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Research Article

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF METRONIDAZOLE AND AMOXICILLIN IN SYNTHETIC MIXTURE BY UV- VISIBLE SPECTROSCOPY

PARTH PATEL[1]*, PRIYA VARSHNEY[1], MINAL ROHIT[1]

[1]Department of Quality Assurance, Pioneer Pharmacy Degree College, Vadodara 390019. Email: patelparth815@rocketmail.com

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ABSTRACT

Two simple, accurate, precise, reproducible, requiring no prior separation and economical procedures for simultaneous estimation of metronidazole and amoxicillin in combined dosage form have been developed. First method employs formation and solving of simultaneous equation using 320 nm and 273 nm as two analytical wavelengths for both drugs in distilled water. The second method is a Q value analysis based on measurement of absorptivity at 320nm and 228nm (as anISO-absorptive point). Metronidazole and amoxicillin at their respective λmax 320 nm and 273 nm and at iso-absorptive point 228 nm shows linearity in a concentration range of 10-30 µg/mL. The results of the analysis have been validated statistically, the relative standard deviation lies in the range of 1.11 – 1.25 for amoxicillin and 1.05 – 1.19 for metronidazole in case of simultaneous equation method and 0.41-1.11formetronidazole and 0.51-1.23 in the case of Q - analysis method.

KEY WORDS Amoxicillin, Metronidazole, UV spectroscopy, Simultaneous equations, Q – analysis

INTRODUCTION

Amoxicillin (6R) -6-(a -D-4-hydroxy- phenylglycylamino) penicillanate and Metronidazole 2-(2-Methyl-5- nitroimidazol-1-yl)are used clinically as independent nonsurgical periodontal therapies for the treatment of periodontal infections. Amoxicillin trihydrate is the most widely used and prescribed medication for oral-dental infections. Amoxicillin trihydrate is a broad spectrum antibiotic and is active against most periodontal pathogens, particularly facultative and aerobic bacteria. It is also activeagainst bacteria that are responsible for periodontal diseases, namely, Bacteroides gingivitis, and anaerobic bacilli. [1]Metronidazole (MTZ), the most potenttetracycline for collagenase inhibition is effective againsta broad-spectrum of microorganisms inhibiting both gram positive as well as gram negative organisms, including the beta- lactamase producing strains.These two drugs with separate antibacterial spectrum areproposed to be formulated as combination therapy to havea wider antibacterial therapy effective against both aerobic and anaerobic periodontal micro flora and hence aperiodontal drug delivery device containing both thedrugs is being developed in our laboratory. In an attemptto develop a periodontal controlled release drug delivery system of metronidazole and amoxicillin, the primary requirement of suitable assay method for simultaneousestimation of both the drugs is the prerequisite. [2]

The review of literature revealed that the combined dosage form has been estimated by spectrophotometric method by using various solvents in the synthetic mixture but no method is yet reported in distilled water. Thus, this method is cost effective. This paper describes two simple, rapid, accurate, precise and economical methods for simultaneous determination of Metronidazole and Amoxicillin in synthetic mixture.

MATERIALS AND METHODS

Reagents & Instruments

A UV-VIS spectrophotometer Shimadzu UV-1800, single pan electronic balance, was used for the experimental purpose. Double distilled water was used throughout the study. Amoxicillin trihydrate and Metronidazole were obtained as a gift sample from Pioneer Pharmacy Degree College, Vadodara.All the other reagents used were of analytical grade.

Determination of absorption maxima

Accurately weighed 10 mg of MTZ was transferred to a 100 ml volumetric flask and volume was made up with the distilled water to get a solution of concentration 100 µg/ml. 3.0 ml of stock solution was diluted to 10 ml to get a concentration of 30 µg/ml. Solution of AMX was also prepared in a similar way to get a concentration of 30 µg/ml. Both the solutions were scanned in the spectrum mode over the range of 200-400 nm. MTZ showed an absorbance peak at 320 nm, whereas AMX at 273 nm. The overlain spectra also showed two iso-absorptive points at 228 nm and 235 nm (Fig. I).

