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Validated Simultaneous Estimation of Ornidazole and Cefixime by RP-HPLC in Pure and Pharmaceutical Dosage Form

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A simple, precise RP-HPLC method was developed for the estimation of ornidazole and cefixime in pure and pharmaceutical dosage forms. The quantification was carried out using a C-18 column 250 × 4.6 mm i.d., 5 µm particle size in isocratic mode, with mobile phase comprising of tetrabutyl ammonium hydroxide and acetonitrile in the ratio of 3:1 (v/v) pH 6.5. The flow rate was 1.5 mL/min and the detection was carried out UV detector at 254 nm. The retention times were 4.75 and 8.98 min for cefixime and ornidazole, respectively. The method produced linear response in the concentration range of 150-350 µg/mL for ornidazole 60-140 µg/mL for cefixime, respectively. The percentage recovery was found to be 99.6 and 99.8 % for ornidazole and cefixime, respectively. Metronidazole used as an internal standard in the present study. The method validated by evaluation of required parameters.

Key Words: RP-HPLC, Cefixime, Ornidazole.

INTRODUCTION

Cefixime is a semi-synthetic cephalosporin antibiotic for oral administration, chemically it is (6R and R)-7-[2-(2-amino-4-thiazolyl)glyoxyl] amino]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid,7^{2}-(2)-[0-carboxymithyl)oxime]trihydrate. Its empirical formula is $C_{16}H_{13}N_5Na_2O_7S_2$[1] and molecular weight is 507.50 as tri hydrate. Cefixime is highly stable in the presence of β-lactamase enzymes. As a result, many organisms resistant to penicillins and some cephalosporins due to the presence of β-lactamases may be susceptible to cefixime.

Ornidazole is an antihelmenthitic drug for oral administration, chemically it is 1-(3-chloro-2-hydroxypropyl)-2-methyl-5-nitroimidazole is used as an antiinfective agent. Its empirical formula is $C_7H_{10}ClN_3O_3$[1] and molecular weight is 219.63.

Ornidazole is used in combination with other fluoroquinolone in the treatment of protozoal infectious diseases (PID) and intra-abdominal infection. A nitro imidazole antiprotozoal agent used in amoeba and trichomonas infections. It is partially plasma bound and also has radiation and sensitizing action. Literature survey[2-9] indicates that no other HPLC method developed for the estimation of cefixime and ornidazole in pure and pharmaceutical dosage forms. In present investigation an attempt was made to develop a simple and economical, validated RP-HPLC with greater precision, accuracy and sensitivity for the estimation of cefixime and ornidazole in bulk as well as in pharmaceutical formulations.

EXPERIMENTAL

Pure standards of cefixime and ornidazole were obtained as gift samples from ATOZ Pharmaceuticals, Chennai. The purities of these standards were 99.5 and 98.6 %, respectively. HPLC grade acetonitrile (qualigens), tetra butyl ammonium hydroxide (qualigens) of AR grade and H_3PO_4 (qualigens), Ornicef Dt (Aristo pharmaceuticals) was employed in the study.

A isocratic, high performance liquid chromatograph with Shimadzu pump LC-10 ATVp equipped with universal injector (Rheodyne) with injection volume 20 µL, ultra violet visible detector (UV-Vis) SPD-10AV_A-Shimadzu series and Shimadzu class Vp software. A thermo hypersil key stone C-18 ODS column 250 × 4.6 mm i.d. with 5 µm particles. Detection was carried out by UV detection at 254 nm.

Chromatographic conditions: Freshly prepared 3:1 (v/v) tetrabutyl ammonium hydroxide and acetonitrile were filtered through 0.45 µm membrane filter and sonicated before used. The flow rate of mobile phase was 1.5 mL/min. The column was maintained at ambient temperature. The detection was carried out by a 254 nm. The injection volume was 20 µL and run time was 10 min.

Preparation of mobile phase: Tetrabutyl ammonium hydroxide and acetonitrile in the ratio of 3:1 (v/v) was used as a mobile phase for present study. Tetrabutyl ammonium hydroxide solution was prepared by taking accurately weighed quantity of 103.792 g of tetrabutyl ammonium hydroxide was taken and dissolved in water and make up to 1000 mL to get 0.4 M of tetrabutyl ammonium hydroxide solution. Finally, the pH of the solution was the adjusted to 6.5 by adding orthophosphoric aicd.

Preparation of buffer solution: In the present study pH-7 buffer was used as a diluent solution. The buffer was prepared by taking 7.1 g of dibasic sodium phosphate in 500 mL water and the pH of the solution was adjusted to 7 with monobasic potassium phosphate. Monobasic potassium phosphate solution was prepared by taking 6.8 g of monobasic potassium phosphate in 500 mL of water.

Preparation of internal standard solution: An accurate weighed amount equivalent to 25 mg of internal standard and transferred into 50 mL volumetric flask. It was dissolved in mobile phase solution and volume made up to 50 mL so as to give concentration about 500 μg/mL (stock solution). Take 1 mL of stock solution made up to the volume with buffer to get concentration about 20 μg/mL. Metronidazole is used as a internal standard in the present study.

Preparation of stock solution of ornidazole and cefixime: About 125 and 50 mg of ornidazole and cefixime was weighed accurately and transferred into 100 mL volumetric flask. They are dissolved in mobile phase solution by shaking for 5 min, the volume was made up to 100 mL with buffer pH 7. Each mL of stock contains 1250 and 500 μg/mL for ornidazole and cefixime, respectively.

