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Development and validation of paracetamol and tramadol tablet in dosage form by Uv and HPLC method

Himanshu Thakur
Assistant Professor
MJ College Bhiali

Rahul Singh
Principal & Associate Professor
MJ College Bhiali

1.1 GENERAL

Pharmaceutical analysis is a specialized branch of analytical chemistry derives its principle from various branches of sciences physics, electronics and biotechnology. It involves separation, identification and quantification of relative amount of the components in a sample matrix. Pharmaceutical analysis plays a vital exceptional role in the assessment of pharmaceutical formulations and bulk drugs regarding quality control and quality assurance. Analytical methods are a measure of quality of drugs and the methods are required for a variety of reasons throughout the drug development process. It has a comprehensive role in drug development and follow-up activities to assure that a drug product meets the established standard to assure the stability of drug product to meet purported quality throughout the shelf life and also to check the absence of impurities, harmful substances and adulterant.

Pharmaceutical analysis plays a critical role in ensuring the safety, efficacy, and quality of medicines. Analytical methods are fundamental tools for the qualitative and quantitative determination of pharmaceutical compounds in bulk drugs, formulations, and biological samples. Among these, Paracetamol and Tramadol are widely prescribed as a combination therapy for effective pain management, especially in moderate to severe pain conditions. This necessitates the development of robust and validated methods to simultaneously quantify these drugs in tablet dosage forms.

1.2 ANALYTICAL METHOD DEVELOPMENT

Rapid increase in the invention, production of drugs and pharmaceutical industries in and around the world bring ahead a rise in inevitable demand to seek novel and systematic analytical techniques to check the quality standards of drugs. As a result, analytical method development became basic and regular activity in analysis. Modern analytical techniques employ a variety of techniques that vary from simple qualitative chemical test to the use of most sophisticated and expensive computer controlled instruments. Hence the development of fast, robust and well-organized methods for drug analysis is an essential process in order to offer chromatographic and spectroscopic profile to quality control and quality assurance sectors. The advancements in the analytical method development diminish the cost and time of analysis and enhance the precision and accuracy of measurements. Techniques pertaining to analysis of drugs are developed and validated for active pharmaceutical ingredients, excipients, related substances, drug products, degradation products and, residual solvents, etc.

Development of analytical methods includes a wide range of simple and instrumental analytical techniques, but the most widely used methods are based on chromatography and spectroscopy. In the present work analytical methods based on HPLC and UV spectrometry were developed.

1.3 HPLC METHOD DEVELOPMENT

Most of the drugs in single and multi-component dosage forms can be investigated by HPLC method because of the numerous advantages like rapidity, specificity, accuracy, precision and ease of automation in this method. HPLC method removes tedious extraction and isolation procedures. High performance liquid chromatography (or high pressure liquid chromatography, HPLC) is a form of column chromatography used commonly in biochemistry and analytical chemistry to separate, identify, and quantify compounds based on their characteristic polarities and interactions with the column's stationary phase. Some of the advantages of HPLC method are speed of analysis (analysis can be accomplished in 20 minutes or less), greater sensitivity (various detectors can be employed), improved resolution (wide variety of stationary phases), reusable columns (expensive columns but can be used for many analysis), ideal for the substances of low volatility, easy sample recovery, handling and maintenance, instrumentation tends itself to automation and quantitation (less time and less labor), precise and reproducible, calculations are done by integrator itself and appropriate for preparative liquid chromatography on a much larger scale

1.3.1 Steps involved in HPLC Method Development

Method development in HPLC is a complex process that involves a number of steps, which are as follows:

1.3.2 Literature search for information retrieval

When developing a HPLC method the first step is always to consult the literature to find out whether separation has been previously performed, if so, under what conditions. This will save time of doing unnecessary experimental work. This information from literature must contain solubility profile, which includes solubility of drug substance in different solvents and at different pH conditions, next analytical profile, which consist of analytical profile of the drug substance, impurity and degradation products and Stability profile, which includes stability profile of the drug substance with respect to the storage conditions.

1.3.3 Selection of Initial Conditions

This step determines the optimum conditions to retain adequately all analysis. No analysis should have the capacity factor of less than 0.5 because poor retention could result in peak overlapping and no analytic should have capacity factor 10 –15 because excessive retention leads to long analysis time and broad peaks.

1.3.4 Selectivity Optimization

The aim of this step is to achieve adequate selectivity (peak spacing). The mobile phase and stationary phase compositions need to be taken into account. To minimize the number of trail chromatograms involved, only the parameters that are likely to have a significant effect on selectivity in the optimization must be examined. To select these, the nature of the analysis must be considered. The optimization of mobile phase parameters is always considered first as this is much easier and convenient than stationary phase optimization

1.3.5 System Optimization

This is used to find the desired balance between resolution and analysis time after satisfactory selectivity has been achieved. The parameters involved for system optimization includes column dimensions, column packing particle size and flow rate. These parameters may be changed without affecting capacity factors or selectivity.

