e-ISSN: 2320-4230, p-ISSN: 2961-6085

Journal of Drug Discovery and Therapeutics

Available Online at www.jddt.in

CODEN: - JDDTBP (Source: - American Chemical Society)

Volume 12, Issue 02; 2024, 84-96

Stability Indicating UPLC Method for Quantifying Assay of Letrozole

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Received: 20-01-2024 / Revised: 17-02-2024 / Accepted: 29-03-2024
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Conflict of interest: No conflict of interest.

Abstract:

Letrozole, an aromatase inhibitor, is widely used in the treatment of hormone receptor-positive breast cancer. Ensuring the stability and accurate quantification of letrozole is crucial for its efficacy and safety in clinical practice. In this study, a stability indicating Ultra Performance Liquid Chromatography (UPLC) method was developed and validated for the quantitative determination of letrozole in pharmaceutical formulations. The UPLC method utilized a reverse-phase C18 column with a mobile phase consisting of acetonitrile and 0.1% formic acid in water, delivered in an isocratic mode at a flow rate of 0.5 mL/min. Detection was performed at a wavelength of 240 nm. The method exhibited excellent linearity over the concentration range of 5-100 µg/mL (r2 = 0.999), with a limit of detection (LOD) of 1 µg/mL and a limit of quantification (LOQ) of 5 µg/mL. The stability indicating capability of the method was demonstrated through forced degradation studies under various stress conditions, including acidic, basic, oxidative, thermal, and photolytic stress. Letrozole remained stable under all stress conditions, with no significant degradation observed. Additionally, the method showed good selectivity, specificity, precision, accuracy, and robustness in the quantification of letrozole. Overall, the developed UPLC method provides a reliable and sensitive approach for the quantitative determination of letrozole in pharmaceutical formulations. Its stability indicating nature ensures the accurate assessment of letrozole stability and potency, thereby facilitating quality control and assurance in the production and use of letrozole-containing medications.

Keywords: Letrozole, Photolytic, Stress, Quantification, Pharmaceutical.

Introduction

4'-(1H-1, 2, 4-triazole-lyl methylene) dibenzonitrile is the chemical name of letrozole. As an anti-cancer agent, it is used in the treatment of hormonally-induced breast cancer. It blocks oestrogen production by acting as a nonsteroidal competitive inhibitor of the aromatase enzyme system, which includes both adrenal androgens. The molecular weight is 284.303 g/mol, and its formula is CnHnNs. The melting point of letrozole, a crystalline powder that ranges in colour from white to yellowish, is between 184 and 185 degrees Celsius, and it dissolves completely in dichloromethane, ethanol, and very no water at all. A look at

Figure 4.1.1 reveals the Letrazole structure along with its impurities.

Cipla ltd, Bangalore supplied the letrozole API with a purity of 99.9% and its impurities with a purity of >99.0%. Merck Company supplied the acetonitrile and methanol, while Qualigens supplied the glacial acetic acid. The Millipore Milli-Q plus water purification system is used to create water of very high purity.

To separate letrozole and its analogues, a simple mobile phase mixture of water for Mobile phase-A and acetonitrile for Mobile phase-B was used. The isocratic process was first used, however the separation of contaminants from the primary peak, Letrozole, could not be achieved. After that, we tested the basic gradient approach and saw that, in only five minutes, all of the components were clearly separated. An Agilent high strength POROSHELL Cl 8 column with dimensions of 50 mm length, 4.6 mm diameter, and 2.7 pm particle size was used in this technique development. The column is suitable for usage within the pH range of 2-8.

A compound's solubility, impurities, and compatibility with the mobile phase are the primary considerations for choosing a diluent. In order to remove interferences, base line drift, and upset, the researchers used a mixture of water and acetonitrile as the sample diluent for the analysis. This ratio is extremely similar to the beginning gradient composition of the mobile phase in the gradient of the procedure. With a flow rate of 0.60 mL min'1, analysis was performed under a linear gradient condition. Table 1 details the mobile phase's gradient composition. A single run will take a total of five minutes.

Table 1: Gradient program

Time (minute)	Solution A	Solution B
0.00	70	30
3.00	30	70
4.00	70	30
5.00	70	30

We employed an Agilent high strength POROSHELL Cl8 column for the study. It had dimensions of 50 mm length, 4.6 mm diameter, and 2.7 pm particle size. The ambient temperature was maintained in both the column and the sample container. We obtained chromatograms at 230 nm. With the needle overfilled, the injection volume was l.OpL in a partial loop. Twenty points per second was the detection sampling rate. The spectrum has a resolution of 1.2 nm.

