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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF PARACETAMOL AND CYCLOBENZAPRINE IN ITS MARKETED FORMULATION BY UV SPECTROPHOTOMETRIC METHOD

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Abstract: For the simultaneous measurement of Paracetamol and Cyclobenzaprine in combined medicinal dose forms, a simple, sensitive, and dependable UV spectrophotometric technique was created. While Cyclobenzaprine is a centrally acting muscle relaxant used in the treatment of muscle spasms, paracetamol is a commonly used painkiller and fever reducer. Quantitative analysis of the combination therapy is crucial for quality control since it is usually administered to treat musculoskeletal pain. Using a UV-visible spectrophotometer, the suggested approach rests on the quantification of absorbance at particular wavelengths. Their strong absorbance in the UV range allows their concurrent quantification free from prior separation. To find the highest absorbance (Amax), standard stock solutions were made and scanned across an appropriate wavelength range. For each medicine, calibration curves were created over a suitable concentration range, showing good linearity. Under ICH criteria, the created technique was confirmed for several criteria including robustness, linearity, precision, accuracy, limit of detection (LOD), and limit of quantification (LOQ). The results acquired fell within reasonable bounds, therefore suggesting that the technique is exact, accurate, and repeatable. The validated approach was effectively used to examine commercially available tablet formulations, and the findings supported its appropriateness for regular quality control analysis. The suggested approach may be successfully used in research labs and pharmaceutical companies given its simplicity, cost-effectiveness, and quick performance

Keywords: Paracetamol, Cyclobenzaprine, UV spectrophotometry, simultaneous estimation, method validation, pharmaceutical analysis

I. INTRODUCTION:

Paracetamol, also known as acetaminophen, is one of the most commonly used drugs for the relief of mild to moderate

pain and fever. It belongs to the class of analgesic and antipyretic agents and is widely preferred due to its effectiveness

and relatively safe profile when used within therapeutic limits. It acts mainly by inhibiting prostaglandin synthesis in the central nervous system, thereby reducing pain perception and body temperature.

Cyclobenzaprine is a centrally acting skeletal muscle relaxant that is primarily used for the treatment

of muscle spasms associated with acute musculoskeletal conditions. It works by acting on the central nervous system, particularly at the brainstem level, to reduce tonic somatic motor activity. This results in muscle relaxation and relief from discomfort caused by muscle stiffness and spasms.

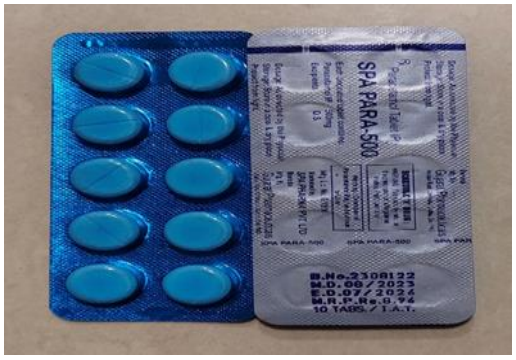


Fig 1: Paracetamol Tablet

Fig 2: Cyclobenzaprine Tablet

The combination of Paracetamol and Cyclobenzaprine is widely prescribed in clinical practice for the effective management of pain associated with muscle spasms, sprains, and injuries. Due to the increasing use of such combination therapies, it is important to develop reliable and efficient analytical methods for their simultaneous estimation in pharmaceutical formulations.

Among various analytical techniques, UV-visible spectrophotometry is widely used in pharmaceutical analysis because of its simplicity, rapidness, accuracy, and cost-effectiveness. It is based on the principle that molecules absorb light at specific wavelengths, and the amount of light absorbed is proportional to the concentration of the analyte, as described by Beer-Lambert's law.

The development of a validated analytical method is essential to ensure the quality, safety, and efficacy of pharmaceutical products. Therefore, the present study focuses on the development and validation of a simple and accurate UV spectrophotometric method for the simultaneous estimation of Paracetamol and Cyclobenzaprine in marketed formulations.

II. NEED OF THE STUDY:

The increasing use of combination drug therapy for musculoskeletal disorders requires reliable methods for simultaneous estimation of multiple drugs. Paracetamol and Cyclobenzaprine are commonly used together for pain and muscle spasm relief, making their accurate quantification essential for ensuring quality, safety, and efficacy.

