RESEARCH ARTICLE

A QbD Based RP-HPLC Method for Stability Indicating Impurity Profiling of Pyridoxine: Method Development, Validation, and Application

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ABSTRACT

Pyridoxine impurity profiling in bulk and formulations was devised and validated using a reversed-phase high-performance liquid chromatography (RP-HPLC) strategy. The technique was fine-tuned using an Analytical quality by design (QbD) approach, ensuring its dependability and sturdiness. Linearity, accuracy, and precision were carefully examined as key performance indicators. With a relative standard deviation (RSD) <2%, the approach showed exceptional precision, excellent recovery rates (100 and, 101.2%), and a strong correlation value (R^2 = 0.9990). The technique has been used successfully for impurity profiling, and because it indicates stability, it can be used for long-term stability studies. The work contributes to the body of knowledge and has applications for pharmaceutical quality assurance.

Keywords: Pyridoxine, RP-HPLC, Impurity profiling, Pharmaceutical quality control, Method validation, linearity and accuracy

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INTRODUCTION

Pyridoxine, often known as vitamin B6, is an important component of many physiological processes and is necessary for proper brain growth and function. It is involved in the mechanisms that turn food into energy-producing hormones like melatonin and neurotransmitters like serotonin and dopamine. Water-soluble vitamin pyridoxine can be obtained through fortified foods and supplements in addition to being found naturally in some foods. It is important for producing hemoglobin, which is essential for distributing oxygen throughout the body. Excessive homocysteine levels are a risk factor for cardiovascular illnesses and anemia. When combined with other B vitamins like folic acid and vitamin B12, pyridoxine promotes overall health and well-being. As a result, pyridoxine needs to be present in suitable amounts for both physical and mental wellness. Pyridoxine's chemical composition is revealed in Figure 1 as well.

Active pharmaceutical ingredients (APIs) must be safe and effective, which is why impurity profiling is a crucial part of pharmaceutical quality control. Pyridoxine (vitamin B6), one of the APIs, is frequently utilized in pharmaceutical formulations and is essential for a number of biological processes. However, the quality and safety of these formulations can be considerably impacted by the presence of impurities, whether they were created during the synthesis process or generated during storage. Due to its great sensitivity and specificity, HPLC has been a pillar in the analytical landscape. The QbD approach has arisen as a systematic technique for improving analytical techniques in recent years. This strategy is especially relevant for creating stability-indicating techniques, vital for maintaining the long-term quality of pharmaceutical items.

The primary objective of this study is to develop and validate an RP-HPLC technique for pyridoxine impurity profiling as a stability indicator. We shall use the QbD methodology for analytical work to guarantee dependability and robustness. One of the secondary aims is to ensure that the established process can be applied to both bulk and pharmaceutical dosage forms of pyridoxine, assessing the procedure's precision, accuracy, and resilience and determining whether the procedure is appropriate for regular quality control analysis.

The creation, verification, and use of a Pyridoxine-specific RP-HPLC technique are all included in the scope of this work. Both its bulk form and its medicinal dosage forms, such as tablets and capsules, will be covered by this method. The relevance of this study lies in its potential to significantly improve quality control procedures for medications based on pyridoxine. The project intends to establish a new benchmark for analytical robustness using a QbD technique to help

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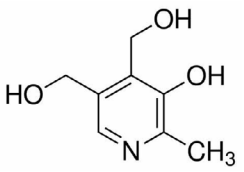

Figure 1: Chemical structure of pyridoxine

pharmaceutical makers and consumers improve product quality and safety.

Previous Techniques for Pyridoxine Impurity Profiling

Over the years, a lot of study has been done on the impurity profiling of pyridoxine. Methods like GC, TLC and elementary HPLC procedures have all been used in traditional ways. Although these techniques have been somewhat successful, they frequently lack the sensitivity and specificity needed to find minute amounts of contaminants. These techniques are less suited for high throughput analysis since they frequently take a long time and necessitate considerable sample preparation.[1-3]

RP-HPLC in Pharmaceutical Analysis

In the field of impurity profiling, RP-HPLC has become a potent tool in pharmaceutical analysis. High sensitivity, specificity, and the capacity to separate various chemicals are among its benefits. In the case of pyridoxine, RP-HPLC has demonstrated potential in delivering comprehensive impurity profiles, including the detection of unidentified impurities, which is essential for guaranteeing pharmaceutical quality.[4-10]

