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Simultaneous Spectrophotometric Estimation of Pioglitazone, Metformin HCl and Glimepiride in Bulk and Formulation

ASHA THOMAS*, SANDIP BODKHE, LATA KOTHAPALLI, SUMITRA JANGAM, MANISHA PATANKAR and A.D. DESHPANDE
Department of Pharmaceutical Chemistry, Padmashri Dr. D.Y. Patil Institute of Pharmaceutical Sciences and Research, Pimpri, Pune-411 018, India
E-mail: dypharmachem@yahoo.co.in

Three sample, accurate and economical spectrophotometric methods *i.e.*, simultaneous equation method, derivative spectroscopy method and area under the curve method have been developed for the simultaneous estimation of pioglitazone, metformin HCl and glimepiride in their combined dosage formulation. Pioglitazone shows absorbance maxima at 267 nm, metformin HCl at 238 nm and glimepiride at 226 nm in methanol. The methods allow rapid analysis of triple drug combination with high degree precision and accuracy. All three drugs exhibit linearity with absorbances in the concentration ranges employed for the methods. Results of the methods were validated statistically and by recovery studies.

Key Words: Spectrophotometric, Pioglitazone, Metformin HCl, Glimepiride.

INTRODUCTION

Pioglitazone[1] (Pio), chemically 5-[[4-[2-(5-ethyl-2-pyridinyl)ethoxy]-phenyl]methyl]-2,4-thiazolidinedione. It decreases insulin resistance in the periphery and liver resulting in increased insulin dependent glucose disposal and decreased hepatic glucose output. Metformin[2] HCl (Met), chemically 1,1-dimethylbiguanide. It decreases hepatic glucose production, decreases intestinal absorption of glucose and improves insulin sensitivity by increasing peripheral glucose uptake and utilization. It lowers plasma glucose.

Glimepiride[3] (Glim), chemically 1-({P-[2-(3-ethyl-4-methyl-2-oxo-3 pyrroline-1-carboxamido)ethyl]phenyl}sulphonyl)-3-(*trans*-4-methyl-cyclohexyl)urea. It is a sulphonyl urea which lowers blood glucose by releasing insulin from pancreas, an effect dependent upon functioning of β cells in pancreatic islets. When administerd chronically, the blood glucose lowering effects persists.

Literature survey reveals several methods such as HPLC[4-6], UV spectrosctropy[7-10] have been reported for the estimation of the individual drugs as well as in combination with other drugs.

However, not a single UV or HPLC method is reported for the simultaneous analysis of Pio, Met and Glim in their combined dosage form. A combination of 15 mg of pioglitazone, 500 mg of metformin HCl and 2 mg of glimpiride is now available in the market. A successful attempt has been made to estimate the three drugs simultaneously by UV spectrophotometric analysis.

EXPERIMENTAL

Shimadzu UV-1700 (Japan): Spectrophotometer was employed with spectral bandwidth of 2 nm, wavelength accuracy of ± 0.5 nm, with automatic wavelength correction and employing a pair of quartz cells. A Shimadzu electronic analytical balance (AX-200) was used for weighing the sample. An ultrasonic cleaner (Art No.400014CL) was used for sonicating the tablet sample solution. Pio, Met and Glim (Micro Laboratories, Bangalore) and methanol AR grade were used in the study.

Preparation of standard stock solution: Standard stock solutions (100 µg/mL) of Pio, Met and Glim were prepared by dissolving separately 10 mg of drug each in 100 mL of methanol. From this appropriate dilutions were made to obtain10 µg/mL Pio, 20 µg/mL Met and 5 µg/mL of Glim. Pio, Met and Glim exhibited λ_{max} at 267, 238 and 226 nm, respectively.

Preparation of sample stock solution: 20 Tablets (GEMER P-2 manufactured by Sun Pharmaceuticals Ltd, Mumbai, India) were weighed and crushed to a fine powder. An accurately weighed powder sample equivalent to 20 mg of Met, was transferred to a 100 mL volumetric flask and dissolved in methanol. After the immediate dissolution, the volume was made up to the mark with methanol. From this 10 mL of solution was transferred to 100 mL volumetric flask and volume was made upto the mark with methanol. The solution was sonicated for about 0.5 h and then filtered through a Whatmann filter paper No. 41. To this solution 9.4 mL of standard Pio (100 µg/mL) and 4.92 mL of Glim (100 µg/mL) was added by standard addition method to give final concentrations of Pio 10, Met 20 and Glim 5 µg/mL.

