Development of Regression Analysis for Determination of Irbesartan in Pharmaceutical Tablets using Fourier Transform Infrared Spectrophotometry

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Abstract

A simple, cost-effective, fast and non-destructive analytical method was developed for the quantification of irbesartan in tablets using Fourier Transform Infrared (FT-IR) spectroscopy. The FTIR quantification of irbesartan in tablets was performed using two chemometric approaches, partial least squares (PLS) and principal component regression (PCR) methods. To compare the predictive ability of these models, the standard errors of prediction (SEP) were calculated. SEP error values in the range of 0.941–1.62% for calibration and validation data sets were obtained for the two procedures applied. A successful quantification of irbesartan in tablets containing 150 mg active ingredient/tablet was performed using the PLS model, with good recovery. The proposed procedure can be a fast, precise and convenient method of irbesartan quantification in commercial tablets.

Keywords: FTIR Spectroscopy; Irbesartan; Angiotensin Receptor Antagonists; Chemometric Methods; Drug Analysis.

Introduction

Irbesartan (2-butyl-3-[p-(o-1H-tetrazol-5-ylphenyl)benzyl]-1,3-diazaaspiro [4.4] non-1-en-4-one) [1], Figure 1, is belongs to non-peptide, orally active angiotensin-II receptor antagonists (ARAs II) used in the treatment of hypertension, congestive heart failure, and chronic renal failure [2,3].

Figure 1: Irbesartan.
According to the literature, many methods have been used to determine this active pharmaceutical ingredient (API). By using UV-spectrophotometry [4-10] the manipulation is simple, the sensitivity is low. VS-Spectrophotometric and spectrofluorimetric [6,11] methods, have high sensitivity, but cumbersome, time-consuming manipulation and poor repeatability. Capillary electrophoresis [12], LC-MS [13-16], HPTLC and HPLC [17-27] methods need costly apparatus and harmful organic solvents. Polarographic methods [28-30] need to use a lot of metal mercury which harms human health.

For the past two decades, Fourier Transform Infrared spectroscopy has undergone significant application in the pharmaceutical analysis field [31,32] due to its main advantages of an easy sample preparation with reduced or no pre-processing steps and the prediction of chemical and physical sample parameters from a single spectrum based on the use of multivariate calibration.

Determination of API with FTIR spectrometry provides an enormous amount of spectroscopic information about a sample. Chemometric methods, such as principal component regression (PCR) and partial least squares (PLS) analysis are commonly used to extract the specific information relevant to the analyte of interest from the full spectrum. To develop a good regression model, the PCR and PLS regression were evaluated; the model with the optimal performance in terms of predictive results based on the lowest Root Mean Square Error (RMSE) was chosen. To enhance the predictive ability of each developed model, different data pre-processing methods, i.e. centering, normalization, Standard Normal Variate (SNV), derivative and Kennard Stone data partitioning algorithm, were tested on the FTIR spectra dataset. They were found to greatly affect the outcome of the data analysis.

The main objective of the present study was the development of a rapid, cheap analytical method for the determination of irbesartan in tablet formulation for routine quality control analysis, based on FT-IR spectroscopy.

**Experimental**

**Reagents and Samples**

Pure Irbesartan (Certified to contain 100.1% w/w) was kindly provided by Medical Union Pharmaceuticals, Abu-sultan, Ismailia, Egypt. Dichloromethane (CH$_2$Cl$_2$) HPLC grade was purchased from Merck, Darmstadt, Germany. X-TENSION® tab (Marcyr1 Company, Egypt) Aprovel® tab (Sanofi-Aventis Egypt), each tablet was labeled to contain 150mg irbesartan.

**Standard Solutions**

A 4.0 mgmL$^{-1}$ irbesartan stock solution was prepared in CH$_2$Cl$_2$, the stock solution was kept protected from light using amber colored flask and refrigerated. Working standard solutions were freshly obtained by diluting the stock standard solution with CH$_2$Cl$_2$ during the analysis day.

**Apparatus and Software**

A Varian 640 FTIR spectrophotometer (Varian Inc., Palo Alto, CA, USA was employed for FTIR spectra acquisition. A demountable liquid cell (Pike Technologies) consisted of two KBr windows (32 mm in diameter, 3 mm thick) separated by a Teflon spacer. To minimize water vapor and CO$_2$ interferences, the system was continuously purged with dry nitrogen. Acquisition time for each spectrum was less than two minutes for both background and spectral measurements. The sample was introduced into the cell by filling ports using a 3 mL syringe. The chemometric analysis (PCA, PCR and PLS) were performed using Unscrambler X version 10.3.0 (CAMO Software AS, Oslo, Norway).

**Recommended Procedures**

Standard irbesartan samples (n= 31) were scanned from 400-4000 cm$^{-1}$ at resolution of 32 cm$^{-1}$ and averaging 18 accumulated scans per spectrum. A background spectrum of CH$_2$Cl$_2$ was measured for each sample; this was used as the background signal subtracted from each sample signal.

