Simultaneous UV Spectrophotometric Estimation Of Paracetamol And Phenylephrine Hydrochloride In Marketed Tablet Formulation Using 0.1 M Hcl–Methanol Solvent System By Vierordt's Method

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Abstract

A simple, precise, and cost-effective UV-visible spectrophotometric method was developed for the simultaneous estimation of Paracetamol (PCM) and Phenylephrine Hydrochloride (PHE) in tablet dosage forms using Vierordt's method. A solvent system comprising 0.1 M HCl and methanol (50:50 v/v) was employed, which provided optimal solubility and ensured stable absorbance readings for both analytes. The wavelength maxima (λmax) were identified as 257 nm for PCM and 274 nm for PHE. The proposed method exhibited excellent linearity within the concentration ranges of 2–20 µg/mL for PCM and 2–15 µg/mL for PHE, with correlation coefficients (r^2) greater than 0.999, confirming strong proportionality between absorbance and concentration. The limits of detection (LOD) and quantitation (LOQ) were determined to be 0.1857 µg/mL and 0.5626 µg/mL for PCM, and 0.1705 µg/mL and 0.5166 µg/mL for PHE, respectively, indicating adequate sensitivity of the method. Accuracy studies demonstrated recovery values within the acceptable range of 98–102%, while precision studies yielded %RSD values below 2%, confirming repeatability and reproducibility of the developed procedure. Assay of marketed formulations revealed drug contents of 99.78% for PCM and 98.00% for PHE, further validating the applicability of the proposed method. Overall, the developed UV spectrophotometric method is accurate, precise, sensitive, and suitable for routine quality control analysis of combined PCM–PHE formulations.

Keywords

Paracetamol, Phenylephrine HCl, UV spectrophotometry, Vierordt's method, simultaneous estimation, 0.1 M HCl, Methanol.

1. Introduction

Paracetamol (PCM) is a widely used analgesic and antipyretic agent classified as a BCS Class I drug due to its high solubility and high permeability. Phenylephrine Hydrochloride (PHE), an α_1-adrenergic agonist used as a nasal decongestant in cold and flu preparations, is categorised as a BCS Class III drug, characterised by high solubility but low permeability. Their combination is commonly included in multi-ingredient tablet formulations intended for the symptomatic relief of upper respiratory tract infections. PCM acts through central inhibition of prostaglandin synthesis, while PHE induces vasoconstriction in the nasal mucosa, providing complementary therapeutic benefits. The chemical structures of both drugs are depicted in **Figure 1.**

Despite their therapeutic significance, simultaneous estimation of PCM and PHE poses analytical challenges due to differences in their physicochemical properties and partial overlap in their UV absorption spectra. Although chromatographic techniques such as HPLC offer high selectivity, they require sophisticated instruments, skilled analysts, and lengthy procedures. UV-visible spectrophotometry serves as a simple, rapid, and economical alternative suitable for routine quality-control laboratories.

Vierordt's method (simultaneous equation method) is particularly appropriate for drug combinations exhibiting distinct λmax values, enabling accurate quantification of each component based on Beer–Lambert's law. Considering the need for a cost-effective and reliable analytical approach, the present study aims to develop a simple and accurate UV spectrophotometric method for the simultaneous estimation of PCM and PHE using 0.1 M HCl–methanol (50:50 v/v). The method was validated as per ICH guidelines to confirm its linearity, accuracy, precision, and suitability for routine quality-control analysis.

Structural Profile of Selected Active Pharmaceutical Ingredients (APIs):

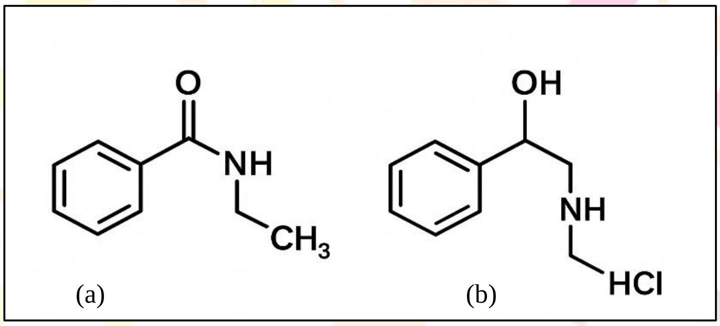


Figure 1. Structural representation of (a) Paracetamol (acetaminophen) (b) Phenylephrine Hydrochloride

