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Development of Stability indicating method for the estimation of Ornidazole in Pharmaceutical Formulations.

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ABSTRACT

For the determination of ornidazole (ONZ) in tablet dosage forms, a simple, specific, accurate, stability-indicating reverse phase liquid chromatographic method was developed.. A stationary phase used was Waters Spherisorb RP-C18, 5 μm column having 250 x 4.6 mm, with a mobile phase containing 20 mM Ammonium Acetate(pH 4.0 with Acetic Acid): acetonitrile (70:30 % v/v) at a flow rate of 1ml/min in isocratic mode. The retention time of ornidazole was 6.8 min. The linearity for ornidazole was in the range of 0.050 – 15 μg/ml. The accuracy of the optimized HPLC method by recovery was found to be in the range of 99.12 – 100.54 %w/w. The detection limit and quantification limit were found to be 0.015 μg/ml and 0.050 μg/ml, respectively. Stress studies were conducted on drugs under ICH ICH-prescribed conditions, viz, hydrolysis, oxidation, photolysis and thermal stress. Ornidazole was exposed to acid, alkali, Neutral, 3% H_2O_2, accelerated temperature and Photolysis. Stress conditions indicate that the drug is susceptible to strong acid, base hydrolysis, oxidative hydrolysis, photolysis in acidic conditions and dry heat degradation. The pure drug peak and the degraded product peaks were clearly separated, and their retention time values varied significantly. The proposed method for the determination of ornidazole in tablet formulations has been validated and successfully applied.

Keywords: Ornidazole, nitroimidazoles, stability-indicating assay and Reverse Phase Liquid Chromatography.

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Introduction

Ornidazole,α-(chloromethyl)-2-methyl-5-nitro-1H-imidazole-1-ethanol-1-(3-chloro-2-hydroxy propyl)-2-methyl-5-nitroimidazole[1,2]. It is an antibacterial medication which inhibit the growth of both anaerobic bacteria and certain anaerobic protozoa, such as Trichomonas, vaginalis, *Entamoeba histolytica* and *Giardia lamblia.*[3]. Ornidazole is used to treat bacterial vaginosis, trichomoniasis, female and male genitourinary infections, amoebiasis, and giardiasis. It also has applications in the treatment of infections caused by anaerobic bacteria, prophylaxis during surgical interventions, particularly those involving the colon, and gynaecological operations[4]. Ornidazole has been successfully employed in combination with other drugs for peptic ulcers, few types of gastritis, stomach cancers, rheumatoid arthritis[5] and in the prophylaxis of Crohn's disease[6]. Ornidazole activity is primarily mediated by the reduction of the nitro group to a more reactive amine that attacks microbial DNA, resulting in the loss of the helical structure of DNA and subsequent DNA fragmentation, thereby inhibiting further synthesis, interfering with transcription, and ultimately causing cell death.[7-8].

Few liquid chromatographic (LC) methods have been reported for the estimation of Ornidazole (ONZ)alone and in combination with other drugs in pharmaceutical formulations [9-17] and in biological fluids [18-21] by using HPLC.

The purpose of this study was to develop a highly selective and sensitive analytical liquid chromatography (LC) method for the estimation of ONZ in medicinal substances and tablets that is suitable for routine quality control and stability studies.

Experimental

Apparatus

The LC system consisted of the following components: Jasco PU 1580 Intelligent pump with Jasco UV 1575 Intelligent UV-Vis detector and Rheodyne injector with 100 µL fixed loop. Integration and data analysis were carried out using Borwin Chromatographic software (Integrator, Jasco Japan). The UV- Vis detector was set at a wavelength of 318 nm. Waters Spherisorb RP-C18 column (250×4.6 mm, 10 mcm) was used for chromatographic Separation. Utilizing a Mettler electronic balance for weighing purposes.

Reagents and Materials

Analytically pure ONZ was obtained as a gift sample from Endoco Pharma Ltd., (Gujarat, India). Acetonitrile, Methanol (Merck Mumbai, India) and Distilled water (Millipore, USA) were of HPLC grade, while Ammonium Acetate and Acetic Acid (Qualigens, Mumbai, India) were of analytical grade used for the preparation of the mobile phase. Tablet formulations A (Onidaz, Medley Pharmaceutical Ltd., India) and B (Ornida, Aristo Pharma Ltd., India) containing 500 mg of ONZ as labeled were acquired from the local market.

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Preparation of Mobile Phase

Ammonium Acetate was weighed (0.154 g) and dissolved in 100 mL of water, Adjust the pH of the solution to 4.0 with Acetic Acid. This solution was mixed with of Acetonitrile in the ratio of 70:30v/v. The solution was sonicated for 10 minutes, filtered through 0.45-m Whatman filter paper, and utilized as the mobile phase.

Preparation of Stock solutions

In order to produce the stock solution, analytically pure ONZ (10 mg) was weighed and transferred to a 10 ml volumetric flask. Methanol was used to reach the required volume, obtaining 1000 g/mL of ONZ. The mobile phase was subjected to additional dilutions.

