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Review

A Review for the Dual Wavelength Method for Simultaneous Determination of Two Drugs in pharmaceutical Mixture

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ABSTRACT

The dual wavelength method's effectiveness in simultaneously determining numerous components in a mixture has drawn a lot of attention in the field of pharmaceutical analysis. The dual wavelength method measures the absorbance values of the medicine of interest at two wavelengths where any other medication in the combination that overlaps with it has absorbance values equal to zero. The dual wavelength method's employment in the quantitative study of two medicines combined is the main topic of this review. The dual wavelength method's guiding principles, benefits, difficulties, and most recent developments are examined. The article highlights the many uses of the dual wavelength approach in pharmaceutical analysis, such as formulation analysis, pharmacokinetic investigations, and quality control.

Keywords: Dual wavelength, Advantages, Quantitative analysis, Simeprevir, Sofosbuvir.

1. Introduction

A method used in many scientific fields, including spectroscopy and analytical chemistry, to improve the specificity and accuracy of results is called dual-wavelength analysis. As the name implies, this technique uses two distinct electromagnetic light wavelengths—typically in the UV-visible range—to examine a sample's characteristics.[1]

Dual-wavelength analysis is based on the idea that a sample behaves differently at two different wavelengths. Researchers can isolate particular components or properties of the sample by comparing the absorbance or emission of light at these two wavelengths, which improves measurement sensitivity and accuracy. [1]. Determining concentration levels in chemical solutions is one typical use for dual-wavelength analysis. Researchers can more precisely determine concentrations by accounting for interferences from other compounds in the sample by measuring the absorbance of light at two wavelengths that are known to be affected differentially by the presence of the target analyte.

The investigation of intricate biological systems, such as protein-protein interactions or enzyme kinetics, is another use for dual-wavelength analysis. Through the utilization of two distinct light wavelengths that have distinct interactions with biomolecules, scientists can more precisely and precisely understand the dynamics of these systems.[1]

In conclusion, dual-wavelength analysis is an effective and adaptable method for examining sample characteristics across a range of scientific domains. This technique allows researchers to obtain deeper insights into the nature of the systems they are studying and extract useful information by taking advantage of the differential reaction of materials to different wavelengths of light.

2. Dual Wavelength analysis in the pharmaceutical field:

In pharmaceutical research and development as well as quality assurance and control procedures, dual-wavelength analysis is essential. This is the application of dual-wavelength analysis in the pharmaceutical industry [2]:

2.1. Quantitative Analysis of Drug Concentrations

Drug Assays: Dual-wavelength analysis is utilized to quantify the concentration of active pharmaceutical ingredients (APIs) in formulations. By measuring absorbance at two wavelengths, interference from excipients or impurities can be minimized, leading to more accurate drug concentration determinations [2].

2.2. Dissolution Testing

dissolving Profiling: Drug formulations can be monitored for dissolving using dual-wavelength analysis. Researchers can evaluate drug release kinetics and dissolution rates by choosing wavelengths that are sensitive to the drug and any excipients. These measurements are crucial for evaluating formulation performance and bioavailability [2].

2.3. Stability Studies

Degradation Kinetics: Dual-wavelength analysis is used in stability studies to track how pharmaceuticals deteriorate over time. Researchers can determine degradation products and evaluate the durability of pharmaceutical formulations under different storage settings by measuring absorbance changes at two wavelengths.

2.4. Impurity Analysis

Dual-wavelength analysis is useful for identifying contaminants in pharmaceutical formulations. Researchers may precisely estimate impurity levels by choosing wavelengths that are particular to the medicine and any potential impurities, assuring adherence to regulatory requirements and recommendations [2].

2.5. Bioanalytical Applications

Pharmacokinetic Studies: Dual-wavelength analysis is employed in pharmacokinetic studies to measure drug concentrations in biological samples such as plasma or urine. By utilizing specific wavelengths for the drug and any interfering compounds, researchers can accurately determine drug concentrations and assess pharmacokinetic parameters [2].

2.6. Quality Control

Batch-to-Batch Consistency: To guarantee the consistency of pharmaceutical products from batch to batch, quality control procedures include dual-wavelength analysis. Manufacturers can

maintain the quality and efficacy of their products by detecting fluctuations in drug concentration or formulation composition by monitoring absorbance at two wavelengths. [3].

2.7. Formulation Development

Formulation Optimization: Dual-wavelength analysis evaluates drug candidates' compatibility with excipients and optimizes formulation parameters, which helps in formulation development. Through examining variations in absorbance at various wavelengths, scientists can pinpoint the best formulations that offer increased stability and bioavailability [3].

2.8. Advantages of Dual-wavelength technique

2.8.1. Improved Specificity: Dual-wavelength analysis can improve measurement specificity by reducing interference from contaminants, excipients, or background signals by employing two wavelengths [4].

2.8.2 Greater Accuracy: Analytes can be quantified and detected more precisely when absorbance or emission at two wavelengths is compared, particularly when interfering chemicals are present [4].

2.8.3 Enhanced Sensitivity: By maximizing contributions from non-specific interactions and background noise, dual-wavelength analysis can increase sensitivity by enhancing signals specific to the target analyte. [4].

2.9. Disadvantages of Dual-wavelength technique:

2.9.1. Complexity of Calibration: Compared to single-wavelength procedures, calibrating a dual-wavelength analytic method may be more difficult because it calls for the establishment of calibration equations or curves for both wavelengths [4].

2.9.2. hardware Requirements: Dual-wavelength analysis necessitates hardware that can choose and measure light at two different wavelengths either concurrently or successively, which could result in more expensive equipment and maintenance needs [4].

2.9.3. Potential Interference: Although interference is reduced through dual-wavelength analysis, overlapping absorbance spectra or spectral artifacts may nevertheless occur and compromise measurement accuracy [4].

2.9.4. Limited Applicability: Not all samples or analytes will benefit from dual-wavelength analysis, especially those with complex spectral characteristics or poorly defined absorption spectra. [4].

2.9.5. Complexity of Data Processing: Compared to single-wavelength techniques, dual-wavelength experiment data analysis may be more complicated, requiring specialist software or algorithms to extract relevant information and correctly interpret results [4].

2.9.6. Sensitivity to Experimental circumstances: To maintain reproducibility, thorough control and optimization are necessary because the results of dual-wavelength analyses may be sensitive to experimental circumstances like temperature, pH, and sample preparation methods [4].

3. Discussion (Applications of Dual-wavelength technique)

3.1. Application of different spectrophotometric methods for quantitative analysis of direct acting antiviral drugs Simeprevir and Sofosbuvir:

Simeprevir and Sofosbuvir are two examples of direct-acting antiviral drugs that are authorized for the treatment of chronic HCV infection. HCV and SARS-CoV-2 have similar structures and mechanisms of replication, according to reports. Therefore, it is suggested that people with COVID-19 take Sofosbuvir and Simeprevir combined since this may help. Spectrophotometric methods have not yet been used to quantitatively evaluate sofosbuvir and mepreprevir combined. For this investigation, the compounds under investigation in their combined state could be quantitatively analyzed thanks to two simple spectrophotometric procedures. The zero-order direct technique could be used to quantitatively assess someprevir at 333 nm, while sofosbuvir showed zero absorbance values, represented in **Figure.1** [5].

