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Journal of Chromatography A, 812 (1998) 249–253

JOURNAL OF
CHROMATOGRAPHY A

Short communication

Determination of meropenem in serum by high-performance liquid chromatography with column switching

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Abstract

A rapid, accurate and sensitive liquid chromatographic assay with on-line solid-phase extraction for determination of meropenem in serum is described. Sample was directly injected onto the extraction column for sample clean-up and extraction. Thereafter, using an on-line column-switching system the drug was quantitatively transferred and separated on a C_{18} analytical column. Ultraviolet absorption at 298 nm was used for detection. The assay was linear from 1 to 100 μg/ml. Recovery was 98.5%. Based on a 20-μl sample volume (serum–water, 1:1, v/v), detection limit was 0.1 μg/ml. An application of the method to study the pharmacokinetics of meropenem is given. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Column switching; Sample preparation; Meropenem; Antibiotics

1. Introduction

Meropenem is a highly active carbapenem antibiotic. It has a broad spectrum of antimicrobial activity in vitro, the majority of Gram-positive and Gram-negative and aerobic pathogens being highly susceptible to the drug. Meropenem has shown clinical and bacteriological efficacy in the treatment of a wide range of serious infections [1].

Several methods have been developed for the determination of the concentration of meropenem in biological fluids, including bioassays [2,3] and liquid chromatographic techniques [4–9]. High-performance liquid chromatography (HPLC) is favoured for the assay of antibiotics in biological specimens because of its speed and specificity, and because it allows the simultaneous analysis of several different drugs or their metabolites. Classical HPLC methods usually involve preparation of the sample before analysis. Sample preparation is often the most time-consuming step in a chemical analysis. Conventional preparation techniques involve numerous steps which increase the potential of introducing a bias into the results. Therefore, extraction procedures which eliminate manual sample manipulation are particularly attractive. The on-line solid-phase extraction (SPE) of drugs in biological samples is gaining widespread application [10].

In this paper we describe a column-switching technique for the determination of meropenem. No pre-treatment and no internal standard are required. The method allows direct injection of serum into the chromatograph. An extraction pre-column produced on-line sample clean-up. Thereafter the fraction of interest was selectively transferred to an analytical C_{18} column where the drug was analysed. The

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PII: S0021-9673(98)00249-0

method requires a small serum volume (10 μl) and it is fast, efficient, simple and easily automated. The on-line sample clean-up technique described has previously been successfully used to determine other antibiotics in biological fluids [11,12].

2. Experimental

2.1. Reagents and chemicals

Methanol was obtained from Merck (Darmstadt, Germany). Tetrabutylammonium hydrogensulfate (TBA HSO_4) was supplied by Sigma (St. Louis, MO, USA). Meropenem was from Zeneca. Water was obtained daily from an Elgastat UHQ PS apparatus (ELGA, High Wycombe, UK). Drug-free serum was obtained from volunteers.

2.2. Apparatus

The HPLC system consisted of a Beckman model 110B HPLC pump (Beckman, Fullerton, CA, USA), a Beckman 126 programmable solvent delivery module and a Beckman 166 programmable ultraviolet detector. The chromatograms were integrated with a System Gold laboratory data system (Beckman). The injector was a Rheodyne Model 7125 manual injection valve equipped with a 20-μl sample loop. The coupled-column system was operated by a pneumatic, six-port, automated, switching valve (Valco, Schencon, Switzerland) controlled by the HPLC system.

2.3. Chromatographic conditions

The extraction column was 5 cm×2.1 mm I.D., dry filled with an NH_2 40-μm silica (Supelclean, LC-NH_2, from Supelco). Chromatography was performed on a C_{18} analytical column (15 cm×4.6 mm I.D.) packed with 3-μm diameter particles (Supelco). The mobile phase 1 (wash solution) consisted of 5% methanol in 0.01 *M* phosphate buffer (pH 7.0). The mobile phase 2 was composed of 30% methanol, 70% 0.01 *M* phosphate buffer (pH 7.0) and 5 m*M* TBA HSO_4. The flow-rate for extraction column was set at 0.3 ml/min. The flow-rate for analytical column was set at 1 ml/min. The effluent from the analytical column was monitored by UV at a wavelength of 298 nm. All analyses were performed at ambient temperature.

