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Development and Validation of Stability Indicating High Performance Liquid Chromatographic Assay for Exemestane In Bulk

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ABSTRACT

A simple reverse phase specific and selective high performance liquid chromatographic method for determination of Exemestane in Exemestane tablets has been developed and validated with Isocratic elution and UV detection. Chromatographic separation was achieved by using Hypersil, C-18, 150 X 4.6mm, 5µ column with a mixture of Acetonitrile and Purified water in the ratio of 35:65, filter and degas the mobile phase same is used as diluent. Detection was at 249 nm. By this method all known and unknown Impurity & Degradation products are well separated from Exemestane main peak. Peak purity factor for Exemestane peak is not less than 99.0%. Both the Precision (System Precision, Method Precision, Intermediate Precision) and Linearity were within acceptable range. Response was a linear function between concentration and area of peak over the range from 50% to 150% of assay concentration for Exemestane. It can be concluded that the Exemestane Peak is found to be degraded more in acid, alkali and peroxide stress condition. Exemestane Peak purity factor was more than 99.0% and all degradation product formed were well separated from Exemestane Peak. By this it was found that this method is robust and system suitability test was established and related parameters are recorded. This method is validated hence this method can be used for routine analysis of stability sample.

Keywords: High performance liquid chromatography, Exemestane, stability, Precision and peak purity.

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INTRODUCTION

Exemestane (Figure 1) is an antineoplastic agent and an irreversible, steroidal aromatase in activator. It is a prescription medicine for the adjuvant treatment of postmenopausal women with estrogen-receptor positive early breast cancer.[1] Chemically it is 6-methylenandrosta-1, 4-diene-3, 17-Dione. Multiple doses of Exemestane ranging from 0.5 to 600 mg/day were administered to postmenopausal women with advanced breast cancer.

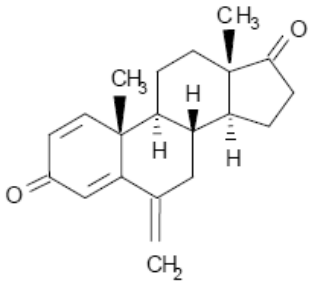

Figure 1: Chemical structure of Exemestane

Exemestane which is recently introduced into the market was selected for the present study for carrying out stability studies by forced degradation (stress testing) method. An extensive literature survey carried out by the writer revealed that several methods have been reported for the individual estimation of this drug. There are however, no reports for separating or estimating exemestane and its degradation products.[2] It becomes essential, therefore, to develop new stability indicating analytical method.

MATERIALS AND METHOD

All the chemicals and reagents used were of analytical grade, The commercial product (Aromasin) tablets containing 25mg Exemestane was procured from laboratory. HPLC Water, N,N Dimethyl formamide and methanol from Merck Specialties Pvt. Ltd., Mumbai, Acetonitrile (HPLC), Sodium hydroxide (AR), Hydrochloric acid (AR) from Thermo Fisher Scientific India Pvt. Ltd., Mumbai, Acetonitrile and double distilled water in the ratio 35:65v/v was used as mobile phase and diluents. Chromatography was performed with Agilent HPLC equipped with UV-Visible detector. The LC separations were performed at ambient temperature and Hypersil, C-18, 150 X 4.6 mm, 5µ column or equivalent and Agilent chemstation software was used for LC Peak integration. The mobile phase was degassed by sonication with an ultrasonic bath (PCI Mumbai).The standard substance was weighed on analytical balance (AX 200 Shimadzu). Mobile phase with flow rate of 1.5ml/min and UV detection was carried out at 249 nm for Exemestane with injection volume 20µl.

Mobile phase:

35:65 v/v of acetonitrile and double distilled water was mixed to get the mobile phase. The mobile phase was then filtered through 0.22µm nylon membrane vacuum filtration and degassed by sonication.

Preparation of standard stock solutions:

Weigh and transfer about 10.0 mg of exemestane standard into 20ml volumetric flask, dissolve and dilute to volume with mobile phase, and mix well. Transfer 5ml of the solution to a 100ml volumetric flask, dilute to volume with mobile phase, and mix well. (or prepare equivalent to 0.025 mg/ ml of exemestane).