The source of pure SMV & SFV powders was the Cairo, Egypt-based Benchmark Health Company. Merospevir® (150 mg SMV per tablet) and Mpiviropack® (400 mg SFV per tablet) were supplied by the neighborhood pharmacy. Each pill contained 150 mg SMV, 400 mg SFV, 20 mg talc powder, 15 mg maize starch, and 7 mg magnesium stearate. The pills were manufactured in a lab. The ethanol was HPLC quality and was supplied by Sigma-Aldrich of Darmstadt, Germany. The El-Nasr Pharmaceutical Chemicals Company, based in Cairo, Egypt, supplied the analytical grade dimethyl sulfoxide (DMSO). [5].

Separately, two sets of 10-mL volumetric flasks were used to hold aliquots equal to 30-450 µg and 30-440 µg of SMV and SFV, respectively, from their standard solutions (100 µg/mL), which were then diluted to volume with ethanol. In the wavelength range of 200 to 400 nm, the produced solutions' absorption spectra were evaluated in relation to ethanol as a blank [5]. Two-wavelength technique: To create the calibration plot, the absorbance values of the SFV spectra at 259.40 and 276 nm were plotted against the SFV concentrations. An equation for regression was created, as shown in **Table 1** and **Table 2.**

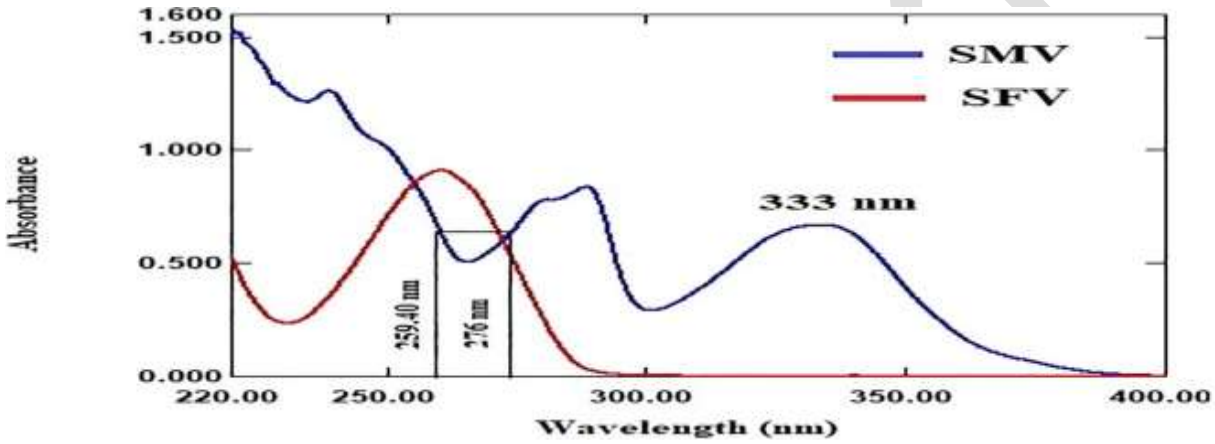

Figure 1. Absorption spectra of SMV (25 µg/mL) and SFV (36 µg/mL).

Table 1. Regression and validation data for quantitative analysis of SMV and SFV by the proposed methods.

Parameters	SMV	SFV
Wavelength (nm)	333	259.40 & 276
Linearity range (µg/mL)	3–45	3–44
Slope	0.0263	0.0130
Intercept	0.0085	−0.0001
Coefficient of determination (r^2)	0.9997	0.9997
LOD (µg/mL)	0.888	0.803
LOQ (µg/mL)	2.692	2.434
Accuracy (%R)[a]	100.25	100.06
Repeatability precision (RSD)[b]	0.768	0.595
Intermediate precision (RSD)[b]	0.927	0.801

[a]Average of 9 determinations (3 concentrations repeated 3 times).

[b]RSD of 9 determinations (3 concentrations repeated 3 times).

Table 2. Application of the proposed methods for the determination of SMV and SFV in their single-ingredient tablets.

Taken (µg/mL)		%Recovery	
Merospevir® tablets	MPIVIROPACK® tablets	SMV	SFV
3	8	100.13	100.10
6	16	99.11	99.66
9	24	99.07	100.99
12	32	100.98	100.50
15	40	99.24	100.02
Mean ± RSD		99.71 ± 0.836	100.26 ± 0.508

Without any interference, the pharmaceuticals under inquiry were successfully identified using the applied methods in the mixes and tablets prepared in the laboratory [5]. We created the first spectrophotometric techniques in this work to analyze SMV and SFV in their mixture quantitatively. Without any interference, the employed procedures successfully identified the pharmaceuticals under research in the laboratory-prepared combinations and tablets.

3.2. Simultaneous determination of Atorvastatin calcium (ATR) and Ezetimibe (EZ) in their bulk powder and pharmaceutical dosage form.

The main task of the developed spectrophotometric methods is to determine the studied drugs in their pharmaceutical dosage form. Since atorvastatine and EZ act as antihyperlipedimic drugs and have been used to treat some serious diseases, such as heart diseases, it is important to develop analytical methods that are not only accurate, precise, fast, simple, and economical[6]. When used with ATR, EZ increases the benefits of statins at lower dosages while lowering their negative effects [6].

ATR and EZ in their binary mixtures can be determined simultaneously using a variety of techniques, including derivative ratio spectrophotometry, HPLC, HPLTLC, and Q-spectrophotometry [7]. The UV-PC personal software version 3.7 and a double beam UV-visible spectrophotometer (SHIMADZU, Japan) model UV-1601 PC with a 1 cm quartz cell were utilized. The scanning speed is 2800 nm/min with a 0.1 nm interval, and the spectral band is 2 nm. [7].

Standard EZ was generously provided by Egyptian Co. for Chemicals and Pharmaceuticals, ADWIA CO, 10th of Ramadan City, Egypt, whereas standard ATR was gratefully provided by Marcyrl Pharmaceutical Industries, El-Obour City, Egypt [7]. ATR and EZ standard stock solutions were made at a concentration of 1 mg/mL in methanol. Spectral properties and wavelength selection: Using methanol as a blank, the absorption spectra of 16 mg/mL of ATR and EZ were recorded at 200–350 nm. In order to choose the appropriate wavelengths for the dual wavelength spectrophotometric approach, the overlay spectra were examined., represented in Figure. 2 [7].

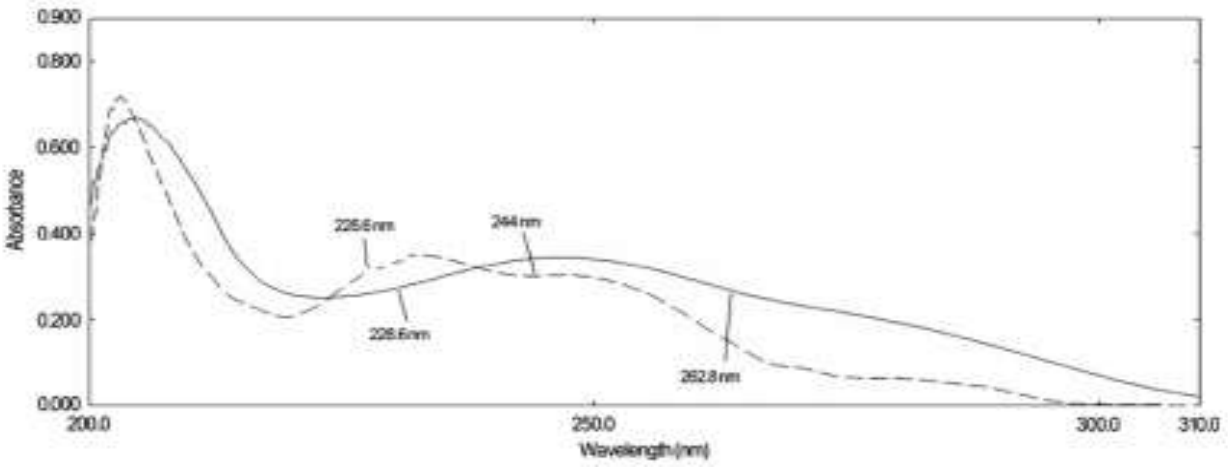

Figure 2. Zero-order absorption spectra of 8 mg/mL each of ATR (—) and EZ (- - -) using methanol as a solvent.

