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DEVELOPING A NEW SPECTROPHOTOMETRIC METHOD USING ORTHOGONAL POLYNOMIAL METHOD FOR SIMULTANEOUS ESTIMATION OF PIOGLITAZONE AND GLIMEPRIDE IN TABLET FORMULATION

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ABSTRACT

Pioglitazone belongs to thiazolidinedione derivative, which potentiate the action of insulin to increase glucose uptake and glucose oxidation in both; muscle and adipose tissue. Glimepride acts primary by stimulating release of insulin from the ßcells. Developing a new spectrophotometric method for simultaneous estimation of pioglitazone and glimepride in tablet formulation facilitates to validate the method as per ICH guidelines. The orthogonal polynomial method is simple, accurate, economical, less time consuming and no prior separation is required for analysis.

Key words: Pioglitazone, Glimepride, Spectrophotometric method, Orthogonal polynomial method.

INTRODUCTION

Orthogonal polynomial function method[1] is a mathematical model for the elimination of irrelevant absorption. This method is based upon the difference in the shape of the spectra of the components in a mixture in the selected wavelength range. The absorption spectrum can be represented in terms of orthogonal function and contribution to the coefficient of the given degree of orthogonal polynomial depends upon the shape of the spectrum and concentration[2]. Thus, a quadratic curve will contribute to coefficients of zero degree, first degree and second degree polynomials; as a linear curve will contribute to coefficients of zero degree polynomial and first degree polynomial and not to that of second degree polynomial. Hence, from the coefficient of second degree polynomial value of sample spectrum, calculated from the wavelength range in which the spectra of one component is linear and the other is quadratic or cubic, it is possible to estimate the content of the second component. Though it is a potential method for the analysis of multi-

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component samples, the method involves complex calculations to select the right combination of degree of polynomial, number of points in the spectrum, interval between the points and optimization of these parameters[3].

Objective of the study

The main objective of this study was to develop a new spectrophotometric method using orthogonal polynomial function analysis for simultaneous estimation of pioglitazone and glimepiride in tablet formulation and to validate the above method as per the ICH guidelines.

EXPERIMENTAL

Materials and methods

Pioglitazone (PIO) and glimepiride (GLM) were procured from Cassel Research Lab, Chennai as a gift sample. Sodium hydroxide (Analytical grade) was procured from SD Fine Chemicals, Mumbai.

Methods: UV spectra[4] of 10 µg/mL solution of PIO in 0.1M sodium hydroxide and 10 µg/mL solution of GLM in 0.1M sodium hydroxide were recorded separately between 200 nm and 400 nm (Fig. 1).