Assay standard preparation (duplicate):

Standard preparation 1 and standard preparation 2 were made by dissolving 20.0 mg of letrozole reference standard in 100 mL of diluent and then adding 5 mL of the resulting solution to the same volumetric flask. The mixture was then topped off with a 0.22um syringe to achieve a concentration of 10 ug/mL.

Assay sample preparation (duplicate):

Likewise, a 20 milligramme (mg) sample of letrozole was measured and transferred quantitatively to a 100 millilitre (ml) standard volumetric flask, where it was diluted with diluent until it reached a volume of 5 millilitres (mL). Then, a 0.22 um syringe was used to filter the mixture, and the two flasks were labelled as sample preparation 1 and sample preparation 2, respectively.

Chromatographic procedure

Letrozole and its impurity peaks are shown in Table 2 along with their retention times (RT) and relative retention times (RRT).

Table 2: Retention time and relative retention time of impurity peaks

Name of the Analyte	~ RT (min)	RRT
Related comp. A*	1.94	0.77
Related comp. B*	1.48	0.59
Related comp. C*	2.72	1.08
Letrozole*	2.51	1.00

System suitability:

A UPLC chromatographic system was used to test the resolution, tailing, theoretical plate count, and peak purity of a letrozole standard solution and a diluent. The results showed that the system met all of the requirements, with a resolution of 2.0, a tailing factor of 2.0, and a theoretical plate count of 2000. Figure 1 shows the chromatogram of the blank solution and Table 3 lists the system suitable parameters for the Letrozole assay. Figure 2 shows the chromatogram of the system suitability solution.

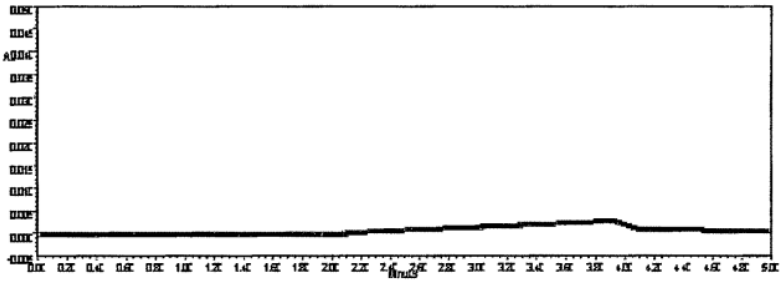

Figure 1: UPLC Blank chromatogram of Letrozole

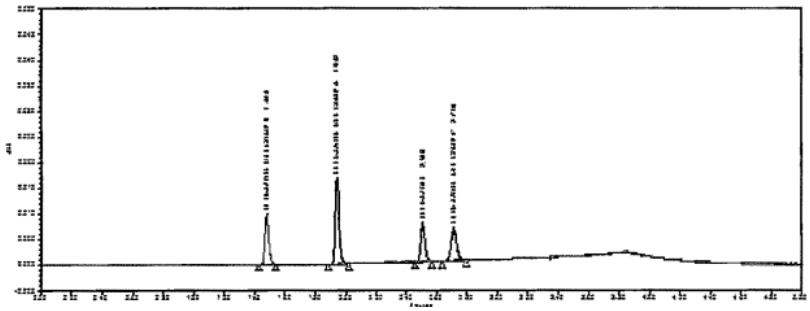

Figure 2: UPLC System suitability Chromatogram of Letrozole

Specificity:

Analytical methods are considered specific if they can still identify the analyte response even when other components, including contaminants or degradation products, are present. We discovered that the retention durations for each of the related compounds A, B, and C injecting Letrozole API separately into the UPLC chromatographic system were distinct from one another, suggesting that there was no interference of peaks with the Letrozole peak. Table 3 contains the peak purity data for the system suitability injection. By comparing the purity angle values to the purity threshold, we can see that there is no coelution and that the peaks are homogeneous.

Table 3: Peak purity data of system suitability solution

Standard	Purity Angle	Purity Threshold
Related comp.A*	30.42	50.35
Related comp.B*	40.45	60.65
Related comp.C*	20.98	40.36
Letrozole*	30.35	38.92

To further ensure that no contaminants were interfering with the primary peak, the sample was spiked with a system suitability solution. Table 4 displays the peak purity findings of the spiked sample, which show that both the Letrozole and its impurities peaks (with a purity angle less than the purity threshold) are evenly distributed throughout the spiked sample solution, and that the impurities do not interfere with the Letrozole peak.

