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DEVELOPMENT AND VALIDATION OF SIMULTANEOUS EQUATION METHOD FOR ESTIMATION OF METRONIDAZOLE BENZOATE AND RELATED IMPURITY IN BULK AND PHARMACEUTICAL FORMULATION

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ABSTRACT

A simple, accurate and precise spectroscopic method was developed for simultaneous estimation of Metronidazole Benzoate and related impurity in bulk and pharmaceutical formulation by simultaneous equation method. The Metronidazole Benzoate shows max absorbance at 310.09 nm, 2-Methyl-5Nitroimidazole show max absorbance at 300 and Benzoic Acid shows max. Absorbance at 225.81 nm. The method was found to be linear (r2>0.999) in the range of 2.0-10 µg/ml. The limit of determination was 0.079 µg/ml and 0.055 µg/ml and0.043 µg/ml for Metronidazole Benzoate, 2-Methyl-5-Nitroimidazole and Benzoic Acid, respectively. The limit of quantification was 0.263 µg/ml and 0.184 µg/ml and 0.132 µg/ml for Metronidazole Benzoate,2-Methyl-5-Nitroimidazole and Benzoic Acid, respectively. The accuracy of this method was evaluated by recovery studies and good recovery result was obtained greater than 99%. The method was successfully applied for simultaneous determination of Metronidazole Benzoate, 2-Methyl-5-Nitroimidazole and Benzoic Acid.

KEYWORDS: Metronidazole Benzoate, 2-Methyl-5-Nitroimidazole, Benzoic Acid, Simultaneous equation method.

INTRODUCTION[1,2]

Metronidazole is an anti-protozoan and antibacterial agent belonging to the class of Nitroimidazoles. Metronidazole is active against a wide range of pathogenic anaerobic Gram-negative micro-organisms notably species of Bacteroides fragilis and spp., Fusobacterium spp, Gardnerella vaginalis, and anaerobic Gram positives such as Peptococcus spp., Peptostreptococcus spp., Clostridium spp. It is also active against protozoaes such as Trichomonas vaginalis, Entamoeba histolytica, Giardia lamblia. Metronidazole was first approved by sanofi-aventis for marketing in France and its International Birth Date (IBD) is 29 July 1959. Since that time it has been registered and arketed worldwide. In Europe, (sanofi-aventis) Metronidazole is currently approved by national procedures and marketed in 19 countries in various formulations, such as tablets, oral suspensions, suppositories, intravenous solution, vaginal pessaries/tablets. Metronidazole is an antimicrobial agent that has been used in clinical medicine for >45 years. Metronidazole has been shown to be carcinogenic in mice and rats. Unnecessary use of the drug should be avoided.

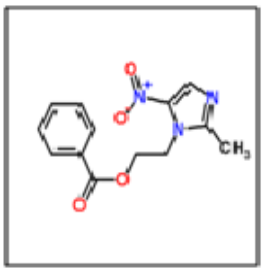

FIG.1 CHEMICAL STRUCTURE OF METRONIDAZOLE BENZOATE

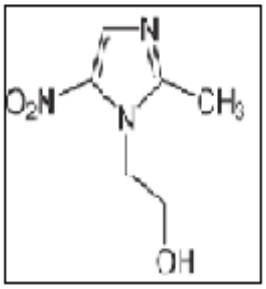

FIG.2 CHEMICAL STRUCTURE OF METRONIDAZOLE

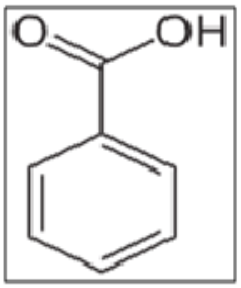

FIG.3 CHEMICAL STRUCTURE OF BENZOIC ACID

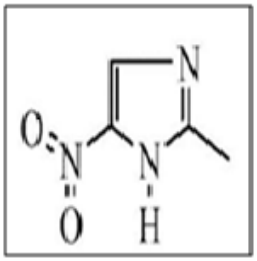

FIG.4 CHEMICAL STRUCTURE OF 2-METHYL-5-NITRO IMIDAZOLE

MATERIAL AND METHOD

A double beam UV/Visible spectrophotometer (Shimadzu model 2450, Japan) with spectral width of 2 nm, 1 cm quartz cells was used to measure absorbance of all the solutions.Spectra were automatically obtained by UV-Probe system software.An analytical balance (Sartorius CD2250, Gottingen, Germany) was used for weighing the samples. Sonicator (D120/2H, TRANS-O-SONIC). All instruments and glass wares were calibrated. Metronidazole Benzoate and 2-Methyl-5-Nitoimidazole raw material was received as gift sample from Dano Pharmacham Pvt.Ltd. Ankleswar. Metronidazole raw material was material was received as gift sample from Mc coy Drug Pvt. Ltd. Sachin.

