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RECENT ADVANCES IN UV-VISIBLE SPECTROPHOTOMETRIC METHOD DEVELOPMENT AND VALIDATION FOR PHARMACEUTICAL DRUG ANALYSIS

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Keywords

C UV-Vis spectrophotometry, atorvastatin, Etoricoxib, simultaneous estimation, pharmaceutical analysis, multivariate calibration.

Abstract

This work set out to meet the requirements of ICH Q2(R1) for the quantitative assessment of paracetamol in both its pure form and its commercial tablet forms by creating and validating a simple, precise, accurate, and cost-effective UV-Visible spectrophotometric technique. The absorbance measurements were taken using a double-beam Shimadzu UV-1800 spectrophotometer at 257 nm, with 0.1 N sodium hydroxide serving as the solvent in the procedure. Over the linear concentration range of 5-25 µg/ml, the created technique complied with Beer-Lambert's law, producing a calibration curve with a remarkable correlation coefficient (r^2) of 0.9995.

The technique's linearity, accuracy, precision, repeatability, intra-day, and inter-day performance, as well as its limit of detection (LOD) and limit of quantitation (LOQ) were all thoroughly tested, and the method was also tough and durable. The recovery tests were conducted at 80%, 100%, and 120% levels of accuracy. The method's dependability and repeatability were confirmed by recovery values that were within the

	permitted range of 98-102% and %RSD values for precision studies that stayed below 2%. Calpol 650 mg, Calpol 500 mg, and Dolo 650 mg were found to have percentage purity findings that fell within the labeled claim by 98-102% when the test was conducted utilizing absorptivity, single-point standard comparison, and calibration curve techniques. The suggested approach is ideal for the regular quality control analysis of paracetamol in pharmaceutical tablet dosage forms and bulk medicinal substances since it is simple, quick, cheap, and does not interact with common excipients.

[1] INTRODUCTION

There is a yearly increase in the number of new medications available to consumers. These medications could be brand-new or slightly altered versions of current ones. The goal of any analytical evaluation is to collect data that is consistent, dependable, and correct. Achieving this objective is greatly assisted by validated analytical procedures. An essential aspect of excellent analytical practice is using the findings of method validation to assess the quality, reliability, and consistency of analytical data. The majority of quality standards and laws that affect labs also need analytical method validation. The inclusion of a medication in pharmacopoeias is typically delayed from the date of commercial debut. There are a number of factors that contribute to this, including concerns about the long-term effects of these medications, the emergence of patient resistance, the release of more effective alternatives, and news of new toxicities that drive their removal from the market. The pharmacopoeias may not have analytical methods or standards for these medications in these circumstances. Newer analytical procedures for these medicines may therefore be developed. The creation and validation of analytical techniques are crucial processes in the pharmaceutical industry, including drug discovery, development, and manufacturing.

Pharmaceutical goods that include several medications, sometimes known as combination products, are created to address patients' needs that have not been fulfilled before. These products need the development and validation of analytical methods in order to harness the combined therapeutic benefits of the pharmaceuticals. Developing and validating analytical techniques may be a challenging task for analytical chemists when dealing with these combination products. Quality control labs employ the approved test procedures that come from these processes to guarantee that drug items are authentic, pure, effective, and safe to use. Impurity identification and quantification is an important step in developing safe and effective pharmaceutical processes. There are a number of potential sources of undesired compounds in pharmaceuticals, known as related components. These might arise during stability testing, formulation, or the aging process for both the active pharmaceutical ingredients (APIs) and the medications that include them. Even trace levels of these undesirable compounds have the potential to compromise the safety and effectiveness of pharmaceuticals. The identification of associated pharmaceutical components makes use of a wide range of analytical techniques. New analytical approaches for evaluating the quality of developing medications are urgently needed.

- **UV-Visible spectroscopy:**

Measurements of light absorption throughout the visible and ultraviolet (UV) portions of the electromagnetic spectrum are made in UV-visible spectroscopy. The ultraviolet (UV, 200–400 nm) and visible (VIS, 400–800 nm) portions of this absorption spectroscopy use electromagnetic radiations within the 200–800 nm range. The underlying idea of UV-Visible spectroscopy is that various spectra are produced when a sample or chemical compound absorbs either visible or ultraviolet light. One kind of electronic energy transition that occurs when a molecule absorbs ultraviolet light is the excitation of its electrons, which leads to a change in the electrons' energy level inside the molecule, from a lower to a higher one. This process gives birth to the ultraviolet emission spectrum. Solvents such as water, methanol, ethanol, ether, chloroform, carbon tetrachloride, cyclohexane, and dichloromethane are often used in ultraviolet spectroscopy. Among the many uses for ultraviolet (UV) spectroscopy are the identification of functional groups, conjugation, geometrical isomers, and contaminants [1].