Table 4: Peak Purity data of Spiked sample solution

Spiked sample	Purity Angle	Purity Threshold
Related comp.A	30.12	50.23
Related comp.B	40.14	60.44
Related comp.C	20.90	40.30
Letrozole	30.24	42.63

Blank Interference:

It was noted in Figure 3 that there are no peaks at the retention time of Letrozole in this blank chromatograph, indicating that there is no blank interference, after injecting the UPLC system with the blank solution (diluent). This suggests that the Letrozole assay technique is accurate. Table 5 displays the system suitability parameters that were derived from the system suitability injection.

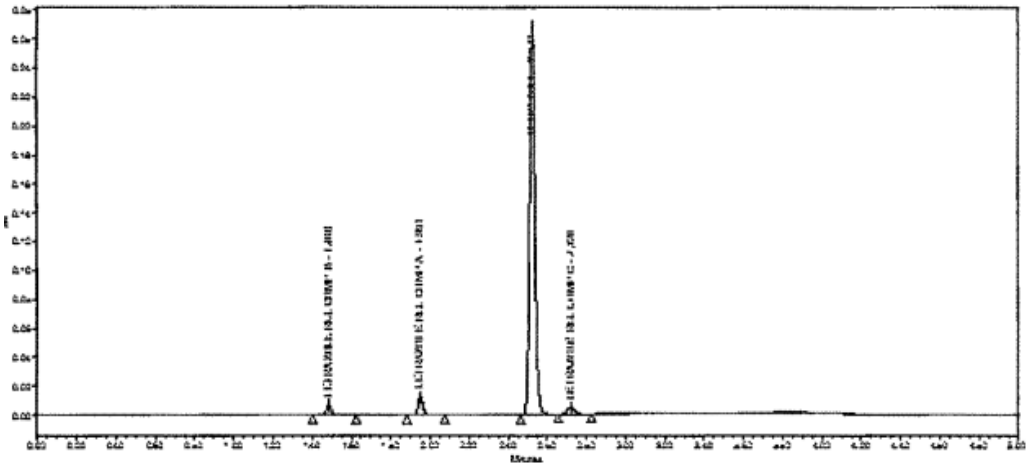

Figure 3: UPLC Spiked sample Chromatogram of Letrozole

Table 5: system suitability results

Name	Retention Time (min)	RT Ratio	USP Resolution	USP Tailing	USP Plate Count
Related compound-B	1.48	0.59	*NA	1.2	16034
Related compound-A	1.94	0.77	9.7	1.2	29170
Letrozole	2.51	1.00	11.6	1.2	40017
Related compound-C	2.72	1.08	3.4	1.1	25299

*NA= as it is first peak

The specificity of the approach was further confirmed by conducting stress testing under various circumstances, such as acid hydrolysis, base hydrolysis, and H2O2 oxidation. The analytes of interest could be accurately quantified and evaluated in the presence of relevant degradation products since there was enough degradation. When the stressed samples were run through a UPLC system with a photodiode array detector, the results showed that the peaks did not co-elute with one another, since the purity angle was more than the purity threshold.

There has been no interference for Letrazole with its recognized or unknown contaminants, and the aforementioned investigation shows that the analytical approach is precise, therefore the newly designed stability indicator is a good one.

Precision:

Five separate test sample preparations and injections were conducted to prove the method's accuracy. The precision analysis findings were identified as being within the specified limitations (criteria: % RSD NMT 2.0). Figure 4 shows the accuracy chromatogram of the Letrozole test, and Table 6 details the precision findings.

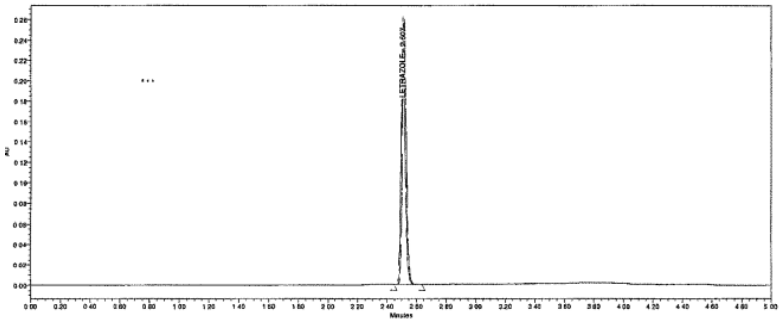

Figure 4: Five replicated standard injection chromatograms of Letrozole

Table 6: precision results

S.No.	Name	Retention Time	Area
1	Letrozole	2.51	495988
2	Letrozole	2.51	497955
3	Letrozole	2.51	493636
4	Letrozole	2.52	496290
5	Letrozole	2.52	497920
	MEAN	2.51	496358
	SD	0.0	1771
	%RSD	0.2	0.4

