SIMULTANEOUS ESTIMATION OF GLIMEPIRIDE, PIOGLITAZONE HYDROCHLORIDE AND METFORMIN HYDROCHLORIDE BY DERIVATIVE SPECTROPHOTOMETRY METHOD

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ABSTRACT
The simple and accurate method of analysis to determine Glimepride (GLM), Pioglitazone hydrochloride (PIO) and Metformin hydrochloride (MET) in combined dosage forms was developed using second-derivative spectrophotometry and. GLM, PIO and MET in combined preparations (tablets) were quantified using the second-derivative responses at 233.4 nm for GLM, 265.4 nm for PIO and 252.6 nm for MET in spectra of their solutions in methanol. The calibration curves were linear [correlation coefficient (r) = 0.9990 for GLM, 0.9990 for PIO and 0.9990 for MET] in the concentration range of 5-25 μg/ml for GLM, 5–25 μg/ml for PIO and 2–12 μg/ml for MET. The method was validated and found to be accurate, precise, and specific. The method was successfully applied to the estimation of GLM, PIO and MET in combined tablet formulations.
KEYWORDS: Derivative spectrophotometry, Glimepiride, Pioglitazone hydrochloride, Metformin hydrochlorie

INTRODUCTION
Glimepiride is identified as 1-[[p-[2-(3-ethyl-4-methyl-2-oxo-3-pyrroline-1carboxamido) ethyl] phenyl]sulfonyl]-3-(trans-4-methylcyclohexyl)urea and an oral blood-glucose-lowering drug of the sulfonylurea class. Pioglitazone is 5-(4-[2-(5-ethylpyridin-2-yl) ethoxy]benzyl)thiazolidine-2,4-dione and of the class thiazolidinedione (TZD) with hypoglycemic (antihyperglycemic, antidiabetic) action. Metformin is *N,N*-dimethylimidodicarbonimidic diamide and oral anti-diabetic drug from the biguanide class. It is the first-line drug for the treatment of type 2 diabetes. Recently, PIO has been combined with MET to obtain a synergistic effect on lowering triglyceride levels. Also, MET inhibits hepatic gluconeogenesis, whereas PIO enhances insulin sensitivity in the muscles. In addition, MET improves peripheral insulin sensitivity, while PIO inhibits gluconeogenesis. In patients where monotherapy with Glimepiride or metformin has not produced adequate glycemic control.[1]
The co-administration of two or more oral antidiabetic drugs may render treatment regimens difficult to follow. Combining oral antidiabetic agents into a single tablet provides a means of intensifying antidiabetic therapy while supporting good patient compliance.[2]

A literature survey regarding quantitative analysis of these drugs revealed that attempts were made to in bulk and formulation. Estimation of PIO in human plasma[3-4] is also reported. As far as MET is concerned, many reports are available for its estimation in bulk and formulation using spectrophotometry [5], potentiometry[6], and ion-pair LC[7]. Estimation of MET in combination with glipizide in tablets using spectrophotometry[8] and with glibenclamide in tablets by LC[9] was also reported. This paper describes second-derivative spectrophotometry method for the determination of GLM, PIO and MET in mixtures without prior separation. Also, the proposed method is shown to be useful in determination of all drugs in combined tablet formulations.

MATERIALS AND METHODS
Apparatus
A double-beam UV VIS Spectrophotometer SL 210 (ELICO Ltd., Hyderabad, India) having 2 matched quartz cell with a 1 cm light path was used for spectrophotometric analysis.
Reagents and Materials
Analytically pure GLM, PIO and MET were procured as gift samples from Torrent Pharmaceutical Ltd. (Baddi, India). Analytical reagent grade methanol (S.D. Fine

Chemicals, Mumbai, India) was used for the preparation of solutions. Tablet formulation A (GLIMADAY-P1, Wockhardt Limited, Mumbai, India) and B (GLIMADAY-P2, Wockhardt Limited, Mumbai, India) were procured from a local market with the labeled amounts of 1mg GLM, 15 mg of PIO and 500 mg of MET.

