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A Qualitative Detection of Phenylephrine Hydrochloride from Pharmaceutical Formulation Using a Multi-Technique Approach

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Abstract

Sympathomimetic drugs, such as Phenylephrine (PHE), are widely used in over-the-counter medications, often combined with other active ingredients and excipients. The qualitative identification of PHE in complex formulations becomes challenging due to low analyte concentration, structural similarity with other sympathomimetic amines, and matrix interference. The present study describes a multi-technique qualitative identification workflow integrating classical presumptive colour test, Thin-Layer Chromatography (TLC) and Reversed-Phase High-Performance Liquid Chromatography Coupled with Photodiode Array Detection (RP-HPLC-PDA). The presumptive identification was carried out using routinely utilised forensic colour tests, such as Chen-Kao, Marquis, Mandelin, Mecke, and Simon reagents, to examine the chromogenic activity of PHE. TLC identification was conducted on silica gel plates using an optimised solvent system with visualisation under Ultraviolet (UV) light (254nm), iodine vapour, and ninhydrin spray; the Rf values were noted and compared among reference standards and samples. RP-HPLC analysis was executed under isocratic conditions using a C18 column (250 x 4.6mm, 5µm) and a mobile phase system comprising buffer (water and triethylamine, adjusted to pH 7.5 with phosphoric acid): acetonitrile (63:37, v/v). The retention time exhibited by phenylephrine was approximately 2.1 minutes and a characteristic PDA absorption maximum at 275nm, which was found to be consistent between the reference standard and pharmaceutical samples.

Major Findings: This work presented a structured qualitative workflow based on the integrated interpretation of presumptive color testing, TLC and HPLC. The consistent chromogenic responses, comparable Rf values of TLC, and HPLC retention and spectral characteristics collectively supported the identification of phenylephrine in complex preparations. The workflow is suitable for primary laboratory identification in forensic chemistry and regulatory screening involving phenylephrine-containing formulations.

Keywords: High-Performance Liquid Chromatography (HPLC), Phenylephrine, Presumptive tests, Qualitative Identification, Thin-Layer Chromatography (TLC)

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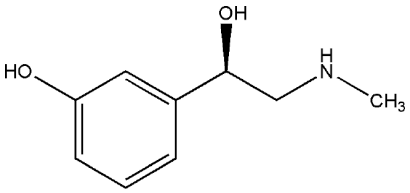
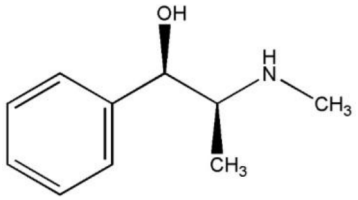
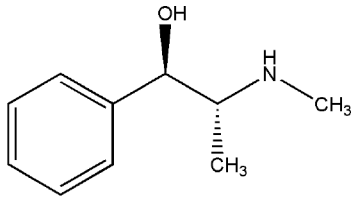
1. Introduction

The surge in the availability and usage of Over-The-Counter (OTC) drugs constitutes a major proportion of medications that are available without prescriptions, particularly those intended for symptomatic relief of respiratory conditions. Phenylephrine Hydrochloride is one of the widely utilised decongestants that induces decongestion by acting as an α-adrenergic agonist. It is often combined with other constituents, including antipyretics, analgesics, antihistamines, and other excipients, resulting in preparations where the target component may be present at relatively low concentration[1-6]. Several research studies based on analytical techniques have been reported for the determination of phenylephrine in multiple dosage

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formulations, including UV spectrophotometry, TLC and HPLC. A stability-indicating RP-HPLC method reported phenylephrine at 4.27 minutes at 273nm in a multi-component tablet, whereas the HPTLC method reported simultaneous resolution of phenylephrine with other ingredients such as chlorpheniramine, ambroxol, paracetamol (Rf=0.67)[7,8]. Green, eco-friendly HPTLC densitometric and HPLC-based methods further advance the determination of phenylephrine in ibuprofen combinations[9-11]. While these methodologies are essential for pharmaceutical analysis, they do not replace the necessity of primary qualitative testing, which remains the strong base of routine forensic analysis. Additionally, colourimetric and spectrophotometric methodologies based on derivatisation reactions, such as oxidation, coupling, and diazotisation, have also been reported for the analysis of PHP, particularly where the rapidity, simplicity and ease of implementation are intended[12,13]. PHP reactions facilitate its detection by converting it into an observable-coloured component. Together, the reported studies indicate that no single analytical approach is universally applicable for all analytical scenarios. The method selection depends upon the nature of the formulation, the objectives and available laboratory resources.