[2] FUNDAMENTALS OF UV-VIS SPECTROPHOTOMETRY

As a standard procedure, analytics often use UV-Vis spectrophotometry to evaluate the sample's absorption of light at both visible and ultraviolet wavelengths. As a result of absorption at wavelengths that coincide with their electronic transition points, the approach is able to operate. Electromagnetic light absorption spectra from 200 to 800 nm provide crucial information on the molecular structure and solution composition of the substances under study [2].

➢ Principle of UV-Vis Spectrophotometry

The principle that electromagnetic radiation interacts with various kinds of materials is the foundation of UV-Vis spectrophotometry. Samples are encountered by UV or visible light beam antennas, with certain wavelengths absorbing the molecules and others allowing them to flow through. In order to facilitate electron transitions between lower and higher energy states, molecules in a solution absorb light at certain frequencies. The absorption level at certain wavelengths is defined by the concentration of absorbing species in a sample, according to Beer-Lambert's law [3-4].

UV-Vis spectrophotometry is based on Beer-Lambert's law, which is sometimes called Beer's law, and is stated as:

$A = \varepsilon \cdot c \cdot l$

Where:

- A is the absorbance of the sample,
- ε is the molar absorptivity (or molar absorption coefficient),
- c is the concentration of the analyse,
- L is the path length through the sample.

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A. Applications in Pharmaceutical Analysis

The fundamental principle upon which UV-Vis spectrophotometry is based is the fact that various kinds of materials are affected by electromagnetic radiation. Some wavelengths of visible or ultraviolet light are absorbed by samples, while others are allowed to flow through when the antennas come into contact with them. Molecules in a solution absorb light at certain frequencies, allowing electrons to move more easily between lower and higher energy levels. According to Beer-Lambert's law, the absorption level at certain wavelengths is defined by the concentration of absorbing species in a sample [6].

- API Quantification: The principle that underlies UV-Vis spectrophotometry is that various forms of materials are affected by electromagnetic radiation. Samples are encountered by UV or visible light beam antennas, with certain wavelengths absorbing the molecules and others allowing them to flow through. In order to facilitate electron transitions between lower and higher energy states, molecules in a solution absorb light at certain frequencies. The absorption level at certain wavelengths is defined by the concentration of absorbing species in the sample, according to Beer-Lambert's law [7].

- Research on Drug Stability: The foundation of ultraviolet-visible spectrophotometry is the interaction of electromagnetic radiation with various substances. Samples are encountered by UV or visible light beam antennas, with certain wavelengths absorbing the molecules and others allowing them to flow through. In order to facilitate electron transitions between lower and higher energy states, molecules in a solution absorb light at certain frequencies. According to Beer-Lambert's law, the absorption level at certain wavelengths is defined by the concentration of absorbing species in a sample [8].
- Developing the Model: The foundation of UV-Vis spectrophotometry is the interaction of electromagnetic radiation with various forms of materials. Samples are encountered by UV or visible light beam antennas, with certain wavelengths absorbing the molecules and others allowing them to flow through. In order to facilitate electron transitions between lower and higher energy states, molecules in a solution absorb light at certain frequencies. The absorption level at certain wavelengths is defined by the concentration of absorbing species in the sample, according to Beer-Lambert's law [9].
- Drug Release Analysis: Dissolution testing, made possible by UV-Vis spectrophotometry, allows for the analysis of drug release patterns from dosage forms. The method allows for the continuous monitoring of drug concentration changes in the dissolving medium, which is crucial for understanding the kinetics of drug release and the availability of pharmaceuticals. Since the creation of controlled-release formulations requires exact regulation of the drug release rate, this becomes crucial to the process [10].
- Impurity and Contaminant Identification: UV-Vis spectrophotometry is a tool for identifying and quantifying contaminants in pharmaceuticals. In order to detect undesirable compounds or degradation products, UV-Vis spectrophotometry analyses the absorption patterns of a pharmacological substance relative to a reference standard. This method must meet regulatory requirements for the safety and purity of pharmacological products [11].
- Applications in Bio analysis: In clinical labs, UV-Vis spectrophotometry is used to analyse drug concentrations inside biological samples such plasma, serum, and urine. Because it gives precise measurements of drug concentrations, the approach is crucial for therapeutic drug monitoring, which is necessary for ensuring the efficacy and safety of medicine administration. Analytical applications of ultraviolet-visible spectrophotometry include tracking the absorption, distribution, metabolism, and excretion (ADME) of drugs in biopharmaceutical studies [12].
- Concurrent Evaluation of Medications: UV-Vis spectrophotometry offers crucial functionality in measuring many medications that are used in treatment regimens. With a single examination, UV-Vis spectrophotometry accurately analyses both the atorvastatin and etoricoxib medicines in combination therapy. Researchers are able to detect medication levels in a mixture without being influenced by other components thanks to a combination of multicomponent analytic approaches, such as derivative spectroscopy and multivariate calibration [13].