Benzoic acid AR Grade. Methanol AR Grade, Distilled water, 0.1 N HCl, 0.1N NaOH were used for development purpose.

PREPARATION OF STANDARD SOLUTIONS

Standard solution of Metronidazole Benzoate (MTZ)

Accurately weighed quantity of Metronidazole Benzoate 10 mg was transferred to 100ml volumetric flask, dissolved and diluted up to mark with Methanol to give a stock solution having strength 100µg/ml.

Standard solution of 2-Methyl-5-Nitroimidazole (MNI)

Accurately weighed quantity of 2-Methyl-5-Nitroimidazole 10 mg was transferred into 100 ml volumetric flask, dissolved and diluted up to mark with Methanol to give a stock solution having strength 100µg/ml.

Standard solution of Benzoic Acid (BA)

Accurately weighed quantity of Benzoic acid 10 mg was transferred to 100ml volumetric flask, dissolved and diluted up to mark with Methanol to give a stock solution having strength 100µg/ml.

Preparation of standard mixture

Pipette out accurately 1.0 ml of Metronidazole Benzoate stock solution (100µg/ml), 1.0 ml of 2-Methyl-5-Nitroimidazole stock solution (100µg/ml) and 1.0 ml of Benzoic acid stock solution (100µg/ml) in 10 ml volumetric flask and make up the volume up to the mark with Methanol. It gives solution containing Metronidazole Benzoate 10µg/ml, 2-Methyl-5-Nitroimidazole 10µg/ml and Benzoic acid 10µg/ml.

RESULT AND DISCUSSION

The standard solution of Metronidazole Benzoate (10µg/ml), 2-Methyl-5-Nitroimidazole (10µg/ml) and Benzoic acid (10µg/ml) were scanned separately between 200-400nm, and zero-order spectra show overlapping peaks.

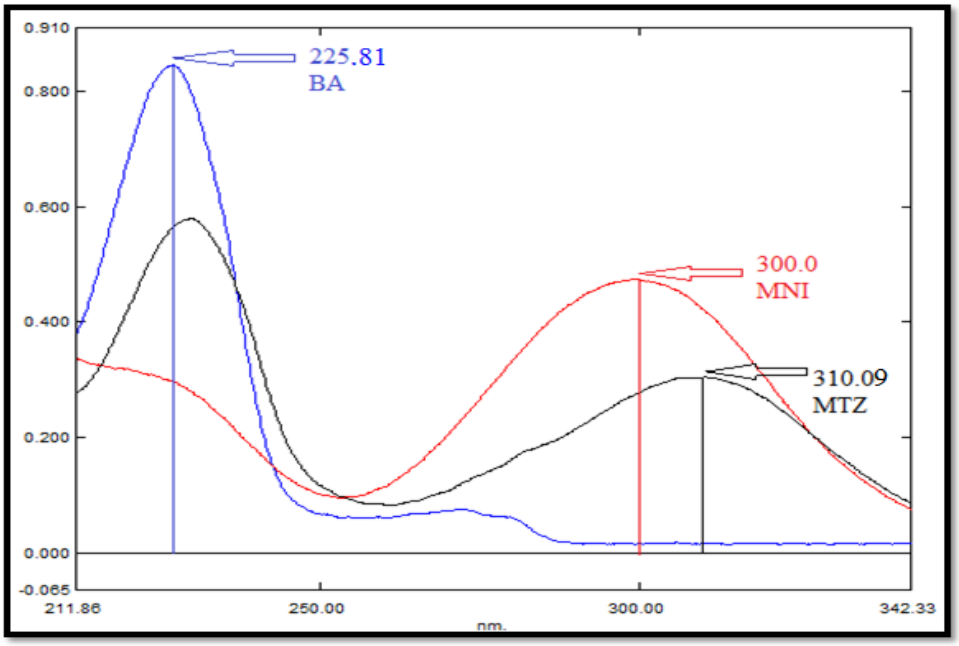

Fig.4: Overlain zero order Spectra of MTZ, MNI And BA (1:1:1) Ratios, Respectively.

