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Development of a UV-Vis Spectroscopic Method for Etodolac Determination and Evaluation of Pharmaceutical Additive Effects

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ABSTRACT
Etodolac (Eto) is an active substance from the nonsteroidal anti-inflammatory drug (NSAID) class and is widely used in the treatment of pain and inflammation. This study aimed to develop and validate a sensitive UV-Vis spectrophotometric method for quantifying etodolac in tablet formulations and to investigate the effects of pharmaceutical excipients on its absorbance. Etodolac showed maximum absorbance at 278 nm. Methanol was optimized as the most suitable solvent in the study, and the method was validated according to these conditions. The limit of detection (LOD) was found to be 0.29 µg mL^{-1}. Additionally, the effects of some pharmaceutical excipients on the UV absorbance of etodolac were investigated. Pharmaceutical excipients can cause changes in the UV absorbance of the active ingredient, which can lead to incorrect dosage determinations and analytical errors. In this context, the effect of mannitol (Man), lactose monohydrate (Lac), and potassium sorbate (PS) on the maximum absorbance of etodolac was evaluated, and possible interferences were examined. In addition, stability studies were carried out for four weeks to test the long-term reliability of the analysis method. Moreover, the recovery of etodolac from the Etotio tablet formulation was measured, and the method's accuracy was evaluated. The data showed that the applied analysis method offers high precision, reproducibility, and reliability. This study presents an optimized, sensitive, and selective method for accurately quantifying etodolac in pharmaceutical preparations.

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Introduction

Etodolac (1,8-diethyl-1,3,4,9-tetrahydropyrano-(3,4-b)-indole-1-acetic acid) is a widely used non-steroidal anti-inflammatory drug (NSAID) known for its analgesic and antipyretic properties. This compound belongs to the pyranocarboxylic acid class and is particularly effective in managing osteoarthritis, rheumatoid arthritis, and acute pain episodes. The mechanism of action of Etodolac primarily involves the inhibition of cyclooxygenase (COX) enzymes—specifically COX-2, which is associated with inflammation and pain [1]. By selectively inhibiting COX-2, etodolac not only mitigates inflammation but also minimizes the gastrointestinal side effects often observed with NSAIDs, making it a favorable option for pain management [2]. Therapeutic drug monitoring (TDM) can be used to monitor the levels of etodolac in the blood and make optimal dose adjustments, thus minimizing side effects while providing effective treatment. In addition, pharmacokinetic and bioavailability studies are used to determine dosage regimens by examining how the drug is distributed, metabolized, and excreted in the body. Assessment of the toxicity risk of etodolac is important, especially in terms of renal and gastrointestinal side effects. In addition, the determination of etodolac plays a critical role in drug interactions and quality control processes, both in terms of patient safety and the accuracy of pharmaceutical manufacturing processes.

In recent years, various optical and electrochemical methods have been developed for the determination of etodolac. The choice of analytical method is influenced by factors such as the required sensitivity, specificity, and the nature of the sample matrix. Optical methods used in drug determination offer significant advantages such as high sensitivity, fast analysis time, environmental friendliness, and often not requiring sample preparation. Fluorescence spectroscopy is highly sensitive in detecting drug components at low concentrations and plays an important role in determining drug metabolites and impurities [3]. Raman spectroscopy is used as an alternative method to IR, especially in the analysis of water-based samples, and provides detailed

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information about the molecular structure of the drug [4]. UV-Vis spectrophotometry provides a fast and reliable analysis opportunity based on the light absorption properties of drug-active ingredients at certain wavelengths. This method is widely preferred especially in the quantitative analysis of colorless or slightly colored solutions [5].