Method-A Simultaneous equation method

Standard Stock solutions (100 µg/mL) of Pio, Met and Glim were prepared by dissolving separately 10 mg of drug in methanol. Glim and Met exhibited λ_{max} at 226 and 238 nm, respectively. But for Pio, 267 nm was chosen as the working λ because at this wavelength, there is minimum interference of Glim and Met. Fig. 1 represents the overlain spectra of Pio,

Met and Glim. For solving the simultaneous equations 267, 238 and 226 nm were selected as the three sampling wavelengths. Pio, Met and Glim exhibited linearity with absorbances in the range of 5-40, 5-40 and 5-50 μg/mL, respectively at their selected wavelengths. Co-efficient of correlations was found to be 0.9970, 0.9991 and 0.9993 for Pio, Met and Glim, respectively. The optical characteristics and validation data are presented in Table-1.

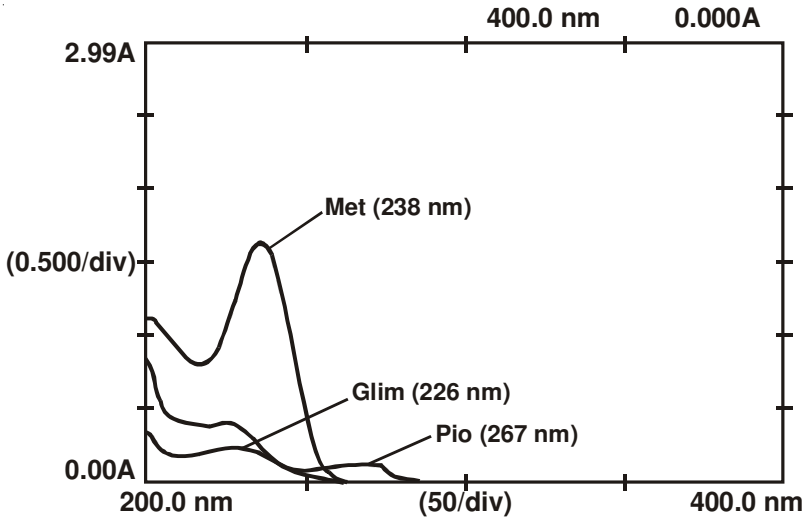

Fig. 1. Overlain spectra of Pio, Met and Glim

A set of three simultaneous equations were established using the mean of the absorptivity coefficients of Pio, Met and Glim at the selected λ's.

$$A_1 = (53.33\ C_{Pio} + 0.165\ C_{Met} + 12.74\ C_{Glim}) \times 10^2 \quad (1)$$

$$A_2 = (83.52\ C_{Pio} + 117.19\ C_{Met} + 226.47\ C_{Glim}) \times 10^2 \quad (2)$$

$$A_3 = (159.21\ C_{Pio} + 75.70\ C_{Met} + 271.56\ C_{Glim}) \times 10^2 \quad (3)$$

where, (1) 53.33, 83.52 and 159.21 are absorptivities of Pio at 267, 238 and 226, respectively, (2) 0.165, 117.19 and 75.70 are absorptivities of Met at 267, 238 and 226, respectively, (3) 12.74, 226.47 and 271.56 are absorptivities of Glim at 267, 238 and 226, respectively, (4) A_1, A_2 and A_3 are absorbances of mixed standard at λ_1, λ_2 and λ_3, respectively, (5) C_{PIO}, C_{MET} and C_{GLIM} are concentrations in g/L.

By solving eqns. 1-3 the concentration of Pio, Met and Glim in standard and tablet sample solution can be obtained.

Method-B First order derivative spectroscopy

Standard solutions (20 μg/mL) of Pio, Met and Glim each were scanned in the spectrum mode of the instrument from 400 to 190 nm. The absorption spectra thus obtained were derivatized from first to fourth order. The

first order derivative spectrum was selected for the analysis. From the overlain derivative spectra obtained, the wavelengths were selected in a manner such that at the zero crossing of one drug, the other drug should have substantial absorbance.