MTZ shows peak at 310.09 nm and 230.13 nm MNI shows at 300.0 nm and BA shows peaks at 225.81 nm. 310.09 nm is selected for Metronidazole Benzoate as difference between 225.81 and 230.13 is less than 10.

Validation Parameters[3,4,5]

1. Linearity and Range

The zero order spectra showed linear absorbance at 310.09 nm for MTZ (2.0-10µg/ml), 300.0 nm for MNI (2.0-10µg/ml) and 225.81 nm for BA (2.0-10µg/ml).This method obeyed beer's law in the concentration range 2.0-10µg/ml for MTZ, MNI and BA. Correlation coefficient (r^2) for calibration curve of MTZ, MNI and BA were found to be 0.9998, 0.9997 and 0.9998, respectively The regression line equation for MTZ, MNI and BA are as following,

y= 0.028x + 0.019 for MTZ

y = 0.040x + 0.066 for MNI

y = 0.071x + 0.113 for BA

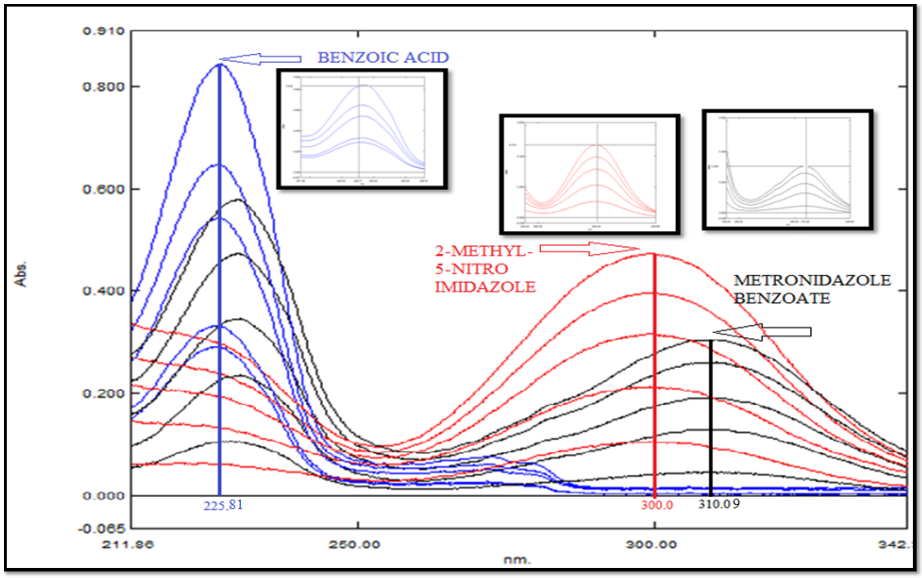

Fig.5: Overlain linear zero order spectra of BA (BLUE), MNI (RED) and MTZ (BLACK) IN 1:1:1 ratio.

