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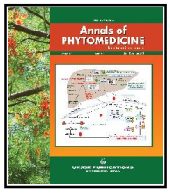

Review Article : Open Access

A comprehensive review of the analytical method for diphenhydramine hydrochloride by chromatographic technique

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Abstract

The diphenhydramine hydrochloride spectrophotometer technique was developed as an environmentally benign, time and money saving alternative to the HPLC method. Due to the high cost of the HPLC-grade solvent required for sample preparation and the extended HPLC run time, the identical procedure performed on an HPLC is quite costly. The technique of diphenhydramine HCl transfer from HPLC to spectrophotometer that saves money and time is the combined acetonitrile and buffer method. A HPLC method approach to the amount of diphenhydramine HCl (DPH) in medications to be created is presented in this publication. To ascertain the form of diphenhydramine, the reversed-phase HPLC (RP-HPLC) method should be employed to record the outcomes. In contrast, the HPLC method's chromatographic analysis was carried out with more accuracy.

1. Introduction

One analytical technique for validating pharmaceuticals and chemicals is high-performance liquid chromatography (HPLC). Diphenhydramine hydrochloride is an antihistaminic medication that is frequently used to treat skin allergies, allergic rhinitis, and the common cold. H_1 receptor antagonist DPH is a white, crystalline powder with a melting point of 168-172°C. Lipophilic in nature, Less than 1 KDa, or 291.82 g/mol, is its molecular weight (Javed *et al*., 2020). A first-generation H_1 antihistamine, diphenhydramine, was initially available in 1946. It has been used to treat a number of ailments, including parkinsonism, allergies, sleeplessness, and motion sickness. Systemic diphenhydramine is being utilized as an adjuvant medication in various therapies to prevent or treat hypersensitivity responses brought on by a variety of substances (Akella Anuradha *et al*., 2023). Specifically, a number of published studies have employed diphenhydramine, either orally or intravenously (IV), as a premedication sequence prior to the administration of immunotherapy or chemotherapy therapies (Dhritimoni Devi and Sumithra, 2023). Adults' diphenhydramine pharmacokinetics for diphenhydramine hydrochloride in single dosages of 25 and 50 mg have been documented. It has some sedative and antimuscarinic properties that are not desirable. [2-(diphenylmethoxy) ethyl] dimethylamine is the chemical name for diphenhydramine. When it comes to binding at HA-receptor sites, diphenhydramine and free histamine compete. As a result, the detrimental consequences of histamine-HA receptor binding are lessened. This counteracts the effects of histamine on HA-receptors (Subhamalar *et al*., 2023). Acetonitrile is a great solvent for the reversed-phase HPLC mobile phase. It is, however, a quite costly solvent that can occasionally be hard to get. As a result, when creating a different HPLC procedure with a different solvent, as the phase of mobility is required, the best substitute for an acetonitrile solvent is methanol. That solvent reduces the danger of solid buffer precipitation since it is more polar than acetonitrile, more affordable, and simpler to obtain. Methanol; however, has a larger back pressure and reduced elution strength. The current study's goal was to create and evaluate a novel reversed-phase HPLC technique (Hayun *et al*., 2017; Amisha Sharma *et al.,* 2021).

1.1 History and discovery

An ethanolamine-based antihistamine is diphenhydramine (DPH), also known as 2-diphenylmethoxy-N, N-dimethylethanamine. It was one of the first antihistaminic drugs to be identified in 1946 and is frequently used to treat the symptoms of common allergies. By inhibiting the effects of histamine at the H_1 receptor sites, DPH works as a central nervous system (CNS) depressant and CYP2D6 inhibitor. It has antiemetic, antitussive, and sedative properties in addition to being an antihistamine (Warren Rodrigues *et al.,* 2012; Sevgi Gezici *et al.,* 2020).

