Simultaneous Estimation of Metformin Hydrochloride, Glimepiride and Pioglitazone using First Generation Chemometric Methods

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ABSTRACT :
In this study, new feasible UV-Visible spectrophotometric and multivariate spectrophotometric methods were described for the simultaneous determination of Metformin hydrochloride (MET), Glimepiride (GLI) and Pioglitazone (PIO) in combined pharmaceutical tablets. Methanol is used as a solvent for analysis and whole UV region has been scanned from 200-400 nm. The resolution has been obtained by using multivariate methods as Classical least square (CLS), Inverse least squares (ILS), Principal Component Regression (PCR), Partial Least Squares Regression (PLSR) applied to the UV spectra of the mixture. The results obtained from all the methods were found within the limit.
Keywords: Classical least square (CLS), Inverse least squares (ILS), Principal Component Regression (PCR), and Partial Least Squares Regression (PLSR).

INTRODUCTION:

Metformin hydrochloride, Glimepiride and Pioglitazone are anti-diabetic drugs used in type 2 diabetes mellitus. Metformin hydrochloride chemically known as N,N-Dimethylimidodicarbonimidic diamide hydrochloride. Metformin is anti-hyperglycemic drug used in type 2 diabetes. It suppresses glucose production by liver ie heapatic gluconeogenisis [1]. Metformin reduces glucose levels primarily by decreasing hepatic glucose production and by increasing insulin action in muscle and fat. These actions are mediated by activation of AMP-activated protein kinase (AMP kinase) which causes insulin signalling and shows inhibitory effect on production of glucose [2]. Metformin is absorbed mainly from the small intestine. The drug is stable, does not bind to plasma proteins, and is excreted unchanged in the urine. It has a elimination half life of 6.2 hours [3].

Glimepiride chemically known as 3-ethyl-4-methyl-*N*-(4-[*N*-((1*R*,4*R*)-4-methylcyclohexylcarbamoyl) sulfamoyl]phenethyl)-2-oxo-2,5-dihydro-1*H*-pyrrole-1-carboxamide belongs to sulfonylurea class. It stimulates insulin release from pancreatic β cells. The acute administration of glimipiride to type 2 diabetes mellitus patients increases insulin release from the pancreas[4]. It has been found that glimipiride act as an insulin secretagouge which act by lowering the blood sugar by increasing the release of insulin by pancreatic β cells and decreases sugar level in the blood [5].

Pioglitazone chemically known as (*RS*)-5-(4-[2-(5-ethylpyridin-2-yl)ethoxy]benzyl)thiazolidine-2,4-dione . It belongs to Thiazolidinediones class and are agonists for the peroxisome proliferator–activated receptor γ (PPARγ). PPARγ, activates insulin-responsive genes that regulate carbohydrate and lipid metabolism. Thiazolidinediones principally act by increasing insulin sensitivity in peripheral tissues and thus are effective only when insulin is present but also may lower hepatic glucose production therefore increases the expense of insulin-dependent glucose; decreases

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withdrawal of glucose from the liver. The thiazolidinediones also activate genes that regulate fatty acid metabolism in peripheral tissue. Adiponectin increases insulin sensitivity, reportedly by elevating AMP kinase, which stimulates glucose transport into muscle and increases fatty acid oxidation[4].

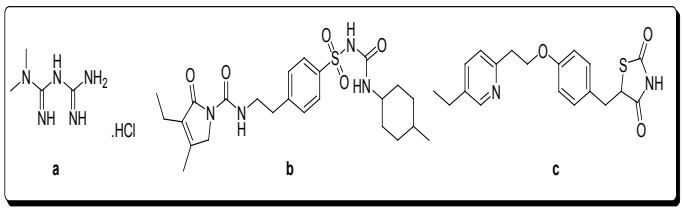


Fig. 1. Chemical structure of Metformin Hydrochloride (a), Glimepiride (b) and Pioglitazone(c).

Very few analytical methods have been available for the evaluation of Metformin hydrochloride, Glimepiride and Pioglitazone in formulation either individually or in combination with other drugs.

Simultaneous estimation of Metformin hydrochloride and Glimepiride in immediate and sustain release tablet is performed by high performance liquid chromatography (HPLC)[6]. Simultaneous estimation of Metformin hydrochloride, Glimepiride and Pioglitazone is done by HPLC using methanol-phosphate buffer (pH 4.3) in the ratio of 75:25 v/v at a flow rate of 1 ml/min [7]. There is variety of methods used for estimation of Metformin hydrochloride, Glimepiride and Pioglitazone individually or in combination, but no method is available for the simultaneous estimation of all these three drugs by UV spectroscopy and application of chemometrics methods.

