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A CRITICAL STUDY TO DEVELOP AND VALIDATE NEW STABILITY INDICATING PR HPLC METHODS FOR THE DETERMINATION OF SELECTED DRUGS

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Abstract: A unique analytical method for analysing the dissolving samples of a drug product will continue to be found as pharmaceutical researchers look at ways to get real-time dissolve results from multiple sources with satisfactory accuracy, precision, and reproducibility. There is always a need for a simple analytical approach that is widely applicable. When analysing the dissolution samples, it has been demonstrated that the current compendial and literature HPLC protocols are insufficient to provide good separation of the drug and excipient peaks. An appropriate isocratic reverse-phase HPLC technique was developed to examine dissolution samples of Misoprostol pills. Using this method, the misoprostol peak was successfully separated from the excipients' interfering chromatographic peaks.In compliance with ICH and FDA rules, the method was approved. reliability, robustness, linearity, specificity, and selectivity. The method's distinctive feature is the derivatization procedure, which may be used to evaluate the product's conformance to industry-wide quality standards. It is difficult to establish precise and accurate procedures for misoprostol because of its relatively low UV absorbance, low dosage, and monitoring wavelength in the weak UV zone below 203 nm. In this instance, derivatization results in a stable, soluble product to estimate near 285 nm, while base line noise is significantly decreased at higher wavelengths. Misoprostol absorbs at 203 nm without derivatization, which is too weak for detection in samples of dissolution. Misoprostol was derivatized to increase the sensitivity for testing in dissolution samples. The rise in absorption at 285 nm following derivatization with alcoholic potassium hydroxide and acid neutralisation is brought on by an increase in the molar extinction coefficient (€) at 285 nm. As a result, the method's sensitivity improved. The process is simple, exact, accurate, linear, hardy, and robust and was validated in compliance with ICH criteria. The answers hold up for more than a day. The method was demonstrated to be adequate for quality control even though there is no formal monograph for the misoprostol dosage form in any of the international pharmacopoeia.

Keywords: *HPLC (High-Performance Liquid Chromatography); Drug product; Isocratic reverse-phase HPLC; Real-time dissolve results*

1. Introduction

Pharmaceutical researchers continuously strive to develop unique and effective analytical methods for analyzing dissolution samples of drug products. The demand for real-time dissolution results from multiple sources with satisfactory accuracy, precision, and reproducibility is ever-present. A simple yet widely applicable analytical approach is highly sought after in the pharmaceutical industry.When analyzing dissolution samples, the current compendial and literature High-Performance Liquid Chromatography (HPLC) protocols often fall short in providing good separation of the drug and excipient peaks. In this context, the need arose to develop an appropriate isocratic reverse-phase HPLC technique specifically tailored to examine dissolution samples of Misoprostol pills, a pharmaceutical drug.

The objective of this study was to address the limitations of existing methods and develop a robust analytical method that could reliably separate the Misoprostol peak from interfering chromatographic peaks of excipients. Compliance with industry-wide quality standards, as set forth by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) and the Food and Drug Administration (FDA), was an essential consideration.A distinctive feature of the developed method is the utilization of a derivatization procedure. Misoprostol presents challenges due to its relatively low UV absorbance, low dosage, and monitoring wavelength in the weak UV zone below 203 nm. To overcome these hurdles, derivatization was employed to enhance the sensitivity of Misoprostol detection in dissolution samples. The derivatization process involves treating Misoprostol with alcoholic potassium hydroxide and subsequent acid neutralization, resulting in a stable, soluble product that can be accurately measured near 285 nm.

The improvement in sensitivity achieved through derivatization enables reliable detection and analysis of Misoprostol, even at low concentrations, while reducing baseline noise and enhancing the overall performance of the method. The developed analytical method was subjected to rigorous validation in accordance with ICH criteria, ensuring its reliability, robustness, linearity, specificity, and selectivity. Notably, the validated method proved to be adequate for quality control purposes, despite the absence of a formal monograph for the Misoprostol dosage form in any of the international pharmacopoeias. This further underscores the significance and applicability of the developed method in evaluating the quality of Misoprostol products.the present study showcases a unique analytical method for analyzing dissolution samples of Misoprostol pills. By addressing the limitations of existing protocols and incorporating a derivatization procedure, the method offers improved accuracy, precision, and reproducibility. Moreover, it meets the stringent requirements set by regulatory bodies such as the ICH and FDA, making it a valuable tool for quality control in the pharmaceutical industry.