UV-Vis spectrophotometry is of great importance in the determination of etodolac due to its fast, sensitive, and economical advantages in drug analysis [6, 7]. This method enables quantitative analysis based on the light absorption property of etodolac at certain wavelengths [8]. It is widely used especially in determining the amount of etodolac in pharmaceutical preparations and biological fluids [9–12]. The UV method accelerates quality control processes in drug production by enabling the analysis to be completed in a short time. In addition, the sample preparation process is simple and fast. Generally, it does not require organic solvents or complex separation processes, which makes the analysis process advantageous in cost. While the low use of chemical reagents offers an environmentally friendly approach, the widespread availability and ease of use of the devices create a practical solution for laboratories. Providing reliable results in terms of sensitivity and accuracy plays a major role in evaluating the effectiveness and stability of the drug [13, 14]. Being a lower-cost and faster method compared to chromatographic methods (HPLC, GC) makes UV spectrophotometry more attractive for routine analysis. In addition, when compared to spectral techniques such as IR and NMR, the low-cost equipment required, and the advantage of simple use are among the factors that make it stand out. There are studies in the literature on the determination of etodolac using UV-Vis spectroscopy. For example, in a study conducted by Insuyu et al., direct determination of etodolac was performed by UV-Vis [14]. Unlike this study, the solvent system was optimized in this study, the effects of common pharmaceutical additives on etodolac absorbance were evaluated, and the stability of the prepared solutions was systematically examined for one month. With these aspects, the study offers an approach that expands the applicability of the method compared to the existing literature and reveals the factors affecting its accuracy. In the analysis of etodolac and similar pharmaceutical compounds, the UV method is considered a critical tool to confirm that drugs are produced in the correct dosage and that safe use is ensured. Encouraged by these factors, we have established an efficient, straightforward, and highly sensitive analytical approach for the precise quantification of etodolac. In this study, the linear range of the developed method for the UV-Vis spectrophotometric determination of etodolac was determined, and a calibration curve equation and correlation coefficient (R^2) were obtained. In addition, in order to evaluate the sensitivity of the method, limits of detection (LOD) and limits of quantification (LOQ) were calculated. The potential effects of excipients such as mannitol, potassium sorbate and lactose monohydrate, which are commonly found in pharmaceutical formulations, on the analytical signal were evaluated; the effects of these additives on peak separation, resolution, and sensitivity were analyzed comprehensively. In order to evaluate the reliability of the method, both intraday and interday reproducibility studies were carried out. In addition, a stability study was carried out for the method by monitoring the changes in UV absorbance of the solutions with time, and the durability of the method against time was evaluated. In order to demonstrate the practical applicability of the developed method, the active substance was successfully extracted from tablet formulations containing etodolac, and validation studies were performed on these samples.

Materials and Methods

Materials

Etodolac was kindly provided by Nobel İlaç (İstanbul, Türkiye) and sourced from Ulkar Kimya (İstanbul, Türkiye). Methyl alcohol was obtained from Sigma Aldrich (St Louis, MO), and ethyl and isopropyl alcohol were obtained from Emir Kimya (Ankara, Türkiye) and Yasin Teknik (İstanbul, Türkiye). Potassium sorbate and mannitol were purchased from Tito Smart Kimya (İzmir, Türkiye). Lactose monohydrate was purchased from Kalipso Kimya (İstanbul, Türkiye). All solutions were freshly prepared and filtered before analysis.

Instrumentation

The UV-Vis absorption spectra were obtained using a Shimadzu UV-1280PC UV-VIS spectrophotometer. The measurements were performed using a quartz cuvette with an optical path of 1.00 cm in methyl alcohol solutions. Standard etodolac solutions were prepared at the specified concentrations. The zero adjustment of the device was performed with a cuvette containing methanol. Then, the absorbance values of the standard solutions and the sample solution were measured at the determined maximum wavelength. A calibration curve was created using the obtained data, and the results were reported by determining the sample concentration.

Preparation of Etodolac Solutions

A precise quantity of 0.0050 g of etodolac was accurately weighed and dissolved in 50 mL of methyl alcohol to prepare the etodolac stock solution. The stock solution was diluted to obtain etodolac solutions at varying

concentrations of 0.5 ppm, 1 ppm, 5 ppm, 15 ppm, 20 ppm, 30 ppm, and 40 ppm, respectively. Each measurement was repeated three times.

