RESEARCH ARTICLE

QbD Approach for Analysis of Tirzepatide in its Bulk and Marketed Formulation by Stability Indicating RP-HPLC

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ABSTRACT

The core intentions of the stated work has been to create and validate a simple, sensitive, specific, precise and cost-effective RP-HPLC method with good performance for the investigation of tirzepatide in API powder and its marketed formulation. HPLC system (WATERS) equipped with DAD detection system was used to develop the current system. The procedure conditions of BDS C_{18} (150 x 4.6 mm,5 m), 0.01N KH_2PO_4: Acetonitrile in the ratio of 41:59 (% v/v), a flow of 0.9 mL/min, and a temperature of 31°C were successfully optimized by central composite design of QbD experiments. The optimized wavelength selected was 250 nm. RT of tirzepatide was observed to be 2.841 minutes with good system suitability. The ICH Q2(R1) standards functioned as a validation for the planned action strategy. Linearity was observed for 5 to 30 µg/mL concentration series of tirzepatide with R^2 of 0.999. The %RSD results of both precisions were found in the range of 0.40 to 0.41.% recovery of Tirzepatide in spiked samples was assessed to be 99.89%. The LoD and LoQ of tirzepatide were calculated to be 0.05 and 0.14 µg/mL, respectively. The results assured that the established procedure was simple, sensitive, specific, accurate and cost-effective. Exploration of tirzepatide under a diversity of FD conditions represents the stability representing the quality of the established HPLC procedure**.** Hence, the anticipated process has significant credit in the pharmaceutical segment.

Key words: Tirzepatide, Sensitivity, Quality by Design, Stability indicating, WATERS HPLC.

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INTRODUCTION

Diabetes and obesity are chronic illnesses that have a high mortality rate in wealthy nations and substantial morbidity everywhere. They are regarded as the century's two biggest epidemics. None of these disorders is simple; they are both complex health problems, including epigenetic, genetic, socioeconomic, lifestyle, and environmental factors.[1-3] Tirzepatide, also known as a "twincretin," is the solitary dual GLP-1 and GIP receptor agonist that can inferior glycemic levels, enhance insulin compassion, start lowering body weight by even higher than 20%, and achieve better lipid metabolic pathway. Tirzepatide is an artificial peptide analog of GIP hormone associated with C20 fatty-diacid fraction by acylation process. Tirzepatide is administered weekly once in a subcutaneous route, consisting of a half-life of 5 days.[2-5] Tirzepatide was officially approved by FDA in May 2022 with brand name of Mounjaro, which Eli Lilly[5] could develop. Chemically tirzepatide is a peptide analog with molecular formula and molecular weight of $C_{225}H_{348}N_{48}O_{68}$ and 4813g/mol, respectively (Figure 1).[6]

As of now, any kind of analytical procedure has been reported for assessment of tirzepatide in both bulk and formulations. All produced products A in the drug industry need to have the highest possible quality in order to pose least risk to patients.[7] Scientists, engineers, and designers use a variety of tests and investigations (including liquid chromatography) to assure their final products will please people. High-performance liquid chromatography has substituted various spectroscopic techniques in the last 10 years for testing the pre- marketing pharmaceutical and drug control. In the product pipeline, having an HPLC method available for routine investigation and evaluation during the production and formulations of substances is highly vital to have in the quality control unit.[7,8] Determining the stability of an analyte, assessing its degree of degradation, and separating its degradants all require stability-demonstrating chromatographic technique.[9-14] The principles outlined in ICH Q8-Q11, also known as Quality by Design (QbD), have also been used in the creation of analytical techniques. Establishing a reversed-phase liquid

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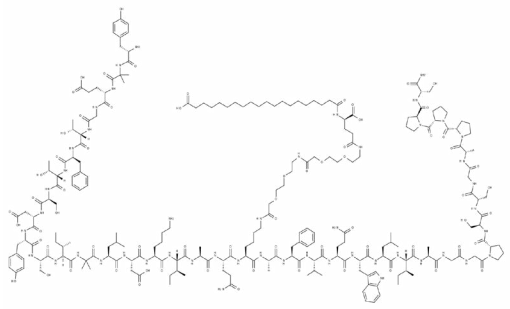


Figure 1: Molecular structures of tirzepatide

chromatography assay and related chemicals medication product approach is used to provide insight into these ideas.[15,16] Applying QbD principles to analytical procedures has many benefits, such as identifying and reducing sources of unpredictability that could lead to insufficient method resilience and ensuring that the method always performs as expected.[15] For this reason, research has been moved frontward to build up a new stability demonstrating RP-HPLC method for analysis of tirzepatide.

