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IMULTANEOUS ESTIMATION OF VALSARTAN, AMLODIPINE BESYLATE AND HYDROCHLOROTHIAZIDE BY FIRST ORDER DERIVATIVE UV SPECTROPHOTOMETRIC METHOD

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Abstract:

Simple first order derivative spectrophotometric method has been developed for simultaneous estimation of valsartan, amlodipine besylate and hydrochlorothiazide in combined dosage form. The method employed was multi-wavelength method for analysis using methanol: water (70:30) as a solvent. The three wavelengths 245, 265 and 279 nm were selected for estimation of valsartan, amlodipine besylate and hydrochlorothiazide respectively. Linearity was observed in the concentration range of 8-80 µg/ml, 1-10 µg/ml and 2-20 µg/ml for valsartan, amlodipine besylate and hydrochlorothiazide respectively. The recovery studies ascertained the accuracy of the proposed method and the results were validated as per ICH guidelines. The method can be employed for estimation of pharmaceutical formulations with no interference from any other excipients and diluents.

Key words: Valsartan, Amlodipine besylate, Hydrochlorothiazide, UV Spectrophotometer.

Introduction:

Literature survey reveals the availability of several methods for estimation of valsartan (VAL), amlodipine besylate (AML) and hydrochlorothiazide (HCZ) includes UV spectrophotometry, RP-HPLC and HPTLC alone or in combination with other drugs. No method has been reported for the estimation of VAL, AML and HCZ in combined dosage form. Present work emphasizes on the quantitative estimation

of VAL, AML and HCZ in their combined dosage form by first order derivative UV spectrophotometric method. [1-7]

The present research work deals with development of validated method of the triad combination of the drugs on UV spectrophotometry. The method was developed using first derivative spectroscopy.

Ultraviolet spectroscopy is the most widely used method from decades. It is the most simple and convenient method for analysis of number of drugs. As there is no method reported for the used triad on UV study has been focused on UV analysis.

Monographs:

Valsartan [8-10]

Valsartan is the INN for the chemical substance (S)-2-{*N*-(1-oxopentyl)-*N*-[[2'-(1H-tetrazol-5-yl)-[1, 1'-biphenyl]-4-yl] methyl]-amino}-3-methyl-butyric acid.

Molecular formula-$C_{24}H_{29}N_5O_3$

Relative molecular mass-435.5

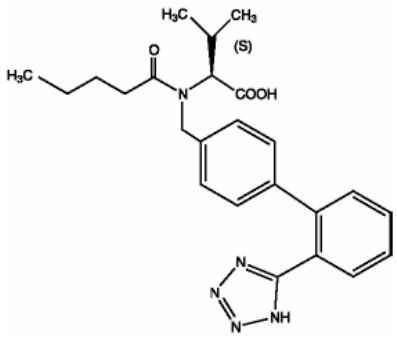


Figure 1: Valsartan

Valsartan is a white to practically white, fine powder, melting at 105-110 °C with decomposition. Its solubility in water is 0.18 mg/ml and in 0.1N HCl 0.084 mg/ml and is freely soluble in methanol, ethanol, acetonitrile. [1-2]

Amlodipine besylate [8-10]

Amlodipine besylate is the INN for the chemical substance, 3-Ethyl-5-methyl(4*RS*)-2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate benzenesulphonate (anhydrous substance).

Molecular formula-$C_{26}H_{31}C_lN_2O_8S$ ($C_{20}H_{25}C_lN_2O_5 \cdot C_6H_5SO_3H$)

Relative molecular mass-567.06

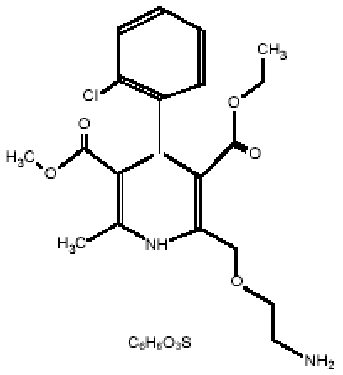


Figure 2: Amlodipine besylate.

The active substance is well known and has been adequately characterised. It is a white or almost white powder. It is slightly soluble in water, freely soluble in methanol, acetonitrile, sparingly soluble in ethanol and slightly soluble in 2-propanol. The compound has 2 pKa's 9 and 0.4 and the distribution coefficient is log Pow 2.76. No solid-state polymorphism of amlodipine besylate is described in the literature. [1]

Hydrochlorothiazide [8-10]

Hydrochlorothiazide is a diuretic and antihypertensive. It is the 3,4-dihydro derivative of chlorothiazide. Its chemical name is 6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide1,1-dioxide.

