

## Stability Indicating RP-HPLC Method for Simultaneous Determination of Atorvastatin and Amlodipine from Their Combination Drug Products

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The study describes development and subsequent validation of a stability indicating reverse-phase HPLC method for the simultaneous estimation of atorvastatin (ATV), and amlodipine (AML) from their combination drug product. The proposed RP-HPLC method utilizes a Lichrospher<sup>®</sup> 100 C<sub>18</sub>, 5  $\mu$ m, 250 mm  $\times$  4.0 mm i.d. column, at ambient temperature, optimum mobile phase consisted of acetonitrile and 50 mM potassium dihydrogen phosphate buffer (60 : 40, v/v), apparent pH adjusted to 3  $\pm$  0.1 with 10% phosphoric acid solution, effluent flow rate monitored at 1.0 ml/min, and UV detection at 254 nm. ATV, AML, and their combination drug product were exposed to thermal, photolytic, hydrolytic, and oxidative stress conditions, and the stressed samples were analyzed by proposed method. The method was applied for the *in vitro* dissolution of marketed combination drug products. The described method was linear over the range of 1–90  $\mu$ g/ml and 1–80  $\mu$ g/ml for ATV and AML, respectively. The mean recoveries were 99.76 and 98.12% for ATV and AML, respectively. The intermediate precision data obtained under different experimental setup, the calculated value of coefficient of variation (CV, %) was found to be less than critical value. The limit of detection for ATV and AML were found to be 0.4 and 0.6  $\mu$ g/ml, respectively and the limit of quantification was 1.0  $\mu$ g/ml for both drugs. The average percentage drug release was found to be more than 70% within 30 min for both drugs. Chromatographic peak purity data of ATV and AML indicated no co-eluting peaks with the main peaks of drugs which demonstrated the specificity of assay method for their estimation in presence of degradation products. The proposed method can be useful in the quality control and *in vitro* dissolution of combination drug products.

**Key words** RP-HPLC; atorvastatin; amlodipine; stability indicating; forced degradation

Atorvastatin<sup>1)</sup> is a synthetic lipid lowering agent which inhibits HMG-CoA reductase and amlodipine<sup>2)</sup> is a calcium antagonist drug effective in hypertension and angina pectoris. The combination drug product of atorvastatin (ATV) and amlodipine (AML) has recently been introduced in the market; co-administration of AML with ATV demonstrated statistically significant dose-related reductions in systolic blood pressure (SBP), diastolic blood pressure (DBP) and LDL-C in patients with co-morbid hypertension and dyslipidemia.<sup>3)</sup> Chemically ATV is [R-(R\*,R\*)]-2-(4-fluorophenyl)- $\beta$ , dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino) carbonyl]-1H-pyrrole-1-heptanoic acid, calcium salt (2 : 1) trihydrate<sup>4)</sup> and AML is 2-[(2-Aminoethoxy)-methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridinedicarboxylic acid 3-ethyl 5-methyl ester.<sup>5)</sup>

Stability testing forms 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 drug product that play an important role in shelf life determination are assay of active drug, and degradation products generated, during the stability study. The assay of drug product in stability test sample needs to be determined using stability indicating method, as recommended by the International Conference on Harmonization (ICH) guidelines<sup>6)</sup> and USP-26.<sup>7)</sup> 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 analytical LC procedure, which would serve as stability indicating assay method for combination drug product of ATV and AML.

