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BIO ANALYTICAL VALIDATED TECHNIQUE FOR THE SIMULTANEOUS ESTIMATION OF AMLODIPINE, ROSUVASTATIN AND VALSARTANIN HUMAN PLASMA BY RP-HPLC METHOD

Gurappa K. Dyade [* 1], Amit S. Lunkad [2] and Ramesh L. Sawant [3]

Department of Postgraduate in Pharmacy [1], SVPM'S College of Pharmacy, Malegaon (BKII) Baramati, Pune - 413115, Maharashtra, India.
Department of Pharm Chemistry [2], ST College of Pharmacy, Shirur, Pune - 411038, Maharashtra, India.
Department of PG and Pharm Chemistry [3], Dr. VVPF'S College of Pharmacy, Vilad, Ahmednagar Maharashtra, India Savitribai Phule Pune University, Pune - 414111, Maharashtra, India.

Keywords:

Amlodipine besylate, Valsartan, Rosuvstatin, Bio analytical technique, Precipitation method

Correspondence to Author:
Gurappa K. Dyade

Department of Postgraduate in Pharmacy, SVPM'S College of Pharmacy, Malegaon (BKII) Baramati, Pune - 413115, Maharashtra, India.

E-mail: gkdyade@gmail.com

ABSTRACT: A reverse phase high-performance liquid chromatographic bio analytical Technique (RP-HPLC) was developed and validated as per ICH 2019, US FDA 2018 guidelines for the quantification of amlodipine besylate, Rosuvastatin and Valsartan in human plasma using RP - C18 column. The mobile phase in composition (acetonitrile: water in the ratio 75:25% v/v, pH adjusted to 4.0 with acetic acid) was pumped and run at a flow rate of 0.8 ml/min, and for monitoring of eluents, a detector was set at wavelength 245 nm. The precipitation method was employed for the separation of the analyte from the plasma. A calibration curve was generated from the response and respective conc of standard solutions in the Concrange of LLOQ to ULOQ. The method was studied and validated per regulatory guidelines for selectivity, specificity, accuracy, precision, and stability study. Statistical data of the calibration curve of these drugs in the biological matrix was found within the prescribed limit. Results of accuracy and precision of quality control samples of these combined drugs were found (104.25 to 107.62 as % nominal conc. and 2.633 to 9.474 as % CV) for amlodipine, (101.01 to 106.45 as % nominal conc. and 4.431 to 13.786 as % CV) for rosuvastatin and (99.91 to 106.03 as % nominal conc. and 2.668 to 12.434 as % CV) for valsartan respectively. The developed bioanalytical method is simple: free from solvent-solvent extraction and solid phase extraction, precise, accurate and consumes less solvent due to less run time. The method suits for quantification of these drugs in plasma is henceforth applied for bioequivalence and bioavailability study in real clinical samples.

INTRODUCTION: Amlodipine besylate (AD), 2 - [(2 - amino ethoxy) - methyl] - 4 - (2 - chloro phenyl) -1, 4 -dihydro - 6 - methyl - 3, 5 - pyridine dicarboxylic acid 3 - ethyl - 5 - methyl ester benzene sulfonate, is a potent dihydro calcium channel blocker [1-4].

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Various analytical methods have been reported for the estimation of AD alone, or in combination with other antihypertensive agents in pharmaceutical dosage form, including accelerated degradation study by RP HPLC [5], RP-HPLC and stability indicating liquid chromatographic method [6-9], Bioanalytical HPLC method [10], HPTLC methods [11-13], stability indicating UPLC method [14], Chemo metric assisted spectroscopic method [15], and UV spectro-photometric methods [16-18].

Valsartan (VAL), a potent angiotensin receptor blocker chemically is, N - (1-oxopentyl) - N - [(2' - (1H - tetrazol-5-yl) (1, 1' -biphenyl) -4-yl) methyl]

- L-valine [1-4]. Techniques such as RP-HPLC methods [19-20], photodegradation study method [21], bio-analytical chromatographic methods [22-23], synchronous spectrofluorimetric bio-analytical method [24] and stability indicating UPLC method [25] have been reported for estimation of VAL alone or in combination with other drugs. One of the more recently introduced statins, Rosuvastatin (RV), chemically 7-[4-(4-fluorophenyl) - 6 - (1-methylethyl) - 2 - (methyl-methylsulfonyl - amino) - pyrimidin-5-yl] 3, 5-dihydroxy-hept-6-enoic acid is HMG-CoA reductase inhibitors [1-3]. The conversion of 3-hydroxy 3 methyl glutaryl (HMG)-COA to mevalonic acid is especially important, a primary control site for cholesterol biosynthesis [4]. Reported methods as stability indicating RP-HPLC [26, 27], UV-spectrophotometric methods [28, 29], LC-MS/MS bioanalytical method [30, 31] are found for quantification of RV alone or in combination with other drugs. Amlodipine, rosuvastatin and valsartan are official in BP [British Pharm] 32 and IP [Indian Pharm, 2018] [33]. The chemical structures of analyte AMB, VAL, and RV are shown in **Fig. 1A, 1B,** and **1C,** respectively).

