MEREDITH WILCOX STORMS
Part I: Stability of Selected Pharmaceuticals in Polypropylene Syringes at Ambient Temperature and 4 Degrees Celsius.
Part II: Development of High-Performance Liquid Chromatography Assays for Selected Pharmaceuticals in Dosage Forms and Human Plasma
(Under the Direction of JAMES T. STEWART)

Part I (Chapters 1-6) of this dissertation describes the use of stability-indicating HPLC methods for ephedrine sulfate, lidocaine hydrochloride, neostigmine methylsulfate, glycopyrrolate, succinylcholine chloride, and tubocurarine chloride stored individually in polypropylene syringes at ambient temperature (24±1°C) or under refrigeration for either 60 or 90 days.

In Part II, HPLC is employed for the analysis of selected pharmaceuticals in dosage forms and human plasma studies.

Chapter 7 reports an assay for the simultaneous determination of guaifenesin-pseudoephedrine-dextromethorphan and guaifenesin-pseudoephedrine in commercially available capsule dosage forms and guaifenesin-codeine in a commercial cough syrup dosage form. For each drug mixture, the separation was achieved within 10 minutes. The method showed linearity for the guaifenesin-pseudoehedrine-dextromethorphan mixture in the 50-200, 7.5-30, and 2.5-10 μg/ml ranges, respectively. The guaifenesin-pseudoephedrine mixture yielded linear ranges of 25-100 and 3.75-15 μg/ml, respectively. The method showed linearity for the guaifenesin-codeine mixture in the 25-100 and 2.5 to 10 μg/ml ranges, respectively. The intra- and inter-day precision and precision for the analytes in each mixture ranged from 0.13-5.04%.

Chapter 8 describes a stability-indicating HPLC assay for the separation and quantitation of epinephrine-prilocaine and epinephrine-procaine combinations in their respective dosage forms. The method showed linearity for the epinephrine-prilocaine mixture in the 0.25 –2.5 and 8-200 μg/ml ranges, respectively. The intra- and inter-day RSDs ranged from 0.26 to 2.05% and 0.04 to 0.61% for epinephrine and prilocaine, respectively. The epinephrine and procaine mixture yielded linear ranges of 0.25-2.0 and 5-100 μg/ml ranges, respectively. The intra- and inter-day RSDs ranged from 0.3 to 1.88 and 0.07 to 0.26% for epinephrine and procaine, respectively. Stability-indicating HPLC assays were also developed for levonordefrin-tetracaine-procaine, levonordefrin-procaine-propoxycaine, and norepinephrine-procaine-propoxycaine drug combinations.

Chapter 9 describes an HPLC method for the determination of amoxicillin, metronidazole, and pantoprazole in human plasma. Solid-phase extraction was employed to extract the analytes along with the internal standard (tinidazole). Limits of detection were 200 ng/ml for amoxicillin and metronidazole and 100 ng/ml for pantoprazole. Recoveries from human plasma ranged from 83-92% for amoxicillin, 81-89% for metronidazole, 85-94% for tinidazole, and 93-101% for pantoprazole. Intra-day (n=5) and inter-day (n=15) precision (%RSD) and accuracy (%error) for analytes ranged from 1.11-5.97% and 0.72-13.5%, respectively.

INDEX WORDS: HPLC, Stability-Indicating, Anesthetics, Polypropylene Syringes, Guaifenesin, Procaine, Pantoprazole, Solid-Phase Extraction (SPE)

# PART I: STABILITY OF SELECTED PHARMACEUTICALS IN POLYPROPYLENE SYRINGES AT AMBIENT TEMPERATURE AND 4 DEGREES CELSIUS

# PART II: DEVELOPMENT OF HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY ASSAYS FOR SELECTED PHARMACEUTICALS IN DOSAGE FORM AND HUMAN PLASMA

by

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## DEDICATION

Lawrence of Arabia, author of Seven Pillars of Wisdom, wrote "All men dream but not in the same way. Those who dream at night…awake in the morning and consider their dream but vanity. But those who dream during the day are dangerous because they can act upon their dream, eyes opened, and make it possible."

This is dedicated to everyone who supported my efforts to further my education.

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# PART I: STABILITY OF SELECTED PHARMACEUTICALS IN POLYPROPYLENE SYRINGES AT AMBIENT TEMPERATURE AND 4 DEGREES CELSIUS

# INTRODUCTION AND LITERATURE REVIEW

From the first reported use by Tswett et al, chromatography has enjoyed a rich history in many facets of science [1]. Although rapid advances have been made in many analytical techniques over the last decade, chromatography continues to be the most frequently used analytical technique for both quantitative and qualitative analysis of drugs in dosage forms [2,3].

Chromatography is a separation method in which the components to be separated are distributed between two phases: (a) mobile phase and (b) stationary phase. More specifically, compounds are separated from each other as they migrate between the mobile phase and stationary phase. The mobile phase may be a liquid or a gas, while the stationary phase may be a liquid or a solid [3]. A distinction between the principal chromatographic methods can be made in terms of the properties of the mobile phase. The most commonly used types of chromatography are: (a) Thin-Layer Chromatography (TLC), (b) Gas Chromatography (GC), and (c) High-Performance Liquid Chromatography (HPLC) [4].

The stationary phase in thin-layer chromatography (TLC) is typically a glass or plastic plate coated with silica gel [2,3]. In TLC, the sample is spotted near one edge of the plate. The plate is then placed into a developing chamber containing a small amount of mobile phase. The mobile phase is allowed to travel up the plate by capillary action. Following development with mobile phase, compounds can be detected by ultraviolet (UV) light using TLC plates, which have been impregnated with a fluorescent material or by location reagents [2].

Gas chromatography (GC) employs either a packed column or capillary column stationary phase. The mobile phase used to carry the sample through the column is a gas, usually helium or nitrogen [2]. The separation mechanism in GC is based on the principle that the compounds to be separated will partition between a stationary phase, usually a high-boiling liquid coated onto an inert support and the carrier gas [3]. Non-polar solutes are dispersed by volatility and elution is typically based on the boiling points of the species under investigation. Polar solutes are often derivatized prior to injection since they have boiling points too high for practical use [2].

High-performance liquid chromatography (HPLC) uses a solid as the stationary phase packed inside an inert column. The column is said to be the "heart" of the chromatographic system [5]. The liquid mobile phase carries the analyte through the column and to the detector [2]. HPLC can be utilized in both the normal- and reversed-phase modes. Since most pharmaceuticals possess polar characteristics, they are typically separated using a reversed-phase system. In this case, analytes elute in decreasing order of polarity [5]. The main advantage of HPLC over GC is that the compounds do not need to be derivatized since HPLC does not require volatile samples [4].

Reversed-phase HPLC (RPLC) is the most commonly employed chromatographic mode [4,5]. Column packings are most often silica-based due to the high surface area and porosity, easy preparation, chemically modifiable, and increased mechanical strength [5]. Although silica-based column packings do offer several advantages, eluents above pH 7 are not recommended due to dissolution of the silica, and below pH 2 due to cleavage of the siloxane linkages. Efforts have been made to minimize sorbent

degradation through endcapping [5] and by manufacturing zirconia-cladded silicas [5,6], alumina-based packings, and by using silica with high bonding density and purity. Polymeric columns may also be utilized in RPLC over a pH range of 1 to 13 [5]. The disadvantages of the polymeric phases include cost as well as the tendency to swell when in contact with lipophilic mobile phases [2,5].

Several detection techniques have been employed to detect and quantitate analytes eluting from an HPLC column. Barth et al. describes over 30 detection techniques that are applicable to chromatography [7]. Detection sensitivity, selectivity, linear range, and baseline noise are some of the factors that are considered when selecting a detection technique for a particular assay [2,5]. Some pharmacopeial assays still rely on direct UV spectroscopy; however, industrial applications typically combine UV spectrophotometry with HPLC [2]. The popularity of UV detection is due to the fact that most pharmaceuticals possess a chromophore. The utilization of UV detection is also enhanced by the fact that the solvents traditionally utilized in HPLC are generally transparent in the UV region, which affords better specificity [3]. However, some drugs lack a chromophore and thus, require an alternative detection method. Other detection methods for pharmaceutical analysis include refractive index, fluorescence, electrochemical, and mass spectrometry. Refractive index (RI) detectors respond to changes of refractive index when the analyte passes through the sample cell in the detector. Although RI detectors are rather inexpensive, their use is limited due to sensitivity to mobile phase composition and temperature [2]. Many pharmaceuticals possess native fluorescence; therefore, fluorimetry yields low limits of detection [3]. One main advantage of fluorescence over UV is its ability to discriminate the analyte from

interferences or background peaks [5]. However, not all pharmaceuticals have native fluorescence and must be derivatized with a reagent that possesses a fluorophore. This process can be somewhat time-consuming and tedious [2,3,5]. Electrochemical detectors are based on the measurement of electric current resulting from the production of electrons when the analyte is oxidized or consumption of electrons when the analyte is reduced. Hence, the presence of an oxidizable or reducible group in the analyte is a prerequisite for this particular detection technique [5]. The use of a mass spectrometer coupled to HPLC (LC-MS) is becoming more common in the pharmaceutical and biotechnology industries for the analysis of drugs. In particular, this detection technique provides information such as the molecular weight and the structure of the analyte [8].

Stability of drugs in pharmaceutical preparations is essential to many pharmaceutical professionals. Pharmaceutical manufacturers routinely perform elaborate, large-scale stability studies on a variety of dosage forms to establish expiration dates and to satisfy regulatory requirements. High-performance liquid chromatography is often employed for the stability studies of such drug mixtures [9]. In fact, establishing a pharmaceutical compound's stability is analogous to establishing its metabolic profile in biological systems. HPLC is often utilized as the separation technique to develop stability-indicating methods due to its ability to separate the drugs of interest from the degradants and to analyze drug levels over a period of time. A stability-indicating method is capable of distinguishing the major pharmaceutical ingredient from any degradation products [10]. In order to obtain degradation products of pharmaceuticals, it is often necessary to force degradation using reactions of hydrolysis, oxidation, and photolysis [10, 11].

Typically, the objective is to force degradation to observe a 10-30% loss of active when compared with the nondegraded compound [5,10].

Stability and compatibility information is typically available in the package insert for most commercial preparations. But, when a dosage form is stored under conditions (i.e. temperature or storage device) other than those described in the package insert, additional stability studies are often warranted. For example, during anesthesia, it is common practice in a hospital emergency room to preload drug solutions in disposable polypropylene syringes for immediate accessibility [12]. In these types of situations, stability information is often unavailable. Since a local hospital pharmacy had the desire to preload polypropylene syringes with selected drugs, it was important to assess the stability of each drug stored in polypropylene syringes at both ambient temperature and 4 degrees celsius. Thus, stability-indicating HPLC methods were developed for the analysis of ephedrine sulfate, lidocaine hydrochloride, neostigmine methylsulfate, glycopyrrolate, succinylcholine chloride, and tubocurarine chloride under these conditions.

Because ephedrine sulfate is not available prepackaged in syringes at a concentration of 5 mg/ml, hospital pharmacies must by dilute ephedrine sulfate 50 mg/ml with 0.9% sodium chloride injection, aseptically preloading ephedrine sulfate solutions into sterile syringes, and storing the resultant dosage form for short periods at ambient temperature ($23 \pm 1°C$) or under refrigeration ($4 \pm 1°$). However, such storage may adversely affect drug potency. Spectrophotometeric methods and HPLC have been used to conduct stability studies; however, stability information at ambient temperature with exposure to light or under refrigeration for up to 60 days is not available [13-15]. Thus, Chapter 1

describes a stability-indicating HPLC assay for the analysis of 5 mg/ml ephedrine sulfate in 0.9% sodium chloride injection stored in 10-ml polypropylene syringes at ambient temperature and under refrigeration over 60 days. Benzyl alcohol, which was added as a preservative, did not interfere with the assay. The separation was achieved on an ODS column using aqueous-organic mobile phase and UV detection at 254 nm [9].

Methods for the determination of lidocaine hydrochloride in pharmaceutical dosage forms are typically based on spectrophotometry, high-performance thin-layer chromatography (HPTLC), TLC-densitometry, and HPLC [16,17]. A stability study has previously been conducted to assess the stability of lidocaine hydrochloride injection (0.2 mg/ml) in 0.9% sodium chloride stored in polypropylene syringes for 24 hours at room temperature and in the dark; however, stability information for lidocaine hydrochloride injection (20 mg/ml) stored at ambient temperature with exposure to light or under refrigeration for up to 90 days is not available [18]. Thus, Chapter 2 describes a stability-indicating HPLC assay for the analysis of lidocaine hydrochloride stored in 12-ml polypropylene syringes at ambient temperature and under refrigeration. The final separation was achieved using an underivatized silica column with a phosphate buffer-organic mobile phase and UV detection at 254 nm. Methylparaben, which was present as a preservative in the injection, did not interfere with the assay.

Chapter 3 addresses the stability of undiluted neostigmine methylsulfate (1.0 mg/ml) injection stored in 6-ml polypropylene syringes. A stability study has previously been conducted to assess the stability of neostigmine methylsulfate injection (0.5 mg/ml) stored in polypropylene syringes for 30 days at room temperature and in the dark which concluded that neostigmine methylsulfate was stable for 30 days under the specified

conditions. Another study also concluded that neostigmine methylsulfate (0.2 mg/ml) in 0.9% sodium chloride injection stored in polypropylene syringes for 24 hours at 4°C and 23°C was stable [19]. However, stability information for neostigmine methylsulfate (1.0 mg/ml) injection stored at ambient temperature with exposure to light or under refrigeration for up to 90 days is not available. A previously reported HPLC method for the determination of neostigmine was modified to determine drug content in each sample [20]. In Chapter 3, the stability-indicating HPLC assay employed an underivatized silica column and a phosphate buffer-organic mobile phase with UV detection at 220 nm. Phenol, which was present as a preservative in the injection, did not interfere with the assay.

Stability studies of glycopyrrolate alone or in combination with other drugs have been investigated; however, stability information for glycopyrrolate injection (0.2 mg/ml) stored at ambient temperature with exposure to light or under refrigeration for up to 90 days is not available [14,21,22]. The USP 24 monograph for glycopyrrolate injection was modified to determine the concentration of glycopyrrolate in each sample [23]. Chapter 4 describes a stability-indicating HPLC method for the analysis of glycopyrrolate stored in 6-ml polypropylene syringes at ambient temperature and 4°C. Benzyl alcohol, which was present as a preservative, did not interfere with the assay. The separation was achieved using an underivatized silica column and a phosphate buffer-organic mobile phase with UV detection at 254 nm.

A stability study previously concluded that succinylcholine chloride (20 mg/ml) in 0.9% sodium chloride or dextrose injection stored in polypropylene syringes and protected from light was stable for 107 days at 4°C and for 90 days at 25°C [24].

Another study concluded that succinylcholine chloride injections (10 mg/ml) containing sodium chloride, methyl-4-hydroxybenzoate, hydrochloric acid, and water prepared by a hospital pharmacy were stable for 5 months at 25°C and 23 months at 4°C [25]. Moreover, another study concluded that succinylcholine chloride injection (10 mg/ml) stored in polypropylene syringes was stable for 45 days at 25°C and 4°C [26]. However, stability studies for undiluted succinylcholine chloride injection (20 mg/ml) stored in polypropylene syringes for 90 days at ambient temperature (under continuous fluorescent lighting) and 4°C is not available. The USP 24 HPLC assay for succinylcholine chloride injection was modified to determine the drug concentration in each sample [27]. Chapter 5 addresses the stability of 20 mg/ml succinylcholine chloride injection stored in 12-ml polypropylene syringes at ambient temperature or under refrigeration. Methylparaben, which was present as a preservative, did not interfere with the assay. The separation was achieved using an underivatized silica column and a phosphate buffer-organic mobile phase with UV detection at 214 nm.

The sorption of tubocurarine chloride injection to plastic intravenous fluid bags has been studied; however, stability studies for tubocurarine chloride injection stored in polypropylene syringes for 90 days at ambient temperature and under continuous fluorescent lighting and 4°C are not available [28,29]. Thus, the aim of Chapter 6 was to investigate the stability of 3-mg/ml tubocurarine chloride injection stored in 3-ml polypropylene syringes at ambient temperature or under refrigeration. The USP 24 HPLC assay for tubocurarine chloride injection was modified to determine the drug content in each sample [30]. The final separation was achieved using an underivatized silica column and a phosphate buffer-organic mobile phase with UV detection at 214 nm.

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# CHAPTER 1

# STABILITY OF EPHEDRINE SULFATE AT AMBIENT TEMPERATURE AND 4°C IN POLYPROPYLENE SYRINGES[1]

[1]ML Storms, JT Stewart and FW Warren. 2001. *International Journal of Pharmaceutical Compounding*. 23(2): 1872-1890. Reprinted here with permission of publisher.

**ABSTRACT**

The stability of 5 mg/mL ephedrine sulfate in 0.9% sodium chloride stored in 10 mL polypropylene syringes stored at ambient temperature and 4°C for up to 60 days was investigated. Concentration levels of ephedrine sulfate were determined at 0, 1, 4, 7, 14, 30, 45 and 60 days after preparation of the syringes using a high performance liquid chromatography (HPLC) stability-indicating assay. Benzyl alcohol, which was added as a preservative did not interfere with the assay. The injections in polypropylene syringes were stable for up to 60 days at both ambient temperature and 4°C. The pH of the ephedrine sulfate injections did not change appreciably in a particular direction over the 60-day study period. These data would support the stability of ephedrine sulfate under the storage conditions investigated in this study.

## INTRODUCTION

Ephedrine sulfate (Figure 1.1) is a potent sympathomimetic that stimulates both α and β receptors and has clinical uses related to both actions. This drug has long been used as a pressor agent, particularly during spinal anesthesia when hypotension frequently occurs.[1] Since ephedrine sulfate is not available prepackaged in syringes with a dose of 5 mg/mL, hospital pharmacies meet the need by diluting 50 mg/mL ephedrine sulfate with 0.9% sodium chloride injection and aseptically preloading ephedrine sulfate solutions into sterile syringes and storing the resultant dosage form for short periods at room, refrigerator, or freezer temperatures. However, such storage possesses the potential to adversely affect drug potency. For anesthetics, the Centers for Disease Control and the Anesthesia Patient Safety Foundation suggest that the storage of drugs in syringes be limited to a 24-hour period. While this recommendation for anesthetics is conservative, prefilled syringes would save time for hospital personnel and decrease cost due to wasted materials.[2,3] In addition, the availability of prefilled syringes would also decrease the delay in drawing up ephedrine sulfate in an emergency situation.[4] Since a local hospital pharmacy had the desire to preload polypropylene syringes with ephedrine sulfate, it was important to assess the stability in polypropylene syringes. Stability studies have been conducted using spectrophotometric methods and HPLC; however, stability information at ambient temperature with exposure to light or under refrigeration for up to 60 days is not available.[5-7] Therefore, the purpose of this study was to investigate the stability of 5 mg/mL ephedrine sulfate prepared in polypropylene syringes and stored either at ambient temperature or under refrigeration (4°±1C) for up to 60 days. Each preparation was

assayed for pH and concentration at 0, 1, 4, 7, 14, 30, 45, and 60 days. A stability-indicating HPLC assay was utilized for this study.

## EXPERIMENTAL

### Chemicals and Reagents

All chemicals were HPLC grade (J.T. Baker, Phillipsburg, NJ). The ephedrine sulfate injection was from commercial lots (200148 and 160376, Ben Venue Lab, Inc., Bedford, OH).

### Equipment

The HPLC chromatographic system consisted of a pump (Model 760, Micromeritics, Norcross, GA), an autosampler (Model 728, Micromeritics, Norcross, GA) equipped with a 50 µL loop, an ultraviolet variable wavelength detector (Model 757, Kratos, Ramsey, NJ) set at 254 nm, and a column (Spherisorb - ODS, 25cm, 4.6mm i.d. 2.5 µm, Alltech, Deerfield, IL). The peak heights were monitored using a Hewlett-Packard Model 3395 integrator (Avondale, PA).

### Chromatographic Conditions

The isocratic elution was performed at 1.0 mL/min with a solution containing acetonitrile – 0.1 M potassium dihydrogen phosphate buffer at pH 2.5 (0.005 M heptanesulfonic acid) (30:70 v/v). The mobile phase was filtered through a 0.45 - µm polymeric membrane filter (Alltech, Deerfield, IL).

### Preparation of Injection for Stability Studies

A pooled sample of 5 mg/mL ephedrine sulfate was prepared by combining 430 mL of 0.9% sodium chloride injection (Lots 66558DK and 66555DK, Abbott Labs, Chicago, IL) with 43 mL of ephedrine sulfate injection (50 mg/mL) in a suitably sized beaker.

After thorough mixing, 30 mL of the solution was removed for 0 hour assay and the remaining 443 mL was divided into 42-10 mL portions by drawing 10 cc into each of 42 polypropylene syringes (Becton Dickinson & Co, Franklin Lake, NJ). The syringes were divided into two groups of 21 syringes each; one group was stored in the refrigerator at 4°C (±1°C) and the other was stored at 25°C ( ± 1°C) under continuous fluorescent lighting. On day 0, the pooled sample was assayed and the pH value was measured by a calibrated pH meter. Drug content in each sample was determined by a stability-indicating high performance liquid chromatographic (HPLC) assay on days 1, 4, 7, 14, 30, 45, and 60 and the pH values were also recorded.

**Preparation of Standard Solutions**

A 1.0 mg quantity of ephedrine sulfate was accurately weighed and added to a 5 mL test tube and 1 mL mobile phase added to give a concentration of 1.0 mg/mL. A 1:20 dilution was made to result in an ephedrine sulfate concentration of 50 µg/mL. Injections of 50 µL were made into the HPLC system for calculation of the response factor (RF) for ephedrine sulfate. The solution was prepared fresh prior to syringe sampling times on the respective days.

**Degradation of Ephedrine Sulfate**

To 1 mL of ephedrine sulfate (1mg/mL), 1 mL of 0.1N HCl was added. The mixture was heated for 10 minutes. It was then cooled, neutralized, diluted to 50 µg/mL and injected into the HPLC. In another experiment, a 1 mL quantity of 0.1N HCl was substituted with 1 mL of 0.1N NAOH solution. In addition, 3% $H_2O_2$ was added to 1 mL of ephedrine sulfate (1.0 mg/mL) for 1 hour and a brief, gentle heating stopped the reaction. The solution was diluted to 50 µg/mL and injected into the HPLC.

**Preparation of Assay Solutions**

A 5 mL aliquot was removed from each of 3 syringes stored at ambient and 4°C at 1, 4, 7, 14, 30, 45, and 60 days. A 1:100 dilution was made using mobile phase as diluent.

**Calculation of Medication Content**

Triplicate injections of analytical samples prepared from syringe mixtures and five injections of the standard solution were made into the HPLC system. Mean response factors (MRF) were calculated for each drug standard using peak heights from the chromatogram. The drug concentration in each analytical sample was then calculated using the appropriate MRF and the peak height of the analyte in the syringe and standard samples. The following calculations were made to determine the drug concentration in each analytical sample:

(A) RF = Drug Standard (mg/mL) ÷ Drug Peak Height of Standard

(B) Calculate Mean RF (MRF) based on 5 replicates of the standard.

(C) Drug Concentration (mg/mL) = MRF x Drug Peak Height of Sample.

**RESULTS**

A previously reported HPLC assay was modified for the determination of ephedrine sulfate and procedures were followed to ensure that this assay was stability-indicating.[8] For example, ephedrine sulfate was forced to degrade under acidic, basic, and oxidative conditions. The objective was to force degradation to observe a 10-30% loss of active when compared with the nondegraded ephedrine sulfate.[9] This target range resulted in small degradation peaks at retention times of 6.4 and 10.2 minutes; however, they did not interfere with the ephedrine sulfate peak which eluted at 2.76 minutes. A typical HPLC chromatogram is shown in Figure 1.2.

