WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 7, 1269-1279. Research Article ISSN 2277– 7105

CHEMOMETRICS ASSISTED SIMULTANEOUS SPECTROPHOTOMETRIC DETERMINATION OF ALOGLIPTIN AND METFORMIN IN PHARMACEUTICAL DOSAGE FORM

Premakumari K. B.*, Sandhyavali M. S., Murugan V. and Shanaz Banu

Department of Pharmaceutical Chemistry, College of Pharmaceutical Sciences, Dayananda Sagar University, Kumaraswamy Layout, Bengaluru, Karnataka- 560 078, India.

Article Received on
07 Feb. 2018,

Revised on 28 Feb. 2018,
Accepted on 21 March 2018,

DOI: 10.20959/wjpr20187-11697

***Corresponding Author**
Premakumari K. B.
Department of
Pharmaceutical Chemistry,
College of Pharmaceutical
Sciences, Dayananda Sagar
University, Kumaraswamy
Layout, Bengaluru,
Karnataka- 560 078, India.

ABSTRACT

Two new methods are designed for the simultaneous determination of Alogliptin(ALG) and Metformin (MTF). The two chemometric assisted spectrophotometric methods are principal component regression (PCR) and partial least squares (PLS). Spectra of Alogliptin and Metformin were recorded at several concentrations within their linear range and the readings so obtained were used to compute the calibration mixture between wavelengths 200 and 400 nm at an interval of 1 nm in methanol. The two methods were successfully applied to quantify each individual drug in the mixture by using the absorption spectra of appropriate solutions. The analytical responses of these chemometric methods were characterized by relative prediction errors and recoveries (%) and were compared with each other. The two methods were found to be satisfactory and successfully applied to a pharmaceutical dosage form and the results were compared.

KEYWORDS: Alogliptin, Metformin, Spectrophotometry, Chemometrics, PCR and PLR.

INTRODUCTION

Alogliptin is 2-[[6-[(3R)-3-aminopiperidin-1-yl]-3-methyl-2,4-dioxypyrimidin-1-yl]methyl] benzonitrile is a selective dipeptidyl peptidase-4 (DPP-4) inhibitor used in combination with diet and exercise in the therapy of Type 2 diabetes either alone or in combination with other oral hypoglycemic agents. Several analytical methods are reported for the quantitative determination of Alogliptin in pharmaceutical formulations or in biological fluids.

Metformin is 1,1- dimethyl biguanide represent the biguanide class of oral hypoglycemic agents employed in the management of non-insulin dependent diabetes mellitus which is not responding to diet. It acts by improving insulin sensitivity and decreasing intestinal absorption of glucose. The determination of metformin has been carried out in tablets by HPLC, Capillary electrophoresis and supercritical fluid chromatography[11], in bulk and solid dosage forms. The structure of Alogliptin and Metformin is represented in Figure 1 & 2.

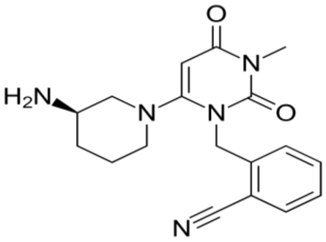

Figure 1: Alogliptin.

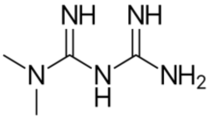

Figure 2: Metformin.

The main objective of the proposed work is to develop and validate new analytical methods for simultaneous determination of alogliptin and metformin in tablet dosage form. This paper gives a simple method which is sensitive and reproducible for the simultaneous estimation of alogliptin and metformin in combined dosage form.

Nowadays, multivariate calibration methods have been reported as reliable techniques that gives best results in terms of complex mixture resolution. The same methods and their algorithms have been successfully employed in the simultaneous spectrophotometric estimation of drugs in pharmaceutical formulations containing two or more compounds with overlapping spectra. Partial least squares regression (PLS) has become the most frequently used method for multivariate calibration because of its high performance calibration models. Chemometrics calibration techniques in spectral analysis are widely employed in the quality control of drugs in mixtures and pharmaceutical formulations containing two or more drugs with overlapping spectra as separation procedures can be avoided in drug determination. In this study, two Chemometric methods for spectral data processing are proposed for simultaneous determination of Alogliptin and Metformin in their binary mixtures and in tablets.