Molecular formula-$C_7H_8ClN_3O_4S_2$

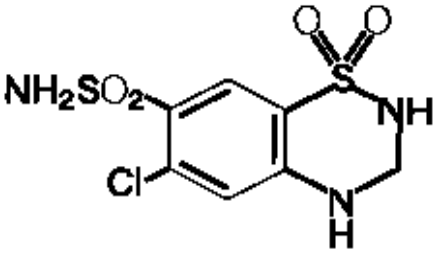


Figure 3: Hydrochlorthiazide.

Hydrochlorothiazide is a white, or practically white, crystalline powder with a molecular weight of 297.74, which is slightly soluble in water, but freely soluble in sodium hydroxide solution, sparingly soluble in ethanol, methanol. [3,4]

MATERIAL AND METHOD

Instrument: UV visible double beam spectrophotometer, JASCO UV- V-550 with matched pair quartz cells corresponding to 1 cm path length.

Chemicals: All chemicals and reagents used were of analytical grade and purchased from Merck Chemicals, India.

Preparation of stock solution: VAL (10mg), AML (Amlodipine besylate equivalent to 10 mg of amlodipine) and HCZ (10 mg) were accurately weighed and transferred to three separate 100 ml amber coloured volumetric flasks and dissolved in methanol: water (70:30) to obtain stock solution of concentration 100 µg/ml each. [11-12]

Selection of λ max: From these stock solutions, working standard solutions were prepared by appropriate dilution of solvent to get final concentration of 10 µg/ml each and were scanned in UV range (Figure 4). The first derivative spectra of VAL, AML and HCZ were showed λ max at 245, 265 and 279 nm respectively (Figure 5).

Figure 4 Overlain spectra of VAL, AML and HCZ (10µg/ml)

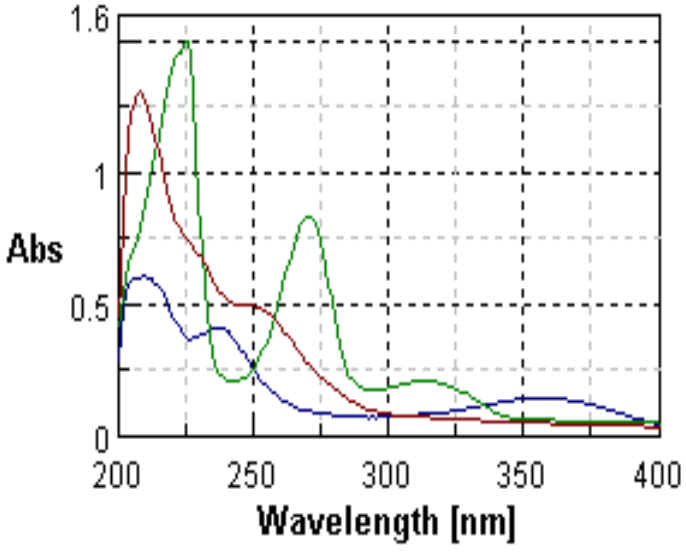
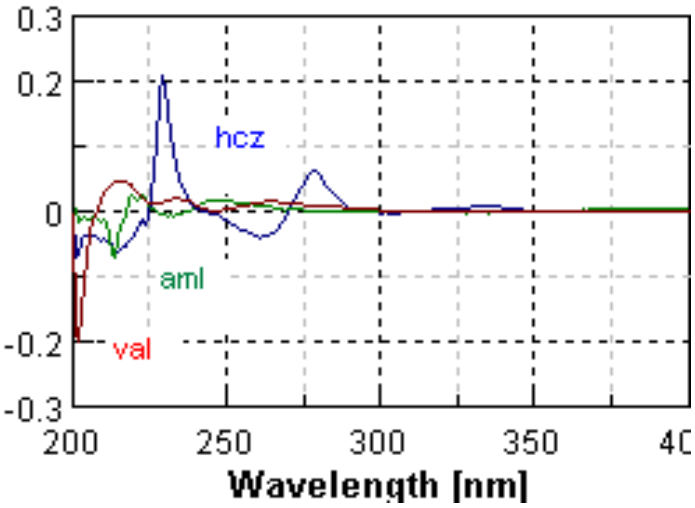


Figure 5 Overlain first order derivative spectra of VAL, AML and HCZ (10µg/ml)



Preparation of sample solution: Marketed tablet formulation containing valsartan 80 mg, amlodipine besylate equivalent to amlodipine base 5mg and hydrochlorothiazide 12.5 mg was analysed using this method. From the triturate of 5 tablets, an amount equivalent to 80 mg of valsartan, 5 mg of amlodipine base and 12.5 mg hydrochlorothiazide was weighed and dissolved in 35 ml of methanol and sonicated for 30 minutes. After 30 min. Sonication the solution was filtered in a 100ml calibrated volumetric flask through whatmann filter paper no.41. The residue was washed three times with 5 ml methanol and then final volume of the solution was made up to 20 ml methanol and 30 ml double distilled water to get a stock solution containing 800 µg/ml VAL, 50 µg/ml AML and 125 µg/ml HCZ. The absorbances of standard and sample solutions were measured at 245,265 and 279 nm using solvent blank. The results were calculated by the formula. [2, 13]

Validation of method: The method was validated in terms of linearity, accuracy, precision and specificity of the sample applications. The linearity of the method was investigated by serially diluting the stock solutions and measured the absorbance at 245, 265 and 279 nm. Calibration curves were constructed by plotting the absorbance against the concentration. All the drugs show linearity in the concentration range as given in table 1 with correlation coefficient of 0.9998.

