Kalpana MJ. et al. / Vol 4 / Issue 7 / 2014 / 415-420.

International Journal of Pharmaceutical Research & Analysis

e-ISSN: 2249 – 7781
Print ISSN: 2249 – 779X

www.ijpra.com

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF SIMULTANEOUS ESTIMATION OF METRONIDAZOLE AND DILOXANIDE FUROATE IN TABLET DOSAGE FORM BY RP-HPLC

Kalpana MJ*, Naga sowjanya G, Ajitha A, Uma Maheshwara Rao V

Department of Pharmaceutical Analysis and Quality Assurance,
CMR College of Pharmacy, Kandlakoya (V), Medchal Road, Hyderabad - 501 401, A.P, India.

ABSTRACT

A simple, precise, rapid, specific and accurate reverse phase high performance liquid chromatography method was developed for simultaneous estimation of Metronidazole and diloxanide furoate in pharmaceutical dosage form. Chromatographic separation was performed on Inertsil ODS 3V, 4.6x250mm, 5µm column, with mobile phase comprising of mixture of buffer (pH 5.5, adjusted with phosphate buffer), acetonitrile and methanol in the ratio of 30:20:50 v/v, at the flow rate 1 ml/min. The detection was carried out at 241 nm. The retention times of Metronidazole and diloxanide furoate were found to be 2.2 and 3.7 mins respectively with a run time of 6 mins, theoretical levels for Metronidazole and diloxanide furoate were 3351 and 5094 respectively, with a resolution of 8.422. As per ICH guidelines the method was validated for linearity, accuracy, precision, limit of detection and limit of quantitation, robustness and ruggedness. Linearity of Metronidazole was found in range of 60-140 µg/mL and that for Diloxanide furoate was found to be 75-175 µg/mL. The correlation coefficient for Metronidazole and diloxanide furoate was 0.99 and 0.99 respectively. The LOD values for Metronidazole and diloxanide furoate were 6.89 and 3.92 µg/mL respectively. The LOQ values for Metronidazole and diloxanide furoate were and 20.87 and 11.88 µg/mL respectively. This demonstrates that the developed method is simple, precise, rapid, selective, accurate and reproducible for simultaneous estimation of Metronidazole and diloxanide furoate tablet dosage form.

Keywords: Metronidazole, Diloxanide furoate, RP-HPLC, Validation.

INTRODUCTION

Metronidazole Fig. (A1) is described chemically as 2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethanoland it is slightly soluble in alcohol with themolecular formula $C_6H_9N_3O_3$and a molecular weight of 171.15g/mol. Diloxanide furoate Fig. (A2) is described chemically as 4-(2,2-dichloro-N-methylacetamido) phenyl furan-2-carboxylate and it is slightly soluble in water, slightly soluble in ether and ethanol with molecular formula $C_{14}H_{11}Cl_2NO_4$ and a molecular weight of 328.147g/mol [1-4].

MATERIALSANDMETHODS

Equipment

Chromatographic separation was performed on HPLC system–Water HPLC grade, UV Detector, equipped with a solvent delivery pump, sample injector and column thermostats. Empower system software was applied for data collecting and processing.

Chemicals and reagents

Acetonitrile (HPLC grade) was used. Buffer used was pH-5.5 (pH adjusted with potassium dihydrogen phosphate). (Reference standards Metronidazole and Diloxanide furoate were obtained from Chandra Labs.

Preparation of standard solutions

100 mg of each metronidazole and 125 mg diloxanide furoate working standard were taken into 100ml

Corresponding Author:-M.J.Kalpana Email:- nutzkalpana5@gmail.com

VF and 70ml diluent added and was sonicated. Make final volume with same diluent. From the above stock pipette out 1ml. Add this 1ml stock into 10ml volumetric flask and make up with diluent.