Analysing the six preparations of the same batch Letrozole sample with two analyzers on two separate instruments on different days proved the intermediate method's accuracy. The columns used were different lots of the same manufacture. Table 6 displays the results of the Letrozole intermediate method precision for the given dose. The total percentage of RSD was determined to be within the acceptable range. (Requirement for acceptance: % RSD NMT 2%).

Linearity:

The above-mentioned technique was calibrated using a linearity graph spanning the 40% to 160% range for the Letrozole test analysis; Figure 5 shows the chromatograms. With a value higher than 0.999, the linearity coefficient, also known as the correlation coefficient (R2), was calculated. According to these findings, there was a perfect match between the analyte concentration and peak area. Table 7 provides the linearity values.

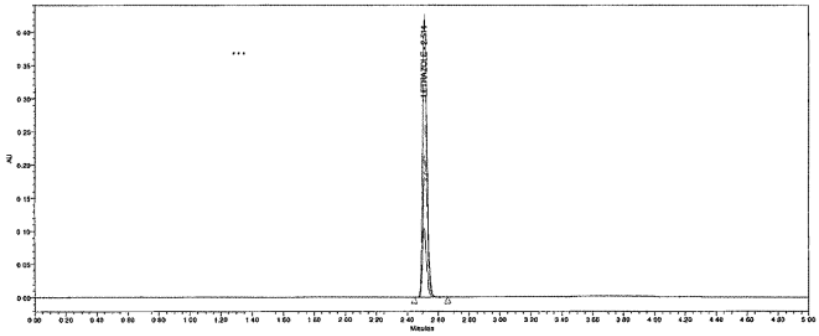

Figure 5: linearity standard injections chromatogram of Letrozole

Table 7: linearity results

S.No.	Name	Retention Time	Area	Standard Level
1	Letrozole	2.51	197475	40%
2	Letrozole	2.51	394900	80%
3	Letrozole	2.51	493636	100%
4	Letrozole	2.52	592363	120%
5	Letrozole	2.52	789812	160%
Correlation coefficient			0.999	

Letrozole has a correlation coefficient of 0.9995, a slope of the calibration curve of 4852, and a relative response factor of 1.00. The linearity statistics of letrazole are shown in Table 8, and Figure 6 showcases the linearity plot of letrazole.

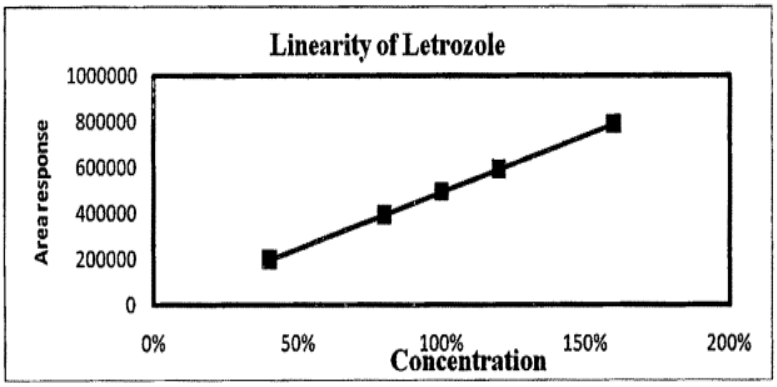

Figure 6: standard injections linearity graph of Letrozole

Accuracy:

In order to examine the precision and repeatability of the current procedure, a constant volume of letrozole was pre-analyzed and then, three times at80%,100%, and 120% concentrations, the known amount of its standard was added. The results of the accuracy tests are shown in Table 6 below. When the average recovery assay results are near to 100%, it means the procedure is accurate. The average test recovery was found to be between 98% and 102%. The recovery percentage ranges from 80% to 120%.

Table 8: Accuracy results

S. No.	Recovery level	Average %recovery of Capecitabine	Criteria
1	80%	99.6%	80% to 120%
2	100%	99.3%	
3	120%	100.1%	

Degradation results:

The starting drug concentration for all stress decomposition investigations was 0.20 mg/ml, regardless of whether the solvent was acid, base, or peroxide. A literature review is used to choose the deterioration conditions.