The ephedrine sulfate injections stored in 10 mL polypropylene syringes were judged to be stable if the drug levels remained > 90% of initial concentrations at the time of preparation.[10] Concentrations (percentage of initial concentration remaining) of ephedrine sulfate are shown in Table 1.1. The data indicated that the ephedrine sulfate stored at both ambient temperature and 4°C remained > 90% of the initial concentration for up to 60 days. In addition to monitoring concentration, the pH of the syringe injections did not change appreciably in a particular direction over the study period. Typically, the pH of the samples ranged from 5.01-5.10. Based on the student's t-test and the assumption that the concentrations are normally distributed at both temperatures, the null hypothesis that there is no evidence that temperature affects the drug concentration or pH is retained.

**CONCLUSION**

These data would support the stability of ephedrine sulfate (5 mg/mL) in 0.9% sodium chloride stored at ambient temperature and under continuous lighting as well as at 4°C over a 60-day period in Becton Dickinson 10 mL polypropylene syringes.

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4. Ducat CM, Merry AF, Webster CS. Attitudes and Practices of New Zealand Anaessthetists with Regard to Emergency Drugs. *Anaesthetic Intensive Care* 2000; 28: 692-697.

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10. Trissel LA. Avoiding Common Flaws in Stability and Compatibility Studies of Injectable Drugs. *Am. J. Hosp. Pharm*. 1983; 40:1159-1160. Editorial.

Table 1.1. Assay results of ephedrine sulfate stored at ambient temperature and 4°C for 60 days in 10 mL polypropylene syringes. *

| Time (Days) | Percent of the Label Claim Based on 100% on Day Zero | | %RSD (n=9) |
|---|---|---|---|
| | 25°C | 4°C | |
| 1 | 100.4 | 97.3 | 1.1/1.1 |
| 4 | 100.3 | 97.3 | 0.5/1.7 |
| 7 | 98.9 | 98.6 | 2.0/2.1 |
| 14 | 98.0 | 98.0 | 1.0/1.9 |
| 30 | 98.0 | 97.8 | 1.7/2.5 |
| 45 | 98.5 | 98.5 | 1.3/2.5 |
| 60 | 97.6 | 97.1 | 2.1/1.6 |

* The injections remained clear and the pH value (5.0) did not change significantly throughout the study. The initial assay indicated that the concentration was 5.11 mg/mL.

RSD, Relative Standard Deviation

Figure 1.1. Chemical structure of ephedrine sulfate.

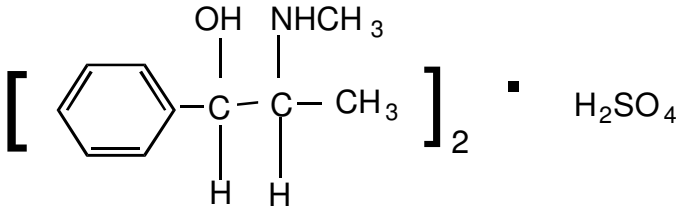
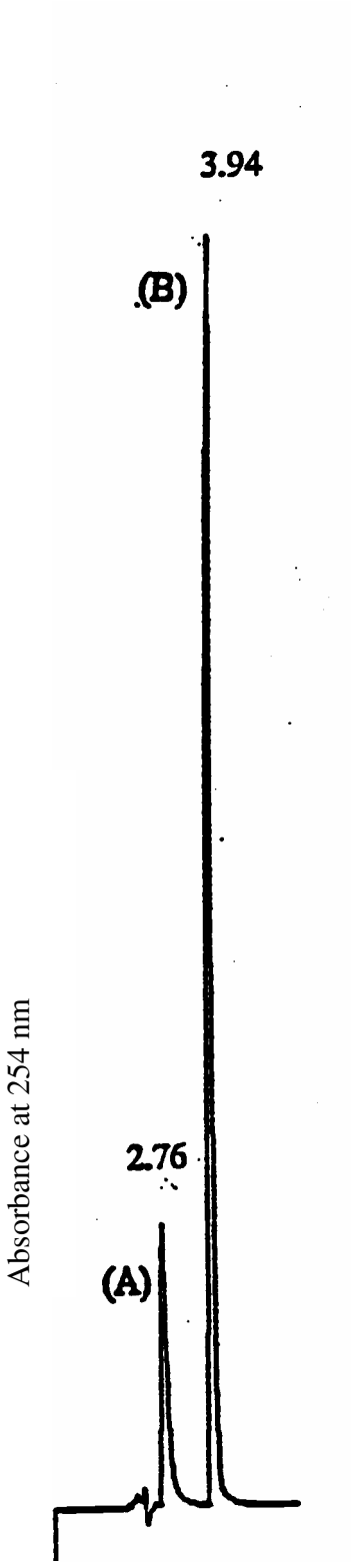

OH
NHCH 3
C
C
CH 3
H
H
2
H2SO4

Figure 1.2. Typical HPLC chromatogram of ephedrine sulfate (A) and benzyl alcohol (B) on a $C_{18}$ column.


3.94
(B)
2.76
(A)
Absorbance at 254 nm
Retention Time, minutes

# CHAPTER 2

# STABILITY OF LIDOCAINE HYDROCHLORIDE INJECTION AT AMBIENT TEMPERATURE AND 4°C IN POLYPROPYLENE SYRINGES[2]

[2]ML Storms, JT Stewart and FW Warren. Accepted to the *International Journal of Pharmaceutical Compounding*, 2001. Reprinted here with permission of publisher.

**Abstract**

The stability of 20 mg/mL lidocaine hydrochloride injection in 12 mL polypropylene syringes stored at ambient temperature and 4°C for up to 90 days was investigated. Concentration levels of lidocaine hydrochloride injection were determined at 0,1, 4, 7, 15, 30, 45, 60 and 90 days after preparation of the syringes using a high-performance liquid chromatography (HPLC) stability indicating assay. Methylparaben, which was present as a preservative did not interfere with the assay. The injections in plastic syringes were stable for up to 90 days at both ambient temperature and 4°C. The pH of lidocaine hydrochloride injection did not change appreciably in a particular direction over the 90-day study period. These data would support the stability of lidocaine hydrochloride injection under the storage conditions investigated in this study.

**Introduction**

Lidocaine (2-diethylamino-2', 6', -xylilide) (Figure 2.1) is a local anesthetic and is an effective antiarrhythmic when give parenterally.[1] Methods for the determination of lidocaine hydrochloride in pharmaceutical dosage forms are typically based on spectrophotometry, high-performance thin-layer chromatography (HPTLC), TLC-densitometry, and HPLC.[2] Since lidocaine hydrochloride injection is not available prepackaged in syringes with 20 mg/mL, hospital pharmacies meet the need by aseptically preloading lidocaine hydrochloride injection into sterile syringes and storing the resultant dosage form for short periods at room, refrigerator, or freezer temperatures. Although such storage possesses the potential to adversely affect drug potency, prefilled syringes would save time for hospital personnel and decrease cost due to wasted materials.[3-5] Since a local pharmacy had the desire to preload polypropylene syringes with lidocaine hydrochloride injection, it was important to assess the stability in polypropylene syringes. A stability study has been conducted to assess the stability of lidocaine hydrochloride injection (0.2 mg/mL) in 0.9% sodium chloride injection stored in polypropylene syringes for 24 hours at room temperature and in the dark; however, stability information for lidocaine hydrochloride injection (20 mg/mL) stored at ambient temperature with exposure to light or under refrigeration for up to 90 days is not available.[6] Thus, the aim of this study was to investigate the stability of 20 mg/mL lidocaine hydrochloride injection prepared in polypropylene syringes. The syringes were prepared at ambient temperature (23±1°C) and were stored either at ambient temperature or under refrigeration (4°C) for up to 90 days. Each preparation was assayed for drug concentration and pH was measured at 0, 1, 4, 7, 15, 30, 45, 60 and 90 days. The USP 24

HPLC assay for lidocaine hydrochloride injection was modified to determine the lidocaine concentration in each sample.[7]

**Methods**

**Chemicals and Reagents**

All chemicals were HPLC grade (J.T. Baker, Phillipsburg, NJ). The lidocaine hydrochloride injection was from lot 69412DK (Abbott Labs, North Chicago, IL). Lidocaine was purchased from Sigma Chemical Company (St. Louis, MO) and the United States Pharmacopeial Convention, Inc. (Rockville, MD).

**Equipment**

The HPLC chromatographic system consisted of a pump (Model 760, Micromeritics, Norcross, GA), an autosampler (Model 728, Micromeritics, Norcross, GA) equipped with a 50 µL loop, an ultraviolet variable wavelength detector (Model 757, Kratos, Ramsey, NJ) set at 254 nm, and a column (silica, 25 cm, 4.6 mm i.d., 3µm, Phenomenex, Torrance, CA). The peak heights were monitored using a Hewlett-Packard Model 3395 integrator (Avondale, PA)

**Chromatographic Conditions**

The isocratic elution was performed at 0.85mL/min with a mobile consisting of methanol – 10 mM potassium dihydrogen phosphate buffer at pH 3.0 (50:50 v/v). The mobile phase was filtered through a 0.45-µm polymeric membrane filter (Alltech, Deerfield, IL).

**Preparation of Injection for Stability Studies**

A pooled sample of 20 mg/mL lidocaine hydrochloride injection was prepared by adding 500 mL lidocaine hydrochloride injection (20 mg/mL) to a suitably sized beaker.

After thorough mixing, 20 mL of the solution was removed for the zero hour assay and the remaining 480 mL was divided into 48-10 mL portions by drawing 10 mL into each of 48 polypropylene syringes (Becton Dickinson, Franklin Lakes, NJ). The syringes were divided into two sets of 24 syringes each; one set was stored in the refrigerator at 4°C (±1°C) and the other set was stored at 25°C (±1°C) under continuous fluorescent lighting. On day zero, the pooled sample was assayed and the pH was measured by a calibrated pH meter. Drug content in each sample was determined by a stability-indicating high-performance liquid chromatography (HPLC) assay on days 1, 4, 7, 15, 30, 45, 60, and 90 and the pH values were also recorded.

**Preparation of Standard Solutions**

A 9.5 mg quantity of lidocaine (equivalent to 10 mg lidocaine hydrochloride) purchased from Sigma Chemical Co. (St. Louis, MO) was added to a 5 mL test tube with 1.0 mL mobile phase added to give a concentration of 9.5 mg/mL lidocaine. A 1:500 dilution was made to result in a lidocaine concentration of 19 µg/mL (equivalent to 20 µg/mL lidocaine hydrochloride). Injections of 50 µL were made into the HPLC system for calculation of the response factor (RF) for lidocaine hydrochloride. The solution was prepared fresh prior to syringe sampling times on the respective days.

The same standard preparation procedure was followed for lidocaine purchased from the United States Pharmacopeial Convention, Inc. (Rockville, MD) to ensure that the two standard solutions were equivalent.

**Degradation of Lidocaine Hydrochloride**

Lidocaine was forced to degrade under acidic, basic, and oxidative conditions.[8] Tin one experiment, 0.1 N HCl (1 mL) was added to 1 mL of lidocaine hydrochloride (1mg/ mL). The mixture was heated for 15 minutes. It was then cooled, neutralized, diluted to 20 µg/mL lidocaine hydrochloride and injected into the HPLC. In another experiment, a 1 mL quantity of 0.1 N HCl was substituted with 1 mL of 0.1N NaOH solution. In addition, 3% $H_2O_2$ was added to 1 mL of lidocaine hydrochloride (1 mg/mL) for 1 hour and a brief, gentle heating stopped the reaction. The solution was diluted to 20 µg/mL and injected into the HPLC.

**Preparation of Assay Solutions**

A 5 mL aliquot was removed from each of 3 syringes stored at ambient and 4°C at 1, 4, 7, 15, 30, 45, 60 and 90 days. A 1:1000 dilution was made using mobile phase as diluent.

**Calculation of Medication Content**

Triplicate injections of analytical samples prepared from syringe mixtures and five injections of the standard solution were made into the HPLC system. Mean response factors (MRF) were calculated for each drug standard using peak heights from the chromatograms. The drug concentration in each analytical sample was then calculated using the appropriate MRF and the peak height of the analyte in the syringe and standard samples. The drug concentration in each analytical sample was then calculated using the following equations:

(A) RF = Drug Standard (mg/mL) ÷ Drug Peak Height of Standard

(B) Calculate Mean RF (MRF) based on 5 replicates of the standard

(C) Drug Concentration (mg/mL) = MRF x Drug Peak Height of Sample

**Results**

The objective of the stability studies under the specified conditions was to force degradation to observe a 10-30% loss of active compound when compared with the nondegraded lidocaine hydrochloride.[8] This target range resulted in a small degradation peak at 4.9 min, however, it did not interfere with the lidocaine hydrochloride peak, which eluted at 8.56 min. A typical HPLC chromatogram of the preservative ($t_r$ = 3.5 min) and lidocaine ($t_r$ = 3.5 min) is shown in Figure 2.2.

The lidocaine hydrochloride injections stored in 12 mL polypropylene syringes were judged to be stable if the drug levels remained >90% of the initial concentration at the time of preparation.[9] Concentrations (percentage of initial concentration remaining) of lidocaine hydrochloride injection are shown in Table 2.1. The data indicated that the lidocaine hydrochloride injection stored at both ambient temperature and 4°C remained >90% of the initial concentration for up to 90 days. In addition to monitoring concentration, the pH of the syringe injections did not change appreciably in a particular direction over the study period. Typically, the pH of the samples ranged from 5.82-5.89. Based on the student's t-test and the assumption that the concentration and pH values are normally distributed at both temperatures, the null hypothesis that there is no evidence that temperature affects the drug concentration or pH is retained.

## Conclusion

These data would support the stability of lidocaine hydrochloride injection (20 mg/mL) stored at ambient temperature and under continuous fluorescent lighting as well as at 4°C over a 90-day period in 12 mL polypropylene syringes.

**References**


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Table 2.1. Assay results of lidocaine hydrochloride injection stored at ambient temperature and 4°C for 90 days in 12-mL polypropylene syringes.*

| Time (Days) | Percent of the Label Claim Based on 100% on Day Zero | |
|---|---|---|
| | 25°C (%RSD)+ | 4°C (%RSD)+ |
| 1 | 100.8 (0.5) | 100.5 (0.9) |
| 4 | 100.8 (0.4) | 101.3 (0.9) |
| 7 | 100.9 (0.5) | 100.7 (0.2) |
| 15 | 100.0 (0.1) | 100.2 (0.3) |
| 30 | 100.6 (0.3) | 100.0 (0.3) |
| 45 | 100.3 (0.2) | 99.8 (0.2) |
| 60 | 99.5 (0.4) | 99.8 (0.3) |
| 90 | 99.7 (0.1) | 99.8 (0.3) |

*The injections remained clear and the pH value (5.84) did not change significantly throughout the study. The initial assay indicated that the concentration was 19.82 mg/mL.

+RSD, Relative Standard Deviation (Based on n=9)

Figure 2.1. Chemical structure of lidocaine hydrochloride.

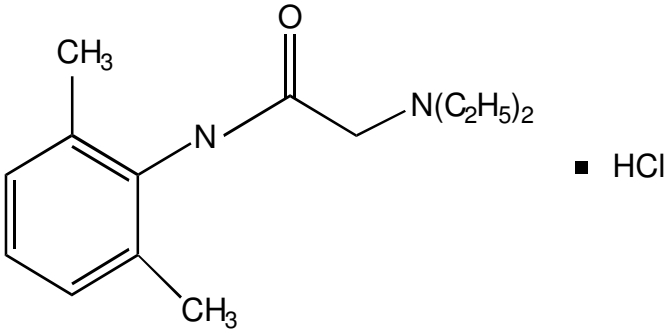
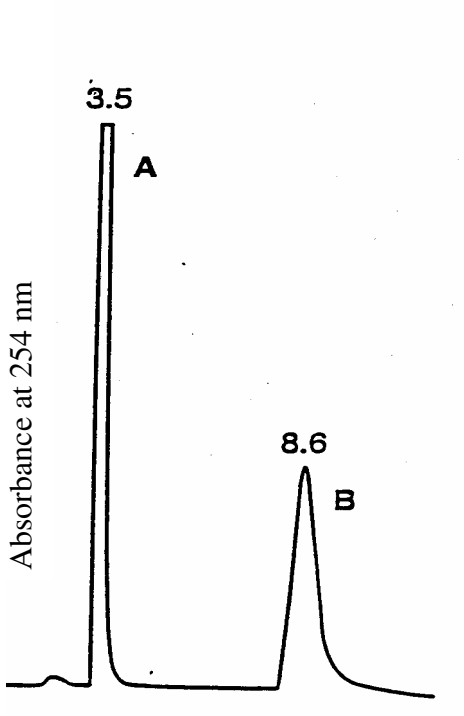

O
CH3
N(C2H5)2
N
HCl
CH3

Figure 2.2. Typical HPLC chromatogram of methylparaben (A) and lidocaine (B) on a silica column.


3.5
A
8.6
B
Absorbance at 254 nm
Retention Time, minutes

# CHAPTER 3

# STABILITY OF NEOSTIGMINE METHYLSULFATE INJECTION AT AMBIENT TEMPERATURE AND 4°C IN POLYPROPYLENE SYRINGES[3]

[3]ML Storms, JT Stewart and FW Warren. Accepted to the *International Journal of Pharmaceutical Compounding*, 2001. Reprinted here with permission of publisher.

**Abstract**

The stability of 1.0 mg/mL neostigmine methylsulfate injection in 6 mL polypropylene syringes stored at ambient temperature and 4°C for up to 90 days was investigated. Concentration levels of neostigmine methylsulfate injection were determined at 0,1, 4, 7, 15, 30, 45, 60 and 90 days after preparation of the syringes using a high-performance liquid chromatography (HPLC) stability indicating assay. Phenol, which was present as a preservative in the injection did not interfere with the assay. The injections in plastic syringes were stable for up to 90 days at both ambient temperature and 4°C. The pH of neostigmine methylsulfate injection did not change appreciably in a particular direction over the 90-day study period. These data would support the stability of neostigmine methylsulfate injection under the storage conditions investigated in this study.

## Introduction

Neostigmine methylsulfate (Figure 3.1) competes with acetylcholine for attachment to acetylcholinesterase at sites of cholinergic transmission to inhibit the hydrolysis of acetylcholine. It is indicated for the prevention and treatment of postoperative distention and urinary retention and reverses the effects of nondepolarizing neuromuscular blocking agents (e.g. tubocurarine, metocurine, etc.).[1] Since neostigmine methylsulfate injection is not available prepackaged in syringes at 1.0 mg/mL, a local hospital meets the need by aseptically preloading neostigmine methylsulfate injection into sterile polypropylene syringes and storing the resultant dosage form for short periods at room, refrigerator, or freezer temperatures. Although such storage possesses the potential to adversely affect drug potency, prefilled syringes would save time for hospital personnel and decrease cost due to wasted materials.[2-4] Thus, it was important to assess the stability of undiluted neostigmine methylsulfate injection in polypropylene syringes. A stability study has been conducted to assess the stability of neostigmine methylsulfate injection (0.5 mg/mL) stored in polypropylene syringes for 30 days at room temperature and in the dark which concluded that neostigmine methylsulfate injection was stable for 30 days under the specified conditions. Another study also concluded that neostigmine methylsulfate (0.2 mg/mL) in 0.9% sodium chloride injection stored in polypropylene syringes for 24 hours at 4°C and 23°C was stable.[5] However, stability information for neostigmine methylsulfate injection (1.0 mg/mL) stored at ambient temperature with exposure to light or under refrigeration for up to 90 days is not available.

Thus, the aim of this study was to investigate the stability of 1.0 mg/mL neostigmine methylsulfate injection prepared in polypropylene syringes. The syringes were prepared

at ambient temperature (23±1°C) and were stored either at ambient temperature or under refrigeration (4°C) for up to 90 days. Each preparation was assayed for drug concentration and pH measured at 0, 1, 4, 7, 15, 30, 45, 60 and 90 days. A previously reported HPLC method for the determination of neostigmine was modified to determine drug content in each sample.[6]

**Methods**

**Chemicals and Reagents**

All chemicals were HPLC grade (J.T. Baker, Phillipsburg, NJ). The neostigmine methylsulfate injection was from lot 302936 (American Pharmaceutical Partners, Inc., Los Angeles, CA). Neostigmine bromide was purchased from Sigma Chemical Company (St. Louis, MO) and neostigmine methylsulfate reference standard was purchased from the United States Pharmacopeial Convention, Inc. (Rockville, MD).

**Equipment**

The HPLC chromatographic system consisted of a pump (Model 760, Micromeritics, Norcross, GA), an autosampler (Model 728, Micromeritics, Norcross, GA) equipped with a 50 µL loop, an ultraviolet variable wavelength detector (Model 757, Kratos, Ramsey, NJ) set at 220 nm, and a column (silica, 25 cm, 4.6 mm i.d., 3µm, Phenomenex, Torrance, CA). The peak heights were monitored using a Hewlett-Packard Model 3395 integrator (Avondale, PA)

**Chromatographic Conditions**

The isocratic elution was performed at 0.85mL/min with a mobile phase consisting of acetonitrile – 10 mM potassium dihydrogen phosphate buffer at pH 3.0 (80:20 v/v). The

mobile phase was filtered through a 0.45-µm polymeric membrane filter (Alltech, Deerfield, IL).

**Preparation of Injection for Stability Studies**

A pooled sample of 1.0 mg/mL neostigmine methylsulfate injection (Figure 1) was prepared by adding 250 mL neostigmine methylsulfate injection (1 mg/mL) to a suitably sized beaker. After thorough mixing, 10 mL of the solution was removed for the zero hour assay and the remaining 240 mL was divided into 48-5 mL portions by drawing 5 mL into each of 48 polypropylene syringes (Becton Dickinson, Franklin Lakes, NJ). The syringes were divided into two sets of 24 syringes each; one set was stored in the refrigerator at 4°C (±1°C) and the other set was stored at 25°C (±1°C) under continuous fluorescent lighting. On day zero, the pooled sample was assayed and the pH was measured by a calibrated pH meter. Drug content in each sample was determined by a stability-indicating high-performance liquid chromatography (HPLC) assay on days 1, 4, 7, 15, 30, 45, 60, and 90 and the pH values were also recorded.

**Preparation of Standard Solutions**

A 0.91 mg quantity of neostigmine bromide (equivalent to 1 mg neostigmine methylsulfate) purchased from Sigma Chemical Co. (St. Louis, MO) was added to a 5 mL test tube with 1.0 mL mobile phase added to give a concentration of 1mg/mL neostigmine methylsulfate. A 1:16 dilution was made to result in a neostigmine methylsulfate concentration of 62.5 µg/mL. Injections of 50 µL were made into the HPLC system for calculation of the response factor (RF) for neostigmine methylsulfate. The solution was prepared fresh prior to syringe sampling times on the respective days.

The same standard preparation procedure was followed for neostigmine methylsulfate reference standard purchased from the United States Pharmacopeial Convention, Inc. (Rockville, MD) to ensure that the two standard solutions were equivalent.

**Degradation of Neostigmine Methylsulfate**

To ensure that the HPLC assay was stability-indicating, 1 mL of 0.1 N HCl was added to 1 mL of neostigmine methylsulfate (1 mg/mL). The mixture was heated for 15 minutes. It was then cooled, neutralized, diluted to 62.5 µg/mL neostigmine methylsulfate and injected into the HPLC. In another experiment, a 1 mL quantity of 0.1 N HCl was substituted with 1 mL of 0.1N NaOH solution. In addition, 3% $H_2O_2$ was added to 1 mL of neostigmine methylsulfate (1 mg/mL) for 1 hour and a brief, gentle heating stopped the reaction. The solution was diluted to 62.5 µg/mL and injected into the HPLC.

**Preparation of Assay Solutions**

A 5 mL aliquot was removed from each of 3 syringes stored at ambient and 4°C at 1, 4, 7, 15, 30, 45, 60 and 90 days. A 1:16 dilution was made using mobile phase as diluent.