Therefore our objective is to develop a new rapid and feasible simultaneous analytical UV/Vis spectrophotometric method combined with multivariate calibration technique for the evaluation of Metformin hydrochloride, Glimepiride and Pioglitazone containing bulk drugs and combined tablet dosage forms.

Materials and Methods

Classical least squares (CLS)

This method assumes Beer's law model with the absorbance at each frequency being proportional to the component concentrations[8]. In matrix notation, Beer's law model for *m* calibration standards containing *l* chemical components with the spectra of *n* digitized absorbance's is given byequation 1:

$$A=C^{*}K+E\_A \quad (1)$$

where*A* is the $m \times n$ matrix of calibration spectra, *C* is the $m \times l$ matrix of component concentration, *K* is the $l \times n$ matrix of absorptivity-path length products, and E_A is the $m \times n$ matrix of spectral errors.

The classical least squares solution during calibration is given by equation (2)

$$K=(C^{T}\,C)^{-1}\,C^{T}{}^{*}A \quad (2)$$

where *K* represents the matrix of pure component spectra at unit concentration and unit path length.

Analysis based on the spectrum of unknown components concentration (samples) is given by equation (3)

$$^{C}0=(KK^{T})^{-1}\,K^{*}A \quad (3)$$

Wherec_0 is vector of predicted concentrations and K^T is transpose of the matrix *K*.

Inverse least squares (ILS)

This method treats these concentrations as a function of absorbance. The inverse of Beer's law model for *m* calibration standards with spectra of *n* digitized absorbance is given by equation(4):

$$C=A^{*}P+E_{C} \quad (4)$$

where*C* and *A* are as before, *P* is the $n \times l$ matrix of unknown calibration coefficient relating the *l* component concentrations of the spectral intensities, and $E_c$is the $m \times l$ vector of errors. The inverse least square solution during calibration for *P* is given by equation(5):

$$P=(A^{T}\,A)^{-1}\,A^{T}{}^{*}C \quad (5)$$

In this method the concentration of analyte in the unknown sample is given by equation (6).

$$C_{0}=a^{T}{}^{*}P \quad (6)$$

Wherec_0 and *a* represents concentration and spectrum of unknown analyte respectively. Since in ILS the number of frequencies cannot exceed the total number of calibration mixtures used, stepwise multiple linear regressions have been used for the selection of frequencies [8].

Principal component regression (PCR)

In the spectral work, the following steps can explain the elemental concept of Principal component regression: (*i*) the original data obtained in absorbances(*A*) and concentration (*C*) of the analyte is reprocessed by mean-centering as A_0 and C_0, respectively. (*ii*) The covariance dispersion matrix of the centered matrix A_0 is computed. The normalized eigenvectors and eigenvalues are calculated starting from the square covariance matrix. The numeral optimal principal component (eigenvectors) is selected by considering only the highest values of the eigenvalues [8]. The other eigenvalues and their corresponding eigenvectors were

eliminated from this study. Using the ordinary linear regression by equation (7):

$$a+b^*A \quad (7)$$

coefficients*a*and *b* are calculated. To reach this objective coefficient *b* is to be estimated first by equation (7a):

$$b = P^*q \quad (7a)$$

where*P* is the matrix of eigenvectors and *q* is the C-loading specified by equation (8):

$$q = D^*T^{T*}A \quad (8)$$

here, T^T is the transpose of the score matrix *T*, *D* is a diagonal matrix having on thecomponents of the selected eigenvalues. Knowing *b*, we can easily find *a* by using the formula (9):

$$a = C_{mean} - A^T{}_{mean}{}^*b \quad (9)$$

whereC_{mean} is the mean concentration of the calibration set*and* A^Tmean represents the transpose of the matrix having the mean absorbance values[8].

Partial least square regression (PLSR)

PLSR is used to analyse strongly collinear and noisy data with numerous *X* variables (independent variables) and also simultaneously model the several response variables i.e *Y* (dependent variables) [9]. MLR in which modelling of *Y* by means of *X* is done as long as when data is few and fairly uncorrelated. However, in modern instrumentation only *X* variables are in larger numbers and also strongly correlated so that they are usually noisy and incomplete. PLSR allows one to inspect more complex problems by handling numerous and collinear *X* variables and response variables *Y* and analyze data in a more rational way[10].

