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Multivariate Chemometric Assisted Analysis of Metformin Hydrochloride, Gliclazide and Pioglitazone Hydrochloride in Bulk Drug and Dosage Forms

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ABSTRACT

Purpose: In this work a numerical method, based on the use of spectrophotometric data coupled to partial least squares (PLS) regression and net analyte preprocessing combined with classical least square (NAP/CLS) multivariate calibration, is reported for the simultaneous determination of metformin hydrochloride (MET), gliclazide (GLZ) and pioglitazone hydrochloride (PIO) in synthetic samples and combined commercial tablets. ***Methods:*** Spectra of MET, GLZ and PIO were recorded at concentrations within their linear ranges (5-25 µg/ml, 0.5-8 µg/ml and 0.5-3 µg/ml respectively) and were used to compute a total of 25 synthetic mixtures involving 15 calibration and 10 validation sets between wavelength range of 200 and 400 nm in 0.1N HCl. The suitability of the models was decided on the basis of root mean square error (RMSE) values of calibration and validation data. ***Results:*** The analytical performances of these chemometric methods were characterized by relative prediction errors and recovery studies (%) and were compared with each other. These two methods were successfully applied to pharmaceutical formulation, tablet, with no interference with excipients as indicated by the recovery study results. Mean recoveries of the commercial formulation set together with the figures of merit (calibration sensitivity, selectivity, limit of detection, limit of quantification etc.) were estimated. ***Conclusion:*** The proposed methods are simple, rapid and can be easily used as an alternative analysis tool in the quality control of drugs and formulation.

Introduction

Oral ingestion has long been the most convenient and commonly employed route of drug delivery due to its ease of administration, least aseptic constraints and flexibility in the design of the dosage form. It is well known that modified release dosage forms may offer one or more advantages over immediate release formulations of the same drug. There are many ways to design modified release dosage forms for oral administration; from film coated pellets, tablets or capsules to more sophisticated and complicated delivery systems such as osmotically driven systems, systems controlled by ion exchange mechanism, systems using three dimensional printing technology and systems using electrostatic deposition technology. The design of modified release drug product is usually intended to optimize a therapeutic regimen by providing slow and continuous delivery of drug over the entire dosing interval whilst also providing greater patient compliance and convenience.[1]

MET, GLZ and PIO are active principles widely used and frequently combined in pharmaceutical preparation. All these three drugs are complimentary to each other. GLZ being an insulin secretagogue helps in insulin secretion from pancreas[2] whereas; insulin secreted under GLZ influence can be utilized by MET for its action. MET not only utilizes the insulin secreted under gliclazide influence but also converts from peripheral tissues.[3] Drawbacks associated with GLZ are weight gain and hypoglycemia.[4] This can easily be overcome by MET. PIO on the other hand is basically responsible for eliminating the problem of insulin resistance occurred on long term uses of sulphonyl ureas.[5]

For the treatment of diabetes mellitus the usual combination of drugs which are available in the market consists of MET and PIO and/or glipizide, or MET and GLZ but all the three drugs are not available in a single formulation. This addition seems to be aimed at improving the antidiabetic efficacy.

Pharmaceutical processing and formulation often introduce various interferants (chemicals other than drug/s under investigation) into the system. When performing quantification these interferants can disturb univariate analysis, but with multivariate analysis the

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quantification can still be performed. Several multivariate techniques of data analysis have been developed and used in the chemometric community by the researchers, out of which PLS and NAP/CLS methods are one of them.[6] PLS regression is a supervised multivariate method with which quantitative analysis of multiple solid forms can be performed even if the differences between the spectra are minor.[7] The method involves a calibration step in which the relation between spectra and component concentrations is estimated from a set of reference samples, and a prediction step in which the results of the calibration are used to estimate the component concentrations in an unknown sample spectrum.[8] NAP/CLS is one of the methods under net analyte signal preprocessing (NAS). The NAS is the part of the signal which is directly related to the concentration predicted by the calibration model. In mathematical terms, it is the part of a spectrum which is orthogonal to the space spanned by the spectra of all analytes except one.[9]

Materials and Methods

Instrument, reagents and softwares

Elico SL 191 double beam UV-Visible Spectrophotometer, with 1 cm path length was used for the absorbance measurement. All the chemicals used were of analytical grade. Pure MET was obtained from Abhilasha Pharma Pvt. Ltd., Gujarat, GLZ was obtained from Kwality Pharmaceuticals, Amritsar and PIO from GMH Laboratories, Baddi.

