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Research Article

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Simultaneous estimation of cefixime trihydrate and ornidazole by high performance thin layer chromatography method

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ABSTRACT

An accurate, simple, precise, specific and robust High Performance Thin Layer Chromatography (HPTLC)method was developed and validated for simultaneous estimation of cefixime trihydrate and ornidazole. The two compounds were separated on TLC plate Silica gel 60 f_{254} using dual run method of mobile phase initially with pure methanol and then with pure ethyl acetate. After development, the plate was evaluated by densitometry, the HPTLC Scanner 3 of CAMAG. The tow compounds separated in a good resolution and R_f values found to be 0.23±0.003 for Cefixime trihydrate and 0.67 ±0.003 for Ornidazole.The method has been validated as per ICH guideline (Q2 R1) for the parameters of Linearity and Range, Accuracy, Precision, Specificity, Limit of Detection (LOD), and Limit of Quantification (LOQ). The method also has been successfully applied for analyzing of two brands of tablet marketed dosage form of combined drugs (MAHACEF OZ& ZIFI-OZ).

Key words: HPTLC, simultaneous estimation, cefixime trihydrate, ornidazole

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INTRODUCTION

Cefixime (as trihydrate) is an orally active 3rd generation cephalosporin which exerts its bactericidal action against both gram positive & gram negative organisms by inhibiting bacterial cell wall synthesis. Cefixime is used in the treatment of the following infections when caused by susceptible strains of the designated microorganisms: (1) uncomplicated urinary tract infections caused by *Escherichia coli* and *Proteus mirabilis*, (2) otitis media caused by *Haemophilusinfluenzae* (beta-lactamase positive and negative strains), *Moraxella catarrhalis* (most of which are beta-lactamase positive), and *S. pyogenes*, (3) pharyngitis and tonsillitis caused by *S. pyogenes*, (4) acute bronchitis and acute exacerbations of chronic bronchitis caused by *Streptococcus pneumoniae* and *Haemophilusinfluenzae* (beta-lactamase positive and negative strains), and (5) uncomplicated gonorrhea (cervical/urethral) caused by *Neisseria gonorrhoeae* (penicillinase- and non-penicillinase-producing strains).[1]

Ornidazole is a nitro imidazole which has broad spectrum cidal activity against Protozoa and some anaerobic bacteria. It is used in the treatment of severe hepatic and intestinal amoebiasis, giardiasis, and trichomoniasis of the uro-genital tract and bacterial vaginosis. It is also used in the treatment and prophylaxis of susceptible anaerobic infections in dental and gastrointestinal surgery. In combination with other drugs, ornidazole is also advocated in the management of *Helicobacter pylori* duodenal ulcers.[2]

The combination of cefixime and ornidazole are available in tablet and suspension dosage forms. It is used for infections of respiratory, urinary and biliary tracts, and *Salmonella* infection.[3]

Literature review [4-33] reveals that there is no valid HPTLC method for analysis of this combination and hence a simple, precise and accurate HPTLC method was developed and validated as per ICH guideline [34].

EXPERIMENTAL SECTION

In this study, the two compounds, cefixime trihydrate and ornidazole, were separated on HPTLC plate (Silica Gel 60 F_{254}) by dual run of mobile phase, initially with methanol up to 1.3 cm, and then following by drying of plates in air, with pure ethyl acetate up to 5.5 cm. This approach of development was chosen because the polarity of two compounds differs such as they cannot be separated easily in isocratic development. The system found to giving compact spots and hence sharp peaks without any tailing or fronting. The R_f found 0.23±0.03 and 0.67±0.03 for cefixime trihydrate and ornidazole respectively. The method has been validated as per ICH guideline (Q2 R1) for the parameters of Linearity and Range, Accuracy, Precision, Specificity, Limit of Detection (LOD), and Limit of Quantification(LOQ). The method also has been successfully applied for analyzing of two brands of tablet marketed dosage form of combined drugs (MAHACEF OZ, & ZIFI-OZ)

- **Method**

- High Performance Thin Layer Chromatography (HPTLC).
- Development mode: Multidevelopment (Dual run) – Manual- Incremental.

- **Instrument:**

CAMAG HPTLC system:

- Spotter : Semi-automatic, Linomat IV.
- Development chamber: CAMAG twin trough chambers, 10x10 cm.
- Syringe: Hamilton 100µl.
- Scanner: CAMAG Scanner 3.
- Software system: CATS4.

- **Reagents:**

Pure APIs of Cefixime trihydrate and Ornidazole received from " Ratnamani Health Care Pvt.Ltd., Mehsana, Gujarat, India" as gift.