Preparation of sample solution:

Crush 20 tablets with mortar and pestle to a fine powder, weigh and transfer about 100 mg of tablet powder (equivalent to 25mg of exemestane) into 50 ml volumetric flask. Dissolve and dilute to volume with mobile phase and mix well. Transfer 5 ml of the solution to a 100 ml volumetric flask, dilute to volume with mobile phase and mix well. Filter this solution through 0.45µm nylon membrane filter.

RESULTS AND DISCUSSION

Method development, optimization and stability testing

In this performed reversed phase liquid chromatography with isocratic elution by uv detection. The uv spectrum of exemestane in mobile phase was noted using uv spectrophotometer. The maximum absorbance was noticed at 249 nm. This wavelength was used for detection of exemestane.

Validation and stability testing of the method

The proposed method was developed by the preferred chromatographic conditions, which would be helpful to conduct a stability study like forced degradation of exemestane, method development for the separation and estimation of exemestane and its degradation products by HPLC method, analysis of degradation products by HPLC method and complete validation study.

The mobile phase was acetonitrile (35) : purified water (65)v/v at 1.5ml/min flow rate and detection wave length 249nm was optimized which gave sharp peak, short run time for exemestane. The retention time for exemestane was found to be 9.869minutes respectively. The proposed method validated as per the guidelines of ICH Q2 (r1).[2] system suitability parameters and optimized chromatographic conditions are shown in table 2. The specificity was studied for the examination of the presence of interfering components, while the comparison of chromatograms

there was no interference from placebo shown in figure 2 and forced degradation of exemestane tablets shall be carried out, to confirm that during stability study or throughout the shelf life. In this perform neutral stressed, acid (figure 5,6) alkali **(**figure 7,8), peroxide, sunlight exposed (figure 9), uv light exposed (figure 10), thermal stressed sample (figure 11). These results are shown in table 4. The exemestane peak is found to be degraded more in acid, alkali and peroxide stress condition. Peak purity factor more than 990.0. Standard chromatogram shown in figure 3**.** The specificity results are summarized in table 3.the chromatographic results for the calibration standards are presented in table 5. The calibration curve for exemestane was found to be linear shown in table 6. The regression equation for exemestane was found to be y= 37797 x -1483.4. With correlation coefficient, r^2=1.000 which indicates this method has good linearity. The data of the regression analysis of the calibration results is shown in table 5 and calibration curve of exemestane shown in figure 12. Precision was studied to find out system, method and intermediate variation in the test methods of exemestane for 6 times on the same day and different days. System, method, intermediate precisions obtained was %RSD (<2.0) indicates that the proposed method is quite precise and reproducible and results are shown in table 7,8,9,10. Recovery studies of the drug were carried out for the accuracy parameters at three different concentration levels that are multiple level recovery studies. A known amount standard was added into pre analyzed sample and subjected them to proposed HPLC method. The % recovery was found to be within the limits as listed in table 11. The mean percentage recovery of exemestane at each level was not less than 98% and not more than 102%. Robustness was done by small changes in chromatographic conditions like flow rate and detection of wave length etc. It was observed that there were no marked changes in chromatograms. In fact the parameters are within the limits which indicates that the method has robustness and suitable for routine use. The robustness results are shown in Table 12. Method validation following ICH guidelines indicated that the developed method had high sensitive.

