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STABILITY INDICATING RP-HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF VALSARTAN AND AMLODIPINE FROM THEIR COMBINATION DRUG PRODUCT

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ABSTRACT: A simple, precise and accurate stability indicating RP-HPLC method has been developed and subsequently validated for simultaneous estimation of Valsartan (VAL) and Amlodipine (AML) from their combination dosage form. A Shimadzu's HPLC (LC-2010-HT, Shimadzu, Singapore) equipped with UV-Visible and Diode Array detectors, with Class-VP software was used. Column used was XTerra® RP18, 5 µm, 150 mm × 4.6 mm i.d., at 25° C. Mobile phase consisted mixture of solution A (1000 mL Water + 0.2 mL Trifluoro Acetic Acid) and solution B (Water: Acetonitrile: Trifluoro Acetic Acid, 400:600:1, v/v/v) with flow rate of 1.5 mL/ min and UV detection was carried out at 237 nm and 265 nm for AML and VAL, respectively. VAL, AML and their combined dosage form were exposed to thermal, photolytic, oxidative, acid-base hydrolytic stress conditions, the stressed samples were analyzed by proposed method. Peak purity results suggested no other co-eluting, interfering peaks from excipients, impurities, or degradation products due to variable stress condition, and the method is specific for the estimation of VAL and AML in presence of their degradation products and impurities. The method was validated with respect to linearity, precision, accuracy, system suitability, and robustness. The described method was linear over the range of 1.6-240 µg/mL and 1-30 µg/mL for VAL and AML, respectively. The mean recoveries were 100.12 and 99.72 % for VAL and AML, respectively. The intermediate precision data were obtained under different experimental conditions and calculated value of the coefficient of variation (CV, %) was found to be less than critical value. The proposed method can be useful in the quality control of pharmaceuticals.

Key words – Valsartan, Amlodipine, RP-HPLC, Stability indicating, Stress condition.

INTRODUCTION

Valsartan (VAL) is a popular Angiotensin II antagonist and chemically it is N-(1-oxopentyl)-N-[[2′-(1H-tetrazol-5-yl)[1,1′-biphenyl]-4-yl]methyl]-L-valine[1]. Amlodipine (AML) is calcium-channel blocker (CCB), and is chemically (3-Ethyl-5-methyl (4RS)-2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3, 5-dicarboxylate benzene sulphonate[1]. The combination of VAL and AML has been shown to be effective in the management of hypertension. The combination was generally more effective than individual drug therapy[2]

Stability testing is an important part of the process of drug product development. The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors, such as temperature, humidity, and light, and enables recommendation of storage conditions, retest periods, and shelf lives to be established. The two main aspects of a drug product that play an important role in shelf life determination are assay of the active drug and degradation products generated during the stability study. The drug product in a stability test sample needs to be

determined using a stability indicating method, as recommended by the International Conference on Harmonization (ICH) guidelines[3] and U.S. Pharmacopia (USP) 26[4]. Although stability indicating methods have been reported for assay of various drugs in drug products, most of them describe assay procedures for drug products containing only one active drug substance. Only few stability indicating methods are reported for assay of combination drug products containing two or more active drug substances. The objective of this work was to develop a simple, precise, and rapid column liquid chromatography (LC) procedure that would serve as stability indicating assay method for combination drug product of VAL and AML.

VAL is official in USP[5], while AML is official in IP[6], BP[7], EP[8] and USP[9] in which HPLC method is describe for both drug in alone. The combination of VAL and AML is not official in any pharmacopoeia.

Literature survey revealed HPLC[10-12], RP-HPLC[13, 14], HPTLC[15, 16], LCMS/MS[17], LC-MS[18] and simultaneous UV Spectrophotometric methods[19, 20] are reported for the estimation of AML alone or in combination with other anti-hypertensive agents. Methods such as HPLC[21-23], LC-MS[24-26], Protein precipitation[27], Capillary electrophoresis[28] and simultaneous UV spectrophotometer methods[29, 30] are reported for estimation of VAL alone or in combination with other agents. stability indicating RP-HPLC assay method for AML alone[31] and in combination with atorvastatine calcium[14] and benazepril hydrochloride[32], respectively are available in literatures but no method is reported for AML and VAL in combination, the aim of the present study was to develop accurate, precise and selective reverse phase HPLC assay procedure for the analysis of AML and VAL in bulk drug samples and in combined dosage formulation.

EXPERIMENTAL

Reagents and Materials:

VAL and AML reference standards were kindly gifted by Torrent Research Centre, Gandhinagar, India, with purity of 98.30% and 99.77%, respectively and were used without further purification for the study. The commercial fixed dose combination product containing 320 mg VAL and 10 mg AML was procured from Torrent Research Centre. Milli-Q water: - (MILLIPORE SAS 67120, France), HPLC grade Acetonitrile (RANKEM, India), AR grades Trifluoro acetic acid (Qualigens fine chemicals, Mumbai), Sodium Hydroxide ("MERCK" Specialties Pvt Ltd, Mumbai), Hydrochloric acid ("RANKEM", RFCL Ltd, New Delhi) and LR grade 30 %, Hydrogen Peroxide (MERCK, India) were used. Water-Acetonitrile in the ratio of 50:50, v/v, was used as diluent.

