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SIMULTANEOUS ESTIMATION OF REPAGLINIDE AND METFORMIN BY UV-VISIBLE SPECTROPHOTOMETRY

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ABSTRACT

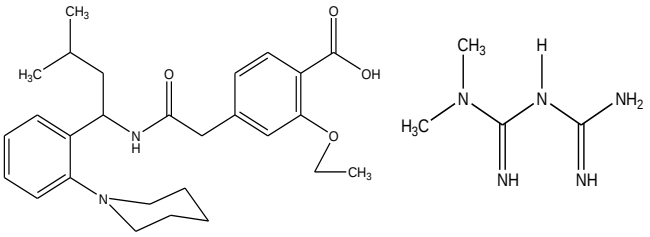
Method for simultaneous estimation of Repaglinide (REP) and Metformin (MET) was developed using methanol as solvent. Both drugs are freely soluble in it. Spectroscopic determination of REP and MET has showed λ_{max} at 248 nm and 237 nm respectively. REP and MET follows Beer-Lambert's law in range of 0.5-3 µg/ml and 50-300 µg/ml respectively. LOD and LOQ values of REP and MET were found to be 0.10 and 0.33 µg/ml and 0.40 and 1.35 µg/ml respectively. The proposed method is recommended for routine analysis since it is rapid, simple, accurate, precise, sensitive and specific.

KEYWORDS: Repaglinide, Metformin, Simultaneous Estimation, UV-Spectrophotometry.

INTRODUCTION

Repaglinide is a new class of non sulphonyl urea oral hypoglycemic agent. It reduces the blood glucose by stimulating the release of insulin from the pancreas. Chemically Repaglinide is (S)-(+)-2-ethoxy-4-[2-(3- methyl-1-[2-(piperidin-1-yl) phenyl] butylamino)-2- oxoethyl] benzoic acid. It is used in the treatment of type II diabetes.

Metformin is 1, 1-Dimethylbiguanidehydrochloride which is used as antidiabitic drug from the biguanide class used in the management of type 2 diabetes. Major action of metformin lay in increasing glucose transport across the cell membrane in skeletal muscle. The structure of Repaglinide and Metformin are given in Figure 1.



(a) (b)

Figure 1. (a) Structure of Repaglinide (b) Structure of Metformin

Standard Repaglinide and Metformin were obtained as a gift sample from Cipla Ltd. and Macleoids Ltd. All chemicals used are of AR grade and were purchased from Qualigens fine Chemicals, Mumbai, India. Marketed formulation Eurepa MF tablet containing REP 1 mg and MET 500 mg was used as sample.

Calibrated glassware's were used throughout the work. For development of analytical method UV- spectrophotometer Model-UV-1800 (Shimadzu, Japan) with spectral bandwidth of 2 nm and 10 mm matched quartz cells were used.

MATERIALS AND METHODS

Preparation of standard stock solutions

An accurately weighed quantity of about 10 mg of pure REP and 50mg of pure MET were dissolved in methanol and diluted to 100 ml in separate volumetric flask. Further dilutions carried out to get final concentration of 100 µg/ml for REP and 500µg/ml for MET.

Selection of analytical wavelengths

Appropriate dilutions were done for each drug from the standard stock solution and scanned in the spectrum mode from 400 nm to 200 nm. REP and MET showed absorbance maxima at 248 nm (Figure 2) and at 237 nm (Figure 3) respectively. Figure 4 represents the overlain spectra of both the drugs.

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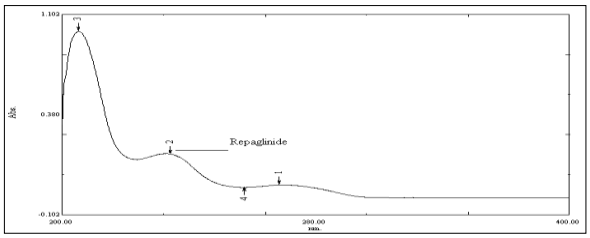

Figure 2. UV spectrum of REP

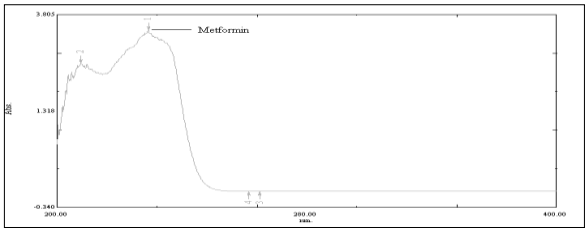

Figure 3. UV spectrum of MET

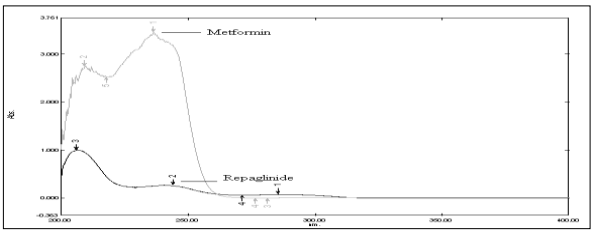

Figure 4. Overlain spectrum of the REP and MET

Selection of analytical concentration ranges

From the standard stock solution of REP, appropriate aliquots were taken in 10 ml volumetric flasks using methanol to obtain working standard solutions of concentrations 0.5- 3.0 µg/ml of REP and 50-300µg/ml of MET. Absorbances for these solutions were measured at 248 nm and 237 nm respectively (Table 2). Calibration curve of absorbance against concentration was plotted for REP (Figure 5) and for MET (Figure 6). Table 1 summarizes the optical characteristics of both the drugs.