Although advanced techniques like HPLC are available, they are costly, time-consuming, and require complex instrumentation. UV spectrophotometry offers a simple, rapid, and economical alternative for

There is a need for analytical methods that are accurate, precise, and easy to perform, especially in quality

control laboratories. Validation as per ICH guidelines is essential to ensure reliability and reproducibility.

Hence, this study aims to develop and validate a simple, accurate, and cost-effective UV spectrophotometric method

for simultaneous estimation of Paracetamol and Cyclobenzaprine in pharmaceutical formulations.

III. DRUG PROFILE AND PHARMACOLOGICAL PROPERTIES:

- Paracetamol:

Paracetamol, also known as acetaminophen, is a widely used analgesic and antipyretic drug. It is chemically

known as N-(4-hydroxyphenyl) acetamide and belongs to the class of para-aminophenol derivatives. It is

commonly used for the relief of mild to moderate pain such as headache, toothache, and musculoskeletal pain,

as well as for the reduction of fever.

Chemical Information:

Molecular Formula: $C_8H_9NO_2$

Molecular Weight: 151.16 g/mol

Paracetamol is rapidly absorbed from the gastrointestinal tract and shows its effect by inhibiting the synthesis

of prostaglandins in the central nervous system. It has minimal anti-inflammatory activity but is preferred due to its

good safety profile when used within therapeutic doses.

Pharmacologically, Paracetamol exhibits analgesic and antipyretic activities. It acts centrally to reduce pain perception

and regulate body temperature. Due to its effectiveness and low incidence of side effects, it is one of the most

commonly used over-the-counter drugs.

- Cyclobenzaprine:

Cyclobenzaprine is a centrally acting skeletal muscle relaxant that is structurally related to tricyclic antidepressants.

It is commonly used in the treatment of muscle spasms associated with acute musculoskeletal conditions such as

sprains, strains, and injuries.

Chemical Information:

Molecular Formula: $C_{20}H_{21}N$

Molecular Weight: 275.39 g/mol

Cyclobenzaprine acts primarily at the brainstem level, where it reduces tonic somatic motor activity influencing both

gamma and alpha motor neurons. This results in muscle relaxation and relief from pain caused by muscle spasms.

Pharmacologically, Cyclobenzaprine exhibits muscle relaxant and mild sedative properties. It helps in improving

mobility and reducing discomfort in patients suffering from musculoskeletal disorders.

Paracetamol and Cyclobenzaprine provide a synergistic effect in the management of pain and muscle spasms. Paracetamol reduces pain and fever, while Cyclobenzaprine relieves muscle stiffness and spasm.

This combination is widely used in clinical practice for enhanced therapeutic effectiveness.

IV. CHEMICAL PROPERTIES AND UV ABSORBING CHARACTERISTICS:

The analytical determination of Paracetamol and Cyclobenzaprine is mainly based on their chemical structure and ability to absorb ultraviolet (UV) radiation. Unlike herbal drugs, synthetic pharmaceutical compounds do not contain phytochemicals, but their pharmacological and analytical behavior depends on specific functional groups present in their molecular structure.

- Paracetamol:

Paracetamol contains important functional groups such as a hydroxyl group (-OH) and an amide group (-$NHCOCH_3$) attached to an aromatic benzene ring. These groups are responsible for its chemical reactivity and UV absorption properties.

- The aromatic ring system allows absorption in the UV region
- The hydroxyl group enhances solubility and contributes to hydrogen bonding
- The amide group plays a role in its stability and pharmacological activity

Paracetamol shows a strong absorbance in the UV region, typically around 240–250 nm, which makes it suitable for spectrophotometric analysis.

- Cyclobenzaprine:

Cyclobenzaprine has a tricyclic structure similar to tricyclic antidepressants, consisting of conjugated double bonds and an amine functional group. These structural features are responsible for its UV absorption characteristics.

- Presence of conjugated aromatic rings enhances UV absorption
- The amine group contributes to its basic nature and solubility
- Extended conjugation results in absorbance at higher wavelengths

Cyclobenzaprine generally exhibits maximum absorbance in the UV region around 280–290 nm.

- Analytical Significance:

The presence of chromophores (aromatic rings and conjugated systems) in both drugs allows them to absorb UV light efficiently. This property is utilized in UV spectrophotometry for their quantitative estimation.

The difference in their absorption maxima (λmax) enables simultaneous estimation using suitable analytical methods such as the simultaneous equation method or absorbance ratio method without the need for prior separation.

