MULTIVARIATE CALIBRATION AND MODELING OF UV-VIS SPECTRA OF GUEST–HOST COMPLEXES FOR THE DETERMINATION OF THE ENANTIOMERIC RATIO OF PROPRANOLOL

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Abstract

The main drawback of the conventional chiral separations techniques is the long method development, analysis and separation times, so there is a need of a new alternative. The key to the present issue is offered by the chemometric interpretation of spectroscopic data (FT-IR, NIR, UV-VIS, Fluorescence, etc.) without the need of a prior sample pretreatment and/or separation.

The potential and performances of the above mentioned method is presented in a rapid chiral analysis of propranolol using β-cyclodextrin as chiral selector with the data mining of UV absorbance spectra (185-400nm). For regression model building and cross-validation, the spectra of calibration samples containing different propranolol enantiomeric ratios at 0.147mM total molar concentration and a fixed concentration of chiral selector (7.51mM β-cyclodextrin) in 100mM formate buffer (pH=3.00) were recorded with the aid of the DAD (diode array) detector of an Agilent G1600 capillary electrophoresis system. The good results obtained on synthetic samples reconfirmed the high analytical value of multivariate data analysis in general, and regression modeling in particular.

Rezumat

Principalul dezavantaj al tehnicilor de separare chirale convenționale îl reprezintă timpii îndelungăți ai dezvoltării metodelor, analizei și separării, de aceea e nevoie de noi alternative. Soluția la această problemă este oferită de către interpretarea chemometrică a datelor spectroscopice (FT-IR, NIR, UV-VIS, Fluorescență, etc.) fără a fi nevoie de un pretratament și/sau separare prelabilă.

Potențialul și performanțele metodei menționate mai sus sunt prezentate într-o analiză chirală rapidă a propranololului folosind β-ciclodextrina ca selector chiral folosind spectrele de absorbanță UV (185-400nm) ale complecșilor supramoleculari formăți. Pentru construirea modelului de regresie și validarea încrucișată, au fost înregistrate spectrele soluțiilor de calibrare conținând proporții enantiomeric diferite de propranolol, dar cu o concentrație molară totală de 0,147mM propranolol și o concentrație fixă a selectorului chiral (7,51mM β-ciclodextrină) în tampon formiat 100mM (pH=3,00). S-a utilizat un detector DAD (diode array detector) al unui sistem de electroforeză capilară Agilent G1600. Rezultatele bune obținute pe probe sintetice reconfirmă valoarea analitică ridicată a analizei multivariate în general și a modelului de regresie în particular.
**Keywords:** chiral analysis, propranolol, β-cyclodextrin, multivariate regression

**Introduction**

Nowadays, increasingly more enantiomeric studies and justification for approval of racemic drugs are required by the regulatory agencies (e.g. The United States Food and Drug Administration) [1-3] because it is well known that one of the enantiomers of racemic drugs often exhibit different pharmacological and toxicological properties. In spite of this, most of the chiral drugs are administered as racemates [4-6]. Determination of the enantiomeric composition of chiral substances evoked the need for analytical procedures for separation and quantitative determination of them [7].

For determination of enantiomeric purity there are widely used many separation techniques, such as gas chromatography, liquid chromatography, capillary electrophoresis, super- and sub-critical fluid chromatography. However, most of these methods involve several manipulation steps before the final result of the analysis is obtained. Other problems using chromatographic methods include expensive instrumentation, time-consuming analyzes and running costs [8-10].

Spectroscopic methods may be an alternative to these techniques, by simplicity, cost effectiveness and relatively short analysis time as compared to the other routine analytical techniques including chromatography. The enantiomeric composition of various chiral guest molecules can be determined with reasonable accuracy by multivariate regression modeling of spectral data based on guest–host interaction [11-20]. These methods are based on the formation of a supramolecular complex between the guest and host compounds, which leads to small changes in their spectra when the total concentration of guest and host compounds is fixed, while the enantiomeric ratio of the guest molecule was varied [21]. Cyclodextrins (CDs) are homochiral barrel-shaped cyclic oligosaccharides able to form host-guest complexes with hydrophobic molecules. There are three naturally occurring cyclodextrins α-, β-, and γ-. The premise behind the approach is that inclusion complex formation between the chiral guest analyte and the homochiral CD host results in the formation of transient diastereomeric inclusion complexes with different physical and spectral properties [11].

