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Anastrozole drug evaluation using the RP-HPLC method in bulk and tablet dosage forms, as well as stability studies.

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ABSTRACT :
For the quantitative determination of anastrozole in pharmaceutical tablet dosage forms an accurate, highly sensitive, precise, simple, efficient, and reproducible isocratic Reversed Phase-High Performance Liquid Chromatography (RP-HPLC) method was developed and validated. Waters Symmetry ODS C18 RP Column (250mmx4.6mm, 5µm) was used in isocratic mode to develop the RP-HPLC method. The mobile phase contained a 75:25 v/v mixture of acetonitrile and phosphate buffer pH-3.00 (pH was adjusted with ortho phosphoric acid). The flow rate was 1.0ml/min, and the effluent was measured at 241nm UV wavelength using an ELICO SL-159 UV-Vis spectrophotometer. Anastrozole's retention time was discovered to be 3.461 minutes. Anastrozole's linearity and range were found to be 0-140 µg/ml, with a correlation coefficient of 0.999. According to ICH guidelines, the method was validated over the analyte concentration range of 30-70 µg/ml, and its accurate accuracies of three concentrations ranged from 98-102%. Specificity, linearity, precision, accuracy, robustness, limit of detection (LOD), and limit of quantitation (LOQ) were all evaluated using the International Conference on Harmonization (ICH) Q2 R1 guidelines. The developed method was successfully used to quantify the bulk and active pharmaceutical ingredient content of tablet dosage form.

KEYWORDS: Anastrozole, Isocratic RP-HPLC, UV-Visible detector, Validation Methods.

I. INTRODUCTION

Anastrozole chemically designated as2-[3-(1-cyano-1-methyl ethyl)-5-(1H-1, 2, 4-triazol-1-yl methyl) phenyl] -2-methyl propanenitrile is a non-steroidal aromatase inhibitor of modern trend with molecularformula $C_{17}H_{19}N_5$[1,2,3]. It selectively inhibits the aromatase enzyme. The primary source of circulating oestrogen (estradiol), which is produced by the adrenal glands and converted to estrone by the action of aromatase found in peripheral tissues [4]. This causes a decrease in serum and tumoroestrogen concentration, resulting in a decrease in tumor mass in women. Anastrozole is used for the treatment of post - menopausal women with breast cancer. It is indicated for adjunctive treatment (surgery) and is also used in metastatic breast cancer. It works by lowering oestrogen hormone levels, which aids in tumor shrinkage and growth [5,6].Anastrozole has been studied for its ability to reduce estrogens, including estradiol, in men. It may also help to lower the risk of stroke, heart attack, chronic inflammation, prostate enlargement, and prostate cancer [7].

According to the literature review, there are only a few analytical methods available for the separation and estimation of anastrozole, such as HPLC, HPTLC, UV-Visible analysis, GC, and LC-MS [8]. In the literature, there are only a few analytical HPLC methods for determining anastrozole in bulk and pharmaceutical dosage forms [9, 10]. So far, the reported HPLC methods in the literature are thought to be cost prohibitive, time consuming, and have poor symmetry. In fact, there is a need for the development of a novel, simple, quick, and reproducible RP-HPLC analytical method for determining anastrozole in bulk and pharmaceutical dosage forms.

The goal of this work is to create a new, simple, and precise method for evaluating anastrozole drug by RP-HPLC method in bulk and tablet dosage forms, as well as studies on its stability. This method had the advantage of a shorter retention time, a shorter runtime, and simple mobile phase preparation.

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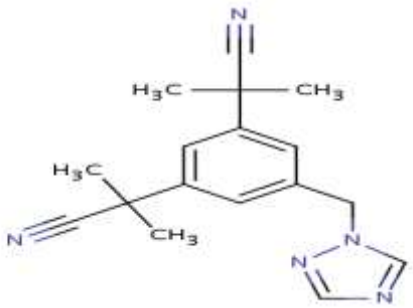

Figure 1 **:**Chemical structure of Anastrozole

II. MATERIALS AND METHODS

MATERIALS

Sd Fine-Chem Ltd. in Mumbai provided the HPLC grade water; dipotassium hydrogen orthophosphate; potassium dihydrogen orthophosphate and orthophosphoric acid. We bought acetonitrile and methanol from LobaChem in Mumbai.

