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Qualitative Chemical Screening of Phenylephrine from Drug Combination: A Forensic Based Approach

Akanksha Behl[1], Rakhi Agarwal[1*]

[1] Research Scholar, School of Forensic Science, Forensic Chemistry and Toxicology Division, National Forensic Science University, Delhi Campus, New Delhi, India

*Corresponding author

akanksha.phdfs21@nfsu.ac.in

[1*] Associate Professor, School of Forensic Science, Forensic Chemistry and Toxicology Division, National Forensic Science University, Delhi Campus, New Delhi, India

rakhi.agarwal@nfsu.ac.in

Abstract

Phenylephrine Hydrochloride is a largely utilized active ingredient in commonly available over-the-counter medications for the treatment of diseases such as cold, sinus clog, and stuffy nose. It has also been classified as a stimulant substance by WADA's monitoring program, rendering it vulnerable to misuse. Pharmacologically, it can cause agitation, hypervigilance, and an increase in blood pressure. It can be found as an additive ingredient in illicit drug complexes. In forensic chemical analysis, established methods are required for the successful and efficient analysis of phenylephrine. The analytical procedure begins with the presumptive testing of the drug which was not reported specifically for phenylephrine. Further, different variants of the Liquid-Liquid Extraction technique were used for the efficient isolation of phenylephrine. Chromatographic techniques such as TLC, HPTLC, and LC-MS/MS were utilized to detect Phenylephrine in the extracted sample. Mass spectrometry analysis of the extracted drug revealed that it underwent degradation, which is indicated through the m/z peaks obtained and fragmentation pattern. This present work incorporates methods for separating and identifying the drug present in a minute quantity from the mixture of drugs, which remains a challenge. The study denotes the significance of identifying the component in different kinds of chemical exhibits. This study aimed to provide an upgraded chemical profile of the Phenylephrine drug. These findings require careful consideration for future studies on the analysis of such components in seized drugs.

Keywords: Phenylephrine, TLC, HPTLC, UV-Visible, Mass Spectrometry

1. Introduction

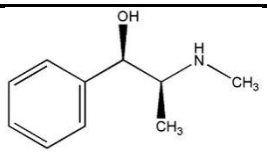
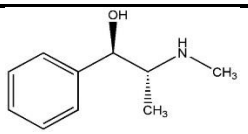
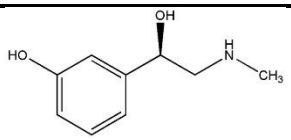
Phenylephrine hydrochloride, also known as [(R)-1-(3-hydroxyphenyl).2-(methylamino) ethanol hydrochloride], is a widely used sympathomimetic agent having psychostimulant properties [1]. It is a functioning component found commonly in over-the-counter (OTC) drugs, used for the treatment of common ailments such as fever, pain, cold, etc. It physically appears as a white crystalline powder and gets freely soluble in water [2,4]. It chemically belongs to the phenylethanolamine group and pharmacologically acts as an alpha-androgenic agonist. Hence, it affects the CNS of the body by exhibiting stimulant effects [3,5-6].

Phenylephrine is claimed to be a safe-to-use replacement for ephedrine/pseudoephedrine. As compared to ephedrine, primarily phenylephrine has an additional an - OH joined to it which leads to an increase in its polarity (Table 1). Ephedrine and Pseudoephedrine have been used as decongestants for a long time, but they have been misused to synthesize methamphetamine [4,7]. These drugs are utilized for the illegal manufacturing of psychotropic substances, hence known as precursor drugs. Ephedrine, pseudoephedrine, and phenylephrine, therefore, belong to the class of drugs known as Amphetamine-type stimulants (ATS) [5,6-7]. Amphetamine and related derivatives consisting of constituents such as ephedrine are consumed in enormous quantities today. They have multiple purposes including medical as well as recreational. ATS drugs are less noticeably used as decongestants [8].

According to World Anti-Doping Agency (WADA), Phenylephrine has been included in the list of monitored drugs in 2021 due to its misuse in sports. It is considered an ergogenic agent which enhances performance by causing an increase in muscle glycogen. It brings about an increment in blood pressure by causing vascular constriction [9, 28]. As per the report of the National Survey on Drug Use and Health 2020, cold medications containing Phenylephrine are being used on a large scale by young adults [10, 30]. It has also been reported to be abused as an active ingredient in cocktail drugs and has been found alongside drugs like methamphetamine, cocaine and xylazine [11, 2-3].

Typically, the commonly utilized pharmaceutical drug contains a combination of salt that has a high measure of acetaminophen with small amounts of phenylephrine hydrochloride and cetirizine hydrochloride [12,13]. The structures of constituents present in pharmaceutical preparation containing phenylephrine are shown in (Table 2). These precursor chemicals are largely important in the forensic context as they have been utilized for the synthesis of psychoactive drugs like amphetamines [14]. Therefore, phenylephrine becomes an essential constituent that has not been explored properly. It can be encountered as an impurity in illicit drug cocktails. Therefore, this work aimed to detect and separate phenylephrine as the active constituent of the drug mixture.

Table 1: Chemical Structure of Ephedrine, Pseudoephedrine, and Phenylephrine

DRUG NAME	STRUCTURE
Ephedrine	
Pseudoephedrine	
Phenylephrine	

2. Materials and Methods

2.1 Pharmaceutical formulation

Pharmaceutical formulation

Pharmaceutical formulations containing phenylephrine were obtained including cold and decongestant tablets. Cold tablet consisted of paracetamol (325mg), phenylephrine hydrochloride (10mg), and cetirizine dihydrochloride (5mg) (Fig 1) with the brand name: Cipla, Lot number: 99317810, Batch Number: GS11718. The decongestant tablet contained only Phenylephrine (10mg) (Fig 1) with the name: Solvin, Lot number: FIFHL3, Batch Number: IHF011004AS.

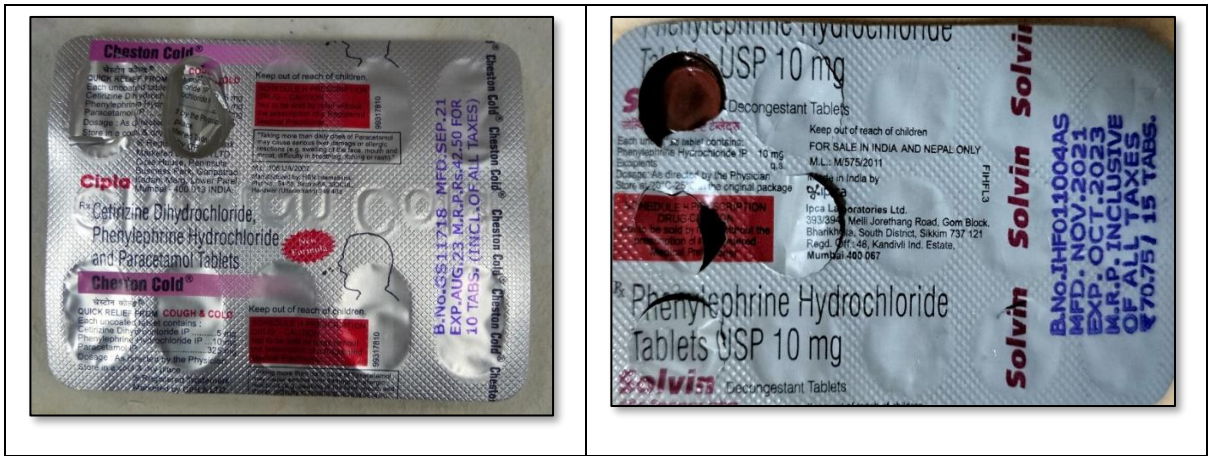
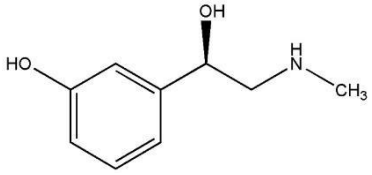
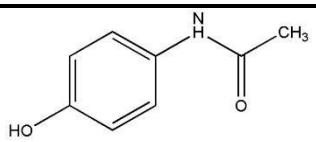
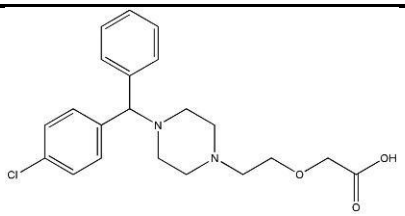


