CHAPTER I

INTRODUCTION

Some oral combination cough-cold syrups designed for symptomatic relief from the discomfort associated with common cold contain drugs having analgesic, antihistamine, antitussive and stimulant effects. Such cough-cold syrups which are commercially available contain active ingredients such as ephedrine hydrochloride, codeine phosphate and promethazine hydrochloride.

Ephedrine is a sympathomimetic amine which resembles adrenaline and amphetamine in its actions. the prolonged administration of ephedrine has no cumulative effect but tolerance may develop. (British Pharmaceutical Codex [BPC], 1973)

Codeine has moderate analgesic and weak cough-suppressant effects (BPC, 1973). It is classified as narcotic analgesic. The inclusion of this drug in medicinal preparation such as cough medicines, has introduced the possibility of abuse (Moore, Tebbett and Scherer, 1989).

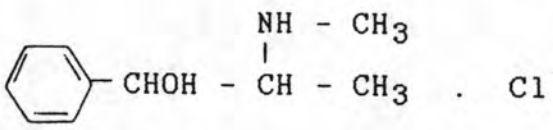
Promethazine is a powerful and long-acting antagonist of histamine, as a sedative and antiemetic (BPC, 1973). It is decomposed primarily by oxidation

and/or photolysis. Aqueous solutions of promethazine hydrochloride stored at room temperature for two days to six months decomposed to promethazine sulfoxide, 9,9 dioxopromethazine, N-demethyl-promethazine and several more unidentified compounds. Phenothiazine was identified as a major degradation product when a solution of promethazine hydrochloride was exposed to sunlight (Shearer and Miller, 1976).

The analysis of a mixture of ephedrine hydrochloride, codeine phosphate and promethazine hydrochloride in syrup dosage form presents many difficulties. Liquid-liquid extraction is normally used for sample preparation prior to instrumental analysis. Traditional liquid-liquid extractions are tedious, time consuming and costly. These methods not only require several sample handling steps but may also present problems of phase emulsions and large solvent volumes to the analyst. The assay of such preparation is also quite tedious because each drug is individually determined by spectrophotometic method. However, these three active ingredients in the original formulation product, Phensedyl$^{(R)}$,(May & Baker,Co.), are simultaneously determined by liquid-liquid extraction prior to Gas-Liquid Chromatographic (GLC) analysis.

Ephedrine Hydrochloride

Ephedrine hydrochloride is the hydrochloride of (-)-2-methylamino-1-phenylpropan-1-ol. Its structure is shown below :



$C_{10}H_{15}NO.HCl = 201.7$

pK_a 9.68 (20°C)

It is odorless, colorless crystals or white crystalline powder with a bitter taste. It is freely soluble in 4 parts of water and in 17 parts of alcohol (96%), very slightly soluble in chloroform and practically insoluble in ether.

The UV spectra show a maximum at 250, 256 and 262 nm. (Ali, 1986).

Stability and Degradation

Decomposition of (-)-ephedrine was less than 1% after the prolonged passage of air through cold (20°C) or refluxing neutral or basic aqueous solution. Significant losses occurred during the extraction of small quantities of ephedrine from aqueous media using either regular or analytical grades of diethyl ether caused

by reaction of ephedrine with aldehydic impurities in the ether. Negligible decomposition was observed on refluxing ephedrine (0.5%) in ether saturated with aqueous 20% NaOH for 8 hours or ephedrine (8%) in ethanolic sodium hydroxide for 3 hours. Ephedrine base stored in ether (100 mg/ml) at room temperature in light for several weeks decomposed to give oxazolidines. The small amount of decomposition occurred upon ultraviolet irradiation of aqueous solution of ephedrine. Solutions of ephedrine base (3% w/v) in ether or benzene were extensively degraded by UV light over 18 hours. (Ali, 1986).

Numerous methods for the assay of ephedrine hydrochloride have been reported including spectrophotometry, GLC and HPLC. The ultraviolet spectrophotometric method is based on the formation of benzaldehyde from the reaction of ephedrine hydrochloride with periodic acid. Benzaldehyde is then extracted with cyclohexane and measured the absorbance at 242 nm (BPC, 1973).