Instrument and conditions:

Chromatography was performed with a Shimadzu's HPLC (LC-2010-HT, Shimadzu, Singapore) equipped with UV-Visible & Diode Array detectors. The LC separations were performed at 25°C on an XTerra® RP_{18}, 5 µm, 150 mm × 4.6 mm chromatographic column and Class-VP software was used for LC peak integration. The mobile phase was degassed by sonication with an Ultrasonic bath (Transonic Digital s, ELMA). The standard substances were weighed on Analytical balance (Mettler Toledo, AG285, Switzerland) Stability studies were carried out in a Photo stability chamber(SVI equipments, Germany) Mobile phase consisted mixture of solution A (1000 mL water + 0.2 mL TFA) and solution B (water: ACN: TFA, 400:600:1, v/v/v) with flow rate of 1.5 mL/ min and UV detection was carried out at 237 nm and 265 nm for AML and VAL, respectively with injection volume of 10 µL.

Preparation of VAL and AML Standard Stock Solutions:

Standard stock solutions of VAL (1600 µg/mL) and AML (200 µg/mL) were prepared separately in diluent. For calibration curve series of mixed working standard solutions were prepared by transferring (0.5, 0.1, 1, 5, 8, 10, 12 and 15 mL) aliquots of standard stock solution of AML and VAL in 100 mL volumetric flasks and diluted to mark with diluent.

Analysis of the Marketed Formulation:

Twenty tablets were accurately weighed, their mean weight was determined, and were ground to fine powder in a glass mortar. An amount of the powder equivalent to 2 tablets was dissolved in 150 mL of diluent, solution was sonicated for 30 minutes with intermittent shaking and diluted to 200 mL with diluent and mixed. The resulted mixture was filtered through 0.22µ nylon filter; first 5 mL of the filtrate was discarded. From the filtrate 5 mL of aliquot was transferred to 100 mL volumetric flask for VAL and 10 mL aliquot was transferred to 50 mL volumetric flask for AML. After dilution 10 µL of both solutions were injected for chromatographic analysis.

Forced Degradation Studies of Standard Drug Solutions:

In three different round bottom flasks, VAL (80 mg) and in three different round bottom flasks, AML (10 mg) were transferred separately; 10 mL of diluent was added in each flask. The above solutions were kept under acid/base hydrolytic and oxidative stress conditions.

For acid hydrolysis, 10 mL of 1M HCl was added in two different round bottom flask containing VAL and AML. Then solutions were refluxed for 6 h and 20 min for VAL and AML, respectively.

For Base hydrolysis, 10 mL of 1M and 0.1M NaOH were added in the round bottom flask containing VAL and AML, respectively. The resulting solutions were refluxed for 12 h for VAL and 45 min for AML.

For peroxide hydrolysis, 10 mL of 30% H_2O_2 was added in two different round bottom flask containing VAL and AML. Then solutions were refluxed for 2 h and 20 min for VAL and AML, respectively.

The above solutions were diluted to achieve the solutions having final concentration of 160 and 20 µg/mL for VAL and AML, respectively.

Forced Degradation Studies of Tablets:

An amount of the tablet powder equivalent to 2.5 tablets was taken in the series of six different round bottom flasks. In each of the flask 15 mL of diluent was added to dissolve active contents. Then after above six flasks were grouped in three sets each of two.

In two flasks of set I, 10 mL each of 1M and 0.1M HCl were added separately for acid degradation of VAL and AML, respectively.

For base hydrolysis, in two flasks of set II, 10 mL each of 1M and 0.1M NaOH were added separately for VAL and AML, respectively.

Finally, in two flasks of set III, 10 mL each of 30% and 10% H_2O_2 were added separately for VAL and AML, respectively.

After that above acidic, basic and oxidized solutions of VAL were refluxed for 2 h, 12 h and 45 min, respectively and solution of AML for 1 h, 20 min and 15 min, respectively.

The tablet powder were also exposed to thermal stress at 105°C in oven for 72 h and photo stability chamber at 1.2 million lux per h.

After sufficient degradation under acid/base hydrolysis, oxidative, thermal and photo degradation stress condition tablet contents were dissolved and diluted to achieve solutions having final concentration 160 µg/mL for VAL and 20 µg/mL for AML.

RESULTS AND DISCUSSION

Method Development:

Literature survey revealed number of reported methods for VAL and AML alone and with combination of other drug, but no stability indicating method was reported for VAL and AML in combine dosage form.

To develop accurate, precise and specific stability indicating RP-HPLC method for simultaneous estimation of VAL and AML using stressed samples various mobile phase with different composition and flow rate were tried. After number of trial experiments, it was established that water-ACN combination have high eluting power compare to acid, base and other phosphate buffer, and still found suitable for closely eluting degradation products.

Initially different ratio of water - ACN was tried but no elution of VAL was found. Then after 0.01 % TFA in water and ACN were tried in gradient program and elution of both drug was found very late. So in further trial, to decrease retention time of drugs, gradient program with different time programming was used and check for peak purity and resolution.

Finally, optimized mobile consisted of solution A (1000 mL Water +0.2 mL TFA) and solution B (Water: ACN: TFA, 400:600:1, v\v\v) was selected with gradient program for stressed samples as well as standard drug solutions because it was found to ideally resolve the peaks of AML (retention time, t_R = 15.3 ± 0.03 min) and VAL (t_R = 25.8± 0.01 min), can give complete separation of both drugs from their degradation products and impurities at a flow rate of 1.5 mL/min. UV detection at 237 nm and 265 nm for AML and VAL, respectively with injection volume of 10 µL and ambient temperature(25 °C) for the column were found to be best for analysis (Figure 1).

As AML was found to be very sensitive drug compare to VAL, when VAL shows countable degradation at the time AML was found to be degrade completely in most of stress conditions. To improve the accuracy of proposed method, degradation condition for both drugs were achieve separately in bulk drug as well as combined dosage forms to get 10 to 30 % degradation.