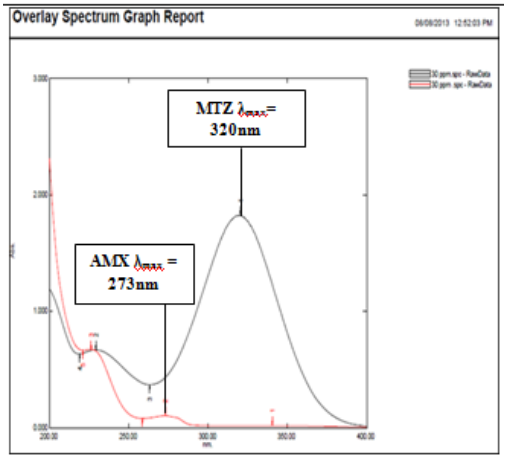

Fig. 1: Spectra showing absorption maxima of MTZ & AMX

Sample Preparation Method

The sample solution was prepared from standard stock solutions (100 µg/ml) in the ratio of 1: 2 (MTZ: AMX). Absorbance of sample solution was simultaneously estimated at 320nm, 273nm, and also at its iso-absorptive point 228nm.

Method I (simultaneous equation method)

Two wavelengths selected for the method are 320 nm and 273nm that are absorption maxima's of MTZ and AMX respectively in distilled water. Standard stock solution(s) of 100 µg/ml each of MTZ and AMX were prepared separately in distilled water. The stock solutions of both the drugs were further diluted separately with distilled water to get a series of standard solutions of 10-30 µg/ml concentrations. The absorbances were measured at the selected wavelengths and absorptivities (A 1%, 1 cm) for both the drugs at both wavelengths were determined as mean of three independent determinations. [3-8] Concentrations in the sample were obtained by using the following equations-

$C_X = A_1 ay_2 - A_2 ay_1 / ax_1 ay_2 - ax_2 ay_1$ ....Eq(I)

$C_Y = A_1 ax_2 - A_2 ax_1 / ay_1 ax_2 - ay_2 ax_1$ ..... Eq (II)

Where, A1 and A2 are absorbances of mixture at 320 nm and 273 nm respectively, ax_1 and ax_2 are absorptivities of metronidazole at λ_1 and λ_2 respectively and ay_1 and ay_2 are absorptivities of amoxicillin at λ_1 and λ_2 respectively. C_x and C_y are concentrations of Metronidazole and amoxicillin respectively.

Method II (absorbance ratio or Q-analysis method)

From the overlay spectrum of MTZ and AMX, two wavelengths were selected one at 228 nm, the Iso-absorptive point for both the drugs and the other at 320 nm, λ_{max} of metronidazole. The absorbances of the standard and sample solutions prepared in a similar manner as in the previous method, were measured and the absorptivity values for both drugs at the selected wavelengths are presented in Table I. The method employs Q values and the concentrations of drugs in sample solution were determined by using the following formula**,** [9, 10]

For Metronidazole for Amoxicillin

$Cx = Q_M - Q_Y / Qx - Q_Y$ ..... Eq (**III**)

$C_Y = C_X - A / ax_1$..... Eq. (**IV**)

Where,

Q_M = Absorbance of sample at 320 nm/ Absorbance of sample at 228 nm

Qx = Absorptivity of Metronidazole at 320 nm/ Absorptivity of Metronidazole at 228 nm

Q_Y = Absorptivity of amoxicillin at 320 nm/ Absorptivity of amoxicillin at 228nm

A = Absorbance of sample at iso-absorptive point

ax_1 = Absorptivity of Metronidazole at iso-absorptive point.

METHOD VALIDATION

The described methods have been validated for the assay of both the major components of bulk drug using following ICH parameters. [10]

Linearity

Linearity was studied by preparing standard solutions at different concentration levels. Calibration curves were prepared using the standard solutions of 10 µg/ml - 30 µg/ml and linear regression analysis was carried out. The regression coefficients are reported in Table I.

Table 1: Linear regression analysis of calibration curves of MTZ & AMX with both the methods reported here

Concentration (µg/ml)	Method 1				Method 2	
	MTZ		AMX		MTZ	AMX
	320 nm	273 nm	273 nm	320 nm	Iso -absorptive Point 228 nm	Iso -absorptive Point 228 nm
10	0.575	0.135	0.034	0.006	0.212	0.236
15	0.832	0.193	0.051	0.009	0.303	0.349
20	1.124	0.259	0.071	0.008	0.404	0.507
25	1.430	0.329	0.078	0.005	0.514	0.590
30	1.819	0.428	0.102	0.013	0.667	0.668
R^2	0.9939		0.9816		0.9939	0.9816
Intercept	0.0784		0.002		0.0784	0.002
$A^{1\%}_{1cm}$	573.98	133.484	33.74	4.466	208.86	236.164

