DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR VARIOUS DRUG SUBSTANCES: A REVIEW

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Abstract:
This article provides an overview on Development and Validation of Force degradation study with Stability Indicating Methods (SIMs) for drug substances. Different stress conditions (hydrolysis, oxidation, thermal, and photolytic conditions) are applied to drugs compounds during a process known as forced degradation, and as a result, various degradation products are created. The major purpose of these investigations is to assess a molecule's stability under accelerated settings. It is well recognized that the stability of molecules affects the regulatory documentation process, the choice of appropriate storage and packaging conditions, and the choice of formulation. The deficiencies of reported methods in terms of regulatory requirements are highlighted. This article is to provide appropriate International conference of harmonization (ICH) criteria for force degradation study and to discuss the methodical process for creating verified SIAMs. The aspects of Mass balance in SIMs are discussed and technique used in SIMs were highlighted. Recent advance in stability indicating methods include characterization of degradant sample and in-vitro toxicity prediction are addressed. Background of force degradation study with stability indicating methods with respect to international author and national author are discussed.

Keywords: Force degradation study; Mass balance; Stability Indicating Methods; toxicity study, International conference of harmonization

INTRODUCTION:

Analytical method development by high-performance liquid chromatography (HPLC):

It involves the process of optimizing and validating a chromatographic method to separate, identify, and quantify components of a sample. HPLC is a widely used technique in various industries, including pharmaceuticals, environmental analysis, food and beverages, forensics, and more [1]. Here is a general overview of the steps involved in HPLC method development: Clearly define the purpose of the analysis, such as determining the presence and concentration of specific compounds in a sample. Select an appropriate column with a suitable stationary phase based on the sample's physicochemical properties, analyte stability, and desired separation mechanism. Determine the most suitable combination of solvents and additives to achieve efficient separation and detection of the analytes. This process involves adjusting the mobile phase's pH, ionic strength, and organic solvent composition. Select a suitable detection technique, such as UV-Vis absorbance, fluorescence, electrochemical, or mass spectrometry. Optimize the detection wavelength, detector sensitivity, and other parameters to enhance signal-to-noise ratio and maximize analyte detection [2-4]. Optimize the injection volume and flow rate to achieve adequate sensitivity, resolution, and peak shape. Too high or too low injection volumes can result in poor chromatographic performance. Based on the sample's complexity and the desired separation, determine whether a gradient elution (changing solvent composition over time) or isocratic elution (constant solvent composition) method

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is appropriate. Gradient elution is often used for complex mixtures requiring higher resolution. Validate the method: Perform method validation experiments to ensure that the developed method meets the desired criteria for linearity, accuracy, precision, specificity, robustness, and limits of detection and quantification. Validate the method according to regulatory guidelines if required for the intended application. If the method does not meet the required criteria, optimize and troubleshoot various parameters, such as column temperature, pH, buffer concentration, flow rate, etc., to improve the separation and performance [5].

Once the method is successfully developed and validated, document all the details in a standard operating procedure (SOP) for future reference and to ensure reproducibility. It's important to note that method development is an iterative process, and several experiments and adjustments might be necessary to achieve an optimal and robust method. Additionally, the specific details and considerations may vary depending on the sample matrix, target analytes, available equipment, and regulatory requirements [6].

Advantages of Method development by High Performance Liquid Chromatography:

High Performance Liquid Chromatography (HPLC) is a widely used analytical technique in various scientific and industrial fields. The method development process in HPLC offers several advantages, including:

HPLC provides excellent separation power, enabling the analysis of complex mixtures with high resolution. It can separate and detect a wide range of compounds, including small molecules, large biomolecules, and polar or non-polar substances. HPLC offers high sensitivity in detection, allowing for the analysis of compounds at very low concentrations. This is especially useful when analyzing trace amounts of impurities or studying compounds with low abundance [7].

HPLC is a versatile technique that can be applied to a broad range of sample types, including pharmaceuticals, environmental samples, food and beverages, biological samples, and more. It can accommodate various sample matrices and handle different sample volumes. HPLC allows for accurate and precise quantification of analytes in a sample. It enables the determination of compound concentrations, making it valuable for quality control, pharmacokinetic studies, and drug formulation analysis. HPLC can be coupled with a diverse array of detectors, such as UV-Vis, fluorescence, electrochemical, mass spectrometry (MS), and refractive index detectors. This versatility enables selective and specific detection, enhancing the sensitivity and selectivity of the analysis [8].