Both the drugs ATV and AML are not official with USP 26. EP 2002<sup>8)</sup> describes an HPLC method for the determination of AML, but does not involve simultaneous determination of ATV. Detailed survey of literature for ATV revealed several methods based on different techniques, *viz.* HPLC<sup>9–11)</sup> and LC-MS<sup>12–14)</sup> for its determination in plasma/serum; HPLC<sup>15)</sup> for its determination in human serum and pharmaceutical formulations; HPLC<sup>16,17)</sup>; HPTLC<sup>18)</sup> for its determination in pharmaceuticals. Similarly, survey of literature for AML revealed methods based on spectrophotometry,<sup>19)</sup> RP-HPLC<sup>20)</sup> using fluorescence detection, HPLC-tandem mass spectrometry,<sup>21,22)</sup> RP-HPLC using UV detection,<sup>23,24)</sup> HPLC<sup>25–29)</sup> in combination with other drugs, Flow injection analysis using UV-detection,<sup>30)</sup> HPTLC,<sup>31,32)</sup> stability indicating HPLC<sup>33)</sup> and stability indicating HPLC<sup>34)</sup> in combination with benazepril hydrochloride have been reported. Spectrophotometric<sup>35)</sup> and HPLC<sup>36)</sup> methods have been reported for simultaneous determination of ATV and AML, but these methods lack stability indicating nature. None of the reported analytical procedures describe a stability indicating method for simultaneous determination of ATV and AML in presence of their degradation products.

This manuscript describes the development and validation of a stability indicating isocratic reversed-phase HPLC method for simultaneous determination of ATV and AML in presence of their degradation products as per ICH guidelines.

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To establish the stability indicating nature of the method, forced degradation of drug substances and drug product was performed under stress conditions (thermal, photolytic, acid/base hydrolytic and oxidative), and stressed samples were analyzed by the proposed method. The proposed LC method was able to separate both drugs from degradation products generated during forced degradation studies. The method was validated as per ICH guidelines<sup>37)</sup> and its updated international convention.<sup>38)</sup> The linearity of response, accuracy and intermediate precision of the described method has been checked.

### Experimental

**Chemicals and Reagents** ATV and AML working standards were generous gifts from Torrent Research Centre, Ahmedabad, India and Excel Laboratory Pvt. Ltd., Mehsana, India, respectively. Combination drug products of ATV and AML (Label claim: atorvastatin calcium equivalent to atorvastatin 10 mg, and amlodipine besylate equivalent to amlodipine 5 mg), A<sup>2</sup>F Tablets (Zydus Cadila, India), Avas<sup>TM</sup> AM Tablets (Micro Labs, India), purchased from local pharmacy. Acetonitrile, methanol, water, potassium dihydrogen phosphate and sodium hydroxide of Rankem used were of HPLC grade. *Ortho*-phosphoric acid used was of analytical reagent grade (S.D. Fine Chemicals Ltd., Mumbai, India). Hydrochloric acid and hydrogen peroxide were from Qualigens Fine Chemicals (Glaxo Ltd.).

**HPLC Instrumentation and Conditions** The HPLC system consisted of Hitachi pump L-7110, Rheodyne universal injector 77251 and Hitachi L-7420 UV-Visible detector. The chromatographic separations were performed using Lichrospher<sup>®</sup> 100 C<sub>18</sub>, 5  $\mu$ m, 250 mm  $\times$  4.0 mm i.d. column, at ambient temperature, eluted with mobile phase at the flow rate of 1.0 ml/min. The mobile phase consisted of acetonitrile and 50 mM potassium dihydrogen phosphate buffer (60:40, v/v), apparent pH adjusted to 3  $\pm$  0.1 with 10% phosphoric acid solution, filtered through 0.45  $\mu$ m nylon filter and degassed in ultrasonic bath prior to use. Wavelength was selected by scanning standard solutions of both drugs over 200 to 400 nm wavelengths using Shimadzu model 1601 double beam UV-visible spectrophotometer with a pair of 10 mm matched quartz cells. Measurements were made with injection volume 20  $\mu$ l and ultraviolet (UV) detection at 254 nm, as both components shows reasonable good response at this wavelength.

**Standard and Sample Preparation** The standard stock solutions 1 mg/ml each of ATV and AML were prepared separately by dissolving working standards in methanol and diluting with the same solvent. Standard calibration solutions of ATV and AML having concentration in the range of 1–100  $\mu$ g/ml were prepared by diluting stock solutions with mobile phase.