Preparation of Standard Solutions
GLM standard solution: 10 mg of standard GLM was weighed and transferred to 100mL volumetric flask and dissolved in methanol and further diluted with the methanol to obtain standard solutions of GLM in range of 5-25µg/ml.

PIO standard solution: 10 mg of standard PIO was weighed and transferred to 100mL volumetric flask and dissolved in methanol and further diluted with the methanol to obtain standard solutions of PIO in range of 5-25µg/ml.

MET standard solution: 10 mg of standard MET was weighed and transferred to 100mL volumetric flask and dissolved in methanol and further diluted with the methanol to obtain standard solutions of MET in range of 2-12µg/ml.

Selection of Wavelengths for Estimation of GLM, PIO and MET
Standard solutions of GLM, PIO and MET were diluted appropriately with methanol to obtain solutions containing 25µg/ml GLM, 25µg/ml PIO and 12µg/ml MET. Spectra of these diluted solutions were scanned in the spectrum mode between 200 and 350 nm, with a bandwidth of 1.8 nm vs methanol as a blank. These zero-order spectra of GLM, PIO and MET were treated to obtain corresponding first- and second-order derivative spectra with an interpoint distance of 5 nm in the range of 200-350 nm.

Derivative Condition
The second-order derivative spectra were overlapped. The zero crossing point (ZCPs) values of MET at which the GLM and PIO showed some derivative response were recorded. The wavelength 233.4 nm was selected for the quantification of GLM (where the derivative response for PIO and MET was zero). Similarly, 265.4 nm was selected for the quantification of PIO (where the derivative response for GLM and MET was zero)and 252.6nm was selected for quantification of MET(where the derivative response for GLM and PIO was zero). Characteristic wavelengths (ZCPs) for GLM, PIO and MET were confirmed by varying the concentration of all drugs.

Calibration Curves for GLM, PIO and MET
The standard solutions of GLM (100µg/ml), PIO (100µg/ml) and MET (100µg/ml) were used to prepare three different sets of diluted standards, series A, B and C as follows.
(a) Series A: This series consisted of GLM solutions of various concentrations (5-25µg/ml) prepared by pipetting appropriate volumes (0.5, 1.0, 1.5, 2.0, and 2.5 ml) of GLM standard solution into 10 ml volumetric flasks, and the volume was diluted to volume with methanol.
(b) Series B: This series consisted of PIO solutions of various concentrations (5-25µg/ml) prepared by pipetting appropriate volumes (0.5, 1.0, 1.5, 2.0 and 2.5 ml) of PIO standard solution into 10 ml volumetric flasks and diluting to volume with methanol.
(c) Series C: This series consisted of MET solutions of various concentrations (2-12µg/ml) prepared by pipetting appropriate volumes (0.2, 0.4, 0.6, 0.8,1.0 and 1.2ml) of MET standard solution into 10 ml volumetric flasks and diluting to volume with methanol.

Method Validation
The method was validated for accuracy, precision, specificity, and robustness by the following procedures[10].
Accuracy: The accuracy of the method was determined by calculating recoveries of GLM, PIO and MET by the method of standard additions. This study was performed by addition of known amounts of Glimepride, Pioglitazone hydrochloride and Metformin hydrochloride to a known concentration of the commercial tablets. The amounts of standard recovered were calculated in the terms of mean recovery with the upper and lower limits of percent relative standard deviation.
Precision: The experiments were repeated three times a day for intra day precision and on three different days for inter day precision.The developed method was found to be precise for intra day and inter day precision on the basis of % RSD values for Glimepride, Pioglitazone hydrochloride and Metformin hydrochloride.
Limit of Detection (LOD) and Limit of Quantitation (LOQ):
LOD and LOQ were measured by taking 10 blank determinations and were calculated as follows:
LOD= (3.3 * SD of analytical blank signals) / slope of calibration curve
LOQ= (10* SD of analytical blank signals) / slope of calibration curve
Linearity and Range
The developed method showed good linearity for Glimepride in the range of 5-25 µg/ml, for Pioglitazone hydrochloride 5-25 µg/ml and for Metformin

hydrochloride 2-12 µg/ml with the co-relation co-efficient 0.999, 0.999 and 0.999 for GLM, PIO and MET respectively