Gupta and Ana (1975) studied a colorimetric determination of ephedrine hydrochloride in cough syrups containing chlorpheniramine maleate and guaiacolsulfonate potassium. Bromthymol blue was used for the assay. Interference from chlorpheniramine maleate was taken into consideration. The standard solutions of ephedrine hydrochloride and ephedrine hydrochloride plus chlorpheniramine maleate were prepared and analysed in order to obtain the corrected absorbance value due to chlorpheniramine maleate. Thus, the absolute

absorbance value of ephedrine hydrochloride in the assay preparation could be determined.

Umbreit (1961) studied the reaction of secondary amine with carbon disulfide to form dithiocarbamic acid. The dithiocarbamic acid reacted with copper (II) to form a yellow-colored salts or complex which was measured spectrophotometrically. Tertiary amines do not interfere in the assay since they do not react with carbon disulfide.

However, these methods were labor intensive and time consuming involving many variable effects and complex liquid-liquid extraction.

Schultz and Churairat (1973) reported the GLC method for the analysis of ephedrine hydrochloride in the preparation of Theophylline, ephedrine hydrochloride, and phenobarbital. Ephedrine hydrochloride was separated by liquid-liquid extraction with chloroform at pH 11. -Naphthylamine was used as the internal standard.

Deavin (1975) recommended a GLC method for the assay of ephedrine hydrochloride in official preparations such as tablets, elixirs and nasal drops. Liquid-liquid extraction was performed for sample preparation prior to GLC analysis.

HPLC retention characteristics of ephedrine and codeine had been measured along with 84 other basic

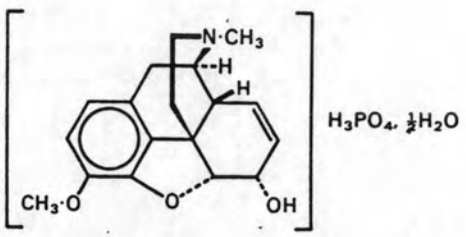
drugs of forensic interest. Chromatography was carried out using 250 x 5 mm i.d. column packed with Spherisorb S5W at 254 nm. The eluent consisted of methanol-aqueous ammonium nitrate buffer (90 : 10), adjusting the pH to 10.1 with ammonia. The retention time of ephedrine was 3.68 min. Because of the alkaline nature of the eluents, a short column, dry packed with silica, was included between the pump and injector to minimise dissolution of the analytical packing material (Gill and Moffat, 1984).

In the British Pharmacopoeia 1988 (BP, 1988), ephedrine hydrochloride in the ephedrine hydrochloride elixir is determined utilizing the HPLC method with Nucleosil C_{18} column. The HPLC method is also employed for the determination of ephedrine hydrochloride in the theophylline, ephedrine and phenobarbital tablet which is official in the United State Pharmacopoeia, XXII (1990). An octadecylsilane column is used for separate determinations of each active ingredients in the formulation.

Although the GLC and HPLC methods described are specific and effective for analysis of ephedrine hydrochloride, they are not suitable for simultaneous determination of ephedrine hydrochloride, codeine phosphate and promethazine hydrochloride due to lack of peak resolution.

Codeine Phosphate

Codeine phosphate is the phosphate of the 3-methyl ether of morphine. Its structure is shown below :



[3-O-Methylmorphine hemihydrate]

$C_{18}H_{21}O_3N.H_3PO_4.1/2\ H_2O$ = 406.4

pK_a 8.2

It is freely soluble in water, very soluble in hot water, slightly soluble in alcohol but more soluble in boiling alcohol. It exhibits a characteristic UV spectrum with a maximum at 285 nm. (Muhtad, 1981)

Codeine linctus is official in the BP (1988). The assay procedure is performed by titration method using dioctyl sodium sulfosuccinate TS as the titrant.

