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Research Article

STABILITY - INDICATING LC METHOD FOR THE DETERMINATION OF RANOLAZINE HYDROCHLORIDE IN THE BULK DRUG AND IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A novel stability – indicating LC assay method was developed and validated for quantitative determination of Ranolazine Hydrochloride (RAN) in bulk drugs and in pharmaceutical dosage form in the presence of degradation products generated from forced degradation studies. An isocratic, reversed phase LC method was developed to separate the drug from the degradation products, using a HiQ Sil C-18 HS, (250mm×4.6mm, 5µm) with methanol–water, 99:1 (%, v/v) as a mobile phase. The detection was carried out at the wavelength of 273nm. RAN was subjected to stress conditions of hydrolysis (acid, base, neutral), oxidation, photolysis and thermal degradation. Degradation was observed for RAN in acid, base and in 30% H_2O_2 conditions. The drug was found to be stable in the other stress conditions attempted. The degradation products were well resolved from the main peak. The percentage recovery of RAN ranged from (99.97 to 100.11 %) in pharmaceutical dosage form. The developed method was validated with respect to linearity, accuracy (recovery), precision, specificity and robustness. The forced studies prove the stability-indicating power of the method.

Keywords: Ranolazine Hydrochloride, RP-HPLC analysis, Method validation, Degradation

INTRODUCTION

Ranolazine Hydrochloride (RAN) is an anti-anginal drug and chemically it is a piperazine derivative. Structurally it is N-(2, 6-dimethylphenyl)-2-[4-[2-hydroxy-3-(2- methoxyphenoxy) propyl] piperazin-1-yl] acetamide (Fig-1). RAN is believed to have its effects via altering the trans-cellular late sodium current. It is by altering the intracellular sodium level that RAN affects the sodium-dependent calcium channels during myocardial ischemia. Thus, RAN indirectly prevents the calcium overload that causes cardiac ischemia[1]. RAN is indicated for the treatment of chronic angina, it may be used with beta blockers, nitrates, calcium channel blockers, antiplatelet therapy, lipid-lowering therapy, ACE inhibitors, and angiotensin receptor blockers.

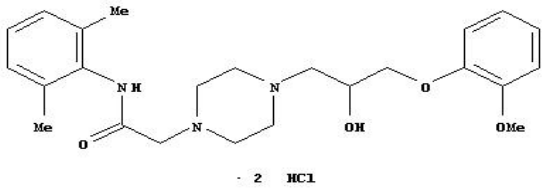


Fig.1: Chemical structure of RAN

The stability of a drug substance or drug product is defined as its capacity to remain within established specifications, i.e. to maintain its identity, strength, quality, and purity until the retest or expiry date[2]. Stability testing of an active substance or finished product provides evidence of how the quality of a drug substance or drug product varies with time under a variety of environmental conditions, for example temperature, humidity, and light. Knowledge from stability studies is used in the development of Manufacturing processes, selection of proper packaging and storage conditions, and determination of product shelf-life [3-6].

Some methods were reported, which were applicable for biological matrices using complex analytical instruments such as mass spectrometer[7-16]. There are two reported stability-indicating analytical method for analysis of RAN in the presence of its degradation products but use of column temperature and buffers as well as gradient LC thereby making it somewhat complex for routine analysis in the QC lab[17-18]. In the absence of official RAN monograph in the pharmacopoeias, including the European Pharmacopoeia, British Pharmacopoeia, and United States Pharmacopoeia, development of such a method may prove all the more useful. The objective of this work was, therefore, to develop a simple, economic, rapid, precise, and accurate stability-indicating HPLC method for quantitative analysis of RAN and to validate the method in accordance with ICH guidelines [19-21].

MATERIALS AND METHODS

Chemicals and Reagents

A sample of RAN was obtained as a gift from Cipla Laboratories, Mumbai, India. HPLC-grade methanol, acetonitrile, and water were purchased from E. Merck, Mumbai, India. RAN tablets of two different brands, Caroza and Ranz-500, containing 500 mg RAN were obtained from Zydus cadila, Ahemdabad, India, and from Torrent Pharmaceuticals, Indrad, India, respectively. All the other chemicals and reagents used were of AR grade and purchased from S.D. Fine Chemicals, Mumbai, India. A stock solution of RAN (1 mg mL^{-1}) was prepared in methanol. Standard solutions were prepared by dilution of the stock solution with methanol to give solutions in the concentration range 10 to 400 µg mL^{-1}.

