UV-Spectrophotometric-Assisted Chemometric Methods for the Simultaneous Determination of Metformin Hydrochloride and Gliclazide in Pharmaceutical Formulations

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Abstract

In this study, the simultaneous determination of Metformin hydrochloride (MET) and Gliclazide (GLZ) in pharmaceuticals by chemometric approaches using UV spectrophotometry has been reported. Spectra of MET and GLZ were recorded at several concentrations within their linear ranges between wavelengths of 200 nm to 400 nm in pH 6.8 phosphate buffer. Partial Least Squares regression (PLS) and Net Analyte Preprocessing combined with Classical Least Square (NAP/CLS) were used for chemometric analysis of data and the parameters of the chemometric procedures were optimized. The recoveries were satisfactory and statistically comparable. The method was successfully applied to pharmaceutical formulation, tablet, with no interference with excipients as indicated by the recovery study results. The proposed methods are simple, rapid and can be easily used in the quality control of drugs as alternative analysis tools.

Keywords: Partial least-squares; Net analyte preprocessing combined with classical least square; Spectroscopy; Metformin hydrochloride; Gliclazide

Introduction

The prevalence of diabetes is readily increasing worldwide specifically in developing countries. Out of the two forms of diabetes viz. Type 1 and Type 2, later is the commonest one. Maintaining blood sugar values as close to the physiological range as possible is essential in a diabetic patient to prevent morbidity and mortality. Oral drugs particularly combination of insulin secretagogue and insulin sensitizer play an important role in the management of type 2 diabetes mellitus after lifestyle changes and monotherapy have failed to control blood sugar [1].

Metformin Hydrochloride (MET) (N, N-dimethylimidodicarbonimidicdiamide hydrochloride) is a biguanide prescribed for the treatment of type II diabetes mellitus, and is the drug of choice in obese patients. It increases glucose transport across the cell membrane in skeletal muscles and can inhibit the formation of advanced glycosylation end-products [2]. Gliclazide (GLZ) (1-(1-azabicyclo [3, 3, 0] oct-3-yl)-3-(p-to-ly sulphonyl) urea) is an oral hypoglycaemic (anti-diabetic) drug and is classified as a second generation sulfonylurea. It acts by stimulating the Ca++ transport across the pancreatic beta cells membrane by reducing conductance of ATP sensitive K+ channel and hence stimulates insulin secretion from pancreas [3]. The combination of gliclazide and metformin hydrochloride complement each other and provide better glycaemia control in management of type II diabetes and probably in the prevention of its associated macro and micro vascular complications [4].

Several spectroscopic methods have been developed for the estimation of MET and GLZ individually and with other active pharmaceutical ingredients [5-12]. Nevertheless, to the best of our knowledge, no simultaneous estimation of these two compounds has been reported in combination using UV spectrophotometry without standard addition method.

Pharmaceutical processing and formulation often introduce various interferents (chemicals other than drug/s under investigation) into the system. When performing quantification these interferents can disturb univariate analysis, but with multivariate analysis the 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 Partial least squares (PLS) regression and net analyte preprocessing combined with classical least square (NAP/CLS) methods are one of them [13]. 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 [14]. 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 [15]. 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 [16].

Experimental

Instrument, reagents and software’s

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 pharmaceutical ingredients. 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 pharmaceutical ingredients. 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 pharmaceutical ingredients. 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 pharmaceutical ingredients. 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 pharmaceutical ingredients.
Abhilasha Pharma Pvt. Ltd., Gujarat and GLZ was obtained from Kwallty Pharmaceuticals, Amritsar. 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.

Procedure

Preparation of standards: 1mg/ml MET and GLZ stock solutions were prepared by dissolving accurately weighed amounts of finely powdered pure MET and GLZ in small quantity of methanol and the final volumes were made respectively with pH 6.8 phosphate buffer. Suitably diluted samples from each stock were utilized for λmax determination of individual component followed by serial dilution of stocks with pH 6.8 phosphate buffers to obtain the aliquots falling in linearity.

Standard solutions for multivariate calibration: The projected Fixed Dose Combination (FDC) of MET and GLZ comprises of 500 mg MET in combination with GLZ ranging from 30-80mg. Therefore, the linearity ranges selected for MET and GLZ are 8-20 µg/ml and 1-5 µg/ml respectively. The calibration and validation mixtures were prepared by mixing MET and GLZ solutions in different ratios varying in their individual linearity ranges. The concentrations of combinations were decided by design expert 8.0.4 software under general factorial design for 2 categories at 5 level of each category. Total 24 sets were prepared out of which 16 sets (Table 1) were utilized as calibration set whereas, the rest 8 served as validation sets (Table 2). All the mixtures were scanned at 220-278 nm range digitized at every 3 nm. The absorbance below 220 nm and above 278 nm was not taken under consideration.

