

Stability Indicating LC Method For the Simultaneous Determination of Amlodipine and Olmesartan in Dosage Form

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Abstract

A simple, rapid, and precise method is developed for the quantitative simultaneous estimation of amlodipine (AM) and olmesartan (OL) in combined pharmaceutical dosage form. A chromatographic separation of the two drugs was achieved with an ACE 5 C₁₈ 25-cm analytical column using buffer–acetonitrile (60:40, v/v). The resolution between OL and AM was found to be more than 12. Theoretical plates for OL and AM were 6970 and 11,841, respectively. Tailing factor for OL and AM was 0.90 and 0.98, respectively. OL, AM, and combination drug product were exposed to thermal, photolytic, hydrolytic, and oxidative stress conditions, and the stressed samples were analyzed by the proposed method. Peak homogeneity data of OL and AM is obtained by photodiode array detector in the stressed sample chromatograms, demonstrating the specificity of the method for their estimation in presence of degradation product. The described method shows excellent linearity over a range of 20–400 µg/mL for OL and 5–100 µg/mL for AM. The correlation coefficient for OL and AM are 0.9995 and 0.9998, respectively. The relative standard deviation for six measurements in two sets of each drug in tablets is always less than 2%. The proposed method was found to be suitable and accurate for quantitative determination and stability study of OL and AM in pharmaceutical preparations.

Introduction

Olmesartan (OL) is an angiotensin II receptor blocker. It works by blocking a substance in the body that causes blood vessels to tighten. As a result, OL relaxes blood vessels. This lowers blood pressure and increases the supply of blood and oxygen to the heart. Amlodipine (AM) is the calcium antagonist that inhibits the transmembrane influx of calcium ions into vascular smooth muscles and cardiac muscles, which in turn affects their contractile process and results in reduced blood pressure. It is used in the treatment of hypertension and angina (1).

AM and OL is a combination of medicines used to treat high blood pressure (hypertension) (2). 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-life to be established. The two main aspects of drug product that play an important role in shelf-life determinations are assay of active drug and degradation product generated during stability study. Although stability-indicating methods have been reported for assay of various drugs in 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 (3–6). The objective of this work was to develop an analytical liquid chromatographic (LC) procedure, which would serve as a stability-indicating assay method for combination drug product of OL and AM.

The literature survey reveals that several methods were reported for the individual estimation of OL and AM. The literature survey reveals that several methods were reported for the estimation of OL in plasma, serum, and in-tablet by high-performance liquid chromatography (HPLC) (7–10). Identification of degradation product in stressed tablets of OL medoxomil by the complementary use of HPLC-hyphenated techniques (11) has described identification of two degradation products forms at accelerated stability condition (40°C and 75% relative humidity). However this article did not describe the forced degradation carried out under thermolytic, photolytic, acid/base hydrolytic, and oxidative stress conditions. The pattern of alkali degradation products in present manuscript is similar with the pattern of identification of degradation product in stressed tablets of OL medoxomil article. European Pharmacopeia 2005 describes an HPLC method for determination of AM but does not involve simultaneous determination of OL. Detailed survey of literature for AM revealed several methods based on different techniques, such as HPLC (12–19), HPTLC (20–21), and UV spectrophotometry (22–33), for its determination from pharmaceuticals. None of the reported analytical procedures describe a method for simultaneous determination of the OL and AM in combined pharmaceutical dosage form in the presence of their degradation product.

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If the reported individual methods are applied for the analysis of the tablets containing OL and AM, it would require double time for analysis and as compared with the method would not be rapid, less expensive, or economical, whereas the simultaneous determination of the ingredients of the tablets would save analysis time and also economy.

In the present study, attempts were made to develop a rapid, economical, precise, and accurate method for the simultaneous estimation of the ingredients of this combination in presence of their degradation product.

Experimental

Chemicals and reagents

OL and AM standards were obtained from Lupin Pharmaceutical (Mumbai, India); glacial acetic acid and acetonitrile (HPLC grade) were obtained from Merck Fine Chemicals (Mumbai, India). Ammonium acetate, liquor ammonia solution 25% (NH₃), sodium hydroxide (NaOH), hydrochloric acid (HCl), and hydrogen peroxide (H₂O₂) were from Qualigens Fine Chemicals (GlaxoSmithKline, Mumbai, India). The 0.45- μ m pump nylon filter was obtained from Advanced Micro devices (Ambala Cantt, India). The drug product of OL and AM (Olmezest AM tablets) (Sun Pharmaceutical Ltd., Mumbai, India.) with a label claim (OL 20 mg and AM 5 mg) were purchased from the market. Double-distilled water was used throughout the experiment. Other chemicals used were analytical or HPLC grade.