Mean centering or scaling of both *X* and *Y* data matrix is performed in PLSR so that it is fitted in such a way that describes the variance of *X* and *Y*. PLSR is a maximum covariance method, because the main aim of PLSR is to predict the y-variables from the x-variables. PLSR finds out the new variables for both *X* and *Y* matrix i.e. X-scores (*T*) and Y-scores (*U*) respectively.

X scores estimate linear combination of variable x_{k-} with coefficient of weight (*W**) in equation (10):

$$T=X.W^* \quad (10)$$

However, the weight *W* can be transformed to *W** which directly relate to *X*.

From the equation 10, W^* can be written as in equation (11):

$$W^*=W(P^T W)^{-1} \quad (11)$$

PLSR model can be supposed to consist of an outer relation and an inner relation where the outer relation describes the *X* and *Y* matrix individually while the inner relation links the two matrixes together. The outer relations are given by following equations (12):

$$X=T.P^T+E \quad (12)$$

$$Y=U.C^T+F \quad (13)$$

whereP^T is the loading matrix of the *X* space, C^T is the loading matrix of the *Y* space. *E* and *F* are the residual matrices of the *X* and *Y* spaces respectively.

X scores (*T*) are also good predictor for *Y* variables i.e. correlated according to equation (14).

$$Y=T.C^T+G \quad (14)$$

By combining equation10 and 14 we can write as:

$$Y=XW^* C^T+G=XB+G \quad (15)$$

$$B=W^* C^T \quad (15a)$$

where*B* represents PLSR coefficient and *G* is residual matrix. The prediction of y-variables of new samples is determined by equation(15):

By putting the value of W^* from the equation 11 in equation 15a:

$$B=W(P^T W)^{-1} C^T \quad (16)$$

The part which is not explained by the model is called residuals. It is useful in determining model applicability which is indicated by residual value[11]. Large residual value indicates that model is poor. When first PLSR component has been calculated then further one can be calculated on the basis of residual matrices. This process continues until we achieve approximately 99 of explained variance. The number of significant PLSR components in a calibration model can be decided by means of cross validation.

Experimental

Chemicals and Reagents

Reference standard of Metformin hydrochloride (MET), Glimepiride (GLI) and Pioglitazone (PIO) are provided from the ISFAL Laboratories, Moga, Punjab. All the working solutions for analytical determination were prepared in analytical grade methanol which is purchased from Loba chemie. Pharmaceutical formulation (tablet) of these three drug combination was

purchased from market (GLIMISTAR-PM2).

Instrumentation and software

The entire UV-Vis spectrophotometric measurements were made with a Perkin Elmer UV-Visible spectrophotometer (Lambda 35) with a fix slit width of 1 nm operated by Perkin Elmer UV Probe software of version 2.31. Complete spectra were saved in CSV (excel file) format and then data was statistically analysed by using unscrambler® 10.3.0.89 software.

