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Research Article

ABSORPTION CORRECTION METHOD FOR THE SIMULTANEOUS ESTIMATION OF AMLODIPINE BESYLATE, VALSARTAN AND HYDROCHLOROTHIAZIDE IN BULK AND IN COMBINED TABLET DOSAGE FORM

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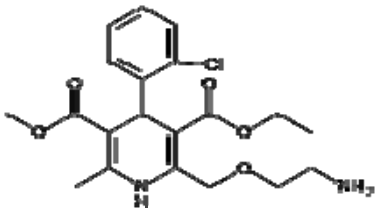
ABSTRACT

A new, simple, accurate and sensitive UV - spectrophotometric absorption correction method has been developed for simultaneous determination of amlodipine besylate, valsartan and hydrochlorothiazide in bulk and in combined tablet dosage form. Methanol and distilled water were used as solvents. The wavelengths selected for the analysis were 365 nm, 250 nm and 315 nm for amlodipine besylate, valsartan and hydrochlorothiazide, respectively. Beer's law obeyed the concentration range of 1 – 32 µg/ ml, 4 – 40 µg/ ml and 2 – 20 µg/ ml for amlodipine besylate, valsartan and hydrochlorothiazide, respectively. The percentage recovery was found in the range of 100.42 - 101.27% for amlodipine besylate, 100.51 - 101.40% for valsartan and 99.25 - 100.35% for hydrochlorothiazide. The developed method was validated statistically and by recovery studies. The % RSD value was found to be less than 2. Thus the proposed method was simple, precise, economic, rapid and accurate and can be successfully applied for simultaneous determination of amlodipine besylate, valsartan and hydrochlorothiazide in bulk and in combined tablet dosage form.

Keywords: Amlodipine Besylate, Valsartan, hydrochlorothiazide, Absorption correction method, ICH guidelines.

INTRODUCTION

Amlodipine besylate (AMB), 2 - [(2 - amino ethoxy) - methyl] - 4 - (2 – chloro phenyl) -1, 4 -dihydro - 6 - methyl - 3, 5 - pyridine di-carboxylic acid 3 - ethyl - 5 - methyl ester, benzene sulfonate (Fig. 1), is a potent dihydro calcium channel blocker[1]. Various analytical methods have been reported for the assay of AMB alone or in combination with other anti - hypertensive agents in pharmaceutical formulations. They include UV spectroscopy[2-4], high performance liquid chromatography[5-8], high performance thin layer chromatography[9, 10], LC - MS[11] and LC - MS/ MS[12].



$C_6H_6O_3S$

Fig. 1: Chemical structure of Amlodipine besylate.

Valsartan (VAL) chemically, N - (1 - oxopentyl) - N - [(2' - (1H - tetrazol - 5 - yl) (1, 1' - biphenyl) - 4 - yl) methyl] - L – valine (Fig. 2), is a potent angiotensin receptor blocker[13, 14]. Methods such as HPLC[15-17], LC - MS[18-20], in plasma[21], Capillary electrophoresis[22] and simultaneous UV spectrophotometric methods[23, 24] are reported for estimation of VAL alone or in combination with other drugs.

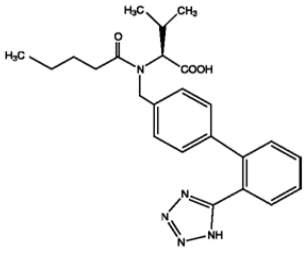


Fig. 2: Chemical structure of Valsartan.

Hydrochlorothiazide (HCT), 6 - chloro - 3, 4 - dihydro - 7 - sulfamoyl - 2H - 1, 2, 4 - benzothia - diazine - 1, 1 – dioxide (Fig. 3), is a thiazide diuretic[25]. It increases sodium and chloride excretion in distilled convoluted tubule. Many analytical methods for HCT alone or in combination with other drugs including spectroscopic and chromatographic methods are also reported in literature[26 - 31].

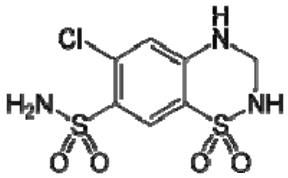


Fig. 3: Chemical structure of Hydrochlorothiazide.