Wavelength Selection and Calibration Curve

The absorption spectrum of etodolac was recorded in the range of 200–400 nm to determine its maximum absorbance wavelength (λ_{max}). The compound exhibited a distinct absorption maximum at 278 nm, which was selected for all subsequent measurements. Calibration standards were prepared in the concentration range of 0.1–40 ppm by appropriate dilution of the stock solution. Absorbance values at 278 nm were recorded for each standard, and a calibration curve was constructed by plotting absorbance versus concentration. The linearity of the method was assessed by calculating the correlation coefficient (R^2) of the regression equation.

Effect of Additives

Model mixtures containing etodolac were prepared with pharmaceutical excipients such as mannitol, potassium sorbate, and lactose monohydrate. The effects of these additives on the absorbance of etodolac at 278 nm were investigated.

Determination of Etodolac Content in Etotio Tablets

The tablet weight of Etotio is 0.907±0.001 g, each tablet contains 400 mg of etodolac, corresponding to a concentration of 4 mg mL^{-1}(4000 ppm) in the prepared solution. The tablet was first powdered, dissolved in 100 mL of methanol, and kept in an ultrasonic bath for 10 minutes. It was then filtered to prepare the sample for analysis. From this solution, etodolac solutions were prepared at different concentrations in 5, 20, and 40 ppm, and absorbance measurements were taken at λmax wavelength, 278 nm, in a UV-Vis spectrophotometer.

Results and Discussion

Determination of Solvent Effect

An optimization study used the UV-Vis method to determine the most suitable solvent for detecting etodolac. This process created calibration curves using three different solvents: ethanol, isopropyl alcohol, and methanol. The determination coefficient (R^2) of the calibration curve was used to evaluate the accuracy and consistency of the method, and the high R^2 value obtained revealed that the measurement results showed a strong fit to the linear model. The effect of each solvent on the R^2 was examined and investigated in Table 1. First, the absorbance values were measured at the determined wavelength for each solvent, the concentration-absorbance relationship was evaluated, and the obtained data were subjected to linear regression analysis. As a result of the comparisons, it was determined that the calibration curve using methanol had the highest linear correlation coefficient of 0.9999 and provided the most reliable result in the analysis. This finding shows that methanol provides more consistent absorbance values by dissolving the etodolac solution more homogeneously. As a result, methanol was optimized as the most suitable solvent and was determined preferred solvent for etodolac analyses.

Table 1 The investigations of the solvent effect on calibration coefficients

Solvent	Calibration equation	Calibration Coefficient
Ethyl alcohol	$y = 0.0281x + 0.0358$	0.9975
Isopropyl alcohol	$y = 0.0285x - 0.0145$	0.9973
Methyl alcohol	$y = 0.0255x - 0.003$	0.9999

Analytical Performance of the UV-Vis Spectroscopic Method for the Determination of Etodolac

Etodolac is active in the UV-Vis region and can absorb light because it contains conjugated double bonds and aromatic rings as indicated in Figure 1. The determination of etodolac was conducted using UV-Vis spectrum, as illustrated in Figure 2. The analytical performance of the UV-Vis method was then evaluated by assessing key validation parameters. A calibration curve was constructed, and the calibration equation was determined as y = 0.0255x-0.003, with a high correlation coefficient (R^2>0.99), indicating good linearity. The method demonstrated a linear working range of 0.88- 40 µg mL^{-1}. The limit of detection (LOD) was found to be 0.29 µg mL^{-1}, while the limit of quantification (LOQ) was determined as 0.88 µg mL^{-1}, ensuring the method's sensitivity. Table 2 presents previously reported LOD values. Comparing our results with those in the literature, we found that the proposed sensor's LOD value is highly satisfactory. These results confirm that the developed UV-Vis method is accurate, precise, and suitable for quantifying etodolac in pharmaceutical formulations.

