# HPTLC Determination of Cefuroxime Axetil and Ornidazole in Combined Tablet Dosage Form

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### Abstract

**A new simple high-performance thin layer chromatographic method for determination of cefuroxime axetil and ornidazole in combined tablet dosage form is developed and validated. Cefuroxime axetil is second-generation cephalosporin used to treat or prevent infections that are proven or strongly suspected to be caused by bacteria. Ornidazole is used to cure protozoan infections. The separation is carried out on Merck precoated silica gel aluminium plate 60 F$_{254}$ using toluene–$n$-butanol–triethylamine (8.5:2:0.5, v/v/v) as mobile phase. Quantitative determination of drugs is carried out by densitometric scanning of plates at 285 nm. The retention factor for ornidazole and cefuroxime axetil is found to be 0.51 ± 0.007 and 0.67 ± 0.009, respectively. The method is validated with respect to linearity, accuracy, precision, and robustness. Response found to be linear in the concentration range of 100–500 ng/band for both cefuroxime axetil and ornidazole. The method has been successfully applied for the analysis of drugs in pharmaceutical formulation. The % assay is found to be 102.36 ± 0.775 and 101.00 ± 1.192 for cefuroxime axetil and ornidazole, respectively.**

## Introduction

Cefuroxime axetil [CA, ($RS$)-1-hydroxyethyl (6$R$, 7R)-7-[2-(2-furyl) glyoxyl-amido]-3-(hydroxymethyl)-8-oxo-5-thia-1-azabi-cyclo [4.2.0]-oct-2-ene-2-carboxylate, 7$^2$-($Z$)-($O$-methyl-oxime), 1-acetate 3-carbamate] is a second-generation cephalosporin used to treat or prevent infections that are proven or strongly suspected to be caused by bacteria (1) whereas ornidazole [OZ, 1-(3-chloro-2-hydroxypropyl)-2-methyl-5-nitroimidazole] is used to cure protozoan infections (2). Structures of CA and OZ are shown in Figure 1.

Literature survey reveals spectrophotometric method (3) and high-performance thin layer chromatography (HPTLC) methods (4–6) for CA determination in combination with other drugs. Stability-indicating (7) and bioanalytical chromatographic methods (8,9) for quantification of CA are also reported. U.S. Pharmacopeia describes an HPLC method for determination of CA (10). For simultaneous determination of OZ with other drugs, reversed-phase (RP)-HPLC (11,12) and HPTLC (13,14) methods are reported. Bioanalytical chromatographic method (15) for OZ determination is also available. No reports were found for deter-mination of CA and OZ by HPTLC method in combined tablet dosage form. The aim of the present work is to develop a simple, economical, rapid, accurate, and precise HPTLC method for deter-mination of these drugs in combined tablet dosage form. The pro-posed method is optimized and validated as per International Conference on Harmonization (ICH) guidelines (16).

## Experimental

### Reagents and chemicals

Analytically pure samples of CA (assay 99.78%) and OZ (assay 100.19%) were kindly supplied by Maxim Pharmaceuticals Pvt. Ltd. (Pune, India) and Cipla Ltd (Mumbai, India), respectively, and were used as such without further purification. The phar-maceutical dosage form used in this study was a CEFAKIND-OZ 250 (Akums Drugs & Pharmaceuticals Ltd., Haridwar, India) tablets labeled to contain 250 mg of CA and 500 mg of OZ.

### Instrumentation and chromatographic conditions

The samples were applied in the form of bands of width 6 mm with space between bands of 5 mm with a 100-µL sample syringe (Hamilton, Bonaduz, Switzerland) on precoated silica gel alu-minium plate 60 F$_{254}$ (10 × 10) with 250-µm thickness (Merck, Darmstadt, Germany) using a CAMAG Linomat 5 sample appli-cator (Muttenz, Switzerland). The plates were pre-washed with methanol and activated at 110°C for 5 min prior to chromatog-raphy. The slit dimensions (5 mm × 0.45 mm) and scanning speed of 20 mm/s were employed during analysis.

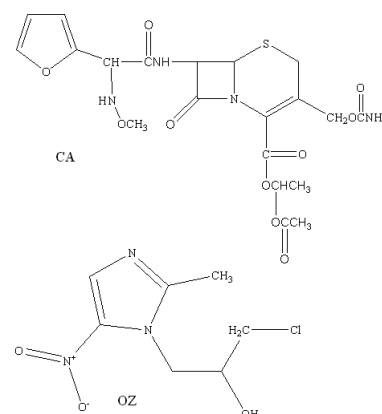The linear ascending development was carried out in a 10 cm × 10 cm twin trough glass chamber (CAMAG, Muttenz,



**Figure 1.** Structures of CA and OZ.

Switzerland) using mobile phase toluene–*n*-butanol–triethylamine (8.5:2:0.5, v/v/v). The optimized chamber saturation time for mobile phase was 15 min. The length of the chromatogram run was 9 cm, and development time was approximately 20 min. HPTLC plates were dried in a current of air with the help of a hair dryer. Densitometric scanning was performed on a CAMAG thin layer chromatography scanner 3 at 285 nm for all developments operated by WINCATS software version 1.4.2. The source of radiation utilized was deuterium lamp emitting a continuous UV spectrum between 200–400 nm.