Acidic conditions—

For AML, standard solution was kept in 1M HCl for 1 h at room temperature, at 70 °C in water bath and at 100 °C in oven but no degradation was found. After number of trials 13 % degradation was achieved in the solution which was refluxed for 20 min in 1M HCl. The same condition was applied for sample in which 80 % degradation was found, so stress condition was reduced to 0.1M HCl and refluxed for 1 h in which 14% degradation was achieved.

For VAL, initially standard solution was kept in 0.1M HCl for 1 h at 70 °C on water bath, 30 min in reflux but no significant degradation was found. So, again standard solution was kept in 1M HCl and refluxed for 1 h in which only 5 % degradation was found. In next trial, sample was further refluxed for 6 h in which 10 % degradation was found. The same condition was applied to sample solution in that degradation was very high so time was reduced to 2 h in which 11 % degradation was achieved. The results are shown in Figure 2.

Alkali conditions.—

For AML, initially standard solution was kept in 0.1M NaOH for 1 h at room temperature, 70 °C in water bath and at 100°C in oven but no sufficient degradation was found. So again standard solution was refluxed in 0.1M NaOH for 45 min in which 16% degradation was achieved for standard solution. Then same condition was applied for sample solution but AML show remarkable degradation so time was reduced to 20 min and 30% degradation was achieved.

For VAL, initially standard solution was kept in 1M NaOH for 1 h at 70 °C on water bath and under refluxed condition for 2 h but no degradation was found. So again solution was further refluxed for 6, 12, 24 h in which no degradation was found that indicate VAL was stable in alkali same is true for sample solution. (Figure 3)

Oxidation conditions.—

For AML, initially standard solution was kept in 10 % H_2O_2 for 1 h at room temperature, 30 min in oven and on reflux for 30 min but no sufficient degradation was found. So again solution was refluxed in 30 % H_2O_2 for 20 min in which 28 % degradation was achieved. Then same condition was applied for sample solution but AML

show remarkable degradation so, concentration of H_2O_2 was reduced to 10 in which 25 % degradation was found. For VAL, initially sample solution was kept in 30 % H_2O_2 for 1 hr at 70 °C on water bath, 1 h at 100°C in oven and refluxed for 30 min but and no degradation was found so again solution was kept in 30 % H_2O_2 and refluxed for 2 h in which 13 % degradation was found. The sample solution was also kept under same condition in that VAL shows high degradation so time was reduced to 45 min in which 13 % degradation was achieved (Figure 4).
Both thermal and photo degradation were performed and no degradation for both sample was found (Figure 5, 6).
The % degradation in stress condition for sample and standard with peak purity results were given in Table 1.

Method Validation:
The described method has been validated, in addition to its specificity, for linearity, system suitability, accuracy, and intermediate precision. The standard solutions for linearity were prepared seven times at different concentration levels. The limit of detection (LOD) and the limit of quantification (LOQ) were determinated according to a signal/noise ratio (S/N) of 3:1 and 10:1, respectively. Characteristic parameters for the regression equation and system suitability are given in Table 2. Repeatability of measurements of peak area evaluated using 7 replicates of VAL (160 □g/mL) and AML (20 □g/mL).The intra- and inter-day variation for the determination of VAL and AML were evaluated at 3 different concentration levels (1.6, 160, and 240 □g/mL for VAL and 1, 20 and 30 □g/mL for AML). The low coefficient of variation (CV) values of within-day and day-to-day variations for VAL and AML revealed that the proposed method is precise (Table 3). Accuracy of method was checked by a recovery study using the placebo addition method at 3 different concentration levels, i.e., a multilevel recovery study. The standards of for both drugs were spiked with 50, 100, and 150% with placebo, and the mixtures were analyzed by proposed method. Results of the recovery study are shown in Table 4. The method was found to be robust even with small variations in flow rate (±0.5 mL/min) and concentration of acetontrile (±5%) in the mobile phase as there was no significant difference in peak area and retention time.

Applicability of the Developed Method to Marketed Formulations:
The assay results of VAL and AML in tablet dosage forms were comparable with the value claimed on the label. The obtained results, presented in Table 5, indicated the suitability of the method for routine analysis of VAL and AML from their combination drug products.

CONCLUSIONS
Based on peak purity results, obtained from the analysis of forced degradation samples using described method, it can be concluded that there is no other co-eluting peak with the main peaks and the method is specific for the estimation of VAL and AML in presence of their degradation products. The method has linear response in stated range and is accurate and precise. Though no attempt was made to identify the degradation products, described method can be used as stability indicating method for assay of VAL and AML in their combined dosage form.

Table 1: Results of forced degradation study using the proposed method

Name of Standard	Stress condition/ duration/ state	% Degradation	Peak Purity Index
AML Standard	Acidic/1M HCl 10 mL/ 20min reflux	12.82%	0.9997
	Alkaline/0.1M NaOH 10 mL/ 45min reflux	15.92%	0.9996
	Oxidative/30 % H_2O_2 10 mL/20 min reflux	28.11%	09993
AML sample	Acidic/ 0.1M HCl / 1 h reflux	14.06%	1.0000
	Alkaline/0.1M NaOH / 20 min reflux	30.18%	0.9999
	Oxidative/10 % H_2O_2 /20 min reflux	25.33%	0.9999
	Thermal/105 °C/72 h/solid	35%	0.9999
	Photo/1.2 million lux h/ solid/ Control	4%	0.9999
VAL Standard	Acidic/1M HCl 10 mL/ 6 h reflux	10.52%	1.0000
	Alkaline/1M NaOH 10 mL/ 12 h reflux	-	1.0000
	Oxidative/30 % H_2O_2 10 mL/2 h reflux	13.09%	1.0000
VAL sample	Acidic/1M HCl 10 mL/ 2 h reflux	11.1%	1.0000
	Alkaline/1M NaOH 10 mL/ 12 h reflux	-	1.0000
	Oxidative/30 % H_2O_2 10 mL/45 min reflux	13.84%	1.0000
	Thermal/105 °C/72 h/solid	NA	1.0000
	Photo/1.2million lux h/ solid/ Control	NA	0.9999