**Calculation of Medication Content**

Triplicate injections of analytical samples prepared from syringe mixtures and five injections of the standard solution were made into the HPLC system. Mean response factors (MRF) were calculated for each drug standard using peak heights from the chromatograms. The drug concentration in each analytical sample was then calculated using the appropriate MRF and the peak height of the analyte in the syringe and standard samples. The following calculations were made to determine the drug concentration in each analytical sample:

(A) RF = Drug Standard (mg/mL) ÷ Drug Peak Height of Standard

(B) Calculate Mean RF (MRF) based on 5 replicates of the standard

(C) Drug Concentration (mg/mL) = MRF x Drug Peak Height of Sample

**Results**

Neostigmine methylsulfate was forced to degrade under acidic, basic, and oxidative conditions. The objective was to force degradation to observe a 10-30% loss of active when compared with the nondegraded neostigmine methylsulfate.[7] This target range resulted in a small degradation peak at 1.9 min, however, it did not interfere with the neostigmine methylsulfate peak, which eluted at 13.6 min. A typical HPLC chromatogram of neostigmine methylsulfate and the preservative, phenol, is shown in Figure 3.2.

The neostigmine methylsulfate injections stored in 6 mL polypropylene syringes were judged to be stable if the drug levels remained >90% of the initial concentration at the time of preparation.[8] Concentrations (percentage of initial concentration remaining) of neostigmine methylsulfate injection are shown in Table 3.1. The data indicated that the neostigmine methylsulfate injection stored at both ambient temperature and 4°C remained >90% of the initial concentration for up to 90 days. In addition to monitoring concentration, the pH of the syringe injections did not change appreciably in a particular direction over the study period. Typically, the pH of the samples ranged from 5.18-5.28. Based on the student's t-test and the assumption that the concentration and pH values are normally distributed at both temperatures, the null hypothesis that there is no evidence that temperature affects the drug concentration or pH is retained.

## Conclusion

These data would support the stability of neostigmine methylsulfate injection (1 mg/mL) stored at ambient temperature and under continuous fluorescent lighting as well as at 4°C over a 90-day period in 6 mL polypropylene syringes.

**References**


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Table 3.1. Assay results of neostigmine methylsulfate injection stored at ambient temperature and 4°C for 90 days in 6-mL polypropylene syringes.*

| Time (Days) | Percent of the Label Claim Based on 100% on Day Zero | | %RSD+ (n=9) |
|---|---|---|---|
| | 25°C | 4°C | |
| 1 | 101.5 | 100.0 | 0.8/0.5 |
| 4 | 100.5 | 101.3 | 0.7/1.2 |
| 7 | 100.5 | 100.5 | 0.8/1.0 |
| 15 | 101.6 | 101.8 | 1.7/1.1 |
| 30 | 100.1 | 100.7 | 0.7/0.7 |
| 45 | 100.1 | 101.2 | 1.0/0.8 |
| 60 | 100.6 | 101.1 | 1.0/0.8 |
| 90 | 98.2 | 98.8 | 1.6/1.1 |

*The injections remained clear and the pH value (5.22) did not change significantly throughout the study. The initial assay indicated that the concentration was 0.99 mg/mL.

+RSD, Relative Standard Deviation

Figure 3.1. Chemical structure of neostigmine methylsulfate.

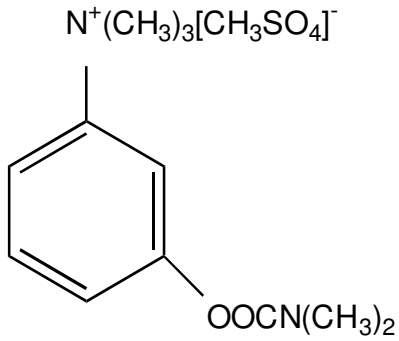
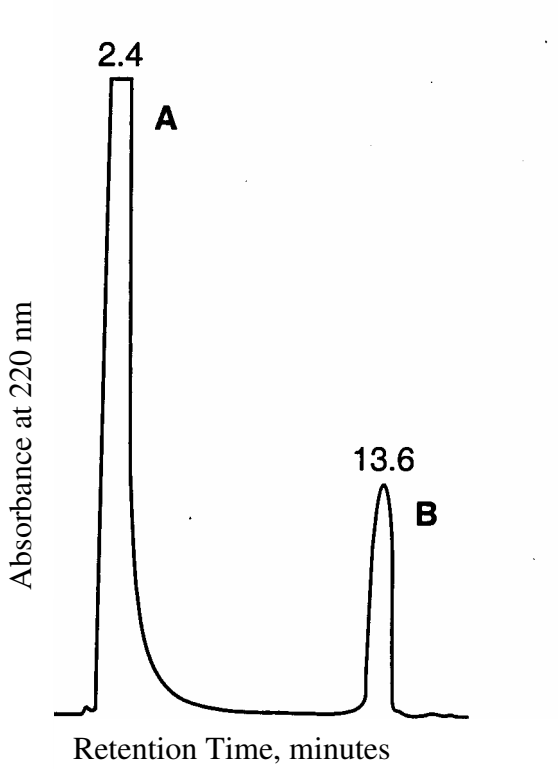

N+(CH3)3[CH3SO4]-
OOCN(CH3)2

Figure 3.2. Typical HPLC chromatogram of phenol (A) and neostigmine methylsulfate (B) on a silica column.


2.4
A
13.6
B
Absorbance at 220 nm
Retention Time, minutes

# CHAPTER 4

# STABILITY OF GLYCOPYRROLATE INJECTION AT AMBIENT TEMPERATURE AND 4°C IN POLYPROPYLENE SYRINGES[4]

[4]ML Storms, JT Stewart and FW Warren. Accepted to the *International Journal of Pharmaceutical Compounding,* 2001. Reprinted here with permission of publisher.

**Abstract**

The stability of 0.2 mg/mL glycopyrrolate injection in 6 mL polypropylene syringes stored at ambient temperature and 4°C for up to 90 days was investigated. Concentration levels of glycopyrrolate injection were determined at 0,1, 4, 7, 15, 30, 45, 60 and 90 days after preparation of the syringes using a high-performance liquid chromatography (HPLC) stability indicating assay. Benzyl alcohol, which was present as a preservative, did not interfere with the assay. The injections in plastic syringes were stable for up to 90 days at both ambient temperature and 4°C. The pH of glycopyrrolate injection did not change appreciably in a particular direction over the 90-day study period. These data would support the stability of glycopyrrolate injection under the storage conditions investigated in this study.

## Introduction

Robinul® Injectable (glycopyrrolate) is a synthetic anticholinergic agent. Glycopyrrolate inhibits salivation and excessive secretions of the respiratory tract when utilized preoperatively and is indicated for the treatment of peptic ulcer when rapid anticholinergic effect is desired.[1] Since glycopyrrolate injection is not available prepackaged in syringes with 0.2 mg/mL, hospital pharmacies meet the need by aseptically preloading glycopyrrolate injection into sterile syringes and storing the resultant dosage form at room, refrigerator, or freezer temperatures. Although such storage possesses the potential to adversely affect drug potency, prefilled syringes would save time for hospital personnel and decrease cost due to wasted materials.[2-4] Since a local pharmacy had the desire to preload polypropylene syringes with glycopyrrolate injection, it was important to assess the stability in polypropylene syringes. Stability studies of glycopyrrolate alone or in combination with other drugs have been investigated; however, stability information for glycopyrrolate injection (0.2 mg/mL) stored at ambient temperature with exposure to light or under refrigeration for up to 90 days is not available.[5-7] Thus, the aim of this study was to investigate the stability of 0.2 mg/mL glycopyrrolate injection prepared in polypropylene syringes. The syringes were prepared at ambient temperature (23±1°C) and were stored either at ambient temperature or under refrigeration (4°C) for up to 90 days. Each preparation was assayed for drug concentration at 0, 1, 4, 7, 15, 30, 45, 60 and 90 days and the pH was measured on each day. The USP 24 monograph for glycopyrrolate injection was modified to determine the glycopyrrolate injection concentration in each sample.[8]

## Methods

### Chemicals and Reagents

All chemicals were HPLC grade (J.T. Baker, Phillipsburg, NJ). The glycopyrrolate injection was from lot 109174 (A.H. Robbins Co., Richmond, VA). Glycopyrrolate reference standard (Lot G) was purchased from the United States Pharmacopeial Convention, Inc. (Rockville, MD).

### Equipment

The HPLC chromatographic system consisted of a pump (Model 760, Micromeritics, Norcross, GA), an autosampler (Model 728, Micromeritics, Norcross, GA) equipped with a 50 µL loop, an ultraviolet variable wavelength detector (Model 757, Kratos, Ramsey, NJ) set at 222 nm, and a column (silica, 25 cm, 4.6 mm i.d., 3µm, Phenomenex, Torrance, CA). The peak heights were monitored using a Hewlett-Packard Model 3395 integrator (Avondale, PA)

### Chromatographic Conditions

The isocratic elution was performed at 0.85mL/min with a mobile consisting of methanol – 10 mM potassium dihydrogen phosphate buffer at pH 3.0 (50:50 v/v). The mobile phase was filtered through a 0.45-µm polymeric membrane filter (Alltech, Deerfield, IL).

### Preparation of Injection for Stability Studies

A pooled sample of 0.2 mg/mL glycopyrrolate injection (Figure 4.1) was prepared by adding 200 mL glycopyrrolate injection (0.2 mg/mL) to a suitably sized beaker. After thorough mixing, 8 mL of the solution was removed for the zero hour assay and the remaining 192 mL was divided into 48-4 mL portions by drawing 4 mL into each of 48

polypropylene syringes (Becton Dickinson, Franklin Lakes, NJ). The syringes were divided into two sets of 24 syringes each; one set was stored in the refrigerator at 4°C (±1°C) and the other set was stored at 25°C (±1°C) under continuous fluorescent lighting. On day zero, the pooled sample was assayed and the pH was measured by a calibrated pH meter. Drug content in each sample was determined by a stability-indicating high-performance liquid chromatography (HPLC) assay on days 1, 4, 7, 15, 30, 45, 60, and 90 and the pH values were also recorded.

**Preparation of Standard Solutions**

A 0.3 mg quantity of glycopyrrolate was added to a 5 mL test tube with 1.0 mL mobile phase added to give a concentration of 0.3 mg/mL glycopyrrolate. A 1:6 dilution was made to result in a glycopyrrolate concentration of 50 µg/mL. Injections of 50 µL were made into the HPLC system for calculation of the response factor (RF) for glycopyrrolate. The solution was prepared fresh prior to syringe sampling times on the respective days.

**Degradation of Glycopyrrolate**

To ensure the HPLC assay was stability-indicating, 1 mL of 0.1 N HCl was added to 1 mL of glycopyrrolate (1 mg/mL). The mixture was heated for 15 minutes. It was then cooled, neutralized, diluted to 50 µg/mL glycopyrrolate and injected into the HPLC. In another experiment, a 1 mL quantity of 0.1 N HCl was substituted with 1 mL of 0.1N NaOH solution. In addition, 3% $H_2O_2$ was added to 1 mL of glycopyrrolate (1 mg/mL) for 1 hour and a brief, gentle heating stopped the reaction. The solution was diluted to 50 µg/mL and injected into the HPLC.

**Preparation of Assay Solutions**

A 4 mL aliquot was removed from each of 3 syringes stored at ambient and 4°C at 1, 4, 7, 15, 30, 45, 60 and 90 days. A 1:4 dilution was made using mobile phase as diluent.

**Calculation of Medication Content**

Triplicate injections of analytical samples prepared from syringe mixtures and five injections of the standard solution were made into the HPLC system. Mean response factors (MRF) were calculated for each drug standard using peak heights from the chromatograms. The drug concentration in each analytical sample was then calculated using the appropriate MRF and the peak height of the analyte in the syringe and standard samples. The following calculations were made to determine the drug concentration in each analytical sample:

(A) RF = Drug Standard (mg/mL) ÷ Drug Peak Height of Standard

(B) Calculate Mean RF (MRF) based on 5 replicates of the standard

(C) Drug Concentration (mg/mL) = MRF x Drug Peak Height of Sample

**Results**

Glycopyrrolate was forced to degrade under acidic, basic, and oxidative conditions. The objective was to force degradation to observe a 10-30% loss of active when compared with the nondegraded glycopyrrolate.[9] A typical HPLC chromatogram of glycopyrrolate and the preservative, benzyl alcohol, is shown in Figure 4.2.

The glycopyrrolate injections stored in 6 mL polypropylene syringes were judged to be stable if the drug levels remained >90% of the initial concentration at the time of preparation.[10] Concentrations (percentage of initial concentration remaining) of glycopyrrolate injection are shown in Table 4.1. The data indicated that the

glycopyrrolate injection stored at both ambient temperature and 4°C remained >90% of the initial concentration for up to 90 days. In addition to monitoring concentration, the pH of the syringe injections did not change appreciably in a particular direction over the study period. Typically, the pH of the samples ranged from 2.34-2.43. Based on the student's t-test and the assumption that the concentration and pH values are normally distributed at both temperatures, the null hypothesis that there is no evidence that temperature affects the glycopyrrolate injection concentration or pH is retained.

**Conclusion**

These data would support the stability of glycopyrrolate injection (0.2 mg/mL) stored at ambient temperature and under continuous fluorescent lighting as well as at 4°C over a 90-day period in 6 mL polypropylene syringes.

**References**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4. Casasin ET, Roca-Mason A, Soy MD. Distribution System of Anesthetic Drugs with Preloaded Syringes: Stability Study. *Farm Hosp*. 1996; 20: 55-59.
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6. Fruin DM, Moy KY, Witte K, et al. Stabilities of Common Anesthesia Drugs in Plastic Syringes. *ASHP Annual Meeting* 1992; 49: P-109R.
7. Jappinen A, KokkiH, Naaranlahti TJ, et al. Stability of Buprenorphine, Haloperidol, and Glycopyrrolate Mixture in 0.9% Sodium Chloride Solution. *Pharm. World Sci.* 1999; 21(6): 272-274.
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10. Trissel LA. Avoiding Common Flaws in Stability and Compatibility Studies of Injectable Drugs. *Am. J. Hosp. Pharm*. 1983; 40: 1159-1160. Editorial.

Table 4.1. Assay results of glycopyrrolate injection stored at ambient temperature and 4°C for 90 days in 6-mL polypropylene syringes.*

| Time (Days) | Percent of the Label Claim Based on 100% on Day Zero | | %RSD+ (n=9) |
|---|---|---|---|
| | 25°C | 4°C | |
| 1 | 100.0 | 100.0 | 0.9/1.0 |
| 4 | 99.8 | 99.7 | 0.7/0.8 |
| 7 | 100.0 | 99.2 | 0.4/0.7 |
| 15 | 99.5 | 99.5 | 0.6/1.1 |
| 30 | 99.8 | 100.3 | 0.4/0.6 |
| 45 | 100.1 | 99.5 | 0.8/0.5 |
| 60 | 99.1 | 99.7 | 1.0/0.8 |
| 90 | 99.2 | 99.7 | 0.6/0.3 |

*The injections remained clear and the pH value (2.42) did not change significantly throughout the study. The initial assay indicated that the concentration was 0.20 mg/mL.

+RSD, Relative Standard Deviation

Figure 4.1. Chemical structure of glycopyrrolate.

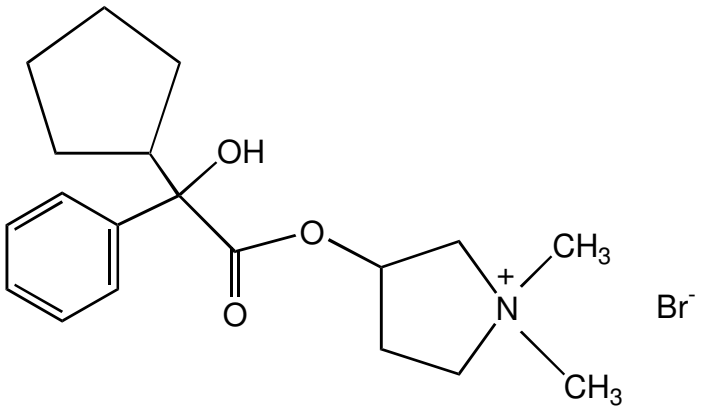
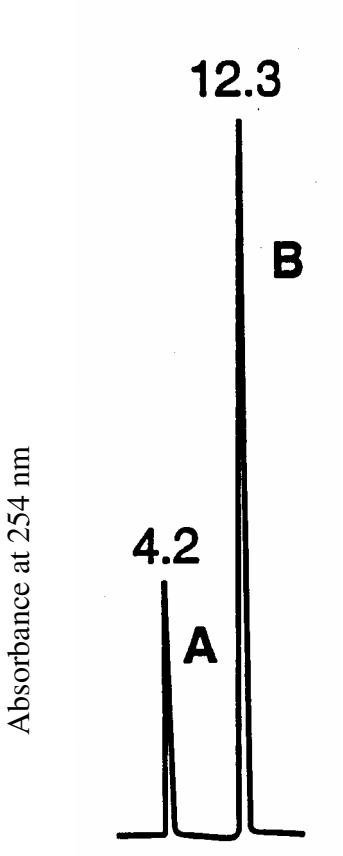

OH
O
O
CH3
+
N
CH3
Br-

Figure 4.2. Typical HPLC chromatogram of benzyl alcohol (A) and glycopyrrolate (B) on a silica column.


12.3
B
4.2
A
Absorbance at 254 nm
Retention Time, minutes

# CHAPTER 5

# STABILITY OF SUCCINYLCHOLINE CHLORIDE INJECTION AT AMBIENT TEMPERATURE AND 4°C IN POLYPROPYLENE SYRINGES[5]

[5]ML Storms, JT Stewart and FW Warren. Submitted to the *International Journal of Pharmaceutical Compounding*, 2001.

**Abstract**

The stability of 20 mg/mL succinylcholine chloride injection in 12 mL polypropylene syringes stored at ambient temperature and 4°C for up to 90 days was investigated. Concentration levels of succinylcholine chloride injection were determined at 0,1, 4, 7, 15, 30, 45, 60 and 90 days after preparation of the syringes using a high-performance liquid chromatography (HPLC) stability indicating assay. Methylparaben, which was present as a preservative in the injection did not interfere with the assay. At 25°C, the loss in potency was less than 10% after 45 days of storage, and at 4°C, it was less than 1% when stored for 90 days. The pH of succinylcholine chloride injection did not change appreciably in a particular direction over the 90-day study period.

## Introduction

Succinylcholine chloride (Figure 5.1) is a depolarizing skeletal muscle relaxant.[1] Since succinylcholine chloride injection (20 mg/mL) is not available prepackaged in syringes, hospital pharmacies meet the need by aseptically preloading succinylcholine chloride injection into sterile syringes and storing the resultant dosage form for short periods at room, refrigerator, or freezer temperatures. Since such storage possesses the potential to adversely affect drug potency, a local hospital pharmacy had the desire to preload polypropylene with succinylcholine chloride injection to save time for hospital personnel and reduce cost due to wasted materials.[2-4] Thus, it was important to assess the stability in polypropylene syringes. A stability study previously concluded that succinylcholine chloride (20 mg/mL) in 0.9% sodium chloride or dextrose injection stored in polypropylene syringes and protected from light was stable for 107 days at 4°C and for 90 days at 25°C.[5] Another study concluded that succinylcholine chloride injections (10 mg/mL) containing sodium chloride, methyl-4-hydroxybenzoate, hydrochloric acid, and water prepared by a hospital pharmacy were stable for 5 months at 25°C and 23 months at 4°C.[6] Moreover, another study concluded that succinylcholine chloride injection stored in polypropylene syringes was stable for 45 days at 25° C and 4°C.[7] However, stability studies for undiluted succinylcholine chloride injection stored in polypropylene syringes for 90 days at ambient temperature and under continuous fluorescent lighting and 4°C is not available. Thus, the aim of this study was to investigate the stability of 20 mg/mL succinylcholine chloride injection prepared in polypropylene syringes. The syringes were prepared at ambient temperature (23±1°C) and were stored either at ambient temperature or under refrigeration (4°C) for up to 90

days. Each preparation was assayed for drug concentration and pH was measured at 0, 1, 4, 7, 15, 30, 45, 60 and 90 days. The USP 24 HPLC assay for succinylcholine chloride injection was modified to determine the drug concentration in each sample.[8]

**Methods**

**Chemicals and Reagents**

All chemicals were HPLC grade (J.T. Baker, Phillipsburg, NJ). The succinylcholine chloride injection, USP (Quelicin®) was from lots 69317DK and 69244DK (Abbott Labs, North Chicago, IL). Succinylcholine chloride reference standard was purchased from the United States Pharmacopeial Convention, Inc. (Rockville, MD).

**Equipment**

The HPLC chromatographic system consisted of a pump (Model 760, Micromeritics, Norcross, GA), an autosampler (Model 728, Micromeritics, Norcross, GA) equipped with a 50 μL loop, an ultraviolet variable wavelength detector (Model 757, Kratos, Ramsey, NJ) set at 214 nm, and a column (silica, 25 cm, 4.6 mm i.d., 3μm, Phenomenex, Torrance, CA). The peak heights were monitored using a Hewlett-Packard Model 3395 integrator (Avondale, PA).

**Chromatographic Conditions**

The isocratic elution was performed at 0.75mL/min. The mobile phase was prepared by combining acetonitrile-methanol (3:2 v/v), water and 25% tetramethylammonium chloride in methanol (27:71:2 v/v/v) and adjusting pH to 4.0 with hydrochloric acid. The mobile phase was filtered through a 0.45-μm polymeric membrane filter (Alltech, Deerfield, IL).

**Preparation of Injection for Stability Studies**

A pooled sample of 20 mg/mL succinylcholine chloride injection was prepared by adding 400 mL succinylcholine chloride injection (20 mg/mL) to a suitably sized beaker. After thorough mixing, 16 mL of the solution was removed for the zero hour assay and the remaining 384 mL was divided into 48-8 mL portions by drawing 8 mL into each of 48 polypropylene syringes (Becton Dickinson, Franklin Lakes, NJ). The syringes were divided into two sets of 24 syringes each; one set was stored in the refrigerator at 4°C (±1°C) and the other set was stored at 25°C (±1°C) under continuous fluorescent lighting. On day zero, the pooled sample was assayed and the pH was measured by a calibrated pH meter. Drug content in each sample was determined by a stability-indicating high-performance liquid chromatography (HPLC) assay on days 1, 4, 7, 15, 30, 45, 60, and 90 and the pH values were also recorded.

**Preparation of Standard Solutions**

A 0.8 mg quantity of succinylcholine chloride reference standard was added to a 5 mL test tube with 1.0 mL phase added to give a concentration of 0.8 mg/mL succinylcholine chloride. A 1:2 dilution was made to result in a succinylcholine chloride concentration of 400 µg/mL. Injections of 50 µL were made into the HPLC system for calculation of the response factor (RF) for succinylcholine chloride. The solution was prepared fresh prior to syringe sampling times on the respective days.

**Degradation of Succinylcholine Chloride**

To ensure that the HPLC assay was stability-indicating, 1 mL of 0.1 N HCl was added to 1 mL of succinylcholine chloride (1 mg/mL). The mixture was heated for 15 minutes. It was then cooled, neutralized, diluted to 400 µg/mL succinylcholine chloride and

injected into the HPLC. In another experiment, a 1 mL quantity of 0.1 N HCl was substituted with 1 mL of 0.1N NaOH solution. In addition, 3% $H_2O_2$ was added to 1 mL of succinylcholine chloride (1 mg/mL) for 1 hour and a brief, gentle heating stopped the reaction. The solution was diluted to 400 µg/mL and injected into the HPLC.

**Preparation of Assay Solutions**

A 5 mL aliquot was removed from each of 3 syringes stored at ambient and 4°C at 1, 4, 7, 15, 30, 45, 60 and 90 days. A 1:50 dilution was made using mobile phase as diluent.