Figure 1: Tablet formulations of phenylephrine

Table 2 Structures of the chemical constituents present in the samples

Compound name	Structure
Phenylephrine. HCl	
Paracetamol	
Cetirizine. HCl	

2.2 Chemicals and reagents

Paracetamol, phenylephrine hydrochloride, ephedrine, and pseudoephedrine were procured from IPC, and all chemicals and solvents used were of analytical grades, such as Acetic acid ethyl ester (Finar), diethyl ether (Fisher Scientific), Chloroform and Acetonitrile (Spectrochem Pvt. Ltd., HPLC Grade), Methanol (Thermo Fisher Scientific Pvt. Ltd., HPLC grade), Sodium Hydroxide Pellets purified (Merck), Sodium Sulphate Anhydrous (Thermo Fisher Scientific Pvt. Ltd.), Resublimed iodine (s d fine-cHEM Limited), Ninhydrin (Merck), Selenious acid (CDH Laboratory reagents) Cupric Sulphate (BDH Chemicals), Sodium nitroprusside (CDH Laboratory reagents), Potassium Iodide (Thermo Fisher Scientific Pvt. Ltd), Acetaldehyde solution (s d fine-cHEM Limited), Ammonium vanadate (CDH Laboratory reagents), Sodium Molybdate (CDH Laboratory reagents), Formaldehyde, Sulfuric acid (Merck), Glacial Acetic Acid (Thermo Fisher Scientific Pvt. Ltd.), and Water purified on the Milli-Q system was used.

Sample preparation

Solutions of reference standard phenylephrine hydrochloride (100 µg/mL and 1000 µg/mL) were prepared for the analysis using HPLC-grade methanol.

Preliminary Test

To detect the presence of the target component, a variety of color tests were applied. The presumptive testing was conducted on the powdered solid samples to obtain clear results. A small amount of sample was taken on a spot plate and a few drops of reagent was added to it. The resultant color changes were carefully observed.

Chen-Kao Reagent Test

The reagent was comprised of 3 main solutions: A, B, and C. Solution A was prepared by dissolving 1mL of glacial acetic acid in 100 mL of distilled water. Solution B was prepared by dissolving 1g of copper (II) sulfate in 100 mL of distilled water. Solution C was prepared by dissolving 8g of sodium hydroxide in 50 mL of distilled water. To obtain the final reagent solution, all the solutions (A, B, and C) were mixed in a ratio of 1:1:1 (Nagy et al., 2005).

Marquis Test

The testing reagent was prepared by adding 10 drops of 40 % formaldehyde solution to 10 mL of concentrated sulfuric acid. (Nagy et al., 2005)

Mandelin's Test

The Mandelin reagent comprised 1g of ammonium vanadate (NH_4VO_3) added to 100mL concentrated sulphuric acid (Nagy et al., 2005)

Mecke's Reagent Test

The solution includes 0.25 grams of selenious acid dissolved in 25 mL of concentrated sulfuric acid(Nagy et al., 2005).

Frohde's Reagent Test

The reagent solution was made up of 50 milligrams of molybdic acid or sodium molybdate mixed with 25 mL of sulfuric acid (Nagy et al., 2005).

Simon's Test

The reagent consisted of 1 gram of sodium nitroprusside mixed in 100 mL of water. Then, 90 mL of the test solution was taken and 10 mL of acetaldehyde was added to it. (Nagy et al., 2005)

2.4 Extraction Procedure

For viable extraction of phenylephrine from the tablet samples, three methods of liquid extraction were optimized: Direct Organic Solvent Extraction with Methanol, One Step Lquid-Liquid Extraction, and Two Step Modified Lquid-Liquid Extraction. The above methods were used to extract phenylephrine from the drug combination (Figure 2).

In the direct solvent extraction with methanol, the powdered sample tablet was extracted with methanol by simply dissolving in it. This is the standard method used for the extraction of chemical components from different matrices. Only a single spot corresponding to paracetamol was obtained on TLC (Fig 1). This indicated that only paracetamol was extracted from methanol.

In single-step alkaline "Liquid-Liquid Extraction", the tablet sample was extracted with water-sodium hydroxide and chloroform. Phenylephrine being an acidic drug, was extracted with strong base. The sample was initially dissolved in water and then turned basic with the addition of 0.1 N NaOH. The resultant solution was extracted with chloroform. The Cholorom layer was collected and used for analysis. Only a single spot corresponding to paracetamol was obtained on TLC (Fig 1). This indicated that only paracetamol was extracted in the chloroform and phenylephrine did not get extracted.

Whereas in two-step alkaline "Liquid-Liquid Extraction", the sample tablet was first dissolved in chloroform and filtered. Then the residue powder was dissolved in water and it was turned basic with the addition of 0.1 N NaOH. The solution was then extracted with chloroform. The aqueous layer that was left was further extracted with ether. On TLC, Spots of both phenylephrine and paracetamol were obtained (Fig 2), indicating the successful extraction of phenylephrine in the ether layer.

2.5 Chromatographic Procedure

Thin Layer Chromatography was performed for the detection and separation of phenylephrine present in the pharmaceutical preparation. TLC plates having aluminum backing, coated with a layer of silica gel 60 F254, 20 × 10 cm (E. Merck) were used as stationary phase. The TLC plates were prewashed with methanol and activated by placing them in the oven at 110 °C for 15 min. Methanol extract, chloroform extract, and ether layer of the extract were used to perform TLC. Spotting of these extracts was done with standard paracetamol and standard phenylephrine on different TLC plates.

2.5.1 Selection of Mobile Phase Composition

Mobile phases having variable ratios of organic solvents were utilized to obtain optimum results. To achieve the best TLC separation, 10 various solvent systems were utilized. The tank was saturated for 15 minutes with each solvent system for 15 min before the TLC plates were run. After using a variety of combinations of solvents, the solvent system consisting of ethyl acetate: methanol (9:1, v/v) was selected. It was found to produce the best result as it generated better separation of the components. However, the desired separation was only achieved in the ether extract. The methanol extract (Table-4) and water-chloroform extract (Table-4) only produced a single spot of paracetamol, which was visible under UV.

