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RP-HPLC method development and validation for quantification of letrozole solid lipid nanoparticle

Sowmya PRIYADARSINI[1] *, Swaroop Rameshwarji LAHOTI[2]

[1] Department of Pharmacy, Y.B Chavan College of Pharmacy, Dr. Rafiq Zakaria Campus, Rauza bag, Aurangabad 431001, Maharashtra, India.
[2] Department of Pharmacy, Y.B Chavan College of Pharmacy, Dr. Rafiq Zakaria Campus, Rauza bag, Aurangabad 431001, Maharashtra, India
* Corresponding Author. E-mail: sowmya639@rediffmail.com (S.P) Tel. +91-915-809-15-52.

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ABSTRACT: A sensitive, simple, rapid, stability-indicating reverse-phase high-pressure liquid chromatographic method was developed for the estimation of letrozole in solid lipid nanoparticles. The developed method was validated as per the International Council of Harmonization (ICH guideline) concerning system suitability, linearity, accuracy, precision, specificity, robustness, and range. The chromatographic separation was achieved on the Zorbax C18 column (250 x 4.6 mm ID; 5.0 µm particle size). Isocratic elution was performed using methanol: 0.1% orthophosphoric acid (60:40) at 0.7 mL/min flow rate. The detection was done using a UV-Vis detector at 240 nm. Linearity for the analytical method was observed at the concentration range of 10 to 50 µg/mL having a correlation coefficient of 0.999. The method was precise, accurate with a relative standard deviation (RSD) ≤ 2.0. The developed validated analytical method can be used as a quality control tool for the quantitative estimation of letrozole in a novel formulated solid lipid nanoparticle.
KEYWORDS: Letrozole; RP-HPLC; Solid lipid nanoparticle; ICH; Validation.

1. INTRODUCTION

Letrozole (Figure 1) is chemically known as 4-[{4-cyanophenyl)-(1, 2,4-triazol-1-yl) methyl] benzonitrile [1]. Letrozole is a third-generation non-steroidal aromatase inhibitor used in the treatment of hormone-responsive positive and metastatic breast cancer in postmenopausal women [2]. Breast cancer is the leading cause of death in women worldwide [3]. Nearly 30% of breast cancer are hormone-responsive and requires hormone for growth. Breast cancer cells have receptors/protein for the hormone estrogen (ER-positive cancer) which acts as a catalyst for the growth and spread of cancer cells. Hence hormone deprivation is the most preferred therapy leading to regress [4, 5]. Estrogen plays a major role in breast cancer [6].

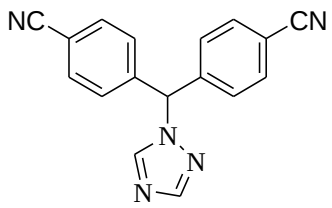

Figure 1. Chemical structure of letrozole.

Before menopause, most estrogen is produced by ovaries. After menopause when the ovary stops functioning a small amount of estrogen is produced by the aromatase enzyme in fat tissues surrounding breast cells [7]. The mechanism of action of an aromatase inhibitor is to block aromatase from producing estrogen [7-8]. Aromatase inhibitors such as letrozole are prescribed as hormone therapy for the treatment and management of breast cancer.

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Currently, letrozole is commercially available as tablets, a conventional oral pharmaceutical formulation. Owing to various disadvantages of conventional dosage forms; novel carriers like solid lipid nanoparticles are gaining superiority due to improved bioavailability, ability to target a specific site, and reducing toxicity [9].

The assay is a major quantitative as well as a qualitative technique used to detect the level of active substance in any pharmaceutical drug product. The higher or lower level of drug substance in any drug product may pose unwanted therapeutic and pharmaceutical effects. Analytical method development is a technique of developing a suitable analytical method to quantify a target analyte in pharmaceutical dosage form and plays a vital role in product lifecycle [10].