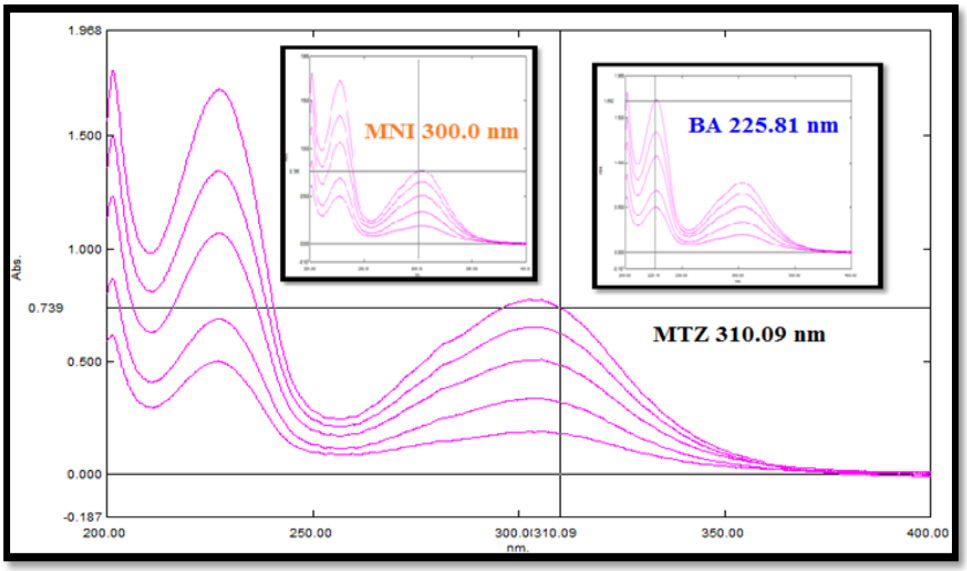

Fig.6: Overlain linear zero order spectra of mixture of MTZ, MNI AND BA in ratio of 1:1:1

Table.1: Calibration data for mixture of MTZ, MNI and BA at 310.09 nm, 300.0 nm AND 225.81 nm, Respectively *(n=6).

Sr. No	Concentration(µg/ml)			Absorbance ± SD*		
	MTZ	MNI	BA	(310.09 nm)	(300.0nm)	(225.81 nm)
1	2.0	2.0	2.0	0.181±0.00057	0.188 ±0.00113	0.501 ±0.00115
2	4.0	4.0	4.0	0.321±0.00115	0.331±0.00115	0.684±0.00059
3	6.0	6.0	6.0	0.485±0.00054	0.503±0.00058	1.061±0.00116
4	8.0	8.0	8.0	0.622± 0.00116	0.646±0.00063	1.341±0.00213
5	10.0	10.0	10.0	0.739± 0.00109	0.766±0.00163	1.690 ±0.00503

Table.2: Calibration Data For MTZ, MNI AND BA AT 310.09 nm, 300.0 nm and 225.81 nm, RESPECTIVELY. *(n=6).

Sr. No	Concentration (µg/ml)			Absorbance ± SD		
	MTZ	MNI	BA	MTZ (310.09 nm)	MNI (300.0 nm)	BA (225.81 nm)
1	02	02	02	0.078±0.00086	0.151±0.00052	0.261±0.00086
2	04	04	04	0.129 ±0.00083	0.221±0.00081	0.392±0.00056
3	06	06	06	0.193 ±0.00075	0.315 ±0.00053	0.541±0.00086
4	08	08	08	0.248 ±0.00051	0.395 ±0.00089	0.673 ±0.00089
5	10	10	10	0.304 ±0.00104	0.473±0.00093	0.831 ±0.00083

