Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Pharmaceutical and Biomedical Analysis
33 (2003) 947–954
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Validation of HPLC and UV spectrophotometric methods for the determination of meropenem in pharmaceutical dosage form

Andreas S.L. Mendez [a,*], Martin Steppe [a,b], Elfrides E.S. Schapoval [a,b]

[a] *Programa de Pós-Graduação em Ciências Farmacêuticas, Faculdade de Farmácia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil*

[b] *Departamento de Produção e Controle de Medicamentos, Faculdade de Farmácia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil*

Received 10 September 2002; received in revised form 7 February 2003; accepted 13 June 2003

Abstract

A high-performance liquid chromatographic method and a UV spectrophotometric method for the quantitative determination of meropenem, a highly active carbapenem antibiotic, in powder for injection were developed in present work. The parameters linearity, precision, accuracy, specificity, robustness, limit of detection and limit of quantitation were studied according to International Conference on Harmonization guidelines. Chromatography was carried out by reversed-phase technique on an RP-18 column with a mobile phase composed of 30 mM monobasic phosphate buffer and acetonitrile (90:10; v/v), adjusted to pH 3.0 with orthophosphoric acid. The UV spectrophotometric method was performed at 298 nm. The samples were prepared in water and the stability of meropenem in aqueous solution at 4 and 25 °C was studied. The results were satisfactory with good stability after 24 h at 4 °C. Statistical analysis by Student's *t*-test showed no significant difference between the results obtained by the two methods. The proposed methods are highly sensitive, precise and accurate and can be used for the reliable quantitation of meropenem in pharmaceutical dosage form.

Keywords: Meropenem; HPLC; UV Spectrophotometry; Validation; Pharmaceutical dosage form

1. Introduction

Meropenem (Fig. 1), chemically (4R,5S,6S)-3-[[(3S,5S)-5-dimethylcarbamoyl pyrrolidin-3-yl]-thio]-6-[(1R)-1-hydroxyethyl]-4-methyl-7-oxo-1-azabicyclo[3,2,0] hept-2-ene-2-carboxylic acid, is a new parenteral carbapenem antibiotic with a very broad spectrum of antibacterial activity against the majority of gram-positive and gram-negative pathogens [1]. It is more active in vitro than imipenem against Enterobacteriaceae and *Pseudomonas aeruginosa*, but less active against gram-positive cocci [2]. Meropenem is more stable to

* Corresponding author. Tel.: +55-51-3316-5214; fax: +55-51-3316-5378.
E-mail address: aslmufrgs@yahoo.com.br (A.S.L. Mendez).

0731-7085/03/$ - see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/S0731-7085(03)00366-2

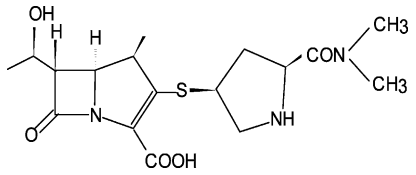

Fig. 1. The chemical structure of meropenem.

ring opening by human renal dehydropeptidase I (DHP-I) than imipenem and consequently does not require concomitant administration of a DHP-1 inhibitor. This antibiotic has shown clinical efficacy in the treatment of a wide range of serious infections such as intra-abdominal infections, urinary tract infections and lower respiratory tract infections including patients with cystic fibrosis [3,4].

A survey of literature has revealed several analytical methods for the determination of meropenem and its main metabolite (ICI-213689) in biological fluids, including high-performance liquid chromatography (HPLC) [5–8], capillary zone electrophoresis [9,10] and microbiological assay [11]. The literature reports few methods for the quantitation of meropenem in pharmaceutical dosage form. HPLC adopted by the United States Pharmacopoeia [12] was based on the mobile phase containing tetrabutylammonium hydroxide as ion-pairing agent, which shortens column life. Moreover, the mobile phase preparation requires tedious procedures. A recent work reports a liquid chromatography using an internal standard [7]. This work does not describe analytical parameters that are very important for the validation of analytical procedure such as accuracy, specificity, robustness, limit of detection (LOD) and limit of quantitation (LOQ). Since this antibiotic is widely used in the antimicrobial therapy, it is important to develop and validate analytical methods for its determination in pharmaceutical dosage form. The HPLC method has been highly used in the quality control of drugs because of their sensitivity, reproducibility and specificity. The UV spectrophotometric (UV) method is very simple, rapid and economical and allows the determination of drugs with sufficient reliability.