Various analytical methods for the estimation of the drug have been found in the literature such as UV-spectrophotometric method, gas chromatography–mass spectrometry (GC/MS) method for identification of letrozole in urine, capillary GC method for the analysis of tamoxifen, anastrozole, and letrozole in their pharmaceutical preparations, Thin layer chromatography (TLC) and high pressure liquid chromatography (HPLC) methods for the estimation of the drug and related components in tablets and HPLC method of the drug and its metabolite in biological fluids with automated liquid-solid extraction and fluorescence detection [11, 12, 13, 14, 15]. Till date no official pharmacopoeial method or literature available for detemination of letrozole in novel injectable drug delivery system. Moreover the reported analytical methods suffers from one or other lacunas such as poor specificity, higher retention time, use of fluorescence detector (Table 1). RP (Reverse-phase)-HPLC for the determination of letrozole in any novel drug delivery system has not been reported in any scientific literature. Along with that, the reliability of other methods to detect analytes in a formulation such as solid lipid nanoparticles was also in dispute. But after considering the widespread use of the RP-HPLC technique it is of utmost importance to develop a simple, accurate method to detect letrozole in drug product [16, 17]. Analytical method development using HPLC is a complex process involving various stages [18].

Table 1. Comparative evaluation of different analytical method.

SNo	Method	Mobile Phase & Detector	Retention time(min)	Linearity (ng/mL)	Remarks	Reference
1	Summary of different mobile phase combination evaluated				Different mobile phase	[11]
2	RP-HPLC	Water: acetonitrile: methanol (UV detector) $\lambda_{max:}$ 240 nm	9.8	0-50000	Use of two solvent and shorter retention time	[12]
3	HPLC	Acetonitrile: phosphate buffer (fluorescence detector) $\lambda_{max:}$ 256 and 585 nm	9.98	50-700	Use of sensitive UV-vis detector and shorter retention time	[13]
4	HPLC	Methanol: tetrabutyl ammonium hydrogen sulfate (UV-Vis detector) $\lambda_{max:}$ 240 nm	10	500-150000	Lower retention time and RP-HPLC method	[14]
5	RP-HPLC	Acetonitrile: water (UV-Vis detector) $\lambda_{max:}$ 265 nm	4.53	160000 - 240000	Linear at lower concentration 10 µg/mL	[15]
6	RP-HPLC	Methanol and 0.1% orthophosphoric acid (60:40) (UV-Vis detector) $\lambda_{max:}$ 240 nm	5.94	10000 -50000	Sensitive, simple, precise stability-indicating, shorter retention time, use of lower quantity of organic solvent, economical and lower risk of environmental hazard	Present method

The present analytical study deals with developing a simple, precise, accurate, stability indicating method and validating as per recommendations set by various guidelines such as the International Council of Harmonization (ICH) Q2, US Food and Drug Administration (FDA), US Pharmacopoeia (USP). The validated method is to be successfully tested for parameters such as specificity, precision (repeatability, intermediate

precision and reproducibility), linearity, accuracy, stability in aqueous solution (SIAS), tests to verify the system.

2. RESULTS

UV-vis detector is the most versatile and widely used detector coupled with RP-HPLC. When UV absorbance was taken, the maximum absorbance was observed at 240 nm. Therefore analysis of letrozole was performed at λ_{max}=240 nm. The HPLC column is the key part involved in analytical separation. After performing the analysis on different types of columns such as C8 (octylsilane) and C18 (octadecylsilane), the C18 column was found to show sharp and well-resolved peaks and hence better separation.

It is of utmost importance to consider the logP value and solubility of the analyte while selecting the mobile phase. Methanol is selected as the mobile phase considering the solubility of the analyte (letrozole) to conduct further analysis. To select the optimum concentration of mobile phase required for effective elution, trial and error methods were used using different ratios of methanol and 0.1% OPA. Methanol and 0.1 % OPA in the ratio of 60:40 at pH=3 was found best suitable.

After performing the trial and error analysis, the optimum flow rate for analyte elution was found to be 0.7 ml/minute. The stability of an analytical solution is one of the parameters that should be taken care of. The standard and sample solutions are found to be stable up to 36 hours at room temperature.

The results for the system suitability test were depicted in Table 2.

Table 2. The values showing suitability criteria for the system used for the analysis of letrozole**.**

Parameter	Value (Mean±SD)*
Symmetry	0.64 ± 0.01
Theoretical plate	8823 ± 130
Retention time	5.94 ± 0.10
Separation factor	1.981 ± 0.20
Capacity factor	17 ± 0.08
Tailing	1.03 ± 0.05
RSD% retention time	0.78
Peak area	5850.88 ± 150

The system was evaluated for its suitability to analyze the drug. After selecting all suitable parameters the analysis was done and the chromatograms were recorded. The peak area for analyte i.e. pure letrozole was observed against blank, placebo, and drug product (Figure 2).