1.3.6 Method Validation

Proper validation of analytical method is important for a developed method in pharmaceutical analysis, because the analytical methods play an important role in ensuring the continuing efficacy and safety of the products manufactured in each batch, relies solely in the determination of quality. Method Validation is generally a one-time process performed after the method has been developed to demonstrate that the method is scientifically sound and that it serves the intended analytical purpose

1.3.7 Optimization of HPLC method

During the optimization stage, the initial sets of conditions that have evolved from the first stages of development are improved or maximized in terms of resolution, peak shape, plate counts, asymmetry, capacity, elution time, detection limits, limit of quantitation and overall ability to quantify the specific analytic of interest. The manual approach of optimization involves varying one experimental variable at a time, while holding all others constant and recording changes in response. The variables might include flow rates, mobile or stationary phase composition, temperature, detection wavelength and pH. The various parameters that include to be optimized during method development include mode of separation, selection of stationary phase, selection of mobile phase and selection of detector.

1.4 AIM

Pharmaceutical research in the previous decades has resulted in the launch of numerous drugs. The advanced and enormously potent drugs have found their applicability in treating different types of diseases. Quality of drug and drug product is very essential as it involves life, it is determined by testing its purity and quality of active substance in the drug and its formulation. Pharmaceutical analysis plays a vital role in quality control and quality assurance of medicines. To check the quality standards of the medicines various analytical methods are used. These analytical methods are needed to characterize the composition of drug substance and drug product, to control the levels of impurities and also for stability evaluation. Development of new discerning analytical methods together with accuracy and precision is obligatory for assurance of safety, quality and efficacy of drugs and pharmaceuticals.

Nowadays abundant pharmaceutical agents are marketed in various dosage forms either as single drug component or in combination with other drug. Therefore, it is absolutely necessary to develop novel methods and to introduce them for controlling the quality of drugs in pharmaceutical dosage forms. Generally analytical methods are developed and validated for analysis of active pharmaceutical ingredient, excipients, drug products, related impurities and degradation products.

In the current circumstances, there is a great need for development of new analytical methods with high sensitivity collectively with precision and accuracy, became mandatory. Hence the prime focus of this work is towards the development of sensitive, accurate, rapid and economical analytical methods for separation, identification and quantification of medicinal compounds in pharmaceutical dosage form. The present analytical research, describes about the development of analytical methods for quantification of drugs in selected pharmaceutical dosage form by using HPLC and Ultraviolet spectroscopic techniques.

1.5 OBJECTIVE OF THE WORK

The main objectives of the work comprise of three major method development parts and validation of the developed methods, which are described below:

Optimization for simultaneous estimation of Paracetamol and tramadol by HPLC method employing response surface design.

HPLC method development for the determination of following drugs in pharmaceutical dosage forms.

Simultaneous estimation of Paracetamol and its impurities in tablet dosage form.

Simultaneous estimation of tramadol and its preservatives in oral liquid dosage form

UV spectroscopic method development for the determination of following drugs in pharmaceutical dosage forms

Simultaneous estimation of Paracetamol and tramadol by simultaneous equation method in combined tablet dosage form.

Validation of the developed methods according to ICH guidelines using the following parameters

Accuracy, Precision (System precision, Method precision, Intermediate precision), Linearity and Range, Limit of detection (LOD), Limit of quantitation (LOQ), Robustness, Specificity and Forced degradation

1.6 Need for Combination Therapy: Advantages in Pain Management

Paracetamol (acetaminophen) is a well-known analgesic and antipyretic agent. It is extensively used for the treatment of mild to moderate pain and fever. Tramadol, on the other hand, is a centrally acting synthetic opioid analgesic with a dual mechanism of action, providing pain relief by inhibiting the reuptake of norepinephrine and serotonin while binding to opioid receptors. The combination of these two drugs offers synergistic pain relief, reducing the need for higher doses of individual agents and minimizing the risk of adverse effects. This makes it a preferred choice in clinical practice for managing moderate to severe pain effectively and safely.

1.7 Importance of Analytical Methods in Pharmaceutical Quality Control

The simultaneous presence of Paracetamol and Tramadol in a single formulation poses challenges for analytical quantification due to their overlapping spectral and chromatographic properties. Analytical methods such as ultraviolet (UV) spectrophotometry and high-performance liquid chromatography (HPLC) are essential for ensuring the quality and consistency of pharmaceutical formulations. These methods provide accurate, reliable, and reproducible results, enabling the detection of impurities, assessment of drug stability, and confirmation of drug content in formulations. The application of validated analytical methods is critical for compliance with regulatory standards and for maintaining patient safety.