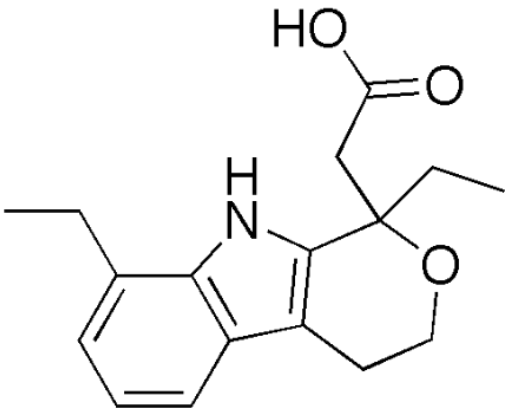

Fig. 1 Chemical structure of Etodolac.

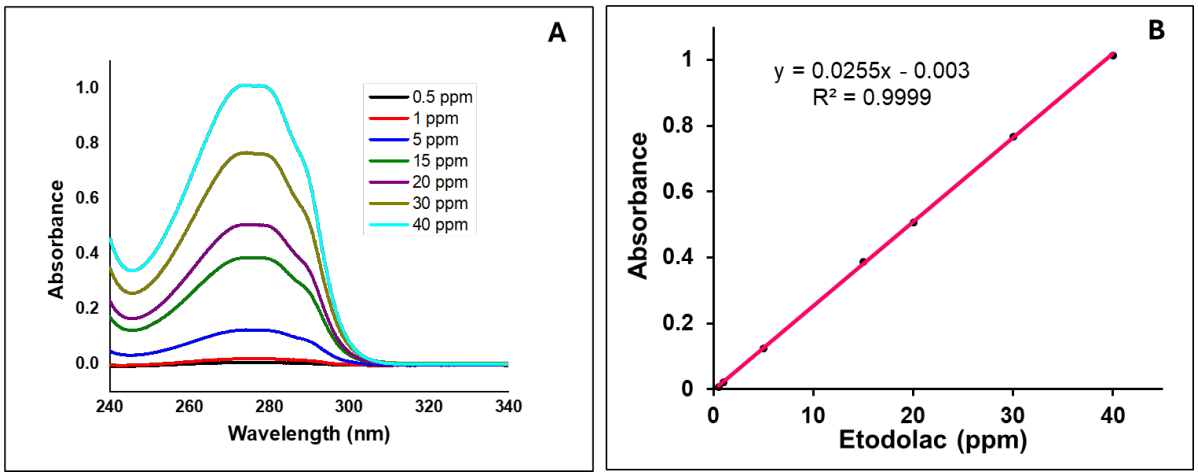

Fig. 2 A) UV-Vis spectra for each etodolac concentration, B) Calibration curve derived from changes in the absorbance signal.

Table 2 Comparison of the developed methods for Etodolac detection

Detection method	LOD	References
UV–Vis	0.49 µg mL^{-1}	[15]
UV–Vis	0.30 µg mL^{-1}	[16]
UV–Vis	0.36 µg mL^{-1}	[17]
LC–MS/MS	0.97 ng mL^{-1}	[18]
DPV	6.8×10^{-7} M	[19]
FS	0.071 ng mL^{-1}	[20]
CV	10.03 ng mL^{-1}	[21]
UV–Vis	0.29 µg mL^{-1}	Our work

The Effect of Pharmaceutical Additives on the UV-Vis Absorbance of Etodolac

To determine the UV-absorbance properties of various drug additives and etodolac 40 ppm solutions of Eto, Man, Lac, and a 2 ppm solution of PS were prepared. Additionally, solutions to these pharmaceutical additives were prepared at the same concentrations in 40 ppm Eto/MeOH. These seven solutions were scanned in the range of 240-400 nm by a UV-Vis spectrophotometer and it was analyzed whether there was any change in the absorbance of etodolac at 278 nm wavelength and results were shown in Figure 3. First, there was an increase in the absorbance of etodolac at 278 nm wavelength in solutions prepared with all three additives. While the absorbance of 40 ppm etodolac at 278 nm wavelength was 1.013±0.004, when mannitol, lactose monohydrate, and potassium sorbate were added, these values became 1.141±0.013, 1.135±0.100, and

1.234±0.004, respectively. Relative Change in Etodolac absorbance was also investigated further using Equation (1) below,

$$Relative\ Absorbance\ Change = \frac{Absorbance_{Final} - Absorbance_{Initial}}{Absorbance_{Initial}} \times 100 \quad (1)$$

