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Research Article

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Analytical Method Development and Validation by RP-HPLC for Simultaneous Estimation of Cefixime and Ornidazole in Combined Tablet Dosage Form

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ABSTRACT

A simple, Accurate, precise method was developed for the simultaneous estimation of the Cefixime and Ornidazole in Tablet dosage form. Chromatogram was run through ODS (250mm 4.6mm, 5 μ). Mobile phase containing Buffer and Acetonitrile and methanol in the ratio of 45:45:10A was pumped through column at a flow rate of 1ml/min. Buffer used in this method was 0.01N KH₂PO₄ buffer at pH 4.6. Temperature was maintained at 30°C. Optimized wavelength for Cefixime and Ornidazole was 220nm. Retention time of Cefixime and Ornidazole were found to be 4.0 min and 2.8 min. %RSD of the Cefixime and Ornidazole were and found to be 0.66 and 0.61 respectively. %Recover was obtained as 101.04% and 100.41% for Cefixime and Ornidazole respectively. LOD, LOQ values are obtained from regression equations of Cefixime and Ornidazole were 0.74ppm, 0.72ppm and 2.23ppm, 2.19ppm respectively. Regression equation of Cefixime is $y = 2829.x + 1707$, and of Ornidazole is $y = 1935x + 866.3$.

Keywords: Cefixime, Ornidazole, RP-HPLC

ARTICLE INFO

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1. Introduction

Pharmaceutical Analysis is the branch of chemistry involved in separating, identifying and determining the International Journal of Research in Pharmacy and Life Sciences

relative amounts of the components making up a sample of matter [1,2,3]. It is mainly involved in the qualitative

identification or detection of compounds and quantitative measurements of the substances present in bulk and pharmaceutical preparation [4,5,6]. This combination of drugs was found to be more effective antibiotics. Cefixime is a third-generation cephalosporin and it is highly stable in the presence of beta-lactamase enzymes [7,8]. The antibacterial effect of cefixime results from inhibition of mucopeptide synthesis in the bacterial cell wall. Used in the treatment of uncomplicated urinary tract infections, otitis, gonorrhoea [9]. Ornidazole is a nitro imidazole which has broad spectrum cidal activity against Protozoa and some anaerobic bacteria. Drug enters the cell by diffusion, the nitro group of drug is reduced by redox proteins present only in anaerobic organisms to reactive nitro radical which exerts cytotoxic action by damaging DNA and other critical biomolecules. Then DNA helix destabilization & strand breakage has been observed. It is an anti-infective; used to treat selected protozoan infections. [10,11]

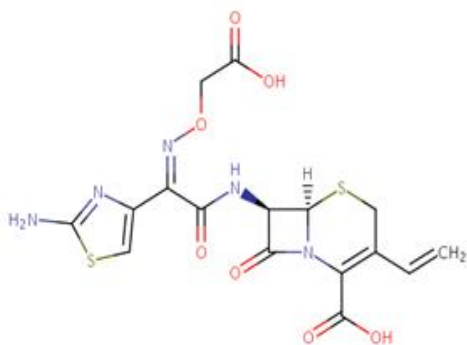


Figure 1: Structure of Cefixime

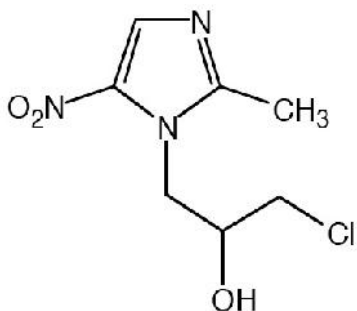


Figure 2: Structure of Ornidazole

2. Materials and Methods

Materials:

Cefixime and Ornidazole, Combination Cefixime and Ornidazole tablets, distilled water, acetonitrile, phosphate buffer, ammonium acetate buffer, glacial acetic acid, methanol, potassium dihydrogen phosphate buffer, tetrahydrofuran, triethylamine, ortho-phosphoric acid etc.

Instrument:

HPLC instrument used was of WATERS HPLC 2965 SYSTEM with Auto Injector and PDA Detector. Software used is Empower 2. UV-VIS spectrophotometer PG Instruments T60 with special band width of 2mm and 10mm and matched quartz was used for measuring absorbance for Cefixime and Ornidazole solutions [12, 13].