Table 2: Regression characteristics and system suitability parameter of proposed RP-HPLC method

Parameter	VAL	AML
Retention time (min)	25.84 ± 0.15	15.38 ± 0.13
Tailing factor	1.08 ± 0.31	1.09 ± 0.25
Asymmetry	1.06 ± 0.71	1.11 ± 0.65
Theoretical plates	63628 ± 0.37	67493 ± 0.34
Linearity range(µg/mL)	1.6-240	1-30
Limit of Detection (LOD) (µg/mL)	0.95	0.80
Limit of Quantification (LOD) (µg/mL)	1.6	1.0
Regression equation (y*=a+bc)		
Slope(b)	15107 ± 673.75	34694 ± 528.64
Intercept (a)	28516 ± 441.19	-2773.5 ± 198.61
Correlation coefficient (r)	0.9994	0.9995

Table 3:-Intra and inter-day precision data of proposed RP-HPLC method

Drug	Con. (µg/mL)	Interday precision(n=7)		Intraday precision(n=7)	
		Peak area ± SD[a]	CV, %	Peak area ± SD[a]	CV, %
VAL	1.6	23858 ± 239.18	1.00	22183 ± 338.98	1.52
	160	2425755 ± 33683.06	1.38	2288916 ± 31509.02	1.37
	240	3714079 ± 33051.65	0.89	3850913 ± 48017.14	1.24
AML	1	33087.7± 301.37	0.91	30620 ± 389.10	1.27
	20	684472.0 ± 5532.36	0.80	693824 ± 7143.85	1.03
	30	1040790 ± 16680.74	1.60	1150790 ± 20803.30	1.80

n= Number of Determination

Table 4: Recovery data for proposed RP-HPLC method (n=3)

Drug	Level of Recovery	Added Amount (µg/mL)	Recovered Amount (µg/mL)	% Recovery	CV, %
VAL	50%	159.48	159.11	99.76	0.71
	100%	320.74	323.08	100.72	1.09
	150%	471.89	471.49	99.91	0.30
AML	50%	10.15	10.08	99.35	1.27
	100%	20.45	20.52	100.36	1.21
	150%	28.49	28.37	100.02	0.93

n= Number of Determination

Table 5: Applicability of the Developed Method to Marketed Formulations (n=5)

Formulations	Labeled amount mg		Amount found (mg)		Assay% ± CV	
	AML	VAL	AML	VAL	AML	VAL
Set 1	10	320	9.97	319.67	99.70 %±1.06	99.98 ± 0.89
Set 2	10	320	10.05	319.98	100.50%±1.15	99.68 ± 1.35

n= Number of Determination

Figure 1: Chromatogram of standard a) Amlodipine at 237nm, b) Valsartan at 265nmand c) mixed spectra on both wavelengths.