All the three drugs are official in USP[32]. Amlodipine besylate and Hydrochlorothiazide are official in IP[33] and BP[34].

Literature survey revealed that there are several methods were reported for the estimation of AMB, VAL and HCT individually as well as in combination with some other drugs. As no method is reported for AMB, VAL and HCT in combination, the aim of the present study was to develop accurate, precise and sensitive method for the simultaneous UV spectrophotometric estimation of AMB, VAL and HCT in bulk and in combined tablet dosage form by absorption correction method. For this purpose marketed tablets Exforge HCT containing 10 mg of AMB, 160 mg of VAL and 25 mg of HCT was used.

MATERIALS AND METHODS

Instrumentation

The present work was carried out on Shimadzu - 1700 double beam UV - Visible spectrophotometer with pair of 10 mm matched quartz cells. Glassware's used were of 'A' grade and were soaked overnight in a mixture of chromic acid and sulphuric acid, rinsed thoroughly with double distilled water and dried in hot air oven.

Reagent and chemicals

Pharmaceutically pure sample of AMB, VAL and HCT were obtained as a gift samples from Caplin point, Chennai. All solvents were of AR grade obtained from Qualigens India Pvt. Limited, Mumbai. A

combination of AMB (10 mg), VAL (160 mg) and HCT (25 mg) in tablet formulation was procured from U.S market (Exforge HCT, Novartis pharmaceutical corporation, Switzerland).

Experimental condition

According to the solubility characteristics, the common solvent for the three drugs was found to be methanol. Hence the stock solution was prepared in methanol and further dilutions were made up with distilled water.

Preparation of standard stock solution

50 mg of AMB, 40 mg of VAL and 20 mg of HCT were accurately weighed and transferred in to 50 ml volumetric flasks separately. Dissolved in methanol and made up to the volume to 50 ml with the same. These solutions were observed to contain 1000 µg/ ml, 800 µg/ ml and 400 µg/ ml of AMB, VAL and HCT, respectively.

Study of spectral and linearity characteristics

The standard stock solutions of AMB, VAL and HCT were further diluted with distilled water to get the concentration of 10 µg/ ml of each and the solutions were scanned between the range 200 - 400 nm in 1cm cell against distilled water as blank and the overlain spectra was recorded.

From the overlain spectrum of AMB, VAL and HCT in methanol followed by distilled water, it was observed that VAL and HCT have zero absorbance at 365 nm, where as AMB has substantial absorbance. Thus AMB was estimated directly at 365 nm without interference of VAL and HCT. At 315 nm, VAL has zero absorbance. For estimation of HCT, the absorbance of AMB was measured at 315 nm using standard solution of AMB (10 µg/ ml). The contribution of AMB was deducted from the total absorbance of sample mixture at 315 nm. The calculated absorbance was called as corrected absorbance for HCT. At 250 nm, these three drugs were showed the absorbance. To estimate the amount of VAL, the absorbance of AMB and HCT were corrected for interference at 250 nm by using absorptivity values. A set of three equations were framed using absorptivity coefficients at selected wavelengths.

$$c_x = \frac{A_1}{a_{x1}}$$

$$c_y = \frac{A_2 - a_{x2}\, c_x}{a_{y2}}$$

$$c_z = \frac{A_2 - (a_{x2}\, c_x + a_{y3} c_y)}{a_{z3}}$$

Where,

A_1, A_2 and A_3 are absorbance of sample solution at 365 nm, 315 nm and 250 nm, respectively.

a_{x1}, a_{x2} and a_{x3}, absorptivity coefficients of AMB at 365 nm, 315 nm and 250 nm, respectively.

a_{y2} and a_{y3}, absorptivity coefficients of HCT at 315 nm and 250 nm, respectively.
a_{z3}, absorptivity coefficient of VAL at 250 nm.

c_x, c_y and c_z are concentrations of AMB, VAL and HCT, respectively in mixture.

