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Simultaneous Estimation and Validation of Ornidazole and Cefixime by HPTLC in Pure and Pharmaceutical Dosage Forms

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A simple, rapid, sensitive high performance thin layer chromatographic method has been developed and validated for simultaneous estimation of ornidazole and cefixime in pure and pharmaceutical dosage form. It was performed on TLC plate precoated with silica gel $60F_{254}$ as a stationary phase using mobile phase composing of methanol and water in the ratio of 60:40 v/v and the detection was carried out in absorbance/reflectance mode at 254 nm showing R_f value 0.95 for ornidazole and 1.15 for cefixime. The percentage estimation of labeled claims of ornidazole and cefixime from marketed tablet was found to be 99.06, 99.48 by height and 99.39, 99.51 by area, respectively. The method was validated in terms of accuracy, precision, specificity and ruggedness. Linearity was observed between 250 and 2500 µg/mL for ornidazole and 100 and 900 µg/mL for cefixime. The recoveries of drugs by standard addition method were found in the range of 98 and 98.4 for both the drugs. The proposed method is precise, accurate and can be used for routine analysis of ornidazole and cefixime tablet.

Key Words: HPTLC, Ornidazole, Cefixime, Validation.

INTRODUCTION

Cefixime (CFX) is a semi-synthetic cephalosporin antibiotic for oral administration, chemically it is (6R and R)-7-[2-(2-amino-4-thiazolyl) glyoxyl]-amido]-8-oxo-3-vinyl-5-thia-l-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid,7^2-(2)-[0-(carboxymethyl)oxime]trihydrate. Its empirical formula[1] is $C_{16}H_{13}N_5O_7S_2Na_2$ and molecular weight is 507.50 as tri hydrate. CFX 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 beta-lactamases may be susceptible to CFX.

Ornidazole (ORN) is an antihelmentic drug for oral administration, chemically it is 1-(3-chloro-2-hydroxypropyl}-2-methyl-5-nitroimidazole and is used as an antiinfective agent. Its empirical formula[1] is $C_7H_{10}N_3O_3Cl$ and molecular weight is 219.63. ORN 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 is partially plasma bound and also has radiation and sensitizing action.

The literature survey[2-10] indicates that ORN and CFX have been determined individually and with combination of other drugs by using UV-spectrophotometry, High performance liquid chromatography and high performance thin layer chromatography in pharmaceutical and biological fluids preparations. No method has been reported for estimation of ORN and CFX simultaneously. In the present investigation an attempt was made to develop a simple and economical validated HPTLC with greater precision, accuracy and sensitivity for the simultaneous estimation of ORN and CFX in pure and tablet dosage form.

EXPERIMENTAL

All chemicals and reagents used were of AR/HPLC grade. Silica gel $60F_{254}$ precoated aluminum plates with thickness of 200 µm, E-Merck, Germany were used as a stationary phase. The instrument used was CAMAG-HPTLC system comprising of CAMAG LINOMAT-IV automatic sample applicator, CAMAG TLC SCANNER III with CATS V 4.01 software, CAMAG-UV cabinet and CAMAG twin trough glass chamber with stainless steel lids. The source of radiation was deuterium lamp emitting a continuous UV spectrum between 190 and 400 nm.

Preparation of standard solution: An accurately weighed quantity of 625 mg of ORN (Ws) and 250 mg of CFX (Ws) was dissolved in methanol make up to 50 mL to obtain a stock solution of 12500 µg/mL of ORN and 5000 µg/ml of CFX.

Mixed standard solution: Solution containing 250 µg/mL of ORN and 100 µg/mL of CFX was prepared and mixed to get mixed standard solution.

Chromatographic conditions: Optimized standard chromatographic conditions required where, stationary phase comprising of TLC aluminum foiled plates precoated with silica gel $60F_{254}$ with thickness of 200 µm methanol and water in the ratio of 60:40 v/v solution was used as a mobile phase and the chamber was saturated for 10 min sample was applied at a constant rate of 10 µL/s having scan speed 10 mm/s with 16 mm band distance the samples were separated by ascending technique. The chamber was maintained at 20 ± 5ºC temperature and 50-60 % relative humidity.

The scanning was carried out by absorbance/reflectance mode with slit dimension 4 × 0.5 mm the detection was carried out at 254 nm. The detection wavelength was selected from overlain spectra of both the drugs in methanol.

Calibration curve: ORN and CFX solutions ranging from 250 to 2500 µg/mL for ORN and 100 to 900 µg/mL for CFX were applied on TLC plate by micro litre syringe with the help of automatic sample applicator. The plates were developed, dried and densiometrically scanned at 254 nm. Peak height and area were recorded for each concentration and curves (concentration/peak height/area) were constructed.

System suitability test: The system suitability test was performed by repeated application of 10 µL of mixed standard solution and development. From the densitograms the mean, standard deviation and coefficient of variance of peak area and peak height were calculated.

Standard laboratory mixtures: Different laboratory mixtures were prepared in the same manner as that of standard solution to get the final concentration as that of standard solution. On TLC aluminum foiled plates, 10 µL of mixed standard solution (duplicate) and laboratory mixture (quadruplet) were applied on TLC plates in the form of 14 mm band. The plates were then developed in presaturated twin trough chamber with mobile phase. After development the plates were dried with the help of hot air dryer and evaluated densitometrically at a wavelength of 254 nm.