According to the relative changes in absorbance of etodolac at 278 nm, additions of mannitol, lactose, and potassium sorbate caused an increase of 12.60%, 11.97%, and 21.81%, respectively. These changes are presented in Figure 4. It also investigated whether there was any shift in the maximum absorbance peak of etadolac in methanol at 278 nm wavelength. The maximum absorbance peaks of Eto-Man, Eto-Lac, and Eto-PS solutions were obtained as 278.6 nm, 278.7 nm, and 269.8 nm, respectively. This indicated that drug additives could cause changes not only in the absorbance intensity but also in the maximum absorption wavelength. Drug additives can cause changes in the UV absorbance of the active substance, which can lead to problems such as loss of accuracy in analyses, incorrect dosage determination, and spectral overlap. Additives can interact with the active substance and change absorbance values or create additional peaks in the UV spectrum, which can cause incorrect determination of the amount of active substances. In addition, they can affect the solubility or stability of the active substance in the solvent environment, causing changes in bioavailability. Therefore, to obtain accurate results in pharmaceutical analyses, the effects of additives should be taken into account, and appropriate analytical methods should be used.

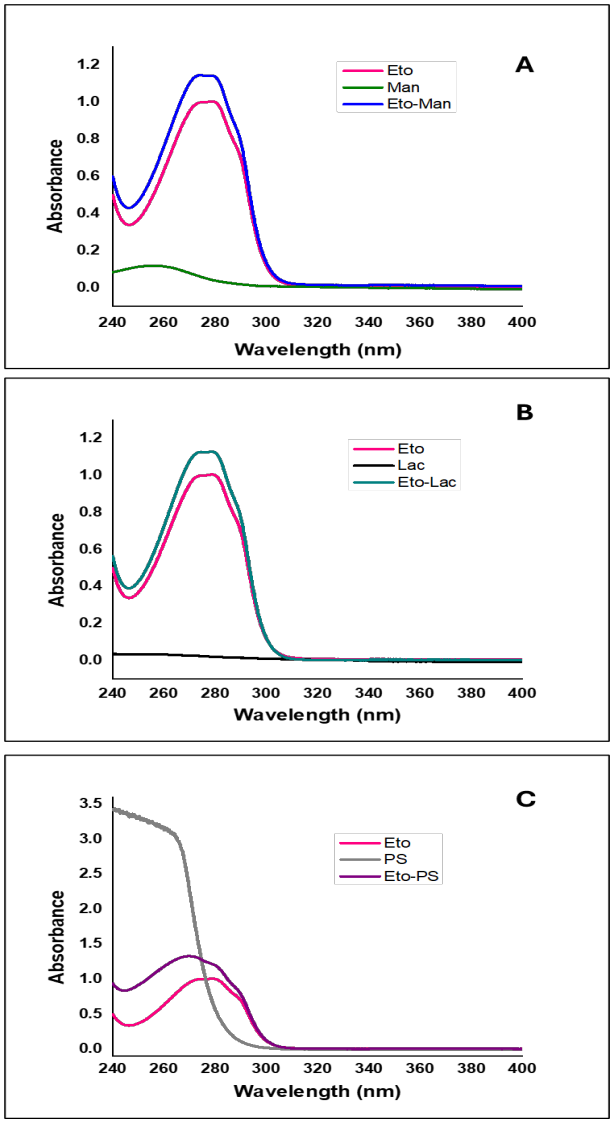

Fig. 3 UV-Vis spectra of A) Eto, Man, and Eto-Man solutions, B) Eto, Lac, and Eto-Lac solutions, and C) Eto, PS, and Eto-PS solutions.

