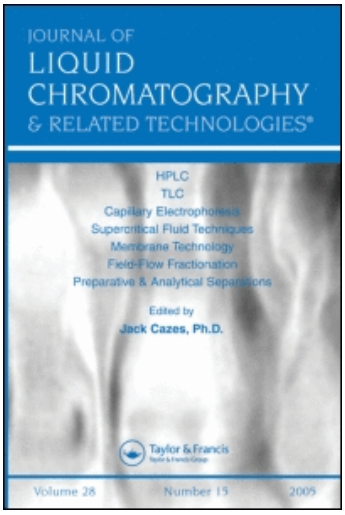

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Simple HPLC Method for the Analysis of Some Pharmaceuticals

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SIMPLE HPLC METHOD FOR THE ANALYSIS OF SOME PHARMACEUTICALS

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ABSTRACT

A simple, selective, and accurate high performance liquid chromatographic method for the determination of cephalexine, cefotaxime, and salbutamol sulphate was developed. The suggested method uses ODS column with methanol-phosphate buffer (pH 7) 6:4 as a mobile phase. The mean percentage recovery ranged from 97.4 to 98.8. The proposed method was applied to the determination of the selected drug in some pharmaceutical preparations. The statistical analysis of the results obtained was compared favourably with those given with the official method.

INTRODUCTION

The cephalosporin group of antibiotics, in general, differ from the penicillins by being stable to acid, so the problem which confronted penicillins administered by the oral route does not in general confront cephalosporins. The first cephalosporin with significant oral activity to be invented was cephalexin which is still used widely. Various methods have been proposed for the investigation of cephalexin including chromatographic[1-9] and spectrophotometric analysis.[10-18]

The other recent advance which seems likely to have produced a compound of considerable importance is the invention of cefotaxime. In this antibiotic we now have an injectable cephalosporin which is certainly safe and considered the first of the extremely potent class of amino-thiazole cephalosporins.

Several methods have been reported for the determination of cefotaxime. These include chromatographic,[19-29] spectrophotometric,[30-35] proton magnetic resonance,[36] and polarographic analysis.[37]

Salbutamol [2-tert-butylamino-1-(4-hydroxy-3-hydroxy methylphenyl) ethanol] is a beta$_2$ sympathomimetic agent that produces relaxation of the smooth muscles of the bronchi. Salbutamol has widespread use in clinical practice due to its prolonged bronchodilator effect. Several methods have been reported for the determination of salbutamol including chromatography,[38-60] spectrophotometry,[61-73] and titrimetric analysis.[74,75]

In this paper, an attempt has been made to develop a simple HLPC based on reversed phase, isocratic elution, and variable wavelength UV detector which can be applied for the determination of cephalexin, cefotaxime, and salbutamol sulphate.

EXPERIMENTAL

Materials

Cephalexin, cefotaxime sodium, and salbutamol sulphate were kindly provided by various manufacturers and were used as received. Pharmaceutical preparations containing the previous active pharmaceuticals were randomly obtained from commercial sources. All reagent chemicals were of analytical grade and used without further purification. Solvents used were of HPLC grade.

HPLC

Apparatus and operating conditions

ACS solvent pump 366, ACS UV detector 750/12, and Kipp & Zonen recorder were used. The column used was supelcosil ODS (4.6 × 150 mm) 5um. The mobile phase composed of 6:4 (v/v) mixture of methanol and phosphate buffer (pH7) and was ultrasonically degassed before use. Detection wave length varied between 215-254 nm. Flow rate was 1 mL/min.

Table 1

Collective Data for the Analysis of the Selected Pharmaceuticals in Pure Form

Compound	Concentration of Stock Solution mg/mL	Concentrations mg/mL	Wave-Length of Detection
Cephalexin	1 mg/mL	(0.1-0.5) mg/mL	222 nm
Cefotaxime sodium	10 mg/mL	(0.1-0.5) mg/mL	254 nm
Salbutamol sulphate	0.4 mg/mL	(0.08-0.4) mg/mL	230 nm

Routinely, the system was allowed to equilibrate (approximately one hour) until a steady base line was observed. Twenty μL volume was injected with a fixed volume loop valve system. The sample was injected until at least two reproducible peaks were obtained. All experiments were done at room temperature.

Sample preparation (pure form)

A stock solution containing the appropriate amount in methanol-water 50/50 (Table 1) was prepared and diluted with methanol to give five different concentrations for each drug.

Sample preparation (dosage form)

Cephalexin (250 mg/capsule)

The average filling weight from the composition of at least 10 capsules has been weighed into 250 mL volumetric. The half volume was filled with 50:50 methanol-water, the resultant suspension well mixed and completed to the final volume with the same previous mixture. This solution was filtered and further diluted with pure methanol to give approximately cephalexin concentrations of 0.1 mg/mL, 0.2 mg/mL, 0.3 mg/mL, 0.4 mg/mL and 0.5 mg/mL.

Cefotaxime sodium (1gm/vial)

Powder equivalent to half gram active ingredient was extracted with 50 mL methanol-water 50:50. After filtration this solution was further diluted with pure methanol to give the following concentration : 0.1 mg/mL, 0.2 mg/mL, 0.3 mg/mL, 0.4 mg/mL and 0.5 mg/mL.

