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DEVELOPMENT AND VALIDATION OF CHROMATOGRAPHIC AND SPECTROSCOPIC METHODS FOR ESTIMATION OF REPAGLINIDE AND METFORMIN HCl IN COMBINED DOSAGE FORM

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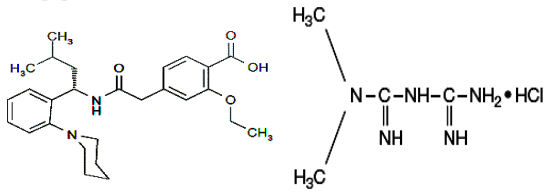
ABSTRACT

Two simple, accurate and reproducible methods have been developed for the determination of repaglinide and metformin as bulk or simultaneously in their combined dosage form. The first is a reversed phase HPLC method using C_{18} column, mobile phase consisting of methanol-0.2% heptane sulphonate sodium (70:30 v/v) at a flow rate of 1ml/min and UV detection at 240 nm. Beer's plot shows good correlations in the concentration range of 1-10 μgml^{-1} for both drugs. The second method is a derivative spectrophotometry, in which the two drugs were quantified using second derivative responses at 287 nm for repaglinide and at 234 or 252 nm for metformin with a linearity range of 5- 40 or 2-12 μgml^{-1} for the two drugs, respectively. The proposed methods were validated and successfully applied to the determination of both drugs in their pharmaceutical formulation with mean recoveries of 99.58-100.45 ± 0.386-0.931. The results obtained were statistically compared to those obtained from a reference method and were found to be in good agreement.

Keywords: Repaglinide, metformin, HPLC, second-derivative spectrophotometry, pharmaceutical formulation.

1. INTRODUCTION

Repaglinide (Rep); 2-ethoxy-4-[2-(3-methyl-1-[2-(1-piperidinyl) phenyl] butylamino)-2-oxoethyl] benzoic acid is a fast acting prandial oral hypoglycemic agent for patients with non-insluin-dependent diabetes mellitus [1]. It reduces the fasting glucose concentrations by increasing the amount of insulin released by the pancreas [2]. Metformin hydrochloride (Met); (1-carbamimidamido-N, N dimethyl methan imidamide is an orally administrated antihyperglycemic. It improves control of glycemia primarily by inhibiting hepatic gluconeogenesis and glucogenolysis [1]. Failure of monotherapy for treating type II diabetes mellitus leads to a switch to combination of various antidiabetic agents. Accordingly, the combined use of metformin and repaglinide improved patient compliance through controlling the basal glycemia and post prandial levels [3].



Repaglinide Metformin HCl

Fig 1: Chemical structure of Rep and Met

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Literature survey of Rep and Met revealed few methods based on UV- spectrophotometry and chromatography have been reported for determination of both drugs in single [4-8] or in their combination [9-13]. The present work describes newly developed and validated HPLC and second-derivative spectrophotometric methods for simultaneous estimation of Rep and Met in bulk and in tablets. So far marketed formulation of both drugs is not available in Egyptian market. Hence, a laboratory sample of tablet dosage form was developed to check the applicability of the developed methods.

2. EXPERIMENTAL

2.1. Instrumentation

- HPLC system (Perkin – Elmer, USA) consists of binary pump, equipped with photo diode array (PDA) detector. Samples were injected (auto sampler injector) into thermo C_{18} column (250 x 4.6 x 5µ particle size). Chromatographic analysis was carried out using Total Chrom software and Shimadzu UV/Vis spectrophotometer (pc-1601, Tokyo, Japan) with 1-cm quartz cell, Jenway 3510 pH meter (USA).

2.2. Pure Samples

- Rep and Met were kindly supplied by Egyptian International Pharmaceutical Industries Co.(EIPICO), 10th of Ramadan City, Egypt. Its purity was found to 99.72 or 99.95%, respectively as stated by the supplier.

- Chlorzoxazone was kindly supplied by Egyptian Company for Chemicals and Pharmaceuticals (ADWIA), 10th of Ramadan City, Egypt.

2.3. Materials and Reagents

- Methanol HPLC grade (Sigma, Aldrich)
- O-phosphoric acid and methanol (El-Nasr).
- Acetonitrile (BDH).
- Heptane sulphonate sodium (sigma)

2.4. Standard Solutions

0.1 mg mL^{-1} standard solutions of the two drugs in methanol were prepared to be used for derivative method. Working methanolic solutions of 0.05mg ml^{-1} Rep and 0.01mg mL^{-1} Met were prepare by further dilution to be used for HPLC method. 0.1 mg ml^{-1} standard chlorzoxazone solution was used as internal standard.

