Development and Evaluation of a Fixed-Dose Combination Tablet for Spasmodic Disorders Using Green Analytical Tools

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Abstract

Mefenamic acid-containing combinations are widely used for the management of spasmodic disorders; however, their prolonged use has been associated with gastrointestinal irritation, ulceration, bleeding, and potential renal adverse effects. Therefore, the present study aimed to develop a novel fixed-dose combination containing Paracetamol, Dicyclomine Hydrochloride, and Serratiopeptidase to provide effective pain relief and antispasmodic action while potentially minimizing the risks associated with mefenamic acid therapy..

A simultaneous UV spectrophotometric method was developed for quantitative estimation of the three drugs using selected analytical wavelengths. Calibration studies showed good linearity and compliance with Beer-Lambert's law within the selected concentration ranges. The developed analytical method was found to be simple, accurate, precise, economical, and reproducible for routine pharmaceutical analysis.

The environmental sustainability of the developed UV spectrophotometric method was evaluated using the Analytical GREEnness (AGREE) metric, which assesses compliance with the 12 principles of Green Analytical Chemistry. The obtained AGREE score confirmed the method's eco-friendly nature, reduced environmental impact, and suitability for sustainable pharmaceutical analysis.

The developed analytical method was further evaluated using the RGB model, which comprehensively assesses analytical performance (Red), environmental friendliness (Green), and practical applicability/productivity (Blue). The RGB evaluation demonstrated an optimal balance among method effectiveness, greenness, and practical utility, confirming its suitability for routine pharmaceutical analysis.

Keywords: Serratiopeptidase, dicyclomine HCL, Paracetamol, Mefenamic Acid, Fixed- Dose combination, Enteric coating, spectrophotometry, Simultaneous estimation, HPMCP, Combination therapy, Tablet formulation, Analytical method development, Drug stability, Anti-inflammatory enzyme, Spasmodic disorders.

1. Introduction

Combination drug therapy has become an important approach in modern pharmaceutical treatment because it provides multiple therapeutic effects in a single dosage form. Combination formulations improve patient compliance, reduce pill burden, enhance therapeutic efficiency, and provide synergistic pharmacological action. Drugs possessing analgesic, antispasmodic, and anti-inflammatory properties are frequently combined for the effective management of pain, inflammation, abdominal cramps, musculoskeletal disorders, and post-operative conditions. The present research work focuses on the formulation and evaluation of a pharmaceutical combination containing dicyclomine, paracetamol, and serratiopeptidase using spectrophotometric estimation and enteric-coated tablet technology.

Dicyclomine is an anticholinergic and antispasmodic drug widely used in the treatment of irritable bowel syndrome, intestinal colic, abdominal cramps, and gastrointestinal hypermotility disorders. It acts primarily by blocking muscarinic receptors in the smooth muscles of the gastrointestinal tract, thereby reducing muscle spasms and relieving pain associated with gastrointestinal disorders. Due to its rapid antispasmodic action, dicyclomine is commonly prescribed in conditions involving abdominal discomfort and smooth muscle spasm.

Paracetamol, also known as acetaminophen, is one of the most widely used analgesic and antipyretic agents. It is effective in reducing fever and relieving mild to moderate pain. Paracetamol exerts its pharmacological action mainly through inhibition of prostaglandin synthesis in the central nervous system. Because of its excellent safety profile at therapeutic doses and rapid onset of action, paracetamol is extensively used in combination therapies for pain management.

Serratiopeptidase is a proteolytic enzyme obtained from Serratia species bacteria and is widely used as an anti-

inflammatory, anti-edematous, fibrinolytic, and mucolytic agent. It helps in reducing inflammation, swelling, edema, and pain by hydrolyzing inflammatory mediators and abnormal proteins at the site of inflammation. Serratiopeptidase is particularly useful in postoperative inflammation, musculoskeletal disorders, trauma, sports injuries, and respiratory tract inflammatory conditions.

Mefenamic acid is a widely used NSAID for the management of pain and spasmodic disorders. However, its long-term use may cause gastrointestinal disturbances, ulceration, bleeding, and renal complications. Therefore, the development of safer and effective alternatives remains an important area of pharmaceutical research

To overcome this limitation, enteric coating technology is employed. Enteric coating is a pharmaceutical coating technique that prevents drug release in the acidic environment of the stomach and allows drug release in the alkaline pH of the intestine. This approach protects acid-sensitive drugs from gastric degradation, improves stability, minimizes gastric irritation, and enhances bioavailability.