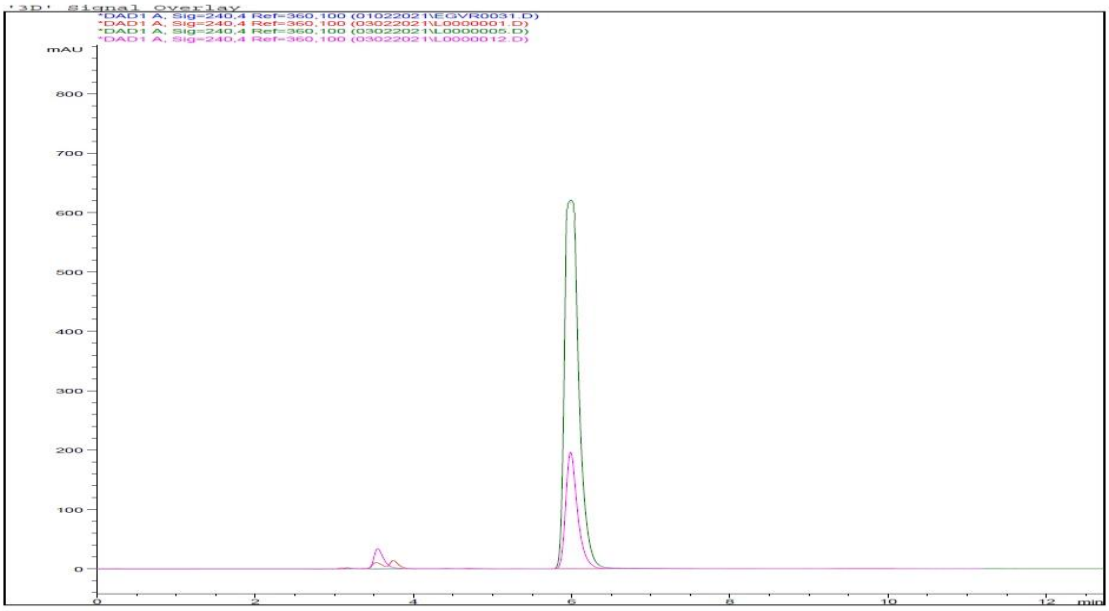

Figure 2. Chromatogram of letrozole, placebo, blank and drug product.

Linearity is the ability of the analytical method to elicit test results directly proportional to the concentration of analyte within the given range. The linearity of the analyte in the solution was observed in range of 10 µg/mL to 50 µg/mL.

The amount of letrozole was quantified and % recovery was calculated from the amount estimated and actual amount added. The results are presented in Table 3**.** The analyte can be recovered in the mobile phases used for analysis.

Table 3. The values of % recovery of analyte from the mobile phases.

Spike level in %	Actual amount added in µg/mL	Amount found in mg	% Recovery	Mean	SD	RSD %
80%	8	8.04	100.5	101.17	0.63	0.62
	8	8.14	101.75			
	8	8.1	101.25			
100%	10	10.2	102	101.03	0.91	0.90
	10	10.02	100.2			
	10	10.09	100.9			
120%	12	12.04	100.3	100.67	0.33	0.33
	12	12.08	100.7			
	12	12.12	101			
Overall Mean					100.96	
Overall SD					0.62	
Overall RSD%					0.62	

The precision of an analytical procedure between a series of measurements obtained from multiple sampling of the same homogeneous sample under prescribed conditions was determined and presented in Table 4**.**

Table 4. The values of precision of the method employed in the analysis of letrozole.

Sr. No.	Concentration (µg/mL)	Standard solution (% of the amount found)		Sample solution (% of the amount found)	
		Intra-day precision	Inter-day precision	Intra-day precision	Inter-day precision
1	10	100.1	100.8	99.5	100.5
2		100.2	99.9	101.4	100
3		100.1	100.7	100.9	100.5
4	20	99.5	100.8	101.7	100
5		100.2	101.3	99.9	100.8
6		99.9	100.8	100.1	99.9
7	50	99.8	100.1	99.4	100.1
8		101.1	99.8	99.8	100.4
9		99.6	100.2	100.5	101.1
Mean		100.1	100.5	100.4	100.4
SD		0.467	0.506	0.825	0.406
RSD%		0.47	0.50	0.82	0.40

The results for specificity were obtained by comparing the representative chromatogram of blank, placebo, and drug products after injection. No peak eluted at retention time of analyte peak from blank and placebo. %difference of assay of sample from method precision sample and a spiked sample was found be 0.5% (less than 2%). The analyte passes the peak purity criteria.