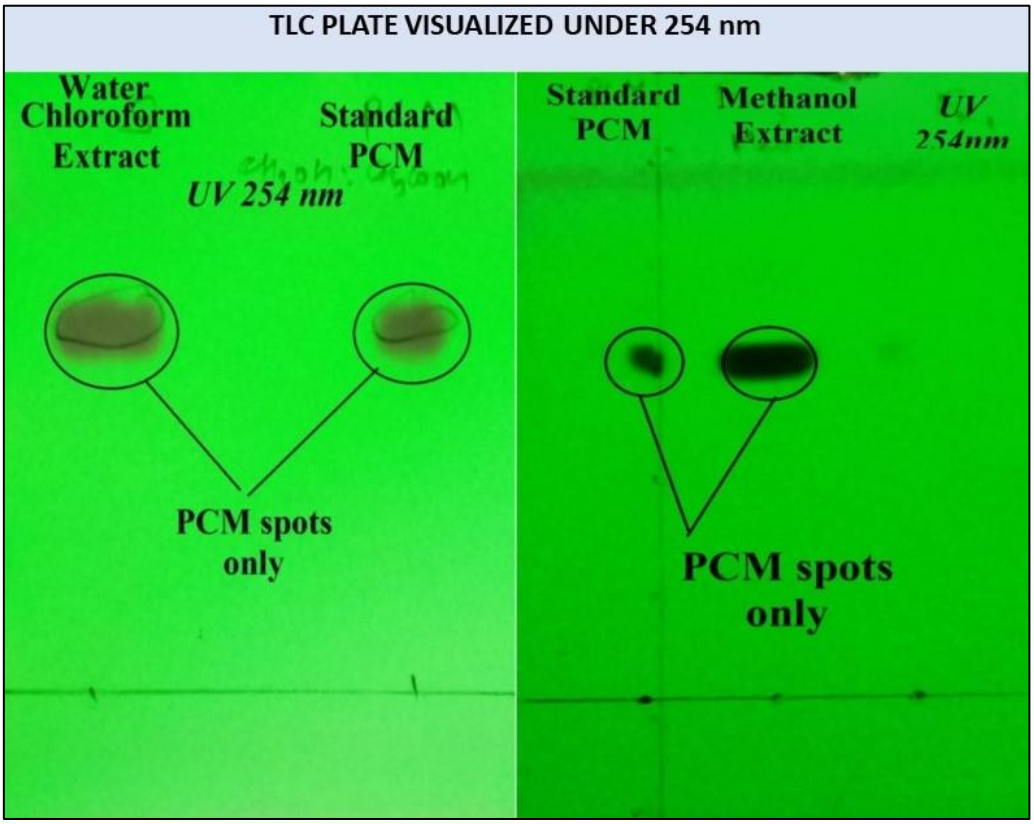
Table: 3 Total solvent systems utilized for the chromatographic separation

S. No.	**Solvent System**	**Ratio**	**Rf for Phenylephrine**			**Observations**
			Methanol Extract	***Water-Chloroform Extract***	***Ether Extract***	
1.	Chloroform: Ethanol	9:1	Not Detected	Not Detected	Not Detected	No spot of Phenylephrine PCM spots only
2.	Chloroform: Methanol	9:1	Not Detected	Not Detected	Not Detected	No spot of Phenylephrine PCM spots only
3.	Chloroform: Methanol	8:2	Not Detected	Not Detected	Not Detected	No spot of Phenylephrine PCM spots only
5.	Chloroform:Methanol:Ammonia	7.5:2:0.5	Not Detected	Not Detected	Not Detected	No spot of Phenylephrine PCM spots only
6.	Methanol:Ammonia	8.5:1.5	Not Detected	Not Detected	Not Detected	No spot of Phenylephrine PCM spots only
7.	Methanol:Acetic acid	9:1	Not Detected	Not Detected	Not Detected	No spot of Phenylephrine PCM spots only
8.	Toluene:Acetone:IPA	7:2:1	Not Detected	Not Detected	Not Detected	No spot of Phenylephrine PCM spots only
9.	Ethyl Acetate: Methanol	1:9	Not Detected	Not Detected	No separation	Two spots without clear separation.
10.	Ethyl Acetate: Methanol	9:1	Not Detected	Not Detected	0.73	Phenylephrine and PCM spots with clear separation.

2.5.2 Visualization of TLC Plates

The developed TLC plate was primarily visualized under UV (254nm short wavelength). Chemically, different spraying reagents were used to detect the presence of phenylephrine. The ones that generated the most favorable results were Ninhydrin and Iodine fuming.

Table 4: TLC Chromatograms of (i) methanol extract and (ii) water-chloroform extract of the sample visualized by UV



2.5.3 HPTLC conditions

Analysis was performed on DESAGA High-Performance Thin layer-Chromatography Systems (HPTLC) DESAGA ProQuant® Windows (32-bit) Software operating under Windows 2000/ XP DESAGA HPTLC DENSITOMETER / SCANNER – Model CD 60. All chemicals were of analytical grade. The experiment was performed on silica gel 60F254 aluminum sheets (20x20 cm) as the stationary phase, using a mixture of ethyl acetate: and methanol (95: 5, v/v) as the mobile phase. The developed and dried TLC plate was scanned between 200 to 300 nm. PDA was used as a detector and the detection wavelength was selected at 290 nm due to the good absorbance of phenylephrine. The slit dimension of 3.00 mm x 0.40 mm (height x width), with evaluation interval set from 5mm to 50mm, the resolution was set at 0.1 mm, step width 10 nm, and the threshold for peak detection 1mm was set with DESAGA ProQuant software.

2.6 Instrumentation

2.6.1 UV-VIS Spectrophotometer

UV Spectroscopic examination was performed on Motras Scientific UV Plus Double beam UV-VIS Spectrophotometer. The background collection and UV spectrum were acquired by Scanalyse Software. Analytical grade methanol was used as a solvent for the experimental procedure. The scanning range was set between 275-315nm.

2.6.2 Mass spectrometry

The preparative TLC procedure was used to separate phenylephrine and paracetamol. The phenylephrine layer on the TLC plate was collected by scraping it out. The scrap was then dissolved in chloroform, filtered, and dried. The extract was then dissolved in methanol and filtered using the 0.45μm Millipore. The direct methanol extract was also used for the mass spectrometric analysis. Mass spectrometric detection was performed using an M/S Thermoscientific ACCELLA TSQ QUANT ACCESS MAX MS/MS operated in Q1MS positive electrospray ionization (ESI) mode. The selected scan range was 30 (first mass) – to 400 (last mass). The collision energy for the experiment was 10eV. The experiment was

performed in full scan–scan type mode. The sample flow rate was selected at 500 μL/min. Data acquisitions of MS and MS/MS were collected by TSQ Tune software.

