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Development and Validation of UV Spectrophotometric Method for Simultaneous Estimation of Quinfamide and Mebendazole in *in-house* Pharmaceutical Formulation

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Abstract: The present work described the development of two simple, accurate, rapid, cost effective and reproducible UV-Spectrophotometric methods for the simultaneous estimation of Quinfamide and Mebendazole in bulk and in laboratory mixture using 0.01M methanolic HCl as a solvent. The absorption maximum for Quinfamide and Mebendazole were found to be at 260.00 nm and 232.40 nm respectively. Beer's - lamberts was followed in concentration ranges of 1 - 6 μg/mL for Quinfamide and 2- 12 μg/mL for Mebendazole. The percentage recovery of Quinfamide and mebendazole ranged from 98.48 to 99.08 and 98.83 to 99.62 (Method I); from 98.14 to 98.93 and 99.16 to 99.35 (Method II) for Quinfamide and Mebendazole. The established methods were sensible for simultaneous quantitative determination of both these drugs in fixed dose combinations. Validation of both these methods was performed as per ICH guidelines. The developed methods can routinely be used for estimation of both these drugs in their combined dosage form.

Keyword: Quinfamide; Mebendazole, Vierodt's Method and Multi-component Mode, UV-Spectrophotometer, Validation.

1. INTRODUCTION

The substantial increase in the demand of combined dosage form for advanced therapeutic achievement has created avenues for enhanced pharmaceutical analysis. The multicomponent formulations provide a greater patient

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acceptability [1]. Simultaneous equation (Vierodt's Method) is relevant for the estimation of those drugs where the spectra of the drugs overlay accurately and multicomponent analysis can be useful to any extent of spectral overlap provided that two or more spectra are not similar exactly [2]. The fundamental development behind these methods is the measurement of some property which is proportional to amount of analyte in sample. Intestinal parasitic diseases are distributed nearly throughout the world, with huge rates in many regions [3]. Invasive amoebiasis and helminthiasis is a chief health and social difficulty in western and South-eastern Africa, South-east Asia, China, and Latin America, specially in Mexico [4]. The combination of Quinfamide (QFN) and Mebendazole (MEB) is used in the treatment of amebiasis and helminthiasis and both drugs give effective and safe results. QFN is chemically [1-(2, 2-dichloroacetyl)-3, 4-dihydro-2*H*-quinolin-6-yl] furan-2-carboxylate [5] (Fig. 1) having antiparasitic properties consist of dichloroacetamide function [6, 7].QFN is an antiamoebic agent used in the intestinal lumen, is absorbed at least possible levels, and is discard within 48 h, has been recognized to be an efficient clinical antiamoebic in 80 to 90% of cases linking a single day of treatment [8, 9]**.** MEB chemically, Methyl-5-benzoyl-2-benzimidazole carbamate (Fig. 2) is broad spectrum antihelmintic agent [10]. It is BCS class II drug showing low solubility and high permeability [11, 12].

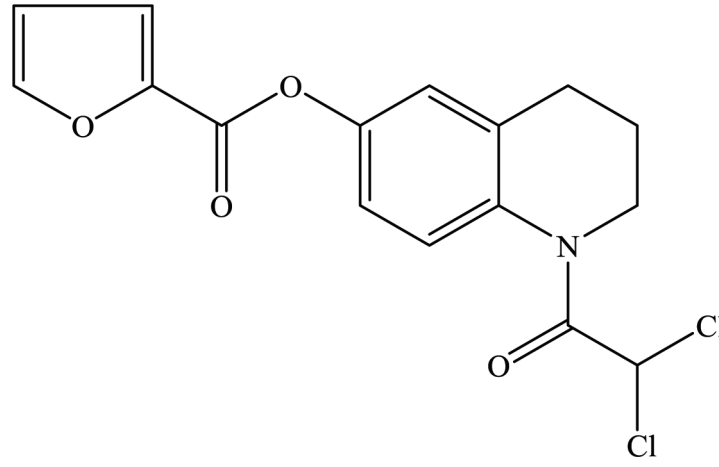

Figure 1: Molecular Structure of Quinfamide.

