AN ABSTRACT OF THE THESIS OF

Huey-Yuh Hsu for the degree of Master of Science in Pharmacy presented on July 22, 1987.

Title: Dissolution and Bioavailability of Immediate and Sustained Release Cold and Cough Dosage Forms

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Abstract approved :_____

A high-pressure liquid chromatography method based on reported literature was developed for the quantitative analysis of chlorpheniramine, pseudoephedrine, phenylpropanolamine and acetaminophen in dissolution fluids and urine. The effects of buffer, pH and different concentrations of organic solvent in the mobile phase on chromatographic separation are described in Chapter I.

Dissolution studies using the USP rotating-basket method were carried out on several commercially available brands of sustained and immediate release products. Dissolution pattern comparisons among products provided preliminary data before conducting a relative bioavailability study.

Relative bioavailability of chlorpheniramine from one immediate and two sustained-release products was determined in a urinary excretion study using 4 normal

healthy subjects. Urinary excretion of acetaminophen was also studied in these four subjects. Bioavailability results were consistent with dissolution results.

Dissolution and Bioavailability of Immediate and Sustained Release Cold and Cough Dosage Forms

By Huey-Yuh Hsu

A THESIS

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Dean of Graduate School

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Dissolution and Bioavailability of Immediate and Sustained Release Cold and Cough Dosage Forms

INTRODUCTION

This thesis deals with evaluation of immediate and sustained release cold and cough dosage forms. Each of the dosage forms evaluated contains chlorpheniramine along with additional ingredients. The chapters constitute a continuum of evaluation in that Chapter 1 deals with high pressure liquid chromatography analysis of ingredients in the dosage forms, Chapter 2 deals with dissolution of ingredients from the dosage forms, and Chapter 3 deals with bioavailability and relative absorption of chlorpheniramine, which is the major active ingredient in the dosage forms.

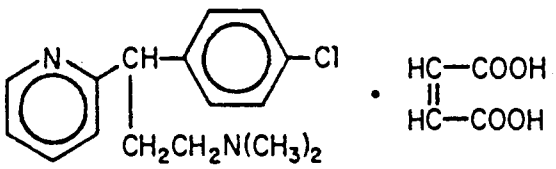
CHAPTER I.

HPLC ANALYSIS OF CHLORPHENIRAMINE AND PSEUDOEPHEDRINE IN SALIVA AND URINE.

INTRODUCTION

This research is part of a larger research project involving comparative bioavailability of non-prescription products. The portion described here had the objective of quantitatively analyzing chlorpheniramine and pseudoephedrine in saliva or urine samples using high-performance liquid chromatography. Several mobile phases and internal standards were investigated. The effects of buffer, pH, and different concentrations of organic solvent (acetonitrile and methanol) in the mobile phase on chromatographic separation were studied. Since both chlorpheniramine and pseudoephedrine have low therapeutic concentrations in saliva and urine, a high sensitivity (able to detect concentrations lower than 20 ng/ml for chlorpheniramine) was desired for biopharmaceutic and pharmacokinetic studies of these two drugs.

The drugs assayed in this study include chlorpheniramine, pseudoephedrine, phenylpropanolamine and acetaminophen. Structures of these compounds are shown below:



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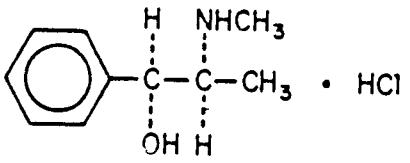

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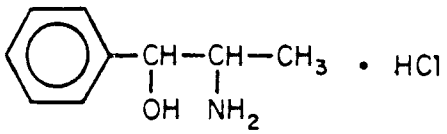
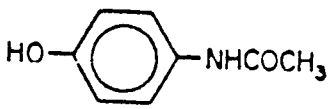


Phenylpropanolamine HCl



Acetaminophen

MATERIALS AND METHODS

Reagents:

Chlorpheniramine maleate and pseudoephedrine HCl were supplied by Warner-Lambert Co. HPLC grade acetonitrile and methanol were obtained from Baker Chemical Co. Most other chemicals were reagent grade.

HPLC instrumentation:

Two HPLC systems were used in this study.

(1) An HPLC solvent delivery pump (Model M-6000A), a sample injector (Model U6K), a 30 cm x 3.9 mm ID μBondapak C-18 column, and a UV detector (Model 480), all from Waters Association (Milford, Mass., U.S.A.).

(2) An HPLC solvent delivery module (Model 110 B), a manual sample injector valve (Model 210 A), and a variable wavelength detector (Model 163), all from Beckman Company. A data module (Model 740) from Waters was used.

RESULTS AND DISCUSSION

Selection of Mobile Phase:

Aqueous solutions of chlorpheniramine were initially chromatographed to select a suitable mobile phase. Binary solvent mixtures consisting of various proportions of methanol-water were tested as mobile phase in the initial studies. First, 10% methanol was used as mobile phase to analyze chlorpheniramine. After an injection of chlorpheniramine maleate, a peak appeared at 1.3 minutes. This peak was maleic acid. Chlorpheniramine maleate dissociates in solution to chlorpheniramine and maleic acid, resulting in the early appearance of the latter on the chromatogram. Chlorpheniramine did not elute in 40 minutes. Hence, if the low polarity molecule chlorpheniramine is to be eluted, a more predominantly organic solvent mobile phase is required.

Thus the mobile phase of Chao et al. (1) was modified. Methanol/H_2O/acetic acid (50:50:1, v/v) was used as mobile phase which had a pH value of 3.4. Dexbrompheniramine was used as internal standard. Unfortunately, both chlorpheniramine and dexbrompheniramine peaks occurred at 13 minutes. Change to a lower flow rate or lower drug concentrations did not separate the two peaks.

The paper of Yacobi et al.(2) was followed. The column was eluted with a mobile phase consisting of

acetonitrile-methanol-H_2O (35:40:25) and potassium nitrate and 1-octanesulfonic acid (0.001N each), pH 5.4, at a flow rate of 1.5 ml/min. In this mobile phase, octanesulfonic acid was used as an ion-pairing reagent. Ion-pair reverse-phase HPLC is a relatively useful technique. General discussions of the method are published (3,4). The separation of two decongestants and one antihistamine was demonstrated using a nonpolar reverse-phase column and heptanesulfonic acid as an ion-pairing agent (2,5). Since most cough-cold preparations contain an antihistamine and a decongestant, this mobile phase-$CH_3CN/CH_3OH/H_2O$ (35:40:25) with 0.001N KNO_3 and 1-octanesulfonic acid was examined for a long time in an effort to make it work.

Effects of Various Mobile Phases:

Mobile phase(I)- $CH_3CN/CH_3OH/H_2O$ (35:40:25) with 0.001N KNO_3 and 1-octanesulfonic acid, pH= 5.4

Internal standard- chlorpromazine.

Flow rate- 1.5 ml/min

Chart speed- 16 inches/hour

Sensitivity: 0.005 Aufs

Retention times of maleic acid (1),pseudoephedrine (2), chlorpheniramine (3), and internal standard (4) were 1.5, 2.5, 3.8, and 4.7 min, respectively (Figure I.1a). However, results were not satisfactory. First, pseudoephedrine always had a double peak (not owing to impurity of the compound as shown later). Second, the

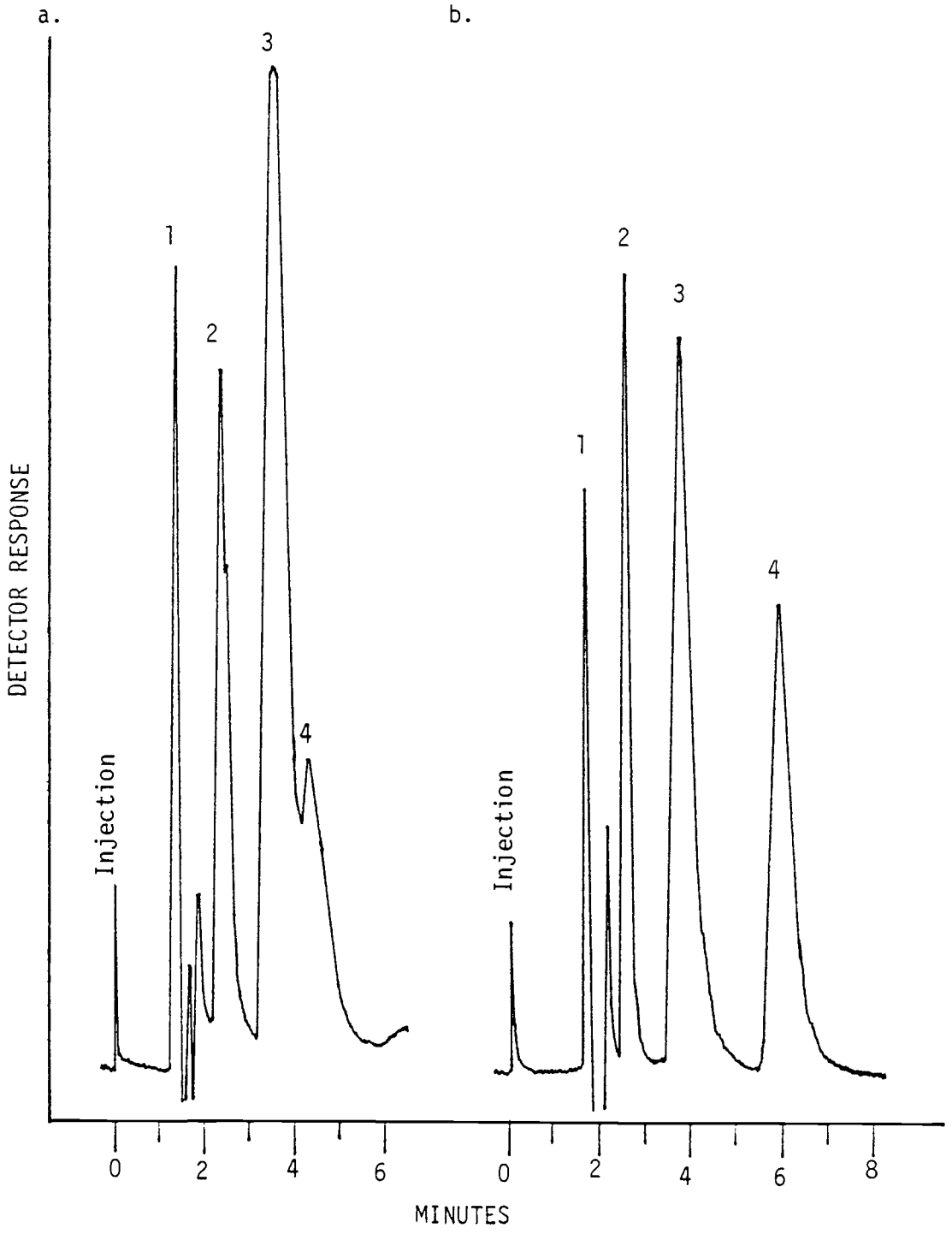


Figure I.1 Chromatographic Separation of 1, Maleic acid; 2, Pseudoephedrine; 3, Chlorpheniramine and 4, Chlorpromazine (IS). (a) Retention Times for 1, 2, 3 and 4 are 1.5, 2.5, 3.8 and 4.7 Minutes, Respectively. (b) Retention Times for 1, 2, 3 and 4 are 1.6, 2.6, 3.8 and 6.2 Minutes, Respectively.

peak for chlorpheniramine was very broad, therefore it did not separate well from the internal standard. Thus, the mobile phase of Yacobi et al. was not satisfactory with this column. Meanwhile, the theoretical plates of this column were calculated. The value was 2000, which was pretty low compared to a new column (10,000).

