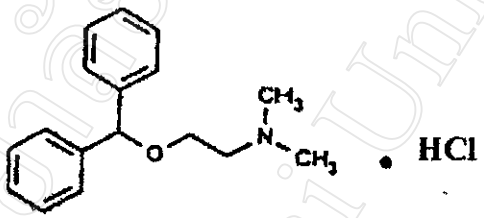
CHAPTER 1
INTRODUCTION

1.1 Diphenhydramine hydrochloride

Diphenhydramine hydrochloride is 2-(diphenylmethoxy)-N,N-dimethylethylamine hydrochloride [1]. Six additional chemical names are listed in the Merck Index [2]. The empirical formula is $C_{17}H_{21}NO.HCl$ with a molecular weight of 291.82. The chemical structure of diphenhydramine hydrochloride is as following.



The CAS Register Number is 147-24-0. It is a white odourless crystalline powder which becomes slowly darkness and faster when exposure to light. It is soluble in water, ethanol, chloroform and practically insoluble in ether [3]. It was first synthesized by Rieveschl in 1947, and the general method involves the reaction of bromodiphenylmethane with the appropriate dialkylamino alcohol in the presence of anhydrous sodium carbonate to form diphenhydramine base and then is converted to the HCl salt [4].

Diphenhydramine hydrochloride is a histamine H_1-receptor antagonist which can block most of the actions of histamine in the body. As with other antihistamines, it has antimuscarinic and pronounces central sedative properties. It is effective used in perrenial and seasonal for the symptomatic relief of hypersentivity reaction including urticaria and angioedema, rhinitis and conjuctivitis due to inhalant allergens and foods, and in pruritic skin disorders. It is also used for its anti-emetic properties, particularly in the prevention and treatment of motion sickness. It is used for its antimuscarinic properties to control parkinsonism including drug induced. It may be used as a hypnotic in the short-term management of insomnia for its central sedative properties. It also has significant antitussive activity; the syrup is used as a cough

suppressant to control cough due to cold or allergy. It is probably effective for using in mild, local allergic reactions to insect bites or physical allergies which are characterized by pruritis.

Nevertheless, numerous side effects are observed by patients on this drug such as drowsiness; nausea; vomitting; diarrhea; blurring of vision; nasal stuffiness; vertigo; headache; thickening of bronchial secretion; dryness of the mouth, nose and throat; and also heaviness and weakness of the hands.

Diphenhydramine is found in many phamaceutical preparations; it is usually given orally in the preparations of tablet, capsule, and elixer, but it may be administered by deep intramuscular injection or by intravenous injection in severe allergies and it is applied topically for local allergic reactions, usually in preparations of lotion and cream containing 1 to 2% [5,6].

1.2 Methods of analysis

1.2.1 Titrimetry

Diphenhydramine hydrochloride can be determined directly by nonaqueous titration with 0.1 M perchloric acid in the presence of mercury(II) acetate using crystal violet as indicator [1]. It can also be determined by an indirectly nonaqueous titrimetric method using an excess perchloric acid in glacial acetic acid which displaces hydrochloride and removes it by boiling. The residual excess perchloric acid is determined by titrating with sodium acetate using either potentiometer or crystal violet as indicator for detection the end point [7]. These methods are suitable only for determination of raw material.

For the assay of diphenhydramine hydrochloride in preparations, some separations are needed prior to titration. By complexometric method [8], diphenhydramine forms insoluble salt with bitmuth in prepared reagent (Na_2H_2L (H_4L-EDTA), KI and $BiCl_3$) and releases an equivalent of EDTA. The liberated EDTA is titrated with $ZnSO_4$ using eriochrome black T as indicator. The slurry method can be used for the separation of diphenhydramine from other ingredients in preparations [9]. The sample is slurred with a mixture of magnesium oxide and siliceous earth (celite 545) and washed with warm chloroform into glacial acetic acid. Diphenhydramine is then titrated with perchloric acid using p-naphtholbenzein as

indicator. In the official method [10], the double extraction of sample with ether has been used,first from acidic medium to eliminate the other interferences and later from alkaline medium to extract diphenhydramine into ether layer. The combined ether is evaporated to dryness and dissolved with a known quantity of acid. The excess acid is titrated with sodium hydroxide solution using methyl red as indicator.