The robustness of the analytical method is evaluated to ensure that the developed analytical method is not affected by small but deliberate variations in method parameters. The RSD% was found to be within predefined acceptance criteria of below 2%.

The range is determined from results of precision, accuracy, and linearity and found to be 10 to 50µg/ml.

3. DISCUSSION

All the physicochemical properties of the active ingredient were found to be following the compendial reference value. The literature study had revealed the hydrophobic nature of the drug. RP chromatography is the method of choice for such drugs. Therefore RP-HPLC method is selected [19].

The trial and error method was used for the selection of mobile phase, flow rate, and column. Better separation was achieved using the C18 column. Longer columns are better retentive due to higher theoretical plate numbers also evidenced from the literature [20, 21, 22]. Hence C18 column was selected, logP and solubility of analyte play a key role in the selection of mobile phase. The pKa helps in deciding the pH of the mobile phase [23]. The pH of the mobile phase is usually selected within ±2 units of pKa of the analyte of choice [23]**.** The pKa of analyte (letrozole) was found to be 2.17 (strongest basic). Methanol and 0.1% OPA in 60:40 ratio pH 3 is selected as suitable solvent system due to better peak resolution and less tailing. The flow rate with the least retention time, better peak symmetry, least backpressure, and better resolution of adjacent peaks shall be selected [24]. Based on these 0.7 ml flow rate is selected.

After performing the photometric estimation of the drug against the standard and blank the maximum ƛmax was selected at 240 nm.

During validation of the method, correlation coefficient was found to be 0.999 which is closer to 1. Hence the developed analytical method was found to be linear in the range of 10 to 50 µg/mL.

The percentage of recovery after spiking at 80%, 100%, and 120% was found to be within the acceptable limit i.e. 100.26% to 101.93% with a relative standard deviation of less than 0.2% as illustrated in Table 3. This indicates that analytical method is accurate. Intra and an inter-day assay of letrozole in solid lipid nanoparticles at three different concentrations were found to be within predefined specification with a relative standard deviation below 2% (Table 4). This indicates that the analytical method is precise to detect the analyte.

There was no interference between peaks and no peak eluted in blank corresponding to the retention time of letrozole. The %difference between assay values was less than 2%. Hence the analytical method was specific to determine letrozole in drug product formulation. The individual and overall RSD% with method precision for flow rate, column oven temperature, least count of mobile phase, pH of buffer in the mobile phase is below 2%. Hence the developed analytical method is robust for the above parameters.

4. CONCLUSION

A sensitive, simple, precise stability-indicating RP-HPLC was developed for the estimation of letrozole in solid lipid nanoparticles. The optimized analytical method was validated as per the ICH Q2 validation of the analytical method. The developed method is found to be superior, reliable and accurate when compared to the reported methods. Additional advantages of method like shorter run time, use of lower quantity of organic solvent, economical, reduction in environmental hazard can be listed. The developed validated method can be envisaged for routine quality control analysis for the determination of letrozole in letrozole-bound solid lipid nanoparticles.

5. MATERIALS AND METHODS

5.1. Materials and reagents

Letrozole working standards were received as a generous gift sample from Beta Drugs, Baddi, India. The components of the mobile phase i.e. methanol and orthophosphoric acid were of analytical grade and were procured from Sigma Aldrich, USA. The other chemicals were of analytical grade, which was available in the analytical laboratory of the Department of Pharmacy, Y.B Chavan College of Pharmacy, Maharashtra, India.

5.2. Instrumentation

HPLC system (Agilent Tech 1100) consisting of a UV detector and installed with chemstation 10.04 software was used for the analysis of letrozole solid lipid nanoparticle (LTR-SLN). Digital Analytical weighing balance, sonicator, Oven, and pH meter were used in these experiments.

5.3. Chromatographic conditions

During analysis, the chromatographic separation was achieved using a Zorbax C18 column (4.6 x 250 mm) having 5.0 µm particle packing at ambient temperature. Elution was performed using methanol and 0.1% orthophosphoric acid (60:40) having 0.7 mL/min flow rate at 240 nm wavelength. The column oven temperature was set as 50°C and sample tray temperature was set as 10°C.

5.4. Preparation of mobile phase

The mobile phase was prepared by mixing the solvents methanol and orthophosphoric acid (OPA; 0.1 %) in a fixed ratio of 60:40 %v/v. The prepared solution was degassed with a suitable method using a vacuum degasser.