A new μBondapak C-18 column with the same mobile phase resulted in a clear separation of each component with no indication of any interference due to maleate or the presence of other molecules resulting from degradation of any of the compounds. The retention times of compounds 1-4 were 1.6, 2.6, 3.8 and 6.2 min, respectively (Figure I.1b). These values show that the entire procedure for simultaneous analyses of chlorpheniramine and pseudoephedrine in the same sample can be completed within 10 min using the (I) mobile phase and a new column.

After repeating the same experiment with all the same conditions, a few problems appeared:

(1) Retention times for chlorpheniramine and internal standard increased after each injection.

(2) The peak height of internal standard became smaller with repeated injections.

Figure I.2a shows retention times for chlorpheniramine and internal standard of 4.3 and 6.5 min. In Figure I.2b, retention times increase to 5.2 and 8.7 min. Table I.1 shows the change of retention time of chlorpheniramine within one day and on different days with

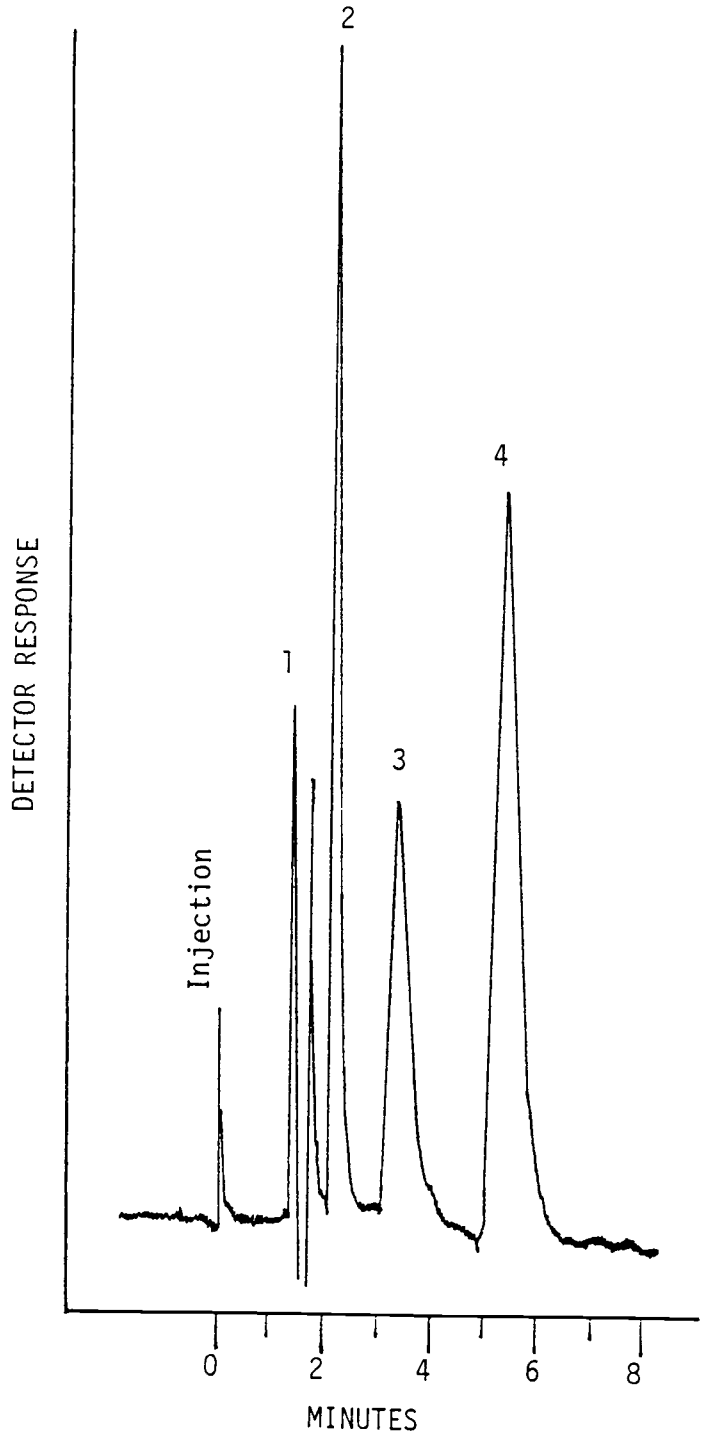


Figure I.2a Chromatographic Separation of 1, Maleic Acid; 2, Pseudoephedrine; 3, Chlorpheniramine and 4, Chlorpromazine (IS).

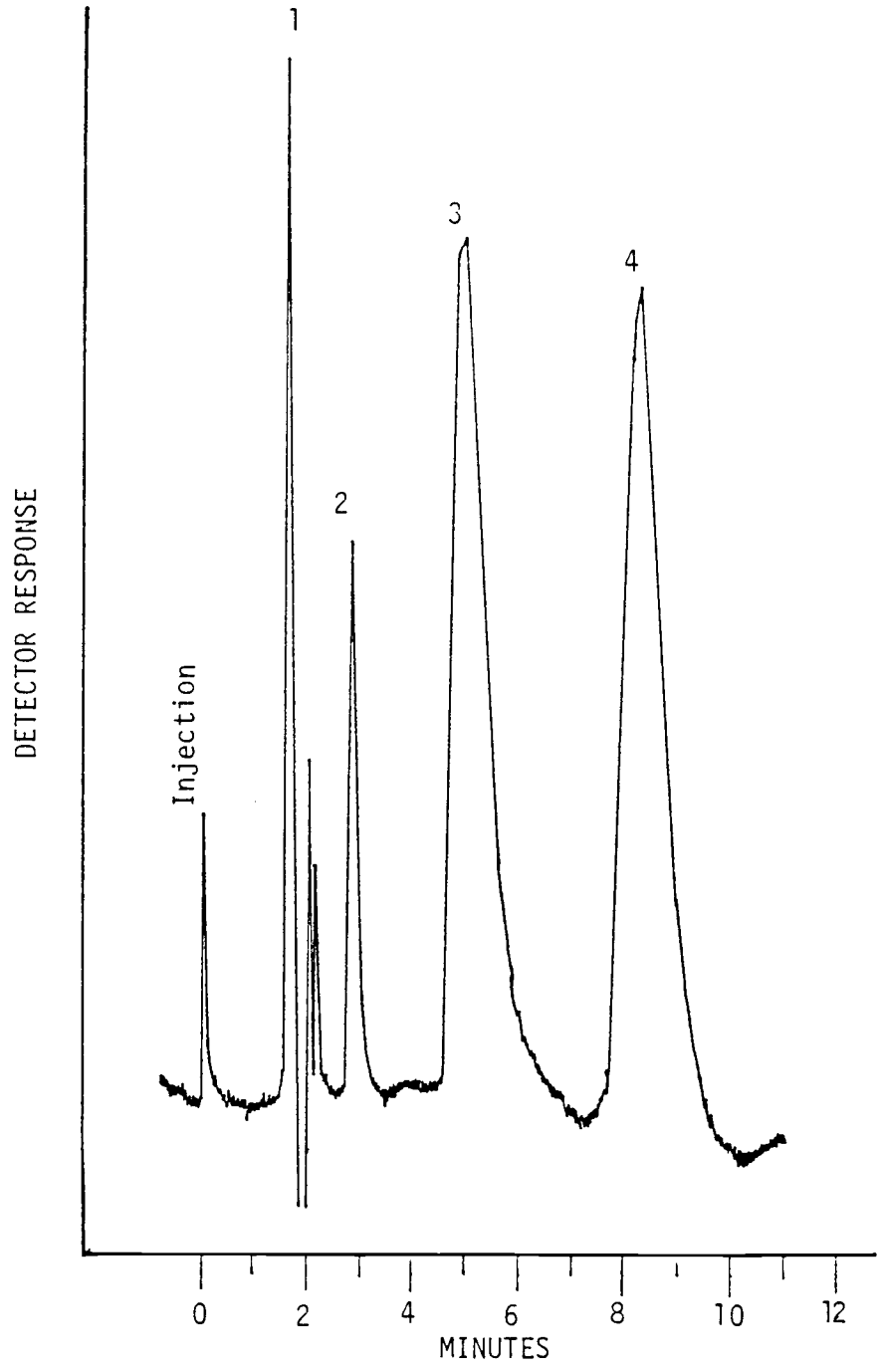


Figure I.2b Chromatographic Separation of 1, Maleic Acid; 2, Pseudoephedrine; 3, Chlorpheniramine and 4, Chlorpromazine (IS).

Table I.1 Retention Time of Chlorpheniramine within One Day and on Different Days.

Sample No.	Retention Time (min)		
	Day 1	Day 2	Day 3
1	4.4	4.4	4.3
2	4.5	4.7	4.6
3	4.8	5.0	4.7
4	4.9	5.2	4.9
5	5.2	5.5	5.2
6	5.5	5.6	5.3
7	5.9	5.9	5.6
8	6.1	6.2	5.9
9	6.5	6.4	6.2
10	6.8	6.8	6.6
11	6.9	6.8	6.8
12	7.2	6.8	7.2

repeated injections. Possible reasons for the problems are:

(1) Instrument reason- precolumn or injector.

(2) Stability of mobile phase.

(3) Stability of chlorpheniramine and chlorpromazine.

Hence, the precolumn and injector were changed which did not solve the problem. Freshly prepared mobile phase had the same result as using a week old mobile phase.

The stability of chlorpheniramine maleate has been studied using accelerated heat and light conditions on solutions of various pH values (6). It has been demonstrated that chlorpheniramine is a stable compound, while chlorpromazine decomposes on exposure to air and light. This may be the reason the chlorpromazine peak became smaller. Therefore, another internal standard- dexbrompheniramine was tried. Unfortunately, dexbrompheniramine can not be separated from chlorpheniramine using mobile phase (I).

During this period, the effect of different normalities KNO_3 were investigated. Too much KNO_3 (> 0.01N) resulted in poor reproducibility of peaks. Insufficient KNO_3 resulted in obvious peak tailing. A 0.002N concentration was selected on the basis of the chromatograms (Figures I.3a, I.3b and I.3c).