Literature survey revealed no single established analytical method for the simultaneous determination of Quinfamide and Mebendazole in pharmaceutical

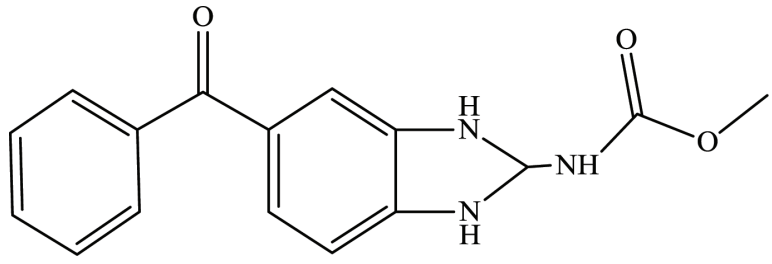

Figure 2: Molecular Structure of Mebendazole.

formulation. Hence, present research work was undertaken with the objective of developing a newer, simple, rapid and cheap simultaneous equation method and multi-component method for the analysis of Quinfamide and Mebendazole in bulk form and laboratory mixture.

2. MATERIALS AND METHODS

2.1 Chemicals

Quinfamide bulk drug was supplied as a gift sample by RPG Life Science Pvt. Ltd., Mumbai, (MS.) India. Mebendazole bulk drug was supplied as a gift sample by Watson Pharmaceuticals Pvt. Ltd., Mumbai, (MS) India. 0.01 M methanolic HCl was used throughout the experimental Work.

2.2 Instrumentation

A UV-Visible spectrophotometer (2450 Shimadzu and UV-1601, software UV Probe 2.21). The spectral bandwidth 1 nm was implemented for all spectroscopic measurements, using a pair of 10 mm matched quartz cells.

2.3 Preparation of Standard Stock Solution

Standard Stock solution of QFN and MEB was prepared by dissolving 10 mg in 100 mL of 0.01M methanolic HCl in different flask to obtain the concentration of 100 μg/mL of each drug.

2.4 Selection of Wavelengths

From these prepared solution, 1mL of QFN and MEB were transferred into two separate 10 mL volumetric flask and volume was made up to the mark using 0.01M methanolic HCl to obtain concentration each of 10 μg/mL; the resultant solution was checked in UV-range (400 - 200 nm) in 1.0 cm cell beside solvent blank. The overlain spectrum was determined absorbance of mixture of two drugs (Fig. 3).

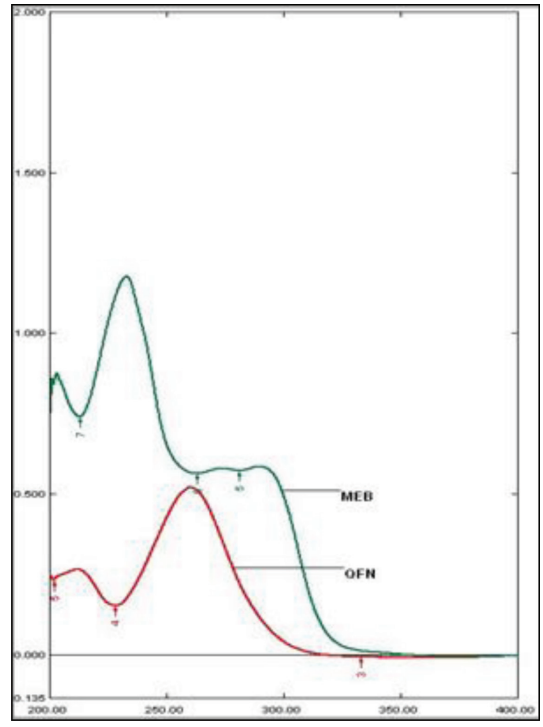

Figure 3: Overlain spectra of Quinfamide and Mebendazole.

