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Assessments of Valsartan in The Presence of Nebivolol or Amlodipine in Solid Formulations and Its Discriminative Dissolution Behavior via RP-HPLC and RP-UPLC Methods

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THE current work provides method development and validation of a rapid, robust, precise and accurate RP-HPLC and RP-UPLC for the assessment of Valsartan (VAL) in the presence of Nebivolol (NEB) or Amlodipine (AML) in their combined solid dosage form, following the ICH instructions. In RP-HPLC, Separation was achieved on an Inertsil column, (C_{18}, 150 mm x 4.6 mm, 5µm) with (30:20:50 v/v%), mobile phase of (buffer pH 3.0: acetonitrile: methanol) in an isocratic mode with a flow rate 1.0 mL/min and using photodiode array (PDA) detection at 281 nm. However, in RP-UPLC, Mobile phase was phosphate buffer pH 3.0 and acetonitrile in a ratio of (55:45 v/v%) at a flow rate 0.3 mL/min on an Eclipse Plus column, (C_{18}, 1.8 µm, 50 mm × 2.1 mm), while detection at 240 nm on a PDA detector. Calibration curves were linear over the concentration ranges of (0.80-72.00,0.05-4.50) and (4.00-144.00, 0.125-4.50) µg/mL, for the assay and *in-vitro* drug release profile of (VAL, NEB) and (VAL, AML), respectively. *In-Vitro* dissolution studies revealed that not less than (NLT) 85% of the labeled amount of VAL and AML was released within 15 min and NLT 85% of the labeled amount of NEB was dissolved within 20 min from their fixed combination solid dosage forms.

Keywords: Amlodipine, Nebivolol and Valsartan; Development, validation and dissolution; RP-HPLC and RP-UPLC.

Introduction

Valsartan (VAL) is an angiotensin II receptor rival, following up on the AT1 subtype. VAL keeps veins from narrowing, which brings down circulatory strain and improves blood stream. VAL is utilized to treat hypertension. It is at times given together with other circulatory strain meds [1]. It additionally starts the arrival of mineralocorticoid aldosterone which is in charge of sodium and water maintenance causing edema and lifts the cardiovascular yield. All of these parts are regarded to be responsible for hypertension. Valsartan antagonizes angiotensin II impacts by preventing hormone binding in the vascular smooth muscle and adrenal gland to AT1 receptors. After dosing, T_{max} is 2 to 4 h. Valsartanvaleryl-4-hydroxy is the main metabolite.

Nebivolol (NEB) is a third-generation ß-adrenergic blocker with FDA-approved vasodilator characteristics for the therapy of hypertension. Apart from being a selective β1-adrenergic blocker it also exhibits NO-mediated vasodilation in vascular smooth muscles. The mean C_{max} is approximately1.5 to 4 h post dosing. Approximately 98% of the drug binds to albumin which accounts for its bioavailability [2]. Combination of NEB and VAL is used as a cardiovascular and β1-adrenergic blocker [3].

Amlodipine besylate (AML) is a long-acting calcium channel blocker utilized as an enemy of hypertensive operator and in the treatment of angina. AML acts by loosening up the smooth muscle in the blood vessel divider, diminishing fringe opposition and subsequently decreasing circulatory strain while in angina, blood flow to

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the cardiac muscle rises [1]. Amlodipine absorbed from the gastrointestinal tract slowly and almost entirely andpeak plasma levels are reached 6-12 hours after oral administration [1]. Amlodipine's estimated bioavailability is 64-90 percent. After 7-8 days of successive daily dosage, steady-state amlodipine plasma concentrations are obtained. Food does not affect absorption.

Literature survey revealed some analytical techniques for determining of VAL, AML and NEB either individually or in conjunction with other medicines, including, Spectrophotometric techniques (UV) [3-7], High-performance thin layer chromatography (HPTLC) [8], High-performance liquid chromatography (HPLC) [1-3, 9-27], Ultra-performance liquid chromatography (UPLC) [28-30], Tandem mass spectrometry LC-MS/MS in plasma matrix [13, 31-43], HPLC providing the *in-vitro* dissolution [1], Some Stability-Indicating method by HPLC [20, 44-48] , by HPTLC [49] and by UPLC [50, 51]. Although the extensive method for analysis of thesis drugs there is no method to determine the valsartan in the presence of NEB or AML in the discriminative dissolution medium. Most of the mentioned methods have limitation like longer run time, low resolution, and narrow linear range.

Herein, we aim at developing two new RP-HPLC and RP-UPLC methods for the quantitative assay, and discriminative dissolution profiles for the assessment of the drugs in powder and solid dosage forms in their binary combination to predict the *in-vivo* efficiency of the mixed product.

Experimental

Materials and reagents

Amlodipine Besylate (AML), purity (99.30%), and Valsartan (VAL), purity (99.70%) was obtained from Hetero drugs limited, Hyderabad, India. Nebivolol HCl (NEB), purity (99.40%) was obtained from Torrent Pharma, Baddi, India. ASTM grade I water, was daily obtained from the central laboratory. Sodium dihydrogen phosphate, Dipotassium hydrogen phosphate, *o*-phosphoric acid, sodium dodecyl sulfate (SDS), and HPLC grade solvents of Acetonitrile and Methanol were supplied by Scharlau, Barcelona, Spain.