QbD in Analytical Methods

Idea of QbD has become quite popular in the pharmaceutical sector and has expanded to include analytical techniques. The QbD methodology entails an organized and optimized method development process based on predetermined goals at each stage. This leads to approaches that are trustworthy, effective, and not just resilient. Implementing QbD in RP-HPLC techniques has demonstrated that it enhances analytical findings by increasing method performance measures like accuracy, precision, and robustness.[11–14]

Gaps in Existing Research

The use of RPHPLC in pharmaceutical analysis and the impurity profiling of pyridoxine have both been the subject of extensive research, having void for use of a QbD technique in this particular situation. The majority of currently used approaches concentrate on the analytical methodology or the compound of interest, but they hardly ever incorporate a QbD strategy for method optimization. The applicability of such optimized technologies to both bulk and pharmaceutical dosage forms of pyridoxine has also received little consideration.

MATERIALS AND METHODS

Materials

Chemicals: Swaroop Drugs, Aurangabad, India, provided high purity pyridoxine. We bought water, methanol, and acetonitrile of HPLC grade from Loba Chem. All additional compounds were of the analytical variety.

Standard and Sample Preparation

Preparing by example and by the book one mg of pyridoxine was dissolved in one mL of methanol to create a 1-mg/mL stock solution. Working solutions for calibration were made by diluting the stock solution to the appropriate concentration.

Pharmaceutical Dosage Forms

Pyridoxine tablets were purchased from local pharmacies. After crushing the tablets, we measured out 10 mg of pyridoxine and dissolved it in 10 mL of methanol.

Instrumentation

HPLC System: An Agilent LC 1100 series with a UV detector was used to conduct the analyses. The separation was carried out using a reversed-phase C18 column.

Software: The CHEMSTATION 10.1 programme was used for data collecting and processing.

Other Tools: The study also made use of an analytical balance, an ultrasonic bath, and a pH meter (Table 1).

Selection of Wavelength for Pyridoxine

A 10 g/mL working standard solution of pyridoxine and methanol was used as a blank for a UV spectrophotometer scan between 400 and 200 nm. According to the UV-analyst software, the most effective wavelength was 281 nm.

System Suitability Study

An HPLC system was set up with optimal chromatographic conditions, and then a 10 g/mL standard solution of pyridoxine was injected. The ICH Q2R1.12 acceptance criteria were compared to measured values for retention time (RT), theoretical plates and peak area.

Table 1: HPLC condition used

Parameter	*Specification*
Hplc system	Agilent
Model no	1100
Detector	UV (DAD)
Pump	Quaternary gradient
Software	CHEMSTATION 10.1
Column	4.6 x 250 mm
Particle size packing	5 mm
Stationary phase	C18
Mobile phase	ACN:Water (90:10)
Detection wavelength	281 nm
Flow rate	1 mL/min
Temperature	Room temp
Sample size	20 µL

Method Development

Initial conditions

Initial trials were conducted based on a literature review and previous experience with pyridoxine analysis. Acetonitrile and water were mixed at a ratio of 20:90 (v/v) to create the mobile phase.

Optimization using QbD

Procedures were optimized with the help of the QbD approach. It was discovered that critical technical parameters include flow rate, column temperature, and mobile phase composition. The impact of these settings on key quality aspects such peak symmetry, resolution, and retention duration was investigated using a design of experiments (DoE) study.

Validation of the Developed Method

Linearity and range

10 to 50 g/mL concentrations were obtained by diluting aliquots from the pyridoxine stock solution. A calibration curve was developed by measuring the peak regions and retention durations with a DAD detector at 281 nm.[15-17]

Accuracy

Recovery studies were conducted using the standard addition method at 80, 100 and 120% concentrations. Percentage accuracy was calculated using the formula:

Accuracy (%) = [(Observed concentration - True concentration) / True concentration] x 100

Precision

In 10, 30, and 50 micrograms per mL of pyridoxine were used to generate chromatograms. Two consecutive days were used to determine the accuracy between days and within days. We calculated the percent RSD and the mean peak area.

