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Analysis of Metronidazole Benzoate by Uv Spectrophotometric Method

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Abstract:
Metronidazole benzoate is the drug that has been prescribed for bacterial disease conditions multiple times a day for longer period of time especially in patient suffering from bacterial disease, some of examples are acne vulgaris, rosacea and bacterial vaginosis. As in pharmaceuticals one of the focuses is to developed and formulate cheap and stable product with great therapeutic effect in order to have improved patient compliance. On data obtained from method development and validation, ICH guidelines were followed, the proposed UV method by using DMSO solvent s precise, reproducible and accurate. Hence this method is easy, feasible, cost effect for daily ongoing quality control testing of metronidazole benzoate by using DMSO as a soluble solvent.

Keywords: Metronidazole benzoate, UV, DMSOMethod development

Introduction:

The chemical name for metronidazole benzoate is 2-(2-methyl-5-nitroimidazol-1-yl) ethyl benzoate, Benzoyl metronidazole. Metronidazole has an antiprotozoal activity. Topical metronidazole has been used with some beneficial response and curable property for the treatment of decubitus and other ulcer and in some case of oral disease. [1] It is a white or slightly yellowish, crystalline powder.Metronidazole Benzoate is the benzoate ester of metronidazole, a synthetic nitroimidazole derivative with antiprotozoal and antibacterial activities.[2] Metronidazole is an artificially substitute of nitromidzole with the property of killing microbes, and reducing inflammation property.[3] The nitromidazole is like a medicine bacteria killing drugs i.e. metronidazole which has property to kill some specified bacteria i.e. protozoa, gram negative, gram positive and those which can survive in the absence of oxygen. For the treatment of decubitis ulcer and other dental condition, topical metronidazole has been successfully proven as a drug of choice for the certain condition as discussed. And study also shows that, it has low toxicity and well tolerated when applied topically. Burning and stinging are the adverse effect which produced in some cases. For the treatment of rosacea; topical metronidazole is the second option to be use. [4, 5, 6]. Topical metronidazole formulations are significantly more effective than placebo when used in the initial treatment of patients with moderate to severe rosacea. Furthermore, some studies show that the topical metronidazole and oral tetracycline has same efficacy against the disorders inflammatory component. Therefore, topical metronidazole is the drug of choice for those patients, who cannot easily accept oral therapy. [7, 8, 9] .

According to Literature survey, it is revealed that Dimethylsulphoxide is used as a best solvent, having an anti-inflammatory property. The chemical formula of Dimethyl sulfoxide (DMSO) $(CH_3)_2SO$, and it is an organic sulphur organic compound.

Materials and Methods:

Instrument:

A double beam schimadzu UV-2800 240V consisting of 2 quartz cells, was taken for measuring absorbance of metronidazole benzoate. Weighing Balance PA21AC used for weighing of ingredients, were utilized in this research.

Chemicals and Reagents:

Pure active drug was gifted by one of my friend in Karachi. The sample metronidazole benzoate containing 1% of labeled claim of metronidazole benzoate purchased from local market. Dimethyl sulphaoxide purchased from Bio Scientific distributor.

Selection of Quantity of Solvent:

Copious trials have been done to evaluate the suitable solvent system for dissolving the metronidazole benzoate. The different ratio of DMSO and water solvents was prepared and tests were performed in order to obtain the best solvent system for the analysis of drug. Metronidazole Benzoate is soluble in solvent in a ratio, 1 part of DMSO and 9 part of distilled water and this solvent system was selected for the experiment of the study.

Selection of Detection of Wave Length:

To estimate the optimum lambda max, Metronidazole Benzoate 500 ppm (50mg/100ml) of the working standard solution was prepared and analyzed in the UV wavelength range of 200-400 nm. At 230 nm, metronidazole benzoate shows accurate peak, it means that at 230 nm the drug has maximum absorbance. So, 230 nm was chosen as the maximum or detection wavelength for the further analysis of metronidazole benzoate.

Preparation of Stock and Working Standard Solution:

Metronidazole Benzoate 500 ppm standard stock solution was done by transferring accurately weighed 50 milligrams of standard metronidazole benzoate to 100 milliliters calibrated flask and dissolved in 1 part of DMSO and 9 part of water. The volume was filled up to the mark with this solvent system (500ppm standard stock solution). Which was treated as the working standard solution.