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Methods:

Preparation of buffer:

Buffer: (0.01KH₂PO₄)

Accurately weighed 2.72gm of Potassium di-hydrogen Ortho phosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water and pH adjusted to 4.6 with dil. OPA Solution[14,15].

Standard Preparation:

Accurately Weighed and transferred 10mg of Cefixime and 25mg of Ornidazole working Standards into a 10ml clean dry volumetric flask, add 3/4th volume of diluent, sonicated for 5 minutes and make up to the final volume with diluents. 1ml from the above two stock solutions was taken into a 10ml volumetric flask and made up to 10ml.

Sample Preparation:

10 tablets was weighed, powdered and then the weight 2500 mg (equivalent to 500 mg of Cefixime and 1250mg of Ornidazole) was transferred into a 100mL volumetric flask, 75mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 0.2ml was pipeted out into a 10 ml volumetric flask and made upto 10ml with diluent.

Linearity:

Linearity solutions are prepared such that 0.25ml, 0.5ml, 0.75ml, 1ml, 1.25ml, 1.5ml from the Stock solutions of Cefixime and Ornidazole are taken in to 6 different volumetric flasks and diluted to 10ml with diluents to get 25ppm, 50ppm, 75ppm, 100ppm, 125ppm, 150ppm of Cefixime and 62.5ppm, 125ppm, 187.5ppm 250ppm, 312.5ppm, 375ppm of Ornidazole.

Standard Preparation:

Accurately Weighed and transferred 10mg of Cefixime and 25mg of Ornidazole working Standards into a 10ml clean dry volumetric flask, add 3/4th volume of diluent, sonicated for 5 minutes and make up to the final volume with diluents. 1ml from the above two stock solutions was taken into a 10ml volumetric flask and made up to 10ml.

Sample Preparation:

10 tablets was weighed, powdered and then the weight 2500 mg (equivalent to 500 mg of Cefixime and 1250mg of Ornidazole) was transferred into a 100mL volumetric flask, 75mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 0.2ml was pipeted out into a 10 ml volumetric flask and made upto 10ml with diluent.

Accuracy:

Standard Preparation:

Accurately Weighed and transferred 10mg of Cefixime and 25mg of Ornidazole working Standards into a 10ml clean dry volumetric flask, add 3/4th volume of diluent, sonicated for 5 minutes and make up to the final volume with diluents. 1ml from the above two stock solutions was taken into a 10ml volumetric flask and made up to 10ml.

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solution 0.2ml was pipeted out into a 10 ml volumetric flask and made upto 10ml with diluent.

50%: 10 tablets was weighed, powdered and then the weight 1250 mg was transferred into a 100mL volumetric flask, 75mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 0.2ml was pipeted out into a 10 ml volumetric flask and made up to 10ml with diluent.

100%: 10 tablets was weighed, powdered and then the weight 2500 mg was transferred into a 100mL volumetric flask, 75mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 0.2ml was pipeted out into a 10 ml volumetric flask and made upto 10ml with diluent.

150%: 10 tablets was weighed, powdered and then the weight 3750 mg was transferred into a 100mL volumetric flask, 75mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 0.2ml was pipeted out into a 10 ml volumetric flask and made upto 10ml with diluent.

