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Stability Indicating RP-HPLC Method for Simultaneous Determination of Atorvastatin and Nicotinic Acid from Their Combined Dosage Form

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Abstract

The study describes development and subsequent validation of a stability indicating reverse-phase HPLC method for simultaneous estimation of atorvastatin (ATR) and nicotinic acid (NTA) from their combined dosage form. The proposed RP-HPLC method utilizes a Phenomenex® C18, 5 µm, 250 mm X 4.6 mm i.d. column, at ambient temperature, optimum mobile phase consisted of acetonitrile and 50mM potassium dihydrogen phosphate buffer (68:32, v/v), apparent pH adjusted to 4.5±0.1 with phosphoric acid solution, effluent flow rate monitored at 0.8 mL min^{-1}, and UV detection at 247 nm. The combination drug product are exposed to thermal, acid/base hydrolytic, humidity and oxidative stress conditions, and the stressed samples were analyzed by proposed method. The described method is linear over the range of 2-10 µg mL^{-1} and 20-100 µg mL^{-1} for ATR and NTA, respectively. The mean recoveries are 100.99 and 102.65% for ATR and NTA, respectively. The intermediate precision data obtained under different experimental setup, the calculated value of coefficient of variation (CV,%) is found to be less than critical value. The limit of detection for ATR and NTA are found to be 0.16 and 0.12 µg mL^{-1}, respectively. Chromatographic peak purity data of ATR and NTA 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 of combination drug products.

Keywords:

Atorvastatin; Nicotinic acid; Rp-HPLC; stability indicating; tablets

1. Introduction

Atorvastatin [1] is a synthetic lipid lowering agent which inhibits HMG-Co A reductase and nicotinic acid [2] is a antihyperlipidemic effective in hypertension and angina pectoris. The combination drug product of atorvastatin (ATR) and nicotinic acid (NTA) has recently been introduced in the market. Chemically ATR is [*R*-(*R**,*R**)]-2-(4-fluorophenyl)-b, dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1*H*-pyrrole-1 heptanoic acid, calcium salt (2 :1) trihydrate [3] and NTA is 2-[(2-Aminoethoxy)-methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridine dicarboxylic acid 3-ethyl 5-methyl ester [4].

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

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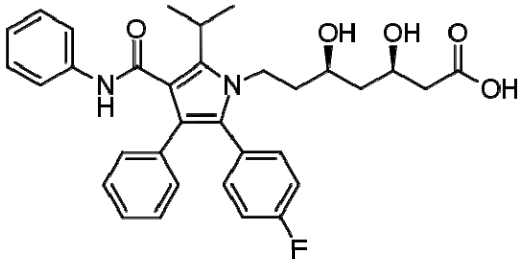
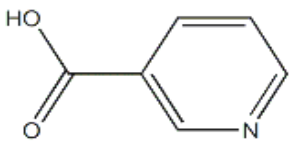
such as temperature, humidity, and light, and enables recommendation of storage conditions, retest periods, and shelf lives to be established. The two main aspects of drug products that play an important role in shelf life determination are assay of active drug, and degradation products generated, during 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 [5] and USP-28 [6]. 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 ATR and NTA.



Atorvastatin calcium



Nicotinic Acid

ATR is not official in any pharmacopoeia while NTA is official with BP 28. BP 2004 [7] describes a titrimetric method for the determination of NTA, but does not involve simultaneous determination of ATR. Detailed survey of literature for ATR revealed several methods based on different techniques, *viz.* HPLC [8-11], HPLC [12] for its determination in human serum; and LC-MS [13-14] for its determination of its metabolites in serum; Capillary electrophoresis [15] HPTLC [16] for its determination in pharmaceuticals. Similarly, survey of literature for NTA revealed methods based on spectrophotometry [17], RP-HPLC [18] using fluorescence detection, HPLC-tandem mass spectrometry [19], RP-HPLC using UV detection [20-21], HPLC [22] in combination with simvastatin, Rp-HPLC with solid phase extraction [23], Reverse Phase ion-pair chromatography [24] and HPLC & capillary electrophoretic determination [25] have been reported. Methods are reported for estimation of atorvastatin in combination with amlodipine besylate [26], pantoprazole [27], Aspirin [28] but none of the reported analytical procedures describe a stability indicating method for simultaneous determination of ATR and NTA 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 ATR and NTA in presence of their degradation products as per ICH guidelines.