Phosphate buffer preparation for the mobile phase.

A phosphate buffer of pH 3.0 was prepared by dissolving 6.80 g sodium dihydrogen phosphate in 900 mL water adjusting the pH to 3.0 with 0.10 M phosphoric acid and diluting with water to 1L in a volumetric flask.

Preparation of phosphate medium for the dissolution studies.

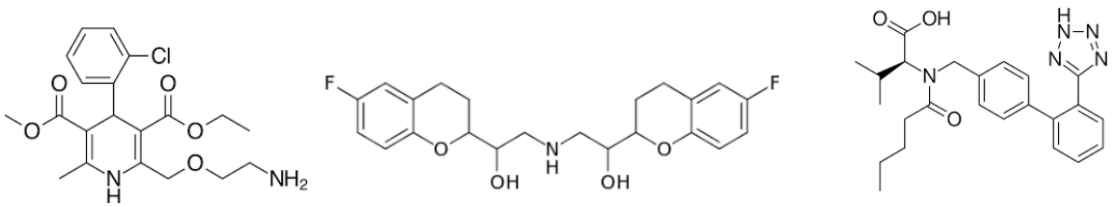
Dissolution medium (pH 6.80, 50 mM phosphate medium) was prepared by dissolving 20.40 g of K_2HPO_4 and 21.18 g of NaH_2PO_4 in 5 L of purified water. The pH of the medium was adjusted to 6.80 (±0.05) with 0.1 M sodium hydroxide solution or 0.1 M phosphoric acid solution, then complete the volume to 6 L with purified water.

Preparation of (67 mM phosphate medium pH 6.80 with 0.5% SDS) for the in-vitro dissolution studies

The dissolution medium was prepared by dissolving 54.67 g of KH_2PO_4 and 30.00 g of SDS in 5 L of purified water. The pH of the medium was adjusted to 6.80 (±0.05) with 0.1 M sodium hydroxide solution or 0.1 M phosphoric acid solution, then complete the volume to 6 L with purified water.

Apparatus

pH meter (Mettler Toledo, 235, MA, USA), Dissolution tester (Electrolab Pvt. Ltd. TDT-08L, Mumbai, India), High-performance liquid chromatography with PDA- detector (Waters, 2690, Milford, UK),Agilent 1290 UPLC system (Deutschland GmbH, Waldbronn, Germany).



Amlodipine **Nebivolol** **Valsartan**

Fig. 1. Chemical structures of VAL, AML and NEB.

Chromatographic conditions

For RP-HPLC, the mobile phase (50 mM sod. dihydrogen phosphate buffer adjusted with 0.1 M o-phosphoric acid to pH 3.0: acetonitrile: methanol) in an isocratic mode with the percentage of (30: 20: 50, v/v%). Using an Inertsil column, (C_{18}, 150 mm x 4.6 mm, 5µm) with flow rate (1.0 mL/min). The wavelength of detection was 281 nm. The temperature of column was 35°C and 100 µl as the injection volume. While for UPLC, The mobile phase (50 mM sod. dihydrogen phosphate buffer adjusted with 0.1 M *o*-phosphoric acid to pH 3.0: acetonitrile) in an isocratic mode with the percentage of (55: 45, v/v%). Using an Eclipse Plus column (C_{18}, 1.8 µm, 50 mm × 2.1 mm) with rate of flow (0.3 mL/min). The wavelength of detection was 240 nm. The temperature of column was 35°C and 5.0 µl as the injection volume.

Preparation of stock and standard solutions

A 160.0 mg of VAL and 10.0 mg NEB were dissolved in 100 mL methanol. Similarly, a 320.0 mg of VAL and 10.0 mg AML were dissolved in 100 mL of methanol. A 1.0 mL from each methanolic solution was separately diluted to the mark with phosphate medium in a 20 mL volumetric flask to yield solutions (A) and (B), respectively. These solutions (A) and (B) were diluted with the mixed mobile phase as needed to prepare different standard solutions over the concentration range of (0.80-72.00, 0.05-4.50) and (4.00-144.00, 0.125-4.50) µg/mL for the assay and *in-vitro* drug release profile of (VAL and NEB) and (VAL and AML)), respectively.

Method validation

The analytical methods were suitably developed and validated [52-56], regarding the accuracy, precision, linearity, Specificity, the limit of detection (LOD) and the quantification limit (LOQ), in agreement to the requirements of ICH guidelines.

Detection limit and quantification limit: LOD is the negligible level of analyte that gives a qualitative response, while LOQ is the lowest concentration that can be quantified reliably with a specified level of recovery and precision. LOD and LOQ were calculated with the following equations as per ICH guidelines.

$$LOD=3.3\times N/S \quad , \quad LOQ=10\times N/S$$

Where N is the standard deviation of the Y-intercepts of the standardization curves and S is the mean slope of the standardization curves.

Selectivity: was evaluated by measuring the response of the blank, placebo, analyst samples and any expected or known species (for example excipients or impurities). It would normally be expected that no response would be obtained that interferes with the response of the analytes.