5.5. Standard stock solution

The standard stock solutions of letrozole were prepared using pre-weight quantities of drug (10 mg) and then transferring the same into 10 mL of volumetric flask. The final volume was made up to 10 mL using HPLC grade methanol to achieve a concentration of 1000 µg/mL. At specified time intervals the aliquots of sample (0.1 mL, 0.2 mL, 0.3 mL, 0.4 mL and 0.5 mL) were collected and volume made up to 10 mL to maintain sink conditions with mobile phase to achieve 10 µg/mL, 20 µg/mL, 30 µg/mL, 40 µg/mL and 50 µg/mL**.**

5.6. Solution stability

The stability testing of LTR-SLN in analytical solution was carried out using autosampler with freshly prepared sample and standard solution as per analytical protocol proposed. The standard and sample solution were stored in an autosampler for 48 hours at 20±5°C and aliquots of the sample were tested as per methodology and compared with initial results. Similarity factor is calculated between standard solution injected at the different period and initial standard solution.

5.7. Method development

The analytical method development is a technique of developing a suitable analytical method to quantify a target analyte in a pharmaceutical dosage form. Analytical method development is a complex process and plays a vital role in product lifecycle. The said method was developed based on literature references and the trial and error method.

5.7.1. Physicochemical properties of the drug

All the physicochemical properties of a drug such as pH, pKa, solubility, polarity must be evaluated to select a method for analytical development [17, 18].

5.7.2. Method selection

A technique of RP-HPLC is widely preferred for pharmaceutical products. Hydrophobic molecules can be separated using RP chromatography with greater recovery and resolution [19].

5.7.3. Detector selection

The chemical nature of the analyte greatly influences the selection of the detector. Other factors like the potential interference, and limit of detection are also considered [18, 19].

5.7.4. Wavelength determination

UV spectrophotometric determination was carried out to select a suitable wavelength. The analyte is dissolved in methanol and screened by UV to determine wavelength showing maximum absorbance.

5.7.5. HPLC column and stationary phase selection

The column is considered the heart of analytical separation. The selection of columns depends on various factors like the nature of the drug molecule, solubility, partition coefficient [19]. C8 (octylsolane) and C18 (octadecylslane) columns mostly preferred for RP-HPLC [25] were evaluated for the separation of the analyte of interest.

5.7.6. Mobile phase optimization

Trials were taken with methanol and 0.1%OPA in 80:20, 70:30, and 60:40 ratios.

5.7.7. Optimization of flow rate

Experimental trials were taken at various flow rates such as 0.5 mL/min, 0.7 mL/min, 0.9 mL/min, 1.1 ml/min and 1.3 mL/min.

5.7.8. Stability in analytical solution

The standard and sample solution were analyzed at 0, 2, 4, 8, 12, 24, 36 and 48 hours upon storage at 20±5°C [26]. The results are accepted if the absolute %difference between the initial result and results obtained at different time intervals should not be more than 2.0. The similarity factor between the initial standard and standard solution at different time intervals should be between 98% to 102%.

5.8. Method validation

The optimized analytical method is validated to provide a high degree of assurance that the product will meet its predefined requirements for the intended purpose. The RP-HPLC method developed for LTR-SLN was validated as per recommendations by various regulatory guidelines such as FDA, EU, USP, ICH Guideline [27, 28]. The various parameters used for analytical method validation as per ICH guidelines are system suitability, linearity, accuracy, precision, specificity, robustness and range are presented below.

5.8.1. System suitability criteria

System suitability test deals with the principle of the contribution of the instrument or device to the system. This is done to evaluate the symmetry, theoretical plates, retention time, separation factor, capacity factor, tailing, retention time and peak area [29, 30, 31, 32]. The system suitability was determined from average of triplicate analysis of three different concentration obtained during precision analysis. Results are shown in Table 2.

5.8.2. Linearity

Linearity is the ability of the analytical method to elicit test results directly proportional to the concentration of analyte within a given range [29, 30, 31, 32]. Linearity of response was determined in 5 concentration of standard solution (10 µg/mL, 20 µg/mL, 30 µg/mL, 40 µg/mL and 50 µg/mL). The regression equation and correlation coefficient were determined using the least square method.The results of a correlation coefficient close to 1 indicates that the selected concentration is linear.