Table 4: Shows the Mass Spectrometry source parameters and conditions of LC

SOURCE PARAMETERS	CONDITIONS OF LC
MS Run Time (min)	5.00
Segment	1
Mobile Phase	Methanol: Water (70:30)
Ion Source Type	HESI
Polarity	Positive
Spray Voltage	4500
Vaporizer Temperature	300
Sheath Gas Pressure	40
Ion Sweep Gas Pressure	0.0
Aux Gas Pressure	10
Capillary Temperature	270

3. Results

3.1 Analysis by Preliminary Colour Tests

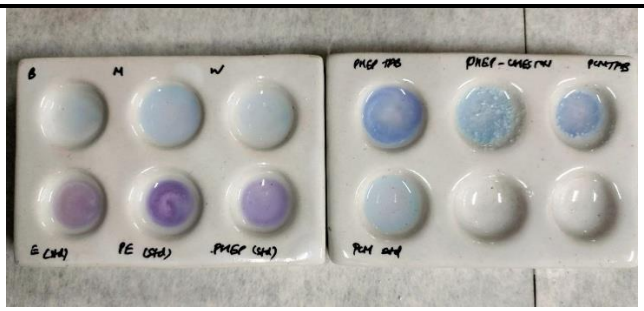
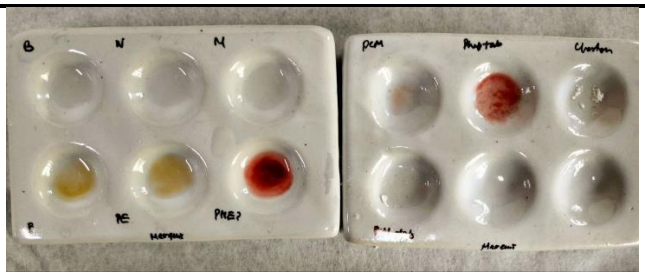
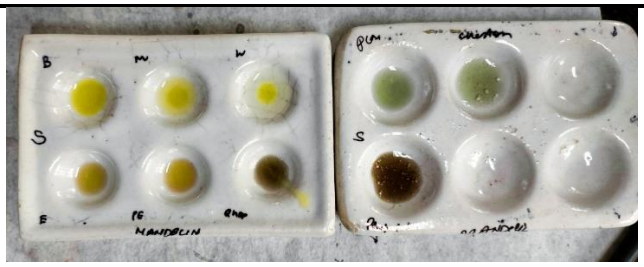
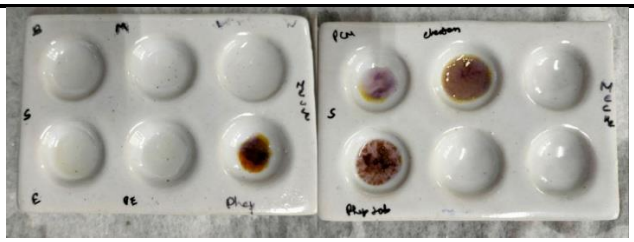
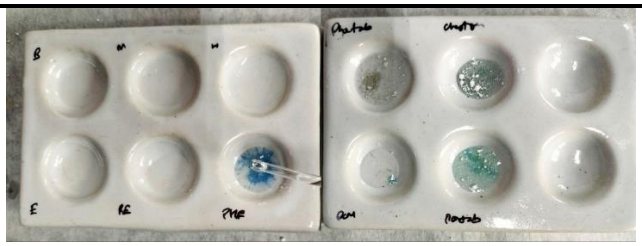
Preliminary color testing is one of the most basic analytical steps in forensic chemistry and toxicological analysis. No such specific tests were reported for the analysis of phenylephrine. Hence, six major tests that are being used for the detection of ATS drugs were employed for the experiment. The Chen Kao test which is specific for the detection of ephedrine and pseudoephedrine generated positive results for the phenylephrine drug. The test works by the development of a chelate complex, which is violet in color. This complex is formed with $CuSo_4$ within a basic medium, making the test exclusive for phenylalkylamines. Marquis test which is utilized for the examination of amphetamines also presented distinguished results for phenylephrine. However, the presumptive tests were not able to show any significant observation regarding phenylephrine present in the drug combination. This is contributed to the fact that a small amount of phenylephrine is present with a significantly larger amount of paracetamol in the pharmaceutical preparation.

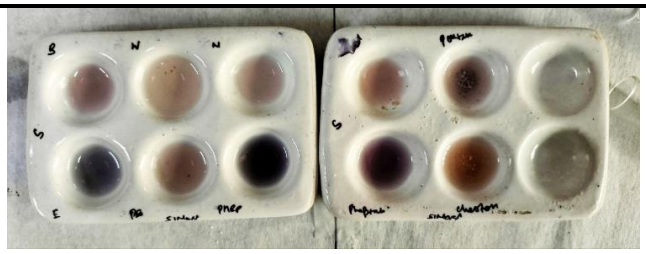
Table 5: A summary of Presumptive tests utilized for the analysis and their observations

Presumptive color tests	**Observations**					
	Ephedrine	Pseudoephedrine	Phenylephrine	Paracetamol	Phenylephrine sample (single component pharmaceutical drug)	Phenylephrine sample (multi-component pharmaceutical drug)
Chen Kao Test	Violet color	Violet color	Dark blue-violet color	Blue color	Dark blue-purple color	Blue color
Marquis Test	Mustard Yellow color	Mustard Yellow color	Red Brown color	No color change	Red Brown color	No color change
Mandelin Reagent	Instant Red color, changed to reddish tint with yellow color	Instant Red color, changed to reddish tint with yellow color	Instant Dark green color, changed to green-brown shade.	Hazy green	Instant Dark green color, changed to green-brown shade.	Hazy green
Mecke's Reagent	No color change	No color change	Instant reddish yellow, changed to dark purple	Purple color developed after a few	Instant reddish yellow, changed to dark purple	Purple color developed after a few minutes

			color	minutes	color	
Frohde's Reagent	No color change	No color change	Green-blue color	No color change	Green-blue color	Dark green-black color
Simon's Reagent	Bluish black	Mauve shade	Bluish black	Light brown shade	Violet black	Brown

Table 6: Observations of performed colour tests

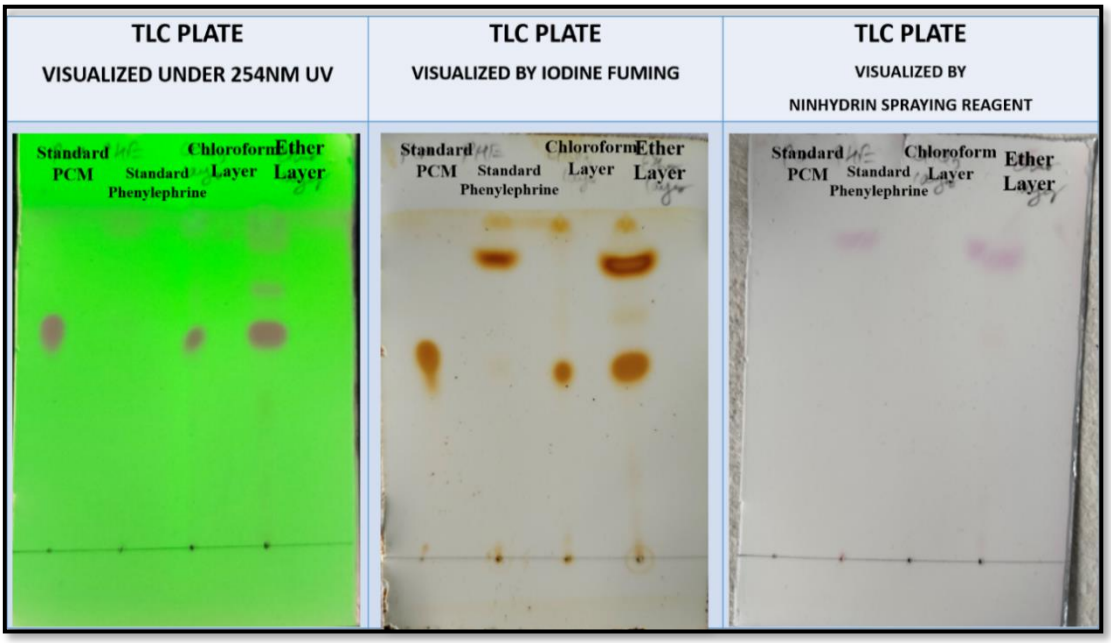
Test Performed	**Observations**
Chen Kao Test	
Marquis Test	
Mandelin's Test	
Mecke's test	
Froehde's Test	