Beta adrenergic receptor blocking agents or popularly known as β-blockers are widely used to treat cardiovascular disorders. They are used in the treatment of high blood pressure, heart failure, angina pectoris, acute myocardial infarction (heart attack) and cardiac arrhythmia [22,23]. Each of
these drugs possesses one or more chiral centers in its molecule. Each isomer possesses a different pharmacological activity, potency, and mode of action. In general, the (S)-enantiomers are more potent than the distomers [24] in 10–500-folds [3] but only few drugs are sold as single enantiomers.

In this study, PLS (partial least squares) regression modeling is applied to UV spectral absorption data from guest–host inclusion complexes between CDs and guest molecules (β-blockers) to perform the determination of the enantiomeric composition of propranolol (PRNL).

**Materials and Methods**

*Sample preparation and spectroscopic determinations*

Racemic propranolol was provided by Zentiva S.A. Romania. Propranolol’s enantiomers (+/-) were purchased from Sigma-Aldrich®, at analytical standard degree. β-cyclodextrin was employed as chiral selector and it was purchased from Cyclolab® Hungary.

For regression model building and cross-validation, the spectra of calibration samples (training sets) containing different propranolol enantiomeric ratios at a total molar concentration (0.147mM) and a fixed concentration of chiral selector (7.51mM β-cyclodextrin) in 100mM formate buffer (pH=3.00) were hydrodynamically injected through a fused silica capillary (total length 32.5 cm, i.d. 50 µm) to the DAD detector of an Agilent G1600 (Agilent Technologies, Germany) capillary electrophoresis system. As a reference solution, before each calibration and validation sample the capillary was flushed by 100mM formate buffer (pH=3.00) and without applying a separation potential an assay was simulated when the detector’s signal was brought to zero. All samples were hydrodynamically injected for 3 minutes and based on the detector’s signal it was made sure that the sample reached the detector. For all the samples the absorbance spectra at 2.5 minutes were recorded on 200-350 nm with a 2nm resolution.

For the testing of the optimized chemometric model simulated aqueous samples of racemic propranolol were analyzed. The propranolol hydrochloride’s concentration was spectrophotometrically determined at 290 nm using a previously assessed linear regression (range 35-140 µg/mL; Absorbance = 0.0074 (µg/mL) + 0.0222, R²=0.9979) on a modular AvaSpec-2048x14 back-thinned typed CCD fiber optic spectrometer (Avantes, Spain) using 1.0 cm pathway quartz cell. By adding the proper aliquots of sample solution the sample was made up in a 2 mL volumetric flask with the aqueous chiral selector’s solution resulting 7.51mM bCD and 0.147 mM total propranolol hydrochloride, serving as real sample for further analysis.
Chiral HPLC

For the confirmation of the obtained results on real samples, chiral separation using high-performance liquid chromatography was employed. Polysaccharide-based, Lux Amylose-2 (5µm, 250 x 4.6 mm, Phenomenex, USA) chiral stationary phase column was used on Agilent Series 1200 HPLC system equipped with a DAD detector. The signal was monitored at 220 nm. The mobile phase consisted of 20mM NH₄HCO₃ with 0.1% (v/v) diethylamine:acetonitrile = 60:40 (v/v) delivered at 0.7 mL/min at 25°C. The injected sample volume was 5 µL. The obtained resolution for the two enantiomers of PRNL was Rs=1.51 (tᵣ= 11.56 min – R-PRNL; tᵣ= 12.12 min – S-PRNL).

Multivariate data analysis

Multivariate analysis of spectral data based on projection methods using Principal Component Analysis (PCA) and Partial Least Squares Projection to Latent Structures (PLS) were exploited for data overview and regression modeling using Simca-p+ 12.0.1 software package (Umetrics, Sweden). PLS regression was performed on the absorbance spectral data using full cross validation of the training set. PLS was used to develop a semi-empirical mathematical model that correlates spectral data over many wavelengths with the enantiomeric composition, expressed as the (R)-(+)propranolol mole fraction (xᵣ). The process involves two steps: (a) the model is trained to predict xᵣ from the calibration set (samples with known enantiomeric composition), and (b) external model validation, in which a second, independent set of samples (validation set, also with known enantiomeric composition) is analyzed over the same range of wavelengths in the same conditions. The predicted enantiomeric composition is then compared with the known reference values.