The present work reports the development and validation of a HPLC method and a UV method for the estimation of meropenem in powder for injection. The stability of meropenem in aqueous solution was studied using the HPLC method.

2. Experimental

2.1. Chemicals

Meropenem reference standard was kindly supplied by Sumitomo Pharmaceuticals Co. Ltd. (Osaka, Japan). Pharmaceutical dosage form (Meronem®) containing meropenem was obtained commercially and was claimed to contain 500 mg (as anhydrous base) of the drug and 104 mg of the anhydrous sodium carbonate as excipient. Acetonitrile for chromatography LiChrosolv®, potassium dihydrogenphosphate p.a. and orthophosphoric acid p.a. were obtained from Merck (Darmstadt, Germany). Ultrapure water was obtained from a Milli-Q® UF-Plus apparatus (Millipore) and was used to prepare all solutions for the HPLC method and distilled water was used to prepare all solutions for the UV method. All solutions were prepared daily.

2.2. Instrumentation and analytical conditions

The HPLC method was performed on a Shimadzu SCL-10A HPLC system, equipped with a model LC-10AD pump, UV–vis detector SPD-10A, Rheodyne injector fitted with a 20 μl loop and a integrator C-R6A chromatopac model (Shimadzu, Kioto, Japan). The method was conducted using a reversed-phase technique. Meropenem was eluted isocratically with a flow rate of 1.0 ml/min using a mobile phase consisting of 30 mM monobasic phosphate buffer and acetonitrile (90:10; v/v), adjusted to pH 3.0 with orthophosphoric acid. The wavelength of the UV–vis detector was set to 298 nm. The mobile phase was prepared daily, filtered through a 0.45 μm membrane filter (Millipore) and sonicated before use. A LiChrospher® 100 RP-18 column (250 mm × 4.0 mm i.d., 5 μm particle size) (Merck) was used. The HPLC system was operated at 25 ± 1 °C.

UV method was performed on a UV–vis Recording Spectrophotometer UV-160A (Shimadzu) at 298 nm and using 1.0 cm quartz cells. SPECTRA MANAGER software was used for all absorbance measurements.

2.3. Preparation of the standard solutions

2.3.1. HPLC method

Accurately weighed 40 mg of meropenem reference standard was transferred to 200 ml volumetric flask and dissolved in ultrapure water (final concentration of 200 μg/ml). From this solution, concentrations of 10, 20, 30, 40, 50, 60 and 70 μg/ml were made in 20 ml volumetric flasks.

2.3.2. UV method

Accurately weighed 25 mg of meropenem reference standard was transferred to 250 ml volumetric flask and dissolved in distilled water (final concentration of 100 μg/ml). From this solution, concentrations of 5, 10, 15, 20, 25, 30 and 35 μg/ml were made in 20 ml volumetric flasks.

2.4. Preparation of the sample solutions

2.4.1. HPLC method

Accurately weighed amount of powder for injection equivalent to 20 mg of meropenem was transferred to 100 ml volumetric flask and dissolved in ultrapure water (final concentration of 200 μg/ml). Aliquot of this solution were diluted in ultrapure water at concentration of 40 μg/ml.

2.4.2. UV method

Accurately weighed amount of powder for injection equivalent to 20 mg of meropenem was transferred to 100 ml volumetric flask and dissolved in distilled water (final concentration of 200 μg/ml). Aliquot of this solution were diluted in distilled water at concentration of 20 μg/ml.

2.5. Method validation

The methods were validated according to International Conference on Harmonisation guidelines [13] for validation of analytical procedures. Analysis of variance (ANOVA) was used to verify the validity of the methods.

2.5.1. Linearity

The calibration curve was obtained with seven concentrations of the standard solution (10–70 μg/ml for HPLC method and 5–35 μg/ml for UV method). The solutions were prepared in triplicate. The linearity was evaluated by linear regression analysis, which was calculated by the least square regression method.