**Analysis of Dosage Forms** Twenty tablets were weighed, their mean weight determined, and crushed in mortar. An amount of powdered mass equivalent to 25 mg of ATV and 12.5 mg of AML was weighed and transferred in conical flask. The drugs from powder were dissolved and extracted with methanol. To ensure complete extraction of drugs it was sonicated for 30 min. The extract was filtered through Whatmann filter paper No. 41 and residue was washed with methanol. The extract and washing were pooled and transferred to a 25 ml volumetric flask and volume was made with methanol. Ten ml aliquot from above solution was transferred in 50 ml volumetric flask and volume was adjusted with mobile phase up to mark (sample stock solution). Appropriate aliquots from sample stock solution were suitably diluted with mobile phase in such a way to achieve concentrations in a range of 1–90  $\mu$ g/ml for both drugs and used for injection on HPLC.

**Procedure for Forced Degradation Study** Forced degradation of each drug substances and the drug product was carried out under thermolytic, photolytic, acid/base hydrolytic and oxidative stress conditions. Thermal and photo-degradation of drug substances and drug product were carried out in solid state. After the degradation stock solutions were prepared by dissolving in methanol to achieve concentration of 1 mg/ml. From these solutions aliquots were diluted with mobile phase to achieve a concentration of 40  $\mu$ g/ml, each of ATV and AML.

For hydrolytic and oxidative degradation, solutions were prepared by dissolving drug substance or drug product in small volume of methanol and later diluted with, hydrochloric acid, sodium hydroxide, or hydrogen peroxide solution, to achieve a concentration of 200  $\mu$ g/ml each of ATV and AML. After the degradation aliquots from these solutions were diluted with mobile phase to achieve a concentration of 40  $\mu$ g/ml each of ATV and AML. Based on the labeled strength of ATV and AML in tablets, the nominal concentration of ATV and AML in its solution was 40 and 20  $\mu$ g/ml, respec-

tively.

For thermal stress, samples of drug substances and drug product were placed in a controlled-temperature oven at 80  $^{\circ}$ C for 48 h.

For photolytic stress, samples of drug substances and drug product, in solid state, were irradiated with UV radiation having peak intensities at 254 and 366 nm.

Acid hydrolysis of drug substance and drug product in solution state was conducted with 0.5 N hydrochloric acid at ambient temperature for 48 h. During the initial forced degradation experiments it was observed that basic hydrolysis was a fast reaction for AML and almost complete degradation occurred when 0.5 N sodium hydroxide solution. Thus, in later experiment, base hydrolysis of drug substance and drug product in solution state was conducted by 0.01 N sodium hydroxide solution at ambient temperature for 48 h. For oxidative stress, sample solutions of drug substance and drug product in 3% hydrogen peroxide were kept at ambient temperature for 48 h. All the samples solution for acid/base hydrolysis and oxidation were kept in a dark to prevent the effect of light.

**In Vitro Dissolution of Tablets** *In vitro* dissolution of six tablets of two different brands containing ATV and AML was performed using triple distilled water as dissolution media at 50 rpm using USP apparatus 2. Dissolution study was carried out in a 900 ml of triple distilled water at 37  $\pm$  0.5  $^{\circ}$ C using paddle method. Five ml of sample was withdrawn and replaced with fresh dissolution medium at time intervals of 15, 30, 45, and 60 min and clarified using cooling centrifuge. The concentrations of ATV and AML in the samples were determined by proposed RP-HPLC method. According to the BP 2005, not less than 70% of the active ingredients of the labeled claim should dissolve within 45 min.

