RESEARCH ARTICLE

# A New Stability Indicating HPLC Method for Related Substances in Dapagliflozin

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***Received: 08th January, 2024; Revised: 09th February, 2024; Accepted: 24th February, 2024; Available Online: 25th March, 2024***

**ABSTRACT**

The requirements established by the International Council for Harmonization (ICH) for the measurement of dapagliflozin from tablets were satisfied by the development and successful implementation of an reversed-phase high performance liquid chromatography (RP-HPLC) technique that is characterized by its speed, precision, and accuracy. In order to separate the samples, an isocratic mode was used on a Princeton C18 column that was operating at a flow rate of 1-mL per minute. The phase was composed of acetonitrile and 0.1% triethylamine in a volume-to-volume ratio of 50:50, with a pH of 5.0 and a detection wavelength of 224 nm. 5.163 minutes was the retention time that was determined to be best under the circumstances of the chromatographic analysis. Based on the correlation value of 0.999, it can be concluded that the procedure adhered to Beer-Lambert's law within the measurement range of 10 to 70 µg/mL. The findings not only verified the claim that was made on the label, but they also validated the mean percent drug concentration that was specified for the commercial tablet formulation, which was 100.57%. Following the completion of an investigation, it was discovered that the approaches that were proposed have sufficient proportions of precision measurements, robustness, accuracy, range, and linearity. As part of the technique for stress testing, a number of criteria were evaluated and evaluated overall. The elements that were considered to be among these were light, humidity, temperature, oxidation, and pH (acid/base).

**Keywords:** Development, Validation, Stability-indicating, Evaluation services are provided for RP-HPLC, Dapagliflozin.

International Journal of Drug Delivery Technology (2024); DOI: 10.25258/ijddt.14.1.53

**How to cite this article:** Chavan B, Birajdar A, Bhusnar H, Yadav J, Badadhe S. A New Stability Indicating HPLC Method for Related Substances in Dapagliflozin. International Journal of Drug Delivery Technology. 2024;14(1):367-372.

**Source of support:** Nil.

**Conflict of interest:** None

## INTRODUCTION

In the treatment of type 2 diabetes, the medicine dapagliflozin, which belongs to the gliflozin family of drugs, is used by medical professionals. It is possible that renal SGLT2 subtype 2 kidneys are capable of reabsorbing at least 90% of the glucose that is present in persons who have diabetes mellitus. This particular subtype 2 is especially inhibited by dapagliflozin. The kidneys are responsible for the excretion of glucose via the generation of urine when transporter activity is compromised. The molecular structure of oxine-3,4,5-triol is composed of -2-(4-chloro-3-[hydroxymethyl (4-ethoxyphenyl) phenyl], while the molecular structure of dapagliflozin is composed of 2S, 3R, 4R, 5S, and 6R. The chemical C21H25ClO6 is contained inside a single molecular unit that is equal to 408.873 grams. Due to the fact that it is soluble in organic solvents such as ethanol, DMSO, and DMF, sorbent gas purging is an essential step in the process of removing dissolved gases. A study was conducted to determine whether or not methanol and dichloromethane are soluble in water.[1-3] The purpose of this project is to develop and validate a reverse phase chromatographic technique for pharmacological medication dosage assessment that is both straightforward and precise. Using the techniques that have been presented, it is feasible to determine the right dose of dapagliflozin pills in a short amount of time. You may see the molecular structure of dapagliflozin, which is shown in Figure 1.

Both UV spectroscopy and reversed-phase high performance liquid chromatography (RP-HPLC) were used in order to ascertain the bulk structure of the medication, as seen in Figure 1 of the dapagliflozin literature survey. Large quantities of dapagliflozin pills may be measured with the help of the RP-HPLC technology, which is not only simple to use but also rapid and inexpensive. A series of tests were carried out on this method in order to ensure that it was capable of meeting the standards that were established by the International Council for Harmonization. During these experiments, the

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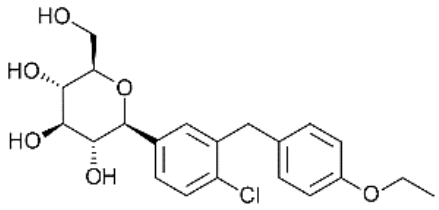


**Figure 1:** Structure of Dapagliflozin

sensitivity, accuracy, precision, and durability of the approach were evaluated thoroughly. The materials were subjected to a battery of tests that comprised varying degrees of heat, light, humidity, oxidation, and acid/base pH.[4,5] These experiments were carried out using a comprehensive stress testing approach. The results of these tests were subjected to a comprehensive examination.