Dual wavelength analysis method

Two distinct sets of 10-mL volumetric flasks were used to hold different aliquots of ATR and EZ, corresponding to 60–260 and 80–400 mg/mL, respectively, that were transferred from their respective standard working solutions (0.1 mg/mL). Methanol was then used to finish the volume. Absorbance values were measured at 226.6 and 244 nm (for ATR) and 228.6 and 262.8 nm (for EZ) after the prepared solutions were scanned in the 200–350 nm range. Plotting the difference in absorbance values at 226.6 and 244 nm (EZ has a difference of zero) against the corresponding concentration allowed for the determination of ATR. Plotting the difference in absorbance values at 228.6 and 262.8 nm (difference is 0 for ATR) against the respective concentrations was similar for determining EZ. [7].

In Figure. 2 directorption spectra for ATR and EZ show significant overlap, which rendered it extremely challenging to determine them directly in their binary mixes. Using their zero order absorption spectra, the dual wavelength approach that has been devised offers a straightforward way to determine both ATR and EZ selectively. This method's basic idea is that, regardless of the interfering component, the absorbance difference between two places on the spectrum is directly proportional to the component of interest [7].

Choosing two wavelengths where the interfering component exhibits the same absorbance value and the component of interest exhibits a notable variation in absorbance with concentration is a prerequisite for this technique [7].

Different wavelengths were tested, including 221.4, 258, 8.8, and 225.4, 252.6 nm for ATR and 233.8, 260.6, and 215, 250 nm for EZ, because choosing the right wavelengths is crucial. When used to determine ATR, the absorbance readings at 226.6 and 244 nm (where EZ has the same absorbance) provided the best selectivity. However, the absorbance values at 228.6 and 262.8 nm were selected in order to determine EZ because they produced the best results. By charting the variation in absorbance values at the chosen wavelengths for every medication against its corresponding concentration, calibration curves for ATR and EZ were created. Beer Lambert's law was followed by ATR and EZ in the concentration ranges of 6–26 and 8–40 mg/mL, respectively with good correlation coefficients. Regression equation parameters are given in **Table 3** [7].

Table 3. Linear regression and analytical parameters of the proposed methods for determination of ATR and EZ.

Parameters	Dual wavelength method	
	ATR	EZ
Linearity range (µg/mL)	6–26	8–40
Slope	0.0092	0.0202
Intercept	0.0097	0.009
Correlation coefficient	0.9997	0.9999
Precision		
Repeatability	0.924	1.071
Intermediate precision	1.273	1.145
Accuracy	100	101.66
Specificity (%)	99.89 ± 1.594	100.87 ± 1.292

In conclusion, the dual wavelength approach that was developed has been effectively used to determine ATR and EZ simultaneously in a combined marketed sample; it is quick, straightforward, accurate, and simple to understand and use. After the equations were created, all that was needed for analysis of the devised dual wavelength approach was measuring the absorbance values of the sample solution at the chosen wavelengths and doing a few quick calculations [7].

3.3. Simultaneously Content Analysis of Sulfadoxine and Pyrimethamine in Tablet Dosage Form by Spectrophotometry Ultraviolet with Dual Wavelength Method

Because it can be administered in a single dosage, Pyrimethamine Sulfadoxine (PS) is an anti-malarial drug combination consisting of sulfonamide group sulfone and nature Skizontosida diaminopirimidine network. Nevertheless, this combination presents a new challenge for the pharmaceutical industry in relation to the development of new analytical methods in the determination of levels [8]. Research is needed to demonstrate that a double wave method may be utilized to assess the amounts of PTN and SDN in tablet dose forms. While the dual wavelength method is one option for evaluating the level of medication.

Shimadzu's 1800 UV-Vis spectrophotometer, along with a set of PCs running UV-Probe 2:42 software, Microsoft Excel and SPSS 20, Matlab® version R2016a, a cuvette measuring one centimeter, glass tools made by Oberoi, a mortar and pestle, an analytical balance made by Boeco, a sonicator made by Branson 1510, a pH meter made by Hannan, and additional tools needed for sample preparation and solution [8].

Unless specified otherwise, all of the reagents used are grade analyses. Raw Materials: Whatman filter paper no. 42, akuabidestilata (PT. Ika Pharmindo), sulfadoxine (BPOM), pyrimethamine (BPOM), methanol (E-Merck), and HCL (E-Merck). Tablet S (Actavis), tablet P (IFARS), and parchment paper [8].

A 50 mL volumetric flask was filled with 50 mg of PTN and SDN, which were diluted with methanol and added to the line mark. The stock standard (stock solution I) has a concentration of 1000 ug/mL. A 50 mL volumetric flask was filled with 5 mL of the stock solution, and the mixture was diluted with methanol by adding a line to indicate that the concentration needed to be 100 ug/mL.[8].

The pipette's PTN (0.9 mL) and SDN (0.6 mL) of stock solution II were transferred to separate 10 mL volumetric flasks. Additionally, methanol is added to the solution to dilute it, and both ends are then combined to create a concentration of 9 mg/mL for PTN and 6 mg/mL for SDN, which is prepared for the dual wavelength technique. A combination of both medications (series C) in the same concentration range prepared for the dual wavelength approach, and the PTN ratio of the absorption spectrum in the range of 5–13 mg/mL (series A) and 3–9 ug/mL for SDN (series B) [8].

PTN demonstrated a same absorbance spectrum at 284.8 nm (λ3) and 258.8 nm (λ4); as a result, both wavelengths were used for SDN investigation. We check all of the series A solutions to make sure there is no difference between λ3 and λ4. In a similar manner, the two wavelengths with the identical absorbance were identified by the solution SDN scan. Two wavelengths were identified, and 291.6 nm (λ2) and 274.2 nm (λ1) were chosen for PTN investigation. To make sure there is no difference between (λ1) and (λ2), all series B answers are scanned. Subsequently, the solution C series is scanned to verify that fluctuations in PTN and SDN concentrations did not impact the absorbance at the chosen wavelength. Difference in absorbance between series C solutions (λ1) and (λ2) used to prepare PTN calibration curves [8].

Studies overlain spectrum and wavelength selection:]

Using the Lambert-Beer law and the proper concentrations, the study was conducted. Using scalable solution concentrations of 9 mg/mL and 6 mg/mL, as well as a mixture of both in the same concentration, the PTN and SDN spectra were overlaid and scanned within a 200–400 nm range, respectively is shown in **Figure. 3** [8].