[3] RESEARCH METHODOLOGY

The technique for estimating paracetamol was developed and verified using UV-Visible spectroscopy in accordance with the recommendations laid forth by ICH Q2(R1). The research used raw paracetamol (API) and three commercially available tablet formulations: Dolo 650 mg, Calpol 500 mg, and Calpol 650 mg. The reagents and chemicals used were all of analytical quality. As a result of the solubility tests, 0.1 N sodium hydroxide (NaOH) was chosen as the solvent. For all absorbance measurements, a Shimadzu UV-1800, a double-beam UV-Visible spectrophotometer, was used. The cells employed in this instrument were 1 cm quartz.

To make a 1000 µg/ml standard stock solution of paracetamol, 100 mg of pure drug was measured and transferred to a 100 ml volumetric flask. Then, 50 ml of 0.1 N NaOH was dissolved in the drug, using sonication if needed, and the remaining capacity was filled with the same solvent. The stock solution was appropriately diluted with 0.1 N NaOH to generate working standard solutions with concentrations ranging from 5 to 25 µg/ml. The solutions' absorbance was measured in the 200-400 nm range using 0.1 N NaOH as a blank to find the wavelength at which the absorbance was at its highest (λmax). All further measurements were conducted at the 257 nm wavelength, which was determined to be the λmax.

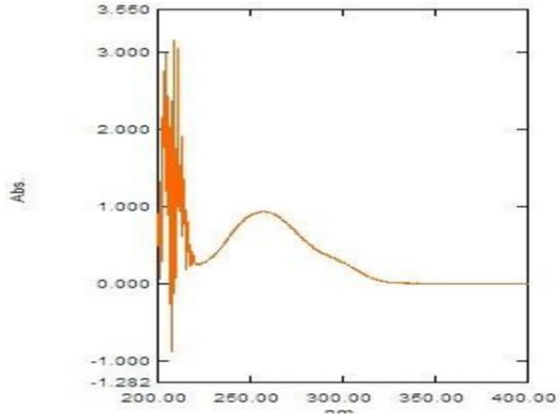
By measuring the absorbance of five different concentrations (5, 10, 15, 20, and 25 µg/ml) in triplicate and creating the calibration curve, linearity was confirmed in order to validate the devised technique. Three levels of recovery investigations were conducted using the conventional addition approach to assess accuracy: 80%, 100%, and 120%. Inter- and intra-day investigations, as well as repeatability (measurements of a 10 µg/ml solution taken six times on the same day), were used to assess precision. Based on the response standard deviation and the calibration curve's slope, the limits of detection (LOD) and quantitation (LOQ) were determined. Deliberately altering experimental circumstances, such as the amount of time between preparation and measurement, allowed for the evaluation of robustness. Three separate analysts using the same tool verified for ruggedness in the results.

The paracetamol tablet assay required twenty weighed and finely powdered pills from each brand. A 100 milliliter volumetric flask was used to hold the powder that was measured to be 100 milligrams of paracetamol. To ensure thorough extraction, sonication was used after adding about 30 ml of 0.1 N NaOH and shaking the liquid manually for 15 minutes. Filtered via What man filter paper, the volume was brought to the mark using 0.1 N NaOH. The filtrate, which had a concentration of 1000 µg/ml, was appropriately diluted to achieve a final concentration of 10 µg/ml. A blank solution of 0.1 N NaOH was used to test the absorbance of this solution at 257 nm. The absorptivity technique, the single-point standard comparison method, and the calibration curve method were used to determine the quantity of paracetamol in the tablets. We computed the percentage of purity for each brand after performing all assays in triplicate.

Statistical validation in accordance with ICH standards confirmed that the created procedure complied with Beer's law within the chosen concentration range. The solutions were kept out of direct sunlight and all experimental procedures were conducted at room temperature.