To establish the stability indicating nature of the method, forced degradation of drug substances and drug product was performed under stress conditions (thermal, humidity, acid/base hydrolytic and oxidative), and stressed samples were analyzed by the proposed method. The method was validated as per ICH guidelines [29] and its updated international convention [30]. The linearity of response, accuracy and intermediate precision of the described method has been checked.

2. Experimental

2.1 Chemicals and Reagents

ATR and NTA working standards were generous gifts from Biocon Limited Bangalore, India and Lupin Laboratory Limited Pune, India respectively. Combination drug

products of ATR and NTA (Label claim: atorvastatin calcium equivalent to atorvastatin 10 mg, and Nicotinic acid 375 mg), Atorin N Tablets (Medley Ltd., India), purchased from local pharmacy. Acetonitrile, methanol and water of Rankem used were of HPLC grade. *Ortho*-phosphoric acid used was of analytical reagent grade (Loba Chemicals Ltd., India). Potassium dihydrogen phosphate and sodium hydroxide, Hydrochloric acid and hydrogen peroxide were from Qualigens Fine Chemicals (Glaxo Ltd.).

2.2 HPLC Instrumentation and Conditions

The HPLC system consisted of Shimadzu HPLC 1100 series consisted of binary pump LC-10 ADvp, Rheodyne universal injector 7725i and Shimadzu SPD-10 UV–Visible detector. The chromatographic separations were performed using Phenomenx® 100 C18, 5 μm, 250 mm X 4.6 mm i.d. column, at ambient temperature, eluted with mobile phase at the flow rate of 0.8 mL/min. The mobile phase consisted of acetonitrile and 50mM potassium dihydrogen phosphate buffer (62:38, v/v), apparent pH adjusted to 4.5±0.1 with phosphoric acid solution, filtered through 0.45 μm 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 μL and ultraviolet (UV) detection at 247 nm, as both components shows reasonable good response at this wavelength.

2.3 Standard and Sample Preparation

The standard stock solutions 0.1 mg mL^{-1} of ATR and 1 mg mL^{-1} of NTA were prepared separately by dissolving working standards in methanol and diluting with the same solvent. Standard calibration solutions of ATR and NTA having concentrations in the range of 2-10 and 20-100 μg mL^{-1} respectively were prepared by diluting stock solutions with mobile phase.

2.4 Analysis of Dosage Forms

Twenty tablets were weighed, their mean weight determined, and crushed in mortar. An amount of powdered mass equivalent to 50 mg of NTA and 1.44 mg of ATR, also 3.56 mg of Atrovastatin working standard 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 50 mL volumetric flask and volume was made with methanol. Five mL aliquot from above solution was transferred in 50 mL volumetric flask and volume was adjusted with mobile phase up to mark and used for injection on HPLC.

2.5 Procedure for Forced Degradation Study

Forced degradation of drug product was carried out under thermolytic, relative humidity, acid/base hydrolytic and oxidative stress conditions. Thermal and relative humidity-degradation of drug product were carried out in solid state. After the degradation stock solutions were prepared by dissolving in methanol. From these solutions aliquots were diluted with mobile phase to achieve a concentration of 10 μg mL^{-1} of ATR and 100 μg mL^{-1} of NTA based on the labeled strength.

For thermal stress, samples of drug substances and drug product were placed in a controlled-temperature oven at 60 °C for 24 h. Acid hydrolysis of drug substance and drug product in solution state was conducted with 1N hydrochloric acid at 50°C for 24 h. Base

hydrolysis of drug product was conducted by 1N sodium hydroxide solution at 50°C for 24 h. For oxidative stress, sample solutions of drug product in 3% hydrogen peroxide were kept at 50°C for 24 h. The humidity effect was carried in solid state, kept at 75% RH at ambient temperature for 24 hrs.