## MATERIAL AND METHODS

### Chemicals and Reagents

SUN Pharmaceuticals Limited made a generous contribution to the state of Maharashtra by supplying it with dapagliflozin (DAPA) of a pharmaceutical quality. An assortment of ingredients, including potassium dihydrogen orthophosphate, acetonitrile, methanol, and orthophosphoric acid, were provided by SD Fine. In addition, we were the ones who provided the water that was of HPLC grade. One of the locations used by Chemical Limited is Mumbai.

### Instruments

*HPLC*

The Shimadzu HPLC series 1100 and the Jasco HPLC PU-2089 Plus are both quite similar.

*Sonicator*

Model 3.5L 100H of the Computer Mumbai model

*Stability chamber*

Senior Member of THERMOLAB, with the number 00002008

### Development of RP-HPLC Method

*Preparation of stock and working standard solutions*

In order to create the stock standard solution, dapagliflozin was first dissolved in deionized water at a concentration of 10 mg/mL. To create five distinct standard solutions, the initial solution was first combined with deionized water, and then the mixture was diluted as necessary. Some of the solutions had values of 1.5, 0.75, 0.375, 0.15, and 2.5 mg/mL. Other solutions had values of none of these. 0.37 mg/mL was one of the other values that was taken. It is possible to get access to a multitude of information and answers that one would need on a daily basis using a method that is both quick and easy.[6-8]

*Preparation of quality control solutions*

Deionized water was used in the production of the dapagliflozin quality control stock solution (QC stock solution) in order to differentiate it from the ordinary stock solution throughout the manufacturing process. Following the production of the quality control stock solution, concentration sample solutions with concentrations of 1.5%, 0.75, and 0.375 mg/mL were created. In order to bring about the formation of these sample solutions, the stock solution was diluted many times with deionized water. Every day, the production of the stock solutions and the samples for quality control was something that was done.

*Selection of wavelength*

Recording the UV spectra of the dapagliflozin solution between 200 and 400 nm allowed for the determination of the wavelength at which the solution could be detected. The objective of this was to determine the wavelength at which the detection occurred. There was a peak in the total absorption at a wavelength that was somewhere between 219 and 275 nm.

*Chromatographic conditions*

Isocratic elution was one of the potential methods that might be used in order to accomplish chromatographic separation at a temperature of 25°C. A length of 4.60 mm and an internal diameter of 150 mm were the dimensions of the Lichrospher® C18 column that was used. This demonstrated that the particle size was 5 µm. There is a wavelength of 275 nm for the ultraviolet detector. It was determined that the mobile phase had two distinct solutions, which were represented by the letters A and B, respectively. On the other hand, acetonitrile was discovered in solution A, while trifluoroacetic acid (TFA) was discovered in solution B at a concentration of 0.1% (v/v) in water. These solutions had a volume-to-volume ratio of 68:32 throughout the experiment. After much deliberation, we decided to go with a discharge rate of 1-mL/minute. Every single injection consisted of the administration of 20 µL of fluid into the body.

### Validation of Proposed HPLC Method

The methodology that was supplied was validated by using the standards established by the USP and the ICH. The recently created RP-HPLC technology was confirmed by comparisons to a wide variety of characteristics in order to guarantee its correctness. Accuracy, specificity, linearity, sensitivity, and stability were some of the characteristics of the analytical solutions that were taken into consideration. These standards are used all across the world.