Thus, the chemical structure and UV absorbing characteristics of both drugs play a crucial role in the development of a simple, accurate, and reliable analytical method.

V. PHARMACOLOGICAL ACTIVITIES OF PARACETAMOL AND CYCLOBENZAPRINE:

a) Analgesic Activity (Paracetamol):

Paracetamol is widely used as an analgesic drug for the relief of mild to moderate pain such as headache, toothache, and musculoskeletal pain. It exerts its analgesic effect primarily by inhibiting prostaglandin synthesis in the central nervous system, thereby reducing pain perception.

b) Antipyretic Activity (Paracetamol):

Paracetamol also possesses strong antipyretic activity. It helps in reducing fever by acting on the hypothalamic heat-regulating center, leading to increased heat dissipation through vasodilation and sweating.

c) Muscle Relaxant Activity (Cyclobenzaprine):

Cyclobenzaprine is a centrally acting skeletal muscle relaxant used for the treatment of muscle spasms. It acts at the brainstem level to reduce tonic somatic motor activity, resulting in muscle relaxation and relief from stiffness and discomfort.

d) Sedative Effect (Cyclobenzaprine):

Cyclobenzaprine exhibits mild sedative properties due to its action on the central nervous system. This effect helps in reducing discomfort and improving rest in patients suffering from painful musculoskeletal conditions.

e) Combination Therapy Effect:

The combination of Paracetamol and Cyclobenzaprine provides a synergistic therapeutic effect. Paracetamol reduces pain and fever, while Cyclobenzaprine relieves muscle spasms. Together, they enhance overall treatment effectiveness in musculoskeletal disorders.

VI. MECHANISM OF ACTION:

The pharmacological actions of Paracetamol and Cyclobenzaprine are based on their distinct mechanisms at the molecular and physiological levels.

- Mechanism of Paracetamol:
  - Inhibits cyclooxygenase (COX) enzymes in the central nervous system
  - Reduces the synthesis of prostaglandins responsible for pain and fever
  - Acts on the hypothalamus to regulate body temperature

- Mechanism of Cyclobenzaprine:
  - Acts centrally at the brainstem level
  - Reduces tonic somatic motor activity
  - Influences both alpha and gamma motor neurons
  - Produces muscle relaxation without directly acting on skeletal muscles

- Overall Mechanism of Combination:
  - Reduction of pain perception (analgesic effect)
  - Decrease in fever (antipyretic effect)
  - Relief from muscle spasms (muscle relaxant effect)
  - Improvement in patient comfort and mobility

VII. MATERIALS AND METHOD:

Chemicals and Reagents:
- Ethanol (analytical grade)
- Distilled Water

Instrument & apparatus
- Analytical Balance (Swisser)
- Sonicator (Toshcon, Toshniwal process instrument pvt. Ltd., Ajmer)
- pH Meter (Systronic – Model no. 335)
- UV – Visible double beam spectrophotometer (Shimadzu model no UV-1780)

UV Instruments specification
- Model no - UV-1780

- Company - Shimadzu corporation, Japan
- Detector - Photo diode array
- Software – UV Probe

VIII. SAMPLE PREPARATION TECHNIQUES:

Sample preparation is a crucial step in the analysis of pharmaceutical formulations, as it ensures accurate and reliable estimation of active ingredients. Proper preparation helps in complete extraction of drugs from the dosage form and eliminates interference from excipients.

Common sample preparation techniques include:

a) Direct Dissolution Method:

In this method, a known quantity of powdered tablet formulation is dissolved in a suitable solvent such as Ethanol or distilled water. The solution is then filtered to remove insoluble excipients, resulting in a clear solution for analysis.

b) Sonication Method:

Ultrasonic waves are used to enhance the dissolution of drug components in the solvent. This method improves extraction efficiency and ensures complete drug release from the formulation.

c) Filtration and Dilution:

The prepared solution is filtered using Whatman filter paper to remove undissolved particles. Further dilutions are made to obtain the desired concentration range suitable for UV analysis.

These techniques ensure that the drug is completely extracted and available in solution form for accurate spectrophotometric measurement.