Chromatographic Conditions

A reverse phase C18 column equilibrated with a mobile phase of 20 mM Ammonium Acetate (pH 4): Acetonitrile (70:30v/v) was employed. The flow rate for the discharge of the mobile phase was kept at 1 mL/min, and effluents were monitored at 318 nm. A 100 L fixed loop was used to inject the sample, and the total run time was 10 minutes.

Calibration curve for ONZ

A linearity study was carried out on Standard solutions having concentrations 0.050, 0.075, 0.125, 0.750, 2.50, 5.00, 10.00, and 15.00 µg/ mL of ONZ were prepared from stock solution having concentrations 25ug/ml by pipetting out appropriate aliquots of stock solution in various 10 mL volumetric flasks. Chromatograms were taken after the solutions were injected using a 100 L fixed loop apparatus. The calibration curve was plotted by average peak area versus concentrations and ONZ regression equation was calculated which was further used for the determination of the content of ornidazole.

Method Validation

The method was validated for accuracy, precision, linearity, specificity, detection limit, quantification limit and robustness.

Linearity

The linearity of the analytical method was determined using 8 concentration levels of ONZ standard solutions in the range of 0.050 to 15.00 µg/mL. The solutions (100 µl) were injected and analyzed using the optimized HPLC method. The linearity was evaluated by the least square regression method.

Precision and Accuracy

The precision study was carried out at intra and interday levels using Quality control standards at low, medium and High concentrations. three different concentrations of ONZ (0.150, 6.00, and 12.00 g/ mL), which represent low, medium, and high concentrations in the analytical range were analyzed six times for confirming intra-day and inter-day precision. The results are reported in terms of relative standard deviation (RSD, Table 2). The accuracy of the method was also calculated by comparison with the theoretical value.

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Specificity

Specificity was carried out by injecting a blank solution and comparing interference at Rt of an analyte. The chromatogram was taken by appropriate dilutions and the quantities of drugs were determined.

Detection limit and Quantification limit

The lowest concentration of an analyte at which background levels may be reliably distinguished is known as the detection limit. The lowest amount of analyte that can be quantitatively measured with enough precision and accuracy is the limit of quantification of a specific analytical method. LOD was the concentration that produced a signal-to-noise ratio (S/N) of 3:1, whereas LOQ produced a S/N of 10:1.

Ruggedness

The robustness of the method was studied by changing the column of make Analyzed using different instruments.

Application of validated method for Assay of ONZ formulation

Twenty tablets were weighed and finely powdered. A volumetric flask measuring 100 mL was filled with a powdery substance weighing precisely 500 mg of ONZ, and 50 mL of methanol was then added. Using a sonicator, the extraction was carried out for 20 minutes. Methanol (solution A, 5000 µg/ml) was used to get the volume up to the required level. The Whatman filter paper (No. 1) was used to filter the aforementioned solution. In a volumetric flask, 1ml of Solution A was diluted to 100ml (Solution B = 50 g/ml). 10ml was created by diluting 1ml of Solution B with Solution C (5.0 g/ml). Under the aforementioned chromatographic conditions, solution C was sonicated for 10 min, injected, and the peak area was calculated.

Accelerated degradation study

Analytically pure ONZ was measured out (10 mg each), put to a 10 mL volumetric flask, and diluted with methanol until the desired consistency was reached. The stock solution, which had a concentration of 10 g/ml, was further diluted in methanol. For forced deterioration studies, this stock solution was utilized.

(a) *Hydrolysis*. -10 mL of stock solution (10 µg/ml) was added to 10mL of 1M HCl, 1M NaOH and Water separately. The resulting mixtures were refluxed on the water bath for 2 hours at 60^{o}C, individually and analyzed further using optimized HPLC method.

(b) *Chemical oxidation.* –3% hydrogen peroxide was added to 10 mL of stock solution. The solution was refluxed for 2 h at 60 ^{o}C.

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(c) *Photolytic Degradation* – 10 mL of stock solution (10 µg/mL) added to 10 mL of Water and 1M HCl separately. The solutions were exposed to UV light at 254 nm for 2days.

(d) *Dry heat degradation.* – Bulk drug was exposed in oven at 50 ^{o}C for 30 days. Then 1 mg of drug was weighed and transferred to 100 mL volumetric flask and volume was made up to the mark with methanol.

Each stressed sample was diluted with mobile phase to obtain final concentration of 5 µg/ mL for ONZ and each solution was analyzed in triplicate.

Results and Discussion

Ornidazole is strong acidic in nature (ionizes in basic medium) so reverse–phase chromatography is considered for method development. Various stationary Phases C_{18} and C_8 were tried. C_{18} column was preferred because C_8 column was giving decreased retention of the drug (2.5 min) which resulted in the merging of solvent and drug peak.