**Calculation of Medication Content**

Triplicate injections of analytical samples prepared from syringe mixtures and five injections of the standard solution were made into the HPLC system. Mean response factors (MRF) were calculated for each drug standard using peak heights from the chromatograms. The drug concentration in each analytical sample was then calculated using the appropriate MRF and the peak height of the analyte in the syringe and standard samples. The following calculations were made to determine the drug concentration in each analytical sample:

(A) RF = Drug Standard (mg/mL) ÷ Drug Peak Height of Standard

(B) Calculate Mean RF (MRF) based on 5 replicates of the standard

(C) Drug Concentration (mg/mL) = MRF x Drug Peak Height of Sample

**Results**

Succinylcholine chloride was forced to degrade under acidic, basic, and oxidative conditions. The objective was to force degradation to observe a 10-30% loss of active when compared with the nondegraded succinylcholine chloride.[9] A typical HPLC

chromatogram of succinylcholine chloride and the preservative, methylparaben, is shown in Figure 5.2.

The succinylcholine chloride injections stored in 12 mL polypropylene syringes were judged to be stable if the drug levels remained >90% of the initial concentration at the time of preparation.[10] Concentrations (percentage of initial concentration remaining) of succinylcholine chloride injection are shown in Table 5.1. The potency of succinylcholine chloride injection decreased to 94.4% after 45 days and to 90.1% after 60 days of storage at 25°C and under continuous fluorescent lighting. After 90 days at 25°, the potency decreased to 88.3%. At 4°C, the loss in potency after 90 days of storage was less than 1%. In addition to monitoring concentration, the pH of the syringe injections did not change appreciably in a particular direction over the study period. Typically, the pH of the samples ranged from 3.50-3.55. Based on the student's t-test and the assumption that the concentration and pH values are normally distributed at both temperatures, the null hypothesis that there is no evidence that temperature affects the drug concentration or pH is retained.

**Conclusion**

Succinylcholine chloride injection (20 mg/mL) stored in 12 mL polypropylene syringes was stable for 45 days at 25°C and for at least 90 days at 4°C.

**References**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3. Ducat CM, Merry AF, Webster CS. Attitudes and Practices of New Zealand Anaesthetists with Regard to Emergency Drugs. *Anaesthetic Intensive Care* 2000; 28: 692-697.
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10. Trissel LA. Avoiding Common Flaws in Stability and Compatibility Studies of Injectable Drugs. *Am. J. Hosp. Pharm*. 1983; 40: 1159-1160. Editorial.

Table 5.1. Assay results of succinylcholine chloride injection stored at ambient temperature and 4°C for 90 days in 12-mL polypropylene syringes.*

| Time (Days) | Percent of the Label Claim Based on 100% on Day Zero | |
|---|---|---|
| | 25°C (%RSD)+ | 4°C (%RSD)+ |
| 1 | 100.1 (1.0) | 100.2 (2.4) |
| 4 | 99.7 (0.7) | 99.7 (1.1) |
| 7 | 98.7 (0.2) | 99.8 (0.5) |
| 15 | 98.0 (0.7) | 99.6 (0.5) |
| 30 | 96.8 (0.3) | 99.5 (0.4) |
| 45 | 94.4 (0.5) | 99.3 (0.5) |
| 60 | 90.1 (0.6) | 99.2 (0.4) |
| 90 | 88.3 (0.3) | 99.5 (0.5) |

*The injections remained clear and the pH value (3.52) did not change significantly throughout the study. The initial assay indicated that the concentration was 20.02 mg/mL.

+RSD, Relative Standard Deviation

Figure 5.1. Chemical structure of succinylcholine chloride.

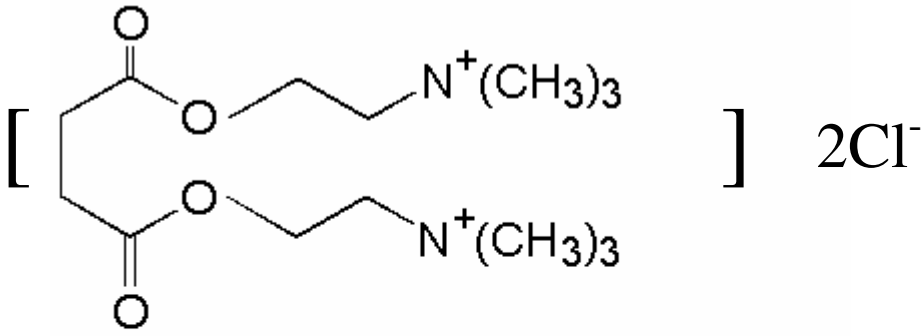
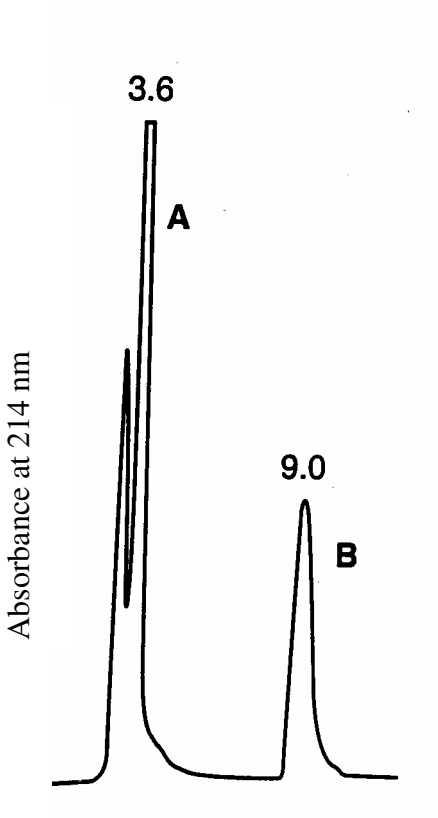

O
O
N+(CH3)3
O
O
N+(CH3)3
2Cl-

Figure 5.2. Typical HPLC chromatogram of methylparaben (A) and succinylcholine chloride (B) on a silica column.


3.6
A
9.0
B
Absorbance at 214 nm
Retention Time, minutes

# CHAPTER 6

# STABILITY OF TUBOCURARINE CHLORIDE INJECTION AT AMBIENT TEMPERATURE AND 4°C IN POLYPROPYLENE SYRINGES[6]

[6]ML Storms, JT Stewart and FW Warren. Accepted to the *International Journal of Pharmaceutical Compounding*, 2001. Reprinted here with permission of publisher.

**Abstract**

The stability of 3 mg/mL tubocurarine chloride injection in 3 mL polypropylene syringes stored at ambient temperature and 4°C for up to 90 days was investigated. Concentration levels of tubocurarine chloride injection were determined at 0,1, 4, 7, 15, 30, 45, 60 and 90 days after preparation of the syringes using a high-performance liquid chromatography (HPLC) stability indicating assay. Benzyl alcohol, which was present as a preservative did not interfere with the assay. At 25°C, the loss in potency was less than 10% after 45 days of storage, and at 4°C, it was less than 1% when stored for 90 days. The pH of tubocurarine chloride injection did not change appreciably in a particular direction over the 90-day study period.

## Introduction

Tubocurarine chloride is a neuromuscular blocking agent.[1] Since tubocurarine chloride injection (3 mg/mL) is not available prepackaged in syringes, hospital pharmacies meet the need by aseptically preloading tubocurarine chloride injection into sterile syringes and storing the resultant dosage form for short periods at room, refrigerator, or freezer temperatures. Since a local hospital pharmacy had the desire to preload polypropylene syringes with tubocurarine chloride injection to save time for hospital personnel and reduce cost due to wasted materials, it was necessary to assess the stability in polypropylene syringes.[2-4] The sorption of tubocurarine chloride injection to plastic intravenous fluid bags has been studied; however, stability studies for tubocurarine chloride injection stored in polypropylene syringes for 90 days at ambient temperature and under continuous fluorescent lighting and 4°C is not available.[5,6] Thus, the aim of this study was to investigate the stability of 3 mg/mL tubocurarine chloride injection prepared in polypropylene syringes. The syringes were prepared at ambient temperature (23±1°C) and were stored either at ambient temperature or under refrigeration (4°C) for up to 90 days. Each preparation was assayed for drug concentration and pH was measured at 0, 1, 4, 7, 15, 30, 45, 60 and 90 days. The USP 24 HPLC assay for tubocurarine chloride injection was modified to determine the drug concentration in each sample.[7]

## Methods

### Chemicals and Reagents

All chemicals were HPLC grade (J.T. Baker, Phillipsburg, NJ). The tubocurarine chloride injection, USP was from lot 69165DK (Abbott Labs, North Chicago, IL). Tubocurarine chloride reference standard was purchased from the United States Pharmacopeial Convention, Inc. (Rockville, MD).

### Equipment

The HPLC chromatographic system consisted of a pump (Model 760, Micromeritics, Norcross, GA), an autosampler (Model 728, Micromeritics, Norcross, GA) equipped with a 50 µL loop, an ultraviolet variable wavelength detector (Model 757, Kratos, Ramsey, NJ) set at 214 nm, and a column (silica, 25 cm, 4.6 mm i.d., 3µm, Phenomenex, Torrance, CA). The peak heights were monitored using a Hewlett-Packard Model 3395 integrator (Avondale, PA)

### Chromatographic Conditions

The isocratic elution was performed at 0.50 mL/min. The mobile phase was prepared by combining acetonitrile-methanol (3:2 v/v), water and 25% tetramethylammonium chloride in methanol (27:71:2 v/v/v) and adjusting pH to 4.0 with hydrochloric acid. The mobile phase was filtered through a 0.45-µm polymeric membrane filter (Alltech, Deerfield, IL).

### Preparation of Injection for Stability Studies

A pooled sample of 3 mg/mL tubocurarine chloride injection (Figure 6.1) was prepared by adding 150 mL tubocurarine chloride injection (3 mg/mL) to a suitably sized beaker. After thorough mixing, 6 mL of the solution was removed for the zero hour

assay and the remaining 144 mL was divided into 48-1.5 mL portions by drawing 1.5 mL into each of 48 polypropylene syringes Becton Dickinson, Franklin Lakes, NJ). The syringes were divided into two sets of 24 syringes each; one set was stored in the refrigerator at 4°C (±1°C) and the other set was stored at 25°C (±1°C) under continuous fluorescent lighting. On day zero, the pooled sample was assayed and the pH was measured by a calibrated pH meter. Drug content in each sample was determined by a stability-indicating high-performance liquid chromatography (HPLC) assay on days 1, 4, 7, 15, 30, 45, 60, and 90 and the pH values were also recorded.

**Preparation of Standard Solutions**

A 1.0 mg quantity of tubocurarine chloride reference standard was added to a 5 mL test tube with 1.0 mL phase added to give a concentration of 1.0 mg/mL succinylcholine chloride. A 1:25 dilution was made to result in a tubocurarine chloride concentration of 40 µg/mL. Injections of 50 µL were made into the HPLC system for calculation of the response factor (RF) for tubocurarine chloride. The solution was prepared fresh prior to syringe sampling times on the respective days.

**Degradation of Tubocurarine Chloride**

Tubocurarine chloride was forced to degrade under acidic, basic, and oxidative conditions. Under acidic conditions, 1 mL of 0.1 N HCl was added to 1 mL of tubocurarine chloride (1 mg/mL). The mixture was heated for 15 minutes. It was then cooled, neutralized, diluted to 40 µg/mL tubocurarine chloride and injected into the HPLC. In another experiment, a 1 mL quantity of 0.1 N HCl was substituted with 1 mL of 0.1N NaOH solution. In addition, 3% $H_2O_2$ was added to 1 mL of tubocurarine

chloride (1 mg/mL) for 1 hour and a brief, gentle heating stopped the reaction. The solution was diluted to 40 μg/mL and injected into the HPLC.

**Preparation of Assay Solutions**

A 1.5 mL aliquot was removed from each of 3 syringes stored at ambient and 4°C at 1, 4, 7, 15, 30, 45, 60 and 90 days. A 1:75 dilution was made using mobile phase as diluent.

**Calculation of Medication Content**

Triplicate injections of analytical samples prepared from syringe mixtures and five injections of the standard solution were made into the HPLC system. Mean response factors (MRF) were calculated for each drug standard using peak heights from the chromatograms. The drug concentration in each analytical sample was then calculated using the appropriate MRF and the peak height of the analyte in the syringe and standard samples. The following calculations were made to determine the drug concentration in each analytical sample:

(A) RF = Drug Standard (mg/mL) ÷ Drug Peak Height of Standard

(B) Calculate Mean RF (MRF) based on 5 replicates of the standard

(C) Drug Concentration (mg/mL) = MRF x Drug Peak Height of Sample

**Results**

Tubocurarine chloride was forced to degrade under acidic, basic, and oxidative conditions. The objective was to force degradation to observe a 10-30% loss of active when compared with the nondegraded tubocurarine chloride.[8] A typical HPLC chromatogram of tubocurarine chloride and the preservative, benzyl alcohol, is shown in Figure 6.2.

The tubocurarine chloride injections stored in 3 mL polypropylene syringes were judged to be stable if the drug levels remained >90% of the initial concentration at the time of preparation.[9] Concentrations (percentage of initial concentration remaining) of tubocurarine chloride injection are shown in Table 6.1. The potency of tubocurarine chloride injection decreased to 92.8% after 45 days of storage at 25°C and under continuous fluorescent lighting. After 60 days at 25°, the potency decreased to 89.4%. At 4°C, the loss in potency after 90 days of storage was less than 1%. In addition to monitoring concentration, the pH of the syringe injections did not change appreciably in a particular direction over the study period. Typically, the pH of the samples ranged from 3.41-3.46. Based on the student's t-test and the assumption that the concentration and pH values are normally distributed at both temperatures, the null hypothesis that there is no evidence that temperature affects the drug concentration or pH is retained.

**Conclusion**

Tubocurarine chloride injection (3 mg/mL) stored in 3 mL polypropylene syringes was stable for 45 days at 25°C and for at least 90 days at 4°C.

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Table 6.1. Assay results of tubocurarine chloride injection stored at ambient temperature and 4°C for 90 days in 3-mL polypropylene syringes.*

| Time (Days) | Percent of the Label Claim Based on 100% on Day Zero | |
|---|---|---|
| | 25°C (%RSD)+ | 4°C (%RSD)+ |
| 1 | 99.9 (0.0) | 99.9 (0.2) |
| 4 | 99.6 (0.2) | 99.8 (0.2) |
| 7 | 98.6 (0.1) | 99.8 (0.3) |
| 15 | 97.1 (0.1) | 99.9 (0.1) |
| 30 | 96.3 (0.2) | 99.9 (0.2) |
| 45 | 92.8 (0.1) | 99.9 (0.4) |
| 60 | 89.4 (0.2) | 99.8 (0.2) |
| 90 | 86.7 (0.1) | 99.8 (0.3) |

*The injections remained clear and the pH value (3.45) did not change significantly throughout the study. The initial assay indicated that the concentration was 3.01 mg/mL.

+RSD, Relative Standard Deviation

Figure 6.1. Chemical structure of tubocurarine chloride**.**

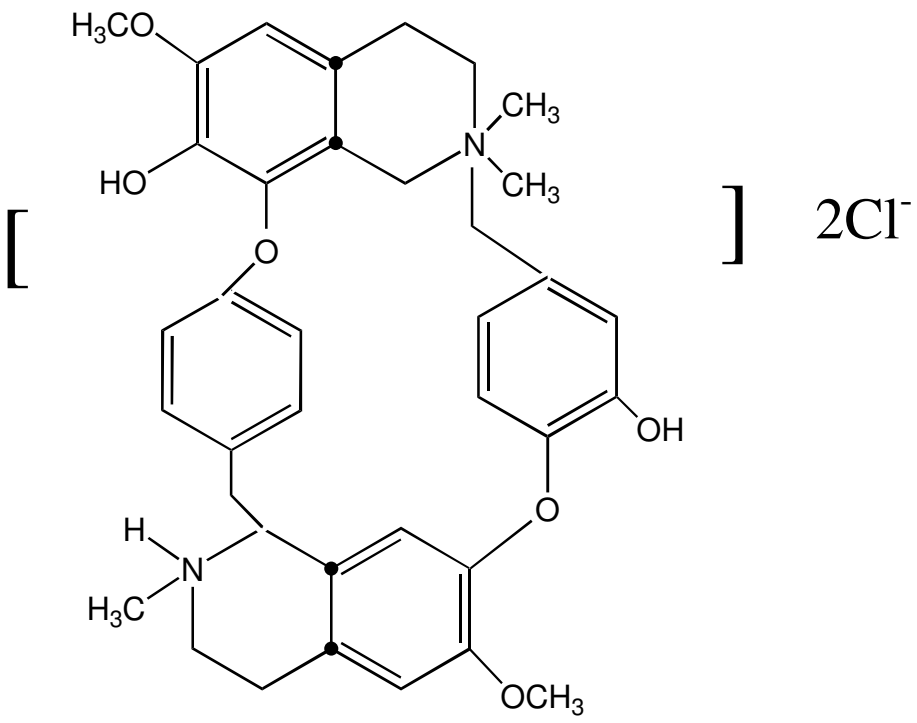
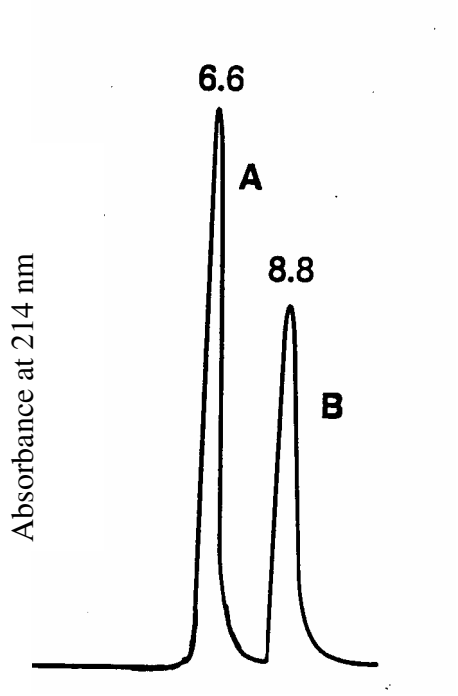

H3CO
HO
CH3
N
CH3
O
OH
O
H
N
H3C
OCH3
2Cl-

Figure 6.2. Typical HPLC chromatogram of benzyl alcohol (A) and tubocurarine chloride (B) on a silica column.


6.6
A
8.8
B
Absorbance at 214 nm
Retention Time, minutes

# DEVELOPMENT OF HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY ASSAYS FOR SELECTED PHARMACEUTICALS IN DOSAGE FORMS AND HUMAN PLASMA

# INTRODUCTION AND LITERATURE REVIEW

Chromatography is frequently employed in several facets of the pharmaceutical industry including drug identification and percentage of a drug in a formulated product, identifying impurities, stability, and the determination of drug concentration in a sample of tissue or biological fluid [1]. The industrial or hospital analytical laboratory plays an important role in many of these stages and well-established techniques for the qualitative and quantitative determination of many drugs are available [2].

## Dosage Form Analyis

HPLC methods which can be routinely employed to provide data to establish the identity, potency, purity, and overall quality of the drug substance and the formulated drug product are important aspects of dosage form analysis [1]. Pharmaceuticals are rarely administered into the body as the active pharmaceutical ingredient alone. Instead, they are formulated with inactive ingredients or excipients into an effective and convenient dosage form. Thus, it is necessary to discriminate interfering peaks from the peak of interest. The detection and assessment of impurities is of prime importance throughout the manufacturing process. HPLC is versatile and capable of detecting similar impurities as well as anti-oxidants or preservatives [2]. Typically, tablets and capsules consist largely of a filler such as lactose, cellulose, starch, and mannitol. The fillers do not absorb UV light so they do not complicate HPLC method development by interfering with the analyte peak. Since lubricants such as magnesium stearate and polyethylene glycol possess weak chromophores, they do not pose a threat for interference with the analyte peak. However, preservatives including benzyl alcohol and the parabens do absorb UV light and must be separated from the analyte peak in order to

provide accurate quantitation of the analyte peak [1]. Although standard methods can often be found in compendia such as he United States Pharmacopeia, it is often necessary to develop faster and more efficient analytical methods for the separation and determination of drugs in dosage form [3].

Stability tests provide evidence on how the quality of an active ingredient or drug product varies with time under the influence of a variety of environmental factors and enables recommended storage conditions and shelf lives to be established. Stability-indicating methods ensure that the assay possesses the ability to detect and separate the intact drug while in the presence of the degradation products [4]. The degradation of a drug substance must be elucidated early in its life history. This is an important factor in determining the formulation, route of administration, and the presence of possible toxic impurities. Stability-indicating methods are useful in monitoring the protection of drug substances from photochemical degradation by various containers as well in the case of those drugs that undergo complex decomposition [2]. This is usually done by degrading the drug under severe stress conditions and observing other peaks in the chromatogram. It is necessary to develop an HPLC assay which is capable of separating the degradation products from the analyte peak [5]. Degradation studies typically use reactions of analyte with acid, base, heat, light, and oxidation [3,5,6]. Stability studies are designed so that 10 to 30% of the original sample degrades. Thus, degradation of the analyte occurs without the risk of secondary degradation products. The data and information collected from the degradation studies is useful for the prediction of “real” degradation peaks and where they may elute [5].

**Biological Matrices**

Since the analysis of drugs in biological fluids is desirable in a variety of situations such as toxicology, drug abuse, therapeutic drug monitoring, and pharmacokinetics, it is essential to employ well-characterized and validated methods to yield reliable results [7]. The plasma concentrations can be used to assess compliance and to adjust the dose for concomitant use of interacting drugs. Monitoring of blood drug concentrations during therapies has significantly reduced drug toxicity and improved treatment outcomes and, hence, patients' quality of life [8]. Therapeutic drug monitoring has been made possible by the development of highly selective and sensitive analytical techniques for a quick determination of plasma levels. HPLC, gas-chromatography (GC), and a variety of mass spectrometry (MS) techniques such as GC-MS and LC-MS are often utilized for the determination of analyte concentrations in a biological matrices [9].

A biological fluid is a complex mixture containing many different components which may complicate HPLC method development. The most commonly analyzed biological fluids are whole blood, plasma, serum, and urine. Whole blood is less often utilized, since the sample preparation of plasma or serum results in less interfering components for most assays. Other biological fluids, which are not as common, include bile, sweat, milk, hair, spinal fluid and saliva [7].

Complex extraction methods or sample preparation are most often applied to bioanalytical procedures. The aim of sample preparation is to result in a sample aliquot with few interferences so that the analyte peak can be accurately quantitated and to protect the HPLC column [5]. Typically, liquid-liquid (LLE) and solid-phase extraction (SPE) are commonly used for liquid samples. SPE is preferred to other traditional

extraction procedures such as LLE since it results in reduced organic solvent consumption, easier collection of analyte fraction, and removal of particulates. However, the variability of SPE cartridges is a disadvantage of this extraction technique. Bonded silicas are most often used, but polymeric and mixed-mode beds are also commercially available [5,10].

Classic SPE involves a sorbent-packed, medical-grade polypropylene syringe barrel with 500 mg of packing in a 3 or 5 ml syringe barrel. Today, SPE cartridges with a smaller mass and volume such as 100 mg of sorbent in a 1 ml syringe barrel is common. Moreover, the presence of smaller sample volumes coupled with improvements in instrument sensitivity is pushing the trend to utilizing smaller packed beds with only 10, 25, and 50 mg of sorbent. SPE pipette tips, discs, and 96- and 384-well SPE plates have also been employed with this extraction method [11,12].