5.8.3. Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scattering) between a series of measurements obtained from multiple sampling of the same homogeneous sample under prescribed conditions [29, 30, 31, 32]. Intra and inter-day precision was carried out using three different concentrations (10 µg/mL, 30µg/mL, 50 µg/mL) in triplicate on the same or different day. RSD was calculated. The results were presented in Table 4. If RSD% is not more than 2% then it falls within acceptance criteria.

5.8.4. Specificity

Specificity is the ability of the analytical method to measure the analyte in presence of interference such as excipients, impurities, degradation products, etc [29, 30, 31, 32]. The specificity is evaluated by comparing the representative chromatogram of injected standard, blank, placebo, and drug products. The %difference in the assay of the sample concerning the mean of method precision results is evaluated. It falls within acceptance criteria if:

a) No peak should elute or interfere at the retention time of analyte peak from blank and placebo
b) Peak purity of analyte peak should pass
c) %difference of assay value should not be more than 2%

The overlay chromatogram is presented in Figure 2.

5.8.5. Robustness

The robustness of the analytical method is evaluated to ensure that the developed analytical method is not affected by small but deliberate variations in method parameters [29, 30, 31, 32]. These deliberate variations include the change in flow rate (±0.1 mL/min), change in wavelength (± 2 nm), change in the least component of mobile phase (± 2% absolute), changing pH of mobile phase (±0.1 unit), changing column oven temperature (± 2°C).

5.8.6. Range

The range of an analytical procedure is the interval between upper and lower concentration having a suitable level of precision, accuracy, and linearity [29, 30, 31, 32]. The range is determined from results of precision, accuracy, and linearity.

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REFERENCES

[1] O Neil MJ, The Merck Index: An enclyopedia of chemicals, drugs and biological, fourtheenth ed., Merck, Whitehouse Station, NJ, 2006.

[2] Maniyar M, Chakraborty A, Kokare C. Formulation and evaluation of letrozole-loaded spray-dried liposomes with PEs for topical application. J Liposome Res. 2020; 30(3): 274-284. [CrossRef]

[3] Torre LA, Islami F, Siegel RL, Ward EM, Jemal A. Global cancer in women: burden and trends. Cancer Epidemiol Biomark Prev. 2017; 26(4): 444-457. [CrossRef]

[4] Trunet PF, Bhatnagar AS, Chaudri HA, Hornberger U. Letrozole (CGS 20267), a new oral aromatase inhibitor for the treatment of advanced breast cancer in postmenopausal patients. Acta Oncol. 1996; 35(sup5): 15-18. [CrossRef]

[5] Miller WR, Larionov AA. Understanding the mechanisms of aromatase inhibitor resistance. Breast Cancer Res. 2012; 14(1): 1-11. [CrossRef]

[6] Johnson PE, Buzdar A. Are differences in the available aromatase inhibitors and inactivators significant?. Clin Cancer Res. 2001; 7(12 suppl): 4360s-4368s.

[7] Fabian CJ. The what, why, and how of aromatase inhibitors: hormonal agents for treatment and prevention of breast cancer. Int J Clin Pract. 2007; 61(12): 2051-2063. [CrossRef]

[8] Mahajan A, Rawal S, Bedi PM, Mahajan N, Sharma A. Aromatase inhibitors: A new hope for breast cancer patients. IJPSR. 2010; 1(6): 27- 40.

[9] Yadav N, Khatak S, Sara UVS. Solid lipid nanoparticles-a review. Int J App Pharm. 2013; 5(2): 8-18.

[10] Panda SS, Ravi Kumar Bera VV, Sahu B. Integrated quality by design (QbD) and quality risk management (QRM) based liquid chromatographic method development and validation for estimation of capecitabine in pharmaceutical dosage form. Anal Chem Lett. 2018; 8(5): 665-676. [CrossRef]

[11] Prathyusa P, Sundarrajan R. A review on different analytical methods:Letrozole. Acta Sci Pharm Sci. 2020; 4(2): 85-88. [CrossRef]

[12] Mondal N, Pal TK, Ghosal SK. Development and validation of RP-HPLC method to determine letrozole in different pharmaceutical formulations and its application to studies of drug release from nanoparticles. Acta Pol Pharm. 2009; 66 (1): 11-17.