2.5. Procedures

Chromatographic Conditions

HPLC system was operated isocratically at ambient temperature, using a mobile phase of methanol – heptane sulphonate sodium (pH adjusted to 3 using O-phosphoric acid) in the ratio 70:30 v/v at a flow rate of 1 mL/min within a run of 20 min. Prior to use, mobile phase was degassed by an ultrasonic bath and filtered by a millipore vacuum filter system equipped with 0.45µm high vacuum filter. Both drugs were detected at 240 nm.

Selection of Wavelengths

Spectra of standard drug solutions (20 µg mL^{-1} Rep and 6 µg ml^{-1} Met) were scanned in the spectrum mode between 200 and 400 nm. These zero order spectra were treated to obtain the corresponding second order derivative spectra with an interpoint distance of 8 nm and scaling factor of 2 using memory channels, the zero crossing point values were recorded. Wavelength of 287 nm was selected for quantitation of Rep (where the derivative response for Met was zero). Similarly, 234 or 252 nm was selected for the determination of Met as the derivative response for Rep was found to be zero.

Construction of Calibration Curves

HPLC Method

Aliquots from standard methanolic drugs solutions (0.1 mg mL^{-1}) equivalent to 0.01- 0.1 mg Rep or Met were transferred into a series of 10-mL volumetric flasks. 0.2 mL of chlorzoxazone standard solution (0.1mg mL^{-1}) was added to each volumetric flask as internal standard and then diluted to the volumes with methanol. Triplicate 20 µL of each obtained solution was injected and different chromatograms were recorded at 240 nm under the previously described chromatographic conditions. Chromatograms were recorded where calibration curves relating peak areas ratio to corresponding drug concentration were constructed.

Derivative Spectrophotometric Method

Appropriate aliquots of 0.05-0.4 mg Rep or 0.02-0.12 mg Met, from their standard solutions (0.1 mg mL^{-1}) were accurately transferred into different 10-mL volumetric flasks and diluted to the mark with methanol. Second order derivative spectra of the drugs were recorded. Calibration curves were constructed relating peak troughs at 287 nm and at 234 for Rep and Met, respectively together with peak amplitude at 252 nm for Met to the corresponding drug concentration.

Consequently, regression equations were computed for both drugs using the two proposed methods from which different concentrations of unknown drugs could be determined.

Application to Laboratory Formulated Tablets

Laboratory tablet sample (2 mg Rep and 500 mg Met) was formulated using directly compressible microcrystalline cellulose as a diluent as well as binder. Magnesium stearate and talc were used as antiadherent and glidant, respectively to get a final tablet of approximate average weight of 700 mg. Five tablets were accurately weighed, powdered and mixed well. A portion of the powder equivalent to one tablet (2 mg Rep and 500 mg Met) were sonicated with 20 mL methanol for 10 minutes then filtered into a 25- mL volumetric flask. The volume was diluted with methanol to obtain a stock solution claimed to contain 0.08 mg mL^{-1} Rep and 20 mg mL^{-1} Met to be used for the analysis of Rep by the proposed methods. 0.5 mL of this solution was diluted to 100 mL methanol giving a concentration of 0.1 mg mL^{-1} be used for the estimation of Met.

3. RESULTS AND DISCUSSION

The present study aimed to develop simple, sensitive, precise and accurate HPLC and derivative spectrophotometric methods for the simultaneous estimation of Rep and Met.

3.1. Method Development

HPLC Method

To optimize various HPLC parameters such as peak shape, peak symmetry and run time, several trials were taken. Suitable mobile phase was determined by performing various trials including methanol-water at varying pH and composition. Mixtures of commonly used solvents like water, methanol and acetonitrile with or without buffers in different concentrations were tested as mobile phases. A mixture of methanol-0.2% heptane sulphonate sodium in the ratio of 70:30 V/V was proved to be the most suitable since the chromatographic peaks obtained were well defined, resolved and free from tailing. During the optimization procedure, several chromatographic conditions were attempted using a C_{18} thermo column. Different flow rates at different detector response were also tried. Optimal flow rate was found to be 1 ml/min and detection was achieved at 240 nm. Drugs were analyzed under the optimized chromatographic conditions and retention times were found to be 14.21 and 3.42 for Rep and Met, respectively, Fig. (2).

