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Validated spectrophotometric methods for simultaneous analysis of glimepiride and metformin in pharmaceutical dosage forms

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ABSTRACT

Two simple, accurate and reproducible spectrophotometric methods have been developed and validated for simultaneous estimation of glimepiride and metformin in combined dosage form. Glimepiride shows maximum absorbance at 222 nm and metformin at 228 nm. First method was based on first-order derivative spectroscopy, in which wavelengths selected were 249 nm (zero crossing of metformin) where glimepiride shows considerable absorbance and 258 nm (zero crossing of glimepiride) where metformin shows considerable absorbance. The second method was based on multicomponent mode technique, in which sampling wavelengths selected were 222 and 228 nm. Linearity for detector response was observed in the concentration range of 3-15 µg/mL for glimepiride and 1.0-5.0 µg/mL for metformin for method I; 2.0-10.0 µg/mL for glimepiride and 0.5-2.5 µg/mL for metformin for method II respectively. Accuracy and precision studies were carried out and results were satisfactory. The proposed methods were validated as per ICH guidelines. The developed methods are simple, precise, rugged and economical. The utility of the methods has been demonstrated by analysis of commercially available formulations.

Keywords: Derivative spectroscopy, Multicomponent mode technique, Metformin, Glimepiride, Validation.

INTRODUCTION

Diabetes mellitus is a most serious and chronic disease caused by an absolute or relative lack of resistance to insulin and is characterized by hyperglycemia in the postprandial and/or fasting state. Incidence rates of diabetes mellitus are increasing with increasing level of obesity and also with aging of the general population over the world [1]. Monotherapy with an oral antidiabetic agent is not sufficient to reach target glycemic goals and multiple drugs may be necessary to achieve adequate control. Two or more antidiabetics agents from different pharmacological classes are often needed to achieve adequate blood glucose control. Combination therapy is an important option that combine efficacy of blood glucose reduction and a low side effect profile with convenient once daily dosing to enhance compliance [2].

Glimepiride (GLI) chemically 3-Ethyl-4-methyl-N-(4-[N-((1r, 4r)-4 methyl cyclohexyl carbomyl) sulfamoyl] phenethyl)-2-oxo-2, 5-dihydro-1H pyrrole-1-carboxamide is a third generation sulphonyl urea used to reduce blood glucose levels by stimulating insulin secretions from the beta cells of pancreas. It also increases peripheral insulin sensitivity thereby decrease insulin resistance. Metformin hydrochloride (MET), chemically 1, 1-Dimethyl biguanidine monohydrochloride is an anti-diabetic drug from the biguanide class of oral hypoglycemic agents, given orally in the treatment of non insulin-dependent diabetes mellitus. The combination of sulfonylurea and metformin is largely used because both the drugs are ancient and large number of studies has demonstrated their synergistic effects. An improvement in blood glucose level and HbA1c was solely observed with the association of both drugs. The structures of GLI and MET are shown in the Fig. 1 & 2. Literature review revealed few methods for the determination of MET and GLI individually or in combination with other drugs which include spectrophotometry

[3-5], HPLC [6-8], LC-MS [9], and HPTLC [10, 11] in pharmaceutical formulation or biological fluids. Till now there was no methods reported for simultaneous quantification of GLI and MET in combined dosage form using spectrophotometric method. The present study reports two simple, rapid, accurate, reproducible and economical methods for the simultaneous determination of MET and GLI in tablet formulation using first order derivative and multicomponent mode analysis technique. The proposed methods were validated as per ICH Guidelines.

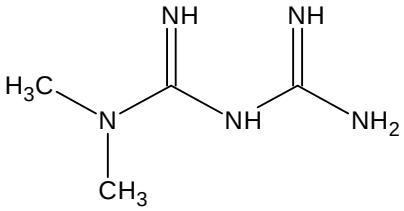

Figure 1: Structure of Metformin

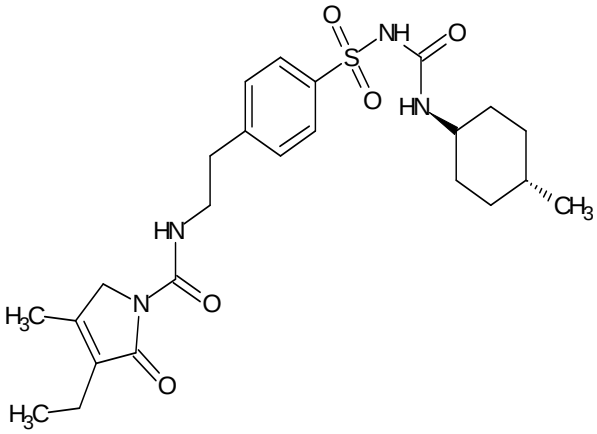

Figure 2: Structure of Glimepiride

EXPERIMENTAL SECTION

Chemicals and Reagents

Metformin and glimepiride were obtained as gift sample from Micro Labs Ltd., Bangalore, India. All chemicals and reagents of analytical grade were purchased from M/s. Merck Chemicals, Mumbai, India. Tablet formulation Gemer 2 (Sun Pharma, Sikkim, India) was procured from a local pharmacy.

