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Simultaneous Spectrophotometric Determination of Alogliptin and Pioglitazone Using Partial Least Squares with and without Genetic Algorithm

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ABSTRACT

Background: Partial least squares-1 (PLS) is a common simple, rapid and selective multivariate method for simultaneous determination of components having overlapping spectra in mixtures. **Objectives:** In the present study, a simple and sensitive chemometric procedure was suggested for the selective determination of alogliptin and pioglitazone without previous separation in pharmaceutical preparation using this powerful multivariate technique with and without variable selection (genetic algorithm). PLS method was run on the calibration data of absorption spectra. Pre-processing of the data was done and the regions below 215 nm and above 290 nm were rejected due to non-linearity, this results in 75 variable. **Results:** Genetic algorithm reduced absorbance matrix to about 61-56% of the original matrix (46 and 42 variables for alogliptin and pioglitazone, respectively). The selected variables were used for running the partial least squares model. **Conclusion:** A comparison between partial least squares and genetic algorithm- partial least squares models was done and the predictive ability of both models were evaluated.

Keywords: *Alogliptin; pioglitazone; multivariate; chemometric; genetic algorithm.*

INTRODUCTION

Alogliptin benzoate, **Figure 1**, is a potent highly selective dipeptidyl peptidase-IV inhibitor which stimulates glucose dependent insulin release. It is used in the treatment of type 2 diabetes[1].

Pioglitazone hydrochloride, **Figure 2**, is a thiazolidine class of antidiabetic drugs[2]. It controls the glucose and lipid metabolism in the muscle, liver and adipose tissue. It also reduces insulin resistance[3, 4].

Combined treatment of alogliptin and pioglitazone at an early stage of diabetes improves metabolic profiles, glycemic control, lipid profiles and increases pancreatic insulin content compared with either alogliptin or pioglitazone mono-therapy[5].

Partial least squares-1 (PLS) is a common simple, rapid and selective multivariate method for simultaneous determination of components having overlapping spectra in mixtures. It has the advantages of minimizing the errors by measuring the absorbance values at many points in the wavelength range of the

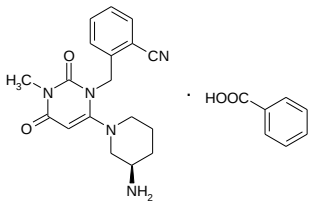

Figure 1. Structural formula of alogliptin benzoate

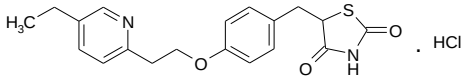

Figure 2. Structural formula of pioglitazone hydrochloride

zero-order and wider range of application[6, 7]. However, with the aim of increasing the quality of the calibration, wavelengths selection was performed, in such a way that uninformative variables were eliminated. This variables selection was carried out using the genetic algorithm procedure[8]. Under computer-controlled instrumentation, PLS method plays a very important role in the analysis of many pharmaceutical mixtures without previous separation by UV–VIS spectrophotometry.[(9-11)]

The review of literature revealed a few methods reported for simultaneous determination of alogliptin and pioglitazone in combined dosage forms including UV spectrophotometric methods; first order derivative[12], dual wavelength[12], area under curve[13], first derivative of ratio spectra[13], ratio difference[13], second- order derivative[14], simultaneous equation[15] and absorption ratio[15] methods as well as chromatographic methods[16-26].

The main purpose of this work is to establish a simple, sensitive, accurate and precise method for the simultaneous determination of alogliptin and pioglitazone in pharmaceutical preparation using this powerful multivariate technique with and without variable selection (genetic algorithm). A comparison between partial least squares and genetic algorithm- partial least squares models was done and the predictive ability of both models were evaluated.

