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Research Article

ABSORPTION CORRECTION METHOD FOR 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. The stock solutions were prepared in methanol followed by the further required dilutions with distilled water. The method is based upon direct estimation of amlodipine besylate at 365 nm, as at this wavelength hydrochlorothiazide and valsartan have zero absorbance and shows no interference. For estimation of hydrochlorothiazide, corrected absorbance was calculated at 315 nm due to the interference of amlodipine besylate and valsartan has zero absorbance at this wavelength. At 250 nm, these three drugs were showed absorbance. To estimate the amount of valsartan, the absorbance of amlodipine besylate and hydrochlorothiazide were corrected for interference at 250 nm by using absorptive values. Beer's law obeyed the concentration range of 1 – 32 mcg/ mL, 4 – 40 mcg/ mL and 2 – 20 mcg/ mL for amlodipine besylate, valsartan and hydrochlorothiazide, respectively. The developed method was validated according to ICH guidelines and it found to be accurate and precise. Thus the proposed method 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, Simultaneous equation method, Method validation.

INTRODUCTION

Amlodipine besylate (AMB), 2 - [(2 - amino ethoxy) - methyl] - 4 - (2 -chlorophenyl) -1, 4 -dihydro - 6 - methyl - 3, 5 - pyridine dicarboxylic acid 3 - ethyl - 5 - methyl ester, benzosulfonate, is a potent dihydro calcium channel blocker[1]. Various analytical methods have been reported for the assay of Amlodipine besylate 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 LCMS/ MS[12]. Valsartan (VAL) chemically, N - (1 - oxopentyl) - N - [(2' - (1H - tetrazol - 5 - yl) (1, 1' - biphenyl) - 4 - yl) methyl] - L - valine, 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 agents. Hydrochlorothiazide (HCT), 6 - chloro - 3, 4 - dihydro - 7 - sulfamoyl - 2H - 1, 2, 4 - benzothia - diazine - 1, 1 - dioxide, 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].

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. 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 in each procedure 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 and Loba Chemie India Limited. 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 of drugs the common solvent for solubility was found to be methanol and further dilutions were made up with distilled water were selected as solvents for analysis.

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 mcg/ mL, 800 mcg/ mL and 400 mcg/ 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 mcg/ 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 (Fig.4), 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 absorptive values. A set of three equations were formed using absorptive coefficients at selected wavelengths.

At λ_1

$A_1 = a_{x1}bc_x$

At λ_2

$A_2 = a_{x2}bc_x + a_{y2}bc_y$

At λ_3

$A_3 = a_{x3}bc_x + a_{y3}bc_y + a_{z3}bc_z$

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}, absorptive coefficients of AMB at 365 nm, 315 nm and 250 nm, respectively; a_{y2} and a_{y3}, absorptive coefficients of HCT at 315 nm and 250 nm, respectively; a_{z3}, absorptive coefficient of VAL at 250 nm; c_x, c_y and c_z are concentrations of AMB, VAL and HCT, respectively in mixture.

The standard stock solutions of Amlodipine besylate (0.1 - 3.2 mL), Valsartan and Hydrochlorothiazide (0.5 - 5 mL) 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 mcg/ mL, 4 - 40 mcg/ mL and 2 - 20 mcg/ mL, respectively at their respective wavelengths by diluting aliquot portions of standard stock solution of each drug.

The correction equations were constructed as follows by using absorptive coefficient values.

At 365 nm

$A_1 = 167.80\ c_x$

At 315 nm

$A_2 = 32.92\ c_x + 111.12\ c_y$

At 250 nm

$A_3 = 318.66\ c_x + 119.09\ c_y + 323.99\ c_z$

Analysis of synthetic mixture of AMB, HCT and VAL

Different mixtures of the three drugs were prepared by transferring different volumes of AMB, HCT and VAL from standard stock solutions into 100 mL volumetric flasks and diluting to volume with distilled water. The concentrations of all the three drugs AMB, HCT and VAL 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, HCT and VAL 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 mcg/ mL solution of VAL which is also contains 2 mcg/ mL of Amlodipine and 5 mcg/mL of Hydrochlorothiazide 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.

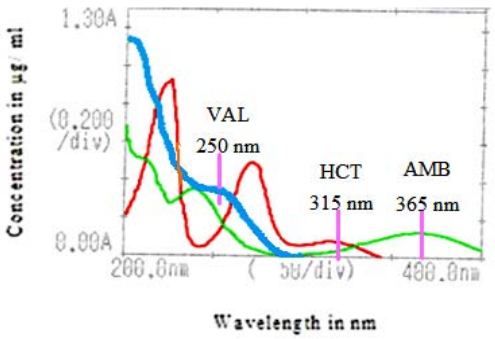

Fig. 1: Overlain UV spectra of AMB, HCT and VAL (10 mcg/ mL).

Validation of methods

The methods were validated with respects to linearity, LOD (Limit of detection), LOQ (Limit of quantization), precision, accuracy and ruggedness[35, 36].

Linearity

Linearity was checked by diluting standard stock solution at six different concentrations. Amlodipine was linear with the concentration range of 1 – 32 µg/ ml at 365 nm, 315 nm and 250 nm. Hydrochlorothiazide was linear in the concentration range of 2 – 20 µg/ ml at 315 nm and 250 nm and Valsartan showed the linearity in the range of 4 – 40 µg/ ml at 250 nm. 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 quantization (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 repeatability of the method was confirmed by the analysis of formulation was repeated for 6 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 methods 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.

Table 1: Spectral and linearity characteristics data.