Equipment

A double beam UV/Visible Spectrophotometer (Shimadzu, Japan) model UV-1700 with quartz cell 1 cm path length, connected to HP computer version 2.21 was used.

Standard stock solution

Standard stock solution (1.0 mg/ml) of GLI and MET was prepared separately by using 0.1M sodium hydroxide. These stock solutions were further diluted to getting working standard stock solutions.

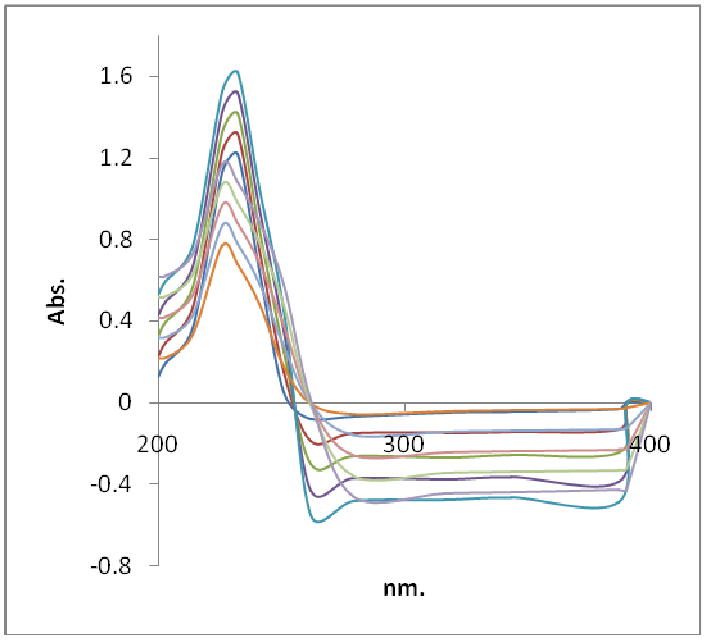

Figure 3: First order derivative spectra for GLI and MET

Sample preparation

Twenty tablets were accurately weighed, pulverized and tablet powder equivalent to 100 mg GLI and MET was transferred into 100 ml volumetric flask, 50 ml 0.1M sodium hydroxide was added. The mixture was sonicated to dissolve, completed to volume with 0.1M sodium hydroxide and filtered through a 0.45 µm membrane filter, discarding first few milliliters. From the above solution suitable aliquots were completed to volume with 0.1M sodium hydroxide to get concentration in the range of 1:250 taking into consideration its amount present in combined tablet formulation.

Method I: First order derivative spectroscopy

The first derivative (D^1) overlain spectra of each drugs (MET, GLI) was found to show zero crossing point assisted in their simultaneous estimation. The first derivative wavelength considered for GLI was 249 and 258 for MET. Calibration curves were constructed with five different concentrations in the range between 3-15 µg/ml and 1-5 µg/ml for GLI and MET respectively (Fig. 3). Each concentration was analyzed thrice. The sample solution of 9 µg/ml of GLI and 3 µg/ml of MET was measured at 249 and 258 nm. The concentration of the drug present in the solution was determined against the calibration curve in quantitation mode [12, 13].

Method II: Multicomponent mode technique

For this method 222.0 nm (λmax of GLI) and 228.0 nm (λmax of MET) were selected as two sampling wavelength for GLI and MET and multicomponent mode of spectrophotometer was used. The data from these scans was used to determine the concentrations of two drugs in tablet sample solutions (Fig. 4). The overlain spectra of five standard binary mixtures (0.20:50, 0.40:100, 0.60:150, 0.80:200, and 1.0:250) were employed to determine the concentration of drug in sample solution by analysis of spectral data of sample solutions with reference to mixture standards [12, 13].

