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Multivariate analytical methods for simultaneous estimation of Atenolol and Hydrochlorothiazide in bulk and tablet dosage form

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ABSTRACT

Aim: The present study aimed to develop simple, accurate and precise FTIR and UV spectrophotometric methods for the quantification of Atenolol and Hydrochlorothiazide in bulk and tablet dosage forms.
Materials and Methods: FT-IR method like classical least squares (CLS) was developed within the range of 2366.69-3433.44; 1564.40-1673.30 cm- UV methods like Cramer's matrix method (method-I) and linear regression analysis (Method II) were developed and they are based upon constructing the matrix set by using molar absorptivity values at 275.60 nm and 270.40 nm.
Results: The assay values for FTIR- CLS method were 102% and 108 % for Atenololand Hydrochlorothiazide respectively. Cramer's matrix method results were found to be 95.15% and 104% for Atenolol and Hydrochlorothiazide respectively and for linear regression method they were found to be 98.50% and 106% (w/w).

1. Introduction

The fixed dose combination of hydrochlorothiazide and atenolol is broadly used for moderate to intense hypertension. Few analytical methods were reported in the literature for this combination and by review it was found that no multivariate method was developed and validated for the simultaneous determination of amlodipine and hydrochlorothiazide. Hence, in the present study an attempt was made to develop multivariate methods like Classical least squares (CLS), Cramer's matrix method, and linear regression analysis. CLS is a multivariate statistical technique that can be used to quantify analyte in IR spectra that are expressed using the Beer-Lambert law for absorbance or equivalent for spectra obtained using DRIFTS. This technique is particularly appropriate when analyzing complex multiple spectra, which contain broad and overlapping bands. [1–5]

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2. Materials and Methods

2.1. Instruments and software

Shimadzu FTIR (IR-SPIRIT) is used for the quantification of multi-variate method (CLS method). Shimadzu UV-1800 spectrophotometer with matched pair of 10mm quartz cells is used for UV quantification. Data acquisition was performed by UV Probe software. Instrument was allowed to stand for 10 minutes to stabilize. Then the instrument parameters viz. start wavelength, end wavelength data interval, scan speed, slit width and sample information were entered in the instrument. Then base line correction was performed by keeping the blank in both the sample and reference compartments.

2.2. Materials and solvents

Working standards of atenolol and hydrochlorothiazide are procured from Raffles Pharmaceuticals, Tirupati. Methanol was used as the solvent for the current UV study and

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potassium bromide was used in IR method. Marketed formulation ATEN-H tablets containing 50 mg atenolol and 12.5 mg hydrochlorothiazide manufactured Zydus cadila Pvt. Ltd was the sample selected for the study.

2.3. Quantitative analysis by using FTIR spectroscopy

The method employed was classical least squares (CLS) method. In this method, different ratios of the two drugs were prepared and their IR spectrums were recorded between the ranges of 2366.69 – 3433.44cm^{-1}and 1564.40 – 1673.30 cm^{-1}using FTIR. Total 16 mixtures were prepared with different concentrations of atenolol and hydrochlorothiazide. From these, 10 mixtures were taken as a calibration set and 6 mixtures was taken as a prediction set or validation set.[6–8]

2.4. Preparation of mixtures

30 mg of atenolol in 300 mg of KBR was taken to get a concentration of 100 μg/ml (stock-1)**.** 30 mg of Hydrochlorothiazide in 300 mg of KBR was taken to get a concentration of 100 μg/ml (stock-1)**.** From this above stock mixtures, different concentrations were prepared to get a series of mixtures.

2.5. Calibration of CLS model

The prepared mixtures were taken in a mortar and pestle and triturated with potassium bromide to get a uniform mixture and those mixtures were subjected to hydraulic press to get pellets. The pellets were subjected to FTIR to record the spectra of mixtures. Calibration of CLS model was done by selecting the proper ranges (2366.69 – 3433.44cm^{-1}and 1564.40 – 1673.30 cm^{-1})using FTIR. By using spectrum values, CLS model was analyzed.

2.6. Assay of marketed formulation

Average weight of 20 tablets was taken and made up with KBR up to 200mcg of total weight. The sample was prepared from the mixture to get a concentration of 15mcg of hydrochlorothiazide and 20 mcg of atenolol.

2.7. UV Spectrophotometric method development

Different Solvents like Water, Methanol, and 0.1N HCl, 0.1N NaOH and Ethanol were employed for the optimization of the method. Methanol gave a single distinct peak with good absorbance for all the five drugs. So, it was employed as the solvent. From trial-and-error method, $\lambda_{max}$of bulk drugs were determined by preparing the solutions in methanol in UV spectrophotometer and the $\lambda_{max\ of}$ atenolol and hydrochlorothiazide was found to be 275.60 nm and 270.4 nm respectively.

