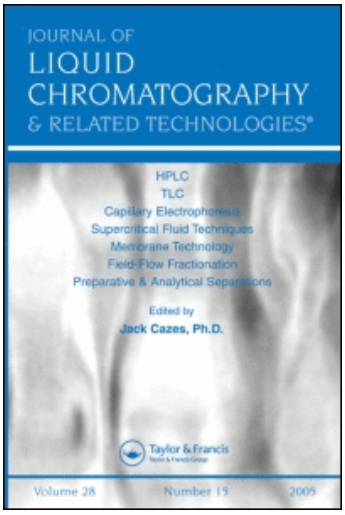

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HPLC ANALYSIS OF A MEROPENEM-OFLOXACIN MIXTURE IN INTRAVENOUS SOLUTIONS USING A NONPOROUS OCTADECYLSILANE COLUMN

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ABSTRACT

A stability-indicating High Performance Liquid Chromatography (HPLC) method utilizing nonporous silica particle technology has been developed for the assay of meropenem and ofloxacin from 5% dextrose in water injection USP and 0.9% sodium chloride injection USP. The separation and quantitation of the mixture were achieved on a column packed with nonporous silica particles chemically bonded with octadecylsilane (3.0 μm particles) using a mobile phase of 94:6 v/v 15 mM potassium dihydrogen phosphate buffer (pH 2.7)-methanol at a flow rate of 0.6 mL/min with UV detection at 270 nm. The separation was achieved in less than 4 min. Meropenem and ofloxacin were subjected to acid, base, peroxide, and ultraviolet degradation and monitored using photodiode detection. None of the products of degradation showed any interference with the peaks of interest. Standard curves were prepared and correlation coefficients were $\geq$ 0.999 for both compounds.

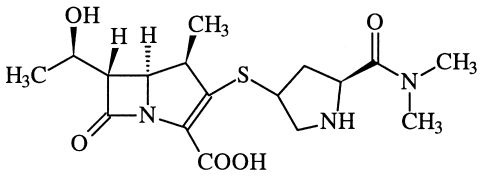
Precision expressed as %RSD was ≤ 2.0% for both analytes. The limits of detection at a signal-to-noise (S/N) ratio of 3 for meropenem and ofloxacin were 312.5 and 300 ng/mL, respectively, using a 10 μL injection.

INTRODUCTION

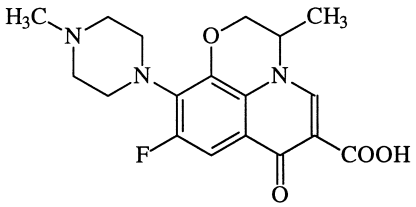
Meropenem is a new antibiotic of the 1-β-methylcarbapenem class that shows activity against Gram-positive and Gram-negative bacteria, including anaerobes. It is considered to be more active *in vitro* than imipenem against Enterobacteriacae and *Pseudomonas aeruginosa.*[1] Ofloxacin is an antibiotic of the fluorinated 4-quinolone class and has broad antimicrobial activity, especially against staphylococci, including methicillin-resistant strains.[2] Meropenem has been determined by HPLC-UV in human plasma,[3] as well as the reported analysis of a carbon dioxide adduct using proton NMR and flow injection quadrupole mass spectrometry.[4] Ofloxacin has been determined by HPLC-UV in plasma,[5,6] and in pharmaceutical dosage forms using UV derivatization.[7] However, there has been no reported stability-indicating HPLC methods for the analysis of meropenem and ofloxacin in 5% dextrose in water injection USP and 0.9% sodium chloride injection USP.