MATERIALS AND METHODS

Pure tirzepatide drug was purchased from Spectrum Labs in Telangana. The chemicals and reagents that were utilized in this investigation were all purchased from Aventar chemicals Pvt Ltd. in India. In order to produce the solutions, borosilicate glassware of the highest grade was utilized.

Standard Solution (30 µg/mL) Preparation

After ensuring that the Tirzepatide API powder was accurately weighed, it was transferred to a volumetric flask with a capacity of 25 mL and then dissolved in diluent to produce a solution with a 200 µg/mL concentration. Taking 1.5 mL of the obtained solution and diluting it to a total volume of 10 mL yielded a solution containing tirzepatide at a concentration of 30 µg/mL at a level of 100%.

Sample Solution (30 µg/mL) Preparation (30 µg/mL)

In order to generate a solution with a concentration of 200 µg/mL,the tablet powder containing 5 mL of tirzepatide was accurately weighed and transferred to a volumetric flask with a capacity of 25 mL before being dissolved in the diluent. Taking 1.5 mL of the obtained solution and diluting it to a total volume of 10 mL yields a solution that contains 30 µg of tirzepatide per mL at a concentration level of 100%.

Method Optimization

The method was optimized using central composite design (CCD). The preliminary trials are required to optimize the ultimate method. Total three factors or critical quality attributes (CQA) such as %organic mobile phase, flow rate and column temperature were needed to be optimized, which can directly affect the method's quality target product profile (QTPP). The QTPP parameters like RT, peak respons, tailing and USP plates were considered to confirm the quality of the methods. So CCD was used to optimize critical quality attributes which were varied over three level (high, mid and low) ranges from 36.59 to 53.41%, 0.01N potassium dihydrogen orthophosphate, column temperature 26.64 and 33.36°C and 0.6636–1.34 mL/min flow rate respectively were taken and counter and 3D surface plot showing the effect of each parameter on RT, plate count and tailing (CQA) were generated. A desirability function is applied to the optimized conditions to predict desirable QTPP parameters (Figure 3).

Method Validation

For the purpose of validating the existing technique, ICH Q2 (R1) standards were taken into consideration.[10]

System suitability test

This was determined by doing an analysis on a standard solution at a concentration of 100% in a series of six repeating samples and then performing an analysis on the acquired chromatograms to determine indicators such as %RSD, peak asymmetry, and plate count.

Linearity

A standard linear plot was generated by completing serial volumetric dilutions of the stock solution (200 µg/mL of Tirzepatide) for the 25 to 150 µg/mL range, including the LoQ level. This plot was then used to determine the relationship between the indicated concentrations and the respective responses. The linearity of the technique was validated by using the R^2 value that was derived from the linear plot.

Precision

Indicative of repetition and repeatability in general, it serves this purpose. The system accuracy of the technique and the method's precision were assessed by analyzing a standard and a sample in six replications, respectively. The %RSD for the produced peak areas and the %assay were assessed to verify the system's and the technique's correctness, respectively. The %RSD for the produced peak areas and the percent assay were assessed to verify the correctness of the system and the technique, respectively.

Accuracy

During the accuracy tests, the conventional approach of addition that is often used was implemented. Standard levels of 50, 100, and 150% were spiked in triplicate to differentiate across the several individual sample sets. Tirzepatide's %accuracy was computed for each level, and the mean % accuracy (n=9) as well as the %RSD emerged.

Specificity

The specificity of the technique is demonstrated when the tirzepatide is effectively identified making use of the described method in the incidence of other compounds but without any interference. During the process, it was necessary to add 0.1 mL of each solution for the standard, the blank, the sample, and the placebo that had been mixed with the standard. The chromatograms that were recorded were analyzed in order to find out whether or not there were interferences at the RT of tirzepatide caused by the solutions that were described before. The research was reinforced further by comparing the chromatograms of several different degradation solutions with

the chromatogram of the fresh Tirzepatide standard solution to detect the interferences from degradants toward Tirzepatide.

Sensitivity

Detection limit (LoD) and quantification limit (LoQ) were computed by make use of standard deviation procedure.

