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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE DETERMINATION OF DICYCLOMINE HYDROCHLORIDE AND DICLOFENAC POTASSIUM BY UHPLC IN SOLID DOSAGE FORMS

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ABSTRACT

In pharmaceutical dosage forms, a new UHPLC method was developed and validated to simultaneously estimate Diclofenac Potassium and Dicyclomine Hydrochloride. By utilizing UHPLC, this method fills a gap in the existing literature. Optimizing various parameters of the chromatographic process was key to developing an effective method for separating and detecting drugs. International guidelines and regulatory requirements were followed for the validation of the method, including specificity, linearity, accuracy, precision, robustness, and suitability for the system. For the injection and analysis of mixed solutions containing Dicyclomine HCl and Diclofenac potassium, a Chromatographic separation was achieved on a Kromasil C18 (4.6 mm × 250 mm), 5µm.a mobile phase ratio consisting of (35:65) Water: Methanol at flow rate 1.0 ml/min, and total run time 8min, The injection volume 20µl. The detection wavelength is 263 nm. Approximately 2000 plates were found, indicating a successful chromatographic separation. With a tailing factor of less than 2 and a well-resolved peak, the peaks appear symmetrical and well-resolved. In order to ensure there were no interfering peaks, the retention times for Dicyclomine Hydrochloride and Diclofenac Potassium were found to be 2.745 min and 3.341min.As for Dicyclomine Hydrochloride, the correlation coefficient (R^2) is 0.9999 and Diclofenac Potassium is 0.9999. This indicates a good linear relationship between drug concentrations and peak areas based on the high correlation coefficients. For Dicyclomine Hydrochloride and Diclofenac Potassium, the %RSD values were 0.87% and 1.08%, respectively, below the acceptable limit of 2%. As a result, the method is accurate and repeatable. In the study of Dicyclomine Hydrochloride, the mean percent recovery was 98.37%, while in the study of Diclofenac Potassium, the mean percent recovery was 98.31%, while the mean percent recovery was 100.34%.Under varied conditions, both Dicyclomine Hydrochloride and Diclofenac Potassium showed %RSD values within the acceptable range of 2%, demonstrating the robustness and reliability of the method. This study concluded that valuable insight is provided into the use of validated UHPLC methods in this study, which contributes significantly to the evolution of pharmaceutical analytical techniques.

KEYWORDS: Diclofenac Potassium, Dicyclomine Hydrochloride, UHPLC, Chromatography, Method validation.

INTRODUCTION

The nonsteroidal anti-inflammatory drug (NSAID) diclofenac potassium, chemically known as potassium [2-(2,6-dichlorophenyl)amino]phenyl]acetate, is widely used to relieve pain and temperature. Dicyclomine Hydrochloride, on the other hand, is an antispasmodic antacid for the digestive tract with the chemical name 2-(diethylamino)ethyl-1-cyclohexylcyclohexane-1-carboxylate HCl. The drug exerts its effects by affecting both acetylcholine receptors and smooth muscle at the same time (musculotropic).[1] For Diclofenac Potassium and Dicyclomine Hydrochloride estimation in pharmaceutical dosage forms, various analytical methods, including UV, HPLC, and HPTLC, have been reported.[2] In bulk drugs and pharmaceutical formulations, however, there has been no reported method for simultaneous estimation of these two drugs with HPLC. To obtain simultaneous measurements of Diclofenac Potassium and Dicyclomine Hydrochloride in pharmaceutical dosage forms, the present study developed and validated a new UHPLC method.

For quality control and batch release of pharmaceutical products, it is important to determine the simultaneous presence of multiple drugs in a formulation. Using this method, a comprehensive analysis is provided of the

composition of the drug, enabling accurate quantification of each component.[3] The high selectivity, sensitivity, and reproducibility of HPLC make it an important technique in pharmaceutical analysis. Diclofenac Potassium and Dicyclomine Hydrochloride are simultaneously measured using chromatographic methods that optimize various chromatographic parameters. These parameters include the choice of a suitable stationary phase, the composition of the mobile phase, the pH, the temperature, and the wavelength of detection.[4]

In this experiment, we aim to separate both drugs adequately, resolve their differences, and detect their effects. It is vital to validate the developed analytical method to ensure its reliability and accuracy. In addition to specificity and linearity, accuracy, precision, robustness, and system suitability are all evaluated.[5] In accordance with international guidelines and regulatory requirements, such as those of the International Conference on Harmonisation and United States Pharmacopeia, validation studies are conducted.