The design expert 8.0.4 software and Matlab 7.5 with MVC1 toolbox were used for construction of binary mixtures and the statistical treatment of the data along application of various multivariate methods.

Preparation of standards

1mg/ml MET, GLZ and PIO stock solutions were prepared by dissolving accurately weighed amounts of finely powdered pure MET, GLZ and PIO in small quantity of methanol and the final volumes were made respectively with 0.1N HCl. Suitably diluted samples from each stock were utilized for λ_{max} determination of individual component followed by serial dilution with 0.1N HCl to obtain the aliquots falling in linearity.

Standard solutions for multivariate calibration

The calibration and validation mixtures were prepared by mixing MET, GLZ and PIO solutions in different ratios varying in their individual linearity ranges viz. 0-25 µg/ml, 0-8 µg/ml, 0-3 µg/ml. The concentrations of combinations were decided by design expert 8.0.4 software under central composite design. Total 25 sets were prepared out of which 15 sets (Table 1) were utilized as calibration set whereas, the rest 10 served as validation sets (Table 2). All the mixtures were scanned at 220-299 nm range digitized at every 3 nm. The absorbance below 220 nm and above 299 nm was not taken under consideration due to too much of noise and diminished responses respectively.

Table 1. Calibration set composition

Runs	MET (µg/ml)	GLZ (µg/ml)	PIO (µg/ml)
C1	5	1	1
C2	25	6	0.75
C3	25	1	3
C4	5	6	3
C5	0	3.5	2
C6	25	3.5	2
C7	15	3	2
C8	15	6	2
C9	15	3.5	0
C10	15	3.5	3
C11	16	4	1
C12	19	0	1
C13	20	8	1
C14	20	4	1
C15	18.5	2	1.6

Table 2. Validation set composition

Runs	MET (µg/ml)	GLZ (µg/ml)	PIO (µg/ml)
V1	25	0	1.3
V2	4.5	2	1.5
V3	6	0.5	3
V4	25	7	2.5
V5	13	6	3
V6	10	8	0.5
V7	25	8	3
V8	5	8	3
V9	16	4	2
V10	10	6	1

Sample preparation

Commercial tablets of MET, GLZ and PIO were analyzed for accuracy. The tablets were processed by taking at least 10 tablets for each and finely crushed to powder in separate mortar-pestles. An equivalent amount of the obtained powder of each drug was weighed, dissolved in methanol, sonicated for 20 min, made up the volume with 0.1N HCl and filtered through a 0.5 µm membrane filter. The final concentrations and analyte ratios in each test solution lied within the corresponding calibration ranges. Each sample solution was prepared in triplicate and measured in random order.

Theory

***PLS-1*:** To start working on PLS-1 using MATLAB, first a data matrix *X* and a concentration vector *Y* need to be identify against J sensors and I samples. Both *X* and *Y* is required for the calculation of singular value decomposition (SVD). On performing PLSSVD on *X* and *Y* matrix, the result will be further 3 matrixes i.e. the singular value matrix (*S*), the right singular value matrix (*V*), and the left singular value matrix (*U*). V matrix can also be termed as loading matrix which helps in the determination of score matrix (T), using the following equation:

$$X \times V = T \qquad \textbf{Eq.1}$$

Reconstruction of original data matrix X is computed by using the preselected numbers of factors as:

$$X_{(estimated\ )} = T \times V' \qquad \textbf{Eq.2}$$

The predicted value of y can be stated as:

$$y_{(estimated\ )} = x_{(estimated\ )} \times b \qquad \textbf{Eq. 3}$$

Where, b is regression vector.[10]

Before finalizing the calibration data, to avoid over fitting, the optimum number of latent variables or factors (A) (figure1) should be selected by applying the cross validation method, leaving one sample at a time.[11]