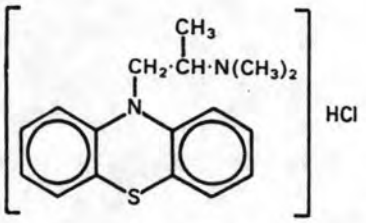
Elixirs codeine with terpin hydrate and codeine with paracetamol are both official in USP XXII (1990). The analysis method for determination of codeine in those two preparations are GLC and HPLC, respectively.

Sisco, W.R et al (1986) studied thermal decomposition of codeine phosphate in acetaminophen with codeine phosphate elixir by placing the elixir in a 60°C oven for two weeks. Codeine phosphate, as well as potential degration products p-aminophenol, codeine N-oxide and codeinone were analysed by HPLC with a diode-array detector at 214 nm, while C_{18} column was heated at 50°C.

Numerous methods for the determination of codeine in pharmaceutical preparations are reported including acid-dye (Matsui and French, 1971; Mathew et al., 1972), GLC methods (Stevens, 1975; Galante, Visalli and Patel, 1979) and several HPLC methods (Gupta, 1980; Halstead, 1982; Sisco et al., 1986). However, these methods are not applicable to analyse the combination of amine ingredients in cough-cold syrups intended for this study.

Promethazine Hydrochloride

Promethazine hydrochloride is 10-(2-dimethylamino-propyl)-phenothiazine hydrochloride. Its structure is shown below:



$C_{17}H_{20}N_2S.HCl$ = 320.9

pK_a 9.1

Promethazine hydrochloride is white to faint yellow, practically odorless, crystalline powder. It is slowly oxidized and acquires a blue color, on prolonged exposure to air.

It is soluble, at 20°, in less than 1 part of water, in 9 parts of alcohol and in 2 parts of chloroform. It exhibits a characteristic UV spectrum with a maximum at 254 nm. (Schearer and Miller, 1976).

Promethazine is known to undergo thermal and photolytic decomposition which is oxidative in character, yielding a wide variety of degradation products including some which are coloured (Cox, Meakin and Davies, 1976).

Promethazine hydrochloride syrup is official in USPXXII(1990) and BP (1988). The drug is assayed using liquid-liquid extraction prior to spectrophotometric method. The method found to be lacked of specificity and

are subjected to interfere from other UV absorbing drugs, coloring and flavoring agents and the oxidation products of the phenothiazine drug.

Davidson (1976) determined phenothiazine drug by a difference spectrophotometric technique based upon the absorbance of the sulfoxide derivative.

Sperling (1967) developed a sample preparation technique using column chromatography to separate the promethazine hydrochloride in official syrups from the interfering degradation products. Promethazine hydrochloride was subsequently identified and determined by IR and UV spectrophotometry, respectively.

The oxidative degradation products of promethazine hydrochloride were investigated and analysed using GLC (Stavechansky, Wallace and Wu, 1983), TLC, GLC as well as spectroscopy (IR, UV and Mass) by Underberg (1978).

Several HPLC methods were developed for determination of promethazine hydrochloride in pharmaceutical preparations (Pound and Sears, 1973; Wallace and Shinuk, 1981; Walker, 1985; Yang, Wilken and Clarke, 1986). These methods were effective for analysis of promethazine hydrochloride but not suitable for simultaneous assay of the combination of active ingredients in this study.

The combination of ephedrine hydrochloride, codeine phosphate and promethazine hydrochloride in cough-cold syrup is not official in pharmacopoeias. Thus, the analysis of such preparation is not official in any pharmacopoeia.

The use of solid phase extraction column as an alternative to liquid-liquid extraction for the isolation of drugs from complex matrices especially biological samples, such as serum and urine, has gained popularity over recent years because of the reported excellent recoveries and ease of operation (Good and Andung, 1981; Stewart et al., 1984; Wilson, 1986).

Other advantages of solid phase extraction include the elimination of time-consuming centrifugation and filtration steps and the elimination of sample loss through emulsion formation.