Diclofenac Potassium and Dicyclomine Hydrochloride can both be measured simultaneously in pharmaceutical dosage forms with the developed and validated UHPLC method. This method can be utilized for routine analysis in quality control laboratories, ensuring the consistency and reliability of drug products on the market. Further, it provides a valuable tool for research and development, allowing the study of interactions between drugs, formulation optimization, and stability studies.[6]

Therefore, the development and validation of an UHPLC method for simultaneous measurement of Diclofenac Potassium and Dicyclomine Hydrochloride fills a gap in the existing literature. By using this method, pharmaceutical formulations can be tested for quality control and batch release with high accuracy and reliability. It supports the development of safe and effective pharmaceutical products by contributing to the advancement of pharmaceutical analysis.

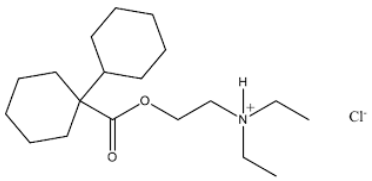

Figure 1: Chemical structure of Dicyclomine Hydrochloride.

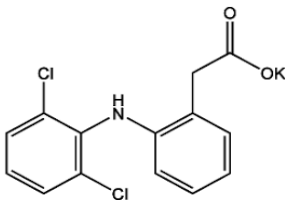

Figure 2: Chemical structure of Diclofenac Potassium.

CHROMATOGRAPHY

UHPLC

Separating, identifying, and quantifying components in a mixture is accomplished using ultrahigh performance liquid chromatography (UHPLC). Analytes are separated more quickly and efficiently using high-pressure liquid chromatography. The Chromatograph-UHPLC is well-known for performing separations with a high degree of resolution and sensitivity. The reason for this is that smaller particles allow for a larger surface area and greater separation efficiency. A high-pressure system can also provide faster elution times and higher flow rates, increasing the overall system throughput.[7]

Chromatography-UHPLC also has the advantage of being versatile. A wide variety of sample types can be analyzed using this device, including biological fluids, environmental samples, and pharmaceuticals. Aside from that, it is useful for both qualitative and quantitative analyses, making it indispensable for a variety of purposes. As a whole, the Chromatograph-UHPLC is an excellent analytical technique that is capable of delivering highly accurate and precise results. For modern analytical laboratories, its versatility, high resolution, and efficiency are essential features.

MATERIALS AND METHODS

Chemicals used

The study was conducted using Methanol, Water of HPLC grade, and Mono Basic Sodium Phosphate of AR grade. A grade of orthophosphoric acid can also be used (AR Grade). An oral tablet containing Dicyclomine HCL (20 mg) and Diclofenac potassium (50 mg) were used.

Solvents used

Solubility of drugs preparations is influenced by many solvents, including water, methanol, acetonitrile, and buffers. There is a partial soluble state in Water: Methanol (95:50) and also a free soluble state in Water: Methanol (20:80), Water: Methanol (35:65). Therefore, we selected Water and Methanol (30:65) as the solvent.

Preparation of Standard stock solution

Weigh accurately and transfer about 40 mg of Dicyclomine Hydrochloridestandard and 50mg of Diclofenac potassium standard into 200 ml volumetric standard flask. Dissolve and makeup to the volume with Methanol.

Preparation of Standard solution

Transfer, 5ml from standard stock solution into 50ml volumetric flask makeup to the volume with Mobile phase.

Preparation of Diluent

As a result of drug solubility, methanol and mobile phase were chosen as diluents

Selection of wavelength

We used 35:65 ratio of water and methanol as solvents. Compounds with absorbance at 263nm can be estimated by UHPLC due to their good absorbance at this wavelength.