Linearity: RP-HPLC, prepare serial dilutions from solution (A) to obtain (2, 5, 20, 60, 100, 140, and 180%) of (VAL and NEB) in 10 mL volumetric flasks, then complete to volume with the mobile phase. Standard solutions over a concentration range of (0.80-72.00) and (0.05-4.50) µg/mL for VAL and NEB. While for RP-UPLC, prepare serial dilutions from solution (B) to obtain (5, 20, 60, 100, 140 and 180%) of (VAL and AML) in 10 mL volumetric flasks, then complete to volume with the mobile phase. Standard solutions over a concentration range of (4.00-144.00) and (0.125-4.500) µg/mL for VAL and AML, each in three replicates, were injected into the system. The method of linear regression was used for data evaluation. Linearity was expressed as a correlation coefficient; the value must be >0.98.

Accuracy: is the proximity of conformity between the true quantity and test result. Accuracy was evaluated as a recovery procedure, by spiking of the active drug to the placebo.

Precision: The precision of the method was checked by injecting a standard solution of VAL and NEB (100%) (40.0, 2.5 µg/mL) and a standard solution of VAL and AML (100%) (80.0, 2.5 µg/mL) six times. Peak areas were determined and compared. percentage relative standard deviation RSD% was used as indicator for precision; results must be less than 2%.

Robustness: Robustness of the optimized method was investigated by assessing the solutions used for precision studies with small changes of the given values in column temperature and Buffer pH value. The quantitative effect of the variables was determined by considering the value of Recovery % and the acceptable limits of ±2 % for peak response and retention times of each analyte.

Solution stability: Stability of sample solutions was assessed by analyzing the known concentrations of the three drugs (100%) (40.0, 2.5 µg/mL) VAL and NEB and (100%) (80.0, 2.5 µg/mL) VAL and AML. Replicates (n = 3) were exposed to different conditions, i.e., lab. temperature (15-25 °C) for 2 days and cooled temperature (2-8 °C) 5 days. Results were investigated by comparing with assays of freshly prepared solutions of reference standards.

Results and Discussion

Optimization of experimental parameters

In the RP-HPLC method, and in order to attain simultaneous elution for the two components (NEB and VAL), initial trials were performed with the aim to select adequate and optimum chromatographic characteristics. Parameters, such as an ideal solvent for NEB and VAL, mobile phase and their proportions, detection wavelength, optimum pH value, different columns and concentration of the targeted standard solutions were carefully studied. Several solvents were tested by using different proportions, such as phosphate buffer with varying strength (10-50) mM was tested. Results show that phosphate buffer 50 mM give a stable baseline and sharp peak shape. In order to study how the pH of buffered mobile phase affect the chromatographic separation, the effect of pH buffer on the retention time of each analyte was investigated over the pH range (2.5-6.0). Results obtained showed that the resolution of VAL, NEB decreases as the pH increases. Therefore, a pH of 3.0 was adopted for further investigations as a resolution for studied compounds was greater than 2.5. Different ratios of methanol and acetonitrile as organic modifiers were evaluated. results show that methanol give longer run time than acetonitrile and when using a mixer from methanol, acetonitrile and buffer give good separation and resolution between the investigated drugs. Flow rate from (0.2-2) mL/min was tried. Finally, The mobile phase (50 mM sod. dihydrogen phosphate buffered solution, adjusted to pH 3.0 with diluted phosphoric acid) : (acetonitrile) : (methanol) in an isocratic technique with the percentage of (30: 20: 50, v/v) was selected. Chromatographic separation of VAL and NEB was evaluated Using an Inertsil column, (C_{18}, 150 mm x 4.6 mm, 5µm) with flow rate (1.0 mL/min).

The UV spectrum of 15 µg/mL VAL and NEB was recorded by scanning the range wavelength of 200 nm to 400 nm. From the UV spectrum, a wavelength of 281 nm was selected. At this wavelength, both the drugs show good absorbance. The column temperature was 35°C and 100 µL as the injection volume.

On the other hand, for the RP-UPLC**,** to study the effect of mobile phase buffer pH on chromatographic separation, the effect of buffer pH on the retention time of each analyte was investigated over the range pH value from (2.5-6.0), Results obtained showed that the resolution of VAL, AML decreases as the pH increases. Therefore, a pH of 3.0 was chosen for further investigations as a resolution for studied compounds was greater than 3. Different ratios of methanol and acetonitrile as an organic modifier was evaluated, results show that methanol gave longer run time than acetonitrile and when using a mixture from acetonitrile and buffer give good separation and resolution between VAL and AML. Flow rate from (0.1-1.0) mL/min was tried. Finally, to improve separation and to reduce the analysis time, the composition of the mobile phase was (50 mM sodium dihydrogen phosphate buffered solution, adjusted with diluted phosphoric acid to pH 3.0) : (acetonitrile) in an isocratic mode with the ratio of (55: 45, v/v%). Chromatographic separation of VAL and AML was evaluated using an Eclipse Plus column (C_{18}, 1.8 µm, 50 mm × 2.1 mm) with flow rate (0.3 mL/min).

The UV spectrum of 10 µg/mL VAL and AML was recorded by scanning in the wavelength range of 200 nm to 400 nm. From the UV spectrum wavelength selected as 240 nm. At this wavelength, both drugs show good absorbance. The column temperature was 35°C and 5 µL was the injection volume.