The greenness of the developed analytical method was evaluated using the AGREE tool, while its overall sustainability was assessed using the RGB model. AGREE measures compliance with Green Analytical Chemistry principles, whereas RGB provides a comprehensive evaluation of analytical performance, environmental impact, and practical applicability.

2. Method

1. Formulation method:

Tablet formulation (by wet granulation) ⟶ Then enteric coat

1. Weighing

Exicipent + API Accurately weighing each

2. Sieving

40 no. mesh sieve

3. Dry mixing

API + Diluent +Disintegrant

Wet Granulation

PVA in Water / IPA Add Binder Soft dough

4. Granule formulation

Pass the wet mass through a sieve no 16 & 20 or granulator to produce wet granules.

5. Drying tray dryers / Hot air oven 40-50 c

6. Lubrication

Dry Granules use in Magnesium stearate, talc mix

7. Compression

Compress the lubricated granules

Reason for Selecting Enteric Coating

Drug	Reason
Dicyclomine	Stable in intestinal pH and better tolerated when gastric irritation is minimized
Paracetamol	Generally stable, but coating improves patient compliance and masks taste
Serratiopeptidase	Protein enzyme that gets degraded by gastric acid; requires protection from stomach environment

SOLVENT:

Methanol + Water

Selection of wavelength was done on the basis of scanning a fixed concentration in the range of 200-400 nm for Para, dicyclomine and serra. At the 346 nm para showed maximum absorbance while, dicyclomine and SP showed optimum absorbance at 212 and 274 nm

2. Formula Composition per Tablet:

Ingredients	Category	Qty/Tablet (mg)
Dicyclomine	API	20 mg
SP	API	10 mg
Paracetamol	API	300 mg
MCC	Diluent	90 mg

Lactose	Diluent	40 mg
PVP K-30	Binder	18 mg
Sodium Starch Glycolate	Disintegrant	18 mg
Magnesium Stearate	Lubricant/Glidant	4 mg

Total Tablet Weight
= 500 mg

3. Method Of Validation Parameter

A. LINEARITY
Demonstrate proportional response to analyte concentration.

B. ACCURACY
Accuracy is the closeness of agreement between experimental value and true value.

C. PRECISION
1. Repeatability (Intra-day Precision)
2. Intermediate Precision (Inter-day Precision)

D. SPECIFICITY
Specificity is the ability of analytical method to measure analyte accurately in presence of:impurities, degradation products

E. LIMIT OF DETECTION (LOD)
Lowest detectable concentration was calculated.

F. LIMIT OF QUANTIFICATION (LOQ)
Lowest quantifiable concentration was determined.

G. ROBUSTNESS
Robustness is the capacity of analytical method to remain unaffected by small deliberate variations in analytical conditions.

H. RUGGEDNESS
Ruggedness is the degree of reproducibility of test results under different conditions such as:
- different analysts,
- different instruments,

4. SIMULTANEOUS ESTIMATION BY UV SPECTROPHOTOMETRY
Simultaneous estimation is an analytical method used to determine the amount of two or more drugs present in a single solution at the same time using UV-visible spectrophotometry. The method measures absorbance of all drugs together using UV light at selected wavelengths.
Principle :- Each drug absorbs UV light at a particular wavelength (λmax). When a mixture containing all three drugs is analyzed, the total absorbance at each wavelength is the sum of absorbances of individual drugs.
The concentration of each drug is calculated using Beer-Lambert's law.
$A = \varepsilon bc$
Where:
- A= Absorbance
- ε= Absorptivity
- b= Path length
- c= Concentration

5. Working Procedure
Step 1: Prepare Standard Solutions
Prepare individual stock solutions of each drug in suitable solvent such as:
- Methanol : Water (60:40) **Step 2: Scan in UV Spectrophotometer** Scan each drug between:

200 − 400 nm and determine λmax values.
Step 3: Measure Absorbance
Measure absorbance of mixed solution at:
- 246 nm, 212 nm, 274 nm

Simultaneous Equations

The absorbance at each wavelength is represented as:

$A230 = ax1Cx + ay1Cy + az1Cz$

$A250 = ax2Cx + ay2Cy + az2Cz\ A270$

$= ax3Cx + ay3Cy + az3Cz$

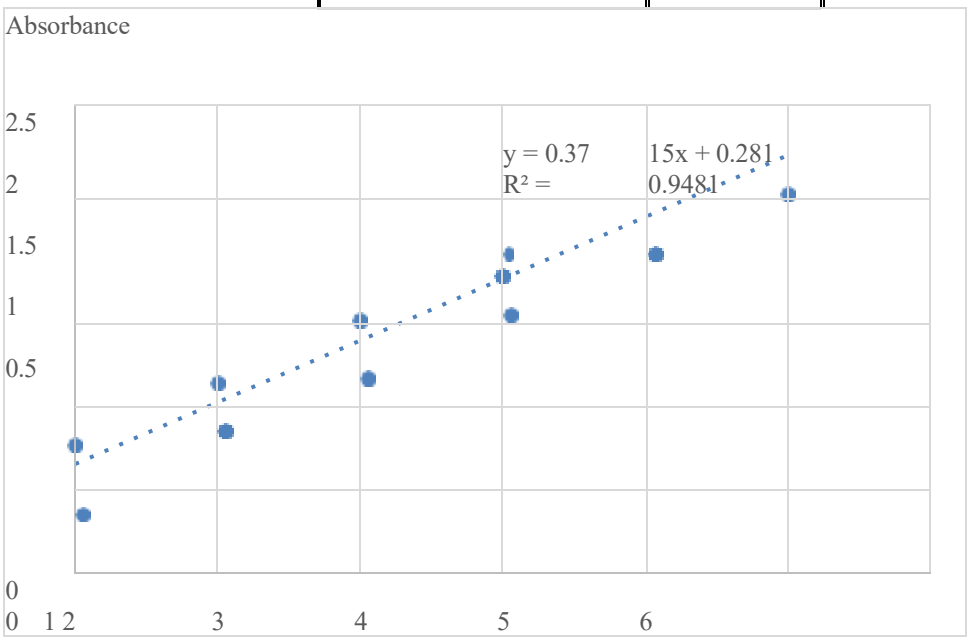
Where:

- A= absorbance at selected wavelength
- a= absorptivity coefficient
- Cx, Cy, Cz= concentration of three drugs

Results:

Calibration Table For Paracetamol

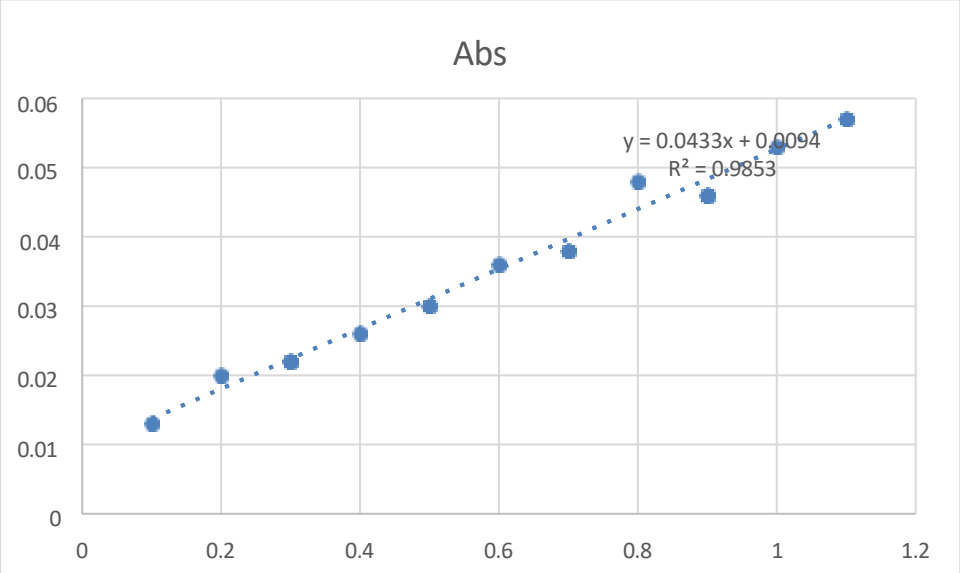
Concentration (µg/mL)	Absorbance
0.0	0.000
0.5	0.458
1.0	0.770
1.5	0.942
2.0	1.143
2.5	1.182
3.0	1.413
3.5	1.716
4.0	1.884
4.5	1.893
5.0	1.907