Preparation of sample solution

Accurately weighed and transferred tablet powder equivalent to 100 mg of Metronidazole and 125mg of diloxanide furoate into a volumetric flask and added about 70mL of diluent. It was sonicated to dissolve completely and made volume up to the mark with the same diluent. Make the final volume with same diluent. From the above Stock pipette out 1ml. Now add this 1ml in to a 10ml volumetric flask and make up with diluent.

Preparation of buffer

Take 1.6250gm of KH_2PO_4 and 0.3000gm of K_2HPO_4, and dissolve in 550ml of HPLC grade water. Then make up with 1000 ml and filtered through 0.45µm nylon membrane filter and degassed.

Optimized chromatographic conditions

Mobile phase: Phosphate Buffer (p^H 5.5):ACN: Methanol(30:20:50v/v); Flow rate:1mL/min
Column: Inertsil ODS 3V, 4.6x250mm, 5µm
Detector wavelength: 241nm
Injection volume: 20µL

METHOD VALIDATION [14]

Linearity

Solutions were prepared under the concentrations of Metronidazole, which corresponding to 60, 80, 100, 120 and 140% respectively, concentrations of Diloxanide furoate which corresponding to 75, 100, 125, 150 and 175% respectively of the test solution concentration. Each solution was injected, linearity was evaluated by linear-regression analysis [1].

Accuracy

Accuracy was determined by the recovery studies at three different concentrations (corresponding to 80, 100 and 120% of the test solution concentration & 100,125 and 150%) of metronidazole and diloxanide furoate by addition of known amounts of standard to pre-analysed sample preparation. For each concentration, three sets were prepared and injected [2].

Precision

Precision of an analytical method is usually expressed as the standard deviation or relative standard deviation (coefficient of variation) of a series of measurements [3].

Robustness

The robustness was evaluated by assaying test solutions after slight but deliberate changes in the analytical conditions. The factors chosen for study were the flow rate (±0.1ml/min), temperature and wavelength [4].

Limit of detection (LOD) and Limit of quantification (LOQ)

LOD and LOQ was calculated from linear curve using formulae

$$LOD=3.3*\sigma/slope,\ LOQ=10*\sigma/slope$$

(Where σ= the standard deviation of the response and S= Slope of calibration curve) [7].

Specificity

Specificity was checked for the interference of impurities in the analysis of blank solution and injecting sample solution under optimized chromatographic conditions to demonstrate separation of both Metronidazole and Diloxanide furoate from impurities [8].

RESULT AND DISCUSSION

Several mobile phase compositions were tried to resolve the peak of Metronidazole and Diloxanide furoate. The mobile phase containing buffer 5.5 (Potassium dihydrogen Ortho phosphate, Dipotassium hydrogen Ortho phosphate): Acetonitrile:Methanol in proportion of 30:20:50v/v was found ideal to resolve the peak of Metronidazole and Diloxanide furoate. Retention time of Metronidazole and Diloxanide furoate were 2.2 and 3.7 min respectively (Figure 2&3). Result of assay is shown in Table 1. The proposed method was found to be linear in concentration range 60-140µg/ml for Metronidazole and 75-175µg/ml for Diloxanide furoate. The data was shown in Table 2 and Figure 4 & 5. System suitability parameters were evaluated and results shown inTable 3, which were within acceptance criteria. The mean percentage recovery for Metronidazole and Diloxanide furoate was found to be 101.8% and 101.5% respectively, which are well within the limit and hence the method was found to be accurate (Table 4). LOD and LOQ values were 6.89µg/mL and 20.87µg/mL for Metronidazole and 3.92µg/mL and 11.88µg/mL for Diloxanide furoate (Table5). Results of precision were shown in the Table 6. The robustness of the method was investigated by varying experimental conditions such as changes in flow rate and temperature. The result obtained implies method is robust for routine qualitative analysis (Table 7).