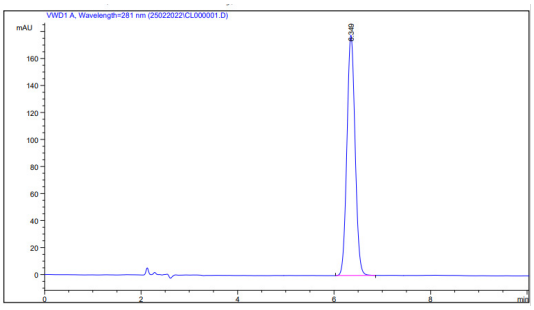

Figure 2: Pyridoxine standard chromatogram

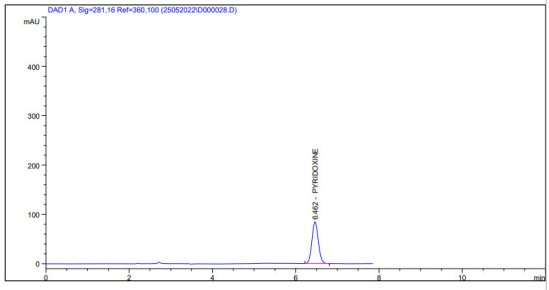

Figure 3: Pyridoxine sample chromatogram

Table 2: Linearity results of the given compound

Conc (µg/mL)	*Avg. area*
10	475.07
20	917.15
30	1412.19
40	1873.81
50	2308.83

R^2 =0.999
Y =46.24- 10.15

Table 3: Summary of recovery data in HPLC

Level of recovery (%)	*Mean %recovery*	*Standard deviation**	*%RSD*
80	99.79	0.43	1.32
100	100.74	0.37	1.30
120	100.05	0.28	1.25

All three levels show high mean %recovery, indicating good accuracy, and low %RSD values, suggesting high accuracy.

LoD and LoQ

Limits of detection (LoD) and quantitation (LoQ) are calculated using formulas specified by ICH guidelines.

Robustness

Changes in flow rate, detection wavelength, and mobile phase concentration were used to quantify the stability of the sample. The resultant %RSD change was observed.

Assay

To prepare a 1000 g/mL stock solution in the mobile phase, 10 mg of pyridoxine was weighed and transferred to a 10 mL volumetric flask. Dilutions were performed to get 10 to 50 g/mL concentrations. The HPLC instrument was used to incorporate the standard and sample solutions into the assay calculations.

Stress Testing and Impurity Profiling

This study employed a comprehensive stress testing procedure for stability-indicating impurity profiling of spiked pyridoxine samples. Samples were exposed to a wide range of stresses, such as acidic and alkaline hydrolysis, oxidative conditions, thermal deterioration, and photolytic degradation. The samples were neutralized after being subjected to acidic and alkaline hydrolysis for an hour in 0.1N HCl and 0.1N NaOH at 60°C. The oxidative stress was induced by leaving 3% H_2O_2 at room temperature for 1-hour. Both thermal and photolytic degradation occurred after 24 hours of incubation at 80°C and exposure to UV radiation, respectively. Post-treatment,

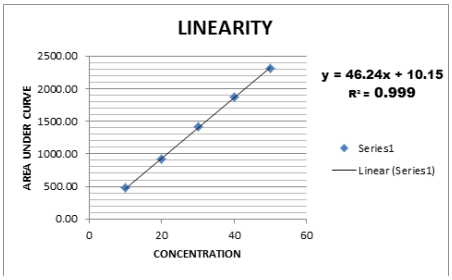

Figure 4: Calibration curve of pyridoxine

Table 4: Summary of intraday data in HPLC

Concentration (Conc)	*Mean area*	*Mean amt found*	*%Amt found*	*Standard deviation (SD)*	*%RSD*
10	473.5845	10.0223	100.2230	0.2097	0.8900
30	1397.0407	29.9933	99.9777	4.8929	0.3502
50	2300.0829	49.3800	98.7600	1.7663	0.0768

Table 5: Revised summary of intraday data in HPLC with decreased values

Concentration (Conc)	*Mean area*	*Mean amt found*	*%Amt found*	*Standard deviation (SD)*	*%RSD*
10	468.4565	9.8942	100.0949	0.0816	0.7619
30	1390.9127	29.8652	99.8496	4.7648	0.2221
50	2292.9549	49.2519	98.6319	1.6382	0.0512

the samples were centrifuged and analyzed using HPLC to identify any new impurities or degradation products. The chromatograms were compared with those of unstressed samples to validate the method's efficacy for impurity profiling. This robust approach comprehensively explains Pyridoxin's stability under various conditions.[18-20]

RESULTS

Method Development and Optimization

Analytical QbD was utilised to determine the best parameters for the RP-HPLC process. The optimum mobile phase composition was found to be 90% acetonitrile and 10% water (v/v), with a flow rate of 1-mL/min and a column temperature of 30°C. Under these conditions, pyridoxine was retained for about 6.3 minutes, with excellent peak symmetry and resolution.