LoD = 3×σ/S

LoQ = 10 σ/S

σ -SD of the intercept

S- Slope of the linear plot

Robustness

Methods are deemed robust if their efficiency does not dramatically change when their parameters are modified little yet purposefully. It was ascertained by evaluating the influence of tiny changes in parameters include mobile phase (±10%), flow rate (± 10%) and pH (± 10%).

Forced degradation studies

These studies purposely applied higher degrees of acute stress than accelerated stability settings to the drug moiety. These investigations were useful in determining the stability of the dug ingredient, which is essential for developing a stable dosage form. According to the ICH's Q1A and QIB regulations, the FD investigations were finished.[11,12]

Acid degradation or hydrolysis

Equal portions of tirzepatide stock and 2N HCl solution were uniformly mixed. This was refluxed for 1-hrs with continuous agitation at 70ºC. NaOH neutralized the produced solution and diuted with suitable solvent to get 30 µg/mL of tirzepatide, which was examined by the HPLC system for 8 hours.

Base degradation or hydrolysis

Equal portions of tirzepatide stock and 2N NaOH solution were uniformly mixed. This was refluxed for 1 hrs with continuous agitation at 70ºC. HCl neutralized the produced solution and diuted with suitable solvent to get 30 µg/mL of tirzepatide, which was examined by the HPLC system for 8 hour.

Oxidative or peroxide degradation

Each 1-mL part of 20% H_2O_2 and tirzepatide stock solutions was mixed thoroughly and kept in dark control for 24 hr. A solution of 30 µg/mL of tirzepatide was got by diluting with a suitable solvent and examined by the HPLC system for 8 hours.

Thermal or Dry heat degradation

50 mg tirzepatide was placed in a watch glass and set aside in a hot air oven at 70ºC for 24 hours. A solution of 30 µg/mL of tirzepatide was got by diluting resultant drug with suitable solvent and examined by the HPLC system

Photo degradation

Total of 50 mg tirzepatide bulk powder was directly exposed to UV light (254 nm) for one day. The stated sample was diluted with suitable solvent to get a 30 µg/mL solution of tirzepatide.

Neutral degradation

In order to carry it out, we combined 10 mL of stock solution with 10 mL of water and sonicated the mixture for ten minutes. After waiting 24 hours, a solution of tirzepatide diluted to 30 µg/mL was created from the abovementioned solution. The percentage of tirzepatide that was degraded over the course of 24 hours in each of the degradation solutions was measured at regular intervals of 6 hours.

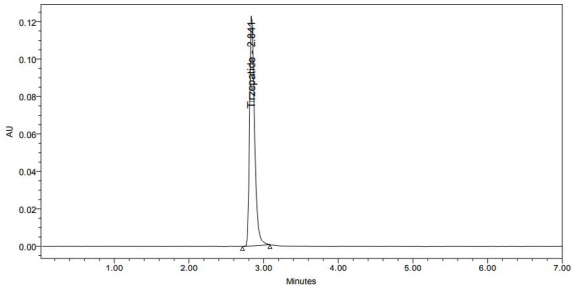
Assay of marketed dosage forms

Assay of the tirzepatide in marketed formulation (Scemblix-20 mg) was assessed by analyzing standard and sample solutions of tirzepatide one after another.

RESULTS AND DISCUSSION

Optimized Method

The process was optimized by doing several trials from the CCD where various solvents and ratios of organic solvent system, column temperature and flow rates were examined to optimize the parameters with satisfactory system suitability (Table 1). Lastly, a process with BDS C18 (150x4.6 mm, 5µ), mobile system of 0.01N KH_2PO_4: Acetonitrile in the 41:59 (%v/v) ratio, a flow rate of 0.9ml/min and column temperature of 31°C was optimized by CCD with quadratic model and surface response plots. The optimized wavelength selected was 250 nm. RT of Tirzepatide was observed to be 2.841 minutes with good system suitability (Figure 2). The mobile phase was used as diluents.