Preparation of Standards Solutions

Standard Stock Solutions

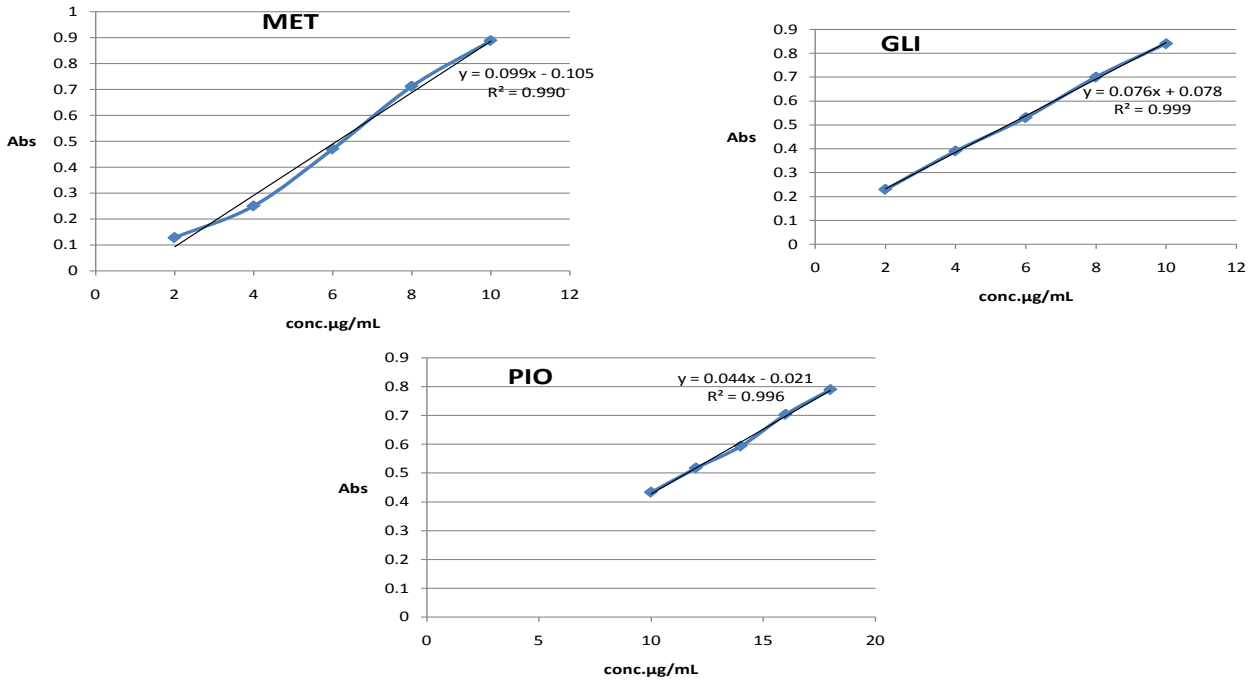
MET, GLI and PIO reference standards (10 mg) were accurately weighed and transferred to 10 mL volumetric flasks separately. They were dissolved and diluted to 10 mL with methanol to obtain a stock solution of with a final concentration of 1 mg/mL (1000 µg/mL).

Working Standard Solutions

Aliquots (1mL) of standard solution of MET, GLI and PIO was pipette out and transferred to 10 mL volumetric flasks separately and diluted to 10 mL with methanol to obtain working standard solution of MET, GLI and PIO with a final concentration of 100 µg/mL.

Calibration curve for UV method

A suitable amount of aliquots of MET, GLI and PIO were pipette out and transferred into a series of 10 mL volumetric flasks. The volume was made up to the mark with methanol to get a concentration of 2-10µg/mL for MET, 2-10 µg/mL for GLI and 10-18 µg/mL for PIO, respectively. All the samples were measured for their absorbances in the UV-VIS spectrophotometer using methanol as blank [12-13]. Standard plot was plotted for MET, GLI and PIO which is given in Figure 2.



Calibration and validation set for analytical estimation

For calibration purpose, training set of 19 mixtures has been prepared by mixing appropriate volume of standard dilutions for 19 different concentration levels in individual 10 mL volumetric flasks (Table 1). For Validation purpose, set of 12 synthetic ternary mixtures were prepared for which measurement has been done in six replicates at each time for evaluating inter and intra-day variations. The UV absorption spectra were recorded over the wavelength range of 200–400 nm. A total of 201 data points were obtained in each and every multivariate tool at an interval of 1 nm. The absorbance data of the calibration set obtained from UV spectra were exported into excel file and then subjected to the Unscrambler® program for the Chemometric tools.

Analysis of marketed formulation

Average weight of 10 tablets were calculated and finely powdered. Powder equivalent to one tablet were weighed properly and transferred into 10 mL volumetric flask. 5-6 mL methanol was added to the volumetric flask followed by sonication for 10 minutes. After sonication volume made up with methanol and solution was filtered through a whatmann filter paper. Filtered solution was measured for its absorbance in UV-VIS spectrophotometer.

Results

Analysis of combination mixtures and pharmaceutical preparations

Ternary mixture of MET, GLI and PIO was scanned by UV-Vis spectrophotometer in the range of 200 – 400 nm at an interval of 1 nm.

Four multivariate tools i.e. Classical least square (CLS), Inverse least squares (ILS), Principal Component Regression (PCR), Partial Least Squares Regression (PLSR) were used to resolve the data of ternary mixtures of MET, GLI and PIO.

the values obtained from Classical least square (CLS), Inverse least squares (ILS), Principal Component Regression (PCR), Partial Least Squares Regression (PLSR) methods were under the ICH limits. Analytical figures of merits and r^2 values were given in Table 3. The marketed formulations obtained from local market has been analysed (Table 4 and 5).

Table 1. Composition of the calibration set (**µg** ml^{-1}).