In another mobile phase the proportions of organic solvents and water were the same as mobile phase (I), only with doubled normality of KNO_3 which resulted in the pH

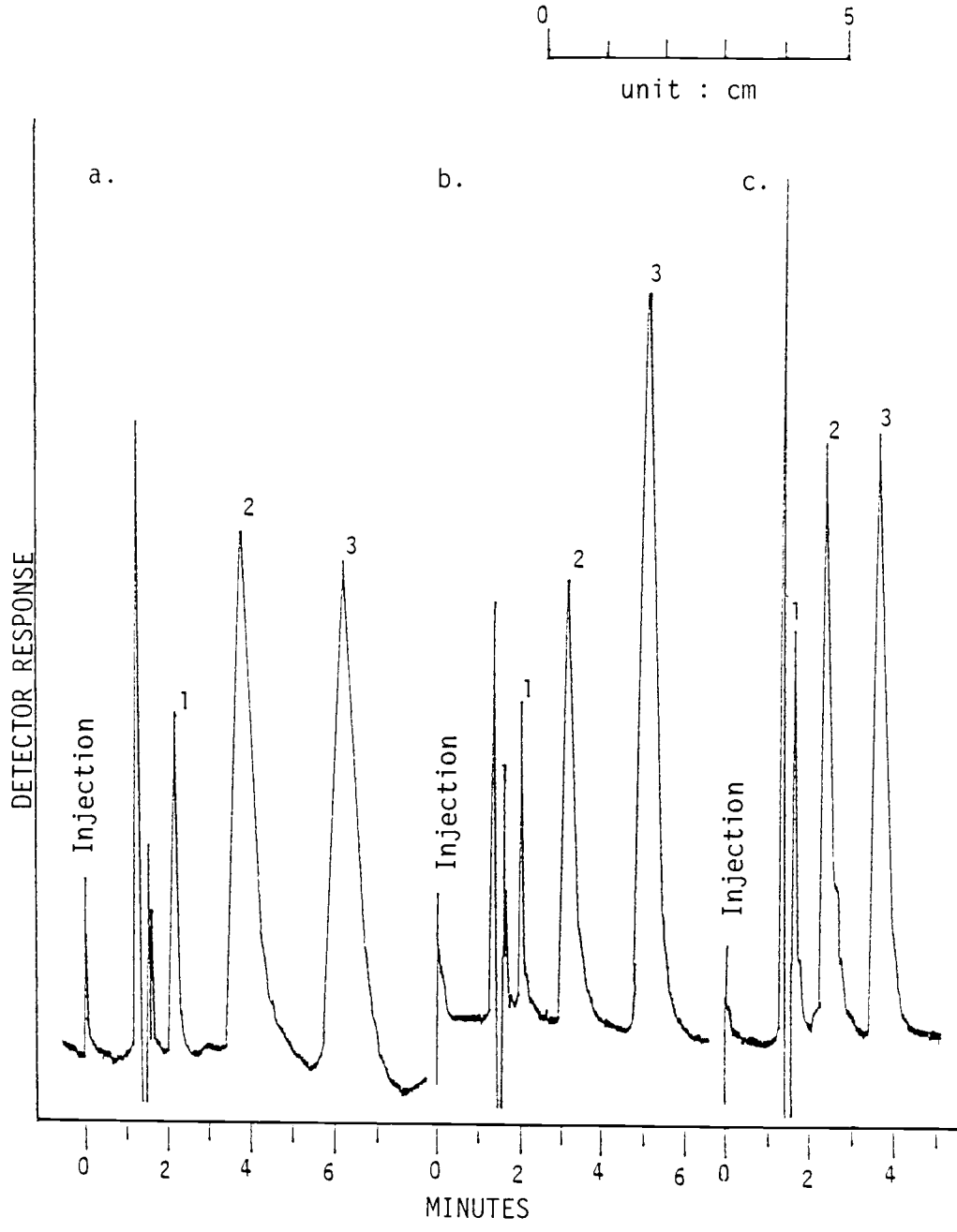


Figure I.3 Chromatographic Separation of (a) 1, Pseudoephedrine (150 mcg/ml); 2, Chlorpheniramine (60 mcg/ml); 3, Chlorpromazine (IS) with 0.001N KNO_3, (b) 1, Pseudoephedrine (30 mcg/ml); 2, Chlorpheniramine (30 mcg/ml); 3, IS with 0.002N KNO_3, and (c) 1, Pseudoephedrine (100 mcg/ml); 2, Chlorpheniramine (30 mcg/ml); 3, IS with 0.01N KNO_3 in the Mobile Phase.

value increasing from 5.4 to 6.2.

Mobile phase (II)- $CH_3CN/CH_3OH/H_2O$ (35:40:25) contains 0.002N KNO_3 and 0.001N octanesulfonic acid, pH = 6.2.

Internal standard- butyl paraben

Flow rate : 1.5 ml/min

Chart speed : 16 inches/hour

Sensitivity : 0.005 Aufs

Retention times and peak heights of butyl paraben for each injection were quite close, but retention times for chlorpheniramine increased with repeated injections. For example, it increased from 5.4 to 7.6 min from the first to the last injection on a certain day (Figures I.4a and I.4b). The retention time for pseudoephedrine also increased, but not so much as chlorpheniramine.

After a few days of experiments, the column back pressure started to increase. Most of the pressure came from the main column. The following method was used to clean the column:

(1) Inject 100 ul of 50% dimethyl sulfoxide every 5 min when using 100ml of deionized water as mobile phase.
(2) Flush with 100ml of methanol.
(3) Flush with 100ml of $CHCl_3$.
(4) Flush with 100ml of methanol.

After the cleaning procedure, the pressure was still pretty high (5000 psi). Another μBondapak C-18 column was purchased. In order to know the effect of buffer and pH in the mobile phase on the chromatographic separation,

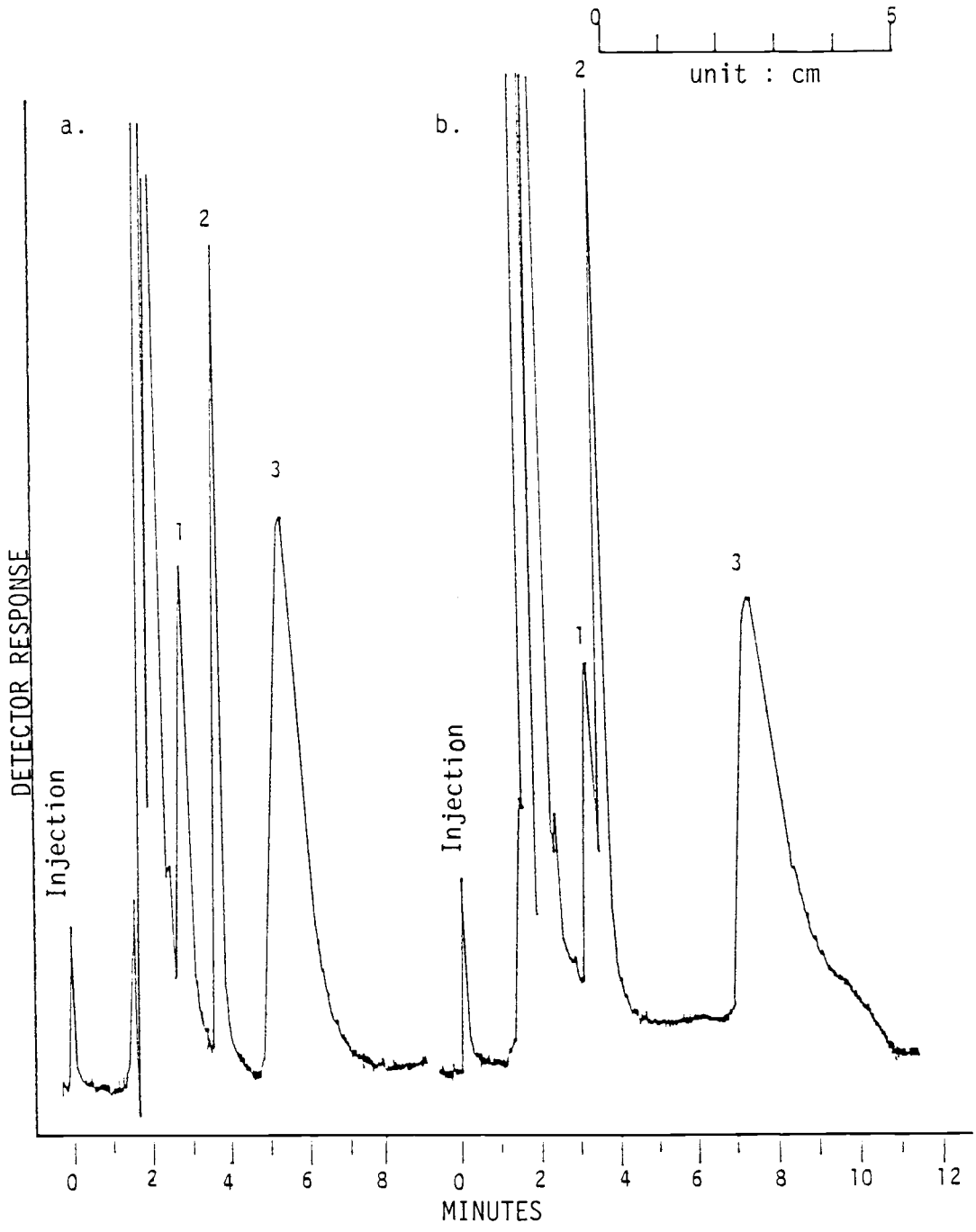


Figure I.4 Chromatographic Separation of 1, Pseudoephedrine; 2, Butyl Paraben (IS) and 3, Chlorpheniramine. Retention Time of Chlorpheniramine is (a) 5.4 min from the First Injection (b) 7.6 min from the Last Injection on a Certain Day.

Walpole's acetate buffer was used instead of H_2O in mobile phase (II). The pH of the mobile phase was decreased from 6.2 to 5.2. For most literature references which refer to the assay of cold-cough mixtures, phosphoric acid was used to adjust the pH of the mobile phase. In order to prevent crystallization of phosphate salts in the HPLC system, Walpole's acetate buffer was used. The peak height for chlorpheniramine increased about 2.5 times (Figures I.5a and I.5b) by using buffer solution at pH 5.2 instead of pure water pH 6.2 in mobile phase (II). The peak height for pseudoephedrine also increased but only 1.2 times. Retention times for pseudoephedrine and chlorpheniramine were 2.2 and 2.9 minutes, respectively with acetate buffer and 2.7 and 4.0 minutes without acetate buffer. Retention time of chlorpheniramine with repeated injection is repeatable using acetate buffer.

From the above results, it seemed when pure chlorpheniramine was chromatographed on a reversed-phase column using nonacidified mobile phase, it was strongly retained. For example, the retention volume for chlorpheniramine and dexbrompheniramine were greater than 60 ml using an eluent of 10% methanol in water. When the mobile phase was acidified, chlorpheniramine was eluted in much smaller retention volume. This is due to the basic property of the compound. These results agree with Athanikar et al.(7). Furthermore, with an acidic mobile phase, the peaks obtained were more symmetrical