Results and Discussion

The training set consisted of nine samples (xᵣ = 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8 and 0.9), whereas five were used as the calibration set (xᵣ = 0.143, 0.286, 0.571 and 0.762) and represented the Y-block of the constructed mathematical model (Tables I and II). Because the total propranolol concentration in both the training and validation set was kept at a fixed value, it was not included in the chemometric model as X variable.

Previous electrochemical assays (data not shown) has proven host-guest type interaction between β-CD and both enantiomers of propranolol in the used 100mM formiate buffer at pH=3, however it could not prove enantiospecificity. Furthermore, propranolol is being known to be most stable at pH=3, whereas with the increase of pH tends to decompose rapidly.
Moreover, formiate buffer has a low molar absorptivity in the UV range being suitable for spectroscopic investigations.

Propranolol has one chiral center and by different spectroscopic techniques (FT-IR, Raman) it has been proven to form 1:1 inclusion complexes with β-CD (spectra not shown) [25-27]. Therefore in all the training, calibration or real samples containing propranolol a fixed concentration of 7.51 mM β-CD as chiral selector was added.

### Table I
Propranolol training set parameters

<table>
<thead>
<tr>
<th>Observation ID identifier</th>
<th>Mole ratio of R- (+)-propranolol (x&lt;R&gt;)</th>
<th>Total concentration of propranolol (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10%</td>
<td>0.1000</td>
<td>0.1470</td>
</tr>
<tr>
<td>20%</td>
<td>0.2000</td>
<td></td>
</tr>
<tr>
<td>30%</td>
<td>0.3000</td>
<td></td>
</tr>
<tr>
<td>40%</td>
<td>0.4000</td>
<td></td>
</tr>
<tr>
<td>50%</td>
<td>0.5000</td>
<td></td>
</tr>
<tr>
<td>60%</td>
<td>0.6000</td>
<td></td>
</tr>
<tr>
<td>70%</td>
<td>0.7000</td>
<td></td>
</tr>
<tr>
<td>80%</td>
<td>0.8000</td>
<td></td>
</tr>
<tr>
<td>90%</td>
<td>0.9000</td>
<td></td>
</tr>
</tbody>
</table>

Spectral data analysis began with a principal component analysis (PCA) to detect outliers and other anomalies in the data. PCA of the unit variance scaled and mean centered X-data gave a three-component model, which explained 91% of the variation (R^2_X = 0.91). Examining the loadings, the first component, accounting for more than 50%, captures the overall variation within the entire spectra, whereas the loading of the second and third component describes mainly the fine features of the spectral variation. The PCA-X model, apart from identifying the outliers (no outliers were identified), can also be useful in giving the first hint of the spectral range loaded with the most useful information considering the chiral analysis. Continuing with the PLS modeling of the entire data set, as part of data pretreatment, the entire spectral data (Figure 2) recorded on the range of 188.5 – 350 nm were mean centered, whereas the mole fraction of (R)- (+)-propranolol (x_R) was unit variance scaled and mean centered. In spite of the fact that the resulting one component PLS model shows a considerable explained variation (R^2_X = 0.909), its predictive power is somewhat poorer (Q^2_Y = 0.732), therefore model optimization was undertaken. Throughout the optimization procedure, in order to enhance the predictive power of the calibration model by eliminating variation in X that is unrelated to Y, the
spectral data was further pre-processed prior to data analysis by the use of different spectral filters. The use of standard normal variate (SNV) signal correction significantly increased the model’s (four principal components) goodness of prediction ($Q^2_Y = 0.885$), but still it was not considered to be satisfactory. The one that offered the best result turned out to be the orthogonal signal correction (OSC) algorithm built in the Simca-p+ software. OSC is a PLS based solution that removes from the X-data variation that is unrelated to Y, the result being a model based on one or more PLS components conveying information about the correction of X. Therefore two OSC components were removed, remaining 20.4% of the original sum of squares in the corrected X-matrix. In the PLS model, the X-block comprised in 151 spectral variables and the Y-block the mole fraction of R-(-)-propranolol ($x_R$).