Analysis of Mixture containing REP and MET

The method was tested by analyzing a solution containing known concentration of both drugs. The mixed standards was prepared in the ratio of 1:500 containing 0.4, 0.5 and 0.6 µg/ml of REP and 200, 250 and 300 µg/ml of MET respectively by diluting appropriate volumes of standard stock solutions. The scanning of mixed standard solutions was carried out in the range of 400 nm to 200 nm in spectrum mode (Table 2). The absorbance of mixed standard solutions was measured at 248 nm and 237 nm. The concentrations of REP and MET present in mixed standards were calculated using simultaneous equation. (Table 3).

Table 1. Optical characteristics and other parameters

Parameters	REP	MET
Working wavelength (nm)	248	237
Linearity range (µg/ml)	0.5-3.0	50-300
Molar absorptivity	132	7.2
Limit of detection (µg/ml)	0.10	0.4
Limit of quantitation (µg/ml)	0.33	1.33
Y= mx + c		
Slope	0.687	0.006
Intercept	0.034	0.0002
Regression Coefficient	0.999	0.999

Table 2. Absorbance of mixed standards containing REP and MET

Sr. No.	Mixed Standards		Abs. at 248 nm	Abs. at 237 nm
	Conc. of REP (µg/ml)	Conc. of MET (µg/ml)		
1.	0.4	200	0.056	1.385
2.	0.5	250	0.068	1.739
3.	0.6	300	0.084	2.081

Procedure for analysis of tablet formulation

For analysis of tablet formulation twenty tablets were weighed and average weight was determined and then triturated to a fine powder. A quantity equivalent to 1 mg of REP and 500 mg of MET was weighed and transferred to a 100 ml volumetric flask containing 70 ml of methanol.

These solution were sonicated for 20 min to dissolve the active ingredients and the volume was made up to 100 ml with methanol and filtered through Whatman filter paper no. 41 Various dilutions of the tablet stock solutions were scanned and the absorbance of these solutions were measured at 248 nm and 237 nm respectively . Concentrations of two drugs in the sample solutions were calculated using simultaneous equation. The analysis procedure was repeated six times. The results of marketed tablet formulation are given in Table 4.

RESULTS AND DISCUSSION

METHOD VALIDATION

Linearity

Both drugs followed the Beer-Lamberts law in the range of 0.1-0.6 µg/ml and 50-300 µg/ml for REP and MET respectively. Regression coefficient for REP and MET was 0.998 and 0.999 respectively.

Accuracy (Recovery studies)

Accuracy was performed by studying recovery using standard addition method at three levels i.e. 80, 100 and 120 % of the tablet label claim as per ICH guidelines. At 80 % level sample containing 1.0 mg of REP and 500 mg of MET was weighed and transferred to a 100 ml volumetric flask. To it, 0.8 mg of standard REP and 400 mg of standard MET was added and mixed thoroughly.

Table 3. Results of mixture containing REP and MET

Sr. No.	Amount Present * (µg/ml)		Amount Found* (µg/ml)		% Amount Found*	
	REP	MET	REP	MET	REP	MET
1.	0.4	200	0.40	199.93	100.00	99.96
2	0.5	250	0.49	249.89	98.00	99.95
3	0.6	300	0.61	299.71	101.66	99.90

*Each value is a mean of six observations

Mixed content transferred to 70 ml methanol and sonicated for 20 min to dissolve the active ingredients completely and the volume was made up to 100 ml with methanol and filtered through Whatman filter paper no. 41.

Similarly to perform recovery studies at 100 % of the test concentration, tablet powder containing 1.0 mg of REP and 500 mg of MET was weighed. To it, 1.0 mg of standard REP and 500 mg of standard MET was added and for 120 % level, 1.2 mg of standard REP and 600 mg of standard MET was added to the tablet powder equivalent to 1 mg of REP and 500 mg of MET. These mixed powder dissolved in 70 ml methanol and sonicated for 20 min to dissolve the active ingredients completely.