Mobile phase (methanol and ethyl acetate) and the solvent used(methanol) , were analytical grade, FINAR brand, manufactured in Ahmedabad, India.

- **Chromatographic conditions:**

– Scanning wavelength : 309 nm (Chosen based on overlay spectrum of the two compounds)
– Mobile phase: 1^{st} run with methanol for 1.3 cm, 2^{nd} run with ethyl acetate up to 5.5 cm.
– Stationary phase: HPTLC precoated plates, silica 60 F254 Merck, Germany
– Measurement mode: Absorption/Reflection
– Slit dimension: 3.0 x 0.45 mm micro
– Room temperature: 27±4 °C
– Spectrum scan speed: 100 nm/s
– Wavelength increment: 1 nm
– Data step resolution: 100 µm
– Monochromator band width: 20 nm
– Display scaling :1000 AU
– Track size: 5mm band shape
– Distance between tracks: 10 mm (between each spotting 5 mm)

RESULTS AND DISCUSSION

Linearity was found in the range of 300-700 ng/spot with correlation coefficient 0.998 for cefixime trihydrate and 750-1750 ng/spot with correlation coefficient of 0.995 for ornidazole. The linearity data shown in tables 1&2 and figures 1,2&3.

Linear regression equation:

- For Cefixime trihydrate: y = 10.917x + 1200.4
- For Ornidazole: y = 5.5817x + 8885.8
- x= concentration in ng y= peak area

Table 1: Linearity data for cefixime trihydrate

Sr.No.	Standard Solution	Concentration	Average Area (n=5)(Mean± S.D.)	% RSD
1	Std. 1	300	4404.18 ±81.74	1.86
2	Std. 2	400	5593.08 ±88.04	1.57
3	Std. 3	500	6808.4 ±126.65	1.86
4	Std. 4	600	7657.28 ±129.18	1.69
5	Std. 5	700	8830.38 ±154.90	1.75