1.8 Significance of the Study

In recent years, UV spectrophotometry and HPLC have emerged as reliable techniques for the analysis of pharmaceutical compounds. UV spectrophotometry is a simple, cost-effective, and rapid method for routine analysis, whereas HPLC provides high specificity, sensitivity, and precision, making it suitable for complex formulations. This study focuses on the development and validation of UV spectrophotometric and HPLC methods for the simultaneous estimation of Paracetamol and Tramadol in tablet dosage forms. Validation of these methods ensures their reliability and reproducibility, addressing key parameters such as specificity, linearity, precision, accuracy, and robustness. The findings of this research will contribute to the pharmaceutical industry by providing validated methods that can be employed in routine quality control and regulatory compliance.

1.9 Overview of Tramadol

A brief history of development: A German pharmaceutical company, synthesized tramadol for the first time in 1962. Originally created as a less dangerous substitute for other opioids, Tramadol was released onto the market in the latter part of the 1970s. Because of its special pharmacological characteristics, which combine inhibition of monoamine reuptake with opioid receptor activation, it became well-known as a useful tool for treating moderate to severe pain. Tramadol has become widely accepted over the years in a number of medical specialties, from the treatment of neuropathic pain to general pain management. The pharmaceutical industry relies heavily on the development and validation of analytical methods to ensure the high quality, safety, and efficacy of the pharmaceuticals produced. Since tramadol is a commonly used opioid analgesic, precise quantification and qualification of its presence in different formulations necessitates the employment of stringent analytical procedures. The process of creating an analytical method for Tramadol entails choosing a suitable methodology, such as gas chromatography (GC) or high-performance liquid chromatography (HPLC), among others. Contrarily, method validation guarantees that the created procedure is dependable, repeatable, and appropriate for the goal for which it was designed. Parameters including accuracy, precision, specificity, linearity, and robustness are evaluated during this process. To achieve consistent production of Tramadol with optimal therapeutic efficacy and to comply with regulatory criteria, method development and validation must be integrated.

2. REVIEW OF LITERATURE

The literature survey was carried out to identify the drugs and drug combinations and their formulations for which the analytical method development was justified. The literature review provides a detailed account on the several analytical methods such as HPLC, HPTLC, LC-MS and Ultra-violet visible spectrophotometry employed for the selected drugs either individually or in combination with other drug in active pharmaceutical ingredient (API), pharmaceutical dosage forms and in the samples from biological origin. The reported methods for selected drugs and drug combinations are described in the following section

Choksi V., Vasava D., et al (2013) Method Development and Validation of Second Order Derivative Spectrophotometric Method for Simultaneous Estimation of Diclofenac Sodium and Thiocolchicoside from its Pharmaceutical Formulation. The objective of this study was to evaluate the utility of derivative spectrophotometric method for analysis of Diclofenac sodium (DIC) and Thiocolchicoside (THIO) in combination. Derivative spectrophotometry has allowed specific determination of Diclofenac sodium at 249 nm with negligible contribution by Thiocolchicoside. Similarly, Thiocolchicoside was determined at 246nm with negligible interference by Diclofenac sodium. Thus from the results obtained it can be concluded that proposed method is simple, rapid and specific.

Kamble D. N., Mahajan S. S., et al (2013) Development and validation of RP-HPLC method for simultaneous estimation of eperisone hydrochloride and diclofenac sodium in bulk and pharmaceutical dosage form. A simple, selective, precise and accurate Reverse Phase High Pressure Liquid Chromatographic (RP-HPLC) method was developed and validated for the simultaneous estimation of eperisone hydrochloride and diclofenac sodium in marketed sustained release (SR) capsules. The method was validated for accuracy, specificity, linearity and robustness as per the ICH guidelines. The method was successfully applied to estimate eperisone hydrochloride and diclofenac sodium in marketed SR capsules since there was no interference from the excipients. 3

Alagar R. M., Godavari S., et al (2013) Analytical method development & validation of Eperisone Hydrochloride and Diclofenac Sodium in Rapisone D SR Capsules by RP-HPLC. The Chromatographic separation was carried out on a Thermo Hypersil C-8 column (250×4.6mm, 5µm) by gradient elution mode. Themobile phase consists of Methanol, Phosphate Buffer (0.1M, pH 6) and Acetonitrile in the ratio of 30:40:30 (v/v/v) pumped at a flow-rate

of 1.0 ml/min. The PDA detector was employed at a wavelength of 261nm. The retention times of Diclofenac Sodium & Eperisone Hydrochloride were found to be 2.21 min and3.6 min respectively. The proposed method is rapid, simple and does not require any separation process and it has been successfully applied to the assay of pharmaceutical dosage form (RAPISONE D SR capsules).