Development of liquid chromatographic method

Various mobile phase compositions were tried to get desired resolution, run time, asymmetric factor and theoretical plates. A 20 mM Ammonium Acetate (pH 4.0) - Acetonitrile (70:30 v/v) mobile phase that produced an ONZ peak that was symmetrical and sharp at a flow rate of 1 mL/min was found to be adequate. The wavelength of detection was selected at 318 nm on the basis of the UV spectrum of ONZ (Figure 1), which revealed that the drug absorbs noticeably at that wavelength. The chromatogram of ONZ revealed a single peak of the drug at 6.8 minutes with an asymmetry of 1.2 under the selected experimental conditions (Figure 2).

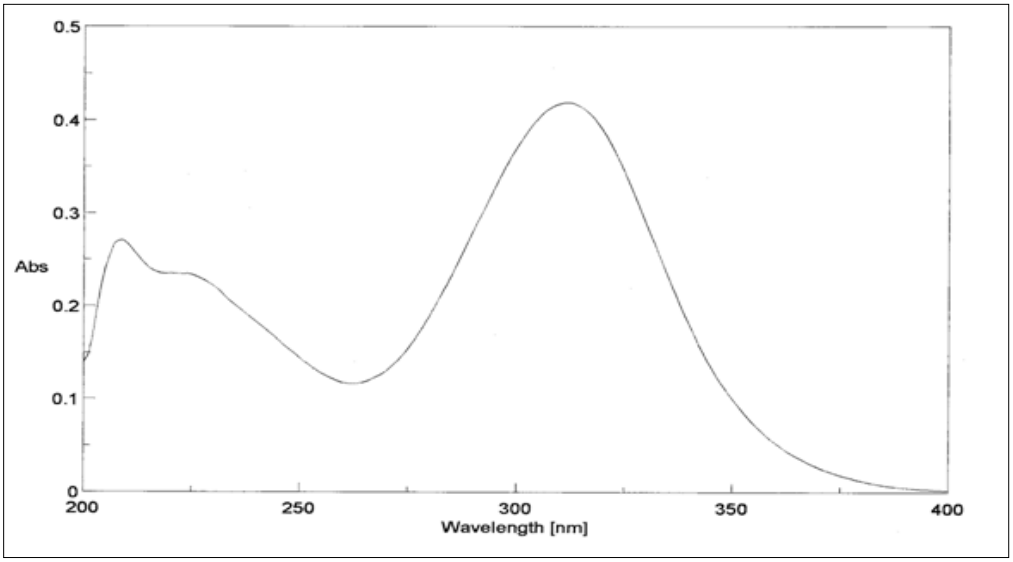

Figure 1: UV spectrum of Ornidazole (ONZ).

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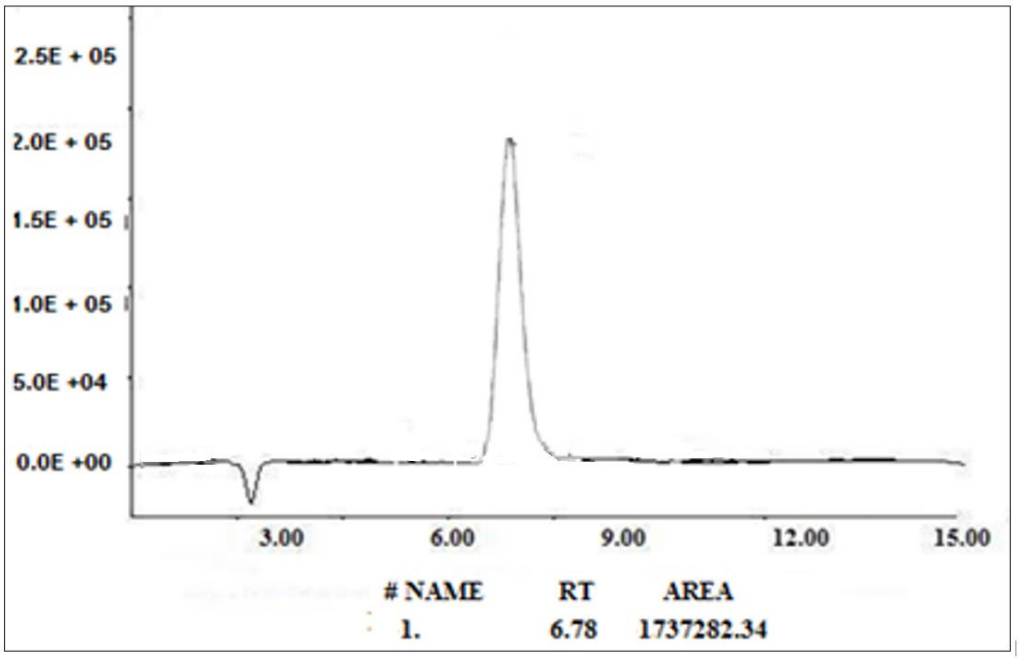

Figure 2: Chromatogram of ONZ in mobile phase 20 mM Ammonium Acetate (pH4.0) – Acetonitrile (70:30v/v)

Validation of the optimized method

Calibration curve for ONZ was discovered to be linear in the range of 0.050 - 15 g/ mL. To determine the calibration equation and correlation coefficient, the least square regression analysis was applied to the peak area and concentrations. Table 1 displays the data for the calibration curve. R was revealed to be 0.9987, demonstrating acceptable linearity. The concentration that produces a signal-to-noise (S/N) ratio of 3:1 was determined to be the LOD value, which is 0.015 g/ mL. The LOQ was 0.50 g/mL with a 10:1 S/N ratio. Three distinct concentration levels (0.150, 6.0, and 12.0 g/ mL) were used for precision studies, and the results are presented in terms of RSD (Table 2).