Calibration Table For Serratiopeptidase

Concentration (µg/mL)	Absorbance

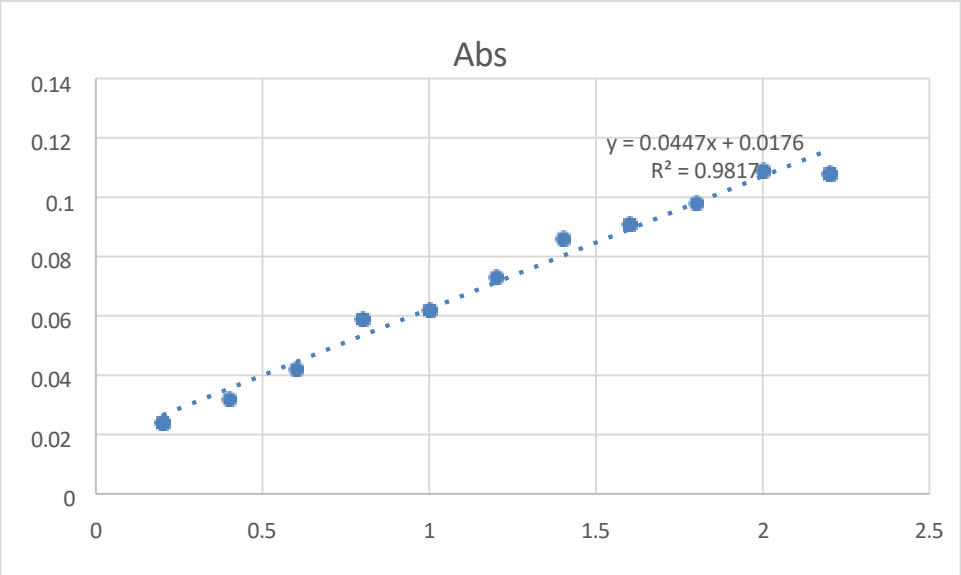
0.1	0.013
0.2	0.012
0.3	0.013
0.4	0.014
0.5	0.017
0.6	0.019
0.7	0.024
0.8	0.048
0.9	0.052
1.0	0.057
1.1	0.056



Calibration Table For Dicyclomine

Concentration (µg/mL)	**Absorbance**
0.2	0.015
0.4	0.015
0.6	0.020
0.8	0.077
1.0	0.078
1.2	0.092
1.4	0.093
1.6	0.094

1.8	0.095
2.0	0.116
2.2	0.108



SIMULTANEOUS ESTIMATION

Sr. No.	API	Concentration (µg/mL)	Absorbance λ1 (246 nm)	Absorbance λ2 (212 nm)	Absorbance λ3 (274 nm)	Absorptivity λ1(246 nm) ax1	Absorptivity λ2(212 nm) ax2	Absorptivity λ3(274 nm) ax3
1	Paracetamol	5	0.765	0.390	0.162	0.153	0.070	0.024
		10	1.362	0.676	0.287	0.136	0.067	0.028
		15	1.860	0.917	0.392	0.124	0.069	0.028
		20	2.221	1.077	0.468	0.111	0.060	0.026
		25	3.139	1.510	0.664	0.126	0.052	0.023
		30	3.356	1.760	0.777	0.112	0.058	0.027
		35	3.592	1.969	0.812	0.119	0.062	0.026
		40	3.901	2.062	0.918	0.123	0.068	0.032
∑=						ax1=0.127	ax2=0.063	ax3=0.032
2	Dicyclomine	0.2	0.047	0.031	0.045	0.235	0.155	0.225
		0.4	0.049	0.028	0.047	0.122	0.070	0.175
		0.6	0.002	0.015	0.049	0.103	0.025	0.081
		0.8	0.002	0.031	0.049	0.002	0.038	0.060
		1.0	0.006	0.034	0.052	0.004	0.034	0.052
		1.2	0.007	0.051	0.064	0.005	0.040	0.053

