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Method Development and Validation of Metronidazole Using UV Spectrophotometer

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Abstract

Metronidazole in METRONIDAZOLE I.V. Infusion 500mg/100ml was determined by Spectrophotometric method. Being a very simple, reliable and sensitive method was developed and adopted to perform the analysis of METRONIDAZOLE I.V. Infusion 500mg/100ml. The Spectrophotometric method described was found to be simple, sensitive and accurate. Therefore this could be applied for the determination of METRONIDAZOLE I.V. Infusion 500mg/100ml.The results obtained confirm the suitability of the proposed method for the precise analysis of the drug. Based on the above results, the test method is found to be specific, linear, precise, reproducible and accurate. So, it has been concluded that test method for METRONIDAZOLE I.V. Infusion 500mg/100mlcan be used for routine testing in Quality Control Laboratory. Since the suggested method is simple and reliable, this can be used for routine analysis of METRONIDAZOLE I.V. Infusion 500mg/100mlInjection in the Quality Control Laboratory to release the commercial batches.

Keywords: Metronidazole; UV Spectroscopy; Assay; Method development; Method validation; Economical method.

Introduction

Chemically, metronidazole (MND) (Figure 1) is 2-methyl-5-nitro-1H-imidazole-1- ethanol [1]. A member of the class of imidazoles substituted at C-1, -2 and -5. It affects tumor cells [2]. The molecular weight of metronidazole is 171.15396 g/mol and Molecular Formula is C6H9N3O3. It is photo and heat sensitive. Resistance to metronidazole develops due to poor cell penetration and decreased nitro reductase activity [3].It is used in combination to treat gastrointestinal tract troubles. Itis used in the treatment of amoebic liver abscesses [4].

There are assays based on the reduction of metronidazole and other drug molecules at λmax = 500 nm [5].Validated methods have been successfully applied with retained accuracy and precision [6]. It was also determined by spectroscopy when it is used with tinidazole at 510 nm [7].In one assay the development and validation criteria are well met and a solution of 1M HCl is used [8]. Its quantification is also done by GC-FID [9] and UPLC-MS [10] techniques.

Aim of our Current Work:

Review of Literature for MND analysis exposed that numerous on hand methods including diverse sophisticated techniques such as HPLC with UV detection, GC-FID, HPLC PDA/MS, UPLC-MS assay for its assay method development and validation. However there is no simple and accurate method reported for the detection of MND in pharmaceutical formulation by UV spectrophotometry. So this vital need has been an aspiration to devise a simple method which not only meets the Assay method development and validation criteria but also caters to be attainable practically in daily practices. The ambition of present effort is to develop and validate a trouble-free, accurate, less time consuming as well as economical assay method for the content of metronidazole using 0.1 N HCl and Spectroscopy which can be easily used in quality control laboratories.

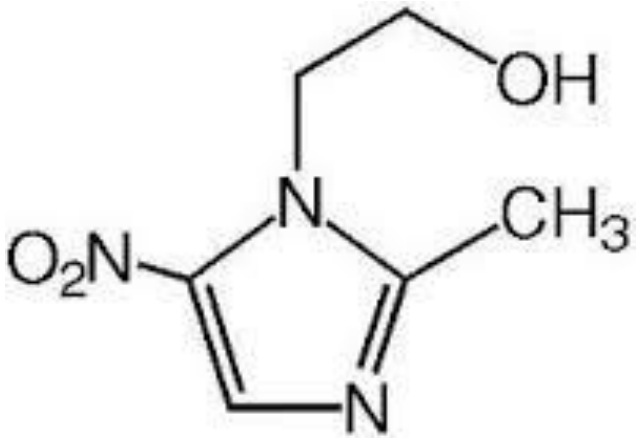

Figure 1: Structure of Metronidazole

Experimental

Required Materials

Metronidazole, 0.1 N HCl, distilled water.

Glass wares used in this experiment are volumetric flasks, stirrer, beakers, pipette and measuring cylinder. All of the glass ware used was made up of Pyrex material. Initially all the glass wares were rinsed with chromic acid then with water and finally washed with freshly prepared distilled water.

Instruments

Weighing Balance used for weighing the drug was 'Shimadzu Japan' and Spectrophotometer 'UV-1601, UV / Visible spectrophotometer, Shimadzu Japan' for the measurement of absorbance of Metronidazole.

Method Development

Selection of Wavelength Detection

By using UV spectrophotometer in the range of 200-400nm the scanning of the solution of Metronidazole has been done. It was examined that metronidazole demonstrated maximum absorbance at 277nm which was selected as the detection wavelength for the drug.