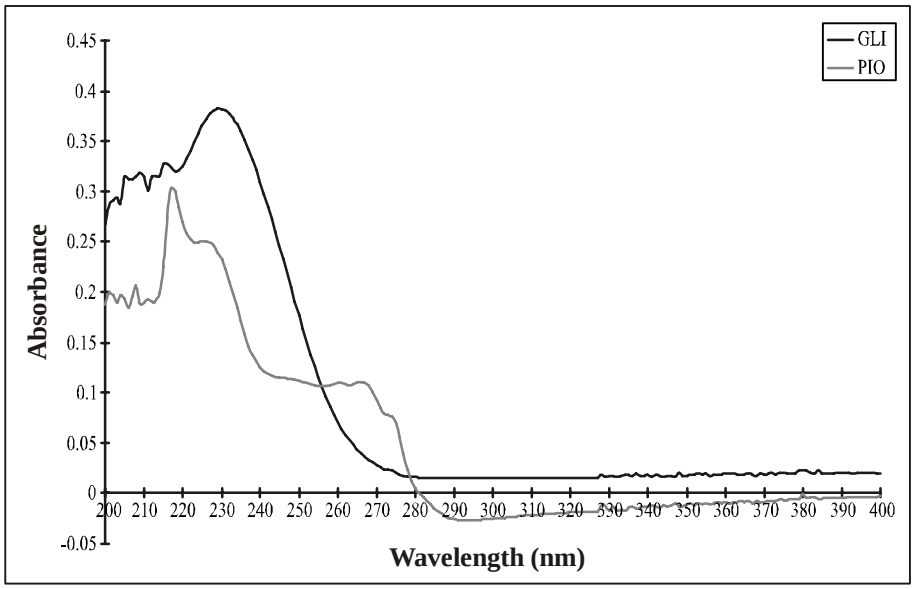

Fig. 1: UV Spectra of PIO and GLM

About 100 mg of PIO powder was accurately weighed and taken in a 100 mL volumetric flask, dissolved in 0.1M sodium hydroxide and volume was made up with 0.1M sodium hydroxide. A series of dilutions were made with 0.1M sodium hydroxide to get concentrations of 5, 10, 15 and 20 μg/mL. Similarly, a series of solutions of GLM in 0.1M sodium hydroxide were made to get concentration of 5, 10, 15 and 20 and 25 μg/mL. For each drug, 6 replicates were made by individual weighing. The spectra were recorded between 200 and 400 nm, using its export function of UV PC software and absorbance at respective λ_{max} (230 for GLM and 226 for PIO) were noted. The calibration graphs were constructed taking mean polynomial values at λ_{max} on Y-axis and concentration on X-axis. The regression coefficient and intercept on Y-axis were calculated. The spectra of the solutions were used for further linearity studies by orthogonal polynomial function method[5].

Table 1: Linearity of pioglitazone in 0.1M sodium hydroxide

Concentration in (μg/mL)	Mean polynomial value
5	0.0420
10	0.0910
15	0.1300
20	0.1814

Table 2: Linearity of glimepiride in 0.1M sodium hydroxide

Concentration (μg/mL)	Mean polynomial value
5	0.1411
10	0.2827
15	0.4296
20	0.5831
25	0.7239

