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Compatibility study of natural coumarin with gabapentin

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ABSTRACT

We investigated the interactions of gabapentin and coumarin using UV Spectrophotometric estimation and molecular docking studies. UV-vis spectrophotometry was utilized to design and validate a method for the simultaneous measurement of the two substances. The absorbance maxima for gabapentin (572 nm) and coumarin (274 nm) were found. The method's specificity, accuracy (% recovery from 98-102 %), precision (intra/inter-day % RSD < 2 %), linearity ($r^2 > 0.99$) and sensitivity (LOD < 0.4 µg/mL) were all validated by ICH guidelines. Swiss Dock molecular docking tests indicated that gabapentin (-4.375 kcal/mol) and coumarin (-5.499 kcal/mol) had relatively favourable binding affinities, indicating a moderate interaction with the enzyme binding site. This study concluded that these chemicals may be compatible and potentially co-formulated to treat neurological aspects of disease conditions.

Keywords: Coumarin, Gabapentin, Method Validation, Molecular Docking, Simultaneous Estimation, UV-vis Spectrophotometry

INTRODUCTION

Coumarins are natural benzopyrone molecules with a wide variety of biological effects, including their anticonvulsant activity. The benzopyrone family also contains chemically, physiologically active compounds called coumarins (1,2-benzopyrones). Coumarins (2H1-benzopyran-2-ones) are made up of an oxygen atom in the α-position connected to the α-pyrone ring by a benzene ring (9). Coumarins make up the majority of secondary plant metabolites with protective qualities against infection, as regulators of growth and as regulators of metabolic change. Coumarin's anti-convulsant effects are related to how it interacts with the γ-aminobutyric acid (GABA) ionotropic receptor (4). Different methodologies have been used in the analysis of coumarins, including liquid chromatography coupled to UV, fluorescence, diode array (DAD), mass spectrometric (MS) detectors, as well as gas chromatographic methods coupled to flame ionization detection (FID) or MS (10). Thin Layer Chromatography (TLC), Gas Chromatography with Mass Spectrometry (GC-MS), High-Performance Liquid Chromatography (HPLC) with UV detection, UV-Visible spectroscopy and sophisticated HPLC techniques combined with multi-stage mass spectrometry (MS) are some of the methods available for coumarin analysis (19). Fig. 1 shows the chemical structure of coumarin and gabapentin (GBP). Neuropathic pain can be treated with GBP (2). Reviews of literature determined a range of methods for determining GBP in biological fluids and pharmacological dosage forms. Several techniques for identifying GBP were mentioned in the literature, including High-Performance TLC (14), GC-MS (12), Liquid Chromatography-Tandem MS (11,20), UV-Vis spectroscopy (3,8) and Reverse phase - HPLC (13). However, no method for concurrent docking studies and determining both compounds in mixed dosage forms was provided. Consequently, the present study uses docking studies and the UV spectroscopic

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method to concurrently investigate gabapentin and coumarin. This study aimed to evaluate the compatibility and interactions of natural coumarin and gabapentin using UV-visible spectroscopy and molecular docking techniques for co-formulation in neurological therapies based upon their distinct pharmacokinetic and pharmacodynamic properties.