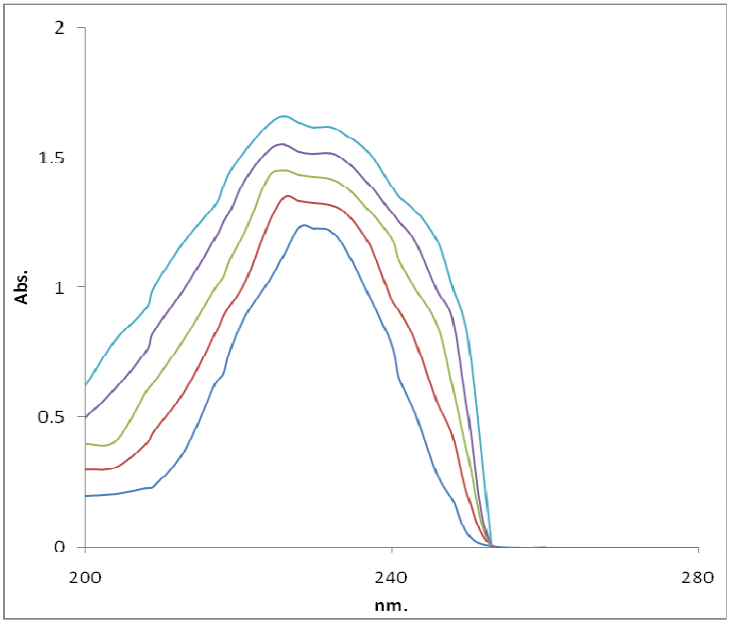

Figure 4: Multicomponent mode spectra for GLI and MET

RESULTS AND DISCUSSION

The aim of this study is to establish and validate simple, sensitive and accurate spectrophotometric method as per the ICH guidelines [14] with satisfactory precision and accuracy.

Linearity and sensitivity

The linearity of method was evaluated thrice by analyzing six concentration of each drug. Linear regression equation was obtained over the concentration range (y = mx+c). Table 1 reveals correlation coefficients, standard deviation of slope (S_b) and intercept (S_a). The sensitivity determined by detection and quantification limit, were calculated based on standard deviation of response and slope.

Table 1: Optical characteristics obtained for GLI and MET by first derivative and multicomponent mode methods

Parameters	First derivative (D^1)		Multicomponent mode	
	GLI	MET	GLI	MET
Range of linearity (µg/ml)	3.0-15.0	1.0-5.0	2.0-10.0	0.5-2.5
S_a	0.00141	0.00071	0.00089	0.00837
S_b	0.00109	0.00089	0.00084	0.00447
Correlation coefficient(r^2)	0.998	0.988	0.997	0.996
LOD (µg/ml)	0.91	0.05	0.52	0.02
LOQ (µg/ml)	2.10	0.20	1.02	0.09

S_a-Standard deviation of slope, S_b-Standard deviation of intercept

Accuracy

The accuracy of the method was determined by method of standard addition. The pre analyzed sample is spiked with 80, 100, 120% of standard drugs and mixtures were analyzed by the proposed methods and the results are re-analyzed. The value for % RSD found to be <1.0 for both the methods, which indicate excellent recoveries as revealed in Table 2.

Table 2: Results of Recovery Study

Drugs	Amount taken (µg/ml)	Amount added (µg/ml)	Amount recovered ± SD*	% RSD	Amount recovered ± SD*	% RSD
			Method I		Method II	
GLI	10	2	11.89±0.018	0.1513	12.11±0.0831	0.6862
		4	14.13±0.013	0.0920	13.72±0.091	0.6632
		6	15.92±0.040	0.2512	16.09±0.054	0.3356
MET	2	0.5	2.47±0.0170	0.6882	2.01±0.0103	0.5124
		1.0	2.92±0.0104	0.3561	2.99±0.014	0.4682
		1.5	3.52±0.009	0.2557	3.49±0.014	0.4011

Method I-First order spectroscopy, Method II-Multicomponent mode,
*SD-Standard deviation, *Mean of three determinations.*

Precision

Precision was ascertained by triplicate estimation of standard drugs for two concentrations (10 µg/ml for GLI and 2 µg/ml for MET) on same day and on three consecutive days. Precision was calculated from the percentage standard deviation (%RSD) for the repeated measurements. The % RSD revealed good precision, Table 3.

Table 3: Result of Precision study

Method	Precision	Amount taken (µg/ml)		% Mean*		% RSD	
		GLI	MET	GLI	MET	GLI	MET
I	Intra-day	10	2.0	99.54	98.95	0.296	0.125
	Inter-day	10	2.0	100.06	101.35	0.357	0.176
II	Intra-day	10	2.0	98.95	99.15	0.845	0.168
	Inter-day	10	2.0	101.07	99.32	0.222	0.482

Method I: First order spectroscopy, Method II: Multicomponent mode
**Mean of three determinations*

Assay of tablet formulation

The assay result of tablet formulation (500 mg MET and 2 mg GLI) for two methods were reported in Table 4. The standard deviation of five replicate analyses for both methods were found to be <1.0. Satisfactory results were obtained, which is given indicate that interference of the excipient matrix is insignificant for the estimation of GLI and MET by proposed methods.

Table 4: Result of Tablet analysis

Parameters	Method I		Method II	
	GLI	MET	GLI	MET
Label claim (mg/tablet)	2	500	2	500
Drug content % ± SD*	98.99±0.15	100.35±0.32	99.65±0.21	100.84±0.13
% RSD	0.15	0.32	0.21	0.13

Method I: First order spectroscopy, Method II: Multicomponent mode
**Mean of five determinations*

CONCLUSION

A simple, accurate, precise and sensitive UV method has been developed for the simultaneous estimation of MET and GLI in tablet dosage form without any interference from excipient. The proposed method is successfully applied for determination of both drugs in tablet dosage form.

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