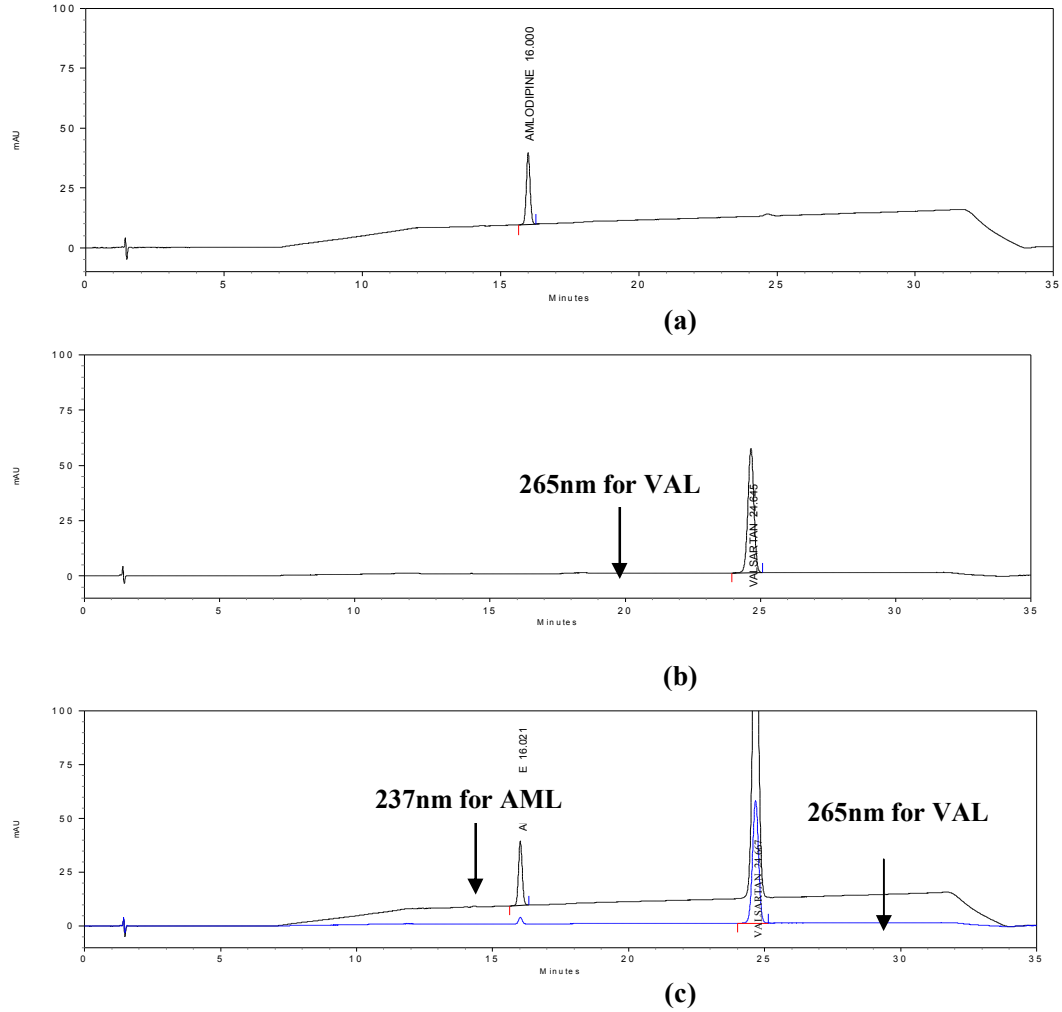
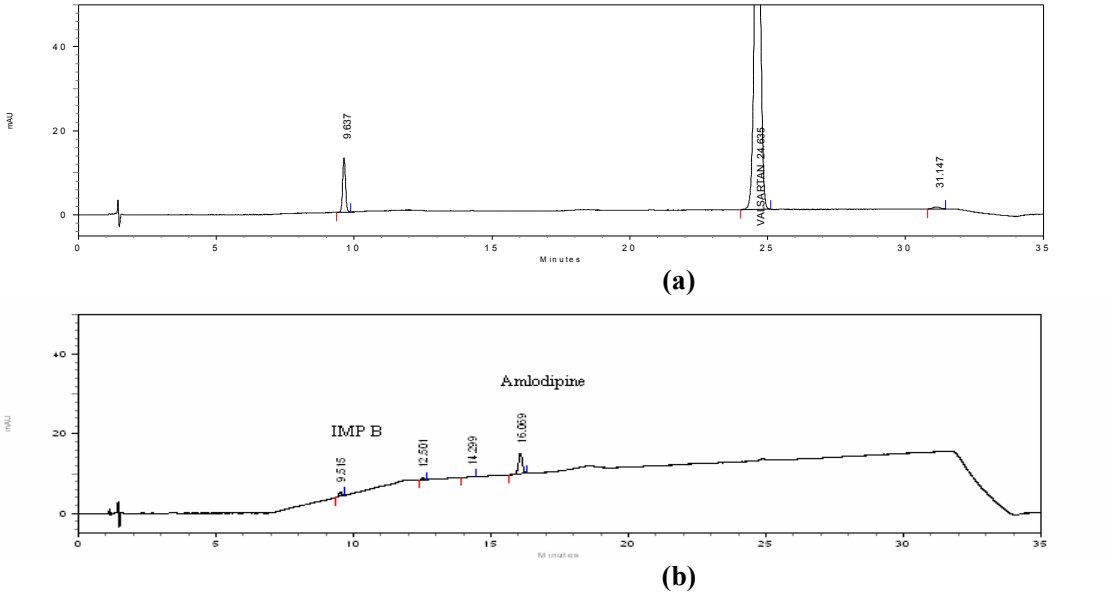
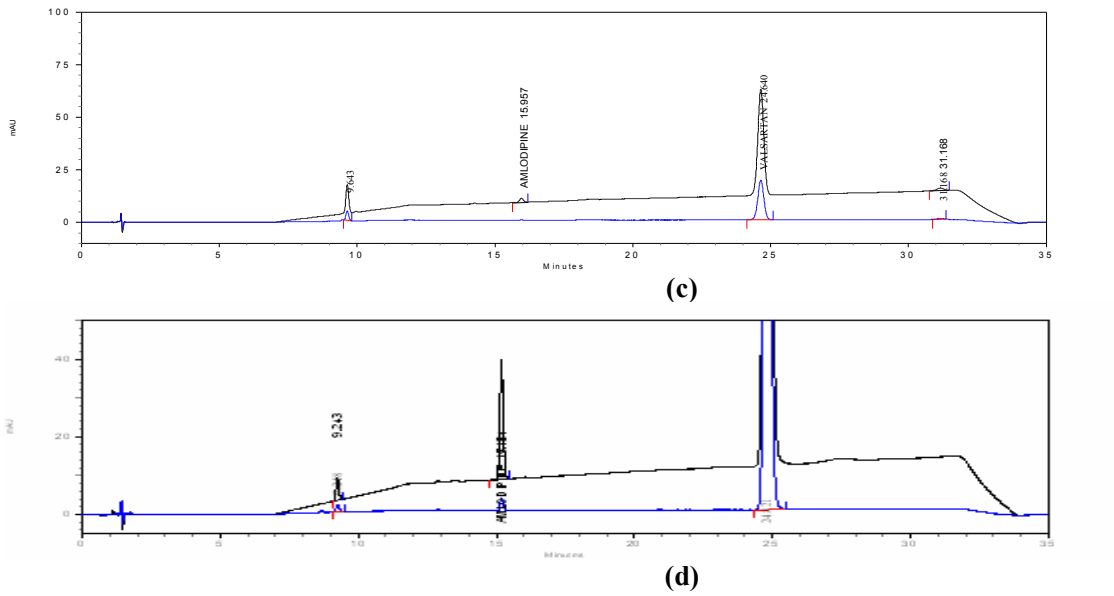


Figure 2: Chromatograms of acid hydrolysis-degraded VAL (a), AML(b), tablet preparation for VAL (c) and AML (d), respectively.