### Preparation of standard stock solutions

Standard stock solution of CA was prepared by dissolving 50 mg of drug in 50 mL of methanol to get a concentration of 1 mg/mL from which 0.5 mL was further diluted to 10 mL to get stock solution of 50 ng/μL. Standard stock solution of OZ was prepared by dissolving 25 mg of drug in 25 mL of methanol to get a concentration of 1 mg/mL from which 0.5 mL was further diluted to 10 mL to get stock solution of 50 ng/μL.

### Selection of detection wavelength

After chromatographic development spots were scanned over the range of 200–400 nm and the spectra were overlain, it was observed that both drugs showed considerable absorbance at 285 nm. So, 285 nm was selected as the detection wavelength (Figure 2).

### Preparation of calibration curve

The standard stock solutions of CA (50 ng/μL) and OZ (50 ng/μL) were applied by overspotting on an HPTLC plate in the range of 2–10 μL with the help of a CAMAG 100-μL sample syringe and use of a Linomat 5 sample applicator. The plate was developed and scanned under the previously established chromatographic conditions. Each standard in five replicates was analyzed, and peak areas were recorded. Calibration curves of CA and OZ were plotted separately of peak area Vs respective to the concentration of CA and OZ.

### Analysis of tablet formulation

Twenty tablets were weighed accurately and finely powdered. A quantity of powder equivalent to 25 mg of CA (50 mg of OZ) was weighed and transferred to a 25-mL volumetric flask containing about 15 mL of methanol, ultrasonicated for 5 min, and volume was made up to the mark with methanol. The solution was filtered using Whatman paper No. 41. From the filtrate, 0.5 mL was further diluted to 10 mL to get sample stock solution of CA 50 ng/μL (OZ 100 ng/μL). From sample stock solution, 2 μL volume was applied on HPTLC plate to obtain final concentration of 100 ng/band for CA and 200 ng/ band for OZ. After chromatographic development peak areas of the spots were measured at 285 nm, concentration of drug in the sample was estimated from the respective calibration curves. Procedure was repeated six times for the analysis of homogenous sample.

### Method validation

As per the ICH guidelines, the method validation parameters checked were linearity, accuracy, precision, limit of detection, limit of quantitation, and robustness.

### Accuracy

To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels 50, 100, and 150%.

### *Precision*

To study intra-day variation, six mixed standard solutions containing CA (100 ng/band) and OZ (200 ng/band) were analyzed on the same day to record any intra-day variation in the results. To study inter-day variation, analysis of three mixed standard solutions of the same concentration was performed on three different days.

### *Specificity and selectivity*

The specificity of the method was ascertained by analyzing standard drug and sample. The spots for both drugs were confirmed by comparing the Rf and spectra of the sample spots with that of standard drugs.

### *Limit of detection and limit of detection*

Limit of detection (LOD) and limit of quantitation (LOQ) were calculated as 3.3 σ/S and 10 σ/S, respectively, where σ is the standard deviation of the response (*y*-intercept) and S is the slope of the calibration plot.

### *Robustness studies*

The robustness of the method was studied during method development by small but deliberate variations in mobile phase composition (± 2%), chamber saturation period (± 10%), development distance (± 10%), time from application to development (0, 10, 20, 30 min), and time from development to scanning (0, 30, 60, 90 min). One factor at a time was changed to study the effect on the peak area of the drugs (concentration level 100 ng/band for CA and 200 ng/band for OZ).

## Results and Discussion

### Method development

Different mobile phases containing various ratios of toluene, methanol, *n*- hexane, and isopropyl alcohol were examined (data not shown). Finally, the mobile phase containing toluene–*n*-butanol–triethylamine (8.5:2:0.5, v/v/v) was selected as optimal for obtaining well-defined and resolved peaks. The optimum
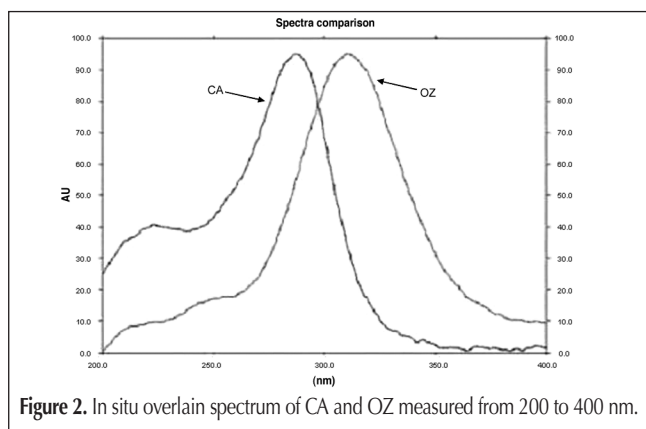