The aliquot portions of standard stock solution of AMB, VAL and HCT were transferred into 100 ml volumetric flasks individually and made up to the volume with distilled water. The absorbance of different concentration solutions were measured at 365 nm, 315 nm and 250 nm for AMB, 315 nm and 250 nm for HCT and 250 nm for VAL. The calibration curves for AMB, VAL and HCT were prepared in the concentration range of 1 - 32 µg/ ml, 4 - 40 µg/ ml and 2 - 20 µg/ ml, respectively at their respective wavelengths by diluting aliquot portions of standard stock solution of each drug.

Analysis of synthetic mixture of AMB, VAL and HCT

Different mixtures of the three drugs were prepared by transferring different volumes of AMB, VAL and HCT from standard stock solutions into 100 ml volumetric flasks and diluting to volume with distilled water. The concentrations of all the three drugs AMB, VAL and HCT were determined by measuring the absorbance of the prepared mixtures at 365 nm, 315 nm and 250 nm. From these absorbance values, the concentrations of AMB, VAL and HCT were determined using absorbance correction method.

Analysis of tablet formulation

Twenty tablets were weighed and average weight was found. The tablets were triturated to a fine powder. An accurately weighed quantity of powder equivalent to 40 mg of VAL was transferred in to 50 ml volumetric flask and added a minimum quantity of methanol to dissolve the substance and made up to the volume with the same. The solution was sonicated for 15 minutes, centrifuged for another 15 minutes at 100 rpm and filtered through Whatmann filter paper No. 41. From the clear solution, further dilutions were made by diluting 4.0 ml into 100 ml with distilled water to obtain 32 µg/ ml solution of VAL which is also contains 2 µg/ ml of AMB and 5 µg/ ml of HCT theoretically. The absorbance of sample solution was measured at all selected wavelengths. The content of AMB, VAL and HCT in sample solution of tablet was calculated. This procedure was repeated for six times.

Validation of methods

The methods were validated with respects to linearity, LOD (Limit of detection), LOQ (Limit of quantitation), precision, accuracy and ruggedness[35].

Linearity

Linearity was checked by diluting standard stock solution at six different concentrations. AMB was linear with the concentration range of 1 – 32 µg/ ml at 365 nm, 315 nm and 250 nm. VAL showed the linearity in the range of 4 – 40 µg/ ml at 250 nm. HCT was linear in the concentration range of 2 – 20 µg/ ml at 315 nm and 250 nm and Calibration curves (n = 6) were plotted between concentration and absorbance of drugs. Optical parameters were calculated.

Sensitivity

The limit of detection (LOD) and limit of quantitation (LOQ) parameters were calculated using the following equations; LOD = 3.3σ/ s and LOQ = 10σ/ s, where σ is standard deviation of y intercept of calibration curve (n = 6) and s is slope of regression equation.

Precision

The precision of the method was confirmed by repeatability and intermediate precision. The repeatability was performed by the analysis of formulation was repeated for six times with the same concentration. The amount of each drug present in the tablet formulation was calculated. The % RSD was calculated. The intermediate precision of the method was confirmed by intraday and inter day analysis i.e. the analysis of formulation was repeated three times in the same day and on three successive days. The amount of drugs was determined and % RSD also calculated.

Accuracy

To check the accuracy of the developed method and to study the interference of formulation excipients, analytical recovery experiments were carried out by using standard addition method in three different concentrations. From the total amount of drug found, the percentage recovery was calculated. This procedure was repeated for three times for each concentration. The % RSD was calculated.

Ruggedness

The ruggedness test of analytical assay method is defined as the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of normal test conditions such as different labs, different analysis, different lots of reagents etc. Ruggedness is a measure of reproducibility of test results under normal expected operational conditions from laboratory to laboratory and from analyst to analyst. In present study, determination of AMB, VAL and HCT were carried out by using different instruments and different analysts.

RESULTS AND DISCUSSION

An attempt has been made to develop a rapid, sensitive, economic, precise and accurate analytical method for simultaneous estimation of AMB, VAL and HCT in pure and in combined tablet dosage form. The proposed method is based on spectrophotometric absorption correction method for the simultaneous estimation of AMB, VAL and HCT in UV region using methanol and distilled water as solvents. The overlain spectra of AMB, VAL and HCT are shown in Fig. 4.