2.5.2. Precision

The precision of the assay was determined by repeatability (intra-day) and intermediate precision (inter-day). Repeatability was evaluated by assaying samples, at same concentration and during the same day. The intermediate precision was studied by comparing the assays on different days (3 days). Six sample solutions (40 μg/ml for HPLC method and 20 μg/ml for UV method) were prepared and assayed.

2.5.3. Accuracy

The accuracy was determined by recovery of known amounts of meropenem reference standard added to the samples at the beginning of the process. For the HPLC method, an accurately weighed amount of powder for injection equivalent to 20 mg of meropenem was transferred to 100 ml volumetric flask and dissolved in ultrapure water (final concentration of 200 μg/ml). Aliquots of 3.0 ml of this solution were transferred into 20 ml volumetric flasks containing 1.0, 2.0 and 3.0 ml of meropenem standard solution (200 μg/ml) and ultrapure water was added to make up to volume to give a final concentrations of 40, 50 and 60 μg/ml. For the UV method, an accurately weighed amount of powder for injection equivalent to 20 mg of meropenem was transferred to 200 ml volumetric flask and dissolved in distilled water (final concentration of 100 μg/ml). Aliquots of 3.0 ml of this solution were transferred into 20 ml volumetric flasks containing 1.0, 2.0 and 3.0 ml of meropenem standard solution (100 μg/ml) and distilled water was added to make up to volume to give a final concentrations of 20, 25 and 30 μg/ml. All solutions were prepared in triplicate and

assayed. The percentage recovery of added meropenem standard was calculated using the equation proposed by AOAC [14].

2.5.4. Specificity

The specificity was determined for the HPLC method. Sample solutions (70 μg/ml) were submitted to accelerated degradation by heat (70 °C for 1.5 h) and by adittion of 0.02 N NaOH for 10 min in order to verify that none of the degradation products of the analyte interfered with the quantitation of drug.

2.5.5. Robustness

The robustness of the HPLC method was determined by analysis of samples under a variety of conditions such as small changes in the pH (3.0–3.6) and in the percentage of acetonitrile (10–8%) in mobile phase and changing the column (Metachem® LC RP-18, with 250 mm × 4.6 mm i.d. and 5 μm particle size). The effect on retention time and peak parameters were studied.

2.5.6. Limit of detection and limit of quantitation

The parameters LOD and LOQ were determined on the basis of response and slope of the regression equation.

2.6. Stability

The stability of meropenem in aqueous solution was studied by HPLC method. Sample solutions of meropenem (70 μg/ml) were prepared in triplicate and stored at 4 and 25 °C for 24, 48 and 72 h. The stability of these solutions was studied by performing the experiment and looking for the change in the chromatographic pattern compared with freshly prepared solutions.

3. Results and discussion

3.1. HPLC method

The development of the HPLC method for the determination of drugs has received considerable attention in recent years because of their importance in routine quality control analysis. A reversed-phase HPLC method was proposed as a suitable method for the estimation of meropenem in pharmaceutical dosage form. The chromatographic conditions were adjusted in order to provide a good performance of the assay. The mobile phases investigated were water and acetonitrile (80:20; v/v), water and methanol (85:15; v/v), 30 mM monobasic phosphate buffer and methanol (88:12; v/v), adjusted to pH 3.0–5.0 with orthophosphoric acid, and 30 mM monobasic phosphate buffer and acetonitrile (90:10; v/v), adjusted to pH 3.0–5.0 with orthophosphoric acid. Mobile phase selection was based on peak parameters (symmetry, tailing), run time, easy of preparation and cost. Fig. 2 shows a typical chromatogram obtained from the analysis of a standard and sample solution of meropenem using the proposed method. As shown in this figure, meropenem was eluted forming symmetrical peak, well separated from the solvent front. The retention time observed (6.84 min) allows a rapid determination of the drug, which is important for routine analysis.