The overlain first order spectrum of the three drugs are given in Fig. 2. The wavelengths selected for first order derivative analysis are 278, 230 and 220 nm for Pio, Met and Glim, respectively.

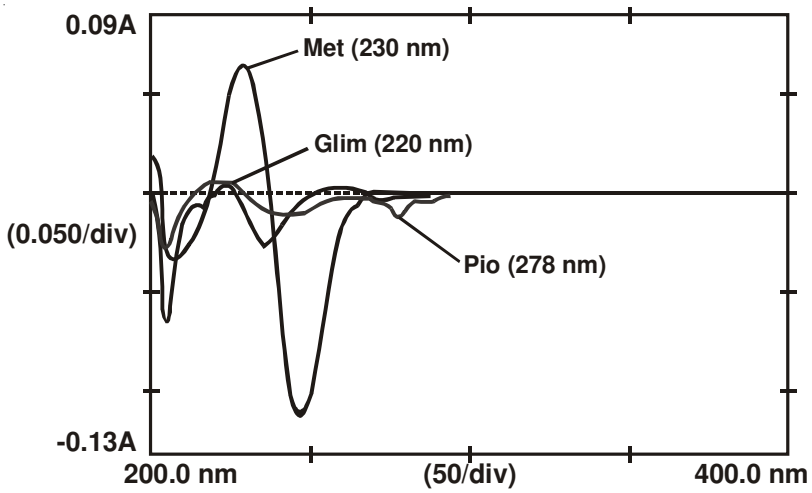

Fig. 2. First order derivative overlain spectra of Pio, Met and Glim

Mixed standards of Pio, Met and Glim were prepared and their absorbances were measured at the selected wavelengths against methanol as blank. These absorbances were plotted against concentration to obtain calibration curves for Pio, Met and Glim. The three drugs exhibited linearity with absorbances in the range of 5-40, 5-40 and 5-50 µg/mL at their respective selected wavelengths. Co-efficient of correlations were found to be 0.9994, 0.9999 and 0.9986 for Pio, Met and Glim, respectively. The optical characteristics and validation data in the first order derivative mode are presented in Table-1.

$$A_1 = (-470.58)\, C_{Pio} + 0.0\, C_{Met} - 98.03\, C_{Glim} \quad (1)$$

$$A_2 = (-431.37)\, C_{Pio} + 471.07\, C_{Met} - 98.03\, C_{Glim} \quad (2)$$

$$A_3 = 0.0\, C_{Pio} + 99.17\, C_{Met} + 588.23\, C_{Glim} \quad (3)$$

where, (1) (-470.58), (-431.37) and 0.0 are absorptivities of Pio at 278, 230 and 220, respectively, (2) 0.0, 471.07 and 99.17 are absorptivities of Met at 278, 230 and 220, respectively, (3) (-98.03), (-98.03) and 588.23 are absorptivities of Glim at 278, 230 and 220, respectively, (4) A_1, A_2 and A_3 are absorbances of mixed standard at 278, 230 and 220, respectively, (5) C_{PIO}, C_{MET} and C_{GLIM} are concentrations in g/L.

TABLE-1
OPTICAL CHARACTERISTICS AND VALIDATION DATA OF PIOGLITAZONE, METFORMIN HCl AND GLIMEPIRIDE FOR METHOD **A** AND **B**

Parameters	Pioglitazone		Metformin HCl		Glimepiride	
	Method **A**	Method **B**	Method **A**	Method **B**	Method **A**	Method **B**
λ_{max} (methanol) (nm)	267	278	238	230	226	220
Beer's law range (μg/mL)	5-40	5-40	5-40	5-40	5-50	5-50
Molar absorptivity (L/mol cm)*	0.59×10^4	-5.09×10^2	1.53×10^4	3.71×10^2	15.56×10^4	18.62×10^2
Precision*						
Intraday	0.3281	0.5492	0.2534	0.3141	0.2426	0.7477
Interday	0.5512	0.7123	0.5869	0.4829	0.5087	0.9821
LOD (μg/mL)*	0.32	0.11	0.19	0.10	0.14	0.14
LOQ (μg/mL)*	0.98	0.35	0.57	0.31	0.43	0.43
Regression values:						
Slope*	0.0109	-0.0016	0.1041	0.0032	0.0503	0.0013
Y-Intercept*	0.0035	0.00013	-0.0208	0.0003	0.0068	0.00016
Regression coefficient (r^2)*	0.9970	0.9994	0.9991	0.9999	0.9993	0.9986

*Average of six determinations where, A is for simultaneous equation method, B is for first order derivative spectroscopic method.

