



## INTERNATIONAL JOURNAL OF PHARMACY & LIFE SCIENCES

### RP-HPLC simultaneous estimation of metronidazole and diloxanide furoate in combination

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#### Abstract

A reverse phase high performance liquid chromatography method was developed for the simultaneous estimation of diloxanide furoate and metronidazole in formulation. The separation was achieved by octadecyl C8 column and a mixture of methanol: acetonitrile: 0.05M phosphate buffer at pH 4.0 (45:25:30 v/v) as eluent, at a flow rate of 1 ml/min. detection was carried out at 277 nm. Quantitation was done by external standard method. The retention time of metronidazole and diloxanide furoate was found to be 3.28 and 6.42 min, respectively. The method has validated for linearity, accuracy and precision. Linearity of metronidazole and diloxanide furoate were in the range of 5-50 µg/ml for both the drugs. The mean recoveries obtained for metronidazole and diloxanide furoate were 100.01% and 99.71%, respectively. The developed method was found to be accurate, precise, selective and rapid for the simultaneous estimation of metronidazole and diloxanide furoate in tablet.

**Key- words:** RP-HPLC, metronidazole, diloxanide furoate, simultaneous estimation

#### Introduction

Metronidazole (MET), chemically 2-(2-methyl-5-nitro-1-H-Imidazole-1-yl)-ethanol<sup>2</sup> is antiprotozoal, antibacterial and antiameobic agent<sup>1</sup>. It is official in I.P., B.P., and non-aqueous titration is an official method for its analysis. Diloxanide furoate (DLX), 4-(N-methyl-2, 2-dichloroacetamido) phenyl -2-furoate<sup>2</sup> is a amoebicidal drug<sup>1</sup>. It is official in IP in which non-aqueous titration is an official method others reports are available in the literature for determination of DLX from commercial dosage form and biological sample including HPLC<sup>4,5,6</sup>, UV etc. several methods are reported determination of MET individually or in combination with other drugs.

A fixed dose combination containing MET and DLX is available in the market as tablet dosage form<sup>7,8,9,10,11</sup>, however there is no method for simultaneous estimation of these drugs in combination in specific condition, hence attempt has been made to develop simple, sensitive, accurate, precise analytical method to estimate metronidazole and diloxanide furoate in tablets using RP-HPLC C8 column. The present communications describe simple liquid.

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chromatographic methods for simultaneous estimation of these drugs from their combined formulation<sup>12, 13, 14</sup>.

### Material and methods

Reference standard of MET and DLX was obtained from J.B.Chemicals and pharma Ltd., Gujarat, India. All the reagent and chemicals were either of AR grade or spectroscopy grade. All the solution were freshly prepared with double distilled water. An isocratic high pressure liquid chromatograph (shimadzu HPLC class VP series) with intelligent pump, variable wavelength programme UV 2401 with detector loop injector (Rheodyne 20  $\mu$ l) was used. The chromatography column used was a reverse phase phenomenax C8 column (250mm x 4.6mm i.e, particle size 10 $\mu$ m). A mixture of methanol, acetonitrile and 0.05M phosphate buffer (adjusted to pH 4.0 using ortho phosphoric acid) in the ratio of 45:25:30 v/v was used as mobile phase and was filter before use through 0.45 $\mu$ m membrane filter. The flow rate of mobile phase was maintained at 1 ml/min detection was carried out at 277 nm at the temperature of 20°

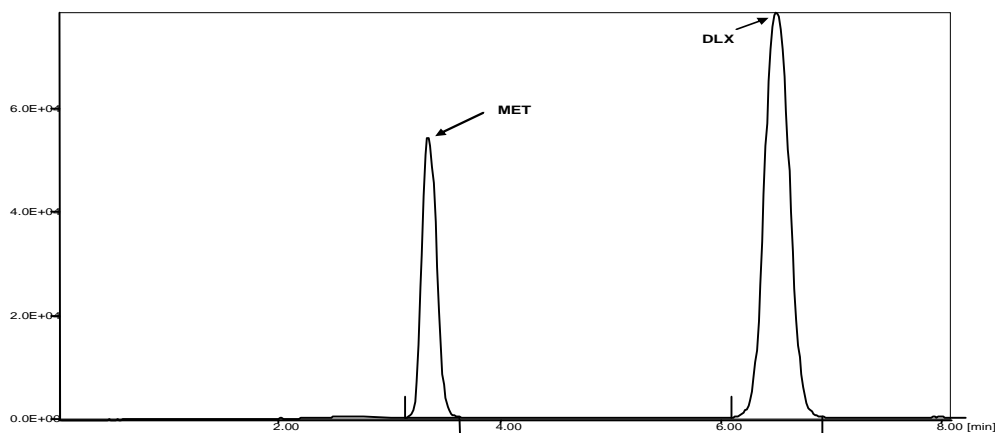
Standard stock solutions of metronidazole and diloxanide furoate (100 $\mu$ g/ml) were prepared in mobile phase. The standard solutions were further diluted in mobile phase containing a mixture of 20  $\mu$ g/ml of metronidazole and 25  $\mu$ g/ml diloxanide furoate. Twenty tablets of Dyrade-M each containing 200 mg of metronidazole and 250 mg of diloxanide furoate were weighed and finely powdered. A quantity of powder equivalent to 20 mg of metronidazole was weighed and transferred in to a 100 ml volumetric flask. The drugs were extracted with the mobile phase. The extracts were made up to the volume (100 ml) with mobile phase and further dilutions were made to get a concentration of 20  $\mu$ g/ml of metronidazole and 25  $\mu$ g/ml of diloxanide furoate. The content were mixed thoroughly and filtered through 0.45 $\mu$ m membrane filter. An aliquot of 20  $\mu$ l of both standard and test solution were injected separately and chromatograms were recorded up to 10 min.