2. Experimental

2.1. Apparatus and software

Digitized UV/Vis absorbance spectra were collected using a UV-Vis Spectrophotometer, Shimadzu, 1700, UV Probe software, with 1 cm quartz cells at a scan rate of 1000 nm min−1 and the slit width of 2 nm. The UV spectra of mixtures were recorded over the wavelength 200–400 nm with one data point per nanometer. All spectral measurements were performed using blank solution as reference. Partial least squares regression, and principal component regression were used for Chemometrics analysis of data, collected. For all calculations, The Unscrambler X for windows (Version 10.4) was used. All programs were run on a Pentium (IV) microcomputer, with windows XP home edition.

2.2. Chemicals

Pure samples of Alogliptin and Metformin were purchased from Vivan Life Sciences and were used without further purification. All solvents and reagents used in the estimation were of analytical reagent grade.

2.3. Pharmaceutical preparation

A commercial pharmaceutical formulation (Kazano tablet) was purchased from Canada and assayed. Its declared content was as follows Alogliptin (12.5mg) and Metformin (500mg) in each tablet.

2.4. Standard solutions

Stock solutions of Alogliptin and Metformin containing1000µgml−1 were prepared in 100 ml volumetric flasks by dissolving 100 mg of each compound in methanol. Working standard solutions were prepared daily by diluting the stock solutions for each drug according to its linear calibration range. Two sets of standard solutions were prepared, the calibration set contained 25 standard solutions and the prediction set contained 9 standard solutions. To a series of 10 ml volumetric flasks, aliquots of Alogliptin and Metformin solutions, containing appropriate amount of these drugs in the range of calibrations, were added and then the solutions were diluted to 10 ml with methanol. UV spectra of the mixtures were recorded in the wavelength range 200–400 nm versus a solvent blank, and digitized absorbance was sampled at1 nm intervals. All the solutions were prepared freshly and were protected from light.

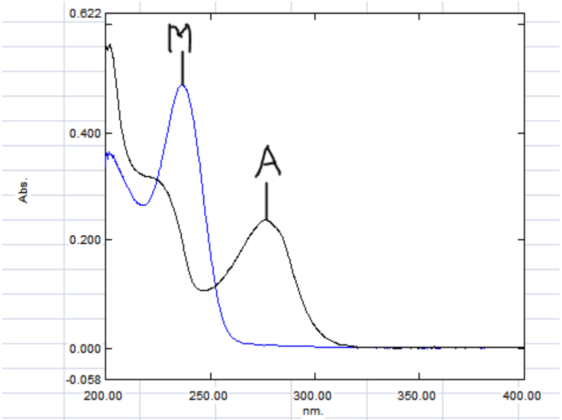

Figure 3: Overlay spectra of Alogliptin and Metformin.

Analysis of Pharmaceutical formulation

Twenty tablets were weighed accurately and powdered. An amount of the powder equivalent to 10 mg equivalent weight of Alogliptin and Metformin was taken in 50mL volumetric flask, 20ml of methanol was added and ultrasonicated for 10 minutes and the volume was made up to 50 mL with methanol and shook well. Then, the solution was filtered through Whatman filter paper No. 41 and the validation set was prepared. Aliquots of these solutions were used in such a way that the concentration of each drug was within the range of the calibration matrix. The diluted solutions were analyzed six times. All the proposed Chemometrics methods were applied, for the data collected.

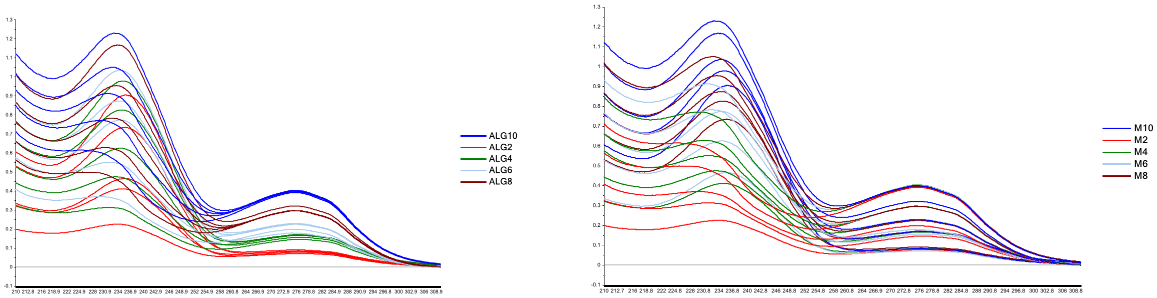

Figure 4: Spectral variation with respect to change in concentration of Alogliptin and Metformin is shown in the plot.