2.5 Preparation of Calibration Curve

Calibration curve of QFN was prepared using standard stock solution by taking appropriate volumes in the range of (0.1 to 0.6 mL) were transferred into series of 10 mL volumetric flask to obtain concentration of 1, 2, 3, 4, 5, and 6 μg/mL. The absorbance of these solutions was recorded initially at 260 nm.

Calibration curve of MEB was prepared using standard stock solution by taking appropriate volumes in the range of (0.2 to 1.2 mL) were transferred to series of 10 mL volumetric and volume were prepared up to the mark using 0.01M methanolic HCl to get concentrations in range of 2, 4, 6, 8, 10 and 12 μg/mL. The absorbance of this solution was measured at 232.40 nm. The overlain spectrum for mixture (10 μg/ ml each) is shown in (Fig. 4).

2.6 Method – I (Vierodt's Method)

Absorbance was measure at the maximum absorption wavelength of two drugs in quantitative determination of two drugs by simultaneous equation. Two wavelengths 260 nm and 232.40 nm for QFN and MEB were selected

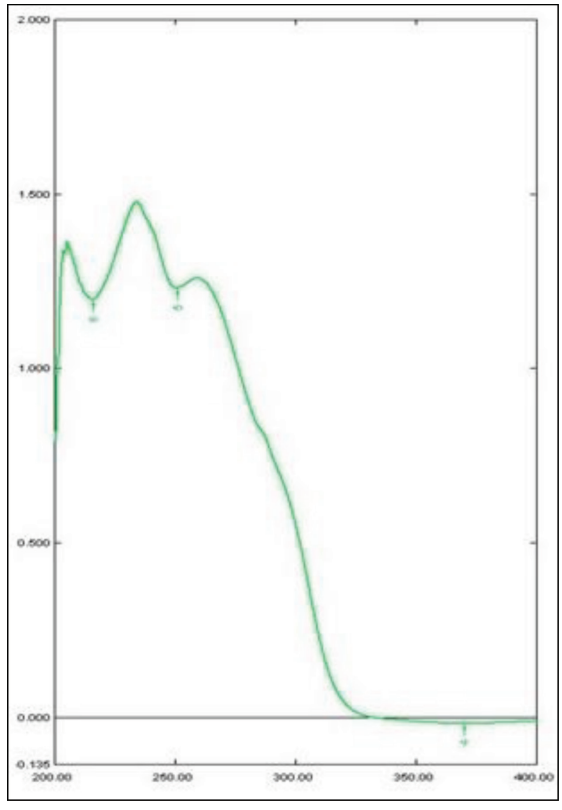

Figure 4: Absorbance maxima of Quinfamide and Mebendazole.

and sixed mixed standards solutions QFN and MEB were prepared in 0.01M methanolic HCl. All prepared standard solution were scanned over the range of 400 – 200 nm in simultaneous mode using two sampling wavelength 260 nm (λ_{max} of QFN) and 232.40 nm (λ_{max} of MEB). The absorbance and the E (1%1, 1cm) at the particular wavelength were calculated and substituted in the following Vierodt's equation. Demographic parameters like slope, intercept, correlation coefficient, standard deviation, relative standard deviation was calculated.

$$Cx = A2\ ay1 - A1\ ay2 / ax2\ ay1 - ax1ay2 \quad (1)$$

$$Cy = A1\ ax2 - A2\ ax2 / ax2\ ay1 - ax1ay2 \quad (2)$$

Where,
Cx and Cy are the concentrations of x (QFN) and y (MEB)

A1 is the absorbance of mixture at λ1
A2 is the absorbance of mixture at λ2
ax1 is the E(1%, 1cm) value of QFN at λ1
ax2 is the E(1 %, 1cm) value of QFN at λ2
ay1 is the E(1%, 1cm) value of MEB at λ1
ay2 is the E(1%, 1cm) value of MEB at λ2
A1 is the absorbance of mixture at 260nm, A2 is the absorbance of mixture at 232.40nm and ax1= (864), ax2= (340), ay1= (530), ay2= (1104) are E (1%, 1 cm) of QFN and MEB at 260 nm and 232.40 nm.