HPLC Instrumentation and Chromatographic Conditions

Chromatography was performed with Jasco, Japan equipment comprising a PU-2089 plus quaternary pump, degasser and a photo diode array detector (Jasco MD-2010 Plus). A Rheodyne injector fitted with a 20 µL loop was also used and data were recorded and evaluated by use of Chrompass software. The chromatographic column used for separation was a HiQ Sil C-18 HS, 250mm×4.6mm id with 5µm particles. The mobile phase consists of methanol and water in the ratio (99:1 v/v). The mobile phase was filtered through a 0.45µm membrane filter and sonicated for degassing. The instrumental setting was at a flow rate 1 $mLmin^{-1}$.The injection volume was 10 µL. The detector wavelength was 273nm.

Method Validation

The analytical method was validated for linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), specificity, robustness, and ruggedness, in accordance with ICH guidelines.

Linearity

Linearity was studied by preparing standard solutions at different concentrations from 10 to 400 µg mL^{-1}, plotting a graph of

concentration against peak area, and determining the linearity by least-squares regression.

Accuracy, as Recovery

Accuracy was evaluated in triplicate, at three different concentrations equivalent to 50, 100, and 150 % of the active ingredient, by adding a known amount of RAN standard to a sample of known concentration and calculating the recovery of RAN, RSD (%), and standard error (SE) for each concentration.

Precision

Precision was studied by measuring intra-day (repeatability) and inter-day (by injection of samples over three consecutive days) variation of the method for three different concentrations of RAN, 40, 80, and100 µg mL^{-1}, each in triplicate.

Limits of Detection (LOD) and Quantification (LOQ)

The limits of detection and quantitation were calculated by the method based on the standard deviation (σ) of responses for triplicate blank injections and the slope (S) of the calibration plot, using the formulae LOD = $3.3\sigma/S$ and LOQ = $10\sigma/S$.

Robustness and Ruggedness

The robustness of the method was determined to assess the effect of small but deliberate variation of the chromatographic conditions on the determination of RAN. Robustness was determined by changing the mobile phase flow rate to 0.9 and 1.1 mL min^{-1}, the concentration of methanol in the mobile phase to 98 and 100% and detector wavelength to 271nm and 275nm. The ruggedness of the method was assessed by comparison of intra-day and inter-day results for assay of RAN performed by two analysts in the same laboratory.

Procedure for Forced Degradation study of RAN

Acidic Degradation

5 mg of RAN was accurately weighed and dissolved in 10 mL of methanol, then 10mL of 0.1N HCl were added and kept at 60^0C about 3 h in a water bath, the solution was allowed to attend ambient temperature then the solution was neutralized by 0.1N NaOH to pH 7 and the volume made up to 50mLwith methanol.

Alkali degradation

5 mg of RAN was accurately weighed and dissolved in 10 mL of methanol, then 10 mL of 0.1N NaOH was added and kept at room temperature for 1 hour. Then the solution was neutralized by 0.1 N HCl to pH 7 and the volume made up to 50mL with methanol.

Neutral degradation

5mg of RAN was accurately weighed and dissolved in 10mL of methanol, then volume was made up to 50mL with HPLC grade water, it was refluxed for 4 hours.

Oxidative degradation

About 5 mg of RAN was accurately weighed and dissolved in 10mL of methanol, then 10 mL of 30 % H_2O_2 solution were added and kept at room temperature for 18 hours then volume was made up to 50 mL with methanol.

Thermal Degradation

50mg of the drug was spread in a borosilicate glass Petri dish and placed in a hot air oven maintained at 60^0C for 48 hours, then the solution was prepared to achieve a final concentration of 100µg/ mL^{-1}.

Photo Degradations

50mg of the drug was spread in a borosilicate glass Petri dish and placed in a light cabinet (Thermo lab, India) and exposed to ≥200 W h m–2 UV irradiation at 320–400 nm, at 25°C, for 10 days. After removal from the light cabinet, all samples were analyzed after preparation of 100 µg mL^{-1} solutions in the mobile phase.