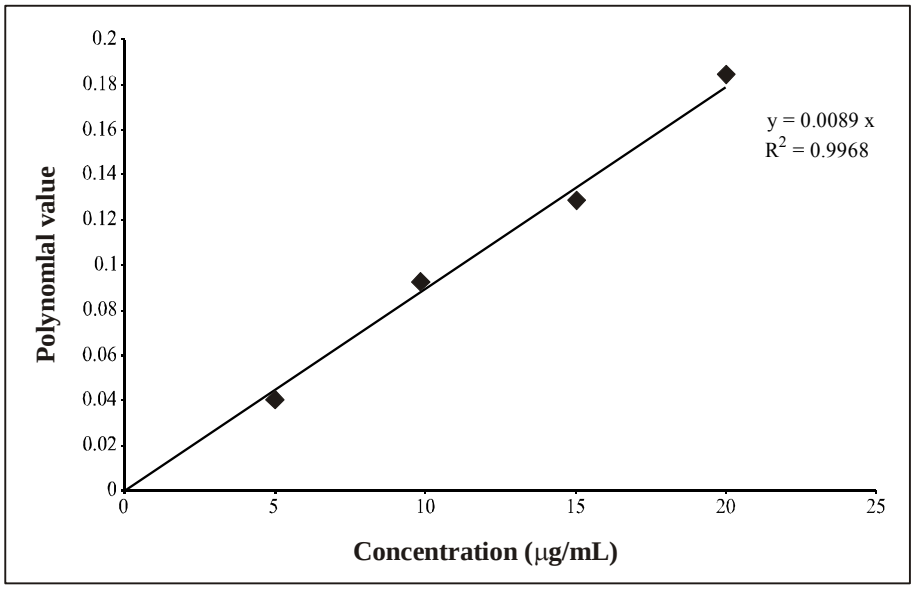

Fig. 2: Linearity of pioglitazone

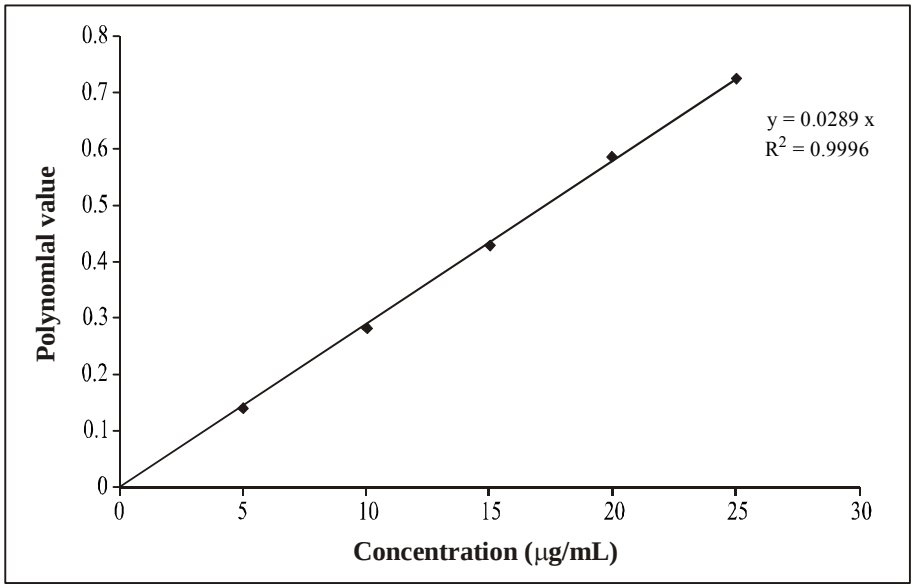

Fig. 3: Linearity of glimepiride

About 100 mg of PIO[6] was weighed accurately and transferred to a 100 mL volumetric flask, dissolved in 0.1M sodium hydroxide volume was made up with 0.1M sodium hydroxide. Further dilutions were made with 0.1M sodium hydroxide to attain a

concentration of 10 μg/mL. Five replicate solutions were prepared by individual weighing. Similarly, five replicate solutions of GLM in 0.1M sodium hydroxide were prepared. The spectra were recorded between 200 nm to 400 nm for all the above solutions and saved in ASCII format[7]. The theoretical concentrations of standard solutions are given in Table 3.

Table 3: Concentration of standard solutions

Replicates	Conc. of PIO (μg/mL)	Conc. of GLM (μg/mL)
1	10.082	10.064
2	10.395	10.195
3	10.220	10.284
4	10.012	10.095
5	10.540	10.080

Analysis of tablet formulation

The average weight of the tablets was determined by weighing twenty tablets and then tablets were powdered. Tablet powder equivalent to 15 mg of PIO (2 mg of GLM) was weighed and transferred to a 100 mL volumetric flask. About 70 mL of 0.1M sodium hydroxide was added and sonicated for 15 minutes for complete dissolution of the drugs. The volume was made up with 0.1M sodium hydroxide[4]. It was filtered through filter paper and spectra were recorded and saved in ASCII format as described earlier. Five replicate analysis were carried out with sample weighed individually (Table 4). The average weight of the tablet was found to be 0.2006 g[8].

Table 4: Weight of the tablet powder for assay

Replicate	Weight taken for analysis (g)
1	0.2009
2	0.2001
3	0.2021
4	0.2000
5	0.2001

Recovery studies

Recovery study was carried out by adding PIO and GLM to pre-analysed tablet powder at three different levels. The levels used for both PIO and GLM were 50%, 100% and 150%. Pre-analysed tablet powder equivalent to 15 mg of PIO (2 mg of GLM) was weighed and required amount of PIO and GLM were added[9]. About 70 mL of 0.1M sodium hydroxide was added, sonicated for 15 minutes to dissolve the drugs, volume was made up and filtered. The spectra of resulting solutions were recorded and stored in ASCII format as described in linearity[10].

RESULTS AND DISCUSSION

Optimization of parameters for the estimation of PIO and GLM

Convoluted graphs were generated, by executing the programme as described in software section of orthogonal polynomial chapter, for various combinations of degree of polynomial (2 or 3, that is quadratic or cubical), number of wavelength points in the spectrum (6 to 12) and interval between the wavelength points (2 nm to 9 nm)[4] using the spectral data of standard PIO and standard GLM in ASCII format recorded as described in spectra of standard section[11]. In total, 112 convoluted graphs each for PIO and GLM were generated. Convoluted graph of PIO were compared with that of corresponding convoluted graph of GLM and the optimum conditions for orthogonal polynomial function method of analysis were selected taking the following points into consideration

(i) The coefficient value (P value) is negligible for one drug and as high as possible for the other[12],

(ii) The wavelength range, where there is steep rise in coefficient value of either drug was avoided, and

(iii) Whenever comparable results are obtained for more than one set of conditions, the one with less number of wavelengths is selected for further studies.