System suitability, the main reason to carry out system suitability was to verify equipment performance; the RSD% for an average area of six replicate injections of working standard was calculated. In the RP-HPLC method, the RSD% for peak area was found as less than 1.5%. Tailing parameter for both the analyte peaks was found to be not more than 1.2 and the theoretical plates discovered were not less than 3000. IN the RP-UPLC, the RSD% for peak area was found as less than 1.2%; the tailing parameter for both the analyte peaks was found to be not more than 1.1 and theoretical plates were found to be not less than 3500.

Selectivity and peak purity, there is no significant peak of placebo as in Figure 2 & 3 at the given retention times. the results demonstrate that the proposed method is specific for quantification of drugs in tablet dissolved into the adopted dissolution medium.

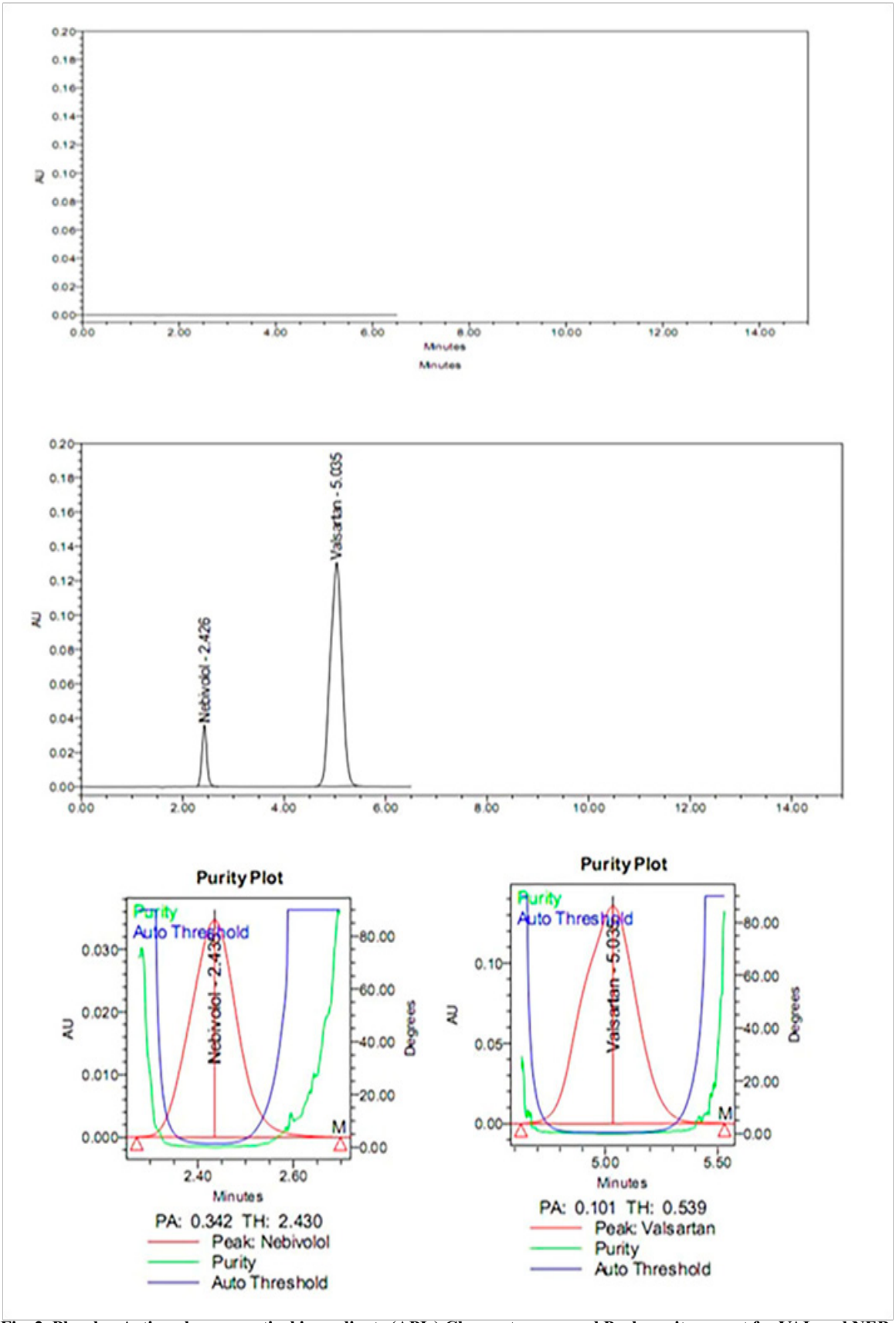


Fig. 2. Placebo, Active pharmaceutical ingredients (APIs) Chromatogram and Peak purity report for VAL and NEB.