Assay Procedure for Tablets

To determine the RAN content of tablet formulations, twenty Caroza and Ranz-500 tablets (label claim 500 mg) were weighed, to determine the average weight of the tablets, and then crushed and mixed using a mortar and pestle. A portion of powder equivalent to 1000 µg mL^{-1} was accurately weighed into each of three 10 mL volumetric flasks and 5 mL methanol was added. Each solution was sonicated for 20 min to achieve complete dissolution of the RAN and the solutions were then diluted to volume with mobile phase, to yield concentrations of 1000 µg mL^{-1}, and filtered through a 0.22-µm Nylon membrane filter. The solution obtained was analyzed by HPLC.

RESULTS AND DISCUSSION

HPLC Method Development and Optimization

A HiQ Sil C-18 HS column was used for method development. The mobile phase, methanol – water 99:1 (v/v) at a flow rate of 1 $mLmin^{-1}$, was selected, after several preliminary investigatory chromatographic runs, on the basis of suitability for drug content estimation and cost, because rapid and economic analysis is becoming increasingly important in pharmaceutical analysis to increase throughput, using for the first time a mobile phase excluding buffer thereby saving cost of analysis and time. Buffer used in the mobile phase may give a sharp peak but simultaneously it reduces the longitivity of column life and also considerable time is spent for washing the column to eliminate the buffered material and subsequent equilibration of the column. Under the experimental conditions described, all peaks were well defined and free from tailing **(**Fig.2).The retention time (RT) and asymmetry factor were 3.4 ± 0.01 min and 1.64± 0.05, respectively.

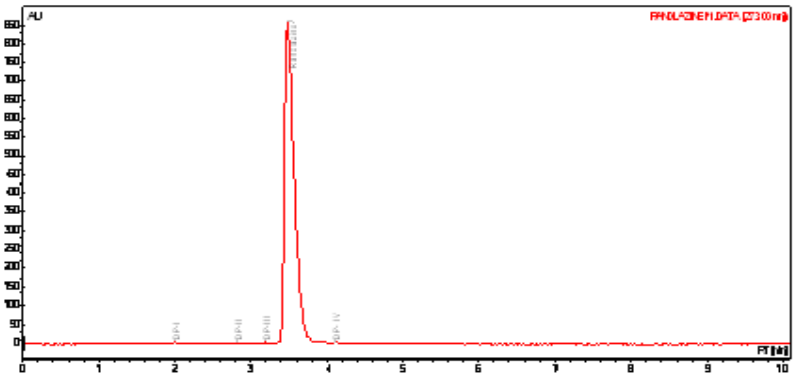


Fig. 2: HPLC chromatogram of RAN

Validation of the Method

Linearity

The linearity of the response of the drug was verified at seven concentration levels, ranging from 10 to 400% of the targeted level (100 µg mL^{-1}), of the assay concentration. Standard solution containing 10 - 400 µgmL^{-1} of RAN in each linearity level was prepared. Linearity solutions were injected in triplicate. The calibration graph was obtained by plotting the peak area versus the concentration data and was treated by least-square linear regression analysis. The equation of the calibration curve for RAN obtained Y = 51573 X - 24119, the calibration graphs were found to be linear in the aforementioned concentrations. The coefficient of determination was 0.9997.

Limits of Detection (LOD) and Quantification (LOQ)

The LOD and LOQ of the method were 0.223 and 0.677 µg mL^{-1}, respectively, which indicates the method is suitable for detection and quantification of RAN over a very wide range of concentrations.

Accuracy, as Recovery, and Precision

By spiking previously analyzed test solution with additional standard drug the recovery of the method was found to be 99.45-100.07%. Values of recovery (%) are listed in Table 1; RSD was always <1% which indicates the method is accurate. The intra-day and inter-day variability or precision data are summarized in **Table 2**. The low value (≤1%) of the RSD is indicative of the repeatability of the method.

Robustness and Ruggedness

There was no significant change in the *R*T of RAN when mobile phase composition, flow rate, and detector wavelength was changed slightly. The low value of the RSD (Table 3) indicates the method is robust. The RSD for intra-day and inter-day assay of RAN performed in the same laboratory by two analysts did not exceed 2.0%, indicating the ruggedness of the method.

Specificity and Degradation Studies

RAN was found to be stable at heat, light and neutral hydrolysis experiments. Degradation was observed at acid hydrolysis, alkali hydrolysis and oxidative stress conditions. RAN degraded into acid, alkali and oxidation (fig 3a, b, d) and forms polar impurities. In acidic condition RAN degraded up to 31.5 %, in basic condition up to 31.6 % and in oxidative condition 95 % degradation was observed for RAN. However our findings (Table 4) indicate that RAN is very susceptible under acidic and basic stress conditions unlike the reported literature[18]. A peak purity result greater than 990 indicates that the RAN peak is homogenous in all stress conditions tested. The mass balance of RAN in stress samples was close to 100% and moreover, the unaffected assay of RAN in tablets confirms the stability – indicating power of the method.

