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

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Research Article

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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 twoprocedures 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-diazaspiro [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].

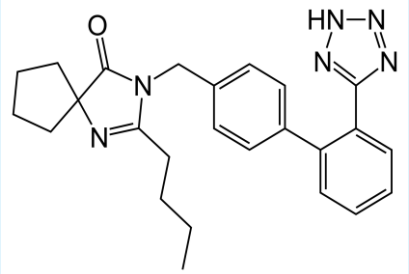

Figure 1: Irbesartan.

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 andPLS 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_2Cl_2) HPLC grade was purchased from Merck, Darmstadt, Germany. X-TENSION® tab (Marcyrl 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_2Cl_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_2Cl_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_2Cl_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/PLS 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 R^2 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 CH_2Cl_2, in the wavenumber region from 400-4000 cm^{-1}.

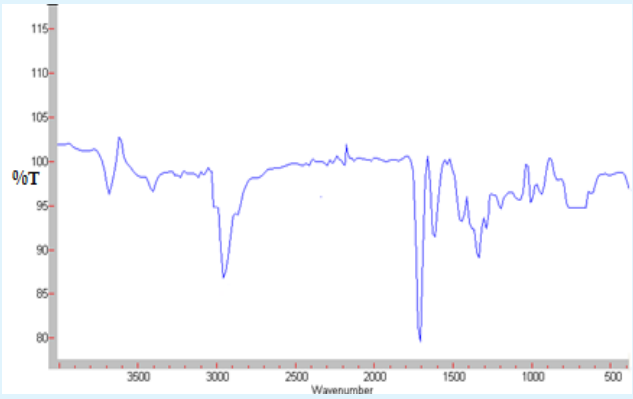

Figure 2: FTIR spectra of pure irbesartan in CH_2Cl_2.

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 1617cm^{-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 1717cm^{-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.

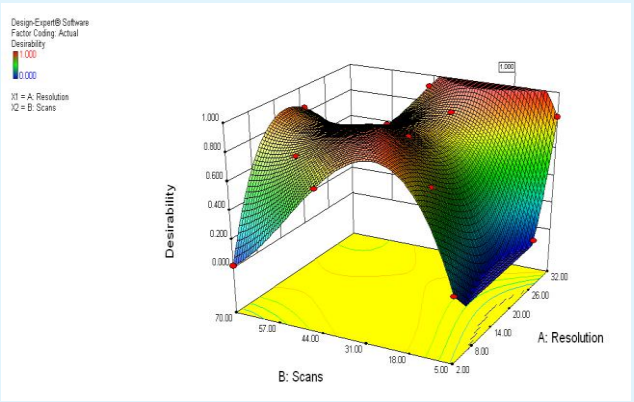

Figure 3: 3-D plots of the desirability function of irbesartan in correlation with a variation of resolution and number of accumulated scans per spectrum.

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).

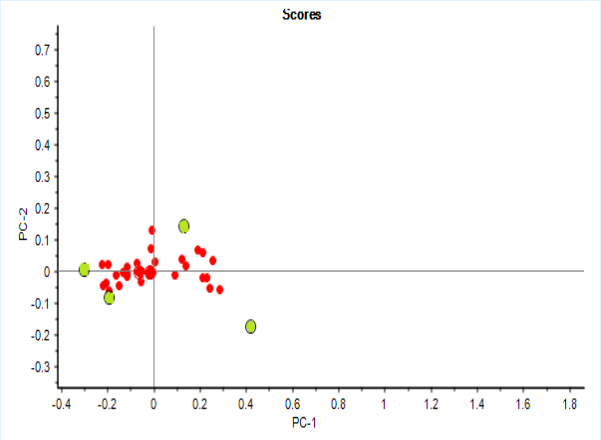

Figure 4: PCA performed on the absorbance spectra of all irbesartan samples (n = 31). The green filled circles are the 4 spectral outliers in the samples.

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.

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}.

