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Short communication

# Simultaneous determination of five antibiotics by ion-pair high-performance liquid chromatography

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**Abstract**

This paper presents new information on the isocratic assay of five antibiotics thereby condensing five different assay procedures to one single report. Five antibiotics, viz., cephalexin and cefaclor (cephalosporins), isoniazid and pyrazinamide (anti-tubercular drugs) and minocycline (tetracycline series) have been separated and estimated from mixtures using a mobile phase consisting of tetrabutylammonium hydrogensulphate (0.025 *M*), methanol–acetonitrile (96:2:2) with pH adjusted to 3.0 with triethylamine. Column used was a Metaphase-CrestPak-ODS (250×4.0 mm, 5 μm), with a flow-rate of 1.5 ml $min^{-1}$, with detection at 248 nm. The retention order was isoniazid (2.68 min), minocycline (5.79 min), pyrazinamide (6.89 min), cephalexin (8.50 min) and cefaclor (12.18 min). The composition and polarity of the mobile phase was found to be critical, not for individual drugs, but for mixtures. The linear dynamic range was 0.30–10.00 μg $ml^{-1}$. Any one of the drugs could be used as internal standard for the other four drugs. All the drugs gave sharp, well-resolved peaks with optimum chromatographic figures of merit. Analytical performance parameters were calculated and were found to conform to the USP standards. 

*Keywords:* Pharmaceutical analysis; Antibiotics

## 1. Introduction

Cephalosporin antibiotics (cephalexin and cefaclor) are active against many gram-positive and -negative bacteria and are being widely used for oral treatment of infectious diseases. Pyrazinamide (PZA) is a drug used in the treatment of pulmonary tuberculosis. This drug has actually a new interest, particularly in the resistance to other major anti-tubercular drugs. Isoniazid (INH) is also an anti-tubercular drug which is normally administered with pyridoxine hydrochloride (vitamin B6). Minocycline (MCY) is a bacteriostatic antibiotic with a wide spectrum of activity and is used in the treatment of a large number of infections caused by susceptible organisms. Several methods [1–4] are available for the estimation of the individual cephalosporins (cefaclor, cephalexin and cephardine) in bulk drug and formulations using spectrophotometric, HPLC methods, etc. The analytical chemistry of PZA and INH also has been extensively studied [5–8]. References on individual/combination of two drugs among the five are numerous. Only some of them are cited here. The present study describes a new, rapid, easy isocratic reversed-phase HPLC method for the separation and estimation of two cephalosporins viz., cephalexin (CPH) and cefaclor (CFC), of two anti-tubercular antibiotics, viz., PZA and INH, and MCY. The primary purpose of this study is to compile HPLC data on the determination of these five antibacterials, even though they belong to two

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groups, the compilation of such HPLC data being useful as reference guide. It was also carried out to study the feasibility of determination of any individual/combination of four drugs using the fifth as the internal standard. The paper thus presents new information on the isocratic assay of five antibiotics, thereby condensing five different assay procedures to one single report.

## 2. Experimental

### 2.1. Materials and reagents

CPH, CFC, PZA, INH and MCY were obtained from M/S. Lupin Labs., Mumbai, India, with certificates of analysis. HPLC-grade methanol and triethylamine were from S.D. Fine Chemicals; HPLC-grade acetonitrile was from Merck, and tetrabutylammonium hydrogensulphate was from Loba-Chemie.

### 2.2. Solutions

Stock standard solutions of the drugs were prepared by dissolving 100 mg of each drug in 100 ml of mobile phase, consisting of 0.025 *M* tetrabutylammonium hydrogensulphate–methanol–acetonitrile (96:2:2, v/v) adjusted to pH 3.0 with triethylamine. The buffer was prepared by dissolving 8.4885 g of tetrabutylammonium hydrogensulphate in distilled water and diluting to 1000 ml in a volumetric flask. The mobile phase was prepared by adding 2% (v/v) of methanol and acetonitrile each and adjusting the pH 3.0 with triethylamine. The mobile phase was filtered through 0.45-μm filter and degassed before use. All further dilutions of the stock drug solutions to the required concentrations were done with the mobile phase.

### 2.3. Apparatus and chromatographic conditions

The apparatus used was a Jasco HPLC-900 Series chromatograph equipped with a PU-980 intelligent pump, a Model 975 UV–visible detector and a Model 7125 Rheodyne injector with a fixed 20-μl external loop. The column was a Metaphase-Crest-Pak-ODS (250×4.0 mm, 5 μm) operating at room temperature. The elution was carried isocratically at a flow-rate of 1.5 ml $min^{-1}$, using the above-mentioned mobile phase. The detector was set at a wavelength of 248 nm. Responses were recorded and integrated using Borwin chromatographic software.

### 2.4. Calibration and calculation

Six different concentrations (0.30, 0.60, 1.25, 2.50, 5.00 and 10.00 μg $ml^{-1}$) of a mixture of the four drugs, PZA, MCY, CFC and CPH, were prepared for linearity studies and assayed ($n=5$ per concentration). INH was added as the internal standard (I.S.) at a concentration of 2.00 μg $ml^{-1}$. Response were measured as peak area and peak area ratios of the drug/I.S. were plotted against concentration. For subsequent assays similar mixtures were prepared using any one of the five drugs as I.S. and the other four as analytes in the above mentioned concentrations.