A set of three simultaneous equations were established using the mean of the absorptivity coefficients of Pio, Met and Glim at the selected λ's. Using eqns. 1-3, the concentration of Pio, Met and Glim can be obtained.

Method-C Area under the curve method

For the simultaneous determination using the area under the curve method, suitable dilutions of the standard stock solutions (100 μg/mL) of Pio, Met and Glim were prepared. The solutions of the drugs were scanned in the range of 200-400 nm. The wavelength ranges selected for the analysis was between 234.5-241.5 nm at which metformin contributes to a larger AUC. The wavelength range selected was between 261-275.5 nm under which only pioglitazone contributes to AUC, whereas wavelength range selected for glimepiride was between 226.5-233 nm. The overlain spectra of Pio, Met and Glim along with its AUC ranges are shown in Fig. 3. Pio and Glim both showed linearity with AUC in the range 5-50 μg/mL at their respective selected wavelength ranges. Met showed linearity in the concentration range of 5-40 μg/mL.

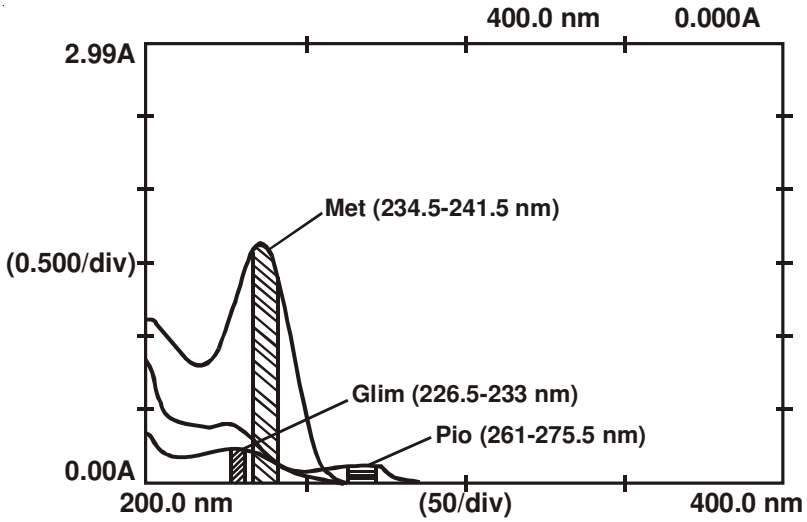

Fig. 3. Overlain spectra of Pio, Met and Glim along with its area under the curve

TABLE-2
OPTICAL CHARACTERISTICS AND VALIDATION DATA OF PIOGLITAZONE, METFORMIN HCl AND GLIMEPIRIDE FOR METHOD **C**

Parameters	Pioglitazone Method **C**	Metformin HCl Method **C**	Glimepiride Method **C**
λ_{max} (methanol) (nm)	261-275.5	234.5-241	226.5-233
Beer's law range (µg/mL)	5-50	5-40	5-50
Molar absorptivity (I/mol cm)*	7.92×10^4	10.69×10^4	15.56×10^4
Precision:* Intraday	0.8214	0.6212	0.4430
Interday	1.021	0.7128	0.5043
LOD (µg/mL)*	0.013	0.99	0.066
LOQ (µg/mL)*	0.95	3.02	0.20
Regression values: Slope*	0.2269	0.6027	0.3184
Y-Intercept*	0.0216	0.1814	0.0093
Regression coefficient (r^2)*	0.9993	0.9995	0.9999

*Denotes average of six determinations where, **C** is for area under the curve method

The co-efficients of correlations were found to be 0.9993 for Pio, 0.9995 for Met and 0.9999 for Glim.