Furthermore, diphenhydramine hydrochloride has been determined by using oxidimetric method [11]: a sample is mixed with the reagent (H_2O, satd.$Na_2H_2P_2O_7$ and 5mM peradipic acid in aq.ethanol), acidified with glacial acetic acid and mixed with 5% KI. The liberated iodine is titrated with $Na_2S_2O_3$ solution.

1.2.2 UV Spectrophotometry

The spectrophotometric determination of diphenhydramine has been carried out by various procedures and needs some separations prior to spectrophotometric assay; most of these involve liquid extraction. From the study of Woo et al. [12], diphenhydramine hydrochloride in various pharmaceutical liquid formulations was extracted from alkaline medium into cyclohexane, reextracted into dilute aqueous acid and determined by measuring the absorbance at 290 nm. Similarly, the official method for the assay of diphenhydramine hydrochloride in capsule, elixir, and injection preparations has been used ether as extracting solvent and measured the absorbance at 258 nm [13].

Column chromatography has been used for separation. Diphenhydramine hydrochloride in cream preparation can be determined by being extracted into chloroform layer and then passed through the column filled with algenic acid. Using acid solution as eluting solvent, the eluate is measured for absorbance at 258 nm [14]. Ion exchange chromatography can also be used; by using celite 545 as packing material and eluting with a mixture of n-heptane and chloroform,the eluate is then extracted into acidic medium and measured for absorbance at 258 nm [15].

As diphenhydramine hydrochloride can be oxidized to benzophenone by dichromate in a sulfuric medium or permanganate in a basic medium, which is separated by extracting into hexane or heptane, it can be determined spectrophotometrically [4]. Besides, it can be converted to chloranil with $FeCl_3$ in hydrochloric acid and hydrogen peroxide, which is extracted and hydrolyzed to

chloranilic acid with potassium hydroxide and then measured for absorbance at 331 nm [16].

Moreover, the determination of diphenhydramine hydrochloride in tablet and capsule formulations can be determined directly by second -derivative ultraviolet spectrophotometry [17]. The sample is diluted to the proper concentration with acid solution and scaned the spectrum in the range of 250-270 nm region. The amplitude is measured at 258 nm without interfering from the other excipients.

1.2.3 Colorimetry

The ion-pair extraction method is commonly used for determination of diphenhydramine hydrochloride. The procedure was first used for determination of diphenhydramine hydrochloride in body tissues by extraction of the DPHH – methyl orange ion pair into chloroform [18]. This method is also used for determination diphenhydramine hydrochloride in pharmaceutical preparations. Various dyes and conditions used are shown in Table 1.1.

Furthermore, a complex formation method is also applied. DPHH is determined by extraction of the complex with cobalt thiocyanate into chloroform [26] or 1,2 dichloromethane [27] and measuring the absorbance in the region 590-620 nm and 625 nm, respectively. It also forms the complex with H ($TiBr_4$) which can be extracted into benzene layer and measured for absorbance at 579 nm [28].

1.2.4 Fluorometry

Diphenhydramine hydrochloride can be determined by measuring the fluorescence in 0.005 M sulfuric acid at 285 nm by exciting at 258 nm and the phosphorescence in absolute ethanol at 375 nm by exciting at 237 nm with the linear range of $4 \times 10^{-8} - 10^{-4}$ M and $3 \times 10^{-7} - 10^{-3}$ M, respectively [29].

1.2.5 Gravimetry

The picrate method and picrolonate method have been used to determine diphenhydramine hydrochloride by forming the insoluble complex with picric acid or picrolonic acid, respectively. The precipitate obtained was washed with water and ether, dried and weighed [30].