The calibration curves for meropenem were constructed by plotting concentration versus peak area and showed good linearity in the 10–70 μg/ml range. The representative linear equation was $y = 14\,608x + 757.47$, with a correlation coefficient ($r = 0.999$) highly significant for the method

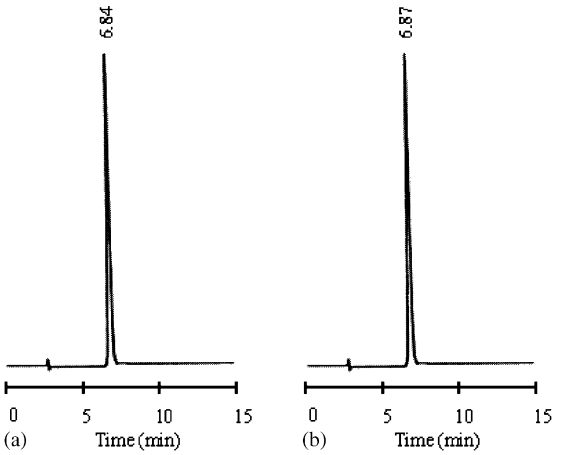

Fig. 2. HPLC chromatograms of meropenem reference standard 40 μg/ml (a) and meropenem powder for injection 40 μg/ml (b).

Table 1
Results of regression analysis of data for the quantitation of meropenem by the proposed methods

Statistical parameters	HPLC Method	UV Method
Regression equation[a]	$y = 14\,608x + 757.47$	$y = 0.0259x + 0.0033$
Correlation coefficient (r)	0.999	0.999
Standard error of slope	1.083×10^{1}	8.83×10^{-5}
Standard error of intercept	5.618×10^{2}	1.24×10^{-3}
Concentration range (μg/ml)	10–70	5–35

y is the peak area (HPLC method) and absorbance (UV method). x is the concentration of the drug in μg/ml (HPLC method and UV method).
[a] Based on three calibration curves.

(Table 1). The LOD and LOQ were found to be 4.24 and 12.85 ng/ml, respectively indicating a high sensitivity of the method. The validity of the assay was verified by means of the ANOVA. According to ANOVA there are linear regression ($F_{\text{calculated}} > F_{\text{critical}}$; $P = 0.01$) and there are no deviation from linearity ($F_{\text{calculated}} < F_{\text{critical}}$; $P = 0.01$).

The precision of the method was determined by repeatability (intra-day) and intermediate precision (inter-day) and was expressed as R.S.D. (%) of a series of measurement. The experimental values obtained for the determination of meropenem in samples are present in Table 2. The result obtained shows R.S.D. of 0.78% indicating good intra-day precision. Inter-day variability was calculated from assays on 3 days and shows a mean R.S.D. of 0.85%. The accuracy of the method was determined and the mean recovery was found to be 99.72% (Table 3) indicating an agreement between the true value and the value found.

The described HPLC method is specific. No interfering peaks were observed in degradated solutions and the degradation products were observed at relative retention time of 2.5 min (Fig. 3). The method was found to be robust when the column and the mobile phase were varied. During these investigations, the retention times were modified, however the area and symmetry of peaks were conserved (Table 4).

3.2. UV method

The proposed UV method allows a rapid and economical quantitation of meropenem in powder for injection without any time-consuming sample preparation. Moreover, the spectrophotometric methods involve simple instrumentation compared with other instrumental techniques. The absorption spectra of meropenem in aqueous solution is shown in Fig. 4. The $\lambda_{\max}$ was found to be 298 nm. These wavelength was used for all measurements. For more accurate analysis, Ringbom curve was constructed and the linear range was observed. Calibration curves were constructed in the range of expected concentrations (5–35 μg/ml). Beer's law is obeyed over this concentration range. The representative equation analysis was $y = 0.0259x + 0.0033$, with a correlation coefficient of 0.999 (Table 1). The LOD and LOQ were found to be 19.48 and 59.05 ng/ml, respectively. According to ANOVA there are linear regression ($F_{\text{calculated}} > F_{\text{critical}}$; $P = 0.01$) and there are no deviation from linearity ($F_{\text{calculated}} < F_{\text{critical}}$; $P = 0.01$).

Table 2 shows the experimental values obtained for the determination of meropenem in samples, indicating a satisfactory intra-day variability (R.S.D. of 0.89%) and inter-day variability (R.S.D. of 0.32%). A good accuracy of the method was verified with a mean recovery of 101.18% (Table 3).