UHPLC Condition criteria

It is important to select the appropriate analytical method depending on the nature of the sample (ionic, ionizable, or neutral molecules), the molecular weight, the pKa value, and the stability of the sample. In the present study, Dicyclomine HCl and Diclofenac potassium were selected as polar drugs. Consequently, ion exchange and reversed-phase chromatography methods are suitable for separating and analyzing them. Due to its simplicity and compatibility with the sample characteristics[8], Ultra-High Performance Liquid Chromatography (UHPLC) was initially selected.A literature survey and knowledge of the properties of the selected drugs led to the selection of the stationary phase and size of 4.6 mm x 250 mm column of C18 (4.6 mm x 250 mm). In order to determine the composition of the mobile phase, different combinations of methanol, water, monobasic sodium phosphate, and orthophosphoric acid were tested.

On the basis of the available data and observations, the initial separation conditions were determined.Chromatographic conditions, including the ratio of mobile phase components, were varied systematically during the method development. In addition to analyzing each trial, chromatograms were also evaluated. After several trials, a mobile phase composition of Water: Methanol in the ratio of 35:65 was found to provide satisfactory separation with the shortest retention time.In addition, the flow rate of the mobile phase was varied from 1 mL to 2 mL for further optimization. With a 1 mL flow rate, sharp peaks were obtained. The subsequent studies were therefore conducted at this flow rate.

A chromatographic system, with a mobile phase consisting of Water:Methanol in a ratio of 35:65 and a flow rate of 1 mL, was developed to inject and analyze mixed solutions containing Dicyclomine HCl and Diclofenac potassium. It was confirmed that a C18 column was capable of separating the two drugs from the chromatograms.

Validation Parameters[9]

System suitability

We determined the system suitability parameters by injecting five times standard solutions of Dicyclomine Hydrochloride & Diclofenac Potassium and measuring peak tailing, resolution, and USP plate count. There should be no more than 2% RSD in the area of five standard injection results.

Specificity

In order to optimize the method, interference must be checked. The retention times of the blank and placebo should not be interfered with at all by the interfering peaks of blank and placebo during this method. It was therefore said that this method was specific.

Precision

Preparation of standard solution

Measure and transfer 40 mg of Dicyclomine Hydrochloride standard to a 200ml volumetric standard flask, and 50 mg of Diclofenac potassium standard to a 100ml volumetric standard flask. Make up to the volume with Methanol after dissolving. Fill the volumetric flask with mobile phase after adding 5ml from standard stock solution.

Preparation of sample solution

Diclofenac potassium and Dicyclomine Hydrochloride sample powder is accurately weighed into 100 ml volumetric flasks equivalent to 50mg. Methanol should be used to dissolve and make up the volume. In a 50ml volumetric flask, dilution 5ml of the above solution with mobile phase is made up to the volume of the flask by diluting 5ml further.

Linearity

In order to determine the linearity of detector response, a graph with concentration versus area is plotted for 40mg of Dicyclomine Hydrochloride and 50mg of Diclofenac potassium, and the correlation coefficient is determined. To manage the concentrations of Diclofenac potassium Dicyclomine Hydrochloride, we prepared a series of solutions and injected them into the HPLC system. These solutions ranged in concentration from 50, 75, 100, 125, and 150 %.

Accuracy[11]

Preparation of Standard stock solution

Measure accurately and transfer about 40 mg of Dicyclomine Hydrochloride to a 200ml volumetric standard flask and 50ng of Diclofenac potassium to a 100ml volumetric standard flask. The solution should be dissolved in Methanol and made up to the volume. Fill a 50ml volumetric flask with 5ml from each stock solution.

Solution Preparation (80, 100 and 120%)

Using a volumetric flask with a 100 ml capacity, weigh accurately equivalent doses of 40 mg, 50 mg, and 60 mg of powdered Diclofenac potassium and 32, 40, and 48 mgof Powdered Dicyclomine Hydrochloride. Methanol should be added to dissolve the solution and make up the volume. Then, filter the solution and add 5ml of mobile phase to 50ml volumetric flask and mix to volume.