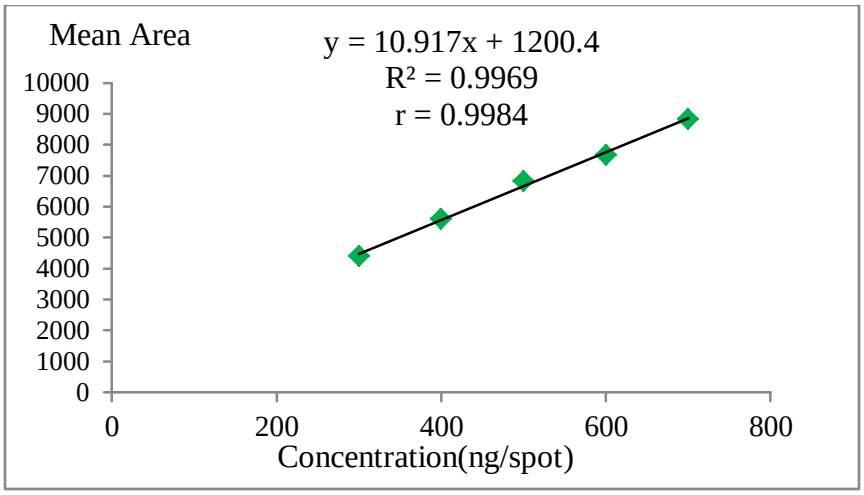

Figure 1: Cefixime trihydrate linear regression

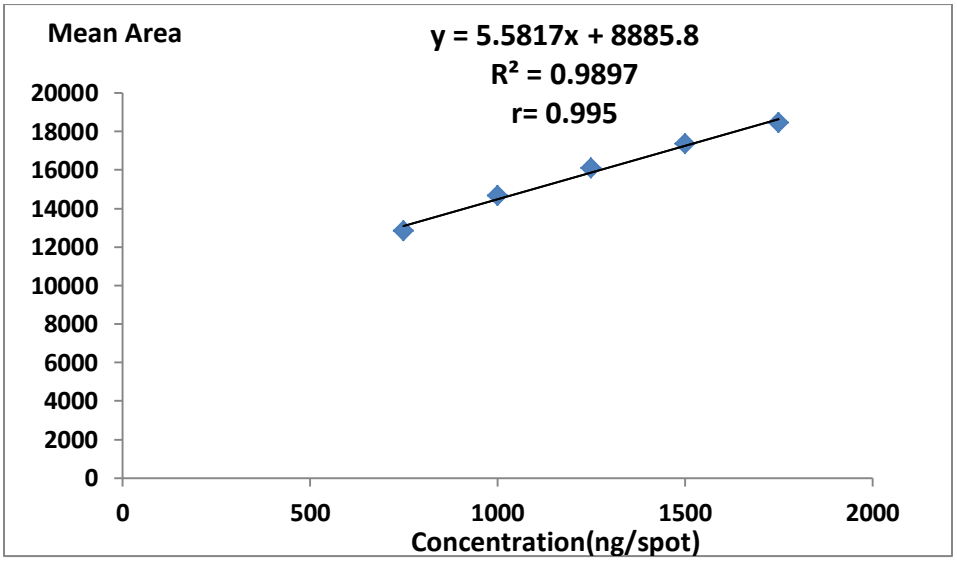

Figure 2: Ornidazole linear regression

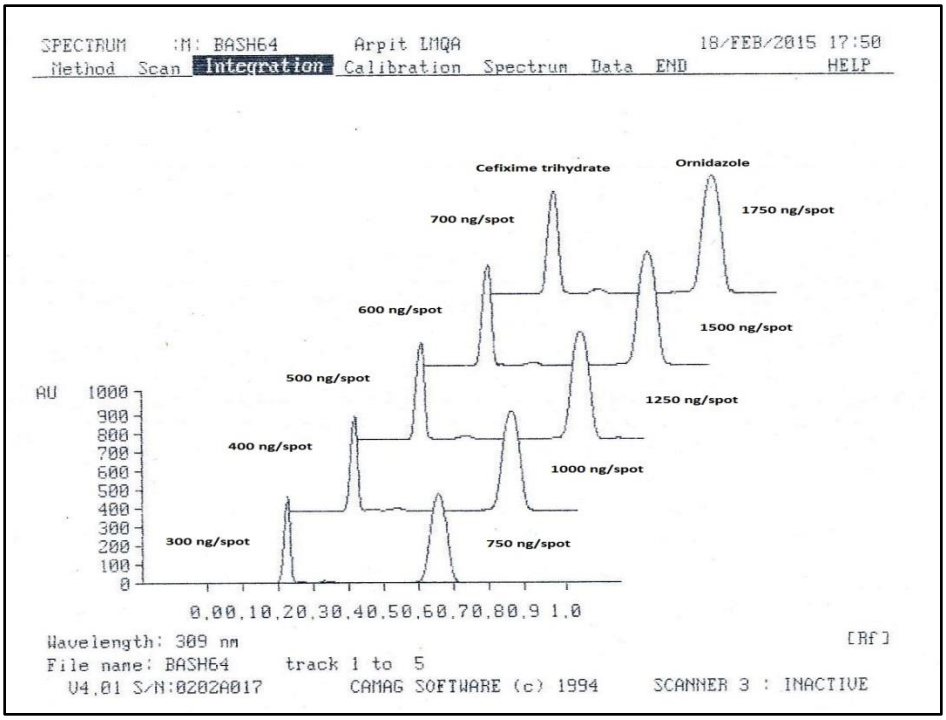

Figure 3: Integration of 5 tracks for cefixime trihydrate and ornidazole in a calibration

Table 2: Linearity data for ornidazole

Sr.No.	Standard Solution	Concentration (ng/spot)	Average Area (n=5) (Mean± S.D.)	% RSD
1	Std. 1	750	12806.56 ±112.91	0.88
2	Std. 2	1000	14652.76±113.64	0.77
3	Std. 3	1250	16072.08±134.59	0.83
4	Std. 4	1500	17346.30±234.07	1.34
5	Std. 5	1750	18436.86±284.25	1.54

The Limit of Detection(LOD)&Limit of Quantification(LOQ) were calculated form deviation of calibration curve, for cefixime trihydrate calculated 35.13 ng/spot & 106.46 ng/spot respectively, and for ornidazole calculated 81.30 ng/spot & 246.36 ng/spot respectively.

The accuracy was found 99.02±0.06 for cefixime trihydrate and 100.34±0.93 for ornidazole. The precision data found, % RSD<1 for repeatability and <2 for intraday and interday reproducibility's.

Specificity of the method was evaluated by measuring peak purity for standard drug and marketed dosage form and found no interference for matrix(peak purity> 0.99). The results are shown in table 3 and figures 4&5.