2. Materials and Methods

2.1 Chemicals

Paracetamol standard (purity 99%) and Phenylephrine Hydrochloride standard (purity 98%) were obtained from certified suppliers. Methanol (AR grade), 0.1 M hydrochloric acid, and distilled water were used throughout the study. All chemicals and reagents employed were of analytical grade

2.2 Instrumentation

A double-beam UV–Visible spectrophotometer equipped with quartz cuvettes of 1 cm path length was used for all absorbance measurements. An analytical balance with high precision was employed for weighing the standards and samples. An ultrasonic bath was utilised to aid in the dissolution of the drug substances. All instruments were calibrated before analysis.

2.3 Preparation of Solvent

A solvent system of 0.1 M hydrochloric acid and methanol was prepared by mixing both components in a 50:50 v/v proportion.

2.4 Standard Stock Solutions

A Paracetamol (PCM) standard stock solution (1000 µg/mL) was prepared by accurately weighing 100 mg of PCM, dissolving it in a small quantity of the solvent mixture, and transferring it to a 100 mL volumetric flask. The volume was made up with 0.1 M HCl–Methanol (50:50 v/v).

A Phenylephrine Hydrochloride (PHE) standard stock solution (1000 µg/mL) was prepared similarly by weighing 100 mg of PHE and making up the volume to 100 mL with the same solvent mixture.

2.5 Working Standards

A series of working standard solutions of PCM (2–20 µg/mL) and PHE (2–15 µg/mL) were prepared by appropriate dilutions of their respective 1000 µg/mL stock solutions using the previously prepared 0.1 M HCl–methanol (50:50 v/v) solvent system.

2.6 Determination of λmax

Standard solutions of Paracetamol (PCM) and Phenylephrine Hydrochloride (PHE) (10 µg/mL each) were scanned in the ultraviolet region (200–400 nm) using the prepared 0.1 M HCl–methanol (50:50 v/v) solvent system as the blank. The absorption spectra obtained for each drug were examined to identify their respective wavelengths of maximum absorbance (λ_max). These λ_max values were then selected as the analytical wavelengths for simultaneous quantification using Vierordt's method.

2.7 Vierordt's Method Equations

Using the absorptivity coefficients of each drug at the selected wavelengths, the concentrations of Paracetamol (PCM) and Phenylephrine Hydrochloride (PHE) in the mixture were calculated by applying Vierordt's simultaneous equations.

The concentration of PCM (Cx) was determined using the following equation:

$$C_x = \frac{(A_2 \times a_{y1} - A_1 \times a_{y2})}{(a_{x2} \times a_{y1} - a_{x1} \times a_{y2})}$$

Similarly, the concentration of PHE (Cy) was calculated using:

$$C_y = \frac{(A_1 \times a_{x2} - A_2 \times a_{x1})}{(a_{x2} \times a_{y1} - a_{x1} \times a_{y2})}$$

Where,

A1 = Absorbance of mixture at wavelength 1 (λ1 = 257 nm)

A2 = Absorbance of mixture at wavelength 2 (λ2 = 274 nm)

ax1 = Absorptivity of PCM at λ1

ax2 = Absorptivity of PCM at λ2

ay1 = Absorptivity of PHE at λ1

ay2 = Absorptivity of PHE at λ2

Cx = Concentration of PCM

Cy = Concentration of PHE

2.8 Sample Preparation

For the assay of PCM and PHE from the tablet formulation, twenty tablets were accurately weighed and finely powdered. A quantity of the powder equivalent to 500 mg of Paracetamol (PCM) and 10 mg of Phenylephrine Hydrochloride (PHE) was transferred to a 100 mL volumetric flask. Approximately 50 mL of the 0.1 M HCl–methanol (50:50 v/v) solvent system was added, and the mixture was sonicated for 15 minutes to ensure complete extraction of the drug components. The resulting solution was filtered through Whatman No. 41 filter paper, and the filtrate was suitably diluted with the same solvent system to obtain final concentrations of 10 µg/mL of PCM and 5 µg/mL of PHE, falling within the validated linearity range. The absorbance of the prepared solution was measured at the selected analytical wavelengths, and the concentrations of PCM and PHE were calculated using Vierordt's method.