SOLUTIONS PREPARATION

STANDARD SOLUTION PREPARATION

A working concentration of around 50 µg/ml is recommended. Transfer 25mg of the working standard anastrozole correctly weighed into a dry, clean 25ml volumetric flask. Then, in order to achieve a concentration of 1000 µg/ml, prepare up to the mark using the same mobile phase or solvent. Finally, prepare the dilutions to get 50 µg/ml concentrations.

MOBILE PHASE PREPARATION

Phosphate buffer, 250 ml (30%) and acetonitrile, 750 ml (HPLC Grade-70%) should be combined and degassed in an ultrasonic water bath for ten minutes. Under vacuum filtration, then filter through a 0.45 micro filter.

SAMPLE SOLUTION PREPARATION

Transfer 0.5ml of the previously produced solution to a clean, dry 10ml volumetric flask. Then use the solvent/mobile phase system to make up to mark.

WAVELENGTH SELECTION

Using a UV spectrophotometer, the UV spectra of several dilute solutions of anastrozole in mobile phase were captured [11,12,13]. At 241 nm, the peak of the highest absorbance was noted. Anastrozole was detected using this wavelength.

METHOD VALIDATION

The devised analytical technique was validated in accordance with ICH for the following criteria: system suitability; specificity; accuracy; precision; linearity; limit of detection (LOD) and limit of quantitation (LOQ); robustness, and ruggedness [14,15,16].

SYSTEM SUITABILITY

Gas and liquid chromatographic techniques as well as other chromatography methods all include the system suitability parameter. The parameters are used to confirm that the chromatographic system's resolution and repeatability are adequate for the analysis [17]. Six injections of the standard solution were made, and the area of each injection was quantified using HPLC. It was discovered that the theoretical plate count and tailing factor were within acceptable bounds. The subsequent Table 1 displays the outcomes that were attained.

SPECIFICITY

The system suitability for specificity was tested to see if any impurities interfered with the analytical peak's retention time [18]. Blank injections were used to conduct the trial.

ACCURACY

When using the developed approach, the accuracy can be tested by injecting 80%, 100%, even 120% of the pure anastrozole medication. It has a 50µg/ml concentration, approximately. We shall determine the % purity of the pure medicine from the administered injections based on these tests [19]. The subsequent Table 2 displays the outcomes that were attained.

PRECISION

Six injections of the standard solution were made, and the area of each injection was determined

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using HPLC. The subsequent Table 3 lists the results.

LINEARITY & RANGE

Theconcentration range studied for linearity was 30ppm to 70ppm. The correlationcoefficient was calculated using the area of each level which is shown in Table 4.

LIMIT OF DETECTION AND LIMIT OF QUANTIFICATION

The smallest concentration of analyte that can be accurately detected by an analytical process is referred to as the limit of detection (LOD) and limit of quantification (LOQ)[20].

The LOQ might be substantially higher in concentration or it might be equal to the LOD.
Equations were used to calculate the LOD and LOQ.
$LOD = 3.3 \times \sigma / S$ and
$LOQ = 10 \times \sigma / S$
Where,
σ is the standard deviation of Calibration curve
S is the average of slope of corresponding Calibration curve

ROBUSTNESS OF THE METHOD

Robustness testing was done for flow rate fluctuations between 0.9 and 1.1 ml/min and mobile phase ratio variations between more and less organic ratio.

RUGGEDNESS OF THE METHOD

The Ruggedness of the methodology was tasted for the five injections of anastrozole on various days and by various analyzers[21].The subsequent Table 5 displays the outcomes that were attained.

FORCED DEGRADATION STUDIES

Forced degradation is a part of stability testing which isfind out by the API and dosage form [22,23]. Here, many forced degradation processes are analyzed which include oxidative, photolytic, thermal, acidic, and basic degradation. Following Table 6 is a summary of these studies on forced degradation.

ACID HYDROLYSIS

Anastrozole was properly weighed out to be 25 mg pure, then put into a 25 ml clean, dry volumetric flask with around 5 ml of diluent, then sonicated to completely dissolve it. The above mentioned solution undergoes acid degradation in 3ml of 0.1N HCl to get the volume up to the required level (Stock Solution-1000µg/ml). After being set aside for 24 hours, the prepared solution is neutralized with 3 ml of 0.1N NaOH. From the above mentioned stock solution, a concentration of 25 µg/ml solution was made, and filtered through a 0.45 micron filter, and then tested.