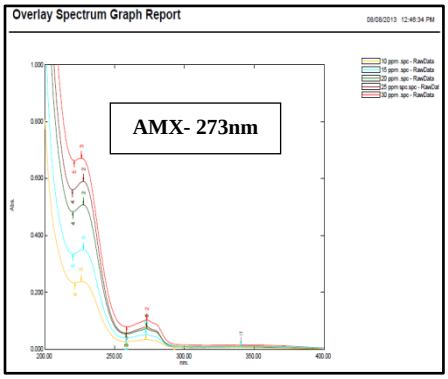

Fig. 2: Linearity spectra of amoxicillin at 273 nm

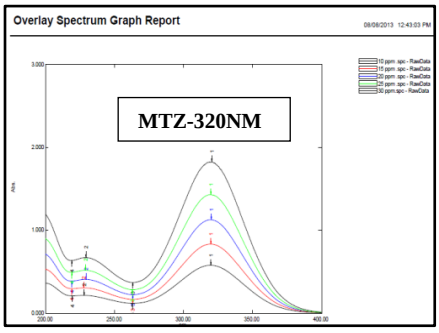

Fig. 3: Linearity spectra of Metronidazole at 320nm

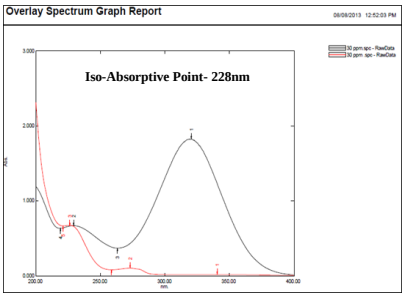

Fig. 4.Q-Absorption spectra at iso absorptive point 228nm

Table 2: Results of precision study (Intra-day and inter-day)

Methods	Inter-Day Precision (n=3) % RSD		Intra-day Precision (n=3) % RSD	
SIMULTANEOUS EQUATION				
	AMX	**MTZ**	**MTZ**	**AMX**
Method – I	1.11	1.05	1.19	1.25
Q- ABSORPTION				
	228 nm	**320 nm**	**228 nm**	**320 nm**
Method – II	0.85	0.41	1.2	1.98

Precision

Precision was studied to find out intra and inter-day variations in the test method of metronidazole and amoxicillin. Calibration curves prepared in medium were run in triplicate in the same day for three

days. %RSD (relative standard deviation) were calculated which should be less than 2 %. The results are tabulated in Table II.

Accuracy

To study the accuracy of the proposed methods, recovery studies were carried out using synthetic mixtures of MTZ and AMX in 1: 2 ratios to be used in the controlled periodontal drug delivery device. Results of recovery studies were presented in Table III.

Table 3: Recovery study of synthetic mixtures of metronidazole and amoxicillin

% Amount added	Actual Concentration taken (ug /ml)		Amount Recovered (ug/ml)		Average % Recovery		% RSD	
	AMX	MTZ	AMX	MTZ	AMX	MTZ	AMX	MTZ
80	9	9	10.33 10.39 10.37	7.20 7.15 7.17	81.48	77.95	0.289	0.348
100	11	11	11.33 11.40 11.38	12.57 12.50 12.59	103.03	97.45	0.263	0.374
120	13	13	14.36 14.31 14.41	14.42 14.49 14.41	115.38	111.30	0.348	0.297

RESULTS AND DISCUSSION

The overlay spectra of MTZ and AMX exhibit λmax at 320 nm and 273 nm for MTZ and AMX respectively which are quite separate from each other. Additionally an iso-absorptive point was observed at 228 nm. Standardcalibration curves for MTZ and AMX were linear with correlation coefficients (r^2) values in the range of 0.9816- 0.9939 at all the selected wavelengths. The method was repeated for the same day and % RSD was found to be <1.21% for MTZ and <2 for AMX, similarly the method was repeated for different days and % RSD was found to be <1.10 for MTZ and <1.151 for AMX. The accuracy of the method was confirmed by recovery studies from synthetic mixtures at three different levels of standard additions.

CONCLUSION

The proposed methods for simultaneous estimation of metronidazole and amoxicillin in combined dosage forms were found to be simple, accurate, precise, economical and rapid. In both the methods percentage recovery was found be to 100% and % RSD found to be less than 2% for both the drugs. Hence, these methods can be employed for routine analysis in quality control laboratory.

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