2.9 Absorptivity Coefficients (a-values)

The absorptivity values (a_1%, 1 cm) for Paracetamol (PCM) and Phenylephrine Hydrochloride (PHE) at the selected analytical wavelengths, λ_1 (257 nm) and λ_2 (274 nm), were experimentally determined. Standard solutions of each drug were scanned individually, and their absorptivity coefficients at both wavelengths were calculated in accordance with Beer–Lambert's law. These experimentally obtained absorptivity values were subsequently utilized for the application of Vierordt's simultaneous equation method.

2.10 Method Validation

The developed UV-visible spectrophotometric method was validated in accordance with the ICH Q2(R1) guidelines and was assessed for linearity, accuracy, precision, robustness, limit of detection (LOD), and limit of quantitation (LOQ).

2.10.1 Linearity

Linearity of the proposed UV-visible spectrophotometric method was evaluated by analyzing a series of standard solutions of PCM (2–20 µg/mL) and PHE (2–15 µg/mL). Calibration curves were constructed by plotting absorbance against concentration for each drug, and the corresponding regression equations were generated from the least-squares analysis. Both PCM and PHE exhibited satisfactory linearity across their respective concentration ranges, demonstrating the suitability of the method for quantitative analysis.

2.10.2 Accuracy

Accuracy of the proposed UV-visible spectrophotometric method was assessed by recovery studies using the standard-addition method at three concentration levels corresponding to 80%, 100%, and 120% of the nominal amounts. Pre-analyzed samples were spiked with known quantities of PCM and PHE, and the resulting mixtures were analyzed in triplicate at each level. The percentage recovery and %RSD values obtained from these studies confirmed the accuracy of the method.

2.10.3 Precision

Precision of the proposed method was evaluated in terms of intra-day and inter-day precision at three different concentration levels for both PCM and PHE. For intra-day precision, each concentration level was analyzed in triplicate within the same day, whereas inter-day precision was assessed by repeating the analysis across three consecutive days. The percentage relative standard deviation (%RSD) values obtained in both cases were within acceptable limits, demonstrating good repeatability and intermediate precision of the method.

2.10.4 LOD and LOQ

The limit of detection (LOD) and limit of quantitation (LOQ) of the proposed UV-visible spectrophotometric method were calculated using the standard ICH equations, where LOD = 3.3 × (SD/Slope) and LOQ = 10 × (SD/Slope). The standard deviation of the y-intercepts and the slope of the calibration curve were used for these calculations. The obtained LOD and LOQ values indicated adequate sensitivity of the method for the quantitative estimation of PCM and PHE.

2.10.5 Robustness

Robustness of the proposed method was evaluated by introducing a deliberate variation in the analytical wavelength by ±1 nm. The prepared samples were analyzed under the modified conditions, and the resulting absorbance and assay values were compared with those obtained under the optimized conditions. No significant variation was observed, and the %RSD values remained within acceptable limits, indicating that the method was robust against small changes in wavelength.

2.11. Assay of PCM and PHE in Marketed Tablet Formulation

For the assay of PCM and PHE in marketed tablet formulations, twenty tablets were accurately weighed, and the average tablet weight was determined. The tablets were finely powdered, and an amount of powder equivalent to 500 mg of PCM and 10 mg of PHE was transferred into a 100 mL volumetric flask. Approximately 50 mL of the 0.1 M HCl–methanol (50:50 v/v) solvent system was added, and the mixture was sonicated for 15 minutes to ensure complete extraction of both drug components.

After sonication, the dispersion was filtered through Whatman No. 41 filter paper. The filtrate obtained represented the primary stock solution, corresponding to 5000 µg/mL PCM and 100 µg/mL PHE in the original flask. A measured volume of this stock was then diluted appropriately with the same solvent system to obtain the working assay solution, containing 10 µg/mL of PCM and 5 µg/mL of PHE, which falls within the established linearity range for both analytes.

The final assay solution was thoroughly mixed to ensure homogeneity and then analysed at the selected analytical wavelengths. Drug concentrations were calculated using the experimentally determined absorptivity coefficients and Vierordt’s simultaneous equation method. This procedure provided accurate quantification of PCM and PHE after batch sampling, excipient removal, and filtration.

