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ECOFRIENDLY SPECTROSCOPIC METHOD DEVELOPMENT FOR SIMULTANEOUS ESTIMATION OF ANTIHYPERTENSIVE DRUGS IN COMBINED DOSAGE FORM

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Abstract:
The present study focuses on the development of an ecofriendly spectroscopic method for the simultaneous estimation of antihypertensive drugs in a combined dosage form. The method was designed to be simple, cost-effective, and environmentally sustainable by avoiding the use of hazardous organic solvents and minimizing chemical waste. Using UV-visible spectroscopy, optimized wavelengths were selected for accurate quantification of the drugs without interference from excipients. The proposed method was validated as per International Conference on Harmonisation (ICH) guidelines for parameters such as linearity, accuracy, precision, specificity, limit of detection (LOD), and limit of quantitation (LOQ). Recovery studies at three concentration levels (80%, 100%, and 120%) confirmed the method's accuracy, with percentage recovery values within the acceptable range for both drugs. The ecofriendly nature of the method not only ensures environmental safety but also offers a sustainable alternative for routine quality control analysis in the pharmaceutical industry.

***Keywords**: Ecofriendly method, UV-visible spectroscopy, simultaneous estimation, antihypertensive drugs, green analytical chemistry, method validation, recovery studies, sustainable analysis.*

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INTRODUCTION:

Hypertension remains a major global health challenge and is commonly managed using combination therapy to achieve better blood-pressure control and reduce cardiovascular risk. Fixed-dose combinations of antihypertensive agents, such as combinations of calcium-channel blockers, angiotensin receptor blockers, beta-blockers, and thiazide diuretics, are increasingly used because they improve adherence and produce synergistic therapeutic effects (Chobanian et al., 2003; Whelton et al., 2018). Accurate, precise, and routine quality-control methods for simultaneous estimation of such combinations are therefore essential for dosage form development, release testing, and stability studies.

Spectroscopic (UV–Vis and derivative, ratio, chemometric) methods are attractive for simultaneous estimation in combined dosage forms because they are fast, cost-effective, require minimal sample preparation, and are readily available in many quality control laboratories. Spectrophotometric techniques including zero and higher-order, derivative, ratio spectra, and multivariate calibration have been successfully applied to resolve overlapping spectra of co-formulated antihypertensives without chromatographic separation, enabling high-throughput analysis suitable for routine QC (Adegoke et al., 2014; Dinc et al., 2002).

Concurrently, analytical chemistry is moving towards sustainability: green analytical chemistry (GAC) principles emphasize reduction of hazardous reagents and wastes, energy minimization, use of safer solvents and reagents, miniaturization, and simpler sample handling. Applying GAC concepts during method development reduces the environmental footprint of routine analyses and improves laboratory safety without compromising method performance. The “12 principles of green analytical chemistry” and modern greenness-assessment tools provide structured guidance for designing greener procedures (Gałuszka et al., 2013; Płotka-Wasylka, 2018).

Practical approaches to “greening” spectroscopic methods include replacing halogenated or highly toxic organic solvents with safer alternatives (water, aqueous ethanol), employing surfactant or micellar media to solubilize poorly soluble drugs, and exploring novel benign solvents such as deep eutectic solvents (DES) or bio-based solvents. These changes can often be implemented while preserving, or even improving, sensitivity and selectivity for UV–Vis-based measurements. The growing literature on DES and other green solvent systems shows their promise as sustainable extraction/dissolution media in analytical workflows (Smith et al., 2014; Florindo et al., 2019).

Any newly developed green spectroscopic method must still satisfy regulatory expectations for analytical method validation. The ICH Q2(R1) guideline defines validation characteristics accuracy, precision, specificity, linearity, range, detection and quantitation limits, robustness, and system suitability that must be demonstrated and documented for methods used in pharmaceutical quality control. Incorporating validation planning (per ICH Q2(R1)) alongside greenness assessment (e.g., Analytical Eco-Scale, GAPI/ComplexGAPI) yields methods that are both fit-for-purpose and environmentally preferable (ICH, 2005; Płotka-Wasylka et al., 2021).

In this study, we therefore develop and validate an ecofriendly UV–Vis spectroscopic method for the simultaneous estimation of antihypertensive drugs in a fixed-dose combination. The method development follows green analytical chemistry principles, uses benign solvents/media where feasible, assesses the method’s greenness with accepted metrics, and validates performance in accordance with ICH Q2(R1). The proposed approach aims to deliver a simple, robust, and sustainable analytical solution for routine quality control of combination antihypertensive dosage forms.