Table 1. Analysis data of formulation

Injection	Label claim(mg)	Assay (%)
Metronidazole	100mg	100.32%
Diloxanide furoate	125mg	100.55%

Table 2. Result of Linearity

S. No	Metronidazole		Diloxanide furoate	
	Conc. (µg/ml)	Peak area	Conc. (µg/ml)	Peak area
1	60	1571.372	75	4338.795
2	80	1839.991	100	4948.486
3	100	2091.759	125	5782.167
4	120	2486.795	150	6858.847
5	140	2763.59	175	7541.702

Table 3. System suitability studies

Parameters	Metronidazole	Diloxanide furoate	Acceptance criteria
Theoretical plates	3351	5094	Not less than 2000
Tailing factor	1.4	1.2	Not more than 2
Resolution	-	8.422	Not less than 2

Table 4.Recovery studies for Metronidazole and Diloxanide furoate

Drug	Spiked level%	Amount taken (µg/ml)	Amount found (µg/ml)	Percent recovery n=3	Mean recovery
Metronidazole	80	85	86.56	101.8	101.7
	100	105	106.1	101.4	
	120	125	127.5	102.0	
Diloxanide furoate	100	106.25	108.38	102.0	101.5
	125	131.25	132.64	101.08	
	150	156.25	158.7	101.5	

n- Number of replicate injections

Table 5. LOD and LOQ for Metronidazole and Diloxanide furoate

Drug	LOD	LOQ
Metronidazole	6.89	20.87
Diloxanide furoate	3.92	11.88

Table 6. Results of Precision

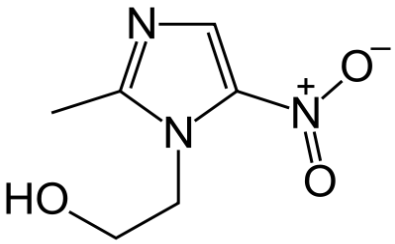
S.No	Sample Weight	MTZ RT	MTZ Area	DF RT	DF Area
1	335.4	2.213	2051.034	3.74	5698.074
2	335.4	2.19	2003.811	3.71	5639.718
3	335.4	2.21	2062.991	3.73	5692.9
4	335.4	2.2	2052.703	3.727	5709.739
5	335.4	2.21	2039.124	3.74	5765.096
6	335.4	2.203	2058.026	3.733	5740.309
Avarage Assay:		2.2043	2044.61	3.730	5707.639
STD		0.0085	21.537	0.011	43.116
%RSD		0.39	1.05	0.30	0.76

Table 7. Results of Robustness study

S. No	Parameter	Condition	Theoretical levels		Tailing factor		Retention time	
			Metroni dazole	Diloxanide furoate	Metroni dazole	Diloxanide furoate	Metronida zole	Diloxanide furoate
1	Flow rate	0.8min/ml	3494	5673	1.4	1.3	2.9	4.9
		1.2min/ml	2986	4607	1.3	1.1	1.7	2.9
2	NM	240nm	3144	5295	1.36	1.2	2.2	3.7
		244nm	3087	5286	1.4	1.2	2.2	3.7

Fig 1. The chemical structure of Metronidazole (A1) And Diloxanide furoate (A2)