Validation of analytical techniques have been carried out in accordance with ICH regulations in order to ensure continued compliance.[9,10]

*Accuracy*

It was necessary to go out recovery tests using the conventional addition method in order to guarantee that the approach that was suggested was as accurate as it could possibly be. After being crushed and examined in advance, the capsule was next put through a weighing process. After this, different percentages of the reference medicine were distributed, ranging from 50 to 150%. Reevaluating the replies that were collected was accomplished *via* the use of the procedure that had been devised in the past. In order to ensure that the homogeneity of the samples was

maintained, we carried out three separate evaluations for each and every sample, regardless of the concentration. In light of the findings, it was clear that the actions that were carried out were appropriate.

*Precision*

Additionally to the standard deviation (SD) of the data, we also used the relative standard deviation (%RSD) of the data in order to evaluate the correctness of the analytical procedure. The accuracy of the DAPA estimate was evaluated using a series of tests that were carried out using the method that was suggested on tablet samples that were similar to one another.

*Ruggedness*

We examined both the analysts and the tools in order to ascertain the extent to which they influenced the minimum level of rigor that was suggested for the procedures. Within the scope of this investigation, dapagliflozin was given at a dose of 40 mg/mL. Throughout the whole of the investigation, two analysts and two recommended methodologies were used, and the limits imposed by the environment and the operations were relatively identical.[11]

*Linearity and range*

On the basis of their weight, the sample pill powders that were evaluated were found to contain 80, 90, 100, 110, and 120% of the quantity that was specified. In order to dilute the commercial product in the suitable manner, we followed the directions that were included with it. Following the injection of the solution one at a time, chromatograms were generated in a sequential fashion based on the results. A graph that displayed the concentration of the drug was constructed with the help of the area under the curve measurement.

*Robustness*

The capacity of a system or process to continue functioning effectively and to maintain its stability in the face of a variety of shocks is what we mean when we talk about robustness. Furthermore, this indicates that the technique must be strong enough to withstand relatively minor modifications to the parameters that have been set for the process. For the purpose of making the analytical technique more efficient, adjustments were made to the pH, the composition of the mobile phase, the detecting wavelength, and the flow velocity.

*Specificity*

In order to guarantee the accuracy and precision of the method, we injected blank samples that were composed of citric acid buffer and deionized water. When this step was taken, verification was the cause behind it. Further evidence was supplied by this that pharmaceutical formulations or standard samples containing dapagliflozin did not have any effect on the elution method.[12]

*Sensitivity*

As a means of determining the sensitivity of the method, the LoD and LoQ of the analytical approach were used throughout the process. The limits of quantification (LoQ) and detection (LoD) were established by the signal-to-noise ratios, which were 3:1 and 10:1, respectively within the context of the experiment. Through the process of calculating the LoQ for dapagliflozin, an exact and quantitative threshold was discovered.[13]

*Stability of analytical solutions*

Through the use of duplicated analysis (N = 3), we were able to determine the stability of the quality control samples in three different scenarios, which was our most significant accomplishment. Re-evaluation of the quality control samples was performed after they had been frozen and thawed every 24 hours between a temperature of twenty and room temperature, after being maintained at a temperature of 25°C for 24 hours, and after being maintained between 2 and 8°C for fortnight. Each of these processes was repeated as many times as was necessary for us to complete them. Throughout the whole of this reanalysis, a fresh reference solution was compared to the samples that were performed previously.[14]

**Assay of Marketed Formulation**

In order to determine whether or not the test technique could be used to the production of commercial products, ten vials containing injectable (2 mg/mL) and infusion (0.75 mg/mL) solutions of Integrilin® were individually examined. For the purpose of determining the total number of injections, a regression equation was used.[15]

## RESULTS AND DISCUSSION

**Selection of Wavelength**

As shown in Figure 2, the ultraviolet spectrum depicts the absorption peak of a dapagliflozin solution at two different wavelengths: 219 and 275 nm. A peak of this kind was seen at these wavelengths.

Figure 3 displays the chromatograms of the sample that were obtained at two different wavelengths, 219 and 275 nm. The wavelength of 275 nm was determined to be the most effective for detection in terms of symmetry, baseline flatness, and resistance to interference from TFA.

