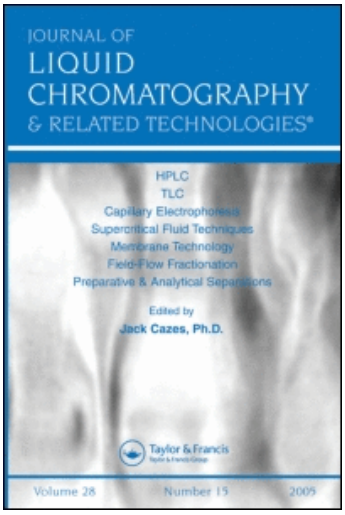

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Determination of a Cefuroxime and Aminophylline/Theophylline Mixture by High-Performance Liquid Chromatography

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DETERMINATION OF A CEFUROXIME AND AMINOPHYLLINE/THEOPHYLLINE MIXTURE BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

A high-performance liquid chromatographic method for the simultaneous determination of cefuroxime and aminophylline/theophylline has been developed. Cefuroxime was separated from aminophylline/theophylline within 8 min using a octadecylsilane column and a mobile phase consisting of a 10:1 mixture of 0.1 M acetate buffer pH 3.4 - acetonitrile at ambient temperature. Orcinol was used as internal standard and the analytes were monitored at 254 nm. The method was free of interference from degradation products related to any of the analytes. Drug to internal standard peak area ratios were linear ($r^2 \geq 0.9998$) for each analyte with good precision (RSD $\leq 2.1\%$) and accuracy ($\leq 2.3\%$).

INTRODUCTION

Cefuroxime is a cephalosporin antibiotic intended for parenteral administration. Cefuroxime for injection is presented as the sterile

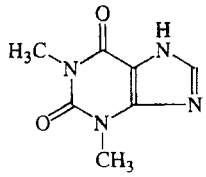
sodium salt in a sealed container and is sometimes admixed by the addition of an appropriate amount of aminophylline or theophylline injection.

Methods for the simultaneous determination of cefuroxime and aminophylline/theophylline in a mixture have not been reported, although there are HPLC methods used separately for the analysis of cefuroxime (1-3) and aminophylline/theophylline (4-6). The HPLC assay method reported herein for cefuroxime and aminophylline/theophylline was modified from a stability-indicating assay for cefuroxime reported in the Code of Federal Regulations (CFR) (1). The HPLC method was shown to be stability-indicating with respect to both cefuroxime and aminophylline/theophylline in a single injection.

EXPERIMENTAL

Reagents and Chemicals

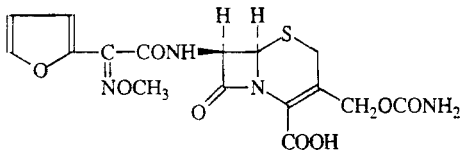
The chemical structures of the analytes are shown in Figure 1. Cefuoxime sodium was a gift from Glaxo, Inc. (Research Triangle Park, NC 27709). Aminophylline and theophylline were obtained from Sigma Chemical Co. (St. Louis, MO 63178). Orcinol monohydrate (5-methylresorcinol, J.T. Baker, Phillipsburg, NJ 08865) was obtained for use as the internal standard. All chemicals and solvents used were the highest grade of commercially available materials. A pH 3.4 acetate buffer was prepared by the addition of 50 mL of 0.1M sodium acetate into a 1000 mL volumetric flask followed by 0.1M acetic acid to volume.



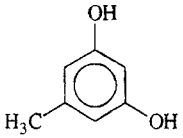
THEOPHYLLINE

$(C_7H_8N_4O_2)_2.H_2NCH_2CH_2NH_2.2H_2O$

AMINOPHYLLINE



CEFUROXIME



ORCINOL

Figure 1. Chemical structures of the analytes.