Table 1: **Composition of the training set of standard synthetic mixtures of three drugs**

Dilutions	VAL(µg/ml)	AML(µg/ml)	HCZ(µg/ml)
1	08.00	0.5	1.25
2	16.00	1.0	2.5
3	32.00	2.0	5.0
4	48.00	3.0	7.5
5	64.00	4.0	10.0
6	80.00	5.0	12.5

Recovery studies were carried out to study the accuracy of the proposed method and ascertained by standard addition method. A known amount of drug was added to pre-analysed tablet sample, at three levels and the percentage recoveries were calculated. Good precision was found with % RSD less than 2. Ruggedness of the proposed method is determined by analysis slot by different analysts using similar operational and environmental conditions (Table 2).

Table 2: **Percent Recoveries of VAL, AML and HCZ.**

Component	100%		80%		120%	
	Mean ±SD	% R.S.D	Mean ±SD	% R.S.D	Mean ±SD	% R.S.D
VAL	102.58 ±2.87	0.1409	80.82 ±1.79	0.1243	120.10 ±2.31	1.2014
AML	98.58 ±1.09	0.1829	80.54 ±1.24	1.1603	120.17 ±1.65	0.159
HCZ	100.06 ± 1.01	0.1257	80.10 ±1.39	0.103	121.05 ± 1.93	1.2164

Specificity study was performed by keeping the sample under various stressed conditions as at 60^{o} by adding 1 ml of 0.1N HCl, exposing with UV light at 245, 265 and279 nm (Table 3).

Table 3: **Result of Analysis:**

Parameters	VAL	AML	HCZ
Beer´s law limit (µg/ml	8-80	1-10	2-20
Correlation coefficient	0.9994	0.9996	0.9998
Regression equation (Y*)			
Slope (B)	0.0425	0.0151	0.0102
Intercept (A)	0.0059	0.0001	0.0012
Limit of Detection(LOD)	0.461µg/ml	0.19µg/ml	0.125 µg/ml
Limit of Quantification(LOQ)	1.291µg/ml	0.63µg/ml	0.417µg/ml
Accuracy	102.58 ±2.87	98.58 ±1.09	100.06 ± 1.01

Y=A+B*C, where C is the concentration in µg/ml and Y is absorbance unit

Results and Discussions:

Tablets were analyzed and amount of drug determined by proposed method was in good agreement with the labelled claim. The results of the marketed formulations were found to be 102.58±0.1409, 98.58 ±0.1829 and 100.06±0.0185 for VAL, AML and HCZ respectively. The proposed method was validated as per the ICH guidelines. Linearity was determined at different concentration, VAL AML and HCZ were showed linearity in the concentration range of 8-80µg/ml, 1-10µg/ml and 2-20µg/ml with correlation coefficient of 0.9994, 0.9996 and 0.9998 respectively. Limit of detection (LOD) and Limit of quantitation (LOQ) were determined by standard deviation of response and slope of calibration curve. LOD and LOQ were found to be 0.461, 1.291 for VAL, 0.19, 0.63 for AML and 0.125, 0.417 for HCZ respectively.

System reproducibility was determined by three replicate applications and five times measurement of a laboratory mixture at the analytical concentration. The reproducibility of sample was expressed in terms of SD and % RSD. There was no interference from the common excipients present in tablets. The recovery of drug was determined at 80, 100 and 120 % levels. The percent recovery was found at 100.053-102.58 for VAL, 98.35-98.58 for AML and 99.63-100.06 for HCZ indicating that method has required accuracy. Ruggedness was performed under three different conditions different days, different analysts and intraday. The results i.e. % RSD <2 % signifies the precision of the method. The proposed method for simultaneous estimation of VAL, AML and HCZ in combined dosage form was found to be simple, accurate and rapid. It can be employed for estimation of pharmaceutical formulations in quality control departments.

Conclusions:

The proposed first order derivative spectrophotometric method is simple, reliable and selective providing satisfactory accuracy and precision with lower limits of detection and quantification. Moreover the shorter duration of analysis for valsartan, amlodipine and hydrochlorothiazide make this method useful for routine quantitative analysis in pharmaceutical dosage forms.

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