Chapter 7 reports a study involving the investigation of two capsule formulations and one cough syrup formulation that are generally recommended for the relief of common cough-cold symptoms. One commercial capsule formulation contained guaifenesin (an expectorant), pseudoephedrine (a nasal and bronchial decongestant), and dextromethorphan (an antitussive agent). The other commercial capsule formulation contained only guaifenesin and pseudoephedrine. The cough syrup in this study contained guaifenesin and codeine (an analgesic). Previous HPLC methods have measured these compounds either individually or in combination. Simultaneous HPLC assays have been described for pseudoephedrine-dextromethorphan [13-15], guaifenesin-dextromethorphan [16-18], and pseudoephedrine-codeine [19] usually along with other components. The determination of guaifenesin-pseudoephedrine-dextromethorphan [20],

guaifenesin-pseudoephedrine [21-24], and guaifenesin-codeine [25,26] was also reported, however, the procedures required the use of more than one column or mobile phase or an increased flow rate which can be time-consuming and uneconomical. For example, in current USP monographs, guaifenesin-pseudoephedrine-dextromethorphan [27] is determined by HPLC in two different mobile phases at a flow rate of 2 ml/min. Thus, Chapter 7 describes an isocratic HPLC assay for the simultaneous determination of guaifenesin-pseudoephedrine-dextromethorphan, guaifenesin-pseudoephedrine, and guaifenesin-codeine each with a single injection. The compounds were separated on underivatized silica using a buffered aqueous acetonitrile eluent. The separation was achieved within 10 min for all analytes in each drug mixture. The HPLC method was employed for the assay of each analyte in the respective dosage forms [28].

Chapter 8 describes stability-indicating HPLC assays for the separation and determination of several local anesthetic mixtures. A review of the literature revealed that a spectrophotometric assay was reported for the determination of epinephrine and procaine hydrochloride [29,30]; however, no HPLC method was available for this combination. For the epinephrine-prilocaine combination, a USP 24 monograph using HPLC is available, but the method involves two different mobile phases as well as two detectors (ECD for epinephrine and UV for prilocaine hydrochloride) [31]. The separation and determination of procaine-tetracaine with other local anesthetics by micellar liquid chromatography [32,33], GC-MS [34], HPLC with UV detection [35], GC-NPD [36], and spectrophotometry [37] have also been reported. Norepinephrine and epinephrine have been determined via HPLC-ECD [31,38], HPLC with fluorescence detection [39], ion-pair HPLC [40], TLC [41], and GC-MS [42]. Levonordefrin has been

determined utilizing ion-pair HPLC [43] and spectrophotometry [44,45]. The existing USP 24 monographs for levonordefrin-tetracaine-procaine involve three different spectrophotometric assays to determine each analyte individually [45]. The USP 24 monographs for levonordefrin-procaine-propoxycaine and norepinephrine-procaine-propoxycaine each involve two different spectrophotometric assays for the determination of the analytes [44,46]. Thus, Chapter 8 describes stability-indicating HPLC assays for the determination of epinephrine-prilocaine, epinephrine-procaine, levonordefrin-tetracaine-procaine, norepinephrine-procaine-propoxycaine, and levonordefrin-procaine-propoxycaine combinations using HPLC with UV detection. The assays were applied to the commercially available epinephrine-prilocaine (4% Citanest Forte) combination and to a laboratory-compounded injection solution of 20 μg/ml epinephrine and 10 mg/ml procaine.

Chapter 9 describes an HPLC method for the determination of amoxicillin, metronidazole, and pantoprazole in human plasma using solid-phase extraction (SPE). A literature survey revealed that several high-performance liquid chromatographic (HPLC) methods have been reported for the individual determination of amoxicillin [47-49] and metronidazole [50-53] in plasma or serum. An HPLC method has been reported for the simultaneous determination of amoxicillin and metronidazole in human plasma using liquid-liquid extraction; however, this method requires the use of two different detector wavelengths and gradient elution analysis [54]. HPLC methods have been reported for the determination of pantoprazole in plasma or serum including enantiomeric separations with direct injection [55,56] and column-switching [57] sample preparation procedures. Thus, Chapter 9 reports an isocratic reversed-phase HPLC method to assay amoxicillin,

metronidazole, and pantoprazole in human plasma using a $C_{18}$ SPE cartridge and UV detection at 230 nm. This combination of SPE and UV detection results in a method with high recoveries and good linearity, accuracy, and precision.

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# CHAPTER 7

# HPLC DETERMINATION OF GUAIFENESIN WITH SELECTED MEDICATIONS ON UNDERIVATIZED SILICA WITH AN AQUEOUS-ORGANIC MOBILE PHASE[7]

[7] ML Storms and JT Stewart, *The Journal of Pharmaceutical and Biomedical Analysis* 23: 909 (2000). Reprinted here with permission from Elsevier Science.

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**ABSTRACT**

A high performance liquid chromatography procedure has been developed for the simultaneous determination of guaifenesin-pseudoephedrine-dextromethorphan and guaifenesin-pseudoephedrine in commercially available capsule dosage forms and guaifenesin-codeine in a commercial cough syrup dosage form. The separation and quantitation are achieved on a 25-cm underivatized silica column using a mobile phase of 60:40% v/v 6.25 mM phosphate buffer, pH 3.0 – acetonitrile at a flow rate of 1 ml $min^{-1}$ with detection of all analytes at 216 nm. The separation is achieved within 10 minutes for each drug mixture. The method showed linearity for the guaifenesin-pseudoephedrine-dextromethorphan mixture in the 50-200, 7.5-30 and 2.5-10 μg $ml^{-1}$ ranges, respectively. The intra- and interday RSDs ranged from 0.23 to 4.20%, 0.18 to 2.85%, and 0.13 to 5.04% for guaifenesin, pseudoephedrine, and dextromethorphan, respectively. The guaifenesin-pseudoephedrine mixture yielded linear ranges of 25-100 and 3.75-15 μg $ml^{-1}$ and intra-and interday RSDs ranged from 0.65 to 4.18% and 0.23 to 3.00% for guaifenesin and pseudoephedrine, respectively. The method showed linearity for the guaifenesin-codeine mixture in the 25-100 and 2.5-10 μg $ml^{-1}$ ranges and RSDs ranged from 0.37 to 4.25% and 0.14 to 2.08% for guaifenesin and codeine, respectively.

Keywords: HPLC; silica; guaifenesin; pseudoephedrine; dextromethorphan; codeine

## 1. INTRODUCTION

Several methods describing the simultaneous determination of a wide variety of active compounds in various cough-cold formulations have been reported. This particular study involved the investigation of two capsule formulations and one cough syrup formulation that are generally recommended for the relief of common cough-cold symptoms. One commercial capsule formulation contained guaifenesin (an expectorant), pseudoephedrine (a nasal and bronchial decongestant), and dextromethorphan (an antitussive agent). The other commercial capsule formulation contained only guaifenesin and pseudoephedrine. The cough syrup in this study contained guaifenesin and codeine (an analgesic). Previous HPLC methods have measured these compounds either individually or in combination. Simultaneous HPLC assays have been described for pseudoephedrine-dextromethorphan [1-3], guaifenesin-dextromethorphan [4-6], and pseudoephedrine-codeine [7] usually along with other components. The determination of guaifenesin-pseudoephedrine-dextromethorphan [8], guaifenesin-pseudoephedrine [9-12], and guaifenesin-codeine [13,14] was also reported, however, the procedures required the use of more than one column or mobile phase or an increased flow rate which can be time-consuming and uneconomical. For example, in current USP monographs, guaifenesin-pseudoephedrine-dextromethorphan [15] are determined by HPLC in two different mobile phases and at a flow rate of 2 ml $min^{-1}$. In this paper, an isocratic HPLC assay is presented that will simultaneously analyze for guaifenesin-pseudoephedrine-dextromethorphan, guaifenesin-pseudoephedrine, and guaifenesin-codeine each with a single injection. The compounds

are separated on underivatized silica using a buffered aqueous acetonitrile eluent. The separation is achieved within 10 minutes for all analytes in each drug mixture.

## 2. EXPERIMENTAL

### *2.1 Reagents and chemicals*

The structure and formulae of the compounds studied are shown in Figure 7.1. Codeine phosphate was purchased from the United States Pharmacopoeial Convention, Inc. (Rockville, MD). Guaifenesin, pseudoephedrine HCl and dextromethorphan HBr were purchased from Sigma Chemical Co. (St. Louis, MO). Cheratussin AC ™ (Lot: 00789A, Expiration: 01/01), Robitussin Cold and Cough Softgels ™ (Lot: 98207, Expiration: 03/01), and Sudafed ™ (Lot: 7E5178, Expiration 10/99) were purchased from a local pharmacy and manufactured by Vintage Pharmaceuticals, Inc., Whitehall-Robins Healthcare, and Warner Lambert Consumer Healthcare, respectively. Acetonitrile (J.T. Baker, Phillipsburg, NJ) was HPLC grade. Monobasic potassium dihydrogen phosphate ($KH_2PO_4$) and concentrated phosphoric acid were Baker analyzed reagents.

### *2.2 Instrumentation*

An Altex Model 110-A pump (Beckman Coulter, Inc., Fullerton, CA), a Rheodyne Model 7125 injection valve equipped with a 20 µl loop (Rheodyne, Cotati, CA), a Waters 486 UV-VIS detector (Waters Corp., Milford, MA), and a Shimadzu C-R3A chromatopac integrator (Shimadzu Corp., Columbia, MD) constituted the HPLC system used in this study. Separation was accomplished on a 25 cm silica column (4.6 mm i.d., 3 µm particle size, Phenomenex, Torrance, CA). The isocratic mobile phase was composed of a buffer solution [6.25 mM potassium phosphate monobasic in water (pH 3.0) - acetonitrile (60:40% v/v)]. The mobile phase was filtered through a 0.45 µm Nylon-66 filter

(Alltech, Deerfield, IL) and degassed by sonication prior to use. The flow rate was set at 1ml $min^{-1}$. The UV detector was set at 216 nm.

*2.3 Preparation of standard solutions*

A combined standard solution containing guaifenesin, pseudoephedrine HCl, and dextromethorphan HBr was prepared by accurately weighing 20 mg, 3 mg, and 1 mg of each powder and transferring to a 10-ml volumetric flask, mixing until dissolved and mobile phase added to volume. Dilutions (1:10, 1:20, and 1:40) were made in the mobile phase from the standard solution to obtain solutions containing 50, 100, and 200 μg $ml^{-1}$ of guaifenesin, 7.5, 15, and 30 μg $ml^{-1}$ of pseudoephedrine HCl, and 2.5, 5, and 10 μg $ml^{-1}$ of dextromethorphan HBr.

A combined standard solution containing guaifenesin and pseudoephedrine HCl was prepared by accurately weighing 10 mg and 1.5 mg of each powder and transferring to a 10-ml volumetric flask, mixing until dissolved and mobile phase added to volume. Dilutions (1:10, 1:20, and 1:40) were made in the mobile phase from the standard solution to obtain solutions containing 25, 50, and 100 μg $ml^{-1}$ of guaifenesin and 3.75, 7.5, and 15 μg $ml^{-1}$ of pseudoephedrine HCl.

A combined standard solution containing guaifenesin and codeine phosphate was prepared by accurately weighing 10 mg and 1 mg of each powder and transferring to a 10-ml volumetric flask, mixing until dissolved and mobile phase added to volume. Dilutions (1:10, 1:20, and 1:40) were made in the mobile phase from the standard solution to obtain solutions containing 25, 50, and 100 μg $ml^{-1}$ of guaifenesin and 2.5, 5, and 10 μg $ml^{-1}$ of codeine phosphate.

Three point calibration curves were constructed for each anlayte in each drug mixture. Additional dilutions (1:13 and 1:27) of the combined standard solutions were prepared in mobile phase to serve as spiked samples for each analyte in each drug mixture to determine accuracy and precision of the method. Quantitation was based on linear regression analysis of analyte peak height versus analyte concentration in μg $ml^{-1}$.

*2.4 Preparation of analytical samples*

*2.4.1 Capsules*

One commercial gelatin capsule containing 200 mg guaifenesin, 30 mg pseudoephedrine HCl, and 10 mg dextromethorphan HBr was carefully cut using a disposable surgical blade. The capsule was placed in a 100 ml volumetric flask, 25 ml mobile phase added, and heated at 90°C over a steam bath for 10 minutes. After the gelatin capsule completely dissolved, the solution was allowed to cool for 45 minutes and mobile phase added to volume. The solution was mixed and sonicated for 10 minutes. Following sonication, a 1:20 dilution was made for analysis.

The same procedure was followed for a commercial gelatin capsule containing 200 mg guaifenesin and 30 mg pseudoephedrine HCl, however, a 1:40 dilution was made for analysis.

*2.4.2 Cough syrup*

A volume of cough syrup equivalent to 5 mg guaifenesin and 0.5 mg codeine phosphate (0.25ml) was transferred to a 100 ml volumetric flask and mobile phase added to volume. The mixture was mixed and sonicated for 10 minutes.

## 3.0 RESULTS AND DISCUSSION

The goal of this study was to develop a single isocratic HPLC assay for the analysis of three typical cough-cold drug mixtures: guaifenesin-pseudoephedrine-dextromethorphan, guaifenesin-pseudoephedrine, and guaifenesin-codeine. Initial studies to develop a single isocratic HPLC method for the analytes in each drug mixture involved the use of $C_{18}$ and phenyl columns with various mobile phases containing acetonitrile- or methanol-aqueous phosphate buffers. In almost every system studied, dextromethorphan showed a retention time of greater than 30 minutes. Furthermore, guaifenesin and codeine were co-eluted.

Thus, our attention turned to the use of an underivatized silica column with a buffered aqueous-organic mobile phase for the separation and quantitation of the analytes in the drug mixtures. This laboratory has previously reported HPLC methods to analyze basic, acidic, and neutral compounds in pharmaceutical dosage forms and biological samples using underivatized silica [16-18]. The separation mechanism for basic drugs with buffered aqueous mobile phases has been ascribed to the interaction of silanols with an amine group to produce a cation exchange mechanism. Since there were no reports describing the separation of our drug mixtures on silica, we investigated chromatographic conditions previously reported by our lab [16]. Despite a pressure drop of 3000 psi, the use of a 25-cm underivatized silica column (3 μm particle size) proved advantageous in the separation of each guaifenesin mixture since guaifenesin behaved as an early eluter with the use of other columns. In addition, dextromethrophan co-eluted with pseudoephedrine with the utilization of other silica columns.

The final HPLC mobile phase consisting of 60:40 v/v phosphate buffer - acetonitrile with pH adjusted to 3.0 and an underivatized silica column, provided chromatograms

(Figures 7.2 and 7.3) with a steady base line and the specificity required for the simultaneous quantitation of guaifenesin-pseudoephedrine-dextromethorphan and guaifenesin-pseudoephedrine in capsule dosage forms. The method also afforded the simultaneous quantitation of guaifenesin-codeine (Figure 7.4) in a commercially available cough syrup dosage form.

*3.1 Linearity*

Linearities were demonstrated for the guaifenesin-pseudoephedrine-dextromethorphan combination from 20 μL injections of solutions containing quantities of guaifenesin (50, 100, and 200 μg $ml^{-1}$), pseudoephedrine (7.5, 15, and 30 μg $ml^{-1}$), and dextromethorphan (2.5, 5, and 10 μg $ml^{-1}$). Linearities were demonstrated for the guaifenesin-pseudoephedrine mixture from injections of 20 μl of solutions containing quantities of guaifenesin (25, 50, and 100 μg $ml^{-1}$) and pseudoephedrine (3.75, 7.5, and 15 μg $ml^{-1}$). Linearities were demonstrated from 20 μl injections of solutions containing guaifenesin (25, 50, and 100 μg $ml^{-1}$) and codeine (2.5, 5, and 10 μg $ml^{-1}$) for the guaifenesin-codeine combination. The resulting data (Tables 7.1-7.3) was plotted as peak height versus concentration and studied by linear regression.

*3.2 Precision*

To obtain intra- and interday precision data for the guaifenesin-pseudoephedrine-dextromethorphan, guaifenesin-pseudoephedrine, and guaifenesin-codeine mixtures, five standard curves for each analyte in each drug mixture was prepared over 3 days. The results of the precision studies are tabulated in Tables 7.1-7.3.

*3.3 Accuracy*

Percent error and precision of the method were evaluated using spiked samples containing each analyte. The results shown in Table 7.4 indicate that the procedure gives acceptable accuracy and precision for all of the analytes in each drug mixture.

*3.4 Assay of commercial dosage forms*

The three combination standards of guaifenesin-pseudoephedrine-dextromethorphan were injected three times each to obtain a standard curve. The correlation coefficients for the curves were 0.9920, 0.9934, and 0.9942 for guaifenesin, pseudoephedrine, and dextromethorphan, respectively (n=9 for each curve). The capsule solution was injected three times and the data subjected to linear regression analysis. The percent label claim for the commercial capsule was found to be 100.51± 1.96% (n=3, RSD=1.95%) or 201.02 mg/capsule for guaifenesin, 103.87±1.34% (n=3, RSD=1.29%) or 31.161 mg/capsule for pseudoephedrine, and 104.63± 1.50% (n=3, RSD=1.43% ) or 10.46 mg/capsule for dextromethorphan.

The three combination standards of guaifenesin-pseudoephedrine were injected three times each to obtain a standard curve. The correlation coefficients for the curves were 0.9995 and 0.9990 for guaifenesin and pseudoephedrine, respectively (n=9 for each curve). The capsule solution was injected three times and the data subjected to linear regression analysis. The percent label claim for the commercial capsule was found to be 101.64±0.58% (n=3, RSD=0.57%) or 203.28 mg/capsule for quaifenesin and 101.63±1.29% (n=3, RSD=1.27%) or 30.49 mg/capsule for pseudoephedrine.

The three combination standards of guaifenesin-codeine were injected three times each to obtain a standard curve. The correlation coefficients for the curves were 0.9936 and

0.9981 for guaifenesin and codeine, respectively (n=9 for each curve). The cough syrup solution was injected three times and the data subjected to linear regression analysis. In quantitation, the percent label claim was found to be 99.38±0.97% (n=3, RSD=0.98%) or 99.38 mg/capsule for guaifenesin and 99.93±0.74% (n=3, RSD=0.74%) or 9.99 mg/capsule for codeine.

**4.0 CONCLUSION**

The proposed HPLC method in this study has the advantage of simplicity, precision, accuracy, and convenience for the separation and quantitation of guaifenesin-pseudoephedrine-dextromethorphan, guaifenesin-pseudoephedrine, and guaifenesin-codeine and can be employed for their assay in dosage forms each with a single injection. Use of the combined method is thus more efficient than analysis of each drug mixture using more than one mobile phase or column. Moreover, the method uses simple reagents, with minimum sample preparation procedures, encouraging its application in routine analysis.

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Table 7.1. Intra-day and inter-day data for guaifenesin, pseudoephedrine, and dextromethorphan.

Guaifenesin [a,b]:

| Day | % RSD (50 μg ml $^{-1}$) | % RSD (100 μg ml $^{-1}$) | % RSD (200 μg ml $^{-1}$) |
|---|---|---|---|
| 1 | 3.05 | 4.20 | 3.22 |
| | 0.23 | 0.89 | 1.02 |
| | 1.23 | 3.32 | 2.15 |
| 2 | 1.23 | 2.45 | 1.82 |
| 3 | 3.55 | 0.81 | 0.37 |

Pseudoephedrine [a,c]:

| Day | % RSD (7.5 μg ml $^{-1}$) | % RSD (15 μg ml $^{-1}$) | % RSD (30 μg ml $^{-1}$) |
|---|---|---|---|
| 1 | 1.44 | 1.11 | 2.85 |
| | 0.35 | 0.18 | 0.76 |
| | 0.96 | 0.75 | 1.30 |
| 2 | 1.84 | 1.15 | 0.35 |
| 3 | 1.12 | 0.38 | 1.44 |

Dextromethorphan [a,d]:

| Day | % RSD (2.5 μg ml $^{-1}$) | % RSD (5 μg ml $^{-1}$) | % RSD (10 μg ml $^{-1}$) |
|---|---|---|---|
| 1 | 1.34 | 1.75 | 5.04 |
| | 0.50 | 0.71 | 1.41 |
| | 0.84 | 0.66 | 0.82 |
| 2 | 1.96 | 2.11 | 0.13 |
| 3 | 1.13 | 0.73 | 0.91 |

[a] Based on n=9 for each curve constructed.
[b] $r^2$ ranged from 0.9921 - 0.9976 (n=9).
[c] $r^2$ ranged from 0.9892 - 0.9935 (n=9).
[d] $r^2$ ranged from 0.9932 - 0.9990 (n=9).

Table 7.2. Intra-day and inter-day precision data for guaifenesin and pseudeoephedrine.

Guaifenesin [a,b] :

| Day | % RSD (25 µg ml $^{-1}$) | % RSD (50 µg ml $^{-1}$) | % RSD (100 µg ml $^{-1}$) |
|---|---|---|---|
| 1 | 0.65 | 1.84 | 0.88 |
| | 2.39 | 0.78 | 0.79 |
| | 4.18 | 0.36 | 2.33 |
| 2 | 1.20 | 1.17 | 2.30 |
| 3 | 1.29 | 1.75 | 1.56 |

Pseudoepedrine [a,c] :

| Day | % RSD (3.75 µg ml $^{-1}$) | % RSD (7.5 µg ml $^{-1}$) | % RSD (15 µg ml $^{-1}$) |
|---|---|---|---|
| 1 | 0.68 | 0.70 | 3.00 |
| | 1.32 | 2.20 | 0.23 |
| | 0.28 | 0.92 | 0.97 |
| 2 | 1.00 | 1.52 | 0.45 |
| 3 | 0.90 | 1.15 | 0.51 |

[a]Based on n=9 for each curve constructed.
[b] $r^2$ ranged from 0.9954 - 0.9999 (n=9).
[c] $r^2$ ranged from 0.9963 - 0.9996 (n=9).

Table 7.3. Intra-day and inter-day precision for guaifenesin and codeine.

Guaifensin [a,b] :

| Day | % RSD (25 μg ml $^{-1}$) | % RSD (50 μg ml $^{-1}$) | % RSD (100 μg ml $^{-1}$) |
|---|---|---|---|
| 1 | 4.25 | 2.91 | 1.25 |
| | 0.55 | 0.04 | 1.90 |
| | 1.92 | 0.37 | 2.49 |
| 2 | 1.30 | 3.69 | 0.76 |
| 3 | 2.30 | 0.46 | 2.01 |

Codeine [a,c] :

| Day | % RSD (2.5 μg ml $^{-1}$) | % RSD (5 μg ml $^{-1}$) | % RSD (10 μg ml $^{-1}$) |
|---|---|---|---|
| 1 | 1.59 | 0.18 | 2.02 |
| | 0.39 | 1.00 | 1.45 |
| | 2.08 | 0.58 | 1.90 |
| 2 | 1.02 | 1.92 | 1.02 |
| 3 | 1.26 | 0.14 | 1.40 |

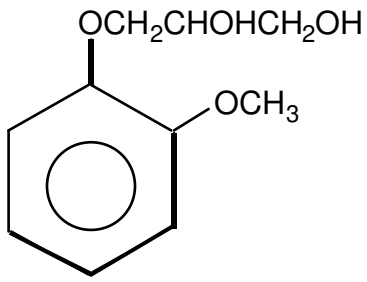
[a] Based on n=9 for each curve constructed.
[b] $r^2$ ranged from 0.9936 - 0.9999 (n=9).
[c] $r^2$ ranged from 0.9981 - 0.9997 (n=9).