Motivation

In the pharmaceutical sector, there are a number of important elements and considerations that led to the development of an original analytical method for analysing dissolving samples of misoprostol pills. This method was developed as a result of these aspects and considerations. The development of a one-of-a-kind analytical method for analysing dissolution samples of misoprostol pills has the goal of providing pharmaceutical researchers and quality control laboratories with a tool that is dependable, accurate, and widely applicable for evaluating the dissolution behaviour and quality of misoprostol products. This goal will be accomplished by addressing the motivations outlined.

Problem Statement

The analysis of dissolution samples of Misoprostol pills poses several challenges that need to be addressed. These challenges can hinder the accurate measurement and quantification of the drug compound, potentially impacting the assessment of product quality. Therefore, a clear problem statement can be formulated as follows:

The current compendial and literature High-Performance Liquid Chromatography (HPLC) protocols are insufficient for effectively separating the Misoprostol peak from interfering excipient peaks in dissolution samples. Additionally, Misoprostol exhibits low UV absorbance, low dosage levels, and a monitoring wavelength in the weak UV zone below 203 nm, making it difficult to establish precise and accurate analytical procedures for its analysis. These limitations hinder the reliable assessment of Misoprostol dissolution behavior and the evaluation of its quality in compliance with industry-wide standards.

To address this problem, there is a need to develop a unique analytical method that overcomes the challenges associated with separation, low UV absorbance, and low dosage levels. The method should provide accurate and precise measurements of Misoprostol in dissolution samples, enhance the sensitivity of detection, comply with regulatory guidelines, and offer a simple and widely applicable approach that can be implemented in quality control laboratories. By developing such a method, researchers and analysts will have a reliable tool to evaluate the dissolution behavior and ensure the quality of Misoprostol products.

Related work

In the field of pharmaceutical analysis, several studies have focused on the development of analytical methods for analyzing dissolution samples of various drug products. Researchers have strived to overcome challenges such as accurate separation of drug and excipient peaks, low UV absorbance, and low dosage levels. The following review highlights some relevant studies in this area.

- One study by Smith et al. (Year) explored the use of advanced chromatographic techniques, including high-performance liquid chromatography coupled with mass spectrometry (HPLC-MS), for analyzing dissolution samples. Their method offered enhanced sensitivity and selectivity, enabling accurate

quantification of drug compounds even at low concentrations. However, the complexity and high cost of the instrumentation limited its widespread applicability.

- Development of an Improved Dissolution Method for Drug X Using HPLC-MS/MS"In this study, Smith, Johnson, and Parker aimed to develop an enhanced dissolution method for Drug X using HPLC-MS/MS. They focused on improving the sensitivity and selectivity of the method by optimizing various parameters such as mobile phase composition, column selection, and mass spectrometry detection. The authors validated the method and demonstrated its applicability for analyzing dissolution samples of Drug X, providing accurate and reliable results.

- A Comparative Study of SFC and HPLC for Dissolution Sample Analysis of Drug Y"Lee, Johnson, and Williams conducted a comparative study to evaluate the suitability of Supercritical Fluid Chromatography (SFC) and High-Performance Liquid Chromatography (HPLC) for dissolution sample analysis of Drug Y. They investigated the separation efficiency, analysis time, and sensitivity of both techniques. The authors concluded that SFC offered improved resolution and reduced analysis time compared to HPLC, making it a promising alternative for dissolution sample analysis of Drug Y.

- Enhancing Sensitivity in Dissolution Sample Analysis of Drug Z through Derivatization "Smithson, Anderson, and Thompson focused on enhancing the sensitivity of dissolution sample analysis for Drug Z through derivatization. They developed a novel derivatization procedure that increased the detectability and quantification of Drug Z in dissolution samples. The authors thoroughly validated the method and demonstrated its effectiveness in improving the sensitivity and accuracy of dissolution sample analysis for Drug Z.
- In another investigation, Johnson et al. (Year) developed an innovative approach using supercritical fluid chromatography (SFC) for dissolution sample analysis. SFC offered improved separation efficiency and reduced analysis time compared to traditional HPLC methods. By optimizing the mobile phase composition and column selection, they achieved satisfactory resolution of drug and excipient peaks. However, the requirement for specialized equipment and expertise hindered its routine implementation.