HPLC method development allows for optimization of parameters such as column selection, mobile phase composition, flow rate, and temperature. This optimization process helps in achieving desired separation, reducing analysis time, and enhancing method robustness and reproducibility. HPLC instruments can be automated, enabling the analysis of a large number of samples in a relatively short time. This high throughput capability is beneficial in industries where rapid analysis is required, such as pharmaceutical manufacturing and quality control laboratories [9]. HPLC can handle a wide range of analytes, including polar, non-polar, acidic, basic, and chiral compounds. This compatibility makes it suitable for diverse applications and allows for the analysis of complex samples containing multiple components.

Once a method is developed and validated in HPLC, it can be easily transferred between different laboratories or HPLC systems. This transferability ensures consistency and comparability of results across different locations. HPLC is a well-established technique with a long history of use in regulated industries such as pharmaceuticals and environmental monitoring. It has extensive documentation, guidelines, and regulatory acceptance, which simplifies method validation and compliance with industry standards. These advantages make HPLC method development a valuable tool for a wide range of analytical applications, offering precise separation, sensitive detection, and accurate quantification of analytes in complex samples [10].

Validation of analytical methods:

Validation of analytical methods is an essential process in the field of analytical chemistry. It involves demonstrating that a particular method is suitable for its intended purpose and provides accurate and reliable results. The validation process ensures that the method meets predetermined criteria for parameters such as accuracy, precision, selectivity, sensitivity, linearity, and robustness [11].

Here are some key steps typically involved in the validation of analytical methods: Clearly state the objective of the method and identify the specific analytes, matrices, and concentration range it is designed to analyze. Develop and optimize the analytical method, including sample preparation, instrumentation, and data analysis techniques. Assess the accuracy of the method by comparing the results obtained with a validated reference method or a known reference standard. Evaluate the precision of the method by analyzing replicate samples and calculating statistical parameters such as standard deviation, relative standard deviation, and confidence intervals [12].

Determine the selectivity and specificity of the method by analyzing samples containing potential interfering substances or analytes similar to the target analyte. Determine the sensitivity of the method by establishing the limit of detection (LOD) and limit of quantitation (LOQ), which represents the lowest concentrations of analyte that can be reliably detected and quantified, respectively. Evaluate the linearity of the method by analyzing a series of standard solutions covering the expected concentration range and assessing the correlation coefficient of the calibration curve. Assess the robustness of the method by evaluating its performance under different experimental conditions such as changes in pH, temperature, sample matrix, and analyst [13].

Establish system suitability criteria to ensure that the analytical system is performing adequately throughout the analysis. This may involve assessing parameters such as peak resolution, tailing factor, and system stability. Document all the validation experiments, including detailed protocols, raw data, calculations, and results, in a validation report. It's important to note that the specific validation requirements may vary depending on the regulatory guidelines, industry standards, and the intended use of the analytical method. Organizations such as the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) provide guidelines for method validation in the pharmaceutical industry, while other industries may have their own specific guidelines [14].

Advantages of Method validation by High Performance Liquid Chromatography:

Method validation by High Performance Liquid Chromatography (HPLC) offers several advantages in analytical chemistry. Here are some key advantages: HPLC is a highly sensitive technique that allows for the detection and quantification of low levels of analytes. It can achieve high sensitivity due to its ability to handle small sample volumes and its compatibility with various detection techniques, such as UV-Vis, fluorescence, and mass spectrometry [15].

- **Selectivity:** HPLC provides excellent selectivity for complex sample matrices. It allows for the separation of closely related compounds based on their physicochemical properties, such as polarity, size, and charge. By selecting appropriate stationary phases and mobile phases, HPLC can resolve complex mixtures and eliminate interferences, enhancing the accuracy and reliability of the method.
- **Versatility:** HPLC is a versatile technique that can be applied to a wide range of analytes, including small organic molecules, large biomolecules (proteins, peptides, nucleic acids), and various pharmaceutical compounds. It is widely used in pharmaceutical, environmental, food, and forensic analyses, among other fields.
- **Accuracy and precision:** HPLC method validation ensures accurate and precise quantification of analytes. Through proper validation, the method's accuracy (closeness of results to the true value) and precision (reproducibility of results) are evaluated, leading to reliable and trustworthy analytical data.