Table 4. Results of marketed tablet formulation

Sr. No.	Label Claim (mg/tab)		Amount Found (mg/tab)		% of Label Claim	
	REP	MET	REP	MET	REP	MET
1	1	500	0.99	499.72	99.00	99.94
2	1	500	0.98	497.91	98.00	99.58
3	1	500	1.00	497.84	100.00	99.76
4	1	500	1.01	499.97	101.00	99.99
5	1	500	0.99	500.12	99.00	100.02
6	1	500	0.99	500.07	99.00	100.01
				Mean*	99.33	99.88
				SD	1.0327	0.1769
				% RSD	1.0396	0.1771

Formulation: *Eurepa MF* (Torrent Pharmaceutical Ltd, Ahmedabad)

Table 5. Standard calibration for REP and MET

Sr. No.	For Repaglinide		For Metformin	
	Conc. (µg/ml)	Abs.* at 248 nm	Conc. (µg/ml)	Abs.* at 237 nm
1.	0.5	0.070	50	0.361
2.	1.0	0.132	100	0.656
3.	1.5	0.211	150	1.037
4.	2.0	0.271	200	1.389
5.	2.5	0.34	250	1.735
6.	3.0	0.418	300	2.086

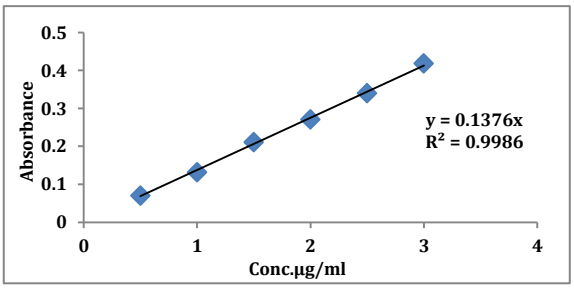

Figure 5. Calibration curve of REP

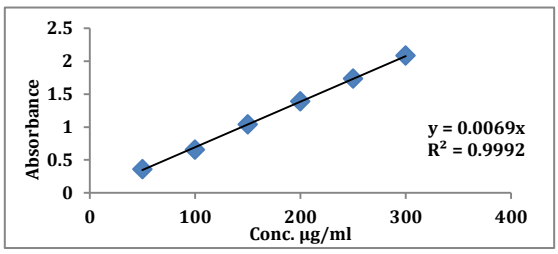

Figure 6. Calibration curve of MET

Volume was made up to 100 ml with methanol and filtered through Whatman filter paper no. 41. At each level suitable aliquots were pipetted out from stock solution and diluted to 10 ml with methanol. Diluted samples were analyzed as per the procedure for Tablet formulations. The results of the recovery studies were validated statistically. The results of recovery studies are given in Table 6.

Precision of method

Precision of the method was done by using stock solutions in the ratio of 1:500 containing 0.2 µg/ml of REP and 100 µg/ml of MET. System repeatability was done by repeating the assay three times of six replicate dilutions of the same concentration after every two hours on the same day for intraday precision. Inter-day precision was carried out by performing the assay of six sample sets after 24 hours and 48 hours. The results of intermediate precision are given in Table 7.

Table 6. Results of recovery studies

Level of (%) Recovery	Amount present (mg/tab)		Amount of standard added (mg)		Total amount recovered (mg)		% Recovery*	
	REP	MET	REP	MET	REP	MET	REP	MET
80	1	500	0.8	400	1.80	900.85	100.18	100.09
100	1	500	1.0	500	1.99	1000.08	99.83	100.00
120	1	500	1.2	600	2.19	1098.81	99.69	99.88
						Mean	99.90	99.99
						SD	0.2060	0.0860
						% RSD	0.2062	0.0860

*Each value is the mean of three observations

Table 7. Results of intermediate precision

Formulation	Parameter	Intra-day precision*	Inter-day precision*
REP	Mean	99.64	100.03
	SD	0.3074	0.7373
	% RSD	0.3085	0.7370
MET	Mean	100.04	99.89
	SD	0.1446	0.2218
	% RSD	0.1445	0.2220

*Each value is a mean of six observations

LOD and LOQ

The limit of detection (LOD) and the limit of quantification (LOQ) of the REP and MET, were calculated using the standard deviation of responses (N) and slopes (S) of respective calibration curves using signal-to-noise ratio.

$$LOD = 3.3 \times N/S$$

$$LOQ = 10 \times N/S$$

Table 8: Results of LOD & LOQ

Parameters	REP	MET
Limit of detection (µg/ml)	0.10	0.4
Limit of quantitation (µg/ml)	0.33	1.33

CONCLUSION

For simultaneous estimation of REP and MET by UV-spectrophotometry novel analytical method was developed in which methanol is used as solvent. REP and MET has shown λ_{max} at 248 nm and 237 nm respectively in methanol. REP and MET follows Beer-Lambert's law in range of 0.5-3.0 µg/ml and 50-300 µg/ml. Commercial formulation containing REP and MET were analyzed by proposed method. Mean assay values in Eurepa MF Plus were found to be 99.33±1.0327 and 99.88±0.1769 respectively. The accuracy of method was determined by recovery studies. Recovery study is performed by standard addition method at three different levels viz. 80, 100, 120% of labeled claims as per the ICH guidelines. Three replicate analyses were carried out at each level. The mean recovery was found to be 99.90±0.2060 % for REP and 99.99±0.0860 % for MET in tablet samples respectively indicating that the method has required accuracy and there was no interference from excipients of tablets. The RSD value below 2% indicated that the method has required precision. LOD and LOQ values at 248 and 237 were found to be 0.10 and 0.4 µg/ml for REP and 0.33 and 1.33 µg/ml for MET.

Thus, the developed method was simple, accurate and precise and can be used for routine analysis of REP and MET in pharmaceutical preparation.

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