**HPLC Method Development**

For the purpose of analyzing dapagliflozin in powdered active pharmaceutical elements (API) and pharmaceutical

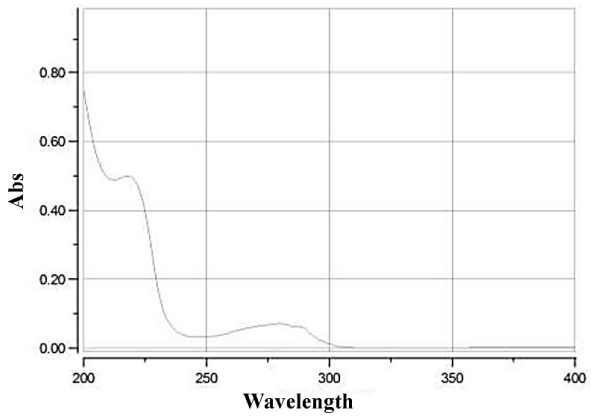


**Figure 2:** UV spectrum of dapagliflozin

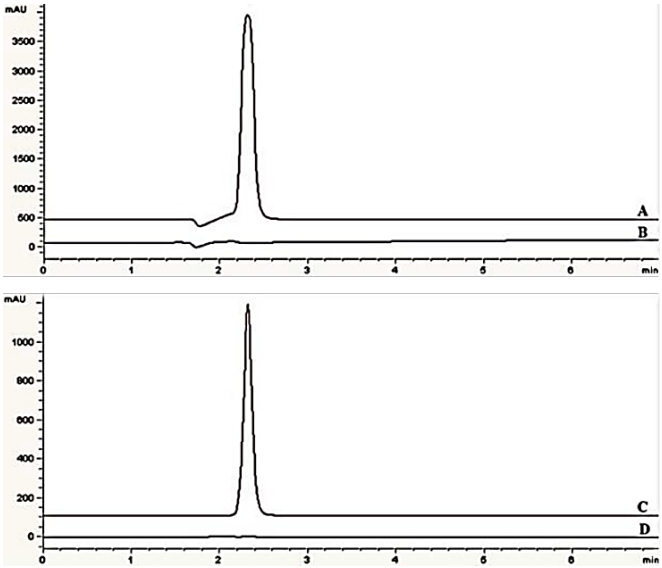


**Figure 3:** shows the chromatograms of the following: (A) Dapagliflozin standard solution (0.75% concentration) in 219 nm, (B) deionized water blank sample in 219 nm, (C) Dapagliflozin standard solution (0.75% concentration) in 275 nm, and (D) blank sample.

formulations, the objective of this study was to discover a method that is not only simple to use but also economical and effective. The retention time of three minutes was shown to be a reliable measure of the effectiveness of the separation achieved by RP-HPLC in comparison to other different techniques. A straightforward sample preparation led to the use of solvents that were both affordable and efficient, and the isocratic C18 column was responsible for providing appropriate resolution. Through the use of quantitative analytical methods, the investigation was able to accomplish both chromatographic responsiveness and a high degree of resolution.

*Specificity*

The chromatograms for the reference sample, the assay sample (an injection solution), and the blank example are superimposed in Figure 4. This figure also includes the data for the blank example. This chart displays the general results that were obtained from the three different samples. It is reasonable to conclude that the drug peak was unaffected by the experimental conditions given that the blank chromatogram does not display any interfering peak throughout the duration of the dapagliflozin retention period. Ascertaining the specificity of the approach may be accomplished by using the peak purity values that are shown on the chromatogram.

*Linearity*

In order to build the calibration curve, graphs were created by making use of the concentration as well as the peak mean area. When we wanted to determine whether or not the calibration curve was linear, we employed linear regression. Within the context of the regression equation, Y is equivalent to 7667X minus 99.948 of the mean value obtained. The correlation coefficient was found to be 0.997, which is considered to be in accordance with the norms that are necessary for the