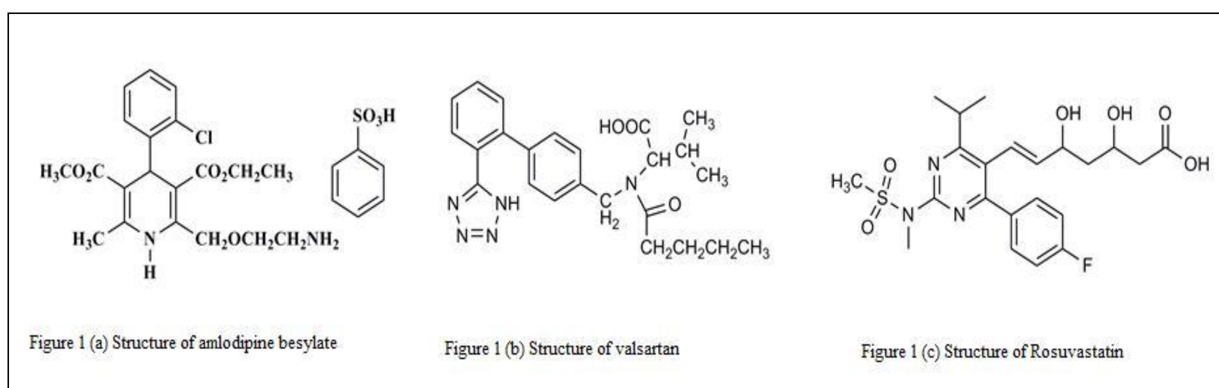

FIG. 1: CHEMICAL STRUCTURE OF (A) AMLODIPINE BESYLATE (B) VALSARTAN (C) ROSUVASTATIN

A literature survey revealed that many analytical methods had been reported for the estimation of AD, VAL, and RV individually and in combination with other antihypertensive or antihyperlipidemic drugs. Yet no bioanalytical method has been reported for estimating these drugs in together or combined form. The combined formulation of these drugs is under clinical trial by pharmaceutical [34], and efficacy and safety of triple therapy, including anti-hyperlipidemic, antihypertensive, and angiotensive inhibiting drugs [35] is revealed during the literature.

In analysis, a good approach is to develop a specific method that avoids interference of other drugs in determination from their combined mixture. Chromatographic methods are selected as efficient inseparation, detectiondue to their selectivity, sensitivity, and specificity. An attempt has been made to develop a simple bioanalytical RP-HPLC method free from extraction for the simultaneous estimation of AD, RV, and VAL analytes from human plasma. Bio-analytical methods must be validated as per regulatory guidelines; hence the proposed bio-analytical RP-HPLC method was validated in accordance with ICH [36], US FDA guidance [37].

MATERIALS AND METHOD:

Instrumentation: The analysis method was accomplished with a Shimadzu (Japan) prominence chromatograph equipped with LC-20AT solvent delivery system, a universal loop injector (Rheodyne 7725) injection capacity of 20 µl, and UV–Visible detector SPD-20A set at 245 nm. A PC workstation with clarity software controlled the instrument. Drugs separations were achieved on the Phenomenex Luna C18 column (250 mm × 4.6 mm i.d., 5-µm particle size) under reversed phase partition conditions. The mobile phase composition was 75: 25 % (v/ v) mixture of Acetonitrile: Water (pH 4.0 ± 0.2, adjusted with Acetic acid). The mobile phase flow rate was kept at 0.8 ml/min with 8 minsrun time. Before the analysis the mobile phase was filtered through a 0.2 µm membrane filter (Pall corporation, Mumbai) and sonicated (Lab man scientific Instruments Chennai) for degassing. For plasma separation from blood-Programmable Microcentrifuge (make by Bioera Life sciences Pvt Ltd) Sr No LQK 236 A was

utilized. For mixing vortex mixer (Make by Yorco sales Pvt Ltd) was utilized.

The identities of the analytes were established by comparing the retention times of drugs in the combined solution with those in standard solutions. Chromatography was performed in an ambient temperature maintained at 40 ±1°C. The overlain UV spectrum of analytes AD, RV and VAL was obtained for selecting the working wavelength of detection on the Shimadzu-1700 double-beam UV-VIS spectrophotometer (Shimadzu, Kyoto, Japan).