BASIC HYDROLYSIS

Similarly, alkaline degradation tests were carried out using an anastrozole concentration of 1000µg/ml in 3 ml of 0.1N NaoH. After being set aside for 24 hours, the solution is neutralized with 3ml of 0.1N HCl. From the above mentioned stock solution, a concentration of 25µg/ml was made, filtered through a 0.45 micron filter, and then tested.

THERMAL DEGRADATION

For thermal degradation studies, 25µg/ml of anastrozole was heated in hot air oven for 24hours at 50^0 C, then cooled and used for testing.

PHOTOLYTIC DEGRADATION

For photo stability testing, the therapeutic solution containing 25µg/ml was exposed to UV light(at 254nm) for 24 hours in a UV light chamber and then tested [24].

OXIDATION WITH (3%) H_2O_2

30ml of 3% H_2O_2 at a concentration of 1000µg/ml was used to make solutions for oxidative stress tests. The stock solution was used to prepare a concentration of 25µg/ml. After being set aside for 24 hours, the final solution was filtered through a 0.45micron filter and then examined [25].

III. RESULT AND DISCUSSION

CHROMATOGRAPHIC CONDITIONS

Several reversed phase columns, including the C8 and C18 columns, were used to evaluate anastrozole. Symmetry ODS C18 RP Column (250mm x 4.6mm, 5µm), among C8 and C18 columns, was chosen. As a column modification, different mixtures of acetonitrile, phosphate buffer, and methanol were examined. This method's mobile phase, is a combination of an acetonitrile and phosphate buffer (pH=3.0) in a ratio of 75:25 v/v, was used to detect UV light at a flow rate of 1.0 mL/min with wavelength 241 nm. The injection volume was 20µL, the temperature was ambient, the runtime was 6 minutes, and the retention duration was 3.461 minutes.

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METHOD VALIDATION

SYSTEM SUITABILITY

Six injections of the standard solution were prepared, and the area of each injection was quantified using HPLC. The Theoretical plate count 3945.667 (N > 2000), tailing factor 1.48 (T ≤ 2) both were within accepted limits.

Table 1: System Suitability Results

Sl. No.	Injection No.	Rt	Peak Area	Height	USP Plate Count	USP Tailing
01	Injection 1	3.458	1065244	4551	3965	1.4
02	Injection 2	3.460	1056565	4560	3954	1.6
03	Injection 3	3.461	1064511	4552	3953	1.5
04	Injection 4	3.458	1065340	4546	3948	1.4
05	Injection 5	3.461	1066841	4558	3941	1.5
06	Injection 6	3.462	1065243	4556	3913	1.5
Mean			**1063957**		**3945.667**	**1.48**
S.D			**3701.051**			
% RSD			**0.347857**			

SPECIFICITY

The system's appropriateness for specificity was tested to see if any contaminants interfered with the analytical peak's retention time. Blank injections were used to conduct the trial.

The specificity test for anastrozole was conducted. There were no further peaks discovered. It was observed that there were no contaminants interfering with the retention time of the analytical peak.

READINGS OF ACCURACY

For anastrozole, the accuracy study was carried out at 80%, 100%, and 120%. Three copies of each level were put into the chromatographic system. Each level's area was used to calculate the recovery percentage.

On the evaluation of accuracy results

The % recovery of 80% concentration was found to be 101.433%

The % recovery of 100% concentration was found to be 101.554%

The % recovery of 120% concentration was found to be 100.242%

Acceptance Criteria: Each level of concentration should have a recovery percentage of98–102%.