## 3. Results and discussion

Of the different columns tried for separation and resolution the ODS column gave the best results. The optimum pH value of 3.0 was reached after studying the effect of pH on separation. A common balanced wavelength value of 248 nm could provide enough sensitivity for all the analytes. Fig. 1 shows a typical chromatogram of the mixture of all the five drugs obtained under the conditions described, the concentrations of INH, MCY and PZA being 10.00 μg $ml^{-1}$, and of CPH and CFC being 20.00 μg $ml^{-1}$. The relative chromatographic resolution parameters are listed in Table 1. The calibration data was analysed by the linear regression least-squares fit method and the results for the four drugs, PZA, MCY, CFC and CPH with INH as the internal standard are given in Table 2. Subsequent assay calculations showed that any one of the five drugs can be used as internal standard for the other four drugs. The minimum quantifiable concentration (MQC) values, for INH, PZA, and MCY were found

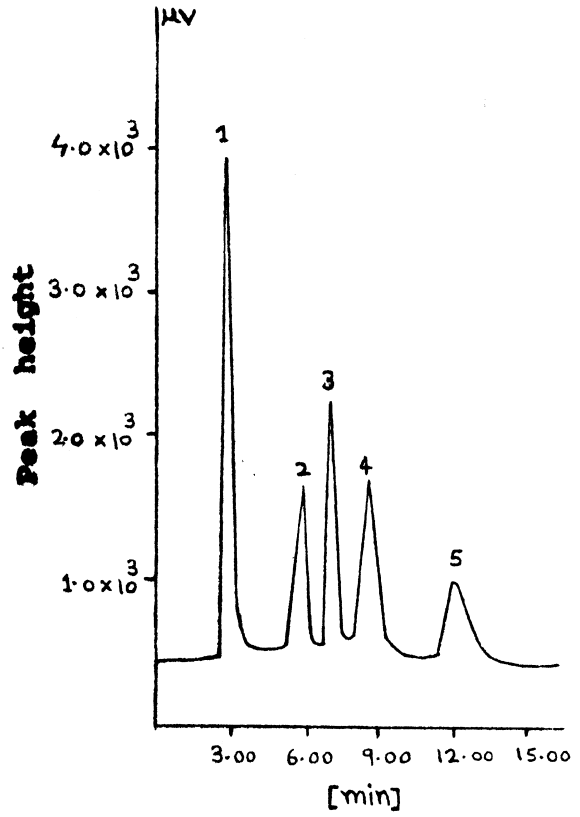


Fig. 1. A typical HPLC chromatogram of the five antibiotic drugs analyzed. (1) Isoniazid (I.S.), 2.68 min; (2) minocycline, 5.79 min; (3) pyrazinamide, 6.89 min; (4) cephalexin, 8.50 min; (5) cefaclor, 12.18 min.

to be 0.15 μg $ml^{-1}$, while for CPH and CFC the value was only 0.30 μg $ml^{-1}$ (defined as the quantity where R.S.D.s exceeded 20%).

Accuracy of the method was evaluated from the values obtained on spiking at three levels known concentrations of each analyte to a known mixture of the drugs. Inter- and intra-day performance studies at each of the three levels revealed that the precision was high and the R.S.D. never exceeded 5%.

## 4. Conclusions

A rapid, easy and accurate ion-pairing HPLC method has been described for the simultaneous determination of isoniazid, pyrazinamide, minocycline, cefaclor and cephalexin by the internal standard method.

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Table 1
Chromatographic resolution parameters for the peaks recorded in analysis of the assayed drugs

| Drug | Relative retention time | Symmetry factor | Resolution | Capacity factor ($k'$) | Number of plates |
|---|---|---|---|---|---|
| Isoniazid | 1.00 | 1.00 | — | 5.00 | 1440 |
| Minocycline | 2.16 | 1.00 | 3.53 | 10.0 | 4840 |
| Pyrazinamide | 2.58 | 1.25 | 1.37 | 13.0 | 5025 |
| Cephalexin | 3.18 | 0.75 | 1.67 | 16.0 | 5146 |
| Cefaclor | 4.56 | 1.25 | 3.70 | 23.0 | 3698 |

Table 2
Linear regression (least-squares fit) calibration data for the drugs ($n=5$) (with INH as internal standard, concentration 2 μg $ml^{-1}$)

| Drug | Conc. range (μg $ml^{-1}$) | Slope, $m \pm t_{CI,V}$ Sm[a] | Intercept | $r^2$ | $S_{yx}$ |
|---|---|---|---|---|---|
| Minocycline | 10.00–0.30 | 0.12±0.03 | 0.020±0.14 | 1.000 | 0.09 |
| Pyrazinamide | 10.00–0.30 | 0.20±0.04 | 0.002±0.20 | 1.000 | 0.12 |
| Cephalexin | 10.00–0.30 | 0.13±0.11 | −0.021±0.54 | 0.999 | 0.34 |
| Cefaclor | 10.00–0.30 | 0.07±0.05 | −0.008±0.25 | 0.999 | 0.16 |

[a]Two-tailed confidence level for Student's distribution with $V$ degees of freedom [9,10].

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