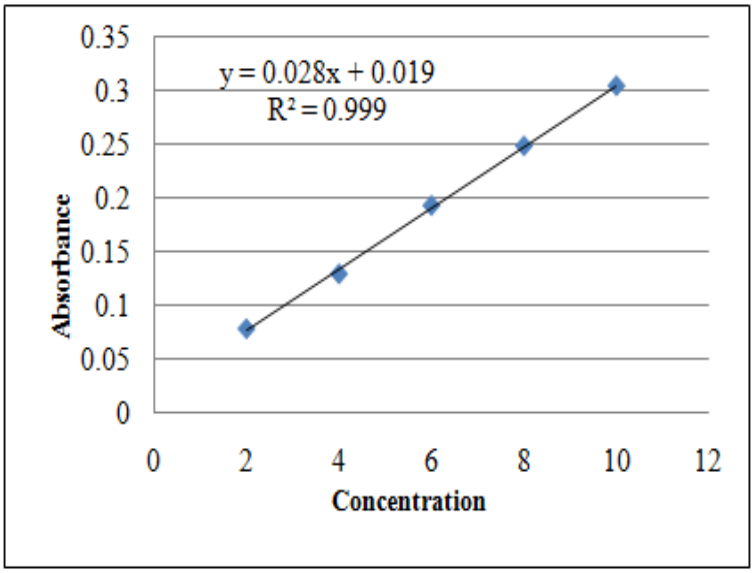

Fig.7: Calibration Curve for MTZ AT 310.04 nm

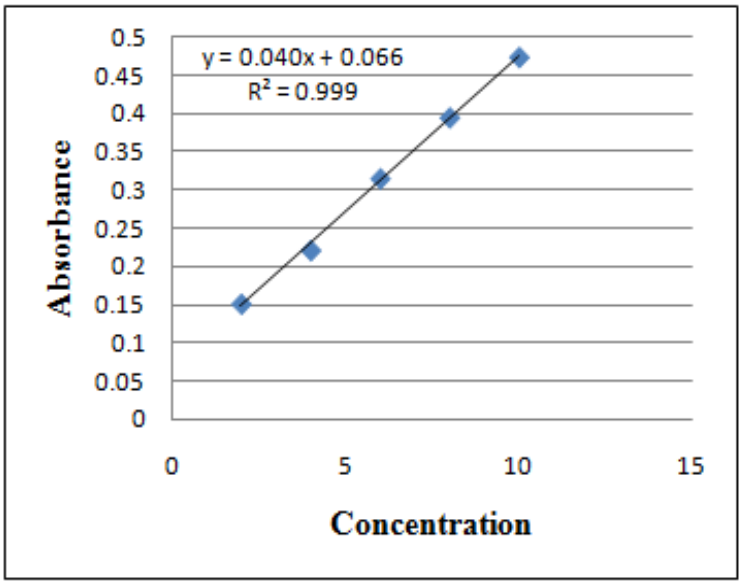

FIG.8: Calibration curve for MNI at 300.0nm.

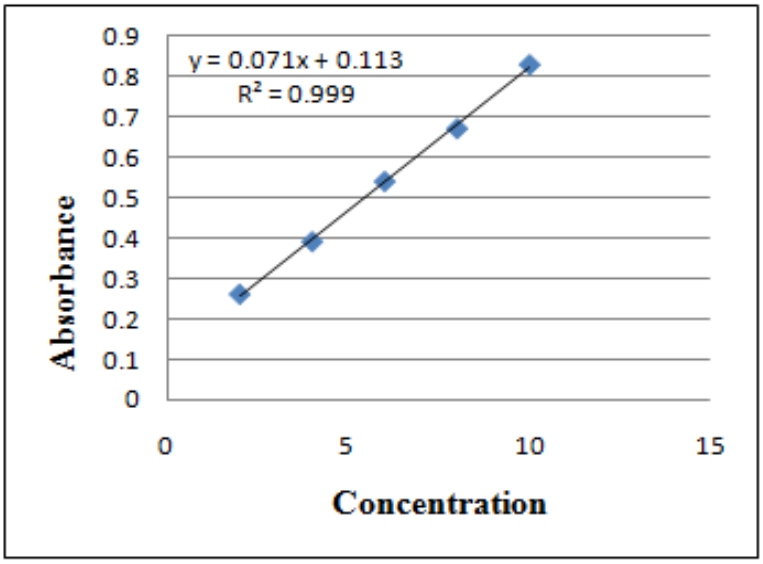

FIG.9: Calibration Curve for BA AT 225.36 nm.

Equations

$A_1^* = A_1 - 0.155$

$A_2^* = A_2 - 0.085$

$A_3^* = A_3 - 0.046$

$$C_x = \frac{0.000162922\ A1^* + 0.000866232\ A2^* + 0.000954954\ A3^*}{0.00000742}$$

$$C_y = \frac{-0.000002A1^* + 0.002742A2^* - 0.001794\ A3^*}{0.00000742}$$

$$C_z = \frac{-0.000001\ A1^* - 4.215\ A2^* + 0.002813\ A3^*}{0.00000742}$$

Where A_1 = Absorbance of test solution at 225.81 nm

A_2 = Absorbance of test solution at 300.0 nm

A_3 = Absorbance of test solution at 310.09 nm

Cx, Cy and Cz are the concentration of Metronidazole Benzoate, 2-Methyl-5-Nitroimidazole and Benzoic Acid, respectively

2. Precision

I. Intraday precision

The data for intraday precision for combined standard solution of MTZ, MNI and BA is presented in Table .The % R.S.D was found to be 0.11 -0.31 % for MTZ, 0.17-0.45 % for MNI and 0.15-0.25 % for BA.These %RSD values were found to be less than ± 2.0 indicated that the method is precise.

Table. 3: Intraday precision data for estimation of MTZ, MNI and BA*(n=3).