Mixed standard solutions of Pio 10 µg/mL, Met 20 µg/mL and Glim 5 µg/mL were prepared by appropriate dilution of stock solutions. The AUC of the mixed standard solutions were recorded at selected wavelength ranges.

The concentration of Pio, Met and Glim in the mixed standard and sample solutions were found by using eqns. 1-3.

$$A_1 = 69.84\ C_{Pio} + 12.53\ C_{Met} + 247.7\ C_{Glim} \quad (1)$$

$$A_2 = 2.16\ C_{Pio} + 80.26\ C_{Met} + 723.3\ C_{Glim} \quad (2)$$

$$A_3 = 7.01\ C_{Pio} + 13.23\ C_{Met} + 178.72\ C_{Glim} \quad (3)$$

where, (1) 69.84, 2.16 and 7.01 are molar absorptivities of AUC for Pio at (261-275.5), (234.5-241.5) and (226.5-233) nm, respectively, (2) 12.53, 80.26 and 13.23 are molar absorptivities of AUC for Met at (261-275.5), (234.5-241.5) and (226.5-233) nm, respectively, (3) 247.7 , 723.3 and 178.72 are molar absorptivities of AUC for Glim at (261-275.5), (234.5-241.5) and (226.5-233) nm, respectively, (4) A_1, A_2 and A_3 are absorbances of mixed standard and sample solution at (261-275.5), (234.5-241.5) and (226.5-233) nm, respectively, (5) C_{PIO}, C_{MET} and C_{GLIM} are concentrations in g/L.

The analysis procedure for method A, B and C was repeated 6 times with same batch of tablet. The results of tablet analysis and its statistical validation are given in Table-3. To check the accuracy of the proposed methods, recovery studies were performed at 80, 100, 120 % of the test concentration of three drugs. The results of recovery studies along with the statistical validation data are given in Tables 4 and 5, respectively.

TABLE-3
STATISTICAL VALIDATION OF FORMULATION

Component	Amount present (mg)	Method	Amount* found (%)	Standard deviation	Co-efficient of variation (%)	Standard error
Pioglitazone	15	A	99.5	0.6429	0.6461	0.3712
	15	B	100.5	0.2000	0.1990	0.1155
	15	C	101.2	0.3786	0.3741	0.2186
Metformin HCl	500	A	100.8	0.2646	0.2625	0.1528
	500	B	101.0	0.3055	0.3022	0.1764
	500	C	99.6	0.3215	0.3227	0.1856
Glimepiride	2	A	100.6	0.4163	0.4138	0.2404
	2	B	101.1	0.3512	0.3473	0.2028
	2	C	100.8	0.1528	0.1515	0.0881

*Denotes the average of six determinations

RESULTS AND DISCUSSION

Analysis of all three method *i.e.* simultaneous equation method, first order derivative spectroscopy and area under the curve method is done. For simultaneous equation method wavelengths selected are 267, 238 and 226 nm with coefficient of correlation 0.9970, 0.9991 and 0.9993 for Pio, Met and Glim, respectively. First order derivative method has 278, 230 and 220 nm with coefficient of correlation 0.9994, 0.9999 and 0.9986 for Pio, Met and Glim, respectively. Area under the curve method has (261-275.5), (234.5-241.5) and (226.5-233) nm with coefficient of correlation 0.9993, 0.9995 and 0.9999 for Pio, Met and Glim, respectively.