Assay procedure: 20 Tablets (Ornicef-DT) labeled to contain 125 mg of ORN and 50 mg of CFX were weighed, powdered an accurately weighed quantity of powder equivalent to 125 and 50 mg (650 mg powder drug) of ORN and CFX was transferred to 100 mL volumetric flask. The contents were dissolved in methanol and volume made up to the mark. The contents were mixed well using ultrasonicator and filtered through Whatmann filter paper no. 42. This was used as a sample solution. After preparation of the sample the same procedure was followed as under laboratory mixture.

The contents of the drugs in average weight of tablet were calculated as follows:

$$\text{Labelled claim (\%)} = \frac{W_E}{W_A} \times 100$$

where W_E = weight of drug estimated (µg), W_A = weight of drug applied (µg) on the basis of labeled claim.

Validation of proposed method: The proposed method was validated for the following parameters:

Accuracy: The accuracy of the proposed method was ascertained by carrying out recovery studies by standard addition method. Accurately known amounts of standard drugs were added to known amount of pre

analyzed tablet powder and it was analyzed by the proposed method to ascertain whether positive or negative interferences from excipients are present in the formulation. The per cent recovery was calculated by using the following formula.

$$\text{Recovery (\%)} = \frac{(A - B)}{C} \times 100$$

where A = total drug estimated in mg; B = amount of drug contributed by tablet powder (as per proposed method); C = amount of pure drug added.

Precision: Replicate estimations of drugs in sample were carried out by the proposed method and SD/RSD value was calculated as a measure of precision.

Ruggedness: Ruggedness was tested under different conditions, *i.e.*, analyzing the samples on different days and by different analysts.

RESULTS AND DISCUSSION

Various pure solvents of varying polarity, *viz.*, ethyl acetate, chloroform, toluene, diethyl ether and their mixtures in different proportions were tried as a mobile phase for development of chromatogram. The mobile phase found to be more suitable was methanol and water 60:40 v/v, it gave the good resolution of two components reasonably good with R_f values of 0.95 for ORN and 1.15 for CFX, respectively. The wavelength was selected as 254 nm for densiometric evaluation of chromatogram as both drugs have sufficient and high absorbance and showing better sensitivity. The per cent estimations of drugs in the laboratory mixture with the ± SD were found to be 99.06 ± 0.3615, 99.39 ± 0.574 and 99.48 ± 0.521, 99.51 ± 0.470 by peak height and peak area for both the drugs and per cent drug estimation in marketed formulation shows 98.78 ± 0.1303, 99.26 ± 0.2073, 98.16 ± 0.2073, 98.2 ± 0.1581 by peak height and peak areas for both drugs, respectively. The results emphasize upon accuracy and precision of the methods. The values were shown in Table-1.

The concentration response plots of drugs show linearity over the concentration range of 250-2500 µg/mL for ORN and 100-900 µg/mL for CFX with coefficient of correlation values 0.9956, 0.9847 and 0.9906, 0.9943 by peak height and area for both the drugs, respectively. The values are shown in Table-2.

The accuracy of the method was evaluated by per cent recovery by standard addition method for both the drugs by peak height and peak area. The results of the methods lying in prescribed limit of 98-102 % show the method is free from influence of excipients. The replicate estimation of both drugs in the same batch of the tablet analyzed by the proposed methods yielded quite concurrent result indicating the reliability of the method.

The values of SD and RSD and coefficient of correlation are within the prescribed limit of 2 % showing high precision of the method. The values are shown in Table-3. The last parameter studied was the ruggedness, which shows that the result of estimation for the proposed methods was reproducible under different conditions like different days and by different analysts. The values are given in Table-4.

TABLE-1
ESTIMATION OF ORN AND CFX

Sample	Statistics	Estimation of labeled claim* (%)			
		ORN		CFX	
		By height	By area	By height	By area
Standard laboratory mixture	Mean	99.060	99.390	99.480	99.510
	± SD	0.3615	0.574	0.521	0.470
	CV	0.3649	0.578	0.524	0.473
Marketed preparation	Mean	98.7800	99.2600	98.1600	98.200
	± SD	0.1303	0.2073	0.2073	0.158
	CV	0.1319	0.2089	0.2112	0.161

*Mean of five values.

TABLE-2
LINEARITY STUDIES

Drug	Linearity range (μg/mL)	Coefficient of correlation		Slope		Y-intercept	
		By height	By area	By height	By area	By height	By area
ORN	250-2500	0.9902	0.9906	14.99	15.12	1499.30	-1505.0
CFX	100-900	0.9919	0.9943	60.01	59.32	602.33	-591.8

TABLE-3
RECOVERY STUDIES

Sample	Statistics	Recovery* (%)			
		ORN		CFX	
		By height	By area	By height	By area
Standard laboratory mixture	Mean	100.2400	98.4600	99.6800	98.1600
	± SD	0.3646	0.1673	0.1303	0.2073
	CV	0.3638	0.1699	0.1308	0.2112

*Mean of five values.

TABLE-4
RUGGEDNESS STUDY OF ORN AND CFX

Sample	Statistics	Labeled claim* (%)			
		ORN		CFX	
		By height	By area	By height	By area
Different days	Mean	98.9700	99.9800	99.6300	98.760
	± SD	0.8692	0.4919	0.3535	0.114
	CV	0.8783	0.4920	0.3548	0.115
Different analysts	Mean	99.1600	99.2700	99.19	99.5800
	± SD	0.2073	0.1581	0.2686	0.3962
	CV	0.2091	0.1592	0.2708	0.3979

*Mean of five values.

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