4. Results

4.1 Determination of λmax

Standard solutions of Paracetamol (PCM) and Phenylephrine Hydrochloride (PHE) (10 µg/mL each) were scanned in the UV region using 0.1 M HCl–methanol (50:50 v/v) as the blank. PCM exhibited a distinct absorption maximum at 257 nm, whereas PHE showed a characteristic λ_max at 274 nm. Although partial overlap was observed in the intermediate wavelength region, both analytes retained well-defined absorption peaks, allowing their simultaneous determination by Vierordt's method.

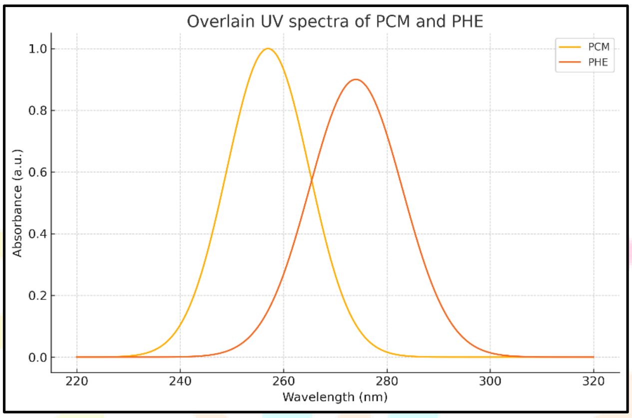
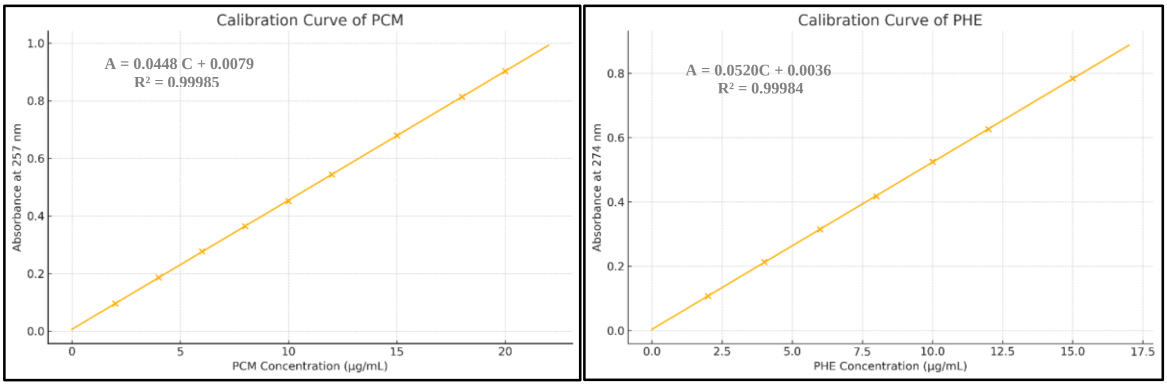


Figure 2: Overlain UV spectra of PCM & PHE (10 µg/mL each)



(a) **(b)**

Figure 3: (a) Calibration Curve of PCM (257 nm) (b) Calibration Curve of PHE (274 nm)

4.2 Absorptivity Coefficients

The absorptivity values (a_1%, 1 cm) for Paracetamol (PCM) and Phenylephrine Hydrochloride (PHE) were experimentally determined at the selected analytical wavelengths, λ_1 = 257 nm and λ_2 = 274 nm. Standard solutions of each drug were scanned individually, and the absorptivity coefficients at both wavelengths were calculated using the slope of the absorbance–concentration relationship in accordance with Beer–Lambert's law.

PCM exhibited higher absorptivity at 257 nm, whereas PHE showed stronger absorption at 274 nm. These experimentally determined absorptivity coefficients were subsequently used for the construction of Vierordt's simultaneous equations for quantitative estimation of both drugs in combination.

The calculated absorptivity values for PCM and PHE at both wavelengths are presented in Table 1.

Table 1. Absorptivity Coefficients (a_x and a_γ) for PCM and PHE

Drug	a_{x1} at 257 nm	a_{x2} at 274 nm	$a_{\gamma 1}$ at 257 nm	$a_{\gamma 2}$ at 274 nm
PCM	0.00185	0.00098	–	–
PHE	–	–	0.00112	0.00221

4.3 Linearity and Calibration Curve

The linearity of the proposed UV–visible method was assessed by constructing calibration curves for PCM and PHE across the concentration ranges of 2–20 µg/mL and 2–15 µg/mL, respectively. Each concentration level was analyzed in triplicate, and the mean absorbance values were used for regression analysis. Both PCM and PHE complied with Beer–Lambert's law within their respective ranges.