**Multivariate Data Analysis Methods**

PCA was performed on all spectra using all the samples to search for outliers and to determine the optimal regions to use in developing the calibration models.

After detection and removal of outliers, the spectra of all samples were divided into 2 groups used for setting up the calibration model and external validation set. The selection of the data for calibration was done by Kennard Stone algorithm [33].

The performance of PCR/PLSR was evaluated for the calibration set by calculating the root mean-squared error of calibration (RMSEC) and the root-mean-squared error of cross validation (RMSECV) were computed by the same software and used as indicators of calibration performance.

The calibration models were then tested using the validation set. The root-mean-squared error of prediction (RMSEP) and coefficient of determination (R$^2$) were calculated as outlined in the literature [34].
For this study, PCR/PLSR models building was an iterative procedure that involved changing the degree of data pre-processing, e.g., standardization, normalization and derivatives, until the best PCR/PLSR models were attained. The final models were chosen based on (1) its ability to minimize the number of PCs required to model the data, (2) optimize the R² statistics, and (3) minimize the RMSEC and RMSEP.

Results and Discussion

Spectral Analysis of Irbesartan

Figure 2 shows the FTIR spectra of 400 µg/mL irbesartan in CH₂Cl₂ in the wavenumber region from 400-4000 cm⁻¹.

The characteristic peak observed at 3412 cm⁻¹ was due to NH stretching vibrations [1]. Peaks observed at 2870-3000 cm⁻¹ was attributed to asymmetric and symmetric CH stretching modes. Peaks observed at 1717 and 1617 cm⁻¹ were due to C=O and C=N stretching [35].

Effect of Measurement Conditions

The effects of the spectral resolution and the number of accumulated scans per spectrum on the signal intensity were evaluated in order to select the most appropriate conditions. With this purpose, optimal response surface experiment with two factors (the resolution and the accumulated scans per spectrum) and single response (peak height values at 1717 cm⁻¹) was performed. An optimal response surface experiment with 17 runs was constructed using Design-Expert® 8.0.7.1 (Stat-Ease Inc., Minneapolis). Desirability function approach was used to get the best conditions, Figure 3 shows, the 3-D plots of the desirability function results, from which the highest results were obtained with resolution of 32 and 18 accumulated scans per spectrum.

Detection of Outliers

A PCA was performed on the FTIR data set (n=31) which enabled to detect 4 spectral outliers in the data (Figure 4, green color filled circles) of irbesartan.

The absorbance spectra of these (spectral outlier) samples were excluded from the PCR and PLS analysis performed to develop a final calibration model (as described next).

Selection of Significant Frequency Regions

The selection of the significant frequency regions in FTIR analysis is very difficult because the chosen regions must be describing the most characteristics analytes to be determined and to provide non-interfered data for the analytes [36]. PCA allows to explore, in an efficient way, the spectra in the full range.
(4000–400 cm\(^{-1}\)) and to identify the most significant regions.

In other way, PLS can be used for selection of the best frequency region from the important regions [37], as the proper frequency region must be has highest value of coefficient of determination (\(R^2\)) and lowest value of root mean standard error of calibration (RMSEC).

The most important region is from 1666 and 1835 with \(R^2\) value of 0.9994 and 0.5372 RMSEC, Table 1.

<table>
<thead>
<tr>
<th>Spectral region cm(^{-1})</th>
<th>(R^2) value</th>
<th>RMSEC</th>
<th>RMSECV</th>
<th>(Q^2) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1218-1527</td>
<td>0.9991</td>
<td>0.6541</td>
<td>0.7653</td>
<td>0.9988</td>
</tr>
<tr>
<td>1542-1666</td>
<td>0.9994</td>
<td>0.5423</td>
<td>0.6375</td>
<td>0.9992</td>
</tr>
<tr>
<td><strong>1666-1835</strong></td>
<td><strong>0.9994</strong></td>
<td><strong>0.5372</strong></td>
<td><strong>0.5781</strong></td>
<td><strong>0.9993</strong></td>
</tr>
<tr>
<td>2792-3039</td>
<td>0.9978</td>
<td>1.02</td>
<td>1.13</td>
<td>0.9975</td>
</tr>
<tr>
<td>1542-1835</td>
<td>0.9984</td>
<td>0.858</td>
<td>0.927</td>
<td>0.9983</td>
</tr>
</tbody>
</table>

Table 1: PLSR analysis of irbesartan spectra using regions which selected by PCA and its combinations.

In the methods below, both normalization, and Standard Normal Variate (SNV), was first performed separately and then together to evaluate their effect. Another way to correct instrumentation errors that was investigated was to calculate the first and second derivative of all spectra to compare results to the results of normalization and standardization (Figure 6). These three methods of pre-processing were tested in quantitative analysis as performance of pre-processing method is not easy to analyze prior to modeling [38].

