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NOVEL SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS DETERMINATION OF ACECLOFENAC AND CURCUMIN IN DEVELOPED DOSAGE FORM

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Keywords:

Rheumatoid arthritis, Aceclofenac, Curcumin, UV-spectrophotometric method, Simultaneous determination, Absorbance correction method

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ABSTRACT: Introduction: Aceclofenac (ACE) and Curcumin (CUR) are anti-inflammatory drugs utilized for osteoarthritis and rheumatoid arthritis. ACE, As NSAID inhibits prostaglandins, while CUR, a natural polyphenol, exhibits antioxidant and anticancer properties. Combining them may offer synergistic benefits for inflammatory diseases. **Objective:** To develop a UV spectrophotometric method for simultaneous determination of ACE and CUR from a developed dosage form, focusing on simplicity, Accuracy and cost-effectiveness. The method employs absorbance correction to calculate drug concentrations based on their absorbance difference at two wavelengths. **Method:** Using a solvent of phosphate buffer pH 5.5: ethanol (1:1), standard solutions of ACE (5-30 µg/mL) and CUR (1-7 µg/mL) were prepared. UV spectrophotometer measured absorbance at 276 nm and 430 nm for both standard and sample solutions. Absorbance correction equations were used for concentration determination. **Result:** Method validation followed ICH guidelines, demonstrating good linearity (correlation coefficients: 0.999 for ACE, 0.998 for CUR), accuracy (recovery values: 92.72% for ACE, For cur) and precision (RSD < 2% for intra-day and inter-day variations). Specificity was confirmed, and method robustness was evaluated by varying buffer pH, solvent ratio, and wavelength, showing no significant impact. Limits of detection (LOD) and quantification (LOQ) were 0.16 µg/mL and 0.49 µg/mL for ACE, and 0.08 µg/mL and 0.25 µg/mL for CUR. **Conclusion:** The proposed UV spectrophotometric method offers a simple, accurate, and cost-effective approach for the simultaneous determination of ACE and CUR from a developed dosage form, suitable for routine analysis in quality control laboratories.

INTRODUCTION: Rheumatoid arthritis (RA) is common and long-lasting joint inflammation disease that can impair and disable adults [1]. About 1% of people worldwide have RA, with more than 3 million new cases every year [2].

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This condition happens when the immune system attacks the body's cells and causes too many inflammatory cells and molecules to harm the joints outside the body's core, such as pain, stiffness, swelling, inflammation of the joint lining, joint deformity, serious damage, and even early death [3].

To ease the symptoms and prevent further harm to the bones and cartilage, RA treatments involve using different drugs, such as painkillers, steroids, anti-inflammatory drugs, and drugs that change how the disease works [4].

However, these drugs can cause very dangerous side effects, such as serious liver damage, lung infection, kidney damage, low white blood cell count, reduced bone marrow function, and scarring of the liver [5]. The main cause of these side effects is that the drugs spread throughout the body and affect other parts that are not the target [6]. To overcome these challenges and address RA and its associated outcomes and symptoms, finding a safe, efficacious, and innovative treatment method is essential [7]. Arthritis may be managed effectively by herbal medicines, which are a potential alternative therapy with promise [8].

Topical formulations are essential for treating rheumatoid conditions as they help alleviate the negative effects linked with oral administration, thus becoming the preferred option for addressing inflammation in these medical conditions [9]. The nanoemulgel, known for its outstanding carrier properties, demonstrates effectiveness in delivering lipophilic drugs [10].

This carrier system showcases improved solubilization efficiency for lipophilic molecules, resulting in notable enhancements in drug loading. As a result, the bioavailability of lipophilic drugs is heightened since they are enclosed within finely dispersed oil droplets, aiding their penetration through the skin and subsequent transfer into the systemic circulation [11].

In this study, Nanoemulsion was developed using the Spontaneous emulsification method. Nanoemulsions, characterized as transparent (or translucent) and thermodynamically stable and mixtures of oil and water, are upheld due to an interfacial film consisting of surfactant and co-surfactant molecules, showcasing droplets with sizes smaller than 100 nm [12].