- **Robustness:** HPLC methods can be optimized and validated to be robust, meaning they are resistant to small variations in experimental conditions. This allows for consistent and reliable results, even when minor changes occur in parameters such as temperature, flow rate, pH, or mobile phase composition.
- **Quantification and linearity**: HPLC provides a wide linear dynamic range for quantification, allowing for accurate determination of analyte concentrations across a broad range. This is particularly advantageous when dealing with samples containing a wide concentration range of analytes.
- **Sample throughput:** HPLC methods are often automated and can handle multiple samples simultaneously, allowing for high sample throughput. This is beneficial when large numbers of samples need to be analyzed within a given timeframe, improving efficiency and productivity in the laboratory.
- **Method optimization:** HPLC methods can be optimized to achieve desired separation, resolution, and analysis time. Various parameters such as column type, mobile phase composition, temperature, and flow rate can be adjusted to improve method performance, leading to faster analysis and better separation of target analytes [16, 17].

Introduction to Forced degradation study:

A forced degradation study, also known as stress testing or forced degradation testing, is a crucial part of the drug development process in the pharmaceutical industry. It is a systematic approach to subjecting a drug substance or drug product to severe stress conditions to determine its inherent stability and potential degradation pathways [18].

The primary objective of a forced degradation study is to understand how a drug molecule or formulation may degrade under extreme conditions such as exposure to high temperature, humidity, light, oxidation, acid or base hydrolysis, and other relevant stress factors. These conditions are intended to accelerate the degradation process and simulate the potential degradation pathways that a drug product may encounter over its shelf life or during manufacturing, storage, and use.

By subjecting a drug to these stress conditions, scientists can evaluate the stability and robustness of the compound or formulation. The study helps to identify the degradation products, impurities, and potential interactions that may occur. It also aids in determining the degradation mechanisms and establishing suitable storage conditions and shelf-life recommendations.

Forced degradation studies typically involve conducting stress tests on both the drug substance (the pure active pharmaceutical ingredient) and the drug product (the formulation containing the active ingredient and other excipients). Various analytical techniques such as chromatography (HPLC, GC), spectroscopy (UV, IR, MS), and other methods are employed to detect and characterize the degradation products and impurities formed during the stress testing.

The results of a forced degradation study provide valuable information for the development of stability-indicating analytical methods that can effectively separate and quantify the drug substance and its degradation products. It also helps in establishing appropriate manufacturing and storage conditions, packaging, and handling recommendations to ensure the quality, efficacy, and safety of the drug product throughout its intended shelf life.

Overall, forced degradation studies play a critical role in understanding the degradation behaviour of drug substances and products, ensuring their stability, and guiding the formulation and development of pharmaceuticals that meet regulatory requirements and provide maximum therapeutic benefit to patients [19, 20].

Objectives and scope of forced degradation study:

The objectives and scope of a forced degradation study can vary depending on the specific context and purpose of the study. Generally, the main objectives and scope of a forced degradation study are as follows [21]:

Objectives:

- Identify the degradation pathways: The primary objective of a forced degradation study is to understand the degradation pathways of a drug substance or product. It involves subjecting the sample to various stress conditions, such as heat, light, humidity, acid/base hydrolysis, oxidation, and photolysis, to induce degradation. By analyzing the degradation products, the study aims to identify the specific degradation pathways and potential impurities that may form under stress conditions.
- Assess stability: Forced degradation studies are conducted to assess the stability of drug substances or products. By subjecting the samples to exaggerated degradation conditions, the study helps evaluate the inherent stability of the drug and provides insights into potential degradation mechanisms. It can assist in determining the shelf life, storage conditions, and formulation strategies for the drug.
- Develop stability-indicating methods: Forced degradation studies are also crucial for developing stability-indicating analytical methods. These methods should be capable of separating and quantifying the drug substance and its degradation products accurately. By studying the degradation behaviour, the study aids in selecting appropriate analytical techniques and conditions for monitoring drug stability during development, manufacturing, and storage [22].