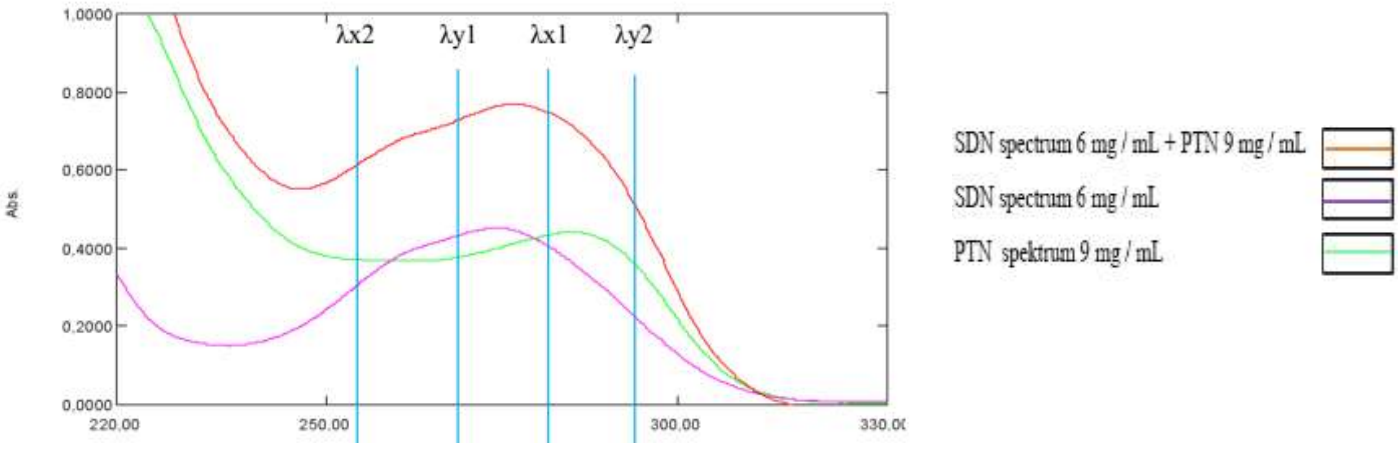

Figure 3. Overlain spectrum of SDN and PTN.

In order to analyze PTN, two spectral wavelengths were selected for the overlay of the study: 274.2 nm and 291.6 nm SDN, where the absorbance difference is zero. PTN 284.8 nm and 258.8 nm were utilized for the analysis of SDN. The spectral ratios for the calibration curve were 5–13 mg/mL for PTN (series A), 3–9 ug/mL for SDN (series B), and 9–6 for a combination of both medications (series C) in the same concentration range. the PTN and SDN calibration readings' outcomes are shown in **Table 4** and **Table 5**.

Table 4. Result of calibration readings for PTN

concentration (ppm) PTN	Differences in absorbance of SDN at 274.2 and 291.6 nm
5	0.0000
7	0.0002
9	0.0000
11	0.0002
13	0.0006

Table 5. Result of calibration readings for SDN.

Concentration (ppm) SDN	Absorbance difference of PTN at 284.8 and 258.8 nm
3	0,000
4.5	-0.003
6	0,001
7.5	0,001
9	0.007

The calibration curves for PTN and SDN were linear between 5–13 ug/mL and 3–9 ug/mL, respectively. The calibration curve's regression equation is Y PTN = 0,0136X - 0.0014, r = 0.9997 for PTN, and Y SDN = 0,0127X + 0.0008, r = 0.9998 for SDN, as shown in **Table 6.** [8].

Table 6. Test for tablet dosage forms that are commercially available.

Preparations Tablets	Component	Content (%)
tablet S	SDN	101,59 ± 1,9309
	PTN	99,89 ± 4,0359

Without the need for pre-separation, the suggested double wave approach provides exact and reliable results for determining the amounts of pyrimethamine and sulfadoxine in the marketed formulation (tablet) and is simple to implement in regular analysis. The dual wavelength method's simplicity and speed are its most intriguing features [8]. The validation process has been proven through a number of linearity, accuracy, and precision tests. The suggested technique was effectively used to identify this medication in a commercial tablet, as shown in **Table 7.** [8].

Table 7. Validation of Dual Wavelength Method.

No.	Parameter	PTN	SDN
1	Analytical wevelengths for determination (nm)	284.8 nm and 258.8 nm	274.2 and 291.6
2	Lamber beer (ug / mL)	3-12	1-12
3	Regression equation	Y = 0.0136X - 0.0014	Y = 0.0127X + 0.0008
4	correlation coefficient	0.9997	0.9998
5	Accuracy (%)	100.27	101.03
6	Precision (RSD) (%)	0.82	0.57
7	Interday (% RSD)	1.29	1.16
8	Intraday (% RSD)	0.47	2.35
9	LOD (ug / mL)	0.39	0.20
10	LOQ (ug / mL)	1.18	0.61

3.4. Application of Ultraviolet Spectrophotometry with Dual Wavelength Method for the Simultaneous Determination of Ecstasy Tablet Content

One kind of narcotic pill that is often used as a stimulant is ecstasy. The primary ingredients are methamphetamine (MA) and methylene dioxymetamphetamine (MDMA), but because there is a wide gap between supply and demand, ecstasy tablets are frequently laced with other substances like paracetamol (PCT), caffeine (KFN), and ephedrine (EFD). Since ecstasy tablets are sometimes mixed with other active ingredients, they can complicate the process of figuring out the amounts of tablets at the Police Forensic Lab. As a result, a quick, easy, and affordable way to figure out the amounts of these tablets is required [9].

Tablets of ecstasy were taken from the evidence that was seized at the forensic laboratory of the North Sumatra Police. The raw materials utilized in this study were methamphetamine (Cerilliant®), paracetamol (Anqiu lu'an), caffeine (Sigma-Aldrich), ephedrine (Malladi), and all other compounds and reagents were of analytical quality [10]. With a computer running UV probe 2.43 software on a UV-Visible Spectrophotometer (Shimadzu 1800), the absorption was measured at a wavelength of 200–400 nm using a 1 cm cuvette and UV-probe software. Glassware, mortar, sonicator (Branson 1510), analytical balance (sartorius), and other necessary tools for sample preparation [10].

50 mg of EFD, KFN, and PCT were carefully weighed, and then they were added to a 50 mL volumetric flask together with methanol and phosphate buffer pH 5 to dissolve it. The concentration of the standard stock solution was 1000 µg/mL. Phosphate buffer pH 5 was added to the line after 5 ml of the parent solution was transferred to a 50 mL volumetric flask and diluted with methanol, yielding a concentration of 100 µg/mL. It is sufficient to drink 18 ml of methamphetamine solution at a concentration of 1000 µg/ml in order to obtain a concentration of 360 µg/ml of MA solution [10].

Methamphetamine solution containing 360 µg/ml, 361 µg/ml, 8.5 µg/ml KFN, and 6.5 µg/ml PCT. A mixture of both medications in the same concentration range was generated for the Dual wavelength approach. The absorption spectrum of MA ratios is in the range 200-520 µg/ml, EFD is in the range 195-527 µg/ml, KFN is in the range 4.5-12.5 µg/ml, and PCT is in the range 3.5-9. 5 µg/ml [10].

The absorbance of MA is similar at 263 nm (λ1) and 250.6 nm (λ2), which is why these two wavelengths were chosen for the KFN analysis. Two wavelengths at 255 nm (λ3) and 259.4 nm (λ4) in the single spectrum EFD have an absorbance difference of zero, making these wavelengths suitable for PCT measurements in drug mixes. Using KFN, it is possible to assess MA in drug combinations at two wavelengths: 263 nm (λ5) and 281.8 nm (λ6), which exhibit negligible absorbance difference in the single KFN spectrum.