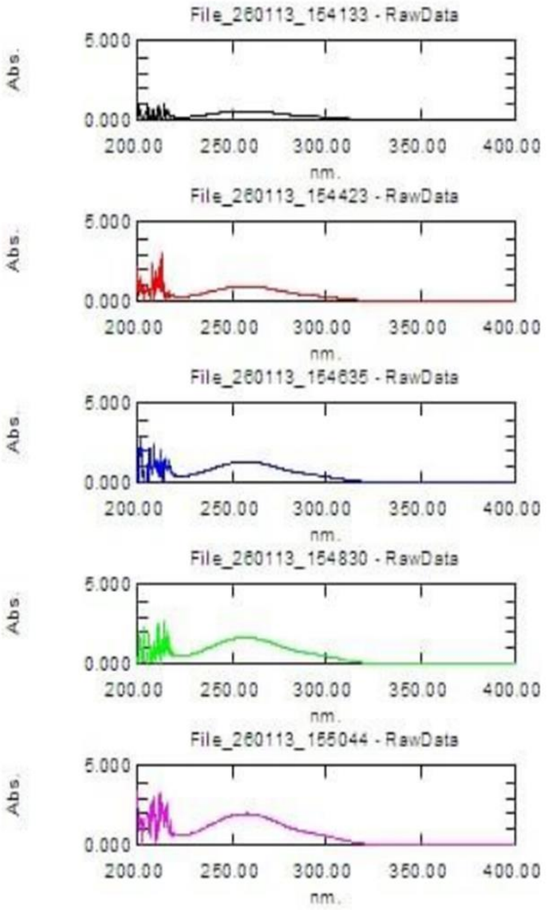
[4] RESULTS

A UV-visible spectrum spanning 200–400 nm was reported for pure paracetamol in 0.1 N NaOH. A sharp maximum absorption (λmax) occurred at 257 nm for the medication. Graph No. 1 shows the pure paracetamol absorption curve.



Graph No: 1 Absorption Curve of Paracetamol (Pure Form)

The absorbance of standard paracetamol solutions at five different concentrations, ranging from 5 to 25 µg/ml, was used to test the linearity of the proposed approach. You can see the results of the absorbance tests in Table No. 1. By comparing absorbance with concentration, the calibration curve was constructed. The method's correlation coefficient (r^2) was 0.9995, indicating good linearity. Graph No. 2 shows the absorbance curve for linearity.



Graph No: 2 Linearity Absorbance Curve

Table No: 1 Linearity Result

Concentration (µg/ml)	Absorbance (nm)
5 µg/ml	0.576 nm
10 µg/ml	0.947 nm
15 µg/ml	1.305 nm
20 µg/ml	1.657 nm
25 µg/ml	1.986 nm

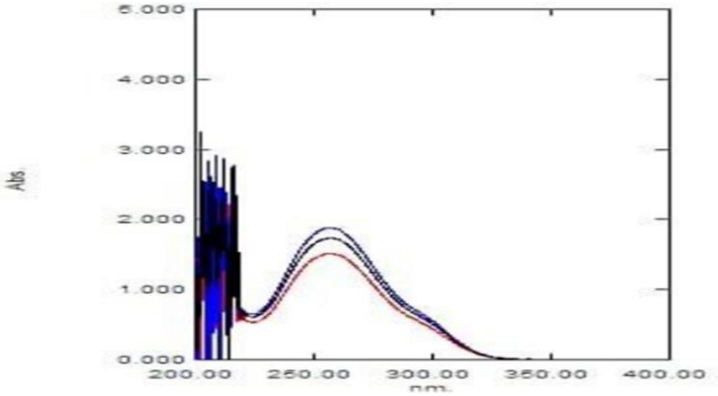
Recovery tests were conducted using the standard addition methodology at three concentration levels (80%, 100%, and 120%) to assess the accuracy of the new method. The computed percentage recovery values were within the permitted range of 98% to 102%. Table 2, Table 3, and Graph No. 3 provide the recovery data and thorough accuracy measurements.

Table No: 2 Accuracy Measures

Absorbance (nm)		
80%	**100%**	**120%**
1.511	1.65	1.8
1.500	1.64	1.78
1.502	1.67	1.8
Average:1.507	1.653	1.793

Table No: 3 Recovery Studies

Level	Sample (Amount Present)	Standard Added	Final Concentration	Amount Found	% Recovery
80%	10 µg/ml	8 µg/ml	18 µg/ml	18.014 µg/ml	100.175%
100%	10 µg/ml	10 µg/ml	20 µg/ml	20.089 µg/ml	100.89%
120%	10 µg/ml	12 µg/ml	22 µg/ml	22.072 µg/ml	100.6%



Graph No: 3 Accuracy Curve

Repeatability, intra-day precision, and inter-day precision were used to evaluate the method's accuracy. A solution with a concentration of 10 µg/ml was measured six times to ensure reproducibility. To determine intra-day accuracy, we analyzed the same concentration at various time intervals on the same day; to assess inter-day precision, we analyzed it across three consecutive days. The %RSD was determined to be less than 2% across the board, which is far lower than the ICH approval threshold. Table No. 4 summarizes the precision findings.

Table No: 4 Precision Results

Precision	Absorbance (nm)	Mean	Standard Deviation	% RSD
Inter-day	0.929 nm	0.945	0.009	0.952
	0.946 nm		0.0005	0.052
	0.960 nm		0.008	0.846
Intra-day	0.929	0.928	0.0005	0.062
	0.931		0.0017	0.186
	0.926		0.0011	0.124

Using the formulae LOD = 3.3 × σ/S and LOQ = 10 × σ/S, where F is the standard deviation of the response and S is the slope of the calibration curve, the limit of quantitation (LOQ) and limit of detection (LOD) were determined. We observed that the LOD was 0.224 µg/ml and the LOQ was 0.680 µg/ml. You may see these outcomes in Table No. 5.

