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METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF NICOTINAMIDE AND METRONIDAZOLE IN GEL USING UV-SPECTROPHOTOMETER

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Keywords:

ICH, Ultraviolet, Sandell's sensitivity, Molar absorptivity, Calibration curve

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ABSTRACT: An easy, precise and accurate spectroscopic technique for the simultaneous estimation of nicotinamide and metronidazole in semi-solid dosage form and in bulk form has been developed. The present method involves the addition of 0.1M hydrochloric acid solution, followed by dilution with water and subjecting the resulting solution to UV spectroscopic assessment. Absorption maximum was found to lie at about 262 nm and 317 nm for nicotinamide and metronidazole respectively. The calibration curve showed linearity as per line equation y=0.0029x + 0.0001 with an R^2value of0.9937 for nicotinamide and y = 0.0072 x + 0.0063 with an R^2 value of 0.9933 for metronidazole. Validation was performed as per ICH guidelines for linearity, accuracy and precision. The assay result was in a good arrangement with the label claim. The mean Sandell's sensitivity values for metronidazole and nicotinamide, respectively, were 0.354 g/cm^2 and 0.134 g/cm^2, showing that the approach is very sensitive and practical for measuring both substances.

INTRODUCTION: Nicotinamide **Fig. 1A** chemically described as Pyridine-3-carboxamide, act as an anti-inflammatory agent [1]. It is very soluble in water and soluble in butanol and chloroform. Metronidazole **Fig. 1B** is chemically described as 2-(2-methyl-5-nitro-1H-imidazol-1-yl) ethan-1-ol. It is active against a wide range of anaerobic bacteria and parasites [2]. The literature review reveals that UV spectroscopy was used to quantify nicotinamide in pure form [1] and in combination [3]. Other methods such as RP- HPLC and HPTLC were also reported for the determination of nicotinamide in combination with other drugs [4, 5].

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Metronidazole in combination form was estimated by the QBD approach for UV-visible spectrophotometry [6], RP-HPLC [7] and pure form by UV-spectrophotometric method [2].

Even though a number of methods were reported for the estimation of these two drugs in pure form and in combination with other drugs, there is no report found for the determination of these two drugs in combination. Hence an attempt was made to analyse these two drugs in combination using a simple spectroscopic method which is more precise and reliable.

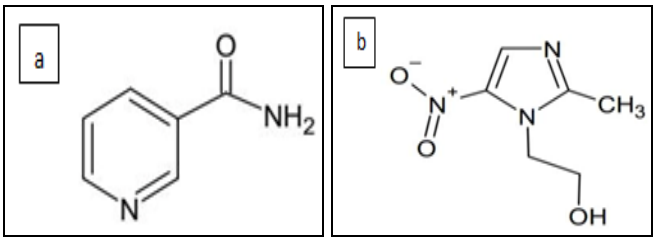

FIG. 1: CHEMICAL STRUCTURE OF A) NICOTINAMIDE, B) METRONIDAZOLE

EXPERIMENTAL: The reference standard metronidazole and nicotinamide were obtained from Yacco Pharma Enterprises, Puducherry. The sample ANERO GEL used in the study was purchased from the local pharmacy. Analytical-grade Hydrochloric acid and methanol were used for the dilutions.

Double beam UV spectrometer (Shimadzu, model-UV-1780), sonicator (I.L.E.co, model-100H), and analytical weighing balance (Sartorius, model-BSA224S-CW) were used to carry out the experiment.

Preparation of Stock Solution of Nicotinamide: Weighed 20 mg of nicotinamide; transferred into a 10 ml standard flask and diluted with 0.01 M hydrochloric acid. The concentration of nicotinamide was 2 mg per ml and named it as the primary stock solution I.

Preparation of Stock Solution of Metronidazole: Weighed 10 mg of metronidazole; transferred into a 10 ml standard flask and diluted volume with 0.01 M hydrochloric acid. The concentration of metronidazole was 1 mg per ml and named it as the primary stock solution II.

Wavelength Selection: The primary stock solution was diluted suitably and scanned in the region of 200-400 nm in UV **Fig. 2**. The wavelength maxima were observed at 262 nm for nicotinamide and 317 nm for metronidazole.