The details of the wavelengths satisfying the above conditions for each of the 56 convoluted graphs for the estimation of PIO and GLM by quadratic polynomial are given in Table 5.

Table 5: Optimised conditions for orthogonal polynomial function method of analysis

Drug	Degree of polynomial	Number of points	Interval between the points	Wavelength (nm)	Average (nm)
PIO	Quadratic	5	2 nm	279, 281, 283, 285 and 287	284
GLM	Quadratic	5	4 nm	249, 253, 257, 261 and 265	259

Calculation of coefficient of polynomial

Coefficient of polynomial is directly proportional to the concentration of analyte and it can be calculated by using equation (1) for PIO and equation (2) for GLM, where the factors are those of five point quadratic polynomials obtained from the text of numerical analysis (Fisher and Yates)

$$P_{PIO} = 5(A_{279})\text{-}1(A_{281})\text{-}4(A_{283})\text{-}4(A_{285})\text{-}1(A_{287})\ 5(A_{289}) \quad \ldots(1)$$

$$P_{GLM} = 5(A_{249})\text{-}1(A_{253})\ \text{-}4(A_{257})\text{-}4(A_{261})\text{-}1(A_{265})\ 5(A_{269}) \quad \ldots(2)$$

Where, P_{PIO} and P_{GLM} are coefficients of polynomial of PIO and GLM, respectively and A is absorbance at respective wavelength. The calculation of coefficient could be carried out either manually from absorbance values at corresponding wavelength or directly from the corresponding out put, when the software was executed[10].