Simon's Reagent Test	

3.2 Analysis by Thin Layer Chromatography

The TLC technique is beneficial for routine analysis because of its attributes such as being a specific, precise, and selective technique. This method has presented a simple approach to separate and quantify phenylephrine directly on TLC plates. The most suitable mobile phase was found to be Ethyl acetate: methanol (9:1). This technique is utilized as often as possible for the determination of the active components present in the samples. The visualizing agents used for the TLC plate were – ninhydrin and iodine fuming. The spraying of Ninhydrin produced a purple-colored spot which led to the conclusion that a secondary amine is present. Whereas, brown-colored spots are produced by iodine fuming, which depicted the separation of phenylephrine from paracetamol as shown in table 3.

TLC Chromatograms of ether extract of the sample visualized by UV, Iodine fuming, and Ninhydrin reagent



3.3 Analysis by HPTLC

HPTLC analysis was conducted for the precise determination of phenylephrine from the pharmaceutical dosage form. With this mobile phase of ethyl acetate: methanol (9:1), the resolution of the peaks with a clear baseline was obtained at 240 and 290 nm. The densitometric determination of phenylephrine was carried out at 290 nm. The phenylephrine was resolved with an Rf value of 0.73.

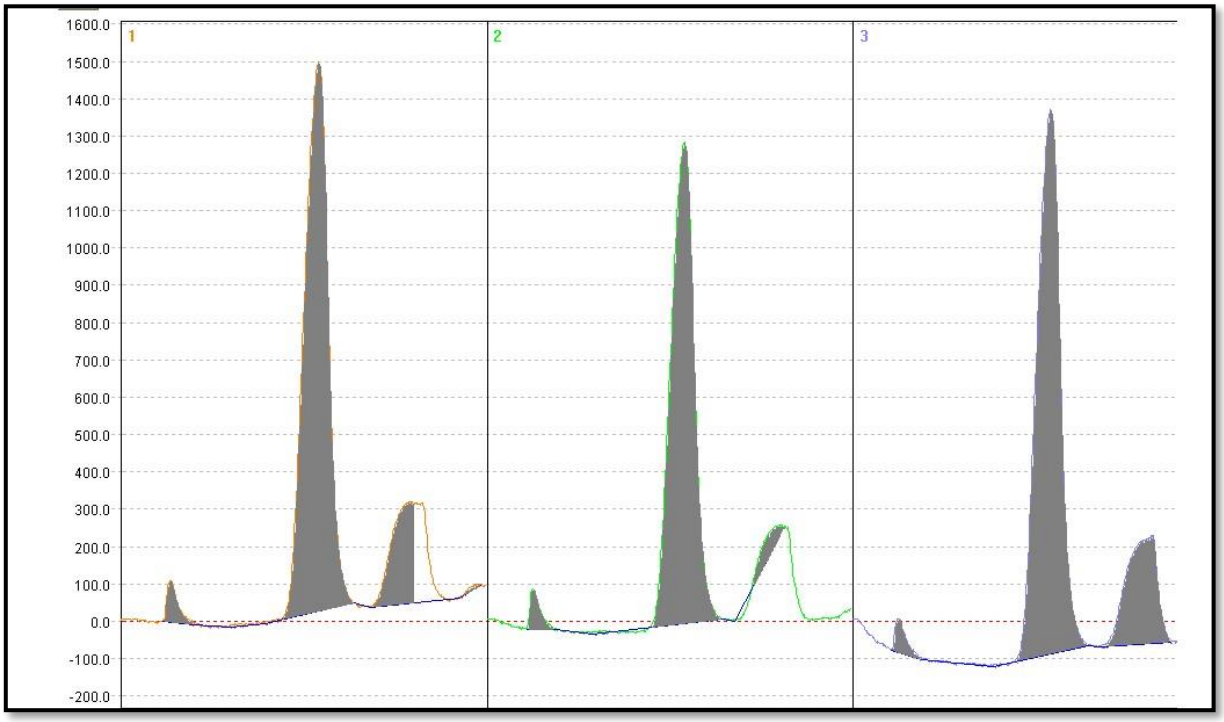


Figure 2: Peaks observed at 240nm wavelength corresponding to PCM

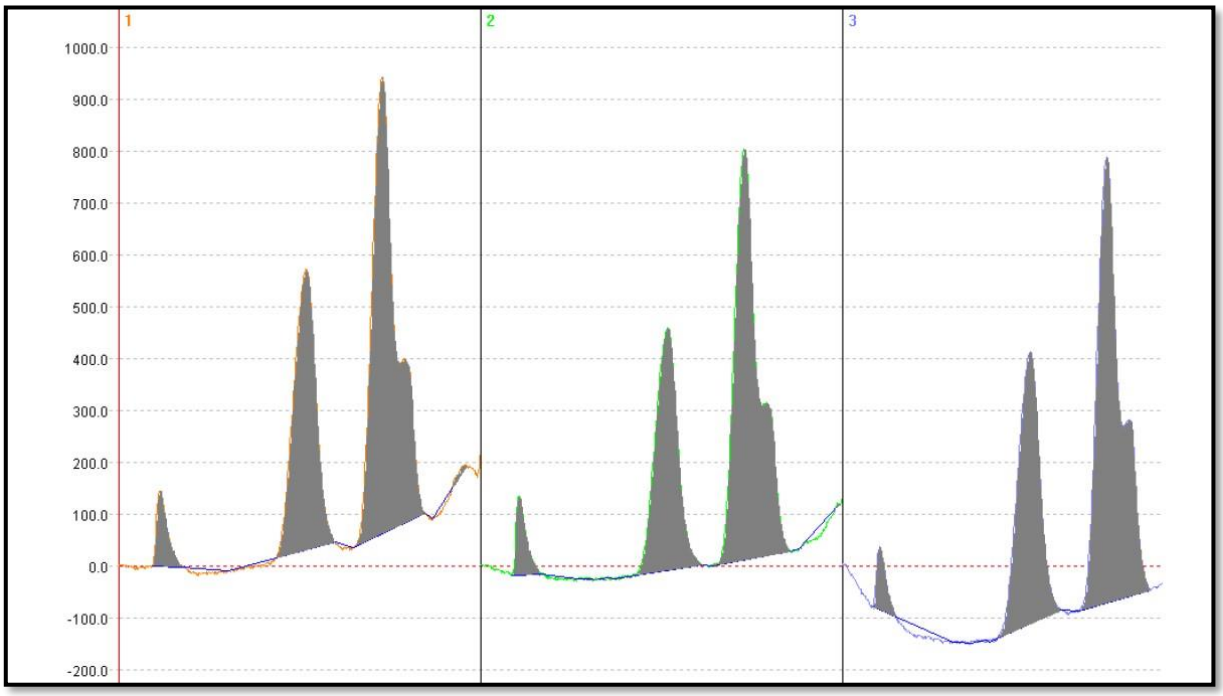


Figure 3: Peaks observed at 290 nm wavelength corresponding to Phenylephrine

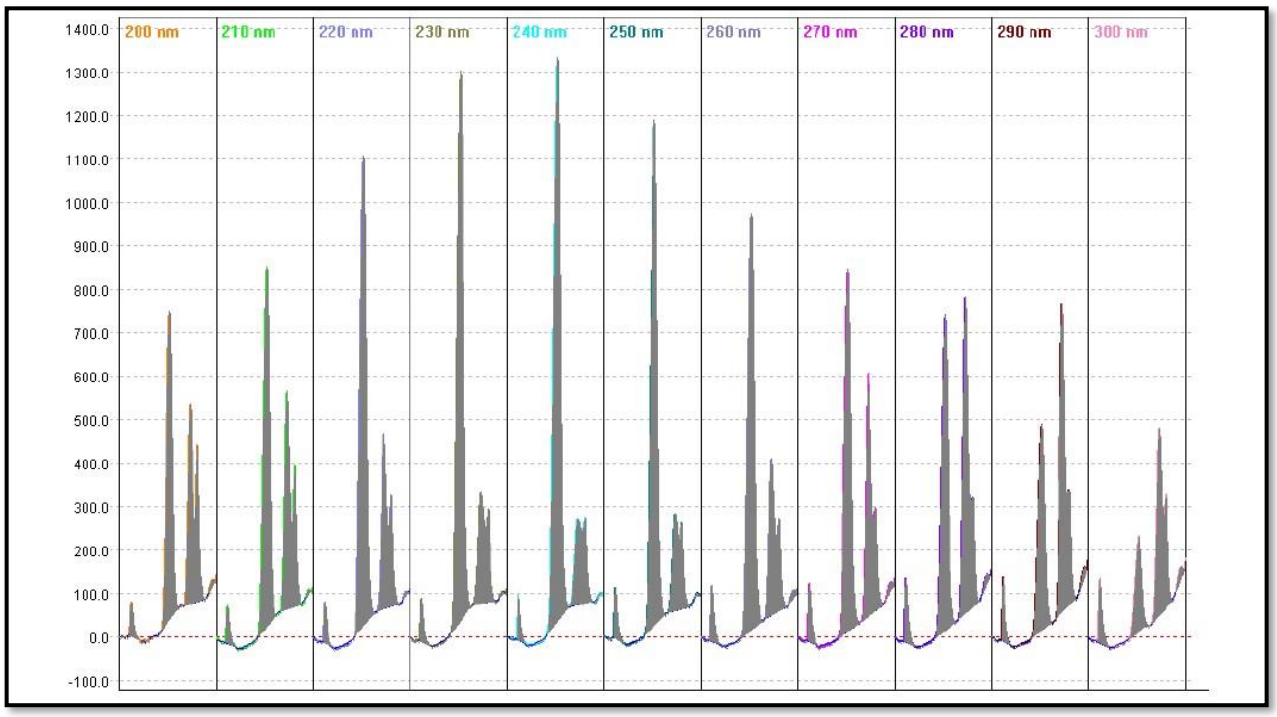


Figure 4: HPTLC Multiwavelength scan from 200-300nm

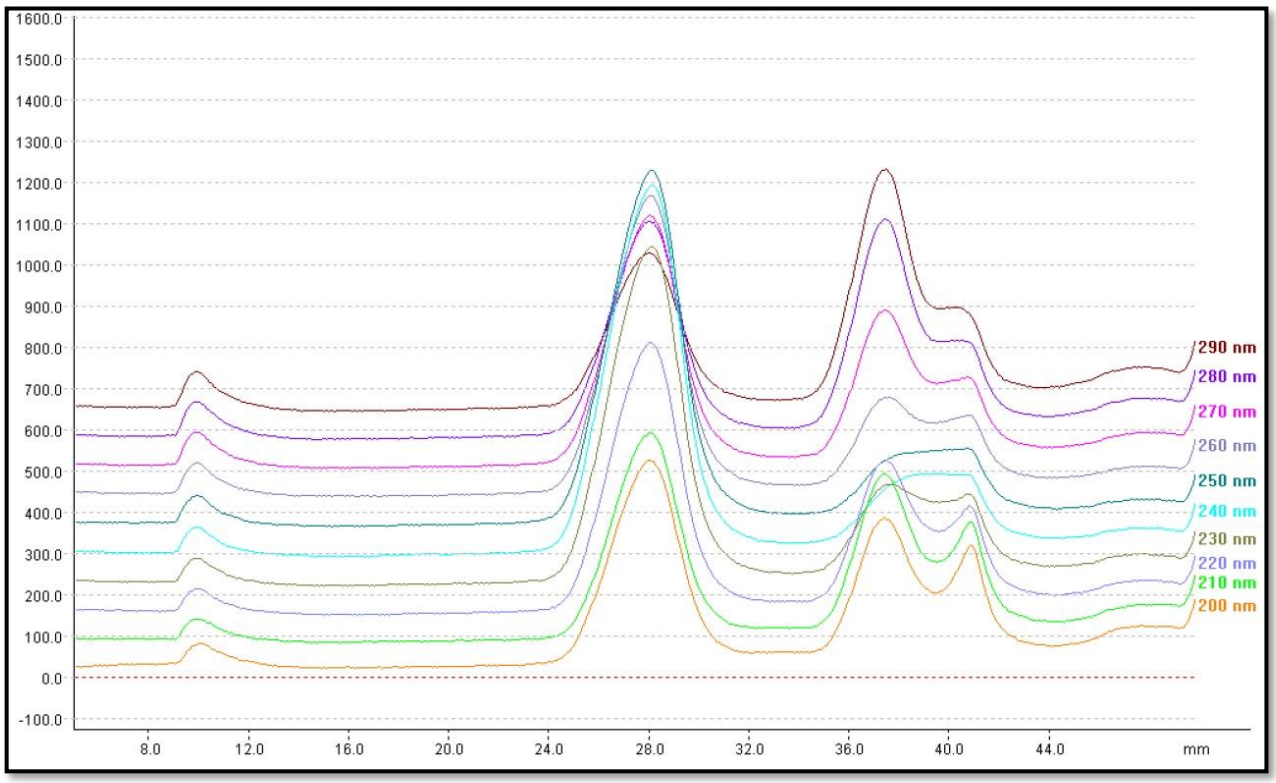


Figure 5: 3D Representation of the HPTLC multiwavelength scan from 200-300nm

3.4 Analysis by UV-Visible Spectrophotometer

To confirm the presence of the desired organic component, the spectrophotometric examination is utilized to determine the number of molecules absorbing the radiation. The maximum UV absorption was found at 292.5nm. This peak indicated the presence of phenylephrine as paracetamol gives maximum UV absorption at 243 nm. The maximum UV absorption value of 0.5901 was obtained at a wavelength of 292nm. The results of both UV and HPTLC examinations confirmed the presence of phenylephrine at 292.5 and 290nm respectively.