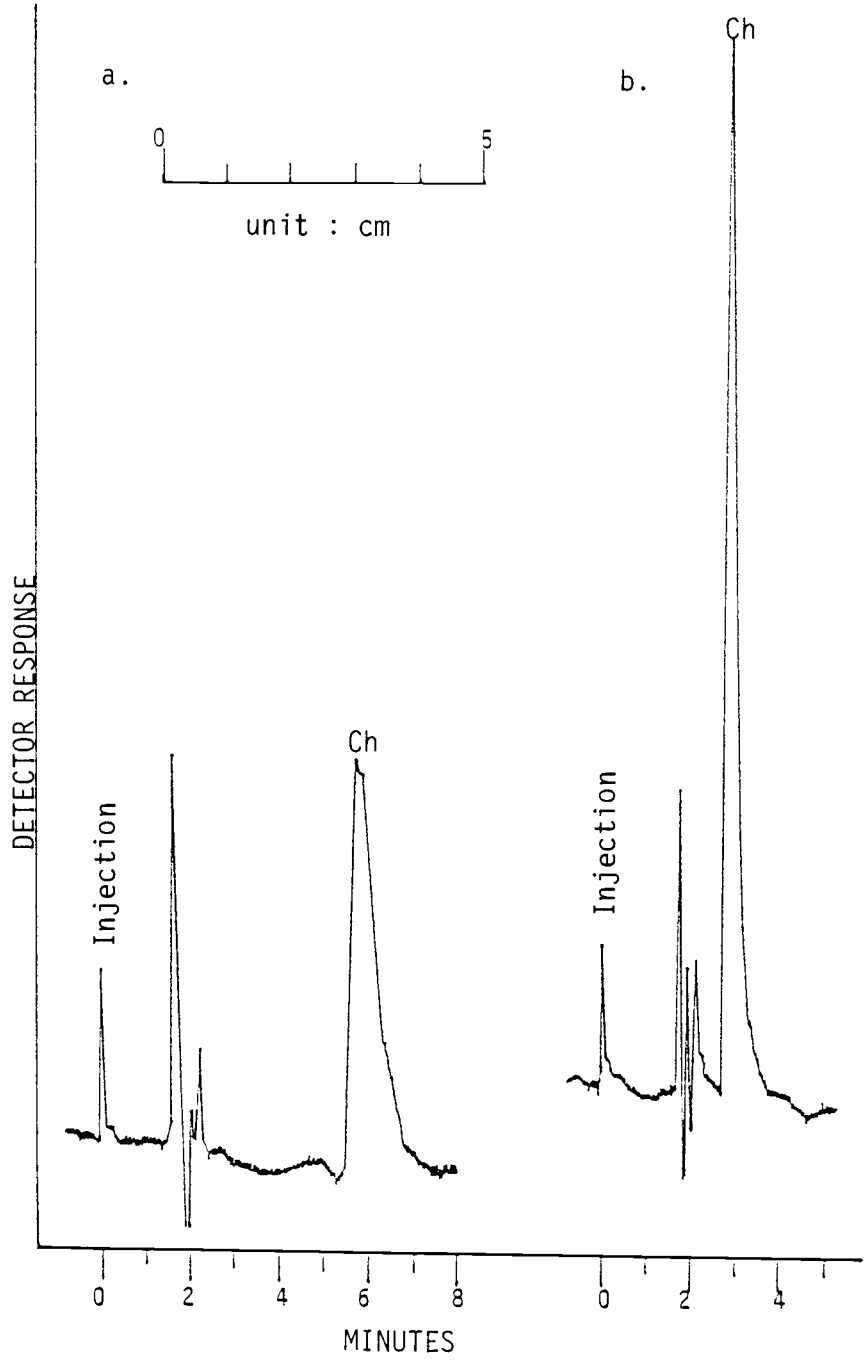


Figure I.5 Chromatogram of Chlorpheniramine (20 mcg/ml) (a) Without Buffer (b) With Buffer in The Mobile Phase.

than peaks obtained with a higher pH mobile phase.

Although separation was good, the retention times for both pseudoephedrine and chlorpheniramine were within 3 min of the solvent front. Since the retention time of saliva or urine solvent front was about 4 min, it was necessary to have longer retention times in order detect chlorpheniramine in biological fluids. According to the investigations of Athanikar et al.(7), incorporation of the inorganic salt, ammonium dihydrogen phosphate in the eluent was found to improve separation. After reading this paper (7), the following mobile phase was used to study the chromatographic behavior of chlorpheniramine and pseudoephedrine.

Mobile phase (III)- acetonitrile/ammonium phosphate buffer (35: 65) with 0.002 N KNO_3.

Internal standard- lidocaine.

Lidocaine was chosen as internal standard (8) instead of butyl paraben. Butyl paraben dissolved in the organic solvent when a pH partitioning extraction procedure was performed, but did not go to the aqueous layer with chlorpheniramine or pseudoephedrine when the pH was acidic. Retention times for chlorpheniramine, pseudoephedrine and lidocaine in this mobile phase (III) containing different concentrations of octanesulfonic acid (OSA) are presented in Table I.2. After evaluating different normalities of octanesulfonic acid (sodium salt) in the mobile phase, 0.005N octanesulfonic acid was chosen.

Table I.2 Retention Times of Chlorpheniramine, Pseudoephedrine, and Lidocaine in Different Concentrations of Octanesulfonic acid.

	No OSA	0.005N OSA	0.01N OSA
Pseudoephedrine	2.1 min	3.2 min	4.1 min
Chlorpheniramine	4.0 min	9 min	13.2 min
Lidocaine	4.2 min	4.8 min	5.1 min

Mobile phase (IV)- 0.01 M Ammonium dihydrogenphosphate solution (7) was prepared in deionized distilled water, and the phase consisted of 65:35 buffer/acetonitrile with 0.005N octanesulfonic acid and 0.002N KNO_3. The final pH was adjusted to 3.0 by adding phosphoric acid. This mobile phase was used in the following studies.

Extraction Procedure(7):

Take 1 ml of chlorpheniramine maleate solution, add 0.1 ml of lidocaine solution as internal standard (IS), basify with 0.1 ml of 1% KOH, and extract with 3 ml of ether. Vortex and centrifuge and freeze (immerse the aqueous solution in dry ice and alcohol). Collect ether layer and pour to another tube containing 0.1 ml 0.5% phosphoric acid. Vortex, centrifuge and freeze, then collect aqueous layer (7).

Standard curve data for chlorpheniramine maleate are shown in Tables I.3a and I.3b. Chromatograms of extracted 2500 and 1250 ng/ml chlorpheniramine prepared from water are shown in Figures I.6a and I.6b. Chromatograms of extracted 3750 and 1250 ng/ml chlorpheniramine prepared from a saliva sample are shown in Figures I.7a and I.7b. Figure I.8 shows that, within the concentration range of 625-5000 ng/ml for both water and saliva samples, the drug-internal standard peak height ratios could be described by a linear relationship.

Table I.3a Standard Curve Data Using Mobile Phase (IV). (Solution Prepared From Water. Injection Volume: 0.01 ml)

Conc.(ng/ml)	[a]PH	[b]PHR	[c]Pred. Conc.	% Actual Conc.
5000	24	1.84	4800	96
3750	9.5	1.56	4100	109
2500	8.9	0.86	2300	92 (Fig I.6a)
1250	3.6	0.34	1140	91 (Fig I.6b)
625	2.0	0.16	700	112

X = 100 % %SD = 9.8 %
% CV = 9.8 %

a. Peak height for chlorpheniramine in centimeters.
b. Peak height ratio of drug to internal standard.
c. Predicted concentration.

Table I.3b Standard Curve Data Using Mobile Phase (IV). (Solution Prepared From Saliva. Injection Volume: 0.01 ml.)

Conc.(ng/ml)	PH	PHR	Pred. Conc.	% Actual Conc.
5000	18	1.83	4870	97.4
3750	11.3	1.51	4010	107 (Fig I.7a)
2500	7.1	0.88	2320	92.8
1250	3.6	0.48	1250	100 (Fig I.7b)
625	2.2	0.24	620	99.1

X = 99.3% %SD = 5.1
%CV = 5

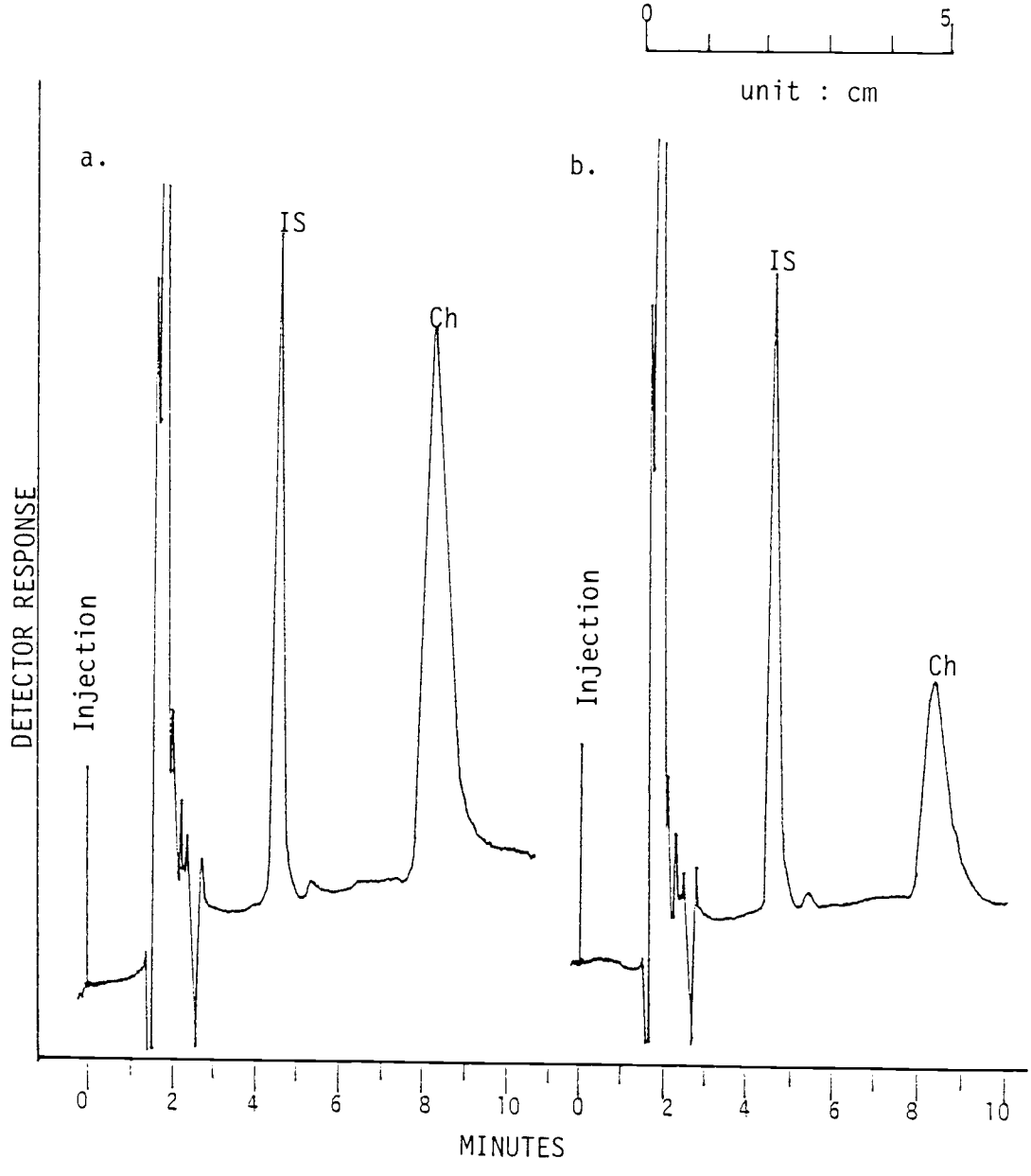


Figure I.6 Chromatogram of an Ether Extract of (a) Chlorpheniramine (2.5 mcg/ml), (b) Chlorpheniramine (1.25 mcg/ml) Obtained from Water.