S.No.	MET	GLI	PIO	So. No.	MET	GLI	PIO
1	5	6	10	12	8	5	15
2	8	5	15	13	7	8	17
3	7	8	17	14	3	9	18
4	3	9	18	15	4	4	19
5	4	4	19	16	3	10	14
6	3	10	14	17	2	4	10
7	2	4	10	18	8	7	17
8	8	7	17	19	8	5	15
9	5	3	19				
10	6	5	16				
11	2	9	13				

Table 2: Composition and results of analysis of prepared mixtures by CLS, ILS, PCR and PLSR.

Mixtures			RESULT FOUND											
			(CLS)			(ILS)			(PCR)			(PLS)		
1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
8	5	15	99.77	98.57	99.47	99.57	99.07	98.37	99.97	101.47	99.97	99.97	101.57	100.37
7	8	17	99.27	97.72	99.67	97.77	99.72	98.47	101.72	99.67	100.27	100.27	101.72	100.22
3	9	18	98.67	99.07	98.77	98.07	98.07	99.77	98.97	101.77	100.57	101.47	99.47	99.87
4	4	19	97.87	97.77	97.17	98.77	97.67	97.87	101.97	101.97	100.27	101.07	100.47	101.07
3	10	14	99.57	98.57	99.27	98.07	98.27	97.57	99.97	99.97	99.87	100.67	99.97	99.97
2	4	10	99.27	99.67	98.30	97.67	98.67	98.67	102.97	101.30	100.67	99.57	99.27	99.87
8	7	17	98.07	98.37	99.01	98.11	98.07	99.37	99.97	99.97	99.97	99.97	99.97	99.97
8	5	15	97.27	98.47	99.77	97.47	99.47	98.07	101.27	99.87	98.97	101.77	100.57	101.17
7	8	17	99.07	99.77	99.27	97.77	97.77	99.47	101.30	100.47	101.97	101.97	100.27	100.63
3	9	18	98.77	97.87	96.27	98.87	97.17	99.07	99.97	99.57	99.97	101.27	99.87	100.67
4	4	19	98.67	98.87	99.27	98.07	93.07	97.87	99.77	99.67	101.27	101.27	100.67	99.57
3	10	14	99.07	99.17	96.07	98.17	99.17	98.87	101.27	99.87	99.67	101.07	100.57	100.97
% Mean recovery			98.78	98.66	98.53	98.20	98.02	98.62	100.76	100.47	100.29	100.86	100.37	100.36
% RSEP			0.836	1.309	1.197	0.501	0.883	1.027	0.431	0.584	0.761	0.422	0.572	0.740

All the values obtained from Classical least square (CLS), Inverse least squares (ILS), Principal Component Regression (PCR), Partial Least Squares Regression (PLSR) methods were under the ICH limits. Analytical figures of merits and r^2 values were given in Table 3. The marketed formulations obtained from local market has been analysed (Table 4 and 5).

Table3. Composition and results of analysis of data of prepared mixtures by CLS, ILS, PCR and PLSR.

Methods	MET			GLI			PIO		
	Range (nm)	r^2	n^a	Range (nm)	r^2	n^a	Range (nm)	r^2	n^a
CLS	210-380	0.9966	-	210-380	0.9951	-	210-320	0.9932	-
ILS	210-380	0.9954	-	210-380	0.9974	-	210-320	0.9914	-
PCR	214-339	0.9988	2	267-385	0.9999	2	230-369	0.9971	2
PLSR	227-350	0.9975	2	267-385	0.9990	2	224-357	0.9976	2

n^a is the number of latent variables employed.

Table 4. Multivariate analytical statistical parameters obtained for MET, GLI and PIO through CLS, ILS, PCR and PLSR.

Parameters	MET				GLI				PIO			
	1	2	3	4	1	2	3	4	1	2	3	4
PRESS	0.158	0.114	0.428	0.238	0.115	0.101	0.117	0.112	0.129	0.117	0.106	0.114
RMSECV	0.084	0.054	0.176	0.12	0.055	0.04	0.057	0.052	0.066	0.056	0.045	0.053
SEL	0.4178	0.4578	0.6678	0.6978	0.4778	0.4978	0.6978	0.6878	0.4378	0.4578	0.6978	0.6978
SEN(ml μg^{-1})	2.409	2.519	2.359	2.559	2.259	2.449	2.209	2.549	2.549	2.409	2.389	2.429
LOD($\mu g\ ml^{-1}$)	0.593	0.262	2.833	0.625	0.261	2.850	0.521	0.219	2.521	0.486	0.208	2.495
LOQ($\mu g\ ml^{-1}$)	1.778	0.785	8.5	1.875	0.782	8.551	1.562	0.658	7.562	1.458	0.625	7.485

Table 5. Determination of MET, GLI and PIO in pharmaceuticals using the proposed methods.

