Validated High Performance Thin Layer Chromatography Method for Simultaneous Estimation of Ofloxacin and Ornidazole in Tablet Dosage Form

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A simple, accurate and precise high performance thin layer chromatographic method has been developed for the estimation of ofloxacin and ornidazole simultaneously in tablet dosage form. The method employed silica gel 60GF254 precoated plates as stationary phase and a mixture of n-butanol: ethanol: ammonia (5:5:4 %v/v/v) as mobile phase. The plate was scanned and quantified at 295 nm using Camag TLC scanner. The method was validated for linearity, accuracy, precision, repeatability and specificity, proving its utility in estimation of ofloxacin and ornidazole in combined dosage form.

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Ofloxacin[1,2] is a broad spectrum, fluorinated quinolone antibacterial drug, and ornidazole[1,2] is a 5-nitro imidazole derivative used as antiprotozoal/antibiotic and antibacterial. A combination of ofloxacin and ornidazole is available in the market, which is highly active against many bacterial infections of enteritis and anaerobic bacteria.

Ofloxacin is official in BP[3], USP[4] and EP[5]. The assay procedure mentioned in these pharmacopoeias uses non-aqueous titration. There are many reported HPLC[6–8], UV[9,10] spectrophotometry and spectroflourimetry[11] methods for the estimation of these drugs from pharmaceutical preparations or biological fluids. Also the analytical methods are available for stability[12] studies of these drugs. Also HPLC[13,14] methods are available for estimation of this combination. There is no reported HPTLC method for the estimation of these two drugs in combination. The present study describes the development and validation of simple, sensitive, accurate, precise and economical HPTLC method for simultaneous determination of ofloxacin and ornidazole in tablet dosage form.

The pure drugs of ofloxacin and ornidazole were obtained as gift samples from Glenmark Pharmaceuticals, Hyderabad. Silica gel 60GF_{254} TLC precoated plates (20 × 10 cm), layer thickness 0.2 mm, E-Merck, were used as the stationary phase. Tablets of ORNID (Ofloxacin - 0.20 g, Ornidazole - 0.50 g) were purchased from local pharmacy. n-Butanol, ethanol, ammonia of AR grade purity were procured from S. D. Fine Chemicals, India. Linomat V sample applicator, twin-trough developing chamber and TLC Scanner 3 (Camag, Muttenz, Switzerland) with WinCATS evaluation, ATS software (version 1.2.6) were used in the studies.

A mixture of standard solution containing 0.01 g of ofloxacin and 0.025 g of ornidazole was prepared in methanol. Further dilutions were made to obtain final concentration of 100 µg/ml and 250 µg/ml of ofloxacin and ornidazole respectively. Twenty tablets of Ornid were crushed and ground to a fine powder. A weight equivalent to 10 mg of ofloxacin was taken and extracted with methanol. The extract was filtered through Whatman filter paper No.1, and the residue was washed with sufficient amount of methanol. The filtrate was further diluted to obtain a sample solution containing 100 µg/ml ofloxacin and 250 µg/ml of ornidazole.

TLC plates were pre-washed with methanol and dried in hot air. Aliquots of 1 µl to 5 µl of standard solutions of ofloxacin and ornidazole were applied as band on the TLC plate. The TLC plate was dried, developed and analyzed. Sample solution measuring 1 µl-5 µl was spotted on to the TLC plate, followed by development and scanning. The analysis was repeated in triplicate. The solvent system offered very good resolution of both the drugs with R_f values of 0.59 ± 0.02 and 0.86 ± 0.01 for ofloxacin and ornidazole respectively (fig. 1). The migration distance was 0.82 cm, and densitometric scanning was done at 295 nm, keeping slit dimension of 0.5 × 0.045 m. A deuterium lamp provided the source of radiation. The content of ofloxacin and ornidazole in tablet formulation calculated as per peak area was found to be 102.27 ± 0.15 and 103.04 ± 0.21% respectively (Table 1).

The method was validated as per ICH guidelines in terms of linearity, accuracy, inter-day and intra-day precision, reproducibility of sample application and specificity. The limit of quantification and limit of detection for both the drugs were also determined. Accuracy of the analysis was evaluated by carrying out recovery studies by adding known concentration (50%) of standard drug to a pre-analyzed tablet sample and percentage recovery was calculated. This study was repeated six times. The good average recovery values obtained indicate that the proposed method is accurate for estimation.