2.8. Construction of calibration curve

Calibration curve for Atenolol was obtained in the linearity range of 30-180μg/mL by measuring the absorbance at 275.60 nm. Calibration curve for hydrochlorothiazide was obtained in the linearity range of 3-15μg/mL by measuring the absorbance at 270.40 nm. The correlation coefficients for atenolol and hydrochlorothiazide were found to be 0.9996 and 0.9999 respectively.

2.9. Application of linear regression analysis method

For linear regression method, two wavelengths (268 nm and 280nm) were considered for the analysis of the component mixture atenolol (ATL) and hydrochlorothiazide (HCTZ). The slope values obtained from the linear regression analysis for each component were used for the formation of the matrix set. The wavelengths selected for the analysis were 268 nm and 280 nm.

The following equation was derived from the linear equation y = mx + c.

The concentrations of the drugs can be calculated by using the following equations.

$$\begin{bmatrix} Amix1 & - & axy1 \\ Amix2 & - & axy2 \end{bmatrix} = \begin{bmatrix} bx1 & by1 \\ bx1 & by2 \end{bmatrix} \times \begin{bmatrix} Cx \\ Cy \end{bmatrix}$$

$$\begin{bmatrix} Cx \\ Cy \end{bmatrix} = \begin{bmatrix} bx1 & by1 \\ bx1 & by2 \end{bmatrix} \times \begin{bmatrix} Amix1 & - & axy1 \\ Amix2 & - & axy2 \end{bmatrix}$$

Where:

- C_x and C_y are the concentrations of X and Y drugs respectively.
- A_{mix1} and A_{mix2} are the absorbance of the mixture of ATL and HCTZ analytes at two wavelengths (268 and 280).
- b_{x1} and b_{x2} are the slope values of ATL.
- b_{y1} and b_{y2} are the slope values of HCTZ.
- axy_1 and axy_2, are the sum of intercepts of the linear regression equation at the two wavelengths (268 and 280).

$$\text{Percent assay} = \frac{calculated\ quantity\ of\ test\ sample(mg)}{Weight\ of\ test\ sample(mg)} \times 100$$

Validation of UV spectrophotometric method:

The method was validated for various validation parameters like linearity, range, precision, LOD, LOQ and accuracy as per the ICH guidelines. The validate method was applied to the dosage form to determine the concentrations of atenolol and hydrochlorothiazide.

3. Results and Discussion

3.1. FTIR-CLS quantitative method

FT-IR method like classical least squares (CLS) was developed with algorithm of MLR evolution via K- matrix for mixtures within the range of 2366.69-3433.44; 1564.40-1673.30 cm^{-1}. The FTIR spectrum of calibration set of mixtures was depicted in Figure 1. The actual and predicted

Table 1: Actual vs predicted values for calibration set in FTIR-CLS method

S.No	Calibration set	ATL		% Recovery	HCTZ		% recovery
		Actual	Predicted		Actual	Predicted	
1	Mixture 1	20	20.930	104.6	20	21.383	106.9
2	Mixture 2	10	9.346	93.5	10	9.170	91.7
3	Mixture 3	10	9.932	99.3	14	13.968	99.77
4	Mixture 4	10	10.352	103.52	16	16.753	104.7
5	Mixture 5	10	8.247	82.42	18	15.486	86.03
6	Mixture 6	10	11.105	111	20	21.839	109.1
7	Mixture 7	14	14.308	102.1	14	14.421	103
8	Mixture 8	18	17.686	98.25	16	16.089	100
9	Mixture 9	18	17.663	98.12	18	16.207	90.03
10	Mixture 10	20	19.950	99.75	10	10.249	102.4
	Mean			99.4	Mean		101.04
	%RSD			0.3901			1.896

Table 2: Actual vs predicted values for validation set in FTIR-CLS method

Validation set	ATL		% Recovery	HCTZ		% recovery
	Actual	Predicted		Actual	Predicted	
Mixture 11	16	16.02	100.1	20	19.398	96.99
Mixture 12	20	22.202	111.01	18	17.020	94.55
Mixture 13	16	16.373	102.33	14	14.536	103.82
Mixture 14	14	14.403	102.87	20	19.247	96.23
Mixture 15	16	15.992	99.95	20	19.086	95.43
Mixture 16	16	15.187	94.91	16	15.336	95.85
Mean			98.36	Mean		97.14
%RSD			8.799	%RSD		1.907

Table 3: Parameters for linear regression method

S.No	Linear equation parameters	Atenolol	Hydrochlorothiazide
1	Linear regression equation	Y=0.0049x+0.012	Y=0.0679x-0.0109
2	R^2	0.9996	0.9999
3	Range	30-180 μg/ml	3-15 μg/ml