In the modern analytical laboratory, there is a need for faster and better performance from HPLC methods. One of the ways to achieve a faster HPLC run, with a greater efficiency, is the use of nonporous silica (NPS) particle technology. Colwell[8] and Giesche et al.[9] provided support for the use of nonporous silica particles and evaluated their use in early HPLC studies. Lee et al.[10] and Jenke[11] performed further investigations with packed HPLC columns utilizing a nonporous silica particle, with a diameter of 1.5 μm, bonded with octadecylsilane (ODS). Both investigators showed that the van Deemter plots comparing reduced plate height versus reduced mobile phase velocities were relatively flat compared to conventional HPLC columns. Nonporous silica particles also tend to have a more uniform particle shape and size compared to most 5μm particles,[12,13] contributing to a more efficient column. However, care must be taken in utilizing nonporous silica columns, as the retention factor is closely related to the organic modifier fraction.[11] There is also the need to reduce extra column effects with nonporous silica columns, such as dissolving the analyte in a solvent closely matched to the mobile phase.

In this paper, an isocratic stability-indicating HPLC method has been developed that will simultaneously quantitate meropenem and ofloxacin in dextrose and saline intravenous solutions utilizing a nonporous ODS column with a single injection in < 4 min at ambient temperature. Limit of detection at a signal-to-noise (S/N = 3) was in the 300 ng/mL range for both analytes.



Meropenem



Ofloxacin

Figure 1. Structural Formulae of meropenem and ofloxacin.

EXPERIMENTAL

Reagents and Chemicals

The structural formulae of the analytes studied are shown in Figure 1. Meropenem was obtained from Zeneca Pharmaceuticals, Inc. (Wilmington, DE 19850). Ofloxacin was obtained from Sigma Chemical Co. (St. Louis, MO 63178).

Methanol (J. T. Baker, Phillipsburg, NJ 08865) was HPLC grade and water was purified by a cartridge system (Continental Water Systems, Roswell, GA 30076).

0.9% Sodium chloride injection USP and 5% dextrose in water USP intravenous solutions were supplied by Baxter Healthcare Corp. (Deerfield, IL 60015; Expiration: 10/98).

Monobasic potassium phosphate, hydrochloric acid, sodium hydroxide pellets, and 3.0% hydrogen peroxide (H_2O_2) were J. T. Baker analyzed reagents (Phillipsburg, NJ 08865).

Instrumentation

The chromatographic separations were performed on an HPLC system consisting of a Micra ODS-IIIE (monomeric and endcapped) column (100 x 4.6 mm i.d.) containing 3.0 um nonporous silica (NPS) microspheres chemically bonded with octadecylsilane (Micra Scientific Northbrook, IL 60062), an Alcott Model 708 autosampler (Norcross, GA 30093) equipped with a 10 μL loop, a Micromeritics Model 760 HPLC Pump (Norcross, GA 30093), a Kratos Spectroflow 757 Absorbance Detector (Ramsey,NJ 07446), a Waters 996 photodiode array detector to confirm the purity of the analyte peaks from forced degradation, and a Hewlett Packard Model 3392A Integrator (Palo Alto, CA 94025). The mobile phase consisted of 94:6 v/v 0.01 M aqueous monobasic potassium phosphate (pH 2.7) (adjusted with conc. phosphoric acid)-methanol at a flow rate of 600 μL/min.

The mobile phase was filtered through a 0.45 um Nylon-66 filter (MSI, Westborough, MA 01581) and degassed. The detector was set at 270 nm to analyze both analytes in the mixtures.

Preparation of Solutions

Individual stock solutions were prepared for meropenem and ofloxacin at concentrations of 0.4 mg/mL. Meropenem stock solutions were made daily and ofloxacin stock solutions were prepared every 3 days. All dilutions were made using 94:6, v/v water:methanol. Standard solutions of the two analytes for linear regression analysis were made by dilution of the respective stock solutions. Meropenem was diluted to 10, 25, 50, 100, and 200 μg/mL, while ofloxacin was diluted to 10, 20, 40, 80, and 160 μg/mL. Four calibration curves were constructed for both analytes in the mixtures. Spiked solutions for accuracy and precision studies were made at two concentration levels: (1) low (meropenem: 18 μg/mL and ofloxacin: 15 μg/mL), and (2) high (meropenem: 150 μg/mL and ofloxacin: 120 μg/mL). Quantitation was based on linear regression analysis of analyte peak versus analyte concentration in μg/mL.