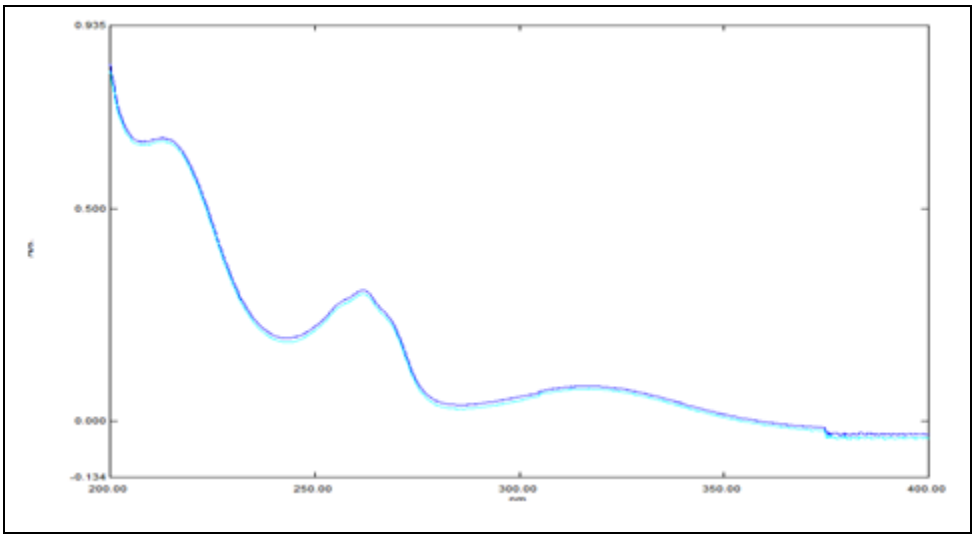

FIG. 2: UV SPECTRUM OF MIXED STANDARDS OF NICOTINAMIDE AND METRONIDAZOLE

Dilutions of Nicotinamide: From the primary stock solution, 1 ml was pipetted out and transferred into a 10 ml standard flask and made up the volume with methanol. The concentration of this solution is 200 µg/ml. From this pipetted out 4 ml and diluted with methanol in a 10 ml standard flask to get a concentration of 80 µg/ml. Serial dilutions were done to get the concentration of 40µg, 20µg, 10µg, and 5µg per ml.

Dilutions of Metronidazole: From the primary stock solution,1 ml was pipetted out and transferred into a 10 ml standard flask and made up the volume with methanol. The concentration of this solution is 100 µg/ml. From this pipetted out 1.5 ml and diluted with methanol in a 10 ml standard flask to get a concentration of 15 µg/ml. Serial dilutions were done to get the concentration of 7.5 µg, 3.75 µg, 1.87 µg and 0.9 µg/ml.

Assay: A quantity of gel equivalent to 500 mg was transferred into a 10 ml standard flask; 5 ml of methanol was added to dissolve and sonicated for five minutes. The volume was made up to the mark using methanol and filtered using Whitman filter paper.

This contains 200 µg/ml of nicotinamide and 35 µg/ml of metronidazole. From this 2 ml was transferred into a 10 ml standard flask and made up the volume with methanol. This solution contains 40 µg of nicotinamide and 7 µg of metronidazole. The absorbance of the sample solutions was measured.

Validation Parameters: The method was validated as per ICH guidelines [8-9]. The following parameters were assessed.

Precision: Precision was determined in terms of repeatability. It was determined by analysing 7 samples of the same concentration, 80 µg of nicotinamide and 15 µg of metronidazole.

Linearity: The standard solution of metronidazole and nicotinamide were prepared and the dilution range was made between 10-100 µg of nicotinamide and 0.9-15 µg metronidazole. By plotting the concentration on X-axis and absorbance on Y-axis the calibration curve was obtained **Fig. 3** & **4**. From the calibration curve, the slope and intercept were determined.

Robustness: The experiment was carried out at two different wavelengths and the capacity of the drugs to remain unaffected by small, deliberate variations in method parameters was observed.

Accuracy: The recovery was carried out in different amounts of 80%, 100%, and 120% The solutions were suitably diluted in the range (64 µg, 80 µg, 96 µg) of nicotinamide and (11.2 µg, 14 µg, 16.8 µg) of metronidazole the absorbance each of these dilutions was measured.