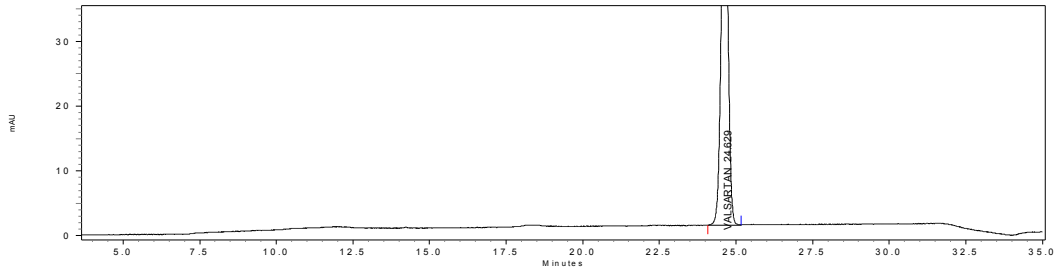




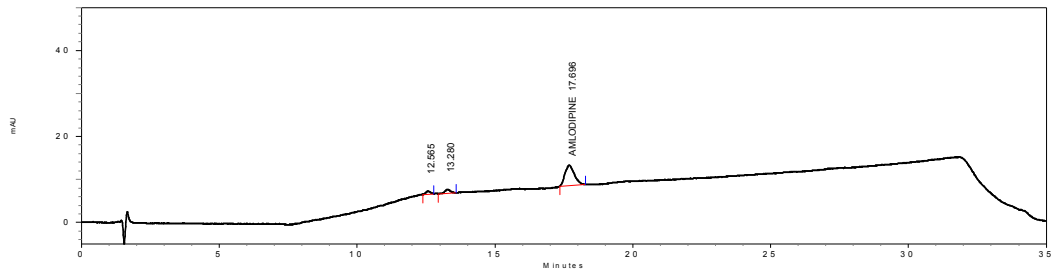
(c)

(d)

Figure 3: Chromatograms of base hydrolysis-degraded VAL (a), AML(b) and tablet preparation for VAL (c) and AML (d), respectively.



(a)



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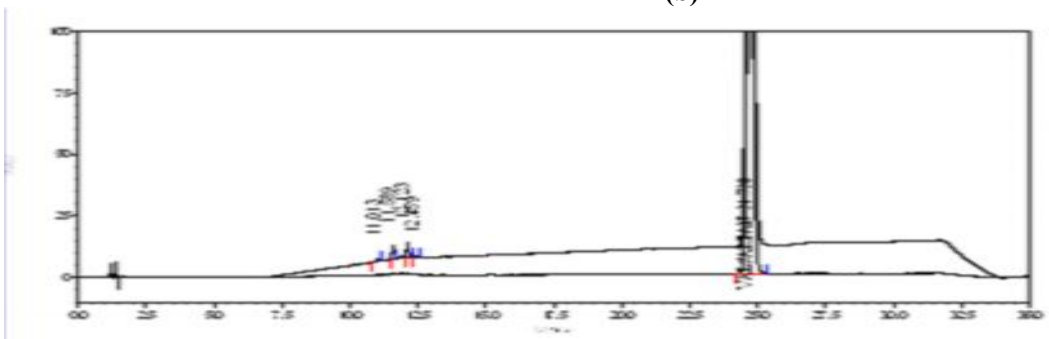

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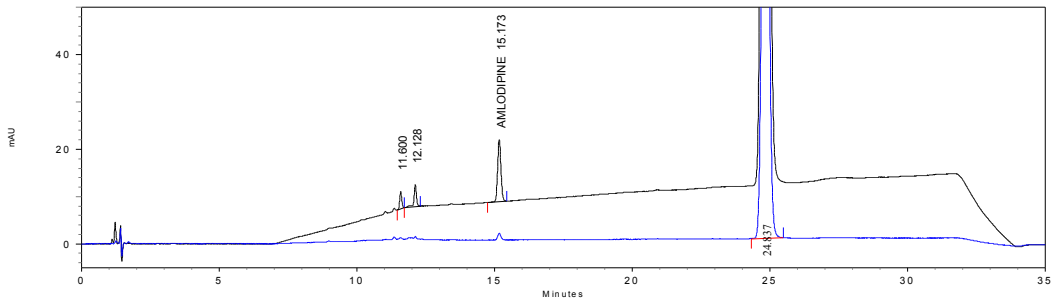
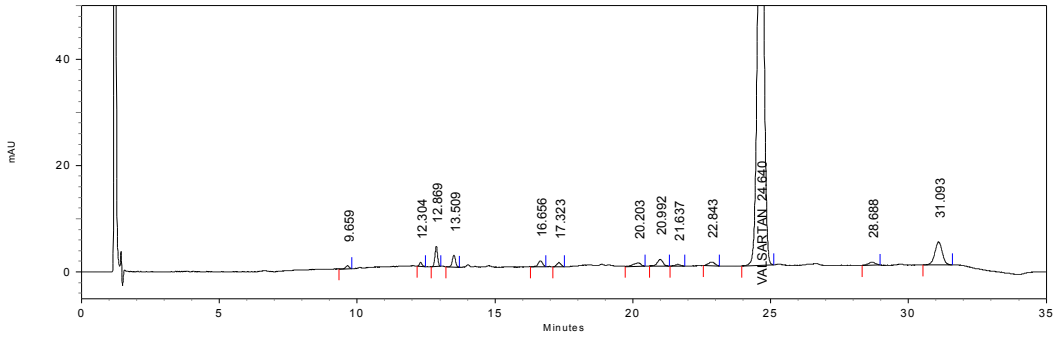
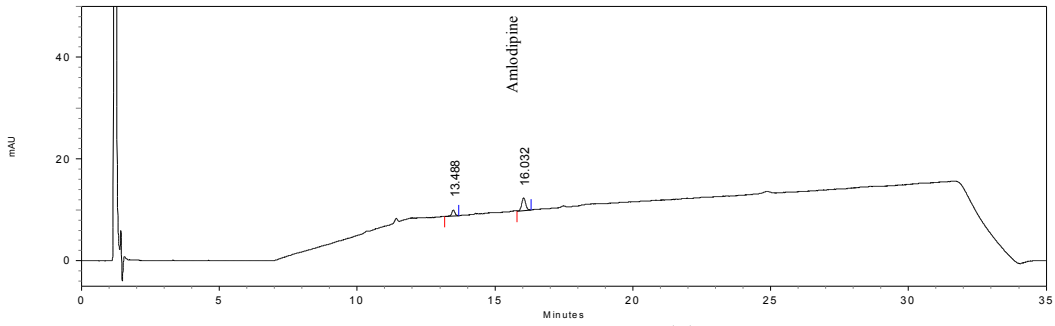