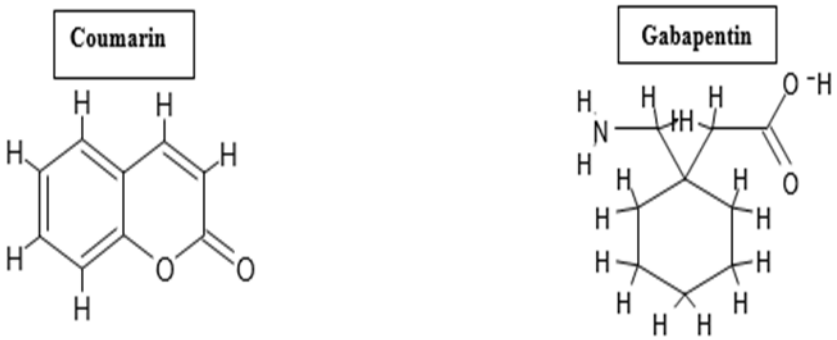

Figure 1. Structures of Coumarin and Gabapentin

3. MATERIALS AND METHODS

3.1. Materials

A JASCO UV-vis spectrophotometer (model UV V-630) with corresponding quartz cells (1.0 cm) was the device used. Analyses were performed using S.D. fine chemicals, including ninhydrin, an analytical reagent and N, N'-dimethylformamide. Furthermore, The Central Drug House, New Delhi, provided the reference samples of gabapentin and coumarin as gifts. All solutions were made with distilled water and ethanol.

3.2. Preformulation Studies of Coumarin and gabapentin

Pre-formulation tests were undertaken to determine coumarin and gabapentin characteristics by using the following physical parameters (7):

3.2.1. Organoleptic properties: The descriptive words were used to identify coumarin and gabapentin properties, including their colour, odour and pH (Table 1).

Table 1. Organoleptic characteristics of coumarin and gabapentin

S. No.	Parameters	Observations of Coumarin	Observations of Gabapentin
1.	Physical form	Fine crystalline powder	White crystalline solid or powder
2.	Colour	White to off-white	Pure White
3.	Odour	Pleasant, sweet (vanilla-like)	Odourless

3.2.2. Solubility: To conduct a solubility test, an excess of coumarin and gabapentin was placed in a conical flask along with distilled water, phosphate buffer 6.8, ethanol and methanol from each solvent. The solution was shaken at room temperature using an orbital shaker. At 12, 24, 36, 48 and 72 h, samples were taken until a consistent result was achieved. After the sample was filtered using Whatman filter paper and suitably diluted with the same solvent, the concentrations were determined using UV-visible spectroscopy (Table 2).

Table 2. Solubility of coumarin and gabapentin

S. No.	Solvent	Solubility of Coumarin	Solubility of Gabapentin
1.	Distilled water	Poorly soluble	Freely soluble
2.	Buffer (PBS 6.8)	Slightly soluble	Freely soluble
3.	Methanol	Moderately soluble	Slightly soluble
4.	Ethanol	Freely soluble	Poorly soluble

3.3. Simultaneous estimation of coumarin and gabapentin

A gabapentin stock standard of 0.1 mg/mL was made in acetonitrile and kept in a cool place. Every day, a fresh ninhydrin reagent (2 mg/mL of ninhydrin in methanol) (1) was used. Deionized water (0.00–10.00 mL) and 2.00 mL of ninhydrin reagent were added and then heated to 70 ± 5 °C for 15 min. After cooling, the resultant solutions were diluted to reach a final concentration of 2-30 mg/mL. The calibration curve was produced by measuring absorbance and plotting the findings (16). A final stock standard coumarin concentration of 1 mg/mL, which was kept at 4-8 °C, was achieved by preparing 50 mg coumarin in ethanol. Fresh working solutions of coumarin (20-100 µg/mL) were made by diluting the stock standard with ethanol. Using a UV-Visible spectrophotometer, absorbance was measured. A calibration curve was prepared by recording and plotting λ_{max} (18).

3.4. Method validation: The International Council for Harmonization (ICH) requirements were followed in the validation of the procedure (17).

3.4.1. Linearity and range: The absorbance was compared to the drug concentration data using linear least-squares regression analysis (Table 3).

Table 3**.** Calibration curves using linear regression analysis data

Specifications	Coumarin	Gabapentin
Wavelength of Detection (nm)	274 nm	572 nm
Limit of Beer's Law (µg/ml)	5 to 30 (µg/ml)	5 to 30 (µg/ml)
Equation of regression	y = 0.0219x + 0.0331	y = 0.0484x - 0.2079
coefficient of correlation (r)	r^2 = 0.9934	r^2 = 0.9904
Intercept (c)	0.0331	- 0.2079
Slope (m)	0.0219	0.0484

3.4.2. Specificity: The fact that neither coumarin nor gabapentin produced any interference was used to assess the specificity of the procedure.