Reagents and Chemicals: Pure drugs AD from Smruthi organics Ltd, RV from Swapnaroop Pharma Aurangabad, and VAL from FDC Mumbai were received and obtained as a gift sample. HPLC grade acetonitrile-water and Glacial acetic acid (HPLC grade) were purchased and obtained from Merck India Pvt Limited, Mumbai, India. Human plasma was procured from an authenticated supplier and also obtained by centrifuging blood.

Collection of Plasma: Plasma was procured from Local blood bank. Also to obtain plasma, a human blood Sample was drawn from the antecubitel fossa of the arm (skill of phlebotomy technique used by local pathological laboratory). Blood was centrifuged (Micro Centrifuge Bio Era life sciences Pvt Ltd) at 4000 rpm for 6 min. The supernatant clear plasma was carefully recovered in a clean and dry eppendorf tube, and stored below -20°C until used.

Preparation of Standard Solution of AD, RV and VAL: The mobile phase was prepared and filtered thrice through 0.2 µ membrane filters (Pall Corporation, Mumbai) and 20 min's sonicated for degassing.

About 5 mg of each AD, VAL and RV were accurately weighed separately and transferred to separate 10 ml volumetric flasks. The drugs were dissolved into solvent acetonitrile and 10 ml volume was made up to the mark into separate 10 ml volumetric flask to obtain stock solutions. Further intermediate stock solution having conc. 10 ppm (µg/ml), 40 ppm (µg/ml) of each analyte were obtained separately by diluting aliquots of stock solution to 10ml. Solutions were stored in a refrigerator below -10°C till use.

Combined Drug Elution in Optimized Mobile Phase: The prepared mobile phase was pumped at a flow rate of 0.8 ml/min, and a combined drug solution containing AD, VAL and RV was prepared by diluting aliquots of these drugs solution for the optimization of mobile phase study. The solution was injected in a column for the mobile phase optimization study.

Calibration Curve: US FDA recommends calibration standards should be prepared in the same biological matrix as the study samples. The calibration range is defined by including the lowest calibration standard LLOQ and the highest calibration standard ULOQ. Calibration curve should be generated for each analyte studied during method validation and for each analytical run. A calibration curve should be generated with a blank sample and six standard solutions including LLOQ and ULOQ.

Freeze-dried plasma was thawed. 100µl plasma was transferred into 2 ml eppendorf tube, added [32] µl of AD of conc. 10 ppm with micropipette homogenized for 5 min on vortex shaker and volume was made up by adding acetonitrile with micropipette to 2ml, again homogenized on vortex mixer. Plasma drug admixture was centrifuged at 7000 rpm for 7 min. The clear supernatant liquid was recovered and filtered through 0.22µ membrane filter of 13 mm diameter, and prepared solution of conc. 160 ng/ml was stored. Similarly, 320ng/ml was obtained by processing 64 µl of 10 ppm AD solution and to prepare standard solutions with conc. 480ng/ml - 960ng/ml for the calibration curve 40 ppm solution of AD was utilized. This process was repeated without a drug to obtain blank plasma. Similarly, drug plasma admixture of RV of conc 160ng/ml – 960 ng/ml and VAL of conc 160ng/ml – 960ng/ml were also prepared from their intermediate stock solutions to prepare the calibration curve. A chromatograph of combined drug solution was also obtained to know any interference during detection. Microsoft Office Excel software was utilized to obtain and analyze standard regression curves and Statistical parameters slope, intercept standard deviations, and coefficient of variance was calculated.

Preparation of Quality Control (QC) Samples in Plasma Matrix: During method validation, the

QCs were prepared at a minimum of 4 concentration levels within the calibration curve range- the LLOQ, within three times of the LLOQ (low QC), around 30-50% of the calibration curve range (medium QC) and at least 75% of the ULOQ (high QC).

Quality Control Samples: QC samples were prepared from the 10 ppm, 40 ppm solutions of AD, RV and VAL. 100 µl of plasma was transferred into 2 ml eppendorf tube, added 32 µl of combined solution of 10 ppm of AD, RV and VAL and homogenized for 5 min on vortex shaker; volume was adjusted to 2 ml with acetonitrile again vortexed for 5 min. Drug plasma admixture was then centrifuged at 7000 rpm for 7 min to separate precipitate; clear supernatant separated liquid was filtered off through 0.22 µ syringe filter and labeled as LLOQ (160 ng/ml). Alongside LQC (400 ng/ml), MQC (560 ng/ml) and HQC (720 ng/ml) were also prepared.

The calibration curve and quality control solutions were degassed in the sonicator and stored below -10°C until injected into the column. 20 µl of each solution was injected in the column to obtain a chromatograph

Validation: Bioanalytical method development and validation is essential to ensure assay performance's acceptability and analytical results' reliability. The validation procedure of bioanalytical method is described in ICH, US FDA 2018 guidelines. The method was validated for selectivity, specificity, calibration curve, accuracy, precision, and stability study.