IX. SAMPLE PREPARATION AND STANDARD SOLUTION PREPARATION:

1. Sample Collection: - Take 20 tablets of Paracetamol and record their average weight.
2. Powdering: - Crush the tablets in a mortar and pestle to obtain a fine uniform powder.
3. Weighing of Powder: - Weigh the powder equivalent to 10 mg of Paracetamol (based on the label claim)
4. Transfer to Volumetric Flask: - Transfer this weighed powder into a 100 mL volumetric flask
5. Dissolution: - Add about 60–70 mL of Ethanol. Shake
6. or sonicate for about 10–15 minutes to dissolve the drug completely
7. Filtration: - Filter the solution using Whatman filter paper No. 41 to remove insoluble tablet excipients
8. Make up the Volume: - Transfer the filtrate into a 100 mL volumetric flask and make up the volume with the same solvent.
9. Final Concentration: - The resulting solution contains 100 µg/mL of Paracetamol (stock solution).
10. Working Solution: - dilute 1 mL of this stock solution to 10 mL with Ethanol to obtain 10 µg/mL working solution for UV analysis

Fig 3: Paracetamol

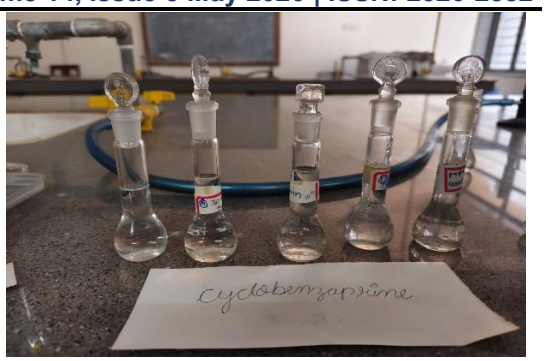


Fig 4: Cyclobenzaprine

X. VALIDATION PARAMETERS:

The developed UV spectrophotometric method was validated according to International Council for Harmonization to confirm that it consistently produces reliable and accurate results. Various validation parameters such as linearity, accuracy, precision, sensitivity, specificity, and robustness were evaluated.

A. Linearity:

Linearity represents the ability of the method to give results that are directly proportional to the concentration of the drug.

It was studied in the range of 5–25 µg/mL by measuring absorbance at 243 nm for Paracetamol and 290 nm for Cyclobenzaprine. A calibration curve was plotted between concentration and absorbance, which showed a straight-line relationship. This indicates that the method follows the Beer–Lambert Law within the selected range.

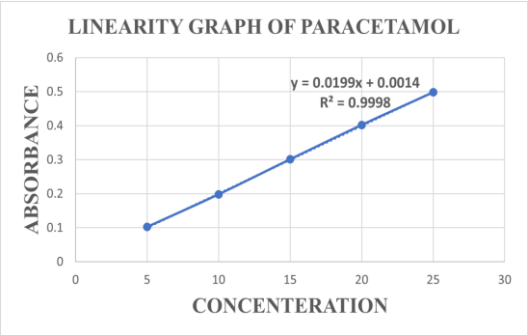
The correlation coefficient (r^2) was found to be close to 1, confirming good linearity.

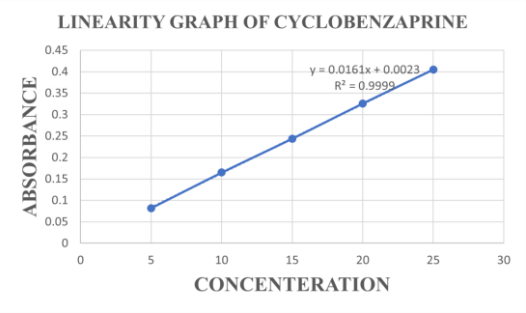
Table 1: Linearity data of PARACETAMOL

SR NO	CONC	ABSORBANCE
1	5	0.102
2	10	0.198
3	15	0.301
4	20	0.402
5	25	0.498

Table 2: Linearity data of CYCLOBENZAPRINE

SR NO	CONC	ABSORBANCE
1	5	0.082
2	10	0.165
3	15	0.244
4	20	0.326
5	25	0.405





B. Accuracy:

Accuracy shows how close the obtained result is to the true value. It was determined by performing recovery studies using the standard addition method at 80%, 100%, and 120% levels. Known amounts of standard drug were added to the pre-analyzed sample, and the total amount was measured. The percentage recovery was calculated and found within the acceptable range of 98–102%. This indicates that the method is accurate and not affected by the presence of excipients.