Chromatographic conditions

The chromatographic system used was an Agilent 1100 series, which comprised a degasser, quaternary pump, auto injector, column compartment, and photodiode array detector, and the system was controlled through Chemstation software. ACE 5 C₁₈ (250 \times 4.6 mm, 5 μ m; Advanced Chromatography Systems, Johns Island, SC) column maintained at 30°C column oven temperature and a mobile phase flow rate of 1.0 mL/min. The mobile phase was composed of buffer–acetonitrile (60:40, v/v). The buffer used in mobile phase contains 0.05 M ammonium acetate in premix double-distilled water and ammonia solution (99.6:0.4, v/v), then pH was adjusted to 7 with glacial acetic acid and filtered through a 0.45- μ m nylon filter before being degassed in ultrasonic bath prior to use. Measurements were made with injection volume 10 μ L and UV detection at 240 nm. For the analysis of forced degradation samples, the photodiode array detector was used in scan mode with a scan range of 200–400 nm. The peak homogeneity was expressed in terms of peak purity and was obtained directly from the spectral analysis report by using the previously mentioned software.

Standard stock solutions

Standard solutions were prepared by dissolving the drugs in the diluent and diluting them to the desired concentration. Diluent used for the standard and sample preparation was composed of acetonitrile and water in the ratio of 70:30 (v/v).

OL standard stock solution

About 25 mg sample of OL (99.95%) was transferred in to a 50-mL volumetric flask and diluted to volume with diluent.

AM standard stock solution

About 25 mg sample of AM (99.96%) was transferred in to a 50-mL volumetric flask and diluted to volume with diluent.

Mixed standard solution

A mixed standard solution was prepared from stock solutions using diluent to give a final concentration of 200 μ g/mL of OL and 50 μ g/mL of AM.

Calibration curve solutions

The solutions which were used for the preparation of the calibration curve were in the concentration range of 20–400 μ g/mL of OL and 5–100 μ g/mL of AM.

Preparation of sample

Ten tablets were weighed and finely powdered. A quantity of powder equivalent to one tablet containing 20 mg of OL and 5 mg of AM was transferred in to a 50-mL volumetric flask. To this flask, 25 mL of diluent was added, and the solution was sonicated for 25 min with intermittent shaking. Then the volume was made up with diluent and centrifuged at 10,000 rpm for 10 min. The centrifuged solution was filtered through a 0.45- μ m filter. From the filtered solution, 5 mL of solution was transferred into a 10-mL volumetric flask and diluted to volume with diluent.

Procedure for forced degradation study of drug substances

Forced degradation of each drug substances and the drug product was carried out under thermolytic, photolytic, acid/base hydrolytic and oxidative stress conditions. The ICH guideline (34) states the minimum desired exposure as 200 Wh/m², which corresponds to a change in absorbance of 0.5 AU of quinine actinometer at 400 nm. This change was observed in 24 h of irradiation. A second photolytic stress test experiment with greater irradiation time of 48 h was carried out. After the degradation, solutions were diluted to achieve concentration of 200 μ g/mL of OL and 50 μ g/mL of AM.

Acidic degradation

Acid hydrolysis was conducted with 1 N HCl for 2 h at 60°C in water bath.

Alkali degradation

Alkali hydrolysis of OL was conducted with 0.01 N NaOH for 5 min at ambient temperature. Alkali hydrolysis of AM was conducted with 1 N NaOH for 1 h at ambient temperature.

Oxidative degradation

Oxidative stress of OL was conducted with 30% H₂O₂ for 2 h at 60°C in a water bath. Oxidative stress of AM was conducted with 3% H₂O₂ for 5 min at ambient temperature.

Thermal degradation

About 100 mg of drug substances were placed in a controlled temperature oven at 80°C for 48 h.

UV-short (254 nm) degradation

About 100 mg of drug substances were exposed to UV short light for 24 h.

UV-long (366 nm) degradation

About 100 mg of drug substances were exposed to UV long light for 48 h.