MATERIAL AND METHODS

Materials

Alogliptin benzoate and pioglitazone hydrochloride pure standards were obtained from El Obour Modern Pharmaceutical Industries in El Obour City - Cairo with purities of 99.76% and 99.52% respectively according to the reported method[13]. **Prandaglim Plus® 25/30 mg tablets** (labeled to contain 34 mg of alogliptin benzoate equivalent to 25 mg alogliptin and 33.06 mg of pioglitazone hydrochloride equivalent to 30 mg pioglitazone) a product of Eva Pharma, Cairo, Egypt, was obtained from local Market.

Chemicals and reagents

Analytical grade methanol, Merck, Germany.

Apparatus and software

Shimadzu UV–Visible 1650 Spectrophotometer, (Tokyo, Japan), equipped with 10 mm matched quartz cells. UV-Probe personal spectroscopy software version 2.1. (Shimadzu). All chemometric models were implemented in Matlab R2013b (8.2.0.701). All models were carried out by PLS toolbox software version 2.1.

Standard solutions

Standard stock solutions (100 µg/ml) of alogliptin benzoate (ALG) and pioglitazone hydrochloride (PIG) were prepared separately by dissolving 10 mg of each drug powder in 50 ml of methanol and the volume was adjusted to 100 ml with methanol.

Procedures

Experimental design

A 5-level, 2-factor design was performed using 5 concentration levels for each of the 2 compounds resulting in 25 mixtures. The design spans the mixture space fairly well. The central level of the design is 15µg/ml for each compound. The chosen concentrations for each compound are based on its linearity (5-30 µg/ml for each compound) and the ratio between the two compounds involved in their pharmaceutical preparation. **Table 1** represents the concentration design matrix. Thirteen mixtures of this design were used as a calibration set and the other twelve mixtures were used as a validation set to test the predictability of the developed multivariate models.

Procedure for pharmaceutical preparation

Five Prandaglim Plus® tablets (34 mg of ALG and 33.06 mg of PIG per tablet) were scratched, and finely powdered. Appropriate weight of the powder equivalent to 100 mg of ALG was accurately weighed, transferred to 100 ml volumetric flask and the volume was made up to 50 ml with methanol. The solution was shaken vigorously for 20 min then sonicated for 30 min and filtered. The volume was completed to 100 ml with methanol to produce a stock solution labeled to contain 1 mg/ml of ALG and 0.972 mg/mL of PIG. Necessary dilutions of the stock solution were made with methanol and analyzed using the general procedure of the described models.

Table 1. Concentrations of ALG and PIG in µg/ml used in the experimental design.

Mixture Number	ALG (µg/ml)	PIG (µg/ml)
1	15	15
2	15	9
3	9	9
4	9	21
5	21	12
6	12	21
7	21	15
8	15	12
9	12	12
10	12	18
11	18	21
12	21	18
13	18	15
14	15	21
15	21	21
16	21	9
17	9	18
18	18	9
19	9	15
20	15	18
21	18	18
22	18	12
23	12	9
24	9	12
25	12	15

The shaded rows represent the validation set.

RESULTS AND DISCUSSION

In the present study, a simple and sensitive chemometric procedure was suggested for the selective determination of ALG and PIG without previous separation. This method was applied with and without variable selection procedure (genetic algorithm) and a comparative study was done between both models.

Spectral characteristics

The zero-order absorption spectra of ALG and PIG, **Figure 3,** show severe overlapping, which does not permit direct determination of both compounds.

In this method, the information from the concentration values is introduced into the calculation of the so-called latent variables, which are linear combinations of the original variables. PLS method was run on the calibration data of absorption spectra. Pre-processing of the data was done and the regions below 215 nm and above 290 nm were rejected due to non-linearity, this results in 75 variable.

To select the number of factors in the partial least squares algorithm, a cross validation method by leaving out one sample at a time was applied using calibration set of 13 calibration samples and root mean squares error of cross-validation was recalculated upon addition of each new factor to the partial least squares.

