Non-destructive detection of adulterated tablets of glibenclamide using solid-phase fluorescence spectroscopy and unfolded partial least squares regression with discriminant analysis

R.S. Fernandes¹, F.S.L. Costa¹, Valderrama, P.², Março, P.H.², D.N. Rutledge³, K.M.G. Lima¹

¹ Federal University of Rio Grande do Norte, Chemical Institute, Research group in applied chemometrics, CEP 59072-970 – Natal-RN-Brazil, kassio@ufnet.br
² Tecnological Federal University of Paraná State, Chemical Departament, Research group in analytical chemistry, BR 369, Km 0.5, CEP 87301-006 – Campo Mourão-PR-Brazil, patriciav@utfpr.edu.br
³ AgroParisTech, Ingénierie Analytique pour la Qualité des Aliments (IAQA), 16 rue Claude Bernard, 75231 Paris Cedex 05, France, rutledge@agroparistech.fr

Keywords: glibenclamide, solid-phase fluorescence spectroscopy, UPLS-DA.

1- Introduction

Glibenclamide is a second-generation sulfonylurea anti-diabetic used in tablet form for the treatment of diabetes mellitus [1]. There is only 5 mg of the active ingredient in each tablet, with the remainder consisting of excipients such as lactose, aerosil, starch and magnesium.

The dissemination of counterfeit drugs is a criminal activity that is escalating in many countries. A significant increase in counterfeit drugs has been noted in Brazil during the last few years. Many of the false products found on the market have altered composition in terms of both the amounts of active and inactive ingredients. According to the World Health Organization (WHO), counterfeit tablets are “a medicine, which is deliberately and fraudulently mislabeled with respect to identity and/or source. Counterfeit products may include products with the correct ingredients or with the wrong ingredients, without active ingredients, with insufficient active ingredients or with fake packaging” [2].

Several analytical methods have been described to detect counterfeit medicines, such as high performance liquid chromatography with UV detection (HPLC-UV) [3], mass spectrometry detection (HPLC-MS) [4], and nuclear magnetic resonance (NMR) [5]. However, despite the reliability of these techniques for the detection of adulterated tablets, they are inherently destructive, expensive and wasteful. In addition, some of these techniques, such as NMR, require specialized reagents and personnel, which limit the use of these techniques beyond the laboratory.

In recent years, excitation and emission fluorescence spectroscopy have been used in conjunction with chemometric methods for the analysis of pharmaceutical formulations by solid-phase molecular fluorescence and second-order multivariate calibration [6,7]. Analysis by molecular fluorescence is an attractive option, due its inherent sensitivity and ease of spectral acquisition. This is especially true for characterization of the solid phase, where reduced sample preparation is necessary. Solid-phase fluorescence spectroscopy allows for fast acquisition of data with minimal reagent consumption and low cost analysis. In addition, it does not generate residues and has good sensitivity and selectivity. Unfortunately, to date, this strategy has been rarely applied, most likely due to a lack of solid samples of pharmaceutical interest.

Several algorithms for the classification of data have been described in the literature, such as: Principal Component Analysis (PCA) [8], Soft Independent Modeling of Class Analogy (SIMCA) [9] and Partial Least Squares Discriminant Analysis (PLS-DA) [10]. In this study, we report on the detection of adulteration in glibenclamide tablets by using Solid-phase fluorescence spectroscopy along with a new purpose of chemometric tools for discriminant analysis of second order data, the Unfolded Partial Least Squares Discriminant Analysis (UPLS-DA).
2- Theory

The PCA method is undoubtedly being the most widely-used unsupervised classification algorithm (the ratings carry no information about the origin of the data) [8]. The properly supervised classification methods SIMCA [9] and PLS-DA [10] are more ideal classification methods, with the main difference between the two being that supervised SIMCA analysis produces more satisfactory results when the variability between groups is greater than the variability within groups. However, in cases where the variability within a group is greater than the variability among several groups, the SIMCA method does not distinguish between groups. For these cases, the PLS-DA method is an alternative. The PLS-DA method is a multivariate method used for the classification of samples when it is necessary to reduce the number of variables and it is unclear whether the differences between groups will dominate the total variability of the samples. When working with fluorescence data, the Unfolded Partial Least Squares (UPLS) method [11] with discriminant analysis may be a better alternative.

The UPLS-DA model was built by unfolding the original data (the matrix containing the fluorescence for each sample), while the array of dependent variables contained the sample classes for different glibenclamide samples (R, G1 and G2). A linear relationship is established in this model between the scores of X (the vectorized matrix values for fluorescence of each sample) and the scores of Y (the matrix containing the different class types R, G1 and G2).

The y block in a UPLS-DA model contains the sample classes and its values, which are established as 0 or 1, indicating if the sample is included (1) or not included (0) in a class. When they have three classes to discriminate, the dependent variable is a matrix with the number of columns equal to the number of classes with values equal 0 or 1. For example, in the case of three classes and one sample included in the class number 2, the y value for this sample will be y = (0 1 0).