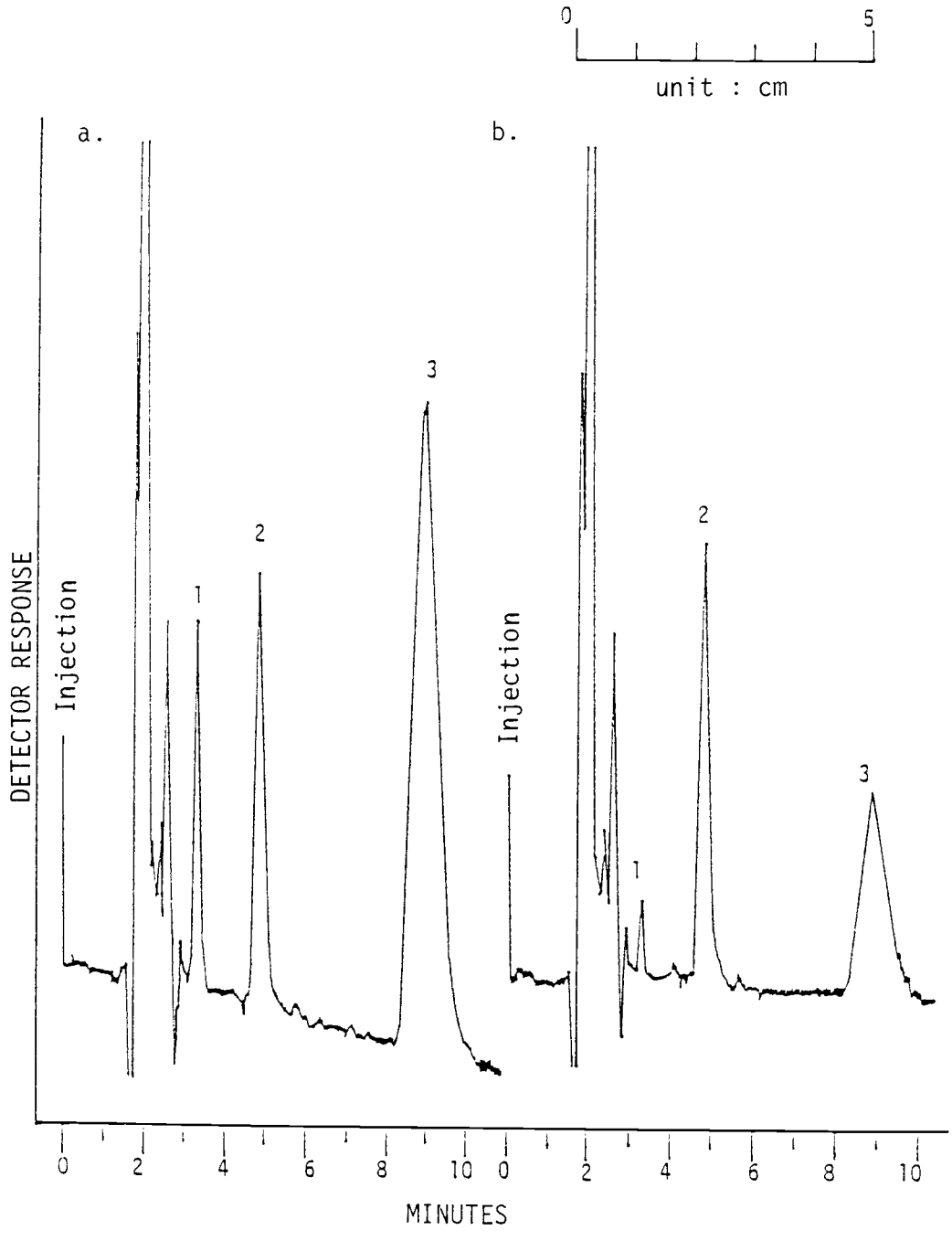


Figure I.7 Chromatogram of an Ether Extract of (a) 1, Pseudoephedrine (10 mcg/ml); 2, Lidocaine (IS); 3, Chlorpheniramine (3.75 mcg/ml), (b) 1, Pseudoephedrine (2.5 mcg/ml); 2, IS; 3, Chlorpheniramine (1.25 mcg/ml) Obtained from a Standard Saliva Sample.

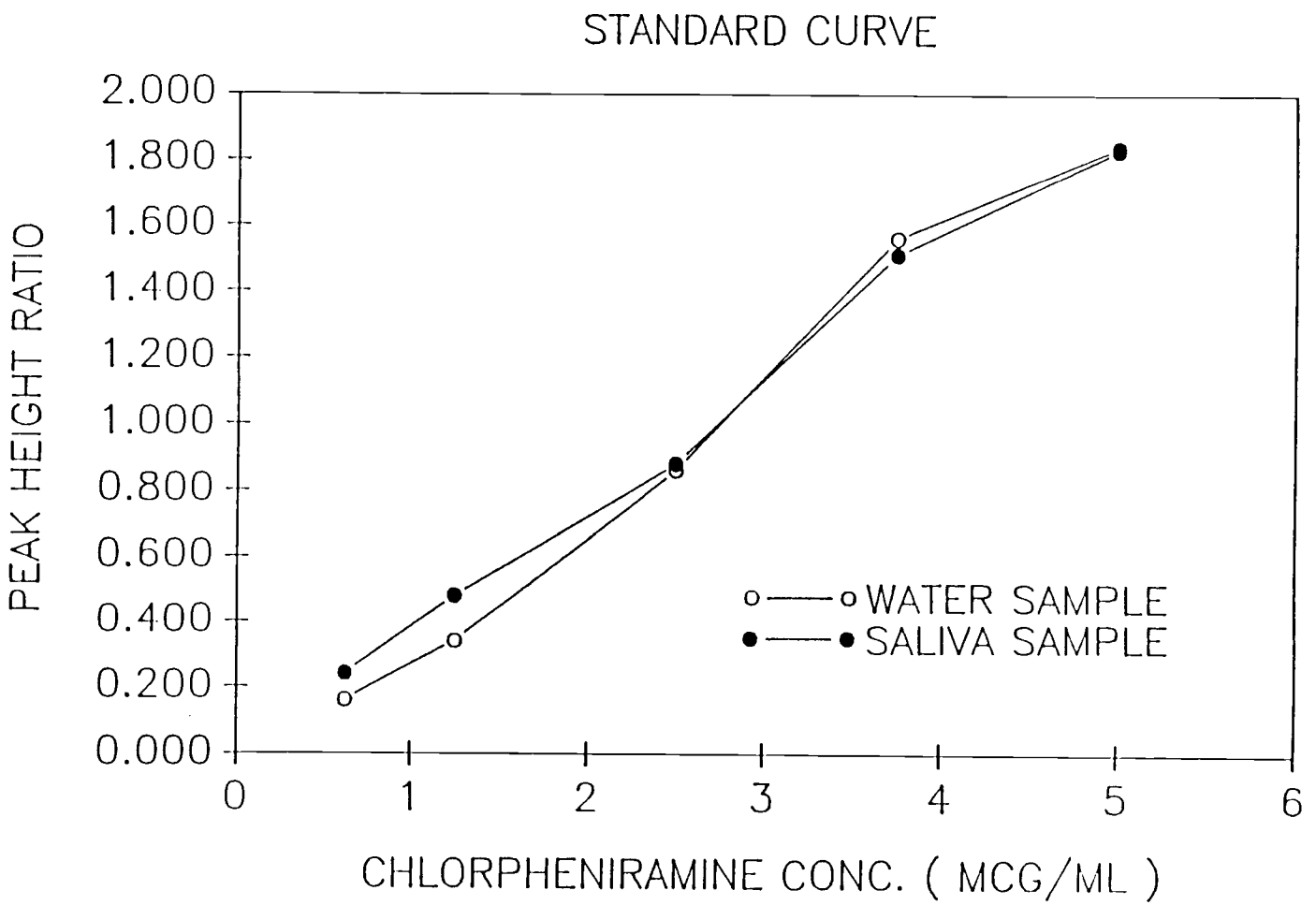


Figure I.8 Standard Curves of Chlorpheniramine Within The Concentration Range of 625-5000 ng/ml Using Waters HPLC Systems.

In order to be certain the drug concentration in saliva is detectable after a normal dose, a healthy normal subject took one tablet of Chlor-Trimeton which contains 4 mg chlorpheniramine maleate and 60 mg pseudoephedrine sulfate. Saliva samples were collected at 0, 1, 2, 3, 4, 5, and 6 hrs. Each sample contained about 5 ml of saliva. Saliva samples were extracted as above. Extracted, concentrated samples (0.01 or 0.05 ml) were injected on HPLC using mobile phase (IV), but no drug peaks were detected. This result suggested that (1) saliva chlorpheniramine concentration is much lower than 600 ng/ml and (2) the sensitivity of this HPLC system was not high enough to detect the saliva drug concentrations. Therefore, a calibration curve with a series of standards spanning the range of lower sample concentrations needed to be constructed. Therefore, a Beckman HPLC system with a data processor was used. First, the same series of standard solutions were evaluated in this system (Tables I.4a and I.4b). Chromatograms of extracted 5000 and 625 ng/ml chlorpheniramine prepared from saliva sample are shown in Figures I.9a and I.9b. Standard curves for water and saliva samples are presented in Figure I.10.

Retention times for chlorpheniramine and lidocaine are 8 and 4.4 minutes respectively. In order to increase the peak height of chlorpheniramine, the next mobile phase (V) was tried.

Table I.4a. Standard Curve Data Using Mobile Phase (IV). (Solutions Prepared From Water. Injection Volume: 0.01 ml. Attenuation 8.)

Conc.(ng/ml)	[a]PH	[b]PHR	[c]Pred. Conc.	% Actual Conc.
5000	6.9	2.3	4914	98
3750	5.9	1.8	3829	102
2500	4.0	1.2	2531	101
1250	2.1	0.6	1254	100
625	0.9	0.3	574	92

X = 98.6 % %SD = 4.0
%CV = 4 %

a. Peak height for chlorpheniramine in centimeters.
b. Peak height ratio of drug to internal standard.
c. Predicted concentration.

Table I.4b. Standard Curve Data Using Mobile Phase (IV). (Solutions Prepared From Saliva. Injection Volume: 0.01 ml. Attenuation 8)

Conc.(ng/ml)	PH	PHR	Pred. Conc.	% Actual Conc.
5000	7.5	2.4	5100	102 (Fig I.9a)
3750	5.4	1.6	3508	93.5
2500	4.2	1.2	2692	108
1250	1.6	0.5	1222	98
625	0.7	0.2	610	98 (Fig I.9b)