The accuracy of the method was determined by the recovery method. Recovery of the standard that had been added to the sample, it was determined to be 99.12 - 100.54%, indicating that the method is accurate. In the specificity study, a single peak of ornidazole was observed which indicates that there was no interference from the excipients used.

The proposed liquid chromatographic method was applied to the determination of ONZ in Tablet formulation A. The results were comparable with the corresponding labeled amounts (Table 3).

Table 1: Regression Analysis of the Calibration Curve for the Proposed Method

Parameters	**ONZ**
Linearity range (mcg/ mL)	0.050 – 15
Slope	180.94
Standard deviation of slope	1.4292
Intercept	14504
Standard deviation of intercept	1101.34
Correlation coefficient (r)	0.9987

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Table 2: Summary of Validation Parameters

Parameters	ONZ
Detection limit (mcg/ mL)	0.015
Quantitation limit (mcg/ mL)	0.050
Accuracy (%)	100.04 - 100.25
Precision (RSD[a])	
Intra-day(n=3)	0.5619 -0.0027
Inter-day(n=3)	0.4392 -0.0032

[a]RSD indicates relative standard deviation

Table 3: Assay Results of Tablet Dosage Form Using Proposed Method

Formulations	Amount of drug taken (mg)[b]	Amount obtained (mg)[b]	% Recovery [b]
A	500	500.19 ± 0.123	100.15 ± 1.23
B	500	500.75 ± 0.030	100.07 ± 0.30

[b]mean value ± standard deviation of three determinations; Tablet formulation A is Onidaz (Medley Pharmaceutical Ltd., India) and B is Ornida (Aristo pharma Ltd., India) containing labeled amount of 500 mg of ONZ.

Accelerated degradation study

The accelerated degradation study was carried out as per ICH guideline[22]. The objective of the study was to find out the likely degradation products, which in turn help in the establishment of degradation pathways and the intrinsic stability of drug molecules.

When Ornidazole was exposed to acid hydrolysis (1 M HCl) the height of the peak decreased with respect to time in 1 M HCl solution, with an additional peak at 6.2 min, Ornidazole was found to be degraded 1.15 % in acidic conditions. The peaks of the degraded products were well resolved from the drug peak (Figure 3). Upon heating the drug in 0.1 M NaOH at 60^0 C, drug was found to be highly labile to alkaline hydrolysis, 15.65% degradation of the drug was found in 2 hours. Two additional peaks at 5.22min and 7.28min (Figure 4). In Neutral hydrolysis, the drug peak area was found to be deceased with respect to time. 1.02% of the drug was found to be degraded. Major degradation product was observed at RT 5.9 and 8.3min, (figure 5).

The drug was found to be degraded in 3% hydrogen peroxide at room temperature. It was decomposed to 2.34%. Major degradation product was observed at RT 6.2 min (Figure 6).

In photolysis in water, minor degradation of about 0.48% was observed at RT 5.8min (Figure 7), in 1M HCl photolysis; drug was degraded into major product at 9.6. Ornidazole was found to be degraded 1.22 %(Figure 8). Ornidazole was found to be degraded in thermal stress. Degradation product was observed at RT 9.3 min with 0.82% degradation (Figure 9).

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Table 4: Degradation of Ornidazole

Condition	Time (hours)	Recovery (%)	Retention time of Degradation product
Acid Hydrolysis (1M HCl)	**2**	1.15%	6.2, 6.7
Base Hydrolysis	**2**	15.65%	5.22, 6.9, 7.28
Neutral Hydrolysis	**2**	1.02%	5.91, 6.8, 8.3
Oxidation (3% H2O2)	**2**	2.34%	6.2, 6.78
Photolytic degradation (Water)	**2**	0.48%	5.8, 6.75
Photolytic degradation (1M HCl)	**2**	1.22%	6.7, 9.6
Thermal Degradation (Dry heat)	**2**	0.82%	6.87, 9.3