The method is based upon direct estimation of AMB at 365 nm, as at this wavelength HCT and VAL have zero absorbance and shows no interference. For estimation of HCT, corrected absorbance was calculated at 315 nm due to the interference of AMB and VAL has zero absorbance at this wavelength. At 250 nm, these three drugs were showed absorbance. To estimate the amount of VAL, the absorbance of AMB and HCT were corrected for interference at 250 nm by using their absorptivity values. The stability was performed by measuring the absorbance of same solution at different time intervals. It was observed that AMB, VAL and HCT were stable for up to 4 hours at their respective wavelengths.

Beer's law obeyed in the concentration range of 1 - 32 µg/ ml at 365 nm, 315 nm and 250 nm, 4 - 40 µg/ ml at 250 nm and 2 - 20 µg/ ml at 315 nm and 250 nm for AMB, VAL and HCT, respectively. The correlation coefficient values were found above 0.999, which shows that absorbance of all the drugs was linear with concentration. The optical characteristics such as Beer's law limits, correlation coefficient, slope, intercept, Sandell's sensitivity and molar absorptivity were calculated and are summarized in Table 1.

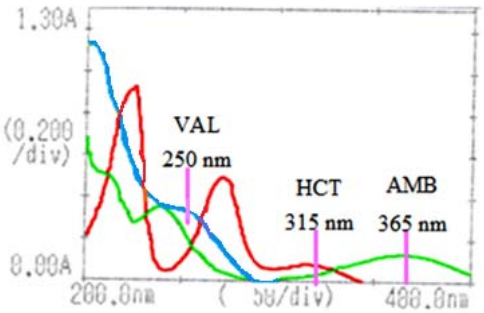


Fig. 4: Overlain UV spectra of AMB, VAL and HCT (10 µg/ ml)

Table 1: Spectral and linearity characteristics data.

Parameters		AMB*	VAL*	HCT*
λ_{max} (nm)		365 nm	250 nm	315 nm
Linearity range (µg/ ml)		1 - 32	4 - 40	2 - 20
Correlation coefficient (r^2)		0.99990	0.99971	0.99985
Molar absorptivity ($L\ mol^{-1}\ cm^{-1}$)		6807.54	14086.53	3325.88
Sandell's sensitivity (µg/ cm^2/ 0.001 A.U)		0.060549	0.030528	0.089152
Slope (m)		0.016511	0.03276	0.011226
Intercept (c)		0.001330	0.004158	0.000556
Regression equation	(y = mx + c)	y = 0.016511x + 0.001330	y = 0.03276x + 0.004158	y = 0.011226x + 0.000556
LOD (µg/ ml)		0.1798	0.2953	0.2460
LOQ (µg/ ml)		0.5451	0.8949	0.7467
Standard Error		0.000409	0.002055	0.000252

* Mean of six observations.

The LOD and LOQ were found to be 0.1798 and 0.5451, 0.2953 and 0.8949, 0.2460 and 0.7467 for AMB, VAL and HCT, respectively. The low values indicated that the sensitivity of the method.

To study the mutual interference, if any, in the simultaneous estimation of AMB, VAL and HCT in synthetic mixture containing various proportions of AMB, VAL and HCT were prepared and the contents were estimated by proposed method. The % recovery varied from 99.59 to 101.12 for AMB, 99.78 to 101.22 for VAL and 99.69 to 101.85 for HCT indicating that there is no mutual interference between these three drugs. The result of analysis of synthetic mixture is shown in Table 2.

The percentage label claim present in tablet formulation was found to be 99.36 ± 1.9111, 99.53 ± 0.3543, 101.72 ± 1.3547 for AMB, VAL and HCT, respectively. Precision of the method was confirmed by the repeated analysis of formulation for six times. The % RSD values were found to be 1.9234, 0.3559 and 1.3318 for AMB, VAL and HCT, respectively. The low % RSD values indicated that all the three drugs showed good agreement with the label claim ensures the precision of the method (Table 3).