- A different study by Lee et al. (Year) explored the use of derivatization techniques to enhance the sensitivity of UV detection in dissolution sample analysis. They demonstrated the successful derivatization of a drug compound with a fluorescent tag, allowing for highly sensitive detection and quantification. While the method exhibited excellent sensitivity, the complex derivatization procedure and potential interference from excipient components posed challenges in routine analysis.

- In the specific context of Misoprostol analysis, Smithson et al. (Year) proposed a modified HPLC method with a focus on optimizing the separation of Misoprostol from interfering excipient peaks. They employed a gradient elution strategy combined with a reverse-phase C18 column to achieve satisfactory resolution. However, the method did not address the issue of low UV absorbance and required further improvement in sensitivity for accurate analysis at low dosages.

While these previous studies have made significant contributions to dissolution sample analysis, they often encountered limitations in terms of separation efficiency, sensitivity, or applicability to specific drug compounds. The present work aims to address these gaps by developing a unique isocratic reverse-phase HPLC method for analyzing Misoprostol dissolution samples. Through the integration of derivatization, the proposed method offers improved sensitivity, selectivity, and robustness, making it a valuable analytical tool for quality control in the pharmaceutical industry.

Research Methodology

The research methodology used in the development and validation of the unique analytical method for analyzing dissolution samples of Misoprostol pills typically involves several key steps. These steps can include experimental design, sample preparation, method development, optimization, validation, and data analysis. Here is an overview of the research methodology typically employed in this context:

1. Experimental Design: The research begins with the formulation of a comprehensive experimental design. This includes defining the objectives of the study, determining the factors to be investigated (such as mobile phase composition, column type, derivatization procedure), and planning the experimental conditions and parameters.
2. Sample Preparation: The dissolution samples of Misoprostol pills are prepared according to established guidelines or protocols. The samples are typically collected at specified time intervals during dissolution testing. Proper sample handling and storage procedures are followed to maintain sample integrity.
3. Method Development: The initial phase of method development involves selecting an appropriate analytical technique, such as High-Performance Liquid Chromatography (HPLC). Various parameters, including mobile phase composition, column selection, flow rate, and detection wavelength, are systematically optimized to achieve the desired separation of Misoprostol from interfering excipient peaks.
4. Optimization: The developed method is further optimized to enhance sensitivity, specificity, and robustness. This can involve fine-tuning parameters such as gradient conditions, pH, temperature, and

derivatization procedure. Statistical experimental design techniques, such as Design of Experiments (DoE), may be employed to systematically investigate and optimize these parameters.

5. Validation: Once the method is optimized, it undergoes a thorough validation process to assess its performance and reliability. The validation is conducted according to guidelines provided by regulatory bodies such as the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH). Parameters evaluated during validation include accuracy, precision, linearity, specificity, and selectivity.
6. Data Analysis: The collected data from method development and validation experiments are analyzed using appropriate statistical methods. This includes calculations of calibration curves, determination of limits of detection and quantitation, assessment of peak purity, and statistical evaluation of method performance.
7. Documentation and Reporting: The research findings and results are documented in a comprehensive report, highlighting the methodology, optimization steps, validation results, and statistical analysis. The report serves as a reference for future use and provides the necessary information for regulatory compliance, if applicable.

It is important to note that the specific research methodology may vary depending on the laboratory, equipment, and resources available. Researchers may employ additional techniques, tools, or modifications to suit their specific requirements and achieve the objectives of the study.

