SHORT COMMUNICATION

ANALYTICAL UV SPECTROSCOPY METHOD DEVELOPMENT AND VALIDATION STUDIES FOR SIMULTANEOUS ESTIMATION OF METFORMIN HCL AND QUERCETIN

ABSTRACT

In the current investigation, we have designed and assessed a simple and swift analytical approach employing UV spectroscopy for the simultaneous quantification of the analytes metformin and quercetin with excellent precision and accuracy. The wavelengths of interest are the wavelengths at which both the drugs show maximum absorbance: 233 nm for metformin and 256 nm for quercetin. Linearity study, conducted in methanol and phosphate buffer, yielded a correlation coefficient (r^2) of 0.99. The validation study for the developed method was conducted in accordance with ICH Q2 R1 guidelines. The percent recovery was 95% to 105%, and the percent relative standard deviation was <2, demonstrating the accuracy and precision of this method. This method can be applied to analysis of the two compounds in fixed dose formulations using simple UV spectroscopy.

Keywords: Metformin HCl, quercetin, UV spectroscopy, ICH Q2(R1)

INTRODUCTION

Glioblastoma (GBM) is a difficult to treat fatal brain tumour and temozolomide (TMZ) is the chemotherapeutic drug of choice used clinically. TMZ was approved by the FDA in 2005, and since then about 50% of patients have reported being nonresponsive to it because of increased expression of O6-methylguanine methyltransferase (MGMT). Additionally, after the treatment with TMZ, *in vitro* investigation has shown a corresponding substantial rise in GBM stem cells (GSCs) in the overall GBM cell population[1]. Due to this resistance, individuals with GBM require novel and more effective treatment approaches. Drug repurposing can be one of the easy and attractive options for the development of effective alternatives of treatment[2,3].

Metformin HCl, is chemically a biguanide antidiabetic drug, used in Type-II diabetes. It has shown selective cancer stem cell targeting and improved response to chemotherapy and radiation in various malignancies, including colon and breast cancers[4–6]. Quercetin is one of the widely distributed bioactive constituents found in several plants[7]. It is reported to prevent the proliferation of different types of cancer[8-10].

Our group is studying the efficacy of combinatorial action of metformin hydrochloride (MET HCL) [11] and quercetin (QUR)[12] in GBM. We have developed hollow gold nanoparticles (HAuNPs) as a carrier system for the co-delivery of MET and QUR. As a part of our research project, we have developed a simple analytical method for the estimation of MET and QUR by UV-spectroscopy. This method will be used for assay and release studies of both the actives in the developed carrier system. This method was validated as per ICH Q2 R1[13–15].

MATERIALS AND METHODS

MET HCL was a gift sample from Aarti Drugs Pvt. Ltd., Mumbai, Quercetin was procured from Otto Chemie Pvt. Ltd., Mumbai. The solvents and chemicals used were of analytical grade.

UV SPECTROSCOPY METHOD DEVELOPMENT

### Selection of detection wavelength and Instrumentation

The spectra of 10ppm solutions of MET in methanol and QUR in methanol each were recorded at 200-400 nm region for determining the maximum absorbance (λmax) of each drug. This λmax was then used for further analytical measurements[16]. This method was developed on a double-beam UV/visible spectrophotometer, Shimadzu 1900.

### Preparations of standard stock solutions and working solutions of MET and QUR

Accurately weighed amounts of MET and QUR (10 mg each) were put into a 10 mL volumetric flask, and the

Table I : Validation studies of MET and QUR in methanol and phosphate buffer pH 7.4
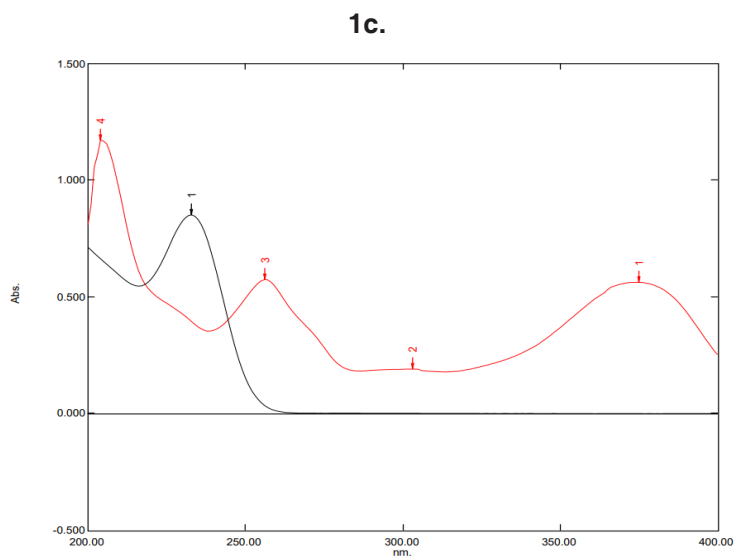
Drug recovery for MET and QUR using LM, SEM and ARM				
Drug	**Concentration (µg mL^{-1})**	**Recovery (µg mL^{-1})**		
		LM	**SEM**	**ARM (λ233)**
Metformin	10	10.07	9.8	9.55
Quercetin	10	10.08	10.23	11.5