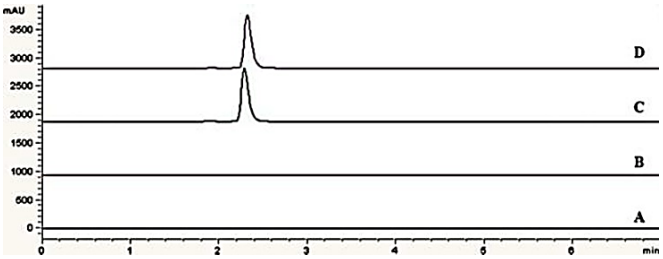


**Figure 4:** Chromatograms showing: (A) a blank sample consisting of deionized water; (B) a blank sample consisting of citric acid buffer as the excipient. (C) a standard solution composed of 0.75 mg/mL Assay sample injection of dapagliflozin (D) (Integrilin® 0.75 mg/mL) from the instrument

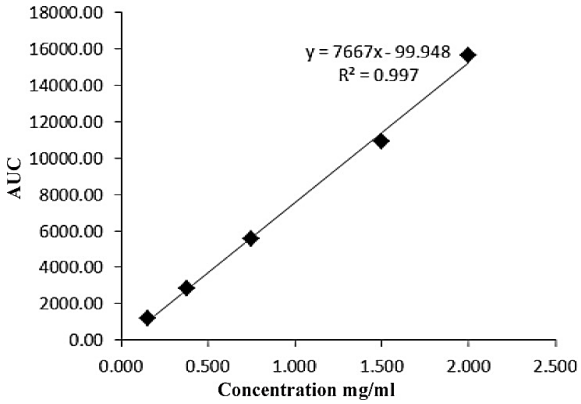


**Figure 5:** The chemical substance dapagliflozin is represented by a linearity plot.

certification of analytical procedures. According to the findings shown in Figure 5, the method demonstrated linearity from a concentration of 0.15 mg/mL all the way up to a concentration of 2 mg/mL.

*Sensitivity*

Based on the signal-to-noise ratio, it was determined that the limits of detection (LoD) and quantitation (LoQ) of the approach were 15 and 45 µg/mL, respectively. Due to the fact that the quantification recovery was only 70% at the limit of quantification (LoQ) concentration level, the conditions for method recovery approval were not satisfied. Following extensive deliberation and decision-making, we arrived at the arbitrary limit of quantification (LoQ) for the testing process, which was set at 0.15 mg/mL. These were chosen because of their capacity to maintain a consistent level of precision and accuracy even when the calibration curve was at its lowest observable level. The selection of these individuals was based on this argument.

*Precision*

Through the use of RSD, we were able to determine the accurateness of the technique both within and across days. According to the findings, the relative standard deviations (RSD) of the quality control sample tests, which were carried out both within and between days, were found to be less than 2%. A fantastic illustration of demonstrating that the results

**Table 1:** The intra-day and inter-day precision and accuracy of dapagliflozin quality control samples as measured by high-performance liquid chromatography are shown

| *Theoretical concentration* | *Calculated concentration (mean ± S.D., n = 3)* | *Precision (R.S.D.) (%)* | *Accuracy Recovery (%)* |
|---|---|---|---|
| *Intra-day* | | | |
| 0.375 | 0.383 ± 0.001 | 0.053 | 102.11 |
| 0.750 | 0.783 ± 0.001 | 0.058 | 98.39 |
| 1.500 | 1.441 ± 0.001 | 0.052 | 96.07 |
| *Inter-day* | | | |
| 1.500 | 1.445 ± 0.003 | 0.204 | 96.36 |
| 0.750 | 0.740 ± 0.003 | 0.364 | 98.60 |
| 0.375 | 0.387 ± 0.002 | 0.598 | 103.18 |