Table 1. Chemical structures of Phenylephrine and other structurally similar components

Chemical Component	Chemical Structure
Phenylephrine	
Ephedrine	
Pseudoephedrine	

From a chemical perspective, PHP belongs to the phenylethanolamine class, and it shares structural similarity with other sympathomimetic amines, including ephedrine and pseudoephedrine[14,15]. These components have been widely reviewed in forensic and regularity studies because of their involvement in illicit manufacturing of drugs, which led to broader usage of phenylephrine in medicinal formulation[16-18] (Table 1). The presence of PHP has been reported in unusual multi-drug formulations, including mixtures containing illicit constituents, further increasing the complexity of analytical detection. Additionally, several phenylephrine-containing fixed-dose combinations have been classified as prohibited in India due to safety concerns, while the compound remains under monitoring in the World Anti-Doping Agency (WADA) 2024 Monitoring program[19-20]. Some neuropsychiatric effects associated with prolonged usage of sympathomimetic-containing cold medications are also reported. Together, these factors emphasise the significance of reliable analytical identification of phenylephrine in complex mixtures[21,22]. The present work proposes a structured qualitative workflow for the identification of phenylephrine hydrochloride in complex formulations. It was hypothesised that phenylephrine could be reliably identified based on consistency through a combination of classical forensic colour tests, thin-layer chromatography and HPLC. While this work demonstrated the procedure working with pharmaceutical tablets, the next essential step would be the application to seized or fake samples. Therefore, this work presents the systematic integration and critical interpretation of these techniques within a single qualitative framework aligned for routine forensic pharmaceuticals screening practice.

2. Materials and Methods

2.1 Chemicals and Reagents

The reference standard of phenylephrine hydrochloride was obtained from the Indian Pharmacopoeia Commission (IPC). Chemicals such as cupric sulphate, sodium nitroprusside, ammonium vanadate, sodium molybdate, selenious acid, and water for HPLC grade were obtained from CDH Laboratory Reagents. Acetaldehyde and resublimed iodine were obtained from SD Fine Chemicals Limited. Ninhydrin, orthophosphoric acid, formaldehyde, and sodium hydroxide pellets were procured from Merck. Sodium sulphate anhydrous,

potassium iodide, acetic acid, diethyl ether, methanol (HPLC grade), and glacial acetic acid were procured from Thermo Fisher Scientific Pvt. Ltd. Chloroform and acetonitrile (HPLC grade) were procured from Spectrochem Pvt. Ltd., whereas triethylamine was purchased from SRL, India. All the chemicals and solvents utilised for presumptive tests, extraction, mobile phase preparations, and HPLC analysis were of analytical grade. The pharmaceutical tablet samples, consisting of phenylephrine hydrochloride as a single dosage and multi-drug combination with other chemical agents, were procured from the pharmacy.

2.2 Standard and Sample Preparation

Stock solutions of PHE standards were prepared in methanol at 1000ppm (10mg/10mL) concentration and subjected to further dilutions to obtain a working concentration range from 100ppm to 5ppm.

2.2.1 Direct Methanolic Extraction

The powdered sample tablet corresponding to 10mg of phenylephrine hydrochloride was dispersed directly in methanol to obtain a concentration of PHP 1000ppm (10mg/10 mL). The solution was then centrifuged for 5 minutes at 6000rpm. The resultant supernatant was collected, stored, and utilised for TLC and HPLC analysis.

2.2.2 Liquid Liquid Extraction (LLE)

The finely powdered specimen tablets were dissolved in water and subjected to liquid-liquid extraction, using chloroform followed by diethyl ether. This was done as a cleanup step to reduce excipients and interfering agents. The organic layers were collected separately and evaluated individually. The primary thin-layer chromatographic evaluation indicated that the ether extract produced cleaner phenylephrine spots with reduced interference. Therefore, the ether layer was selected for further analysis. The extract was prepared after being centrifuged and filtered by a 0.45µm membrane filter.