The intra-day precision was determined by analyzing

TABLE 1: ASSAY OF OFLOXACIN AND ORNIDAZOLE

Label claim	Amount found ± SD (mg)*	% Assay ±SD*	% CV
Ofloxacin (200 mg)	204.54 ± 0.92	102.27 ± 0.15	0.13
Ornidazole (500 mg)	515.20 ± 0.17	103.04 ± 0.21	0.59

Average value ± SD of six determinations. Ornid contains 0.20 g of ofloxacin and 0.50 g of ornidazole

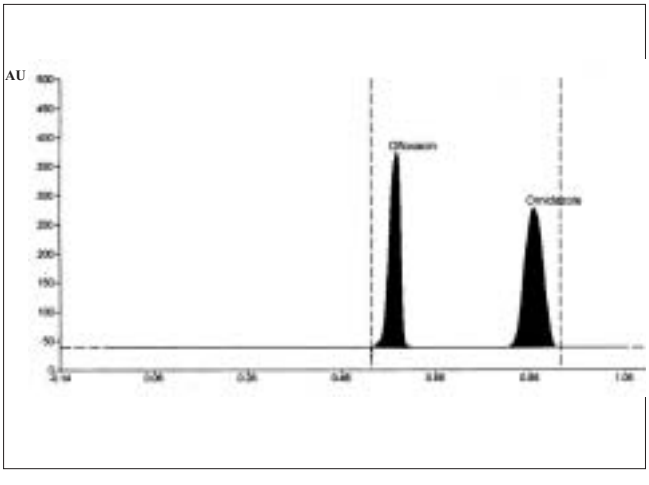

Fig. 1: Typical chromatogram of ofloxacin and ornidazole Ofloxacin – 0.5 µg/spot, ornidazole – 1.25 µg/spot. For chromatographic conditions, see text. R_f value of ofloxacin is 0.59 and that of ornidazole is 0.86

TABLE 2: METHOD VALIDATION PARAMETERS

Parameters	Result	
	Ofloxacin	Ornidazole
Linearity range (ng/spot)	1-5	2.5-12.5
Correlation coefficient (r)	0.9655	0.9927
Limit of detection (ng/spot)	0.1	0.2
Limit of quantification (ng/spot)	1	2
Accuracy (% CV)	1.2	1.62
Precision		
Repeatability of application % RSD (n=6)	0.16	0.30
Repeatability of measurement % RSD (n=6)	0.16	0.25
Intra-day (n=6)	0.2 to 1.9%	0.5 to 1.5%
Inter-day (n=6)	0.3 to 1.3%	0.2 to 1.4%

CV is coefficient of variation. RSD is relative standard deviation

standard drug solutions containing 1 µg/spot of ofloxacin and 2.5 µg/spot of ornidazole on the same day, while inter-day precision was determined by analyzing corresponding standards daily for a period of 6 d. Repeatability of measurement of peak areas was determined by spotting 1 µl of standard drug solution on a TLC plate and developing the plate. The separated spot was scanned six times without changing the position of plates, and relative standard deviation (RSD) for measurement of peak area was calculated. Repeatability of sample application was assessed by spotting 3 µl of standard drug solution six times on a TLC plate by semiautomatic spotter. The intra-day and inter-day coefficients for two drugs were found to be in the range of 0.2-1.9%, 0.5-1.5% and 0.3-1.3%, 0.2-1.4% respectively.

Lower values of intra-day and inter-day variations in analysis indicate that the method is precise. The RSD for repeatability of measurement of peak area and RSD for repeatability of sample application were found well below the instrumental specifications, ensuring proper functioning of HPTLC system. It was observed that excipients present in formulation did not interfere with peaks of ofloxacin (0.59 ± 0.02) and ornidazole (0.86 ± 0.01). Different validation parameters for the proposed HPTLC method for the determination for ofloxacin and ornidazole have been summarized in Table 2. The proposed HPTLC method was found to be rapid, simple, specific, sensitive, precise and accurate. Thus it can be employed for the routine quality control of ofloxacin and ornidazole tablets.

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