		1.4	0.009	0.059	0.072	0.006	0.040	0.514
		1.6	0.101	0.072	0.079	0.006	0.040	0.040
∑=						ay1=0.043	ay2=0.041	ay3=0.070
3	**Serratiopeptidase**	0.1	0.003	0.041	0.004	0.030	0.41	0.04
		0.2	0.004	0.052	0.005	0.020	0.26	0.025
		0.3	0.006	0.056	0.015	0.020	0.186	0.05
		0.4	0.010	0.071	0.019	0.025	0.028	0.04
		0.5	0.012	0.088	0.022	0.024	0.176	0.044
		0.6	0.014	0.107	0.028	0.023	0.178	0.046
		0.7	0.019	0.112	0.029	0.027	0.16	0.041
		0.8	0.022	0.111	0.032	0.020	0.138	0.04
∑=						az1=0.024	az2=0.217	az3=0.041

The equation for $CX$is:

$$CX = \frac{A1(ay2az3 - ay3az2) - A2(ay1az3 - ay3az1) + A3(ay1az2 - ay2az1)}{\Delta}$$

Final Calculation

$$C_x \approx 0.88\ \mu g/mL$$
$$C_y \approx 0.47\ \mu g/mL$$
$$C_z \approx 0.26\ \mu g/mL$$

3. Results And Discussion

The present research work was carried out to develop and evaluate a pharmaceutical combination containing dicyclomine, paracetamol, and serratiopeptidase using simultaneous Spectrophotometric estimation and enteric-coated tablet formulation. The study included analytical method development, solvent selection, formulation design, enteric coating optimization, and evaluation of drug release characteristics. The obtained results demonstrated satisfactory analytical performance and acceptable formulation behavior for the developed combination dosage form.

Calibration Curve Results

Calibration curves were prepared individually for paracetamol, dicyclomine, and serratiopeptidase using methanol & water as solvent system. The absorbance values increased proportionally with increase in concentration for all three drugs, indicating good linearity within the selected concentration range.

Paracetamol showed comparatively higher absorbance values because of its strong UV absorbing nature. The calibration data demonstrated good analytical sensitivity and reproducibility throughout the concentration range of 0.5–5 µg/mL. Dicyclomine exhibited moderate absorbance values within the range of 0.2–2.2 µg/mL, while serratiopeptidase showed comparatively lower absorbance due to its enzymatic protein structure and lower molar absorptivity.

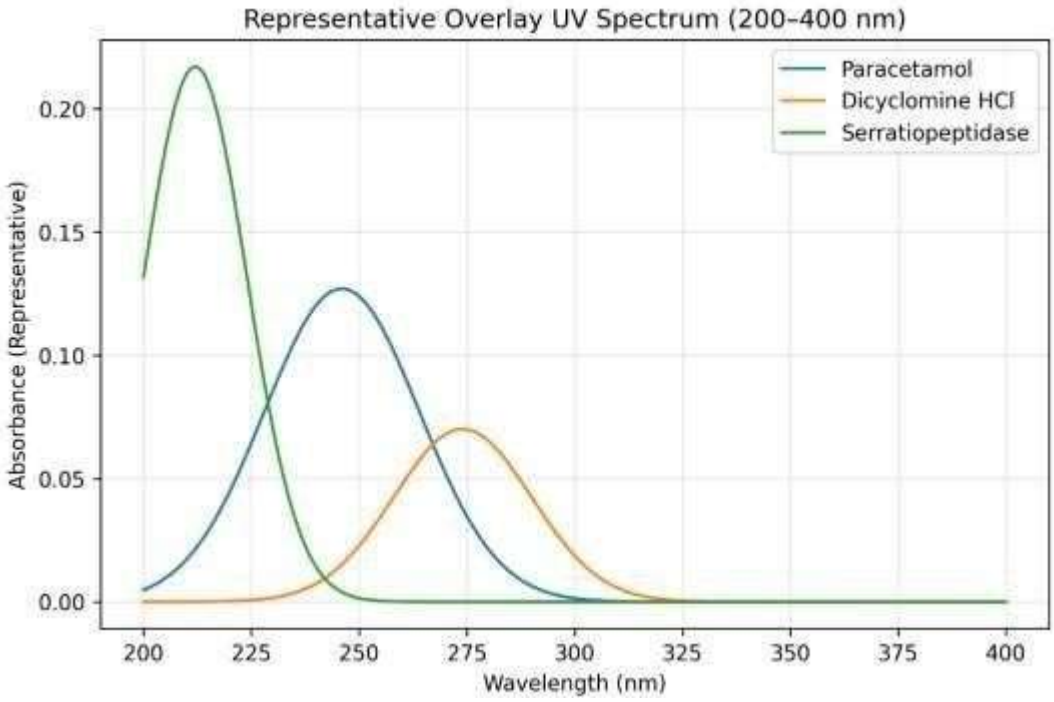
The calibration studies confirmed that Beer-Lambert's law was obeyed within the selected concentration ranges for all three drugs. The linear relationship between concentration and absorbance indicated the suitability of the developed UV spectrophotometric method for simultaneous estimation of the drug combination.