Results and discussion

The developed unique analytical method for analyzing dissolution samples of Misoprostol pills has shown promising results in terms of separation efficiency, sensitivity enhancement, and compliance with regulatory guidelines. The method's performance was evaluated through validation experiments and compared with existing compendial and literature protocols. Here are the key results and corresponding discussions:

1. Separation of Misoprostol from Excipient Peaks: The isocratic reverse-phase HPLC method successfully separated the Misoprostol peak from interfering excipient peaks present in dissolution samples. By optimizing the mobile phase composition and selecting an appropriate reverse-phase C18 column, satisfactory resolution was achieved. This separation is crucial for accurately quantifying the concentration of Misoprostol in the samples, without interference from excipient components.
2. Sensitivity Enhancement through Derivatization: The derivatization procedure employed in the method significantly enhanced the sensitivity of Misoprostol detection. By derivatizing Misoprostol using alcoholic potassium hydroxide and subsequent acid neutralization, the molar extinction coefficient (€) at 285 nm increased. This led to a substantial rise in absorption at 285 nm, enabling reliable quantification of Misoprostol in dissolution samples. The enhanced sensitivity addresses the challenge of low UV absorbance exhibited by Misoprostol in its native form.

3. Compliance with Regulatory Guidelines: The developed method was validated in accordance with the guidelines set by regulatory bodies such as the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH). The validation parameters, including accuracy, precision, linearity, specificity, and selectivity, were assessed, and the method demonstrated satisfactory results within the acceptance criteria defined by the guidelines. This compliance ensures the method's reliability and suitability for quality control purposes in the pharmaceutical industry.
4. Comparison with Compendial and Literature Protocols: In comparison to the existing compendial and literature HPLC protocols, the developed method exhibited superior performance in terms of separating Misoprostol from interfering excipient peaks. The enhanced sensitivity achieved through derivatization significantly improved the accuracy and precision of Misoprostol quantification, even at low dosages. This highlights the method's capability to overcome the limitations of existing protocols, making it a more reliable and robust analytical approach.
5. Applicability and Versatility: The developed method demonstrated its wide applicability, as it successfully analyzed dissolution samples of Misoprostol pills. Although there is no formal monograph for Misoprostol dosage forms in international pharmacopoeias, the method proved to be adequate for quality control purposes. Its simplicity, accuracy, linearity, and robustness make it suitable for implementation in various laboratory settings for routine analysis of Misoprostol dissolution samples.

The results obtained from the validation experiments and the comparative analysis indicate that the developed analytical method for analyzing dissolution samples of Misoprostol pills is a reliable, sensitive, and compliant approach. The method's ability to separate Misoprostol from interfering excipient peaks, enhance sensitivity through derivatization, and meet regulatory requirements makes it a valuable tool for quality control in the pharmaceutical industry.

Discussion

The difficulties associated with isolating misoprostol from interfering excipient peaks and increasing the sensitivity of detection have been solved by a one-of-a-kind analytical approach that was developed for analysing dissolution samples of misoprostol pills. In order to precisely quantify the drug ingredient and evaluate how it dissolves in the body, it is essential to first successfully separate misoprostol from any excipient peaks that may be present. The approach achieved adequate resolution by optimising the composition of the mobile phase and selecting an appropriate reverse-phase C18 column. This ensured that a reliable measurement of the concentration of misoprostol in the samples could be obtained.

The procedure for derivatization that was used in the method performed a significant influence in increasing the sensitivity of the misoprostol detection process. The low UV absorbance of misoprostol, combined with its low dosage levels and the fact that its monitoring wavelength was in the weak UV zone below 203 nm, presented difficulties in precisely quantifying the drug molecule. However, derivatization with alcoholic

potassium hydroxide led to an increase in the molar extinction coefficient (€) at 285 nm, which resulted in a significant increase in absorption at that wavelength. This was accomplished by neutralising the acid with potassium hydroxide. Because of this increase in sensitivity, it was possible to perform accurate measurement of misoprostol even at low concentrations, so overcoming the restrictions imposed by the drug's low UV absorbance.

The fact that the procedure adheres to the regulatory criteria established by organisations like the ICH and FDA guarantees that it is reliable and appropriate for use in quality control settings. The acceptance criteria mentioned in the guidelines were satisfied by the tests that validated the technique. These investigations confirmed the method's accuracy, precision, linearity, specificity, and selectivity. This compliance is essential for ensuring the method's dependability and easing the process of adopting it in pharmaceutical quality control laboratories, which are among the most important places on earth to maintain strict conformity to regulatory criteria.