The regression analysis yielded the following equations:

- PCM: $A = 0.0448C + 0.0079$ ($R^2 = 0.99985$)
- PHE: $A = 0.0520C + 0.0036$ ($R^2 = 0.99984$)

The high correlation coefficients confirmed excellent linearity for both analytes.

The optical characteristics and regression parameters for both drugs are defined in Table 2.

The detailed linearity absorbance data for all concentrations are listed separately in Table 3.

Table 2. Optical Characteristics & Regression Data

Parameter	**PCM**	**PHE**
λmax (nm)	257	274
Beer's law range (µg/mL)	2–20	2–15
Regression equation	$A = 0.0448\ C + 0.0063$	$A = 0.0520\ C + 0.0036$
Slope	0.0448	0.0520
Intercept	0.0079	0.0036
R^2	0.99985	0.99984
LOD (µg/mL)	0.1857	0.1705
LOQ (µg/mL)	0.5626	0.5166

Table 3. Linearity Data (PCM & PHE)

Conc (µg/mL)	PCM Abs ± SD	%RSD	PHE Abs ± SD	%RSD
2	0.0955 ± 0.0029	3.04	0.1083 ± 0.0030	2.76
4	0.1871 ± 0.0034	1.82	0.2103 ± 0.0020	0.94
6	0.2801 ± 0.0031	1.11	0.3121 ± 0.0026	0.83
8	0.3641 ± 0.0033	0.90	0.4217 ± 0.0031	0.73
10	0.4612 ± 0.0043	0.94	0.5184 ± 0.0028	0.54
12	0.5438 ± 0.0029	0.53	0.6202 ± 0.0029	0.47
15	0.6754 ± 0.0035	0.52	0.7821 ± 0.0022	0.28
18	0.8115 ± 0.0032	0.39	–	–
20	0.9078 ± 0.0034	0.37	–	–

4.4 Accuracy (Recovery)

Accuracy of the proposed method was evaluated by the standard-addition technique at three concentration levels corresponding to 80%, 100%, and 120% of the nominal analyte concentration. Pre-analyzed samples of PCM and PHE were spiked with known quantities of standard drug, and the resulting mixtures were analyzed in triplicate. The obtained recovery values for both drugs were within the acceptable range of 98–102%, demonstrating the accuracy of the method and indicating that excipients present in the tablet formulation did not interfere with the analysis.

For PCM, recovery values ranged from 98.30% to 99.12% with %RSD values below 1.20, while PHE showed recovery values between 98.02% and 101.08% with %RSD values below 1.05, as shown in Table 4(a) and Table 4(b), respectively.

Table 4(a). Recovery Study for PCM

Level(%)	Sample(µg/ml)	Added(µg/ml)	Total(µg/ml)	Found(µg/ml)	%Recovery	%RSD
80%	10	8	18	17.84	99.12	0.79
100%	10	10	20	19.71	98.56	1.02
120%	10	12	22	21.63	98.30	1.18

Table 4(b). PHE Recovery

Level(%)	Sample(µg/ml)	Added(µg/ml)	Total(µg/ml)	Found(µg/ml)	%Recovery	%RSD
80%	7.5	6	13.5	13.65	101.08	0.48
100%	7.5	7.5	15	14.70	98.02	1.03
120%	7.5	9	16.5	16.64	100.83	0.96

4.5 Precision

Precision of the proposed method was evaluated in terms of intra-day and inter-day variation at three concentration levels (low, medium, and high) for both PCM and PHE. Six replicates were analyzed for intra-day precision, while inter-day precision was assessed over three consecutive days.

For intra-day precision, PCM showed %RSD values ranging from 0.43–1.39, whereas PHE exhibited %RSD values between 0.29–0.94. Inter-day precision results demonstrated %RSD values of 0.43–1.13 for PCM and 0.10–1.21 for PHE.

All precision values were below 2% RSD, confirming excellent repeatability and intermediate precision of the method. The detailed intra-day and inter-day precision results are presented in Table 5(a) and Table 5(b), respectively.