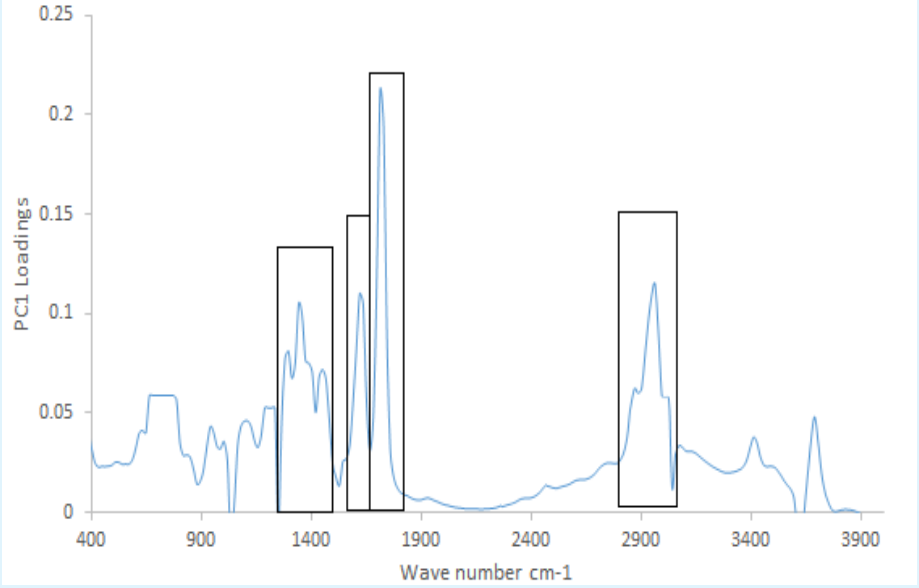

Figure 5: A PCA-loadings plot performed on the 400-4000 cm-1 range of FTIR spectra of irbesartan

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.

Spectral region cm^{-1}	R^2 value	RMSEC	RMSECV	Q^2 value
1218-1527	0.9991	0.6541	0.7653	0.9988
1542-1666	0.9994	0.5423	0.6375	0.9992
1666-1835	**0.9994**	**0.5372**	**0.5781**	**0.9993**
2792-3039	0.9978	1.02	1.13	0.9975
1542-1835	0.9984	0.858	0.927	0.9983

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

Data pre-processing

The development of useful models requires both appropriate methods (PLSR, PCR etc.) and high quality data. In many situations, choice of data is limited due to the specific physicochemical properties of the samples, the availability of instruments, and so on. However, the data quality could be improved by appropriate pre-processing methods. Although it is common for pre-processing methods to be used before multivariate modelling, there is no set standard approach and the results of multivariate analysis are affected by the methods used. This also means that data pre-processing is often revisited to ascertain optimal solutions before performing any computational analysis.

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

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.