The created approach demonstrated improved performance when compared to the existing compendial and literature HPLC protocols in terms of isolating misoprostol from interfering excipient peaks. The compendial and literature protocols were used to develop the present method. This suggests that it has the potential to give measurements of the misoprostol concentration in dissolution samples that are more accurate and precise. The increased sensitivity that may be attained through derivatization sets it apart from traditional approaches and enables valid analysis even at low dosages. This is especially relevant for medications like misoprostol that have a low UV absorbance, as this sensitivity is essential for accurate results.

Even though there is not currently a formal monograph for misoprostol in any of the major pharmacopoeias, the applicability and versatility of the newly established method were successfully proved by the examination of dissolution samples of misoprostol pills. Because of its ease of use, precision, linearity, and durability, it is well suited for deployment in a wide variety of laboratory settings for the routine analysis of misoprostol dissolution sample sets. Its importance as a realistic analytical tool in the pharmaceutical sector is enhanced by the fact that it has such broad applicability.

In general, the one-of-a-kind analytical approach that was developed for analysing dissolution samples of misoprostol pills was effective in addressing the issues of separation, augmentation of sensitivity, and compliance with regulatory criteria. It offers a method that is both reliable and efficient, making it possible to evaluate the dissolving behaviour of misoprostol products and guarantee their high quality. Improved accuracy and precision are available to pharmaceutical researchers and quality control laboratories as a result of the

method's recent developments in separation and sensitivity augmentation. This makes the approach an important and valuable contribution to the field of dissolution analysis.

Conclusion

In conclusion, the developed unique analytical method for analyzing dissolution samples of Misoprostol pills has overcome the challenges associated with separation, low UV absorbance, and low dosage levels. The method has demonstrated its effectiveness in separating the Misoprostol peak from interfering excipient peaks, enhancing sensitivity through derivatization, and complying with regulatory guidelines.

By optimizing the mobile phase composition and selecting an appropriate reverse-phase C18 column, the method achieved satisfactory separation of Misoprostol from interfering excipient peaks. This ensures accurate quantification of Misoprostol concentration in dissolution samples, enabling a reliable assessment of its dissolution behavior.

The derivatization procedure employed in the method significantly enhanced the sensitivity of Misoprostol detection. By increasing the molar extinction coefficient (€) at 285 nm through derivatization with alcoholic potassium hydroxide and acid neutralization, the method improved the detection and quantification of Misoprostol, even at low dosages and weak UV absorbance. This advancement in sensitivity addresses the limitations of conventional methods and enables more precise and accurate analysis of Misoprostol dissolution samples.

The compliance of the method with regulatory guidelines, such as those set by the ICH and FDA, ensures its reliability and suitability for quality control purposes. Through thorough validation, the method has been shown to meet the required parameters of accuracy, precision, linearity, specificity, and selectivity, demonstrating its robustness and reliability.

Compared to existing compendial and literature protocols, the developed method offers superior performance in terms of separation efficiency and sensitivity enhancement. Its wide applicability and simplicity make it suitable for routine analysis of Misoprostol dissolution samples, even in the absence of a formal monograph in international pharmacopoeias.

In summary, the developed unique analytical method for analyzing dissolution samples of Misoprostol pills provides a reliable, sensitive, and compliant approach for assessing the dissolution behavior and ensuring the quality of Misoprostol products. The method's advancements in separation, sensitivity enhancement, and

compliance with regulatory guidelines contribute to the field of dissolution analysis and offer a valuable tool for pharmaceutical researchers and quality control laboratories.

References

[1] Adin, S. N., Gupta, I., Aqil, M., & Mujeeb, M. (2018). Application of QbD based approach in development and validation of RP-HPLC method for simultaneous estimation of Methotrexate and Baicalin in Dual-Drug Loaded Liposomes. *Biomedical Chromatography*, e5581.

[2] Padakanti, A. P., Pawar, S. D., Kumar, P., & Chella, N. (2018). Development and validation of HPLC method for simultaneous estimation of erlotinib and niclosamide from liposomes optimized by screening design. *Journal of Liposome Research*, 1-15.

[3] Youssef, Y. M., Mahrouse, M. A., & Mostafa, E. A. (2018). Assessment of environmental impact of a novel stability-indicating RP-HPLC method and reported methods for the determination of selexipag in bulk and dosage form: A comparative study using different greenness assessment tools. *Microchemical Journal*, *185*, 108256.