Table 2.Accuracy Results of Anastrozole

Sample ID	Concentration (µg/ml)		Rt	Peak Area	USP Plate Count	USP Tailing	% Recover Of pure Drug	Statistical Analysis
	Amount Injected	Amount Found						
S_1 : 80 %	40	40.711	3.458	935683	3452	1.0	101.775	Mean= 101.433%
S_2 : 80 %	40	40.270	3.469	925688	3464	1.1	100.66	% R.S.D.= 0.66175%
S_3 : 80 %	40	40.745	3.462	936524	3447	1.0	101.865	
S_4 : 100 %	50	50.776	3.460	1165242	3965	1.4	101.554	Mean= 101.554%
S_5 : 100 %	50	50.783	3.462	1165381	3984	1.3	101.567	%R.S.D.= 0.01182%
S_6 : 100 %	50	50.770	3.458	1165120	3957	1.4	101.543	
S_7 : 120 %	60	59.558	3.463	1365481	3796	1.6	99.266	Mean= 100.242%
S_8 : 120 %	60	60.435	3.462	1385462	3747	1.6	100.725	%R.S.D. = 0.84379%
S_9 : 120 %	60	60.445	3.465	1385644	3788	1.6	100.737	

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PRECISION

The peak areas and retention time determined by real estimation of 6 replicates of a constant dose of anastrozole (API).

Acceptance Criteria: The RSD percentage should not be greater than 2% for the area of six standard injection results.

Based on the review of the aforementioned results, it can be said that the RSD % was found to be 0.4%, which does not significantly damage the procedure and thus shows that the method is accurate.

Table 3.Readings of Repeatability

HPLC Injection Replicates of Anastrozole	Rt	Peak Area	USP Plate Count	USP Tailing
Replicate – 1	3.462	1065244	3987	1.5
Replicate – 2	3.459	1056841	3955	1.4
Replicate – 3	3.458	1065340	3986	1.5
Replicate – 4	3.462	1064513	3927	1.4
Replicate – 5	3.461	1056864	3963	1.5
Replicate – 6	3.458	1056844	3950	1.4
Mean	**3.46**	**1060941**	**3961.3**	**1.45**
Standard Deviation	**0.001897**	**4490.958**		
% RSD	**0.05482**	**0.42329**		

LINEARITY AND RANGE

The concentration range studied for linearity was 30ppm to 70ppm. The correlation coefficient was calculated using the area of each level.

Results: With a correlation value (R^2) of 0.999, the calibration curve for anastrozole (API) demonstrated good linearity in the range of 0-70 µg/ml. For anastrozole, the standard calibration curve has the regression equation y = 22802x + 7400.

Acceptance Criteria: The correlation coefficient for the results of the area of five standard injections must be greater than or equal to (>=) 0.999.

Table 4.Results of Co-relation Coefficient

Sl. No.	Concentration (µg/ml)	Peak Area
01	0ppm	0
02	30ppm	668665
03	40ppm	899410
04	50ppm	1128425
05	60ppm	1365425
06	70ppm	1594288
Mean		**1131243**
Co-relation Coefficient		**0.999**

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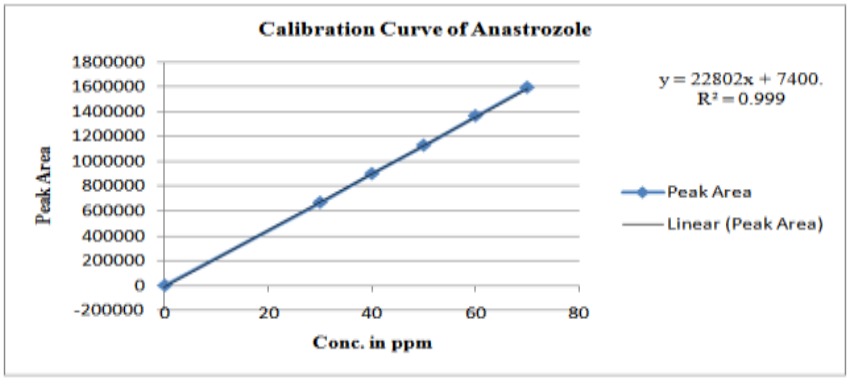

Figure 2.Calibration curve of Anastrozole (API)

On, **X-Axis**-Concentration (in ppm)
Y-**Axis**- Peak Area

LOD (LIMIT OF DETECTION) AND LOQ (LIMIT OF QUANTIFICATION)

Anastrozole's minimum concentration levels at which it can be reliably detected (LOD) and quantified (LOQ) were found to be 0.16µg/ml and 1.64µg/ml, respectively, indicating that the method's sensitivity is high.