Mixtures of meropenem and ofloxacin were also prepared in dextrose and saline intravenous solutions. Meropenem solutions were prepared at 5 and 10 mg/mL, and ofloxacin was made up at 4 mg/mL in each injection. From these, one mL of each intravenous solution was diluted to volume in a 100 mL volumetric flask using 94:6 v/v, water:methanol.

Solutions of 0.1 N HCL, 0.1 N NaOH, deionized water, and 3% H_2O_2 were utilized in degradation studies. In each case, 1 mg (or 2 mg with acid and base degradation) were dissolved in the appropriate degradation solution and

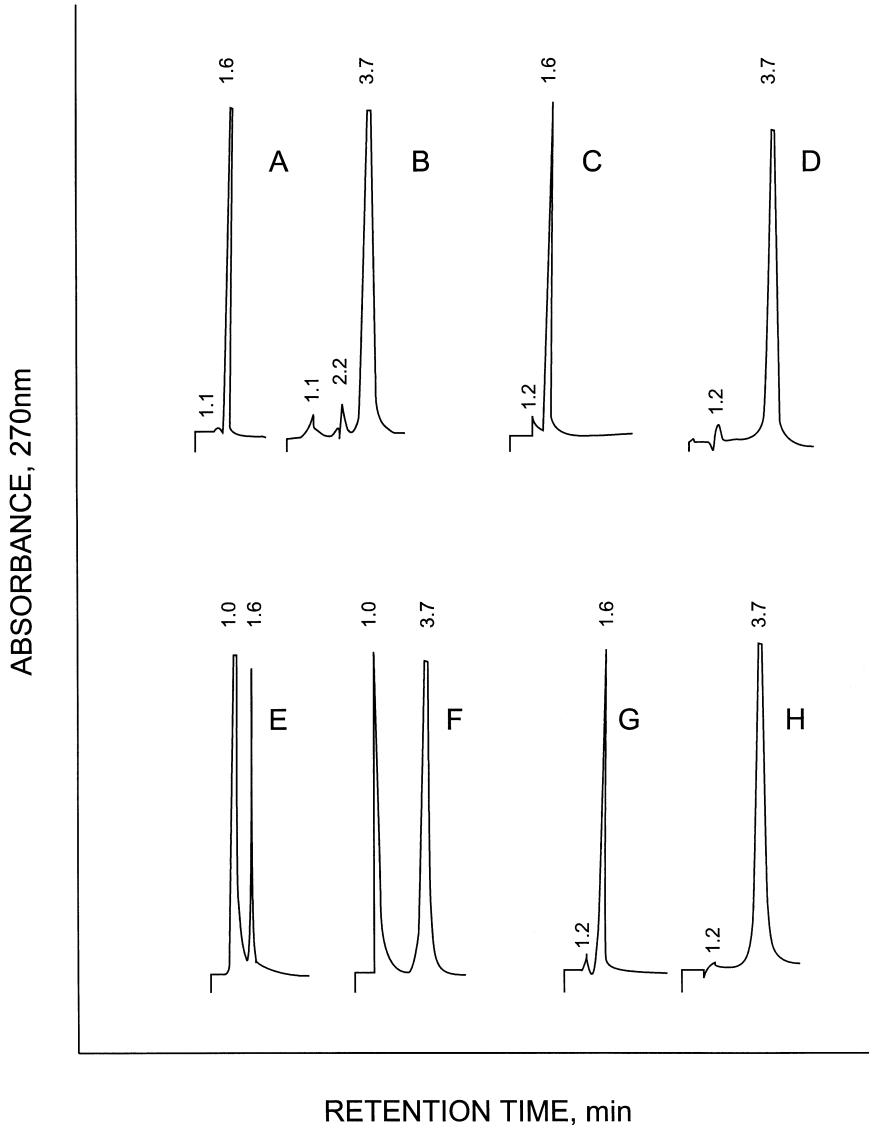


Figure 2. HPLC chromatograms of (A) meropenem degraded with 0.1N HCI; (B) ofloxacin degraded with 1.0 N HCI; (C) meropenem degraded with 0.1 N NaOH; (E) meropenem degraded with 3% hydrogen peroxide, (F) ofloxacin degraded with 3% hydrogen peroxide; (GA) meropenem degraded with 254 nm UV light; and (H) ofloxacin degraded with 254 nm UV light. Retention times of 1.0, 1.1, 1.2 and 2.2 min are possible degradation products.