### Results and Discussion

The reported spectrophotometric<sup>35)</sup> and HPLC<sup>36)</sup> methods for simultaneous determination of ATV and AML from their binary mixture and combination drug product, respectively does not give data on specificity for their estimation in the presence of degradation products or impurities. The reported LC method describes RP-HPLC procedure employing a C18 column and mobile phase comprising acetonitrile and 0.03 M phosphate buffer pH 2.9, in the ratio of 55:45, v/v. The EP 2002 monograph for AML<sup>8)</sup> also states a reversed-phase HPLC method using C18 column for its assay and related compounds. The mobile phase composition in this method is buffer-acetonitrile-methanol (50:15:35, v/v/v) with pH of buffer adjusted to 3.0. Using these methods it could not be possible to separate ATV and AML and the degradation products generated during forced degradation studies. In case of RP-HPLC various columns are available, but as the main aim of the method is to resolve both the compounds in presence of degradation products and impurities, C18 column (250 mm  $\times$  4.0 mm i.d., 5  $\mu$ m particle size) was preferred over other columns. A Lichrospher<sup>®</sup> 100 C18 column was preferred as it has high carbon loading with very closely packed material to give high resolution over other C18 columns. To develop a precise, accurate, specific and suitable stability indicating RP-HPLC method for the simultaneous estimation

Table 1. Results of Forced Degradation Study Samples Using Proposed Method, Indicating Percentage Degradation of ATV and AML

Stress condition/duration/state	(% ) Degradation	
	ATV	AML
Thermal/80 $^{\circ}$ C/48 h/solid	6.9	2.4
Photo/UV 254 and 366 nm/48 h/solid	4.8	6.56
Acidic/0.5 N HCL/48 h/solution	89.3	25.83
Alkaline/0.01 N NaOH/48 h/solution	8.9	55.99
Oxidative/3.0% H <sub>2</sub> O <sub>2</sub> /48 h/solution	9.96	73.7

of ATV and AML, different mobile phases were employed and proposed chromatographic condition was found appropriate for the quantitative determination in presence of degradation products and impurities. The optimum mobile phase consisted of acetonitrile and phosphate buffer (60 : 40, v/v), apparent pH adjusted to  $3 \pm 0.1$  with 10% phosphoric acid solution, selected because it was found to ideally resolve the peaks of ATV ( $t_R = 5.02$  min) and AML ( $t_R = 2.79$  min), with

clear line separation in presence of their degradation products and impurities at effluent flow rate of 1.0 ml/min. UV detection wavelength at 254 nm, injection volume  $20 \mu\text{l}$ , ambient temperature for column and HPLC system was found to best for analysis.

Singh and Bakshi, in their article on stress testing,<sup>39)</sup> suggested a target degradation of 20–80% for the establishing stability indicating nature of the assay method, as even inter-

