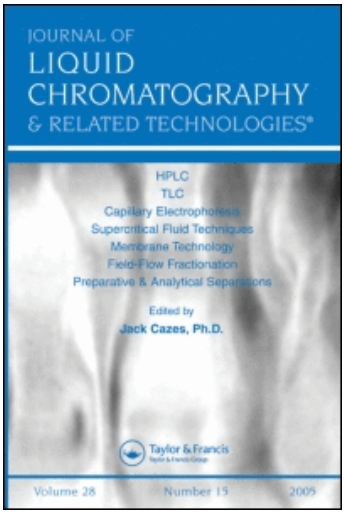

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HPLC DETERMINATION OF AN AQUEOUS CEFEPIME AND METRONIDAZOLE MIXTURE

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HPLC DETERMINATION OF AN AQUEOUS CEFEPIME AND METRONIDAZOLE MIXTURE

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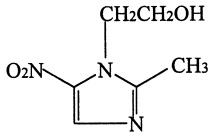
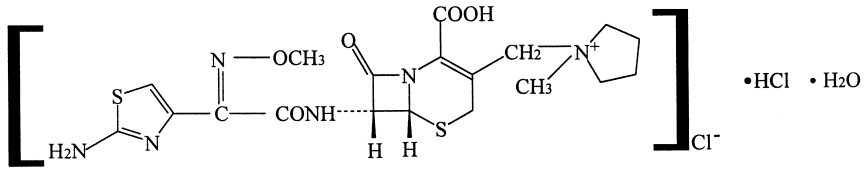
ABSTRACT

A high performance liquid chromatographic procedure has been developed for the assay of a cefepime and metronidazole mixture in aqueous solution. The separation and quantitation were achieved on a phenyl column at ambient temperature using a mobile phase of 94.5:5.5 v/v water-acetonitrile containing 0.015 M pentane sulfonic acid sodium salt (adjusted to pH 3.4 with glacial acetic acid and then 4.0 with 45% potassium hydroxide) at a flow rate of 1.5 mL/min with detection of both analytes at 280 nm. The separation was achieved within 10 min with sensitivity in the ng/mL range for each analyte. The method showed linearity for cefepime and metronidazole in the 18.77 - 300.2 and 9.39 - 150.1 μg/mL ranges, respectively. Accuracy and precision were in the 0.52 - 2.40 and 0.63 - 2.77% ranges, respectively, for both analytes. The limits of detection for cefepime and metronidazole were 125 and 63 ng/mL, respectively, based on a signal to noise ratio of 3 and a 10 μL injection.



METRONIDAZOLE



CEFEPIME HYDROCHLORIDE MONOHYDRATE

Figure 1. Chemical structures of compounds studied.

INTRODUCTION

A cefepime and metronidazole mixture is administered as a safe and effective therapy for human patients with severe intra-abdominal infections. Interest in our laboratory in the stability and compatibility of the drug mixture over time required the development of an HPLC method. A search of the literature indicated that HPLC methods were not available to assay the two compounds in a single injection. Cefepime has been analyzed by HPLC,[1,2] second derivative spectrophotometry,[3] and diffuse reflectance IR and powder X-ray diffraction techniques.[4] Metronidazole has been determined in various matrices using HPLC,[5-8] spectrophotometry,[9] and electrochemistry.[10,11]

In this paper, an HPLC method is reported for the concurrent determination of a cefepime-metronidazole mixture in an aqueous solution. The procedure provides good accuracy and precision and should be applicable to future stability and compatibility studies in aqueous solution.

EXPERIMENTAL

Reagents and Chemicals

The structural formulae of the compounds studied are shown in Figure 1. Cefepime (Batch #CCB4U0189) was a gift from Bristol-Myers Squibb and

Metronidazole was purchased from Sigma Chemical Company (St. Louis, MO 63178). Pentane sulfonic acid sodium salt HPLC grade was obtained from Fisher Scientific (Fair Lawn, NJ 07410).