Partial least squares (PLS).–In the UV-Vis spectra, the absorbance data (A) and concentration data (C) are mean centered to give the data matrix A_0 and vector C_0. The orthogonalized PLS algorithm has the following steps. The scores and the loading figures are given in figure 5.

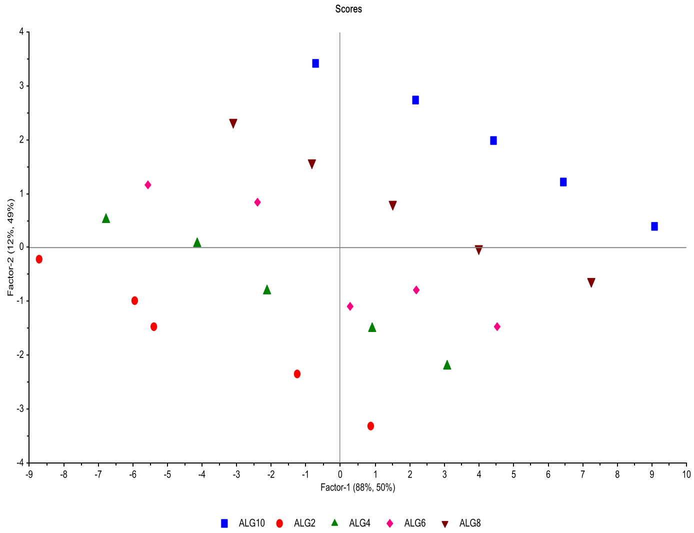

Figure 5: Scores Plot for Alogliptin.

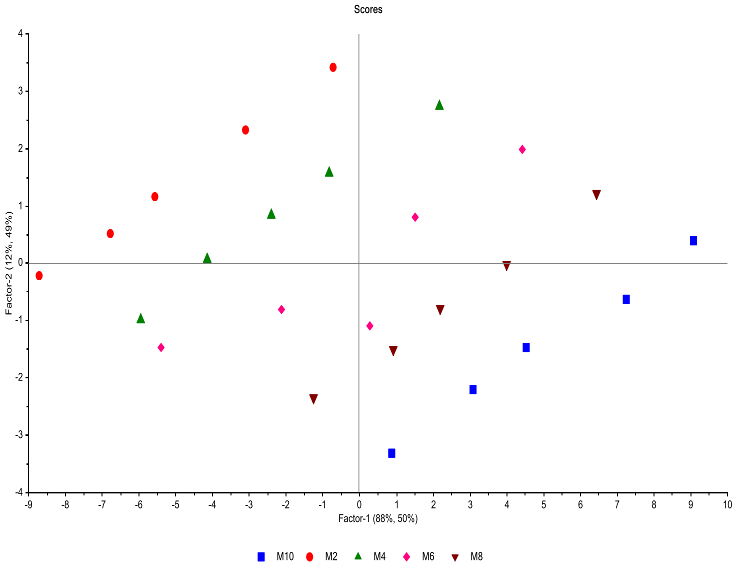

Figure 6: Scores Plot for Metformin.

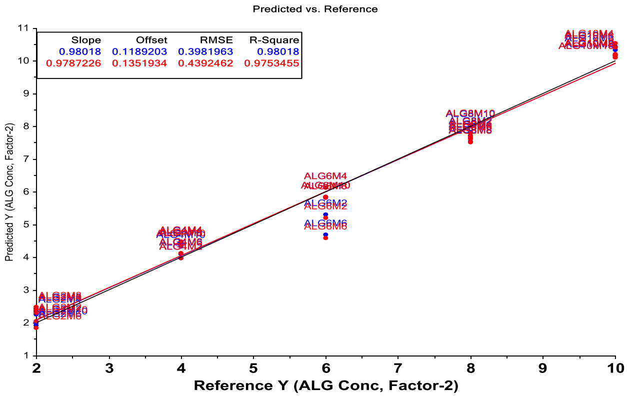

Figure 7 (i): Plot of Actual Vs Predicted value of Alogliptin.