Standard Preparation

Weigh accurately 50.0 mg metronidazole standard in 100ml volumetric flask , dissolve in 0.1 N HCl and make up the volume with 0.1 N HCl. Take 1ml from the above dilution into 50ml volumetric flask and make up the volume with 0.1 N HCl and mix well.

Sample Preparation

Accurately transfer 10ml of the Mezole I.V. Infusion 5mg/ml into 100ml volumetric flask, add about 50ml 0.1 N HCl and sonicate for about 30 minutes. After completion of time makes up the volume with 0.1 N HCl and mix well and filter the solution with Whatsman filter no.1. Take 1ml from the above filter solution into 50ml volumetric flask and make up the volume with 0.1 N HCl and mix well.

Measure the absorbance of both standard and sample preparation on a suitable spectrophotometer at the wavelength of 277nm, using 0.1 N HCl as a blank. (See Table 1)

Wavelength:

Absorbance at 277nm

Method Validation:

Specificity

Product Name : Metronidazole I.V. Infusion 500mg /100ml

Active Ingredient : Metronidazole

Technique : Spectrophotometric Method

Specificity:

a. Identification

The identity of Metronidazole by Spectrophotometric method was accomplished by subjecting the Standard preparation, Sample preparation and Blank for analysis. It was observed that the Standard and Sample absorbed at the same wavelength. None of conflicting peak was seen at the wavelength of Metronidazole.

b. Placebo

Placebo containing all ingredients except Metronidazole was prepared and analyzed on Spectrophotometer. No interference was observed at the wavelength of active ingredients.

Table 1: Concentration and Absorbance Range

S.No.	% Level Solution	Concentration of Standard mcg/ml	Absorbance of Standard		
			Absorbance 01	Absorbance 02	Average
1	80%	8.0080	0.302	0.301	0.3015
2	90%	9.0490	0.340	0.343	0.3415
3	100%	10.070	0.371	0.371	0.371
4	110%	11.071	0.406	0.406	0.406
5	120%	12.020	0.444	0.445	0.4445

	Concentration mcg / ml	Absorbance
1	8.008	0.302
2	9.049	0.342
3	10.070	0.3710
4	11.071	0.4060
5	12.020	0.445