Table 3: Accuracy data for PARACETAMOL and CYCLOBENZAPRINE

	%level	stock solution of tablet	Amount of std. spiked (µg/ml)	Total conc we get	conc. Found	%recovery
SPA PAR A	50%	10	5	15	14.92	99.46
	100%	10	10	20	19.88	99.40
	150%	10	15	25	24.98	99.80
CYC LOBE NZAP RINE	50%	10	5	15	14.89	99.26
	100%	10	10	20	19.91	99.55
	150%	10	15	25	24.93	99.72

C. Precision:

Precision indicates the reproducibility of the method when repeated under the same conditions. It was evaluated in two ways:

Intraday precision: The same sample was analyzed multiple times within a single day.

Interday precision: The analysis was carried out on different days.

The results obtained were very close to each other. Precision was expressed in terms of %RSD (Relative Standard Deviation), which was found to be less than 2%. This confirms that the method is precise and gives consistent results.

Table 4: Precision of PARACETAMOL and CYCLOBENZAPRINE

DRUG	CONCENTRATION (µg/ml)	Intraday absorbance			Inter day absorbance		
		Mean Abs.	SD	%RSD	Mean Abs.	SD	%RSD
PARACETAMOL	10	0.198	0.0015	0.75	0.197	0.0018	0.91
	15	0.301	0.0021	0.69	0.300	0.0025	0.83
	20	0.402	0.0028	0.70	0.401	0.0030	0.75
	Mean			0.71%			0.83%
CYCLOBENZAPRINE	10	0.165	0.0012	0.72	0.164	0.0015	0.91
	15	0.244	0.0018	0.73	0.243	0.0020	0.82
	20	0.326	0.0023	0.70	0.325	0.0026	0.80
	Mean			0.72%			0.84%

D. Limit of Detection (LOD):

LOD is the lowest amount of drug that can be detected by the method but may not be quantified accurately. It was calculated using the standard deviation of the response and the slope of the calibration curve. The low LOD value obtained indicates that the method is sensitive enough to detect small quantities of the drug.

E. Limit of Quantification (LOQ):

LOQ is the lowest concentration that can be measured accurately and precisely. It was also calculated using calibration

curve data. The low LOQ value shows that the method can be used for quantitative analysis even at low concentrations.

Table 5: LOD and LOQ data for PARACETAMOL and CYCLOBENZAPRINE

DRUG	LOD	LOQ
PARACETAMOL	0.32	0.98
CYCLOBENZAPRINE	0.41	1.24

F. Specificity:

Specificity is the ability of the method to measure the analyte accurately in the presence of other components like excipients. The absorbance of the tablet sample was compared with the standard solution, and no interference was observed. This confirms that the method is specific for the drugs.

Table 6: Specificity data for PARACETAMOL and CYCLOBENZAPRINE

Drug	Absorbance (A1)	Absorbance (A2)	Difference	SD	%RSD
PARACETAMOL	0.301	0.303	0.002	0.0014	0.47
CYCLOBENZAPRINE	0.244	0.246	0.002	0.0015	0.61

G. Robustness:

Robustness indicates how reliable the method is when small changes are made in experimental conditions. Minor variations such as slight changes in wavelength (±2 nm) and solvent conditions were introduced. The results remained consistent, showing that the method is robust and unaffected by small changes.

H. Ruggedness:

Ruggedness evaluates the reproducibility of the method under different conditions such as different analysts or instruments. The same procedure was performed by different analysts, and similar results were obtained. This indicates that the method is rugged and reliable.

I. Suitability:

System suitability tests were performed before analysis to ensure that the instrument is functioning properly. Parameters like absorbance stability and baseline noise were checked and found to be satisfactory.

This ensures that the system is suitable for accurate analysis.

XI. CONCLUSION:

This study developed a simple, rapid, and cost-effective UV spectrophotometric method for the simultaneous estimation of Paracetamol and Cyclobenzaprine in combined dosage forms. Compared to conventional techniques like HPLC, the method does not require complex instrumentation or prior separation. It is based on UV absorption and enables accurate quantification of both drugs. The method was validated as per ICH guidelines and showed acceptable linearity, accuracy, precision, LOD, and LOQ, along with good reproducibility and reliability. It demonstrated minimal interference from excipients and proved effective when applied to marketed formulations.

Overall, the method is precise, economical, and suitable for routine quality control analysis, with potential for further application to other combination drugs.

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