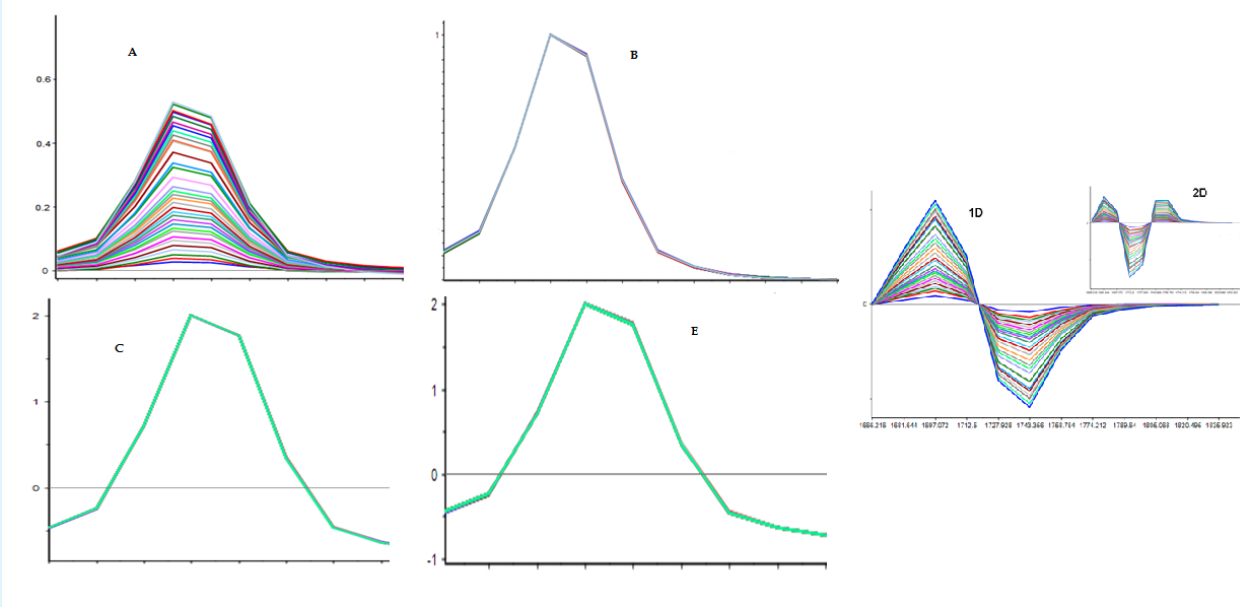

Figure 6: FTIR Spectra of irbesartan before scaling (A), after normalization (B), after SNV (C), after SNV on normalized data and after 1st, 2nd derivatives.

Data Selection Scheme

From the 'X' and 'Y' matrices of the calibration set of irbesartan in CH_2Cl_2, 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 modelwas constructed and subsequently validated. Figure 7 represent the KS selections split into calibration set and test set.

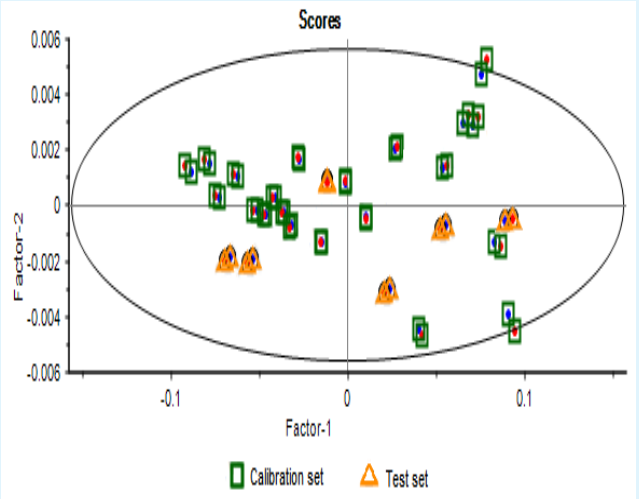

Figure 7: Selected samples of irbesartan used in the calibration set and the test set after application of the Kennard Stone selection algorithm.

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).

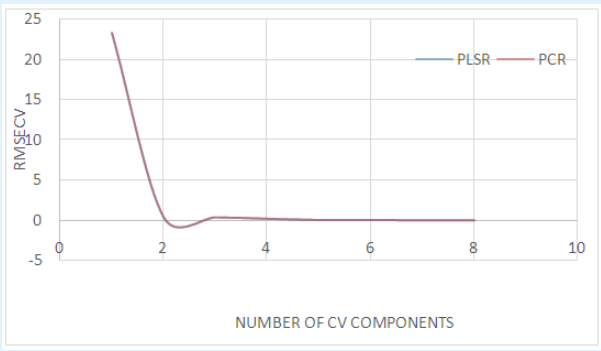

Figure 8: Plot of RMSECV versus number of components for a calibration set prediction using cross-validation of PLSR/PCR for irbesartan.

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 R^2 together with RMSEC were used for calibration criteria.

PLS with normalized spectra was selected because it produced the lowest RMSEC (0.360) and highest R^2 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 R^2 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.

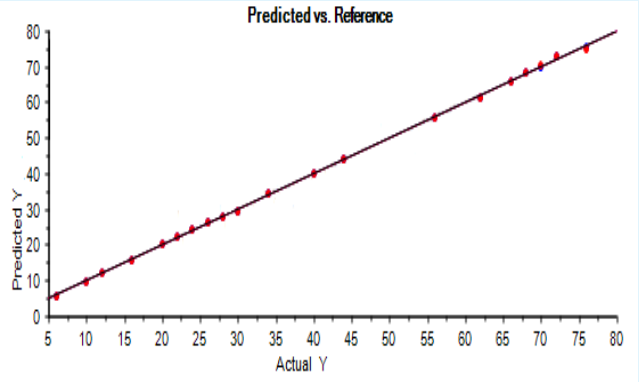

Figure 9: Calibration curve for the analysis of irbesartan content obtained from PLS model based on normalized spectra.