Selectivity: Selectivity is defined as the ability of an analytical method to differentiate and quantify the analyte in the presence of other components of the blank biological matrix. Evidence was provided by injecting blank plasma (20 µl) in column to obtain a chromatograph.

Specificity: Specificity is the ability of a bio analytical method to differentiate and detect the analyte from other substances, for specificity study mixed solution of RV, AD and VAL was used; and injected to obtain a chromatograph.

Plot of Calibration Curve of Drugs in Plasma Matrix: Calibration curve of each drug was generated by preparing standard solutions in conc. range 160-960 ng/ml. All solutions were degassed and injected (20 µl) into the column to obtain a chromatograph. Peak areas were measured from the chromatograph, and a calibration curve was made by plotting peak areas against their respective concentrations. Standard regression curve analysis was obtained by usage of Microsoft Office Excel software, and correlation coefficient, slope, and standard deviation were obtained.

Accuracy and Precision: The accuracy was determined by replicate analysis of samples containing the analyte of known amounts. Accuracy and precision both were studied by preparing and measuring three determinations of four different QC samples in each analytical run. The conc. of quality control samples 160 ng/ml, 400 ng /ml, 560 ng /ml and 720 ng/ ml were selected for drug accuracy and precision study.

Analyte peak response should be identifiable, discrete and reproducible and the back-calculated concentration should have the overall accuracy at every concentration level within ±15% of the nominal concentration, except at the LLOQ, where it should be within ±20%. The methods precision was studied by calculating (%CV) at each level of QC sample and it should not exceed 15% at each conc. level, except at the LLOQ, where it should be within 20%.

Sensitivity: It is well defined as the lowest analyte concentration that can be measured with acceptable accuracy and precision. Drugs solution having conc. LLOQ was injected to obtain data and chromatography.

Reproducibility: Reproducibility of the method is judged by the replicate determination of the QCs and is usually included in the study of precision and accuracy.

Stability: The stability of the analyte in the studied matrix was evaluated and carried out using low and high-concentration stability QCs. Stability analysis of aliquots of the low and high QCs is done at time zero and after the applied storage conditions to be evaluated. Three stability QCs were prepared and analyzed per concentration level/storage condition/time point.

The chemical stability of an analyte in the biological matrix under specific conditions; for specified time intervals was assessed in below ways mentioned.

As per guidelines, at least three replicates should be assessed at each low and high concentration. Stability sample results should be within 15% of nominal concentrations.

Freeze and Thaw Stability: The study aims to mimic the intended sample handling conditions to be used during sample analysis. Prepared Quality Control samples (QCS) were freeze and thawed, and three freeze-thaw cycles were carried out to study.

Bench-top Stability: At laboratory temp, stability of QCS was achieved, mimicking the design of expt and covering the laboratory handling conditions expected for study samples.

Long-term Stability: The QCS were stored, and storage time in a long-term stability evaluation was equal to the time from the first sample collection to the date of the last sample analysis.

Stability testing should evaluate the drug's or analyte's stability during sample collection and handling, after long-term (frozen at the intended storage temperature) and short-term (bench top room temp) storage, and after freeze and thaw cycles and the analytical process. The procedure should also include an evaluation of analyte stability in the stock solution.

Carry Over: Carry-over is a variation of a measured concentration due to residual analyte from a previous sample that remains in the analytical instrument. Carry-over should be assessed and minimized during analytical method development. Carry-over should be evaluated during validation by analyzing blank samples after the higher conc solution ULOQ of the calibration standard.

RESULTS AND DISCUSSION:

HPLC Method Development and Optimization: The multi-component formulations are advantageous as they have gained a lot of importance as more patient acceptability, improved potency, and decreased side effects. AD, RV and VAL are being used in managing hypertension as antihypertensive drugs, antihyperlipidemic agents and angiotensin receptor blocker agents respectively. This combined drug formulation is under study by pharmaceuticals. This research was concentrated on optimizing the chromato-graphic conditions for the simple, rapid, and low-cost effective bio analysis, including selecting the suitable column or mobile phase to obtain satisfactory results.

The variation in the solvent type determined chromatographic conditions for the well separation, solvent strength (volume fraction of organic solvent(s) in the mobile phase and pH of the solution), detection wavelength and flow rate. The mobile phase conditions were optimized and found no interference from solvent and excipients. Other criteria include time required for analysis, appropriate k range ($1 < k < 10$) for eluted peaks, assay sensitivity, solvent noise, and use of the same solvent system for extraction of the drugs from the bulk mixture during drug analysis were also considered.