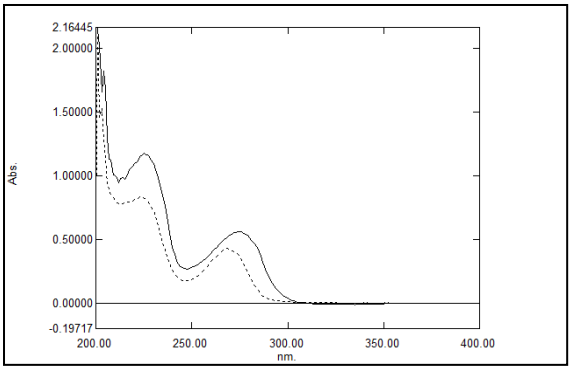

Figure 3. Zero order absorption spectra of ALG, 20 µg/ml, (—) and PIG, 20 µg/ml, (.....)

The method developed by Haaland &Thomas[27] was used for selecting the optimum number of factors. It involves selecting the model including the smallest number of factors that results in an insignificant difference between the corresponding root mean squares error of cross-validation and the minimum root mean squares error of cross-validation. It was found that 5 latent variables are sufficient for modeling ALG. On the other hand, 7 latent variables are needed to model PIG, as shown in **Figures (4 &5)** respectively.

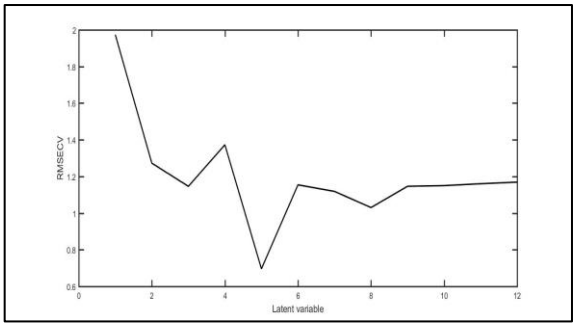

Figure 4. RMSECV plot of the cross validation results of the calibration set as a function of the latent variables (LVs) used to construct the PLS model for ALG

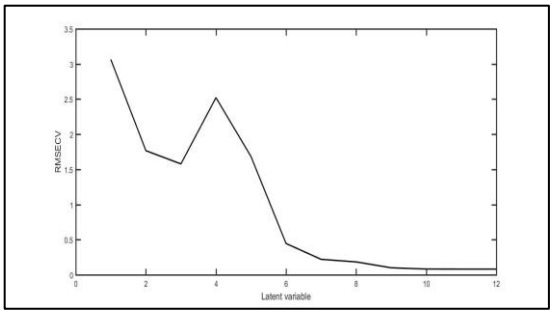

Figure 5. RMSECV plot of the cross validation results of the calibration set as a function of the latent variables (LVs) used to construct the PLS model for PIG

Table 2. Parameters of the genetic algorithm used for variable selection applied to ALG and PIG raw data

Parameter	Value	
	ALG	PIG
Population size	44	44
Maximum generations	300	
Mutation rate	0.005	
The number of variables in a window (window width)	2	
Per cent of population the same at Convergence	100	
% Wavelengths used at initiation	50	50
Crossover type	Double	Double
Maximum number of latent variables	5	7
Cross validation	Random	
Number of subsets to divide Data into for cross validation	13	
Number of iterations for cross validation at each generation	2	

Table 3. Percentage Recoveries, means, SD, RMSEC and RMSEP for ALG and PIG in the calibration and the validation samples by PLS and GA-PLS models.