### Result and discussion

The mobile phase was optimized with methanol, acetonitrile and 0.05M phosphate buffer (pH 4.0) in the proportion 45:25:30 v/v with above mentioned composition of mobile phase, sharp peaks with good resolution between metronidazole and diloxanide furoate was achieved with reasonable short run time of 8 min the criteria employed for assessing the suitability of above said solvent system were cost, time required for analysis, solvent noise, preparative steps involved in the use of same solvent system for the extraction of the drugs from the formulation excipient matrix for the estimation of drug content. UV detection was carried out at 277 nm as metronidazole and diloxanide furoate showed good absorbance at this wavelength.

The retention time of metronidazole and diloxanide furoate was found to be 3.28 and 6.42 min, respectively. A typical chromatogram of test solution is shown in fig. 1. The capacity factors (k) of metronidazole and diloxanide furoate were found to be 1.32 and 3.54, respectively. The peak shapes of both the drugs were symmetrically and asymmetrically factor was less than 2. The response factor of both the standard and the test solution was calculated. The proposed method was validated as per the standard analytical procedure. Each of the samples was injected five times and the retention time was observed in all the cases. Precision of the proposed method was found to be 0.353% for metronidazole and 0.108% for diloxanide furoate. The low %  $\pm$ SD values indicated that the proposed method has good precision. Linearity experiments were performed tens for both the component and the response was found to be in range of 5-50  $\mu$ g/ml for both drugs. Linearity of metronidazole and diloxanide furoate was plotted by a graph of peak area versus concentration. The correlation coefficient r values (n=10) for both metronidazole and diloxanide furoate were 0.9995 and 0.9998 respectively. Accuracy of the method was calculated by recovery studies at three levels. Standard drugs solution containing drugs in the range 80%, 100% and 120% of nominal concentration (20  $\mu$ g/ml of metronidazole and 25  $\mu$ g/ml diloxanide furoate) was added to previously analysed test solution. Amount of drug recovered at each level was calculated. Table 1 shows the data from recovery study for metronidazole and diloxanide furoate were 99.68% and 99.56% respectively. The sample recovery in the formulation was in good agreement with the label claim. High percentage recovery showed that the method was free from interference of the excipients used in the formulations. System suitability parameters of metronidazole and diloxanide furoate are given in table 2.

Assay of the combination in tablet dosage form was found to be 100.45% of metronidazole and 99.09% of diloxanide furoate. The method was simple and had short run time of 8 min, which makes the method rapid. The result of the study indicates that the proposed HPLC method is simple, precise, accurate and less time consuming.



**Fig.1: Typical chromatogram of the sample solution**  
 Chromatogram showing sample solution of metronidazole and diloxanide furoate at the approximate concentration of 20 µg/ml of metronidazole and 25 µg/ml of diloxanide furoate.

**Table No. 1: Recovery Studies**

Drug	Amount added (µg/ml) (n=3)	Amount recovered (µg/ml) (n=3)	Recovery (%)	Average recovery ±SD (%)
Metronidazole	16.00	15.881	99.26	99.68±0.383
	20.00	20.002	100.01	
Diloxanide furoate	24.00	23.884	99.77	
	20.00	19.886	99.43	
	25.00	24.927	99.71	
	30.00	29.862	99.54	

**Table No. 2: System Suitability Parameter**

Parameter	MET	DLX
Peak area	221193.52	551499.66
No. of theoretical plates	4318.972	4125.116
Retention time (min)	3.28	6.42
Asymmetry	0.0242	0.0245
Capacity factor	1.3272	3.5492
Selectivity	2.6696	
Resolution	10.43	

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