**Table 2:** presents the results of the quality control sample stability research, including the mean concentration, relative standard deviation, and recovery percentage

| *Condition* | *Theoretical concentration* | *Calculated concentration (mean ± S.D., n = 3)* | *Precision (R.S.D.) (%)* | *Accuracy Recovery (%)* |
|---|---|---|---|---|
| Short term storage | 0.375 | 0.385 ± 0.001 | 0.103 | 102.73 |
| | 0.750 | 0.737 ± 0.002 | 0.225 | 98.29 |
| | 1.500 | 1.472 ± 0.001 | 0.054 | 98.12 |
| long term storage | 0.375 | 0.387 ± 0.001 | 0.154 | 103.23 |
| | 0.750 | 0.400 ± 0.001 | 0.103 | 98.70 |
| | 1.500 | 1.549 ± 0.009 | 0.603 | 103.27 |
| Freeze and thaw | 0.375 | 0.384 ± 0.001 | 0.175 | 102.53 |
| | 0.750 | 0.739 ± 0.001 | 0.148 | 98.58 |
| | 1.500 | 1.511 ± 0.011 | 0.742 | 100.74 |

were correct was provided here. These findings are highlighted in Table 1.

*Accuracy*

In order to determine the degree of precision of the technique, quality control (QC) samples were subjected to a selection of recovery tests. The recovery rate was found to be anywhere between 96.36 and 103.18%, according to the findings of the assessment of the quality control samples.

*Stability of analytical solution*

The following is a summary of the stability tests that were carried out on quality control samples that were stored in a variety of different circumstances (Table 2). According to the findings, the quality of the quality control samples was unaffected by a period of 24 hours at the temperature of the surrounding environment, 14 days in the refrigerator, or three cycles of freezing and thawing.

**Application of the Method for the Analysis of Dapagliflozin injection Formulation (Integrilin ®)**

Validation of the recommended method was performed in order to ensure that injectable dapagliflozin (Integrilin®) concentrations of 0.75 and 2 mg/mL were accurately evaluated. Based on the results from a sample of ten individuals, it was determined that the average test value for injectable formulation solutions was 103.446 ± 0.001, while the average test value for infusion formulation solutions was 100.100 ± 0.003. As a result of its high level of accuracy, short analytical time, and low relative standard deviation (RSD), this approach is suitable for the routine testing of commercial injectable Dapagliflozin formulations. Due to these characteristics, the method is an excellent choice for doing routine analysis.[16]

## CONCLUSION

Through the use of a well-established, efficient, user-friendly, isocratic RP-HPLC method with UV detection, dapagliflozin was identified and quantified in both its pharmaceutical and pure forms. Through the use of statistical data, we evaluated the recommended technique in terms of its accuracy, precision, sensitivity, linearity, and selectivity. Throughout the whole process of validation, the adherence to the technical standards that have been established by the International Council for Harmonization (ICH) for medicines that are designed for use by humans was at the forefront of the discussion. This method, which has been described in earlier publications, may also be used to evaluate the stability of analytical solutions, as shown by the previously mentioned articles. It is possible that pharmaceutical firms will be able to use the technology that is currently available as a quality control device for the dapagliflozin test. This is justified by the fact that there is a pressing need to shorten the detention periods that are involved in routine drug investigations. RP-HPLC, which stands for reversed-phase high-performance liquid chromatography, is the specific method that is used in the process of analyzing the dosage of dapagliflozin tablets. During the course of the exhaustive inquiry, the assessment of the technique generated results that were unambiguous, sensitive, accurate, and straightforward. Once the dapagliflozin has been extracted from the tablets, it may then be subjected to the normal analytical techniques. As shown by the outcomes of controlled settings testing, the active component of the method was successfully separated from the breakdown products that it produced in response to the stress that was imposed by the environment. When conducting pharmaceutical analyses, it is necessary to do so on a consistent basis utilizing methods that are not influenced by degradation products, whether they are in tablet or bulk form. When tablets and bulk drug combinations are analyzed using classic HPLC techniques, it has been shown by a number of studies that accurate findings may be achieved.