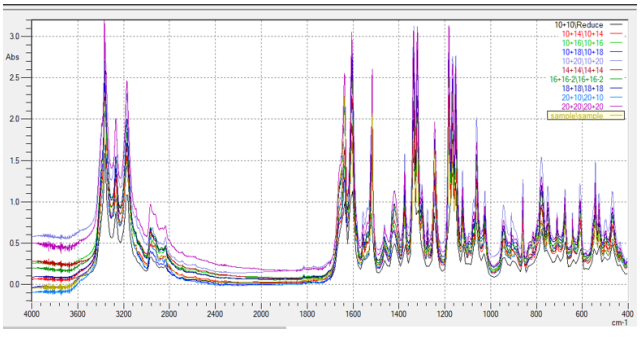

Fig. 1: Spectrum for calibration set of mixtures

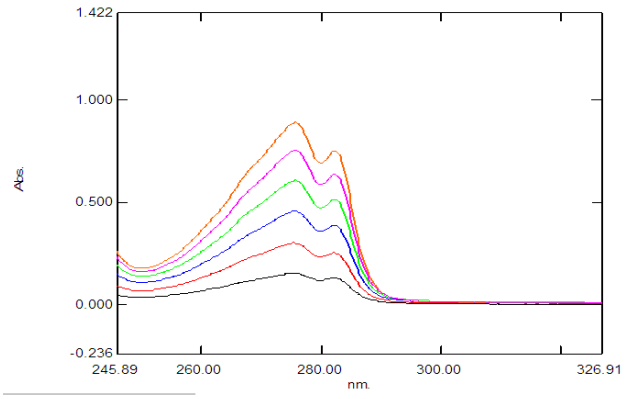

Fig. 2: Calibration curve for Atenolol

values obtained for the calibration set and validation set mixtures were presented in Tables 1 and 2 respectively. The values obtained were found to be within the range and minimum PRESS values were obtained. Hence this CLS calibration set is optimized and applied to analyze the marketed formulation. The assay values for FTIR- CLS method were found to be 102% and 108 % for Atenolol and Hydrochlorothiazide respectively.

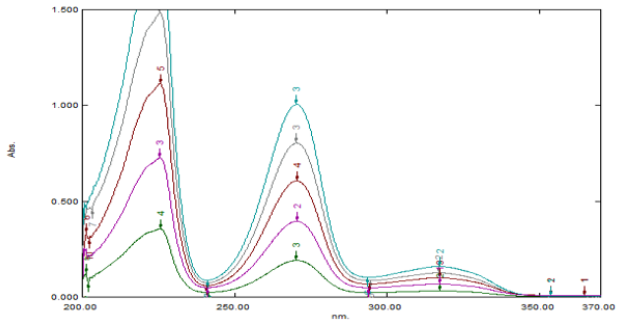

Fig. 3: Calibration curve for hydrochlorothiazide

3.2. UV Spectrophotometric method

Methanol was selected as solvent keeping solubility, spectral characteristics, cost and environmental safety in view. The UV absorption spectra of the standard solutions of atenolol and hydrochlorothiazide were recorded in the UV region between 200-400 nm and the obtained spectra showed a maximum absorbance at 275.6 nm for atenolol (ATL) and 270.4 nm for Hydrochlorothiazide (HCTZ) respectively. The two wavelengths ($\lambda 1$ and $\lambda 2$) considered for ATL (X) and HCTZ (Y) were 275.6 nm and 270.4 nm respectively. The linear regression equations were obtained by using the absorbencies measured at two wavelengths against the concentrations of standard solution for each drug. The calibration curve was constructed in the concentration range of 30-180 μg /mL of ATL and 3-15 μg/mL for HCTZ and the overlay spectra were represented in Figures 2 and 3. The linear regression equation was established between concentration and absorbance of various working standard solutions of ATL individually as shown in the Table 3. All the regression parameters showed that a good linear relation exists between the concentration and absorbance. Hence, the method was found to be linear in the range of 30-180 μg/mL for ATL and 3-15μg /mL for HCTZ. The repeatability results showed a % RSD value of less than 2 for both the drugs, which is acceptable and hence the method was precise in terms of repeatability. The assay was calculated from the regression equations that were constructed for both the drugs and the result was found to be 98.5 % (w/w) for ATL and 106% (w/w) for HCTZ. From the overall results for all the analytical performance characteristics, it can be inferred that the developed Linear regression analysis method was simple, precise, accurate and economical.

4. Conclusion

An attempt was made to develop and validate FTIR spectroscopy and UV Spectrophotometric methods for the multivariate analytical methods for simultaneous estimation of Atenolol and Hydrochlorothiazide in bulk and tablet dosage form. The developed FTIR method was classical least squares method (CLS) and UV spectrophotometric method was linear regression method for the simultaneous estimation of Atenolol and Hydrochlorothiazide in bulk and tablet dosage form. The developed methods were found to be accurate and precise. All the developed methods were validated as per the ICH guidelines and can be used for regular analysis of Atenolol and Hydrochlorothiazide.[9]

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6. Conflict o f Interest

The authors declare no conflict of interest.

7. Source of Funding

None.

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