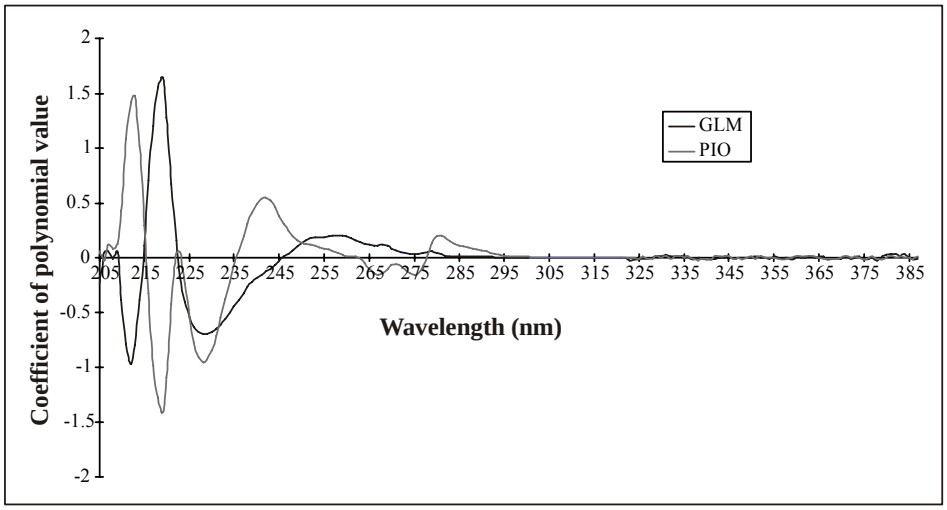

Fig. 4: Convoluted graph for the estimation of pioglitazone

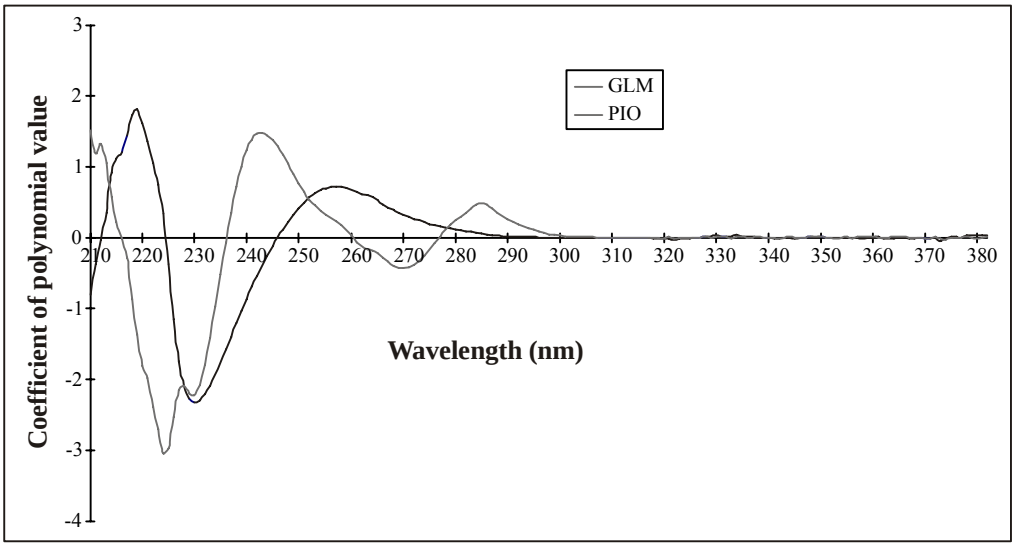

Fig. 5: Convoluted graph for the estimation of glimepiride

Determination of $P_{1\,cm}^{1\%}$

The $P_{1\,cm}^{1\%}$ is a constant, which represents the coefficient corresponding to the absorbance of 1% solution kept in 1 cm cell. It can be used for the calculation of concentration of sample similar to the use of $A_{1\,cm}^{1\%}$ in conventional spectrophotometry. Using the spectral data of standard solutions recorded, the coefficients of polynomials at the optimized conditions were computed for PIO as well as GLM. From the coefficient of polynomial values and the concentration of corresponding solution, $P_{1\,cm}^{1\%}$ values were calculated and the results are given in Table 6.

Table 6: $P_{1\,cm}^{1\%}$ for PIO and GLM

Conc. of PIO (μg/mL)	P Value for PIO	$P_{1\,cm}^{1\%}$ for PIO	Conc. of GLM (μg/mL)	P Value for GLM	$P_{1\,cm}^{1\%}$ for GLM
10.082	0.0485	56.4	10.064	0.2827	222.2
10.395	0.0496	49.5	10.195	0.2956	282.7
10.220	0.0490	51.73	10.284	0.2955	286.4

Cont…

Conc. of PIO (μg/mL)	P Value for PIO	$P_{1\,cm}^{1\%}$ for PIO	Conc. of GLM (μg/mL)	P Value for GLM	$P_{1\,cm}^{1\%}$ for GLM
10.012	0.0478	49.90	10.095	0.2702	291.55
10.540	0.0512	52.10	10.080	0.2768	289.56
Mean		51.9			274.4
SD		3.568			7.63
% RSD		6.874			2.78
P value is coefficient of polynomial value					

Analysis of PIO and GLM in tablet formulations

P values were calculated by executing the programme. The results are given in Table 7.

Table 7: Analysis of tablet formulation

No.	Pioglitazone		Glimepiride	
	mg/tab	% Label claim	mg/tab	% Label claim
1	15.49	103.2	1.99	99.5
2	14.94	99.6	2.08	104.4
3	15.12	100.8	2.03	101.5
4	15.28	101.8	20.1	100.5
5	15.11	100.7	1.97	98.5
Mean		101.2		100.8
SD		1.007		7.547
% RSD		0.995		7.487
Label claim: Each tablet contains 15 mg PIO and 2 mg GLM				

Recovery study

Using the spectral data of solution prepared for recovery studies, P values were

calculated by executing the programme. The PIO and GLM contents were calculated as described for synthetic mixtures[13]. From the PIO and GLM contents, percentage recoveries were determined and are given in Tables 8 and 9.