S. Bhati, H. Padaliya et al (2013) developed and validated RP-HPLC Method for Simultaneous Estimation of Eperisone Hydrochloride and Diclofenac Sodium in Bulk and Pharmaceutical Dosage form. The sample was analyzed using 50mM ammonium acetate buffer containing 0.2% triethylamine (pH-4.0 adjusted with glacial acetic acid): Acetonitrile (40:60, v/v), as a mobile phase at a flow rate of 1.0 ml/min. and detection at 273 nm. The method was validated in terms of accuracy, precision, linearity, limit of detection, limit of quantitation, robustness and ruggedness as per ICH guidelines.

Vemula Babu R. V., Sharma K. P. (2013) developed and validated RP-HPLC method for simultaneous estimation of Diclofenac and Tolperisone in tablet dosage form. Chromatography was carried out isocratically at 30°C ± 0.5°C on an XDB C-18 column (4.6 x 150mm, 5µ particle size) with a mobile phase composed of acetonitrile -phosphate buffer pH-3.4 (30:70% v/v) at a flow rate of 1.0 mL/min. Detection was carried out using a PDA detector at 260 nm. Validation parameters were studied as per ICH guidelines. This method is fast, accurate, precise and sensitive hence it can be employed for routine quality control of tablets containing both drugs in industries.

M Sharaf El-Din, Eid M., et al (2013) developed Simultaneous determination of methocarbamol and aspirin by RP-HPLC using fluorescence detection with time programming: its application to pharmaceutical dosage form. The analysis was performed at a flow rate of 1.0 mL/min with fluorescence detection at 277/313 nm for MET and 298/410 nm for ASP using real-time programming. The proposed method was successfully applied for the analysis of both MET.

Chaitanya Prasad M., Mamillapalli V., et al (2013) developed a simple UV spectrometric for the estimation of Methocarbamol in Bulk and its formulation. The objective of the work is to obtain an analytical method for analysis of active substance in bulk drug and in the finished product. From the literature survey it was found that Methocarbamol was used extensively in Pharmaceutical solid unit dosage form as alone or with combination with other active ingredient and excipient, but till now no experiment is conducted on analysis of methocarbamol by UV spectroscopy using propylene glycol as solvent.

Nataraj K.S., Reddy S., et al (2013) developed and validated RP-HPLC method development and validation for the simultaneous estimation of Ibuprofen- Methocarbamol Caplets. The degradants formed were well separated from the main peaks. The separation was achieved by isocratic elution on Inertsil ODS-3V Column (150 mm x 4.6mm x 5 µm) and 0.1MPhosphate buffer pH 3.5: methanol: acetonitrile and tetrahydrofuran (20:25:55:0.3) used as a mobile phase at a flow rate of 1ml/min. Developed RP-HPLC method was found to be accurate, precise, selective and rapid for simultaneous estimation of Ibuprofen and Methocarbamol in tablets.

M. Sharaf El-Din, Eid M. & Zeid A.M., et al (2013) developed and validated Simultaneous determination of methocarbamol and ibuprofen in their binary mixtures using HPLC method with fluorescence detection: application to combined tablets. The separation of these compounds was achieved within 7.0 min on a CLC Shim-pack C8 column (250 × 4.6 mm, 5-µm particle size) using isocratic mobile phase containing a mixture of methanol and 0.05 Mdihydrogen phosphate buffer (75:25, v/v) at pH 6.5. The analysis was performed at a flow rate of 1.0 mL/min with fluorescence-detection at 295 nm after excitation at 224 nm. The results were favorably compared to those obtained by the USP reference methods.

Ashokan A. S., Mathew M., et al (2013) developed and validated of UV spectrophotometric methods for simultaneous estimation of tolperisone hydrochloride and diclofenac sodium in tablet dosage form. This present

study reports the simultaneous quantification of Tolperisone hydrochloride and Diclofenac sodium in the bulk drug and tablet dosage form employing simultaneous equation and absorbance ratio method. The accuracy and reliability of the method was assessed by linearity, precision (intra-day % RSD and inter-day % RSD of Tolperisone hydrochloride and Diclofenac sodium) and specificity in accordance with ICH guidelines.

Sabitha M., Mahaboobsubhani, et al (2014) developed and validated Analytical method for simulataneous estimation of diclofenac sodium and thiocolchicosde in tablet dosage form by using RP-HPLC. A Waters Symmetry ShieldeRp18, (250*4.6*5µ) column with mobile phase containing water pH 9.2adjusted with di-Potassium Hydrogen Phosphate: Methanol in the ratio of (60: 40, v/v) was used. The flow rate was 1.0 mL/min, column temperature was 30°C and effluents were monitored at 223 nm. The proposed method was validated with respect to linearity, accuracy, precision, specificity, and robustness. Recovery of Diclofenac Sodium and Thiocolchicoside in formulations was found to be in the range of 97-103% and 97-103%respectively confirms the non-interferences of the excipients in the formulation.