3. Results and Discussion

To develop a precise, accurate, specific and suitable stability indicating RP-HPLC method for the simultaneous estimation of ATR and NTA, different mobile phases were employed and proposed chromatographic condition was found appropriate for the quantitative determination in presence of degradation products. The optimum mobile phase consisted of acetonitrile and phosphate buffer (62:38, v/v), apparent pH adjusted to 4.5±0.1 with phosphoric acid solution, selected because it was found to ideally resolve the peaks of ATR (*t*R_5.31 min) and NTA (*t*R_2.877 min), with clear line separation in presence of their degradation products at effluent flow rate of 0.8 mL/min. UV detection wavelength at 247 nm, injection volume 20µl, ambient temperature for column and HPLC system was found to best for analysis.

Singh and Bakshi, in their article on stress testing [31] 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 some cases even after exposure for prolonged duration. ATR showed extensive degradation in nearly all the hydrolytic condition except in alkali where the peak shape was quite broad while NTA in humid condition. Table 1 indicates the extent of ATR and NTA under various stress conditions. Fig. 1 show normal chromatogram while 2a-e shows the chromatogram of forced degraded samples. No other co-eluting peak was found with the main peaks suggested the specificity of the method for the simultaneous estimation of ATR and NTA in presence of degradation products.

Table 1. Results of Forced Degradation Study Samples Using Proposed Method, Indicating Percentage of label claim of ATR and NTA

Stress condition/ duration/state	% of Label claim estimated	
	ATR	NTA
Alkaline/ 1 N NaOH/ 24h/solution	126.10	108.26
Acidic/ 1 N HCl/ 24h/ solution	1.80	104.65
Oxide/ 3% H_2O_2/24h/ solution	14.90	101.95
Thermal/60°C / 24h/ solid	28.52	94.35
Humidity/ 75% RH/ 24h/ solid	37.36	82.88

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 concentration (10 & 100 µg mL^{-1} of ATR and NTA respectively). The intra- and inter-day variation of the method was carried out for one concentration level. The low % CV values of within a day, day to day variations and analyst to analyst variation for ATR and NTA revealed that the proposed method is precise.

Table 2. Regression Characteristics and System suitability parameters

Parameters	ATR	NTA
Retention Time (min)	5.310	2.877
Asymmetry	1.619	2.154
Theoretical plates	5405	3184
Linearity range (µg mL^{-1})	2-10	20-100
Limit of detection (µg mL^{-1})	0.16	0.12
Correlation coefficient (r)	0.9992	0.9999
Method Precision (CV, %) (n=7)	0.290	0.330