Table 8: Recovery study for PIO

Level	PIO content in pre-analysed sample	PIO Added (mg)	Total PIO (mg)	Amount found (mg)	% Recovery	Mean ± SD
50%	15	7.5	22.5	23.24	103.28%	23.04 0.694
	15	7.5	22.5	23.08	102.57%	
	15	7.5	22.5	22.80	101.33%	
100%	15	15	30	30.85	102.83%	30.57 0.784
	15	15	30	30.22	100.73%	
	15	15	30	30.66	102.2%	
150%	15	22.5	37.5	37.05	98.8%	36.83 1.056
	15	22.5	37.5	36.25	96.6%	
	15	22.5	37.5	37.19	99.1%	

Table 9: Recovery study for GLM

Level	GLM content in pre-analysed sample	GLM added (mg)	Total GLM (mg)	Amount found (mg)	% Recovery	Mean ± SD
50%	2	1	3	3.04	101.3%	3.03 1.98
	2	1	3	2.94	98 %	
	2	1	3	3.11	103.6%	
100%	2	2	4	3.94	98.5%	3.98 2.34
	2	2	4	3.88	97%	
	2	2	4	4.12	103%	
150%	2	3	5	5.27	105.4%	5.07 3.01
		3	5	5.11	102.2%	
	2	3	5	4.85	97%	

UV spectra of 10 μg/mL solution of PIO in 0.1M sodium hydroxide and 10 μg/mL solution of GLM in 0.1M sodium hydroxide were recorded separately between 200 nm and 400 nm (Fig. 1). These spectral properties make this an ideal combination for orthogonal polynomial function analysis. Analytical conditions were optimised by the help of the software[5]. A total of 112 convoluted graphs were obtained each from the absorbance spectra of PIO and the absorbance spectra of GLM. Out of the convoluted graphs under 112 different conditions, 6 graphs exhibited considerable coefficient values for PIO and almost negligible coefficient values for GLM. All the five conditions could be used for the estimation of PIO[13]. The one having the least number of wavelength points and lesser wavelength range was selected for further studies. Since it will be much easier for routine analysis particularly, when the calculations are done manually or by a calculator. Condition chosen for estimation of PIO is 6 point quadratic polynomial covering the wavelength range from 279 to 289 nm. The UV spectrum of PIO is parabolic whereas the spectrum of GLM in the same wavelength range is a straight line[2]. Due to this property of the spectrum, the coefficient of quadratic polynomial is negligible for GLM. whereas PIO exhibited considerable coefficient values under the same conditions. Hence, under this condition, PIO can be estimated with out interference from GLM.

A similar study was carried out to optimize the conditions for estimation of GLM. The optimized condition was 6 points quadratic polynomials covering the wavelength range from 249 to 269 nm. Orthogonal polynomial function method for GLM estimation was attempted since the estimation could be carried out conveniently using the software. Further orthogonal polynomial function method of analysis would estimate interference from formulations excipients, if any. Orthogonal polynomial function method is based upon the measurement of absorbance at many wavelength points whereas conventional spectrophotometric method is based upon measurement of absorbance at single wavelength i.e. at λmax. Hence, a separate linearity study was carried out to establish the linearity of coefficient value with concentration although absorbance at single wavelength; that is respective λmax of PIO and GLM exhibited linearity. Ideally in spectrophotometric method, the concentration of analyte is determined by comparing the absorbance of analyte with that of standard solution of known concentrations[4].

Similarly, for the orthogonal polynomial function method of analysis, the concentration of analyte can be calculated by comparing P value of sample solution with that of standard solution of known concentration. However, for routine analysis, it will be more convenient to calculate analyte concentration using some constant. In the case of conventional spectrophotometric method, $A_{1cm}^{1\%}$ is the constant used for the calculation of analyte concentration. Similarly, $P_{1cm}^{1\%}$ is coefficient of polynomial, when concentration is

1% w/v and the measurement is taken using 1 cm cuvete. To establish $P_{1cm}^{1\%}$, the P value of 6 replicates standard solutions were determined and the average is calculated as $P_{1cm}^{1\%}$ separately for PIO and GLM 51.90 and 274.4, respectively. The method was used for analysis of marketed formulations. Tablet assay result shows that mean percentage is 101.2% with RSD 0.995 for PIO and 100.8% with RSD 7.487 for GLM. The accuracy of the method was established by recovery study, as per ICH guidelines, at three different levels viz 50%, 100% and 150%. The recovery was within the limits, in all the three levels, prescribed by ICH guidelines.

CONCLUSION

Selected tablet formulation containing pioglitazone and glimepiride could be analysed by orthogonal polynomial function method with required accuracy and precision. The proposed method is simple, accurate, economical, less time consuming and no prior separation is required for analysis.

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