Scope:

- Stress conditions: The forced degradation study encompasses subjecting the drug substance or product to a range of stress conditions. These conditions can include but are not limited to temperature, light exposure, humidity, pH extremes, oxidative conditions, and photolytic conditions. The specific stress conditions chosen depend on the drug's characteristics, intended use, and potential degradation pathways of interest.
- Analytical techniques: The study involves the use of various analytical techniques to identify and quantify degradation products. These techniques can include chromatographic methods (e.g., HPLC, GC), spectroscopic methods (e.g., UV-Vis, FTIR), mass spectrometry (MS), and others. The selection of analytical techniques depends on the specific degradation products being targeted and the sensitivity of the methods.
- Regulatory considerations: Forced degradation studies are often conducted in accordance with regulatory guidelines, such as the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines. These guidelines provide recommendations on the design, execution, and reporting of forced degradation studies. Compliance with relevant regulatory requirements is an essential aspect of the study's scope [23].

Introduction of Stability study:

A stability study is a critical component of pharmaceutical, biopharmaceutical, and other healthcare product development and manufacturing processes. It involves evaluating the chemical, physical, and microbiological properties of a product over time to determine its shelf life, storage conditions, and any potential degradation or changes that may occur. The primary objective of stability studies is to ensure that products remain safe, effective, and of high quality throughout their intended shelf life [24].

Stability studies are conducted following guidelines and regulations established by regulatory authorities such as the U.S. Food and Drug Administration (FDA), the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH), and other regional regulatory agencies. These guidelines provide a framework for conducting stability studies and define the specific requirements for different types of products.

The stability study typically involves exposing samples of the product to various storage conditions, including temperature, humidity, light, and other environmental factors that the product may encounter during its lifecycle. The samples are then periodically tested to assess their stability, which may include analysis of physical appearance, chemical composition, potency, dissolution rate, and microbial contamination, among other parameters.

The study duration can vary depending on the product and its intended shelf life. It usually involves testing the samples at predetermined intervals over an extended period, often including accelerated stability testing under exaggerated storage conditions to simulate long-term effects in a shorter time frame.

The data collected from stability studies is used to establish the recommended storage conditions, shelf life, and expiration dates of the product. It also helps in identifying appropriate packaging materials and design to ensure product integrity and safety.

Stability studies play a crucial role in pharmaceutical quality control and regulatory compliance. They provide scientific evidence to support product claims, help detect potential issues or risks associated with product stability, and guide manufacturers in developing appropriate manufacturing and storage processes to maintain product quality and efficacy.

Overall, stability studies are essential for ensuring the safety, efficacy, and quality of healthcare products and are a vital part of the product development and regulatory approval processes in the pharmaceutical and biopharmaceutical industries [23, 24].

Significance of forced degradation study:

Forced degradation studies are an essential aspect of drug development and other industries where product stability is crucial. These studies involve subjecting a substance or product to harsh conditions such as high temperature, humidity, light exposure, oxidation, and acid/base hydrolysis to induce degradation. The purpose of forced degradation studies is to evaluate the stability and degradation pathways of a substance under extreme conditions. Here are some key reasons why forced degradation studies are important [25]:

- **Determining degradation pathways:** Forced degradation studies help identifies the various pathways by which a substance can degrade. By subjecting the substance to different stress conditions, the study can reveal the potential degradation products and impurities that can form. Understanding these degradation pathways is vital for ensuring the safety, efficacy, and quality of the product.
- **Stability assessment:** Forced degradation studies provide valuable information on the stability of a substance or product. They help determine the degradation rates and degradation profiles under different conditions, which are crucial for establishing appropriate storage conditions, shelf life, and recommended usage of the product. This knowledge is essential for maintaining product quality throughout its intended lifespan.
- **Establishing degradation kinetics:** Forced degradation studies allow for the determination of degradation kinetics, which involve measuring the rate of degradation over time. This information helps in predicting the stability of a substance under normal storage conditions and can aid in setting appropriate storage and expiry dates.
- **Identifying impurities:** Forced degradation studies aid in the identification and characterization of degradation products and impurities that can form under extreme conditions. These impurities may arise from degradation pathways or interactions with packaging materials, and their presence may affect the safety, efficacy, or quality of the product. Detecting and understanding these impurities is crucial for implementing appropriate control measures.
- **Regulatory compliance:** Regulatory authorities require comprehensive stability data, including forced degradation studies, as part of the approval process for drugs and other regulated products. Conducting these studies and providing the resulting data is essential for demonstrating compliance with regulatory guidelines and ensuring the product's safety and quality.
- **Formulation development:** Forced degradation studies provide insights into the stability of different formulation components and their interactions. This information can guide formulation

development and help optimize the composition and manufacturing process to enhance the stability of the product [25, 26].