(d)

Figure 4: Chromatograms of oxidation-degraded VAL (a), AML(b) and tablet preparation for VAL (c) and AML (d), respectively.



(a)



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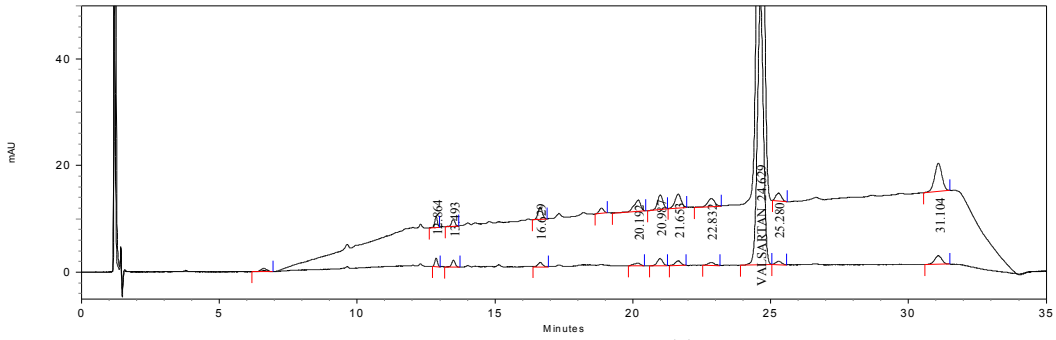

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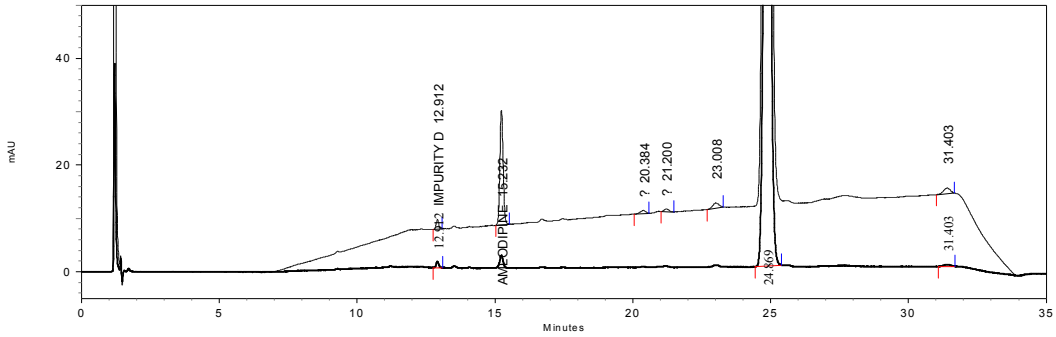
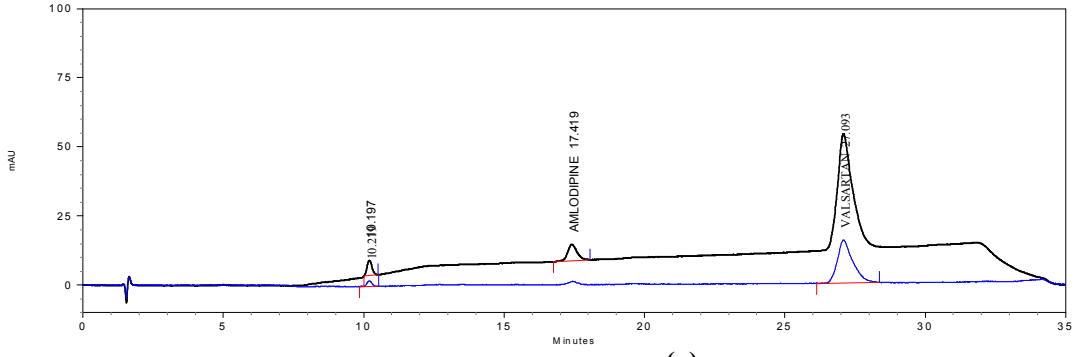


(d)

Figure 5: Chromatograms of thermal-degraded tablet preparation for VAL (a) and AML (b), respectively