2. Drug profile

Table 1: Drug profile of diphenhydramine hydrochloride

Generic name	Diphenhydramine Hydrochloride
Brand name	Benadryl
Type	Small molecule
Group	Approved, investigational
Chemical formula	$C_{17}H_{21}NO$
Weight	Average: 255.3547
IUPAC name	2-(diphenylmethoxy)-N,N-dimethylethanamine

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2.1 Physicochemical properties

Table 2: Physicochemical properties of diphenhydramine hydrochloride

Description	It is a white, crystalline powder in appearance. It tastes numbingly harsh and has no smell.
Solubility	It dissolves in water and forms acidic aqueous solutions.
Melting and boiling points	166-169°C and 150-165°C at 2E0 mmHg. At 37°C, diphenhydramine dissolves in water at a rate of 3060 mg/l.
Density	There is 1.013-1.012 density.
Log p	3.27
pKa	8.98
Log S	3.5
Polarizability	29
Refractivity	79.93

2.2 Structure of diphenhydramine hydrochloride

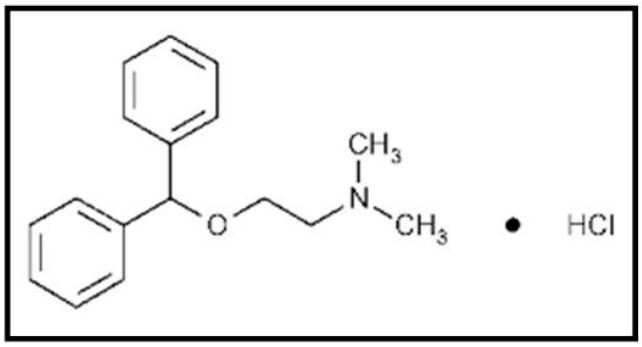

Figure 1: Structure of diphenhydramine hydrochloride.

2.3 Pharmacokinetic properties

2.3.1 Absorption

Following oral treatment, diphenhydramine is rapidly absorbed, reaching peak action in about 60 min. Diphenhydramine oral bioavailability is reported to be between 40 and 60, and 180 min following ingestion is when its peak plasma concentration occurs.

2.3.2 The amount of distribution

It is present in many parts of the body, including the central nervous system. The volume of distribution after a diphenhydramine 50 mg oral dose varies from 3.3 to 6.8 l/kg.

2.3.3 Binding of proteins

Diphenhydramine's protein binding has been reported in some prescribing material to be about 78%, whereas in other reports, it has been estimated that the drug is between 80 and 85% bound to proteins in the plasma (Raghavi *et al.,* 2023).

2.3.4 The metabolism

The first-pass metabolism of diphenhydramine is quick and widespread. Specifically, there are two consecutive N-demethylations that take place. The metabolite N-desmethyldiphenhydramine is created when diphenhydramine is demethylated. It is the demethylated form of N,N-didemethyldiphendramine, which is a N,N-didemethyl metabolite. The amino moiety of an N-didemethyl metabolite 8, then produces an acetyl metabolite such as N-actyl-N-desmethyldiphenhydramine. Furthermore, oxidation of an N-didesmethyl metabolite results in the diphenylmethoxyacetic acid metabolite. Diphenhydramine, taken in part, is excreted in an unaltered proportion in urine, which is the result of the metabolites' further conjugation with glutamine and glycine.

2.3.5 Route of elimination

Glycine and glutamine are combined to form diphenhydramine metabolites, which are then eliminated in urine. Urine contains just around 1% of a single dosage that is eliminated undisturbed. In the end, the kidneys gradually eliminate the medication from the body, primarily as inactive metabolites (Nivetha *et al*., 2023).

2.3.6 Half-life

The rate of elimination half-life in healthy individuals ranges from 2.4 to 9.3 h. The final elimination half-life is prolonged in liver cirrhosis.

2.3.7 Toxicity

Overdosing is expected to result in adverse effects similar to those frequently associated with diphenhydramine usage, including anticholinergic effects, hyperpyrexia, and drowsiness. Mydriasis, fever, flushing, anxiety, tremor, dystonic responses, hallucinations, and abnormal ECG readings are possible extra-overdose symptoms. Rhabdomyolysis, convulsions, delirium, toxic psychosis, arrhythmias, coma, and circulatory collapse can all result from a high dose. Larger doses may potentially result in coma or circulatory collapse; moreover, in youngsters in particular, they may show indicators of CNS excitation, including convulsions and hallucinations (Varuni *et al*., 2023; Falguni *et al*., 2019).