3.4.3. Limits of quantification (LOQ) and detection (LOD): The Limit of Detection (LOD) and Limit of Quantitation (LOQ) were determined using the following formulae to determine the minimum quantity of the material that our approach can accurately detect and measure:

$$LOD = 3.3 \times (\sigma/S)$$
$$LOQ = 10 \times (\sigma/S)$$

Where, S: Calibration curve's slope, it indicates how significantly the response varies with concentration,

σ : Instrument's response standard deviation, a measure of reading fluctuation.

3.4.4. Accuracy (percentage of recovery studies): The recoveries of coumarin and gabapentin were determined using the normal additions method as a means of showing the accuracy of the methods (Table 4).

Table 4. Recovery (%) data for coumarin and gabapentin

Drugs	Level (%)	Spiked Amount (µg/mL)	Recovered Amount (µg/mL)	Recovery (%)	Result
Coumarin	80%	8	8.01	100.12%	Acceptable
Coumarin	100%	10	9.92	99.20%	Acceptable
Coumarin	120%	12	11.85	98.75%	Acceptable
Gabapentin	80%	8	7.92	99.00%	Acceptable
Gabapentin	100%	10	9.95	99.50%	Acceptable
Gabapentin	120%	12	11.88	99.00%	Acceptable

3.4.5. Precision: It appears in the lab as variations in tools or analysis from day to day. Intra-day and intraday precision measurements were made (Table 5).

Table 5. Repeatability and intermediate precision of coumarin and gabapentin

Intra-day (Repeatability)

Drug	Concentration (µg/mL)	Mean Absorbance	% Relative Standard Deviation (RSD)
Coumarin	10	0.253	1.24%
Gabapentin	10	0.300	1.10%

Inter-day (Intermediate Precision)

Drug	Concentration (µg/mL)	Mean Absorbance	RSD (%)
Coumarin	10	0.251	1.35%
Gabapentin	10	0.298	1.25%

3.5. Molecular docking

To study the interaction binding energy dynamics of the binding site of both drugs, gabapentin and coumarin, molecular docking in silico was performed as a complement to the experimental work (Table 6). The Swiss Dock software's Autodock Vina docking tool

was used for sub-studies in this investigation. The degree of interaction can lead to a stronger potential for the drug candidate to bind to the enzyme's binding site (15). Ligand preparation for Swiss Dock 2024 requires formats like Tripos Molecular 2 File Format, Protein Data Bank Partial Charges and Torsions, or Simplified Molecular Input Line Entry System, while the target is uploaded as a Protein Data Bank or Protein Data Bank Partial Charges and Torsions file (5,6)**.**

RESULTS AND DISCUSSION

4.1 Pre-formulation research

4.1.1. Characteristics of an organoleptic: The organoleptic characteristics confirmed that both coumarin and gabapentin possess these features, which help in quality control and raw material identification (Table 1).

4.1.2. Solubility: The gabapentin was highly soluble in water and phosphate buffer, while coumarin showed better solubility in ethanol. These results are crucial for selecting the appropriate solvent in combined formulation strategies (Table 2).

4.2. Analytical wavelength selection

After scanning a few dilutions, absorbance maxima of 274 nm and 572 nm were chosen to measure the gabapentin and coumarin, respectively (Fig. 2a). The two separate absorbance maxima confirm the suitability of the method for simultaneous estimation without spectral interference. This non-overlapping λ_{max} is critical for accurate quantification in mixed drug formulations.

4.3. Calibration curve for working standards

For gabapentin and coumarin, the corresponding coefficients of correlation (r^2) were 0.9904 and 0.9934, respectively (Figs. 2b and 2c). These correlation demonstrates method reliability and consistency across a defined concentration range.