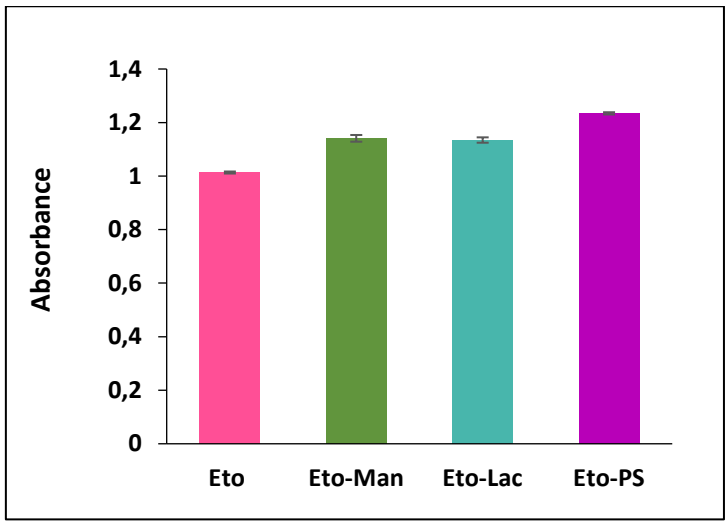

Fig. 4 Change in absorbance of etodolac at 278 nm wavelength in the presence of mannitol, lactose monohydrate, and potassium sorbate.

Interday and Intraday Repeatability of the Method

Intraday and interday repeatability are critical to the reliability and accuracy of the method for drug determination. Intraday repeatability evaluates the consistency between measurements made on the same day, indicating the short-term stability of the method. Within the scope of the intraday repeatability study, 1 ppm, 15 ppm, and 30 ppm etodolac solutions were analyzed, and etodolac recoveries of 0.95±0.08 ppm, 14.98±0.38 ppm, and 29.33±0.24 ppm were obtained, respectively. These results show that the method offers high accuracy and reliability, with minimal deviations between measurements. In addition, high repeatability increases the efficiency of analytical processes by showing that the margin of error is low, and the method is suitable for routine analysis.

Interday repeatability determines whether analyses made on different days yield similar results, indicating how reliable the method is in the long term. For this purpose, 1 ppm, 15 ppm, and 30 ppm etodolac solutions were analyzed on the 1st day, 7th, and 14th days, and etodolac recoveries of 1.06±0.08 ppm, 15.45±0.31 ppm, and 30.37±0.33 ppm were obtained, respectively. These results show that the method provides consistent and reliable results across days and is not affected by time-dependent changes. Table 3 presents the measured etodolac concentrations along with the Relative Standard Deviations (RSD%) for intraday and interday repeatability assessments.

Table 3 The precision and accuracy results of the proposed methods for Etodolac detection

	Intraday (n=3)		Interday (n=3)	
Eto (ppm)	**Eto found (ppm)**	**RSD, %**	**Eto found (ppm)**	**RSD, %**
1	0.95±0.08	8.73	1.06±0.08	7.55
15	14.98±0.38	2.51	15.45±0.31	1.99
30	29.33±0.24	0.81	30.37±0.33	1.10

Stability of the Developed UV-Vis Method for Etodolac Determination

UV-vis absorbance measurements of 1, 15, and 30 ppm etodolac solutions were performed to evaluate the four-week stability of the method. The stability study was conducted by storing the solutions at +4 °C in the dark for four weeks in airtight containers. During the study period, the sensitivity, repeatability, and accuracy of the method were examined and possible changes that may occur over time were recorded. According to the results given in Figure 5, it was observed that the method maintained its stability for three weeks and the UV-Vis absorbance was not affected. However, especially for the 1 ppm solution, the fourth-week measurements

could not be obtained successfully. In this case, it was shown that low-concentration etodolac solutions could maintain their stability for a maximum of three weeks. In addition, the changes in absorbance were statistically analyzed and the long-term usage potential of the method was evaluated. There was an increase in absorbance values for 15 ppm and 30 ppm solutions for different days. This can be seen due to precipitation or particle formation in the solutions. Such situations increase the optical density of the solution and the formed precipitates or particles can absorb light more. These findings demonstrate that the three-week stability of the method is at a level that will provide reliable and consistent results across application areas.