2.3.8. Mechanism of action

Diphenhydramine has several modes of action, but its primary mode of action is antagonistic activity on the H_1 (histamine 1) receptor. The uterus, cardiac tissue, respiratory smooth muscles, vascular endothelial cells, immune cells, and central nervous system (CNS) neurons are among the tissues with H_1 receptors. In these tissues, stimulation of the H_1 receptor results in a wide range of effects, such as enhanced vascular permeability, flushing-causing vasodilation, shortened conduction time of the AV node, activation of the smooth muscles in the bronchi, and GIT, which causes coughing, and eosinophilic chemotaxis, and also causes an allergic reaction. Diphenhydramine acts as an inverse agonist at the H_1 receptor, counteracting the effects of histamine on capillaries and reducing the symptoms of allergic responses. First-generation antihistamine diphenhydramine inhibits the medullary cough center and makes you drowsy by inversely agonizing the H_1 CNS receptors. It crosses the blood-brain barrier with ease. The H_1 receptor is similar to muscarinic receptors. Since diphenhydramine is a competitive antagonist of the muscarinic acetylcholine receptor and has antimuscarinic properties as well, it is utilized as an antiparkinson medication. Finally, the intracellular sodium channel blocker activity of diphenhydramine produces local anesthetic effects. Diphenhydramine is metabolized by the liver *via* CYP450. Its half-

life is between 3.4 and 9.2 h, and it is eliminated in the urine unaltered. The medication takes 120 min to peak in serum (Gelotte *et al*., 2018).

Table 3: The weight-age diphenhydramine HCl dosage schedule

Weight	Range age (Years)	Dose (mg)
24-35	2-3	6.25
36-47	4-5	12.5
48-59	6-8	18.75
60-71	9-10	25
72-95	11	31.25
Not applicable	12-17	50

3. Analytical method

Using pseudoephedrine as the internal standard, we developed a sensitive, specific liquid chromatography-tandem mass spectrometry (LC-MS/MS) approach for the quantitative detection of d-amphetamine and diphenhydramine, the two primary effective components of the QAAMC. The analyses and internal standard were extracted using a simple liquid-liquid extraction method from 200 µl plasma samples (LLE). The Zorbas SB-C18 column (100 mm × 3.0 mm, 3.5 µm) was used for reverse-phase HPLC separation at a flow rate of 0.2 ml/min. The mobile phase consisted of methanol, water, and formic acid (65:35:0.5, v/v/v). The chromatographic run time of the technique was five min. The precursor-to-product ion transitions, m/z 136.0 → 91.0 (d-amphetamine), were monitored in selective reaction monitoring (SRM) mode using a variable 1200 l electro spray dual-mass spectrometer fitted with an electro spray ionization source. The quantification ranges that were employed were 256.0 → 167.0 (diphenhydramine) and 166.1-148.0 (IS). Strong linearity was shown in the range of 0.5-200 mg/ml for d-amphetamine -and 1-500 mg/ml for diphenhydramine (r^2 >0.9990), with the lowest level of quantization (LLOQ) being 0.5 mg/ml for d-amphetamine and 1 mg/ml for diphenhydramine. Every validation data point, including inter-day repeatability, accuracy, and precision, fell within the necessary bounds (Chen Wang *et al.,* 2007).

The chromatographic investigation was conducted using the HPLC-UV system. The arcus EP-C18 Ion Pac column measured 4.6 mm by 250 mm, or 5 µm. Methanal, acetonitrile, water, 10 mm heptane sulfonate, and 13 nm triethylamine (10:26:64) at pH 3.3 made up the mobile phase. The flow rate was 1.0 ml/min. The UV detection wavelength in the HPLC system is 254 nm. The technique met all acceptable standards for particularity, affectability, correctness, exactness, and linearity. The maintenance of the diphenhydramine took 9.9 min. Diphenhydramine HCl (DPH) alignment graphs showed linear trends for the focus ranges of 1-4 µg/l. The quantitation limit was 3.16585 µg/ml, while the detection limit was 1.04473 µg/ml. The present HPLC-UV technique, which was used for the examination of commercial pharmaceutical samples, was granted a trademark by the ministry of health in Iraq. It was established that the recommended analytical procedure was valid. Quality, accuracy, and precision are all within reasonable limitations, according to all research findings. Additionally, the results indicate that there is no statistically significant difference between the qualities obtained using the recommended approach (Al-Salman, 2020).

