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EXPLORING UV-SPECTROPHOTOMETRIC TECHNIQUES FOR ANALYSIS OF MULTICOMPONENT DRUG FORMULATIONS

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Keywords:

Cilnidipine, Chlorthalidone, Ratio Subtraction,Spectrum Subtraction, Absorption Factor, ICH Q2 (R2) guidelines, ComplexGAPI, AGREE

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ABSTRACT: The objective of the current research work includes development and validation of novel, precise and accurate UV-visible spectrophotometric techniques for the analysis of Cilnidipine and Chlorthalidone without the need of prior separation by employing mathematical spectrophotometric manipulations, which utilize absorbance relationships to resolve overlapping spectral components. Two methods, namely Ratio Subtraction Coupled with Spectrum Subtraction (RS-SS) and Absorption Factor Method (AFM) were developed for the simultaneous estimation of Cilnidipine (CIL) and Chlorthalidone (CHLOR). In the RS-SS method, CIL and CHLOR were quantified at 365 nm and 275 nm, respectively, using a divisor concentration of 8 µg/mL of CIL. In the AFM method, CHLOR concentration was quantified directly by recording the absorbance at 275 nm. For the estimation of CIL, an absorption factor was calculated from the absorbance values at two selected wavelengths, 275 nm and 365 nm. These values were applied in the AFM equation to compute the absorbance attributable to CIL in the mixture. The developed methods were validated in accordance with ICH Q2 (R2) guidelines. Linearity was established over the concentration ranges of 4–12 µg/mL for CIL and 5–15 µg/mL for CHLOR, with good correlation coefficients. Precision was confirmed through intraday and interday studies, with % RSD values below 2%. Robustness was evaluated by small deliberate variations in wavelength, solvent composition, divisor concentration and absorption factor with %RSD <2%. Accuracy was demonstrated by recovery studies conducted at three concentration levels (80%, 100%, and 120%), with recovery results ranging between 98% and 102%, confirming the reliability and robustness of the methods. Greenness was evaluated using Complex GAPI and AGREE software.

INTRODUCTION: Cilnidipine is chemically 1, 4-Dihydro-2, 6-dimethyl-4-(3-nitrophenyl)-3, 5-pyridinedicarboxylic acid 2-methoxyethyl (2E)-3-phenyl-2-propenyl ester, Cilnidipine is a dihydropyridine calcium channel blocker with action on both N- and L-type calcium channels used to treat hypertension by blocking the incoming calcium and suppressing the contraction of blood vessels.

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A light yellow, crystalline powder which is Soluble in acetone; sparingly soluble in ethanol (95 %); very slightly soluble in water. It dissolves in dilute solutions of alkali hydroxides and structure of Cilnidipine is depicted in **Fig. 1A** [1-3].

Chlorthalidone is chemically (RS)-2-chloro-5-(1-hydroxy – 3 – oxoisoindolin – 1 - yl) benzene-sulphonamide, Chlorthalidone acts by inhibiting the Na^+/Cl^- symporter in the cortical diluting segment of the ascending limb of the loop of Henle, thereby blocking the reabsorption of sodium and chloride. As more sodium reaches the distal renal tubule, potassium excretion is indirectly increased through the sodium-potassium exchange mechanism. The enhanced diuresis ultimately lowers plasma and

extracellular fluid volume, which in turn reduces cardiac output and contributes to a decrease in blood pressure. A white to yellow, crystalline powder that dissolves in methanol but is insoluble in water, ether and chloroform. Structure of Chlorthalidone is depicted in **Fig. 1B** [4-6].

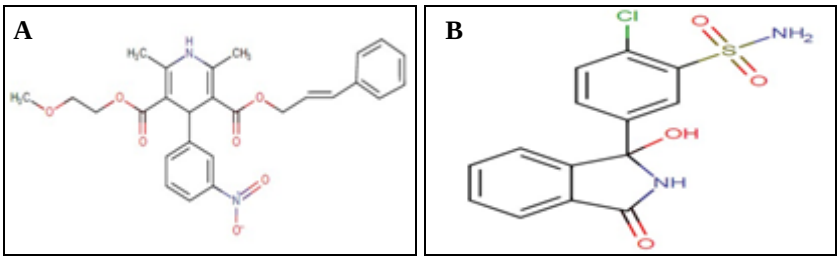

FIG. 1: MOLECULAR STRUCTUREOF (A) CILNIDIPINEAND (B) CHLORTHALIDONE

Literature survey reveals that several analytical methods developed for Cilnidipine [7-12] alone and in combination with Chlorthalidone [13-16] and with other drugs. Reported Spectrophotometric methods are UV [17, 18], RP-HPLC [19-21].

MATERIALS AND METHODS:

Instrumentation: The analysis was conducted using a UV-Visible double beam spectrophotometer (SHIMADZU, model UV-2700) equipped with a pair of 1 cm matched quartz cells (spectral bandwidth of 1 nm, scan speed of 400 nm/min and data interval 0f 0.5nm). All weighing procedures were performed on Wensar electronic balance (model MAB 220) and calibrated glassware was used consistently throughout the study. Baseline correction was performed using solvent blank prior to each scan.

Reagents and Chemicals: Pure drug samples of Chlorthalidone and Cilnidipine were generously provided as gift sample by CTX Life Sciences Pvt. Ltd., Sachin, Surat and Pure and Cure Healthcare Pvt. Ltd., Haridwar, Uttarakhand respectively. Methanol HPLC grade and Distilled water were employed as solvents throughout the study.

Marketed Formulation: The commercial formulation, CTD – C tablet, produced by IPCA Laboratories Ltd. Each tablet comprises 10mg of Cilnidipine and 12.5mg of Chlorthalidone (1:1.25).

Preparation of Solutions:

Preparation of Standard Stock Solution of CIL (1000 µg/mL): An accurately weighed 100 mg of CIL was transferred into a 100 mL volumetric flask. To ensure complete dissolution, 50 mL of methanol was added initially. The solution was then diluted up to the mark by adding methanol to get a standard stock solution with a concentration of 1000 µg/mL.

Preparation of Working Standard Solution of CIL (100 µg/mL): A 10 mL aliquot of the standard stock solution of CIL was precisely transferred into 100 mL volumetric flask. To ensure uniform mixing, 50 mL of methanol was added initially. The solution was then diluted to volume with the same solvent to prepare a working standard solution with a concentration of 100 µg/mL.

Preparation of Standard Stock Solution of CHLOR (1000 µg/mL): An accurately weighed 100 mg of CHLOR was transferred into a 100 mL volumetric flask. To ensure complete dissolution, 50 mL of methanol was added initially. The solution was then diluted up to the mark by adding methanol to get a standard stock solution with a concentration of 1000 µg/mL.