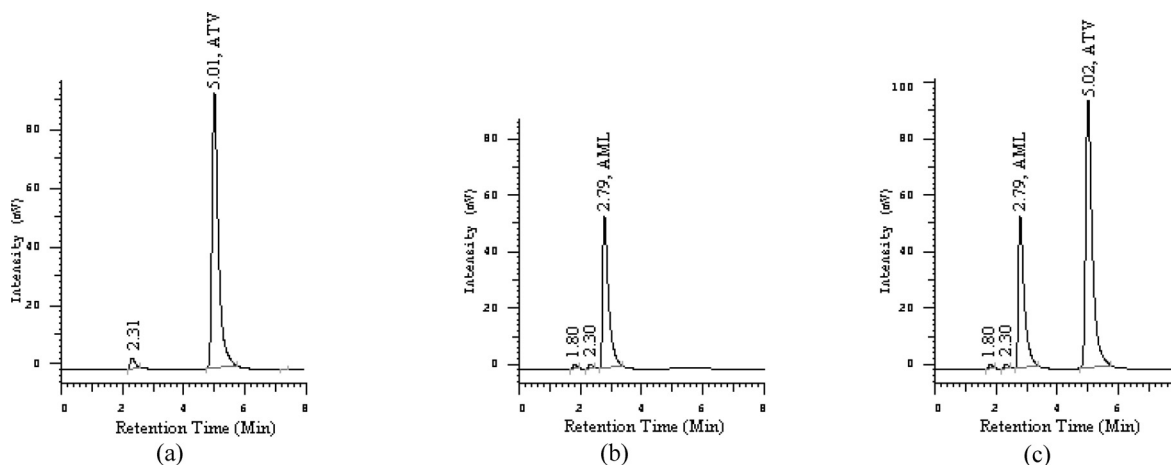


Fig. 1. Chromatograms of Untreated (a) ATV, (b) AML and (c) Tablet Solutions

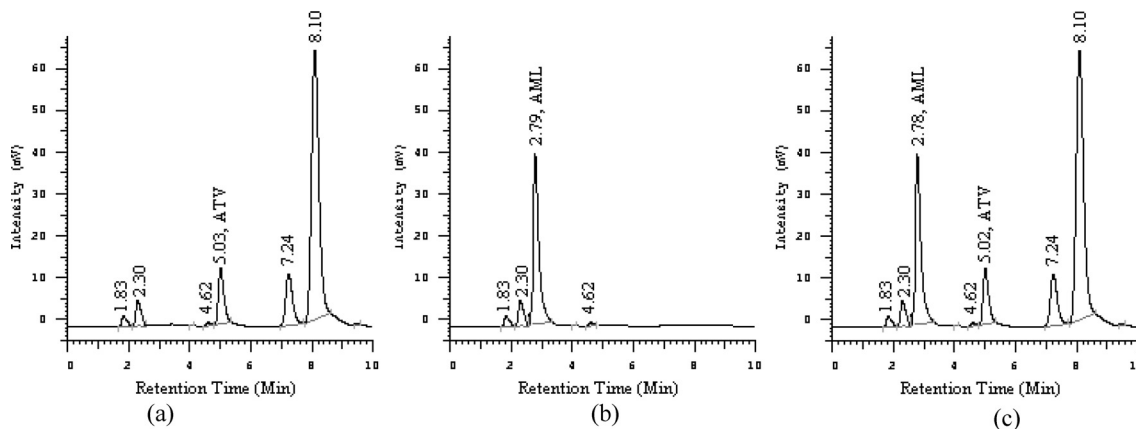


Fig. 2. Chromatograms of Acid Hydrolysis-Degraded (a) ATV, (b) AML and (c) Tablet Solutions

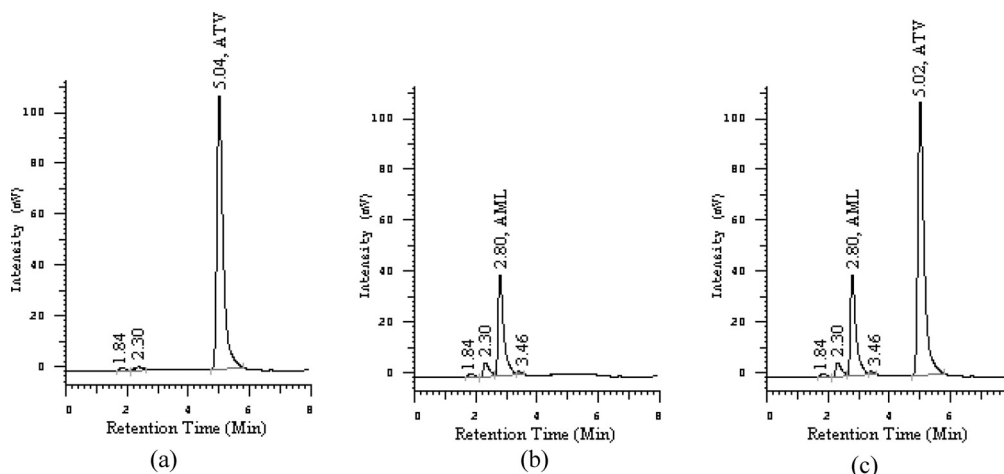


Fig. 3. Chromatograms of Base Hydrolysis-Degraded (a) ATV, (b) AML and (c) Tablet Solutions