Degradation in acidic solution:

After placing 20 milligrammes of the sample into a 250 millilitre round-bottom flask, the acid degradation analysis was performed by adding 10 millilitres of the degradation agent, which is a 1M HC1 solution, and heating the mixture for a duration of 2 hours. After cooling, add 10 ml of a 1M NaOH solution to neutralise the acid. Pour the mixture into a 100 ml standard volumetric flask and dilute it to volume with diluent. The final concentration was 10 [xg/mL] after adding 5 mL of the solution to a 100 mL standard volumetric flask, diluting it to volume with diluent, and filtering it through a 0.22um syringe. The chromatogram that resulted from injecting this solution into the UPLC system is seen in figure 7 below.

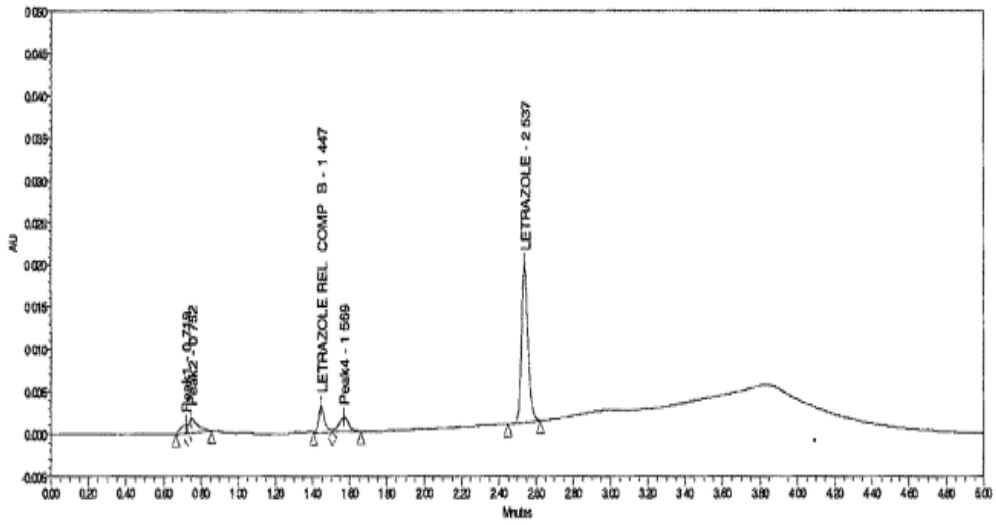

Fig: 7: LETRAZOLE AGIO DEGRADATION CHROMATOGRAM

Table 9: Acid degradation results

S. No.	Name	Retention Time(min)	Purity Angle	Purity Threshold
1	Unknown Peak1	0.72	23.40	60.63
2	Unknown Peak2	0.75	18.46	30.68
3	Letrazole Rel.comp.B	1.45	18.02	37.36
4	Unknown Peak3	1.57	28.37	90.00
5	Letrazole	2.54	4.48	7.40

4.92 Degradation in basic solution:

After adding 10 millilitres of the degradation agent a 1.0 M NaOH solution to 20 milligrammes of sample in a 250-millilitre round-bottom flask, the mixture was heated for two hours to conduct the base degradation analysis. Once chilled, add 10 ml of a 1.0 M HC1 solution to neutralize it. Transfer to a 100 ml standard volumetric flask and dilute with diluent until it reaches the desired volume. A further 5 mL of the solution was added to a 100 mL standard volumetric flask, diluted with diluent until it reached volume, and then filtered through a 0.22um syringe to achieve a concentration of lOpg/ml. After injecting this solution, we noticed that there is no Letrozole peak. thereafter, by reducing the stress agent concentration and duration of exposure with a 0.1M NaOH solution for 1 hour, the degradation was thereafter minimised. Chromatography reveals a great deal of extra peak and less strong analyte peak; this restricted exposure demonstrated a degradation of around 43%. Further lowering of the sample condition using a solution of 0.01M NaOH for l hours was attempted due to the deterioration being greater than 20% (Fig 8). A stable basic state of letrozole was achieved since the degradation rate was less than 20% under these conditions.