Table 7.4. Accuracy and Precision Using Spiked Drug Samples

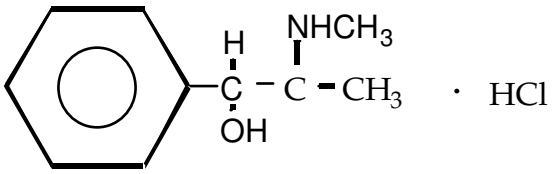
| Analyte | Concn Added ($\mu g\ ml^{-1}$) | Concn Found[a] ($\mu g\ ml^{-1}$) | Percent Error | %RSD |
|---|---|---|---|---|
| Guaifenesin-Pseudoephedrine-Dextromethorphan: | | | | |
| Guaifenesin | 153.85 | 152.35±3.29 | 0.98 | 2.16 |
| | 74.07 | 72.36±0.36 | 2.31 | 0.50 |
| Pseudoephedrine | 23.08 | 23.61±0.88 | 2.30 | 3.72 |
| | 11.11 | 11.45±0.04 | 3.06 | 0.35 |
| Dextromethorphan | 7.69 | 7.94±0.14 | 2.73 | 1.77 |
| | 3.70 | 3.81±0.04 | 2.97 | 1.05 |
| Guaifenesin-Pseudoephedrine: | | | | |
| Guaifenesin | 76.92 | 73.11±0.24 | 4.95 | 0.33 |
| | 37.04 | 36.27±0.10 | 2.08 | 0.29 |
| Pseudoephedrine | 11.54 | 11.41±0.09 | 1.13 | 0.80 |
| | 5.56 | 5.36±0.10 | 3.60 | 1.87 |
| Guaifenesin-Codeine: | | | | |
| Guaifenesin | 76.92 | 76.44±1.47 | 0.62 | 1.92 |
| | 37.04 | 38.24±0.60 | 3.24 | 1.56 |
| Codeine | 7.69 | 8.06±0.13 | 4.81 | 1.62 |
| | 3.70 | 3.60±0.03 | 2.70 | 0.83 |

[a] Based on n=3.

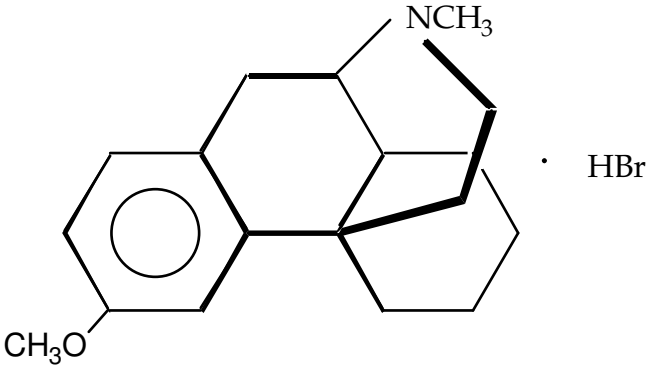
Figure 7.1. Chemical structures of compounds studied.



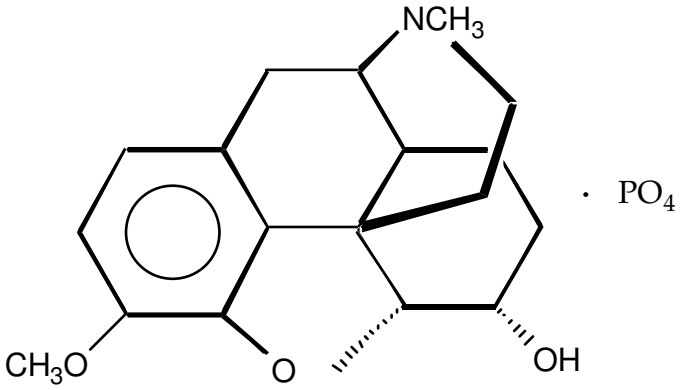

Guaifenesin




Pseudoephedrine HCl




Dextromethorphan HBr




Codeine $PO_4$

Figure 7.2. Typical HPLC chromatogram of guaifenesin (A), pseudoephedrine (B), and dextromethorphan (C) on underivatized silica with acetonitrile - aqueous phosphate buffer pH 3.0 mobile phase.

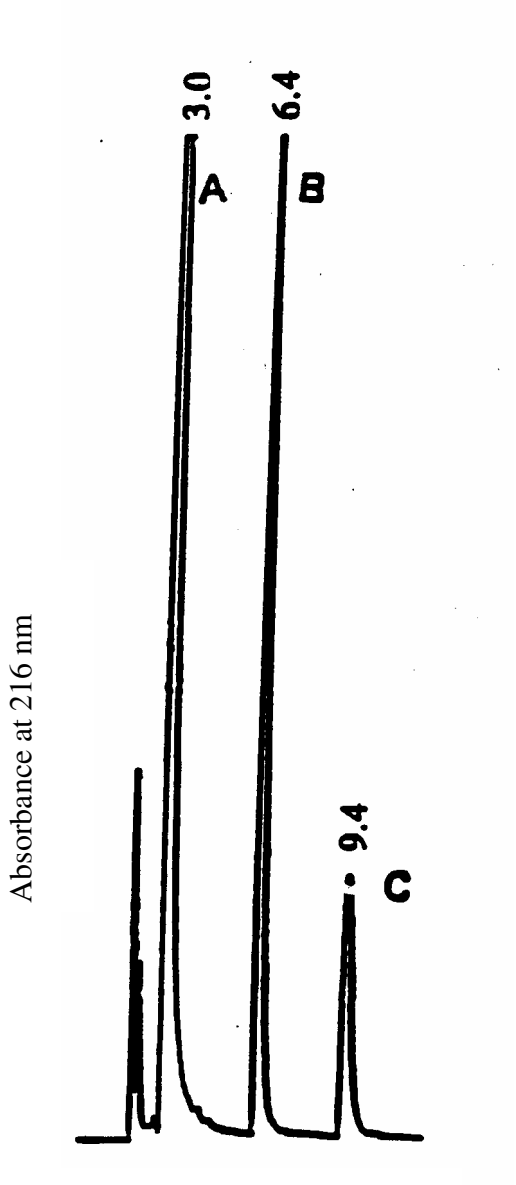

3.0
6.4
A
B
9.4
C
Absorbance at 216 nm
Retention Time, minutes

Figure 7.3. Typical HPLC chromatogram of guaifenesin (A) and pseudoephedrine (B) on underivatized silica with acetonitrile - aqueous phosphate buffer pH 3.0 mobile phase.

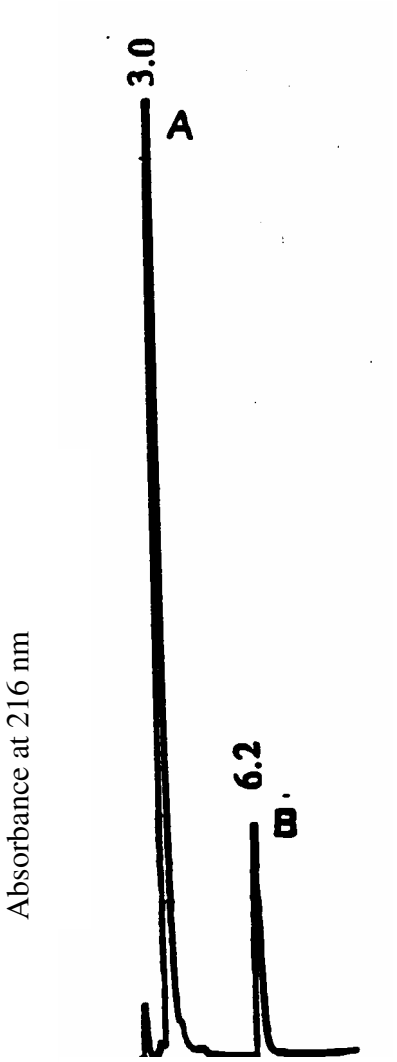
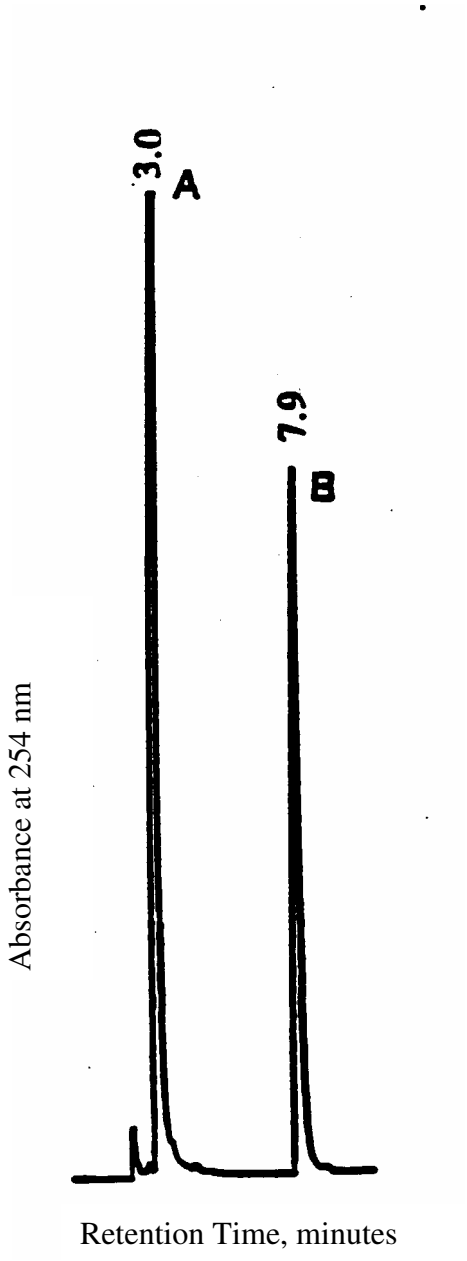

3.0
A
6.2
B
Absorbance at 216 nm
Retention Time, minutes

Figure 7.4. Typical HPLC chromatogram of guaifenesin (A) and codeine (B) on underivatized silica with acetonitrile - aqueous phosphate buffer pH 3.0 mobile phase.


3.0
A
7.9
B
Absorbance at 254 nm
Retention Time, minutes

# CHAPTER 8

# STABILITY-INDICATING HPLC ASSAYS FOR THE DETERMINATION OF PRILOCAINE AND PROCAINE DRUG COMBINATIONS[8]

[8]ML Storms and JT Stewart. Accepted to *The Journal of Pharmaceutical and Biomedical Analysis*, 2002. Reprinted here with permission of Elsevier Science.

**Abstract**

Stability-indicating, reversed phase high-performance liquid chromatographic methods have been developed for the determination of several procaine hydrochloride and prilocaine hydrochloride combinations. The separation and quantitation of epinephrine-prilocaine and epinephrine-procaine drug combinations were achieved on a phenyl column using a mobile phase of 80:20 v/v 25 mM phosphate buffer (pH 3.0) containing 50 mM heptanesulfonic acid sodium salt – acetonitrile at a flow rate of 1 mL $min^{-1}$ and UV detection at 254 nm. The method showed linearity for the epinephrine and prilocaine hydrochloride mixture in the 0.25-2.5 μg $mL^{-1}$ and 8-200 μg $mL^{-1}$ ranges, respectively. The intra-and inter-day RSDs ranged from 0.26-2.05% and 0.04-0.61% for epinephrine and prilocaine hydrochloride, respectively. The epinephrine and procaine hydrochloride mixture yielded linear ranges of 0.25-2.0 μg $mL^{-1}$ and 5-100 μg $mL^{-1}$ intra- and inter-day RSDs ranged from 0.23-1.88% and 0.07-0.26% for epinephrine and procaine hydrochloride, respectively. The assays were shown to be suitable for measuring epinephrine-prilocaine and epinephrine-procaine combinations in their respective injection dosage forms. Stability-indicating HPLC assays were also developed for several other procaine drug combinations since their monographs are present in the USP 24; however, quantitation was not investigated since these combinations are not commercially available. A mobile phase consisting of 80:20 v/v 25mM phosphate buffer (pH 3.0) containing 50mM heptanesulfonic acid – acetonitrile was utilized for the levonordefrin-tetracaine-procaine drug combination while a mobile phase consisting of 70:30 %v/v 25mM phosphate buffer (pH 3.0) containing 50 mM heptanesulfonic acid sodium salt – acetonitrile was utilized for the separation of levonordefrin-procaine-

propoxycaine and norepinephrine-procaine-propoxycaine. All separations were achieved on a phenyl column at a flow rate of 1 mL $min^{-1}$ and UV detection at 254 nm.

**Keywords:** Prilocaine, Procaine, Epinephrine, Norepinephrine, Levonordefrin, Propoxycaine, Tetracaine, HPLC, Stability-indicating

## 1. Introduction

Local anesthetics play an important role clinically in dentistry and minor surgery for the temporary relief of pain. Local anesthetics may be applied either topically or parenterally to a localized area to produce a state of local anesthesia by reversibly blocking the nerve conductances that transmit the feeling of pain [1]. Prilocaine hydrochloride, procaine hydrochloride, tetracaine, and propoxycaine hydrochloride contain in their chemical structure a secondary or tertiary amino group connected to an aromatic residue. The aromatic residue is connected to the intermediate group by an ester or amide linkage for which local anesthetics are typically classified. The type of linkage along with other structural changes in the molecule affects potency, duration of action, rate of metabolism, and toxicity [2]. Since the duration of action of a local anesthetic is proportional to the time during which it is in contact with the nerve, vasoconstrictors are often combined with local anesthetic preparations to decrease the rate at which the local anesthetics are absorbed into circulation [1]. Thus, epinephrine, norepinephrine, and levonordefrin have all been used in conjunction with a number of local anesthetics by various medical professions.

A literature survey revealed that a spectrophotometric assay was reported for the determination of epinephrine and procaine hydrochloride [3,4]; however, no HPLC method was available for this combination. For the epinephrine-prilocaine combination, a USP 24 monograph using HPLC is available, but the method involves two different mobile phases as well as two detectors (ECD for epinephrine and UV for prilocaine hydrochloride) [5]. The separation and determination of procaine-tetracaine with other local anesthetics by micellar liquid chromatography [6,7], GC-MS [8], HPLC with UV

detection [9], GC-NPD [10], and spectrophotometry [11] have also been reported. Norepinephrine and epinephrine have been determined via HPLC-ECD [5,12], HPLC with fluorescence detection [13], ion-pair HPLC [14], TLC [15], and GC-MS [16]. Levonordefrin has been determined utilizing ion-pair HPLC [17] and spectrophotometry [18,19]. The existing USP 24 monographs for levonordefrin-tetracaine-procaine involve three different spectrophotometric assays to determine each analyte individually [19]. The USP 24 monographs for levonordefrin-procaine-propoxycaine and norepinephrine-procaine-propoxycaine each involve two different spectrophotometric assays for the determination of the analytes [18,20].

In this paper, stability-indicating assays have been developed for the determination of epinephrine-prilocaine, epinephrine-procaine, levonordefrin-tetracaine-procaine, norepinephrine-procaine-propoxycaine, and levonordefrin-procaine-propoxycaine combinations using HPLC with UV detection. The compounds are separated on a phenyl column using an ion-pair mobile phase. The assays were applied to the commercially available epinephrine-prilocaine (Citanest® Forte) combination and to a laboratory-compounded injection solution of epinephrine-procaine. The separations were achieved within 20 min for all analytes in each drug mixture. The HPLC separations for the other procaine drug combinations are also shown since their monographs are included in the USP 24 [18-20].

## 2. Experimental

### *2.1 Reagents and chemicals*

The structures and formulae of the compounds studied are shown in Figure 8.1. Tetracaine, propoxycaine hydrochloride, procaine hydrochloride, and levonordefrin reference standards were purchased from the United States Pharmacopoeial Convention, Inc. (Rockville, MD). Norepinephrine bitartrate, epinephrine bitartrate, prilocaine hydrochloride and heptanesulfonic acid sodium salt were purchased from Sigma Chemical Co. (St. Louis, MO). 4% Citanest® Forte (prilocaine hydrochloride and epinephrine injection, USP) (Lot: 009061, Expiration: 09/01) was purchased from Astra (Westborough, MA). Acetonitrile (J.T. Baker, Phillipsburg, NJ) was HPLC grade. Monobasic potassium dihydrogen phosphate ($KH_2PO_4$) and concentrated phosphoric acid were Baker analyzed reagents.

### *2.2 Apparatus*

A Beckman Model 110-B pump (Fullerton, CA), a Rheodyne Model 7125 injection valve equipped with a 20 µL loop (Rheodyne, Cotati, CA), a Waters 486 UV-VIS detector (Waters Corp., Milford, MA), and a Shimadzu C-R3A chromatopac integrator (Shimadzu Corp., Columbia, MD) constituted the HPLC system used in this study. Separations were accomplished on a µ-Bondapak™ phenyl column (300 x 3.9 mm i.d., Waters, Millford, MA).

### *2.3 Chromatographic conditions*

Chromatographic analysis was carried out at ambient temperature. The isocratic separations of the epinephrine-prilocaine, epinephrine-procaine, and levonordefrin-tetracaine-procaine combinations were achieved with a buffered ion-pair mobile phase

[25 mM potassium phosphate monobasic in water (pH 3.0) with 50 mM heptanesulfonic acid sodium salt – acetonitrile (80:20 %v/v)]. The mobile phase for the separation of norepinephrine-procaine-propoxycaine and levonordefrin-procaine-propoxycaine drug combinations was composed of a buffer solution [25 mM potassium phosphate monobasic in water (pH 3.0) with 50 mM heptanesulfonic acid sodium salt – acetonitrile (70:30 %v/v)]. Each mobile phase was filtered through a 0.45 μm nylon-66 filter (Alltech, Deerfield, IL) and degassed by sonication prior to use. The flow rate was set at 1 ml $min^{-1}$. The UV detector was set at 254 nm.

*2.4 Standard solutions*

A combined standard solution containing epinephrine and prilocaine hydrochloride was prepared by accurately weighing 1.0 mg of epinephrine and transferring to a 5-mL volumetric flask with mobile phase added to volume. A 1:40 dilution was made in mobile phase to give a 5 μg $mL^{-1}$ epinephrine solution. Then, 40 mg of prilocaine hydrochloride was added to result in the combined standard solution. Appropriate dilutions were made in mobile phase to obtain solutions containing 2.5, 1, 0.5, 0.33, and 0.25 μg $mL^{-1}$ of epinephrine and 200, 80, 40, 20, and 8 μg $mL^{-1}$ of prilocaine hydrochloride.

A combined standard solution containing epinephrine and procaine hydrochloride was prepared by accurately weighing 1 mg of epinephrine and transferring to a 5-mL volumetric flask with mobile phase added to volume. A 1:10 dilution was made in mobile phase to result in a 20 μg $mL^{-1}$epinephrine solution. Then, 10 mg of procaine hydrochloride was added to result in the combined standard solution. Appropriate dilutions were made in mobile phase to obtain solutions containing 2, 1, 0.8, 0.5, 0.25 μg $mL^{-1}$ of epinephrine and 100, 50, 20, 10, and 5 μg $mL^{-1}$ of procaine hydrochloride.

A combined standard solution containing levonordefrin, tetracaine, and procaine hydrochloride was prepared by accurately weighing 250 μg levonordefrin, 7.5 mg tetracaine, and 100 mg procaine hydrochloride and transferring to a 5-mL volumetric flask with mobile phase added to volume. For the separation, a 1:150 dilution was made in mobile phase prior to the HPLC injection.

A combined standard solution containing levonordefrin, procaine hydrochloride, and propoxycaine hydrochloride was prepared by accurately weighing 250 μg of levonordefrin, 100 mg procaine hydrochloride, and 20 mg propoxycaine hydrochloride. A 1:150 dilution was made in mobile phase prior to separation by HPLC.

A combined standard solution containing norepinephrine, procaine hydrochloride, and propoxycaine hydrochloride was prepared by accurately weighing 167 μg of norepinephrine, 100 mg procaine hydrochloride, and 20 mg propoxycaine hydrochloride and transferring to a 5-mL volumetric flask with mobile phase added to volume. For the separation, a 1:150 dilution was made in mobile phase prior to the HPLC injection.

*2.5 Application of the proposed method to dosage forms*

A 1 mL aliquot of the commercially available injection (Citanest® Forte) equivalent to 5 μg epinephrine and 40 mg prilocaine hydrochloride was transferred to a 5 mL test tube. For the analysis of epinephrine, a 1:12 dilution was made utilizing mobile phase to result in an epinephrine concentration of 0.42 μg $mL^{-1}$. A 1:800 dilution in mobile phase resulted in a concentration of 50 μg $mL^{-1}$ for the analysis of prilocaine hydrochloride.

Since epinephrine and procaine hydrochloride are not commercially available prepackaged in the United States, an injection solution was prepared in the laboratory. This solution was prepared by accurately weighing 100 μg epinephrine and 50 mg of

procaine hydrochloride USP reference standard and transferring to a 5-mL volumetric flask and filled to volume with mobile phase. A 1:5 dilution was made in mobile phase to result in 10 mg $mL^{-1}$ procaine hydrochloride and 20 μg $mL^{-1}$ epinephrine. For the analysis of epinephrine, a 1:22 dilution was made in mobile phase. A 1:300 dilution in mobile phase was utilized for the analysis of procaine hydrochloride.

**3. Results and Discussion**

The goal of this study was to develop stability-indicating HPLC assays for the analysis of five local anesthetic drug combinations: epinephrine-prilocaine, epinephrine-procaine, levonordefrin-tetracaine-procaine, levonordefrin-procaine-propoxycaine, and norepinephrine-procaine-propoxycaine. Initial studies to develop HPLC assays involved the use of $C_{18,}$ $C_{8,}$ and phenyl columns with various mobile phases containing acetonitrile- or methanol-aqueous phosphate buffers. In addition, a bare silica column with a buffered aqueous-organic mobile phase was investigated since this laboratory has previously reported HPLC methods to analyze basic, acidic, and neutral compounds in pharmaceutical dosage forms and biological samples using underivatized silica [21-24]. In each study, epinephrine, levonordefrin and norepinephrine eluted too close to the solvent front. However, the phenyl column was chosen for further ion-pair studies since it produced sharp and symmetrical peaks.

Thus, our attention turned to the use of heptanesulfonic acid sodium salt as an ion-pair reagent to increase the retention time of epinephrine, norepinephrine, and levonordefrin. An HPLC assay utilizing an ion-pair mobile phase for the determination of levonordefrin has been reported [17]. In addition, the USP 24 monograph for the epinephrine and prilocaine injection uses an ion-pair mobile phase for the determination

of epinephrine by HPLC-ECD [5]. Since there were no reports describing the separation of the selected drug mixtures utilizing HPLC with UV detection, we investigated various ion-pair concentrations in the mobile phase with the phenyl column.

The final selective HPLC mobile phase consisting of 80:20 v/v phosphate buffer (pH 3.0) with heptanesulfonic acid sodium salt – acetonitrile and a phenyl column, provided chromatograms (Figures 8.2-8.4) with a steady base line and the specificity required for the separation of epinephrine-prilocaine, epinephrine-procaine, and levonordefrin-tetracaine-procaine drug combinations. The HPLC mobile phase consisting of 70:30 v/v phosphate buffer (pH 3.0) with heptanesulfonic acid sodium salt – acetonitrile and a phenyl column, provided chromatograms (Figures 8.5 and 8.6) with the specificity required for the separation of norepinephrine-procaine-propoxycaine and levonordefrin-procaine-propoxycaine drug combinations.

*3.1 Linearity*

Linearities were demonstrated for the epinephrine-prilocaine combination from 20 µL injections of solutions containing quantities of epinephrine (2.5, 1, 0.5, 0.33, and 0.25 µg $mL^{-1}$) and prilocaine hydrochloride (200, 80, 40, 20, and 8 µg $mL^{-1}$). Linearities were demonstrated for the epinephrine-procaine combination from 20 µL injections of solutions containing quantities of epinephrine (2,1, 0.8, 0.5, and 0.25 µg $mL^{-1}$) and procaine hydrochloride (100, 50, 20, 10, and 5 µg $mL^{-1}$). Linearities were demonstrated for the levonordefrin-tetracaine-procaine combination from 20 µL injections of solutions containing quantities of levonordefrin (2.5, 1.25, 1, 0.66, 0.5, and 0.33 µg $mL^{-1}$), tetracaine (75, 37.5, 30, 20, 15, and 10 µg $mL^{-1}$), and procaine hydrochloride (100, 50, 20, 10, and 5 µg $mL^{-1}$). Linearities were demonstrated for the levonordefrin-procaine-

propoxycaine combination from 20 μL injections of solutions containing quantities of levonordefrin (2.5, 1.25, 1, 0.66, 0.5, and 0.33 μg $mL^{-1}$), procaine hydrochloride (100, 50, 20, 10, and 5 μg $mL^{-1}$), and propoxycaine (20, 10, 5, 3.33, and 2 μg $mL^{-1}$). Linearities were demonstrated for the norepinephrine-procaine-propoxycaine combination from 20 μL injections of solutions containing quantities of norepinephrine (3.33, 1.67, 1.11, 0.55, and 0.33 μg $mL^{-1}$), procaine hydrochloride (100, 50, 20, 10, and 5 μg $mL^{-1}$), and propoxycaine (20, 10, 5, 3.33, and 2 μg $mL^{-1}$). The resulting data was plotted as peak height versus concentration and studied by linear regression analysis.