2.7 Method- II (Multi-component mode of analysis)

Mixed standard solutions of QFN and MEB were prepared six times in 0.01M methanolic HCl. In the multicomponent mode, all the standards were scanned over the range of 400 - 200 nm, using two sampling wavelength 260 nm (λ max of QFN) and 232.40 nm (λmax of MEB). The scanning data were used to resolve the concentrations of two drugs in solution of laboratory mixture (Fig. 5).

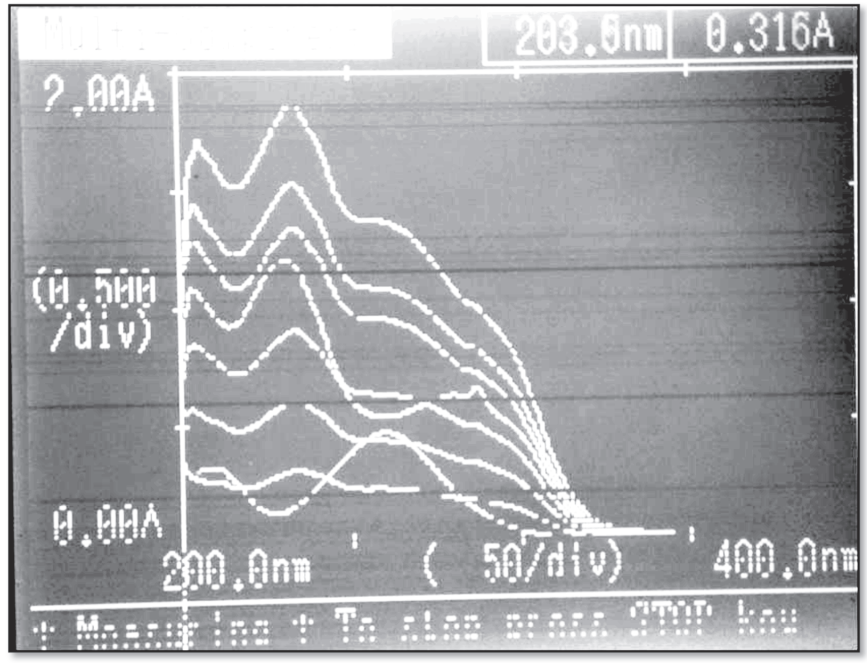

Figure 5: Overlain spectra of Quinfamide (QFN) and Mebendazole (MEB).

2.8 Analysis of Laboratory Formulation

The laboratory formulation was prepared containing 150 mg of QFN and 300 mg of MEB along with commonly used excipient, a quantity of powder drug equivalent 10 mg of QFN and 20 mg of MEB was transferred into 100 mL volumetric flask containing 60 mL 0.01M methanolic HCl, sonicated for 20 min and the aggregate was load up to marking and filtered through Whatmann filter paper (no.41). Further, the solution was diluted with 0.01M methanolic HCl to obtain the final concentration 4 μg/mL for QFN and 8 μg/mL for MEB. Absorbance of this solution was measured at 260 nm and 232.40 nm. The quantitative estimation of both these drugs were performed solving simultaneous equation (1) & equation (2) (Method I). The equivalent quantities of solutions were regulated to investigate in the multicomponent mode present in the instrument (UV-Spectrophotometer 1601). The solution was studied over the wavelength the range of 400 - 200 nm; the concentration of drugs were stored by analysis of spectral information of the sample solution with reference to the mixed standards (Method II). The analysis procedure was repeated six times with laboratory formulations (Table 1).