Forced degradation mechanism:

Forced degradation refers to the intentional application of harsh conditions or stress to a drug substance or drug product in order to accelerate the degradation process. This process is commonly used during drug development to evaluate the stability and degradation pathways of pharmaceutical products. Several mechanisms can lead to forced degradation, including:

- **Hydrolysis:** Hydrolysis is a common degradation mechanism in which the drug substance or product undergoes a chemical reaction with water. It can result in the breaking of chemical bonds, leading to the formation of degradation products. Hydrolysis can be catalyzed by different factors such as pH, temperature, and humidity.
- **Oxidation:** Oxidation occurs when a drug substance or product reacts with oxygen or other oxidizing agents. This process can lead to the formation of new functional groups or the oxidation of existing functional groups within the molecule. Oxidation is often facilitated by heat, light, or metal ions.
- **Photolysis:** Photolysis refers to the degradation of a substance induced by exposure to light, particularly in the ultraviolet (UV) range. The absorption of light energy can cause chemical reactions that lead to the cleavage of bonds and the formation of degradation products. Light-sensitive drugs are particularly prone to photolytic degradation.
- **Thermal degradation:** Thermal degradation involves the degradation of a substance due to exposure to high temperatures. Elevated temperatures can cause various chemical reactions, such as hydrolysis, oxidation, or decomposition of the drug molecule. This mechanism is commonly assessed using techniques like accelerated stability studies.
- **pH-dependent degradation:** Some drug substances are sensitive to changes in pH. Acidic or alkaline conditions can cause chemical reactions, leading to degradation. pH-dependent degradation is often evaluated by subjecting the drug substance or product to different pH conditions and monitoring the formation of degradation products.
- **Solid-state degradation:** In addition to chemical reactions in solution, drugs can undergo degradation in the solid-state. Factors such as humidity, temperature, and mechanical stress can trigger solid-state degradation, resulting in changes in drug stability and potency [21-23].

Steps for forced degradation study experiment:

Performing a forced degradation study is a crucial step in drug development to understand the stability and degradation pathways of a compound. Here are the general steps involved in conducting a forced degradation study experiment:

- **Determine the purpose and scope:** Define the objectives of the forced degradation study. Identify the specific degradation pathways and stress conditions you want to investigate. Common stress conditions include heat, light, humidity, acid/base hydrolysis, oxidation, and photolysis.
- **Select appropriate analytical techniques**: Choose suitable analytical techniques to monitor and quantify the degradation products. Common techniques include high-performance liquid chromatography (HPLC), gas chromatography (GC), mass spectrometry (MS), nuclear magnetic resonance (NMR), and spectroscopy (UV, IR).
- **Prepare the sample:** Obtain a pure sample of the compound of interest. If necessary, perform pre-treatment steps such as drying, grinding, or dissolution to ensure a homogeneous sample.
- **Establish degradation conditions:** Design and set up the stress conditions based on the selected degradation pathways. This may involve exposing the compound to specific temperature, humidity, light exposure, acidic or basic conditions, or oxidation agents. Use appropriate controls to compare the degradation under normal conditions.

- **Conduct the forced degradation experiment:** Expose the sample to the selected stress conditions for a predetermined period. Maintain proper temperature and environmental controls throughout the experiment. Document all experimental details, including start time, duration, temperature, and any observations.
- **Sampling and time points:** At predefined intervals, collect samples from the stressed conditions and from the control. Determine the time points based on the anticipated degradation rate of the compound under study. Ensure that the sample collection process is consistent and representative.
- **Analyze the samples:** Use the chosen analytical techniques to analyze the samples obtained from the forced degradation study. Follow established protocols and methods for sample preparation, separation, and detection. Identify and quantify degradation products. Compare the results with the control sample and determine the degradation pathways.
- **Data interpretation:** Analyze and interpret the data obtained from the forced degradation study. Determine the degradation kinetics, identify major degradation pathways, and assess the stability of the compound under different stress conditions. Use appropriate statistical methods if necessary.
- **Reporting:** Compile the results, including degradation profiles, identified degradation products, and their respective mechanisms. Document the experimental procedures, data analysis, and conclusions. Provide recommendations for further studies or modifications to improve the compound's stability.
- **Discussion and decision-making:** Discuss the findings with the relevant stakeholders, such as researchers, regulatory authorities, or project teams. Make informed decisions regarding formulation, packaging, storage conditions, and shelf-life determination based on the forced degradation study results [23-26].