For the purpose of measuring EFD in drug mixes, two wavelengths, 255 nm (λ7) and 236 nm (λ8), are obtained in PCT that exhibit a zero absorbance difference in the single spectrum of PCT [10].

Validation test

Linearity

The wavelength points that were chosen for the standard solution of MA, EFD, KFN, and PCT for the absorption spectra were 263 nm and 250.6 nm for MA, 255 nm and 259.4 nm for EFD, 263 nm and 281.8 nm for KFN, and 255 nm and 236 nm for PCT. The regression equation for each component at the chosen wavelength is derived from the difference between the absorbance values of the two wavelengths [10], as represented in **Figure 4.**

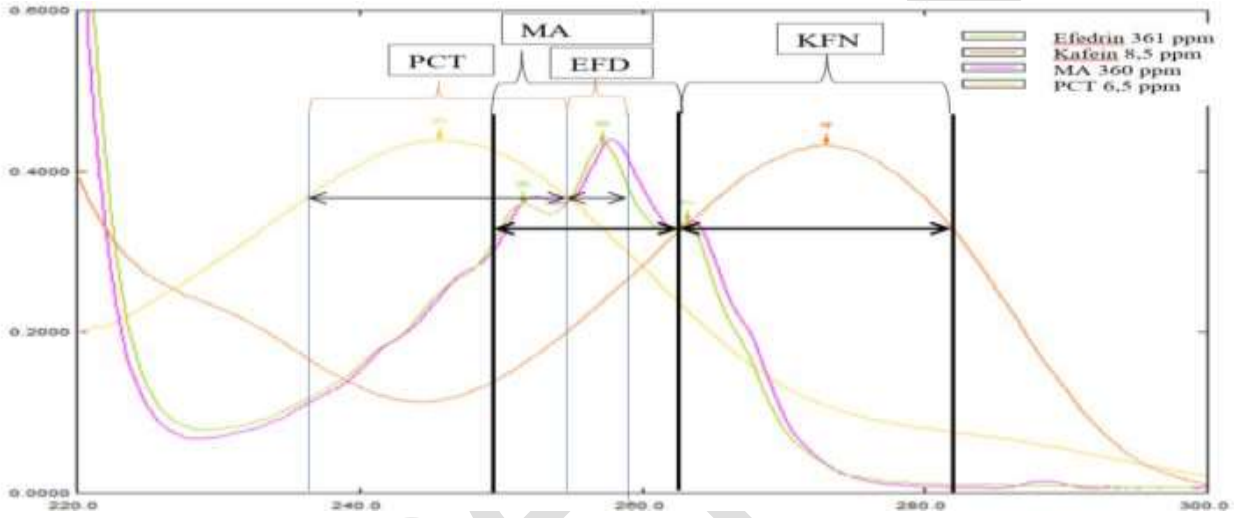

Figure 4. Overlain spectrum of MA, EFD, KFN and PCT.

In a research including the appropriate concentrations of the drugs methamphetamine (MA), ephedrine (EFD), caffeine (KFN), and paracetamol, the concentrations of each were tested in accordance with the Lambert-Beer law (PCT). With a range of 200-400 nm1, the concentrations of 360 µg/ml, 361 µg/ml, 8.5 µg / ml, and 6.5 µg/ml, as well as their mixes in the same concentration, were scanned. The solubility overlaid spectra of MA, EFD, KFN, and PCT that were observed are shown in **Figure. 4**, **Table 8.** and **Table 9.**

Table 8. Result of simultaneous estimation of MA, EFD, KFN and PCT by Dual Wavelength spectrophotometry method.

Component	Content (%)
MA	40.05
EFD	1.63
KFN	38.11
PCT	20.21

Table 9. Optical characteristics of the proposed methods and results of formulation analysis & precision study

No.	Parameter	MA	EFD	KFN	PCT
1	Analytical wavelengths for determination (nm)	263 and 281.8	255 and 236	250.6 and 263	259.4 and 255
2	Lamber beer (µg/mL)	200-520	195-527	4.5-12.5	3.5-9.5
3	Regression equation	Y_{MA} = 5.3581X - 0.0003	Y_{EFD} = 8.1966X – 0.0002	Y_{KFN} = 0.0894X - 0.0189	Y_{EFD} = 0.0799X – 0.0128
4	Correlation coefficient	0.9990	0.9993	0.9990	0.9990
5	Accuracy (%)	101.12	100.16	99.84	100.10
6	Presisi (RSD) (%)	1.59	18.41	0.55	1.82
9	LOD (µg/mL)	30.07	25.50	0.71	0.55
10	LOQ (µg/mL)	91.13	77.29	2.15	1.68

In conclusion, the suggested multiple wavelength approach is conveniently implemented for regular analysis and yields precise and accurate findings for the determination of MA, EFD, KFN, and PCT in tablet mix formulations without prior separation. The dual wavelength method's simplicity and speed are its most alluring qualities. Several linearity, accuracy, and precision tests have validated the validation process. The suggested technique is effectively used to identify this medication in tablets [10].

3.5. Simultaneous estimation of amoxycillin and potassium clavulanate in injection formulation by dual wavelength spectrophotometry

For the purpose of simultaneously determining the dosage formulation of amoxycillin (AMX) and potassium clavulanate (PTC), a straightforward, quick, and accurate dual wavelength approach has been developed.

The absorbance difference between two points on the mixture spectra is directly proportional to the concentration of the component of interest" describes the dual wavelength approach. Based on the aforementioned idea, the two-wavelength data processing program can be used extensively and simply to determine the concentration (unknown) of a specific of interest in a mixture [11].

It is common to find amoxycillin and potassium clavulanate together on the market. After a survey, it was discovered that several UV and HPLC techniques have been published in the literature, either alone or in conjunction with other medications. Single point measurement or simultaneous estimation of many medications were the approaches employed [12]. No technique utilizing 0.1M NaOH as a solvent has been published. Therefore, the purpose of the proposed work was to use UV spectrophotometry to establish a straightforward, quick, accurate, and cost-effective method for simultaneously analyzing amoxycillin and potassium clavulanate in injection dose form, and to validate the method in accordance with ICH guidelines [12].

Alkem Labs Baddi graciously provided a complimentary sample of pure amoxycillin and potassium clavunate. For the study, the commercial formulation (Clavum-150), which comprises 25 mg of potassium clavunate and 125 mg of amoxycillin, was used. For analysis, a double beam UV/visible spectrophotometer (Model UV-1700, Shimadzu, Japan) with a resolution of 1 nm and a 0.5 mm slit width was utilized [13].

Accurately weighed 100 mg each of amoxycillin and potassium clavulanate were then put into separate 100 ml volumetric flasks and dissolved in freshly manufactured 0.1 M NaOH, with the volume increased to 100 ml to obtain a concentration of 1000µg/ml (Stock-A) for both medications. In 100 ml volumetric flasks, 10 ml aliquots of stock-A of amoxycillin and potassium clavulanate were collected separately, and each was diluted up to 100 ml with 0.1 M NaOH to get a 100µg/ml (Stock-B) concentration for both medications. Ultimately, various concentrations of 10, 20, 30, 40, and 50µg/ml of Amoxycillin were produced from stock solution-B. [13].