allowed to stand for 5 min up to 10 hr at ambient temperature, according to stability testing procedures discussed by Weiser.[14] In the case of samples degraded with peroxide and UV irradiation (254 nm), samples were directly injected into the HPLC system. In the case of acid and base stressed samples, 0.5 mL of a sample was neutralized with an equal volume of either acid or base prior to injection into the LC system.

RESULTS AND DISCUSSION

The objective of this investigation was to develop a stability-indicating method for the analysis of meropenem and ofloxacin in dextrose and saline intravenous solutions using nonporous silica technology. None of the HPLC methods in the literature describes the concurrent analysis of meropenem and ofloxacin in an intravenous mixture.

The initial phase of the study entailed examining the degradation products associated with meropenem and ofloxacin. Upon exposure to 0.1 N HCL, meropenem showed 10% degradation at 30 min, with a degradant peak at the solvent front. Ofloxacin needed 30 min exposure to 1.0 N HCL to achieve 10% degradation, with a degradant peak eluting at 2.19 min. The acid degradant peaks were easily resolved from the meropenem and ofloxacin peaks. With 0.1N NaOH, meropenem showed 20% degradation at 3 min, while ofloxacin showed 10% degradation at 45 min. All base degradation peaks eluted at the solvent front. With 3% peroxide, meropenem showed 15% degradation at 15 min and ofloxacin showed 10% degradation at 45 min. Again, all peroxide degradation products eluded at the solvent front.

Finally, the analytes were subjected to degradation with UV irradiation at 254 nm. Meropenem demonstrated 10% degradation at 4 hr, and ofloxacin showed about 5% degradation at 10 hr, and all degradant peaks eluted with the solvent front. Typical chromatograms of the degraded solutions are shown in Figure 2.

The second part of the study entailed the development and validation of the method. During development, two types of nonporous ODS columns were examined, both with the dimension of 100 mm x 4.6 mm i.d., with 3.0 μm particles. It was determined that an endcapped nonporous ODS column gave the best efficiency and was used in the method.

Various mobile phases were tested, ranging from 5 to 30% methanol content. It was determined that 6% methanol provided the optimized separation. Limits of detection were 312.5 ng/mL for meropenem and 300 ng/mL for ofloxacin based on a S/N of 3. A typical chromatogram of the separation of meropenem and ofloxacin is shown in Figure 3. The analytical figures of merit for each analyte on the nonporous ODS column are shown in Table 1.