Slope (m)	=	0.03
Intercept (b)	=	0.06
Correlation Coefficient	=	0.945

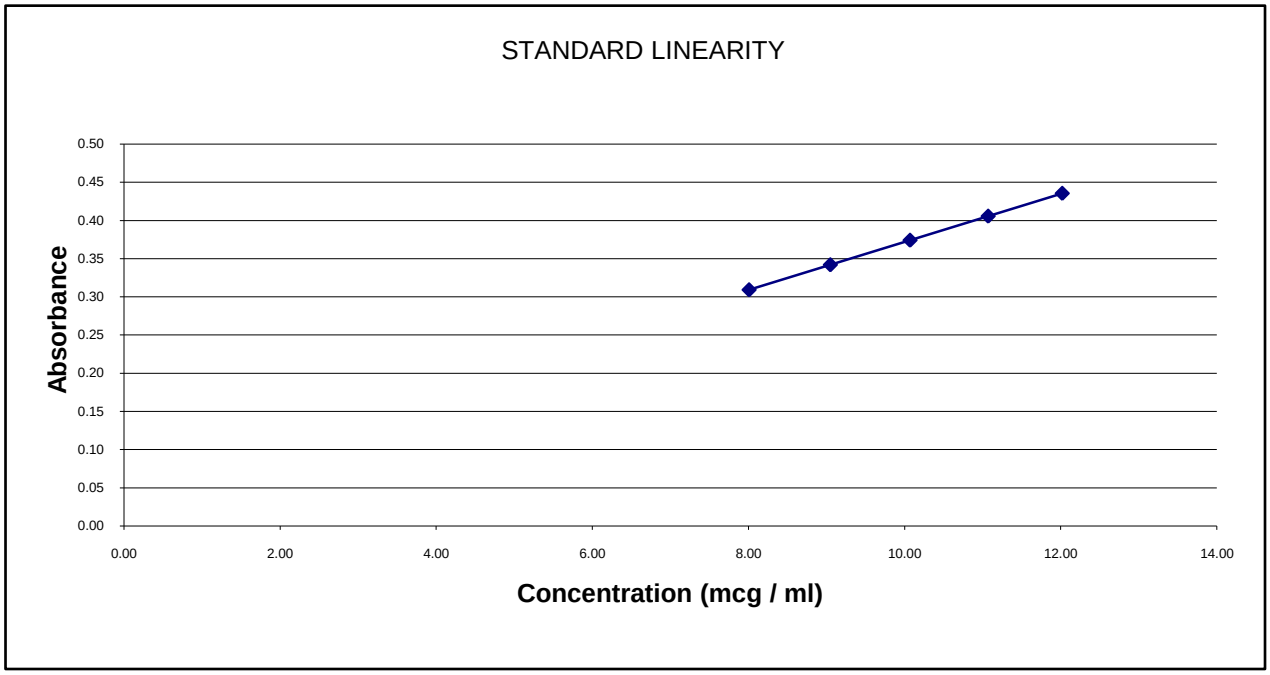

Figure 2: Linearity of the Developed Method

Table 2: Accuracy / Recovery of the Developed Method

GRAND AVERAGE OF 3 SAMPLES			
SR.NO.	LEVEL(%)	Metronidazole(%)	LIMIT±3(%)
1	50	51.62%	47.00-53.00
2	100	102.16%	97.00-103.00
3	150	152.12%	147.00-153.00

AVERAGE OF SAMPLE # 1			
SR.NO.	LEVEL(%)	METRONIDAZOLE	LIMIT±4(%)
1	50	51.59 %	46.00-54.00
2	100	102.48%	96.00-104.00
3	150	151.11%	146.00-154.00

AVERAGE OF SAMPLE # 2			
SR.NO.	LEVEL(%)	METRONIDAZOLE	LIMIT±4(%)
1	50	51.70%	46.00-54.00
2	100	102.27%	96.00-104.00
3	150	152.26%	146.00-154.00

AVERAGE OF SAMPLE # 3			
SR.NO.	LEVEL(%)	METRONIDAZOLE	LIMIT±4(%)
1	50	51.59%	46.00-54.00
2	100	101.75%	96.00-104.00
3	150	152.99%	146.00-154.00

Repeatability

Procedure: One batch of the finished product was analyzed by an analyst six times under same condition in accordance with the analytical testing instructions described in test methods. (See Table 3).

Table 3: Repeatability of the Developed Method

Sample #	Result in 500mg/100ml	Result in %
1	508.23mg/100ml	101.64%
2	509.93mg/100ml	101.98%
3	509.93mg/100ml	101.98%
4	507.55mg/100ml	101.51%
5	508.57mg/100ml	101.71%
6	508.23mg/100ml	101.64%
Mean Value	508.74mg/100ml	101.74%
Standard Deviation	0.1944	
RSD	0.2282%	
RSD Limit	3%	
Result	Within range	

Reproducibility

The reproducibility of an analytical method is demonstrated by performing the analysis of finished product by two analysts under same condition. The RSD; calculated indicates the degree of precision for the analytical method. Closeness of the agreement between the results of measurements of the same measured carried out under change conditions of measurement. A valid statement of reproducibility requires specification of the conditions changes. The changes conditions may include principle of measurement, method of measurement, Observer, measuring instrument, reference standard, location, conditions of use, time.

Procedure:

Same batch of the finished product was analyzed by two analysts under same conditions in accordance with the analytical testing instructions described in test methods. (See Table 4).

Table 4: Reproducibility of the Developed Method

Analyst	Sample	Result in %	Average %
Analyst 1	Sample 01	101.71%	102.04%
	Sample 02	102.52%	
	Sample 03	101.91%	
Analyst 2	Sample 01	101.51%	101.66%
	Sample 02	101.50%	
	Sample 03	101.98%	
Mean Value		101.85%	
Standard Deviation		0.3813	
Relative Standard Deviation		0.5171%	
RSD Limit		3%	
Result		Within range	

Results

Assay method of METRONIDAZOLE I.V. Infusion 500mg/100mlfor the content of metronidazole (by Spectrophotometer) has been developed using 0.1 N HCl and validated as per the parameters given below. The method stands validated, as it is observed that:

1. **Specificity**

   There is no interference observed in analyte area response and other matrix present in the METRONIDAZOLE I.V. Infusion 500mg/100ml**.** No absorbance detected of placebo and diluents at the wavelength of 277nm.

2. **Linearity**

   The method is observed to be linear in the range of 80% - 120% concentration of metronidazole. Correlation coefficient for Metronidazole = 0.945 (Figure 2).

3. **Accuracy / Recovery**

   The accuracy of the method that is the results of recovery and percent recovery values are observed to be within limits. The study proves that test method is accurate for quantitative analysis of assay of analyte in range of 50% to 150% of target concentrations.

Conclusion

It has been concluded that developed method for metronidazole I.V. Infusion 500mg/100mlcan be used for routine testing in Quality Control Laboratory. Since the suggested method is simple and reliable, this can be used for routine analysis of metronidazole I.V. Infusion 500mg/100mlInjection in the Quality Control Laboratory to release the commercial batches.

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