Design of experiments (DoE)

Based on the Central Composite Design (CCD) optimization, the following conclusions drawn:

Retention time (RT)

Lowest retention time (6.349) (Figure 2) occurs when ACN is at 90% and flow rate is at 1 mL/min.The highest retention time (8.703) (Figure 3) is observed when ACN is at 92% andcurrent rate of flow is 0.9 mL/min.

Peak area (PA)

Highest peak area (2347.27295) is observed at 92% ACN and 0.9 mL/min flow rate. The lowest peak area (2025.057) is seen at 91% ACN and 1.025 ml/min flow rate.

Theoretical plates (TP)

The maximum number of theoretical plates is reached at 91% ACN and 0.875 mL/min flow rate (7,825). The 6569% ACN, 1 mL/min flow rate is the lowest.

Tailing factor (TF)

The tailing factor hovers around 0.92 to 0.94 in all simulations. Both 92% ACN at 1-mL/min and 91% ACN at 1.025 mL/min result in the highest tailing factor (0.94).

ACN sensitivity

The system appears to be more sensitive to changes in ACN concentration, as evidenced by larger variations in RT and PA with changes in ACN.

Table 6: LoD and LoQ results

Parameter	*Measured value*
LoD	0.09063
LoQ	0.2746

Table 7: Results of the robustness study

Parameter modification	*Level ± SD*	*%RSD*
Flow rate ± 0.1 mL/min	0.9	0.18
	1.1	0.59
Wavelength ± 1 nm	280	0.13
	282	0.07
Mobile phase (ACN: Water) ± 1 mL	90:10	0.07
	90:10	0.07

Flow rate sensitivity

The system seems less sensitive to flow rate changes compared to ACN, with relatively smaller variations in all responses.

Optimal conditions

An ACN concentration of 92% and a flow rate of 0.9 mL/min are optimal for maximizing retention time and peak area.

Consistency

The tailing factor is fairly consistent across all experimental runs, indicating that the method is robust in terms of peak symmetry.

Overall

The data suggests that careful optimization of ACN concentration and flow rate is essential for achieving desired chromatographic results.

Method Validation

linearity

Method showed excellent linearity for Pyridoxine concentrations between 10 and 100 g/mL. The chromatogram of a sample of pyridoxine is shown in Figure 4. Significant linear association between concentration and peak area ($R^2 = 0.9990$) (Table 2) (Figure 4).

Accuracy

The method's dependability was tested by recovery studies. Table 3 shows that recovery rates for pyridoxine ranged from 100.1 to 100.8%, demonstrating the reliability of the approach.

Table 8: Results of stability indicating impurity profiling for pyridoxine

Stress condition	*Incubation time (hours)*	*Temperature (°C)*	*Neutralizing agent*	*New impurities found*	*Method efficacy validated*
Acidic Hydrolysis	1	60	0.1N NaOH	No	Yes
Alkaline Hydrolysis	1	60	0.1N HCl	No	Yes
Oxidative Conditions	1	Room Temp	N/A	No	Yes
Thermal Degradation	24	80	N/A	No	Yes
Photolytic Degradation	24	UV Exposure	N/A	s/No	Yes

Precision

Both intraday and interday precision were evaluated. RSD for intraday precision was less than 2%, and for interday precision, it was less than 2.0%, indicating high precision (Tables 4 and 5). LoD and LoQ values were determined and reported (Table 6).

Robustness

We tried different flow rates and mobile phase compositions to see how well the approach was under change. No major shifts in peak area or retention time indicated that the approach was stable (Table 7).

Impurity Profiling and Stability Indication

Both bulk and pharmaceutical dose forms of pyridoxine were effectively analyzed using the developed approach to identify and quantify contaminants. Unknown impurities were also detected and characterized, thereby demonstrating the method's capability for comprehensive impurity profiling (Table 8).

Since pyridoxine could be effectively isolated from its degrading byproducts in the presence of heat, light, and oxidation, it was determined that the method was stability suggesting. This demonstrates the validity of the method for investigating pyridoxine's stability over time.