Preparation of Calibration Curve:

From the above prepared metronidazole benzoate stock solution, appropriate dilutions were prepared to get the eventual concentration of 50 ppm, 25ppm, 12.5ppm, 6.25ppm, 3.125ppm,1ppm,0.5ppm and absorbance was taken at lambda max 230 nm. Calibration curve was plotted by taking averages of five sets of values for standard calibration plot. Calibration curve was done by plotting the values in such a way that the absorbance of metronidazole benzoate on Y-axis and concentration of the drug on X-axis. Calibration data are shown in table. 1. The calibration curve is exhibited in figure 2.

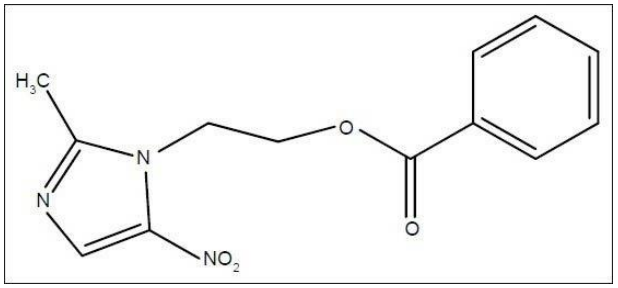

Figure. 1: Chemical structure of Metronidazole Benzoate

Table .1: Calibration data of Metronidazole Benzoate

CONCENTRAION	ABSORBANCE
50 ppm	0.430
25ppm	0.232
12.5ppm	0.133
6.25ppm	0.065
3.125ppm	0.097
1ppm	0.031
0.5ppm	0.009

Table. 2. Linear regression data

PARAMETER	RESULTS
Detection Wavelength	230nm
Regression equation (Y=mx+c) : slope (b)	0.0079+0.0394
Standard error slope (Sb)	0.9793
Intercept (a)	0.039
Correlation Coefficient (r2)	0.9794

Method Development and Validation:

A number of solvents were analyzed, including different ratio of DMSO and water at different ppm. Nevertheless metronidazole benzoate was completely dissolved stable for minimum 6 hours at room temperature and the absorbance was slightly decreased after twenty four hours in same solvent system which was prepared, i.e. DMSO and water. Hence DMSO and water solvent system was used for the determination of detection of wavelength and preparation of standard and working concentration. In order to check and proposed method to the pharmaceutical formulation, an assay of Metronidazole benzoate 1% was utilized at working concentration. Assay for working concentration of sample at 230nm. According to ICH guidelines [10, 11] , it has been estimated that this process has characteristics performance which has been established by laboratory studies. By following the guidelines, the UV method has been developed and it is concluded that the proposed method was validated for all the parameters such as linearity, specificity, precision, accuracy, robustness, ruggedness, LOD and LOQ.

Precision:

System Precision:

At 230nm 10ug/ml concentration of six replicate recordings were observed on the same day and corresponding responses were studied. The mean, SD and % RSD were calculated.

Method Precision:

Method precision was determined by performing assay of the sample under the test of repeatability (intraday precision) and intermediate precision performed during two consecutive days by two different working concentrations. Eventually the mean, SD and & Relative standard deviation were counted. The intermediate precision results, i.e., inter day and intraday precision of metronidazole benzoate were tabulated in tablets. 3-5.

Table .3 Results of system Precision

S.No	Absorbance
1	0.430
2	0.43
3	0.430
4	0.431
5	0.431
Mean	0.4303

Standard deviation	0.0006
% Relative Standard deviation	0.13416

Table . 4 Results of method Precision (Intraday Precision)

Concentration (ug/ml)	Sample absorbance	Mean absorbance + S.D	% RSD
25	0.232 0.232 0.232	0.233+ 0.0017	0.7433
12.5	0.133 0.135 0.133	0.1337+0.0012	0.8638
6.25	0.065 0.067 0.065	0.0657+0.0012	1.7584

Table. 5 Results of method Precision (Inter day Precision)

Concentration (ug/ml)	Sample absorbance	Mean absorbance + S.D	% RSD
25	0.233 0.236 0.237	0.2353+ 0.0021	0.8845
12.5	0.138 0.136 0.137	0.137+0.001	0.7299
6.25	0.062 0.063 0.064	0.063+0.001	1.587

ACCURACY (RECOVERY STUDIES):

Recovery studies of metronidazole benzoate were carried out but utilizing standard addition method in which estimation of % mean recovery of sample by % method at 3 different levels (80%, 100%, 120%) i.e. 30 ppm, 12.5ppm, 7.5ppm. These 80 to 120 levels of sample solution were prepared as per the procedure given in the methods from the dilution used for linearity .At each level, 3 analyses were performed % mean recovery was calculated as shown in table 6. The accepted limits of recovery are 98% -102%. In fact from the amount of Metronidazole benzoate found % recovery was evaluated. The results are presented in table 6.