Method	Pre-processing	NO. component	RMSEC	R^2	RMSECV	Q^2
PLS	**Norm**	**PC2**	**0.36**	**0.9998**	**0.447**	**0.9996**
PCR	Norm	PC2	0.37	0.9997	0.457	0.9996
PLS	SNV	PC2	0.383	0.9997	0.462	0.9996
PCR	SNV	PC2	0.385	0.9997	0.463	0.9995
PLS	Norm+SNV	PC2	0.383	0.9997	0.461	0.9996
PCR	Norm+SNV	PC2	0.385	0.9997	0.463	0.9996
PLS	1st D	PC2	0.653	0.9991	0.77	0.9986
PCR	1st D	PC2	0.655	0.9991	0.772	0.9979
PLS	2nd D	PC2	0.534	0.9994	0.635	0.9992
PCR	2nd D	PC2	0.542	0.9994	0.645	0.992

Table 2: Results for PCR and PLSR methods on preprocessed spectra of irbesartan.

Validation of the Method

The validation based on traditional chemometric parameters such as Q^2 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.

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

Method	Range µg/mL	r	Pre-processing	Intercept	Slope	SEP	RMSEP	R^2
PLS	6.0-76.0	0.9999	Norm	0.004	0.9997	0.941	1.03	0.9973
PCR		0.9999	Norm	0.004	0.9997	0.941	1.13	0.9963
PLS		0.9999	SNV	0.006	0.9997	0.968	1.07	0.9971
PCR		0.9999	SNV	0.006	0.9997	0.968	1.27	0.9951
PLS		0.9999	Norm+SNV	0.006	0.9997	0.968	1.07	0.9971
PCR		0.9999	Norm+SNV	0.006	0.9997	0.968	1.17	0.9961
PLS		0.9996	1st D	0.026	0.9991	1.31	1.45	0.9948
PCR		0.9996	1st D	0.0282	0.9992	1.62	1.48	0.9958
PLS		0.9997	2nd D	0.017	0.9994	1.2	1.31	0.9957
PCR		0.9997	2nd D	0.019	0.9994	1.21	1.33	0.9956

Table 3: Mathematical modeling for calibration of irbesartan by FTIR method using PLS and PCR.

Analyte	Concentration (µg/mL)	Intra-day precision			Inter-day precision		
		Found (Conc.± SD)	Accuracy (R%)	Precision (RSD%)	Found (Conc.± SD)	Accuracy (R%)	Precision (RSD%)
IRS	10	9.96±0.054	99.63	0.55	9.96±0.048	99.61	0.49
	40	39.66±0.066	99.16	0.17	39.84±0.289	99.62	0.73
	70	69.79±0.75	99.7	1.09	69.70±0.155	99.57	0.22

Table 4: Inter-day and intra-day precision and accuracy for captopril and irbesartan using PLS (normal) method.

Analysis of Pharmaceutical Tablets

Ten tablets of each pharmaceutical were weighed, separately, finely powdered. An accurately weighed quantity of drug powder (equivalent to 150 mg of irbesartan) was dissolved, separately, in 80 mL of CH2Cl2, and was sonicated for 20 min. after cooling, the flasks were filtered through a 0.45-µm (Nylon 66-membrane) filter into 100 mL volumetric flask and made up to the mark with CH_2Cl_2 to obtain a concentration of irbesartan as 1.5 $mgmL^{-1}$, respectively.

The resulted solution was diluted with CH_2Cl_2 to produce the desired concentrations. Five replicates determination were made and satisfactory results were obtained in agreement with the label claim, where no interference from excipients and additives was observed as shown in Table 5. 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

Drug	Pharmaceutical Dosage forms	% Recovery ± S.D.		*t*-value*	F-value*
		Proposed method (n=5)	Reported method (n=5)		
Irbesartan	Approvel™ tablets	99.62±0.57	99.41±0.51	0.61	1.22
	X-TENSION® tablets	99.74±0.55		0.97	1.15

Table 5: Application of the proposed FTIR method for analysis of the studied drug.
*Tabulated values of t= 2.31 and F = 6.39 at (P = 0.05)

Conclusion

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

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