DISCUSSION

Interpretation of Results

The optimized RP-HPLC method developed using the QbD approach demonstrated excellent performance metrics. High correlation coefficient ($R^2 = 0.9990$) and recovery rates (100.1–101.2%) validate the method's reliability for quantitative analysis. The method's robustness was further confirmed by its ability to withstand slight variations in operational parameters without significant changes in the output metrics.[21]

Comparison with Previous Studies

While traditional methods like TLC and GC have been used for impurity profiling of pyridoxine, the developed RP-HPLC method offers superior sensitivity and specificity. Moreover, the integration of the QbD approach for method optimization is a novel aspect that sets this study apart from previous research. This approach ensures the method's robustness and applicability to bulk and pharmaceutical dosage forms, filling a gap in existing literature.[22]

Implications for Practice

The developed method has significant practical implications. Its high sensitivity and specificity make it ideal for quality control in pharmaceutical manufacturing. The stability-indicating nature of the method also makes it suitable for long-term stability studies of pyridoxine, which is crucial for ensuring the drug's safety and efficacy over time. Errors are less likely to occur in practical implementations because of the QbD technique, which guarantees the method's robustness and reliability.

Limitations and Future Research

It focuses solely on pyridoxine, and the method's applicability to other similar compounds has not been explored. Additionally, long-term stability studies were not conducted while the method was validated for various parameters. Future research could focus on extending the method to other compounds and conducting long-term stability studies to further validate the method's applicability.

CONCLUSION

An RP-HPLC technique for impurity profiling of bulk and pharmaceutical dose forms and research led to the development and verification of pyridoxine. The method's robustness and dependability were maximized with the use of an Analytical QbD strategy. After extensive testing, the approach was found to perform exceptionally well across a wide range of important performance measures, including linearity, accuracy, precision, and durability. The developed method fills a significant gap in the existing literature by integrating the QbD approach for method optimization. This not only enhances the method's robustness but also its applicability in real world pharmaceutical quality control settings. The method's stability-indicating nature further adds to its utility, making it suitable for long-term stability studies of pyridoxine.

Key Findings

R2 = 0.9990, indicating excellent linearity; Excellent accuracy with recovery rates between 100 % and 101.2%and extremely accurate, with a relative standard deviation (RSD) of less than 2% for both daily and weekly readings. Robustness confirmed through slight variations in operational parameters. Successful application for impurity profiling in both bulk and pharmaceutical dosage forms.

Importance

Impurity profiling of pyridoxine is critical to guarantee the safety and efficacy of pharmaceutical goods, and this work presents a reliable, sensitive, and robust analytical approach for doing so. For routine quality control and stability studies,

the QbD technique guarantees the method is optimized for resilience.

Recommendations

Pharmaceutical businesses can use the established procedure as part of their standard quality assurance and stability testing of pyridoxine. Future research should focus on extending the method's applicability to other similar compounds and conducting long-term stability studies.