*3.2 Precision and accuracy*

To obtain intra- and inter-day precision data for the epinephrine-prilocaine and epinephrine-procaine combinations, five standard curves for each analyte in each drug mixture were prepared over 3 days. The results are presented in Table 8.1. The intra- and inter-day RSDs ranged from 0.26-2.05% and 0.04-0.61% for epinephrine and prilocaine hydrochloride, respectively. The epinephrine and procaine hydrochloride mixture yielded intra- and inter-day RSDs of 0.23-1.88% and 0.07-0.26% for epinephrine and procaine hydrochloride, respectively. Percent error was also evaluated for both the epinephrine-prilocaine and epinephrine-procaine combinations using spiked samples containing each analyte. Table 8.1 shows that the percent error of the method ranged from 0.02-3.23% for both the epinephrine-prilocaine and epinephrine-procaine combinations; therefore, it was concluded that the procedure gives acceptable accuracy and precision for all of the analytes in each drug mixture.

*3.3 Stability*

To show that the methods are stability-indicating, it was necessary to subject the analytes to extreme conditions to cause them to degrade [21]. In each case, 1 mg/mL of each analyte was mixed with the appropriate degradation solution such as 0.1 N HCl (ambient temperature and 90°C), 0.1 N NaOH (ambient temperature and 90°C), and 3% $H_2O_2$ (ambient temperature and 90°C). Acid and base degraded samples were first neutralized with equal volumes and concentrations of either acid or base and diluted prior to injection into the HPLC system. Samples degraded with peroxide were diluted and injected into the HPLC. For comparison, a 1 mL aliquot of each stock solution was heated to 90°C without the addition of acid, base, or peroxide. Results are shown in Tables 8.2 and 8.3.

*3.5 Assay of dosage forms*

The combination standards of epinephrine-prilocaine were injected in duplicate to obtain a standard curve for each analyte. The correlation coefficients for the curves were 0.9996 and 0.9998 for epinephrine and prilocaine hydrochloride, respectively (n=10 for each curve). The injection solution was injected three times and the data subjected to linear regression analysis. The percent label claim for the commercial injection (Citanest® Forte) was found to be 98.41 ± 1.37% (n=3, %RSD = 1.39) or 4.92 μg $mL^{-1}$ for epinephrine and 100.03 ± 0.04% (n=3, %RSD = 0.04) or 40.01 μg $mL^{-1}$ for prilocaine hydrochloride.

The combination standards of epinephrine-procaine were injected in duplicate to obtain a standard curve for each analyte. The correlation coefficients for the curves were 0.9994 and 0.9996 for epinephrine and procaine hydrochloride, respectively (n=10 for

each curve). The laboratory prepared injection solution was injected three times and data subjected to linear regression analysis. The percent label claim for the laboratory prepared injection was found to be 101.10 ± 1.10% (n=3, %RSD = 0.95) or 20.22 μg $mL^{-1}$ for epinephrine and 100.02 ± 0.05% (n=3, %RSD = 0.05) or 10.00 μg $mL^{-1}$ for procaine hydrochloride.

**4. Conclusion**

The proposed stability-indicating HPLC methods in this study have the advantage of simplicity, precision, accuracy, and convenience for the separation and quantitation of prilocaine-epinephrine and procaine-epinephrine drug combinations and can be employed for the assay of their respective dosage forms. Although different dilutions are necessary to quantitate prilocaine hydrochloride and epinephrine in the mixtures, this method is advantageous over the existing USP 24 HPLC assays, which employ different mobile phases as well as two detectors [5]. The method is also an advantage over the existing USP HPLC assays for the procaine hydrochloride combinations since these methods typically involve spectrophotometric assays which require a sequence of sample preparation steps as well as the preparation of a variety of reagents [4,18-20]. Moreover, the proposed stability-indicating HPLC methods use simple reagents, with minimal preparation procedures, encouraging its application in routine analysis.

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Table 8.1. Accuracy and precision using spiked drug samples.

| Analyte | Concn added ($\mu g\ mL^{-1}$) | Concn found[a] ($\mu g\ mL^{-1}$) | Percent error | %RSD |
|---|---|---|---|---|
| Prilocaine-Epinephrine: | | | | |
| Prilocaine | 100.00 | 100.04 ± 0.02 | 0.04 | 0.02 |
| | 25.00 | 25.02 ± 0.04 | 0.20 | 0.16 |
| | 10.00 | 10.01 ± 0.02 | 0.10 | 0.20 |
| Epinephrine | 1.25 | 1.26 ± 0.03 | 0.80 | 2.56 |
| | 0.63 | 0.62 ± 0.02 | 1.59 | 3.23 |
| | 0.31 | 0.32 ± 0.00 | 3.23 | 1.82 |
| Procaine-Epinephrine: | | | | |
| Procaine | 62.50 | 62.51 ± 0.02 | 0.02 | 0.03 |
| | 25.00 | 24.99 ± 0.04 | 0.04 | 0.16 |
| | 6.25 | 6.26 ± 0.02 | 0.16 | 0.24 |
| Epinephrine | 1.33 | 1.32 ± 0.02 | 0.75 | 1.24 |
| | 0.67 | 0.69 ± 0.02 | 2.99 | 1.19 |
| | 0.40 | 0.41 ± 0.01 | 2.56 | 2.56 |

[a] Each value represents mean ± S.D. of three replicates.

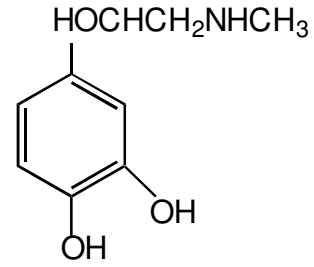
Table 8.2. Stability-indicating nature of assays.

| Drug | Treatment | Duration | %Δ in Peak Height |
|---|---|---|---|
| **Epinephrine-Prilocaine:** | | | |
| Epinephrine | 0.1N HCl (90°C) | 1 h | -25% |
| | 0.1N NaOH | 45 min | -15% |
| | 3% $H_2O_2$ | 2 h | -12% |
| Prilocaine | 0.1N HCl (90°C) | 1 h | -20% |
| | 0.1N NaOH | 30 min | -15% |
| | 3% $H_2O_2$ (90°C) | 1 h | -17% |
| **Epinephrine-Procaine:** | | | |
| Epinephrine | 0.1N HCl (90°C) | 1h | -18% |
| | 0.1N NaOH | 45 min | -11% |
| | 3% $H_2O_2$ | 2h | -16% |
| Procaine | 0.1N HCl (90°C) | 1h | -25% |
| | 0.1N NaOH (90°C) | 15 min | -10% |
| | 3% $H_2O_2$ (90°C) | 1h | -14% |
| **Levonordefrin-Tetracaine-Procaine:** | | | |
| Levonordefrin | 0.1N HCl | 1h | -25% |
| | 0.1N NaOH | 1 h | -10% |
| | 3% $H_2O_2$ | 2h | -11% |
| Tetracaine | 0.1N HCl (90°C) | 1h | -15% |
| | 0.1N NaOH (90°C) | 30 min | -20% |
| | 3% $H_2O_2$ | 45 min | -13% |
| Procaine | 0.1N HCl (90°C) | 1h | -22% |
| | 0.1N NaOH (90°C) | 15 min | -13% |
| | 3% $H_2O_2$ (90°C) | 1h | -16% |

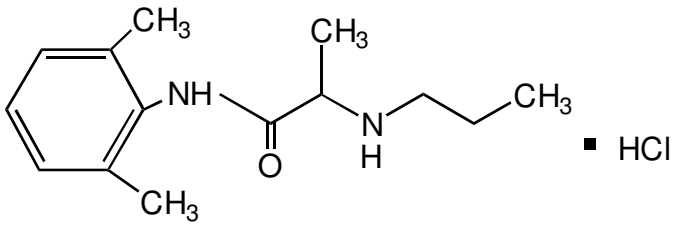
Table 8.3. Stability-indicating nature of assays.

| Drug | Treatment | Duration | %Δ in Peak Height |
|---|---|---|---|
| **Levonordefrin-Procaine-Propoxycaine:** | | | |
| Levonordefrin | 0.1N HCl | 1h | -25% |
| | 0.1N NaOH | 1 h | -10% |
| | 3% $H_2O_2$ | 2h | -11% |
| Procaine | 0.1N HCl (90°C) | 1h | -18% |
| | 0.1N NaOH (90°C) | 30 min | -20% |
| | 3% $H_2O_2$ (90°C) | 1h | -14% |
| Propoxycaine | 0.1N HCl (90°C) | 1h | -25% |
| | 0.1N NaOH (90°C) | 45 min | -20% |
| | 3% $H_2O_2$ | 1h | -12% |
| **Norepinephrine-Procaine-Propoxycaine:** | | | |
| Norepinephrine | 0.1N HCl (90°C) | 1h | -12% |
| | 0.1N NaOH | 30 min | -25% |
| | 3% $H_2O_2$ | 2h | -10% |
| Procaine | 0.1N HCl (90°C) | 1h | -21% |
| | 0.1N NaOH (90°C) | 45 min | -24% |
| | 3% $H_2O_2$ (90°C) | 1h | -16% |
| Propoxycaine | 0.1N HCl (90°C) | 1h | -21% |
| | 0.1N NaOH (90°C) | 30 min | -16% |
| | 3% $H_2O_2$ | 1h | -13% |

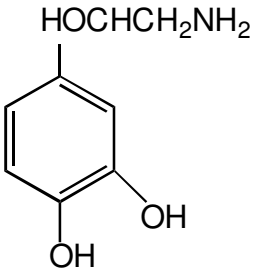
Figure 8.1. Chemical structures of compounds studied.



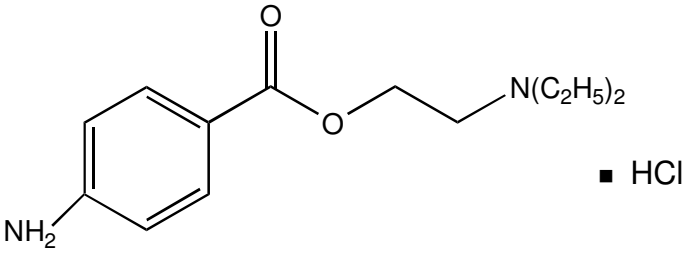

Epinephrine



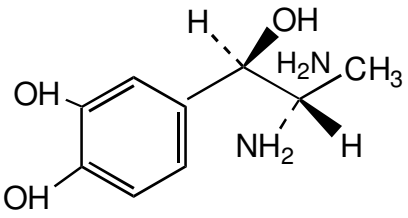

Prilocaine hydrochloride



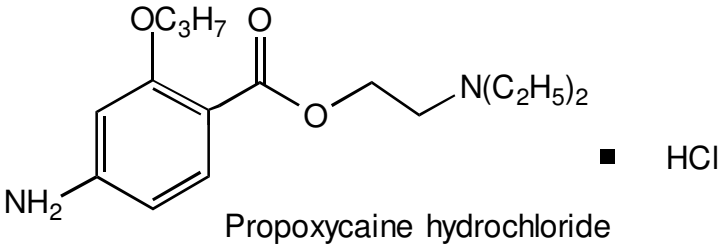

Norepinephrine



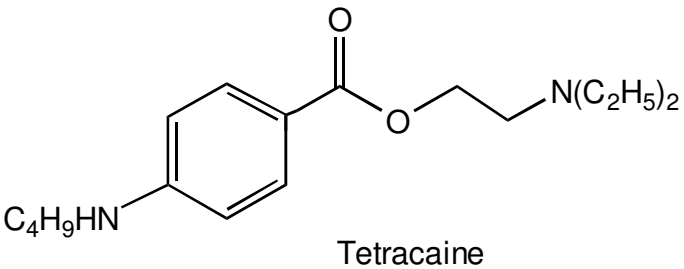

Procaine hydrochloride




Levonordefrin



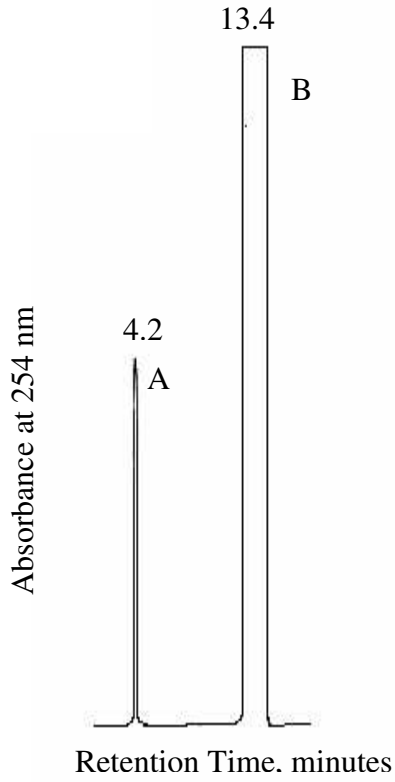

Propoxycaine hydrochloride




Tetracaine

Figure 8.2. Typical HPLC chromatogram of epinephrine (A) and prilocaine Hydrochloride (B). (I) Typical HPLC chromatogram for quantitation of A. (II) Typical HPLC chromatogram for quantitation of B.

I.


13.4
B
4.2
A
Absorbance at 254 nm
Retention Time. minutes

II

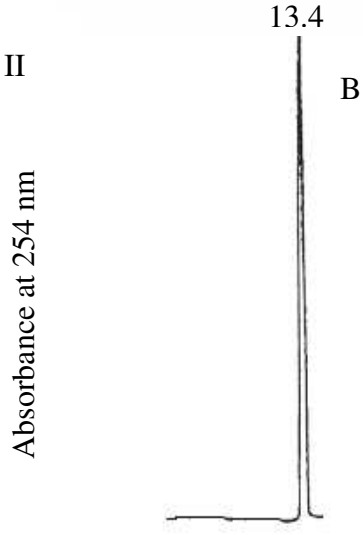
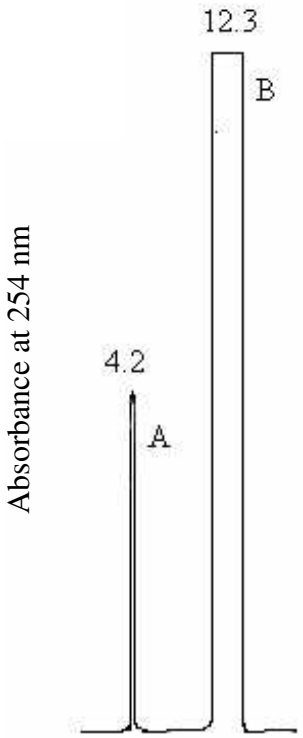

13.4
B
Absorbance at 254 nm
Retention Time, minutes

Figure 8.3. Typical HPLC chromatogram of epinephrine (A) and procaine hydrochloride (B). (I) Typical HPLC chromatogram for quantitation of A. (II) Typical HPLC chromatogram for quantitation of B.

I.


12.3
B
4.2
A
Absorbance at 254 nm
Retention Time, minutes

II.

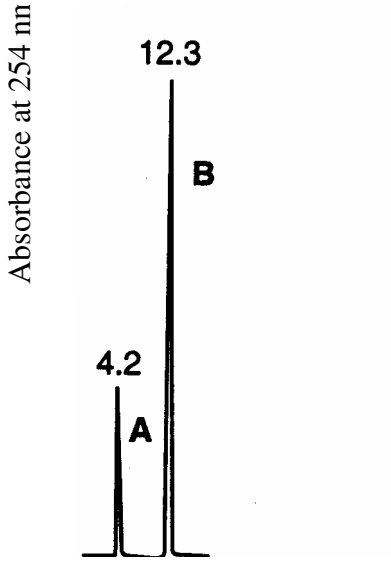

12.3
B
4.2
A
Absorbance at 254 nn
Retention Time, minutes

Figure 8.4. Typical HPLC chromatogram of levonordefrin (A), tetracaine (B), and procaine hydrochloride (C) based on a 1:150 dilution.

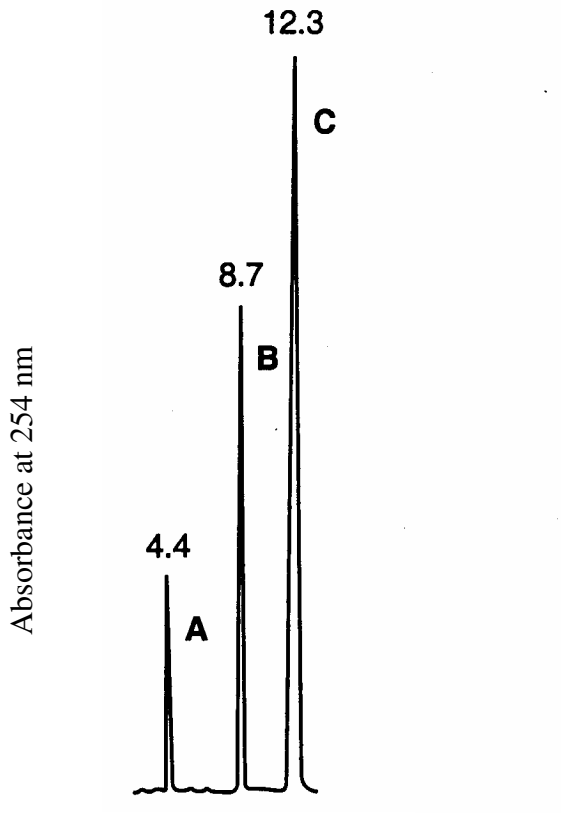

12.3
C
8.7
B
4.4
A
Absorbance at 254 nm
Retention Time, minutes

Figure 8.5. Typical HPLC chromatogram of levonordefrin (A), procaine hydrochloride (B), and propoxycaine hydrochloride(C) based on a 1:150 dilution.

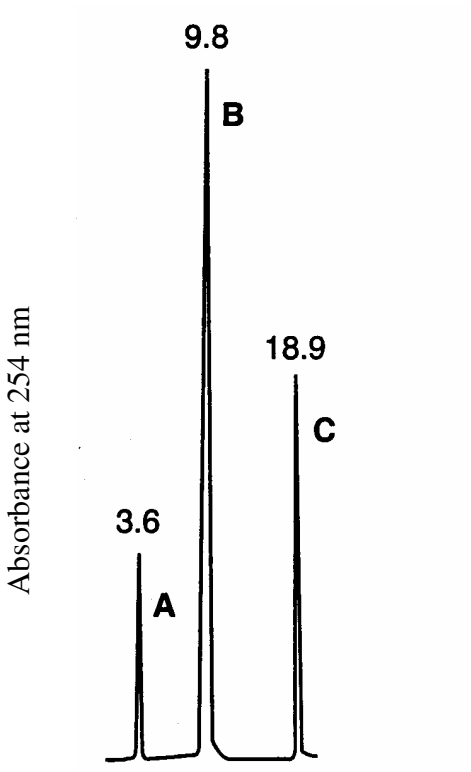
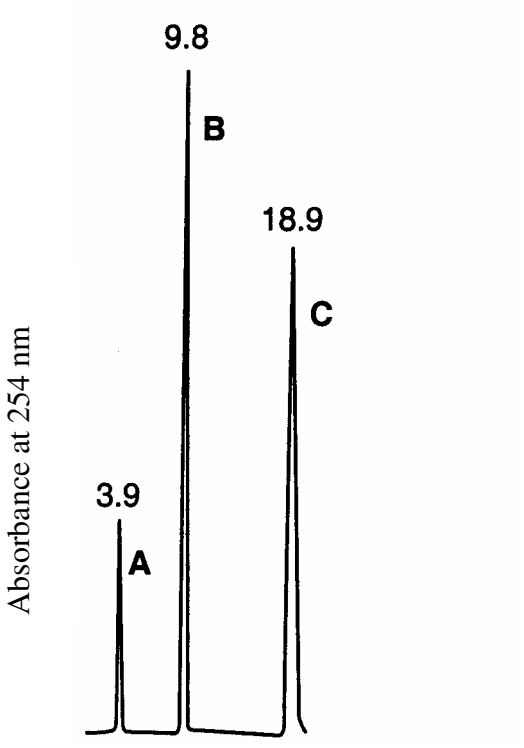

9.8
B
18.9
C
3.6
A
Absorbance at 254 nm
Retention Time, minutes

Figure 8.6. Typical HPLC chromatogram of norepinephrine (A), procaine hydrochloride (B), and propoxycaine hydrochloride (C) based on a 1:150 dilution.


9.8
B
18.9
C
3.9
A
Absorbance at 254 nm
Retention Time, minutes

# CHAPTER 9

# DEVELOPMENT OF A REVERSED-PHASE LIQUID CHROMATOGRAPHIC METHOD FOR THE ANALYSIS OF AMOXICILLIN, METRONIDAZOLE, AND PANTOPRAZOLE IN HUMAN PLASMA USING SOLID-PHASE EXTRACTION[9]

[9]ML Storms and JT Stewart. Submitted to *Journal of Liquid Chromatography and Related Technologies*, 2002.

ABSTRACT

A high-performance liquid chromatography method has been developed and validated for the simultaneous determination of amoxicillin, metronidazole, and pantoprazole in human plasma. Solid-phase extraction (SPE) was utilized to extract the analytes along with the internal standard, tinidazole. Baseline resolution was achieved using a 30:70 v/v acetonitrile - 25 mM potassium phosphate buffer containing 0.25% triethylamine (pH 6.5) mobile phase at a flow rate of 0.5 ml/min. A 5 μm phenyl column equipped with a guard column with detection at 230 nm constituted the HPLC system. The method yields retention times of 3.7, 4.8, 6.9, and 16.2 min for amoxicillin, metronidazole, tinidazole (internal standard), and pantoprazole, respectively. Limits of detection were 200 ng/ml for amoxicillin and metronidazole and 100 ng/ml for pantoprazole. Recoveries from human plasma ranged from 83-92% for amoxicillin, 81-89% for metronidazole, 85-94% for tinidazole, and 93-101% for pantoprazole. Intra-day (n=5) and inter-day (n=15) precision (%RSD) and accuracy (%error) for all analytes ranged from 1.11-5.97% and 0.72-13.5%, respectively.

## INTRODUCTION

Helicobacter pylori (Hp) is a common bacterial infection of the gastric mucosa and is widely accepted as the most common cause of peptic ulcer disease and gastritis (1). Current treatment for the eradication of Hp most often involves multi-drug therapy consisting of two antibiotics and a histamine-2 antagonist or proton pump inhibitor (PPI) (2). More specifically, PPI triple therapies which use the twice-a-day combination of a PPI plus metronidazole, 500 mg twice a day and amoxicillin, 1 g twice a day yield cure rates of 95-99% when patients with duodenal ulcer are prescribed triple therapy for 10 to 14 days (1).

A review of the literature revealed that several high performance liquid chromatographic (HPLC) methods have been reported for the individual determination of amoxicillin (3-5) and metronidazole (6-9) in plasma or serum. Although an HPLC method has been reported for the simultaneous determination of amoxicillin and metronidazole in human plasma using liquid-liquid extraction, the method requires the use of two different detector wavelengths and gradient elution analysis (10). HPLC methods have been reported for the determination of pantoprazole in plasma or serum including enantiomeric separations with direct injection (11-12) and column-switching sample preparation procedures (13). However, no HPLC method has been developed for the simultaneous determination of amoxicillin, metronidazole, and pantoprazole in human plasma or gastric fluid using solid-phase extraction (SPE).

In this paper, we report an isocratic reversed-phase HPLC method to assay amoxicillin, metronidazole, and pantoprazole in human plasma using a $C_{18}$ SPE extraction cartridge and UV detection at 230 nm. This combination of SPE and UV

detection results in a method with high recoveries and good linearity, accuracy, and precision.

