained from aqueous standard solutions with the same concentration. The recovery was 92.8 ±2.5% at 0.5 mg/L and 95.4 ±2.9 % at 16.0 mg/L (n =3).

Chromatograms

Figure 1 shows chromatograms obtained from extracts of plasma spiked with 16 mg/L meropenem (Figure 1a), plasma sample obtained in a rat 15 min. after the administration of a single 100 mg/kg IV dose of meropenem (Figure 1b), and blank plasma (Figure 1c). Concentrations were calculated by comparing the ratio of peak areas of samples (MERO/IS), with daily calibration curves. Meropenem and IS were eluted in 7.3 and 5.8 min., respectively.

Limit of Quantitation

Only H. Elkaïli et al. estimated a limit of quantitation (LQ) of 0.25 mg/L using a plasma sample of 0.5 mL,[9] whereas all other authors only described a limit of detection.

In our method the limit of quantitation of meropenem was 0.125 mg/L (Figure 2), using 0.1 mL of plasma. The limit was taken as a chromatographic peak corresponding to the lowest point of the calibration curve (14), giving good accuracy (100.4%) and precision (CV=13%, n =6). The assay is sufficiently sensitive to be used for the analysis of plasma samples in pharmacokinetic studies both in rats and humans.

Table 2

Between-Day Variability and Accuracy in Measured Meropenem Concentrations in Plasma

Concentration Spiked (mg/L)	Concentration Found (mg/L) mean ± SD	Accuracy (%)	CV (%)
0 (n=6)	ND*	----	----
0.125 (n=6)	0.126 ± 0.016	100.4	13.0
2.0 (n=6)	1.90 ± 0.18	95.2	9.2
4.0 (n=6)	4.02 ± 0.39	100.5	9.7
64.0 (n=6)	60.22 ± 1.04	94.1	1.7

ND = Not detected.

Accuracy and Precision

The within-day variability of the method was characterized by coefficients of variation lower than 10 % (Table 1). The inter-day variability in plasma was assessed over thirty days from four series of rat plasma samples containing 0.125, 2.0, 4.0, 64.0 mg/L of meropenem (Table 2). The CV for inter-day analysis was less than 13 % and the accuracy within 94.1 and 100.5 %.

Specificity

Since this assay was essentially developed for pharmacokinetic studies in small laboratory animals, but could also be used in human study, possible chromatographic interference with others drugs was carefully investigated. Table 3, lists the retention times of this different drugs.

For most compounds, retention times were sufficiently different from those of meropenem and IS. However, cefsulodin and ceftizoxime may give a chromatographic interference after SPE.

Stability

With the earliest available carbapenem : imipenem, the important lack of stability (in biological fluids) was due to both a natural hydrolysis of lactam ring and a metabolism by the dipeptidase dehydropeptidase I, located on the brush

Table 3

Retention Time for Different Drugs Potentially Co-Administered with Meropenem Treatment

Drug	RT min.	Drug	RT min.
Cefamandole	43.9	Rifampicin	ND
Valproic acid	ND	Pipemidic acid	5.1
Methotrexate	16.8	Clozapine	ND
Temazepam	6.5	Ofloxacin	ND
Oxazepam	6.5	Hydroxyquinidine	9.6
Flurazepam	ND	Caffeine	23.1
Dflunitrazepam	3.7	Theophylline	15.4
Imipenem	5.2	Itraconzaole	ND
Bamifylline	18.4	Mexiletine	ND
Piroxicam	63.5	Cloxacillin	ND
Nitrofurantoin	20	Pefloxacin	ND
Flucytosine	5.9	Ciprofloxacin	ND
Ornidazole	19.5	Ceftizoxime	7.0
Azactam	6.2	Ampicillin	ND
Metoprolol	ND	Cefoxitin	17.8
Pindolol	26.2	Amoxycillin	6.4
Atenolol	24.4	Teicoplanin	ND
Propranolol	ND	Indomethacin	ND
Sotalol	9.9	Clonazepam	ND
Tazobactam	ND	Clobazam	ND
Vancomycin	9.9	Desmethyldiazepam	7.0
Cefalexin	19.6	Diazepam	6.9
Cefotaxime	14.3	Triazolam	ND
Cefazolin	16.3	Cefsulodin	5.3
Ceftriaxone	7.8	β-Hydroxytheophylline	15.3