3. Results and Discussion

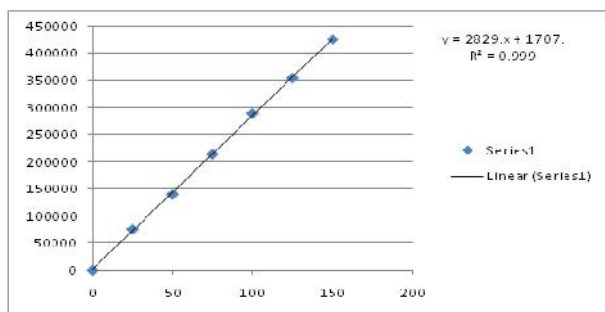


Figure 3: Calibration curve of Cefixime

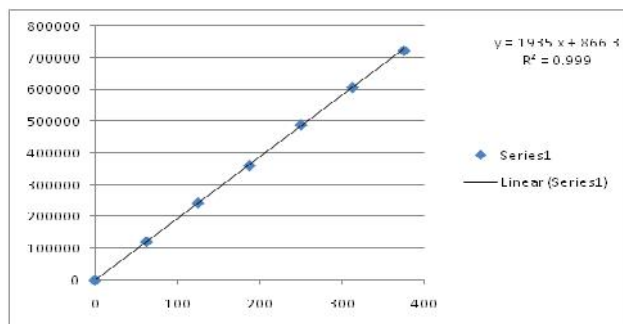


Figure 4: Calibration curve of Ornidazole

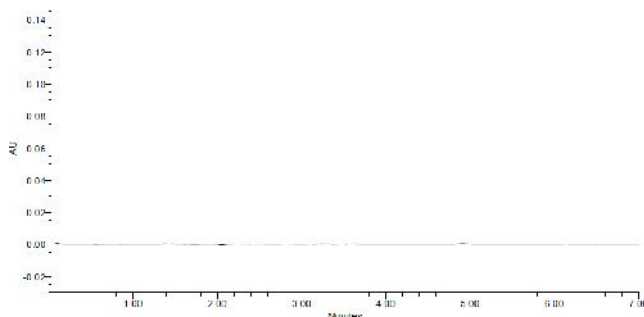


Figure 5: Chromatogram of blank

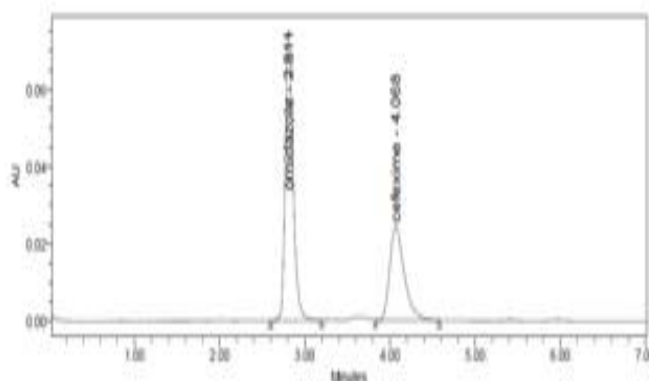


Figure 6: Typical chromatogram of Cefixime and Ornidazole

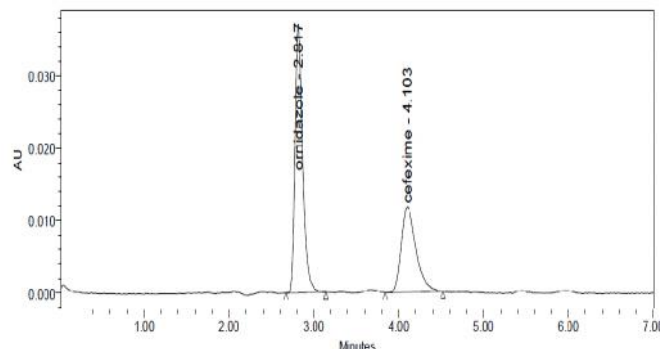


Figure 7: Linearity 50% Chromatogram of Cefixime and Ornidazole method

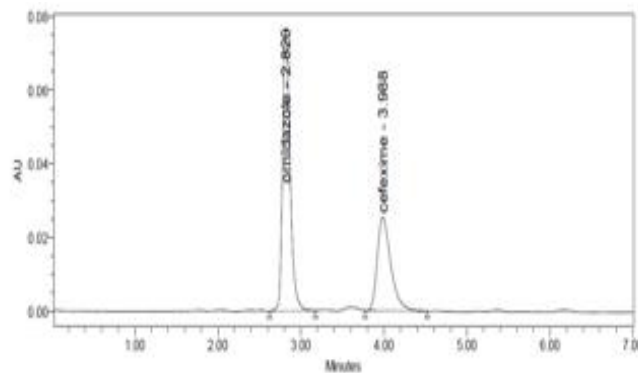


Figure 8: Linearity 100% Chromatogram of Cefixime and Ornidazole method

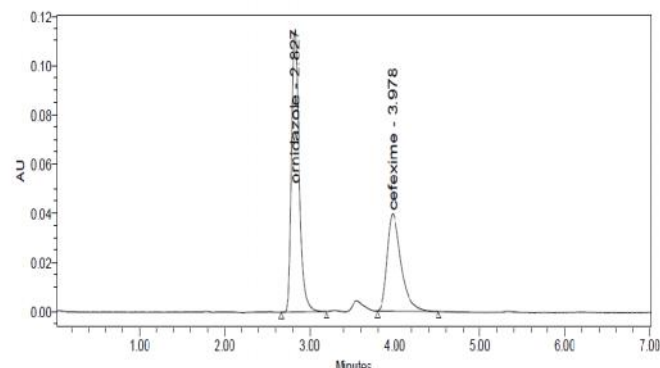


Figure 9: Linearity 150% Chromatogram of Cefixime and Ornidazole method

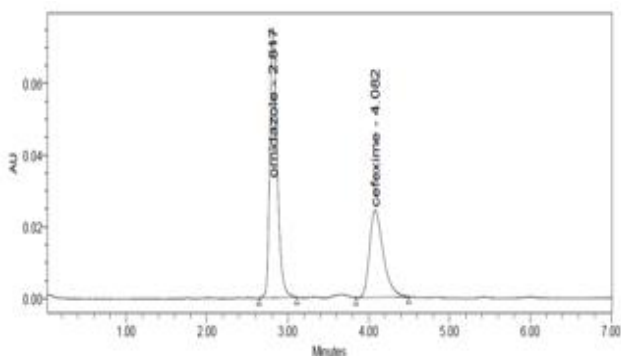


Figure 10: Repeatability Chromatogram of Cefixime and Ornidazole method

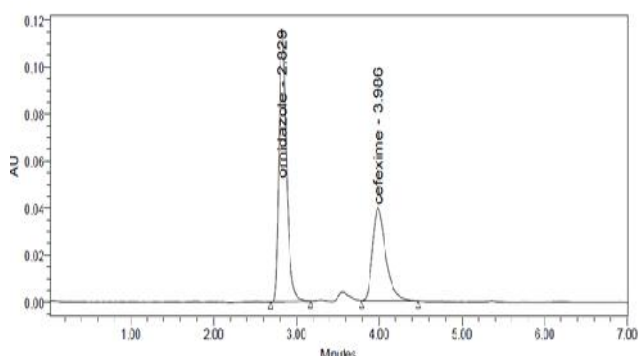


Figure 14: Accuracy 150% Chromatogram of Cefixime and Ornidazole method

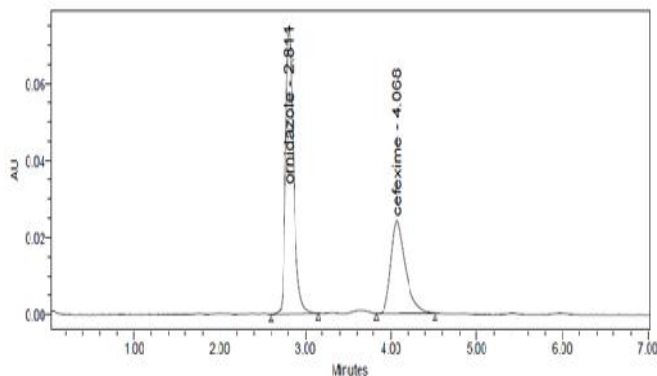


Figure 11: Inter Day precision Chromatogram of Cefixime and Ornidazole method

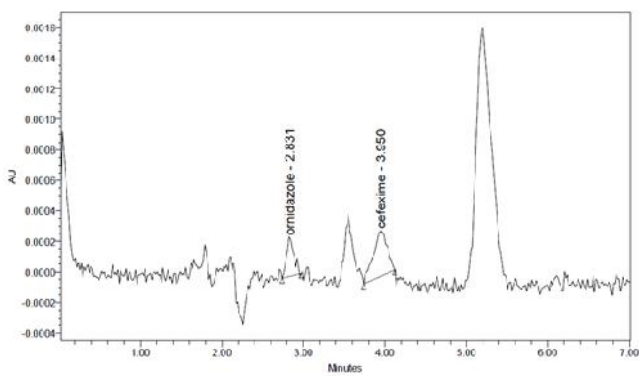