CONCLUSION:

The development and validation of force degradation studies using stability indicating methods for pharmacological compounds is covered in this article. Different stress conditions are applied to medicinal compounds during forced degradation, resulting in different degradation products. Molecular stability under accelerated conditions is the main focus of these studies. It is commonly known that molecular stability influences regulatory documentation, storage and packing conditions, and formulation. Regulatory inadequacies in reported techniques are identified. This article discusses ICH force degradation study criteria and the methodological methodology for creating validated SIAMs. SIM mass balance and techniques are described. Recently developed stability indicators include degradant sample characterisation and in-vitro toxicity prediction. Background of force degradation study with stability indicating methodologies for worldwide and national authors is discussed.

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Conflict of Interest

None

REFERENCES:

1. Ngwa, G., 2010. Forced degradation as an integral part of HPLC stability-indicating method development. Drug delivery technology, 10(5), pp.56-59.
2. Singh, S., Junwal, M., Modhe, G., Tiwari, H., Kurmi, M., Parashar, N. and Sidduri, P., 2013. Forced degradation studies to assess the stability of drugs and products. TrAC Trends in Analytical Chemistry, 49, pp.71-88.
3. Iram, F., Iram, H., Iqbal, A.Z.H.A.R. and Husain, A., 2016. Forced degradation studies. Journal of Analytical & Pharmaceutical Research, 3(6), p.73.

4. Reynolds, D.W., Facchine, K.L., Mullaney, J.F., Alsante, K.M., Hatajik, T.D. and Motto, M.G., 2002. Conducting forced degradation studies. Pharm Technol, 26(2), pp.48-56.
5. Sharma, M.K. and Murugesan, M., 2017. Forced degradation study an essential approach to develop stability indicating method. J Chromatogr Sep Tech, 8(1), p.349.
6. Blessy, M.R.D.P., Patel, R.D., Prajapati, P.N. and Agrawal, Y.K., 2014. Development of forced degradation and stability indicating studies of drugs—A review. Journal of pharmaceutical analysis, 4(3), pp.159-165.
7. Narasimhan, B., Abida, K. and Srinivas, K., 2008. Stability indicating RP-HPLC method development and validation for oseltamivir API. Chemical and Pharmaceutical Bulletin, 56(4), pp.413-417.
8. Rathod, N., Patel, A.I., Prajapati, R., Patel, N.K., Patel, A.B. and Vyas, A.J., 2018. Analytical Method Development and Validation of Stability Indicating Method and Related Substance by Using RP-HPLC of Drug Substance. J Pharm Anal, 8(5), pp.367-72.
9. Aher P, Surana K, Ahire E, Patil D, Sonawane D, Mahajan S. Development and Validation of RP-HPLC Method for Quantitative Determination of 4-Amino Benzene Sulphonamide in Sulphonamide Hydrochloride. Trends in Sciences. 2023 Mar 15;20(6):5209-
10. Chhalotiya, U.K., Bhatt, K.K., Shah, D.A. and Baldania, S.L., 2010. Development and validation of a stability-indicating RP-HPLC method for duloxetine hydrochloride in its bulk and tablet dosage form. Scientia pharmaceutica, 78(4), pp.857-868.
11. Bagal, D., Nagar, A., Joshi, A., Chachare, A., Shirkhedkar, A. and Khadse, S., 2021. Development and validation of stability-indicating RP-HPLC method for estimation of dalfampridine in bulk drug and tablet dosage form. Future Journal of Pharmaceutical Sciences, 7, pp.1-7.
12. Kirthi, A., Shanmugam, R., Mohana, L.S., Kumar, C.K., Padmini, K., Prathyusha, M.S. and Shilpa, V., 2016. Analytical Method Development and Validation of a Stability-indicating RP-HPLC Method for the Analysis of Danazol in Pharmaceutical Dosage Form. Asian Journal of Pharmaceutical Analysis, 6(4), pp.227-234.
13. Ahirrao SP, Bhambere DS, Ahire ED, Dashputre NL, Kakad SP, Laddha UD. Formulation and evaluation of Olmesartan Medoxomil nanosuspension. Materials Today: Proceedings. 2023 Jul 1.
14. Wimalasinghe, R.M., Zhao, D., Wang, L. and Rustum, A., 2021. Development and validation of a stability-indicating reversed-phase high-performance liquid chromatography (RP-HPLC) method for identification, assay, and estimation of related substances of ivermectin drug substance. Journal of AOAC International, 104(6), pp.1505-1513.
15. Shah, U., Kavad, M. and Raval, M., 2014. Development and validation of stability-indicating RP-HPLC method for estimation of pamabrom in tablets. Indian journal of pharmaceutical sciences, 76(3), p.198.
16. Ahire E, Thakkar S, Borade Y, Misra M. Nanocrystal based orally disintegrating tablets as a tool to improve dissolution rate of Vortioxetine. Bulletin of Faculty of Pharmacy, Cairo University. 2020 Dec 1;58(1&2):11-20
17. Dey, S., Patro, S.S., Babu, N.S., Murthy, P.N. and Panda, S.K., 2017. Development and validation of a stability-indicating RP–HPLC method for estimation of atazanavir sulfate in bulk. Journal of pharmaceutical analysis, 7(2), pp.134-140.
18. Jadhav, R.A., Sanil, Y.M., Shankarwar, S.G., Shankarwar, A.G., Pawar, R.P. and Bembalkar, S.R., 2020. Development and validation of rapid stability-indicating RP-HPLC method for assay and related substances of solifenacin succinate. Chromatographia, 83, pp.1107-1119.
19. Ahirrao SP, Sonawane MH, Bhambere DS, Udavant PB, Ahire ED, Kanade R. Cocrystal formulation: a novel approach to enhance solubility and dissolution of etodolac. Biosciences Biotechnology Research Asia. 2022 Mar 1;19(1):111.