Conc. (µg/ml)			MTZ(310.09 nm)	MNI(300.0 nm)	BA(225.81 nm)
MTZ	MNI	BA	Avg.± RSD*	Avg.±RSD*	Avg. ± RSD*
2.0	2.0	2.0	0.181 ±0.31	0.188 ±0.45	0.501 ±0.19
4.0	4.0	4.0	0.321 ±0.35	0.331 ±0.34	0.684 ±0.25
6.0	6.0	6.0	0.485 ±0.11	0.503 ±0.17	1.061 ±0.15

II. Interday precision

The data for interday precision for combined standard solution of MTZ, MNI and BA is presented in Table. The % R.S.D was found to be 0.13-0.54 % for MTZ, 0.19-0.30 % for MNI and 0.18-0.23% for BA. These %RSD values were found to be less than ± 2.0 indicated that the method is precise.

Table.4: Interday precision data for estimation of MTZ, MNI and BA*(n=3).

Conc. (µg/ml)			MTZ(310.09 nm)	MNI(300.0 nm)	BA(225.81 nm)
MTZ	MNI	BA	Avg.± RSD*	Avg.±RSD*	Avg. ± RSD*
2.0	2.0	2.0	0.182 ±0.54	0.188 ±0.30	0.502 ±0.23
4.0	4.0	4.0	0.322 ±0.35	0.332 ±0.53	0.684 ±0.29
6.0	6.0	6.0	0.486 ±0.13	0.503 ±0.19	1.062 ±0.18

3. Accuracy

Accuracy of the method was determined by recovery study from dosage form at three levels (80%, 100%, and 120%) by spiking method. The % recovery values are tabulated in Table. Percentage recovery for MTZ, MNI and BA by this method was found in the range of 100.02 to 100.09 %, 100.05 to 100.76 % and 100.01 to 100.95 %, respectively. The value of %RSD within the limit indicated that the method is accurate and percentage recovery shows that there is no interference from the excipients.

TABLE.5 Recovery data of MTZ *(n=3)

Conc. of MTZ from formulation (µg/ml)	Amount of Std.MTZ added (µg/ml)	Total amount of MTZ (µg/ml)	Total amount of MTZ found (µg/ml) Mean ± SD*	% Recovery (n=3)	% RSD MTZ
8.0	6.4	14.4	14.42 ± 0.0038	100.02	0.26
8.0	8.0	16	16.09 ± 0.0133	100.09	0.82
8.0	9.6	17.6	17.61 ± 0.0322	100.08	0.18

Table.6: Recovery data of MNI*(n=3).

Conc. of MNI from formulation (µg/ml)	Amount of Std.MNI added (µg/ml)	Total amount of MNI (µg/ml)	Total amount of MNI found (µg/ml) Mean ± SD*	% Recovery (n=3)	% RSD MNI
0.0	0.64	0.64	0.644 ± 0.0041	100.76	0.64
0.0	0.8	0.8	0.807±0.0043	100.94	0.51
0.0	0.96	0.96	0.960±0.0082	100.05	0.24

Table.7: Recovery data of BA*(n=3).

Conc. of BA from formulation (µg/ml)	Amount of Std. BA added (µg/ml)	Total amount of BA (µg/ml)	Total amount of BA found (µg/ml) Mean ± SD*	% Recovery (n=3)	% RSD BA
0.0	0.64	0.64	0.646±0.0038	100.95	0.81
0.0	0.8	0.8	0.802±0.0075	100.32	0.93
0.0	0.96	0.96	0.961±0.0011	100.01	0.12

4. Limit of Detection and Quantification

The LOD for MTZ, MNI and BA was conformed to be 0.079µg/ml, 0.055µg/ml and 0.043 µg/ml respectively. The LOQ for MTZ, MNI and BA was conformed to be 0.263 µg/ ml, 0.184µg/ml and 0.132 µg/ml respectively. The obtained LOD and LOQ results are presented in Table

$$LOD = 3.3 \times \frac{SD}{Slope}$$

$$LOQ = 10 \times \frac{SD}{Slope}$$

Table.8: LOD and LOQ data of MTZ, MNI and BA*(n=10).