Table 1.1 Ion-pair extraction methods for the determination of diphenhydramine hydrochloride.

Formulation	Reagent	pH	Extracting solvent	λ,nm	Ref.
Capsule, injection	Bromocresol green	2.0-5.0	$CHCl_3$	415	19
Capsule, injection, Elixir	Dipicrylamine	5.0	$CHCl_3$	420	20
Tablet, Capsule, injection, Cream	Methyl orange	5.0	1,2-Dichloroethane	422	21
Ointment	Tetrabromophenolphthalein Ethyl Ester	7.0-9.0	1,2-Dichloroethane	594	22
Tablet, Solution	Tetrabromofluorescein	2.7	Measured the absorbance of ion-pair compound in aqueous solution, using polyvinyl alcohol to stabilize the product solution.	540	23
Tablet	Eosin	4.5-7.5	$CHCl_3$	582	24
Tablet, Capsule	Bromocresol purple	5-6	Benzene	410	25

1.2.6 Electrochemical Analysis

Diphenhydramine hydrochloride in syrup can be determined by using the diphenhydramine-sensitive membrane electrode based on poly(vinyl chloride) matrices, consisting of the diphenhydramine cation tetraphenylborate ion-pair

compound, PVC and dioctyl phthalate and using Ag/AgCl as reference electrode. The dynamic range was $10^{-4} - 10^{-2}$ M. [31].

1.2.7 Chromatography

Diphenhydramine hydrochloride has been determined by TLC-densitometry [32]. After dissolving a powdered tablet with 95% ethanol, the sample solution was analyzed on silica gel GF_{254} plate by using benzene-methanol-diethyl amine (40:9:1) as mobile phase. The spot was scanned at 265 nm. The recovery of DPHH in tablet was 99.8 to 104.3%, with relative standard deviation of 1.9% (n=5)

DPHH in cough-cold syrup can also be determined simultaneously by capillary gas-chromatography with FID, operated with temperature programming from 100°C to 250°C at 10°C min^{-1} and He as carrier gas [33].

Moreover, it can be determined by high performance liquid chromatography (HPLC) using Zorbax 300-SCX cation exchanger column, with acetonitrile-ethylenediamine sulphate buffer solution of pH 4.52 as mobile phase and detection at 216.2 nm [34].

Since 1990, the official method [35] for determination of diphenhydramine hydrochloride in capsule, elixir and injection preparations is by HPLC using nitrile (CN) column, with acetonitrile-water-triethylamine solution of pH 6.5 as mobile phase and detection at 254 nm.

1.2.8 Flow Injection Analysis

The FIA spectrophotometric methods have been applied for determination of diphenhydramine hydrochloride based on ion-pair formation [36] or UV absorption of its free base[37].

By on-line extraction-FIA method can determine diphenhydramine hydrochloride and 8-chlorotheophylline, the active ingredients in dramamine tablets simultaneously. At the pH higher than pK_a (9.1) of diphenhydramine hydrochloride, the neutral free base, diphenhydramine was extracted into cyclohexane whereas 8-chlorotheophylline remained in aqueous layer. The two phases were separated by the dual-membrane separator, diphenhydramine in cyclohexane was monitored at 254 nm and 8-chlorotheophylline in aqueous phase at 300 nm.

As spectrophotometric assay, the reactions commonly base on ion-pair formation. Diphenhydramine hydrochloride formed ion-pair with bromophenol blue which was monitored at 650 nm by turbidimetric determination without solvent extraction.

1.3 Ion pair formation [38,39,40]

The formation of ion pairs with colored reagent and subsequent extration is one of the most important methods used for the analysis of spectrophotometrically inactive (or only UV active) organic acids, bases and quarternary ammonium salts. For quantitative analysis of organic bases such as amines,the acid-dye technique is frequently used. In this approach the amine in its protonated state forms an ion pair with an anionic species to form a neutral ion pair which can be extracted into an organic solvent.