Applicability of the Developed Method to Marketed Formulations

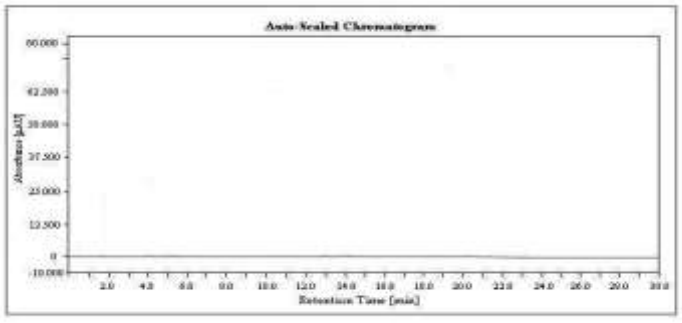

Figure 2: Chromatogram of blank

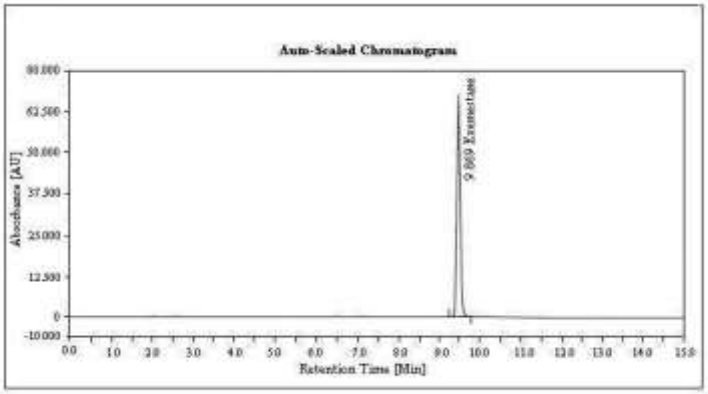

Figure 3: Standard Chromatogram of Exemestane

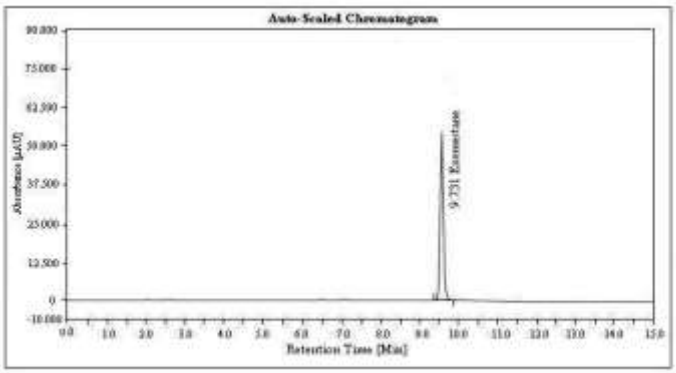

Figure 4: Chromatogram of sample assay for Exemestane Area responce

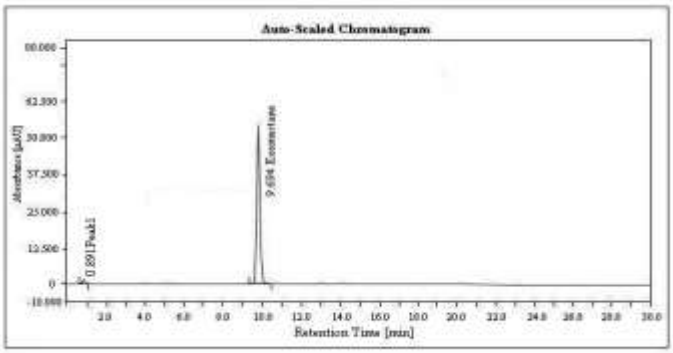

Figure.5 :HPLC Chromatogram of Acid stressed (0.1N HCl) Sample

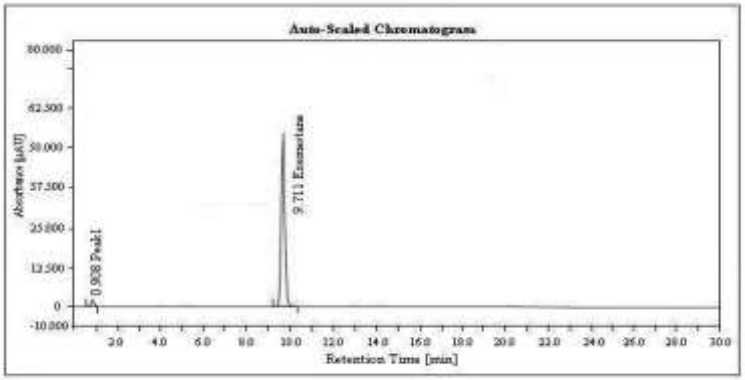

Figure 6: HPLC Chromatogram of Acid stressed (1N HCl) Sample

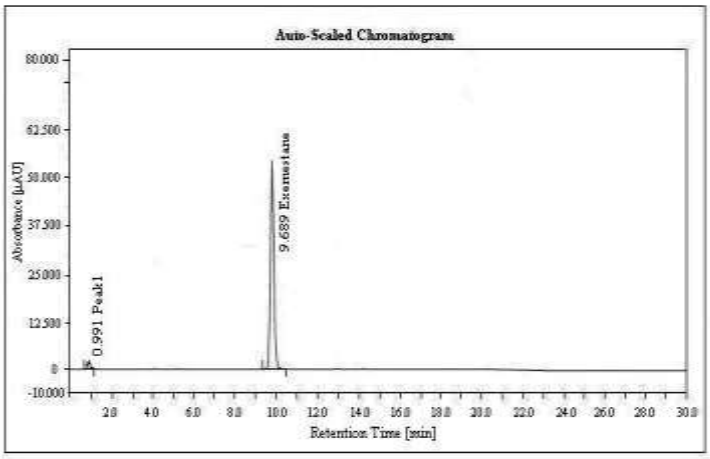

Figure.7: HPLC Chromatogram of Alkali stressed (0.1N NaOH) Sample

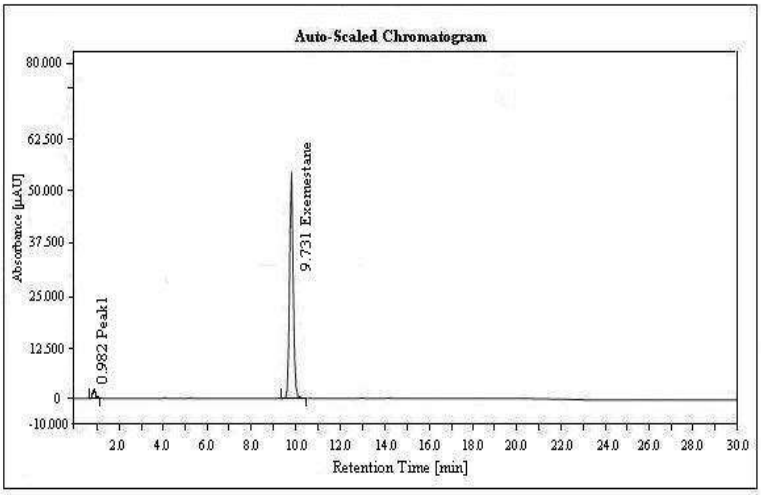

Figure 8: HPLC Chromatogram of Alkali stressed (1N NaOH) Sample