Procedure

The developed analytical method was evaluated by preparing standard solutions of Dicyclomine HCl and Diclofenac potassium at 80%, 100%, and 120% of target concentration. As a result, these solutions were injected into the chromatographic system. In order to calculate the recovery values, the amount of each drug in the sample was determined and compared with the amount

added. Multiple injections of Dicyclomine HCl and Diclofenac potassium were used to determine their mean recovery values. In order to assess the accuracy of the method, the mean recovery values were compared to theoretical values (80%, 100%, and 120%).Dicyclomine HCl and Diclofenac potassium were simultaneously estimated using the accuracy study, revealing its reliability and precision. There should be a 98.0% to 102.0% recovery rate for each level.

Robustness[12]

A variety of method conditions were intentionally introduced to test the robustness of the method. We performed the analysis under four different flow rates: "Flow minus," "Flow plus," "Wavelength decreasing," and "Wavelength increasing." The samples were injected twice with these variations. In spite of these robustness conditions, the chromatographic system suitability parameters did not change. A robust and reliable method was demonstrated by meeting all system suitability parameters. In addition, the relative standard deviation (%RSD) values, a measure of precision, were well within acceptable limits. In spite of robustness conditions, Dicyclomine HCl and Diclofenac potassium were found to be consistent, further demonstrating the method's reliability.

Assay of Marketed Preparation[13]

Preparation of Standard solution

In a 50 ml volumetric flask, pipette out 5 ml of standard stock solution and fill it with mobile phase to a volume of 25 ml. Record the peak area response for each of the replicate six preparations and calculate it.

Preparation of Sample solution

Make up a volumetric flask to 25 ml with mobile phase by pipetting out 5 ml of the stock solution into the flask. Record the peak area response from the replicate six preparations.

RESULTS AND DISCUSSION

Table 1: Validation Parameters for Dicyclomine Hydrochloride and Diclofenac Potassium.

Parameters		LIMIT	DICYCLOMINE HYDROCHLORIDE	DICLOFENAC POTASSIUM
Linearity Regression equation (Y=mx+c)		R^2not less than 0.999	R^2 = 0.9999	R^2 = 0.9999
Assay (% mean assay)		90.0% -110.0%	99.85%	99.40%
Specificity		No interference of any peak	Complies	Complies
Method Precision %RSD		NMT 2.0%	0.87%	1.08%
Intermediate Precision Day-01 %RSD		NMT 2.0%	0.61%	1.00%
Intermediate Precision Day-02 %RSD		NMT 2.0%	0.59%	0.72%
Accuracy %		98-102%	98.11% to 100.18%	98.31% to 100.34%
Robustness	FM	RSD NMT 2.0%	0.61%	0.62%
	FP	RSD NMT 2.0%	0.73%	1.05%
	WM	RSD NMT 2.0%	0.77%	1.13%
	WP	RSD NMT 2.0%	0.90%	0.62%

*FM-Flow rate minus; FP- Flow rate Plus; WM- Wavelength Minus and WP- Wavelength Plus

Table 2: Assay of marketed formulation.

Sample	Label claim	Peak area*	Amount obtained*	Percent label claim% w/w*	SD	%RSD
Dicyclomine HCL	20 mg	3746.036	19.969	99.85%	7.05	0.19
Diclofenac potassium	50mg	1546.953	4.699	99.40%	14.32	0.93