Figure 15: LOD Chromatogram of Cefixime and Ornidazole method

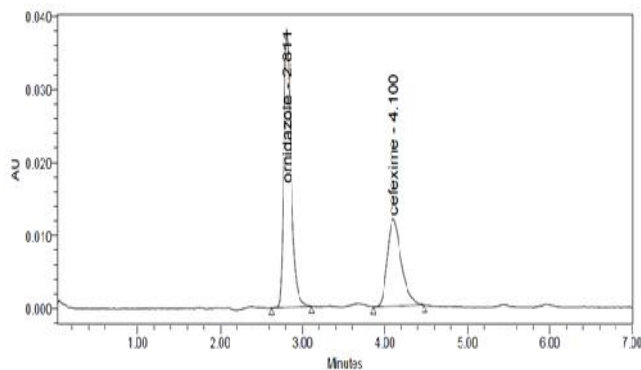


Figure 12: Accuracy 50% Chromatogram of Cefixime and Ornidazole method

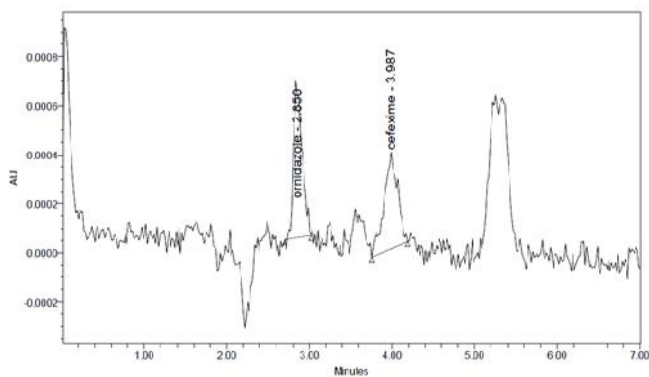


Figure 16: LOQ Chromatogram of Cefixime and Ornidazole method

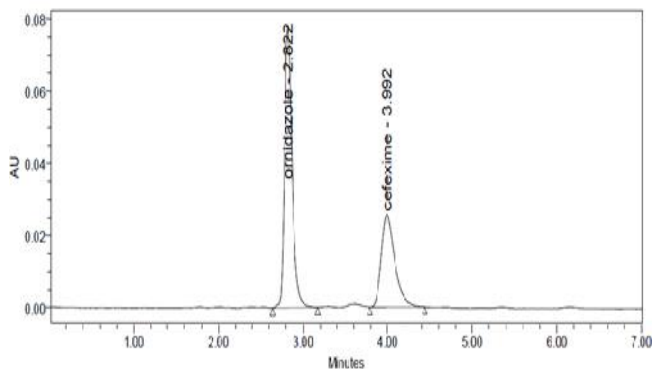


Figure 13: Accuracy 100% Chromatogram of Cefixime and Ornidazole method

4. Conclusion

The method was found to be precise, accurate and linear over the linear concentration range. The method developed is unique in determining the impurities even at low levels than that of specifications. The analytical method validation of Cefixime and Ornidazole in tablet dosage form by RP-HPLC was found to be satisfactory and could be used for the routine pharmaceutical analysis of Cefixime and Ornidazole in tablet dosage form. Method was validated as per ICH guidelines like system suitability, accuracy, precision, linearity, specificity, forced degradation studies, ruggedness, robustness and solution stability, Therefore, this HPLC method can be used as a routine analysis of these drugs in pharmaceutical formulations.

Table 1: Calibration data of Cefixime and Ornidazole method