(A1) **(A2)**



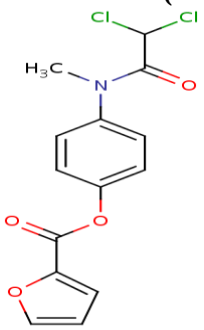


Fig 2. Chromatogram of Metronidazole and Diloxanide furoate sample

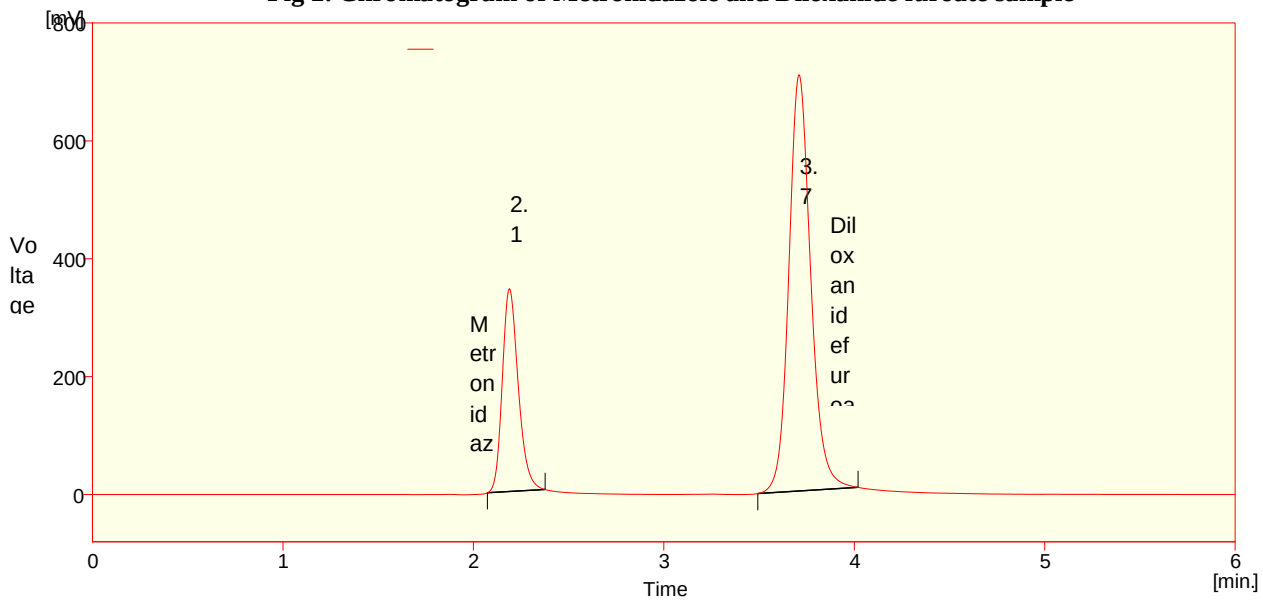


Fig 3.Chromatogram of Metronidazole