RT - Retention time.
ND = Not detected.

border of the proximal tube requiring the co-administration of a dehydropetidase I enzyme inhibitor such as cilastatin.[13] With the meropenem, its seems that this precaution is not required and only H. Elkhaïli et al.[9] reported a non-enzymatic degradation of meropenem in urine and plasma samples at room temperature. The ring-open lactam (ICI 213,689), which is a natural metabolite of meropenem, can also be produced *ex vivo* by chemical hydrolysis of meropenem according to a first order reaction with an half-life of 11 h at 37°C.[15]

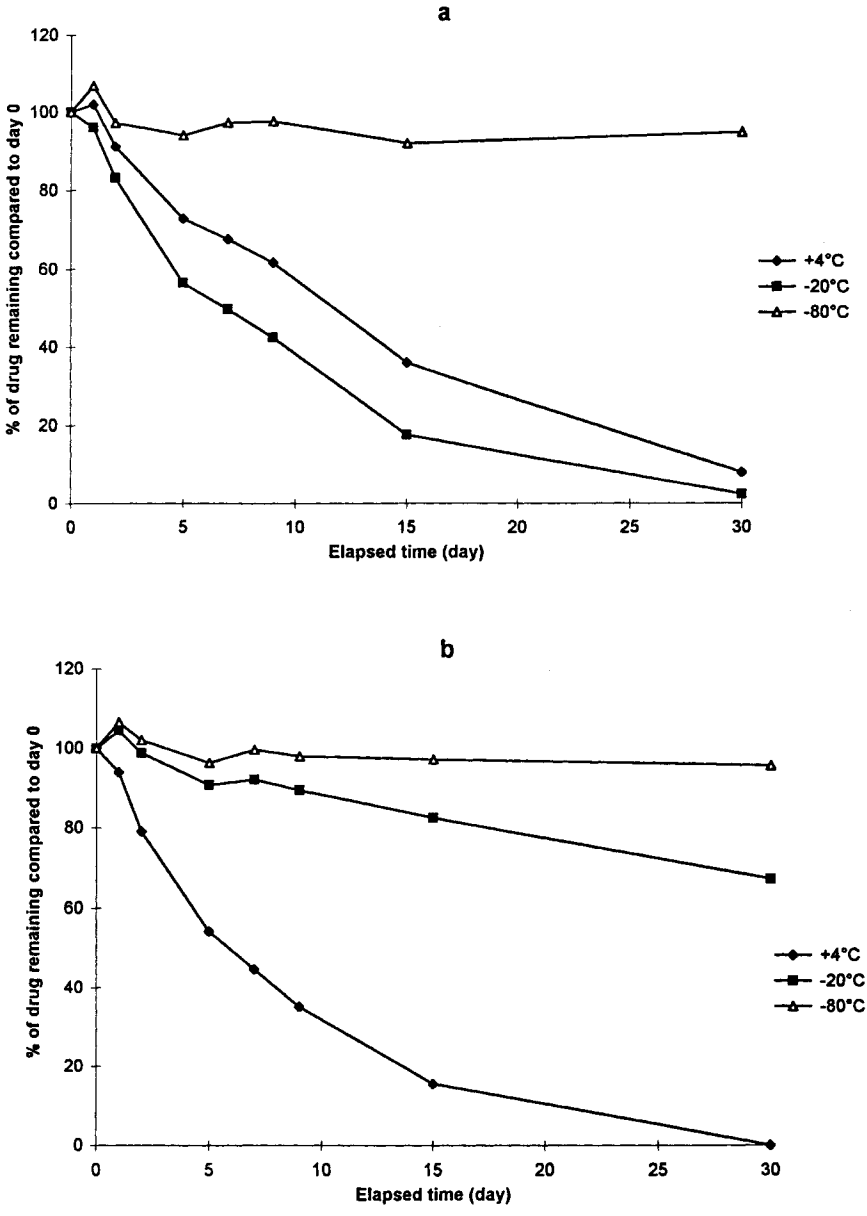

Figure 3. Curves of meropenem stability (a) plasma samples stored at +4°C, -20°C, -80°C during 1, 2, 5, 7, 9, 15, and 30 days, respectively. (b) plasma samples diluted with a stabilization solution (1:1, v/v) and stored in the same conditions.

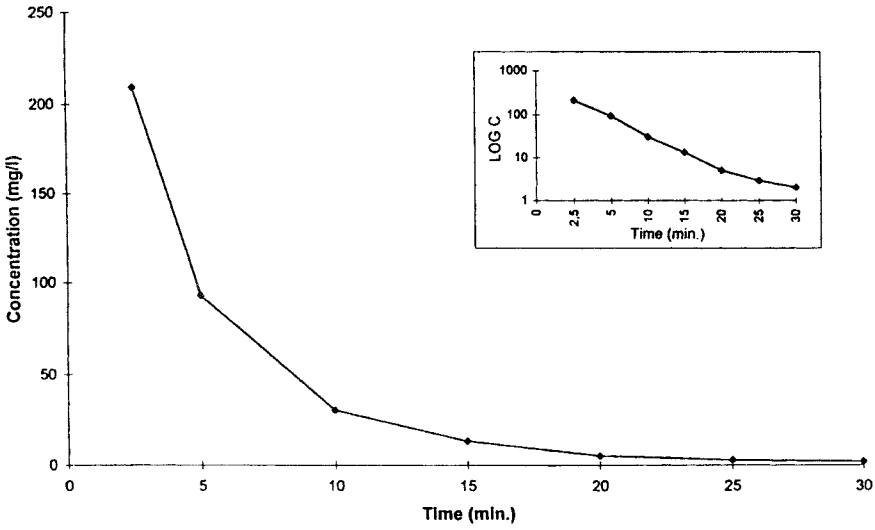

Figure 4 : Plasma levels of meropenem with time, following single IV administration (100 mg/kg) in rat.

Therefore, we evaluated the stability of meropenem (expressed as percentage of drug remaining) in both plasma and plasma diluted with a stabilizer (1:1, v/v): [0.5 M HEPES pH 6.8/ethylene glycol/HPLC grade water, (1:0.5:0.5, v/v/v)] after a storage at +4°C, -20°C, -80°C during 1, 2, 5, 7, 9, 15, and 30 days (Figure 3a and 3b). No significant degradation of meropenem in plasma was observed after 30 days at -80°C. In contrast meropenem was unstable at -20°C and at +4°C (Figure 3a). The storage of meropenem in a mixture plasma/stabilizer allowed a good stability at -80°C, and it slowed down the degradation at -20°C (Figure 3b).

Pharmacokinetic Application

The applicability of this assay was examined in a preliminary pharmacokinetic study in rats. A typical plasma concentration-time curve of meropenem is shown in Figure 4. Meropenem concentrations could be quantified for up to 30 min. post dosing, which corresponds to about six elimination half-lives of the drug ($t_{1/2}$ = 4.8 min.), indicating that the assay is suitable for pharmacokinetic analysis.

CONCLUSION

The HPLC assay described here demonstrated good reproducibility, accuracy and selectivity. It is more sensitive than the HPLC methods reported previously and suitable to be used both in animal or human pharmacokinetic studies, and therapeutic drug monitoring.

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