2.4. Column-switching procedure

The scheme for the switching procedure is shown in Fig. 1. Serum samples were diluted 1:1 (v/v,) with purified water to avoid clogging of column frits. Twenty μl of the diluted sample were injected directly onto the extraction column. Mobile phase 1, which passed through the column and was directed to waste, was delivered by pump 1, while pump 2 delivered mobile phase 2 to the analytical column (switching valve at the initial position (Fig. 1A)). Matrix components were removed whilst the drug was trapped in the column. After a flushing period of 1.1 min the valve was switched and mobile phase 2 from pump 2 eluted the analyte of interest to the analytical column (Fig. 1B). The connection time of the extraction column to the analytical column was 1.3 min. Thereafter the valve was switched to the initial position and pump 1 delivered mobile phase 1 to the extraction column to prepare it for the next

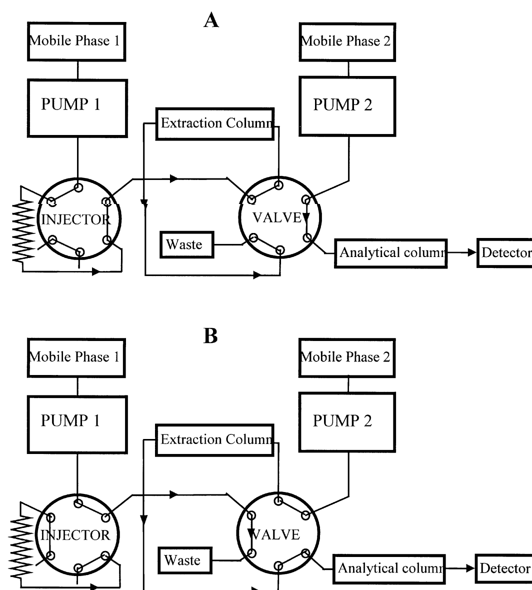

Fig. 1. Scheme of column switching.

sample, while pump 2 maintained the flow of mobile phase 2 through the analytical column where meropenem was separated and detected by UV.

2.5. Quantification

Stock solutions were prepared by dissolving meropenem in purified water and storing at −80°C. Working solutions were prepared daily by dilution of stock solutions. Calibration standards were prepared by spiking control serum at concentration of meropenem ranging from 1 to 100 μg/ml.

Each concentration was measured eight times. Peak areas were plotted versus drug concentrations and the resulting calibration curve was used to calculate the drug concentrations of the unknown samples. The detection limit was defined as the analyte concentration yielding a peak three times the noise level. The separation was examined for interference from a number of drugs which could be administered with the antibiotic (fluconazole, acyclovir, theophylline, digoxin, ranitidine, paracetamol, vancomycin). Recovery was determined by comparing the peak area resulting from extracted spiked serum standard to the peak area resulting from an aqueous solution at the same drug concentration injected directly onto the analytical column.

3. Results

Fig. 2 shows chromatograms obtained after injecting both a blank and a spiked serum. The absence of interference demonstrates the clean-up efficiency. Meropenem had a retention time of 4.2 min and was well separated from the other detectable components in serum at the selected wavelength. Using the criterion of minimum detectability as three times the system noise, the detection limit with 10 μl of serum was 0.1 μg/ml. The calibration curves obtained were linear over the working interval 1–100 μg/ml of meropenem. The regression line obeyed the equation $y=(0.5364\pm0.00198)x+(-0.119\pm 0.09)$, the correlation coefficient being $r^2=0.999$. The accuracy was between −6.50 and +11.50% for the concentration range 1–100 μg/ml. Values for precision (relative standard deviation, R.S.D.) were

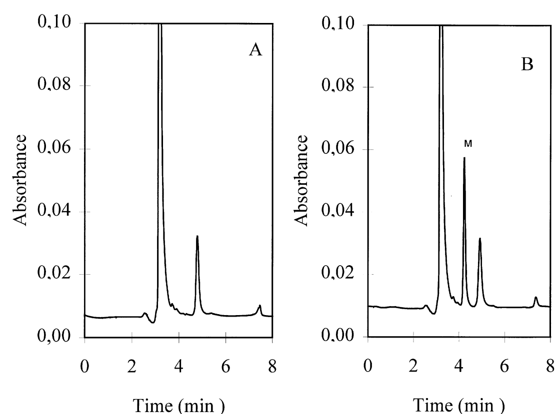

Fig. 2. Chromatograms of (A) drug-free serum and (B) serum spiked with 10 μg/ml of meropenem (M).

between 2.14 and 9.80%. The results are shown in Table 1. The column-switching procedure described gave nearly complete recovery of the investigated drug from serum. Recovery for the spiked serum samples was 98.5%. Meropenem was extracted and analysed in the presence of several commonly administered drugs. No interference was seen from any of the compounds tested: fluconazole, acyclovir, theophylline, digoxin, ranitidine, paracetamol, vancomycin.

NH_2 packing was chosen for sample clean-up because it gave reduced background from endogenous component when compared with C_8 and C_{18}. Moreover, alkylamino material is more selective for meropenem. In fact the functional group is positively charged at the pH used in the assay, and it can bind the carboxylic group of the drug that is negatively charged. TBA, in the mobile phase, acting to both displace the drug from the cationic sites on the cartridge and to form an ionpair with the carboxy group of meropenem, make the elution of the analyte easy, and the peak was well resolved.