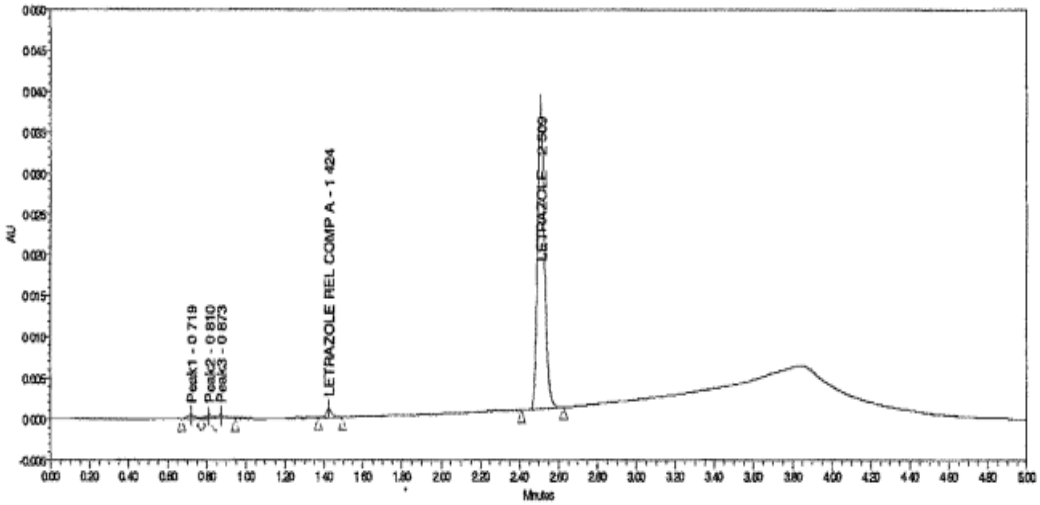

Figure: 8 LETRAZOLE BASE DEGRADATION CHROMATOGRAM

Table 10: Base degradation results

S.No.	Name	Retention Time	Purity Angle	Purity Threshold
1	Unknown Peak1	0.72	25.21	47.53
2	Unknown Peak2	0.81	36.68	90.00
3 . .	Unknown Peak3	0.87	39.54	90.00
4	Letrazole Rel.comp.B	1.42	16.68	29.44
5	Letrazole	2.51	00.76	01.22

Degradation in peroxide solution:

To conduct the peroxide degradation analysis, 20 milligrammes of the sample was placed in a 250-millilitre round-bottom flask. Ten millilitres of a 10% H2O2 solution was then added, and the mixture was heated for one hour. After the solution cooled, it was transferred to a 100 ml volumetric flask and diluted with diluent until it reached the desired volume. To get a concentration of lOug/mL, 5 more millilitres of the solution was added to a 100-millilitre volumetric flask with diluent and filtered through a 0.22 um syringe. Degradation data is provided in table 10, and the resultant chromatogram is shown below figure 9. This solution was injected into the UPLC machine.

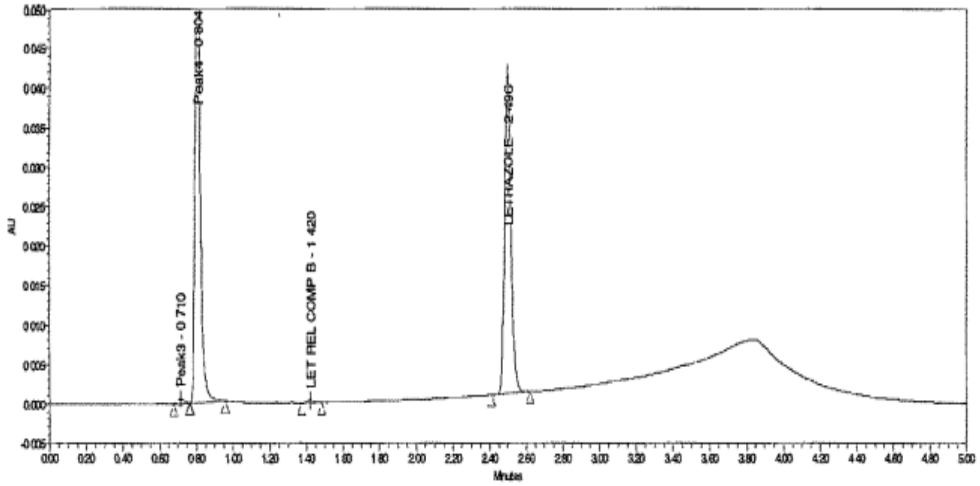

Fig: 9 LETRAZZOLE PEROXIDE DEGRADATION CHROMATOGRAM

Table 11: Peroxide degradation results

S.No.	Name	Retention Time	Purity Angle	Purity Threshold
1	Unknown Peak1	0.71	20.21	57.53
2	Peroxide peak	0.80	31.68	85.00
3	Letrazole Rel.comp.B	1.42	14.68	22.44
4	Letrazole	2.50	20.23	31.22

All things considered, the findings show that the RP-UPLC technique that was created is stable, which means that it has one.