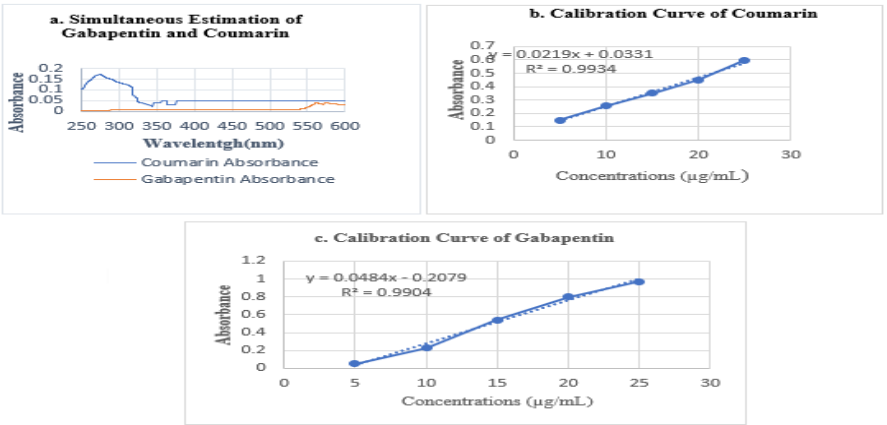

Figure 2. a. Simultaneous Estimation of Gabapentin and Coumarin; b. Calibration Curve of Coumarin; c. Calibration Curve of Gabapentin

4.4. Method of validation

The procedure was verified following ICH guidelines:

4.4.1. Linearity and range: The method showed good linearity with a high r^2 value. Low intercept and proper slope confirm accurate and consistent response across the tested range (Table 3).

4.4.2. Specificity:

(i). No interference was observed at 274 nm (Coumarin) and 572 nm (Gabapentin).
(ii). Blank and placebo solutions showed no absorbance at the selected wavelengths.
(iii). Result: The Method is specific to coumarin and gabapentin.

4.4.3. LOD and LOQ:

Coumarin:

- σ (Standard deviation of intercept) = 0.0025
- S (Slope) = 0.0219
- LOD = 0.376 µg/mL
- LOQ = 1.14 µg/mL

Gabapentin:

- σ = 0.0050
- S = 0.0484
- LOD = 0.341 µg/ML
- LOQ = 1.03 µg/mL

Result: Both LOD and LOQ values are within acceptable limits, indicating good sensitivity.

4.4.4. Accuracy (% Recovery study): This ensures that the analytical method can accurately measure the actual drug content without interference from excipients or solvents (Table 4).

Results: All values fall within the acceptable recovery range of 98-102 %.

4.4.5. Precision: This indicates that low % RSD values for intra-day and inter-day measurements show high reproducibility and reliability (Table 5).

Inter-day (Intermediate Precision):

Result: % RSD < 2% for both intra- and inter-day → Precision is acceptable.

4.5. Molecular docking of coumarin and gabapentin

The molecular docking studies revealed that coumarin showed a higher binding affinity (-5.499 to -4.681 kcal/mol) compared to gabapentin, which ranged from -4.375 to -3.820 kcal/mol (Table 6). This suggested that coumarin forms stronger interactions with the

Table 6. Binding affinity of coumarin and gabapentin

Model	Binding Affinity of Coumarin	Binding Affinity of Gabapentin
1.	-5.499	-4.375
2.	-5.431	-4.316
3.	-5.066	-4.269
4.	-5.016	-4.144
5.	-4.813	-4.099
6.	-4.783	-4.032
7.	-4.742	-3.998
8.	-4.707	-3.975
9.	-4.694	-3.925
10.	-4.681	-3.820

target, likely due to its planar aromatic structure enhancing binding stability. In summary, coumarin showed stronger receptor binding, whereas gabapentin offered better solubility, suggesting their combination may be beneficial in enhancing both efficacy and pharmacokinetics.