	Peak Name	RT	Area	USP Plate Count	USP Tailing	% Area	Purity1 Angle	Purity1 Threshold
1	Tirzepatide	2.841	575086	8829	1.4	100.00	0.298	0.351

Figure 2: Chromatogram of optimized method

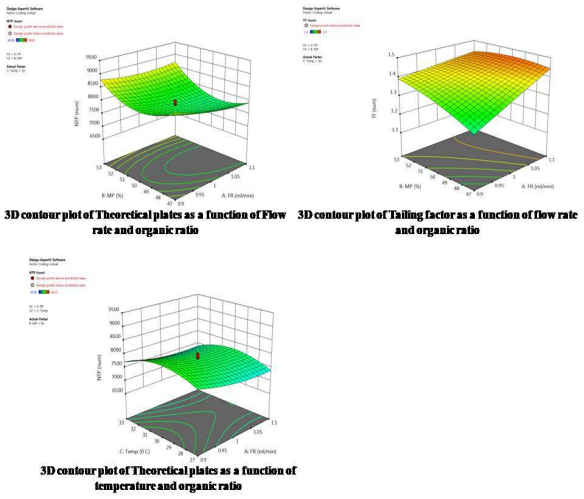


Figure 3: 3D surface plots for theoretical plates and tailing factor

Table 1: CCD experimental design with three independent variables with responses

	Factor			*Response*		
	1	*2*	*3*	*1*	*2*	*3*
Run	*Flow rate (A)*	*Organic phase (B)*	*Temperature (C)*	*RT*	*Tailing*	*Plates*
	ml/min	%	0 C	min	number	number
1	0.9	47	27	3.016	1.1	8288
2	1.1	47	27	2.495	1.4	7842
3	0.9	53	27	2.98	1.3	8257
4	1.1	53	27	2.449	1.5	7847
5	0.9	47	33	3.006	1.2	7785
6	1.1	47	33	2.48	1.3	6935
7	0.9	53	33	2.952	1.3	8887
8	1.1	53	33	2.435	1.2	9085
9	0.83	50	30	3.247	1.3	8512
10	1.16	50	30	2.324	1.5	7897
11	1	44.95	30	2.666	1.3	9354
12	1	55.04	30	2.667	1.4	9433
13	1	50	24.9	2.698	1.2	7031
14	1	50	35.04	2.702	1.1	6576
15	1	50	30	2.685	1.4	7874
16	1	50	30	2.691	1.4	7607
17	1	50	30	2.691	1.4	7884
18	1	50	30	2.692	1.4	7802

Method Validation

The results that are reported in Table 2 were assurance that the technique followed the ICH principles and was suitable for the system. The tailing, plate count, and %RSD values were determined to be correspondingly $\leq 2, \geq 2000, \leq 2$, respectively.

The R^2 value of the tirzepatide was determined to be 0.999 for the given concentration range stand solutions of Tirzepatide **(**Figure 4**)** which confirmed that the QbD RP-HPLC method has significant linearity for stated concentrations. The observed mean % of tirzepatide recovery over a range of spiked solutions (50, to 150%) was found to be 100% ± 2 limits (Table 3). This eloquently demonstrates both the linearity and precision of the newly developed approach. The %RSD of repeated samplings of a solution containing 100% tirzepatide was calculated to be less than two (Table 4); this was the case for both precisions. The ascertained results substantially confirm the precision of the method. The obtained %RSD values ensured that the method's performance was unaffected by the deliberate and small changes to the method's parameters (Table 5). As a result, the approach is very robust as of ICH. The lack of interference from placebo, degradants, and blanks during the RT of tirzepatide verified the method's exclusivity for tirzepatide (Figure 5). The LoD and LoQ were assessed to be 0.05 and 0.14 µg/mL, respectively.

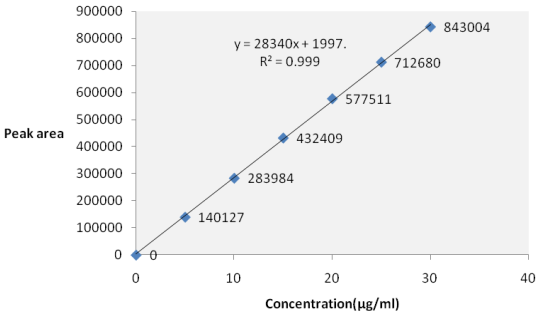


Figure 4: Linearity results of Tirzepatide solution

Table 2: Results of system suitability test of Tirzepatide at 100% level

S. No.	*Peak response*	*Plate count*	*Tailing factor*
1	572538	8820	1.41
2	570044	8829	1.45
3	574275	8622	1.44
4	579185	8714	1.45
5	571435	8923	1.36
6	574019	8624	1.47
Mean	573583		
SD	3171.2		
%RSD	0.6		