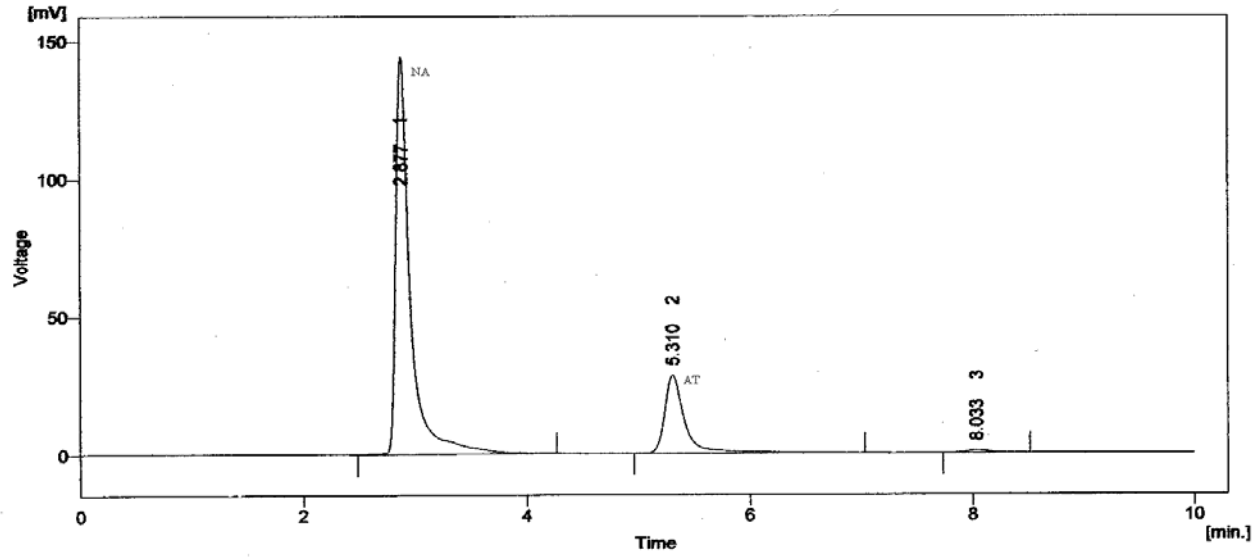


Fig. 1. Chromatogram of untreated marketed formulation: 1) NTA, 2) ATR

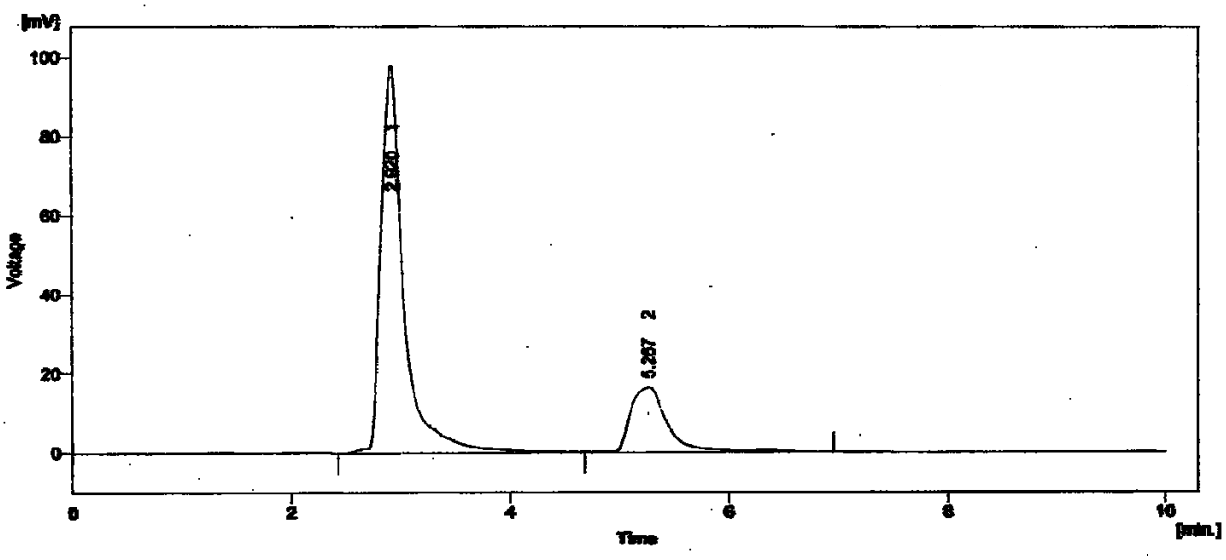


Fig. 2a. Chromatograms of alkali degraded sample: 1) NTA, 2) ATR

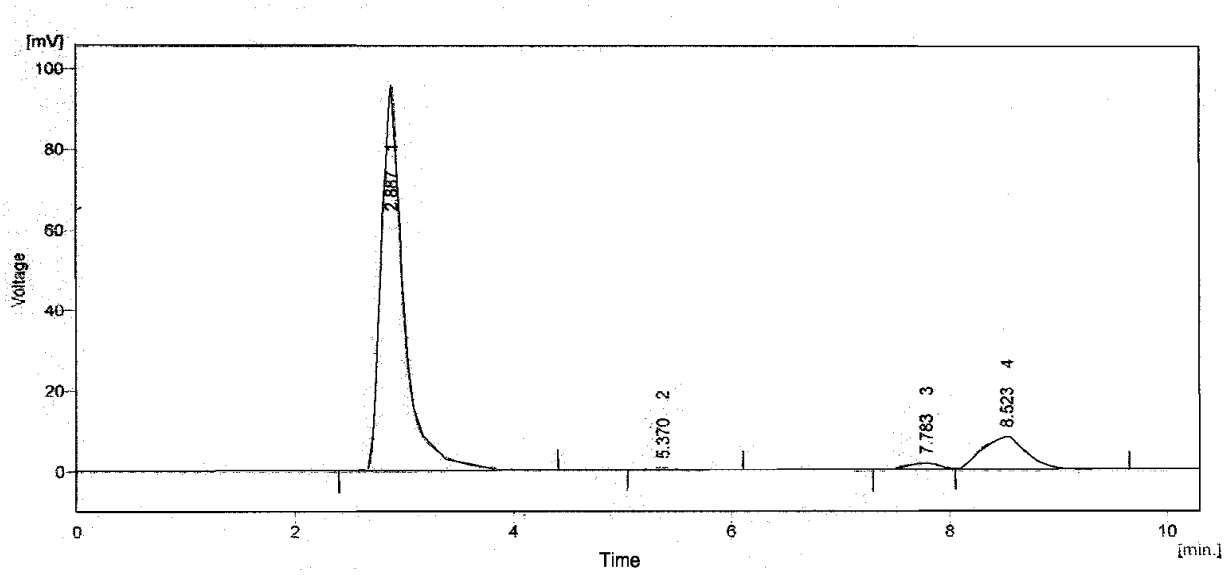


Fig. 2b. Chromatogram of Acid degraded sample

1) NTA 2) ATR, 3 & 4 ATR degraded products

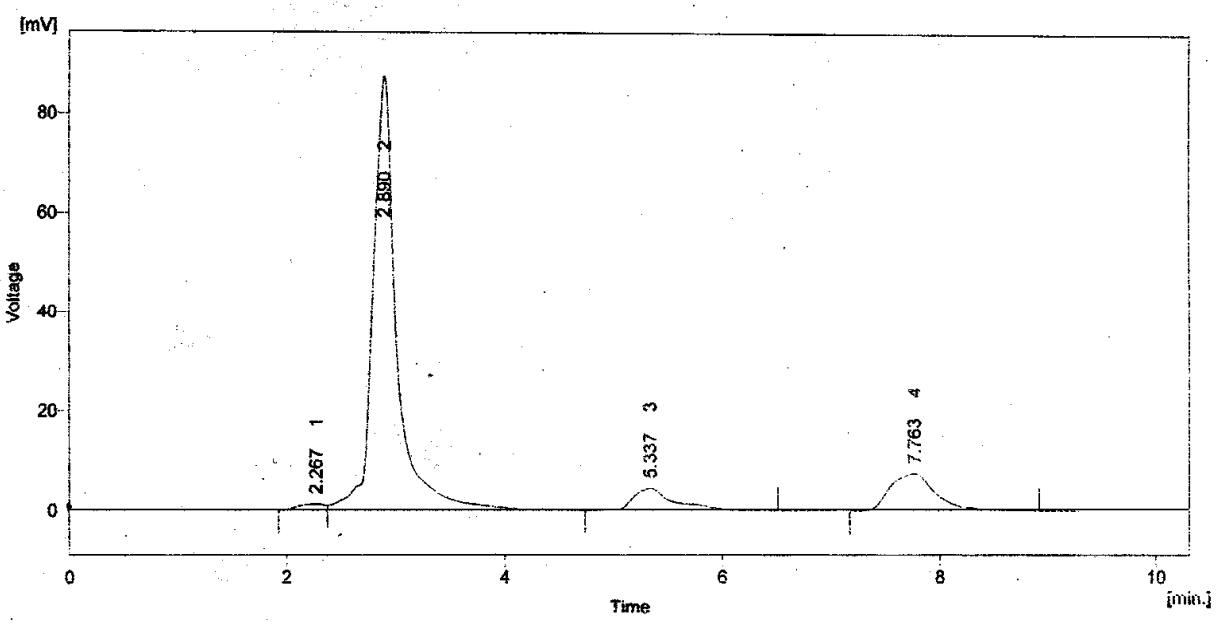