Table 5(a). Intra-day Precision

Drug	Level	Mean Abs	SD	%RSD
PCM	Low	0.1852	0.0026	1.39
PCM	Medium	0.4551	0.0044	0.97
PCM	High	0.8152	0.0035	0.43
PHE	Low	0.2103	0.0020	0.94
PHE	Medium	0.4177	0.0015	0.36
PHE	High	0.7843	0.0023	0.29

Table 5(b). Inter-day Precision

Drug	Level	Day 1	Day 2	Day 3	%RSD
PCM	Low	0.1858	0.1843	0.1817	1.13
PCM	Medium	0.4547	0.4519	0.4557	0.44
PCM	High	0.8108	0.8170	0.8112	0.43

PHE	Low	0.2107	0.2135	0.2084	1.21
PHE	Medium	0.4199	0.4226	0.4153	0.88
PHE	High	0.7835	0.7837	0.7850	0.10

4.6 Robustness

Robustness of the proposed method was evaluated by introducing a deliberate variation in the analytical wavelength of ±1 nm from the optimized values. The prepared samples were analyzed under these modified conditions, and the resulting absorbance, %Assay, and %RSD values were compared with those obtained at the nominal wavelength. No significant variations were noted in any of the parameters.

For PCM, %Assay values ranged from 99.69% to 100.77%, while PHE exhibited %Assay values between 99.17% and 100.21%. In all cases, %RSD values remained below 1%, confirming the robustness of the method against minor wavelength variations.

The detailed robustness results for both analytes are presented in Table 6.

Table 6. Robustness (±1 nm)

Drug	Nom λ(nm)	Var λ(nm)	Conc(µg/mL)	Abs	%Assay	%RSD
PCM	257	256	10	0.4465	99.69	0.93
PCM	257	258	10	0.4513	100.77	0.98
PHE	274	273	8	0.4100	99.17	0.36
PHE	274	275	8	0.4143	100.21	0.21

4.7 LOD and LOQ

The sensitivity of the proposed method was established by determining the Limit of Detection (LOD) and Limit of Quantification (LOQ) for both PCM and PHE. These parameters were calculated using the IUPAC-recommended equations, where LOD = 3.3 × (SD/Slope) and LOQ = 10 × (SD/Slope). The standard deviation of the response and the slope of the calibration curve were used for the calculations.

The LOD values obtained were 0.1857 µg/mL for PCM and 0.1705 µg/mL for PHE, whereas the LOQ values were 0.5626 µg/mL and 0.5166 µg/mL, respectively. In all cases, the LOQ values exceeded the corresponding LOD values, indicating acceptable method sensitivity and compliance with analytical validation requirements. The calculated LOD and LOQ values for both analytes are presented in Table 7.

Table 7. LOD and LOQ values for PCM and PHE.

Drug	SD of Response	Slope	LOD (µg/mL)	LOQ (µg/mL)
PCM	0.00252	0.04479	0.1857	0.5626
PHE	0.00267	0.05168	0.1705	0.5166

4.8 Assay of Marketed Tablets

The validated UV–visible spectrophotometric method was applied for the quantitative analysis of a marketed PCM–PHE combination tablet. The assay results demonstrated that the method accurately determined the drug content in the formulation. PCM was found to contain 498.9 mg/tablet (99.78%), whereas PHE was found to contain 9.8 mg/tablet (98.00%).

The %RSD values for both analytes were within acceptable limits, confirming the precision and reliability of the assay procedure.

Table 8 presents the complete assay profile for PCM and PHE, showing the labeled claim, measured content, %Assay, and associated %RSD values.

Table 8. % Assay determination

Drug	Label (mg)	Found (mg)	%Assay(Mean ± SD)	%RSD
PCM	500	498.9	498.9 ± 1.95	0.39
PHE	10	9.8	9.8 ± 0.10	1.02

5. Discussion

The proposed method demonstrated excellent linearity for both drugs with R^2 close to 0.999. Accuracy fell within the acceptable range of 98–102%, and precision showed %RSD < 2%, confirming reliability. The LOD and LOQ values indicate high sensitivity. Assay results were within pharmacopeial limits, demonstrating suitability for routine QC.

6. Conclusion

A simple, precise, and economical UV spectrophotometric method was successfully developed for the simultaneous estimation of PCM and PHE using Vierordt's method. The method is sensitive, accurate, and suitable for routine analysis in pharmaceutical laboratories.

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