Apparatus

The liquid chromatography system consisted of a Beckman Model 110B Pump (San Ramon, CA 94583), an Alcott Model 738 Autosampler (Norcross, GA 30093) and an ABI Model 759A UV/VIS Absorbance Detector (Foster City, CA 94404). Separation was accomplished on an Beckman Ultrasphere Octadecylsilane (C18) column (150 mm x 4.6 mm i.d., 5 μm) equipped with a 50 mm C18 guard column. The mobile phase consisted of a 10:1 v/v mixture of 0.1M acetate buffer, pH 3.4 and acetonitrile. The mixture was filtered through a 0.45 μm membrane filter and degassed by sonication prior to use. The operating conditions for this system were as follows: flow rate, 2 mL/min; detector

wavelength, 254 nm; injection volume, 50 μL; and column temperature, 23 ± 1°C. Data acquisition and reduction were performed on an HP Model 3394A Integrator (Hewlett-Packard Company, Avondale, PA 19311).

Preparation of Standard and Sample Solutions

An internal standard solution was prepared by dissolving a weighed amount (45 mg) of orcinol monohydrate in 50 mL of water to give a 900 μg/mL solution. The solution was filtered, protected from light, and prepared fresh every week. A combined cefuroxime and aminophylline/theophylline standard solution was prepared by accurately weighing 18 mg of cefuroxime sodium and 2.4 mg of aminophylline or 1.9 mg of theophylline, transferring the powders to a 100-mL low actinic volumetric flask, water added to volume, and manual shaking for 30 sec. The solutions were stored in a refrigerator (4°C) and used within one week (7). Accurately pipetted volumes of 62.5, 125, 250, 500, and 1000 μL of the combined standard solution were each mixed with 500 μL of the internal standard solution and diluted to 1.5 mL with water to give concentrations in the range of 7.5 -120 μg/mL for cefuroxime sodium, 1 - 16 μg/mL for aminophylline and 0.8 - 13 μg/mL for theophylline. Five point calibration curves were constructed for each analyte. Additional dilutions of the combined standard solution were prepared in water to serve as spiked samples for each analyte to determine accuracy and precision of the method. Samples to be assayed

were mixed with 500 μL of the internal standard solution and diluted to 1.5 mL with water. Standards and samples were placed in autosampler vials and 50 μL aliquots were injected into the HPLC system. Quantitation was based on linear regression analysis of peak area ratios of each drug to internal standard versus their concentration in μg/mL.

RESULTS AND DISCUSSION

The aim of this study was to modify the CFR isocratic HPLC assay for cefuroxime to determine both cefuroxime and aminophylline/theophylline in a single injection. The method would need to be free of interference from degradation products related to either analyte. The HPLC procedure for cefuroxime reported in the CFR was initially investigated for use in this study (1). The cefuroxime mobile phase consisted of a 10:1 v/v mixture of 0.1M acetate buffer pH 3.4-acetonitrile and a column packed with 5 μm hexylsilane (C6). It was found that the cefuroxime and aminophylline peaks overlapped (2.35 vs 2.26 min, respectively) on the C6 column. When the column was changed to a 5 μM octysilane (C8), both drugs were still not well separated (5.82 vs 5.51 min, respectively). A 10 μm octadecylsilane (C18) column was then placed in the HPLC system and showed suitable resolution of the cefuroxime and aminophylline peaks (4.01 vs 5.50 min, respectively). Further investigations of various octadecylsilane columns from different manufacturers indicated that the best peak resolution and peak shapes were obtained using a Beckman ultrasphere octadecylsilane

column. A typical chromatogram from the ultrasphere C18 column is shown in Figure 2-I.

Determination of both cefuroxime and aminophylline/theophylline was performed using internal calibration. Calibration curves were generated by least-square regression of the drug/internal standard peak area ratios versus the concentration of each drug. The regression analysis showed linearity for cefuroxime over the 7.5 - 120 μg/mL range and for aminophylline/theophylline over the 1-16/0.8-13 μg/mL range with correlation coefficients $\geq$ 0.9998 (n = 5).

Percent error (accuracy) and precision of the method were evaluated using spiked samples. The results shown in Tables 1 and 2 indicate that the procedure gives acceptable accuracy and precision.