Fig. 2c. Chromatogram of oxide degraded sample 2) NTA 3) ATR 4) ATR degraded product

Accuracy of method was checked by recovery study using standard addition method, at four different concentration levels, *i.e.* multilevel recovery study. The pre-analyzed samples were spiked with standard ATR and standard NTA and the mixtures were analyzed by proposed method. Recovery of standard drugs added was found to be 100.29—101.45% for ATR and 101.56—103.59% for NTA with the value of % CV less than 2 indicating proposed method is accurate for the simultaneous estimation of ATR and NTA from their combination drug products in presence of their degradation product(s). Results of recovery study are shown in Table 3.

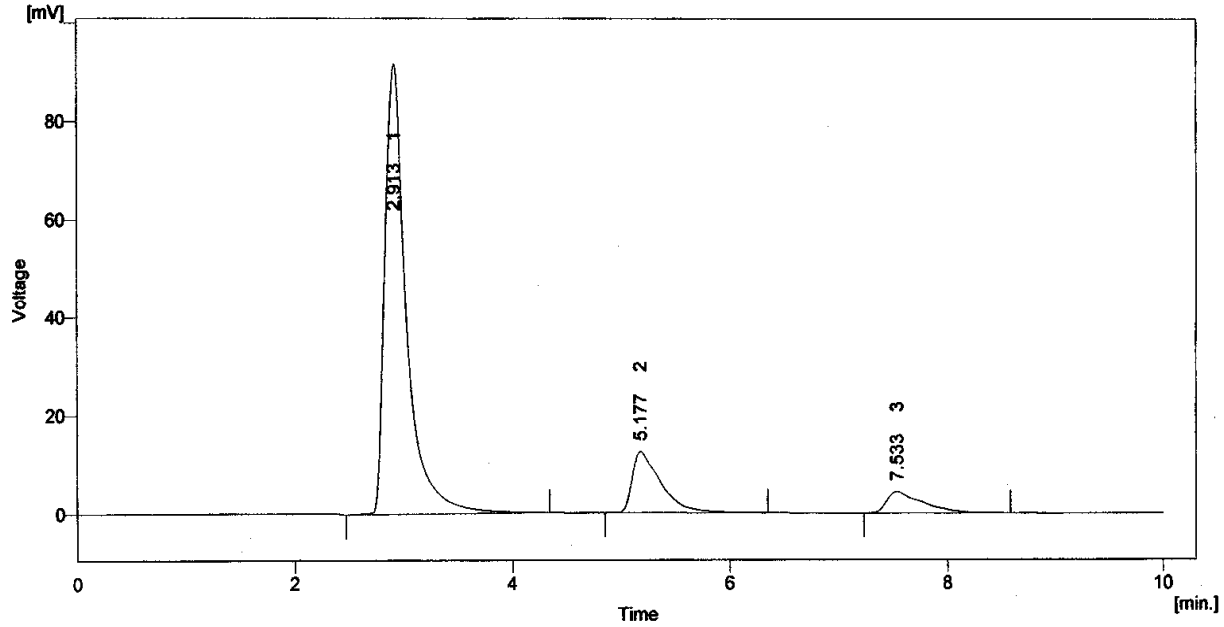


Fig. 2d. Chromatogram of Thermal degraded sample 1) NTA 2) ATR 3) ATR degraded product