Table 3: %Recovery outcomes of tirzepatide

% Level	*Spiked (Added) concentration in µg/mL*	*Recovered concentration in µg/mL*	*%Recovery*	*Average %Recovery*
	10	9.93	99.33	
50	10	9.91	99.07	
	10	9.91	99.06	
	20	20.18	100.92	
100	20	20.03	100.16	99.89%
	20	20.11	100.55	
	30	30.24	100.79	
150	30	30.00	99.99	
	30	29.73	99.11	

Table 4: Precision results of tirzepatide

	Precision	
Parameter	*System*	*Method*
Peak area (n=6)	576880	100.27
SD	2418.5	0.420
RSD (%)	0.4	0.41
Acceptance criteria RSD (%)	≤ 2	

Table 5: Results of robustness of tirzepatide

Parameter	*Flow rate (0.9 mL/min)*		*Mobile phase (Buffer: Acetonitrile 41:59 v/v)*		*Temperature 32℃*	
	Plus	*Minus*	*Plus*	*Minus*	*Plus*	*Minus*
Mean peak area (n=3)	574311	573605	575371	574868	577638	572113
SD	2731.4	2194.8	3763.3	4485.9	1451.9	1714.9
%RSD	0.5	0.4	0.7	0.8	0.3	0.3

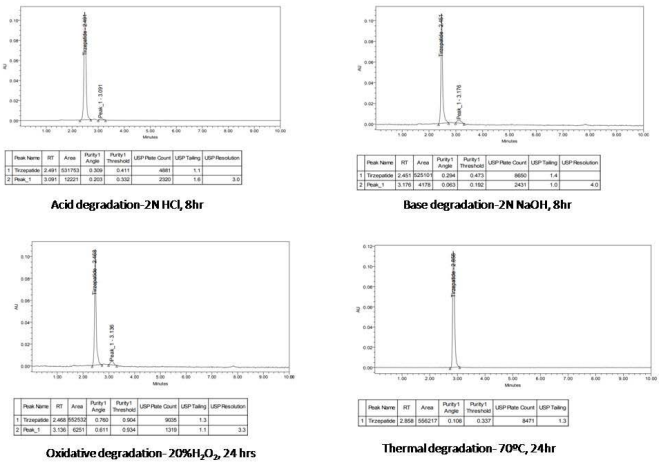


Figure 5: %Degradation of Tirzepatide in stressed standard solution

Table 6: %Degradation of tirzepatide in various forced degradation conditions

S.No.	*Condition of degradation*	*%Tirzepatide degraded*
1.	2N HCl, 8 hours	7.57
2.	2N NaOH, 8 hours	8.73
3.	Oxidative degradation-20% H_2O_2,24 hours	0.74
4.	Thermal degradation, 70ºC, 24 hours	3.32
5.	Photodegradation- 254nm, 24 hours	1.68
6.	Neutral hydrolysis – Water, 24 hours	0.73

Table 7: %Assay of the marketed tablets tirzepatide

Name	*Peak*	*Peak response (n=6)*	*Assay(%) ± SD*	*%RSD*	*Acceptance range*
Tirze-patide	Standard	2439168	100.6% ± 0.42	0.41	(100 ± 2%)
	Test	2441224			

Up to 20% drug degradation is thought to be more important in most stability-indicating procedures.[12,13] By contrasting the peak regions obtained from freshly processed and under-stress standard solutions, the percentage of tirzepatide degradation was calculated. Figure 5 showed the chromatograms consist of purity angle and threshold results of Tirzepatide under various stressful situations. The purity of the tirzepatide peak was guaranteed relative to the detected degradants peaks since the obtained peaks' purity threshold values were higher than the purity angle. The measured percent degradation of tirzepatide in FD experiments clearly demonstrates the technique's stability representativeness (Table 6). According to the data, Tirzepatide is very sensitive to acidic and basic conditions. The assay of the marketed formulation was determined to be 100.6 ± 0.42 (Table 7).

In general, stability representing LC procedure is essential for the evaluation of drug moiety qualitatively and quantitatively.[12,13] No single liquid chromatographic procedure has been accessible solely for the tirzepatide in bulk and formulation. For this rationale, research was moved frontward to build up a new stability demonstrating RP-HPLC

method for analysis of tirzepatide. In the established method, a mobile phase composition of 0.01N KH_2PO_4: Acetonitrile in the 41:59 (%v/v) used with isocratic elution mode and the RT of tirzepatide was noticed at 2.84 minutes. Due to simple solvent systems and faster elution times, these were demonstrating the methods' inexpensive nature and quicker analysis times. The assessment of degradants of tirzepatide considerably ensures the method's stability representing the character. With the current technique, a speedy assessment of more samples should be completed. The advancement of the method includes good sensitivity, accuracy, and considerable specificity to tirzepatide, according to the static data of the verified parameters.