Table 3: Specificity data for peak purity

Name	Type/Brand name	$R_{start, apex}$	$R_{apex, end}$
Cefixime trihydrate	Standard	0.997831	0.994410
Cefixime trihydrate	MAHCEF OZ	0.999777	0.994567
Cefixime trihydrate	ZIFI-OZ	0.998091	0.989774
Ornidazole	Standard	0.999913	0.999785
Ornidazole	MAHCEF OZ	0.999962	0.999847
Ornidazole	ZIFI-OZ	0.999956	0.999819

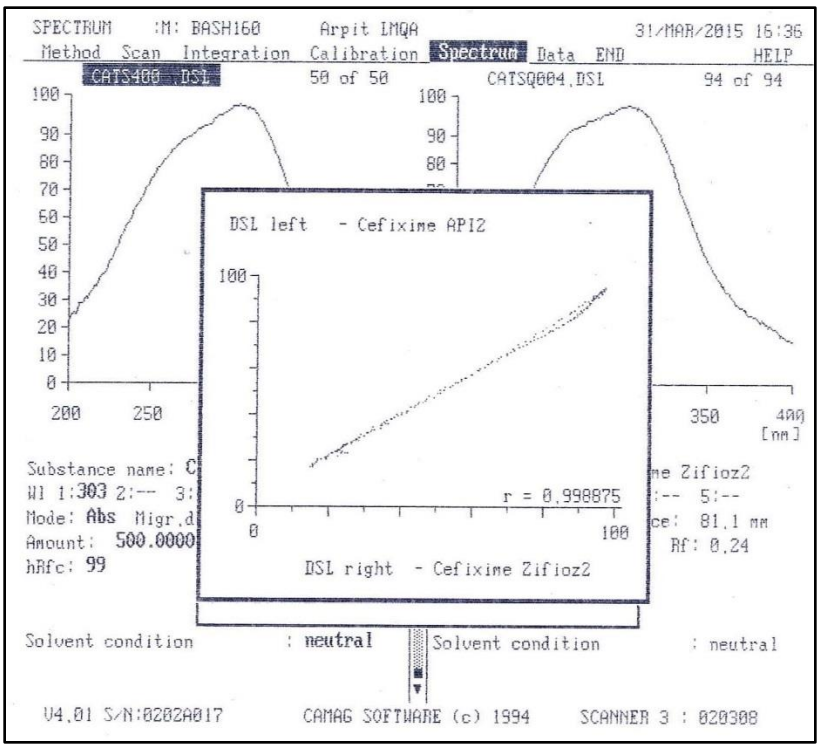

Figure 4: Peak correlation of cefixime trihydrate in API and tablet

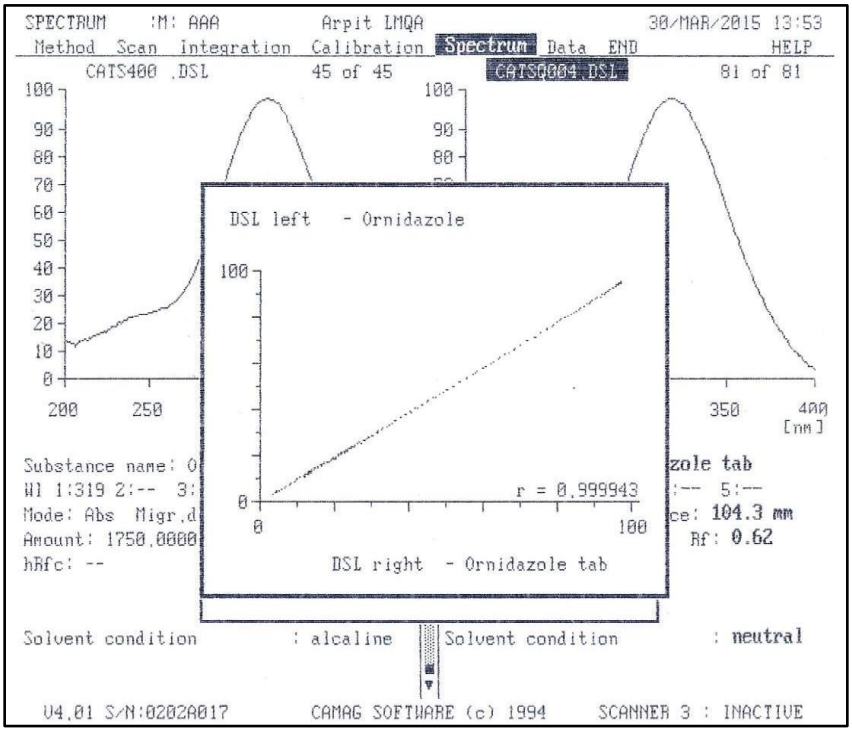

Figure 5: Peak correlation of Ornidazole in API and tablet

CONCLUSION

The method successfully validated as per ICH guideline and applied for analyzing of two brands of tablet dosage form. As the HPLTC method is simple, cost effective and the validation data shows that it satisfactory& can be used for routine analysis of the combination of Cefixime trihydrate and Ornidazole in bulk and marketed dosage forms.

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