3.3. Comparison between HPLC method and UV method

The proposed analytical methods were compared using statistical analysis. The Student's t-test was applied and does not reveal significant difference between the experimental values obtained in the sample analysis by the two methods. The calculated t-value ($t_{\text{calc}} = 1.201$) was found to

Table 2
Results of the determination of meropenem in powder for injection by the proposed methods

Method	Sample (mg) (powder for injection)	Experimental amount[a] (mg)	Purity (%)	R.S.D. (%) Intra-day	R.S.D. (%) Inter-day[b]
HPLC	500	502.42 (0.59%)	100.48	0.78	0.85
		501.91 (0.27%)	100.38		
		497.21 (0.64%)	99.44		
		498.62 (0.40%)	99.72		
		493.83 (0.69%)	98.76		
		493.13 (0.39%)	98.62		
UV	500	496.25 (0.21%)	99.25	0.89	0.32
		507.45 (0.10%)	101.49		
		504.95 (0.0%)	100.99		
		505.90 (0.0%)	101.18		
		506.50 (0.10%)	101.30		
		509.00 (0.10%)	101.80		

[a] Mean of three determinations. R.S.D. are listed in brackets.
[b] 3 days.

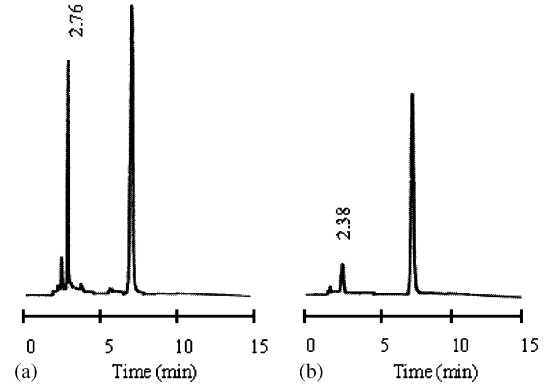

Fig. 3. Chromatograms from accelerated degradation studies of meropenem aqueous solution (70 μg/ml). In (a), meropenem at 70 °C for 1.5 h (degradation product at retention time of 2.76 min). In (b), meropenem in 0.02 N NaOH for 10 min (degradation product at retention time of 2.38 min).

be less than the critical t-value ($t_{crit} = 2.228$) at 5% significance level.

3.4. Stability

The stability of meropenem in aqueous solution was evaluated to verify that any spontaneous degradation occur when the samples were prepared. Fig. 5 shows the stability profile at 4 and 25 °C for 24, 48 and 72 h. The results were expressed as percentage of drug remaining. The data obtained showed that sample solutions were stable during 24 h when stored at 4 and 25 °C with a degradation less than 5%. Meropenem was less stable at 25 °C with a degradation of 12.7% after 72 h.

Table 3
Experimental values obtained in the recovery test for meropenem in powder for injection by the proposed methods

Method	Sample concentration (μg/ml)	Concentration of added standard (μg/ml)	% Recovery[a] ± R.S.D. (%)
HPLC	30.0	10.0	99.11 ± 0.77
	30.0	20.0	100.10 ± 0.61
	30.0	30.0	99.97 ± 0.63
UV	15.0	5.0	100.76 ± 1.16
	15.0	10.0	101.78 ± 0.57
	15.0	15.0	101.02 ± 1.15

[a] Mean of three determinations.

Table 4
Robustness of the method observed by varying the mobile phase (pH and % of acetonitrile) and the column

Sample concentration	Mobile phase pH 3.6		8% acetonitrile in mobile phase		Column	
(μg/ml)	rt	Peak area	rt	Peak area	rt	Peak area
40	5.30	590 212 (0.59%)	10.69	590 433 (1.13%)	10.25	594 202 (0.99%)
40	5.29	602 929 (0.58%)	10.79	597 756 (0.25%)	10.16	601 538 (0.75%)
40	5.35	585 820 (0.91%)	10.72	588 950 (0.49%)	10.19	598 969 (0.11%)

This parameter was analysed by the HPLC method. Each sample was injected three times. R.S.D. are listed in brackets.