and Diloxanide furoate standard

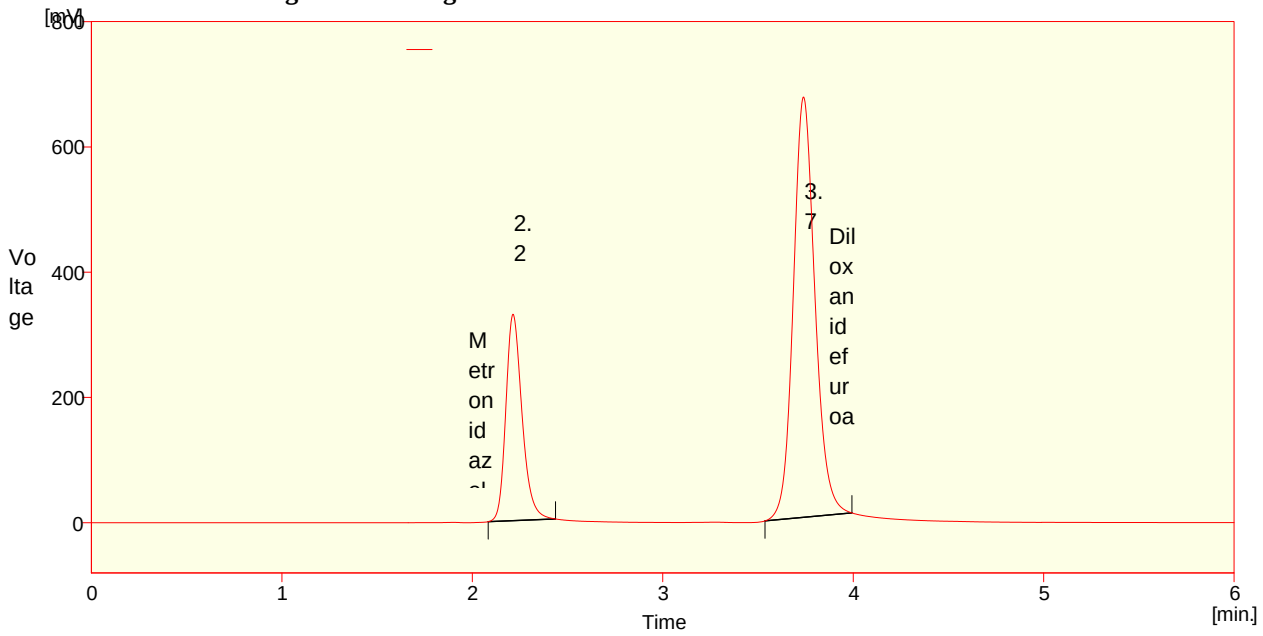


Fig 4. Calibration curve for Metronidazole

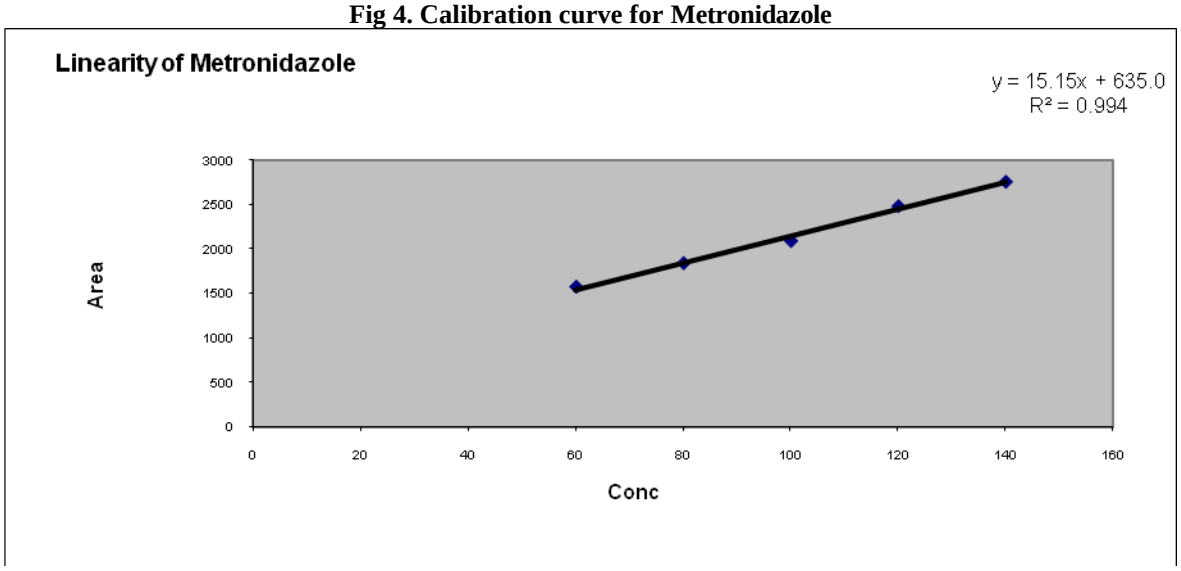
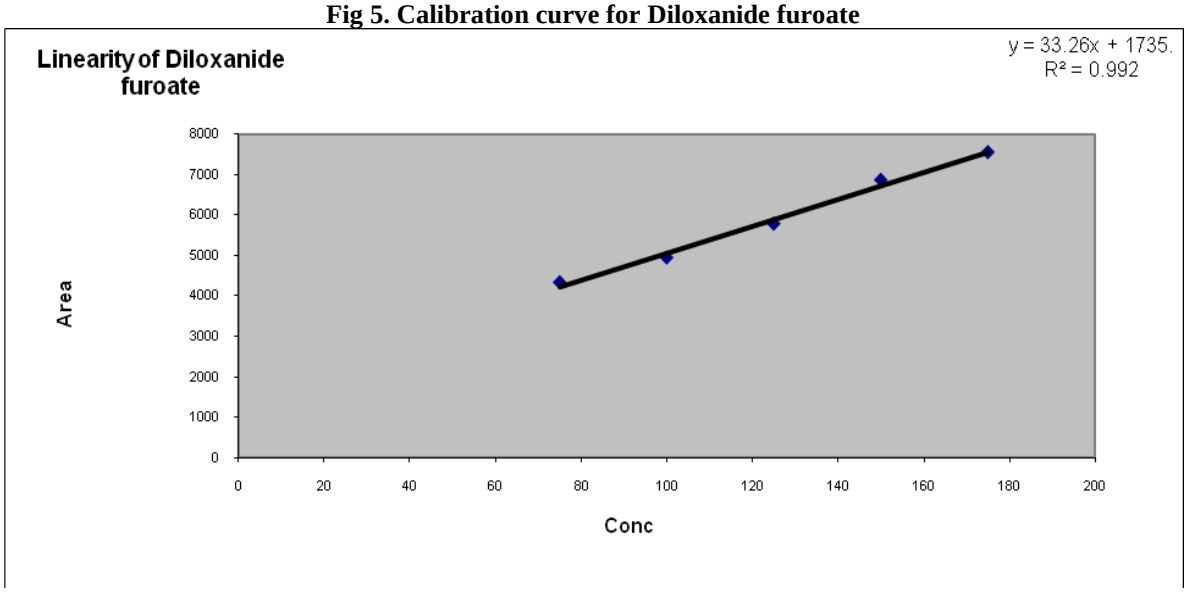