Procedure for forced degradation study of drug products

Acid, thermolytic, and photolytic degradation of drug product was conducted as described for drug substances. Alkali degradation and oxidative degradation of drug product was conducted with 1 N NaOH and 30% H₂O₂.

Results and Discussion**Optimization of the chromatographic conditions**

To develop a stability-indicating method different stationary phases like C₁₈, CN, different mobile phases containing buffers like phosphate, ammonium acetate, acetic acid, and trifluoroacetic acid with different pH (3–7), and organic modifier (acetonitrile) were used.

Our objective of chromatographic method development was to achieve peak tailing factor < 2, retention time between 3 to 10 min, along with resolution between OL and AM > 2.

The chromatographic separation was achieved using ACE 5 C₁₈ (250 × 4.6 mm i.d.) column. Changing the composition of mobile phase optimized the chromatographic method. To develop a stability-indicating method assessing the effect of change of proportion, the pH of mobile phase was maintained at 7.0. OL and AM were well-resolved from degradation products at mobile phase composition of buffer–acetonitrile (60:40, v/v), whereas when the proportion of buffer–acetonitrile (55:45, v/v) was used to reduce the run time of method it did not succeed as the degradants generated in acid degradation of AM interfered with OL peak. The Resolution between OL and AM was observed on any C₁₈ or CN (YMC Pack ODS A, YMC Pack AM, Alltima CN) column, but it was difficult to separate both drugs degradation product on these columns. ACE 5 column shows better performance as compared to other columns due to high carbon loading 15.5% and surface area 300 m²/g.

From the development studies, it was determined that 0.05 M ammonium acetate in premix double-distilled water and ammonia solution (99.6:0.4, v/v), then pH adjusted to 7 with glacial acetic acid and acetonitrile in the ratio of 60:40 (v/v), the flow rate of mobile phase 1.0 mL/min, and column temperature 30°C was optimal. The analytes of this combination had adequate retentions, peak shape, less tailing, more resolution and the chromatographic analysis time was less than 12 min. In optimized conditions OL, AM, and their degradation product were well-separated. Typical retention times of OL and AM were about 5.1 and 8.7 min, respectively. Resolution between OL and AM founds to be 12.7.

Singh and Bakshi in their article on stress testing (35) suggested a target degradation of 20–80% for the establishing stability indicating nature of the assay method, as even inter-

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 the case of thermal and photolytic degradation even after exposure for prolonged duration. During the initial forced degradation experiments, it was observed that basic hydrolysis was a fast reaction for OL, and almost complete degradation occurred when 0.1N NaOH solution is used. Oxidative degradation was a fast reaction for AM, and almost complete degradation occurred when 30% H₂O₂ solution is used. For drug substances, diluted NaOH (0.01 N) and 3% H₂O₂ was used to achieve 20–80% degradation. In drug product degradation, it was difficult to show degradation of AM with 0.01 N NaOH and degradation of OL with 3% H₂O₂ because at alkali AM is stable than OL and at oxidative condition OL is stable than AM. Table I indicates the extent of degradation of OL and AM under various stress conditions (drug substances). Chromatographic peak purity data was obtained from the spectral analysis report, and a peak purity value greater than 990 indicates a homogeneous peak. The peak purity values for analyte peaks, OL and AM, were in the range of 999–1000 for drug substance and in the range of 998–1000 for tablets, indicating homogeneous peaks and thus establishing the specificity of assay method. Figure 1 shows the chromatograms of tablet solution, drug substances, and drug product forced degradation.