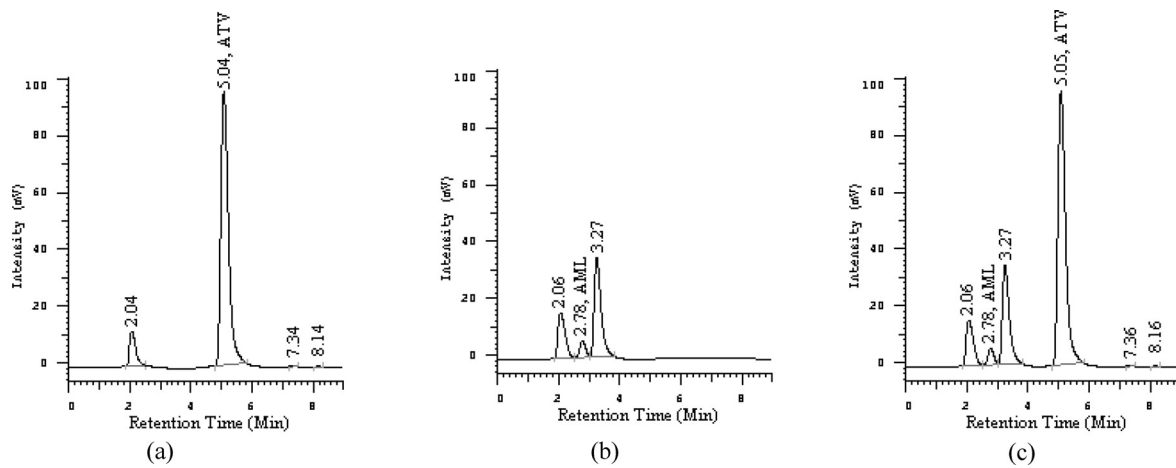


Fig. 4. Chromatograms of Oxidative-Degraded (a) ATV, (b) AML and (c) Tablet Solutions