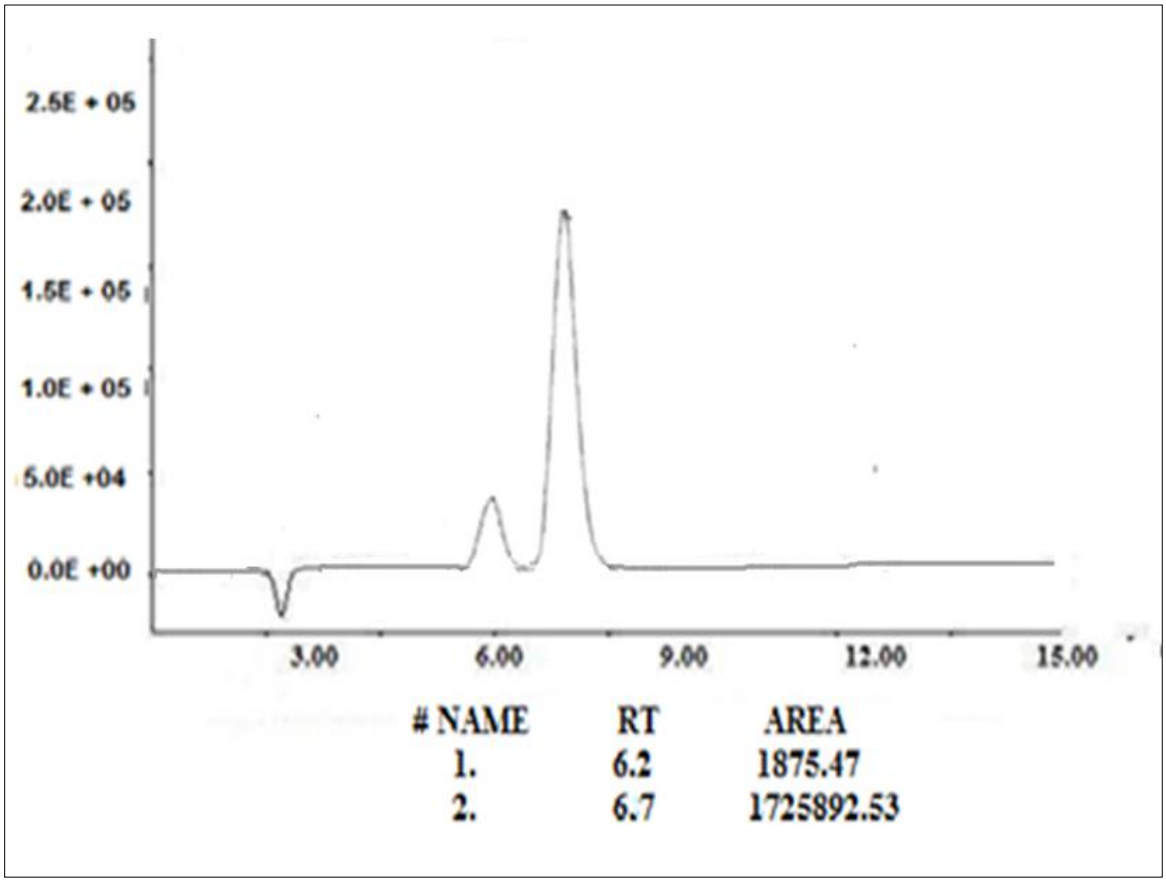

Figure 3: Chromatogram of ONZ subjected to acid hydrolysis (0.1N HCl at 60 ^{o}C for 2 hours).