For every medication, stock solutions containing 1000 µg/ml were produced in 0.1M NaOH. Amoxycillin and potassium clavulanate were produced from stock solutions at a concentration of 25µg/ml. The samples were scanned over a wavelength range of 200–400 nm, and overlaying spectra were seen.

It was shown that the maximum absorbance of amoxycillin is 246.8 nm, while the highest absorbance of potassium clavulanate is 257.6 nm. Since the absorbance maxima of potassium clavulanate and amoxycillin are near to one another and interfere with each other, these two medications can be evaluated simultaneously using the dual wavelength approach [13].

From overlay spectra, it was found that although amoxycillin does not have absorbance on potassium clavulanate maxima, potassium clavunate interferes with it at these locations. As a

result, potassium clavulanate was estimated as a single component, but amoxycillin was determined by selecting two wavelengths at which potassium clavulanate shows similar absorbance (zero absorbance) and amoxycillin was determined by selecting two wavelengths at which potassium clavulanate shows similar absorbance given in **Table 10** [13].

Table 10. Selection of dual wavelength.

Drug	Selected wavelength	
	λ Max (nm)	λ Max (nm)
AMX	261nm	226nm
PTC	279nm	225nm

Analysis of injection sample:

Twenty dry injections were made, the average weight was calculated, and the fine powder was well blended. In a 100 ml volumetric flask, the equivalent of 100 mg of amoxycillin and 100 mg of potassium clavulanate was taken. After dissolving in 0.1M NaOH, this was sonicated for three minutes. 0.1M NaOH was used to bring the volume up to mark, and Whatman filter paper (number 41) was used to filter the mixture. To acquire the final concentration of both medications on the working range, the filtrate was further diluted with solvent. At particular wavelengths, the responses of the final dilutions were recorded, and the concentrations were acquired using the regression equation. To accentuate the process's validation, it was carried out three times. The statistical data given in **Table 11.** and **Table 12.** [13].

Table 11. Analysis of Injection Sample.

S.No.	Conc. Present (µg/ml)		Absorbance (nm)		Conc. Found (µg/ml)		% Conc. Found (µg/ml)	
	PTC	AMX	PTC	AMX	PTC	AMX	PTC	AMX
1	2	10	0.120	0.069	1.94	9.7	97.0	97.1
2	4	20	0.255	0.134	4.04	18.8	101.0	94.4
3	6	30	0.372	0.201	5.90	28.3	98.4	94.3
4	8	40	0.508	0.271	8.06	38.1	100.7	95.4
5	10	50	0.635	0.342	10.0	48.1	100.01	96.3

Table 12. Results of statistical validation of injection formulation.

Drug	% Mean	S.D.	%RSD
AMX	95.50	1.210	1.27
PTC	99.42	1.686	1.70

For AMX and PTC, a variety of dilutions were made with concentrations between 10 and 50 mg/ml and 2 and 10 mg/ml, respectively. The concentration versus response of AMX and PTC were plotted on separate calibration curves, and the values of the slope, intercept, and correlation coefficient (r2) were calculated. The medications' respective slope, intercept, and correlation coefficient value (r2) are given in **Table 13.[**13].

Table 13. Validation Parameters

Method	Parameter	AMX	PTC
Linearity	Slope	0.0066	0.065
	Y-Intercept	0.0025	0.004
	r^2	0.9993	0.9991
Accuracy	% Recovered	99.32	**99.47**
	S.D.	0.3111	**0.193**
	R.S.D	0.630	**0.923**
Precision	S.D.	0.047	0.041
	R.S.D	0.139	0.563
Repeatability	S.D.	0.080	0.045
	R.S.D	0.228	0.600
Day to day	S.D.	0.466	0.020
	R.S.D	1.199	0.233
Analyst to analyst	S.D.	0.047	0.041
	R.S.D	0.139	0.563

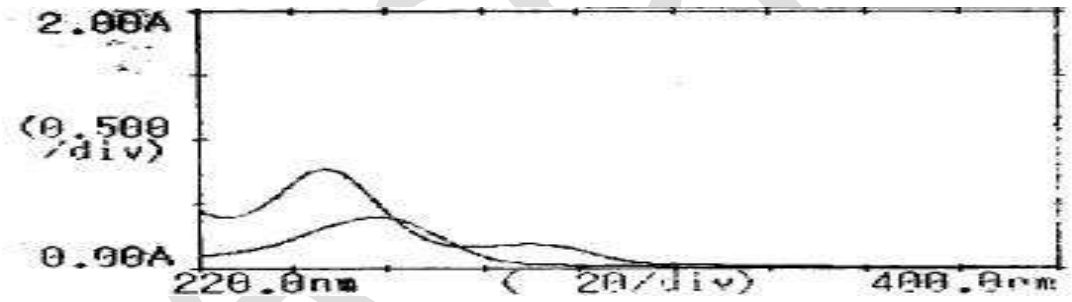

Figure 5. Overlay spectra of Amoxycillin and Potassium clavulanate in 0.1M NaOH.

In the 0.5M NaOH solution, the absorbance maxima for AMX and PTC were discovered to be 246.6 nm and 257.6 nm, respectively. Due to the close proximity of these two medications' absorption maxima, the dual wavelength approach was chosen for their simultaneous assessment. Due to the drug's identical absorbance at these wavelengths, two wavelengths—261 nm, 226 nm

and 279 nm, 225 nm for AMX and PTC, respectively—were chosen for the dual wavelength approach, as shown in **Figure 5.** [13].

3.6. Spectrophotometric Methods for Determination of Salicylamide and Ascorbic acid in their binary mixture

Cidal C® tablets include a combination of salicylamide and ascorbic acid to treat common cold symptoms that include fever and soreness in the muscles. For the purpose of determining SAD and ASC in their binary combination, only one HPLC method has been referenced in the literature [14].

This study's objective is to create and validate various spectrophotometric techniques that can measure SAD and ASC in their binary mixture. The suggested techniques were validated in accordance with ICH requirements [14].

Accurately, aliquots comprising 20–200 µg of each SAD and ASC solution were transferred from their working solutions into two distinct sets of 10 ml volumetric flasks. Double-distilled water was then added to complete the capacity. Double-distilled water was used as a blank to capture the absorption spectra of 10 µg ml-1 of SAD and ASC in the 200–400 nm region [15]. Different aliquots of SAD 50-200 µg and ASC 20-100 µg were transferred from their respective working solutions (100 µg ml-1) in double distilled water to prepare mixtures of different ratios of SAD and ASC. The concentration of each drug was then obtained by following the instructions listed under each suggested method. The mixtures were then placed into 10 ml volumetric flasks [15].

Ten Cidal C® tablets should be well-powdered and mixed. An precisely weighed part of the powdered tablet, equal to 100 mg of SAD and 10 mg of ASC, should be deposited into a 100-ml volumetric flask. Add 75 ml of methanol, sonicate for 30 minutes, filter, and then add more methanol to the flask to reach its full content [15]. This solution serves as the working solution for ASC (100 µg ml-1), and double-distilled water is utilized as a solvent to dilute a portion of the solution to create the working solution for SAD (100 µg ml-1). The steps outlined under each suggested approach were used to assess SAD and ASC, and the relevant regression equations were used to determine each drug's concentration [15].