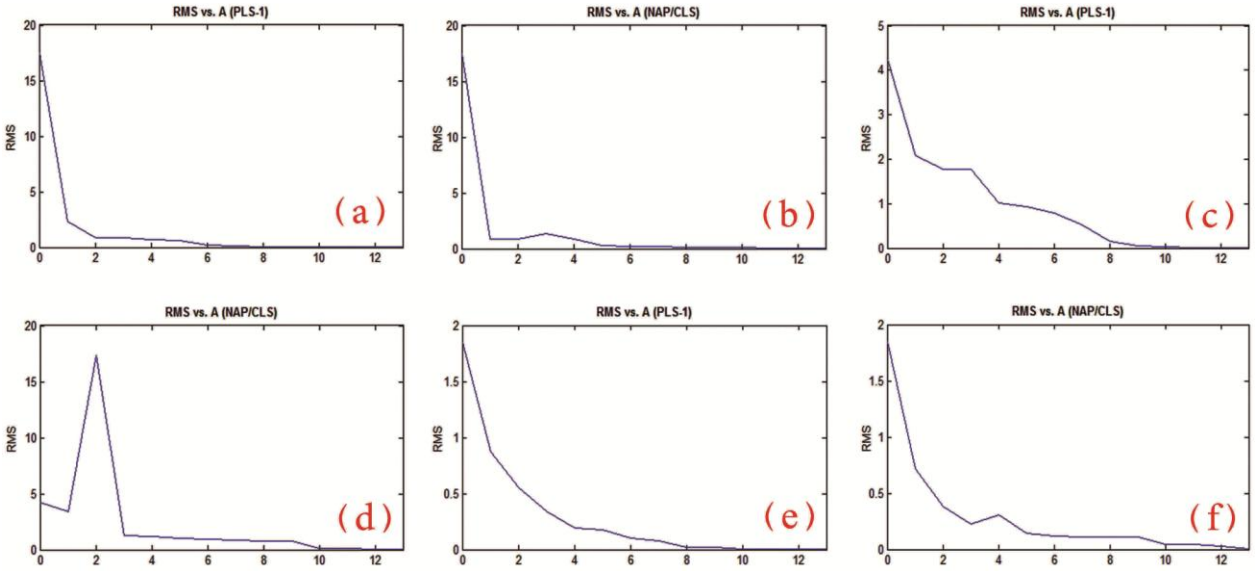

Figure 1. Plot of RMS(CV) vs factor number for calibration set prediction using cross validation of (a) MET PLS-1, (b) MET NAP/CLS, (c) GLZ PLS-1, (d) GLZ NAP/CLS, (e) PIO PLS-1, (f) PIO NAP/CLS

***NAP/CLS*[12]:** In contrast to PLS-1, the concept of NAS based calibration utilizes the contribution of two types of analyte signals, Y_ki.e. the analyte of interest and Y_{-k}, signals developed by sources of variability. The virtual signals obtained are a sum of these two and can be presented as:

$$Y = Y_k + Y_{-k} \qquad \textbf{Eq.4}$$

For unit concentration of k the J×1 vector can be denoted as $s_k$hence

$$Y = x_k s_k{}' + Y_{-k} \qquad \textbf{Eq.5}$$

Both sides of equations when multiplied with an appropriate filtering or preprocessing J×J matrix, named, M_{NAP} which in turn is supposed to be orthogonal to Y_{-k},the eq.5 get converted to:

$$YM_{NAP} = x_k s_k{}' M_{NAP} \qquad \textbf{Eq.6}$$

Eq.6 can also be presented as:

$$Y^{\$} = x_k \left(s_k^{\$}\right)' \qquad \textbf{Eq. 7}$$

Where, $Y^{\$}$ is matrix of net analyte calibration spectra and $s_k^{\$}$ is net sensitivity for analyte k.

The filtering matrix in eq.6 as mentioned above is orthogonal to Y_k and can be calculated as

$$M_{NAP} = L - (Y_{-k})^p Y_{-k} \qquad \textbf{Eq. 8}$$

Where, L is J×J unitary matrix and $(Y_{-k})^{p}$ is pseudo-inverse of Y_{-k}. Pseudo-inverse of Y_{-k} can be calculated by applying singular value decomposition (SVD) at factor A:

$$M_{NAP} = [L - UU'] \qquad \textbf{Eq. 9}$$

The applied filter M_{NAP} removes all sources of variability except k. The new generated problem can be resolved by applying classical least square (CLS) method in combination with NAS and that leads to the generation of equation 10.