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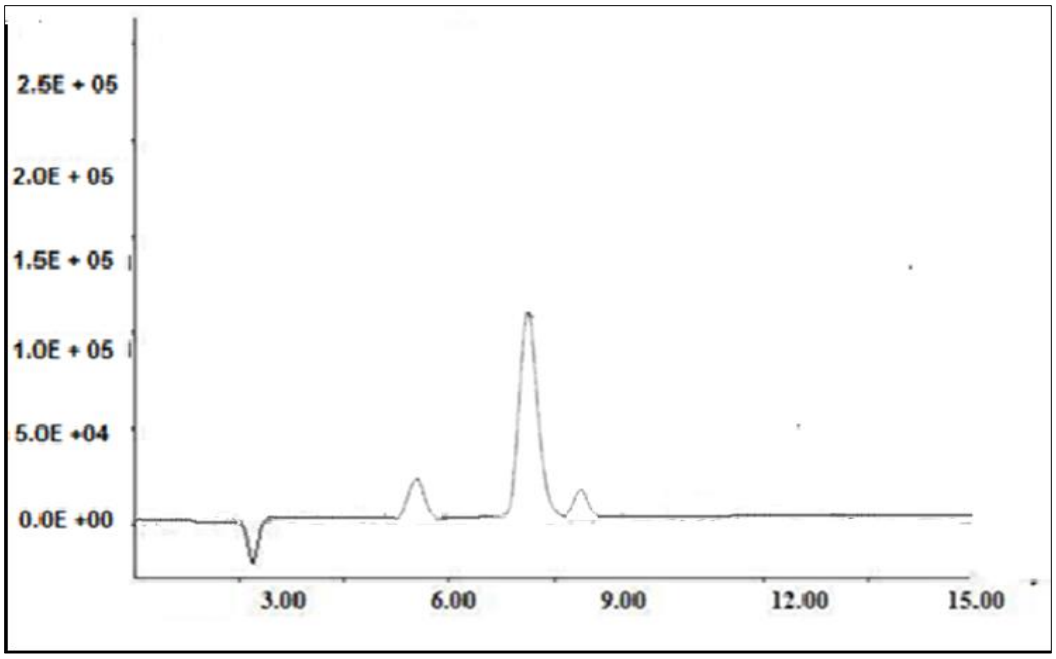
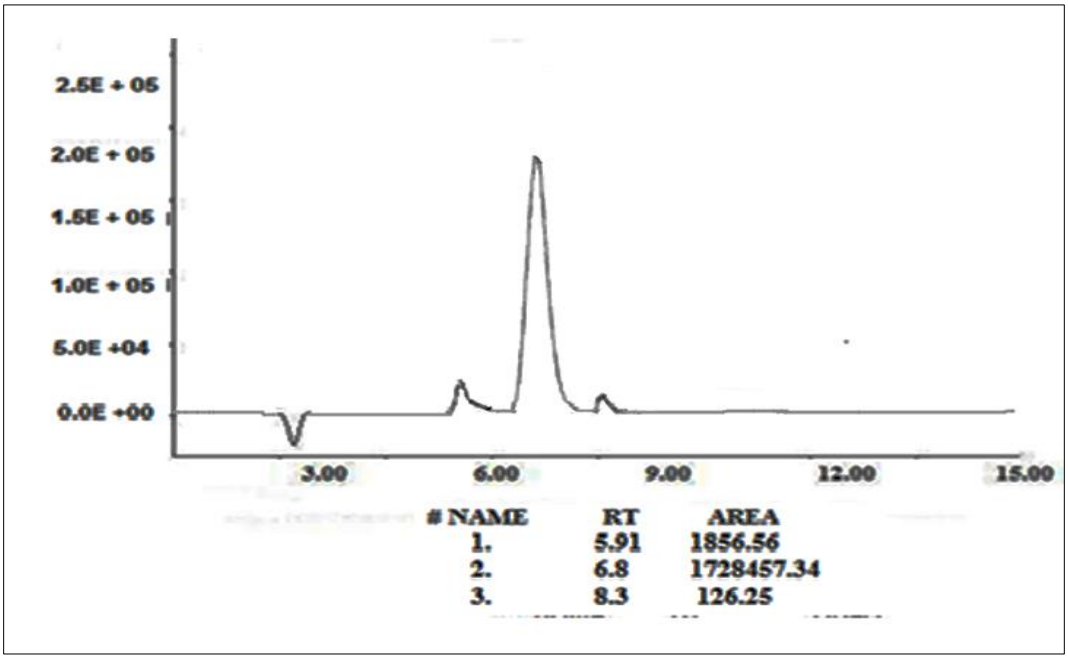


#	NAME	RT	AREA
1.		5.22	1869.56
2.		6.9	1473632.23
3.		7.28	1475.38

Figure 4: Chromatogram of ONZ subjected to Base hydrolysis (0.1 N NaOH at 60 ^{o}C for 2 hours).



#	NAME	RT	AREA
1.		5.91	1856.56
2.		6.8	1728457.34
3.		8.3	126.25

Figure 5: Chromatogram of ONZ subjected to Neutral hydrolysis (Water at 60 ^{o}C for 2 hours).

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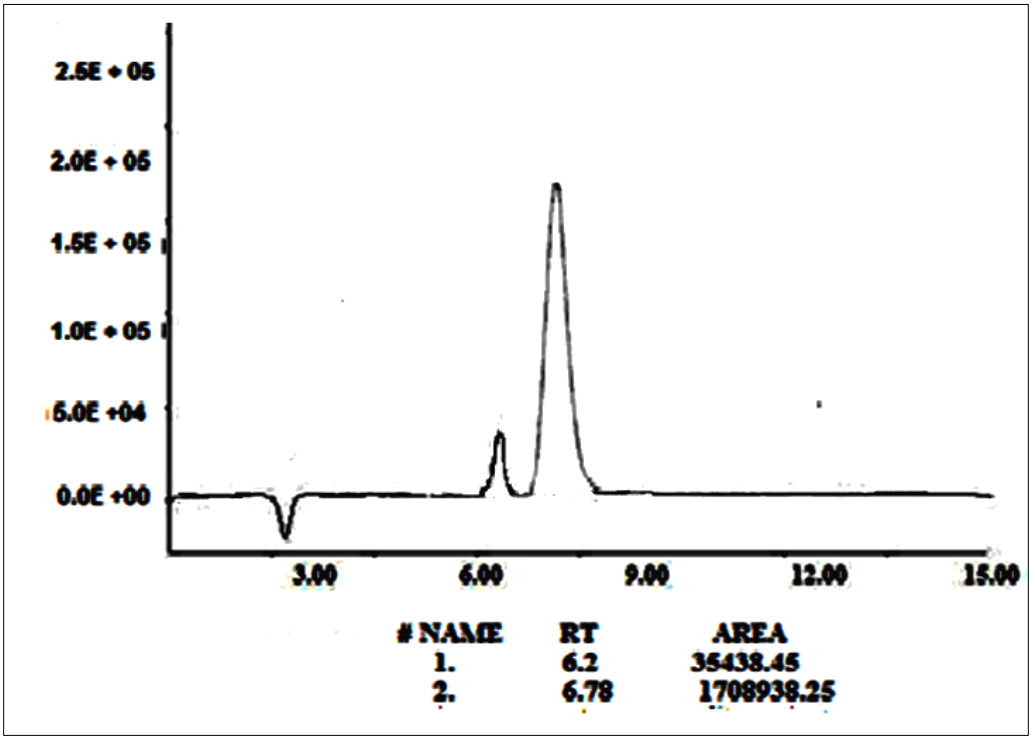