Sivakumar N., Chenthilnathan A., et al (2014) developed and validated of RP-HPLC method for the quantitative determination of diclofenac sodium in pharmaceutical dosage form. The chromatography was carried out by using HPLC system (Shimadzu LC2010HT) with UV- Visible dual absorbance detector (PDA), Phenomenex C18, 25 cm X 4.6 mm, 5 µm column. The mobile phase consisting of Acetonitrile and Buffer (1.0 g of Potassium dihydrogen orthophosphate in 40 ml of water and adjusted the pH to 3.0 with dilute orthophosphoric acid) in the ratio of 60:40. Validation parameters such as system suitability, specificity, linearity, accuracy, precision robustness, ruggedness and stability were performed according to the ICH guidelines for the proposed method and the results obtained were within the limits. Hence, the method could be successfully applied for routine analysis of diclofenac sodium in pharmaceutical dosage forms.

Jigar P., Pinak P., (2014) developed and validated RP-HPLC method for the estimation of Diclofenac sodium, Tramadol Hydrochloride and Chlorzoxazone form their combined tablet dosage form. The objective of the current study was to develop and validate the RP-HPLC method for the simultaneous estimation of Tramadol Hydrochloride, Chlorzoxazone and Diclofenac sodium from their combined tablet dosage form. The mobile phase used was Acetonitrile: 0.05M Disodium Hydrogen Phosphate buffer pH 3.5 adjusted with 10% v/v Ortho Phosphoric acid (50:50 v/v) and Hypersil ODS C18 (250 mm x 4.6 mm, 5.0 µ particle sizes) was used as a stationary phase with detection wavelength of 220 nm. The method was found to be simple, accurate, precise, and suitable for the estimation of Tramadol Hydrochloride, Chlorzoxazone and Dicofenac sodium from their combined tablet dosage form.

Patel R.H., Joshi S.H., et al (2014) developed and validated of spectrophotometric methods for simultaneous estimation of diclofenac sodium and serratiopeptidase in pharmaceutical dosage form. The methods employed were (A) Q – Absorbance ratio and (B) Area under curve (AUC). Method – A involves measurement of absorbance at 242.20 nm (iso-absorptive point) and 272.40 nm (λmax of Diclofenac sodium). Method – B involves measurement of area at two wavelength range 258.0 – 268.0 nm and 280.0 – 290.0 nm. In both methods linearity for detector response for Diclofenac sodium and Serratiopeptidase were found over the concentration range of 7 – 35 µg/ml and 100 – 500 µg/ml respectively. The method was validated according to ICH guidelines.

Priyanka M., Pritosh P., et al (2014) developed and validated stability indicating HPLC assay method for methocarbamol in Bulk formulation. A suitable HPLC having a isocratic system equipped with manual injector, UV detector is used or this work. The HPLC separation was achieved on HITACHI L2130 with D 2000 Elite Software with Isocratic with UV-Visible Detector (L-2400), stationary phase was Develosil ODS HG -5 RP 150mm × 4.6 mm 5µm particle size. The mobile phase used in this analysis consists of phosphate Buffer and Acetronitrile in a ratio of 70:30. Stock sample is prepared by using aectonitrile and buffer working sample used is about 50

ppm. Flow rate maintained is about 0.8 ml/minute and wave length is about 225 nm. Sample colour is Ambient. Injection volume injected about 5µL with run time 06 minutes.

Vijayasree P., Devika G.S., et al (2014) developed a Simple UV Spectrophotometric Estimation of Methocarbamol by Co–Solubilization Technique. The present work attempts to minimize the time consumption and cost by simple spectrophotometric method by co- solubilization technique based on the use of acetone and 0.1N sodium hydroxide solution used in ratio of 1:9 as a solvent system. Here acetone acts as a co solvent. The drug has an absorption maximum at 267 nm and obeys Beer-Lambert's law in the concentration range of 5–25 µg/ml with correlation coefficient value of 0.999. The proposed method was successfully applied to the determination of methocarbamol in bulk and pharmaceutical dosage forms.

Hirpara M., Patel P., Patel N., et al (2015) developed and validated of analytical method for simultaneous estimation of diclofenac sodium and benzocaine in gel dosage form. The present work involves the development and validation of RP-HPLC method and UV Visible Spectroscopic method for the estimation of Diclofenac Sodium and Benzocaine in gel dosage form. Benzocaine and Diclofenac Sodium has absorbance at 321.5 nm and 233.5nm respectively The linearity was obtained in concentration range 0.6-3.0 µg/ml and 4-20 µg/ml for Diclofenac Sodium and Benzocaine respectively for all UV-Visible method.

Mali A. D., Jadhav S., et al (2015) developed and validated of UV Spetrophotometric estimation of Diclofenac sodium bulk and tablet dosage form using area under curve method. A simple, precise, accurate and economical UV visible spectrophotometric method has been developed for estimation of Diclofenac sodium drug by AUC method. The standard and sample solutions were prepared by using double distilled water as a solvent. The proposed method has been validated as per ICH guidelines.