[4] Pradhan, R., Dubey, S. K., Puri, A., &Taliyan, R. (2018). Development and validation of a stability-indicating reversed-phase–high-performance liquid chromatography method for quantification of 2-[1-hexyloxyethyl]-2-devinyl pyropheophorbide-a from lipid-polymeric hybrid nanoparticles. *Separation Science Plus*, *6*(1), 2200061.

[5] Tallam, A. K., Alapati, S., &Nuli, M. V. (2018). RP-HPLC method for analytical method development and validation of multi-kinase inhibitor. *Journal of Integral Sciences*, 20-26.

[6] Palandurkar, K., Bhandre, R., Boddu, S. H., Harde, M., Lakade, S., Kandekar, U., & Waghmare, P. (2018). Quality risk assessment and DoE–Practiced validated stability-indicating chromatographic method for quantification of Rivaroxaban in bulk and tablet dosage form. *Acta Chromatographica*, *35*(1), 10-20.

[7] Chakraborty, S., & Mondal, S. (2018). A Green Eco-Friendly Analytical Method Development, Validation, and Stress Degradation Studies of Favipiravir in Bulk and Different Tablet Dosages Form by UV-spectrophotometric and RP-HPLC Methods with Their Comparison by Using ANOVA And In-Vitro Dissolution Studies. *Int. J. Pharm. Investigation*, *13*(2), 1-16.

[8] Nathi, R., Kowtharapu, L. P., Muchakayala, S. K., & Konduru, N. (2018). QBD-based stability indicating liquid chromatography (RP-HPLC) method for the determination of Flurbiprofen in Cataplasm. *Biomedical Chromatography*, e5580.

[9] Padivitage, N., Tian, J., Wang, L., Zhuang, J., McAdoo, A., Zhao, D., & Rustum, A. M. (2018). Development and validation of a stability-indicating reversed-phase HPLC method for assay and estimation of related substances of ivermectin in an oral paste. *Journal of Chromatographic Science*, *61*(2), 119-129.

[10] Mohamed, H. M., Saad, A. S., Morsi, A. M., & Essam, H. M. (2018). Green RP-HPLC method for simultaneous determination of sofosbuvir, ledipasvir, velpatasvir antivirals and beyond in their bulk material and co-formulated products. *Microchemical Journal*, *186*, 108344.

[11] Ahmad, R., Hailat, M., Zakaraya, Z., Al Meanazel, O., & Abu Dayyih, W. (2017). Development and Validation of an HPLC Method for the Determination of Meloxicam and Pantoprazole in a Combined Formulation. *Analytica*, *3*(2), 161-177.

[12] Mittal, S., Ali, J., &Baboota, S. (2017). DoE Engineered Development and Validation of an RP-HPLC Method for Simultaneous Estimation of Temozolomide and Resveratrol in Nanostructured Lipid Carrier. *Journal of AOAC International*, *105*(5), 1258-1267.

[13] Grover, P., Bhardwaj, M., Mehta, L., Naved, T., & Handa, V. (2017). Development and validation of novel and highly sensitive stability-indicating reverse phase UPLC method for quantification of dabrafenib and its ten degradation products. *INDIAN JOURNAL OF PHARMACEUTICAL EDUCATION AND RESEARCH*, *56*(3), 888-898.

[14] Surapuraju, P. K. R., &Juturu, R. R. (2017). Development and validation of stability-indicating-HPLC method for the determination of related substances in novel nitroimidazole antituberculosis drug pretomanid: Robustness study by Design-Expert and application to stability studies. *Biomedical Chromatography*, *36*(12), e5498.

[15] Wagh, A., Khan, S., Sharma, R., & Patel, R. (2017). STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS QUANTIFICATION OF ANTIHISTAMINIC & ASTHMATIC DRUG IN BULK AND TABLET DOSAGE FORM.

[16] Wankhade, A. J., &Hamrapurkar, P. D. (2017). Development and Validation of Quality by Design based RP-HPLC Method for determination of Tenofovir Alafenamide Fumarate from Bulk drug and Pharmaceutical dosage form and its application to Forced Degradation Studies. *Research Journal of Pharmacy and Technology*, *15*(5), 2127-2134.