Preparation of Working Standard Solution of CHLOR (100 µg/mL): A 10 mL aliquot of the standard stock solution of CHLOR was precisely transferred into 100 mL volumetric flask. To ensure uniform mixing, 50 mL of methanol was added initially. The solution was then diluted to volume with the same solvent mixture to prepare a working standard solution with a concentration of 100 µg/mL.

Method Development for Simultaneous Estimation of Cilnidipine and Chlorthalidone in Water: Methanol Mixture:

Ratio Subtraction with Spectrum Subtraction [22, 24]: This method is designed for the analysis of binary mixtures of drugs Chlorthalidone and Cilnidipine with overlapping spectra, where one component Cilnidipine exhibits a more extended

absorption spectrum than the other Chlorthalidone. The λ_{max} of CHLOR and CIL in water and methanol mixture was found to be 275nm and 365nm, respectively.

To determine Drug CHLOR,

Step 1:

(CHLOR + CIL) / CIL' = CHLOR + CIL / CIL' = CHLOR / CIL + CONSTANT / CIL'

The plateau region of the resulting spectrum, where the contribution from X is negligible, is used to measure the constant value CIL/CIL'.

Step 2:

CHLOR + CONSTANT – CONSTANT / CIL'

This constant is subtracted from the entire ratio spectrum, and the resulting curve is then multiplied by the divisor CIL'. This operation reconstructs the original zero-order absorption spectrum of CHLOR, free from interference by CIL.

Step 3:

CHLOR × CIL'= CHLOR / CIL'

To determine Drug CIL (the more extended drug), the Spectrum Subtraction approach is applied. The previously obtained zero-order spectrum of CHLOR is subtracted from mixture (CHLOR +CIL) recovering the zero-order absorption spectrum of CIL.

Step 1:

(CHLOR + CIL) – CHLOR = CIL

This stepwise mathematical manipulation allows for the selective and accurate determination of each drug in the mixture without prior separation, even in the presence of spectral overlap.

Determination of Chlor by RS Method:
Division:

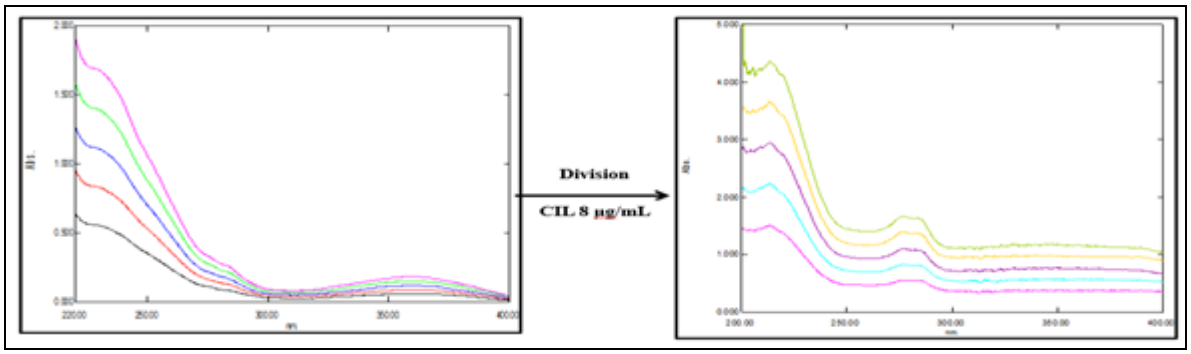

FIG. 2: ZERO ORDER SPECTRA OF DIFFERENT LABORATORY PREPARED MIXTURES OF CHLOR (5-15 MG/ML) AND CIL (4-12MG/ML)

FIG. 3: RATIO SPECTRA OF DIFFERENT LABORATORY PREPARED MIXTURES OF: CHLOR (5-15 MG/ML) AND CIL (4-12 MG/ML) USING CIL 8 MG/ML AS A DIVISOR (Y')

Subtraction of Constant and Multiplication:

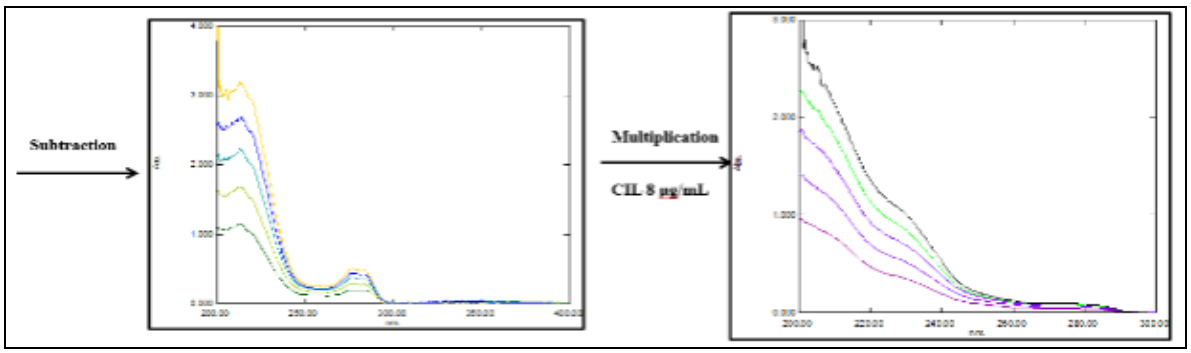

FIG. 4: RATIO SPECTRA OF DIFFERENT LABORATORY PREPARED MIXTURES OF CHLOR AND CIL AFTER SUBTRACTION OF CONSTANT

FIG. 5: ZERO ORDER SPECTRA OF CHLOR

Determination of CIL by Spectrum Subtraction Method:

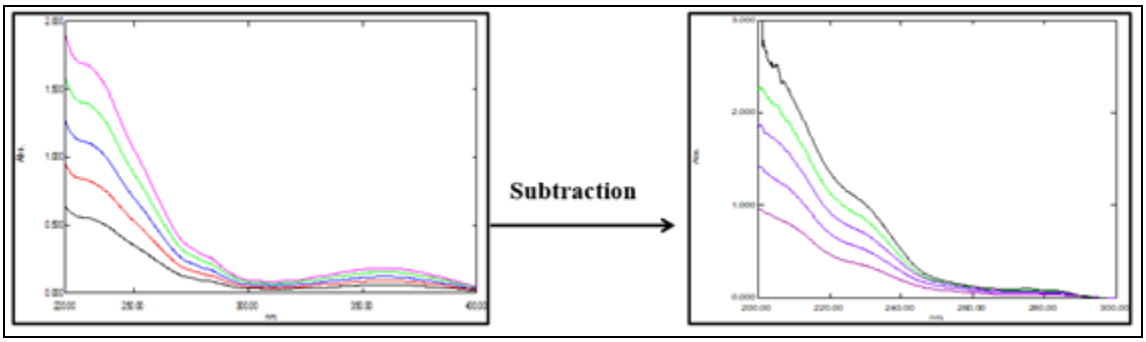

FIG. 6: ZERO ORDER SPECTRA OF DIFFERENT LABORATORY PREPARED MIXTURES OF: CHLOR 5-15 MG/ML) AND CIL (4-12 MG/ML)