Syed Mujtaba A., Priyadarsini L.R., et al (2015) RP-HPLC method development and validation for simultaneous Estimation of diclofenac sodium and serratiopeptidase in Tablet dosage form. A novel, simple, sensitive and rapid Chromatographic (RP-HPLC) method has been developed for simultaneous estimation of NSAIDS (Serratiopeptidase and Diclofenac sodium) from pharmaceutical formulation. The developed method was validated in terms of accuracy, precision, linearity, limit of detection, limit of quantitation and solution stability. The proposed method can be used for the estimation of these drugs in combined dosage forms.

Nagamani M., Ramana H., et al (2015) developed a UV- Visible method for the estimation of methocarbamol in bulk and pharmaceutical formulation. Methocarbamol used extensively in pharmaceutical solid unit dosage form as alone or combination with other active ingredient for the estimation of Methocarbamol in UV at λmax274nm by using methanol as a solvent. Detector response was linear in the concentration of 10-50µg/ml. The interday and intraday variation was found to be less than 1%. The mean recovery of the drug from the solution was 100.24%.

Maslarska V.N., Tsvetkova B.G., et al (2015) developed and validated RP-HPLC method for simultaneous determination of Paracetamol, Diclofenac sodium and Famotidine in Tablet Dosage form. The chromatography was carried out on a C18 (250 mm x 4.6 mm, 10 µm) column with acetonitrile: water: 0.5% trimethylamine: o-phosphoric acid (60:20:5:15v/v) as mobile phase, at a flow rate of 1.0 ml/min, with detection at 240 nm. Separation was completed in less than 10 min. The results of the studies showed that the proposed RP-HPLC method is rapid, precise and accurate, which can be applied for the routine assessment of described drugs in pharmaceutical dosage forms.

N.P. Patel et al (2016) developed and validated UV Spectroscopic method for simultaneous estimation of Methocarbamol and Diclofenac sodium in injection dosage form using solvent composed of methanol: water (10:90). Estimation of Methocarbamol and Diclofenac sodium was carried out at 274.11 nm (max. wavelength of

MET) and 282.45 nm (isoabsorptive point) for Absorbance Ratio method and 223.92 nm for Diclofenac sodium (ZCP of Methocarbamol) and 281.69 nm for Methocarbamol (ZCP of Diclofenac sodium) for First order derivative spectroscopy method. Methocarbamol and Diclofenac sodium were found to be linear over the range of 12-60 µg/ml and 2-10 µg/ml respectively for both the methods. The method was validated statistically and studied for linearity, precision, accuracy, LOD and LOQ. The obtained results proved the method can be employed for the routine analysis of Methocarbamol and Diclofenac sodium in injection dosage form.

N.P. Patel et al (2016) developed and validated RP-HPLC method for simultaneous determination of Methocarbamol and Diclofenac sodium in injection dosage form. The chromatography was carried on BDS Hypersil C18 (250 mm x 4.6 mm, 5µm) column and with Mobile phase Composition of Phosphate buffer: Methanol (30:70 v/v), pH 4.5 with Orthophosphoric acid at a Flow rate of 1ml/min and detection was carried out at 281 nm. RP- HPLC method was found to be linear over the range of 3-9 µg/ml for Diclofenac sodium and 18-54 µg/ml for Methocarbamol. The method has been validated for Linearity, Precision, Accuracy, LOD, LOQ and System suitability according to ICH Q2 (R1) guideline

Bhavani Podili109 et al. (2017) have developed in this study, high performance chromatographic method has been developed and validated for the estimation of Paracetamol (PC), Aceclofenac (AF), and Serratio peptidase (SP) in combined tablet dosage form. The chromatography was carried out on a phenomenex C18 (Luna) coloumn (250mmX 4.6mm, 5µm) with a mobile phase consisting of buffer containing 1gm heptanesulfonic acid in 1lit water (adjusted to pH 2.5 with ortho phosphoric acid) acetonitrile (90:10 v/v) at a flow rate of 1.0 mL/min and ultraviolet detection at 226 nm. The retention time of PC, AF and SP were 3.119, 7.196 and 13.560 minutes respectively.