Solid phase adsorption extraction is most often performed on bonded silica phases. Through careful choice of the bonded phase from the variety available commercial, improvements can be made in selectivity of the extraction over liquid-liquid partition extraction procedures. The sorbent can be devided into three classes according to the nature of the interaction between isolates and solvents : non-polar, polar and ion-exchanger.

All sorbents contain groups of atoms known as functional groups, which determine their chemical and physical properties. For examples.

Non-polar : Octadecyl, octyl, butyl, cyclohexyl, phenyl and XAD-2 resin.

Polar : Cyano, amino, alumina, silica gel.

Anion exchanger : primary, secondary-amino, quarternary amine.

Cation exchanger : Carboxylic acid, sulfonic acid. (J.T. Baker, 1984)

Amberlite XAD-2 resin.

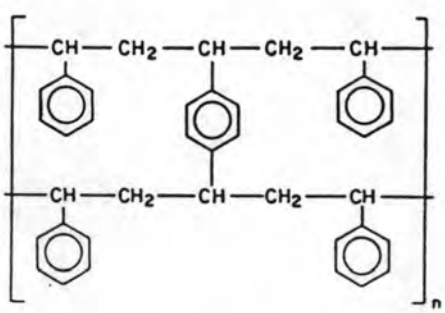
The XAD-2 resin has a macroreticular structure of styrene divinylbenzene copolymer with high surface area. It has the ability to adsorb many water-soluble organic compounds by van der Waals interaction. The resin has a nonionic structure and does not contract or expand as do ion exchange resin upon hydration. It is stable at all pH, from 0 to 14 in aquous solution and in most organic solvents. It had been rinsed with 5% sodium chloride and 1% sodium carbonate solution to control bacteria and mold growth. It could be used for the simultaneous extraction of acidic, neutral which basic drugs in one step save time and effort (Fujimoto and Wang, 1970).

Amberlite XAD-2 is obtained commercially as hard, white spheres, nominally 20 to 50 mesh, compatible with

virtually all solvents and relatively strong adsorbent properties (Baum, R.G; Saetra, R. and Cantwell, F.F., 1980).

Before use, the resin is washed thoroughly to remove any extraneous organic materials that might interfere with the measurement step. Two typical resin preparation schemes are as follows : (1) wash the resin with four column volumes of acetone, three times with three column volumes of methanol, and three times with three column volumes of distilled water or (2) wash the resin with 5% sodium chloride and 1% sodium carbonate solution to control bacteria and mold growth, and then wash with four bed volumes of distilled water. The resin is then ready to use.

The structure of styrene-divinyl benzene copolymer (XAD-2) is shown below. The degree of cross-linking is dictated by the proportion of divinyl benzene used in the polymerization reaction.



(Smith and Stewart, 1981)

Weissman, Lowe, Beatie and Demetriou (1971) described a method for the detection in human urine of

three major classes of drugs of abuse : amphetamines, barbiturate and alkaloids. The drugs are adsorbed from urine by a column of non-ionic resin (Amberlite XAD-2), eluted with methanol, and chromatographed on a thin-layer silica gel plate. The use of the non-ionic resin column permits essentially complete recovery of drug in a small volume of eluate.

Mohammed, H.Y. and Cantwell, F.F. (1978) studied the chromatographic retention, on a column of Amberlite XAD-2 resin, of 29 drugs which are common compositions of cough-cold preparations, using acidic, neutral and alkaline mobile phases, each containing various ratios of water and methanol. The moderate efficiency, compatibility with solvents of all pH, long column life and low cost of such column make it an attractive alternative to low efficiency liquid-liquid partition system for many applications. When combined with suitable pre-column, the use of this resin eliminate the need for syrup clean-up prior to injection.

The purpose of this study was to develop a simple, convenient and effective analytical method for simultaneous analysis of ephedrine hydrochloride, codeine phosphate and promethazine hydrochloride in syrup dosage form. The method utilized the solid phase extraction (SPE) and isocratic reversed-phase high-performance liquid chromatographic (RP-HPLC) techniques. The Amberlite XAD-2 was chosen to be a solid adsorbent in the solid phase extraction procedure.