## EXPERIMENTAL

### Reagents and chemicals:

The structures and formulae of the compounds studied are shown in Figure 9.1. Amoxicillin, metronidazole, and triethylamine were purchased from Sigma Chemical Co. (St. Louis, MO). Pantoprazole was kindly provided by Wyeth Laboratories (Pearl River, NY). Acetonitrile (J.T. Baker, Phillipsburg, NJ) was HPLC grade. Monobasic potassium dihydrogen phosphate ($KH_2PO_4$) and concentrated phosphoric acid were Baker analyzed reagents. Varian bond-elut™ $C_{18}$ SPE cartridges and the VAC-ELUT™ vacuum manifold were obtained from Varian Sample Preparation Products (Harbor City, CA). Oasis HLB cartridges were purchased from Waters Corp (Milford, MA). Strata™ C-18E SPE cartridges were purchased from Phenomenex (Torrance, CA). Drug-free human plasma used in this study was purchased from Bioreclamation Inc. (Hicksville, NY) and stored at -20°C until the assay.

### Chromatographic system:

The HPLC chromatographic system consisted of a pump (Model 760, Micromeritics, Norcross, GA), autosampler (Model 728, Micromeritics, Norcross, GA) equipped with a 50 µL loop, and an ultraviolet variable wavelength detector (Model 481, Waters Corp., Milford, MA). Chromatographic separations were achieved on an XTerra™ phenyl column (5 µm, 15 cm X 4.6 mm i.d., Waters, Milford, MA) equipped with an XTerra™ Sentry™ guard column.

Chromatographic conditions:

Chromatographic analysis was carried out at ambient temperature (24 ± 1°C). The isocratic mobile phase was composed of a buffer solution [acetonitrile - 25 mM potassium phosphate monobasic in water containing 0.25% triethylamine (pH 6.5) (30:70% v/v)]. The pH was adjusted with phosphoric acid. The mobile phase was filtered through a 0.45 µm nylon-66 filter (Alltech, Deerfield, IL) and degassed by sonication prior to use. The mobile phase flow rate was 0.5 mL/min and the detection wavelength was set at 230 nm. Under the chromatographic conditions described, amoxicillin eluted at 3.7 min, metronidazole eluted at 4.8 min, tinidazole (internal standard) eluted at 6.9 min, and pantoprazole eluted at 16.2 min.

Preparation of standard solutions:

Stock solutions of amoxicillin, metronidazole, tinidazole (internal standard) and pantoprazole were prepared by dissolving appropriate amounts of each drug in water to obtain final drug concentrations of 100 µg/ml. Working solutions were prepared by further diluting these stock solutions in 25 mM potassium phosphate buffer (pH 6.5). Calibration standards for all samples were prepared by spiking 100 µl of drug-free human plasma with appropriate volumes of each standard solution and 20 µl of tinidazole internal standard solution to obtain amoxicillin, metronidazole, and pantoprazole concentrations of 0.5-50 µg/ml (0.5, 1, 5, 10, 25, and 50 µg/ml) and a 2.0 µg/ml tinidazole concentration. The spiked plasma standards were then extracted from plasma.

Extraction procedure:

Extraction cartridges (Phenomenex Strata™ C18-E, 3 ml, 500 mg) were placed on a vacuum elution manifold (VAC-ELUT™, Varian Sample Preparation Products, Harbor City, CA). Each 500 mg $C_{18}$ SPE cartridge was pre-conditioned with methanol (2 x1 ml) and distilled water (2 x 1 ml). Care was taken to ensure that the cartridges did not run dry. One milliliter of the spiked plasma samples was loaded onto the cartridges and allowed to pass through the cartridge under mild vacuum. The cartridge was washed with water (2 x 1 ml) and dried under vacuum for 5 min. After discarding the eluent, the analytes were eluted with 1 mL methanol (4 x 250 μL) into clean 1.5-mL microcentrifuge tubes. Eluents from the cartridges were then dried at 40°C using the vacuum centrifuge (Model SC110A, Savant Instruments, Holbrook, NY, USA) and reconstituted in 1 mL mobile phase. The samples were then transferred to 1 mL injection vials where 50 μL of sample was injected onto the HPLC column.

Assay validation:

Samples were quantified using peak area ratios of analyte to internal standard (tinidazole). The calibration curves showed good linearity in the range of 0.5–50 μg/ml for amoxicillin, metronidazole, and pantoprazole in human plasma. The regression coefficients ($r^2$) of calibration curves of each drug were higher than 0.99.

The limits of detection (LODs) for each analyte in plasma were demonstrated by analysis of standard-spiked samples gradually decreasing in concentration. The LODs were determined as the concentration at which the signal/noise ratio was ~3.

The method accuracy (% error) was obtained by comparing the concentrations calculated from the calibration curves versus concentrations added. Precision was

calculated as percent relative standard deviation (%RSD). The intra-day accuracy and precision of the assay were determined by assaying three quality control samples at low (2 μg/ml, n=5), medium (10 μg/ml, n=5), and high (40 μg/ml, n=5) concentrations for amoxicillin, metronidazole, and pantoprazole in three analytical runs within the same day. The inter-day accuracy and precision samples were analyzed on three different days.

To investigate the extraction efficiency of amoxicillin, metronidazole, tinidazole, and pantoprazole from human plasma, standard-spiked plasma samples were subjected to extraction and then analyzed. The resulting peak areas were compared to peak areas of samples containing equal amounts of each analyte in mobile phase.

Both processed sample and freeze-thaw stability were evaluated for amoxicillin, metronidazole, tinidazole and pantoprazole. The stability of all four analytes stored on the autosampler tray was assessed by injecting replicate standard-spiked plasma samples at concentrations of 1 and 10 μg/ml at evenly spaced intervals over a 24-hour period. Freeze-thaw stability studies were also conducted to investigate the influence of freezing and thawing on the four analytes at concentrations of 1 and 10 μg/ml in spiked drug-free plasma. The spiked drug-free plasma samples were assayed in duplicate and the remainder placed into a -20°C freezer for 24 hours. After 24 hours, samples were thawed and a further aliquot was assayed in duplicate. This was repeated until three freeze-thaw cycles were completed.

## RESULTS AND DISCUSSION

The goal of this study was to develop an isocratic HPLC assay for the analysis of amoxicillin, metronidazole, and pantoprazole in human plasma and gastric fluid. Since pantoprazole is more lipophilic than either amoxicillin or metronidazole, it was more

challenging to obtain a short run time under isocratic conditions. Initial studies to develop an isocratic HPLC method for this mixture involved the use of $C_{18}$ and $C_8$ columns with various mobile phases containing acetonitrile- or methanol-aqueous phosphate buffers. In almost every system studied, amoxicillin and metronidazole eluted at the solvent front with poor peak symmetry while pantoprazole eluted after 30 min.

Our attention turned to the use of a Waters Xterra™ phenyl column (Milford, MA) since it takes advantage of both silica and polymer packing materials; thereby, separating compounds with widely different physical and chemical properties. Also, Xterra™ particles replace one third of the surface silanol groups with methyl groups resulting in the most homogenous coverage of any reversed-phase material yielding sharp, symmetrical peaks for basic compounds. It was found that the phenyl column reduced the retention of pantoprazole to about 16 min while resolving amoxicillin and metronidazole away from the solvent front under the described conditions. Although the phenyl column did improve peak shape for the basic analytes, it was still necessary to use an amine modifier (0.25% triethylamine) in the mobile phase to obtain sharp, symmetrical peaks. Repeated injections could be made in less than 20 min allowing a reasonably high sample throughput. The described HPLC conditions also separated amoxicillin, metronidazole, and pantoprazole from the endogenous materials in human plasma (Figure 9.2). Several different classes of drugs and the sulphone metabolite of pantoprazole were tested by the described HPLC conditions to see if they interfered with the analysis (Table 9.1).

Solid-phase extraction using a series of different extraction cartridges, such as Varian Bon-Elut™, C18, Oasis™ HLB, and Phenomenex Strata™ C18-E were evaluated for the plasma and gastric fluid sample clean-up procedure. It was determined that under the

conditions described in the experimental section, Strata™ cartridges produced the highest recoveries of the drugs (>80%), as well as cleaner assay samples. An attempt was made to employ the extraction procedure for gastric fluid samples; however, the recovery of amoxicillin and metronidazole in gastric fluid was lower than in plasma (30% vs. 80%). Recovery of pantoprazole in gastric fluid could not be determined due to its instability at pH 2.

Using the described solid-phase extraction procedure and HPLC method, calibration curves were linear over the 0.5-50 μg/ml concentration range with $r^2$ values greater than 0.99 for amoxicillin, metronidazole, and pantoprazole in human plasma. The LODs were found to be 200 ng/ml for amoxicillin and metronidazole and 100 ng/ml for pantoprazole. The intra-day (n=5) precision and accuracy for amoxicillin (spiked concentrations of 2, 10, and 20 μg/ml) were in the range of 1.80-5.97% (RSD) and 0.73-13.5% (error), respectively, and for metronidazole (spiked concentrations of 2, 10, and 20μg/ml) 1.86-2.81% (RSD) and 1.32-8.90% (error), respectively. At the same spiked concentrations, intra-day (n=5) precision and accuracy for pantoprazole were 1.16-1.77% (RSD) and 4.85-6.70% (error), respectively. The plasma recoveries were high and reproducible, ranging from 83-92% for amoxicillin, 81-89% for metronidazole, 85-94% for tinidazole, and 93-101% for pantoprazole. At 2, 10, and 20 μg/ml, inter-day (n=15) precision and accuracy for amoxicillin ranged from 1.93-5.34% (RSD) and 4.12-6.39% (error), data for metronidazole ranged from 1.11-1.95% (RSD) and 3.05-7.77% (error), and pantoprazole ranged from 1.18-2.54% (RSD) and 4.77-9.54% (error). The results from the validation of the method in human plasma are shown in Figure 9.2. The stability of each drug stored on the autosampler was assessed up to 24 hr. The lack of instability for this period

of time allows a large batch of samples to be processed in one assay. Moreover, none of the drugs degraded after three freeze-thaw cycles.

## CONCLUSION

A sensitive and efficient method for the extraction and simultaneous analysis of amoxicillin, metronidazole, and pantoprazole in human plasma has been developed and validated. This method yields high recoveries, good linearity, precision and accuracy within the range of 0.5-50 μg/ml. Although not necessary for concentrating our samples, the solid-phase extraction procedure provided excellent sample clean up. Thus, the method is applicable for pharmacokinetic studies after single of multiple doses or each drug.

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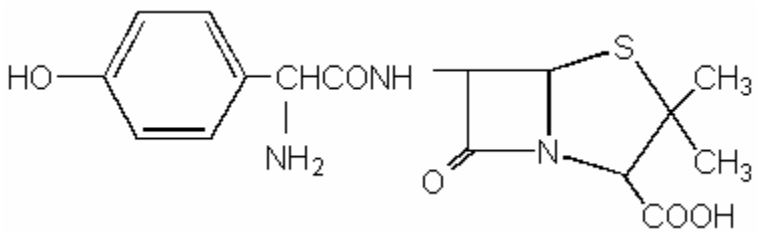
Table 9.1. Retention times of amoxicillin, metronidazole, tinidazole, pantoprazole and other drugs.

| Drug | Retention Time (min) | Drug | Retention Time (min) |
|---|---|---|---|
| Amoxicillin | 3.7 | Ibuprofen | >20 |
| Metronidazole | 4.8 | Acetaminophen | 5.2 |
| Tinidazole | 6.9 | Naproxen | 12.6 |
| Pantoprazole | 16.2 | Nifedipine | >20 |
| P-sulphone metabolite | 8.9 | Ranitidine | 4.1 |
| Guaifenesin | 7.8 | Cimetidine | 5.9 |
| Theophylline | 4.4 | Famotidine | 5.4 |
| Codeine | 4.9 | | |

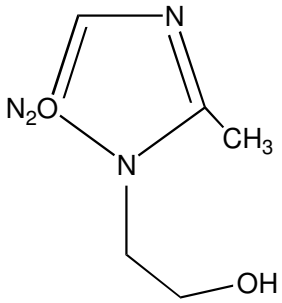
Table 9.2. The intra- and inter-day precision (% RSD) and accuracy (% Error) of amoxicillin, metronidazole, and pantoprazole in human plasma.

| Analyte | Conc. Added (ug/ml) | Intra-day (n=5) Conc. Found (ug/ml) | Intra-day (n=5) % RSD | Intra-day (n=5) % Error | Inter-day (n=15) Conc. Found (ug/ml) | Inter-day (n=15) %RSD | Inter-day (n=15) %Error |
|---|---|---|---|---|---|---|---|
| Amoxicillin | 2 | 1.90±0.11 | 5.97 | 5.05 | 2.16±0.04 | 1.93 | 6.39 |
| | 10 | 11.35±0.28 | 2.48 | 13.5 | 9.96±0.53 | 5.34 | 4.12 |
| | 20 | 19.86±0.37 | 1.85 | 0.72 | 19.64±0.84 | 4.26 | 4.76 |
| Metronidazole | 2 | 2.03±0.06 | 2.81 | 1.32 | 2.03±0.04 | 1.82 | 3.05 |
| | 10 | 9.11±0.24 | 2.64 | 8.90 | 9.54±0.19 | 1.95 | 3.29 |
| | 20 | 21.26±0.40 | 1.86 | 6.32 | 21.73±0.24 | 1.11 | 7.77 |
| Pantoprazole | 2 | 2.10±0.03 | 1.65 | 4.94 | 2.17±0.03 | 1.18 | 9.54 |
| | 10 | 9.51±0.17 | 1.77 | 4.85 | 10.29±0.26 | 2.54 | 4.77 |
| | 20 | 18.66±0.22 | 1.16 | 6.70 | 19.27±0.39 | 2.01 | 5.01 |

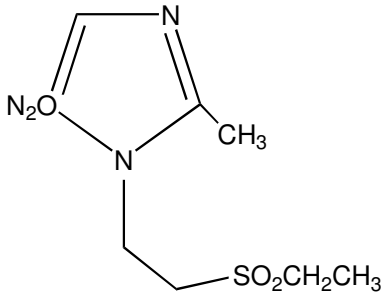
Figure 9.1. Chemical structures of compounds studied.



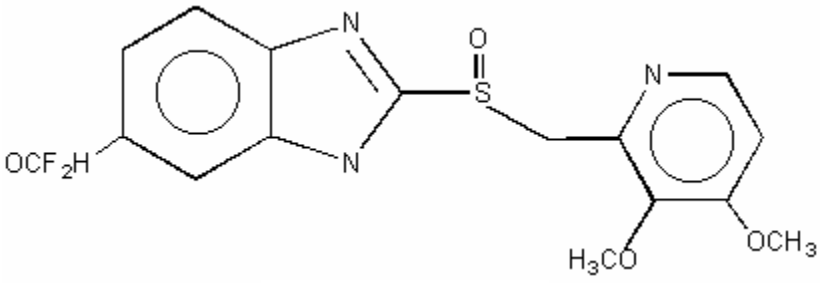

Amoxicillin




Metronidazole



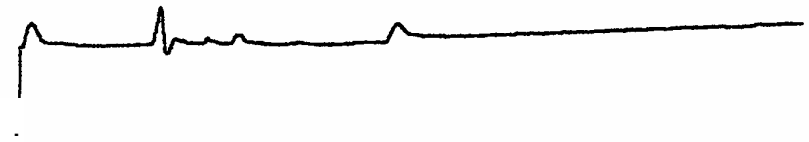

Tinidazole



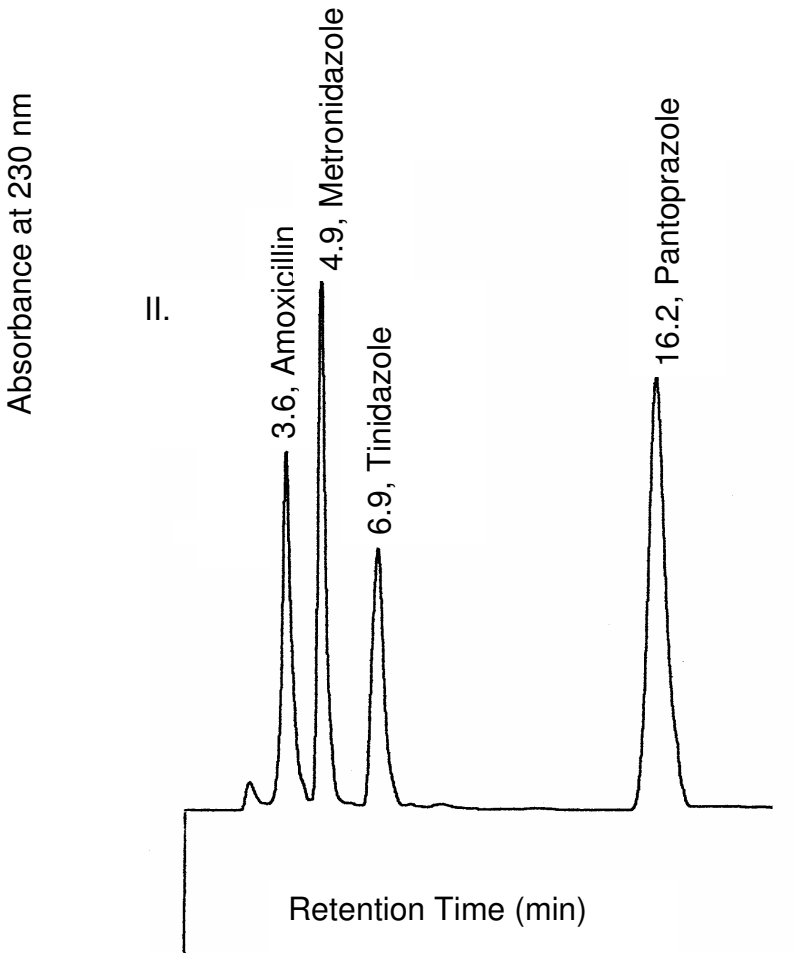

Pantoprazole

Figure 9.2. Representative chromatograms of blank plasma (I) and human plasma spiked with 5 µg/ml of amoxicillin, metronidazole, and pantoprazole (II). Tinidazole was used as the internal standard.

I.



II.


Absorbance at 230 nm
3.6, Amoxicillin
4.9, Metronidazole
6.9, Tinidazole
16.2, Pantoprazole
Retention Time (min)

# CONCLUSION

In this dissertation, HPLC methods for selected pharmaceuticals in various dosage forms and human plasma were discussed. Part I described the development of stability-indicating HPLC methods for ephedrine sulfate, lidocaine hydrochloride, neostigmine methylsulfate, glycopyrrolate, succinylcholine chloride, and tubocurarine chloride. The respective HPLC assays were applied to each drug stored individually in polypropylene syringes at ambient temperature (24±1°C) for up to 60 or 90 days.

Part II described HPLC method development and validation for selected pharmaceuticals in various dosage forms and human plasma. An underivatized silica column with an aqueous-organic mobile phase offered a solution to isocratically separate and quantitate several cough cold preprations including two capsule formulations (guaifenesin-pseudoephedrine-dextromethorphan and guaifenesin-pseudoephedrine) and one cough syrup formulation (guaifenesin-codeine). Ion-pair HPLC was employed to separate and quantitate several local anesthetic combinations under isocratic conditions. The combinations included epinephrine-prilcoaine, epinephrine-procaine, levonordefrin-tetracaine-procaine, norepinephrine-procaine-propoxycaine, and levonordefrin-procaine-propoxycaine. The respective methods were applied to the commercially available epinephrine-prilocaine (4% Citanest Forte) combination and to a laboratory-compounded injection solution of 20 μg/ml epinephrine and 10 μg/ml procaine. A bioanalytical method which employed HPLC with UV detection was also described for the separation and determination of amoxicillin, metronidazole, and pantoprazole in human plasma

using solid-phase extraction (SPE). This combination of SPE and UV detection results in a method with high recoveries and good linearity, accuracy, and precision.

# APPENDIX

Figure 10.1. Typical HPLC chromatogram of levonordefrin (A), tetracaine (B), and procaine (C).

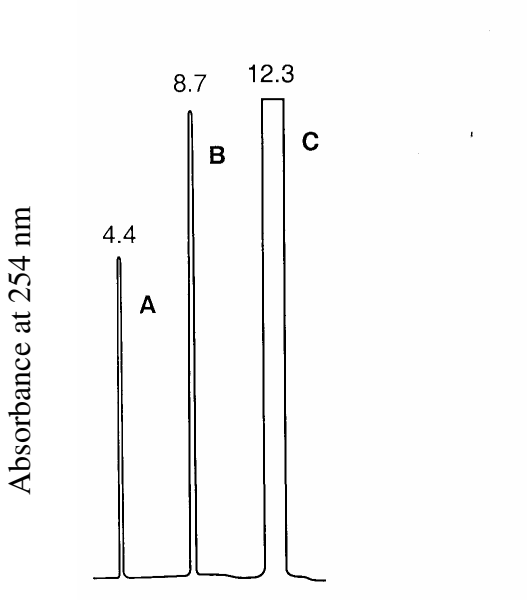

8.7
12.3
B
C
4.4
A
Absorbance at 254 nm
Retention Time, minutes

Figure 10.2. Typical HPLC chromatogram of levonordefrin (A), procaine (B), and propxycaine (C).

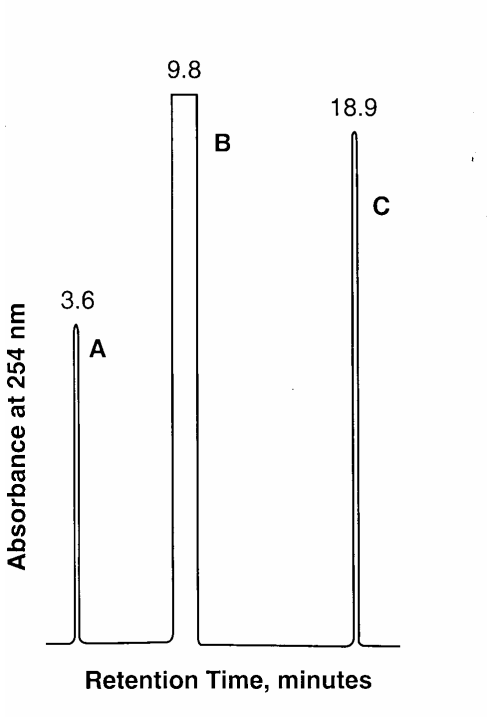
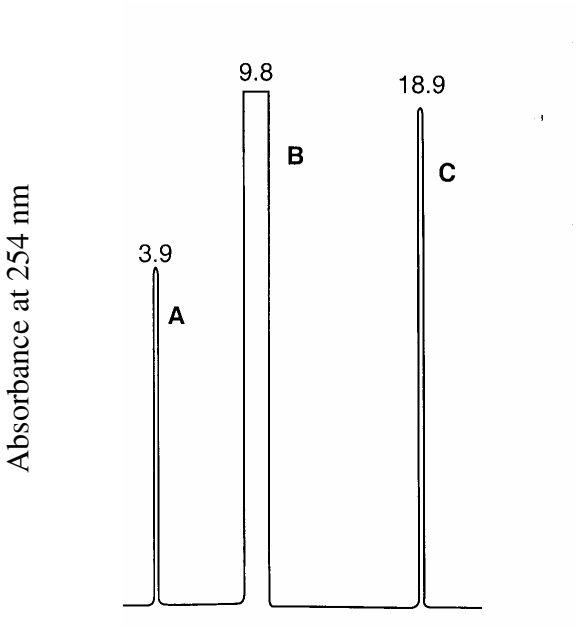

9.8
18.9
B
C
3.6
A
Absorbance at 254 nm
Retention Time, minutes

Figure 10.3. Typical HPLC chromatogram of norepinephrine (A), procaine (B), and propoxycaine (C).


9.8
18.9
B
C
3.9
A
Absorbance at 254 nm
Retention Time, minutes