MATERIAL AND METHODS:

Solubility Studies

The solubility of PNL and HCZ was determined at 25 ± 1°C. Accurately weighed 10 mg of each drug was transferred into separate 10 mL volumetric flasks containing different solvents. The flasks were placed on a mechanical shaker for 8 h to facilitate dissolution. After shaking, each solution was filtered through Whatman filter paper No. 41. The filtrates were suitably diluted and visually analyzed.

Determination of Solubility Enhancement by UV–Vis Spectroscopy

Solubility enhancement studies were performed using distilled water, 2 M sodium acetate, 8 M urea, 2 M sodium citrate, 2 M sodium benzoate, 2 M ammonium acetate, binary solvent systems (e.g., 2 M sodium acetate : 2 M sodium benzoate, 2 M urea : 2 M sodium acetate, 2 M sodium citrate : 8 M urea, 2 M ammonium acetate : 2 M sodium citrate) at 25 ± 2°C.

An excess amount of each drug was added to 100 mL of solvent in screw-capped glass vials. The vials were shaken mechanically for 48 h at 25°C until equilibrium was achieved. The solutions were then filtered through a 0.45 µm membrane filter, and the filtrates were analyzed spectrophotometrically to determine solubility.

The mixed hydrotropic solution 2 M ammonium acetate : 2 M sodium citrate (1 : 1) showed the highest solubility enhancement more than 17-fold for PNL and more than 21-fold for HCZ compared to water. This enhancement was attributed to the hydrotropic solubilization phenomenon.

Stability Profile

The stability of PNL and HCZ in the optimized mixed hydrotropic solvent (2M ammonium acetate: 2 M sodium citrate, 1:1) was evaluated. Solutions of 5µg/mL PNL and 10µg/mL HCZ were scanned in time-scan mode for 30 min. The spectra showed no significant change, indicating both drugs were stable in the selected solvent system.

Linearity Range and Calibration Graph

Preparation of Standard Stock Solution (Stock A)

100 mg of each drug was separately dissolved in 80 mL of mixed hydrotropic solution (2 M ammonium acetate : 2 M sodium citrate, 1 : 1) in a 100 mL volumetric flask. The mixture was sonicated for 10 min to achieve complete dissolution, and the volume was adjusted to 100 mL with the same solvent, yielding a concentration of 1000 µg/mL.

Preparation of Sub-stock Solution (Stock B)

From Stock A, 2.5 mL was pipetted into a 25 mL volumetric flask and diluted to the mark with RO water, obtaining a 100 µg/mL solution.

Preparation of Working Standards

PNL: Aliquots of 1.0, 2.0, 3.0, 4.0, and 5.0 mL from Stock B were transferred to separate 10 mL volumetric flasks and diluted to volume with RO water to obtain concentrations of 10–50 µg/mL.

HCZ: Aliquots of 0.5, 1.0, 1.5, 2.0, and 2.5 mL from Stock B were similarly diluted to obtain concentrations of 5–25 µg/mL.

Selection of Wavelength for Linearity

Solutions of 5 µg/mL PNL and 10 µg/mL HCZ were scanned over 200–400 nm. The λmax values were found at 280.0 nm (PNL) and 260.0 nm (HCZ). Both drugs showed good linearity within the tested ranges (PNL: 10–50 µg/mL; HCZ: 5–25 µg/mL). Calibration curves were constructed by plotting absorbance versus concentration.

Simultaneous Equation Method

Overlay spectra were recorded for standard solutions of 5µg/mL PNL and 10µg/mL HCZ in the selected solvent system. PNL showed maximum absorbance at 280.0 nm, HCZ at 260.0 nm, and an isoabsorptive point was observed at 272.0 nm. The two wavelengths selected for the method were 280.0 nm (λ1) and 260.0 nm (λ2).