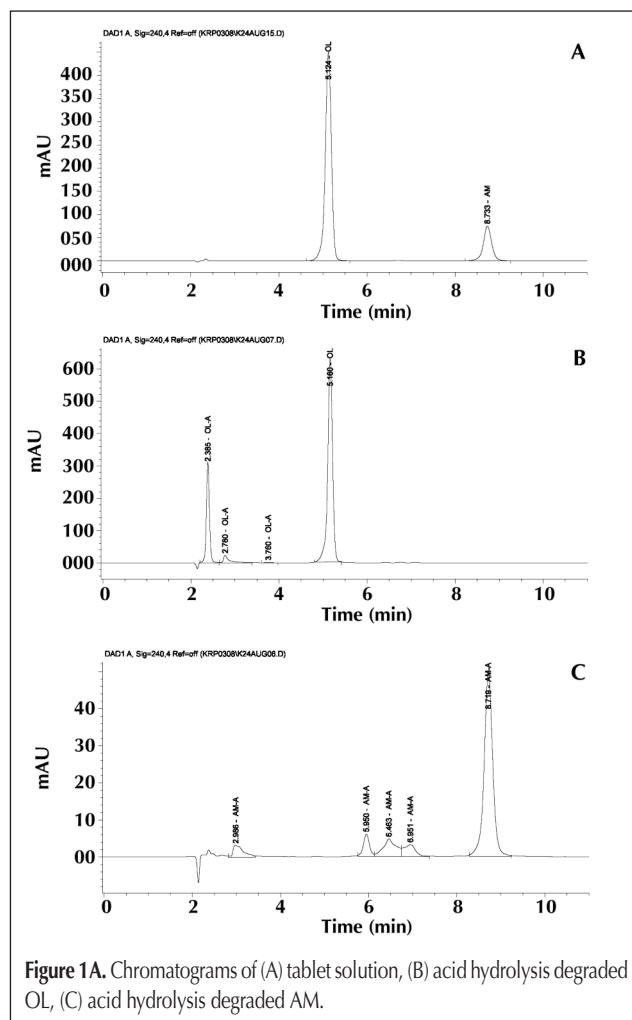


Figure 1A. Chromatograms of (A) tablet solution, (B) acid hydrolysis degraded OL, (C) acid hydrolysis degraded AM.

Method Validation

Specificity

Photodiode array detection was used as an evidence of the specificity of the method and to evaluate the homogeneity of the drug peak. The peak purity values are more than 998 for drug substances as well as drug products, which shows that the peaks of analyte were pure and also that formulation excipients and degradation product were not interfering with the analyte peaks.

Calibration and linearity

Linearity the method was tested from 10 to 200% of the targeted level of the assay concentration (OL 200 µg/mL and AM 50 µg/mL) for both analytes. Mixed standard solutions contained 20–400 µg/mL of OL and 5–100 µg/mL of AM.

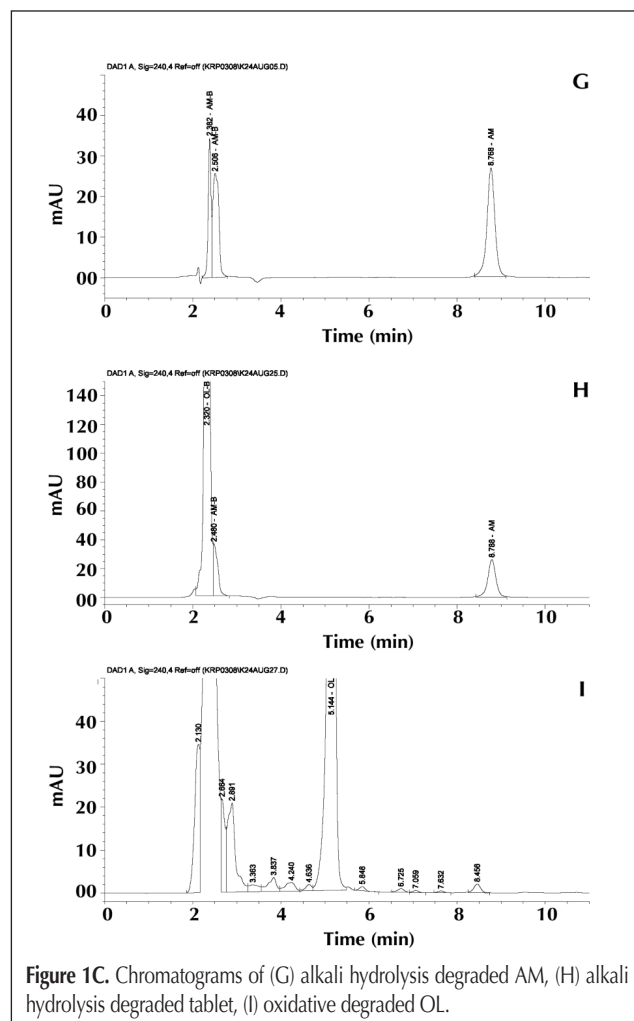
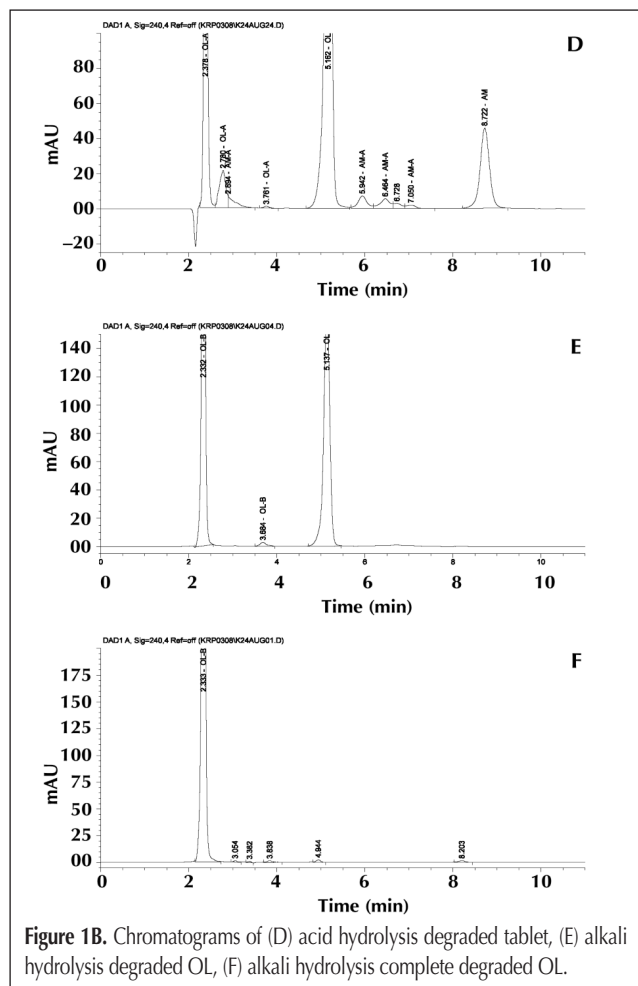
Linearity solutions were injected in triplicate. The calibration graphs were obtained by plotting peak area against the concentration of the drugs. The equations of the calibration curves for OL and AM obtained were $y = 88.539x - 0.112$ and $y = 4.931x - 1.352$, respectively. In the simultaneous determination, the calibration graphs were found to be linear in the aforementioned concentrations with correlation coefficients 0.9995 and 0.9998 for OL and AM, respectively. Relative standard deviation (%RSD) for slope of OL and AM were 0.23 and 0.19, respectively.