Vijaya Kumar Meher solanki et al. (2022) In this review articles, the development, formulation, and manufacture of drugs, analytical method development & validation play a critical role. Methods are developed for ensuring purity, identity, potency, and performance of pharmaceutical products. Methods should be applied to the extent that they are sufficient for their intended purpose. Throughout the life cycle of a drug product and substance, a range of activities are associated with developing and validating methods. An objective of method validation is to prove that the procedure can be used as intended. Once the method is developed, validation is performed. Different national and international committees have defined the parameters for method validation. The International Conference on Harmonization attempted to harmonize pharmaceutical applications. In accordance with the ICH, other organizations define Linearity, Selectivity/Specificity, Range, Accuracy, Precision (repeatability, intermediate precision, and reproducibility), Limit of quantitation, Limit of detection, Ruggedness, and Robustness

Yuvrajsinh solanki et al. (2023) Sildenafil citrate and Tramadol hydrochloride used in treatment of Premature ejaculation. Tramadol HCl is thought to exert its therapeutic action in PE patients through one or more of the following mechanisms: weak µ-opioid effect, 5-HT2 receptor antagonist effect, N-Methyl-D-aspartate receptor antagonist effect, serotonin and norepinephrine reuptake inhibitory effect, and acetylcholine receptor antagonist effect. On the other hand, Sildenafil citrate is thought to play a therapeutic role in treating PE though the following mechanisms: peripheral delay of ejaculation through modulation of contractions of the vas deferens, seminal vesicles, prostate and urethra, increasing the duration of erection , central decrease of the sympathetic output via modulation of NO activity in the medial pre-optic area , peripheral analgesic effect, peripheral analgesic effect, increasing patient confidence, and improving the perception of ejaculation control and sexual satisfaction.

Zakira Chaudhary et al. (2024) Acute lymphoblastic leukemia (ALL) can affect both children and adults, with a peak incidence between ages one and four. Most cases occur in otherwise healthy individuals, with few having identifiable risk factors. The disease is characterized by chromosome abnormalities and genetic changes that

affect lymphoid precursor cells.Outcomes have improved significantly for children and young adults due to tailored treatment strategies, but older adults and those with relapsed or refractory ALL still face poor prognosis. New immunotherapy options, like CAR T-cell therapy and monoclonal antibodies, are being developed to enhance treatment.ALL is part of a broader group of lymphoid cancers, and distinguishing it from other cancers involves analyzing its specific morphological, immunophenotypic, and genetic traits. Current aggressive chemotherapy regimens cure about 85-90% of children and 40-50% of adults, but results can vary based on the disease's genetic subtype and clinical features at diagnosis. Monitoring minimal residual disease is essential for assessing prognosis and optimizing treatment.

3. SCOPE AND PLAN OF WORK

3.1 Scope

Analytical methods are a measure of quality of drugs, which play a comprehensive role in drug development process, formulation development process and in the follow-up activities. The analytical method developments are needed to assure that a drug product meets the established standard, it is stable and also to guarantee that the drug will continue to meet the purported quality right through its shelf life. The determination of drugs in single or multi-component dosage form using extraction methods or by other simple analytical procedures are too difficult, due to the presence of different additives, excipients and their interference with the results. To overcome the above constrains, instrumental methods of analysis were employed in the recent decades. Generally analytical methods for determination of drugs require accurate, sensitive, and precise results, shorter analysis time and also economy of the conduct of the study. Hence to fulfill all the above mentioned requirements and also for the reason of economy and easy availability, few optimized analytical methods were designed by exploiting the advantages of chromatographic and spectroscopic techniques. In the present research, modern analytical instruments like HPLC and UV spectrometry were selected to develop novel analytical methods which enable to achieve specificity, sensitivity, accuracy, reproducibility, reliability, rapidity and economy of the study. These instrumental methods have wide scope to overcome all the insufficiency and drawbacks of drug analysis.

3.2 Justification for the selection of drugs and methods

Selection of drug combination for method development by optimization The literature survey revealed that few HPLC methods have been reported for the simultaneous estimation of the drugs, Paracetamol, and tramadol. However, no chemo metric method development for optimization of chromatographic system by HPLC was reported up to now. Hence in the current work an attempt was made to develop, optimize and validate an accurate and sensitive and robust HPLC method for the simultaneous determination of selected drug combination in tablet dosage form by using response surface methodology

3.3 Selection of drugs and drug combinations for HPLC method development

The literature survey revealed that few analytical methods have been reported for estimation of dapagliflozin alone or in combination with other drugs by UV spectrometry, and HPLC. However, there is no method was reported about the separation and determination of dapagliflozin impurities. Hence an attempt was made to develop a simple, accurate, precise and sensitive HPLC method for the estimation of dapagliflozin in presence of its impurities.

Paracetamol and tramadol The literature survey revealed that few HPLC methods are available for the simultaneous estimation of Paracetamol and tramadol peptidase in combination dosage form. But there is no UV spectrophotometric method is reported for the drug combination. Hence in the present work a simple and sensitive UV spectrophotometric method was developed for the simultaneous estimation of Paracetamol and tramadol in combination dosage form.

4. PLAN OF WORK

4.1 Plan

The plan of work was divided into following steps

Literature survey The literature survey was carried out for the selection of drugs and consequently to acquire the information about the physic-chemical properties of the selected drugs such as solubility, polarity, molecular weight and ionic characters etc.