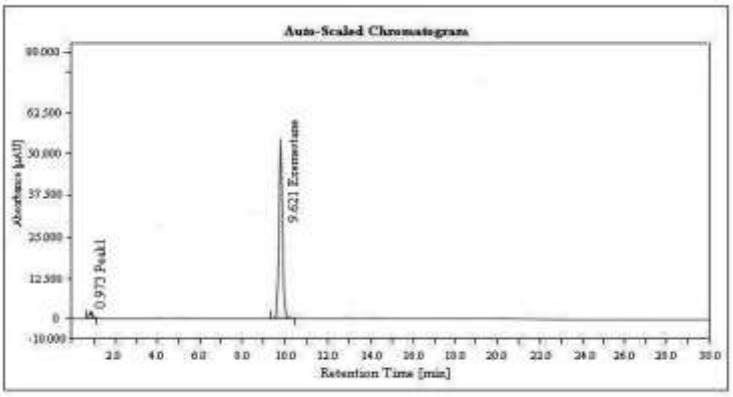

Figure 9: HPLC Chromatogram of Sunlight exposed Sample

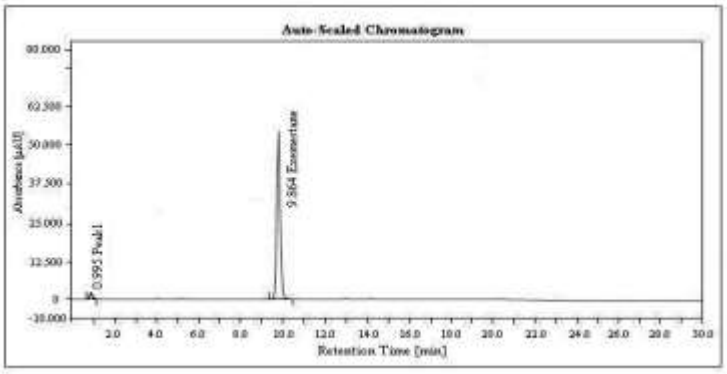

Figure 10: HPLC Chromatogram of UV light exposed Sample

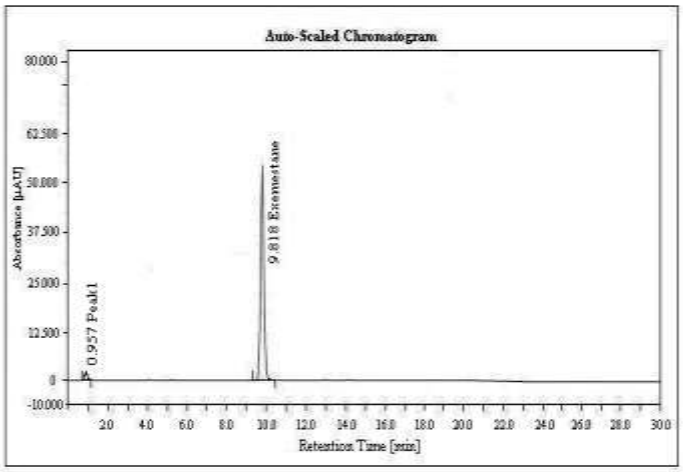

Figure 11: HPLC Chromatogram Thermal stressed Sample

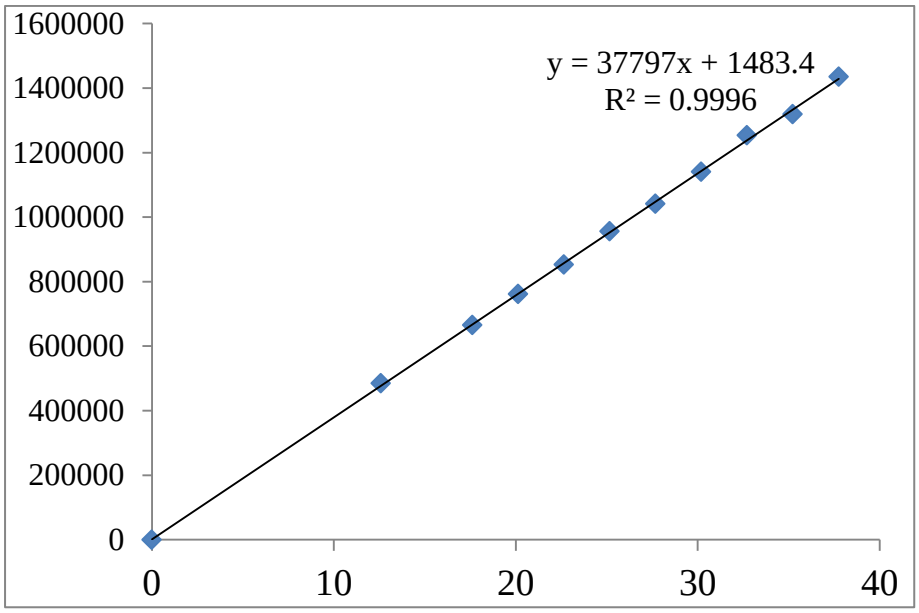

Figure 12: Calibration curve of Exemestane

Table 2: Optimized chromatographic conditions and system suitability parameters

Parameter	Chromatographic conditions
Instrument	AGILENT
Column	Hypersil, C-18,150 X 4.6mm, 5µ column or equivalent
Detector	Diode array detector
Diluents	Acetonitrile : purified water (35:65, v/v)
Mobile phase	Acetonitrile : purified water (35:65, v/v)
Flow rate	1.5ml/min.
Detection wave length	UV at 249nm.
Run time	15 minutes
Temperature	(40^0C)
Injection Volume	20µl
System suitability	Exemestane
Retention time (t_R)	9.869min.

Table 3: Results of specificity study

Name of the solution	Retention time, (t_R) min.
Mobile phase	No peaks
Placebo	No peaks
Solution containing a Exemestane.	Peak at 9.869min for Exemestane respectively.

Table 4: Results for Forced Degradation Study

Stress Condition	As Such	Neutral	0.1N NaOH	1N NaOH	0.1N HCl	1.0N HCl	Thermal	H_2O_2	Sun light	UV Light
Peak Purity**	P	P	P	P	P	P	P	P	P	P
Peak purity factor	999.9	999.9	999.9	999.9	999.9	999.9	999.9	999.9	999.9	999.9
% Assay	99.9	99.8	97.5	96.5	97.9	95.4	98.7	96.0	99.0	99.5

Peak purity **; 'P' indicates Exemestane peak is pure which is confirmed by Diode array detector and Agilent Chemstation software.