Precision (repeatability)

The precision of the method was studied by determining the concentrations of each drug in the tablets six times. The area %RSD for OL and AM were 0.10 and 0.13, respectively. The average assay% for six determinations was 99.94% and 100.34%, respectively, for OL and AM. %RSD of assay values was 0.43 and 0.52, respectively, for OL and AM. The results of the precision study indicate that the method is reliable (%RSD < 2).

Accuracy (recovery test)

Accuracy of the method was studied by recovery experiments. The recovery experiments were performed by adding known amounts of the drugs in the placebo (pregelatinized starch, microcrystalline cellulose, croscarmellose sodium, magnesium stearate, and opadry TT). The recovery was performed at three levels, 80%, 100%, and 120% of the label claim of the tablet (20 mg of OL and 5 mg of AM). Placebo equivalent to one tablet was transferred into a 100-mL volumetric flask, and the amounts of OL and of AM at 80%, 100%, and 120% of the label claim of the tablet were added. The recovery samples were prepared as per the procedure mentioned earlier. Three samples were prepared for each recovery level. The solutions were then analyzed, and the percentage recoveries were calculated from the calibration curve. The recovery values for OL and AM ranged from 98.89 to 100.98% and 98.86 to 100.89%, respectively. The average



recovery of three levels (nine determinations) for OL and AM were 100.08% (0.68) and 99.94% (0.60), respectively, with %RSD shown in parenthesis. The results are shown in Table II.

Intermediate precision

Intermediate precision of the method was determined by analyzing the samples six times on different days by different chemists using different analytical columns of the same make and different HPLC systems. The percentage assay was calculated using calibration curves. The assay results of chemist 2 for OL and AM were 99.87% and 100.38%, respectively. %RSD of assay values was 0.51 and 0.49, respectively, for OL and AM.

Robustness

The robustness of a method is the ability of method to remain unaffected by small changes in parameters. To determine robustness of the method, experimental conditions were purposely altered, and chromatographic resolution between OL and AM were evaluated.