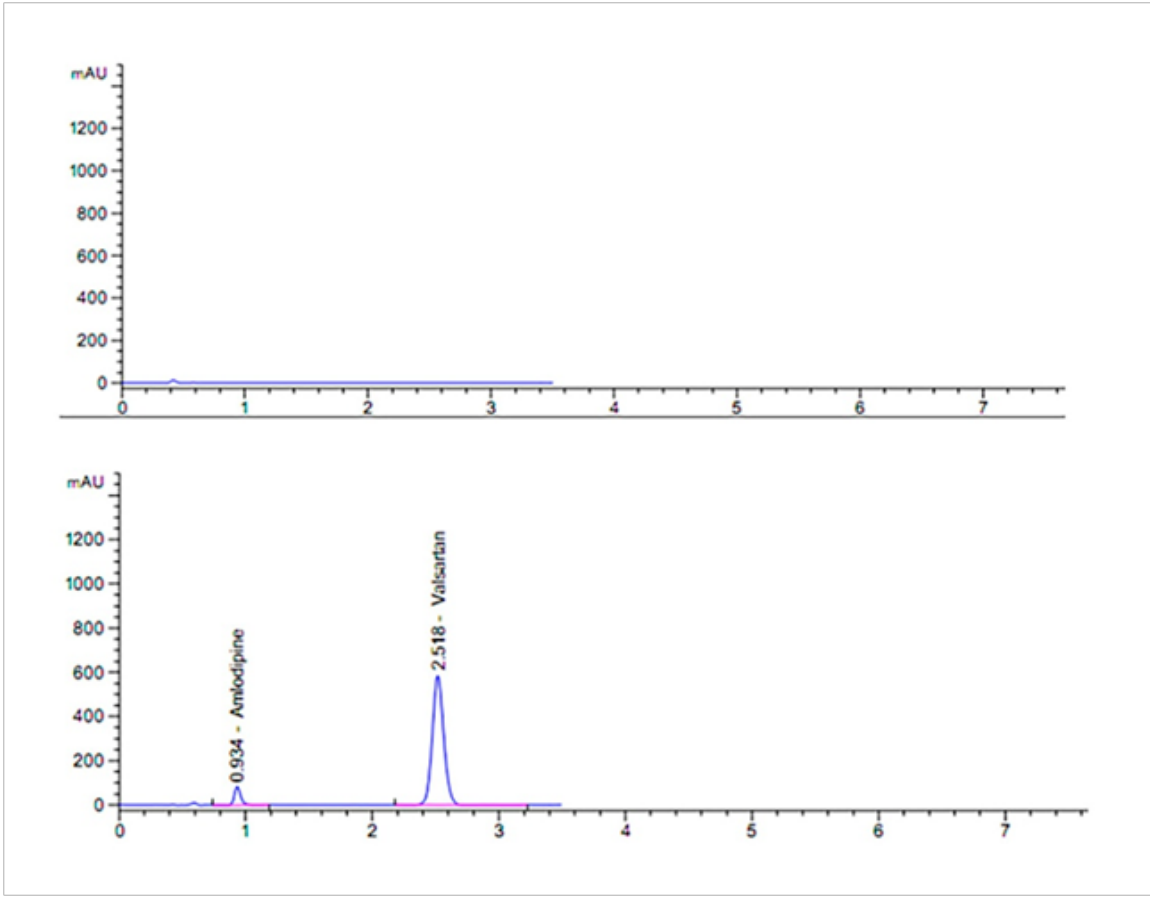


Fig. 3. Placebo and APIs Chromatogram of AML and VAL.

Linearity and range, the standard calibration curves for the drugs were linear over the investigated range with RSD% of less than 2% based on three successive readings. Validation parameters for VAL and NEB are in Table 1, a determination coefficient of 0.999 suggested that the developed RP-HPLC method had an excellent linearity over the concentration ranges (0.80-72.00) and (0.05-4.50) µg/mL for VAL and NEB respectively. While a determination coefficient of 0.999 suggests that the developed RP-HPLC method had an excellent linearity over the concentration range (4.00-144.00) and (0.125-4.500) µg/mL for VAL and AML, respectively.

Accuracy and recovery: The accuracy of the method was assessed by carrying out recovery studies at different spiked levels. At each level, three determinations were performed and the results recorded. The amounts recovered and the percent recovery values were calculated, results are in Table 2. The percentage recovery was found to be within the limit (99.0 -101.0%) with acceptable RSD%.

Precision

Results for precision tests performed on the standard solution of concentration (100%) of drugs showed that the % RSD was in the acceptable range for the standard solution. Therefore, the system precision is confirmed to be satisfactory, results are tabulated in Table 3.

Robustness: The findings in Table 4 indicate that minor modifications in the values provided in chromatographic circumstances do not influence the outcomes for recovery and retention of drugs.

Stability of solution: Stability of drugs solutions was investigated and shown in Table 5. The results confirmed that the solutions were stable for 2 days at room temperature (15-25 °C) and for 5 days at cool temperature (2-8 °C) stored in amber colored flasks protected from light.

TABLE 1. Validation parameters.

Method	RP-HPLC		RP-UPLC	
Parameters	**VAL**	**NEB**	**VAL**	**AML**
Linear range (µg/mL)	(0.80-72.00)	(0.05-4.50)	(4.00-144.00)	(0.125-4.50)
Determination Coefficient (R^2)	0.999	0.999	0.999	0.999
Equation of calibration curve	y = 52,765.93x - 2,057.74	y = 96,521.54x - 268.94	y = 71.49x - 2.18	y = 221.39x - 2.12
SD for the slope of the calibration curve	1581.67	260.47	3.57	2.09
LOD (µg/mL)	0.099	0.009	0.165	0.031
LOQ (µg/mL)	0.299	0.027	0.499	0.095

TABLE 2. Recovery results.