Table No: 5 LOD & LOQ Result

Drug	Absorbance (nm)	Standard Deviation	Slope	LOD	LOQ
Paracetamol (10 µg/ml)	0.947nm	0.0159	0.0706	0.743 µg/ml	2.25 µg/ml

To test the method's robustness, we purposefully introduced tiny changes to one of the analytical parameters (the amount of time we measured after solution preparation at5,10, and 15 minutes). The method's robustness was shown by the absorbance readings maintaining consistency with low %RSD.

In order to measure ruggedness, three separate analysts used the same tool to conduct the study. The acquired %RSD values were within acceptable bounds, indicating that the suggested approach is robust.

The three commercially available tablet formulations of paracetamol (650 mg Calpol, 500 mg Calpol, and 650 mg Dolo) were all accurately assayed using the established methodology. We used 0.1 N NaOH to extract 100 mg of paracetamol from 20 powdered pills of each brand. We used the absorption technique, the single-point standard comparison method, and the calibration curve method to determine the purity %. All brands' percentage purity results were within the specified range of 98% to 102%.

The findings show that the UV-Visible spectrophotometric technique is easy to use, quick, exact, sensitive, strong, and not easily broken. In the concentration range of 5-25 µg/ml, it follows Beer's Lambert's rule and is often used for estimating paracetamol in both bulk medication and pharmaceutical tablet forms.

[5] ADVANTAGES OF ANALYTICAL METHOD VALIDATION

Validating methods gives both the developer and the user peace of mind. While validation may seem like a waste of time and money at first, it really ends up saving money, doing away with annoying repeats, and improving time management.

Even though it's evident that factors like reagent supplier or grade and analytical setup are subject to change, method validation mitigates the impact of these shocks and returns more investment than the process itself.

The "Q.C. Department" of pharmaceutical organizations will find the to-be-developed methodologies useful for their regular analyses.

More modern medications and their details, whether in single or combined dosage forms, are calculated and promoted over the long term. The time it takes for a medicine to get from being introduced to the market to being considered for inclusion in pharmacopoeias is always longer. Therefore, developing novel diagnostic techniques for these drugs becomes very important.

In order to determine what a material is made of and in what quantities, it is necessary to conduct a formulation analysis to get information on its qualitative and quantitative composition. The improvement of the drug's therapeutic activity and production processes depend on this data.

- **Selection of Analytical Wavelength: [14]**

The drug solution in the mobile phase was scanned in a UV-visible spectrophotometer within the 200–400 nm range to determine the optimal wavelength. A drug's analytical wavelength, or lambda max, is the wavelength at which its absorption is at its highest.

- **Specificity: [15]**

The ability to precisely measure the amount of the analytic of interest, even when additional additives that are likely to be present in the sample matrix are present, is known as specificity. The level of influence from such topics may be measured by its specificity. To guarantee a peak response, it might include various active ingredients, excipients, contaminants, and breakdown products. The resolution, plate count, and tailing factor are used to assess specificity within the study, and this information is then reported.

The capacity to distinguish between compounds with similar structural features or to compare to known reference materials demonstrates specificity when used for identification purposes. The specificity of an assay or impurity test may be shown by determining which two chemicals in the sample eluted to the highest concentration. In most cases, the molecules are both an essential element and an undesirable byproduct. It must be shown and shown that the inclusion of spiking items, such as contaminants or excipients, does not impact the assay if the sample contains them. We compare the test findings to another well-established process to ensure the absence of contaminants. The two outcomes are contrasted in order to conduct the test. To conduct impurity testing, it is necessary to compare the impurity profiles.

Measurements and documentation of specificity in a separation are done using the resolution, plate count efficiency, and tailing factor. Additionally, current photodiode array detectors may be used to assess specificity by quantitatively comparing spectra gathered throughout a peak, which indicates the homogeneity of that peak. Specificity is also used by ICH, which classifies it as either identification tests or assay testing.

Ensuring that the test is unaffected by the presence of contaminants or excipients is crucial for demonstrating specificity. To put this into reality, one may show that the assay remains unaffected by the addition of suitable amounts of contaminants or excipients to the medicinal compounds or product. It is possible to prove specificity in the absence of degradation product impurity standards by comparing the outcomes of one well-characterized process to those of another when testing materials that include contaminants or degradation products.

By comparing the chromatograms of the test, standard, and blank samples, the specificity of the target analytic may be shown. No diluent or placebo peaks were detected at the primary peaks. The excipients were shown to not interfere with the analysis, according to the specificity testing. The chromatogram did not show any signs of excipient interference. The results show that the strategy is targeted.