Sandell's Sensitivity: It was the recommended technique for determining the sensitivity and it was calculated by

Sandell's sensitivity = Concentration of the drug (µg/ml) × 0.001 / Absorbance

Molar Absorptivity: The molar absorptivity determines the concentration by plotting the calibration curve. According to Beer-Lambert law, the absorbance was proportional to the concentration; so the molar absorptivity was given as **A= €cl**

Where, A =Absorbance, € =Molar absorptivity, C =Molar concentration l =path length

RESULTS AND DISCUSSION:

Precision: The precision of an analytical method was investigated in terms of repeatability at a concentration of 80 µg/ml for nicotinamide and 15 µg/ml for metronidazole. The results of the precision testing were expressed as the Relative standard deviation % RSD. The results obtained are given in **Table 1.**

TABLE 1: PRECISION OF NICOTINAMIDE AND METRONIDAZOLE

Nicotinamide			Metronidazole		
Concentration (µg/ml)	**Absorbance**	**% RSD**	**Concentration (µg/ml)**	**Absorbance**	**% RSD**
80	0.247		15	0.102	
80	0.245		15	0.101	
80	0.244		15	0.100	
80	0.246	0.714 %	15	0.102	1.6 %
80	0.247		15	0.103	
80	0.249		15	0.095	
80	0.242		15	0.098	

Linearity: Nicotinamide was found to be linear between the concentration ranges of 10 to 100 µg/ml **Fig. 3** and Metronidazole **Fig. 4** concentration was found to be linear between 1.8 to 18.5 µg/ml. A Promising linear relationship (R^2=0.9937) and (R^2=0.9933) was observed between the concentration of nicotinamide and metronidazole and the corresponding absorbance**.**

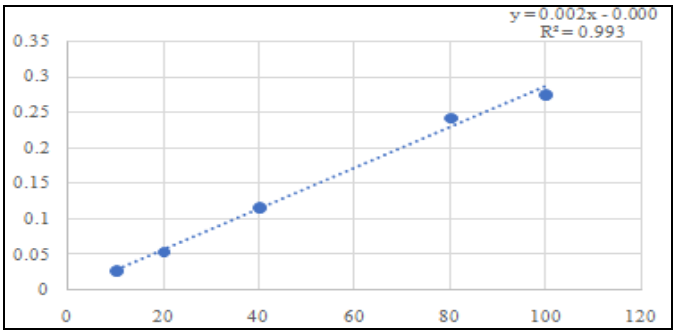

FIG. 3: LINEARITY OF NICOTINAMIDE

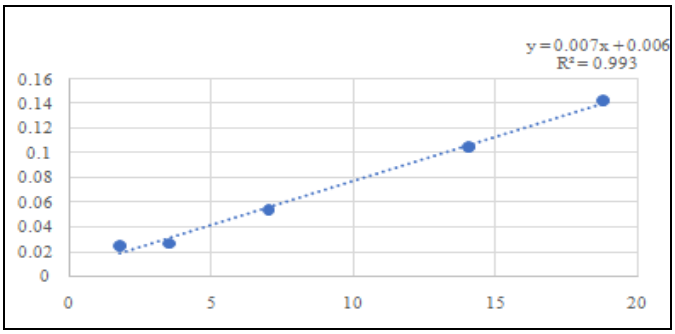

FIG. 4: LINEARITY OF METRONIDAZOLE

Assay: The amount of metronidazole and nicotinamide present in the formulation was calculated by comparing the absorbance of the sample with standard absorbance. The content of metronidazole and nicotinamide in gel formulation determined by the developed method **Table 2**, **Fig. 5** was found to be compatible with the label claim.

TABLE 2: ASSAY OF NICOTINAMIDE AND METRONIDAZOLE

Drug	Label claim	Concentration (µg/ml)	Absorbance	% purity	Average (%)	% RSD	Drug
			0.242	98.77			
			0.240	98.36			
Nicotinamide	4 %	80	0.241	98.74	99.51	1.12%	Nicotinamide
			0.243	99.59			
			0.245	100.40			
			0.247	101.22			
			0.098	98.98			
			0.099	100.00			
Metronidazole	0.75%	15	0.101	102.02	101.17	1.47%	Metronidazole
			0.100	101.01			
			0.102	103.03			
			0.105	102.02			