These nanoemulsions function as efficient drug delivery vehicles, increasing the drug loading and solubility of weakly soluble and permeable drugs to increase their bioavailability [13]. Because of their diminutive size, Nanoemulsions can easily enter the uneven surface of the skin, facilitating deeper drug permeation into the skin layers. Moreover, Nanoemulsions can act as reservoirs for sustained drug release, preventing excessive drug concentrations in the bloodstream over an extended period [1]. Aceclofenac is a strong nonsteroidal anti-inflammatory medication (NSAID) that has several issues because of its low solubility [14, 15]. Since, it is essentially water-insoluble, using a nanoemulsion is essential to increasing its solubility. An approach that shows its potential for enhancing medication solubility is nanoemulsion technology. Aceclofenac acts by inhibiting the cyclooxygenase enzyme, which lowers the generation of prostaglandins [16]. Encapsulating aceclofenac in Nanoemulsion (NE) technology can potentially increase skin permeability, which would improve the efficacy of treating rheumatoid arthritis. Aceclofenac is often given for the symptomatic alleviation of inflammation & pain in rheumatoid arthritis. Moreover, the gel formulation leads to better therapy since, in comparison to other topical formulations, it increases skin surface moisture and promotes better drug penetration through the skin.

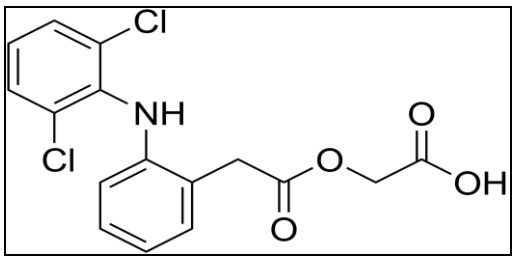

FIG. 1: CHEMICAL STRUCTURE OF ACECLOFENAC

Curcumin, derived from a herbal source, faces solubility challenges in water and necessitates enhancement. Curcumin has a variety of functions, such as anti-inflammatory properties, but its main ones include inhibiting of cyclo-oxygenase, lipoxygenase, metabolsing of arachidonic acid, cytokines (tumour necrosis factor and interleukins), nuclear factor-KB, and release of steroidal hormones [17]. Recent research has illustrated the efficacy of using nanoemulsion or nanoemulsion-loaded gel as a strategy to increase the effectiveness of formulations containing curcumin [18].

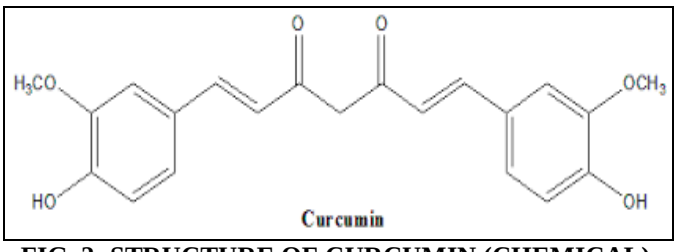

FIG. 2: STRUCTURE OF CURCUMIN (CHEMICAL)

Hence, the combination of aceclofenac and curcumin will have a combined effect as they both inhibit the prostaglandin biosynthesis and inhibition of the Cyclooxygenase (COX) enzyme [19]. In the present research work a novel spectro-photometric method was optimized for simultaneous determination of Aceclofenac and Curcumin *via* the Absorbance correction Method. Formulations containing a combination of these two drugs are currently not available in the market. Individual analysis of drugs has been performed and this combination has not been done before hence with this method of absorbance correction method it is an easy and convenient method to analyze both drugs at a time with time saving and cost-effective [20].

METHODS & MATERIALS

Materials: Aceclofenac was sourced from Kaushik Therapeutics (P) Ltd in Mumbai, and Curcumin was received as gift samples from Sunpure Extracts Pvt Ltd. Ethanol (AR Grade) was procured from S D Fines Mumbai (India) Ltd. Additionally, Potassium dihydrogen phosphate, Disodium hydrogen orthophosphate, Buffer salts, and Hydrochloric acid were also obtained from S.D. Fines Mumbai (India) Ltd. All the chemicals and solvents utilized in the experimentation were of analytical grade

Instrumentation: The UV-visible spectro-photometry was performed using a Shimadzu 1900 spectrophotometer having spectral bandwidth of 1 nm. Spectroscopic measurements were conducted using a set of 1.0 cm engaged quartz cells in between the wavelength ranging of 200-800 nm. Furthermore, pH measurements were performed using a Labman Scientific Instruments digital pH meter and sample weighing was done using a Citizen analytical weighing balance.

Standard Stock Solution Preparation: An appropriately measured amount of Aceclofenac (10mg) and Curcumin (10mg) was shifted into a 10 mL volumetric flask.

The flask was then filled with a mixture of phosphate buffer (pH5.5) and ethanol (1:1) to create a standard solution with concentrations of Aceclofenac (1000 µg/mL) and Curcumin (1000 µg/mL). Subsequently, this solution was moreover diluted to achieve concentrations of 10 µg/mL for both Aceclofenac and Curcumin.