Table 1: Results of *in-house* tablets for Method I and II.

Method	Amount Taken (μg/mL)	Amount Found (μg/mL)	% Amount Found	% RSD
I	4(QFN) 8(MEB)	3.89	98.29	0.71
		7.91	98.93	1.03
II	4(QFN) 8(MEB)	3.91	98.95	1.07
		7.94	99.29	0.64

N = Number of determinations (N = 6)

2.9 Method Validation Parameters

The method was developed and validated according as per ICH guideline [13].
All parameter such as accuracy, precision, linearity, DL and QL and for the analytes were found to be within the limit and satisfactory.

2.10 Accuracy

The accuracy of the suggested method was confirmed by recovery investigation with the addition of known amounts to tablet, at three contrasting percentage

levels (80%, 100%, and 120%) in a period of the area of linearity for both the drugs (Table 2 and 3).

Table 2: Results of accuracy study (Method I).

Initial Amount (μg/mL)	Amount Added (μg/mL)	Total Amount	Amount Recovered (μg/mL)	% Recovery	% RSD
QFN 2	1.6	3.6	3.52	98.77	0.80
	2	4	3.96	99.08	0.31
	2.4	4.4	4.33	98.48	1.08
MEB 4	3.2	7.2	7.17	99.62	0.17
	4	8	7.97	99.62	0.30
	4.8	8.8	8.43	98.83	1.47

N = Number of determinations (N = 3)

Table 3: Results of accuracy study (Method II).

Initial Amount (μg/mL)	Amount Added (μg/mL)	Total Amount	Amount Recovered (μg/mL)	% Recovery	% RSD
QFN 2	1.6	3.6	3.53	98.14	0.96
	2	4	3.92	98.16	0.66
	2.4	4.4	4.35	98.93	0.47
MEB 4	3.2	7.2	7.15	99.35	0.34
	4	8	7.94	99.33	0.42
	4.8	8.8	8.72	99.16	0.37

N = Number of determinations (N = 3)

2.11 Precision

Assay method precision (intra-day precision) was established by carrying out four assay of test samples by the same analyst with the same equipment; whereas inter-day precision effect evaluation of modification in analysis when the method is used on different days. The % RSD values of the response was found to be less than 2 % (Table 4 and 5) for intra-day and inter-day precision, respectively.

Repeatability of the scanning device and injection was studied by applying and analyzing samples six times. The % RSD values were less than 2.0 (Table 6 and 7), representing that the method is repeatable and irreducible.

Table 4: Results of precision studies (Method I).

Drugs	Concentration (μg/mL)	Intra day Amount found (μg/mL)		Inter day Amount found(μg/mL)	
		Mean ± SD	% RSD	Mean ± SD	% RSD
QFN	2	1.94 ± 0.013	0.34	1.94 ±0.024	1.03
	3	2.98 ±0.005	0.17	2.98 ±0.007	0.81
	4	3.91 ±0.030	0.38	3.91 ±0.024	0.40
MEB	4	3.95 ±0.018	0.21	3.95±0.018	0.56
	6	5.96±0.007	0.11	5.95±0.012	0.75
	8	7.91±0.299	0.25	7.90±0.076	1.47

N = Number of determinations (N = 3)

Table 5: Results of precision studies (Method II).

Drugs	Conc. (μg/mL)	Intra day Amount found (μg/mL)		Inter day Amount found (μg/mL)	
		Mean ± SD [n= 3]	% RSD	Mean ± SD [n= 3]	% RSD
QFN	2	1.93 ± 0.011	0.59	1.94 ±0.020	1.06
	3	2.98 ±0.020	0.70	2.95 ±0.049	1.66
	4	3.90 ±0.049	1.26	3.92 ±0.037	0.96
MEB	4	3.95 ±0.017	0.438	3.95±0.032	0.81
	6	5.91 ±0.020	0.35	5.94 ±0.020	0.35
	8	7.93 ±0.060	0.767	7.89 ±0.105	1.33

N = Number of determinations (N = 3)

Table 6: Results of repeatability study.