**Figure 2.** In situ overlain spectrum of CA and OZ measured from 200 to 400 nm.

wavelength for detection and quantitation used was 285 nm. The retention factors for OZ and CA were found to be $0.51 \pm 0.007$ and $0.67 \pm 0.009$, respectively. Densitogram of mixed standard solution of CA and OZ is shown in Figure 3.

### Validation

The standard calibration curves were found to be linear over a concentration range of 100–500 ng/band for both drugs with correlation coefficients of $0.9989 \pm 0.0015$ for CA and $0.9991 \pm 0.0052$ for OZ. Summary of validation data is given in Table I, which gives linearity range, linear regression equation, correlation coefficient, LOD and LOQ for both the drugs. For CA, the recovery study results ranged from 99.81 to 101.81% with percent relative standard deviation (% RSD) values ranging from 0.499 to 0.754%. For OZ, the recovery results ranged from 98.88 to 100.59% with % RSD values ranging from 0.264 to 0.738%. Results of recovery studies are reported in Table II. The proposed method was also evaluated by the assay of commercially available tablets containing CA and OZ. Six replicate determinations were performed on the accurately weighed amounts of tablets. The %
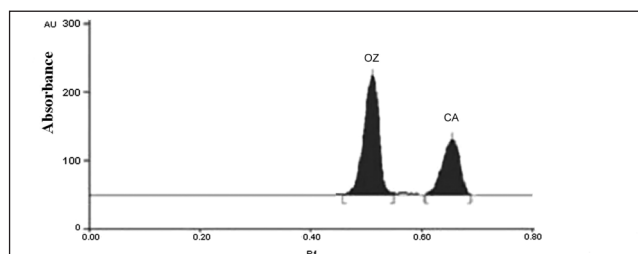
assay for CA was found to be $102.36 \pm 0.775$ and for OZ $101.00 \pm 1.192$. Intra-day variation, expressed as % RSD, was found to be 0.491 for CA and 0.750 for OZ with standard error of 0.289 and 0.441, respectively. Inter-day variation was found to be 0.822 for CA and 1.033 for OZ with standard error of 0.488 and 0.598, respectively. The spectra acquired for CA and OZ extracted from the tablet were also compared with those acquired from CA and OZ standards; correlation was good, which indicates specificity of the method. Robustness of the method checked after deliberate alterations of the analytical parameters showed that areas of peaks of interest remained unaffected by small changes in the operational parameters (% RSD < 2).

## Conclusion

The validated HPTLC method employed here proved to be simple, fast, accurate, precise, and robust, and thus can be used for routine analysis of CA and OZ in combined tablet dosage form.

**Figure 3.** Standard densitogram of OZ (200 ng/band, Rf = 0.51 ± 0.007) and CA (100 ng/band, Rf = 0.67 ± 0.009).

### Table I. Regression Analysis of Calibration Curves

| Parameter | CA | OZ |
|---|---|---|
| Detection Wavelength (nm) | 285 | |
| Beer's Law Limit (ng/band) | 100–500 | 100–500 |
| Correlation Coefficient (r) | 0.9989 | 0.9991 |
| Linear Regression Equation* | | |
| (y = mx + c) | | |
| Intercept (c) | 378.42 | 527.64 |
| Slope (m) | 10.698 | 12.336 |
| LOD (ng/band) | 0.398 | 0.369 |
| LOQ (ng/band) | 1.208 | 1.118 |

\* With respect to y = mx + c, where y is the peak area and x is the concentration (ng/band).

### Table II. Recovery Studies of CA and OZ

| Drug | Amount taken (ng/band) | Amount added (ng/band) | Total found (ng/band) | % Recovery | % RSD* |
|---|---|---|---|---|---|
| CA | 100 | 50 | 152.103 | 100.319 | 0.754 |
| | 100 | 100 | 203.193 | 101.250 | 0.563 |
| | 100 | 150 | 253.123 | 100.786 | 0.499 |
| OZ | 200 | 100 | 302.823 | 99.223 | 0.326 |
| | 200 | 200 | 403.669 | 100.035 | 0.264 |
| | 200 | 300 | 503.215 | 99.872 | 0.738 |

\* Average of three determinations.

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