Table 1: Results from recovery studies (*n* = 3)

Sample	Amount taken (µg mL^{-1})	Amount %	Amount added (µg mL^{-1})	Mean amount present (µg mL^{-1})	Mean amount found ± SD	Recovery %
Ranz-500	80	50	40	120	119.35± 0.42	99.45
	80	100	80	160	159.78± 0.16	99.86
	80	150	120	200	199.93± 0.08	99.96
Caroza	80	50	40	120	120.09± 0.13	100.07
	80	100	80	160	159.95± 0.06	99.96
	80	150	120	200	199.97± 0.13	99.98

Table 2: Results from determination of the precision of the method (*n* = 3)

Concentration (µg mL^{-1})	Intra-day precision		Inter-day precision	
	Mean area ± SD	RSD (%)	Mean area ± SD	RSD (%)
40	2098741±1210.71	0.057	2098237±2122.04	0.10
80	3980440 ±3593.08	0.090	3977297±1464.08	0.036
100	4954928± 1527.57	0.030	4954597±2084.02	0.042

Table 3: Results from testing the robustness of the method

Condition	Modification	Mean area ± SD	RSD (%)	Mean *R*T (min) ± SD
Mobile phase composition (v/v)	98:2	3981804 ± 6722.20	0.168	3.435 ± 0.028
	99:1	3982446 ± 6074.14	0.152	3.404 ± 0.020
	100	3981839 ± 5789.90	0.145	3.412 ± 0.068
Mobile phase flow rate (mLmin^{-1})	0.9	3976113 ± 1985.16	0.049	3.413 ± 0.012
	1	3977556 ± 1564.72	0.039	3.402 ± 0.014
	1.1	3976879 ± 2280.28	0.057	3.399 ± 0.011
Detector wavelength (nm)	271	3977897 ± 1283.97	0.032	3.420 ± 0.018
	273	3979964 ± 4668.73	0.117	3.421 ± 0.015
	275	3978631 ± 5134.66	0.129	3.415 ± 0.010

(*n* = 3, concentration = 80 µg mL^{-1})

Table 4: Summary of forced degradation results

Forced degradation results				
Stress conditions	**Time**	**% Degradation**	**% Assay of active substance**	**Mass balance (% Assay+ % DP)**
Acid hydrolysis 0.1 N HCl 60^0C	3 hrs	31.507	68.093	99.6
Base hydrolysis 0.1 N NaOH 60^0C	1 hrs	31.67	67.830	99.5
Oxidation 30% H_2O_2	18 hrs	95.096	4.504	99.6
Neutral hydrolysis 100^0C	4 hrs	1.173	98.527	99.7
Thermal hydrolysis 60^0C	48 hrs	0.673	98.927	99.6
Light (photolytic degradation)	10 days	0.465	99.035	99.5

Solution Stability

To demonstrate the stability of standard solutions and tablet sample solutions during analysis, they were analyzed over a period of 3 days at room temperature. The results showed that for both solutions the retention time and peak area of RAN remained almost unchanged (RSD less than 0.01 and 0.57%, respectively) and no significant degradation was observed during this period, suggesting that both solutions were stable for at least 3 days, which was sufficient for the whole analytical process .

Use of the Method for Analysis of Marketed RAN Formulations

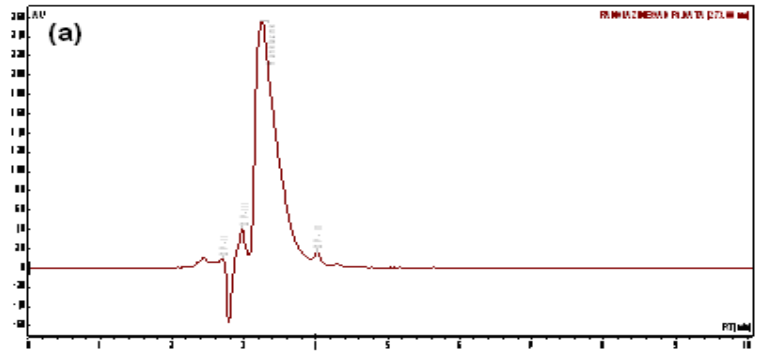
The validated method was used to estimate the RAN content of two commercially available brands of tablet containing 500 mg RAN-Caroza and Ranz 500, manufactured by Zydus cadila Ahmadabad, India. Torrent Pharmaceuticals, Indrad, India, respectively. Satisfactory results were obtained. Recovery of RAN from Caroza and Ranz 500 tablets was 99.55% (RSD 0.0305%) and 99.86% (RSD 0.0364%), respectively, and the amounts measured were in good agreement with the label claims.