Fig 5. Calibration curve for Diloxanide furoate



CONCLUSION

The proposed RP-HPLC method was validated as per International Conference on Harmonization (ICH) guidelines, and found to be applicable for routine quality control analysis for the simultaneous estimation of Metronidazole and diloxanide furoate using isocratic mode of elution. The results of linearity, precision, accuracy and specificity, proved to be within the limits. The proposed method is highly sensitive, reproducible, reliable, rapid and specific.

ACKNOWLEDGEMENT

The authors are thankful to Chandra Labs, Hyderabad.

REFERENCES

1. Abbas SS, Wagieh NE, Abdelkawy M, Abdelrahman MM. Simultaneous determination of diloxanide furoate and metronidazole in presence of diloxanide furoate degradation products. *Journal of AOAC International*, 94(5), 2011, 1427-1439.
2. Saeed-ul-Hassan, Shafique HMTA. Quantitation of Metronidazole in Pharmaceutical Suspension Using High Performance Liquid Chromatographic Method. *Pakistan Journal of Zoology*, 43(5), 2011, 909-914.
3. Danao KR, Hiradeve SM, Moon RS, Kasture AV, Yeole PG. RP-HPLC simultaneous estimation of metronidazole anddiloxanide furoate in combination. *International Journal of Pharmacy and Life Sciences*, 1(2), 2010, 82-85.

4. Mohamed R. El-Ghobashy, Nisreen F. Abo-Talib. Spectrophotometric methods for the simultaneous determination of binary mixture of metronidazole and diloxanide furoate without prior separation. *Journal of Advanced Research, Cairo University*, 1, 2010, 323-329.
5. Nora H. Al-Shaalan. Determination of Diloxanide furoate and Metronidazole in Binary MixtureUsing First Derivative of the Ratio-Spectra and High-Performance Liquid Chromatography-UV Methods. *American Journal of Applied Sciences*, 4(2), 2007, 66-72.
6. Mishal A, Sober D, Stability indicating reversed-phase liquid chromatographic determination of metronidazole benzoate and diloxanide furoate as bulk drug and in suspension dosage form. *Journal of pharmaceutical and biomedical analysis*, 39(3-4), 2005, 819-823.
7. Qureshi H, Ali A, Baqai R, Ahmed W. Efficacy of a combined diloxanide furoate-metronidazole preparation in the treatment of amoebiasis and giardiasis. *Journal of international medical research*, 25(3), 1997, 167-170.
8. Hasan NY, Elkawy MA, Elzeany BE, Wagieh NE. Stability indicating methods for the determination of diloxanide furoate. *Journal of Pharmaceutical and Biomedical Analysis*, 28(2), 2002, 187-190.