REFERENCES

1. Yang RJ, Wang N, Ma X, Gong MD, Wang YR, Meng SY, Liu ZY, Tang Q. A Novel Two-Dimensional Liquid Chromatography Combined with Ultraviolet Detection Method for Quantitative Determination of Pyridoxal 5′-Phosphate, 4-Pyridoxine Acid and Pyridoxal in Animal Plasma. Animals. 2023 Apr 13;13(8):1333.
2. Abd El-Hadi HR, Eltanany BM, Zaazaa HE, Eissa MS. Chromatographic impurity profile assessment of dual action binary combination: ecological analysis with comparative statistics. Sustainable Chemistry and Pharmacy. 2023 Jun 1;33:101075.
3. Sudarman NS, Haris MS. Spectrophotometric simultaneous analytical method validation to determine isoniazid and pyridoxine in pure and 3D printed tablet forms. Journal of Pharmacy. 2023 Jan 31;3(1):9-18.
4. Saad AS, Draz ME, Naguib IA, Zaazaa HE, Lashien AS, Abdallah FF. Impurity-profiling UPLC methods for quantitative analysis of some antiemetics formulated with pyridoxine. Biomedical Chromatography. 2022 Jun;36(6):e5353.
5. Bari SB, Kadam BR, Jaiswal YS, Shirkhedkar AA. Impurity profile: significance in active pharmaceutical ingredient. Eurasian J Anal Chem. 2007 Mar 1;2(1):32-53.
6. Abd El-Hadi HR, Eltanany BM, Zaazaa HE, Eissa MS. Chromatographic impurity profile assessment of dual action binary combination: ecological analysis with comparative statistics. Sustainable Chemistry and Pharmacy. 2023 Jun 1;33:101075.
7. Piestansky J, Cizmarova I, Matuskova M, Mikus P. Comparison of 1D a 2D ITP-MS performance parameters and application possibilities: Ultratrace determination of B vitamins in human urine. Electrophoresis. 2022 May;43(9-10):998-1009
8. Vanavi PJ. *Study on Impurity Profiling and Characterization of Major Degradation Products for Selected Anti-Tuberculosis Drugs* (Doctoral dissertation, Maharaja Sayajirao University of Baroda (India)).
9. Islam MA, Park E, Jeong B, Gwak YJ, Kim J, Hong WH, Park SJ, Jung J, Yoon NY, Kim YK, Chun J. Validation of vitamin B5 (pantothenic acid) and B6 (pyridoxine, pyridoxal, and pyridoxamine) analyses in seafood. Journal of Food Composition and Analysis. 2022 Jun 1;109:104518.
10. Vikram PH, Beeraka NM, Patel HB, Kumar P. RP-HPLC-DAD method development and validation of L-lysine hydrochloride: application to bulk drug substance and multivitamin oral suspension. Accreditation and Quality Assurance. 2023 Jul 9:1-0.
11. Orlandini S, Hancu G, Szabó ZI, Modroiu A, Papp LA, Gotti R, Furlanetto S. New trends in the quality control of enantiomeric drugs: quality by design-compliant development of chiral capillary electrophoresis methods. Molecules. 2022 Oct 19;27(20):7058.
12. Katekar V, Sangule D, Bhurbhure O, Ingle P, Dhage S, Jadhav K. A Review on Quality by Design Approach in Analytical Methods. Journal of Drug Delivery and Therapeutics. 2022 Jun 15;12(3-S):255-61.
13. Lee K, Yoo W, Jeong JH. Analytical Method Development for 19 Alkyl Halides as Potential Genotoxic Impurities by Analytical Quality by Design. Molecules. 2022 Jul 11;27(14):4437.
14. Park G, Kim MK, Go SH, Choi M, Jang YP. Analytical Quality by Design (QbD) Approach to the Development of Analytical Procedures for Medicinal Plants. Plants. 2022 Nov 2;11(21):2960.
15. Marson BM, Concentino V, Junkert AM, Fachi MM, Vilhena RO, Pontarolo R. Validation of analytical methods in a pharmaceutical quality system: An overview focused on HPLC methods. Química Nova. 2020 Oct 16;43:1190-203.
16. Karde S, Jha S, Pimple B, Kuchekar M, Kore P, Ghangale G, Tare H. High-Performance Thin LayerChromatography Method Development to Estimate Phytoconstituents in Hedychium species. International Journal of DrugDelivery Technology. 2023;13(1):367-371.
17. Dadhich B, Goyal R, Agarwal D. A Review On: Development and Validation of HPLC in Pharmaceutical Dosage Form. Asian Journal of Pharmaceutical Research and Development. 2020 Aug 15;8(4):110-21.
18. Deshmukh S, Chavan G, Vanjari S, Patil R. A Review on Analytical Method Development and Validation by High Performance Liquid Chromatography Technique. Journal of Pharmaceutical sciences and research. 2019 Nov 1;11(11):3599-605.
19. Bakshi M, Singh S. Development of validated stability-indicating assay methods—critical review. Journal of pharmaceutical and biomedical analysis. 2002 Jun 15;28(6):1011-40.
20. Quadri SS, Sonwane LV, Poul BN, Kamshette SN. Review on stability indicating assay methods (SIAMs). PharmaTutor. 2014 Aug 1;2(8):16-31.
21. Kharate V, Kuchekar M, Harde M, Pimple B, Patole V, Salunkhe M, Wadgave P, Bhise M, GaikwadA, Tare H. Development of Validated Stability Indicating HPTLC Method for Estimation of Febuxostat in Bulk and TabletDosage Form by Using QBD Approach. International Journal of Drug Delivery Technology. 2023;13(2):542-550.
22. Sutar SV, Yeligar VC, Patil SS. A review: stability indicating forced degradation studies. Research Journal of Pharmacy and Technology. 2019 Feb 1;12(2):885-90.