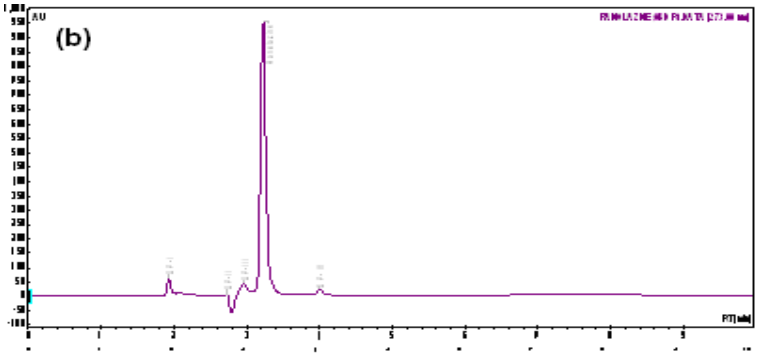
The respective chromatograms also indicate that the decomposition products present in the formulations are satisfactorily separated and their amounts are well within regulatory specifications (<2%).

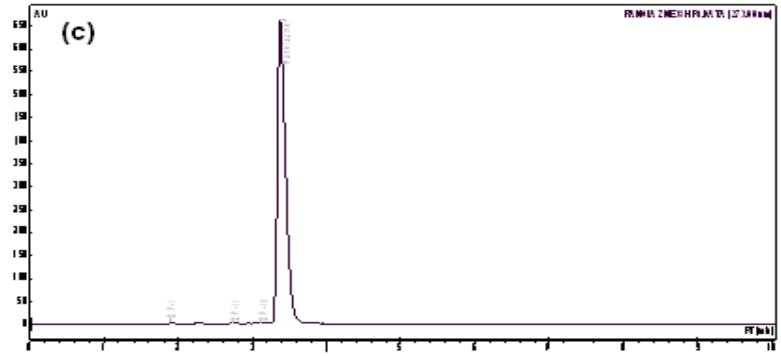
The results of the assay indicated the method is selective for analysis of RAN without interference from the excipients present (Table 5 and Figs 3g and h).

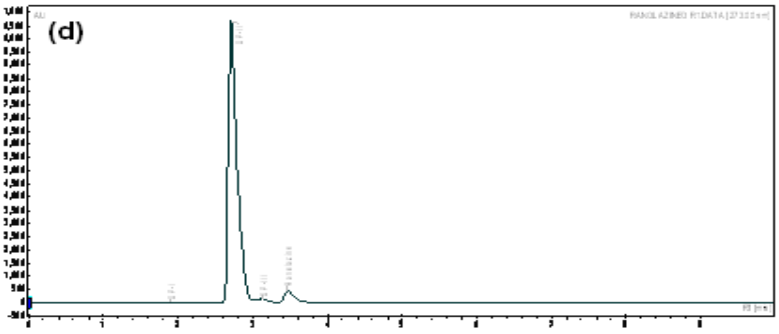
Table 5: Results from assay of RAN tablets (*n* = 3)

Formulation	Mean concentration ± SD	RSD (%)	SE	Recovery (%)
Ranz-500	499.32 ± 0.1823	0.0364	0.105	99.86
Caroza	497.76 ± 0.1527	0.0305	0.088	99.55