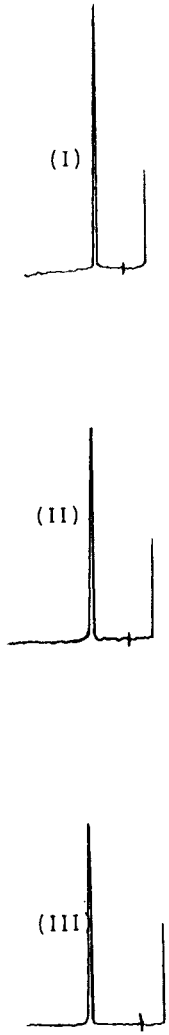

Figure 1. Chromatograms of cephalexin (I), cefotaxime (II), and salbutamol (III).

Salbutamol sulphate (syrup 0.4 mg/mL)

5 mL was diluted to 25 mL with methanol-water 50/50, mixed well and finally diluted with pure methanol to get different concentration of 0.08 mg/mL, 0.16 mg/mL, 0.24 mg/mL, 0.32 mg/mL, and 0.40 mg/mL.

Table 2

Some Chromatographic Parameters for the Selected Pharmaceuticals

Compound	k'	N	H
Cephalexin	1.13	2360	0.064 mm
Cefotaxime sodium	1.63	2756	0.054 mm
Salbutamol sulphate	2.13	3086	0.049 mm

k': Capacity factor.
N: Number of theoretical plates.
H: Height equivalence of theoretical plates.

Official Methods

The procedure for the official method was that of British pharmacopeia (1988) for cephalexin and solbutamal sulphate. For cefotaxine sodium, the official method was that of U. S. pharmacobea (1995).

RESULTS AND DISCUSSION

A comprehensive study of a quality control system is exercised. In this work, we tried to establish a high performance liquid chromatographic system which can be generally applied for the identification and quantitation of cephalexin, cefotaxime sodium, and solbutamol sulphate. The investigation was done for the previous drugs in pure form and in pharmaceutical preparations.

As the experiments were conducted in a constant temperature environment, the remaining important factors that govern the retention were studied. In this study the selectivity of different organic solvents: methyl alcohol, acetonitrile, and tetrahydrofuran have been tested separately after mixing with phosphate buffer with different ratios at different pH's. All the tested analytes gave highly resolved sharp peaks with methyl alcohol-phosphate buffer at pH 7 6:4 as mobile phase.

The effect of hydrophobicity has been investigated; the results clearly show that the shorter the alkyl chain of the bonded phase (RP-8) the worse the resolution and the RP-18 column still gave the best resolution if the retention times on each column were comparable. However, to overcome the problem of obtaining deviating results due to the use of different commercial stationary phase, a single column was used throughout all the experiments.

Table 3

Resolution and the Relative Retention of Some Mixtures of the Tested Drugs

The Selected Mixture	Rs	α
Cephalexin-cefotaxime	1.33	1.24
Cephalexin-salbutamal sulphate	2.5	1.47
Cefotaxime sodium-salbutamal sulphate	1.18	1.19

α: Relative retention.
Rs: Resolution factor.

Table 4

Comparative Analytical Results of the Proposed and Official Method for the Tested Drugs in Pure Form

Compound	% Recovery Proposed Method*	Official Method
Cephalexin	97.420 ± 0.826	98.06 ± 0.702
Cefotaxime sodium	97.416 ± 1.037	98.87 ± 1.133
Salbutamol sulphate	97.725 ± 1.126	98.22 ± 0.696

* Average of five separate determinations.

Table 2 shows the efficiency of the applied system where the values of the theoretical plate (N) and the height equivalent of the theoretical plate (H) indicate the good selectivity of this method for the tested drug (Figure 1). The efficiency for separating some mixtures has been tested. The resolution (Rs) and the separating factor (relative retention) (α) illustrated in Table 3 indicate the high resolution efficiency.

The statistical data of the analytical results obtained by the proposed method and the official methods for the tested drugs in pure form has been illustrated in Table 4. As evidenced, there is no significant difference between the two methods as regard to the accuracy and precision.

Table 5

Comparative Analytical Results of the Proposed and Official Method for the Tested Drugs in Some Pharmaceutical Preparations

Compound	% Recovery	
	Proposed Method*	Official Method
Cephalexin	98.510 ± 0.474	98.75 ± 0.433
Cefotaxime sodium	97.866 ± 1.023	98.186 ± 0.859
Salbutamol sulphate	97.984 ± 0.926	98.396 ± 0.832

* Average of five separate determinations.

The results obtained were encouraging to apply the proposed method for the determination of these tested drugs in some pharmaceutical preparations. The results listed in Table 5 show an agreement with those given with the official methods (within ± 1.02%). The commonly used excipients, colors, and preservatives were found to offer no positive interference by the proposed method, thus making the methods more reliable, less time consuming, and more suitable for routine analysis in dosage form.

In summary, the proposed method has been shown to be simple, rapid, sufficiently sensitive for the determination of cephalexin, cefotaxime sodium, and salbutamol sulphate.

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