	Method I		Method II	
Drugs	% Amount found ± SD	% RSD	% Amount found ± SD	% RSD
QFN	98.08 ± 1.10	1.12	97.79 ± 1.17	1.20
MEB	99.08 ± 0.52	0.52	99.10 ± 0.49	0.50

N = Number of determinations (N = 6)

Table 7: Validation Parameters for Quinfamide and Mebendazole.

Parameters	QFN		MEB	
	Method I	Method II	Method I	Method II
Working Wavelengths(nm)	260	260	232.40	232.40
Linearity Range (μg/mL)	1 – 6	1– 6	2 – 12	2–12
Precision(%RSD)				
Inter–day (n = 3)	0.40 –1.03	0.96 – 1.66	0.56 – 1.47	0.35 – 1.33
Intra–day (n = 3)	0.17–0.38	0.59 – 1.26	0.11 – 0.25	0.35 – 0.76
Repeatability (n = 6)	1.12	1.20	0.52	0.50
Ruggedness [%RSD]				
Analyst I (n = 6)	0.89	0.88	1.36	0.23
Analyst II (n= 6)	0.85	0.55	0.53	0.91
% Recovery (n = 3)				
%RSD	98.77 – 99.48 0.73	98.14 – 98.93 0.30	98.83 – 99.62 0.73	99.16 – 99.35 1.03

2.12 Sensitivity

Sensitivity of the method was predicted as Detection Limit (DL) and Quantification Limit (QL). The DL and QL were estimated by the use of the comparison DL = 3.3 X ASD/S and QL = 10 X ASD/S; where, 'ASD' is Average standard deviation of the peak height and areas of the drug (n = 3), taken as a measure of noise, and 'S' is the slope of the corresponding calibration plot. The procedure was repeated in triplicate. In method I LOD and LOQ for QFN was 0.26 and **0.80** μ**g** as well as for MEB 0.26 and 0.79 μ**g** and in method II for QFN was 0.30 and 0.91 μ**g** as well as for MEB 0.25 and 0.77 μg, respectively.

3. RESULTS AND DISCUSSION

Quinfamide and Mebendazole depicted good linearity over the concentration range of 1 - 6 μg/ml and 2 - 12 μg/ml using 0.01M methanolic HCl for these

two methods at their particular λ max with coefficient correlation. Laboratory formulations were analyzed. The amounts of QFN and MEB determined by 'Method I' was found to be 98.29 and 98.93, respectively; while, by 'Method II', it was found to be 98.95 and 99.29, respectively. The proposed method was validated as per ICH guideline. The percentage recovery was decisive by designing by mean percentage recovery. It was studied at 80, 100 and 120 %. Precision was determined as repeatability (% RSD is less than 2.0) and inter-day- intra-day deviation (% RSD is less than 2.0) for both drugs. The ruggedness of the process was examined by two particular analysts keeping same operative and environmental conditions. Sensitivity of the method was examined as limit of detection and limit of quantification. The analyzed data like, % recovery, repeatability data, ruggedness data are presented in details (Table 7).

CONCLUSION

The developed methods were found to be simple, rapid, reproducible and precise and can be used for quality control analysis of Quinfamide and Mebendazole in bulk and may be conveniently extended towards determination of drug in pharmaceutical formulations.

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Abbreviations

- QFN-Quinfamide
- MEB-Mebendazole
- UV- Ultra Violet
- VIS- Visible
- Mg/mL- Micro Gram/ Milliliter
- mL-Milliliter
- DL-Detection of Limit
- QL-Quantification of Limit
- ASD-Average Standard Deviation
- % RSD-Percentage Relative Standard Deviation
- SD-Standard Deviation
- ICH-International Council on Harmonization