The PLS modeling yielded a one-component model, which according to cross-validation gave an explained variation of $R^2_Y = 0.939$ and an excellent predicted Y-variation according to internal cross-validation (goodness of prediction) of $Q^2_{intY} = 1$. In order to obtain an estimate of the significance of the $Q^2_Y$-value a response permutation was carried out, which consists in developing a number of parallel models based on fit to randomly re-ordered Y-data, and evaluates the real $Q^2_Y$ in the light of a distribution of $Q^2_Y$-values of the re-ordered response data, giving a statement of the statistical significance of the estimated predictive power,
ruling out any overfitted model ($R^2_Y= -0.0808$, $Q^2_Y= -0.274$; Crit.: $R^2_Y<0.3$-$0.4$, $Q^2_Y<0.05$). Predictive validation by means of cross-validation and response permutation testing in many ways provide a reasonable first approximation of the predictive ability of the PLS model. However, a more demanding and rigorous way of testing predictive performance consists of computing predictions for an independent set of test observations (validation set). The results of the external validation of the obtained PLS model are presented in Table II. The very high value of goodness of prediction ($Q^2_{extY} = 0.9997$), the low Root Mean Square Error of Prediction (RMSEP = 0.009080) and the good correlation ($y=1.036x-0.01162$, $r^2=0.9997$) between real and predicted mole fraction values of R-(+)-propranolol from the validation set (Figure 3) all prove the high analytical value of the obtained model.

The chemometric analysis of the real sample of propranolol (synthetic solution of racemic propranolol) gave a molar fraction of R-propranolol $x_R = 0.5021$ in a very close agreement with the one of the racemic propranolol ($x_R = 0.5$), fact also confirmed by chiral HPLC separation (Table III, Figure 4).

### Table II

Results for the external validation of the PLS model

<table>
<thead>
<tr>
<th>Mole ratio of R- (+)-propranolol ($x_R$)</th>
<th>Error of prediction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Real</td>
<td>Predicted</td>
</tr>
<tr>
<td>0.14285</td>
<td>0.14958</td>
</tr>
<tr>
<td>0.285714</td>
<td>0.284124</td>
</tr>
<tr>
<td>0.571429</td>
<td>0.569491</td>
</tr>
<tr>
<td>0.761905</td>
<td>0.74303</td>
</tr>
</tbody>
</table>

### Table III

Mole fraction of R-propranolol enantiomer in the real sample

<table>
<thead>
<tr>
<th>Synthetic sample (racemic propranolol solution)</th>
<th>Mole ratio of R- (+)-PRNL ($x_a$)</th>
<th>Error (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Real</td>
<td>Obtained</td>
</tr>
<tr>
<td>Chemometry</td>
<td>0.5000</td>
<td>0.5021*</td>
</tr>
<tr>
<td>Chiral HPLC</td>
<td>0.5000</td>
<td>0.4798**</td>
</tr>
</tbody>
</table>

* n=2, RSD = 0.30%; ** n=2, RSD = 0.46%
Conclusions

The effective determination of the enantiomeric ratio of propranolol enantiomers based on the entire UV–VIS spectra (188.5-400.5 nm) of guest–host inclusion complexes was achieved in this work. Small changes in the UV-VIS spectra due to the formation of the supramolecular complex between the chiral selector and the studied chiral analyte can be resolved by using chemometric methods with the PLS analysis providing the corresponding regression models for chiral purity determination.

The obtained results demonstrate once again the high analytical value of multivariate data analysis in general, and regression modeling in particular, offering cost and time-effective alternatives in the chiral analysis of bioactive chiral compounds in comparison with the much more expensive chiral separation techniques (chromatographic and electrophoretic).

Acknowledgements

This work was supported by CNCSIS-UEFISCSU, project number PN II-RU 469/2010, "Iuliu Hațieganu" University of Medicine Cluj-Napoca, project number 27020/2/2011 and EU funded POS-DRU project no. 56949.

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