The predicted values for UPLS-DA will be ideally 0 and 1, nevertheless, in practice, these values are very close to but not exactly 0 or 1. A limit value is calculated between the predicted values and when the value is higher than the limit, it indicates that this sample is included in the modeled class. Values which are smaller than the calculated limit indicates that this sample is not included in the modeled class. The model also presents the probability of the sample to be included in the modeled class.

3- Material and methods

Tablets

The glibenclamide tablet that was used as the standard reference tablet was purchased from Sigma-Aldrich and had a purity greater than 99% (HPLC grade). Ethanol (99%, Merck) was used to prepare solutions of glibenclamide used for the construction of a calibration curve. A total of 366 tablets, all declared as having a glibenclamide content of 5 mg, were purchased from local traders in Natal-RN-Brazil. Amongst this group, 57 tablets were called reference (R) (Daoill®, 3 different batches), 58 were called generic 1 (G1, 3 different batches) and 251 tablets were called generic 2 (G2, 3 different batches).

Instrumentation

The excitation/emission fluorescence data were acquired in the range of wavelengths of 300–400 nm for excitation and 405–600 nm for emission, with steps of 5 and 2 nm for excitation and emission, respectively. A Perkin Elmer model LS-55 fluorescence spectrometer was used. The excitation and emission slits were set at 5 nm, the speed scan was set to the fastest mode, the photomultiplier tube was set to the medium level and a cell with a fiber optic reflectance probe was used. For model building, a range of 300 to 400 nm for excitation and 405 to 600 nm for emission was used, with steps of 25 and 0.5 nm used for excitation and emission, respectively. This resulted in a data matrix size of 5x392 for each sample. The instrument noise level was estimated based on 15 measurements of a blank solution.

An Agilent HP8453 UV/VIs spectrophotometer with a single beam, automated variation in wavelength, and a quartz cuvette (10mm path length) was used for characterization of the purchased glibenclamide tablets. In this case, 45 tablets were analyzed (12 R, 9 G1, and 24 G2), all of which were declared by their manufacturers as having a glibenclamide content of 5 mg. For the analysis, tablets were chosen randomly among the total set of 366 tablets for determination of drug content. A calibration curve made from standard solutions of glibenclamide in methanol was made based on first-order derivative spectrophotometry at 315 nm. Based on this UV characterization method, the R and G1 class tablets had concentrations of glibenclamide of 4.65 ± 0.22 and 4.73 ± 0.16 mg, respectively. The tablets referred to
as class G2 had a concentration of 2.53 ± 0.32 mg. Recovery for the reference tablets was above 98%, with a relative standard deviation of less than 1%.

**Software**

Importing of the data, pre-treatment (the results from smoothing and the Savitzky-Golay filter), and the construction of chemometric classification models UPLS-DA was performed in MATLAB version 6.5 (Math-Works, Natick, USA) using the PLS-toolbox (Eigenvector Research, Inc., Wenatchee, WA, USA, version 6.01).

## 4- Results and discussion

The Figure 1 shows the excitation/emission contour map obtained by solid-phase molecular spectroscopy for samples of three classes of glibenclamide. The results are identical, not allowing a visual identification of the samples in a determined class.

![Figure 1](image1.png)

*Figure 1 : Contour map obtained by solid-phase fluorescence spectroscopy for a sample of the class (A) G1; (B) R and (C) G2.*

To built second-order models for the purpose of classification, the UPLS-DA model was developed from solid-phase molecular fluorescence data of all 194 tablets separated in two groups: calibration and validation, with 129 and 65 samples, respectively. Each sample (matrix) was unfolded into its corresponding vector.

Figure 2 shows the separation between the three classes of the investigated pills. The scores in this figure shows that there is a clear distinction from the adulterated tablets (class G2) to the original ones (class R). Figure 3 confirms the correct classification and shows the probability of classification for the three classes evaluated in this study. In addition, a set of 45 tablets, randomly chosen, within the total set was analyzed by derivative UV spectroscopy to investigate the composition of glibenclamide. In this case, samples belonging to class G2 (where the detected levels of glibenclamide were below 5 mg) showed that the UPLS-DA scores resulting from the application of this technique to the solid-phase fluorescence spectroscopy data were able to detect adulteration.

![Figure 2](image2.png)

*Figure 2 : Scores from UPLS-DA model. “o” calibration set; “x” validation set.*
CONCLUSION

Solid-phase fluorescence spectroscopy in combination with chemometric tools proved to be highly effective for detecting adulterated glibenclamide tablets. The method is advantageous over traditional analysis methods as fluorescence spectroscopy is non-destructive, offers feasible quick analysis, and do not create large amounts of waste. The results from this study are promising and suggest that the use of solid-phase fluorescence spectroscopy in combination with chemometric methods could be used as a satisfactory approach for the classification and identification of counterfeit pharmaceuticals.
References


Acknowledgements

R.S. Fernandes and F.S.L. Costa thanks Propesq-UFRN for financial support.
P. Valderrama and P.H. Março thanks Fundação Araucária