S.No	Concentration Cefixime (µg/ml)	Response	Concentration Ornidazole (µg/ml)	Response
1	0	0	0	0
2	25	75603	62.5	121461
3	50	140100	125	243322
4	75	213893	187.5	361563
5	100	288926	250	489440
6	125	353978	312.5	607549
7	150	425093	375	722840

Table 2: Repeatability results for Cefixime and Ornidazole

S. No	Cefixime	Ornidazole
1	294201	486989
2	294612	483539
3	290375	478706
4	292645	485166
5	293548	486319
6	290061	483940
AVG	292573.7	484109.8
STDEV	1944.5	2961.135
%RSD	0.66	0.61

*Average of six determinations

Table 3: Inter day precision results for Cefixime and Ornidazole

S. No.	Cefixime	Ornidazole
1	286674	477643
2	286121	477108
3	285495	478876
4	287515	477917
5	285969	478116
6	283293	478007
AVG	285844.5	477944.5
STDEV	1428.5	581.7906
%RSD	0.50	0.12

Table 4: Accuracy results of Cefixime and Ornidazole

Sample	Amount added (µg/ml)	Amount Recovered (µg/ml)	Recovery (%)	% RSD
Cefixime	50	49.82	99.64	0.21
	100	99.15	99.15	0.08
	150	152.49	101.66	0.27
Ornidazole	125	124.12	99.30	0.2
	250	248.60	99.44	0.04
	375	375.60	100.16	0.78

5. Acknowledgement

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