Acetonitrile (J.T. Baker, Phillipsburg, NJ 08865) was HPLC grade and water was purified by a cartridge system (Hydro Picotec Water System, Research Triangle Park, NC 27709). Potassium hydroxide and glacial acetic acid were Baker analyzed reagents.

Instrumentation

The chromatographic separation was performed on an HPLC system consisting of a Beckman Model 110B pump (San Mateo, CA), an Alcott Model 738 autosampler (Norcross, GA 30093) equipped with a 10 µL loop, a Waters Associates Model 461 variable wavelength UV/VIS detector (Milford, MA 01757) and a Hewlett-Packard Model 3394A integrator (Palo Alto, CA). Separation was accomplished on a 100 x 4.6 mm i.d. Brownlee-Perkin Elmer phenyl column (5 µm particle size). The mobile phase consisted of 94.5:5.5 v/v water-acetonitrile containing 0.015 M pentane sulfonic acid (pH adjusted to 3.4 with glacial acetic acid and then 4.0 with 45% potassium hydroxide). The mobile phase was filtered through a 0.45 µm nylon-66 filter (MSI, Westborough, MA 01581) and degassed by sonication prior to use. The flow rate was set at 1.5 mL/min. The detector was set at 280 nm.

Preparation of Standard Solutions

Separate standard solutions containing 600.4 and 300.2 µg/mL of cefepime and metronidazole, respectively, were prepared by accurately weighing the pure reference powders, transferring to a 10 mL volumetric flask, and adding mobile phase to volume. Aliquotes of each solution were combined to give mixtures containing 18.77-300.2 µg/mL and 9.39-150.1 µg/mL of cefepime and metronidazole, respectively. Additional dilutions of each standard were prepared to give spiked samples from which accuracy and precision of the method were calculated. Quantitation was based on linear regression analysis of analyte peak area versus concentration in µg/mL.

RESULTS AND DISCUSSION

The goal of this project was to develop an isocratic HPLC assay for the analysis of a cefepime and metronidazole mixture in water. The mixture is typical of a therapeutic regimen that would be administered to a human patient. Stability studies of the mixture would require an assay method that would detect and quantitate each analyte with reasonable accuracy and precision.

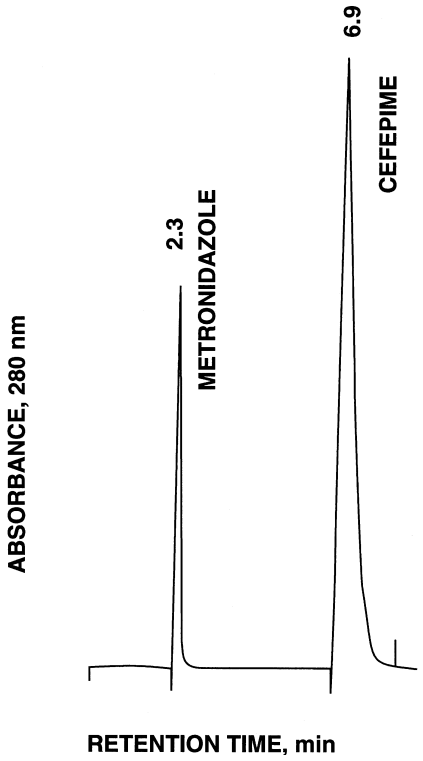


Figure 2. Typical HPLC chromatogram of metronidazole (2.3 min) and cefepime (6.9 min) on a phenyl column with a mobile phase of acetonitrile-aqueous acetate buffer pH 4.0 containing 0.015 M pentane sulfonic acid sodium salt. See experimental section for assay conditions.

There were no reports in the scientific literature describing a separation of these two analytes in a single mixture. Initial studies to develop a single isocratic HPLC method for the compounds involved the use of octylsilane and octadecylsilane columns with various mobile phases consisting of acetonitrile-aqueous acetate buffers pH 4.0 containing pentane sulfonic acid sodium salt. Changes in the mobile phase composition did not significantly improve the resolution of the two peaks on either column. The cefepime peak was retained longer on the octadecylsilane column than metronidazole with a retention time of about 10 min. Retention of the analytes on the octylsilane columns showed a peak reversal with cefepime eluting first (= 5 min) followed by metronidazole at 7 min. While both analyte peaks were resolved, greater resolution was desired.