2.3 Preliminary Colour Tests

Presumptive identification of phenylephrine was performed using six classical colour tests: Chen-Kao, Marquis, Mandelin, Mecke, and Simon's. All tests were conducted in spot plates using small amounts of the powdered tablet directly and prepared reagents. The reagents were used to examine the general chromogenic activity of PHP. Reagent formulations were as follows:

- **Marquis Reagent**: A few drops (8-10) of 40% formaldehyde in 10mL concentrated H_2SO_4.
- **Simon's Reagent**: 1g sodium nitroprusside dissolved in 100mL water. 90mL of the reagent was prepared with 10mL acetaldehyde.
- **Chen-Kao Reagent**: Solution A - 1mL of Glacial Acetic Acid (GAA) in 100mL H_20, Solution B - 1 gram copper sulfate in 100mL H_20 and Solution C - 8 grams sodium NaOH in 50mL H_20. The final testing solution is combined in a 1:1:1 ratio.
- **Mecke's Reagent**: 0.25 gram of selenious acid dispersed in 25mL concentrated H_2SO_4.
- **Mandelin Reagent**: 1 gram of ammonium vanadate mixed with 100mL concentrated H_2SO_4[23-29].

2.4 Thin Layer Chromatography (TLC)

TLC analysis was conducted on Silica gel 60 F_{254} aluminium plates. For this analysis, several solvent systems were evaluated, and ethyl acetate: methanol (9;1, v/v) was selected as it provided optimal migration and spot clarity for phenylephrine in both standard and samples. The developed TLC plates were visualised under UV light at 254nm, iodine vapor and ninhydrin spray reagent. The Rf values for phenylephrine hydrochloride were calculated across all the developed plates[30].

2.5 HPLC Conditions

The Shimadzu Prominence-I RP-HPLC system equipped with a C18 column (250mm × 4.6mm, 5µm), a column oven, a quaternary pump, a Photodiode Array Detector (PDA) detector, and Liquid Chromatography (LC) Solutions software for data acquisition was employed for the HPLC examination. The mobile phase consisted of buffer solution (water containing triethylamine, pH adjusted to 7.5 with phosphoric acid) and acetonitrile in a ratio of 63:37 (v/v). Before analysis, the mobile phase was processed via filtration by a 0.45µm filter and degassed via an ultrasonicator for 10 minutes to eliminate air bubbles and ensure baseline stability. The system was operated at isocratic mode with a flow rate of 1.0 mL/min throughout the analysis. The column temperature was held constant at 25°C. The injection volume for all standards and samples was 10µl. Detection was performed at 275nm, corresponding to the absorption maximum of phenylephrine hydrochloride. The solution was run

in triplicate to assess consistency of chromatographic observations under identical conditions[31].

3. Results and Discussion

The presumptive colour tests applied in this procedure were used to evaluate the general chromogenic properties of phenylephrine with widely used forensic chemical tests. These approaches were carefully selected for their utility in forensic laboratories for rapid, low-cost primary screening. The observed responses were interpreted as presumptive class -level reactions indicative of functional group reactivity associated with phenylethanolamines. These tests were utilised solely as an initial screening step before subsequent chromatographic analysis.

The colour development observed with reagents such as Marquis, Mandelin and Mecke is reported in forensic studies attributed to non-specific reactions occurring under acidic and oxidative conditions, which promote the formation of coloured compounds from aromatic and nitrogen-containing organic compounds. PHP, as a phenylethanolamine derivative, consists of an aromatic ring and has amine functionality. Hence, it depicted chromogenic responses under these conditions. Similar colour responses were observed for both the standard and the sample formulation, which indicated comparable class-level chemical behaviour rather than compound-specific identification. The Chen Kao test produced a blue violet coloured response for both the standard and sample. This response is consistent with the reported chromogenic behaviour of certain sympathomimetic and phenylethanolamine derivatives in the presence of copper ions under alkaline conditions. Simon's reagent test depicted variable dark colouration. This test is originally designed particularly for secondary amines, but here the observed colour can be interpreted as a chromogenic interaction that may arise from reagent chemistry or matrix effect[26-29] (Figure 1, Table 2).