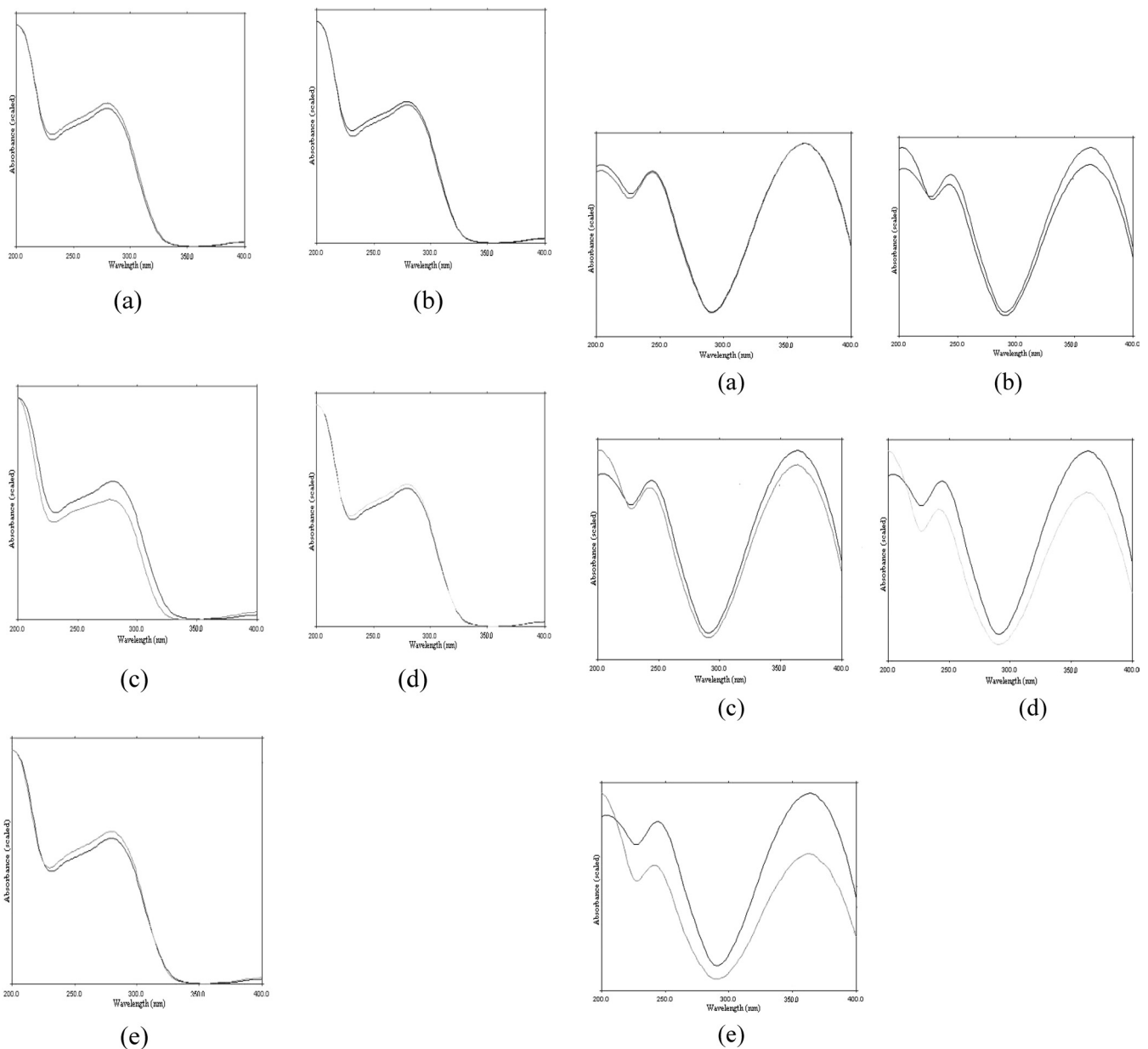


Fig. 5. Spectral Overlay of Standard ATV with (a) Thermal Degraded Sample, (b) Photo Degraded Sample, (c) Acid Degraded Sample, (d) Base Degraded Sample and (e) Oxidative Degraded Sample of ATV

Fig. 6. Spectral Overlay of Standard AML with (a) Thermal Degraded Sample, (b) Photo Degraded Sample, (c) Acid Degraded Sample, (d) Base Degraded Sample and (e) Oxidative Degraded Sample of AML

mediate degradation products should not interfere with any stage of drug analysis. Though conditions used for forced degradation were attenuated to achieve degradation in the range of 20–80%, this could not be achieved in some cases even after exposure for prolonged duration. AML showed extensive degradation in basic hydrolytic condition while ATV in acidic hydrolytic condition. Table 1 indicates the extent of degradation of ATV and AML under various stress conditions. Figs. 1a–c to 4a–c show the chromatograms of forced degraded samples. The peak purity of ATV and AML were assessed by comparing the shape of spectra of standard drugs and degraded samples at the upslope, the apex and at the downslope of the peak<sup>40</sup> as shown in Figs. 5a–e and 6a–e. No other co-eluting peak was found with the main peaks suggested the specificity of the method for the simultaneous estimation of ATV and AML in presence of degradation products and impurities.

The described method has been validated, apart from specificity, for linearity, system suitability, accuracy, and intermediate precision. The standard solutions for linearity were prepared five times at different concentration levels. Characteristic parameters for regression equation and system suitability are given in Table 2.

Repeatability of measurements of peak area was carried out using seven replicates of same concentration (20 µg/ml for each drugs). The intra- and inter-day variation of the method was carried out at four different concentration levels, (10, 20, 30, 40 µg/ml). The low % CV values of within a day and day to day variations for ATV and AML revealed that the proposed method is precise (Table 3).

Accuracy of method was checked by recovery study using standard addition method, at three different concentration levels, *i.e.* multilevel recovery study. The pre-analyzed samples were spiked with extra 25, 50, 75% of the standard ATV and 50, 100, 200% of standard AML and the mixtures were

analyzed by proposed method. Recovery of standard drugs added was found to be 98.95–100.39% for ATV and 97.92–98.60% for AML with the value of % CV less than 2 indicating proposed method is accurate for the simultaneous estimation of ATV and AML from their combination drug products in presence of their degradation product(s) and impurities. Results of recovery study are shown in Table 4.