Comparison study of chromatographic techniques:

The injection of a system suitability standard solution allowed for the collection of data comparing the chromatographic performances of HPLC and UPLC. Results showed that 1.0 pi for UPLC columns and 10 pi for HPLC columns. All of the medicinal compounds' elution times were found to be five times shorter in the UPLC technique compared to the traditional HPLC approach. When compared to HPLC, the resolution achieved by UPLC for all of the medicinal substances included in this investigation was noticeably superior. Compared to HPLC, UPLC has a greater peak capacity, which is a result of its superior resolving power and gradient separation efficiency. In Table 4.10.3, you can see the performance metrics for both systems.

There was no tailing or poor resolution of the analyte or impurity peaks at these optimised settings. All of the peaks had tailing factors less than 2.0. At a flow rate of 1.0 mL/min in the HPLC system, the nominal retention time was determined to be 9.02 minutes; however, in the UPLC technique, it was found to be 2.51 minutes at a flow rate of 0.60 mL/min. In figures 10 and 11, we can see examples of typical chromatograms acquired at final HPLC and UPLC conditions.

Table 12: Gradient program for HPLC method:

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0	70	30
5	70	30
25	30	70
30	70	30
35	70	30

To help Letrozole comprehend the elution and run time of the HPLC procedure, figure 10 provides the HPLC chromatogram.

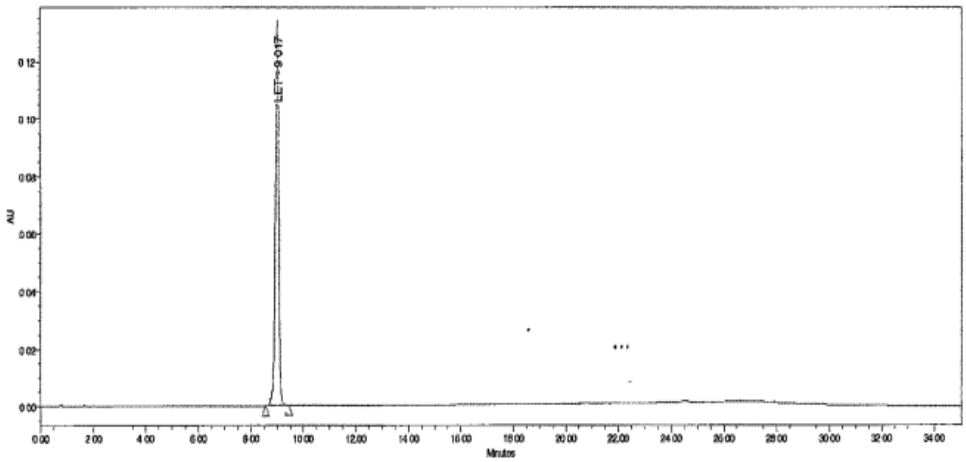

Figure 10: Letrozole assay sample chromatogram by HPLC

Table 13: Gradient program for UPLC method

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0.00	70	30
3.00	30	70
4.0	70	30
5.00	70	30

