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First derivative spectrophotometric method for the simultaneous estimation of valsartan and hydrochlorthiazide in their combined dosage form

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Abstract
The present manuscript describe simple, sensitive, rapid, accurate, precise and cost effective First derivative spectrophotometric zero crossing point method for the simultaneous determination of Valsartan and Hydrochlorthiazide in combined dosage form. The utility of first derivative data processing program is its ability to calculate unknown concentration of components of interest in a mixture containing an interfering component. The first order derivative absorption at 250.20 nm (zero cross point of Valsartan) was used for Hydrochlorthiazide and 270.60 nm (zero cross point of Hydrochlorthiazide) was used for Valsartan. The linearity was obtained in the concentration range of 4-20 µg/ml for Valsartan and 2-14 µg/ml for Hydrochlorthiazide. The method was successfully applied to pharmaceutical dosage form because no interference from the mixture excipient was found. The suitability of this method for the quantitative determination of Valsartan and Hydrochlorthiazide was proved by validation. The proposed methods were found to be simple and sensitive for the routine quality control application of Valsartan and Hydrochlorthiazide in pharmaceutical dosage form. The results of analysis have been validated statistically and by recovery studies.

Key-Words: Valsartan, Derivative spectrophotometric method, Hydrochlorthiazide, Drug analysis, Validation, Recovery

Introduction
Valsartan (VAL) is chemically N-(1-oxopentyl)-N-[[2'-(1H-tetrazol-5-yl) [1,1'-biphenyl]-4-yl]methyl]-L-valine[4]. It is official in IP. IP[1] describe Liquid Chromatography method and Thin Layer Chromatography Method for its estimation. Literature survey reveals Solid-phase UV spectrophotometric method [7], HPLC [8] method and HPTLC [9] method for determination of VAL in pharmaceutical dosage forms as well as in biological fluids. Literature survey also reveals RP-HPLC[10] and UV- spectrometry [11] method and Spectoflurimetric method [12] and for determination of VAL with other drugs in combination. Hydrochlorthiazide (HCTZ) is chemically 6-chloro-3,4-dihydro-2H-1,2,4-benzo-thiazidiazine-7 sulfonamide 1,1-dioxide[5].

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Hydrochlorthiazide is official in IP[2], USP[3] and describe liquid chromatography method and HPLC method for its estimation. Literature survey reveals HPLC[13],UV Spectrophotometry[14] method and LC-MS[15] method for the determination of HCTZ. Literature survey also reveals HPLC[16] ,UV Spectrophotometry[17] and HPTLC[18] method for determination of HCTZ with other drugs in combination. The combined dosage forms of VAL and HCTZ is used as anti-hypertensive drug[6]. The combination of these two drugs is official in Indian pharmacopoeia but no official method is available for the simultaneous estimation of VAL and HCTZ in their combined dosage forms. Literature survey does not reveal any simple Spectrophotometric method for simultaneous estimation of VAL and HCTZ in combined dosage forms. The present communication describes simple, sensitive, rapid, accurate, precise and cost effective Spectrophotometric method based on First derivative Spectrophotometric method for simultaneous estimation of both drugs in their combined dosage form.

Material and Methods

A Shimadzu model 1700 (Japan) double beam UV/Visible spectrophotometer with spectral width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cell was used to measure absorbance of all the solutions. Spectra were automatically obtained by UV-Probe system software. A Sartorius CP224S analytical balance (Gottingen, Germany), an ultrasonic bath (Frontline FS 4, Mumbai, India) was used in the study. VAL and HCTZ bulk powder was kindly gifted by Lupin Pharmaceuticals Ltd. J&K, India. Methanol (AR Grade, S. D. Fine Chemicals Ltd., Mumbai, India) were used in the study.

Preparation of Standard Solutions

A 10 mg of standard VAL and HCTZ were weighed and transferred to 100 ml separate volumetric flasks and dissolved in methanol. The flasks were shaken and volumes were made up to mark with methanol to give a solution containing 100µg/ml each of VAL and HCTZ.

Methodology

The working standard solutions of VAL and HCTZ were prepared separately in methanol having concentration of 10µg/ml. They were scanned in the wavelength range of 200-400 nm against solvent methanol as blank. The absorption spectra thus obtained were derivatised from first to fourth order. First order derivative spectrum was selected for the analysis of both the drugs. From the overlain spectra of both the drugs (figure 3) wavelengths selected for quantitation were 250.20 nm (zero cross point of Valsartan) was used for Hydrochlorthiazide and 270.60 nm (zero cross point of Hydrochlorthiazide) was used for Valsartan.