- **Linearity and Range: [16]**

If the technique can provide test findings that are proportionate to the analytic concentration within a certain range, then it is considered linear. In most cases, the variance of the slope of the regression line is used to indicate linearity. The range is the interval between the highest and lowest analytic levels that may be measured using the given technique with good precision, accuracy, and linearity. In most cases, the range is given in the same units as the method's test findings. The International Conference on Harmonization (ICH) standards provides specific ranges and at least five concentration levels.

By using seven distinct calibration standards, the linearity of the suggested UV technique was confirmed. We used the results of the calibration standard study to create calibration curves that plot absorbance against concentration and then ran them via linear least square regression. To prove that the suggested approach was linear, the R-squared value was thought to be a crucial metric. The stated range of the suggested UV technique is the interval between the upper and lower concentration limits with satisfactory linearity.

A concentration between 80% and 120% of the target is required to conduct the test. The absolute minimum for an impurity test is the range from the impurity reporting levels to 120% of the specification.

- **Accuracy: [17]**

Analytical precision, or the degree to which the result agrees with a commonly acknowledged reference or conventional real value, is what we mean when we talk about accuracy. The percentage of analytic recovered by assay, as determined by spiking samples in a blind trial, is the unit of measurement. Measurements of accuracy are achieved for the drug substance test by comparing the findings to either standard reference material analysis or a second, well-characterized approach. To ensure precision in the drug product test, synthetic mixes spiked with known amounts of components are analyzed.

Data should be collected from at least nine determinations over at least three concentration levels encompassing the defined range (e.g., three concentrations with three replicates each) in order to demonstrate accuracy, according to the ICH methodological guideline. You may provide the data as a percentage of the known, additional amount's recovery rate or as the difference between the mean and real value with confidence intervals. Since the findings' percentage recoveries fall squarely inside the acceptable range, we may conclude that the procedure is valid.

- **Precision: [18]**

Typically, the percentage relative standard deviation for a statistically significant number of samples is used to describe precision, which is a measure of the degree of repeatability of an analytical technique under normal operation. Repeatability, intermediate precision, and reproducibility are the three tiers of accuracy that the ICH recommends. When a technique runs under the same circumstances of inter-assay precision over a short period of time, it becomes repeatable. For example, three levels with three repeats each, or six determinations at 100% of the test or target concentration would enough to cover the given range of the method. Results from within-lab variance owing to random occurrences (e.g., various days, analysts, equipment, etc.) provide intermediate precision. It is recommended to use experimental design while calculating intermediate precision in order to monitor the impact of the various factors.

- **Limit of Detection: [19]**

When analyzing a sample, the limit of detection is the lowest concentration of the analytic that can be detected, but not necessarily quantified. A limit test determines whether an analytic is more than or less than a predetermined value. At concentrations close to the limit of detection (LOD), the formula LOD = 3.3 (S.D/A) may be used to get the LOD from the response standard deviation and the slope (S) of the calibration curve. Using the residual standard deviation of the regression line—the standard deviation of the blank—as a foundation, we can calculate the standard deviation of the answer. On the other hand, you may find it by calculating the standard deviation of the y-intercept of the regression lines. The process used to establish LOD must be documented and supported. To ensure the established technique is valid, it is necessary to evaluate a sufficient number of samples.

- **Limit of Quantitation: [19]**

The smallest quantity of analytic that can be detected in a sample is called the limit of quantitation (LOQ). This parameter provides the concentration of an analytic in a sample that can be accurately and reliably measured under the method's specified operating parameters. In order to determine the LOQ, the response's standard deviation (S.D.) and the calibration curve's slope (A) are used. A proper calculation would be LOQ = 10 (S.D/A). The response's standard deviation is calculated by taking the square root of the blank (S.D.), the regression line's residual standard deviation, or the standard deviation of the y-intercepts of the regression lines in the plot. Just like LOD, LOQ should likewise have a published and supported mechanism. In order to verify the established approach, it is necessary to examine a sufficient number of samples at the limit.

- **Robustness: [19]**

A robust technique is one that can withstand intentional but minor changes to its parameters with little to no impact on the outcome. A number of technique factors, such as the amount of organic solvent, pH, ionic strength, or temperature, are used to assess a method's robustness by seeing how they impact the method's outcomes. Depending on the research type, robustness evaluations should be addressed throughout development. It has to demonstrate that the analysis is reliable with respect to intentional changes to the technique parameters. The findings were computed as a percentage change to conduct a robustness test, which included making tiny changes to the chromatographic conditions at a concentration equivalent to the standard concentration. Specifically, here, we tested resilience by varying the detector's wavelength, mobile phase flow rate, and mobile phase ratio.

- **System Suitability Testing: [19]**

An essential component of several analytical processes is system appropriateness testing. The idea that the apparatus, electronics, analytical procedures, and samples to be examined form a whole that can be assessed in this way is the foundation of the tests. The validation process type determines the system suitability test parameters that need to be set for that method. For further information, see the pharmacopoeias.