Preliminary studies The properties like solubility, melting point and UV absorption behavior of the reference standards of selected drugs were determined.

3. Development of analytical methods

I. HPLC method development by Chemometric optimization

Selection of chromatographic key factors for optimization

Selection of levels of factors using central composite design

Execution of trials

Response surface analysis of selected factors on the observed responses.

Statistical and graphical analysis of the results

II. HPLC method development

Selection of chromatographic mode of separation, stationery and mobile phase, detection wavelength and elution method.

Optimization of chromatographic conditions by conducting trials

III. UV spectrophotometric method development

Selection of solvent for drugs or common solvent for drug combinations by conducting solubility study

Determination of -max by scanning the standard solution in the range of 200-400 nm.

4. Validation of the developed analytical methods The developed methods were validated as per ICH guidelines

5. Forced degradation studies (Stability indicating method) For the conduct of forced degradation the samples were exposed to the following conditions

Acid hydrolysis

Base hydrolysis

Oxidation with peroxide

Exposure to light and heat

MATERIALS AND METHODOLOGY

5.1 Materials

Signoflam tablet paracetamol 500 mg lupin ltd, mumbai, and Tramadol has a bioavailability of approximately 75% when taken orally. It is quickly absorbed and goes through first-pass metabolism when taken orally. Chemo metric approaches were applied for simultaneous optimization of chromatographic parameters and for better understanding of the interaction of several chromatographic factors on the separation quality. In the present work chemo metric optimization was conducted to reduce the overall assay development time and to obtain essential information regarding the sensitivity of different chromatographic separation attributes. And also to develop accurate and specific analytical method for the determination of selected complex mixture of drug sample the novel chemo metric optimization method was employed.

5.2 Selection of common solvent

The solubility study was conducted by using various solvents. Finally, phosphate buffer pH 6.8 was selected as a common solvent for all the three drugs.

5.2.1 Preparation of standard stock solutions

Accurately weighed amount of Paracetamol, Aceclofenac and Serrati peptidase each 100 mg were transferred to separate 100 ml volumetric flask. All the drugs were dissolved and diluted to 100 ml with phosphate buffer to obtain the standard stock solution of 1000 µg/ml concentration of each drug.

5.2.2 Preparation of working standard solutions

From the paracetamol stock solution 2.5 ml, was transferred to the 100 ml volumetric flask and diluted to the volume with phosphate buffer. From the aceclofenac stock solution 1 ml was transferred to the 200 ml volumetric flask and diluted to the volume with phosphate buffer. From the serrati peptidase stock solution 15 ml was diluted to 200 ml and from the resulting solution 1 ml was diluted to 100 ml with phosphate buffer. After the dilutions the concentrations of paracetamol, aceclofenac and serratiopeptidiase working standards were found to be 25 µg/ml, 5 µg/ml and 0.75 µg/ml respectively.

5.2.3 Sample solutions

Twenty tablets were weighed and powdered. A quantity of tablet powder equivalent to 100 mg of paracetamol was weighed, dissolved in 50 ml of phosphate buffer, sonicated for 15 minutes, the volume was made up to 100 ml with the same solvent and filtered. From the above solution 2.5 ml was transferred to a 100 ml volumetric flask and the volume was made with the same solvent. This solution contains 25 µg/ml, 5 µg/ml and 0.75 µg/ml of paracetamol and tramadol respectively.

6. CONCLUSION

This review article has provided a comprehensive analysis of paracetamol and Tramadol, highlighting its pharmacological properties, therapeutic applications, and the importance of validated analytical methods in its analysis.

Tramadol's dual mechanism of action, involving both opioid receptor agonism and the inhibition of neurotransmitter reuptake, underpins its effectiveness in treating a variety of pain conditions, particularly neuropathic pain. Despite its classification as a weak opioid, Tramadol remains a vital option in pain management due to its favorable safety profile compared to stronger opioids. Clinical Relevance: In clinical practice,

Tramadol continues to be a valuable analgesic, particularly for patients who require pain relief but are at risk of opioid dependence or have contraindications to stronger opioids. The drug's efficacy is closely tied to its pharmacokinetic and pharmacodynamics properties, which vary among individuals, emphasizing the need for personalized dosing strategies. The review of analytical methods underscores the necessity of robus

Validation processes to ensure the accuracy and reliability of paracetamol and Tramadol measurements in both pharmaceutical formulations and biological matrices.

In conclusion, Tramadol's unique pharmacological profile, combined with the rigor of validated analytical methods, ensures its continued relevance in pain management. However, its use must be carefully managed to maximize therapeutic benefits while minimizing potential risks.

The evolving landscape of pain management and opioid regulation will undoubtedly influence the future role of Tramadol in clinical practice

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