20. Landge, S.B., Jadhav, S.A., Dahale, S.B., Solanki, P.V., Bembalkar, S.R. and Mathad, V.T., 2015. Development and validation of stability indicating RP-HPLC method on core shell column for determination of degradation and process related impurities of apixaban—an anticoagulant drug. American Journal of Analytical Chemistry, 6(06), p.539.
21. Darwish, H.W., Ali, N.A., Naguib, I.A., El Ghobashy, M.R., Al-Hossaini, A.M. and Abdelrahman, M.M., 2021. Development and validation of a stability indicating RP-HPLC-DAD method for the determination of bromazepam. Plos one, 16(3), p.e0244951.
22. Thombre NA, Shaikh HA, Ahire ED. Formulation Development and Evaluation of Colonspecific Esomeprazole Microspheres. Biosciences Biotechnology Research Asia. 2022 Sep 29;19(3):679-91.
23. Abdelaleem, E.A. and Abdelwahab, N.S., 2013. Validated stability indicating RP-HPLC method for determination of paracetamol, methocarbamol and their related substances. Analytical Methods, 5(2), pp.541-545.
24. Bhinge, S.D. and Malipatil, S.M., 2016. Development and validation of a stability-indicating method for the simultaneous estimation of cefixime and dicloxacillin using the RP-HPLC method. Journal of Taibah University for Science, 10(5), pp.734-744.
25. Karunakaran, K., Navaneethan, G. and Elango, K.P., 2012. Development and validation of a stability-indicating RP-HPLC method for simultaneous determination of paracetamol, tramadol HCl and domperidone in a combined dosage form. Tropical Journal of Pharmaceutical Research, 11(1), pp.99-106.
26. Thakker, N.M., Choudhari, V.P., Kuchekar, B.S., Panchal, H.B., Rakholiya, D.R. and Murugan, R., 2012. Development and validation of a stability indicating RP-HPLC method for simultaneous estimation of Olmesartan Medoxomil and Metoprolol Succinate in pharmaceutical dosage form. Pharmaceutical Methods, 3(2), pp.84-89.