To optimize the mobile phase, a chromatogram of these drugs was obtained in mobile phase acetonitrile: water in the ratio 80:20% v/v **Fig. 2**; all three drugs eluted but the resolution was not proper. Then the proportion of acetonitrile was decreased to 75%, resulting in all drugs elution within 10 min. Also, the mobile phase composition was changed to methanol: water in proportion 75:25% v/v, and the result was increased retention time. To determine the appropriate wavelength for simultaneous measurement of AD, RV and VAL solutions of these analyte in mobile phase were scanned in the range of 200 – 400 nm.

From the overlaid UV spectra, the suitable wavelength considered for monitoring this analyte was 245 nm **Fig. 3**. Solution of each analyte was also injected in the column for HPLC analysis, and the responses (peak area) were recorded at 245 nm.

It was observed that all analytes absorbed well at 245 nm, and at this wavelength, no interference from the mobile phase or smooth baseline was found. And therefore, it was concluded that 245 nm was the most appropriate wavelength for analyzing drugs with suitable sensitivity.

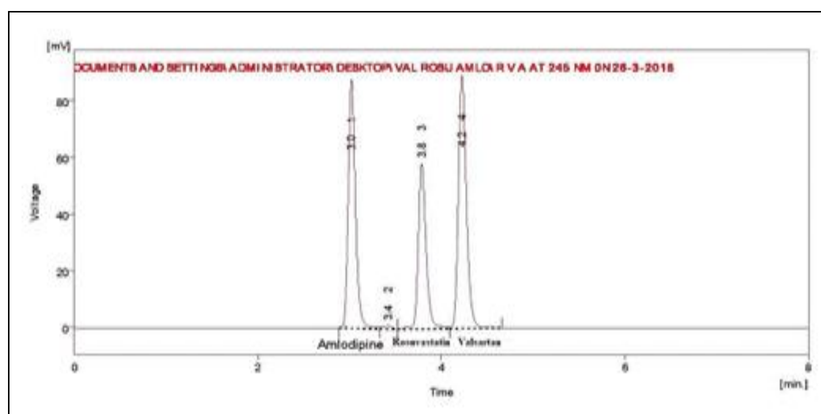

FIG. 2: CHROMATOGRAPH OF COMBINED DRUGS IN ACETONITRILE: WATER 80: 20

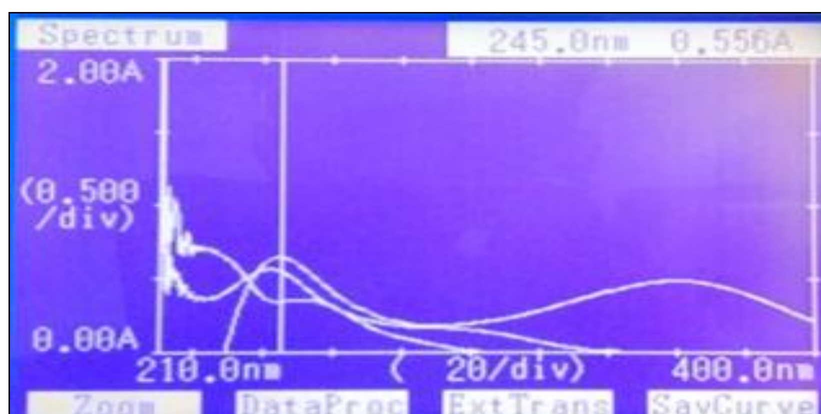

FIG. 3: OVERLAIN SPECTRA OF AD, RV AND VAL

Therefore Mobile Phase Acetonitrile: Water in composition 75:25 % v/v was selected, and pH of mobile phase was adjusted to 4.0with acetic acid. The result was elution of this analyte with reasonable retention factor and symmetry in peak. Under these chromatographic conditions well-resolved peaks were obtained for AD, RV, and VAL with the retention time 2.21, 4.03 min and 5.04 min, respectively. The optimized chromatogram of analyte AD, RV, and VAL is shown in Fig. 4. The resolution (RS) between AD and VAL was 6.715; and between RV and VAL 5.222 was found. Chromatographic conditions were unaffected by varying conc. of drugs in mixture and shown in chromatogram.

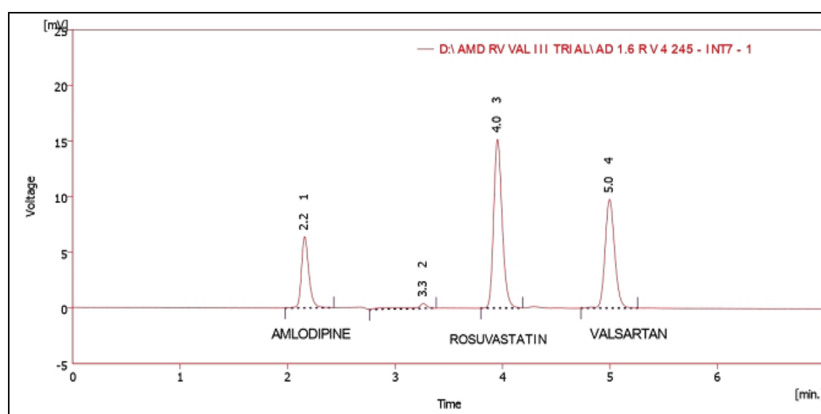

FIG. 4: CHROMATOGRAPH OF COMBINED DRUGS IN ACETONITRILE: WATER 75:25

Method Validation: The system suitability parameters like capacity factor, number of theoretical plates, and tailing factor for all the analytes were found to be within the limit indicating the suitability of the system **Table 1**. The values obtained for k′ (1 < k′< 10) and RS (>2) showed these chromatographic conditions are appropriate for separation and quantification of all