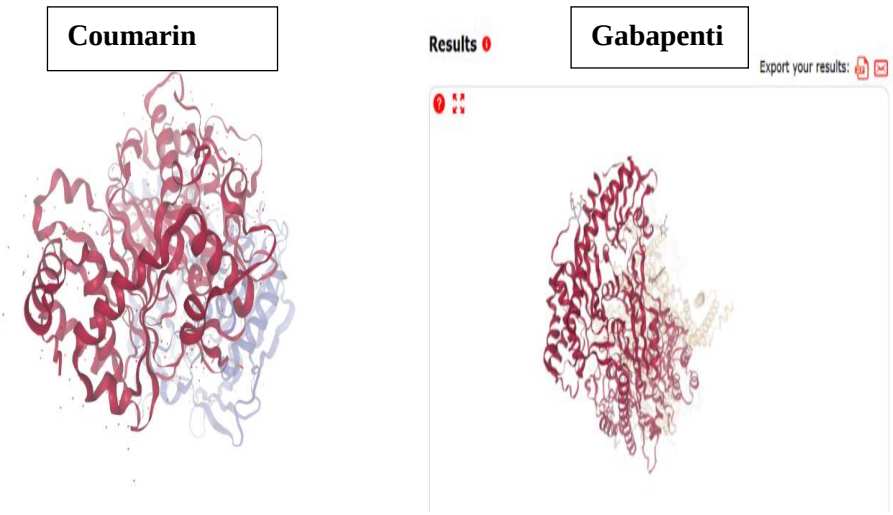

Figure 3. Docked Poses of Coumarin and Gabapentin

The search space can be customized either by using the molecular viewer itself or by entering centre coordinates and dimensions. The 3D structure can also be used to view molecular interactions and the protein surface with the buttons below the viewer (Fig. 3).

5. CONCLUSIONS

By using molecular docking techniques and UV-visible spectroscopy, this study was able to measure and compare gabapentin and coumarin at the same time. It was determined that the UV spectrophotometric approach met all validation requirements and was straightforward, sensitive, accurate, exact and specific based on the ICH recommendations. Since coumarin and gabapentin had different λmax values at 274 nm and 572 nm, respectively, it was possible to measure them precisely without any spectral interference. In terms of absolute binding affinity, molecular docking studies showed that coumarin binds to the chosen protein target more strongly than gabapentin. Nonetheless, gabapentin showed significant increases in water solubility, indicating a superior pharmacokinetic profile (at least for gabapentin). The two could work in concert to create a therapeutic impact and comparatively higher bioavailability. Overall, by developing a validated analytical method of detecting a dual-drug in mixed dosage units and a molecular rationale for combination medication use, the current study lays the groundwork for future formulation and *in-vivo* studies. Optimizing bioavailability and targeted delivery may require thorough formulation research, including the creation of co-loaded delivery systems (such as transferosomes, nanoformulations, or intranasal gels). Together, *in-vivo* pharmacodynamic and pharmacokinetic research are crucial to prove the safety, therapeutic potential and synergistic effectiveness of treating neurological conditions. Together, these studies provide a scientific foundation for the practical translation of this combination into a suitable dosage form in the future.

AUTHOR'S CONTRIBUTIONS

RPM conceived of the present idea for the article. VC performed the literature search, data analysis, and wrote the manuscript. SSV and RKM provided critical feedback and helped to shape the final draft.

DECLARATION

Each author has made a substantial contribution to this manuscript, we confirm. No author who contributed significantly to this was overlooked in the manuscript. The ethical norms of each of our institutions have been followed.

CONFLICT OF INTEREST

No conflicts of interest are disclosed by the authors.

ETHICAL APPROVAL

The study was conducted in accordance with scientific ethics and conduct, according to the authors. However, as no animals were used in this investigation, the relevant committee has not granted ethical approval.

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