Method	API	Added µg/mL	Found µg/mL	Recovery %	RSD %
RP-HPLC	**VAL**	8.0	8.03	100.38	0.02
		24.0	23.99	99.96	0.18
		40.0	39.92	99.79	0.06
		56.0	56.09	100.18	0.02
	NEB	0.5	0.49	99.54	0.45
		1.5	1.51	100.82	0.05
		2.5	2.51	100.54	0.09
		3.5	3.49	99.96	0.05
RP-UPLC	**VAL**	16.0	15.87	99.20	0.22
		48.0	47.97	99.95	0.09
		80.0	80.12	100.15	0.05
		112.0	112.23	100.21	0.03
	AML	0.5	0.49	98.62	1.87
		1.5	1.51	100.92	0.90
		2.5	2.53	101.08	0.45
		3.5	3.52	100.63	0.32

TABLE 3. Precision results.

Method	API	parameters	Analyst I	Analyst II	Day I	Day II
RP-HPLC	**VAL**	**Mean %**	99.57	100.95	99.90	100.26
	40 µg/mL	**RSD %**	0.16	0.11	0.05	0.22
	NEB	**Mean %**	99.77	100.82	100.16	100.22
	2.5 µg/mL	**RSD %**	0.11	0.04	0.06	0.12
RP-UPLC	**VAL**	**Mean %**	99.99	100.31	99.99	100.17
	80.0 µg/mL	**RSD %**	0.06	0.06	0.07	0.04
	AML	**Mean %**	100.63	99.00	99.93	100.72
	2.5 µg/mL	**RSD %**	0.58	0.67	0.57	0.59

TABLE 4. Robustness results.

Method		RP-HPLC		RP-UPLC	
Parameter/ API		**VAL %**	**NEB %**	**VAL %**	**AML %**
Column temp.	**35°C**	99.95	100.03	99.95	100.11
	32°C	99.94	100.08	99.86	99.39
	38°C	99.95	100.11	99.89	99.75
Buffer pH	**3.00**	99.95	100.09	100.00	98.85
	2.95	99.94	100.03	100.06	99.57
	3.05	99.94	100.07	100.02	99.21

TABLE 5. Solution stability results.

Method	API	Added (µg/mL)	2 days at (15-25)°C, %	RSD%	5 days at (2-8)°C, %	RSD%
RP-HPLC	**VAL**	24.0	100.06	0.36	100.11	0.04
		40.0	100.12	0.04	100.02	0.05
		56.0	100.19	0.01	100.17	0.03
	NEB	1.5	100.29	0.18	100.25	0.08
		2.5	100.14	0.07	100.03	0.04
		3.5	100.21	0.03	100.11	0.02
RP-UPLC	**VAL**	48.0	100.23	0.07	100.21	0.06
		80.0	100.00	0.05	99.92	0.06
		112.0	100.15	0.03	100.19	0.05
	AML	1.5	100.32	0.30	99.31	0.77
		2.5	99.93	0.48	98.79	0.46
		3.5	100.29	0.39	99.86	0.19

Applications of the proposed methods

Potency determination for VAL and NEB by-RP-HPLC method.

Standard solution preparation

160.0 mg of VAL and 10.0 mg NEB were dissolved in 100 mL methanol. A 1.0mL of this methanolic solution was transferred into 20 mL volumetric flask that was completed with phosphate medium to yield solution (A). 5.0mL from solution (A) was diluted into 10 mL volumetric flask and the volume was diluted with the mixed mobile phase to yield solution (100 %) (40.0 and 2.5) µg/mL (VAL and NEB).

Test solution preparation

Ten tablets were grinded to fine powder and the average weight equivalent to (80 and 5) mg (VAL and NEB) was dissolved into a 100 mL volumetric flask containing 50 mL methanol then sonicated for 15 min and diluted to volume with methanol. A 1.0mL of this methanolic solution was diluted in a 10 mL volumetric flask using the phosphate medium to yield solution (B). A 5.0mL from solution (B) was diluted in a 10 mL volumetric flask with the mixed mobile phase to give conc. (40.0 and 2.5) µg/mL (VAL and NEB).

Potency determination for VAL and AML by RP-UPLC method

Standard solution preparation

A 320.0 mg of VAL and 10.0 mg AML were dissolved in methanol in a 100 mL volumetric flask. A 1.0mL of this methanolic solution was diluted in a 20 mL volumetric flask using the phosphate medium to yield solution (B). A 5.0mL from solution (B) was diluted in a 10 mL volumetric with the mixed mobile phase to obtain conc. (80.0 and 2.5) µg/mL (VAL and AML).

Test solution preparation

Ten tablets were grinded and the average weight equivalent to (160 and 5) mg (VAL and AML) was dissolved in methanol a 100 mL volumetric with sonication for 15 min. A 1.0 mL of this methanolic solution was dilutedin a 10 mL volumetric flask with the phosphate medium to yield solution (B). A 5.0mL from solution (B) was diluted into a 10 mL volumetric flask with the mixed mobile phase solution to obtain conc. (80.0 and 2.5) µg/mL (VAL and AML).