$$s_k^{\$} = (Y_k^{\$})' x_k (x_k' x_k)^{-1} \qquad \textbf{Eq.10}$$

Hence unknown concentration x_k is determined by:

$$x_k = (s_k^{\$'} s_K^{\$})^{-1} s_K^{\$'} y_k^{\$} \qquad \textbf{Eq.11}$$

The usual statistical parameters giving an indication of the quality of fit of all data are the root mean square difference (RMSECV), square of the correlation coefficient (R^2) and relative error of prediction (REP%). The expressions of these parameters are:

$$RMSECV = \left[\frac{1}{m}\sum_1^m \left(c_{act} - c_{pred}\right)^2\right]^{1/2} \qquad \textbf{Eq. 12}$$

$$R^2 = 1 - \frac{\sum_1^m (c_{act} - c_{pred})^2}{\sum_1^m (c_{act} - c)^2} \qquad \textbf{Eq.13}$$

$$\text{REP\%} = \frac{100}{c}\left[\frac{1}{m}\sum_1^m \left(c_{act} - c_{pred}\right)^2\right]^{1/2} \qquad \textbf{Eq.14}$$

$$\text{Bias} = \left[\frac{1}{m}\sum_1^m \left(c_{act} - c_{pred}\right)\right] \qquad \textbf{Eq.15}$$

Where c_{act} and c_{pred} are the actual and predicted concentrations during the cross validation process, m is number of samples used in cross validation and validation.[7] The goodness of data fit can be visualized in figure 2.

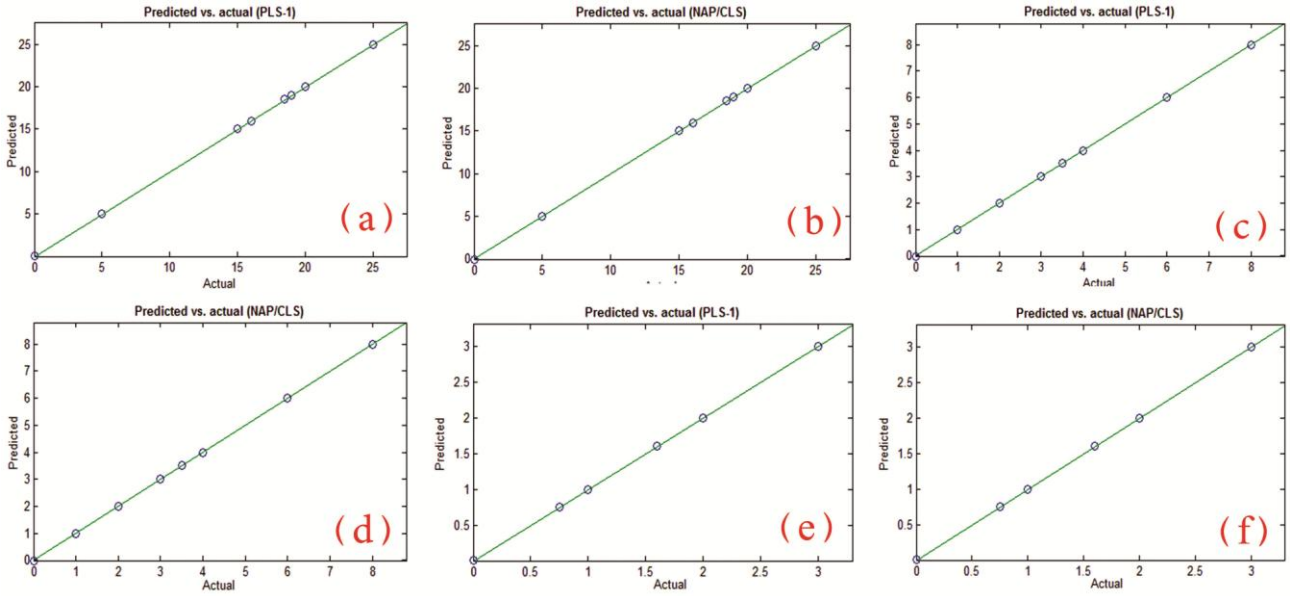

Figure 2. Plots of actual vs predicted values for (a) MET PLS-1, (b) MET NAP/CLS, (c) GLZ PLS-1, (d) GLZ NAP/CLS, (e) PIO PLS-1, (f) PIO NAP/CLS

Along with the above said statistical formulae, another preferred method for assessing the relative accuracy of the studied models is the linear regression analysis of actual verses predicted data by comparing the results of the estimated slope and intercept with their ideal value of 1 and 0. If the point (1, 0) is inside the EJCR (elliptical joint confidence region) for cross validation data, it can be concluded that constant and proportional bias are absent (figure 3).