The fundamental equilibria involved in an ion pair extraction of amines are

$$B^+_{(aq)} + A^-_{(aq)} \rightleftharpoons BA_{(aq)} \qquad (1)$$

$$BA_{(aq)} \rightleftharpoons BA_{(org)} \qquad (2)$$

Where B^+ is the protonated primary, secondary or tertiary amine cation, A^- is the organic or inorganic acid anion, and BA is the neutral ion pair.

The equilibrium conditions of the extraction can be defined by the extraction constant,K_{ex}:

$$K_{ex} = \frac{BA_{(org)}}{B^+_{(aq)}.A^-_{(aq)}} \qquad (3)$$

Where $BA_{(org)}$ is the concentration of the ion pair transferred into the organic phase, $B^+_{(aq)}$ and $A^-_{(aq)}$ are the concentrations of the protonated base and anionic dye after extraction in the aqueous phase.

The efficiency of extraction of the protonated base by means of an appropriate counter ion, A^-, can be defined by the partition ratio as:

$$P_B = \frac{BA_{(org)}}{B^+_{(aq)}} = K_{ex}.A^-_{(aq)} \qquad (4)$$

As seen from Equation (4), the efficiency of the extraction of protonated bases is directly proportional to the concentration of the anionic dye and the extraction constant.

There are several factors affecting the extraction constant. First, the structure of the anionic dye is mentioned which the bigger of non-polar molecular fragments, the higher the extraction constant. However, the extraction constant is reduced by the subsequent non-ionizing polar groups. Another important factor is the choice of water immiscible solvent. The great capability of hydrogen bonding is particularly significant in the extraction of bases of which benzene, chloroform, and dichloromethane are used most frequently. It is found that a large extraction constant for the ion pair is obtained when strong hydrogen bonds are formed between the base and anion of the pair, and between the ion pair and the solvent. Nevertheless, the greatest selectivity is obtained when the anionic dye rather than the base is the component of the ion pair that hydrogen bonds to the extraction solvent. The third, very important factor affecting the ion pair extraction of amines is their protolytic equilibrium state in the aqueous phase which is determined by the pK_a values of the amines to be extracted, the counter ion and the pH of the solution.

The condition of ion pair formation is that the amine drug is presented in protonated form only over a limited pH range which usually is selected to be a pH at least one unit lower than the pK_a of the drug. However, the reduction of pH is limited by the condition that the anionic dye should be in a dissociated state in the solution to be extracted, so that the pH of the extraction should be optimized carefully. Besides, the anionic dye should be water soluble at the pH of the extraction and poorly soluble in the organic solvent chosen, so that only the ion pair formed with the analyte is transferred into organic phase.

Of course, the wavelenghts and intensities of the absorption maxima depend on the base to be analyzed, the anionic dye applied and the extraction solvent.

Several anionic dyes have been employed including bromothymol blue, methyl orange, picrate(trinitrophenolate), dipicrlyamine, bromocresol purple, anthracene-2-sulfonate, and bromocresol green. Since the protonated form of the amine forms the ion pair, quarternary ammonium compounds can also be analyzed by this method.

1.4 Flow injection analysis (fia)

1.4.1 Theory and principle

The automation measurement technique based on the segment continuous flow was first introduced by Skeggs in 1957 and marketed by Technicon under the tradename Autoanalyzer [41]. The samples are aspirated sequentially, and air bubbles separate the flow. Given the presence of air bubbles, the system is complex and poses a series of problems. Unsegmented-flow methods which first described by Ruzicka and Hansen in 1975, are referred to as flow-injection analysis (fia), and solve the problems caused in segmented-flow method.