The concentrations of each drug in the mixture were determined using the following equations:

$$\mathrm{C\ PNL} = \frac{\mathrm{A1ay2} - \mathrm{A2ay2}}{\mathrm{ax1ay2} - \mathrm{ax2ay1}}.$$

$$\mathrm{C\ HCZ} = \frac{\mathrm{A1ax2} - \mathrm{A2ax2}}{\mathrm{ax1ay2} - \mathrm{ax2ay1}}.$$

Where, A1 and A2 are absorbances of mixture at 280.0 nm and 260.0 nm respectively, ax1 and ax2 are absorptivities of PNL at λ1 (280.0 i.e. λmax of PNL) and λ2 (260.0 i.e. λmax of HCZ) respectively and ay1 and ay2 are absorptivities of HCZ at λ1 and λ2 respectively. CHCZ and CPNL are concentrations of PNL and HCZ respectively.

Validation of the Simultaneous Equation Method (ICH; 2005)

Linearity

Linearity was evaluated by calculating the response ratio (absorbance/concentration) and plotting it against concentration for each drug.

Accuracy

Accuracy was assessed via recovery studies at 80%, 100%, and 120% levels by adding known amounts of standard drugs to pre-analyzed tablet solutions. Recovery analysis was performed in triplicate at each level.

Precision

Precision was studied at three levels:

Repeatability: Analysis of the same concentration in five replicates.

Intermediate precision: Day-to-day and analyst-to-analyst variations.

Reproducibility: Independent replication of the method.

Analysis of Tablet Formulation

Twenty marketed tablets containing PNL and HCZ were weighed, powdered, and a portion equivalent to 10 mg PNL (and 5 mg HCZ) was transferred into a 10 mL volumetric flask. Eight milliliters of the mixed hydrotropic solution were added, and the mixture was sonicated for 10 min. The volume was adjusted with the same solvent, and the solution was filtered through Whatman filter paper No. 41. The filtrate was suitably diluted with RO water to bring both drugs within the working range. Absorbances were measured at 280.0 nm and 260.0 nm, and concentrations were calculated using the simultaneous equation method. The procedure was performed in quintuplicate.

RESULTS AND DISCUSSION:

The overlay spectra of propranolol (PNL) and hydrochlorothiazide (HCZ) (Figure 1) revealed distinct absorption maxima at 280 nm and 260 nm, respectively. These well-separated λmax values allowed for direct spectrophotometric measurement without interference between the two drugs, forming the basis for simultaneous estimation in the combined dosage form.

Linearity

The calibration curves for PNL and HCZ (Table 1) showed excellent linearity within the concentration ranges of 10–50 µg/mL for PNL and 5–25 µg/mL for HCZ. The correlation coefficients (r^2) were 0.998 for PNL and 0.999 for HCZ, indicating a strong linear relationship between absorbance and concentration. The low intercept values (0.003 for both drugs) and well-defined slopes further confirm the suitability of the method for quantitative analysis.

Accuracy (Recovery Studies)

Recovery studies (Table 3) conducted at 80%, 100%, and 120% of label claim levels demonstrated mean recoveries close to 100% for both drugs ranging from 99.09% to 99.23% for PNL and from 98.50% to 98.90% for HCZ. The low standard deviation (SD) values indicate good reproducibility and confirm that the method is free from interference from excipients present in the marketed formulations.

Precision

The precision results (Table 4) show that the method has excellent repeatability and intermediate precision. For PNL, the % mean values ranged between 99.221 ± 0.106 and 99.592 ± 0.015, while for HCZ they ranged between 98.354 ± 0.106 and 99.666 ± 0.039. The low SD values across repeatability, day-to-day, analyst-to-analyst, and reproducibility studies confirm that the method consistently produces reliable results under varied conditions.

Assay of Tablet Formulations

The assay results for marketed formulations (Table 5) indicated that the method accurately quantified PNL and HCZ within their respective label claims. PNL was found to be 79.95 mg (99.94% of label claim) and HCZ was found to be 24.92 mg (99.68% of label claim), both with low SD and %RSD values (<0.15%). These results meet typical acceptance criteria for assay and further confirm the accuracy and robustness of the method. The developed ecofriendly UV–Vis spectrophotometric method satisfies the International Conference on Harmonisation (ICH) Q2(R1) validation parameters for linearity, accuracy, precision, and assay. The use of non-toxic solvents and simple sample preparation aligns with the principles of green analytical chemistry, making the method both sustainable and practical for routine quality control of combination antihypertensive dosage forms.