Sample preparation: Commercial FDC tablets of MET and GLZ were analyzed. The tablets were analysed as per the following process: at least 10 tablets were taken for each FDC and finely crushed to powder. A suitable amount of the obtained powders of each FDC were 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 PLS-SVD 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:

\[ X \times V = T \]  

(1)

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

\[ X_{(estimated)} = T \times V' \]  

(2)

The predicted value of y can be stated as:

\[ y_{(estimated)} = x_{(estimated)} \times b \]  

(3)

Where, b is regression vector [17].

Before finalising the calibration data, to avoid over fitting, the optimum number of latent variables or factors (A) (Figure 1) should be selected by applying the cross validation method, leaving one sample at a time [18].

NAP/CLS

In contrast to PLS-1, [19] the concept of NAS based calibration utilizes the contribution of two types of analyte signals, Yk i.e. the analyte of interest and Yk,i signals developed by sources of variability. The virtual signals obtained are a sum of these two and can be presented as:

\[ y = y_k + y_{-k} \]  

(4)

For unit concentration of k the Jx1 vector can be denoted as \( s^k \)

\[ y = x_k s^k + y_{-k} \]  

(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 equation 5 get converted to:

\[ y M_{NAP} = x_k s^k M_{NAP} \]  

(6)

Equation 6 can also be presented as:

\[ y^s = x_k (s^k)^{T} \]  

(7)

Where, \( y^s \) is matrix of net analyte calibration spectra and \( s^k \) is net sensitivity for analyte k.

The filtering matrix in equation 6 as mentioned above is orthogonal to \( y_k \) and can be calculated as

\[ M_{NAP} = L - (Y_{-k})^T Y_{-k} \]  

(8)

| Runs | MET (µg/ml) | GLZ (µg/ml) |
|------|------------|-------------|
| C1   | 20         | 4           |
| C2   | 11         | 1           |
| C3   | 17         | 3           |
| C4   | 20         | 1           |
| C5   | 14         | 4           |
| C6   | 20         | 5           |
| C7   | 11         | 3           |
| C8   | 11         | 5           |
| C9   | 14         | 2           |
| C10  | 14         | 3           |
| C11  | 17         | 5           |
| C12  | 8          | 5           |
| C13  | 8          | 1           |
| C14  | 8          | 2           |
| C15  | 20         | 2           |
| C16  | 17         | 1           |

Table 1: Calibration set composition.

| Runs | MET (µg/ml) | GLZ (µg/ml) |
|------|------------|-------------|
| V1   | 14         | 5           |
| V2   | 11         | 4           |
| V3   | 17         | 4           |
| V4   | 11         | 2           |
| V5   | 17         | 2           |
| V6   | 20         | 3           |
| V7   | 8          | 3           |
| V8   | 14         | 1           |

Table 2: Validation set composition.
Where, $L$ is $J \times J$ unitary matrix and $(Y_k)^r$ is pseudo-inverse of $Y_k$. Pseudo-inverse of $Y_k$ can be calculated by applying singular value decomposition (SVD) at factor $A$:

$$M_{NAP} = [L - UU']$$ (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.

$$s^k = (Y_k^r)^{-1}$$ (10)

Hence unknown concentration $x_k$ is determined by:

$$x_k = (s_k^r s_k^r)^{-1}(s_k^r y_k)$$ (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:

$$RMSECV = \left[ \frac{1}{m} \sum_{i=1}^{m} (c_{act} - c_{pred})^2 \right]^{1/2}$$ (12)

$$R^2 = 1 - \frac{\sum_{i=1}^{m} (c_{act} - c_{pred})^2}{\sum_{i=1}^{m} (c_{act} - c)^2}$$ (13)

$$REP% = 100 \frac{1}{c} \left[ \frac{1}{m} \sum_{i=1}^{m} (c_{act} - c_{pred})^2 \right]^{1/2}$$ (14)

$$Bias = \frac{1}{m} \sum_{i=1}^{m} (c_{act} - c_{pred})$$ (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 [14]. The goodness of data fit can be visualized in Figure 2.

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).

**Result and Discussion**

![Figure 1](image1.png)

**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.

![Figure 2](image2.png)

**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.

![Figure 3](image3.png)

**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.
UV-Vis spectra of MET, GLZ and mixture

Figure 4 shows the individual absorption spectra of MET and GLZ along with their mixture in pH 6.8 phosphate buffer between 200 and 300 nm.

Results of PLS-1 and NAP/CLS

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.

Analysis of commercial sample

Commercial mixture products were analysed using the proposed spectrophotometric methods. Results are summarised 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 and GLZ 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 two drugs in both synthetic and commercial mixture. The results obtained confirm the suitability of the proposed method for accurate analysis of metformin hydrochloride and gliclazide 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.

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