*Each value is a Mean of six readings

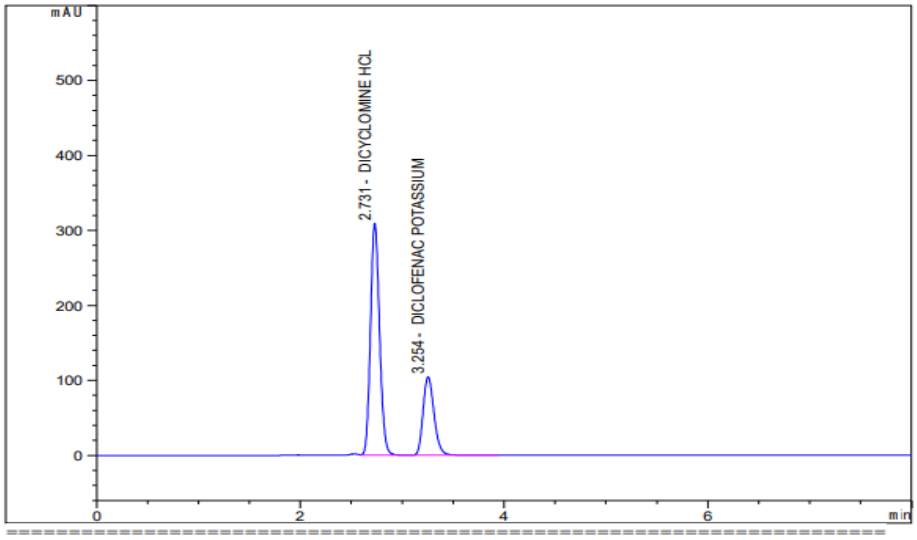

Figure 3: Typical Chromatogram.

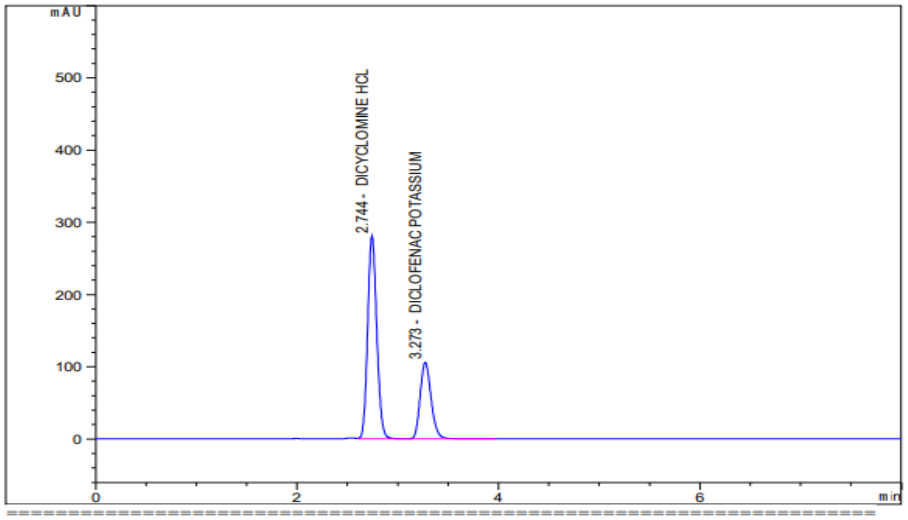

Figure 4: Chromatogram of Standard

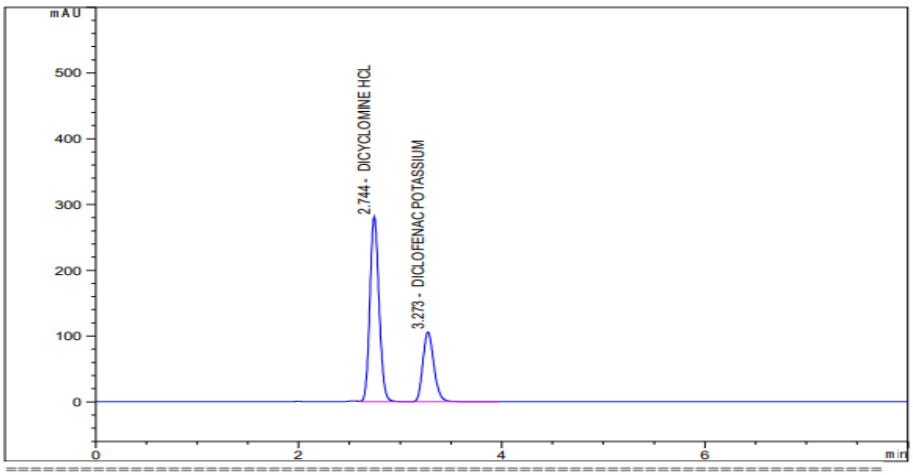

Figure 5: Chromatogram of Sample.