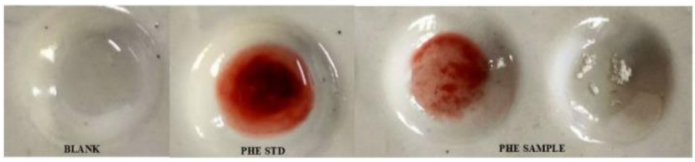
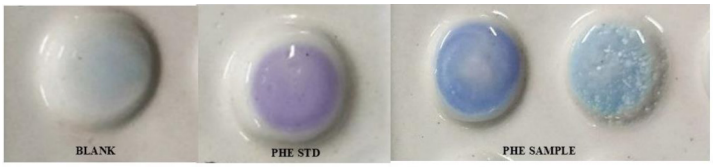
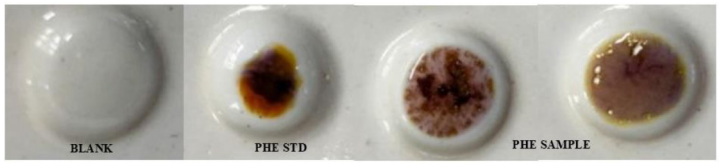
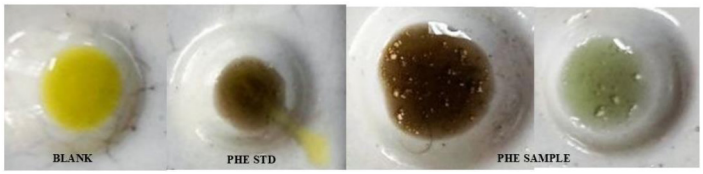
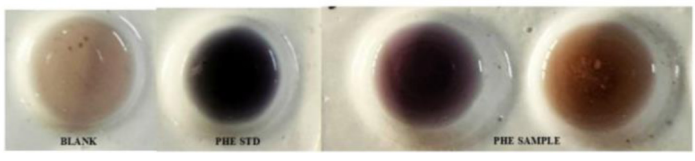
Test Performed	Results
Marquis Test	
Chen Kao Test	
Mecke's test	
Mandelin's Test	
Simon's Reagent Tests	

Figure 1. Visualised results of colourimetric tests.

Table 2. Colour test responses of phenylephrine hydrochloride

Reagent / Test	Observed Colour Response	Interpretation
Marquis	Red–brown	Acid-driven colour reaction related to aromatic amine–containing compounds
Mandelin	Dark green → green–brown	Oxidative colour reaction related to aromatic and phenolic structures
Mecke	Reddish-yellow → dark purple	oxidative response under highly acidic conditions
Chen–Kao	Blue–violet	Reaction pattern reported for phenylethanolamine and related sympathomimetic components
Simon's	Variable dark colouration	Inconsistent colour response reflecting limited applicability for this compound

The thin-layer chromatographic procedure was implemented as an initial technique to assess the migration characteristic of phenylephrine hydrochloride in reference standards in pharmaceutical formulations. The TLC provides a rapid qualitative tool for the evaluation of chromatographic consistency between standards and samples before instrumental analysis.

In thin layer chromatographic procedure, multiple solvent systems with varying polarity were evaluated to examine the migration characteristics of PHP in both standards and formulations. In several solvent systems, PHP demonstrated limited or no migration, and other components hindered the clear visualisation. Among the other mobile phase systems, ethyl acetate: Methanol (9:1, v/v) showed consistent migration and visualisation of phenylephrine by producing a distinct spot that can be compared between the standard and samples. Therefore, this solvent system was selected to facilitate the qualitative comparison of PHP migration. The PHP standard exhibited Rf values of 0.86 in iodine vapour and 0.88 under UV and ninhydrin spray. The sample extract demonstrated similar RF values of 0.87 in UV, 0.86 in iodine vapour, and ninhydrin spray. Visualisation under UV light 254nm, iodine fuming, and ninhydrin spray reagent showed consistent spot position and appearance between the standard and sample extract (Figure 2 and Table 3). These observations were interpreted as presumptive screening data and were used as support for HPLC analysis[30].

RP HPLC coupled with photodiode array detection was utilised as a qualitative chromatographic technique to assess the chromatographic and spectral behaviour of phenylephrine hydrochloride. The chromatographic conditions were adopted from the reported methodology and were utilised in the present work for qualitative comparison[31]. The reference standard was examined to

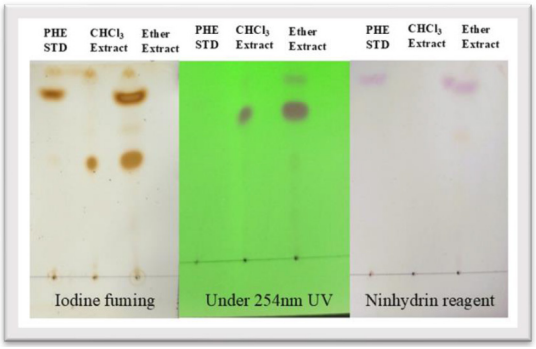


Figure 2. Visualised TLC plates.