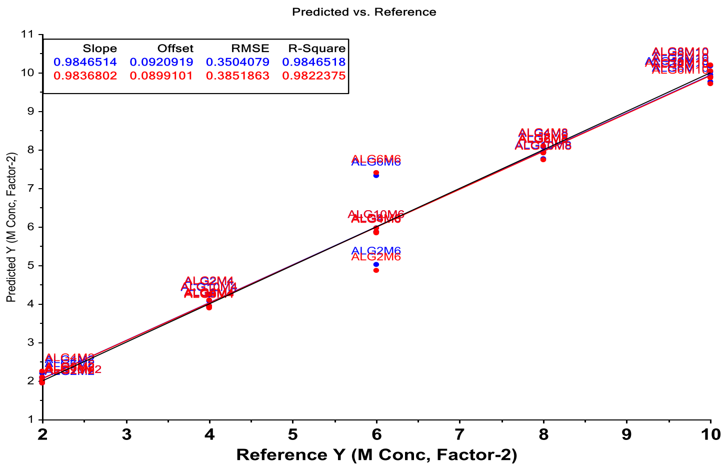

Fig7(ii): Plot of Actual Vs Predicted value of Metformin

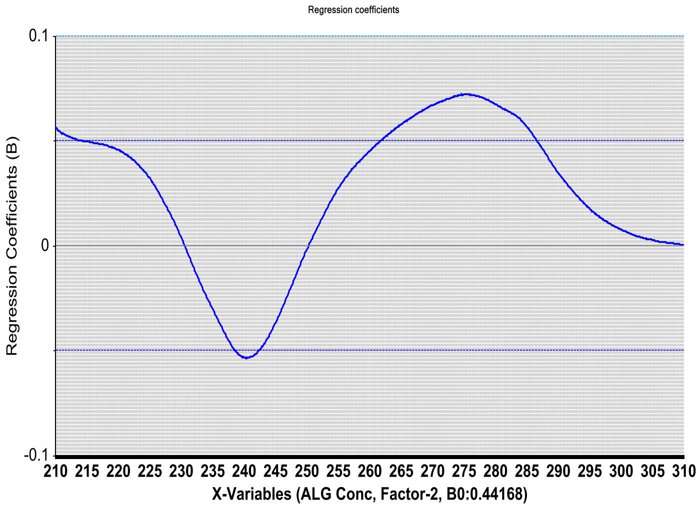

Figure 8(i): X-variable for Alogliptin

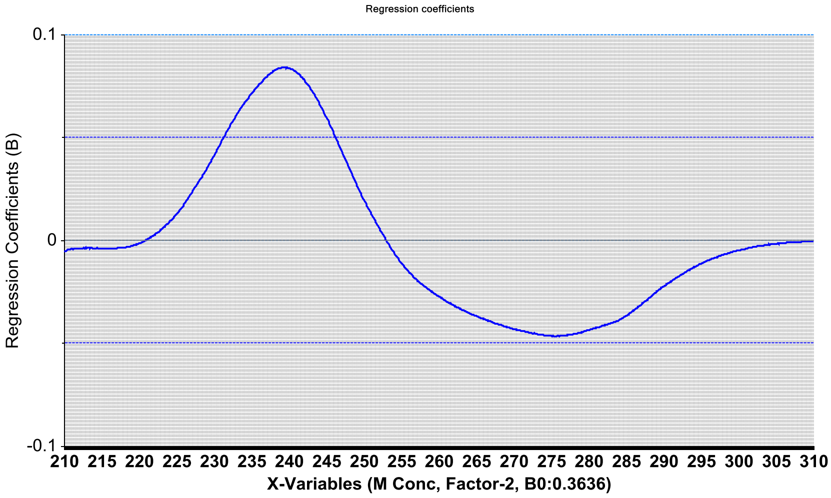

Figure 8(ii) for X-variable M conc

Table 1: The matrix and vector of the residuals in *A*0 and *C*0.

Q Residual Limits							
	Factor-0	**Factor-1**	**Factor-2**	**Factor-3**	**Factor-4**	**Factor-5**	**Factor-6**
0.10%	258.4459	31.49245	0.1417225	0.02173162	0.01199685	0.009795	0.000433817
0.50%	180.3999	22.29034	0.09657416	0.01633005	0.009042311	0.006885	0.000307354
1%	149.5226	18.56347	0.07939561	0.01410666	0.007807039	0.005719	0.000256068
5%	84.80301	10.55419	0.04478396	0.00917947	0.005026242	0.003239	0.000145682
10%	60.2485	7.43437	0.0321696	0.007151969	0.003864774	0.002285	0.000102609
25%	31.37902	3.71182	0.01765105	0.004534986	0.00235546	0.001156	0.000051152

From the general linear equation, the regression coefficients were calculated by:

$b = W\ (W)^{-1}q$

$a = C_{mean}-{}_{meanb}$

The built calibration equation is used for the estimation of the compounds in the samples.