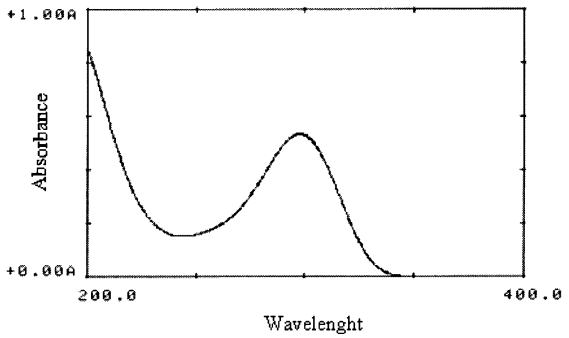

Fig. 4. UV spectrum of meropenem reference standard in aqueous solution (20 μg/ml).

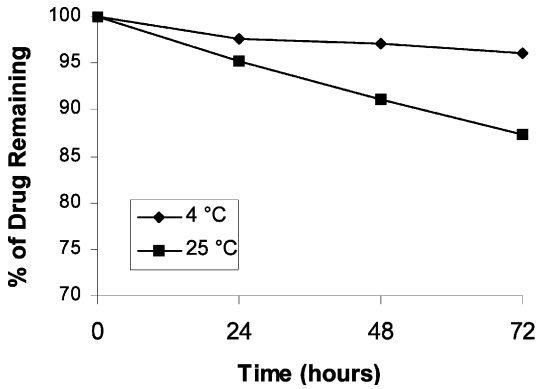

Fig. 5. Curves of meropenem aqueous solution (70 μg/ml) stability stored at 4 and 25 °C during 24, 48 and 72 h.

4. Conclusions

The HPLC method and the UV method for the determination of meropenem in powder for injection were found to be simple, rapid, precise, accurate and sensitive. Moreover, the HPLC method is suitable for the investigation of the chemical stability of meropenem. In summary, the proposed methods can be used for the drug analysis in routine quality control.

Acknowledgements

We would like to thank Sumitomo Pharmaceuticals Co. Ltd and LEPCQ. This study was supported by CNPq program.

References

[1] M.A. Pfaller, R.N. Jones, Diagn. Microbiol. Infect. Dis. 28 (1997) 157–163.
[2] J.L. Blumer, Int. J. Antimicrob. Agents 8 (1997) 73–92.
[3] L.R. Wiseman, A.J. Wagstaff, R.N. Brogden, H.M. Bryson, Drugs 50 (1995) 73–101.
[4] J.S. Bradley, Pediatr. Infect. Dis. J. 16 (1997) 263–268.
[5] S. Bompadre, L. Ferrante, M. De Martinis, L. Leone, J. Chromatogr. A 812 (1998) 249–253.
[6] M. Ehrlich, F.D. Daschner, K. Kümmerer, J. Chromatogr. B 751 (2001) 357–363.
[7] Y. Özkan, I. Küçükgüzel, S.A. Özkan, H.Y. Aboul-Eneim, Biomed. Chromatogr. 15 (2001) 263–266.
[8] C. Robatel, T. Buclin, P. Éckert, M.D. Schaller, J. Biollaz, L.A. Decosterd, J. Pharmac. Biom. Anal. 29 (2002) 17–33.
[9] Y. Mrestani, R. Neubert, F. Nagel, J. Pharmac. Biom. Anal. 20 (1999) 899–903.
[10] S. Taniguchi, K. Hamase, A. Kinoshita, K. Zaitsu, J. Chromatogr. B 727 (1999) 219–225.

[11] M.A. Al-Meshal, M.A. Ramadan, K.M. Lotfi, A.M. Shibl, J. Clin. Pharm. Therap. 20 (1995) 159–163.
[12] The United States Pharmacopoeia, 25 ed., Rockville, United States Pharmacopoeial Convention (2002) 1083–1084.
[13] Validation of analytical procedures, Proceedings of the International Conference on Harmonisation (ICH). Commission of the European Communities (1996).
[14] AOAC, Official Methods of Analytical Chemists of AOAC, 15 ed., XVII (1990).