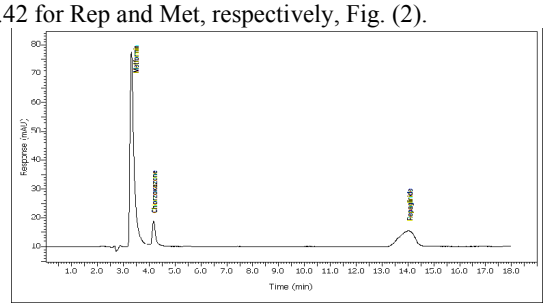

Fig.2: HPLC Chromatogram of a mixture of Rep and Met with chlorzoxazone (internal standard)

Second Derivative Spectrophotometric Method

The second method eliminating the interference in spectra is the second derivative method. The zero-order spectra of the two cited drugs showed some overlapping; Fig. (3). However, conversion of zero-order spectra to second derivative spectra permitted the determination of Rep at 287 nm and Met at 234 or 252 nm; Fig. (4).

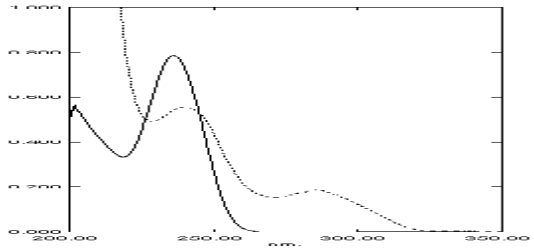

Fig 3: Zero order spectra of Rep (—) and Met (-----) in methanol

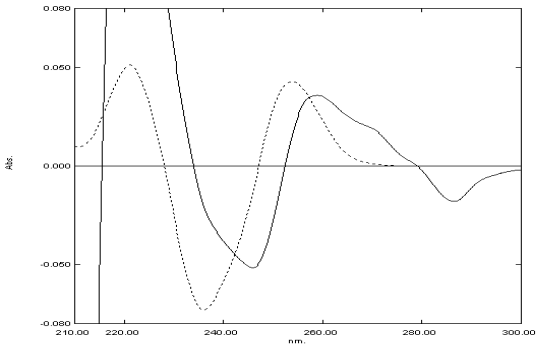

Fig. 4: Second derivative spectra of Rep (—) and Met (-----) in methanol

3.2. Method Validation

The methods were validated as per ICH guidelines [14].

System suitability:

System suitability test was carried out to perform the reproducibility of the system for the analysis to be performed. Parameters measured in this study are retention time, theoretical plates, tailing factor and resolution; table (1).

Table 1: Systems suitability parameter for the proposed HPLC method

Parameter	Rep	Met
Retention time (min)	14.21	3.42
Theoretical plates	6832	4761
Tailing factor	1.240	1.091
Resolution	13.24	

Linearity:

The linearity was determined in the range of 1-10 µg ml^{-1} for the two drugs using HPLC method. For derivative method calibration curves were found to be linear in the range of 5-40 µg ml^{-1} for Rep and 2-12 µg ml^{-1} for Met. Correlation coefficients were calculated and ranged from 0.9903 to 0.9982; table (2).

Accuracy:

The accuracy of the proposed methods was demonstrated by analyzing different concentrations covering the points in the calibration range for Rep and Met. The average percentage recovery at each concentration was determined and found to range from 99.05 to 100.58% for both drugs using the two proposed methods; table (2).

Precision:

Intraday and interday precision (calculated as % RSD) of the proposed methods were determined by estimating the corresponding responses of three different concentrations with-in linearity range on the same day and on three different days, respectively. % RSD values for the two methods were found to be less than 2% indicating good precision of the developed methods; table (2).

LOD and LOQ:

The experimental limit of detection (LOD) and limit of quantitation (LOQ) were estimated according to ICH [14] using two criteria; the slope of the calibration curve and the standard deviation of the blank; table (2).