Validation of the proposed method

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines.[19]

Linearity (calibration curve)

Appropriate aliquots from the standard stock solutions of VAL and HCTZ were used to prepare two different sets of dilutions: Series A, and B as follows. Series A consisted of different concentration of VAL (4-20 µg/ml). Aliquot from the stock solution of VAL (100 µg/ml) was pipette out in to a series of 10 ml volumetric flask and diluted with methanol to get final concentration in range of 4-20 µg/ml (0.4, 0.8, 1.2, 1.6 and 2.0 ml). It is shown in figure 1. Series B consisted of varying concentrations of HCTZ (2-14 µg/ml). Appropriate volume of the stock solution of HCTZ (100 µg/ml) was transferred into a series of 10 ml volumetric flask and the volume was adjusted to the mark with methanol to get final concentration in range of 2-14 µg/ml (0.2, 0.4, 0.6, 0.8, 1.2, and 1.4 ml). It is shown in figure 2. The calibration curve were constructed by plotting drug concentration versus the absorbance values of first derivative spectrum 250.20 nm for HCTZ and 270.60 nm for VAL. Statistical data for calibration curves is depicted in Table 1. The concentration of individual drugs present in the mixture was determined from the calibration curves in quantitation mode.

Method precision (repeatability)

The precision of the instrument was checked by repeated scanning and measurement of absorbance of solutions (n = 6) for VAL and HCTZ (8 µg/ml for both drugs) without changing the parameter of the proposed Spectrophotometry method.

Intermediate precision (reproducibility)

The intraday and interday precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 3 different concentrations of standard solutions of VAL and HCTZ (4, 8,12 µg/ml for VAL and 4, 8, 12 µg/ml for HCTZ). The result was reported in terms of relative standard deviation (% RSD).

Accuracy (recovery study)

The accuracy of the method was determined by calculating the recoveries of VAL and HCTZ by the standard addition method. Known amounts of standard solutions of VAL and HCTZ were added at 80, 100 and 120 % level to prequantified sample solutions of VAL and HCTZ (8µg/ml for VAL and 1.25µg/ml for HCTZ). The amounts of VAL and HCTZ were estimated by applying obtained values to the respective regression line equations.

Limit of detection and limit of quantification

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) using the following equations designated by International Conference on Harmonization (ICH) guidelines.

$$LOD = 3.3 \times \sigma/S$$

$$LOQ = 10 \times \sigma/S$$

Where, σ = the standard deviation of the response and
S = slope of the calibration curve.

Analysis of sample

The sample solution containing VAL (8 µg/ml) and HCTZ (1.25 µg/ml) was prepared in the methanol. After making the solution absorbance was taken at 250.20 nm and 270.60 nm for HCTZ and VAL, respectively.

Results and Discussion

The standard solutions of VAL and HCTZ were scanned separately in the UV range and First-order spectra for VAL and HCTZ were recorded. The first order derivative absorption at 250.20 nm (zero cross point of Valsartan) was used for Hydrochlorthiazide and 270.60 nm (zero cross point of Hydrochlorthiazide) was used for Valsartan. These two wavelengths can be employed for the determination of VAL and HCTZ without any interference from the other drug in their combined formulations.

Linear correlation was obtained between absorbances and concentrations of VAL and HCTZ in the concentration ranges of 4-20 µg/ml and 2-14 µg/ml, with R^2 value 0.998 at both the wavelength respectively. The linearity of the calibration curve was validated by the high values of correlation coefficient of regression. The regression analysis data and summary of validation parameters for the proposed method is summarized in Table 1.

The recovery experiment was performed by the standard addition method. The recoveries of VAL and HCTZ were found to 100.22 ± 1.33 and 96.15 ± 1.83 for Valsartan and Hydrochlorthiazide, respectively. The results of recovery studies indicate that the proposed method is highly accurate [Table 2]. The validation parameters are summarized in [Table 1]. The proposed validated spectroscopic method was successfully applied to combined dosage form. The results obtained for VAL and HCTZ were comparable with the corresponding label claim percentage [Table 3]. No interference of the excipients with the absorbance of interest appeared; hence the proposed method is applicable for the routine simultaneous estimation of VAL and HCTZ in pharmaceutical dosage forms.

The proposed Spectrophotometric method was found to be simple, sensitive, accurate and precise for determination of VAL and HCTZ in the combined dosage form. The method utilizes easily available and cheap solvent for analysis of VAL and HCTZ hence the method was also economic for estimation of VAL and HCTZ from the combined dosage form. The common excipients and other additives are usually present in the synthetic mixture do not interfere in the analysis of VAL and HCTZ in method, hence it can be conveniently adopted for routine quality control analysis of the drugs in combined pharmaceutical formulation.