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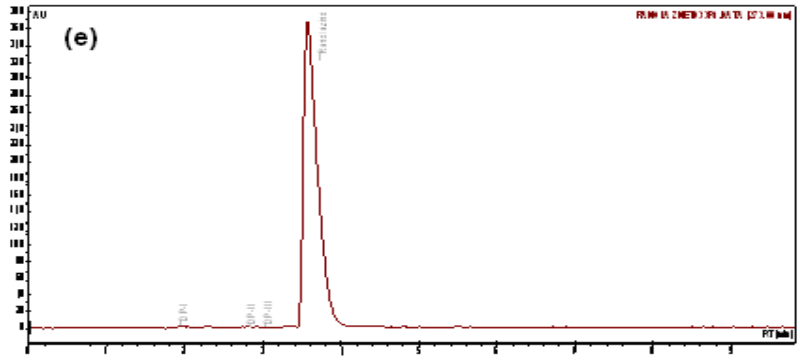
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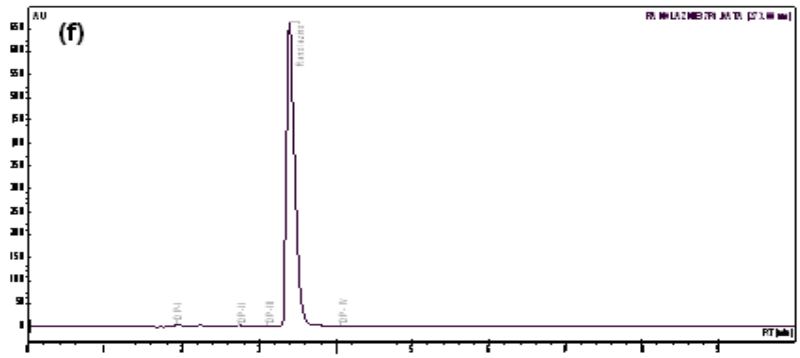
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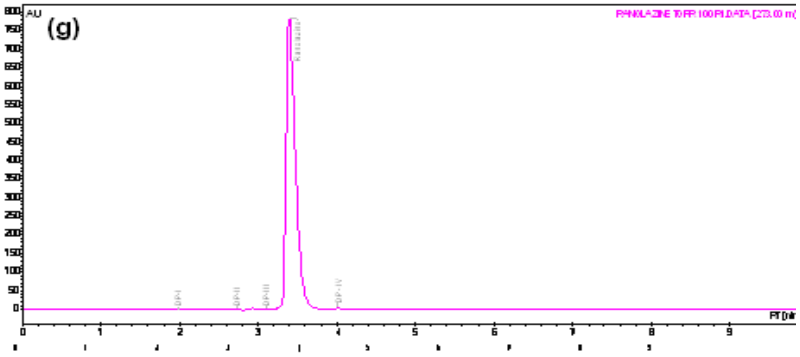
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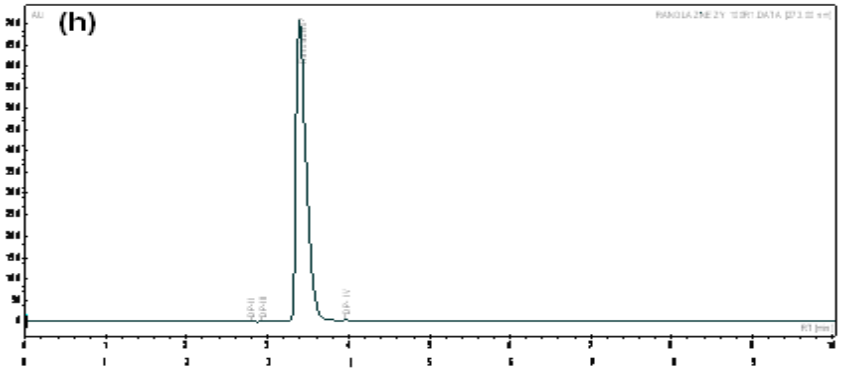

Fig. 3: Typical chromatograms obtained from API degraded by (a) acid hydrolysis,(b) alkaline hydrolysis, (c) neutral hydrolysis, (d) oxidation, (e) dry heat, (f) Photo degradation (g) Formulation I, (h) Formulation II

CONCLUSION

A simple, rapid, accurate, and precise stability indicating RP- HPLC analytical method with PDA detection has been developed for the determination of RAN in active pharmaceutical ingredient and two different brands of tablet dosage forms. The method was validated in accordance with ICH guidelines. The retention time (3.4 min) enables rapid determination of the drug, which is important for routine analysis. The low detection and quantification limits achieved mean the method is highly sensitive, stress testing showed that all degradation products were well separated from RAN, confirming its stability-indicating capability. The method seems to be suitable for the quality control in the pharmaceutical industry because of its sensitivity, simplicity, and selectivity.

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