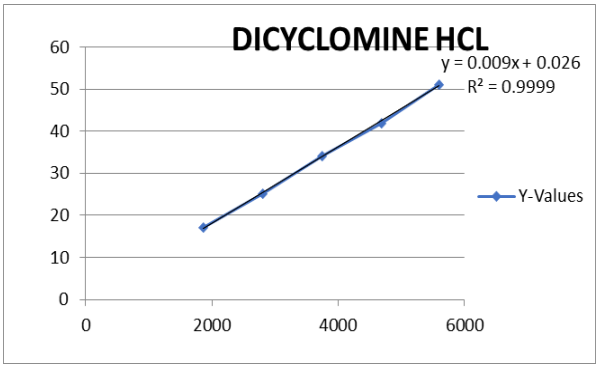

Figure 5: Linearity profile of Dicyclomine HCL.

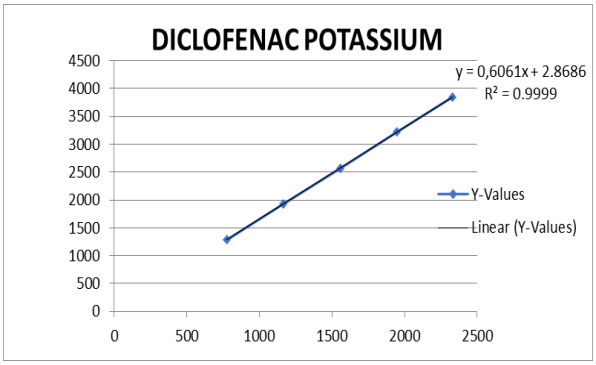

Figure 6: Linearity profile of Diclofenac Potassium.

In accordance with the International Council for Harmonisation's guidelines, a method was developed for the simultaneous estimation of Diclofenac Potassium and Dicyclomine Hydrochloride in pharmaceutical dosage forms.In terms of system suitability parameters such as plate count, tailing factor, and resolution, the parameters met the guidelines' acceptable limits. It was found that there were more than 2000 plates, indicating that chromatographic separation was efficient. It appears the peaks are well-resolved and symmetrical with a tailing factor lower than 2. As a result of the resolution being greater than 2, the analytes were well separated. A robust and reliable method has been developed as a result of these results.

Dicyclomine Hydrochloride and Diclofenac Potassium retention times were tested for specificity to ensure there were no interfering peaks. Neither blank nor placebo samples showed interfering peaks, demonstrating the specificity of the method.Dicyclomine Hydrochloride and Diclofenac Potassium were injected in duplicate at five different concentrations to determine the linearity of the method. In order to calculate the linearity equations, we used the average areas obtained.

There is a correlation coefficient (R2) of 0.9999 for Dicyclomine Hydrochloride and 0.9999 for Diclofenac Potassium. Based on these high correlation coefficients, it is clear that drug concentrations and peak areas have an excellent linear relationship. By injecting multiple volumes of the working standard solution into a volumetric flask, the precision of the method was assessed. Dicyclomine Hydrochloride and Diclofenac Potassium were measured for average area, standard deviation, and percent relative standard deviation. For Dicyclomine Hydrochloride and Diclofenac Potassium, the %RSD values were 0.87% and 1.08%, respectively, below the acceptable limit of 2%. As a result, the method is accurate and repeatable.Three levels of accuracy were assessed using the different sample weight method to assess the accuracy of the method. Each level was tested in duplicate, and the mean %Recovery was determined. In the study of Dicyclomine Hydrochloride, the mean percent recovery was 98.37%, while in the study of Diclofenac Potassium, the mean percent recovery was 98.31%, while the mean percent recovery was 100.34%. Based on these values, the method indicates satisfactory accuracy and the ability to recover the desired amount of drugs.By changing the flow rate and wavelength of the method, robustness testing was conducted[14]. In these robustness conditions, multiple injections were evaluated for system suitability parameters and %RSD values were calculated. Under varied conditions, both Dicyclomine Hydrochloride and Diclofenac Potassium showed %RSD values within the acceptable range of 2%, demonstrating the robustness and reliability of the method[15].

CONCLUSION

In conclusion, the developed UHPLC method for the simultaneous estimation of Dicyclomine Hydrochloride and Diclofenac Potassium in pharmaceutical dosage forms meets the requirements outlined in the ICH guidelines. The method exhibits good specificity, linearity, precision, accuracy, and robustness. It provides a reliable and efficient approach for routine analysis and quality control of these drugs in various pharmaceutical formulations.

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