Figure 6: Chromatogram of ONZ subjected to chemical oxidation (3% H_2O_2 at 70 ^oC for 2 hours).

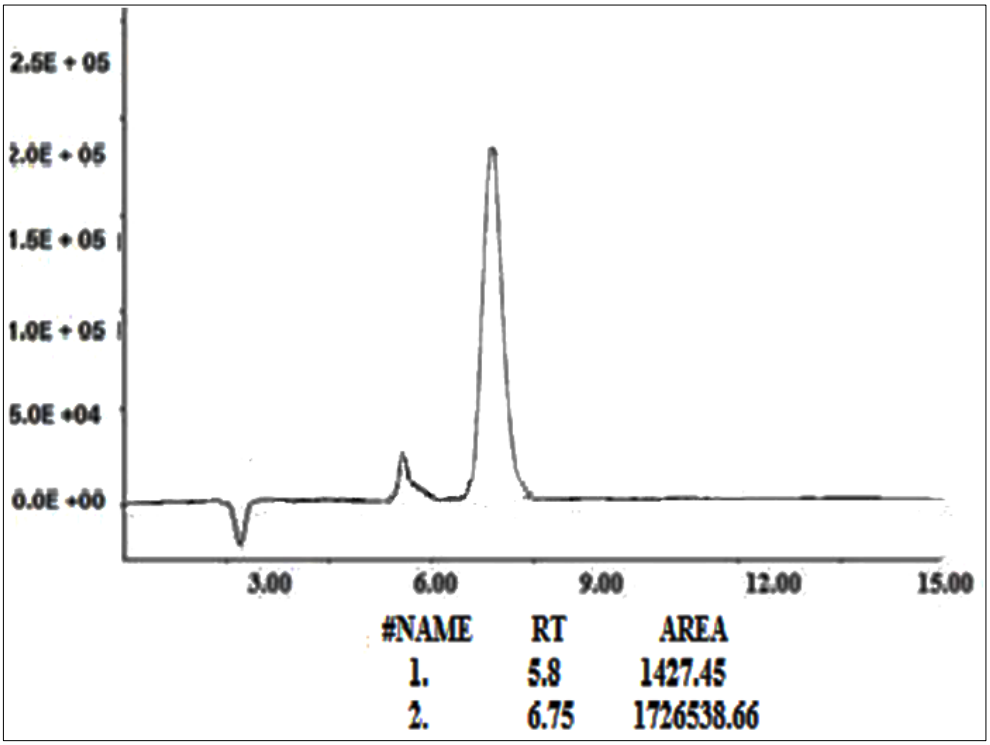

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Figure 7: Chromatogram of ONZ subjected to Photolysis (Water exposure to UV light at 254nm for 2 days).

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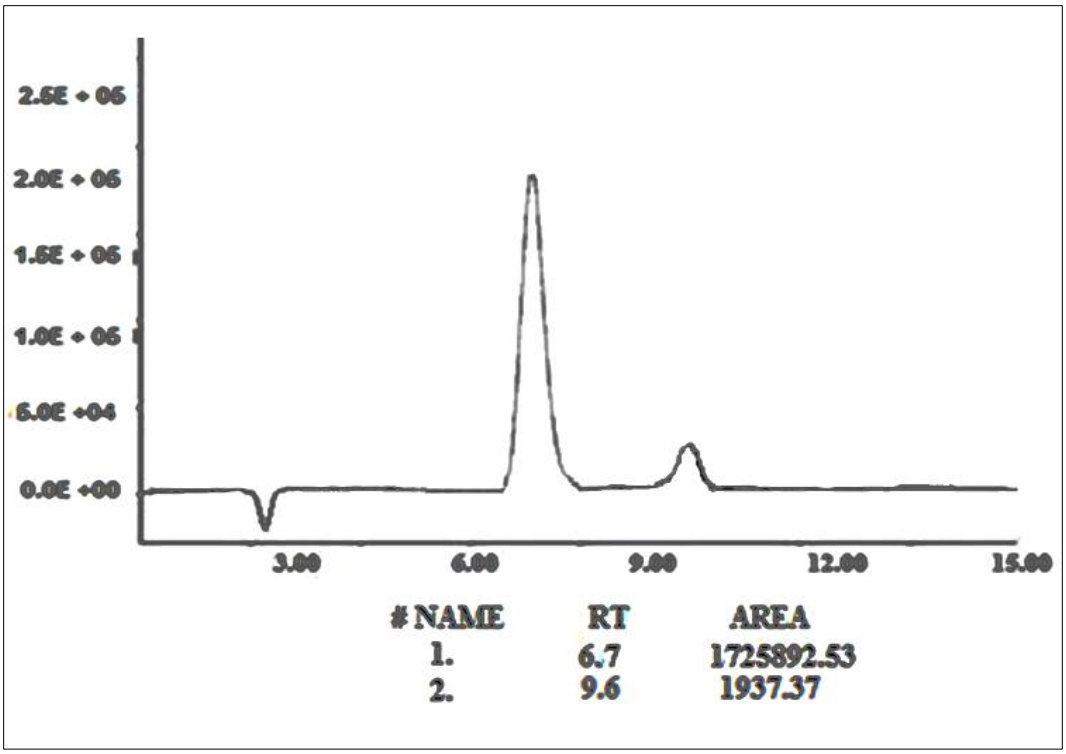