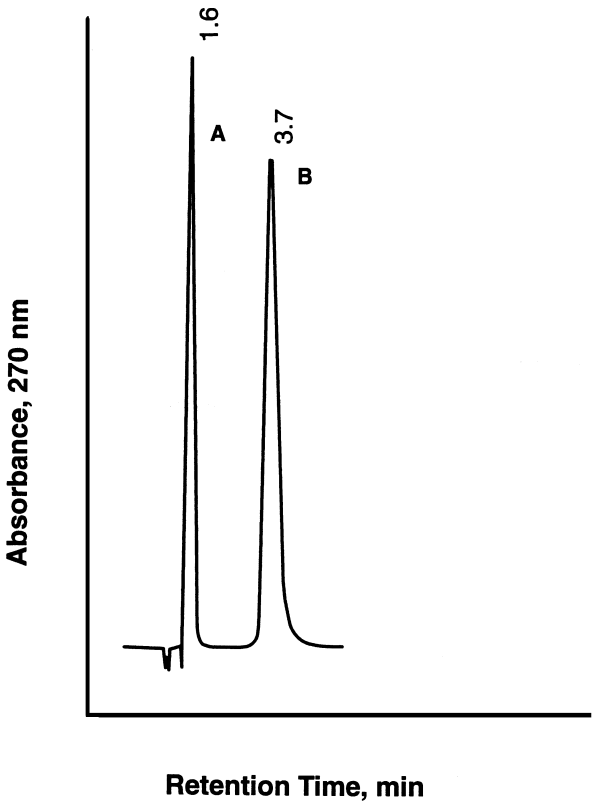


Figure 3. HPLC chromatogram of meropenem (A) and ofloxacin (B) on a nonporous ODS column (100 x 4.6 mm i.d.). See Experimental Section for details.

Table 1

Analytical Figures of Merit for Meropenem and Ofloxacin on the Nonporous Column

Analyte	k	N[a]	T_f[b]	R_s	α
Meropenem	0.67	312	0.63	4.86	3.82
Ofloxacin	2.56	227	0.83		

[a] Calculated as $5.54\ (t_r/t_w)^2$. [b] Calculated as 5% peak height.

Table 2

Inter- and Intra-Day Linearity Data

Analyte	r^2 (Day 1)	r^2 (Day 2)	r^2 (Day 3)
Meropenem[a]	0.9999	0.9999	0.9998 (Run 1) 0.9996 (Run 2)
Ofloxacin[b]	0.9999	0.9999	0.9999 (Run 1) 0.9999 (Run 2)

[a] Range examined from 10-200 μg/mL (based on n = 25).
[b] Range examined from 10-160 μg/mL (based on n = 25).

Table 3

Accuracy and Precision Data for Samples Spiked With Analytes

Analyte	Conc. Added (μg/mL)	Conc. Found (μg/mL)[a]	Percent Error	% RSD
Meropenem	18	18.40 ± 0.01	2.22	0.81
	150	147.67 ± 0.18	1.55	1.24
Ofloxacin	15	14.63 ± 0.01	2.47	0.75
	120	121.56 ± 0.06	1.30	0.51

[a] Mean ± std. deviation based on n = 3.

Inter- and intra-day linearity of the assay are shown in Table 2. The correlation coefficients calculated from day to day were very good for each component in the mixture. Inter- and intraday precision ranged from 0.22-1.90% for meropenem to 0.36-1.97% for ofloxacin. Accuracy and precision were also evaluated for each component in the spiked samples. Recoveries were determined for mixtures of meropenem and ofloxacin spiked in the various intravenous solutions. The recovery data shown in Tables 3 and 4 indicate that the method gave acceptable accuracy and precision for each analyte in spiked and intravenous solutions.

In summary, a nonporous ODS column with a 94:6 v/v aqueous phosphate buffer (pH 3.0)-methanol mobile phase was developed for the analysis of meropenem and ofloxacin in dextrose and saline intravenous

Table 4

Accuracy and Precision Data for Samples Spiked in Intravenous Solutions

Analyte	IV Solution	Conc. Added (mg/mL)	Conc. Found (mg/mL)	% RSD
Meropenem	0.9% NaCl	5.00	5.03 ± 0.03	0.64
Ofloxacin		4.07	3.93 ± 0.04	1.12
Meropenem	0.9% NaCl	10.01	10.58 ± 0.09	0.87
Ofloxacin		3.95	3.86 ± 0.03	0.88
Merepenem	5% Dextrose	4.99	4.94 ± 0.05	0.98
Ofloxacin		4.08	4.06 ± 0.03	0.76
Merepenem	5% Dextrose	10.00	10.15 ± 0.20	1.94
Ofloxacin		4.10	4.02 ± 0.05	1.24

[a] Mean ± Std. Deviation based on n =3.

mixtures. The run times were $\leq$ 4.0 min, solvent usage was substantially reduced, and the column showed good efficiencies for both analytes. In using these column types, an analyst must be certain to reduce dead volumes in the HPLC system and use small injection volumes ($\leq$ 10 μL) to achieve optimal results. Nonporous ODS columns have been shown to provide good analytical results with a reduction in time and solvent waste.

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