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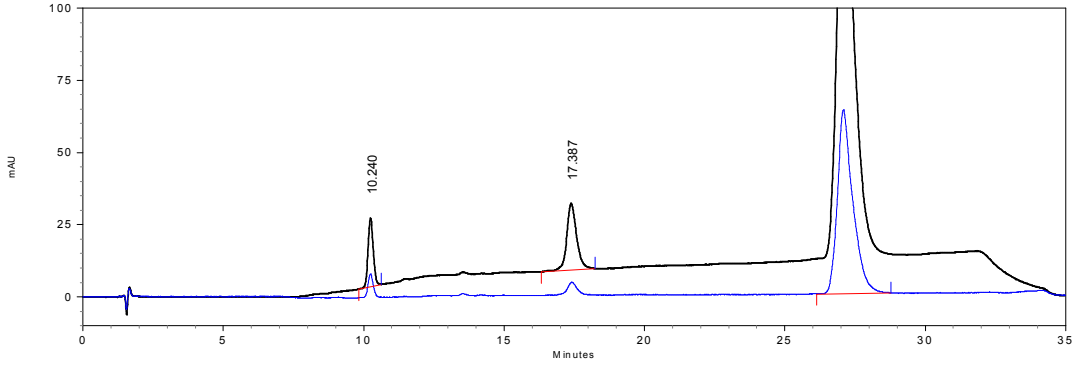

(b)

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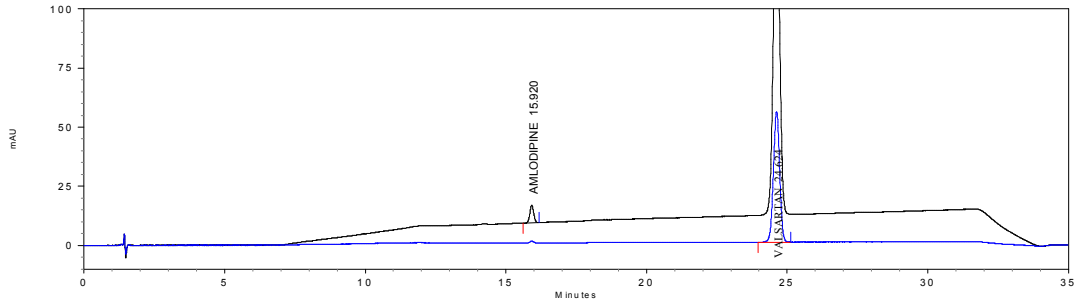
Figure 6: Chromatograms of photo-degraded (control) tablet preparation for VAL (a) and AML (b), (uncontrolled) tablet preparation for VAL (c) and AML (d), respectively.



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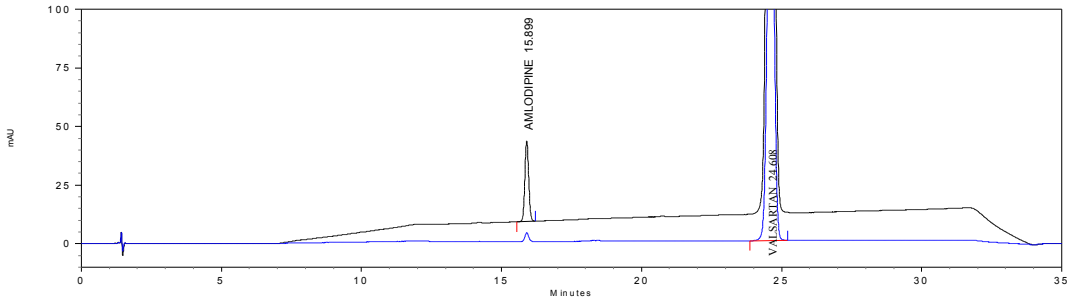

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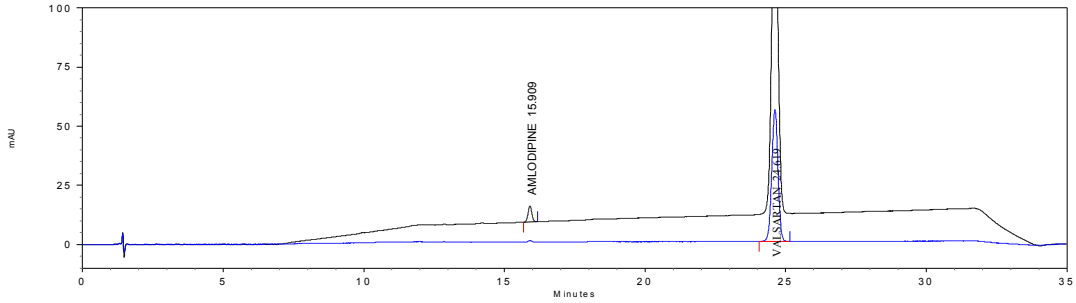

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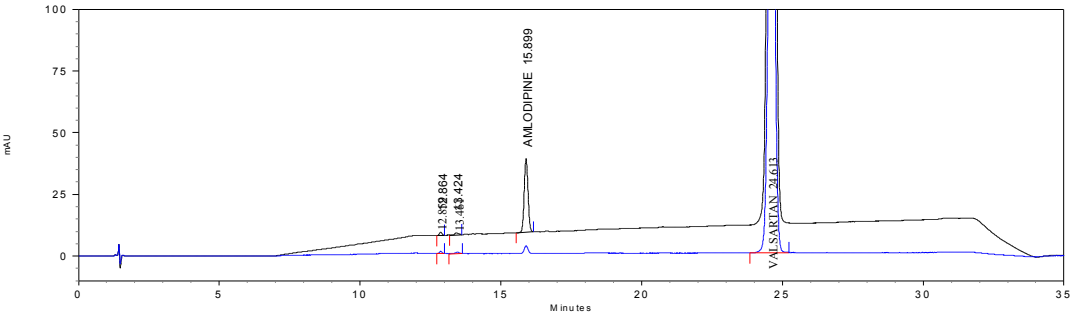

(d)

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