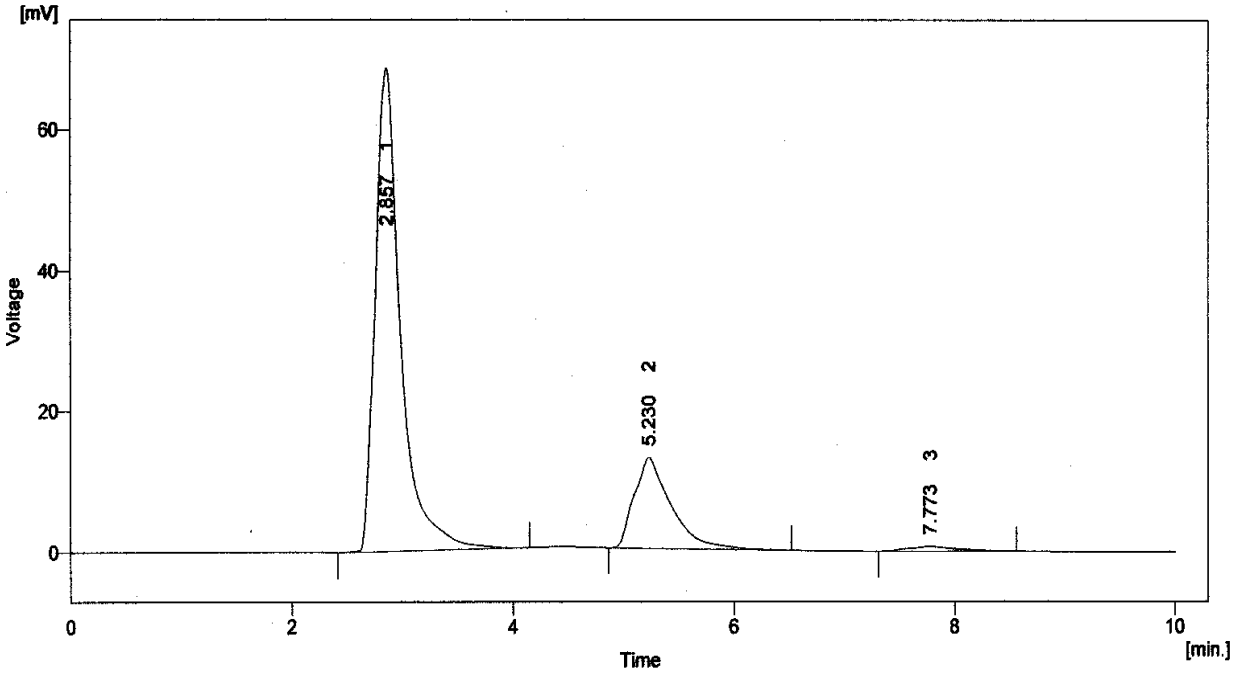


Fig. 2e. Chromatogram of Humidity degraded sample- 1) NTA 2) ATR

Table 3. Recovery data of the proposed HPLC method

Drug	Level	Amount of pure drug spiked	Amount of pure drug recovered	Recovery, (%)
ATR	I	4.1	4.112	100.29
	II	4.5	4.572	100.96
	III	5.0	5.073	101.45
	IV	5.7	5.772	101.27
NTA	I	5.1	5.18	101.56
	II	10.1	10.45	103.46
	III	15.3	15.85	103.59
	IV	20.0	20.40	102.00

3.1. Assay of ATR and NTA from Its Tablet Dosage Forms

The assay results of ATR and NTA in tablet dosage forms were comparable with the value of label claimed. The results presented in Table 4 indicate the suitability of the method for routine analysis of ATR and NTA from their combination drug products.

Table 4. Analysis of ATR and NTA by proposed method (n=5)

Drug	Labeled Amount, mg	Amount found, mg	Assay (%) ± CV (%)
ATR	10	10.135	101.35 ± 0.29
NTA	375	376.58	100.42 ± 0.33

4. 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 ATR and NTA 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 ATR and NTA in their combination drug product. The proposed method can also be conveniently adopted for dissolution testing of tablets containing ATR and NTA.

5. Acknowledgements

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