[13] Kaplan C, Unal D. A validated method without derivatization for the determination of Letrozole by high-performance liquid chromatography-fluorimetric method for pharmaceutical preparation. Istanbul J Pharm. 2018; 48(2): 38-42. [CrossRef]

[14] Annapurna MM, Mohapatro C, Narendra A. Stability-indicating liquid chromatographic method for the determination of Letrozole in pharmaceutical formulations. J Pharm Anal. 2012; 2(4): 298-305. [CrossRef]

[15] Shrivastava A, Chakraborty AK, Rambhade SK, Patil UK. Development of validated RP-HPLC method for determination of letrozole in bulk and its pharmaceutical dosage forms. Pharm Sin. 2011; 2(2): 263-269.

[16] Validation of analytical procedures: text and methodology: Q2 (R1). https://database.ich.org/sites/default/files/Q2%28R1%29%20Guideline.pdf (assessed on 08 Jan 2022).

[17] Blessy M, Patel RD, Prajapati PN, Agrawal YK. Development of forced degradation and stability indicating studies of drugs—A review. J Pharm Anal. 2014; 4(3): 159-165. [CrossRef]

[18] Sabir AM, Moloy M, Parminder B. HPLC method development and validation: A review. Int Res J Pharm. 2013; 4(4): 39-46. [CrossRef]

[19] Kumar SD, Kumar DRH. Importance of RP-HPLC in analytical method development: a review. IJPSR. 2012; 3(12): 4626-4633. [CrossRef]

[20] Reverse phase chromatography, principles and method, Amersham Pharmacia Biotech, 1999.

[21] Viana OD, Medeiros FP, Grangeiro Junior S, Albuquerque MM, Soares MF, Soares-Sobrinho JL, Alves LD, Rolim LA, Silva KE, Rolim-Neto PJ. Development and validation of a HPLC analytical assay method for efavirenz tablets: A medicine for HIV infections. Braz J Pharm Sci. 2011; 47(1): 97-102.

[22] Bakshi M, Singh S. Development of validated stability-indicating assay methods—critical review. J Pharm Biomed Anal. 2002; 28(6): 1011-1040. [CrossRef]

[23] Kaushal CK, Srivastava B. A process of method development: a chromatographic approach. J Chem Pharm Res. 2010; 2(2): 519-545.

[24] Ranganathan HP, Govindrajulu GE, Palaniyappan VE. Forced degradation study of letrozole—a validated stability-indicating HPLC assay for bulk and tablet dosage form. J Pharm Biomed Anal. 2012; 4(2): 582-586.

[25] Zuvela P, Skoczylas M, JayLiu J, Bączek T, Kaliszan R, Wong MW, Buszewski B. Column characterization and selection systems in reversed-phase high-performance liquid chromatography. Chem Rev. 2019; 119(6): 3674-3729. [CrossRef]

[26] Prasad H, Govindrajulu G, Palaniyappan V. Forced degradation study of letrozole a validated stability indicating HPLC assay for bulk and tablet dosage form. Int J Pharm Sci. 2012; 4(2): 582-586.

[27] Chan CC, Lee YC, Lam H, Zhang XM. Analytical method validation and instrument performance verification, First ed., Wiley- interscience, Newyork, 2004.

[28] Lavanya G, Sunil M, Eswarudu MM, Chinna Eswaraiah M, Harisudha K, Spandana BN. Analytical method validation: An updated review. Int J Pharm Sci Res. 2013; 4(4): 1280-1286. [CrossRef]

[29] Palleria S, Prabhakar B. Development and validation of Temozolomide solid lipid nanoparticle formulation by RP-HPLC method. J Pharm Biomed Sci. 2015; 05(09): 745-749.

[30] Ahmad S, Mohammed Usman MR. Analytical method development and validation for the simultaneous estimation of emtricitabine and tenofovir by reversed phase high performance liquid chromatography in bulk and tablet dosage forms. Asian J Pharm Clin Res. 2017; 10(11): 59-64. [CrossRef]

[31] Managuli RS, Kumar L, Chonkar AD, Shirodkar RK, Lewis S, Koteshwara KB, Reddy MS, Mutalik S. Development and validation of a stability-indicating RP-HPLC method by a statistical optimization process for the quantification of asenapine maleate in lipidic nanoformulations. J Chromatogr Sci. 2016; 54(8): 1290-1300. [CrossRef]

[32] Ramani VD, Jani GK, Sen AK, Sailor GU, Sutariya VB. Development and validation of RP-HPLC method for pitavastatin calcium in bulk and formulation using experimental design. J App Pharm Sci. 2019; 9(10): 75-83. [CrossRef]