Determination of GLM, PIO and MET in Their Combined Dosage Forms Using Second-Derivative Spectrophotometry

(a) Sample preparation: Twenty tablets were weighed and finely powdered. Powder equivalent to 1 mg GLM, 15 mg PIO and 500 mg MET was accurately weighed and transferred to a volumetric flask. Methanol (15 ml) was transferred to the volumetric flask and sonicated for 5 min. The flask was shaken, and the volume was diluted upto 100ml with same mixture. (stock solution)

The above stock solution was filtered through Whatman filter paper (No. 41). A 1 ml aliquot was taken and transferred to a 10 ml volumetric flask. The volume was diluted to the mark with of methanol to give a solution containing 1 µg/ml GLM, 15 µg/ml PIO and 500 µg/ml MET (Solution 1), which was used for the estimation of GLM. From stock solution, 1 ml was transferred to a 20 ml volumetric flask. The volume was diluted to the mark to give a solution containing 0.5 µg/ml GLM, 7.5 µg/ml PIO and 250 µg/ml MET (Solution 2), which was used for the estimation of PIO. From solution 2, 1 ml was transferred to a 25 ml volumetric flask. The volume was diluted to the mark to give a solution containing 0.02 µg/ml GLM, 0.3 µg/ml PIO and 10 µg/ml MET (solution 3), which was used for the estimation of MET. Due to the large difference in content of these 3 drugs in the formulation, they were estimated in different dilutions, first GLM at lower dilution and then MET at higher dilution. This avoids any need for addition of standard PIO to the sample solution.

(b) Estimation of GLM, PIO and MET.-The derivative response of Solution 1 was measured at 233.4 nm, solution 2 at 265.4nm and of Solution 3at 252.6nm for quantification of GLM, PIO and MET, respectively. The amounts of PIO and MET present in the sample solution were determined by fitting the derivative responses into the equation of the straight line representing the calibration

RESULTS AND DISCUSSION

Figure 1 shows overlaid zero-order spectra of GLM, PIO and MET, and the spectra were found to be similar in nature and overlapping. Hence, the derivative graphical method was used to estimate GLM, PIO and MET in presence of each other.

The overlaid first-derivative spectra of PIO and MET showed a ZCP of MET where PIO could be analyzed, but the ZCP of PIO shifted with an increase in concentration; hence, the first-derivative method could not be used for estimation of both the drugs. The second-derivative spectra (D2) of GLM, PIO and MET were found to be appropriate for the determination of GLM, PIO and MET by having separated ZCPs in methanol. At 233.4 nm GLM gives significant derivative response and PIO & MET has zero absorbance. At 265.4nm PIO gives significant derivative response and GLM & MET has zero absorbance. At 252.6nm MET gives significant derivative response and GLM & MET has zero absorbance. Therefore, 233.4 nm was selected for the estimation of GLM, 265.4 nm was selected for PIO and 252.6 nm was selected for the estimation of MET (Figure2).

It was also observed that with the increase in PIO concentration, the derivative response at 227.55 nm increased. The responses for GLM were found to be linear in the concentration range of 5-25[micro]g/mL, with a correlation coefficient (r) of 0.9990. Similarly, the derivative responses for PIO and MET at 265.4 nm and 252.6 nm were linear in the concentration range of 5-25 [micro]g/mL and 2-12 [micro]g/mL, with r = 0.9990 and r= 0.9990 respectively. The limits of detection for GLM, PIO and MET were 0.116, 0.069 and 0.045 [micro]g/mL, respectively.

The recoveries of GLM, PIO and MET were found to be 99.30%, 99.73% and 99.63% respectively, which are satisfactory. Excipients used in the specificity studies did not interfere with derivative response of either of the drugs at their respective analytical wavelengths. Also, no significant change in derivative response of GLM, PIO and MET was observed after 24 h. Hence, the method is specific and accurate for estimation of GLM, PIO and MET. The validation parameters are summarized in Table-1.