Linearity study of MET and QUR in methanol		
Parameter	**Metformin**	**Quercetin**
Linearity range	2-10 µg mL^{-1}	2-12 µg mL^{-1}
Regression equation	y = 0.0821x - 0.0028	y = 0.0689x - 0.0037
Coefficient of correlation (R^2)	R^2 = 0.998	R^2 = 0.9975
Slope (m)	0.0821	0.0689
Intercept (c)	-0.0028	-0.0037

Linearity study of MET and QUR in phosphate buffer pH 7.4		
Parameter	**Metformin**	**Quercetin**
Linearity range	2-10 µg mL^{-1}	2-16 µg mL^{-1}
Regression equation	y = 0.0884x + 0.011	y = 0.0518x - 0.0156
Coefficient of correlation (R^2)	R^2 = 0.9991	R^2 = 0.9975
Slope (m)	0.0884	0.0518
Intercept (c)	0.0011	-0.0156

Precision study of MET and QUR				
Parameter	**Metformin**		**Quercetin**	
	Mean Abs. ± SD	%RSD	Mean Abs. ± SD	%RSD
Repeatability	1.244 ± 0.022	1.744	0.546 ± 0.008	1.505
Inter-day precision	1.276 ± 0.023	1.829	0.536 ± 0.008	1.596
Intraday precision	1.184 ± 0.012	1.043	0.550 ± 0.006	1.155

Accuracy study of MET and QUR							
Drug	**Level**	**Std amount (µg mL^{-1})**	**Amount added (µg mL^{-1})**	**Total amount (µg mL^{-1})**	**Amount recovered (µg mL^{-1}) ± SD**	**% Recovery**	**% RSD**
Metformin	80%	10	8	18	18.141 ± 0.26	100.78	1.41
	100%	10	10	20	19.261 ± 0.31	96.31	1.58
	120%	10	12	22	21.312 ± 0.09	96.87	0.45
Quercetin	80%	10	8	18	17.097 ± 0.20	94.98	1.63
	100%	10	10	20	19.701 ± 0.29	98.50	1.56
	120%	10	12	22	22.792 ± 0.36	103.60	1.59