3.5 Analysis by LC-MS/MS

The sample and the certified reference standard of phenylephrine were analyzed using the LC-MS/MS and conditions were as mentioned in table no. 5. The mass spectrum of phenylephrine hydrochloride in positive ion mode. At m/z = 168, the protonated molecule ion [M+H]+ of the phenylephrine base was identified. Surprisingly, another ion signal at m/z = 150 was found in full scan mode without the presence of fragmentation energy, which was employed in MS/MS investigations. In addition to the drug molecule's innate instability, ESI in-source fragmentation must be taken into consideration to account for this finding. To make this procedure easier, no extra source of fragmentation collision energy has been applied.

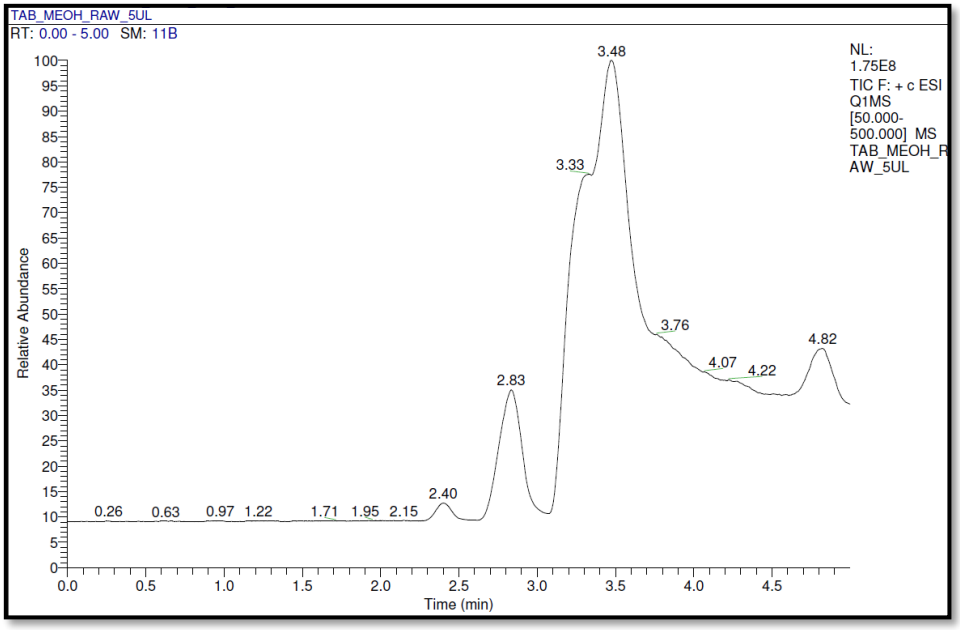


Figure 10: Shows the presence of three components eluted at 2.40, 2.83, and 3.48.

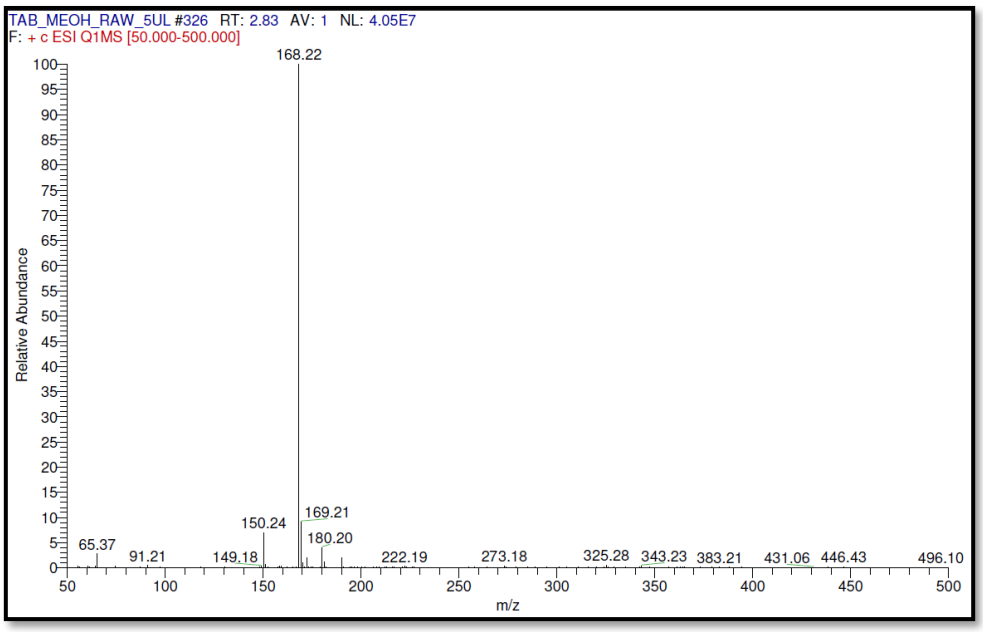


Figure 11: Shows at 2.83 the m/z was 168.22 which is for phenylephrine.

For further analysis and confirmation, MS/MS was performed at the same peak which was eluted at 2.83. The m/z = 168.2 tandem mass spectrum. In an MS2 experiment, further fragmentation of the m/z = 168 ion produced peaks at m/z = 150.10, m/z = 135.09, m/z = 119.13, m/z = 109.14, and m/z=91.15.

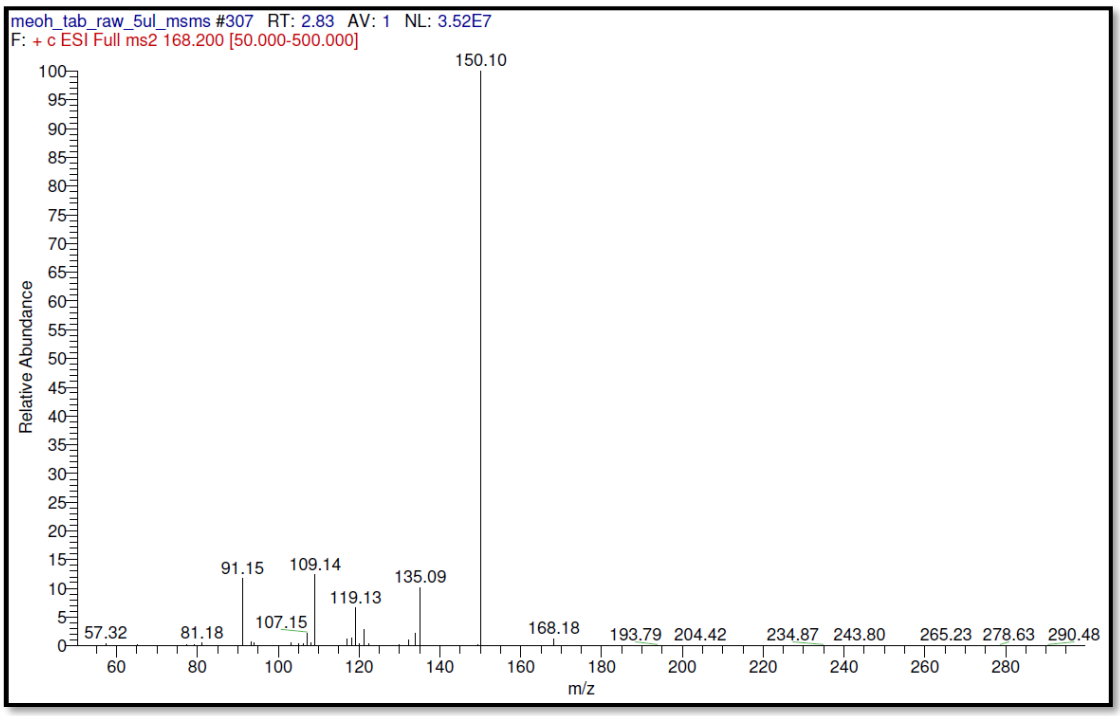


Figure 12: Shows the MSMS fragmentation of phenylephrine at 150.10, 135.09, 109.14, 119.13, and 91.15