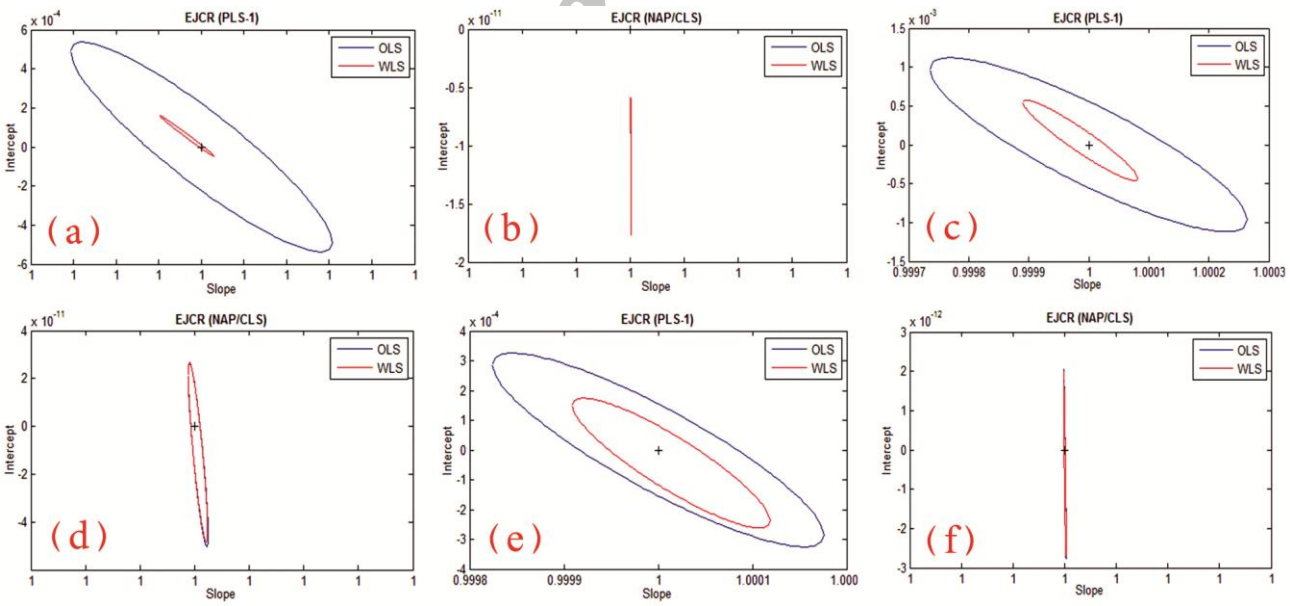

Figure 3. Ellipticle joint confident region for slope and intercept corresponding to regressions of the actual vs predicted concentrations of (a) MET PLS-1, (b) MET NAP/CLS, (c) GLZ PLS-1, (d) GLZ NAP/CLS, (e) PIO PLS-1, (f) PIO NAP/CLS

Results and Discussion

UV-Vis spectra of MET, GLZ, PIO and mixture

Figure 4 shows the individual absorption spectra of MET, GLZ and PIO along with their mixture in 0.1N HCl between 200 and 300 nm.

PLS-1 and NAP/CLS Results

The statistical parameters obtained after applying PLS-1 and NAP/CLS to the spectrophotometric data of cross validation and validation are shown in Table 3. The results suggest that the present method is accurate in concern to the validation samples, as suggested by the low RMSE and REP value for this validation set.

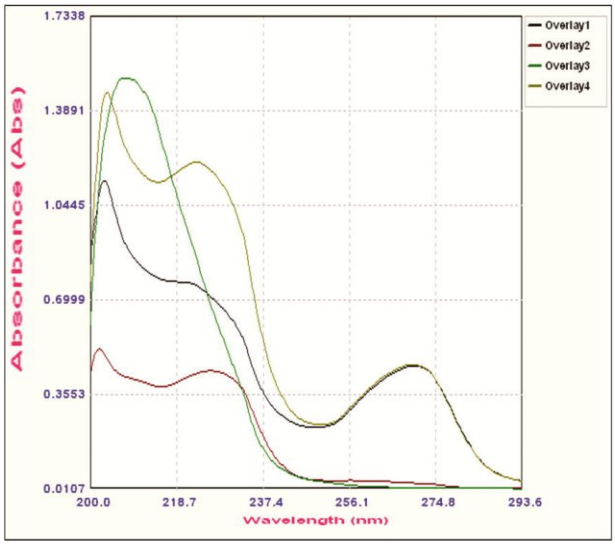

Figure 4. Overlay of MET, GLZ, PIO and Mixture.