compounds. The number of theoretical plates and the tailing factor were within the acceptance criteria of >2000 and ≤ 1.5, respectively, indicating good column efficiency and optimum mobile phase composition. The method was evaluated by determination of the correlation coefficient and intercept, calculated in the corresponding statistical study correlation coefficient r^2 values > 0.999 and intercepts very close to zero confirmed the good linearity of the method.

TABLE 1: SYSTEM SUITABILITY PARAMETERS OF THE CHROMATOGRAPHIC CONDITION

Parameters	AD	VAL	RV
Retention time*	2.21	4.03	5.04
Tailing factor*	1.158	1.136	1.198
Asymmetrical factor*	1.264	1.204	1.216
Number of Theoretical plates	6193	14792	11984
Resolution	-	6.715	5.222
Flow rate ml/min	0.8 ml/min	0.8 ml/min	0.8 ml/min

Selectivity for Analyte: Evidence of the selectivity was provided by testing blank plasma in the absence of an analyte. The evaluation of selectivity from the chromatograph was demonstrated that no significant response attributable to interfering components was observed at the retention time(s) of the analyte in the blank samples shown in **Fig. 5.**

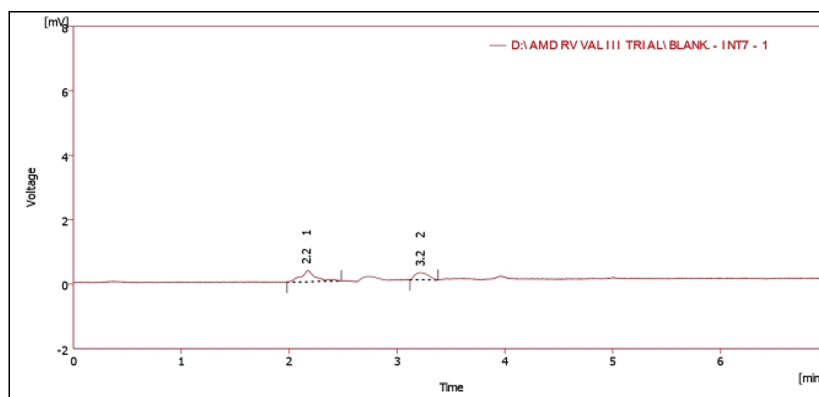

FIG. 5: CHROMATOGRAPH OF BLANK PLASMA (ABSENCE OF DRUG)

Specificity: Specificity is the ability of a bioanalytical method to detect and differentiate the analyte from other substances, for specificity study mixed drug solution of RV and AD; and RV and VAL were used; and injected to obtain chromatograph **Fig. 6.**

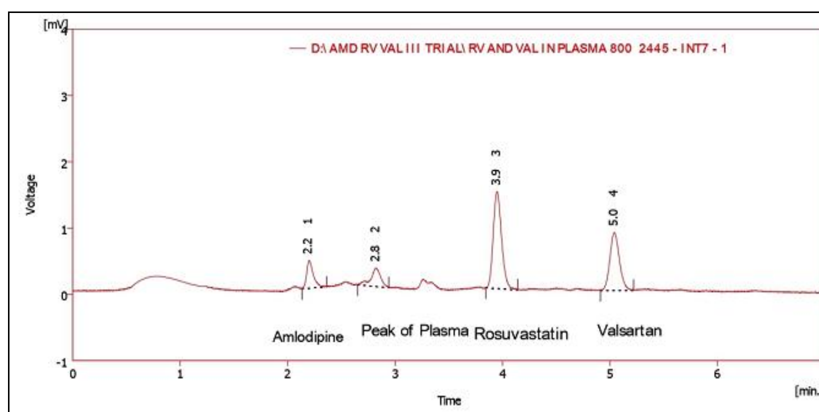

FIG. 6: CHROMATOGRAPH OF COMBINED DRUG IN THE PRESENCE OF PLASMA

Calibration Curve of Drugs in Plasma Matrix and Plot of Calibration Curve: As per guidelines calibration curve for these drugs was established in conc. range RV (160-960ng/ml), AD (160-960 ng/ml), and VAL (160-960 ng/ml) shown in **Fig. 7**. The regression equation obtained for the standard curve with a coefficient of regression (r^2) and slope for these drugs were given in **Table 2.**