Conc. (µg/ml)			MTZ(310.09 nm) (n=10)		MNI(300.0 nm) (n=10)		BA(225.81 nm) (n=10)	
MTZ	MNI	BA	Avg. ± SD*	% RSD	Avg.± SD*	% RSD	Avg. ± SD*	% RSD
2.0	2.0	2.0	0.182±0.00073	0.41	0.187±0.00082	0.43	0.502±0.00094	0.18
LOD(µg/ml)			0.079		0.055		0.043	
LOQ(µg/ml)			0.263		0.184		0.132	

5. Robustness and Ruggedness

The obtained Ruggedness and Robustness results are presented in table 6.2.10.

The % R.S.D was found to be 0.31 - 0.84 % for MTZ, 0.34 – 0.96 % for MNI and 0.25 - 0.83 % for BA. These %RSD value was found to be less than ± 2.0 indicated that the method is precise. No significant changes in the spectra were observed, proving that the

developed method is rugged and robust.For different stock solutions for MTZ,MNI and BA stock-1 is 100 µg/ml and stock-2 is 50 µg/ml.

Table.9: Robustness and Ruggedness data of MTZ, MNI and BA*(n=3).

Drugs	Concentration (ppm)	Mean ±% RSD(n=3)		Mean ±% RSD(n=3)	
		Stock Solution I	Stock Solution II	Instrument I	Instrument II
Metronidazole Benzoate	02	0.181±0.84	0.183±0.54	0.182±0.37	0.183±0.62
	04	0.322±0.47	0.324 ±0.61	0.324 ± 0.61	0.323±0.64
	06	0.488±0.31	0.486±0.33	0.487±0.35	0.489 ±0.41
2-Methyl-5-Nitroimidazole	02	0.104±0.55	0.104 ±0.96	0.105 ± 0.93	0.107±0.54
	04	0.213±0.71	0.214 ±0.71	0.215±0.70	0.216±0.96
	06	0.317±0.48	0.317 ±0.34	0.317±0.48	0.317±0.48
Benzoic Acid	02	0.501±0.34	0.503 ±0.51	0.504 ± 0.59	0.502± 0.83
	04	0.685±0.25	0.684±0.62	0.684±0.48	0.686± 0.53
	06	1.061±0.40	1.064 ±0.36	1.063± 0..34	1.065 ±0.46

Application of the proposed method for analysis of metronidazole benzoate oral suspension

Metronidazole Benzoate oral suspension contains **200mg/5 ml** and Pipette out accurately 1.0 ml of suspension in to 100 ml volumetric flask make up the volume with Methanol, sonicate for 15 min**.** Filter it with Nylon membrane filters (0.22 µm, 20 mm D) Pipette out 1.0 ml dillute solution into 100 ml volumetric flask make up the volume using Methanol Measure absorbance at 310.09 nm, 300.0 nm and 225.81 nm Determine the concentration of MTZ using equation.

Table.10: Analysis data of commercial formulation *(n=3).

DRUGS	% Assay ± % RSD(n=3)	IP LIMIT
Metronidazole Benzoate	100.84 ±0.47	98.0-101.0%

Table.11 Summary of Validation Parameter

Parameter	Metronidazole Benzoate	2-Methyl-5-Nitroimidazole	Benzoic Acid
λ_{max}	310.09 nm	300.0 nm	225.81 nm
Concentrationrange(µg/ml)	2.0 – 10	2.0 – 10	2.0– 10
Regression equation	y = 0.028x + 0.019	y = 0.040x + 0.066	y = 0.071x +0.113
Correlation coefficient (r^2)	0.999	0.999	0.999
Accuracy (%Recovery) (n=3)	100.24	100.58	100.44
Intra-day Precision (%RSD) (n=3)	0.11 – 0.31	0.17 – 0.45	0.15 – 0.25
Inter-day precision (%RSD) (n=3)	0.13 – 0.54	0.19 – 0.53	0.18 – 0.29
LOD (µg/ml)	0.079	0..055	0.043
LOQ (µg/ml)	0.263	0.184	0.132
Ruggedness and Robustness	0.31–0.84	0.34– 0.96	0.25– 0.83
% Assay	100.84	–	–

CONCLUSION

The developed UV-spectroscopy method was proved to be simple, rapid & reproducible. The validation data indicate good specificity, precision, accuracy & reliability of the method. The developed method offers several advantages in terms simultaneous determination of Metronidazole Benzoate and related impurity in bulk and pharmaceutical formulation.

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