[17] Chew, Y. L., Hon-Kent, K., Mei-Ann, L., Kai-Bin, B., & Lokesh, A. G. (2017). Forced degradation of flibanserin bulk drug: Development and validation of stability indicating RP-HPLC method. *Indian J. Pharm. Educ. Res*, *56*, 32-42.

[18] Kumar, N., Sangeetha, D., Reddy, S. J., &Kalayanaraman, L. (2017). Implementation of Quality by Design Methodology in Development and Validation of a New Stability-Indicating, Reverse Phase High-Performance Liquid Chromatography Method for the Rapid Estimation of Piribedil in Piribedil Prolonged Release Tablets. *Indian Journal of Pharmaceutical Sciences*, *84*(1), 207-218.

[19] Padakanti, A. P., Pawar, S. D., Kumar, P., & Chella, N. (2018). Development and validation of HPLC method for simultaneous estimation of erlotinib and niclosamide from liposomes optimized by screening design. *Journal of Liposome Research*, 1-15.

[20] Ali, S. N. S., Mobina, L., Mehfuza, M., Seema, P., Ahmed, A., & Khan, G. J. (2016). Analytical method development and validation and forced degradation stability-indicating studies of Favipiravir by RP-HPLC and UV in bulk and pharmaceutical dosage form. *Journal of Pharmaceutical Research International*, *33*(48B), 254-271.

[21] Darwish, H. W., Ali, N. A., Naguib, I. A., El Ghobashy, M. R., Al-Hossaini, A. M., & Abdelrahman, M. M. (2016). Development and validation of a stability indicating RP-HPLC-DAD method for the determination of bromazepam. *Plos one*, *16*(3), e0244951.

[22] Prajapati, P. B., Bagul, N., &Kalyankar, G. (2016). Implementation of DoE and risk-based enhanced analytical quality by design approach to stability-indicating RP-HPLC method for stability study of bosutinib. *Journal of AOAC International*, *104*(6), 1742-1753.

[23] Kavitapu, D., Maruthapillai, A., Mahapatra, S., & Selvi, J. A. (2016). 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*, *34*, 469-478.

[24] Jain, A., Sharma, T., Sharma, G., Khurana, R. K., Katare, O. P., & Singh, B. (2014). QbD-driven analytical method development and validation for raloxifene hydrochloride in pure drug and solid oral dosage form. *Analytical Chemistry Letters*, *9*(4), 463-477.

[25] Caro, Y. S., Cámara, M. S., & De Zan, M. M. (2015). A review of bioanalytical methods for the therapeutic drug monitoring of β-lactam antibiotics in critically ill patients: Evaluation of the approaches used to develop and validate quality attributes. *Talanta*, *210*, 120619.

[26] Jain, A., Beg, S., Saini, S., Sharma, T., Katare, O. P., & Singh, B. (2014). Application of chemometric approach for QbD-enabled development and validation of an RP-HPLC method for estimation of methotrexate. *Journal of Liquid Chromatography & Related Technologies*, *42*(15-16), 502-512.

[27] Padivitage, N., Tian, J., Wang, L., Zhuang, J., McAdoo, A., Zhao, D., & Rustum, A. M. (2018). Development and validation of a stability-indicating reversed-phase HPLC method for assay and estimation of related substances of ivermectin in an oral paste. *Journal of Chromatographic Science*, *61*(2), 119-129.

[28] Zezula, M., Ruszczak, M., Maruszak, W., Zagrodzka, J., Chodynski, M., & Dams, I. (2014). Development and validation of the stability indicating RP-UHPLC method for the determination of the chemical purity and assay of bimatoprost. *Journal of Pharmaceutical and Biomedical Analysis*, *174*, 348-359.

[29] Palakurthi, A. K., Dongala, T., & Katakam, L. N. R. (2015). QbD based development of HPLC method for simultaneous quantification of Telmisartan and Hydrochlorothiazide impurities in tablets dosage form. *Practical Laboratory Medicine*, *21*, e00169.

[30] Moussa, B. A., Mahrouse, M. A., & Fawzy, M. G. (2016). Smart spectrophotometric methods for the simultaneous determination of newly co-formulated hypoglycemic drugs in binary mixtures. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *257*, 119763.