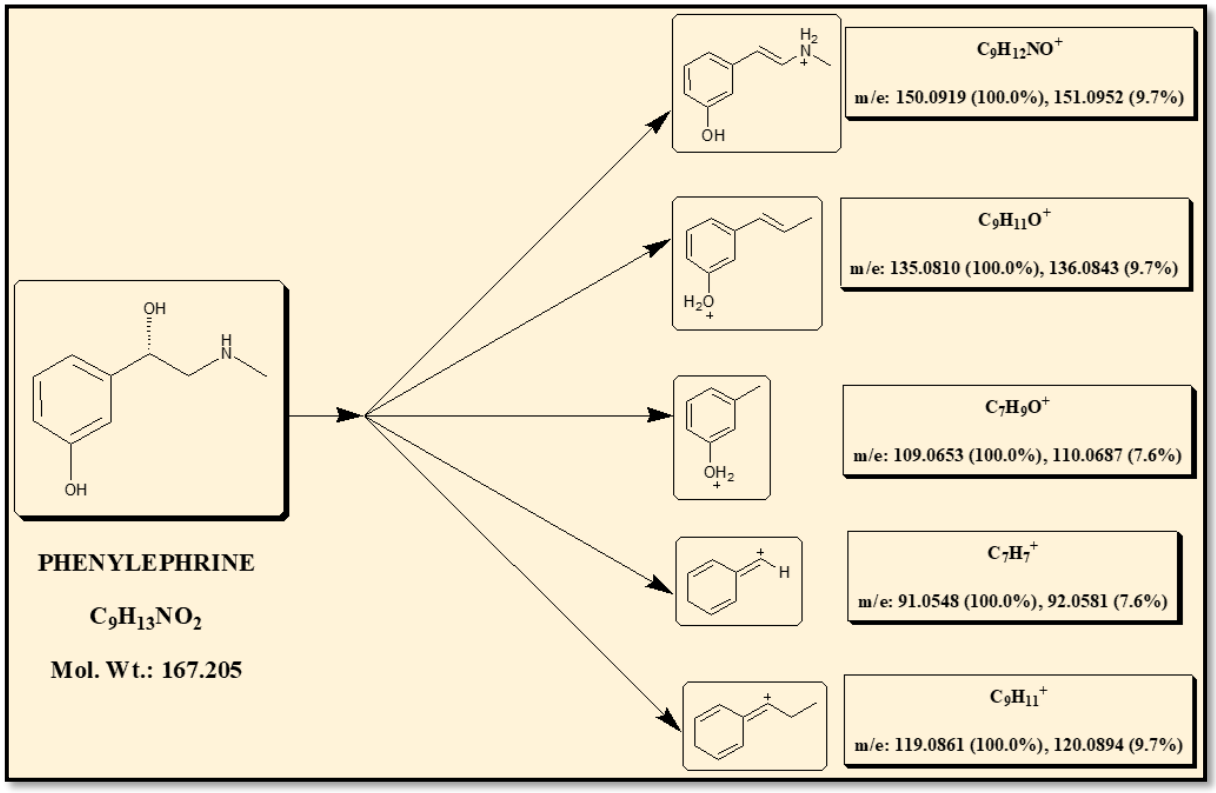


Figure 13: Shows fragmentation pattern of Phenylephrine

4. Discussion

Sympathomimetics are commonly included in over-the-counter (OTC) cold and flu medications as a systemic nasal decongestant. Because of its widespread availability and potential for misuse, phenylephrine was examined using a variety of analytical instruments in this work. Most studies are based on validation for the determination of phenylephrine with little focus on the analytical properties of the drug. For phenylephrine solely, detailed data regarding

the preliminary color test is not available so far. In this study, six reagents were used to detect and analyze phenylephrine as a single drug and as a combination.

The extraction and separation of phenylephrine having low concentrations along with other components create an issue. For this reason, three different extraction procedures were used for the isolation of phenylephrine from the drug combination. Phenylephrine was best extracted with ether by "two-step alkaline extraction" due to its higher solubility in ether. "One-step alkaline extraction" did not yield good results as the solubility of phenylephrine was found to be poor in chloroform. The primitive technique used for the examination is TLC, which is majorly used in forensic chemistry and toxicological analysis. It produced the separation of the drug from the complex matrix with immense clarity. The procedure didn't require any derivatization technique. Ninhydrin and Iodine fuming turned out to be the most appropriate visualizing agents. Ninhydrin gave a purple spot which indicated the presence of a compound containing secondary amine. Iodine fuming produced dark brown spots indicative of the presence of organic compounds.

HPTLC method provided a fast, simple, precise, and rapid way to determine phenylephrine from pharmaceutical preparations. The created chromatograms can be authenticated, maximum absorbance can be obtained and UV spectra can be acquired. The spectrophotometric procedure is a basic, speedy, and efficient technique and is appropriate for the analysis of small amounts of components. The procedure was applied to detect phenylephrine from different tablet formulations. Both UV and HPTLC techniques were successfully employed for the determination of phenylephrine at the wavelength of 292.5 and 290nm. Finally, the results of the previous examinations were confirmed by LC-MS/MS. It successfully identified Phenylephrine at m/z 168 from the tablet combination. The resultant peaks for Phenylephrine were properly defined with good resolution, and free from tailing.

In this study, we report basic preliminary tests, spectroscopic, and chromatographic analysis for the precise identification of phenylephrine from different drug combinations. Phenylephrine is still an underrated component in terms of forensic analytical chemistry even after being a largely consumed drug having multiple effects. It can be encountered as cutting agent in illicit drugs. The complex arrangement of OTC drugs makes it harder to detect and separate the low-concentration components. Every single technique that is used for the analysis in the present work demonstrates its exclusive advantages. Firstly, TLC has been an effective method to understand the structural behaviour of the compound and help develop the separation. In forensic chemistry analysis, TLC is the primary technique used for the examination of chemical evidence. Secondly, the HPTLC technique is preferred as it is a cost-effective technique with minimal run time. According to the results obtained, it provided well-resolved peaks for phenylephrine hydrochloride. Moreover, the results of HPTLC were verified with that of the UV spectroscopic examination. Lastly, the LC-MS/MS was able to confirm the structure and provide the conclusive fragmentation pattern and analysis of the pharmaceutical formulation. This study provides a concise summary of analytical data (TLC, HPTLC-UV, LC-MS/MS) on the psychostimulant sympathomimetic drug Phenylephrine, which is abundantly available in many medicines in combined dosage formulations.

List of Abbreviations

LC-MS/MS Liquid Chromatography Tandem Mass Spectrometry, TLC Thin Layer Chromatography, UV Ultraviolet, HPTLC High Performance Thin Layer Chromatography

Compliance with ethical standards

Not Applicable

Conflict of interest

Not Applicable

Ethical statement

Not Applicable

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