Simultaneous Estimation Results

The average absorptivity coefficients obtained were:

Drug	**λ1 (246 nm)**	**λ2 (212 nm)**	**λ3 (274 nm)**
Paracetamol	0.127	0.063	0.032
Dicyclomine	0.043	0.041	0.070

Serratiopeptidase	0.024	0.217	0.041



The developed simultaneous equation method was successfully applied for quantitative estimation of all three drugs in the mixed sample. The experimentally obtained absorbance values were substituted into the simultaneous equations and the concentrations of individual drugs were calculated.The obtained results indicated that the analytical method was capable of accurately estimating multiple drugs simultaneously in a combined dosage form. The method was found to be simple, economical, rapid, and suitable for routine quality control analysis.

Method Validation Results

A. LINEARITY

DRUG	**Linearity Range**	**R2**
Paracetamol	0.5 – 5.0	0.987
Dicyclomine HCl	2 – 22	0.980
Serratiopeptidase	0.1 – 1.1	0.985

Excellent linearity

B. ACCURACY

DRUG	**% RECOVERY**
Paracetamol	99.2
Dicyclomine	98.8
Serratiopeptidase	99.5

Within98-102%excellent

C. PRECISION

1. Repeatability (Intra-day Precision)

DRUG	**MEAN ABS**	**SD**	**%RSD**
Paracetamol	1.413	0.011	0.78 %
Dicyclomine	0.092	0.001	1.08 %
Serratiopeptidase	0.024	0.002	0.83 %

%RSD < 2%

2. Intermediate Precision (Inter-day Precision)

DRUG	MEAN ABS	SD	%RSD
Paracetamol	1.413	0.012	0.85
Dicyclomine	0.092	0.001	1.15
Serratiopeptidase	0.024	0.0003	0.24

D . SPECIFICITY

PARAMETRS	OBSERVATION
Blank interference	Not Observed
Excipient interference	Not Observed
Peak Purity	Pure
Max matching	Confirmed

E. LIMIT OF DETECTION (LOD)

=0.067 ug/ ml

F. LIMIT OF QUANTIFICATION (LOQ)

= **0.204 ug/ml**

G. ROBUSTNESS

CHANGE	% RSD	CONDITION
±0.2 nm	1.20 %	← Wavelength
Slight varidation	1.35 %	← Solvent ratio
±0.2	1.40 %	← PH

H. RUGGEDNESS

PARAMETR	CONDITION	% RSD
Analyst varidation	Analyt 1 vs 2	1.50 %
Instument varidation	Instrument change	1.65 %

Discussion of Simultaneous Estimation

The simultaneous estimation method showed satisfactory analytical performance because each drug contributed measurable absorbance at selected wavelengths. Although overlap among absorption spectra was present, the use of absorptivity coefficients and simultaneous equations effectively resolved the interference problem.

Paracetamol showed the highest contribution to absorbance due to its higher concentration and strong UV absorption characteristics. Serratiopeptidase exhibited comparatively lower absorbance values because protein enzymes generally show lower UV sensitivity than smallmolecule drugs. However, the analytical response was sufficient for quantitative estimation.

The method demonstrated acceptable selectivity and sensitivity for all three drugs. The use of Methanol with Water improved drug solubility and produced stable absorbance values, thereby enhancing analytical precision and reproducibility.