Standard normal variates (SNV) which involve centering the data and scaling at the same time to give a correlation of the samples in the dataset. Comparing the SNV plot to the plot of the normalized spectra in Figure 5 represent PCA-loadings plot of spectra of irbesartan samples shows that the most important regions were 1218-1527, 1542-1666, 1666-1835, and 2792-3039 cm\(^{-1}\).
6. It is obvious that SNV gives tighter groupings of the measurements and a better attempt at peak separation. In fact, SNV has been shown to do better with infrared spectroscopic data of this sort; however, Normalization (range scaling) also keeps the data close to their original form as opposed to SNV, but SNV centered the data better than Normalization.

![FTIR Spectra of irbesartan](image)

**Figure 6:** FTIR Spectra of irbesartan before scaling (A), after normalization (B), after SNV (C), after SNV on normalized data and after 1st, 2nd derivatives.

**Data Selection Scheme**

From the 'X' and 'Y' matrices of the calibration set of irbesartan in CH₂Cl₂, 20 spectra were used to develop the calibration models, and 7 spectra were used for validation set. Kennard Stone algorithm (KS) did this selection [33].

KS is a well-known method for the selection of a sample subset for calibration. It chooses objects that are uniformly distributed in the X-matrix by assigning a sample to the calibration set, closest to the mean of the entire sample; the next sample is then chosen based on the square distance to the sample already assigned [39]. The sample furthest from the already selected sample is added to the calibration set, etc.

In this way, variations of the dataset are chosen up front. The algorithm was set to select 20 standards representing the dataset, with the rest left for validation. The calibration model was constructed and subsequently validated. Figure 7 represent the KS selections split into calibration set and test set.

![KS Selections](image)

**Figure 7:** Selected samples of irbesartan used in the calibration set and the test set after application of the Kennard Stone selection algorithm.
**PLS and PCR Modeling**

For our data calibration, PLS and PCR cross-validation were plotted for comparison. These plots are shown in Figure 8. The plot suggest that as few as 2 PCR/PLS components may be adequate to interpret the information in the predictor (X) which is related to the response (Y).

As the object of this comparison is to show the difference in fitting between PLSR and PCR with similar components, this was done using selected spectral regions for irbesartan and pre-processed as described before. The values of R² together with RMSEC were used for calibration criteria.

PLS with normalized spectra was selected because it produced the lowest RMSEC (0.360) and highest R² value (0.9998) which after cross-validation, gave an RMSECV value of 0.447 with only 2 components. The RMSEP of the test set was 1.03 with an R² of 0.9973. Figure 9 shows the predicted vs actual plots for irbesartan calibration from the selected region of the FTIR spectra using the same method.

Table 2 summarizes PCR/PLSR model results for irbesartan for the different pre-processing methods when using the selected spectral ranges. Among the different transformation methods, the best PLSR model result is indicated for models based on spectra combined with normalization.

**Validation of the Method**

The validation based on traditional chemometric parameters such as Q² and RMSEP is insufficient towards pharmaceutical regulatory requirements [40]. So the selected model was validated in accordance with the International Conference on Harmonisation ICH using parameters usually recommended: accuracy, precision and linearity.

A straight line calibration was obtained by plotting of FTIR predicted values by PLS model versus reference values. Table 3 presents the performance data and statistical parameters including linear regression
equations, concentration ranges, correlation coefficients and slope.

Intra-day accuracy ranged from 99.16 to 99.70 for irbesartan, while inter-day accuracy ranged from 99.57 to 99.62.

The small values of RSD % indicate high precision of the method.

In Table 4, the intra-day precision (repeatability) ranged from 0.17 to 1.09, while inter-day precision (intermediate precision) ranged from 0.22 to 0.73, as shown in Table 4. The results were compared with reported methods [41,42]. Regarding the calculated student’s t-test and variance ratio F-test, there is no significant difference between the proposed and the reference methods regarding accuracy and precision.

Table 5 shows the application of the proposed FTIR method for analysis of the studied drug.

Table 5: Application of the proposed FTIR method for analysis of the studied drug.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Pharmaceutical Dosage forms</th>
<th>% Recovery ± S.D.</th>
<th>t-value</th>
<th>F-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irbesartan</td>
<td>Approval™ tablets</td>
<td>99.62±0.57</td>
<td>9.61</td>
<td>1.22</td>
</tr>
<tr>
<td></td>
<td>X-TENSION® tablets</td>
<td>99.41±0.51</td>
<td>0.61</td>
<td>1.22</td>
</tr>
</tbody>
</table>
Conclusion

FT-IR spectrometry is capable for the analytical quantification of irbesartan in pharmaceutical tablets. Commercial software involving chemometric approaches, the method proposed is simple, precise and not time-consuming compared to the chromatographic methods that exist in literature.

References