CONCLUSION

The assessment of tirzepatide in pure powder and tablet form, a cost-efficient, perceptive, exact, trouble-free reverse phase liquid chromatographic process with better responsiveness, was proposed by the help of QbD studies. The stability representativeness of the approach is confirmed by research on tirzepatide under various stressful conditions. The proposed method successfully and with superior resolution separated the drug tirzepatide from any conceivable byproducts it may make when under stress. The predicted technology will be highly regarded in the pharmaceutical industry because it has a shorter retention period for tirzepatide.

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REFERENCES

1. Chavda VP, Ajabiya J, Teli D, Bojarska J, Apostolopoulos V. Tirzepatide, a new era of dual-targeted treatment for diabetes and obesity: a mini-review. Molecules. 2022 Jul 5;27(13):4315.
2. Nauck MA, D'Alessio DA. Tirzepatide, a dual GIP/GLP-1 receptor co-agonist for the treatment of type 2 diabetes with unmatched effectiveness regrading glycaemic control and body weight reduction. Cardiovascular diabetology. 2022 Dec;21(1):1-6.
3. Chavda VP, Ajabiya J, Teli D, Bojarska J, Apostolopoulos V. Tirzepatide, a new era of dual-targeted treatment for diabetes and obesity: a mini-review. Molecules. 2022 Jul 5;27(13):4315.
4. Chavda VP, Ajabiya J, Teli D, Bojarska J, Apostolopoulos V. Tirzepatide, a new era of dual-targeted treatment for diabetes and obesity: a mini-review. Molecules. 2022 Jul 5;27(13):4315.
5. Jastreboff AM, Aronne LJ, Ahmad NN, Wharton S, Connery L, Alves B, Kiyosue A, Zhang S, Liu B, Bunck MC, Stefanski A. Tirzepatide Once Weekly for the Treatment of Obesity. New England Journal of Medicine. 2022 Jun 4.
6. PubChem. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; 2004-. bChem Compound Summary for CID 156588324, Tirzepatide; [cited 2022 Dec. 4]
7. Erni F. Use of high-performance liquid chromatography in the pharmaceutical industry. Journal of Chromatography A. 1990 May 16;507:141-9.
8. Nikolin B, Imamović B, Medanhodzić-Vuk S, Sober M. High perfomance liquid chromatography in pharmaceutical analyses. Bosn J Basic Med Sci. 2004 May;4(2):5-9
9. Ngwa G. Forced degradation as an integral part of HPLC stability-indicating method development. Drug delivery technology. 2010;10(5):56-9.
10. Godela R, Gummadi S. A simple stability indicating RP-HPLC-DAD method for concurrent analysis of Tenofovir Disoproxil Fumarate, Doravirine and Lamivudine in pure blend and their combined film coated tablets. InAnnales Pharmaceutiques Françaises 2021; 79, (6):640-651.
11. Blessy M, Patel RD, Prajapati PN, Agrawal YK. Development of forced degradation and stability indicating studies of drugs—A review. J Pharm Anal 2014;4:159–65.
12. Mohan Goud V, Rao AS, Ranjith kumar S. Method development and validation of RP-HPLC for simultaneous determination of Lamivudine and Tenofovir in tablet dosage form. International journal of pharmacy and pharmaceutical sciences 2013; 5 (suppl. 3): 215-218.
13. Hotha KK, Reddy SPK, Raju VK, Ravindranath LK. Forced Degradation Studies: Practical Approach - Overview Of Regulatory Guidance And Literature For The Drug Products And Drug Substances. Int Res J Pharm 2013;4:78-85.
14. Bakshi M., Singh S. Development of validated stability-indicating assay methods—critical review. Journal of Pharmaceutical and Biomedical Analysis. 2002;28(6):1011–1040.
15. Reid GL, Morgado J, Barnett K, Harrington B, Wang J, Harwood J, Fortin D. Analytical quality by design (AQbD) in pharmaceutical development. Am. Pharm. Rev. 2013 Aug;144191.
16. Hanna Brown M, Barnette K, Harrington B. Using quality by design to develop robust chromatographic methods. Pharm Tech. 2014 Sep 2;38(8):48-64.