Absorption Correction Method: In the process of determining the λmax (wavelength of maximum absorbance), we analyzed working standard solutions across the range of 200 to 800 nm. Specifically, for Aceclofenac, the λmax was identified at 276 nm, while for Curcumin, it was observed at 430 nm. Interestingly, Curcumin exhibited interference at 276 nm, whereas Aceclofenac remained unaffected at 430 nm. To create Beer-Lambert plots for Aceclofenac and Curcumin, we prepared a stock solution of both drugs in a 1:1 mixture of phosphate buffer (pH 5.5) and ethanol. Additionally, we obtained Beer-Lambert plots for a mixture containing both Aceclofenac and Curcumin in a 1:1 ratio at various concentrations.

The quantitative estimation of Aceclofenac and Curcumin in the mixture was conducted using the following equation:

$$c = A/ab.$$

In this equation, a represents the absorbance, a corresponds to the absorbance at a wavelength of 276.0 nm with a path length of 1 cm (1% solution), and b is the path length (which is 1 cm). The concentration (c) is expressed in micrograms per millilitre (µg/ml) or grams per 100 millilitres. To determine the corrected absorbance of Aceclofenac, we subtracted the absorbance of Curcumin at 276 nm from the absorbance of the mixture. Additionally, we calculated Cx as the corrected absorbance of Aceclofenac at 276 nm divided by the absorptivity of Aceclofenac at the same wavelength. Similarly, Cy represents the concentration of Curcumin at 430 nm divided by the absorptivity of Curcumin at 430 nm.

Validation of Method: The analytical procedure was validated following the ICH Q2B guidelines. This validation process covered essential parameters such as Linearity, Accuracy, Precision, Robustness, Limit of detection (LOD), and Limit of quantitation (LOQ). To evaluate the accuracy and standard addition method was employed, assessing the percentage recovery for both Aceclofenac and Curcumin.

Range and Linearity: As per the guidelines established by the International Council for Harmonisation (ICH), the linearity using an analytical method is determined by its capacity to produce test results that directly correspond to the concentration or amount of the analyte in the sample.

The analytical procedure covers both the highest and lowest concentrations or quantity of the analyte in the sample, indicating the method's suitability in terms of precision, accuracy, and linearity. With respect to these established guidelines, the linearity of an analytical method is characterized by its ability to generate test outcomes that exhibit a direct relationship with the concentration or specified range. The range of an analytical procedure represents the span that encompasses the highest and lowest concentrations or analyte amounts in the sample. This range serves as a showcase for the analytical method's suitability in terms of precision, accuracy, and linearity.

In this study, we established standard curves for curcumin and aceclofenac across specific concentration ranges. Specifically, the concentration range for curcumin spanned from 1 to 7 µg/ml at a wavelength of 430 nm, while for aceclofenac, it extended from 5 to 30 µg/ml at 276 nm. Additionally, we evaluated the linearity of a mixture containing both drugs: at 430 nm (within the range of 1-7 µg/ml) and at 276 nm (within the range of 3-18 µg/ml). To achieve this, we conducted the experimental procedure three times, calculating the standard curve equation and regression coefficient using the average absorbance value at each concentration

Accuracy: The accuracy of an analytical procedure intends to the broader agreement between the determined value and an accepted reference or true value. This aspect of accuracy is often referred to as trueness. Percent recovery is a measure used to express accuracy, and it is determined by multiplication of the ratio of the measured drug concentration to the anticipated drug concentration by 100, yielding the percentage recovery. This calculation provides insight into how closely the analytical results align with the expected or true values, offering a quantitative measure of accuracy in the analytical procedure.

The % recovery by the proposed method was calculated by using the formula as below.

$$\text{Recovery} = (A-B) / C \times 100$$

Where A = Amount of drug estimated (mg). B = Amount of drug present on a pre-analyzed basis (mg). C = Sufficient amount of bulk drug added (mg).

Precision: The validation process of assay tests and determination of quantitative impurities involves a thorough investigation of precision.

Repeatability: The assessment of repeatability involves conducting 6 determining tests at 100% of the test concentration. The relative standard deviation (RSD) should not exceed 2%, which serves as an indicator of good repeatability. Additionally, the precision of the method was evaluated through both intraday (within the same day, at three different times) and interday (on different days, at varying time intervals) measurements.

Robustness: The method's resilience was evaluated by analyzing the phosphate buffer under different pH conditions specifically at pH 5.5, 5.3, and 5.7.