The flow rate of the mobile phase was 1.0 mL/min. To study the effect of flow rate on resolution of OL and AM, it was changed to 0.1 units from 1.0 to 1.1 mL/min and 0.9 mL/min. The effect of column temperature on resolution was studied at 28°C and 32°C instead of 30°C, while other mobile phase components were held constant. The effect of mobile phase composition on resolution of OL and AM was studied with buffer–acetonitrile at 58:42 (v/v) and 62:38 (v/v). The effect of buffer pH on resolution of OL and AM was studied at pH 6.8 and 7.2.

At all conditions resolution between OL and AM was found to be more than 12.

Determination of limits of quantification and detection

The limit of detection (LOD) and limit of quantitation (LOQ) for OL and AM were determined at a signal-to-noise ratio of 3:1 and 10:1, respectively, by injecting a series of dilute solutions with known concentration (36). The LODs for OL and AM were

0.08 and 0.11 µg/mL, respectively, and the LOQs were 0.26 and 0.35 µg/mL, respectively, for 10-µL injection volume.

Solution stability

The stability of the standard solution was tested at intervals of 30 and 48 h. The stability of solutions was determined by comparing results of area%, resolution, and peak purity of OL and AM. The area% values were within 0.5 % after 48 h. The results indicate that the solutions were stable for 48 h at ambient temperature as there was no formation of any unknown peak and solution remains stable. The RSD of peak area% was 0.21 and 0.33%, peak purity was 999.984 and 999.962, asymmetry factor was 0.99 and 1.03, and capacity factor was 2.38 and 4.06 for OL and AM, respectively. The resolution between OL and AM was 12.72.

Conclusion

The isocratic reversed phase-LC method developed for analysis of binary mixture of OL and AM in their pharmaceutical preparations is precise, accurate, and with short run time. The method was fully validated showing satisfactory data for all the method validation parameters tested. The developed method is a stability indicating, separates degradation product, and can be conveniently used by quality control department to determine the assay of pharmaceutical preparations and also stability samples.

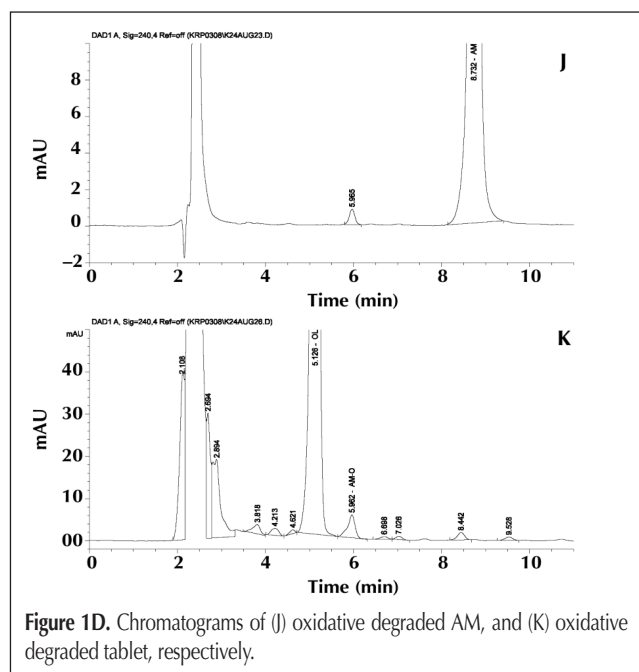


Table I. Results of Forced Degradation Study Samples Using Proposed Method

Stress condition	OL (%) degradation	Peak purity*	AM (%) degradation	Peak purity*
Acidic	27.54	999.964	28.5	999.542
Alkali	60.15	999.508	52.35	999.387
Oxidative	22.32	998.517	12.64	999.429
Thermal	0.36	999.916	0.25	999.746
UV-short	ND	999.657	0.16	999.843
UV-long	ND	999.678	2.05	999.518

* Peak purity values in the range of 990–1000 indicate a homogeneous peak.

Table II. Results of Recovery Tests for the Drugs

Level of addition (%)	Ingredient	Amount added (n = 3) (mg)	% Recovery*	% Average recovery†
80	OL	16.0	100.15 (0.33)	100.08 (0.68)
	AM	4	99.84 (0.19)	
100	OL	20.0	99.36 (0.41)	99.94 (0.60)
	AM	5	99.39 (0.48)	
120	OL	24.0	100.72 (0.35)	
	AM	6	100.58 (0.34)	

* RSD shown in parenthesis.
† Average recovery = the average of three levels, nine determinations.

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