[6] RECENT ADVANCES AND INNOVATIONS

Because pharmaceutical analysers need quick and accurate analytical methods with wider application, UV-Vis spectrophotometry has seen a plethora of recent major advancements. Scientists have developed novel methods for UVVis spectrophotometry, which improve sensitivity performance while decreasing interference, and address basic problems with multiple components analysis. UV-Vis spectrophotometry, which is used to examine complicated combinations of atorvastatin and etoricoxib, has been made more precise and reliable by strategies using novel methods and methodologies [20] [21].

- **Novel Approaches in UV-Vis Spectrophotometry**

1. Derivative Spectrophotometry: One new development in UV-Vis spectrophotometry is derivative spectrophotometry, which allows for the resolution of overlapping peaks in the analysis of multicomponent mixtures. The technique relies on the differentiation of standard absorbance spectra to improve the separation of specific components. Even when the peaks are close together, this technique can still improve peak separation by revealing the change in absorbance via first or second derivatives. For more accurate determinations of atorvastatin and etoricoxib concentrations, especially in cases where their spectra overlap, derivative spectrophotometry improves the method's sensitivity and specificity [22].
2. Multivariate Calibration: Multivariate calibration methods, such as Principal Component Regression (PCR) and Partial Least Squares (PLS), have enhanced ultraviolet-visible spectrophotometry for the analysis of numerous components. The analytical approach can analyse whole spectrum data, rather than only picking certain wavelengths, which allows it to identify various components. These techniques handle overlapping absorption bands and spectral interferences by extracting important features from the whole spectrum. When it comes to detecting substances like atorvastatin and etoricoxib, which overlap during absorption, the strong multivariate calibration approach works effectively. Even when dealing with sample interruptions induced by other medications in the system or by interfering components and excipients, these procedures allow for exact drug measurement. Their work lays the groundwork for drug concentration analysis prediction systems [23].

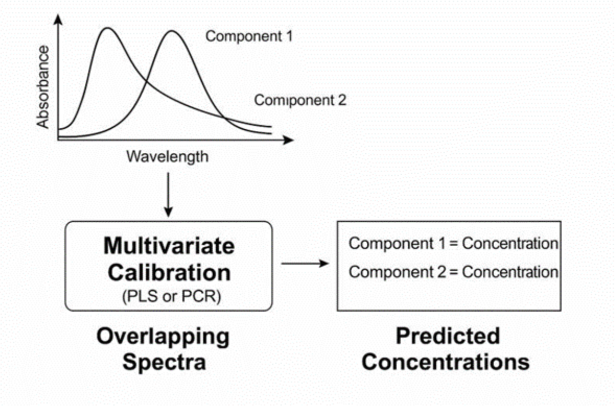


Figure 2: This graphic is included after the "Multivariate Calibration" section because it demonstrates how spectra from multi-component systems are separated using multivariate calibration techniques like PLS or PCR. Despite the fact that atorvastatin and etoricoxib have overlapping spectra, the visual display shows that they may be quantitatively measured using different approaches.

3. Use of Chemo metrics: Significant advancements in UV-Vis spectrophotometric analysis have resulted from the application of statistical and mathematical analytic approaches to the examination of chemical data. In order to find patterns of connection between different measurement elements, such as drug concentration or solvent impact or interference effects, researchers utilize Cluster Analysis and Multivariate Analysis of Variance (MANOVA) to examine distinct datasets. For the purpose of multiple drug quantification in a single sample, chemo metric methods enable researchers to choose appropriate experimental settings, improve data interpretation, and decrease errors depending on human analysis.

4. Microfluidic Devices and Lab-on-a-Chip Technology: The latest advancement in UV-Vis spectrophotometry involves integrating microfluidic devices with LOC technology. The system reduces analytical times and reagent use while performing accurate readings on small samples. Rapid analyse mixing, separation, and detection are all possible because to the microfluidics system's tiny channels and reaction chambers. Because of their higher analytical capabilities and real-time operational potential, these systems improve the evaluations provided by UV-Vis spectrophotometry. Because they allow for the critical, rapid, and accurate assessment of medication concentrations, these developments are particularly valuable in clinical and point-of-care diagnostics [24].