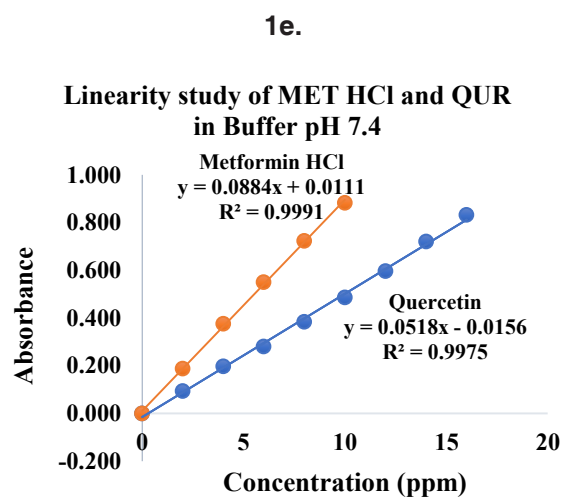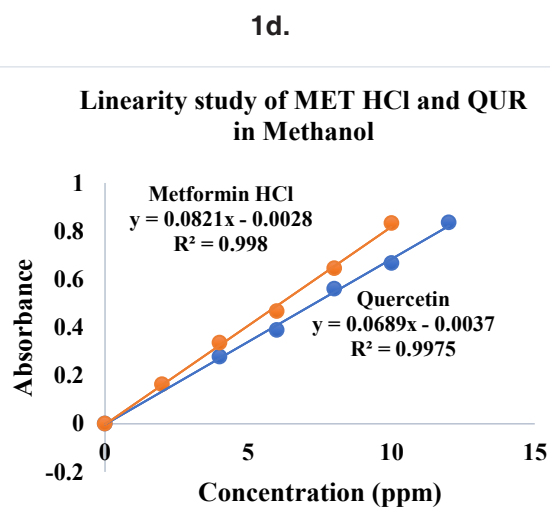| Robustness study of MET HCL and QUR						
Variable	**Metformin**			**Quercetin**		
	λmax (nm) and pH	Mean Abs. ± SD	% RSD	λmax (nm) and pH	Mean Abs. ± SD	% RSD
Variation in wavelength λmax (nm)	232	1.163 ± 0.023	1.978	254	0.548 ± 0.011	1.92
	233	1.277 ± 0.020	1.574	256	0.556 ± 0.009	1.55
	235	1.138 ± 0.012	1.011	258	0.544 ± 0.007	1.25
Variation in pH	7.2	0.550 ± 0.010	1.914	7.2	0.562 ± 0.009	1.70
	7.4	0.548 ± 0.006	1.574	7.4	0.562 ± 0.009	1.55
	7.6	0.558 ± 0.01	1.128	7.6	0.565 ± 0.006	0.96
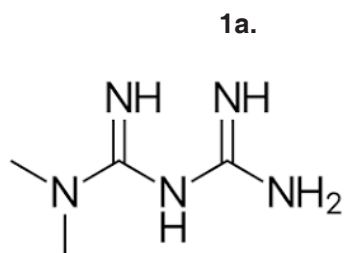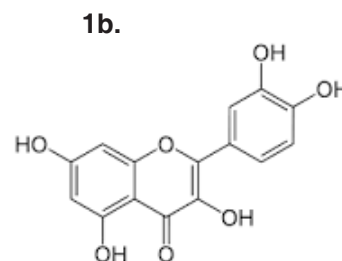
Fig.: 1a. Structure of MET, 1b. Structure of QUR, 1c. Overlay spectra of MET and QUR, 1d. Linearity study of MET and QUR in methanol, 1e. Linearity study of MET and QUR in phosphate buffer pH 7.4

remaining volume was filled with methanol. The solutions were then sonicated for 10 min to give 1000 µg mL⁻¹ stocks of MET and QUR, respectively. The solutions were scanned at 200 and 400 nm, and the maximum wavelengths of absorption for MET and QUR, were 233 nm and 256 nm, respectively. 1 mL aliquots from each stock solution (1000 µg mL⁻¹) of MET and QUR were taken in a 10 mL volumetric flask and volume adjusted with methanol upto 10 mL to give a 100 µg mL⁻¹ solution. Subsequently, working solutions of MET (2 -10 µg mL⁻¹) and QUR (4-14 µg mL⁻¹) were prepared in methanol.

METHOD DEVELOPMENT STUDIES

Simultaneous equation method

The estimation of analyte by simultaneous approach is based on the absorption of the analytes MET and QUR at their maximum wavelengths. The λmax of MET and QUR in phosphate buffer (pH-7.4) was found to be 235 and 256 nm, respectively. The absorptivity values of MET were 0.0882 (a_{x1}), 0.0033 (a_{x2}), and for QUR were 0.0408 (a_{y1}), 0.0482 (a_{y2}) at 233 and 256 nm, respectively. Equations (1) and (2) were used to calculate the concentration of MET and QUR.

$$C_x = \frac{[A_2 a_{y1}] - [A_1 a_{y2}]}{[a_{x2} a_{y2}] - [a_{x1} a_{y2}]} \ldots\ldots\ldots\ldots\ldots (1)$$

$$C_y = \frac{[A_1 a_{x2}] - [A_2 a_{x1}]}{[a_{x2} a_{y2}] - [a_{x1} a_{y2}]} \ldots\ldots\ldots\ldots\ldots (2)$$

where,

A₁ and A₂ are the absorbances of sample solutions at 233 and 256 nm, respectively

a_{x1} and a_{x2} are the absorptivity of MET solutions at 233 and 256 nm, respectively

a_{y1} and a_{y2} are the absorptivity of QUR solutions at 233 and 256 nm, respectively

Cx and Cy represent concentration of MET and QUR in sample solution[17,18]

Absorbance ratio method

This approach involves measuring the absorbances of MET and QUR at two specific wavelengths. One of the wavelengths is $λ_1$, the maximum wavelength for either analyte, while $λ_2$, represents the iso-absorptive wavelength of both analytes. In the absorbance equation, we selected $λ_1$ as 245 nm (the iso-absorption

point) and $λ_2$ as 233 nm for substitution. Equations (3) and (4) were used to calculate the concentration of MET and QUR[19].