Potency Results

In-Vitro dissolution studies

Dissolution studies on the fixed combination tablet formulation (80.0 mg VAL and 5.0 mg NEB) were conducted using USP Apparatus I (basket method) at 100 rpm with six replicates at 37±0.5°C. Phosphate buffer (pH 6.8) with 0.5% SDS was used as the medium for dissolution (900 mL) [53]. At predetermined time intervals (5, 10, 15, 20 and 30 min), while dissolution studies on the fixed combination tablet formulation (160.0 mg VAL and 5.0 mg AML) were conducted using USP Apparatus II (paddle method) at 75 rpm with six replicates at 37±0.5°C. Phosphate buffer (pH 6.8) was used as the medium for dissolution (1000 mL). At programmed time intervals (5, 15, 30 and 45 min); the 10 mL aliquot was removed and substituted with an equivalent amount of new medium to preserve a steady complete quantity. After the dissolution samples are filtered by 0.45 µm nylon syringe filter discarding first 5 mL, 500 µL samples were mixed with 500 µL of the mobile phase. The concentrations of (VAL, NEB) and (VAL, AML) in these samples were determined simultaneously by the developed and validated RP-HPLC and RP-UPLC methods. The percentage drug released at each point of moment was calculated using the following equation.

%Dissolved = (Amount dissolved/Labeled amount) *100

Dissolution profiles results

The validated RP-HPLC method was productively used for the determination of the average percentage of drugs released within 30 min for the *discriminative* dissolution of tablets containing fixed combination of drug product. The *in-vitro* discriminative dissolution behavior revealed that NLT 85% of labeled amounts of VAL and NEB from their fixed combination solid dosage form were released within 20 min as in Figure 6. At the end of the dissolution test (30 min), the released% of the drugs were found as 97.93% and 96.79% for VAL and NEB, respectively. While, the developed RP-UPLC method was successfully used for the determination of the average percentage of drugs released within 45 min for *in-vitro* dissolution of tablets containing fixed combination of drug product. The *in-vitro* dissolution studies revealed that NLT 85% of labeled amounts of VAL and AML from their fixed combination tablet dosage form were released within 15 min. At the end of the dissolution test (45 min), the released% of the drugs were found as 97.19 and 95.78% for VAL and AML, respectively. The discriminative dissolution pattern is in agreement with the FDA Guidance standards indicating the suitability of the proposed optimized method for the dissolution study of the drugs.

TABLE 6. Potency results for RP-HPLC and RP-UPLC method.

Method	RP-HPLC		RP-UPLC	
EXP.#	**VAL assay %**	**NEB assay %**	**VAL assay %**	**AML assay %**
I	99.00	101.18	101.34	102.46
II	98.99	100.98	101.24	101.92
III	98.99	101.03	101.31	102.82
Average	99.00	101.07	101.30	102.40
RSD %	0.01	0.10	0.05	0.44

TABLE 7. Comparison between the present work and the previously published about the tested drugs is described.

Method	API	Detection nm	Linear range µg mL^{-1}	Recovery % Mean ± RSD%	LOQ µg mL^{-1}	Run Time min	Reference #
HPLC	Amlodipine	240	0.1-50	98.5±0.8	0.1	9	**1**
	Valsartan		50 - 0.05	99.6±0.6	0.05		
HPLC	Valsartan	210	48-112	100.21±0.42	9.557	6	**2**
	Nebivolol		3-7	100.03±0.64	3.154		
HPLC	Valsartan	275	5-30	99.58	0.095	12	**3**
	Nebivolol		2-12	100.22	0.38		
UV	Amlodipine	239	1-32	100.63±1.01	0.29	NA	**5**
	Valsartan	250	4-40	99.37±0.67	0.89		
	HCT	272	2-20	100.08±1.31	0.58		
UV	Nebivolol	284	20-80	99.90±0.17	1	NA	**7**
HPTLC	Nebivolol	271	0.50-2.50	99.79±0.08	0.135	0.9	**9**
	Amlodipine		0.25-1.25	99.48±0.11	0.094		
HPLC	Valsartan	289	160-400	99.08±1.05	53.29	15	**12**
	Nebivolol		10-250	99.05±1.01	6.92		
HPLC	Valsartan	230	1-200	101.2±0.32	1	30	**15**
	Ezetimibe			99.6±0.55			
HPLC	valsartan	210	10-100	99.76±0.17	0.06	20	**17**
HPLC	Valsartan	281	0.80-72.00	99.79±0.06	0.299	6.0	**Current methods**
	Nebivolol		0.05-4.50	100.54±0.09	0.027		
UPLC	Valsartan	240	4.00-144.00	100.15±0.05	0.499	3.0	
	Amlodipine		0.125-4.50	101.08±0.45	0.095		