These chemicals were separated on a nucleour gravity C18 column (250 × 4.0 mm, 5 µm) in 37.9 min. These chemicals were separated using an isocratic mobile phase in a single chromatographic run. The mobile phase was a room-temperature, 0.75 ml/min flow rate of a methanol-buffer mixture (38:62 v/v). At 210 nm, UV absorption was detected. 2,4,6-trimethoxybenzaldehyde (ISTD) was used as an internal standard. The following elements were examined as part of the approach validation: accuracy, precision within and between days, linearity of a calibration, forced degradation tests, and accuracy. It has correlation values above 0.9993. The relative standard deviations between days were all less than 4%. The suggested liquid chromatography technique performed well when used to regularly evaluate these substances in a variety of cough and cold remedies, including syrups and tablets (Caglar *et al.,* 2014; Dandu *et al.,* 2022).

The chromatographic conditions comprised an isocratic apparatus including a C18 column (250 mm x 4.6 mm, 5 µm), a 1.0 ml/min flow rate, and a UV detector set at 254 nm. In addition, acetonitrile-triethylamine (70:30:0.3), v/v, pH 3.0, and a 50:50 KH_2PO_4 buffer combination were used in the mobile phase. The process's selectivity, linearity, detection limit, LOQ, precision, and accuracy were all confirmed. The retention times of IBU and DPH were 15.6 min and 4.3 min, respectively. The method showed good selectivity; the calibration curves were linear over the concentration range of 10-100 µg/ml for DH and 50-500 µg/ml for IB, with a correlation coefficient of 0.9996 and 0.9997, respectively, the precision (RSD) was less than 1.0, and the accuracy ranges were 99.46-100.20 and 100.01-101.05% for DPH and IBU, respectively. In summary, this method's sensitivity, accuracy, precision, high degree of selectivity, and precision make it suitable for the simultaneous measurement of DPH and IBU in tablet dosage form. Since methanol is less expensive than acetonitrile, using the procedure might result in a reduction in analytical costs (Ahmed and Seemaa Hameed, 2020).

Separate measurements of pseudoephedrine and diphenhydramine hydrochloride in cough syrup are now easy, quick, and precise thanks to gas chromatography. This process uses nitrogen as a carrier gas at a flow rate of 30 ml/min and a 10% OV 1 SS column on chromocarb W-HP (80-100 mesh). The temperature of the oven was programmed to begin at 135°C for one minute, then rise by 10°C per minute until 250°C (a temperature that was held for five min). The injector and detector port temperatures were maintained at 280°C. The method of detection was carried out using the Flame Ionization Detector. Guaiphenesin was the internal standard that was used. The test and recovery study data were evaluated statistically for accuracy and precision (Raj *et al.,* 1998; Vimal Raj and Sumithra, 2023).

An established and verified HPLC/GC-MSD method may be used to quantify diphenhydramine in rabbit whole blood. The UV absorbance detection in this method is measured at 258 nm, and it involves liquid-liquid extraction and reversed-phase chromatography. When HPLC eluant fractions were recovered, re-extracted, and subjected to GC-MSD analysis, they included diphenhydramine and the internal standard, orphenadrine. Reducing the amount of sample required and increasing the sensitivity of the assay were the results of using whole blood. The range of 1 to 1000 mg/ml is the diphenhydramine concentration found in whole blood (Walters Thompson and Mason, 1992; Yamina Bouatrous, 2019).

4. Conclusion

In conclusion, this review article has given a thorough analysis of diphenhydramine hydrochloride and illuminated its pharmacological characteristics, therapeutic applications, possible side effects, and important concerns. As an antihistamine, diphenhydramine hydrochloride has shown promise in treating allergic responses and symptoms of a number of illnesses, such as allergic rhinitis and sleeplessness. The accomplishment of developing and validating this analytical method for diphenhydramine HCl has demonstrated its usefulness in pharmaceutical research, quality control, and regulatory compliance; in the end, this has supported the more general objectives of guaranteeing the safety and effectiveness of products containing diphenhydramine HCl.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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