$$Q_m = \frac{A_1}{A_2} \quad Q_x = \frac{a_{x2}}{a_{x1}} \quad Q_y = \frac{a_{y2}}{a_{y1}}$$

$$C_x = \frac{Q_m - Q_y}{Q_x - Q_y} \times \frac{A_1}{a_{x1}} \ldots\ldots\ldots\ldots\ldots (3)$$

$$C_y = \frac{Q_x - Q_m}{Q_x - Q_y} \times \frac{A_1}{a_{x1}} \ldots\ldots\ldots\ldots\ldots (4)$$

where, C_x and C_y represent the concentrations of MET and QUR, respectively.

Validation studies

This developed method was examined for validation parameters as per the ICH Q2 R1 guidelines.

A 1000 µg mL⁻¹ solution of MET and QUR in phosphate buffer pH 7.4 and ethanol (1:1) was prepared for the linearity study. Phosphate buffer was used to make a stock solution of 100 µg mL⁻¹, and working solutions containing 1–16 µg mL⁻¹ of each analyte were prepared in phosphate buffer. Repeatability, intraday and interday precision studies were conducted to evaluate the precision of the developed method. Three independent samples of a 10 µg mL⁻¹ combination of the MET and QUR solutions were examined thrice on the same day at different times to get intermediate intra-day precision. By checking three distinct samples of working solutions containing a 10 µg mL⁻¹ combination of MET and QUR on three consecutive days, inter-day precision was ascertained. 10 µg mL⁻¹ combination of MET and QUR solution was prepared in separate samples (n = 6) for the repeatability investigation, and percent RSD was calculated. The working solution was spiked with a known concentration of analyte stock solution (100 µg mL⁻¹) at three levels (80%, 100%, and 120%), and the percent recovery was determined. This study was conducted in triplicate. The robustness study of the developed analytical method was conducted by deliberate variation of pH of the buffer and wavelength. The phosphate buffer at three different pH levels (7.2, 7.4, and 7.6) and the variation in absorbance values was measured. The robustness study was conducted at 233±2 nm for MET HCL and 256±2 nm for QUR and the % RSD was determined. The LOD and LOQ were calculated using the equations- LOD = 3.3 × σ /S; LOQ = 10 × σ /S; where, σ is standard deviation and S is slope of the regression line.

RESULTS AND DISCUSSION

MET HCL exhibited maximum absorption (λmax) at 233 nm, and QUR at 256 nm and their overlay spectra indicated the iso-absorptive point to be 245 nm (Fig. 1a). Table I represents the results of linearity studies of analytes in methanol and phosphate buffer (pH 7.4), and precision, accuracy, robustness and recovery studies. It also represents the results of recovery studies conducted using the linearity method (LM), the simultaneous equation method (SEM), and the absorbance ratio method (ARM).

VALIDATION STUDIES OF THE DEVELOPED ANALYTICAL METHOD

Six different concentrations of MET (2–10 µg mL^{-1}) and six different concentrations of QUR (2–12 µg mL^{-1}) in methanol were used in the linearity study. Fig. 1c depicts the calibration curves of MET and QUR in methanol. The coefficients of regression were 0.998 and 0.9975, respectively. The calibration curves in phosphate buffer (pH 7.4) are shown in Fig. 1d; the coefficients of regression were 0.9991 and 0.9975, respectively.

The developed analytical method was found to be accurate; the percent relative standard deviation (SD) was under 2% in repeatability, intra-day accuracy, and inter-day precision studies. The mean recovery of MET at QUR was computed at three distinct percentages of addition (80, 100, and 120%). The recovery percentage was observed to be in range from 95% to 105%. The results indicated that the developed analytical method was robust and slight variation in pH and in wavelength did not significantly alter the results. The LOD of MET and QUR were 0.97 and 1.01 µg mL^{-1}, respectively and LOQ of MET and QUR were 2.94 and 3.08 µg mL^{-1}, respectively (Table I)[20].

CONCLUSION

A convenient and simple analytical method for the simultaneous estimation of metformin and quercetin was developed using UV spectroscopy. This method was precise, quick and highly specific. We have developed simultaneous estimation and absorption ratio methods for the assessment of MET HCL and QUR with no obvious difference in percent recovery between the two. The entire validation study was in accordance with the ICH Q2 R1 guidelines. This method is suitable for use in the quantitative estimation of MET and QUR in combination products with high precision and accuracy.

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