ROBUSTNESS OF THE METHOD

Robustness testing was done for flow rate fluctuations between 0.9 and 1.1 ml/min and mobile phase ratio variations between more and less organic ratio.

On the basis of analysis, it can be said that the flow rate variation has little to no impact on the methodology.
Therefore, it demonstrates that the method is reliable even when the flow rate changes by ± 0.1 ml/min.

RUGGEDNESS OF THE METHOD

For the five injections of anastrozole, the robustness was tested on various days and by various analyzers.
Acceptance Criteria: The %RSD for the outcomes of five standard injections shouldn't be higher than 2%.
According to the analysis, the%RSD reported to be 0.01% and 0.08% do not significantly damage the method, indicating that the methodology is accurate.

Table 5.Results of Ruggedness

Sl. No.		Rt	Area	Height	USP Plate Count	USP Tailing
01		3.462	1065241	4553	3894	1.5
02	**Day 1 &**	3.460	1065342	4556	3988	1.4
03	**Analyst 1**	3.462	1065425	4565	3898	1.6
04		3.462	1065127	4550	3983	1.5
05		3.458	1065641	4553	3814	1.4
Mean			**1065355**		**3915.4**	**1.48**
S.D			**194.8363**			
% RSD			**0.018288**			
01		3.465	1065242		3888	1.5
02	**Day 2 &**	3.463	1063253		3983	1.6
03	**Analyst 2**	3.461	1065257		3897	1.5
04		3.463	1065342		3964	1.4
05		3.460	1065477		3913	1.5
Mean			**1064914**		**3929**	**1.5**
S.D			**933.3148**			
% RSD			**0.087642**			

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FORCED DEGRADATION STUDIES

The results of the tests indicate how specific the developed method performs. Anastrozole was partly stable under oxidative and basic stress conditions, as well as fully stable in acidic and thermal conditions.

Table6. Results of force degradation studies of Anastrozole API

Stress Condition	Time	Assay of active substance	Assay of degraded products	Mass Balance (%)
Standard drug	-----	100	-----	100.0
Acid Hydrolysis (0.1N HCl)	24Hrs.	99.05	0.95	100.0
Basic Hydrolysis (0.IN NaOH)	24Hrs.	90.52	9.48	100.0
Thermal Degradation (50 °C)	24Hrs.	99.15	0.85	100.0
Photolytic Degradation (UV 254nm)	24Hrs.	89.55	10.45	100.0
Oxidation (with 3% Hydrogen peroxide)	24Hrs.	94.52	5.48	100.0

IV. CONCLUSION

The findings of this investigation demonstrate that, under chromatographic conditions, the flow rate, wavelength, and retention time are, respectively, 1.0 mL/min, 241 nm, and 3.461 min. The theoretical plate count and system suitability tailing factor, respectively, are 3945.667 and 1.48. The LOQ is 1.64 µg/ml, while the LOD is 0.16 µg/ml. According to force degradation investigations, anastrozole was mostly stable under oxidative and basic stress conditions and completely stable under acidic and thermal conditions. From above we can conclude that, in the present work, we have successfully developed analytical method namely HPLC for anastrozole in bulk and formulation. The validation by HPLC is performed; the HPLC method is simple, efficient, rapid and precise.

REFERENCES

[1]. Drug today medical journal, Lorina publication (India) Inc, Delhi-91, 2012; 78(1): 742.

[2]. M J. O'Neil, Royal Society of Chemistry, Cambridge, UK. The Merck Index: An Encyclopedia of Chemicals, Drugs, and Biologicals, 2013; 74(5):283-340.

[3]. Sweetman, et al. Martindale. The Complete Drug Reference. Pharmaceutical Press, 2011; 37:528.

[4]. Keri Wellington & Diana M. Faulds. Anastrozolein Early Breast Cancer. Springers Link, 2012; 62:2483-2490.

[5]. K Mokbel, Taylor &Fransics. Focus on anastrozole and breast cancer. Current medical research and opinion, 2008; 683-688.

[6]. J Geisler, S. Detre, H. Berntsen, L. Ottestad, B.Lindtjørn, M. Dowsett, and P.E. Lønning. Influence of neoadjuvantanastrozole (Arimidex) on intratumoral estrogen levels and proliferation markers in patients with locally advanced breast cancer.Clin Cancer Res. PubMed, 2001; 7(5):1230-6.