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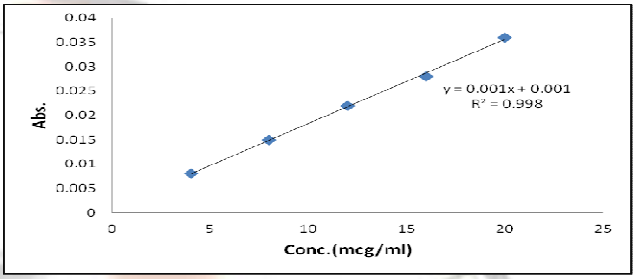

Fig. 1: Calibration curve of VAL

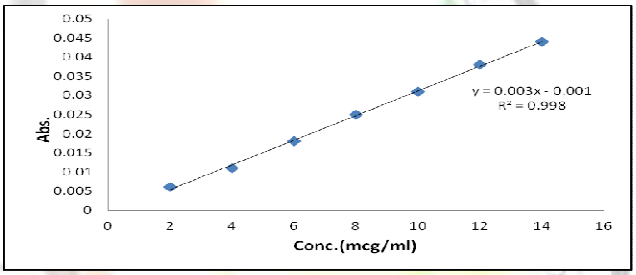

Fig. 2: Calibration curve of HCTZ

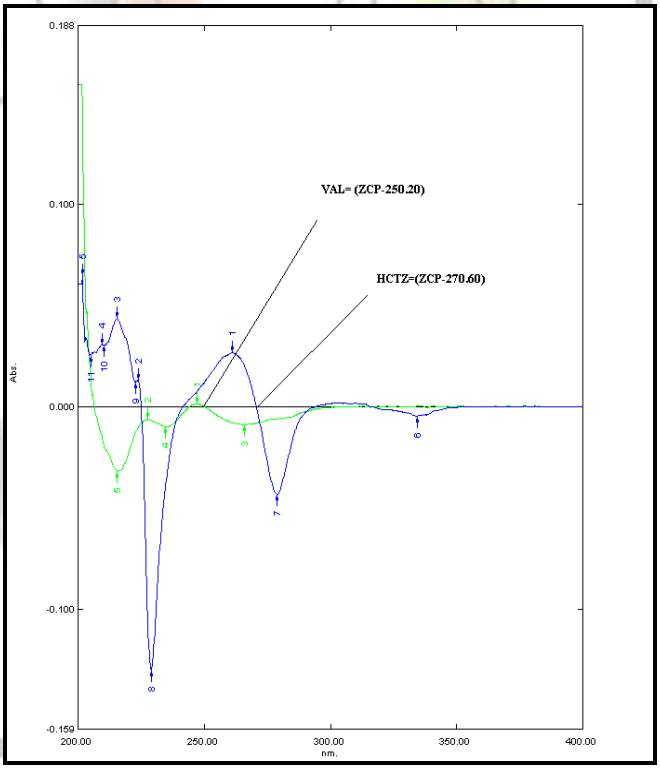

Fig. 3: Overlain First order Derivative absorption spectra of VAL and HCTZ in methanol

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Table 1: Regression Analysis Data and Summary of Validation Parameters for VAL and HCTZ by First Derivative Spectrophotometric Method

Parameters	VAL	HCTZ
Wavelength (nm)	270.60	250.20
Beer's law limit (µg /ml)	4-20	2-14
Regression equation (y = a + bc) Slope (b) Intercept (a)	0.001x + 0.001 0.001 0.001	0.003x – 0.001 0.001 0.003
Correlation coefficient (r^2)	0.998	0.998
LOD[a] (µg/ml)	1.157	0.634
LOQ[b] (µg /ml)	3.50	1.92
Repeatability (% RSD[c], n =6)	1.86	1.37
Precision (%RSD, n = 3) Interday Intraday	 1.86 – 1.97 1.75 – 1.94	 1.37 – 1.98 1.35 – 1.96
Accuracy ± S.D[d]. (%Recovery, n= 5)	100.22 ± 1.33	96.15 ± 1.83

[a]LOD = Limit of detection, [b]LOQ = Limit of quantification, [c]RSD = Relative standard deviation. [d]S.D. = Standard deviation

Table 2: Recovery Data of VAL and HCTZ by Spectrophotometric Method

Drug	Amount taken (µg/ml)	Amount added (%)	%Recovery ± S. D. (n=5)		
VAL	8 8 8	80 100 120	96.66 ± 1.75 98.99 ± 1.98 100.22 ± 1.33		
HCTZ	1.25 1.25 1.25	80 100 120	97.53 ± 1.34 100.14 ±1.16 96.15 ± 1.83		

Table 3: Analysis of VAL and HCTZ by Spectrophotometric Method

Mixture	Label Claim (mg)		Amount Found (mg)		% Label Claim ± S.D. (n=6)	
	VAL	HCTZ	VAL	HCTZ	VAL	HCTZ
I	80	12.5	82.5	11.96	99.15 ± 0.42	99.30 ± 0.48