Table 5: Chromatographic results showing linearity of the proposed method for Exemestane

Level	Linearity stock solution added (In ml)	Total volume with diluent (In ml)
1	2.50	20
2	3.50	20
3	4.00	20
4	4.50	20
5	5.00	20
6	5.50	20
7	6.00	20
8	6.50	20
9	7.00	20
10	7.50	20

Table 6: Results of linear regression analysis

Level	Concentration in PPM	Area of Exemestane
1	0.0000	0.000
2	12.5838	485080.038
3	17.6173	665156.344
4	20.1340	761492.860
5	22.6508	852474.413
6	25.1675	955753.000
7	27.6843	1041431.520
8	30.2010	1140326.956
9	32.7178	1253729.316
10	35.2345	1319109.324
11	37.7513	1434934.835
Slope		37797.363
Correlation Coefficient		1.000
Intercept		1483.542
% Intercept		0.2%

Parameter	Exemestane
Detection wavelength(λ_{max})	UV at 249 nm
Regression equation (Y = ax - b)	Y = 37797x -1483.4.
Slope	37797.363
Intercept	1483.542
% Intercept	0.2%
Correlation Coefficient	1.000

[#]Average of above determinations

Table 7: Results for System Precision

Injection No.	Exemestane tablets	
	Retention time	Area response
1	9.610	933864.879
2	9.589	933629.704

3	9.585	933653.982
4	9.585	933249.013
5	9.584	933602.769
6	9.585	933366.299
Mean	9.590	933561.108
%RSD	0.1	0.0

Table 8: Results for Method Precision

Set No.	Exemestane in %
1	104.6
2	104.7
3	104.6
4	104.3
5	104.5
6	104.2
Mean	104.5
%RSD	0.2

Table No.9: Results for Intermediate Precision

Set No.	Exemestane in %
1	104.0
2	105.8
3	104.6
4	104.8
5	106.0
6	105.9
Mean	**105.2**
%RSD	**0.8**

Table 10: Comparison of the results obtained in Method precision and Intermediate precision

Study	Set No.	Exemestane in %
Method Precision	1	104.6
	2	104.7
	3	104.6
	4	104.3
	5	104.5
	6	104.2
Intermediate Precision	1	104.0
	2	105.8
	3	104.6
	4	104.8
	5	106.0
	6	105.9
	Mean	**104.8**
	% RSD	**0.6**

Table.11 Results for Recovery of Exemestane

S.No	Level (about)	Area Response	*mg added(Actual)	mg Recovered	% Recovery	Mean % Recovery	% RSD
1	50%	484015.635	12.7840	12.8887	100.8	101.2	0.5
2		479141.219	12.5340	12.7589	101.8		
3		480570.333	12.6610	12.7970	101.1		
4	100%	932526.586	25.0100	24.8320	99.3	99.7	0.5
5		931047.068	24.8700	24.7926	99.7		
6		933440.395	24.8000	24.8564	100.2		
7	150%	1402287.148	37.4600	37.3412	99.7	99.8	0.2
8		1400076.144	37.3600	37.2823	99.8		
9		1400192.901	37.2800	37.2854	100.0		
% RSD for 3 X 3 Levels							

*potency is considered

Table 12: Results for Robustness

Acceptance Criteria	% RSD
Original Conditions	0.0
Decreased Flow rate (1.3mL/min)	0.1
Increased Flow rate (1.7mL/min)	0.0
Decreased Column Temp (-5°C)	0.1
Increased Column Temp (+5°C)	0.0
Decreased Organic ratio (-2.0%)	0.1
Increased Organic ratio (+2.0%)	0.2

CONCLUSION

The proposed HPLC method for estimation of % Assay in the drug product of Exemestane Tablets is validated as per analytical method. The method is found to be specific. The method is found to be linear in the specified range for exemestane. Accuracy of the method is established for Exemestane Tablets. The method is found to be robust. A system suitability test is established and related parameters are recorded. Hence this method is validated and can be used for routine and stability sample analysis.

REFERENCES

1. Suresh Kumar R, Narasimha Naidu M, Srinivasulu K, Raja Sekhar K, Veerender M, Srinivasu MK. “Development and validation of a stability indicating LC method for the assay and related substances determination of Exemestane, an aromatase inhibitor”. Journal of Pharmaceutical and Biomedical Analysis. 2009; 50(5): 746-752.
2. PC Kamboj. “Pharmaceutical analysis’’. Vallabh prakashan, New Delhi. 2003; Vol I, p 1.