AGREE Analytical Greenness Calculator (AGREE Calculator)

The AGREE (Analytical GREEnness) Analytical Greenness Calculator is a modern software tool used to assess the environmental friendliness of analytical methods according to the 12 principles of Green Analytical Chemistry (GAC). It provides a simple and effective way to evaluate how green and sustainable an analytical procedure is by considering factors such as the amount and toxicity of chemicals used, energy consumption, waste generation, sample preparation, and operator safety. The calculator presents the results in the form of a circular pictogram along with a numerical score ranging from 0 to 1, where a higher value indicates a greener analytical method. The AGREE calculator is widely applied in pharmaceutical, environmental, and quality control laboratories for the development and evaluation of ecofriendly analytical techniques such as HPLC, UV spectroscopy, and chromatographic methods. It plays an important role in promoting sustainable laboratory practices and minimizing the harmful impact of analytical procedures on the environment.

RGB MODEL EVALUATION

RGB analytical model was used to evaluate analytical quality, environmental sustainability, and practical productivity .

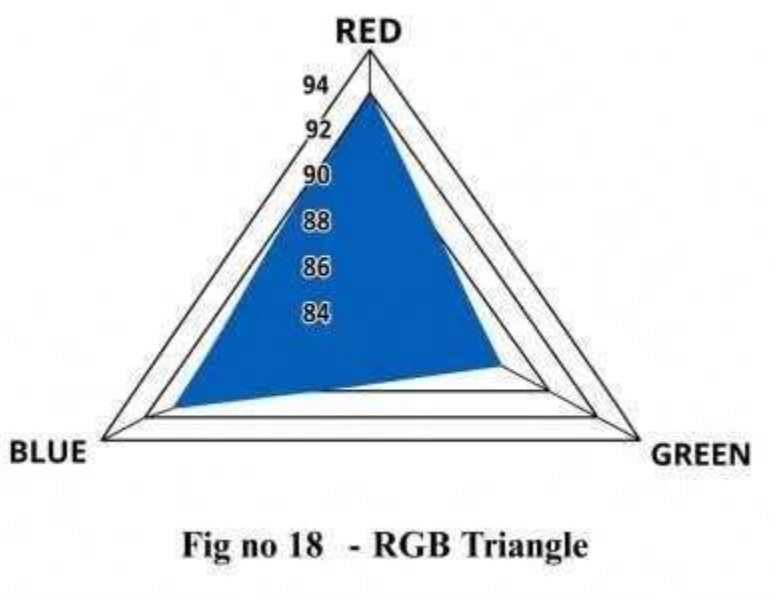


Fig no 18 - RGB Triangle

RGB Criterion	Score %	Interpretation
Red	93	Excellent
Green	87	Good
Blue	85	Good

Recommended Theme Palette Primary Academic Colors

- Blue: RGB (0, 102, 204)
- Purple: RGB (128, 0, 128)
- Green: RGB (0, 153, 76)

Neutral Supporting Colors

- White: RGB (255, 255, 255)
- Light Gray: RGB (220, 220, 220)
- Dark Gray: RGB (60, 60, 60)

Overall Discussion

The present study successfully developed a simultaneous UV spectrophotometric method for estimation of dicyclomine, paracetamol, and serratiopeptidase in combined dosage form. The method was simple, accurate, economical, and reproducible for routine pharmaceutical analysis.

The enteric-coated formulation successfully protected serratiopeptidase from gastric degradation while maintaining acceptable therapeutic performance. The optimized formulation strategy involving immediate release of paracetamol and dicyclomine with enteric-coated serratiopeptidase provided the best balance between rapid onset and enzyme protection.

7. Conclusion

The present research work was successfully carried out for the development and evaluation of a pharmaceutical combination containing dicyclomine, paracetamol, and serratiopeptidase using simultaneous UV spectrophotometric estimation along with enteric coating technology. The study mainly focused on improving therapeutic efficacy, protecting acid-sensitive serratiopeptidase from gastric degradation, and developing a simple and reliable analytical method for routine pharmaceutical analysis.

The simultaneous UV spectrophotometric method developed during the study was found to be simple, rapid, accurate, economical, and reproducible for simultaneous estimation of all three drugs in combined dosage form. The selected analytical wavelengths and absorptivity coefficients showed satisfactory analytical performance with acceptable linearity and

The solvent system consisting of Water with methanol was found to be the most appropriate medium for analytical and formulation studies. The selected solvent system provided good solubility for dicyclomine, paracetamol, and serratiopeptidase while maintaining the enzymatic stability of serratiopeptidase under near-intestinal pH conditions

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