Table 2: Regression analysis and validation parameters for the determination of Rep and Met by the proposed methods

Parameter	HPLC method		Derivative spectrophotomeric method		
	Rep	Met	Rep	Met	
				λ_{234}	λ_{252}
Linearity (µg/mL)	1-10	1-10	5-40	2-12	2-12
Slope ± S.D.	0.4124	1.1936	-0.0009 ± 4.89E-05	0.011 ± 4.7E-04	0.011 ± 5.2E-04
Intercept ± S.D.	0.0642	0.1674	-0.0003 ± 12E-03	-0.010 ± 4E-03	-0.004 ± 3E-03
Correlation coefficient (r)	0.9982	0.9978	0.9927	0.9928	0.9903
LOD	0.14	0.28	1.01	0.32	0.39
LOQ	0.47	0.93	3.13	1.06	1.24
Accuracy (%)	99.99	100.58	100.21	99.74	99.05
Precision (% RSD)					
Intraday	1.23	0.52	1.33	0.95	1.26
Interday	0.96	0.69	0.84	0.76	0.91

Specificity:

Laboratory prepared mixtures of the two studied drugs at different ratios were analyzed by the proposed methods applying the recommended procedures previously mentioned. Good recoveries of both drugs ranging from 99.28 to100.69 ± 0.48-0.87 were obtained; table (3).

Table 3: Determination of Rep and Met in their laboratory prepared mixtures by the proposed methods

Rep/Met Ratio	HPLC method		Derivative spectrophotomeric method		
	Recovery %		Recovery %		
	Rep	Met	Rep	Met	
				λ_{234}	λ_{252}
1 : 2	100.76	99.94	101.22	99.21	98.62
1 : 1	99.52	100.31	100.21	99.52	99.59
2 : 1	101.62	100.37	100.35	99.79	99.67
3 : 1	100.89	101.53	99.65	100.52	99.24
Mean ± S.D.	100.69 ± 0.87	100.54 ± 0.69	100.36 ± 0.65	99.68 ± 0.48	99.28 ± 0.49

Robustness:

System suitability parameters and results of assay under the changed experimental conditions were found within acceptable limits. In case of HPLC method, changes in mobile phase ratio, flow rate and detection wavelength was adopted, whereas for UV-method robustness was assessed by changes in detection wavelength and source of methanol; table (4).

Table 4: Robustness study for the determination of Rep and Met by the proposed

Parameter	Changed condition	% RSD (Rep)	% RSD (Met)	
HPLC method				
Flow rate	± 10%	0.68 - 0.89	1.18 - 1.70	
Mobile phase ratio	± 2%	0.96 - 0.85	0.14 - 0.77	
Derivative spectrophotometric method				
			λ_{234}	λ_{252}
Wavelength of detection	±2 nm	0.21 - 0.63	0.74 - 0.98	0.39 - 0.62
Solvent source	Sigma and El-Nasr	0.87 - 1.52	0.41 - 0.59	0.90 -1.23

Stability of Standard Solutions – Solution stability was checked by analyzing samples kept at room temperature and at refrigerator. Difference in results less than 2% when compared with freshly prepared standard solution of both drugs by both HPLC and derivative methods indicates the solutions can be analyzed within 2 days at room temperature or 7 days at refrigerator.

3.3. Assay of Laboratory Formulated Tablets

The proposed methods were successfully applied to determine the two drugs in their formulation. Satisfactory results were obtained for each drug in good agreement with the label claim (mean recovery ranging from 99.58 to 100.45%). Results obtained were statistically compared with those obtained by the reported method [9]. Calculated t and F values at 95% confidence level were less than the tabulated ones confirm accuracy and precision of the proposed methods; table (5)

Table 5: Results obtained for determination of Rep and Met in their formulation compared with reported method [9]

Parameter	HPLC method		Derivative spectrophotometric method			Reported method[9]	
	Rep	Met	Rep	Met		Rep	Met
				λ_{234}	λ_{252}		
Linearity range (µg/mL)	1-10	1-10	5-40	2-12	2-12	10-50	2-12
N	6	6	6	6	6	5	5
Mean %	99.77	100.45	99.58	99.92	99.99	100.21	99.17
S.D.	0.475	0.386	0.931	0.743	0.865	0.990	0.830
t	1.245 (2.262)	0.636 (2.262)	1.086 (2.262)	1.582 (2.262)	1.595 (2.262)		
F	4.344 (5.91)	4.626 (5.91)	1.131 (5.91)	1.248 (5.91)	1.086 (5.91)		

- Figures in parenthesis are the theoretical t and F values at p = 0.05.
- Reported method is a UV difference spectroscopic method depends on measuring the absorbance of repaglinide at 304 nm and metformin HCl at 234.5 nm in 0.1 N NaOH versus 0.1 N HCl.

4. CONCLUSION

The proposed study describes an HPLC and second derivative spectrophotometric methods for estimation of repaglinide and metformin HCl in bulk or in their combination. The methods were validated and found to be simple, sensitive, accurate, precise and devoid from any potential interference. Hence, they can be effectively applied for the routine analysis of both drugs.

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