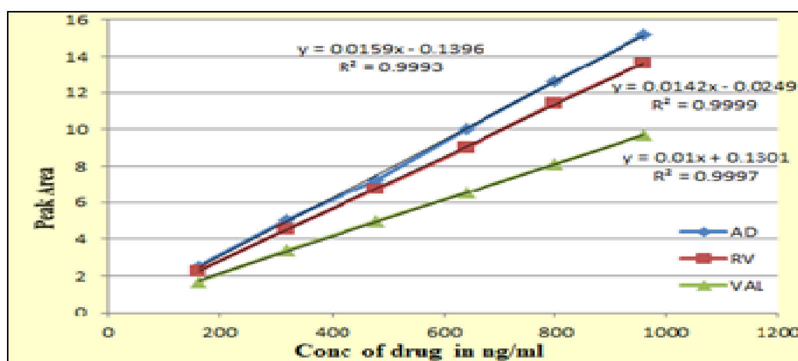

FIG. 7: CALIBRATION CURVE OF AD, RV AND VAL

TABLE 2: RESULTS OF CALIBRATION CURVE OF AD, RV AND VAL

Parameters	AD	VAL	RV
Detection wavelength	245 nm	245 nm	245 nm
Calibration curve range (ng/ml)	160-960 ng/ml	160-960 ng/ml	160-960 ng/ml
Correlation coefficient (r)	0.9993	0.9997	0.9999
Regression equation (y = mx + c)	Y=0.0159X-0.1396	Y=0.01X+0.1301	Y=0.0142X+0.0249
Slope (b)	0.0159	0.01	0.0142
Intercept (a)	0.1396	0.1301	0.0249

Accuracy and Precision: The accuracy and precision within the batch was calculated and results were tabulated in **Table 3**. Precision was measured as percentage of coefficient of variation or relative standard deviation (% CV) and accuracy was determined as percent of nominal conc. (% Nominal). The within batch % CV was found in the range 4.431-13.786 for RV, 2.633-9.474 for AD, and 2.668-12.434 for VAL at all conc. levels. The data of % nominal conc. were varied between 101.01-106.45 for RV, 104.25-107.62 for AD and 99.91-106.03 for VAL at all conc. levels. All the obtained values were within the acceptable limit as per guidelines. The chromatography of MQC and LLOQ are shown in **Fig. 8** and **9.**

TABLE 3: RESULTS OF ACCURACY AND PRECISION

Parameter	Concentration of QC samples											
	LLOQ 160 ng/ml			LQC 400 ng/ml			MQC 560 ng/ml			HQC 720 ng/ml		
	AD	RV	VAL	AD	RV	VAL	AD	RV	VAL	AD	RV	VAL
	Within batch n=5											
Mean*	171.33	170.33	169.66	417.66	409.66	399.66	602.66	565.66	575	760.66	740	735.66
% CV	6.469	4.431	2.668	2.633	12.218	12.434	6.917	13.786	7.152	9.474	9.731	9.291
% RE	7.08	6.45	6.03	4.25	2.41	-0.09	7.62	1.01	2.67	5.55	2.77	2.17
% Nominal	107.08	106.45	106.03	104.25	102.41	99.91	107.62	101.01	102.67	105.55	102.77	102.17