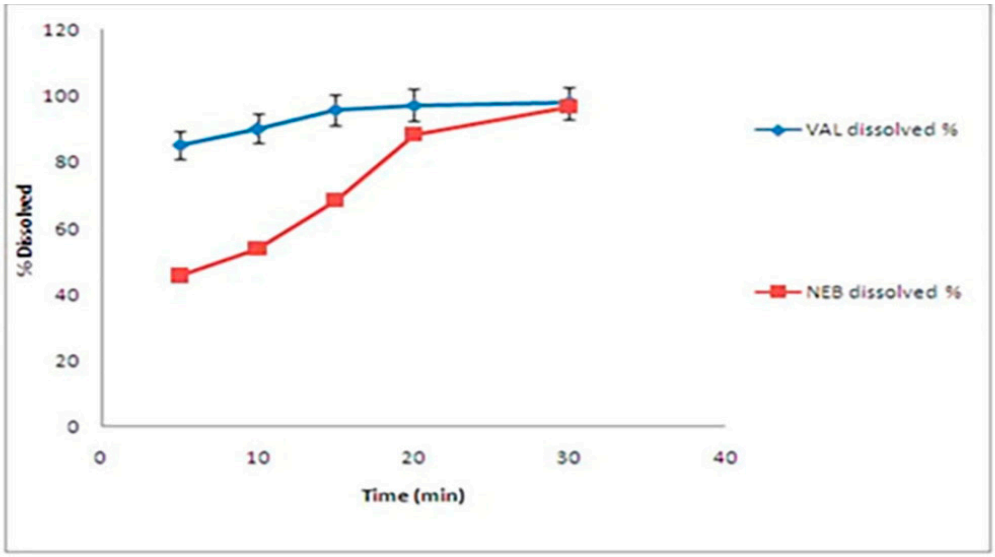


Fig. 4. Dissolution profiles result for VAL and NEB.

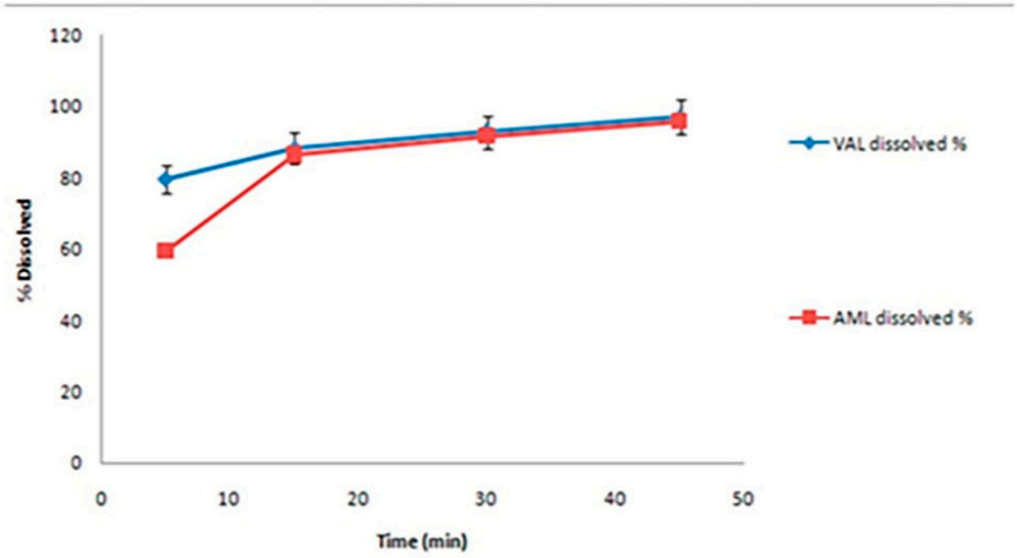


Fig.5. Dissolution profiles result for VAL and AML.

Conclusion

In this work, simple, fast and reliable RP-HPLC and RP-UPLC methods were optimized and validated according ICH guidelines for the simultaneous determination of (VAL, NEB) and (VAL, AML) in discriminative dissolution samples. The optimized methods demonstrate excellent efficiency in linearity, sensitivity, recovery, precision, and selectivity. The established methods were effectively implemented to analyze the studied drugs in dissolution samples suggesting that this can be applied successfully to the routine work of quality control.

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تعيين نسبة الفالسارتان في وجود النيبيفالول او الاملوديبين في الجرعة الدوائيه الصلبه وتطبيقها لتقدير نسبة معدل الذوبان باستخدام RP-HPLC & RP-UHPLC

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RP-HPLC لتحديد في وقت واحد نسبة كلا من النيبيفالول NEB **وفالسارتان** VAL **في شكل جرعة الدواء وتطبيقها لتقدير نسبة معدل الذوبان.**

كانت الطريقة المقترحة خطية على مدى تركيز (72.00-0.80) و (4.50-0.05) ميكروغرام / مل لل VAL و NEB على التوالي. كشفت دراسات الذوبان أنه تم إصدار ما لا يقل عن 85% من كمية VAL في غضون 15 دقيقة ، وأطلق سراح ما لا يقل عن 85% من كمية NEB في غضون 20 دقيقة من شكل جرعات قرص ثابت مزيج. تم التحقق من صحة طريقة RP-HPLC المطورة بما في ذلك هذه العناصر (الانتقائية والخطية والدقة) وفقًا لإرشادات (ICH).

RP-UHPLC لتحديدفي وقت واحد نسبة كلا من أملوديبين AML **وفالسارتان** VAL **في شكل جرعة قرص وتطبيقها على دراسات معدل الذوبان.**

كانت الطريقة المقترحة خطية على مدى تركيز (144.000-4.000) و (4.500-0.125) ميكروغرام / مل ، لل VAL و AML على التوالي. كشفت دراسات معدل الذوبان أن ما لا يقل عن 85% من كمية VAL و AML تم إطلاقها في غضون 15 دقيقة من شكل جرعة الدواء الثابتة المركبة. تم التحقق من صحة طريقة RP-UHPLC المطورة بما في ذلك هذه العناصر (الانتقائية والخطية والدقة) وفقًا لإرشادات (ICH).