FIG. 7: ZERO ORDER SPECTRA OF CHLOR

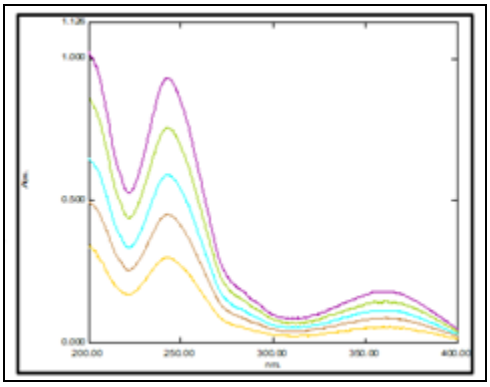

FIG. 8: ZERO ORDER SPECTRA OF CIL

Note* - the selection of divisor spectra were based on correlation coefficients approaching unity, and therefore 8 µg/mL of CIL and 10 µg/mL of CHLOR were employed as the optimal divisor concentrations. This concentration provided an optimal signal-to-noise ratio and yielded smooth ratio spectra with stable and flat plateau region suitable for measurement. The divisor spectrum was recorded under the same instrumental and experimental conditions as those used for mixture spectra.

Absorption Factor Method [23, 24]: The Absorption Factor Method is used to analyse a binary mixture containing two components, CHLOR and CIL, whose absorption spectra overlap. This method is particularly useful when CIL interferes with CHLOR at CHLOR's maximum absorbance wavelength (λ_1), whereas CHLOR does not interfere with CIL at a different wavelength (λ_2). At λ_1, the total absorbance $A\lambda_1$ arises from both CHLOR and CIL, while at λ_2, only CIL contributes to the absorbance. The Absorption Factor (A.F.) is defined as:

$$Ay, \lambda_1 / Ay, \lambda_2 = \text{Absorption factor of pure y'}$$

Using this, the corrected absorbance due to CHLOR at λ_1 can be determined by subtracting the contribution of CIL at λ_1 (which is inferred from λ_2 using A.F.) from the total absorbance at λ_1:

$$Ax, \lambda_1 = Ay1\,(x+y) - Ay, \lambda_1 / Ay, \lambda_2 \times Ay_2\,(x+y)$$

Where,

λ_1 = wavelength where both x and y components absorb

λ_2 = wavelength where only y absorbs

$A\lambda_1\,(x+y)$ = absorbance of mixture at λ_1

$A\lambda_2\,(x+y)$ = absorbance of mixture at λ_2

Ay, λ_1 = absorbance of pure component y at λ_1

Ay, λ_2 = absorbance of pure component y at λ_2

Ax, λ_1 = corrected absorbance of component x at λ_1

This equation isolates the absorbance due to CHLOR by correcting for CIL's interference, enabling accurate quantification of CHLOR in the presence of CIL.

Specificity Study: Specificity of the developed methods was assessed by comparing the spectral profiles of a laboratory-prepared mixture of CIL and CHLOR (1:1.25) with that of a mixture extracted from a commercial fixed-dose combination tablet (CTD-C), both containing CIL 8 µg/mL and CHLOR 10 µg/mL. The laboratory mixture was prepared by accurately pipetting 0.8 mL of CIL (100 µg/mL) and 1 mL of CHLOR (100 µg/mL) into a 10 mL volumetric flask and diluting to volume with water and methanol mixture.

To prepare the sample from the tablet formulation, 20 CTD-C tablets (total weight: 3.74 g) were powdered, and an amount equivalent to a single tablet (0.193 g, containing 12.5 mg CHLOR and 10 mg CIL) was transferred to a 100 mL volumetric flask. Methanol (50 mL) was added, and the mixture was sonicated for 20 minutes for extraction, then diluted to volume with methanol, yielding a solution containing 100 µg/mL CIL and 125 µg/mL CHLOR.

No significant differences observed between laboratory mixture and tablet extract.

Linearity and Range Study: Linearity for CIL and CHLOR were evaluated over the concentration ranges of 4–12 µg/mL and 5–15 µg/mL, respectively. For CIL, five concentrations (4, 6, 8, 10, and 12 µg/mL) were prepared by pipetting 0.4–1.2 mL aliquots from a 100 µg/mL working standard into 10 mL volumetric flasks, followed by dilution with solvent. Similarly, for CHLOR, concentrations of 5, 7.5, 10, 12.5, and 15 µg/mL were prepared by pipetting 0.5–1.5 mL from a 100 µg/mL working solution (1:1.25). Spectra were recorded in the 200–400 nm range using a UV-Vis spectrophotometer.

For RS-SS absorbance of zero-order spectra were measured at 365 nm (CIL) and 275 nm (CHLOR), and calibration curves (absorbance vs. concentration) were plotted to obtain regression equations. For AFM, zero-order absorbance were recorded at 365 nm for CIL and 275 nm for CHLOR. Calibration curves were similarly established and corresponding regression equations computed.

Precision Study: Precision was evaluated at the assay concentration level using CIL (8 µg/mL) and CHLOR (10 µg/mL) for both intra-day and inter-day studies.

Twenty CTD-C tablets were powdered, and a quantity equivalent to one tablet (0.193 g, containing 10 mg CIL and 12.5 mg CHLOR (1:1.25)) was transferred into a 100 mL volumetric flask. To this, 50mL methanol was added, and the mixture was sonicated for 20 minutes to facilitate drug extraction. The volume was then adjusted to 100mL with methanol, resulting in a stock solution containing 100 µg/mL CIL and 125 µg/mL CHLOR. This solution was centrifuged at 3800 rpm (~1600 x g) for 30 minutes and the supernatant was carefully collected by filtering using Whatman filter paper grade 1. Suitable aliquots of the supernatant were then transferred into a 10 mL volumetric flask and diluted with the chosen solvent to obtain final assay concentrations of CIL (8 µg/mL) and CHLOR (10 µg/mL) (1:1.25). The resulting solutions were scanned using a UV-Visible spectrophotometer across the wavelength range of 200–400 nm. Spectral data were recorded and precision was assessed as follows:

RS-SS Method: CHLOR was quantified at 275nm using 8 µg/mL CIL as the divisor; CIL was determined by subtracting obtained CHLOR from mixture and absorbance measurements were specifically recorded at 365 nm.

AFM: CHLOR absorbance was measured at 275 nm and CIL at 365 nm, without interference. Each analysis was performed in six replicates, and the % RSD was calculated to assess method precision.

Accuracy Study: Accuracy was assessed using the standard addition method at three concentration levels - 80%, 100%, and 120% relative to the assay concentrations (8 µg/mL CIL and 10 µg/mL CHLOR (1:1.25)). Percent recovery was determined for each level to evaluate method accuracy.