3. International Conference on Harmonization (ICH) harmonized tripartite guideline Validation of analytical procedures: text and methodology Q2 (R1) ICH, Geneva, Nov (2005).
4. PV Pawar, PD Gaikwad, VH Bankar, SP Pawar. "Development and validation of UV-HPLC method on tablet dosage form: A Review". International Journal of Pharmaceutical Research and Development. 2011; Vol-3, Issue-1: 180-192.
5. Willard HH, Merritt LL. "Instrumental methods of analysis''. CBS publishers and distributors, New Delhi. 2001; p. 1-12, 97-106, 118-136, 513-534, 580- 629.
6. Shah K, Kumar S, Upmanyu N, Mishra P. "Evaluation of an Analytical Method". International Journal of Pharmaceutical Chemistry Research. 2012; 1(1): 6-12.
7. VOGEL's, "Textbook of Quantitative Chemical Analysis", Fifth edition, Great Britain by Bath Press, 1-14.
8. Skoog, DA Holler, FJ Nieman. "T.A. Principles of Instrumental Analysis'', Thomson Brooks/Cole. Fifth edition. 1998: 329-335.
9. Sharma BK. "Instrumental Methods of Chemical Analysis''. Goel Publishing House, Meerut. 1991; Eleventh edition: 1-9.
10. Kennedy JH. "Analytical Chemistry –Principles''. Saunders College Publishing, New York. 1990; Second edition: 1-8.
11. Unilever, Modern Chemical Techniques: Chapter 5 Chromatography, The Royal Society Of Chemistry: 116-159.
12. Remington. "The science and practice of pharmacy", 21st edition, Lippincott Williams & Wilkins – P - 615
13. U.S. Pharmacopoeia 32 Chapter <621>
14. ICH Q2B, Harmonized Triplicate Guideline, Validation of analytical Procedure Methodology. IFPMA, Proceeding of the International Conference on Harmonization. Geneva, March 1996.
15. ICH Q2A, Harmonized Tripartite Guideline, Validation of analytical Procedure Methodology. IFPMA, Proceedings of the International Conference on Harmonization. Geneva. March 1994.
16. Hou S, Hindle M, Byron PR. "Stability Indicating HPLC Assay Method for Budesonide". Journal of Pharmaceutical & Biomedical Analysis. 2001; 24(3): 371-380.
17. Mao YP, Tao XL, Lipsky PE. "Analysis of the Stability and Degradation Products of triptolide". Journal of Pharmacy & Pharmacology. 2000; 52(1): 3-12.

18. Tipre D.N. Vavia P.R,. "Oxidative Degradation Study of Nitrendipine Using Stability Indicating, HPLC, HPTLC and Spectrophotometric Method". Journal of Pharmaceutical & Biomedical Analysis. 2001; 24 (4): 705-714.
19. Cenacchi V, Barette S, Cicioni P, Frigerio E, Long J, James J. "LC-MS-MS determination of Exemestane in human plasma with heated nebulizer interface following solid phase extraction in the 96 well plate format". Journal of Pharm Biomed. 2000; Anal 22: 451-460.
20. USP 25–NF 20, Validation of Compendial Methods Section (1225). (United States Pharmacopeial Convention, Rockville, Maryland, USA, 2002); 225.
21. ICH Stability Testing: Photostability Testing of New Drug Substances and Products. (International Conference on Harmonization, Geneva, Switzerland, November 1996).
22. Monika Bakshi, Saranjit Singh. "Development of validated stability-indicating assay methods—Critical Review". Journal of Pharmaceutical and Biomedical Analysis. 2002; 28 : 1011–1040.
23. Bhoomi P. Shah, Suresh Jain, Krishna K. Prajapati, Nasimabanu Y. Mansuri. "Stability Indicating HPLC Method Development: A Review". IJPSR, 2012; 3(9): 2978-2988.
24. Michael J. Smela: "Regulatory Considerations for Stability Indicating Analytical Methods in Drug Substance and Drug Product Testing". American Pharmaceutical Review. 2005; 8(3): 51-54.
25. Swartz M, Krull I. "Developing and Validating Stability- Indicating Methods". LCGC North America. 2005; 23(6):586- 593.
26. Monika Bakshi, Saranjit Singh. "Development of validated stability-indicating assay methods—Critical Review". Journal of Pharmaceutical and Biomedical Analysis. 2002; 28: 1011–1040.