Table.6. Accuracy of results

Level %	Absorbance	% Recovery	Mean % Recovery
80	0.429	99.72	99.90
80	0.430	100.82	
80	0.429	99.17	
100	0.133	98.78	99.52
100	0.135	99.34	
100	0.134	100.44	
120	0.051	99.45	

120	0.054	100.18	99.99
120	0.052	100.36	

Ruggedness:
Ruggedness is done by performing proposed method on different instruments. In addition to that this methods is carried out by two different analyst and performing the method on different days to find the reproducibility. The results obtained are shown in table 7.

Table. 7: Results of Ruggedness

Analyst	Sample Absorbance	Mean Absorbance + S.D	% RSD
Analyst 1	0.430 0.435 0.432	0.432+0.001	0.433
Analyst 2	0.439 0.431 0.438	0.436+0.001	0.437

Robustness:
Robustness was estimated by performing the same proposed method on different wavelength. The analysis showed % RSD less than 2 which shows the proposed method are robust. The Robustness of Metronidazole is tabulated in 8.

Table. 8: Results of Robustness

Wavelength (nm)	Sample Absorbance	Mean Absorbance + S.D	% RSD
230nm	0.430 0.435 0.432	0.432+0.001	0.433
319 nm	0.262 0.260 0.261	0.261+0.001	0.262

Analysis of commercial Formulation:
The validated method was applied to the estimation of metronidazole benzoate gel. 1 gm were assayed and the results are represented in table 9 which indicates that the amount of drug in the gel sample was in good agreement with the label claim of the formulation as indicated by percentage recovery

Table. 9 Results of assay of Pharmaceutical Formulation

Concentration (ug/ml)	Mean Absorbance + S.D	% RSD	% Recovery (Amount Found)
Revomet gel 6ug/ml	0.133 +0.001	0.7299	99.93

Mean of 3 determinations

Results and Discussion:
The ultra violet spectra of metronidazole benzoate were scanned in the region between 200-400 nm. The best spectra of metronidazole benzoate at different concentrations were absorbed maximum at 230nm, which was selected as a detector wave length. The response of metronidazole benzoate was found to be linear in the concentration range of 2ug-10ug/ml with good correlation coefficient of r= **0.9794** and the figure 2 shows the metronidazole benzoate

Linearity calibration curve and the table 1 shows the calibrations data. Table 2 shows the linear regression data of the proposed UV method by using suitable solvent that is DMSO. The system precision and method precision of the method with interday and intraday precision was found to be good with %RSD less than 2, which indicated that the method was precise and the results are presented in table 3 to table 5. At three different concentration levels (80%, 100%, and 120%). accuracy studies were carried out by recovery study using a standard addition method. %). The mean percentage recovery at each level should be 98.0- 102.0 %. All the results are well within the acceptance criteria (99.1-99.99) and the result indicates that the method is accurate. Results of accuracy study shows in table 6. Ruggedness' was performed to check the reproducibility which showed the % RSD less then which indicates that the method was rugged (Table 7). By changing two different wavelengths robustness was performed. Even though by changing the minor modification, the % RSD got less than 2 which shows that the method developed is robust. (Table 8). The developed method was applied for the quantification of metronidazole benzoate in gel base. The means % assay values were found to be 99.93. The amount of drug in the gel sample was in good agreement with the label claim of the formulation. The assay results are shown in table 9. According to the result of linearity, accuracy and precision it has been conclude that the proposed method is within the limits of guidelines and the proposed method is qualitative.

Conclusion:

Sensitive, simple, precise method was found for the analysis of metronidazole benzoate by using UV- spectrophotometer. Hence the analyzed method can be used for the daily analysis of metronidazole benzoate in pharmaceutical preparations. Moreover, this UV method offers time saving, cost effective for an HPLC method of analysis for metronidazole benzoate from formulations.

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