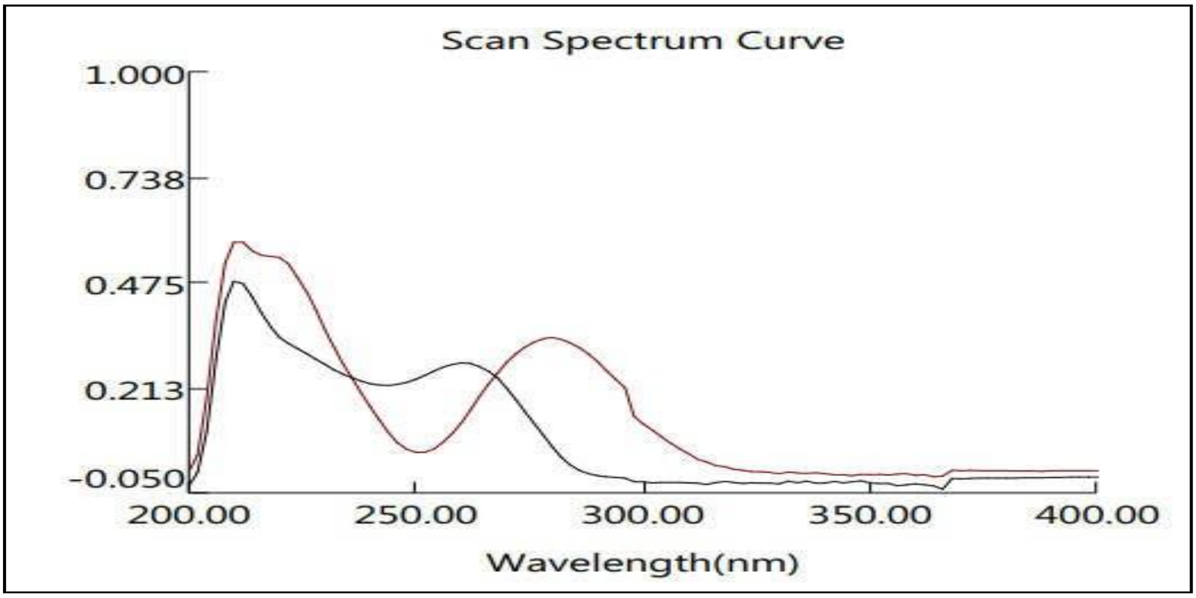

Figure 1: Overlay Spectra of PNL and HCZ

Table 1: Linearity data for Propranolol (PNL) and Hydrochlorothiazide (HCZ)

Parameter	PNL	HCZ
Working λmax	280 nm	260 nm
Beer's law limit (µg/ml)	10–50	5–25
Correlation coefficient (r^2)*	0.998	0.999
Slope (m)*	0.014	0.022
Intercept (c)*	0.003	0.003

Table 3: Recovery studies of marketed formulations

Recovery Level (%)	% Recovery (Mean ± SD)* PNL	% Recovery (Mean ± SD)* HCZ
80	99.23 ± 0.464	98.50 ± 0.667
100	99.17 ± 0.334	98.59 ± 0.486
120	99.09 ± 0.343	98.90 ± 0.949

Table 4: Precision and validation results (Mean ± SD)*

Parameter	PNL	HCZ
Precision		
Repeatability	99.221 ± 0.106	98.354 ± 0.106
Day-to-Day	99.592 ± 0.015	99.205 ± 0.015
Analyst-to-Analyst	99.235 ± 0.151	99.245 ± 0.058
Reproducibility	99.504 ± 0.114	99.666 ± 0.039

Table 5: Analysis of tablet formulation containing PNL and HCZ

Drug	Label Claim (mg)	Amount Found (mg)	% of Label Claim	SD	% RSD
PNL	80	79.95	99.94	0.115	0.135
HCZ	25	24.92	99.68	0.142	0.148

CONCLUSION:

The present study successfully developed and validated a simple, accurate, precise, and ecofriendly spectroscopic method for the simultaneous estimation of PNL and HCZ in marketed formulations. The method demonstrated excellent linearity, accuracy, and precision within the tested concentration range. Recovery studies confirmed the reliability and reproducibility of the method with minimal standard deviation values. The approach is cost-effective, does not require sophisticated instrumentation, and reduces the use of harmful organic solvents, making it environmentally friendly. The proposed method is also rapid, with minimal sample preparation steps, allowing for efficient routine analysis in pharmaceutical quality control laboratories. This validated method is suitable for application in regular analytical procedures for the quality assessment of combined dosage forms containing PNL and HCZ.

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