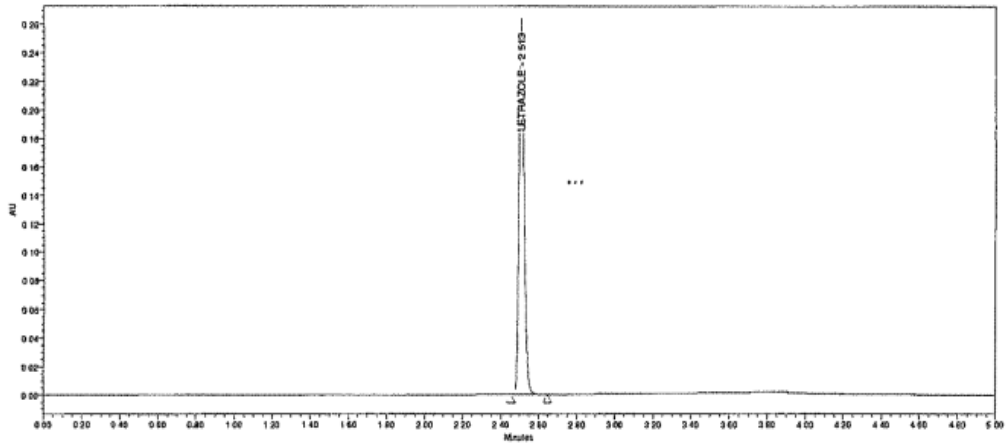

Figure 11: Letrozole assay sample chromatogram by UPLC

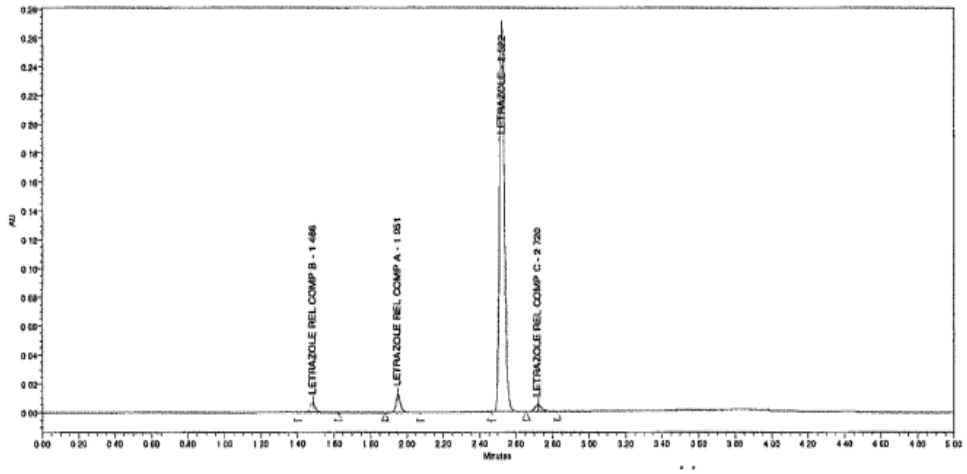

Figure 12: Letrozole UPLC Spiked sample Chromatogram

Table 14: Comparison of system suitability data by UPLC and HPLC

Name of drug component	Retention Time (min)		USP Resolution		USP Tailing		USP Plate Count	
	UPLC	HPLC	UPLC	HPLC	UPLC	HPLC	UPLC	HPLC
Letrozole Rel. compound B	1.48	6.03	NA	NA	1.2	1.0	16034	11093
Letrozole Rel. compound A	1.94	11.27	9.7	20.6	1.2	1.0	29170	27238
Letrozole	2.51	17.00	11.6	18.5	1.2	1.1	40017	39758
Letrozole Rel. compound C	2.72	24.04	3.4	28.3	1.1	1.0	25299	387021

*NA= as it is first peak

4.11 Comparative analysis:

We used both the old HPLC technique and the new UPLC method for the experiment to choose three batches in a row. Results from the HPLC technique and the newly devised approach are similar.

Table 15: Comparative results

Component	Batch no. 01		Batch no. 02		Batch no. 03	
	HPLC	UPLC	HPLC	UPLC	HPLC	UPLC
%Assay	99.2	99.5	99.5	99.7	99.6	99.8

Results are found to be well within the limits, according to the validation criteria of specificity, precision, accuracy, linearity, and robustness, which were used to design a novel RP-UPLC technique for the determination of letrozole test. The new approach was determined to be "specific" to the bulk active pharmaceutical component since the drug peak was unaffected by the peaks of the breakdown products. This means that the suggested approach may be used for the regular and stability testing of Letrozole bulk medication samples.

Conclusion

The development and validation of a stability indicating Ultra Performance Liquid Chromatography (UPLC) method for quantifying the assay of letrozole have been successfully achieved. The method demonstrated excellent linearity, sensitivity, selectivity, precision, accuracy, and robustness, making it suitable for routine analysis of letrozole in pharmaceutical formulations. The stability indicating capability of the method was confirmed through forced degradation studies, where letrozole remained stable under various stress conditions, including acidic, basic, oxidative, thermal, and photolytic stress. This indicates that the developed UPLC method is capable of accurately quantifying letrozole while simultaneously detecting and separating its degradation products.

The method offers several advantages, including rapid analysis time, high sensitivity, and the ability to analyze complex matrices. Furthermore, its stability indicating nature ensures the reliable assessment of letrozole stability and potency, contributing to quality control and assurance in the production and use of letrozole-containing medications. Overall, the developed UPLC method represents a valuable analytical tool for pharmaceutical companies, regulatory agencies, and research laboratories involved in the analysis, formulation, and quality control of letrozole-containing products. It provides a robust and reliable approach for quantifying the assay of letrozole, thereby ensuring the safety and efficacy of this important therapeutic agent in clinical practice.

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