Principle component regression (PCR).: In the spectral work, the following steps can explain the fundamental concept of PCR. The original data obtained in absorbances (A) and concentrations (C) of analytes were reprocessed by mean-centering as $A0$ and $C0$, respectively.

Using the ordinary linear regression:

$$C = a + b \times A \qquad (6)$$

The coefficient b is, $b = P\text{x}q$, where P is the matrix of eigen vectors and q is the C loadings given by $q = D \times T^T\text{x}A_0$. Here, T^T is the transpose of the score matrix T. D is a diagonal matrix having on components the inverse of the selected eigenvalues.Knowing b one can easily find a by using the formula $a = C$ mean $\text{x}A^T_{mean}$ x b, where A^T_{mean} represents the transpose of the matrix having the entries of the mean absorbance values, and C_{mean} is the mean concentration of the calibration set.

RESULTS AND DISCUSSIONS

The first step in simultaneous determination of the binary mixture of drugs by multivariate calibration methods involves constructing the calibration matrix for binary mixture of Alogliptin and Metformin. Twenty five binary mixtures were selected by random design as the calibration set. The composition of the samples was designed by applying multilevel multifactor design in order to obtain non correlated concentration profiles. The UV absorbance data were obtained by measuring the absorbance in the region of 200-400nm The selected wavelength was 205-300nm and shown in **Figure 3 & 4.**

The calibration model in each chemometric method was validated with 9 synthetic mixtures set containing the drugs under study in different proportions selected randomly. The predictive abilities of PLS and PCR were examined for simultaneous determination of Alogliptin and Metformin in sample mixtures. The common requirement for all the mentioned methods is that the unknown samples and standards be of the same nature. The most commonly employed validation criterion is to divide the dataset into two subsets, a calibration set and a validation set. The calibration model is calculated using the calibration set. Then, the root mean square errors of calibration and validation, RMSEC – root mean square error of calibration and RMSECV – root mean square error of cross validation, are calculated by using the calibration model under investigation to predict the samples in the calibration set and validation set, respectively

Selection of the optimum number of factors for PCR and PLS

For PCR and PLS methods, 25 calibration spectra were used for the selection of the optimum number of factors by using the cross-validation with the leave-out-one technique. This allows modeling of the system with the optimum amount of information and avoidance of over-fitting or under-fitting. The cross-validation procedure consisted of systematically removing one of a group of training samples in turn and using only the remaining ones for the construction of latent variable factors and applied regression. The predicted concentrations were then compared with the actual ones for each of the calibration samples and the root mean square error of prediction (RMSEP) was calculated. The RMSEP was computed in the same manner each time, and then a new factor was added to the PCR and PLS model. The selected model was that with the smallest number of factors such that its RMSECV values were not significantly greater than that for the model, which yielded the minimum RMSECV. A plot of RMSECV values against the number of components(Figs. 2 and 3) indicates that the latent variable factor 3 was optimum for PCR and PLS selected based on the RMSEC and RMSECV, respectively, for the estimation of the titled drugs. At the selected principal component of PCR and PLS, the concentrations of each sample was then predicted and compared with the known concentration and the RMSEP was calculated:

The results are tabulated in **Table 1, 2 & 3.**

Table 2: Composition of the Calibration set for applying PLS and PCR.

Number of calibration sample	Alogliptin (μg ml^{-1})	Metformin (μg ml^{-1})
1	2	2
2	2	4
3	2	6
4	2	8
5	2	10
6	4	2
7	4	4
8	4	6
9	4	8
10	4	10
11	6	2
12	6	4
13	6	6
14	6	8
15	6	10
16	8	2
17	8	4
18	8	6
19	8	8
20	8	10
21	10	2
22	10	4
23	10	6
24	10	8
25	10	10

Table 3: Statistical parameters of Chemometric methods – PLS and PCR in the Calibration set.

Method	Parameter	Alogliptin	Metformin
PLS	RMSEC	0.39	0.35
	r^2	0.9801	0.9846
PCR	RMSEC	0.302	0.1058
	r^2	0.9885	0.9987

PLS – Partial Least Squares, PCR – Principal Component regression, RMSEP – Root Mean Square Error of Prediction, RMSECV – Root Mean Square Error of Cross Validation. In order to test the proposed techniques, the validation set of synthetic mixtures(from standards) containing the two drugs variable ratios was carried out. The maximum values of the mean percent errors corresponding to PCR and PLS for the same mixtures were completely acceptable because of their very small numerical values (below 0.2).