Table 3. Calculated Rf values

Visualisation Method	Rf (Standard)	Rf (Sample)
Iodine vapour	0.86	0.86
Ninhydrin spray	0.88	0.86
UV (254nm)	0.88	0.87

establish the retention behaviour and PDA UV spectral characteristics under these selected chromatographic conditions. The reference standard produced a distinct chromatographic peak at a retention time of approximately 2.1 minutes and demonstrated a characteristic UV absorption maximum at 275nm. Furthermore, the sample extract was analysed under the same conditions, and the sample chromatogram showed a peak at the corresponding retention time, and the PDA UV spectrum of this peak displayed the absorption characteristic consistent with those observed with the reference standard. All injections were conducted in triplicate to ensure the consistency of observations under the identical experimental conditions (Figure 3). While the workflow demonstrates retention (Rt 2.1 min) and spectral (λ_max 275nm) consistency for phenylephrine, definitive identification of closely

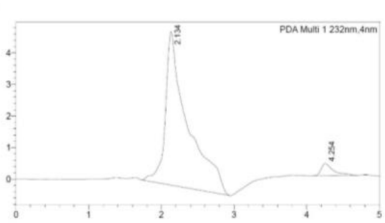
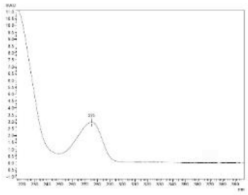
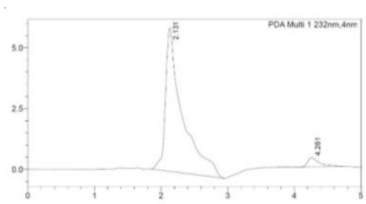
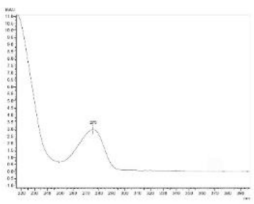
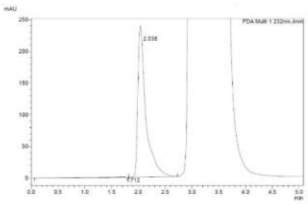
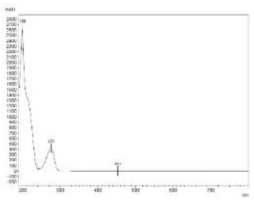
Sample	HPLC Chromatogram	PDA Spectra
PHE Standard 5ppm		
PHE Standard 10ppm		
PHE in sample		

Figure 3. HPLC chromatograms and PDA spectral data of the standard and the sample of Phenylephrine.

related phenylethanolamine analogues typically relies on confirmatory techniques such as mass spectrometry[32]. The findings of the HPLC PDA analysis were interpreted in combination with the outcomes obtained from the presumptive colour tests and TLC. The consistency in the observations across these techniques supports the qualitative detection of phenylephrine hydrochloride in a complex formulation. This integrated approach is intended for preliminary detection and screening purposes.

4. Conclusion

The routine forensic analysis involves preliminary detection of the target component through colour tests and TLC, followed by further examination through instrumental techniques. Hence, in such a context, qualitative identification constitutes a major part of the forensic laboratory workflow, especially during initial testing. The present work described a structured qualitative workflow for the detection of phenylephrine hydrochloride in complex formulations using an integration of classical forensic chemical tests, TLC and HPLC. The approach was formulated to assess the consistency in analytical characteristics across the multiple routinely available techniques. Presumptive forensic chemical tests were applied to evaluate the colour response activity of phenylephrine under routine conditions. TLC generated supportive qualitative evidence through a consistent pattern of migration and similar Rf values of reference standards and samples. RP-HPLC examination further corroborated qualitative detection through a comparable retention pattern and alignment of UV spectral characteristics between standard and sample. The findings are confined to qualitative screening and preliminary detection and demonstrate the combined application of independent instrumental methods. The integrated technical approach within the scope offers a practical workflow for qualitative testing in forensic laboratory analysis. The workflow presented here is intended for qualitative identification in multi-component preparations, with further specificity assessment and validation required for regulatory applications.

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Author Contributions

Conceptualisation, A.B. and R.A.; methodology, A.B. and R.A.; experiments, A.B. and R.A; writing—original draft preparation, A.B. and R.A.; writing—review and editing, A.B., P.B., and R.A.; supervision, R.A. All authors have read and agreed to the published version of the manuscript

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