Procedure for assay: From the stock solution the suitable volume (3-7 mL) of drug solution was transferred into 10 mL volumetric flask. These solutions are spiked with and 1 mL of internal standard solution make up to volume of 25 mL with diluent (buffer pH-7) to get 150-350, 60-140 and 2 μg/mL of ornidazole, cefixime and metronidazole, respectively. Five sets of cefixime and ornidazole solution were prepared to get concentrations of 60, 80, 100, 120, 140 and 150, 200, 250, 300, 350 μg/mL, respectively, with 20 μg/mL metronidazole as an internal standard. The solutions were prepared as above were filtered through 0.45 μm membrane filter and each of the samples were injected five times into the column and flow rate was 1.5 mL/min. The ratio of drug peak are to that of internal standard for each of the drug concentrations was calculated. The regression of the drug content over the ratio of drug peak area to that of internal standard was obtained.

Estimation of ornidazole and cefixime in tablet dosage forms: About 10 tablets were pulverized and an accurately weighed sample of powdered tablets equivalent to 125 and 50 mg of ornidazole and cefixime was taken in 100 mL volumetric flask. Add 50 mL of methanol and extracted by sonification to ensure complete solubility of drug. The mixture was made up to volume 100 mL with diluent solution (buffer pH 7). The insoluble portion was filtered through a 0.45 μm membrane filter. The filtrate was diluted to get required dilutions. The filtrate was diluted to get the concentrations about 150-350 and 60-140 μg/mL for ornidazole and cefixime, respectively. The solutions were spiked with suitable volume of the internal standard solution, such that the concentration of internal standard in each was 20 μg/mL. Each of these solutions was then injected 5 times in to the column. From the peak areas, the drug content in the tablets was quantified using the regression equation obtained from the pure sample.

RESULTS AND DISCUSSION

The present study carried out to develop a simple, rapid, accurate and precise RP-HPLC method for the analysis of cefixime and ornidazole in pharmaceutical dosage forms. The retention times for cefixime and ornidazole were 4.75 and 8.98 min, respectively.

The ratio of the peak area of the cefixime and ornidazole to peak area of internal standard for different concentrations set up as above were calculated and the average values of five such determinations given in below Table-1. By using peak area ratios LOD, LOQ of ornidazole and cefixime were calculated.

TABLE-1
CONCENTRATION *vs.* MEAN PEAK AREA RATIO OF ORNIDAZOLE AND CEFIXIME

Drug	Drug conc. (µg/mL)	Mean peak area ratio (n = 5)*	Coefficient of variance (%)
Ornidazole	150	4.450	0.356
	200	6.031	0.216
	250	7.452	0.153
	300	8.903	0.570
	350	10.530	0.240
Cefixime	60	0.220	0.580
	80	0.294	0.380
	100	0.358	0.300
	120	0.429	0.550
	140	0.511	0.220

*Mean of 5 values.

A good linear relationship (r = 0.9998) was observed between the ornidazole and respective peak area ratio. The calibration equation was found to be Y = 0.0299X - 0.02679 (where Y is the ratio of peak area of drug to that of internal standard, X = concentration of ornidazole.

A good linear relationship (r = 0.9990) was observed between the cefixime and respective peak area ratio. The calibration equation was found to be Y = 0.0036X - 0.01512 (where Y is the ratio of peak area of drug to that of internal standard, X = concentration of cefixime.

The intra-day and inter-day variations of the method were determined using five replicate injections of three different concentration which were prepared and analyzed on the same day and three different days over a period of 2 weeks, a low coefficient of variation was observed in Table-2.

TABLE-2
PRECISION OF METHOD

Drug	Conc. (μg/mL)	Observed concentration (n = 5)			
		Intra-day	CV (%)	Inter-day	CV (%)
Ornidazole	150	150.06	0.195	150.08	0.197
	200	199.98	0.005	200.02	0.007
	250	250.09	0.196	250.06	0.197
Cefixime	60	60.02	0.016	60.04	0.015
	80	80.99	0.012	80.97	0.010
	90	90.06	0.011	90.08	0.013

To ensure the reliability and accuracy of the method recovery studies were carried out by mixing a known quantity of drug with pre-analyzed sample and contents were reanalyzed by the proposed method. The values were given in Table-3.

About 99.9 % of cefixime and ornidazole could be recovered from the pre-analyzed samples indicating the high accuracy of the proposed HPLC method.

TABLE-3
RECOVERY STUDIES

Drug	Amount added (mg)	Amount present (mg)	Mean amount found (n = 5)*	Mean recovery (%)
Ornidazole	50	150	149.79	99.57
	100	200	198.82	99.08
	150	250	249.82	99.68
Cefixime	10	60	59.90	98.40
	30	80	79.90	99.40
	50	100	99.90	99.80

*Mean of 5 values.

It can be concluded that the proposed HPLC method is simple, sensitive, rapid and reproducible for the analysis of cefixime and ornidazole in pharmaceutical dosage forms.

TABLE-4
ESTIMATION OF AMOUNT PRESENT IN TABLET DOSAGE FORM

Brand name	Label claim (mg)	Amount estimated (mg)	Mean (±SD) mean (mg) found by the proposed method (n = 5)*	Mean (±SD) % labelled amount (n = 5)*
Ornidazole	50	50.29	50.09 ± 0.0125	100.58 ± 0.04
Cefixime	125	132.91	132.62 ± 0.0004	106.28 ± 0.02

*Mean of 5 values.

TABLE-5
SYSTEM SUITABILITY PARAMETERS

Parameters	Cefixime	Ornidazole
Resolution factor	10.575	10.575
Theoretical plates	4011.10	5160.98
Linearity range (μg/mL)	60-140	150-350
Limit of detection (LOD) (μg/mL)	0.1019	0.2377
Limit of quantitation (LOD) (μg/mL)	0.3397	0.7925
Relative standard deviation (RSD)	0.2891	0.2848

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