Limit of Detection: The detection limit (DL) is the minimum amount of analyte in a sample that can be found but is not always measured precisely. To find the DL, we analyze samples with known analyte amounts and see the lowest level that we can detect reliably.

$$DL = (3.3\sigma / S)$$

Where σ is the standard deviation of the response and S is the slope of a calibration curve.

Limits of Quantification: The quantitation limit for an individual analytical procedure denotes the minimum amount of analyte in a sample that can be measured precisely and accurately. It acts as an indicator of the quantitative method's capability to detect low levels of compounds in sample matrices, especially impurities and degradation products. To establish the quantitation limit (QL), we analyze samples with known analyte quantities and pinpoint the lowest concentration level that we can measure with both precision and accuracy.

$$QL = (10\sigma/S)$$

Where σ is the standard deviation of the response and S is the slope of a calibration curve.

Preparation and Analysis of Nanoemulgels Containing Aceclofenac and Curcumin: To prepare a nanoemulgel, Aceclofenac and Curcumin were integrated into a Nanoemulsion. This Nanoemulsion was subsequently combined with Carbopol® Ultrez 10 NF, serving as the gelling agent. Additional components included meglumine, a preservative, and deionized water (q.s). For analysis, 0.1g of the formulation containing gel.

Aceclofenac and Curcumin was transferred into a 100 ml of volumetric flask. The sample underwent dissolution in phosphate buffer at pH 5.5, using a 1:1 mixture of ethanol by sonication for 20 minutes. After filtration, the solution was utilized for the determination of Aceclofenac and Curcumin. A placebo gel treatment in the same way served as a blank to prevent interference from other gel ingredients during spectrophotometric analysis.

RESULTS AND DISCUSSION: The above research, it has endeavoured to create UV-spectroscopic techniques in concurrently assessing ACE (Aceclofenac) and CUR (Curcumin) in pharmaceutical dosage forms. We successfully devised straightforward spectroscopic methods to estimate ACE and CUR simultaneously from their respective pharmaceutical formulations. Additionally, we employed the Absorbance Correction method to analyze diffusion samples. Our developed methods underwent validation according to the ICH guidelines for various parameters.

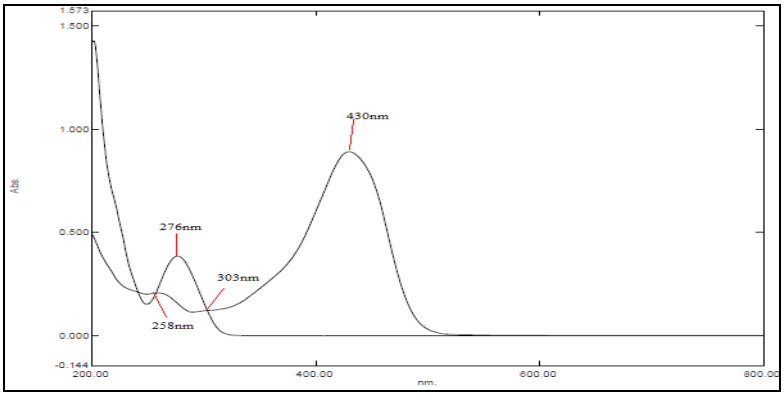

FIG. 3: SPECTRAL OVERLAY OF ACECLOFENAC AND CURCUMIN

The standard curve for Aceclofenac and Curcumin was constructed using UV-spectrophotometry at absorption maxima of 276nm and 430nm, respectively, in a solvent mixture of Ethanol (1:1): PBS 5.5 (n=3). When examining the UV spectra of these drugs in the phosphate buffer pH 5.5: ethanol (1:1) solution, it's observed that two of them Aceclofenac and Curcumin absorbed at wavelength of 276nm, while just Curcumin exhibited absorption at wavelength of 430nm consequently, the Absorption correction method employed. The calibration curves for Aceclofenac and Curcumin were linear within the concentration ranges of 5-30 µg/mL and 1-7 µg/mL, respectively.