X = 99.9 % %SD = 5.4
%CV = 5.4

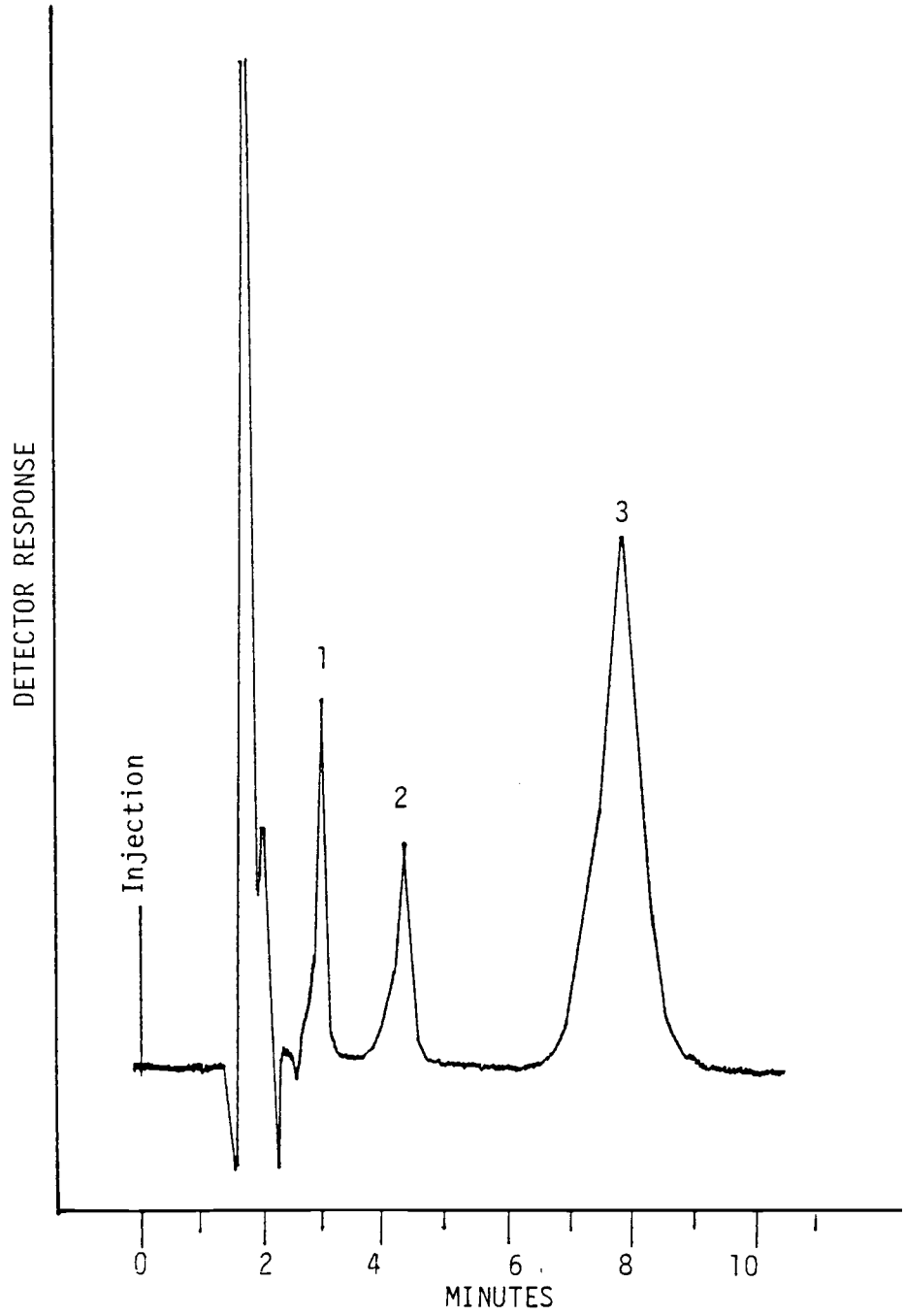


Figure I.9a Chromatogram of Ether Extract of 1, Pseudoephedrine (15 mcg/ml) ; 2, Lidocaine; 3, Chlorpheniramine (5 mcg/ml) Obtained from a Standard Saliva Sample Using Mobile Phase (IV). Recorder Attenuation: 8.

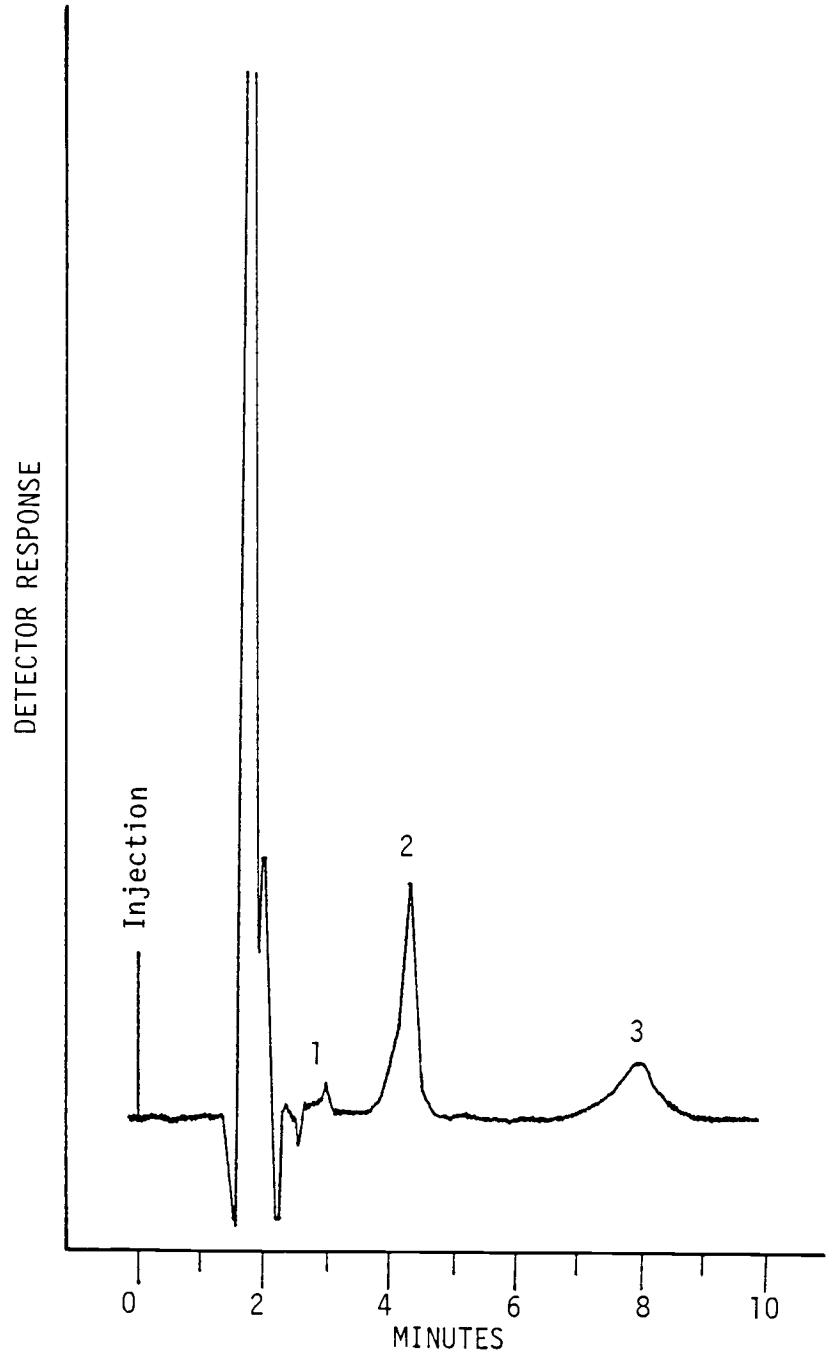


Figure I.9b Chromatogram of Ether Extract of 1, Pseudoephedrine (1.25 mcg/ml) ; 2, Lidocaine; 3, Chlorpheniramine (625 ng/ml) Obtained From a Standard Saliva Sample Using Mobile Phase (IV). Recorder Attenuation: 8.

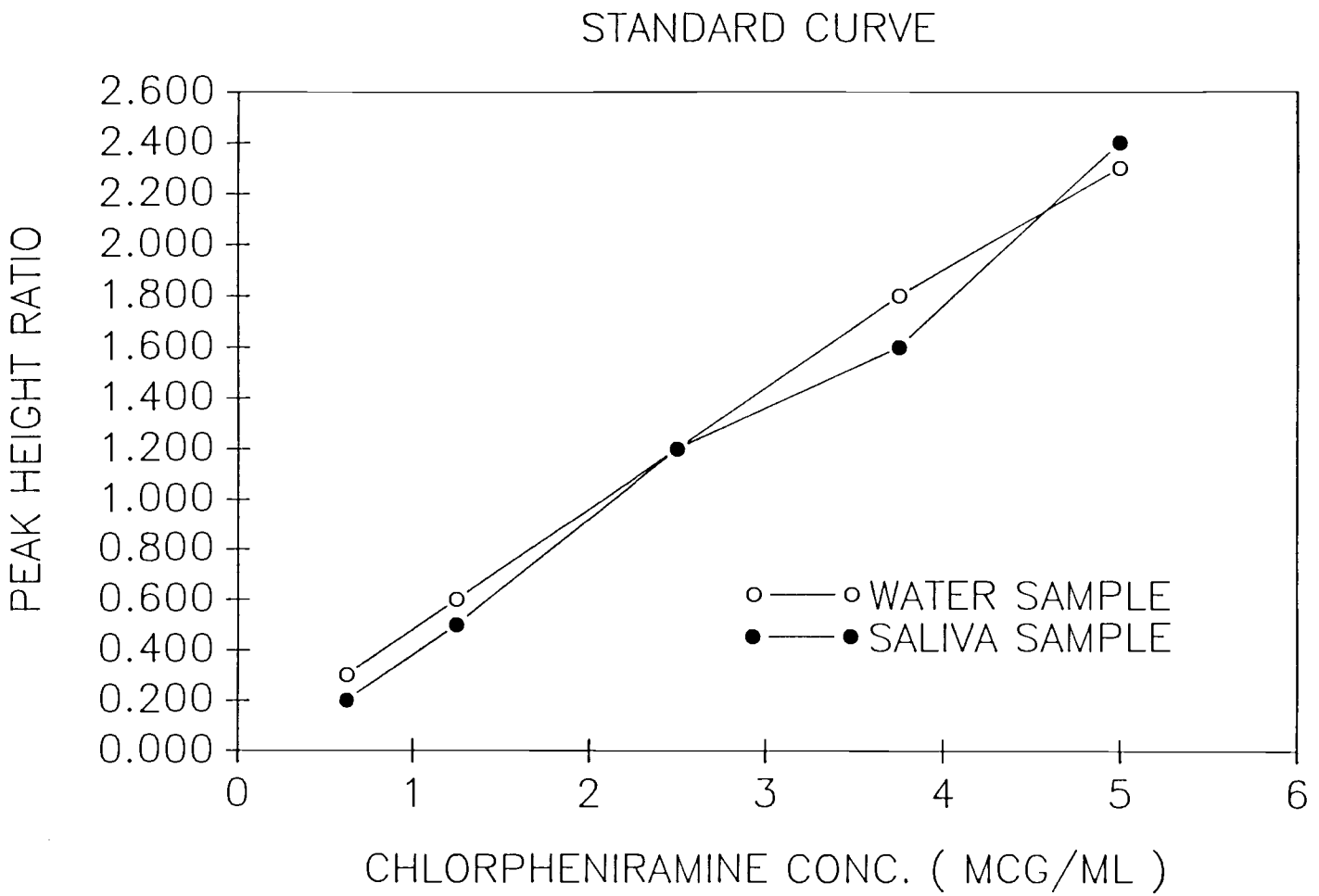


Figure I.10 Standard Curves of Chlorpheniramine Withhin the Concentration Range of 0.625-5 mcg/ml Using Beckman HPLC System.

Mobile phase (V):

All the ingredients are the same as (IV) except 0.075 M ammonium dihydrogenphosphate solution was used instead of 0.01 M $NH_4H_2PO_4$. Retention times for chlorpheniramine and internal standard were 4.0 and 3.4 minutes (Figures I.11a and I.11b). With this mobile phase, it is not possible to detect pseudoephedrine HCl, but chlorpheniramine produces a sharper peak which should allow detection of lower quantities. Standard curve data for chlorpheniramine maleate prepared from saliva using mobile phase (V) are shown in Table I.5.

Using this mobile phase the peak for chlorpheniramine is sharper and 2.5 times higher than using mobile phase (IV). Therefore, this mobile phase was chosen to run a standard curve for lower chlorpheniramine concentrations (Tables I.6a and I.6b). Chromatograms of 50 and 5 ng/ml of extracted chlorpheniramine prepared from water sample are shown in Figures I.12a and I.12b. Chromatograms of 100 and 10 ng/ml of extracted chlorpheniramine prepared from saliva sample are shown in Figures I.13a and I.13b. These two standard curves were not satisfactory. The coefficient of variation was too high. It has been demonstrated that a significant fraction of chlorpheniramine at low concentration (5-100 ng/ml) was adsorbed by various types of glassware from its aqueous solution (7). This may be the reason for the low precision of the standard curve.