A batch of twenty CTD-C tablets, with a total weight: 3.74 g was finely powdered, and a portion

equivalent to one tablet (0.193 g, containing 10 mg CIL and 12.5 mg CHLOR (1:1.25)) was transferred into a 100 mL volumetric flask. Methanol (50 mL) was added, and the mixture was sonicated for 20 minutes to facilitate drug extraction. The volume was then made up to 100mL with methanol, resulting in a solution containing 100 µg/mL CIL and 125 µg/mL CHLOR. The solution was centrifuged at 3800 rpm (~1600 x g) for 30 minutes, and the clear supernatant was collected by filtering using Whatman filter paper grade 1 into a separate volumetric flask for further analysis. From this0.4 mL was withdrawn to obtain a base concentration of CIL (4 µg/mL) and CHLOR (5 µg/mL), which was then analysed using RS-SS and AFM methods. To prepare 80%, 100%, and 120% assay levels, standard additions of 3.2, 4.0, and 4.8 mL for CIL and 4.0,5.0, and 6.0 mL for CHLOR (from their respective 100 µg/mL working standards) were added to the base solution. All prepared solutions were scanned in the range of 200–400 nm using a UV – Visible spectrophotometer.

RS-SS Methods: CHLOR was quantified at 275nm using 8 µg/mL CIL as the divisor; CIL was determined by subtracting obtained CHLOR from mixture and absorbance was measured at 365 nm

AFM: CHLOR was determined at 275 nm, and CIL at 365 nm without CHLOR interference. Each level was tested in triplicate. Percent recovery and %RSD were calculated to assess accuracy.

Assay: The assay of CIL and CHLOR in the marketed formulation (CTD-C) (1:1.25) was carried out to determine the percent label claim.

Twenty CTD-C tablets, with a total weight of 3.74g, were weighed accurately and finely powdered. A portion equivalent to one tablet (0.193 g, containing 10 mg CIL and 12.5 mg CHLOR (1:1.25)) was transferred into a 100 mL volumetric flask. Methanol (50 mL) was added, and the mixture was sonicated for 20 minutes to facilitate drug extraction. The resulting solution was centrifuged at 3800 rpm (~1600 x g) for 30 minutes and the clear supernatant was collected by filtering using Whatman filter paper grade 1 into a separate volumetric flask. An aliquot 0.8 mL from the supernatant was pipetted into a 10 mL volumetric flask and diluted to volume using the selected solvent, yielding a final solution of CIL (8 µg/mL) and CHLOR (10 µg/mL). The solution was analysed and spectra were recorded in the wavelength range of 200-400 nm using UV – Visible spectrophotometer. Assay determination was performed using the following analytical methods:

RS-SS Method: CHLOR was determined at 275 nm using 8 µg/mL CIL as the divisor.

CIL was determined at 365 nm by spectrum subtraction method.

AFM: CHLOR was quantified by measuring absorbance at 275 nm.

CIL was quantified at 365 nm without interference from CHLOR.

All measurements were performed in triplicate, and the %RSD was calculated to assess the precision of the assay.

Robustness Study: The robustness of the proposed methods was evaluated by introducing small deliberate variations in analytical parameters such wavelength (±2 nm), solvent composition (±2% v/v), divisor concentration (±0.5 µg/mL) for the RS-SS and absorption factor (±2%) stability across concentration range for the AFM. The analysis was carried out under modified conditions and %RSD were calculated. The method was considered robust when %RSD was less than 2%

RESULTS AND DISCUSSION [9]:

Selection of Solvent System: Preliminary investigations were carried out with distilled water and methanol alone, but both exhibited instability in the individual solvents. Hence stability studies were performed to select a suitable solvent system (distilled water and methanol mixture) ensuring spectral stability at the selected wavelength.

During method development using UV – Visible spectroscopy, CIL was found to exhibit maximum absorbance at a wavelength of 365 nm, while CHLOR showed its maximum absorption at 275 nm. To evaluate the accuracy of the analytical approach, comparative percent recovery data for both drugs that is CIL and CHLOR using two

distinct methods: ratio subtraction coupled with spectrum subtraction (RS SS) and absorption factor method (AFM).

These techniques were applied to assess the accuracy and precision of quantifying CIL and CHLOR in combined formulations.

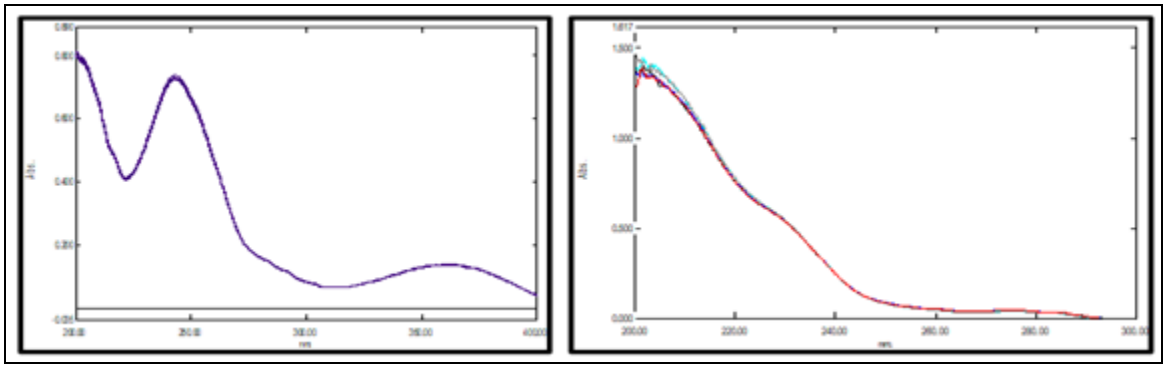

FIG. 9: ZERO-ORDER SPECTRA OF (A) CIL AND (B) CHLOR IN DISTILLED WATER AND METHANOL MIXTURE RECORDED AT 0, 2, 4, 6 AND 24 HR

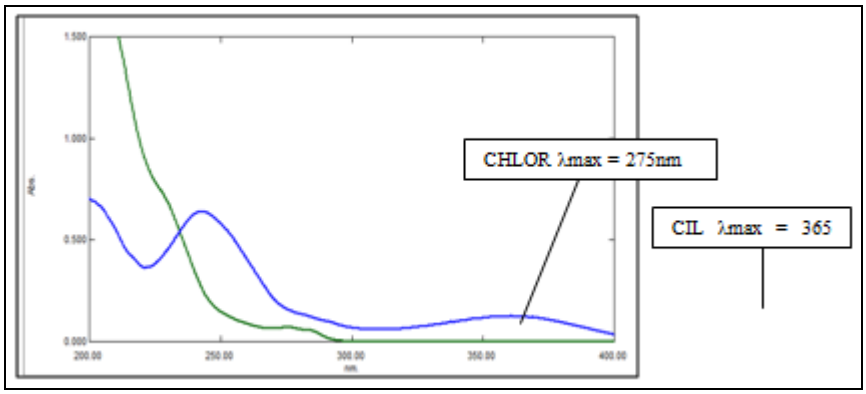

FIG. 10: OVERLAINSPECTRAOF CIL AND CHLOR WITH THEIR WAVELENGTH MAXIMA

Validation Studies of the Developed Analytical Method:

Method 1:

Ratio Subtraction (RS) Coupled with Spectrum Subtraction Method:

Specificity: Specificity of the analytical method was evaluated by comparing the UV spectra of the laboratory – prepared mixture with those of the tablet formulation. The observed overlapping spectra confirmed no interference from excipients present in tablet, thereby confirming the method's specificity for their determination of CIL and CHLOR. The correspond results are illustrated in the figure below

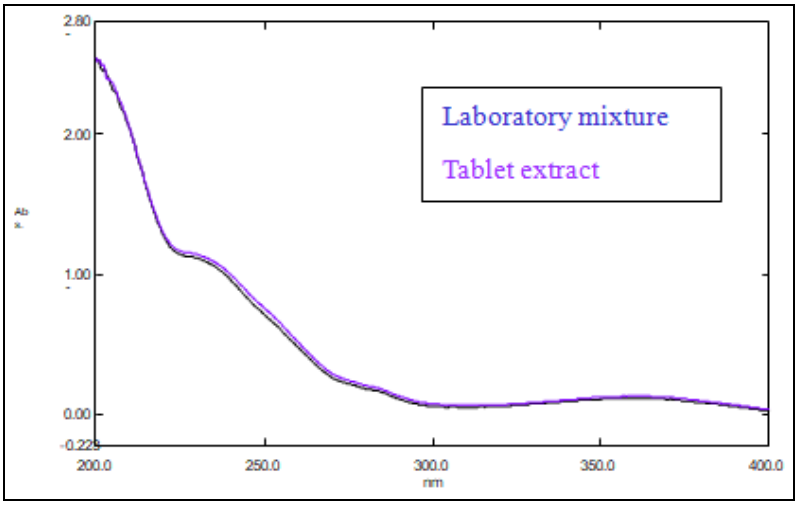

FIG. 11: OVERLAIN SPECTRA OF LABORATORY PREPARED MIXTURE AND TABLET FORMULATION OF CIL (8 µG/ML) AND CHLOR (10 µG/ML) FOR RS-SS METHOD (1:1.25)

TABLE 1: ASSAY COMPARISON OF TABLET EXTRACT AND LABORATORY MIXTURE

Drug	Sample	Wavelength (nm)	Label claim (µg/mL)	Found (µg/mL)	%Label claim
CIL	Laboratory mixture	365	8	7.86	98.25
CHLOR	Laboratory mixture	275	10	10.03	100.30
CIL	Tablet extract	265	8	8.06	100.70
CHLOR	Tablet extract	275	10	10.14	101.40

Note: The assay limit as per IP for Cilnidipine is NLT 90 % and NMT 110 % than of the stated amount and for Chlorthalidone is NLT 92.5 % and NMT 107.5 % than the stated amount.

Linearity and Calibration Curve: Linearity was evaluated by analysing five increasing concentrations of CIL (4–12 µg/mL) and CHLOR (5–15 µg/mL), each in triplicate.

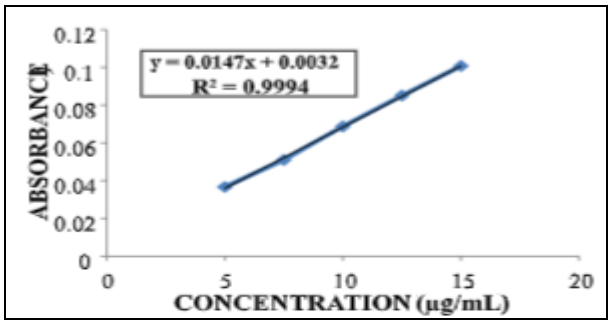

FIG. 12: CALIBRATION CURVE OF CIL BY RS-SS METHOD

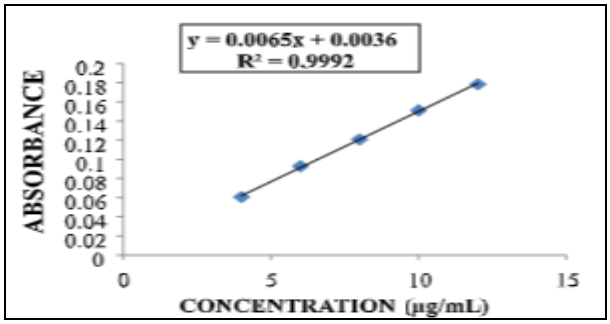

FIG. 13: CALIBRATION CURVE OF CHLOR BY RS-SS METHOD

TABLE 2: LINEARITY STUDY OF CIL AND CHLOR IN DISTILLED WATER AND METHANOL MIXTURE

Parameters	Cilnidipine	Chlorthalidone
Linearity range	4-12 µg/mL	5-15 µg/mL
Regression equation	y = 0.0147x + 0.0032	y = 0.0065x + 0.0036
Coefficient of correlation (R^2)	0.9994	0.9992
Slope (m)	0.0147	0.0065
Intercept (c)	0.0032	0.0036

Precision Study: The Precision of the proposed method was assessed at the assay concentration level through both intra-day and inter-day studies, utilising standard solutions of CIL and CHLOR. Each study comprised of six replicate measurements. The percentage relative standard deviation (% RSD) for both analytes was found to beless than 2%, indicating that the method demonstrates satisfactory precision.

TABLE 3: PRECISION STUDY OF CIL AND CHLOR (N = 6)

Parameters	Cilnidipine		Chlorthalidone	
	Mean Conc. ± SD	%RSD	Mean Conc. ± SD	%RSD
Intra-day	8.183 ± 0.095	1.167	10.02 ± 0.069	0.691
Inter-day	8.220 ± 0.153	1.865	10.151 ± 0.132	1.304

Accuracy Study: Accuracy was assessed using the standard addition method at three concentration levels (80%, 100%, and 120%), and percent recovery of the added standard drug was calculated at each level.

TABLE 4: ACCURACY STUDY OF CIL AND CHLOR (N = 3)

Level of assay concentration	Amount of base concentration (µg/mL)		Amount of standard drug added (µg/mL)		Final concentration (µg/mL)		Amount of standard drug recovered (µg/mL)		Recovery (%) means ± SD, RSD (%) (n = 3)	
	CIL	CHLOR	CIL	CHLOR	CIL	CHLOR	CIL	CHLOR	CIL	CHLOR
80%	4.00	5.00	3.20	4.00	7.20	9.00	3.21	4.05	100.40 ±0.80, 0.79	101.30 ± 0.69, 0.69
100%	4.00	5.00	4.00	5.00	8.00	10.00	4.03	5.09	100.86	101.90

120%	4.00	5.00	4.80	6.00	8.80	11.00	4.81	6.03	±0.78, 0.77 100.03 ±1.11, 1.11	±1.15, 1.14 100.61 ±0.98, 0.97

Assay: The validated RS – SS method for the simultaneous estimation of CIL and CHLOR were further applied to the analysis of the marketed formulation (CTD-C). The assay results obtained are presented in the table below.