[7]. Faloon, William, "Dangers of Excess Estrogen In the Aging Male". Life Extension Magazine,2008; 1.

[8]. M B. Abubakar and S. H. Gan. A Review of chromatographic Methods used in the Determination of Anastrozole Levels. Indian J Pharm Sci, 2016; 78(2); 173-181.

[9]. PerumallaBharati, AryaVinodini, Ala Reddy and Potturi Devi. Development and validation of a planar chromatographic method with reflectance scanning densitometer for quantitative analysis of Anastrozole in the

bulk material and tablet formulations. Journal of planar Chromatography, 2010; 23:79 – 83.
[10]. D.Sathis Kumar, A.Harani, D.Sridhar, David banji, KNV Rao, Guruviah and Yogeswaran. Development and Validation of a HPLC Method for Determination of Anastrozole in Tablet Dosage Form. E-Journal of Chemistry, 2011; 8(2):794-797.
[11]. Vidya. K, Lakshmi. PK, Padmavathi. Y.Development and Validation of UV Spectrophotometric method for in-vitro studies of AnastrozoleInvasomes. J. Pharm. Sci. & Res, 2019; 11(5):1727-1730.
[12]. Mrityunjay Banerjee, TejaswiniKumari Dash, AnkitaKumari and SwapneswarKhatua.Optimized UV-Vis spectrophotometric method for estimation of anastrozolein pharmaceutical solid dosage form. Der PharmaChemica, 2014; 6(3):140-144.
[13]. Pavia D, Lampman G, Krix G. Introduction to Spectroscopy. Bloomington, IN: Indiana University, 2001.
[14]. International Conference on Harmonization, Q2B: Guideline on the Validation of Analytical Procedures: Methodology and Availability. Federal Register, 1997; 62(96):27463–27467.
[15]. P Ravisankar and G. DevalaRao. A novel validated RP-HPLC method for the determination of anastrozole in bulk and pharmaceutical tablet dosage forms. Der PharmaChemica, 2013; 5(3):51-62.
[16]. Hema, Swati Reddy G. A review on new analytical method development and validation by RP-HPLC. Int Res J Pharm Biosci, 2017; 4:41-50.
[17]. Patil R, Deshmukh T, Patil V, Khandelwal K. Review on analytical method development and validation. Res Rev J Pharm Anal, 2014; 3:1-10.
[18]. Pathuri R, Muthukumaran M, Krishnamoorthy B, Nishat A. A review on analytical method development and validation of the pharmaceutical technology. Curr Pharm Res, 2013; 3:855-70.
[19]. K R Sreejith, P.L Rajagopal, K Premaletha. Analytical Method Development and Validation of Anostrozole in Pure and Tablet Dosage Form by UV Spectroscopy. Research J. Pharm. and Tech, 2017; 10(4):1015-1019.
[20]. Shivani Sharma, SwapnilGoyal, KalindiChauhan. A review on analytical method development and validation. International Journal of Applied Pharmaceutics, 2018; 10(6):8-15.
[21]. Prafulla Kumar Sahu, NageswaraRaoRamisetti, Teresa Cecchi, SuryakantaSwaind, Chandra SekharPatroa, Jagadeesh Panda. An overview of experimental designs in HPLC method development and validation. Journal of Pharmaceutical and Biomedical Analysis, 2018; 147:590–611.
[22]. Madhusudhan Reddy Bethi, PrasannaBethanamudi. Analytical method development and validation of impurity profile in anastrozole. IJIPSR, 2018; 6(03):143-154.
[23]. BlessyMn ,Ruchi D. Patel, Prajesh N. Prajapati, Y.K. Agrawal. Development of forced degradation and stability indicating studies of drugs. Journal of Pharmaceutical Analysis, 2014; 4(3):159-165.
[24]. M Allwood, J. Plane. The wavelength dependent degradation of vitamin A exposed to ultraviolet radiation. Int. J. Pharm, 1986; 31:1–7.
[25]. K M. Alsante, A. Ando, R. Brown, et al. The role of degradant profiling in active pharmaceutical ingredients and drug products. Adv. Drug Deliv. Rev, 2007; 59(1): 29–37.