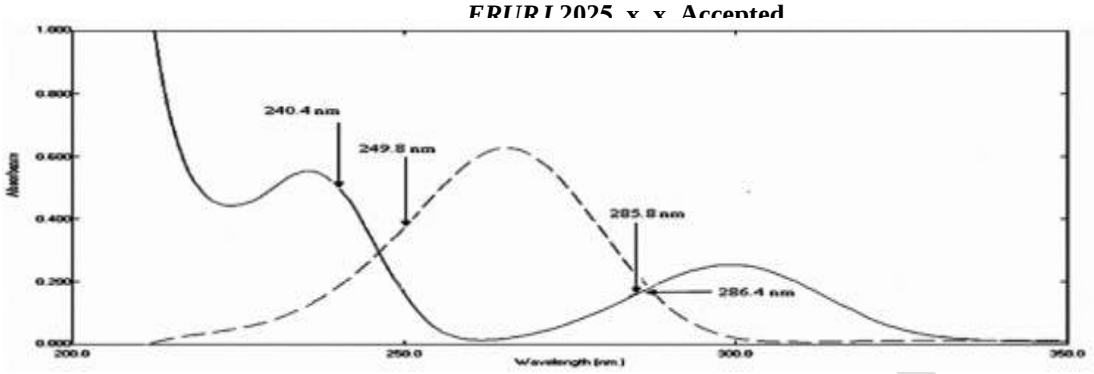

Figure 6. Zero order absorption spectrum of 10 µg mL^{-1} of each of Salicylamide (___) and Ascorbic acid (----) using double distilled water as a solvent showing the selected wavelengths.

Measurements were made of the absorbance values at 240.4 and 286.4 nm for SAD in the range of 2–20 µg ml–1, and at 249.8 and 285.8 nm for ASC in the same range. Plotting the absorbance difference at 240.4 and 286.4 nm (zero difference for ASC) against the corresponding concentration allowed for the determination of SAD. Plotting the difference in absorbance at 249.8 and 285.8 nm (zero difference for SAD) against the respective concentrations was similar for determining ASC. Each drug's calibration curve was created, and regression equations were then computed, as shown in **Figure.6** and **Figure. 7** [15].

The absorbance difference at two places on the spectrum, independent of the interfering component, is directly proportional to the component of interest according to the dual wavelength method's guiding principle. To a large extent, it may be used with little difficulty to determine the unknown concentration of the component of interest in a combination. The selection of two wavelengths where the interfering component exhibits the same absorbance and the component of interest exhibits a notable variation in absorbance with concentration is a prerequisite for the dual wavelength approach, as shown in **Table 14**. And **Table 15.** [15].

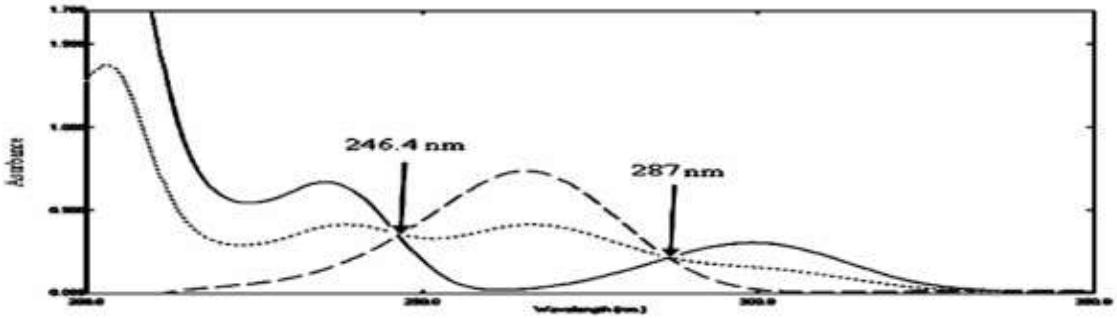

Figure 7. Zero order absorption spectrum of 12 µg mL^{-1} Salicylamide (___) and 12 µg mL^{-1} Ascorbic acid (----) and mixture (salicylamide + Ascorbic acid 6 µg ml^{-1} of each) using double distilled water as a solvent.

Table 14. Results of assay validation.

Parameters	Dual wavelength method	
	SAD	ASC
Range		
Slope	0.0324	0.0144
Intercept	-0.0030	0.0209
Correlation coefficient	0.9997	0.9998
Accuracy (mean ± SD)	99.85 ± 1.183	100.02 ± 1.322
Selectivity (mean ± SD)	100.25 ± 1.457	100.83 ± 1.829
Precision (%RSD)		
Repeatability*	1.30	1.24
Intermediate precision*	1.65	1.55
LOD** (µg mL^{-1})	0.57	0.62
LOQ** (µg mL^{-1})	1.72	1.86

Table 15. Statistical comparison of the results obtained by dual wavelength for the determination of pure Salicylamide and Ascorbic acid

Items	Dual wavelength method	
	SAD	ASC
Mean	99.85	100.02
SD	1.183	1.322
%RSD	1.185	1.322
N	10	10
Variance	1.404	1.748
Student's t-test (1.761)**	0.544	0.279
F-test (3.482)**	1.186	1.244

Ethopabate binary mixture:

Ethopabate and amprolium hydrochloride are frequently used to treat and prevent chicken coccidiosis. Since both are frequently combined, it's critical to create straightforward spectrophotometric techniques to measure them both at the same time [16].

Amprolium hydrochloride is an antiprotozoal that is used in veterinary medicine to treat coccidiosis in pigeons and poultry, either by itself or in combination with other medications like etopabate [16]. Ethopabate is an antiprotozoal that is used in veterinary medicine in conjunction with other medications, including amprolium, to treat coccidiosis in chicken [16].

Apparatus:

Spectrophotometer: IBM compatible PC and HP-600 inkjet printer are linked to the dual beam

UV-visible SHIMADZU UV-1601 PC spectrophotometer, which has two matching 1-cm quartz cells. The absorption is processed using Bundled UV-PC Personal Spectroscopy software version (3.91). The wavelength scanning speed is 2800 nm min^{-1} and the spectral band width is 0.1 nm [17]. The feed additive Amprolium & Ethopabate PREMIX 25%® (Batch No. 1203322), made by Adwia Co. S.A.E. 10th of Ramadan city, Egypt, is labeled to contain 250 gm of Amprolium hydrochloride and 16 gm of Ethopabate per kilogram [17].

Standard solutions: AMP stock standard solution: 200 lg/mL in methanol. ETH stock standard solution: 200 lg/mL in methanol. Different aliquots equivalent to 20–320 mcg of AMP and 10–140 mcg of ETH is accurately transferred from their standard stock solutions (200 mcg/mL) into two separate series of 10-mL volumetric flasks then completed to volume with methanol. Using the scanned spectra, absorbance values at both 235.3 and 308 nm (for AMP) and at both 244 and 268.4 nm (for ETH) are measured. AMP is determined by plotting the difference in absorbance at 235.3 and 308 nm (difference is zero for ETH) against its corresponding concentration. Similarly, for determination of ETH, the difference in absorbance at 244 and 268.4 nm (difference is zero for AMP) is plotted against its corresponding concentration. The concentrations of the two drugs are calculated each from the corresponding calibration curve equation. Similar to this, the absorbance difference at 244 and 268.4 nm (which is 0 for AMP) is plotted against the relevant concentration to determine ETH. Each drug's concentration is determined using the matching calibration curve equation, **Figure. 8.** [17].