TABLE 5: ASSAY OF CIL AND CHLOR

Parameter	Acceptance criteria	CHLOR	CIL
Assay	(n=3)	Average ± SD	
% content	CHLOR: 92.5– 107.5% CIL: 90 – 110%	100.7±0.64	101.1±0.45

Method II:

Absorption Factor Method: The basic idea underlying the absorption factor method (AFM) is the measurement of one drug (CHLOR) at its λ_{max} (λ_1), when the interfering drug (CIL) also exhibits some absorbance at that wavelength (λ_1). The interfering drug (CIL) is directly determined from the mixture spectra at its λ_2 because at λ_2 the other drug i.e., CHLOR do not show any absorbance.

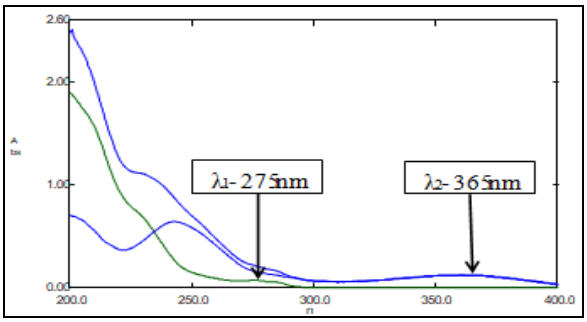

FIG. 14: OVERLAIN SPECTRA OF CIL (8µG/ML) AND CHLOR (10µG/ML) AND MIXTURE (8+10µG/ML) (1:1.25)

TABLE 6: EVALUATION OF ABSORPTION FACTOR CONSTANCY ACROSS DIFFERENT CONCENTRATIONS OF CILNIDIPINE

Sr. no.	Concentration (µg/mL)	Absorbance At λ_1 (275nm)	Absorbance At λ_2 (365nm)	Absorption Factor ($A\lambda_1/A\lambda_2$)
1.	4	0.083	0.060	1.383
2.	6	0.130	0.097	1.340
3.	8	0.166	0.120	1.383
4.	10	0.208	0.155	1.341
5.	12	0.250	0.178	1.404

Mean A.F. ± SD = 1.370 ± 0.0284

Analytical Method Validation:

Specificity Study: Specificity was assessed by comparing the spectra of the laboratory-prepared mixture with that of the tablet formulation. The overlapping spectra confirmed no interference from excipients, indicating the method is specific.

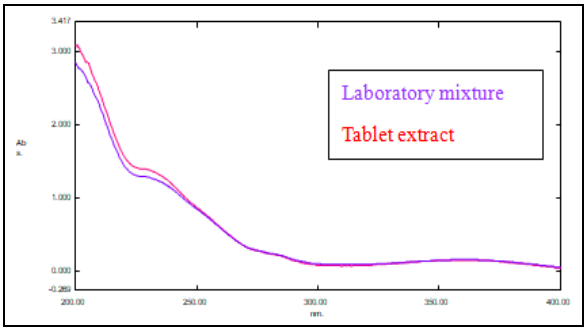

FIG. 15: OVERLAIN SPECTRA OF LABORATORY PREPARED MIXTURE AND TABLET FORMULATION OF CIL (8 µG/ML) AND CHLOR (10 µG/ML) FOR AFM (1:1.25)

TABLE 7: ASSAY COMPARISON OF TABLET EXTRACT AND LABORATORY MIXTURE

Drug	Sample	Wavelength (nm)	Label claim (µg/mL)	Found (µg/mL)	%Label claim
CIL	Laboratory mixture	365	8	8.00	100.00
CHLOR	Laboratory mixture	275	10	10.07	100.70
CIL	Tablet extract	265	8	8.13	101.62
CHLOR	Tablet extract	275	10	10.17	101.70

Note: The assay limit as per IP for Cilnidipine is NLT 90 % and NMT 110 % than of the stated amount and for Chlorthalidone is NLT 92.5 % and NMT 107.5 % than the stated amount.

Linearity and Range: Linearity was evaluated by analysing five concentrations of CIL (4–12 µg/mL) and CHLOR (5–15 µg/mL), each in triplicate. The method showed good linearity across the tested ranges, and results are presented in the tables below.

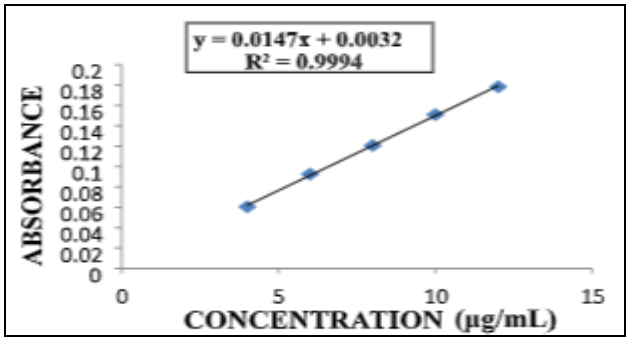

FIG. 16: CALIBRATIONCURVEOF CIL FOR AFM

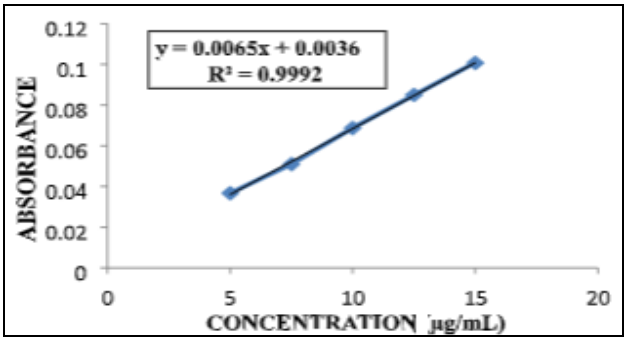

FIG. 17: CALIBRATIONCURVEOF CHLOR FOR AFM

TABLE 8: LINEARITY STUDYOF CIL AND CHLOR IN DISTILLED WATER AND METHANOL MIXTURE

Parameters	Cilnidipine	Chlorthalidone
Linearity range	4-12 µg/mL	5-15 µg/mL
Regression equation	y = 0.0147x + 0.0032	y = 0.0065x + 0.0036
Coefficient of correlation (R^2)	0.9994	0.9992
Slope (m)	0.0147	0.0065
Intercept (c)	0.0032	0.0036

Precision Study: Precision of the proposed method was assessed at the assay concentration level by conducting both intraday and inter-day studies using standard solutions of CIL (8 µg/mL) and CHLOR (10 µg/mL). Each precision study involved six replicate measurements. The percentage relative standard deviation (% RSD) for both analytes remained below 2%, confirming that the method exhibits an acceptable level of precision.