TABLE-4
RECOVERY STUDIES AND ITS STATISTICAL VALIDATION DATA FOR METHOD **A** AND **B**

Recovery (%)	Component	Amount present (mg)	Amount standard added (mg)	% Recovery* ± SD		% Coefficient variation*		Standard error*	
				A	B	A	B	A	B
80	Pioglitazone	15	12.0	100.5 ± 0.4041	100.70 ± 0.4509	0.4020	0.4477	0.23	0.44
	Metformin HCl	500	400.0	100.0 ± 0.2082	100.00 ± 0.2082	0.2082	0.2082	0.12	0.20
	Glimepiride	2	1.6	99.9 ± 0.2000	100.03 ± 0.4933	0.2004	0.4931	0.11	0.49
100	Pioglitazone	15	15.0	99.3 ± 0.6505	101.00 ± 0.7000	0.6550	0.6930	0.37	0.69
	Metformin HCl	500	500.0	101.0 ± 0.3512	100.50 ± 0.3000	0.3477	0.2985	0.20	0.29
	Glimepiride	2	2.0	100.3 ± 0.6807	101.10 ± 0.5033	0.6786	0.4978	0.39	0.49
120	Pioglitazone	15	18.0	99.2 ± 0.3606	99.40 ± 0.7371	0.3635	0.7415	0.20	0.74
	Metformin HCl	500	600.0	100.5 ± 0.4000	99.60 ± 0.5508	0.3980	0.5530	0.23	0.55
	Glimepiride	2	2.4	101.3 ± 0.1528	100.60 ± 0.4726	0.1508	0.4697	0.08	0.46

*Average of three determinations

TABLE-5
RECOVERY STUDIES AND ITS STATISTICAL VALIDATION DATA FOR METHOD **C**

Recovery (%)	Component	Amount present (mg)	Amount standard added (mg)	% Recovery* ± SD	% Coefficient variation*	Standard error*
80	Pioglitazone	15	12.0	100.3 ± 0.7767	0.7743	0.44
	Metformin HCl	500	400.0	100.6 ± 0.7810	0.7763	0.45
	Glimepiride	2	1.6	99.1 ± 0.7387	0.7387	0.42
100	Pioglitazone	15	15.0	100.5 ± 0.7937	0.7897	0.45
	Metformin HCl	500	500.0	99.3 ± 0.7095	0.7145	0.40
	Glimepiride	2	2.0	100.3 ± 1.1060	1.1060	0.63
120	Pioglitazone	15	18.0	100.4 ± 0.4041	0.4029	0.23
	Metformin HCl	500	600.0	101.1 ± 0.4726	0.4674	0.27
	Glimepiride	2	2.4	100.6 ± 0.5132	0.5101	0.29

*Average of three determinations

The results of the analysis of tablet formulation are in good agreement with the label claim of the formulation. The value of the standard deviation and coefficient of variation calculated both for tablet analysis and recovery studies were satisfactorily low, indicating the high degree of precision and accuracy of the proposed methods. The results of these proposed methods were also evaluated using the t-test and F-test to determine if there exists any significant difference between these methods for the analysis of Pio, Met and Glim which are given in Table-6. It is concluded that the proposed methods are new, simple, accurate, precise, and economical and can successfully employed in the routine simultaneous estimation of Pio, Met and Glim in formulation.

TABLE-6
STATISTICAL SIGNIFICANCE OF DIFFERENCE
BETWEEN THREE METHODS

(I) DIFFERENCE BETWEEN METHOD **A** AND **B**

Parameters	Pioglitazone	Metformin HCl	Glimepiride
t value	0.304	2.04	2.17
F value	1.140	1.90	0.22

t =0.304,t=2.04, t =2.17 for Pio, Met and Glim respectively, at 16 degrees of freedom are < 16.02
F=1.14, F =1.90, F =0.22 for Pio, Met and Glim respectively, at 7 degrees of freedom are < 7.05

(II) DIFFERENCE BETWEEN METHOD **B** AND **C**

Parameters	Pioglitazone	Metformin HCl	Glimepiride
t value	1.08	0.15	1.28
F value	0.25	0.44	2.56

t =1.08, t=0.15, t =1.28 for Pio, Met and Glim respectively, at 16 degrees of freedom are < 16.02
F=0.25, F =0.44, F = 2.56 for Pio, Met and Glim respectively, at 7 degrees of freedom are < 7.05

(III) DIFFERENCE BETWEEN METHOD **A** AND **C**

Parameters	Pioglitazone	Metformin HCl	Glimepiride
t value	1.32	3.33	4.04
F value	1.82	0.18	1.02

t =1.08, t=0.15, t =1.28 for Pio, Met and Glim respectively, at 16 degrees of freedom are < 16.02
F=0.25, F =0.44, F = 2.56 for Pio, Met and Glim respectively, at 7 degrees of freedom are < 7.05

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