Parameters	AMB*	HCT*	VAL*
λ_{max} (nm)	365 nm	315 nm	250 nm
Linearity range (mcg/ mL)	1 - 32	2 - 20	4 - 40
Correlation coefficient (r^2)	0.99990	0.99985	0.99971
Molar absorptivity ($L\ mol^{-1}\ cm^{-1}$)	6807.54	3325.88	14086.53
Sand ell's sensitivity ($\mu g/ cm^2/ 0.001$ A.U)	0.060549	0.089152	0.030528
Slope (m)	0.016511	0.011226	0.03276
Intercept (c)	0.001330	0.000556	0.004158
Regression equation (y = mx + c)	y = 0.016511x + 0.001330	y = 0.011226x + 0.000556	y = 0.03276x + 0.004158
LOD (mcg/ mL)	0.179881	0.246005	0.295343
LOQ (mcg/ mL)	0.545096	0.746684	0.894981
Standard Error	0.000409	0.000252	0.002055

* Mean of six observations.

RESULTS AND DISCUSSION

An attempt has been made to develop a fast, sensitive, precise, reproducible and economical analytical method for simultaneous estimation of AMB, VAL and HCT in their combined dosage form. The proposed method is based on spectrophotometric absorbance correction method for the estimation of AMB, VAL and HCT in UV region using methanol and distilled water as solvent. The overlain spectral analysis involves the estimation of AMB, HCT and VAL using 365 nm, 315 nm and 250 nm, respectively (Fig.1).

This method involves the construction and solving of three equations using absorptive coefficient 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 mcg/ mL at 365 nm, 315 nm and 250 nm, 4 - 40 mcg/ mL at 315 nm and 250 nm and 2 - 20 mcg/ mL at 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 values, Sand ell's sensitivity and molar absorptive values were calculated and are summarized in Table I.

The LOD and LOQ were found to be 0.0989 and 0.2999, 0.2953 and 0.8950, 0.1932 and 0.5855 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, HCT and VAL in synthetic mixture containing various proportions of AMB, HCT and VAL were prepared and the contents were estimated by proposed method. The % recovery varied from 99.59 to 101.12 for AMB, 99.69 to 101.85 for HCT and 99.78 to 101.22 for VAL indicating that no mutual interference up to the ratio of 5 : 12.5 : 24 for all the drugs. The result of analysis of synthetic mixture is shown in Table II.

Table 2: Results of analysis of synthetic mixtures

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

Table 3: Results of analysis of tablet formulation

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

* Mean of six determinations.

Table 4: Intermediate precision and ruggedness of the method

Parameters	% Label claim estimated* (Mean ± %R.S.D.)		
	AMB	HCT	VAL
Intraday Precision (n=3)	97.89 ± 1.1232	99.57 ± 0.3524	103.31 ± 1.0467
Inter day Precision (n=3)	102.91 ±1.7541	98.84 ± 0.3712	102.19 ± 0.1837
Different instruments (n=6)			
Instrument I	101.27 ± 1.8950	99.01 ± 0.2938	101.84 ± 0.4623
Instrument II	99.59 ± 1.9421	99.59 ± 0.1821	102.57 ± 0.0487
Different analysts (n=6)			
Analyst I	103.80 ± 0.6633	99.07 ± 0.2071	102.02 ± 0.1181
Analyst II	102.28 ± 0.7459	99.38 ± 0.1006	103.55 ± 0.3690

Table 5: Recovery studies

Drug	Amount present (mcg/ mL)	Amount added (mcg/ mL)	Amount found* (mcg/ mL)	Amount recovered (mcg/ mL)	% Recovery*	S.D	% R.S.D
AMB	2.0508	1.9979	4.0224	1.9716	98.68		
	2.0508	3.9914	6.0672	4.0164	100.63	1.0981	1.0151
	2.0508	6.0050	7.9826	5.9318	98.78		
HCT	31.4828	1.1297	32.6092	1.1264	99.71		
	31.4828	2.0865	33.5562	2.0734	99.37	0.6742	0.6747
	31.4828	3.0587	34.5621	3.0793	100.67		
VAL	5.0465	1.9967	7.0740	2.0275	101.54		
	5.0465	3.9964	9.0459	3.9994	100.08	1.3129	1.3105
	5.0465	6.0006	10.9823	5.9358	98.92		

*Mean of three observations

The percentage label claim present in tablet formulation was found to be 102.54 ± 1.8929, 99.04 ± 0.2273, 102.93 ± 1.2331 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 1.8461, 0.2295 and 1.1980 for AMB, VAL and HCT, respectively. The low % RSD values indicated that all the three drugs showed good agreement with the label claim. Hence the precision of the method was confirmed. The result of analysis of formulation is shown in Table III.

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 1.1232 and 1.7541 for AMB, 0.3524 and 0.3712 for VAL and 1.0467 and 0.1837 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. Hence the ruggedness of the method was confirmed. The results of analysis of intermediate precision and ruggedness are shown in Table IV.

In order to check the accuracy of the developed method, known quantities of standard drugs of AMB, VAL and HCT in three different concentrations was added to its preanalysed sample and analyzed by the developed method. The percentage recovery was found to be in the range of 98.68 - 100.63% for AMB, 99.37 - 100.67% for VAL and 98.92 - 101.54% for HCT. The results of recovery studies are shown in Table V. The % RSD values for AMB, VAL and HCT were found to be 1.0151, 0.6747 and 1.3105, respectively. The low % RSD value ensures that there is no interference due to excipients used in formulation. Hence the accuracy of the method was confirmed.

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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