Further, the precision of the method was confirmed by Intraday and Inter day analysis. The % RSD values for intraday and inter day analysis was found to be 0.4078 and 1.5058 for AMB, 0.1140 and 0.3459 for VAL and 0.5056 and 1.7086 for HCT, respectively. Hence the precision of the method was further confirmed.

The developed method was validated for Ruggedness. The analysis of formulation was done by using different instruments and different analysts. The % RSD values were found to be less than 2 indicating that the method was more rugged. The results of analysis of intermediate precision and ruggedness are shown in Table 4.

Table 2: Results of analysis of synthetic mixtures

Conc. of AMB (µg/ ml)		% recovery	Conc. of VAL (µg/ ml)		% recovery	Conc. of HCT (µg/ ml)		% recovery
Theoretical	Experimental		Theoretical	Experimental		Theoretical	Experimental	
1	1.0112	101.12	16	15.9648	99.78	2.5	2.5463	101.85
2	2.0134	100.67	18	17.9832	99.91	5	4.9843	99.69
3	2.9876	99.59	20	20.0190	100.10	7.5	7.5219	100.29
4	3.9945	99.86	22	22.0467	100.21	10	9.9757	99.76
5	5.0189	100.38	24	24.2938	101.22	12.5	12.5631	100.50

Table 3: Results of analysis of tablet formulation

Parameters	AMB	VAL	HCT
Labeled	10	160	25
Claim (mg)	99.36	99.53	101.72
% Assay*	1.9111	0.3543	1.3547
SD	1.9234	0.3559	1.3318
% RSD			

* Mean of six determinations.

Table 4: Intermediate Precision and Ruggedness of the method

Parameters	% Label claim estimated (Mean ± %R.S.D.)		
	AMB	VAL	HCT
Intraday Precision (n=3)	101.60 ± 0.4078	99.62 ± 0.1140	102.22 ± 0.5056
Inter day Precision (n=3)	101.03 ±1.5058	99.31 ± 0.3459	100.73 ± 1.7086
Different instruments (n=6)			
Instrument I	100.74 ± 1.7280	101.12 ± 1.8160	99.26 ± 0.1792
Instrument II	99.58 ± 0.6867	99.81 ± 0.1161	102.36 ± 1.5743
Different analysts (n=6)			
Analyst I	98.15 ± 0.7864	102.32 ± 0.3606	99.80 ± 0.2469
Analyst II	101.47 ± 1.3209	99.31 ± 0.3130	101.88 ± 0.4397

In order to check the accuracy of the developed method, known quantities of standard drugs of AMB, VAL and HCT in three different concentrations were added to its preanalysed sample and analysed by the developed method. The percentage recovery was found to be in the range of 100.42 - 101.27% for AMB, 100.51 - 101.40% for VAL and 99.25 - 100.35% for HCT. The results of recovery studies are shown in Table 5. The % RSD values for AMB, VAL and HCT were found to be 0.4845, 0.4464 and 0.0551, respectively. The low % RSD values confirm that there is no interference due to the excipients used in formulation. This ensures the accuracy of the method.

Table 5: Recovery studies

Drug	Amount present (µg/ ml)	Amount added (µg/ ml)	Amount found* (µg/ ml)	Amount recovered (µg/ ml)	% Recovery*	S.D	% R.S.D
AMB	1.9872	1.9906	4.0030	2.0158	101.27		
	1.9872	3.9931	5.9954	4.0082	100.43	0.4879	0.4845
	1.9872	5.9896	8.0021	6.0149	100.42		
VAL	31.8496	1.1296	32.9917	1.1421	101.40		
	31.8496	2.0865	33.9588	2.1092	101.08	0.4508	0.4464
	31.8496	3.0587	34.9240	3.0744	100.51		
HCT	5.0068	2.0068	7.0224	2.0156	100.35		
	5.0068	3.9957	8.9933	3.9865	99.77	0.5503	0.0551
	5.0068	6.0025	10.9641	5.9573	99.25		

*Mean of three observations

From validation, the developed method was found to be simple, rapid, economical, precise, accurate and rugged. Hence the proposed method could be effectively applied for the routine analysis of AMB, VAL and HCT in bulk and in combined tablet dosage form.

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