#### Assay of ATV and AML from Its Tablet Dosage Forms

The assay results of ATV and AML in tablet dosage forms were comparable with the value of label claimed. The results presented in Table 5 indicate the suitability of the method for routine analysis of ATV and AML from their combination drug products.

**In Vitro Dissolution of Tablets** The average percentage drugs released within 60 min as detected by proposed RP-HPLC method after *in vitro* dissolution of tablets of two different brands containing combination drug product are depicted in Table 6. The dissolution pattern complies with the Pharmacopoeial standards indicating suitability of proposed

Table 2. Regression Characteristics and System Suitability Parameters of Proposed RP-HPLC Method

Parameters	ATV	AML
Retention time (min)	5.03	2.79
Tailing factor	1.28	1.20
Asymmetry	1.23	1.30
Theoretical plates	1677.3	727.8
Linearity range (µg/ml)	1–90	1–80
Limit of Detection (LOD) (µg/ml)	0.4	0.6
Limit of Quantification (LOQ)	1.0	1.0
Regression equation ( $y^* = a + bc$ )		
Slope ( <i>b</i> )	43161	17964
Intercept ( <i>a</i> )	+8107.1	–21144
Correlation coefficient ( <i>r</i> )	0.9996	0.9998
Method precision (CV, %) ( <i>n</i> = 7)	0.716	0.728

$y^* = a + bc$ , where *c* is the concentration.

Table 3. Intra- and Inter-day Precision Data of Proposed RP-HPLC Method

Concentration added (µg/ml)	ATV		AML	
	Mean ± S.D. ( <i>n</i> = 7)	CV (%)	Mean ± S.D. ( <i>n</i> = 7)	CV (%)
Intra-day precision				
10	422946 ± 3198.07	0.756	162333 ± 1937.47	1.193
20	876047 ± 6901.77	0.788	327574 ± 2721.38	0.831
30	1336116 ± 9702.27	0.726	503763 ± 4670.82	0.927
40	1828253 ± 12758.0	0.698	694284 ± 5873.69	0.574
Inter-day precision				
10	423786 ± 4520.68	1.067	161975 ± 2001.03	1.235
20	877469 ± 8296.16	0.945	327289 ± 3110.73	0.950
30	1336037 ± 7963.10	0.596	501354 ± 4542.80	0.906
40	1831253 ± 13908.9	0.759	697387 ± 7509.29	1.077

Table 4. Recovery Data for the Proposed RP-HPLC Method (*n* = 5)

Drug	Level	Amount of sample taken (µg/ml)	Amount of standard spiked (µg/ml)	Amount of standard recovered (µg/ml)	Recovery (%)	CV (%)
ATV	I	20	5	4.95	98.95	0.659
	II	20	10	9.99	99.95	0.693
	III	20	15	15.06	100.39	0.828
AML	I	10	5	4.89	97.92	1.721
	II	10	10	9.86	98.60	1.121
	III	10	20	19.57	97.85	0.515

Table 5. Analysis of ATV and AML from Their Combination Drug Products by Proposed RP-HPLC Method (n=5)

Formulations	Labeled amount (mg)		Amount found (mg)		Assay (%)±CV (%)	
	ATV	AML	ATV	AML	ATV	AML
Set-1	10	5	10.02	4.99	100.17±0.73	99.78±1.54
Set-2	10	5	9.99	4.95	99.99±0.72	98.95±1.44

Table 6. Average Percentage Drug Release in Water

Time (min)	Average % drugs release			
	ATV		AML	
	Brand I	Brand II	Brand I	Brand II
15	63.75	80.74	95.34	86.97
30	78.64	89.88	101.91	89.21
45	83.78	92.22	105.71	89.51
60	88.54	91.69	106.23	95.45

method for the dissolution study of the two drugs.

### Conclusion

Based on the peak purity results, obtained from the analysis of forced degraded 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 ATV and AML in presence of degradation products and impurities. 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 ATV and AML in their combination drug product. The proposed method can also be conveniently adopted for dissolution testing of tablets containing ATV and AML.

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