Pharmaceutical Product	Mean recovery ± S.D			
	CLS	ILS	PCR	PLSR
MET	98.23 ± 0.73	98.35 ± 0.71	99.20 ± 0.76	100.27 ± 0.64
GLI	98.32 ± 0.69	99.26 ± 0.62	100.43 ± 0.65	100.32 ± 0.41
PIO	98.33 ± 0.74	98.13 ± 0.39	100.27 ± 0.71	99.12 ± 0.68
% Recovery[a]	99.34 ± 0.56	99.11 ± 0.35	100.12 ± 0.34	100.13 ± 0.45
	99.43 ± 0.87	100.00 ± 0.25	100.03 ± 0.57	100.04 ± 0.61
	99 .01± 0.54	99.01 ± 0.23	100 .01± 0.44	100.09 ± 0.57

[a] For standard addition of 100% at nominal.

CONCLUSION

Four multivariate tools were adopted for simultaneous estimation of MET, GLI and PIO in combined pharmaceuticals without prior separation. All four methods were found to be accurate, precise and having good recoveries. The results obtained from PCR and PLSR method gives better results as compared to CLS and ILS methods individually. These multivariate tools are time saving, less laborious and inexpensive than other analytical techniques i.e. HPLC, HPTLC, GC, LC-MS, GC-MS etc. Multivariate tools adopted for the simultaneous estimation confirm acceptable to the label claim and signify the high accuracy and precision of the proposed method when applied to tablets.

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REFERENCES :

1.Kirpichnikov D, McFarlane SI, Sowers JR. Metformin: an update. Ann Intern Med. 2002;137(1):25-33.

2.Zhou G, Myers R, Li Y, Chen Y, Shen X, Fenyk-Melody J, et al. Role of AMP-activated protein kinase in mechanism of metformin action. J Clin Invest. 2001;108(8):1167.

3.Squibb B-M, Food U, Administration D. Glucophage (metformin hydrochloride tablets) Label Information. August; 2008.

4.Hilal-Dandan R, Brunton L. Goodman and Gilman manual of pharmacology and therapeutics: McGraw Hill Professional; 2013.

5.Nissen SE, Nicholls SJ, Wolski K, Nesto R, Kupfer S, Perez A, et al. Comparison of pioglitazone vs glimepiride on progression of coronary atherosclerosis in patients with type 2 diabetes: the PERISCOPE randomized controlled trial. JAMA. 2008;299(13):1561-73.

6.Pawar SP, Meshram GA, Phadke MU. Simultaneous LC estimation of glimepiride and metformin in glimepiride immediate release and metformin sustained release tablets. Chromatographia. 2008;68(11-12):1063-6.

7.Jain D, Jain S, Jain D, Amin M. Simultaneous estimation of metformin hydrochloride, pioglitazone hydrochloride, and glimepiride by RP-HPLC in tablet formulation. J Chromatogr Sci. 2008;46(6):501-4.

8.Kumar N, Bansal A, Sarma G, Rawal RK. Chemometrics tools used in analytical chemistry: An overview. Talanta. 2014;123:186-99.

9.Espinosa-Mansilla A, Salinas F, Paya IDO. Simultaneous determination of sulfadiazine, doxycycline, furaltadone and trimethoprim by partial least squares multivariate calibration. Anal Chim Acta. 1995;313(1):103-12.

10.Lorber A, Faber K, Kowalski BR. Net analyte signal calculation in multivariate calibration. Anal Chem. 1997;69(8):1620-6.
11.Wold S, Sjöström M, Eriksson L. PLS-regression: a basic tool of chemometrics. Chemometr Intell Lab. 2001;58(2):109-30.
12.Sujana K, Swathi Rani G, Bhanu Prasad M, Saheethi Reddy M. Simultaneous estimation of pioglitazone hydrochloride and metformin hydrochloride using UV spectroscopic method. J Biomed Sci Res. 2010;2(2):110-5.
13.Game MD. First Order Derivative Spectrophotometric Method for Simultaneous Estimation of Glimepiride and Pioglitazone HCl in Combined Dosage Form. J Pharm Res. 2011;4(11).

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