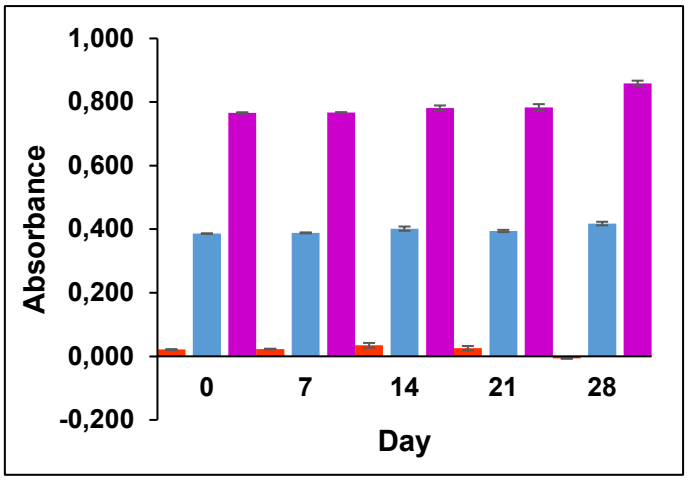

Fig.5 Four-week stability results of 1 (orange), 15 (blue), and 30 (purple) ppm Etodolac solutions.

Etodolac Recovery in Etotio Tablet

The absorbance values of the tablet solutions were measured, and their concentration was determined from the calibration curve equation. The recovery values obtained because of the measurements were determined as 85%, 106%, and 103% for 5, 20, and 40 ppm, respectively. If the analyte concentration is between 1 and 100 ppm, the acceptable recovery percentage is between 80–110% [22]. For this reason, the obtained recovery values indicate that the method is within the accuracy limits. In addition, the accuracy of the method was evaluated by calculating the percent relative standard deviation (RSD), as shown in Table 4. It was observed that lactose monohydrate increased the absorbance of etodolac, which may indicate a potential interference effect.

Table 4 Recovery values of Etodolac in Etotio tablets

Etodolac in Etotio (ppm)	Found Etodolac (ppm)	RSD, %	Recovery (%)
5	4.3±0.2	1.3	85.0±4.3
20	21.4±0.4	1.7	106.8±1.8
40	41.1±0.5	5.1	102.8±1.3

Conclusion

The findings of this study illustrate a fast, simple, and cost-effective method for the determination of etodolac using UV-Vis spectroscopy, which does not require complex equipment or lengthy processing times. This analytical method offers a remarkable degree of accuracy and reproducibility, ensuring consistent and reliable results across multiple measurements and experimental conditions. In addition, this study investigated the effect of common pharmaceutical additives on absorbance in the determination of etodolac. In particular, potassium sorbate was observed to have the most significant effect. These findings suggest that the effect of additives should be considered in etodolac analysis and that appropriate corrections are important to increase the accuracy of quantitative determinations. Intraday and interday repeatability results show that there are minimal deviations between measurements made on the same day and different days. The stability results show that the method provides reliable and consistent results for three weeks, but the fourth-week measurements, especially for low-concentration (1 ppm) solutions, cannot be successfully performed. The obtained recovery

rates from Etotio (85%, 106%, and 103%) are within the acceptable recovery range of 80–110%, indicating that the method is within the accuracy limits. These results reveal that the developed method provides a reliable, accurate, and reproducible analysis method for the determination of etodolac. Future research will focus on enhancing the method's precision by optimizing experimental conditions, validating its applicability in complex biological matrices, and comparing it with alternative analytical techniques such as HPLC to ensure broader applicability.

Abbreviations
NSAID: Nonsteroidal anti-inflammatory drug, UV-Vis: Ultraviolet-visible spectroscopy, HPLC: High Performance Liquid Chromatography, GC: Gas chromatography, IR: Infrared, NMR: Nuclear magnetic resonance, DPV: Differential pulse voltammetry, LC–MS/MS: Liquid chromatography–Mass spectrometry/ Mass spectrometry, FS: Fluorescence spectroscopy, CV: Cyclic voltammetry

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Data Availability statement
Please contact the corresponding author for any data requests.

Compliance with ethical standards
Conflict of interest
The authors declare no conflict of interest.

Ethical standards
The study is proper with ethical standards.

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