The most important advantages gained by elimination of the air bubbles are: [41,42]

1. simpler and inexpensive equipment
2. high analysis rates (high sample throughput)
3. rapid start-up time and shut-down time, and enhanced response time
4. more reproducible flow rate and sample volume
5. possible incorporated separation steps
6. wide application and analytical potential

The fia bases on the important three principles including reproducible timing, sample injection and controlled dispersion with the basic components as shown in Fig 1.1

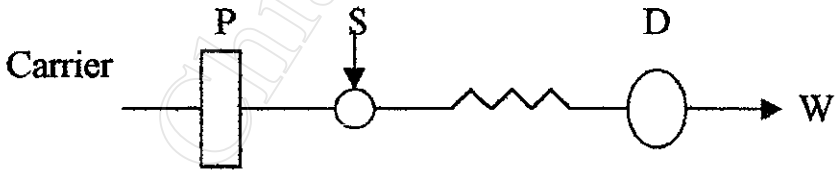


Fig 1.1 A single-line fia manifold

P = pump, S = sample injection point

D = flow through cell detector, W = waste

Dispersion was defined as the amount that the chemical signal is reduced by injecting the sample plug into a system. This is represented mathematically by: [42, 43]

$$D = C^0/C^{max}$$

Where D is the dispersion coefficient at the peak maximum produced by the ratio between C^0 the sample concentration before, and C^{max}, the sample concentration after tranfer from the injection valve to the detector flow-cell. Since the concentration of sample is direct proportional to the detector signal, peak height, the dispersion coefficient can be defined as the ratio between the signals produced before and after the sample disperses. Therefore, the previous concentration ratio becomes a height ratio, $D = h^0/h$. In order to study the factors influencing dispersion, the results are normally obtained by univariate method which monitor changes in not only the peak height, but also its width, the residence time and the start-up time.

Factors Influencing Dispersion [43, 44]

The fi experimental parameters which may influence dispersion include:

- sample volume
- carrier flow-rate
- travelled distance between the injection and detection
- halting the flow (in order to increase the reaction yield and sensitivity and to determine the reaction rate)
- reaction rate
- geometrical dimensions and configurations of manifold conponents
- viscosity of the fluids (usually affecting in mixing chambers)
- temperature

1.4.2 Basic components of a fia system [41, 43]

A fia system includes the following essential parts:

1. The propulsion system

Fluids can be propelled through fia manifolds by various mechanisms such as gravity-based; gas pressure-based; peristaltic pumps; high-pressure pump; and an alternative type pump, electro-osmotic flow. The peristaltic pumps are the most frequently used types of propulsion systems in fia.

2. The sample introduction system

This part is intended to insert reproducibly and accurate sample volumes into a carrier solution without altering its flow-rate. Sample introduction methods can be divided into two broad categories according to whether they rely on insertion of a fixed volume or during a fixed time. Syringe injection and rotary valve injection are

commonly used in fixed volume method. The second pump used to aspirate the sample at a constant, known flow rate over a preset time interval for loading into an adsorbent column, combining with the switching valve, is an alternative route used in the fixed time method.

3. The sample transport and reaction system

This part is intended both to carry the flowing stream along the manifold and to interconnect the various parts making up the working system by means of tubing, connectors and reactors. The extent of mixing and dispersion is governed by the length and diameters of tubing and type of reactors. There are various reactors such as opened tubing; coiled tubing; packed reactors which are normally filled with chemically inert or active material; glass bead columns; mixing chambers and knitted reactors. One or a combination of any of these can be used in the transport system.

4. The detection system

Conventional analytical techniques in fia use a wide variety of detectors. The optical detectors are the most commonly used including uv-vis, atomic absorption spectrophotometer, fluorescence and chemiluminescence detectors, infrared and near infrared spectrometers. The other detectors are such as electrochemical and radiometric detectors.

5. Data acquisition and processing unit

The data output from detector is recorded as a peak by means of either a chart recorder, microprocessor or computer.

1.5 Scope and aims of the study

This study is dealing with the method development for determination of diphenhydramine hydrochloride in various pharmaceutical preparations which can be applied for routine work by using the simple and economic fia system.

The aims of this study are summarized as following:

1. To investigate the optimum conditions for extraction (reagent concentration, pH of reagent and extraction time).

2. To investigate the optimum conditions for spectrophotometric fia system (carrier solution, flow rate, sample volume, mixing coil length and wavelength for monitoring)