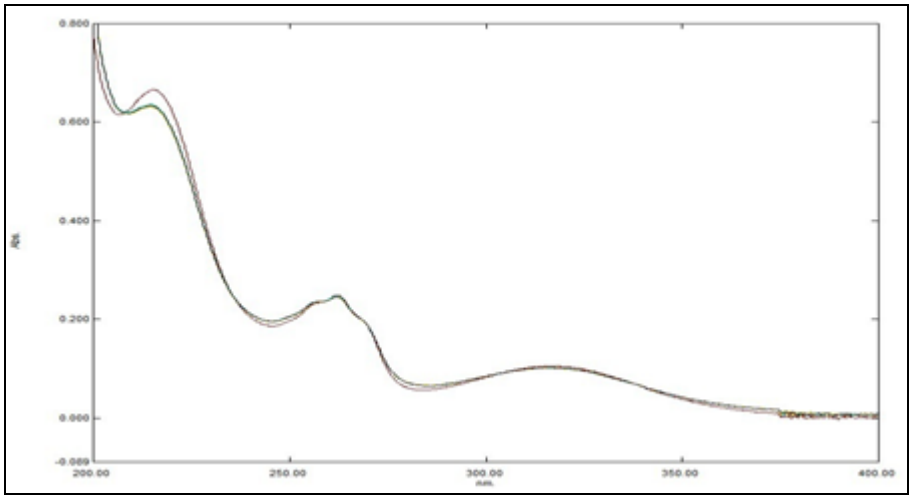

FIG. 5: ASSAY OF NICOTINAMIDE AND METRONIDAZOLE

Recovery: The accuracy of the method was studied by recovery studies. Absorbance of Nicotinamide and metronidazole were successfully measured at 262 nm and 317 nm, respectively. Three repetitions of each of the three levels 80 %, 100 %, and 120 % were examined at the aforementioned wavelengths. **Table 3** and **4** show the relative standard deviation % RSD and the percentage recovery for 3 levels of nicotinamide and metronidazole.

TABLE 3: RECOVERY OF NICOTINAMIDE

Levels	Absorbance	Concentration (µg/ml)	% RSD	% Recovery
	0.185	63.68		
80 %	0.190	65.41	1.34%	102.5 %
	0.188	64.72		
	0.233	80.24		
100 %	0.235	80.93	1.08%	100.3 %
	0.230	79.20		
	0.280	96.44		
120 %	0.279	96.10	1.16 %	99.3 %
	0.274	94.37		

TABLE 4: RECOVERY OF METRONIDAZOLE

Level	Absorbance	Concentration (µg/ml)	% RSD	% Recovery
	0.085	11.41		
80 %	0.082	11.01	1.83 %	99.5 %
	0.083	11.14		
	0.103	13.85		
100 %	0.102	13.71	1.48 %	98.4 %
	0.105	14.12		
	0.122	16.41		
120 %	0.125	16.82	1.24 %	98.2 %
	0.124	16.68		

Sandell's Sensitivity: The mean Sandell's sensitivity of nicotinamide and metronidazole was 0.354 µg/cm^2 and 0.134 µg/cm^2 respectively confirming that the method was very sensitive and effectively used for quantification of metronidazole and nicotinamide.

Molar Absorptivity: Molar absorptivity was the unit concentration of the sample and the average molar absorptivity of metronidazole and nicotinamide was found to be 1.277x10^5 and 3.439x10^5 respectively.

Optical Parameters of the Method: The correlation coefficient, intercept and slope for the calibrated data were summarized and the apparent molar absorptivity and Sandell's sensitivity, linearity, and precision values were calculated in **Table 5.**

TABLE 5: DETERMINATION OF OPTICAL PARAMETERS

Parameters	Observations	
	Metronidazole	Nicotinamide
λ_{max}	317 nm	262nm
Slope	0.0072	0.0029
Intercept	0.0063	0.0001
Regression equation (y=mx+c)	0.0072x+0.0063	0.0029x+0.0001
Linearity	1.8 to 18.5 µg/ml	10 to 100 µg/ml
Correlation Coefficient(R^2)	0.9933	0.9937
Sandell's sensitivity	0.134 µg/cm^2	0.354 µg/cm^2
Molar absorptivity	1.277x10^5	3.439x10^5
Precision % RSD	1.6 %	0.714 %

CONCLUSION: The simultaneous quantification of nicotinamide and metronidazole combination in the gel form has been effectively established and verified using a straightforward and trustworthy UV spectrophotometric approach.

The method's linearity, precision, accuracy, assay, molar absorptivity, and Sandell's sensitivity were all evaluated. The analysis of nicotinamide and metronidazole in gel form using this method demonstrated that the excipients had no impact on the results.

This procedure doesn't involve any extra chemicals or prolonged analysis times. The purity of the medicine can also be ascertained using this method. The developed method was advantageous in terms of sensitivity, simplicity, cost-effectiveness, time consumption, and the absence of any tedious experimental settings

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CONFLICTS OF INTEREST: The authors have no conflicts of interest in this work.

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