Figure 8: Chromatogram of ONZ subjected to Photolysis (1 M HCl exposure to UV light at 254nm for 2 days).

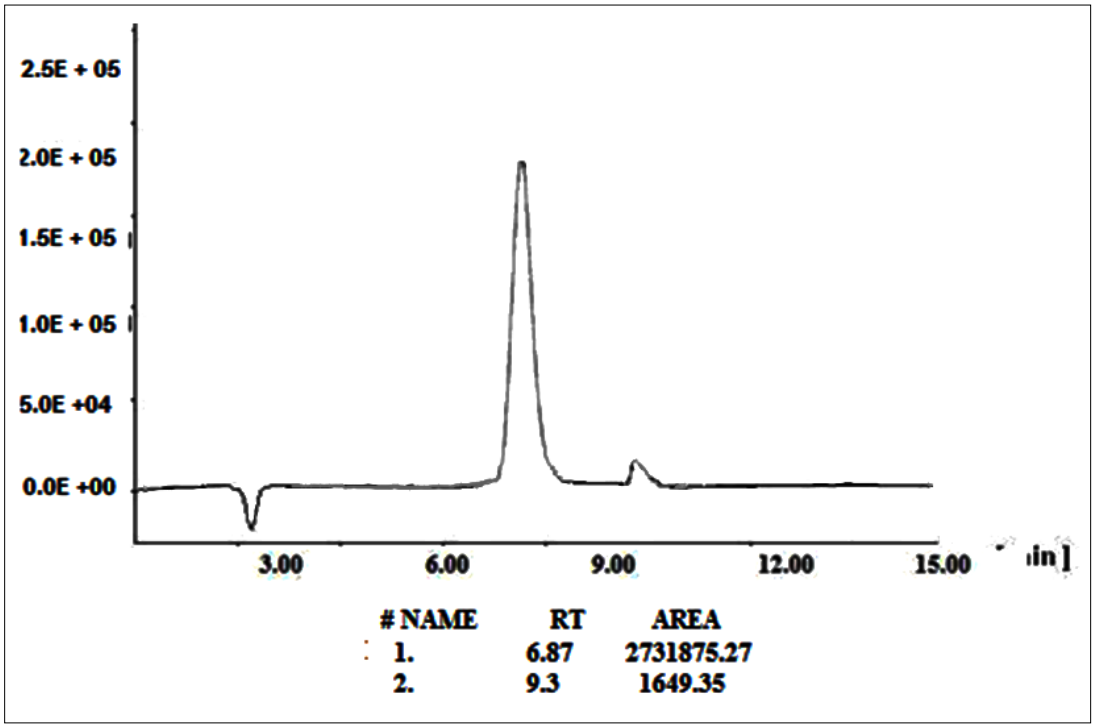

Figure 9: Chromatogram of ONZ subjected to thermal stress at 50^0C for 30 days.

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In summary, forced degradation study under described condition showed that, ONZ is susceptible to strong acid, base hydrolysis, oxidative hydrolysis, photolysis in acidic conditions and dry heat degradation (Table 4). The proposed method can be employed for monitoring the stability of Ornidazole drug substance, because the peak of the parent drug is not interfered by the peak of the degraded products.

Conclusions

In proposed study, analytical method has been developed for determination of ONZ in tablet dosage form and the method was further applied for force degradation studies. The method was validated and found to be simple, sensitive, accurate and precise. Statistical analysis proved that method was repeatable and selective for the analysis of ONZ without any interference from the excipients. The method was successfully used for determination of drugs in their pharmaceutical formulations and also for force degradation studies viz. acid, base, neutral, chemical, photolytic and dry heat degradation study.

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