* Mean of three

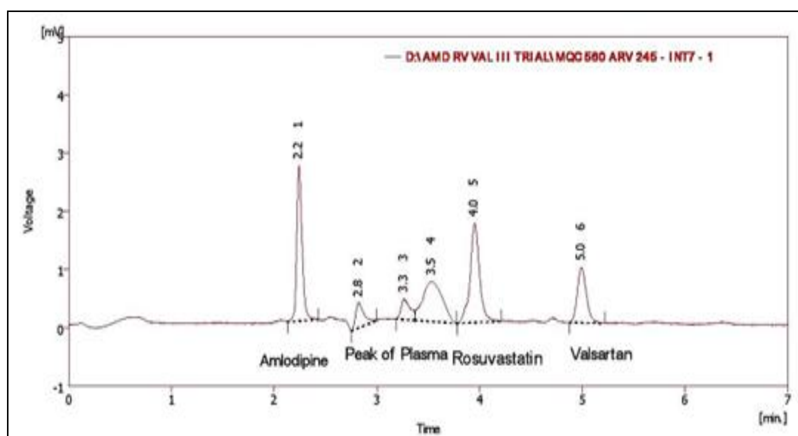

FIG. 8: CHROMATOGRAPH OF QC SAMPLE MQC 560 ng/mL

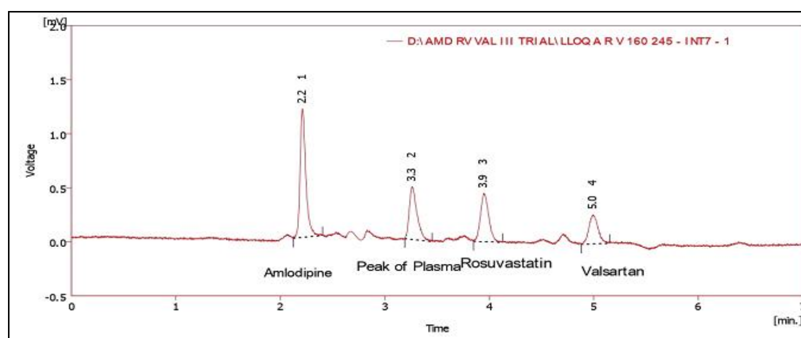

FIG. 9: CHROMATOGRAPH OF QC SAMPLE LLOQ 160 ng/mL

Reproducibility: Reproducibility of the method is assessed by replicate measurements using the samples, including quality controls samples. Accuracy and precision results were shown reproducibility of the method.

Stability Study: From the stability results it was observed that no substantial degradation observed in the samples stored at different conditions **Fig. 10**. It was concluded that sample was stable at 12 Hrs (Short term stability), for three freeze-thaw cycles and for 30 days at-20° C (Long term stability). The data of % CV were found in the acceptable range of 7.132-12.503 for AD, 4.931-10.242 for RV and 3.936-10.895 for VAL at all conc. levels; and % of nominal conc. were varied from105.78-107.33 for AD, 101.38-104.41 for RV, and 100.69-103.33 for VAL at all conc. levels. The results are shown in **Table 4**, and were found within the prescribed limit mentioned in the guidelines.

TABLE 4: RESULTS OF STABILITY STUDIES FOR AD, RV AND VAL

Parameter	Bench top stability					
	LQC 400 ng/ml			HQC 720 ng/ml		
	AD	RV	VAL	AD	RV	VAL
Mean	428.33	417.66	410.66	771.66	730	725
% CV	7.132	7.188	3.936	11.741	4.931	9.931
% Nominal	107.08	104.41	102.66	107.17	101.38	100.69

Parameter	Freeze thaw stability					
	LQC 400 ng/ml			HQC 720 ng/ml		
	AD	RV	VAL	AD	RV	VAL
Mean	428.33	415	412.66	765	734.33	735
% CV	7.132	9.638	10.895	8.150	9.110	6.116
% Nominal	107.08	103.75	103.16	106.25	101.99	102.08

Parameter	Long term stability					
	LQC 400 ng/ml			HQC 720 ng/ml		
	AD	RV	VAL	AD	RV	VAL
Mean	429.30	411.66	413.33	761.66	731.66	736.66
% CV	12.503	7.637	6.110	10.916	10.242	7.464
% Nominal	107.33	102.91	103.33	105.78	101.62	102.31

*Mean of three

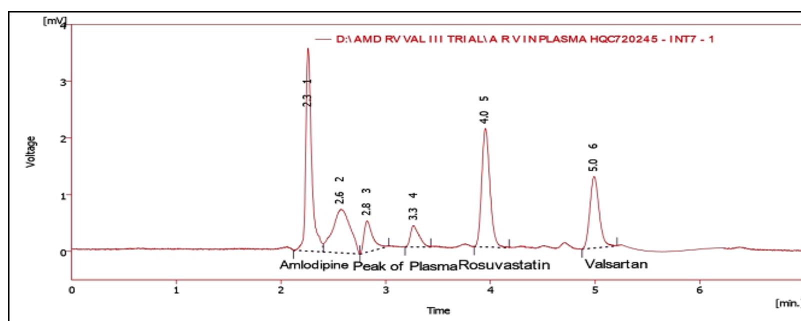

FIG. 10: CHROMATOGRAPH OF QC SAMPLE HQC 800 ng/mL IN STABILITY STUDY

Carry Over: It was not found residual analyte from a preceding sample that remains in the analytical instrument. The blank plasma injected after calibration study and obtained chromatogram showed no significant peak or response at retention time of analyte.

CONCLUSION: A simple isocratic RP - HPLC method with UV detection has been developed for quantitative estimation of AD, RV and VAL in the presence of plasma. The method was free from solid-phase extraction or liquid-liquid extraction. The run time was relatively short (8 min), which enables rapid quantification of many samples within short span.

Thus the proposed method was rapid, selective, and specific and required a simple sample preparation procedure. Drug-drug interaction between the antihypertensive, anti-hyperlipidemic and angiotensin receptor blocking agent was not observed during detection from biological matrix plasma. The bioanalytical method can be used to detect and quantify these drugs in plasma, hence applied for bio equivalence and bioavailability study in real clinical samples.

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