Table I.5 Standard Curve Data Using Mobile Phase (V). (Solution Prepared From Saliva. Injection Volume: 0.01 ml. Attenuation 8.)

Conc.(ng/ml)	PH	PHR	Pred. Conc.	% Actual Conc.
3750	12.7	3.1	3652	97.4 (Fig I.11a)
2500	9.8	2.3	2740	109.6
1250	3.9	0.9	1179	94.3
625	1.8	0.4	606	97.0 (Fig I.11b)

X = 99.6 % %SD = 6.8
%CV = 6.9

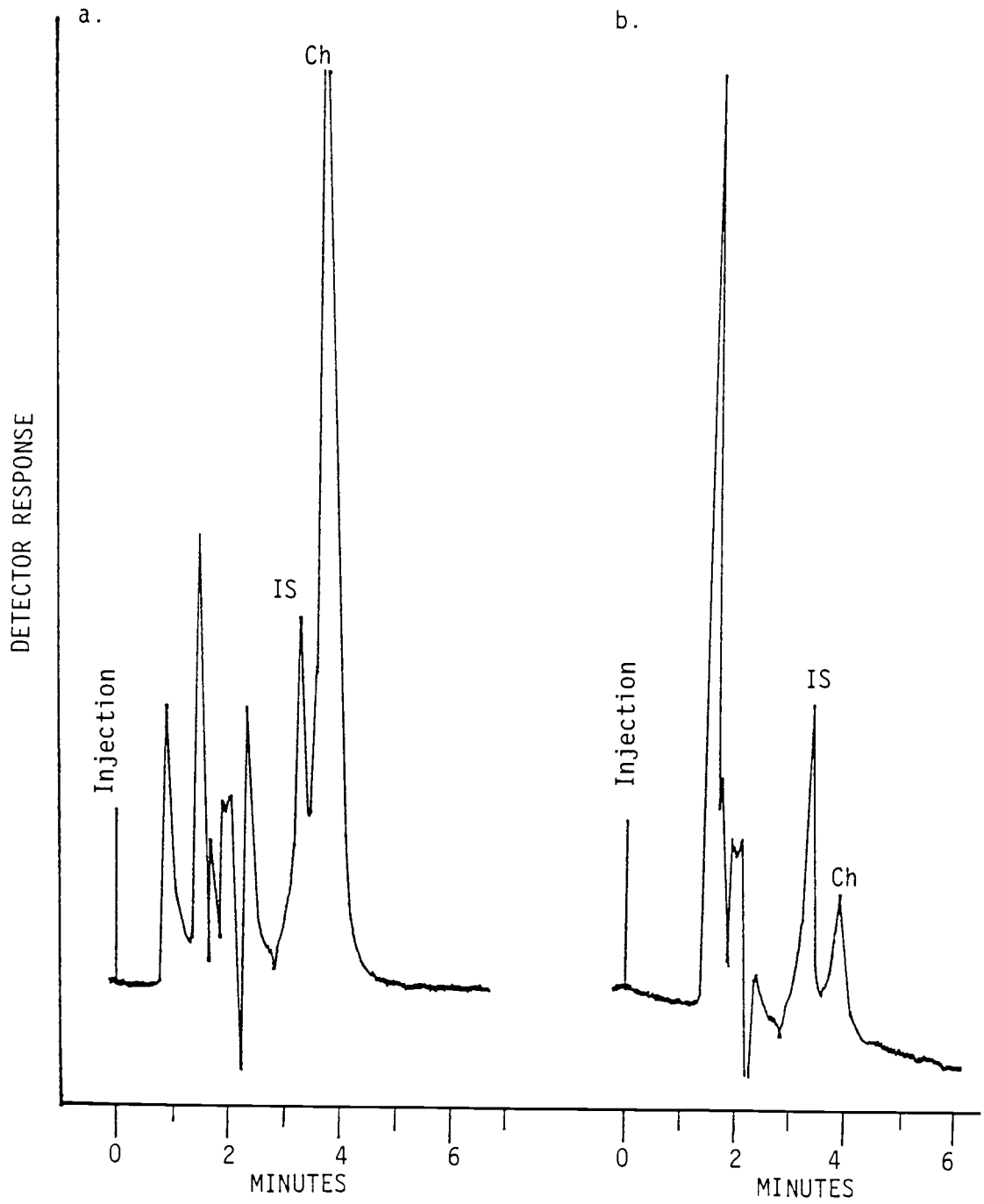


Figure I.11 Chromatogram of an Ether Extract of (a) Chlorpheniramine (3.75 mcg/ml), (b) Chlorpheniramine (625 ng/ml) Obtained from a Standard Saliva Sample Using Mobile Phase (V). Recorder Attenuation: 8.

Table I.6a Standard Curve Data Using Mobile Phase (V). (Solutions Prepared From Water. Injection Volume: 0.02 ml. Attenuation 2.)

Conc.(ng/ml)	[a]PH	[b]PHR	[c]Pred. Conc.	% of Actual Conc.
100	3.2	0.5	97.6	97.6
50	2.5	0.3	57.1	114 (Fig I.12a)
25	0.9	0.1	20.8	83.2
5	0.3	0.04	4.6	93 (Fig I.12b)

X = 97.0 % %SD = 12.86
%CV = 13.2

a. Peak height for chlorpheniramine in centimeters.
b. Peak height ratio of drug to internal standard.
c. Predicted concentration.

Table I.6b. Standard Curve Data Using Mobile Phase (V). (Solutions Prepared From Saliva. Injection Volume: 0.02 ml. Attenuation 2.)

Conc.(ng/ml)	PH	PHR	Pred. Conc.	% of Actual Conc.
100	2.8	0.4	100.3	100.0 (Fig I.13a)
50	1.4	0.2	48.1	96.2
25	1.0	0.14	25.9	103.6
10	0.6	0.10	15.5	155.0 (Fig I.13b)

X = 113.7 % %SD = 27.7
%CV = 24

In general, the concentration of chlorpheniramine in urine are reported to be as much as 50-100 times higher than in saliva (7). Therefore, standard curves for chlorpheniramine solutions from urine were prepared.

Mobile phase (VI) : Acetonitrile-phosphate (0.05M $NH_4H_3PO_4$) solution (20 : 80 V/V) in 0.11 % H_3PO_4. pH = 3.0 (6).

Extraction procedures were performed the same as described on page 19 except that urine samples were basified with 0.2 ml of 10% potassium hydroxide before adding the extraction solvent. Standard curve data using mobile phases (VI) and (V) are shown in Tables I.7 and I.8. Representative chromatograms of chlorpheniramine using mobile phase (VI) and (V) are shown in Figures I.14a, I.14b and I.15a, I.15b. Both standard curves (Figure I.16) over the range of 125-2500 ng/ml could be considered linear. Since mobile phase (V) gave sharper peaks, this mobile phase was used to evaluate urine samples.

Two subjects were used, each took a tablet of Chlor-Trimeton. Selected urine and saliva samples were collected. The results are shown in Tables I.9a and I.9b.

This preliminary study shows urine samples have much higher concentrations of chlorpheniramine than saliva samples (Figures I.17 and I.18). Thus, an HPLC method has been found which can assay chlorpheniramine in urine after administration of a single dose of 4mg.

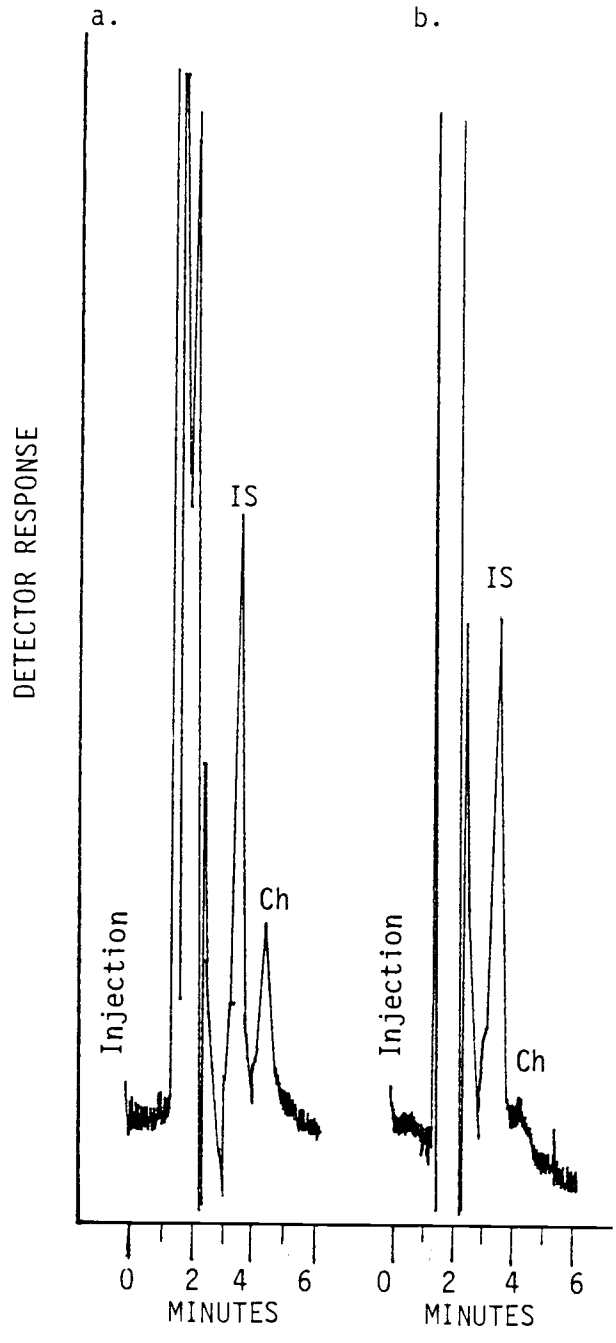


Figure I.12 Chromatogram of an Ether Extract of (a) Chlorpheniramine (50 ng/ml), (b) Chlorpheniramine (5 ng/ml) Obtained from Water Using Mobile Phase (V). Recorder Attenuation: 2.