The extraction column was replaced after 100 injections. No deterioration of the analytical column was observed during the study. It was washed at the end of each day with water–methanol (1:1, v/v). The six-port valve and the loop were washed with sodium dodecyl sulfate (SDS) every day. No blocking of frits, phase shrinkage or back-pressure problem was experienced.

Table 1
Linearity, precision and accuracy for meropenem $y=(0.5364\pm0.00198)x+(-0.119\pm0.09)$

Nominal (μg/ml)	Actual value (mean±S.D., n=8) (μg/ml)	Precision (%)	Accuracy (%)
1	1.11±0.09	9.80	11.50
2	1.87±0.15	7.25	−6.50
5	4.74±0.32	6.44	−5.20
10	10.43±0.34	3.40	4.30
20	20.45±0.78	3.90	2.25
50	51.32±2.16	4.32	2.64
100	97.13±2.14	2.14	−2.87

Several HPLC methods have been used for the determination of meropenem and various approaches to sample handling have been proposed. The methods used by Bax et al. [4] and Nilsson-Ehle et al. [5] are very briefly outlined in papers aimed at studying meropenem pharmacokinetics. They reported detection limits of 0.4 and 0.06, respectively. Bax et al. used an HPLC method based on SPE, but this procedure was carried out off-line, and therefore does not eliminate the need of sample manipulation. The only authors which describe in detail an HPLC method for meropenem are Elkhaili et al. [8]. They deproteinized the samples with acetonitrile, the excess of the solvent was then removed with methylene chloride. The quantitation limit reported is lower (0.25 mg/l) than that determined by us, but the extraction procedure is prone to complication because it involves separate steps which not only make the method time consuming, but also increase the potential to introduce bias in the results. The advantages of the proposed method are: minimum sample handling, considerable saving of both time and chemicals, sample size requirements, and applicability to automation. The remarkable simplicity of the procedure does not require the preparation or maintenance of costly apparatus or skilled operators.

3.1. Application

The HPLC assay described was applied to determine serum concentrations of meropenem after 1 g intravenous administration to three male volunteers who gave their informed consent. Serum levels of the antibiotic were fitted to a two compartment open model by means of an iterative, nonlinear least-squares technique. Fig. 3 shows serum levels of meropenem from a volunteer after administration of a 1-g dose by intravenous injection over 5 min. The main pharmacokinetic parameters (mean±S.D.) calculated in this study, terminal half-life ($t_{1/2\beta}$), area under the serum concentration–time curve (AUC), volume of distribution of the central compartment (V_1), serum clearance (Cl_s) and mean residence time in the central compartment (MRT_1) are given in Table 2. Our results are in agreement with those previously reported [9].

4. Conclusions

The method described is advantageous for simplicity of execution, saving chemicals, speed of analysis, efficiency and it can easily be automated.

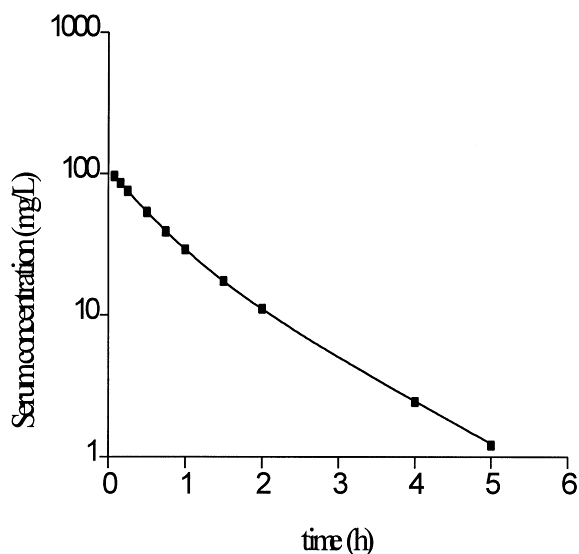

Fig. 3. Serum concentration–time curve of meropenem in a volunteer after an intravenous 1-g dose.

Table 2
Pharmacokinetic parameters (mean±S.D.) of meropenem after i.v. administration of a 1-g dose

$t_{1/2\beta}$(min)	AUC (mg h/l)	Cl_s (ml/min)	V_1 (l)	MRT_1 (min)
61.0±8.0	92.2±9.3	180.7±20.1	9.1±1.7	65.0±9.9

SPE provides excellent background serum interference removal and quantitative antibiotic recovery. Manual sample preparation was reduced to an absolute minimum: for the serum sample only water was added prior to injection into the chromatograph. Furthermore, the small sample volume required makes this assay particularly useful for specimens from children and neonates. The assay is suitable for pharmacokinetic studies and clinical purposes.

Acknowledgement

This work was supported by a grant of MURST 60%.

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