## REFERENCES

1. Patil K, Narkhede S, Nemade M, Rane S, Chaudhari R, Dhobale G, Tare H. A Validated Sensitive Stability Indicating HPLC Method for the Determination of Etoricoxib in Bulk and Formulation. International Journal of Pharmaceutical Quality Assurance. 2023;14(2):352-7.
2. Ahmad S, Aakanksha D, Patil M, Bhise M, Barde L, Tare H. Development and Validation of HPLC And HPTLC for

Simultaneous Analysis of E and Z Guggulsterone, A-11–KBA And 11–KBA from Herbal Formulation. International Journal of Pharmaceutical Quality Assurance. 2023;14(2):393-396.
3. Kharate V, Kuchekar M, Harde M, Pimple B, Patole V, Salunkhe M, Wadgave P, Bhise M, Gaikwad A, Tare H. Development of Validated Stability Indicating HPTLC Method for Estimation of Febuxostat in Bulk and Tablet Dosage Form by Using QBD Approach. International Journal of Drug Delivery Technology. 2023;13(2):542-550.
4. Wagh K, Bakhshi A, Ahmad S, Tare H, Deore S. LC-MS/MS Method Development and Validation for Human Plasma Canagliflozin Determination. International Research Journal of Multidisciplinary Scope (IRJMS), 2024; 5(1): 441-451
5. Mane V, Killedar S, More H, Salunkhe S, Tare H. Development and Validation of a Novel Bioanalytical Method for Estimating Epigallocatechin 3 Gallate in Wistar Rat Plasma by RP-HPLC Employing Gradient Elution Techniques. Journal of Research in Pharmacy. 2023 May 1;27(3).
6. Shivatare RS, Jangra S, Gaikwad A, Kewatkar S, Bhutale N, Suryavanshi DS, Tare H. Development and Validation of HPLC Method Along With Anti-Aging Activity For Ximenynic Acid In *Santalum album* Linn. Indian Drugs. 2023 Apr 1;60(4).
7. Pund S, Nalawade S, Rajurkar V, Jayatpal S, Deshmukh N, Tare H. A brief review on recent advances in reverse phase HPLC. Multidisciplinary Reviews. 2024 Jan 18;7(4):2024072.
8. Mujawar T, Ahmad S, Tajane P, Sable V, Gaikwad A, Tare H. Development and validation of ultraviolet spectrophotometric method for Saquinavir estimation in bulk and dosage form. Multidisciplinary Science Journal. 2023 Apr 9;5(2):2023020.
9. Vijayakumar EK, Samel MA, Bhalekar SB, Pakhale SM. A new stability indicating HPLC method for related substances in zolmitriptan. Indian Journal of Pharmaceutical Sciences. 2010 Jan;72(1):119.
10. Luo Z, Liu A, Liu Y, Wang G, Chen X, Wang H, Li M, Zhang H, Qiu Y, Zhai H. Development of a stability–indicating HPLC method for simultaneous determination of ten related substances in vonoprazan fumarate drug substance. Journal of Pharmaceutical and Biomedical Analysis. 2018 Feb 5;149:133-42.
11. Gu P, Li Y. Development and validation of a stability-indicating HPLC method for determination of voriconazole and its related substances. Journal of chromatographic science. 2009 Aug 1;47(7):594-8.
12. Luo Z, Deng Z, Liu Y, Wang G, Yang W, Hou C, Tang M, Yang R, Zhou H. Development and validation of a novel stability-indicating HPLC method for the quantitative determination of eleven related substances in ezetimibe drug substance and drug product. Talanta. 2015 Jul 1;139:67-74.
13. Zhu S, Meng X, Su X, Luo Y, Sun Z. Development and validation of a stability-indicating high performance liquid chromatographic (HPLC) method for the determination of related substances of micafungin sodium in drug substances. International Journal of Molecular Sciences. 2013 Oct 24;14(11):21202-14.
14. Kavitapu D, Maruthapillai A, Mahapatra S, Selvi JA. New stability indicating RP-HPLC method for the determination of Abiraterone acetate, its related substances and degradation products in bulk and dosage form. Materials Today: Proceedings. 2021 Jan 1;34:469-78.
15. Kasawar GB, Farooqui M. Development and validation of a stability indicating RP-HPLC method for the simultaneous determination of related substances of albuterol sulfate and ipratropium bromide in nasal solution. Journal of pharmaceutical and biomedical analysis. 2010 May 1;52(1):19-29.
16. Verma MV, Patel CJ, Patel MM. Development and stability indicating HPLC method for dapagliflozin in API and pharmaceutical dosage form. International Journal of Applied Pharmaceutics. 2017;9(5):33-41.