CONCLUSION

The most striking features of spectrophotometric method are its simplicity and rapidity without requiring time-consuming sample preparation. Chemometric calibration techniques in spectral analysis are widely used in quality control of drugs in mixtures and multicomponent pharmaceutical formulations with overlapping spectra, as separation procedures in the drug determinations are not required. A comparative study of the use of PLS and PCR for the simultaneous spectrophotometric determination of Alogliptin and Metformin has been accomplished. Results also showed that the developed methods can be applied to a routine, quality control analysis in both raw material as well as in pharmaceutical dosage form.

ACKNOWLEDGMENT

We would like to thank RGUHS for providing financial assistance. And also like to thank Dean and management of Dayananda Sagar College of Pharmacy, for providing the facilities required for carrying out this research.

REFERENCES

1. Joshi SS, Nahire RR, Shastri NR, Surendranath KV, Satish J. Validated stability indicating RP-HPLC, UV method for simultaneous determination of metformin and repaglinide. Acta Chromatogra., 2012; 24: 419-432.
2. Abdul S, Poddar SS. A flexible technology for modified release of drugs: multi layered tablets. J Control Release., 2004; 97: 393-405.
3. Campbellt DB, Laviellez R, Nathan C. The mode of action and clinical pharmacology of gliclazide: A review. Diabetic Res Clin Pract., 1991;,14: S2l–36.
4. Dunn CJ, Peters DH. A review of its pharmacological properties and therapeutic use in non-insulin-dependent diabetes mellitus. Drugs., 1995; 49(5):,721–49.
5. Ziegler O, Drouin P. Hemobiological Properties of Gliclazide. J DiabGnnp., 1994; 8: 235–9.
6. Waugh J, Keating GM, Plosker GL, Easthope S, Robinson DM. Pioglitazone: A Review of its Use in Type 2 Diabetes Mellitus. Drugs., 2006; 66(1): 85–109.
7. Olivieria AC, Goicoechea HC, Inon FA. MVC1: an integrated MatLab toolbox for first-order multivariate calibration. ChemomIntell Lab Syst., 2004; 73: 189–97.
8. Ni Y, Wang Y, Kokot S. Multicomponent kinetics spectrophotometric determination of pefloxacin and norfloxacin in pharmaceutical preparations and human plasma samples

with the aid of chemometrics. Spectrochim Acta A Mol Biomol Spectrosc., 2008; 70(5): 1049–59.

9. Culzoni MJ, Zan MMD, Robles JC, Mantovani VE, Goicoechea HC. Chemometrics-assisted UV-spectroscopic strategies for the determination of theophylline in syrup. J Pharm Biomed Anal., 2005; 39(5): 1068–74.
10. Goicoechea HC, Olivieria AC. A comparison of orthogonal signal correction and net analyte preprocessing methodsTheoretical and experimental studies. Chemometr Intell Lab Syst., 2001; 56: 73–81.
11. Santosh V Gandhi, Priyanka S Ghone, Shreeyash R Tapale. Chemometric-assisted UV Spectrophotometric method for determination of diacerein and aceclofenac in pharmaceutical formulation. J of Chem and Pharm Res., 2016; 8(7): 912-17.
12. Lakshmi KS, Rajesh T, Sharma S (2009) Simultaneous determination of metformin and pioglitazone by reversed phase HPLC in pharmaceutical dosage forms. Int J Pharmacy Pharm Sci., 2009; 1: 162-166.
13. Khan G, Sahu D, Agrawal YP, Sabarwal N, Jain A, et al. Simultaneous estimation of Metformin and Sitagliptin in tablet dosage form. Asian J Biochem Pharm Res., 2011; 1: 352-358.
14. Jain D, Jain S, Amin M. Simultaneous estimation of metformin hydrochloride, pioglitazone hydrochloride, and glimepiride by RP-HPLC in tablet formulation. J Chromatogr Sci., 2008; 46: 501-504.
15. Ni Y, Wang Y, Kokot S. Multicomponent kinetics Spectrophotometric determination in pharmaceutical preparations and human plasma samples with the aid of Chemometrics. Spectrochim Acta A Mol Biomol Spectrosc., 2008; 70: 1049-1059.