- **New Techniques for Simultaneous Estimation**

- UV-Vis Spectrophotometry with Dual Wavelengths: Dual wavelength UV-Vis spectrophotometry, which allows for simultaneous estimation, shows promise since it is able to detect the maximum absorbance of each analyse separately at various wavelengths. This method allows for the simultaneous counting of two substances with little interference from each other. Atorvastatin and etoricoxib both show strong individual peaks, hence the dual-wavelength UV-Vis spectrophotometric approach may identify two distinct wavelengths for absorption of both medications. Individual medication concentrations may be calculated by measuring absorbance values at the selected wavelengths. By measuring many substances simultaneously using dual-wavelength spectrophotometry, a fast and cheap technique is developed [25].
- Multi-Wavelength Spectrophotometry: Dual-wavelength spectrometry principles may be expanded to allow for simultaneous analysis utilizing multi-wavelength spectrophotometry. In order to collect a large amount of data for analysis, multi-wavelength spectrophotometry takes readings at several wavelengths. For the simultaneous analysis of medicines with widely separated absorption peaks, such as atorvastatin and etoricoxib, multi-wavelength spectrophotometry is still an invaluable technique. Researchers may differentiate between component spectra even when they overlap using multivariate calibration techniques or several chemos metric data processing approaches.
- Spectroscopy using the Fourier Transform UV-Vis Technology: Although initially designed for infrared (IR) and nuclear magnetic resonance (NMR) spectroscopy, the Fourier Transform (FT) technique has now found a home in ultraviolet-visible spectrophotometry. Compared to traditional UV-Vis methods, the FT-UV-Vis spectroscopic approach outperforms them thanks to faster data collection, better signal clarity, and higher signal strength ratios. Up until Fourier transforms provide typical absorption spectra, UV-Vis absorbance spectra are obtained as sequences of interferograms using FT-UV-Vis technology. Due to its very sensitive detection, FT-UV-Vis spectroscopy allows for the simultaneous identification of many components at low concentrations, making it ideal for complex sample solutions [26].
- UV-Vis Coupled with Surface Plasmon Resonance (SPR): Utilizing novel methods, surface plasmon resonance (SPR) technology integrates with UV-Vis spectrophotometry. Since SPR accurately measures changes in refractive index close to metallic surfaces, it may be used by researchers as an optical tool to identify molecule binding events. When atorvastatin and etoricoxib are analysed together, the measurement sensitivity is improved by combining SPR and UV-Vis spectrophotometry. Bio analytical applications benefit from the combined method's outstanding potential to identify trace drug concentrations in challenging sample matrices.

- Handheld and Portable UV-Vis Spectrometers: Advances in technology allowed for the creation of portable handheld UV-Vis spectrometers, which were used to evaluate pharmaceutical samples on the go. Small and portable, these analytical devices provide field testing and quality control in a way that is comparable to that of traditional laboratory equipment. Portable UV-Vis spectrometers are useful for assessment operations in remote areas, such as medical institutions and manufacturing sites, since they can quickly and accurately evaluate drug concentrations [27].

[7] CONCLUSION

In its widest sense, the term "drug" encompasses "all chemicals other than food that affect living processes." A substance is considered medicinal if its effects have a positive impact on the body. In addition, it may be further developed as a chemical and pharmacological tool for the internal or external treatment, alleviation, or prevention of illnesses in humans or animals. Their identification is of utmost relevance due to the growing number of chemotherapeutic and pharmacodynamics drugs used in pharmaceutical formulation. By applying the fundamental principles of chemistry, pharmaceutical chemists investigate medicinal substances and their effects on living organisms, as well as their physical and chemical characteristics, quality control procedures, and storage requirements.

To guarantee that people have access to safe and effective medication formulations, it is crucial to ensure and manage the quality of pharmaceutical substances and formulations. Factors such as pharmacological activity, stability, potency, homogeneity, and purity make up a drug's quality. The onus is on the producer to keep the product's quality high while turning out safe, effective, and non-toxic medication forms. Therefore, whether it's the business or regulatory bodies certifying meds and their formulations, pharmaceutical analysis plays a crucial part. For the purpose of determining the drug in its pharmaceutical formulations, researchers benefit from being aware of all these important aspects of a medication, as they aid in both the creation of an accurate experimental design and the scientific interpretation of results.

The current study makes heavy use of visible spectrophotometric methods. We have selected five medications for the creation of novel analytical techniques. We will use their physical and chemical properties, as well as the basic moieties and functional groups contained in their compounds, to determine which chromogenic reagents will be most suited for their test.

[8] AUTHOR(S) CONTRIBUTION

Dr. Shine Sudev comprehended and conducted the study, as well as evaluated and interpreted the results. Ms. Kunathu Kamarunisa wrote and updated the main manuscript. All authors read and approved the final version of the manuscript.

[9] LIMITATIONS

The size of the sample was very small.

The study was completely conducted on senior citizens.

[10] RECOMMENDATIONS

Needs to conduct in Tai-chi exercise to assess the physical problems in old age people.

Comparison research may be done to discover changes in adults and old age

Recommend to do this study as qualitative research.

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[13] PLAGIARISM POLICY

The authors declare that any kind of violation of plagiarism, copyright, and ethical matters will be handled by all authors. Journalists and editors are not liable for the aforesaid matters.

[14] CONFLICT OF INTEREST

The authors declared that no potential conflicts of interest concerning the research, authorship, and/or publication of this article.

[15] PROTECTION OF RESEARCH PARTICIPANTS

This study do not involve any such criteria or condition.

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