Analysis of commercial sample

Commercial mixture products were analyzed using the proposed spectrophotometric methods. Results are summarized in Table 4. As can be seen, satisfactory results were obtained by the proposed methods.

Conclusion

A comparative study with the use of PLS-1 and NAP/CLS for the separation and simultaneous estimation of MET, GLZ and PIO in a binary mixture has been accomplished, showing that this spectrophotometric method provides a good example of the high resolving power of these techniques. In other words, almost comparable results were obtained for these three drugs in both synthetic and commercial mixture. The results obtained confirm the suitability of the proposed method for accurate analysis of MET, GLZ and PIO in pharmaceutical preparations. These methods were applied directly to the commercial mixture preparations without previous treatment. In addition the proposed methods are suitable for application without interference of the excipients as well.

Conflict of Interest

There is no conflict of interest in this study.

Table 3. Statistical parameters for the optimized models

Parameters		MET		GLZ		PIO	
		PLS-1	NAP/CLS	PLS-1	NAP/CLS	PLS-1	NAP/CLS
Calibration set results	No. of factors	6	6	9	11	8	10
	Press	0.6673	0.4725	0.0502	0.1022	0.0087	0.0273
	RMSE(µg/ml)	0.2110	0.1776	0.0579	0.0826	0.0241	0.0427
	REP%	1.3271	1.1172	1.5817	2.2535	1.4876	2.6345
	Slope	0.9996	1.0003	1.0000	1.0006	0.9987	0.9988
	R^2	0.9991	0.9994	0.9992	0.9984	0.9992	0.9976
	Bias	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Validation set results	Press	0.5330	0.4973	0.1179	0.2057	0.0455	0.0904
	RMSE(µg/ml)	0.2309	0.2230	0.1086	0.1434	0.0674	0.0951
	REP%	1.6549	1.5986	2.1939	2.8977	3.2416	4.5721
	Slope	1.0010	1.0089	1.0018	1.0101	1.0193	0.9847
	R^2	0.9993	0.9993	0.9988	0.9979	0.9954	0.9884
	Bias	0.0677	0.0137	-0.0186	0.0141	0.0112	0.0121
Figure of merits	LOD(µg/ml)	0.1861	0.1564	0.0510	0.0727	0.0213	0.0377
	LOQ(µg/ml)	0.5639	0.4741	0.1546	0.2205	0.0646	0.1142
	SEM	0.0563	0.0474	0.0154	0.0221	0.0064	0.0114

Table 4. Prediction results on recovery samples

Commercial Sample (nominal content)	Metformin HCl*		Gliclazide*		Pioglitazone HCl*	
	PLS-1	NAP/CLS	PLS-1	NAP/CLS	PLS-I	NAP/CLS
MET-500mg, GLZ-30mg, PIO-15mg	500.33 (2.08) (100.06%)	501.33 (2.08) (100.26%)	30.33 (2.08) (101.11%)	29.00 (1.00) (96.66%)	14.33 (2.08) (95.55%)	16.66 (1.52) (111.11%)
MET-500mg, GLZ-30mg, PIO-45mg	500.33 (2.51) (100.06%)	496.66 (1.15) (99.33%)	31.00 (2.00) (103.33%)	29.33 (2.51) (97.77%)	45.33 (2.08) (100.74%)	45.00 (2.00) (100.00%)
MET-500mg, GLZ-80mg, PIO-15mg	496.66 (1.52) (99.33%)	500.00 (2.00) (100.00%)	79.00 (2.00) (98.75%)	82.66 (1.52) (103.33%)	15.00 (2.00) (100.00%)	14.33 (1.54) (95.55%)
MET-500mg, GLZ-80mg, PIO-45mg	498.66 (3.05) (99.73%)	498.33 (1.52) (99.66%)	82.00 (1.00) (102.50%)	79.33 (1.52) (99.19%)	46.00 (1.73) (102.22%)	44.33 (1.53) (98.51%)

*The results are averages of three replicates and are given in mg per sample. ±S.D. is in parenthesis.

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