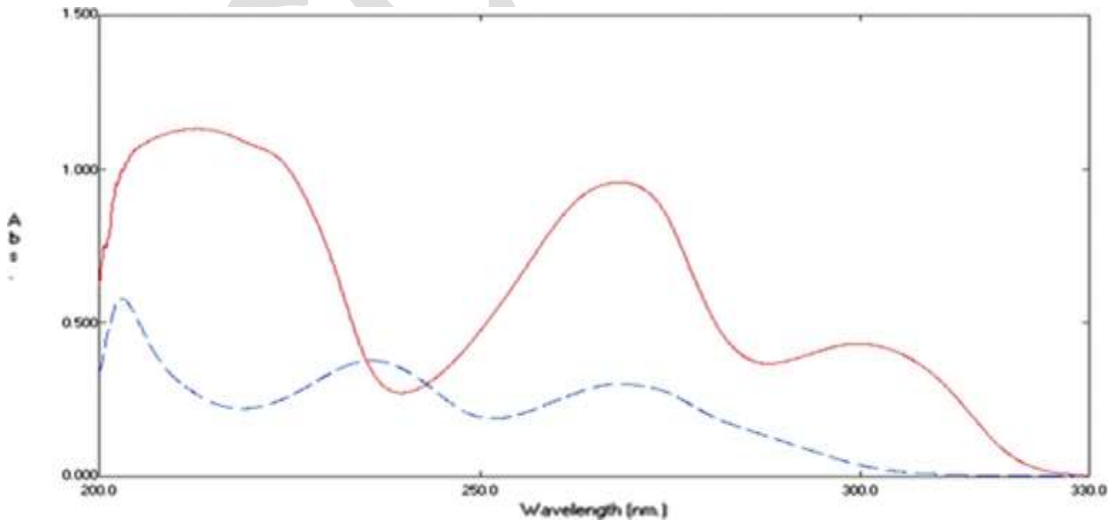

Figure 8. Zero order absorption spectra of 10 mcg/mL AMP (- - - - -), 10 mcg/mL ETH (___), using methanol as blank.

The absorbance difference at two places on the spectrum, independent of the interfering component, is directly proportional to the component of interest according to the dual wavelength method's guiding principle. The selection of two wavelengths where the component of interest exhibits a considerable difference in absorbance and the interfering component exhibits the same absorbance is a prerequisite for the dual wavelength approach. **Figure. 9** shows the testing of various sets of wavelengths for ETH determination. Similarly, the absorbance values of ETH are the same at wavelengths 235.3 and 308 nm; for this reason, these two wavelengths are chosen to determine AMP.

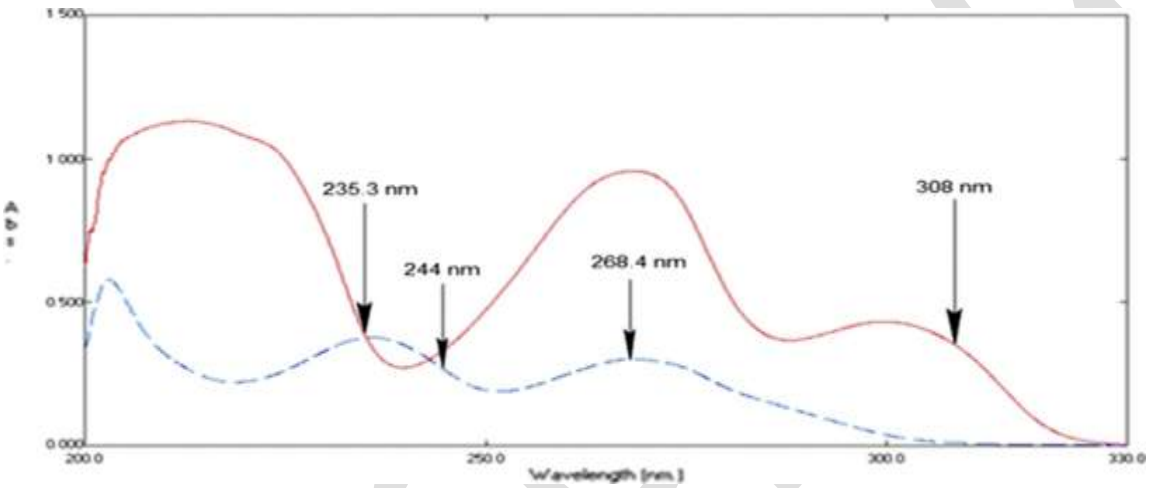

Figure 9. Zero order absorption spectra of 10 mcg/mL of AMP (- - - -) and 10 mcg/mL of ETH (—) showing the selected wavelengths for dual wavelength method using methanol as a blank.

Plotting the difference in absorbances for AMP and ETH at 235.3 and 308 nm versus their respective concentrations shows that the former is more concentrated in the range of 2–32 mcg/mL, while the latter is more concentrated in the range of 1–14 mcg/mL. The following regression equations can be used to determine the concentration of AMP and ETH:

$$\Delta A_{AMP} = 0.0409C - 0.0029 \quad r = 0.9999$$

$$\Delta A_{ETH} = 0.0637C - 0.0141 \quad r = 0.9999$$

where the absorbance difference at 235.3 and 308 nm is represented by ΔAAMP. The absorbance difference between 244 and 268.4 nm is expressed as ΔAETH. C represents the AMP and ETH concentration in mcg/mL. The correlation coefficient is denoted by r, as shown in **Table 16.** and **Table 17**.

Table 16. Results of assay validation.

Parameters	Dual wavelength method	
	AMP	ETH
λ (nm)	Difference at 235.3 and 308	Difference at 244 and 268.4
Concentration range (μg/mL)	2–32	1–14
Linearity		
Slope Intercept Correlation coefficient (r)	0.0409 0.0029 0.9999	0.0637 0.0141 0.9999
Accuracy (mean ± S.D.)	99.30 ± 1.097	100.03 ± 1.065
Specificity	99.95 ± 0.830	99.93 ± 0.652
Precision (%RSD)		
Repeatability[a] Intermediate precision[b]	0.504 1.204	0.229 0.602
LOD[c] (μg/ml)	0.049	0.08
LOQ[c] (μg/ml)	0.149	0.244

Table 17. Statistical comparison of the results obtained by dual wavelength for the determination of pure Amprolium hydrochloride and Ethopabate.

Value	Dual wavelength method	
	AMP	ETH
Mean	99.30	100.03
SD	1.097	1.065
RSD%	1.105	1.065
N	6	6
Variance	1.203	1.134
Student's *t*-test[a]	1.351 (2.228)	0.648 (2.228)
F value[a]	2.021 (5.050)	2.098 (5.050)

4. Conclusion

In conclusion, the dual wavelength method proves to be a robust and efficient technique for the simultaneous determination of two drugs in pharmaceutical mixtures. Through a comprehensive review of existing literature and methodologies, this paper highlights the advantages of employing this method, including its simplicity, accuracy, and cost-effectiveness.

By utilizing two wavelengths, it allows for the quantification of both drugs without the need for complex sample preparation or separation techniques. Furthermore, the method offers excellent selectivity, sensitivity, and precision, making it suitable for various pharmaceutical applications. However, it is essential to consider the potential limitations and challenges associated with this approach, such as interference from excipients or overlapping absorption spectra.

All things considered, the dual wavelength approach offers a viable way to analyze drug mixes and gives researchers and pharmaceutical analysts a useful tool for dosage calculation and quality assurance. Additional investigation and advancement in this domain may result in ongoing improvements and more extensive utilization of this approach in the study of pharmaceuticals.

- **Conflict of Interest**

The Authors declare no conflict of interest.

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