A study of any interferences from degradants of each drug was conducted. Solutions containing 90 μg/mL cefuroxime and 12 μg/mL aminophylline or 10 μg/mL theophylline in water were allowed to degrade by standing at ambient temperature (23 $\pm$ 1°C) or under room lighting for 9 days, or by heating for 6 hours in an 80°C oven. The chromatogram from the degraded sample shown in Fig. 2-II demonstrates that there were no interferences noted at the retention times of cefuroxime, aminophylline/theophylline, or orcinol (internal standard).

The chromatographic assay thus provides a rapid, precise and selective method for the simultaneous determination of both cefuroxime

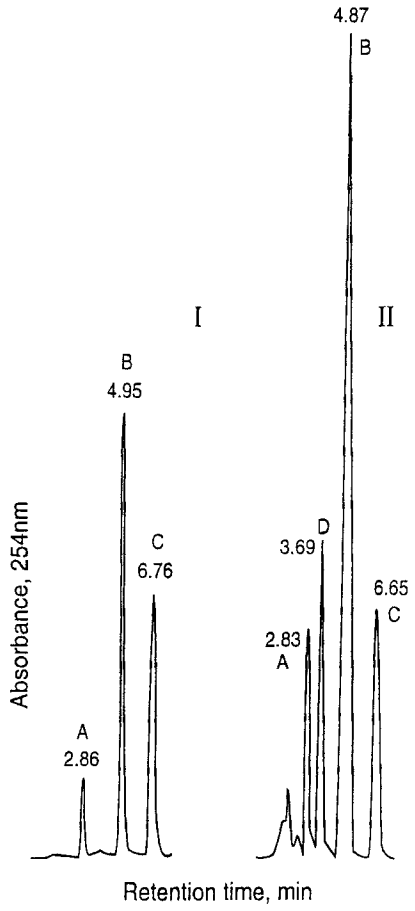


Figure 2. Typical chromatograms of a typical mixture (I) and a degraded mixture (II) containing aminophylline/theophylline (A), cefuroxime (B), orcinol (C) and an unknown degradation product. (D).

Table 1 - Inter-day Precision and Accuracy of Cefuroxime and Aminophylline /Theophylline

Concentration added, (μg/mL)	Concentration found, (μg/mL)	n	Relative Error (%)	RSD (%)
Cefuroxime Sodium				
15.00	14.84 ± 0.18	6	1.1	1.2
45.00	44.16 ± 0.47	5	1.9	1.1
90.00	89.28 ± 0.50	6	0.8	0.6
Aminophylline				
2.028	1.981 ± 0.033	6	2.3	1.6
6.085	6.034 ± 0.032	5	0.8	0.5
12.17	12.02 ± 0.102	6	1.3	0.8
Theophylline				
1.616	1.593 ± 0.025	6	1.4	1.6
4.848	4.830 ± 0.044	6	0.4	0.9
9.695	9.705 ± 0.091	6	0.1	0.9

Table 2 - Intra-day Precision and Accuracy of Cefuroxime and Aminophylline/Theophylline

Concentration added, (μg/mL)	Concentration found, (μg/mL)	n	Relative Error (%)	RSD (%)
Cefuroxime Sodium				
15.00	14.73 ± 0.11	5	1.8	0.7
45.00	43.97 ± 0.13	5	2.3	0.3
90.00	89.00 ± 0.08	5	1.1	0.1
Aminophylline				
2.028	1.985 ± 0.042	5	2.1	2.1
6.085	6.027 ± 0.047	5	0.9	0.8
12.17	11.94 ± 0.131	5	1.9	1.1
Theophylline				
1.616	1.609 ± 0.032	5	0.4	2.0
4.848	4.899 ± 0.012	5	1.1	0.3
9.695	9.746 ± 0.080	5	0.5	0.8

and aminophylline/theophylline in a single injection. The procedure is suitable for the analysis of freshly manufactured materials and it also provides a reliable estimate of the potency of stored samples.

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