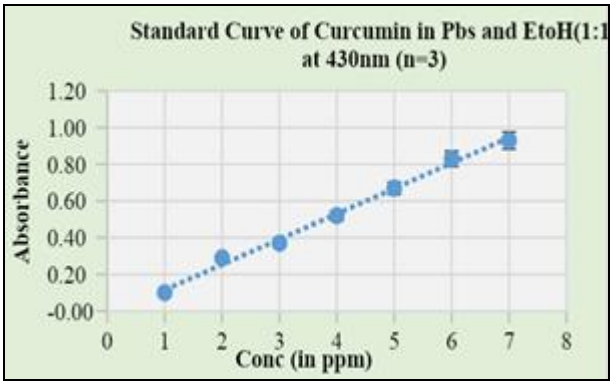

FIG. 4: LINEARITY OF CURCUMIN AT 430NM

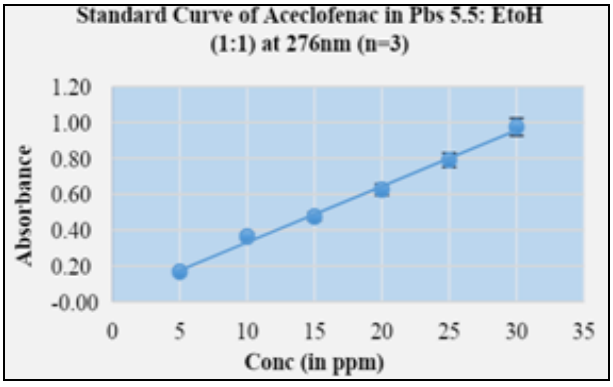

FIG. 5: LINEARITY OF ACECLOFENAC AT 430NM

The analytical method successfully assessed the combination of Aceclofenac and Curcumin in a single dosage form, demonstrating precise separation of these drugs from other substances. The limit of detection (LOD) for aceclofenac and curcumin were 0.4672 µg/ml and 0.2950 µg/ml, respectively. Additionally, the limit of quantification (LOQ) found to be 1.415 µg/ml for aceclofenac and 0.894 µg/ml for curcumin. The method had an accuracy range of 95.72% to 98.40%, affirming its reliability. Furthermore, its stability and consistency were confirmed using repeatability, intra-day, and inter-day precision tests, all of which yielded relative standard deviations (RSDs) below 2%. Notably, the method remained robust even when pH values were intentionally altered. For detailed validation results, please refer to **Table 1.**

TABLE 1: REGRESSION ANALYSIS DATA WITH SUMMARY OF THE VALIDATION PARAMETER

S. no.	Parameters (units)	Aceclofenac	Curcumin
1	Concentration range(µg/ml)	5-30 µg/mL	1-7 µg/mL
2	Regression equation	y= 0.0312x+0.019	y=0.1382x+0.0229
3	Slope	0.0312	0.1382
4	Intercept	0.019	0.0229
5	Correlation coefficient (r^2)	0.9951	0.9946
6	LOD (µg/ml)	0.4672	0.2950
7	LOQ (µg/ml)	1.415	0.894
8	Accuracy (% Recovery) (n=6)	97.83-98.40	95.72-97.43
9	Robustness	Robust	Robust
10	Repeatability (% RSD)	1.479	0.956
	Precision (Method precision, % RSD)		
11	Interday (n=3)	0.978	1.376
12	Intraday (n=3)	0.845	1.263

The method developed using simultaneous determination of Aceclofenac and Curcumin was validated according to the ICH guidelines. This method was accurate and precise. The drug content of Aceclofenac and Curcumin in the gel formulation was determined simultaneously to evaluate the validity of the method. The recovery was 96.6% (0.185% RSD) for Aceclofenac and 95.4% (0.095% RSD) for Curcumin respectively, as shown in **Table 2**. The assay method showed no interference of excipients for the analysis of aceclofenac and curcumin. The accuracy and validity of the method were verified by recovery studies.

TABLE 2: SUMMARY OF ESTIMATION OF ACECLOFENAC AND CURCUMININ NANOEMULGEL FORMULATION

Sr. no.	Drug	Total amount of drug added (µg/ml)	Total amount of drug recovered (mean ± SD)	% RSD	% Recovery
1	Aceclofenac	10	9.912±0.011	0.116	99.12
2	Curcumin	10	9.857±0.026	0.269	98.57

The findings indicate that the suggested UV-spectrophotometric approach (Absorbance correction method) for concurrently assessing Aceclofenac and Curcumin is straightforward, dependable, and cost-effective. Consequently, this technique is suitable for determining both drugs, whether in large quantities or within the dosage formulation, without any impact on the commonly employed excipient and related substances.

CONCLUSION: The method stands out due to its simplicity, speed, and minimal time requirement. The newly developed absorbance correction technique demonstrated sensitivity, accuracy, and precision, making it suitable for routine analysis of both Aceclofenac and Curcumin. Validation was conducted following ICH guidelines, and statistical analysis confirmed the method's repeatability and selectivity for the simultaneous determination of these compounds in combination and pharmaceutical formulations.

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