Table 1

Analytical Figures of Merit for Cefepime and Metronidazole

Analyte	r^{2a}	System Suitability[b]	LOD ng/mL[c]	k	Theoretical Plates[d]	Tailing Factor[e]	R_s
Cefepime	0.9999	0.62	125	6.2	1947	1.38	9.39
Metronidazole	0.9999	0.41	63	1.5	2061	1.0	

[a] Range examined from 18.77 - 300.2 μg/mL cefepime (n=6) and 9.39 - 150.1 μg/mL metronidazole (n=6) mobile phase consisted of 94.5:5.5 v/v water-acetonitrile containing 0.015 M pentane sulfonic acid sodium salt adjusted to 3.4 with glacial acetic acid and to pH 4.0 with 45% potassium hydroxide at 1.5 mL/min with detection at 280 nm. [b] RSD % of 6 replicated injections at 201 μg/mL cefepime and 50 μg/mL metronidazole at 280 min. [c] Limit of detection, S/N = 3. [d] Calculated as $N = 16 (t/w)^2$. [e] Calculated at 10% peak height.

Our attention turned to the use of a phenyl column for better resolution of the two analytes. Using an acetonitrile-acetate buffer pH 4.0 mobile phase containing pentane sulfonic acid sodium salt, the best separation and resolution of the analytes were obtained using a 5.5:94.5 v.v acetonitrile-aqueous acetate buffer pH 4.0 containing 0.015 M pentane sulfonic acid sodium salt with retention times of 2.3 and 6.9 min for metronidazole and cefepime, respectively. A typical chromatogram showing the separation is shown in Figure 2.

In the acetonitrile-acetate buffer mobile phase, the absorption maxima for metronidazole and cefepime were 280 and 254 nm, respectively. It was decided to use 280 nm as the detection wavelength for the mixture since the metronidazole concentration in the mixture would generally be less than cefepime and this wavelength provided the best accuracy and precision data for both analytes.

The HPLC method showed concentration versus absorbance linearity for cefepime and metronidazole in the 18.77 - 300.2 μg/mL and 9.39 - 150.1 μg/mL ranges, respectively, at 280 nm. Table 1 gives other analytical figures of merit for each analyte. A photodiode array detector (Model 996 Water Associates, Milford, MA 01757) was used to verify that none of the degradation products of the two analytes would interfere with the quantitation of each drug at 280 nm.

These experiments were performed on each analyte after they had been degraded for 30 min at 80 °C in 1 N acid, 1 min at ambient temperature in 0.2 N base, and 30-60 min at ambient temperature in 30% hydrogen peroxide. Percent degradation for both analytes were in the 10-30% range.

Table 2

Accuracy and Precision Using Spiked Analyte Samples

	Concn. Added (μg/mL)	Conc. Found (μg/mL)[a]	Percent Error	RSD (%)
Cefepime	37.53	37.90 ± 1.05	0.99	2.77
	150.10	150.88 ± 0.95	0.52	0.63
Metronidazole	18.77	18.32 ± 0.28	2.40	1.53
	75.05	74.22 ± 0.68	1.11	0.92

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

Percent error and precision of the method were evaluated using spiked samples containing both analytes. The results shown in Table 2 indicate that the procedure gives acceptable accuracy and precision for the two compounds. Intra day variabilities of the assay for cefepime and metronidazole expressed as % RSD were 1.1 and 0.85% (n=18), respectively. Inter-day variabilities of the assay were 1.15 and 0.68% (n=18 or 3 days), respectively.

In summary, a phenyl column with a mobile phase of acetonitrile-aqueous acetate buffer pH 4.0 containing pentane sulfonic acid sodium salt has been shown to be amenable for the separation and quantitation of a cefepime-metronidazole mixture in water. This study suggests that the HPLC method can be used to investigate the chemical stability of the two drugs in sterile water for injection or other water based injections.

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