TABLE 9: PRECISION STUDY OF CIL AND CHLOR (N = 6)

Parameters	Cilnidipine		Chlorthalidone	
	Mean Conc. ± SD	%RSD	Mean Conc. ± SD	%RSD
Intra-day	8.19 ± 0.089	1.088	10.12 ± 0.202	1.909
Inter-day	7.90 ± 0.122	1.548	10.07 ± 0.187	1.957

Accuracy Study: The standard addition method was used to determine accuracy at three different concentration levels (80, 100 and 120 %) and the percent recovery of the standard drug added at each level was calculate.

TABLE 10: ACCURACY STUDY OF CIL AND CHLOR (N = 3)

Level of assay concentration	Amount of base concentration (µg/mL)		Amount of standard drug added (µg/mL)		Final concentration (µg/mL)		Amount of standard drug recovered (µg/mL)		Recovery (%) means ± SD, RSD (%) (n = 3)	
	CIL	CHLOR	CIL	CHLOR	CIL	CHLOR	CIL	CHLOR	CIL	CHLOR
80%	4.00	5.00	3.20	4.00	7.20	9.00	3.22	4.04	100.80	100.91 ±

									±1.85, 1.83	1.89, 1.87
100%	4.00	5.00	4.00	5.00	8.00	10.00	4.05	4.98	101.45	99.79
									±0.72, 0.71	±1.67, 1.67
120%	4.00	5.00	4.80	6.00	8.80	11.00	4.85	6.03	101.22	100.53
									±1.88, 1.86	±1.52, 1.51

Assay: The validated AFM method for the simultaneous estimation of CIL and CHLOR were further applied to the analysis of the marketed formulation (CTD-C) (1:1.25). The assay results obtained are presented in the table below.

TABLE 11: ASSAY OF CIL AND CHLOR

Parameter	Acceptance criteria	CHLOR	CIL
Assay	**(n=3)**	**Average ± SD**	
% content	CHLOR: 92.5– 107.5%, CIL: 90 – 110%	101.4±0.85	100.2±1.78

TABLE 12: ROBUSTNESS STUDY FOR RS-SS AND AFM METHOD FOR CILAND CHLOR

Sr. no.	Parameter	Nominal condition	Variation applied	Drug	Mean % Assay (n=3)	SD	%RSD
1	Wavelength	365nm	363nm	CIL	100.60	0.82	0.81
				CHLOR	101.24	0.74	0.73
			367nm	CIL	100.99	0.76	0.75
				CHLOR	101.08	0.68	0.67
2	Wavelength	275nm	273nm	CIL	100.52	0.79	0.78
				CHLOR	100.80	0.65	0.64
			277nm	CIL	100.75	0.72	0.71
				CHLOR	101.31	0.70	0.69
3	Divisor concentration (RS-SS)	8 µg/mL	7.5 µg/mL	CIL	100.40	0.84	0.83
				CHLOR	101.12	0.71	0.70
			8.5 µg/mL	CIL	100.87	0.77	0.76
				CHLOR	100.96	0.69	0.68
4	Absorption factor (AFM)	Calculated AF (275/365nm)	-2%	CIL	100.64	0.73	0.72
				CHLOR	101.00	0.66	0.65
			+2%	CIL	100.70	0.71	0.70
				CHLOR	101.28	0.63	0.62
5	Solvent composition	Methanol: water (70:30)	68:32	CIL	100.57	0.82	0.81
				CHLOR	100.91	0.74	0.73
			72:28	CIL	100.80	0.78	0.77
				CHLOR	101.19	0.69	0.68

Acceptance Criteria: %RSD not more than 2%:

Greenness Assessment for the Drug Combination: Green chemistry is a widely recognized approach in modern chemistry that aligns with the principles of sustainable development. It emphasizes conducting chemical processes in a manner that promotes environmental protection, resource efficiency, and economic viability. The integration of green chemistry concepts is now common in both analytical and manufacturing practices, driving a shift in attitudes toward more sustainable chemical development.

Evaluating the "greenness" of an analytical method can be challenging due to the multiple factors involved. It is essential to provide concrete evidence of the method's actual environmental impact to substantiate its sustainability claims. In the present study involving the combination of Cilnidipine and Chlorthalidone, the greenness of the developed analytical methods was assessed using two established tools:

- Complex GAPI
- AGREE

These tools provide a comprehensive evaluation of environmental friendliness based on various criteria, ensuring that the method adheres to green analytical chemistry principles [25].

Complex GAPI:

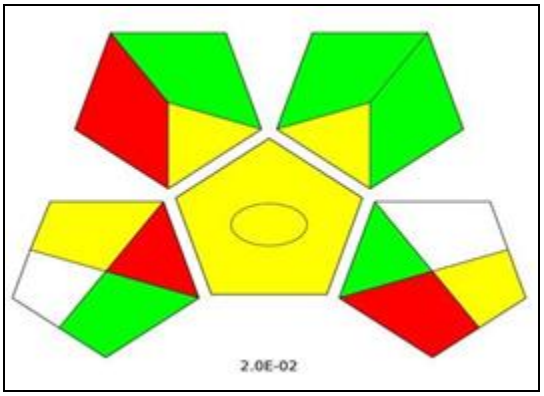

FIG. 18: GREENNESS ASSESSMENT (RS-SS AND AFM) BY COMPLEX GAPI

For the combination of Cilnidipine and Chlorthalidone, novel UV spectrophotometric method namely Ratio Subtraction Coupled with Spectrum Subtraction (RS-SS) and the Absorption Factor Method (AFM) were developed. The greenness of these methods was evaluated using the Complex GAPI tool. The resulting E-factor was found to be 0.2, which is close to zero, indicating minimal waste generation. The greenness assessment suggests that the proposed methods exhibit acceptable environmental compatibility.[26]

AGREE:

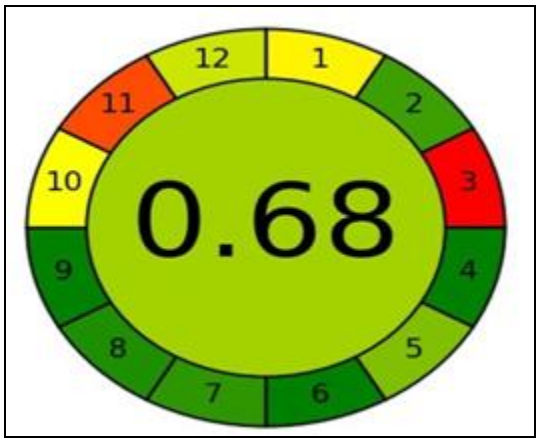

FIG. 19: GREENNESS ASSESSMENT (RS-SS AND AFM) BY AGREE

For the drug combination of Cilnidipine and Chlorthalidone, novel UV spectrophotometric methods i.e. Ratio Subtraction Coupled with Spectrum Subtraction (RS-SS) and Absorption Factor Method (AFM) were developed. The greenness of these methods was evaluated using the AGREE tool. The resulting score was 0.68, which is close to 1, indicating moderate to good environmental compatibility. This value confirms that the developed methods align well with green chemistry principles and can be considered environmentally friendly [27, 28].