Sets	Calibration set				Validation set			
Drug	ALG (%R)		PIG (%R)		ALG (%R)		PIG (%R)	
Method	PLS	GA-PLS	PLS	GA-PLS	PLS	GA-PLS	PLS	GA-PLS
	103.09	100.85	99.86	100.00	100.24	100.75	100.03	101.03
	97.57	99.31	100.09	100.16	103.13	100.01	101.90	101.37
	101.26	99.61	100.01	99.95	101.35	102.54	103.94	102.23
	99.72	100.74	100.16	100.11	98.01	101.14	101.11	101.53
	100.36	100.07	99.98	99.96	103.38	101.26	98.93	98.57
	101.46	99.75	100.06	100.35	98.48	99.82	97.89	98.28
	98.37	99.29	99.65	99.46	102.06	99.98	99.41	98.55
	99.80	100.16	99.96	99.82	97.13	100.33	98.49	98.64
	102.02	99.25	100.06	99.92	98.20	100.00	98.72	98.59
	97.69	100.87	99.90	100.03	100.54	100.51	98.38	98.19
	97.52	99.58	100.15	100.24	97.54	99.92	97.94	98.32
	97.35	99.99	100.21	100.70	98.71	99.33	102.02	101.98
	99.34	100.40	99.98	99.58	—	—	—	—
Mean	99.657	99.99	100.01	100.02	99.89	100.47	99.89	99.77
SD	1.9053	0.589	0.147	0.317	2.195	0.861	1.932	1.668
RMSE	0.2705[a]	0.0865[a]	0.0214[a]	0.0445[a]	0.3218[b]	0.1203[b]	0.3285[b]	0.2636[b]

[a]*RMSEC*
[b]*RMSEP*

However, with the aim of increasing the quality of the calibration, wavelengths selection was performed, in such a way that uninformative variables were eliminated. This variables selection was carried out using the genetic algorithm procedure. The genetic algorithm was run on 75 variables for ALG and PIG using a partial least squares with the maximum number of latent variables determined by cross-validation on the model containing all the variables. The adjusted genetic algorithm parameters were shown in **Table 2.**
Genetic algorithm reduced absorbance matrix to about about61-56% of the original matrix (46 and 42 variables for ALG and PIG, respectively). The selected variables were used for running the partial least squares model.

Percent recoveries, mean, SD, RMSEC and RMSEP for ALG and PIG in both models were shown in **Table (3).**

Table 4. Determination of ALG and PIG in PrandaglimPlus® tablets by the proposed PLS, GA-PLS models and the reported method:

Parameters	ALG			PIG		
	PLS	GA-PLS	Reported method*	PLS	GA-PLS	Reported method[13]*
n**	5	5	5	5	5	5
% R	98.82	98.42	98.24	101.16	100.76	100.24
SD	1.574	0.749	0.830	1.155	0.756	0.842
t*** (2.306)	0.729	0.360	—	1.439	1.016	—
F*** (6.388)	3.594	1.228	—	1.879	1.242	—

**Spectrophotometric method depends on measuring the first derivative of the ratio spectra at 300 nm & 277 nm for ALG & PIG respectively.*
***Number of experiments. *** The values in parenthesis are tabulated values of "t "and "F" at (P = 0.05).*

GA-PLS is considered more robust, accurate and precise model compared to PLS since it has lower RMSEC, RMSEP and SD. This shows the advantage of using the variable selection procedure before construction of the partial least squares model.

Compared to the reported spectrophotometric methods, there was no significant difference between them in terms of linearity, sensitivity and selectivity.

Pharmaceutical applications

The proposed procedures were applied for the determination of ALG and PIG in **Prandaglim Plus®** tablets. Satisfactory results were obtained in good agreement with the label claim, indicating no interference from excipients and additives. The obtained results were statistically compared to those obtained by the reported method[13]. No significant differences were found by applying t-test and F-test at 95% confidence level[28] indicating good accuracy and precision of the proposed methods for the analysis of the cited drugs in their pharmaceutical dosage form, as shown in **Table 4.**

CONCLUSION

Simple, sensitive, accurate and precise spectrophotometric methods were developed for simultaneous determination of alogliptin and pioglitazone in pharmaceutical preparation using partial least squares chemometric model with and without variable selection (genetic algorithm). The methods were successfully applied for quantitative analysis of both drugs in their combined dosage form without interference from tablet excipients which extended its application to quality control laboratories.

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Non.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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