The proposed second-derivative spectrophotometry method was applied to the determination of GLM, PIO and MET in their combined tablet formulations. The results obtained for GLM, PIO and MET were comparable with the corresponding labeled amounts (Table-2).

The proposed method was found to be simple and rapid for the determination of GLM, PIO and MET in the presence of each other. The method was validated and found to be simple, sensitive, accurate, and precise. In spite of the low content of GLM, method was successfully used to estimate the amount of GLM, PIO and MET present in tablet formulations. Thus the developed method was successfully utilized for the

simultaneous determination of labeled drugs without any prior separation or pretreatment.

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Table1: Summary of validation parameter

Parameters	Second derivative zero-crossing spectrophotometry		
	GLM	PIO	MET
Wavelength	233.4 nm	265.4 nm	252.6 nm
Range	5-25 µg mL^{-1}	5-25 µg mL^{-1}	2-12 µg mL^{-1}
Linearity	0.999	0.999	0.999
Slope	0.02	0.039	0.054
Accuracy	99.49%	99.55%	99.66%
Intra day precision	% RSD < 2	% RSD < 2	% RSD < 2
Inter day precision	% RSD < 2	% RSD < 2	% RSD < 2
LOD	0.116 µg mL^{-1}	0.069 µg mL^{-1}	0.045 µg mL^{-1}
LOQ	0.353 µg mL^{-1}	0.209 µg mL^{-1}	0.136 µg mL^{-1}

Table 2: Analysis of market formulation

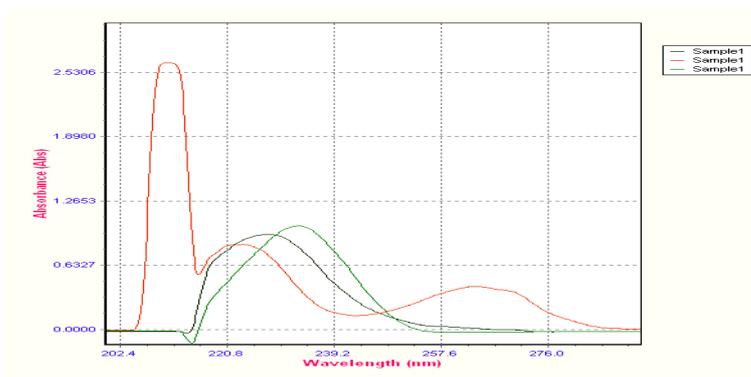| Formulation	Drug	Labeled (mg/tab.)	Amt[*]. Found (mg/tab.)	% Labeled claim.
GLIMADAY-P1	GLM	1	0.997	99.70±0.356
	PIO	15	15.06	100.42 ± 0.512
	MET	500	501.5	100.75 ± 0.282
GLIMADAY-P2	GLM	2	1.96	98.72 ± 0.421
	PIO	15	15.08	98.82 ± 0.426
	MET	500	499.72	99.94 ± 0.356

Figure1: Overlay of GLM-25mcg, PIO-25mcg, MET-12mcg
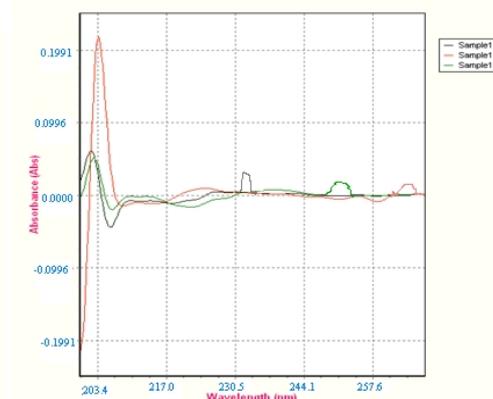
Figure 2: 2nd derivative overlay of GLM-25mcg, PIO-25mcg, MET-12mcg