CONCLUSION: The proposed UV spectrophotometric methods i.e. RS-SS, and AFM were developed based on their underlying principles and successfully applied to the analysis of Cilnidipine–Chlorthalidone combinations. These methods require neither advanced instrumentation nor expensive solvents, rendering them well – suited for routine application in quality control laboratories. They offer good selectivity and sensitivity for binary mixtures without the need for complex sample preparation or interference from excipients.

The methods are simple, precise, accurate, specific, and costeffective, with no need for prior separation. Furthermore, adhering to the principles of green analytical chemistry, the consumption of organic solvents was kept to a minimum. The validation outcomes affirmed the reliability and suitability of the developed methods for routine quality control of the studied drugs.

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CONFLICT OF INTEREST: The authors affirm that they have no conflict of interest to disclose.

REFERENCES:

1. MRMed. Cilnidipine and Chlorthalidone: Uses, Side Effects, and Interactions.
2. Indian Pharmacopoeia Commission. Indian Pharmacopoeia. Ghaziabad: Indian Pharmacopoeia Commission 2022; 2: 1535-1537.
3. DrugBank Online. Cilnidipine: Uses, Interactions, Mechanism of Action.Available from: https://go.drugbank.com/drugs/DB09232
4. Apotex Inc. PrAPO-CHLORTHALIDONE (Chlorthalidone Tablets) [Product Monograph]. Toronto: Apotex Inc.Available from: https://pdf.hres.ca/dpd_pm/00069909.PDF
5. DrugBank Online. Chlorthalidone: Uses, Interactions, Mechanism of Action. Available from: https://go.drugbank.com/drugs/DB00310
6. National Center for Biotechnology Information. PubChem Compound Summary for CID 2732, Chlorthalidone. Bethesda (MD): National Library of Medicine (US). Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Chlorthalidone
7. Safhi MM: Spectrophotometric method for the estimation of cilnidipine in bulk and pharmaceutical dosage forms. Orient J Chem 2013; 29(1): 131.
8. Jadhav RS, Ubale MB and Bharad JV: A simple, significant UV-spectroscopic analytical method development and validation for estimation of formulation drug product-cilnidipine tablet. Int J Pharm Biol Sci 2018; 8(3): 187-194.
9. Ravi V, KS K, Kavitha J, Seetharaman R and Lakshmi KS: Multivariate UV spectrophotometric quantification of cilnidipine in bulk drug and pharmaceutical formulations. J Med Pharm Allied Sci 2022; 11(2): 4672-4678.
10. Sankar PR, Swathi V and Babu PS: Development and validation of novel UV and RP-HPLC methods for determination of cilnidipine (a new generation Ca channel blocker) in pharmaceutical dosage form. Int J Pharm Sci Res 2019; 10(4): 1886-1894.
11. Kasimala BB, Anna VR and Mallu UR: Stability-indicating reversed-phase HPLC method for the separation and estimation of related impurities of cilnidipine in pharmaceutical formulations. Indian Drugs 2018; 55(12): 54-79.
12. Balakrishna T, Shirsat MK and Amol K: Development and validation of stability indicating RP-HPLC method for estimation of cilnidipine. J Drug Deliv Ther 2020; 10(1): 97-100.
13. Ingle SU, Patil PA, Kulkarni VC, Patil SV, Salunke PA and Wagh RS: Development and validation of UV spectrophotometric method for chlorthalidone in bulk and pharmaceutical dosage forms. World J Pharm Res 2014; 3(9): 958-963.
14. Abdullah NS, Hassan MA and Hassan RO: Spectrophotometric determination of chlorthalidone in pharmaceutical formulations using different order derivative methods. Arab J Chem 2017; 10: 426-433.
15. Kharat C, Shirsat VA, Kodgule YM and Kodgule M: A validated RP-HPLC stability method for the estimation of chlorthalidone and its process-related impurities in an API and tablet formulation. Int J Anal Chem 2020; 2020: 359-380.
16. Gandhi SV and Sanklecha AP: HPLC method development and validation for estimation of chlorthalidone in tablet dosage form. J Drug Deliv Ther
17. Chanduluru HK, Sugumaran A and Kannaiah KP: Multiple spectrophotometric determinations of chlorthalidone and cilnidipine using propylene carbonate – a step towards greenness. Anal Biochem 2022; 657: 114-890.
18. Kalyankar GG, Patel J, Bodiwala KB, Lodha SR and Mistry V: Development and validation of first order UV derivative spectroscopy method for simultaneous estimation of cilnidipine and chlorthalidone in their combined tablet dosage form. Pharma Sci Monit 2019; 10(2): 101-111.
19. Kudumula N and Prasad YR: Development and validation of RP-HPLC method for the simultaneous estimation of chlorthalidone and cilnidipine in bulk and combined tablet dosage form. Pharmacophore 2014; 5(4): 442-540.
20. Pawar VT, Pawar SV, More HN, Kulkarni AS and Gaikwad DT: RP-HPLC method for simultaneous estimation of cilnidipine and chlorthalidone. Res J Pharm Technol 2017; 10(11): 3990-3996.
21. Sawaikar L and Kapupara P: Development and validation of a stability indicating RP-HPLC method for the estimation of chlorthalidone and cilnidipine in combined pharmaceutical dosage form. Res J Pharm Technol 2020; 13(5): 2376-2380.
22. Mohammad MA, Mahrouse MA, Amer EA and Elharati NS: Simultaneous determination of enalapril maleate and nitrendipine in tablets using spectrophotometric methods manipulating ratio spectra. Spectrochim Acta A Mol Biomol Spectrosc 2021; 244: 118-894.
23. Malathi DS, Pallavi M and Sunil Kumar: Instrumental Methods of Analysis (English Edition). Lucknow: Thakur Publication Pvt Ltd 2020.

24. Kamal AH, El-Malla SF and Hammad SF: A review on UV spectrophotometric methods for simultaneous multicomponent analysis. EJPMR 2016; 3(2): 348-365.
25. American Chemical Society. 12 Principles of Green Chemistry. Washington DC: ACS 2024.
26. Complementary green analytical procedure index (ComplexGAPI) and software. Green Chem.
27. Plotka-Wasylka J and Wojnowski W: Complementary green analytical procedure index (Complex GAPI) and software. Green Chem 2021; 23(21): 8657-8665.
28. Pena-Pereira F, Wojnowski W and Tobiszewski M: AGREE – Analytical GREEnness Metric approach and software. Anal Chem 2020; 92: 10076-10082.

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