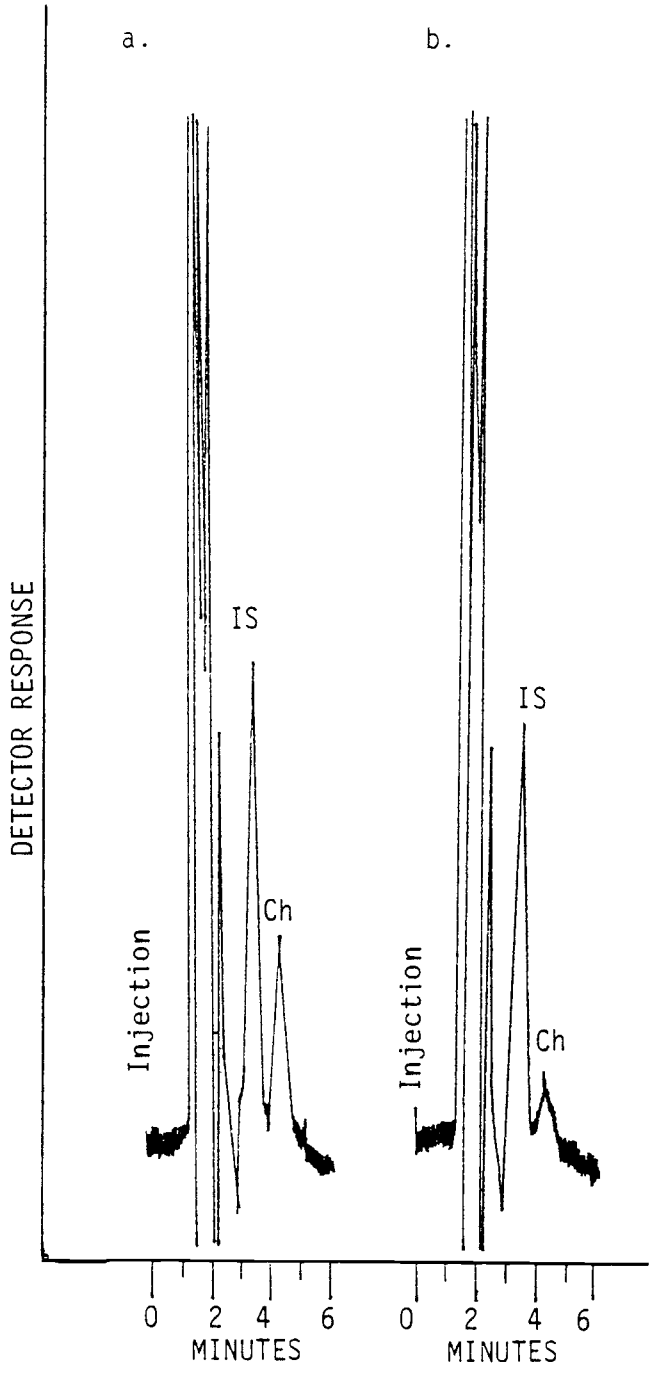


Figure I.13 Chromatogram of an Ether Extract of (a) Chlorpheniramine (100 ng/ml), (b) Chlorpheniramine (10 ng/ml) Obtained from a Standard Saliva Sample Using Mobile Phase (V). Recorder Attenuation: 2.

Table I.7 Data From The Chromatographic Experiment Using Mobile Phase (VI) (Solution Prepared From Urine Sample. Injection Volume: 0.02 ml. Attenuation 8).

Conc.(ng/ml)	PH	PHR	Pred. Conc.	% of Actual Conc.
2500	5.6	0.8	2507.7	100.3 (Fig I.14a)
625	1.6	0.18	588.2	94.1
250	0.6	0.08	278.6	111.4
125	0.2	0.03	123.8	99.1 (Fig I.14b)

X = 101.2% %SD = 7.3
%CV = 7.2

Table I.8 Data From The Chromatographic Experiment Using Mobile Phase (V) (Solution Prepared From Urine Sample Injection Volume: 0.02 ml. Attenuation 4.)

Conc.(ng/ml)	PH	PHR	Pred. Conc.	% of Actual Conc.
2500	9.6	0.8	2457.6	98.3
1250	5.5	0.41	1275.8	102.1 (Fig I.15a)
625	2.1	0.19	609.1	97.5
250	0.7	0.066	233.3	93.3
125	0.1	0.02	93.9	75.2 (Fig I.15b)

X = 93.3 % %SD = 9.5
%CV = 10.1

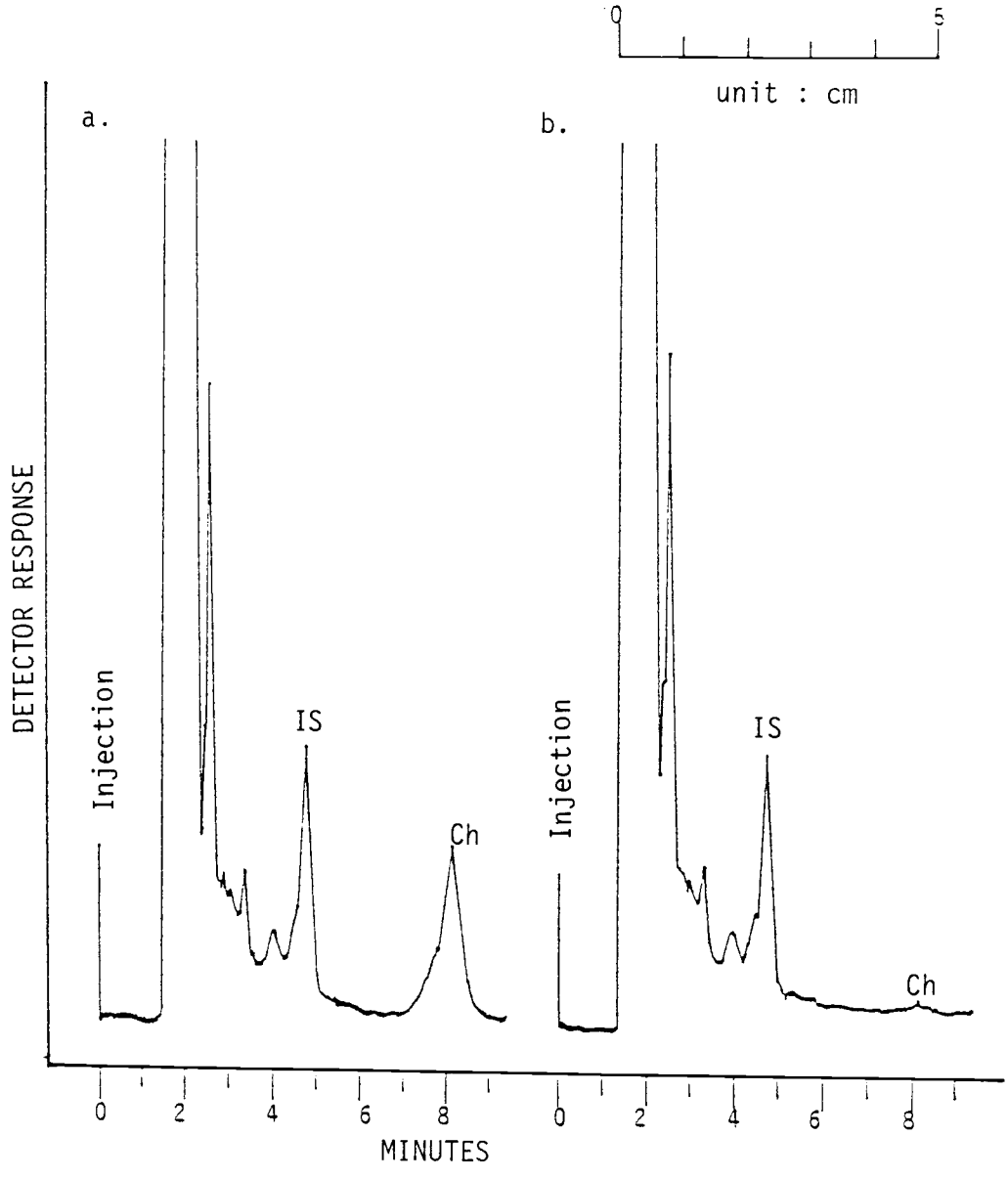


Figure I.14 Chromatogram of an Ether Extract of (a) Chlorphenirmaine (2.5 mcg/ml), (b) Chlorpheniramine (125 ng/ml) Obtained from a Standard Urine Sample Using Mobile Phase (VI). Recorder Attenuation: 8.

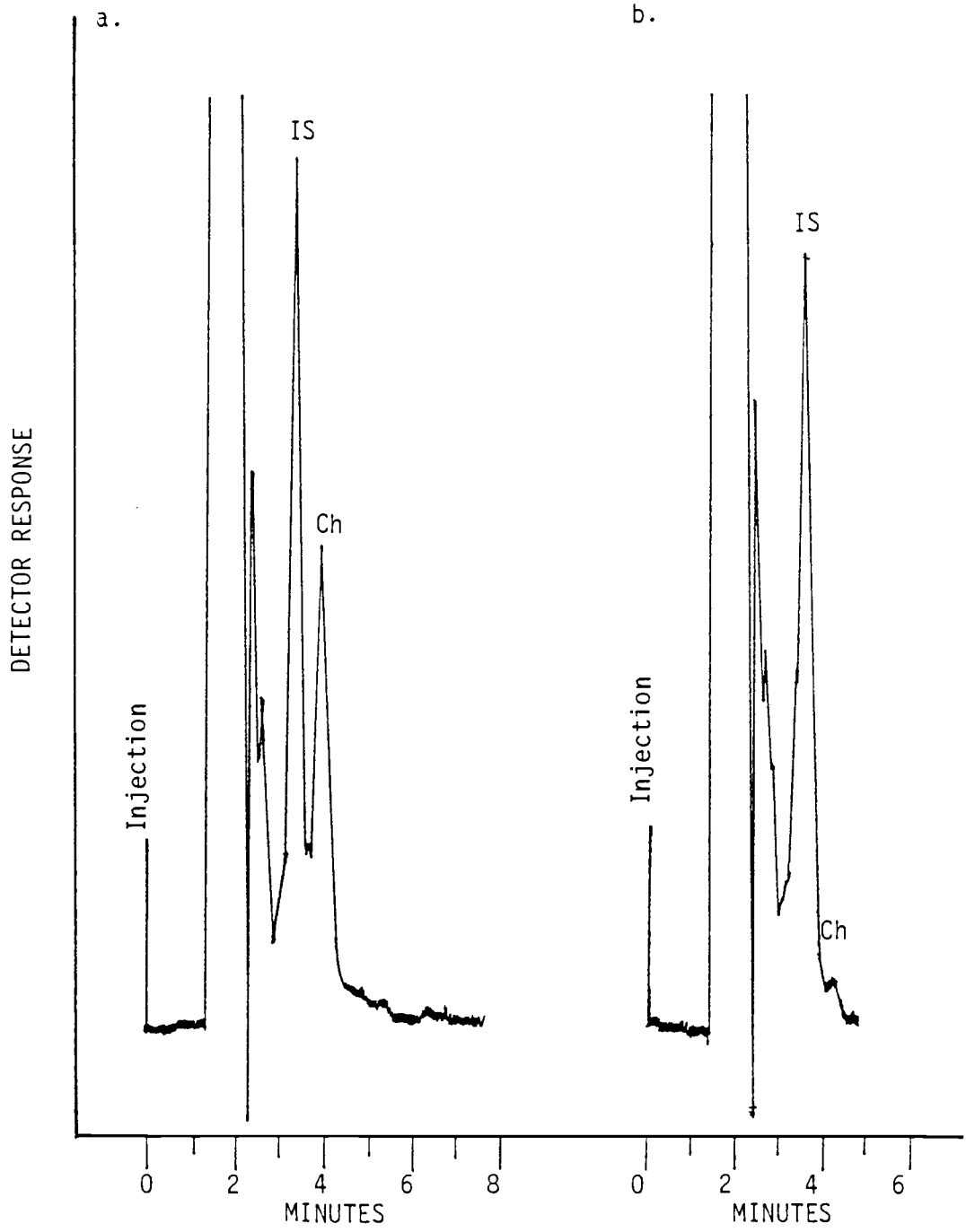


Figure I.15 Chromatogram of an Ether Extract of (a) Chlorpheniramine (1.25 mcg/ml), (b) Chlorpheniramine (125 ng/ml) Obtained From a Standard Urine Sample Using Mobile Phase (V). Recorder Attenuation: 4.

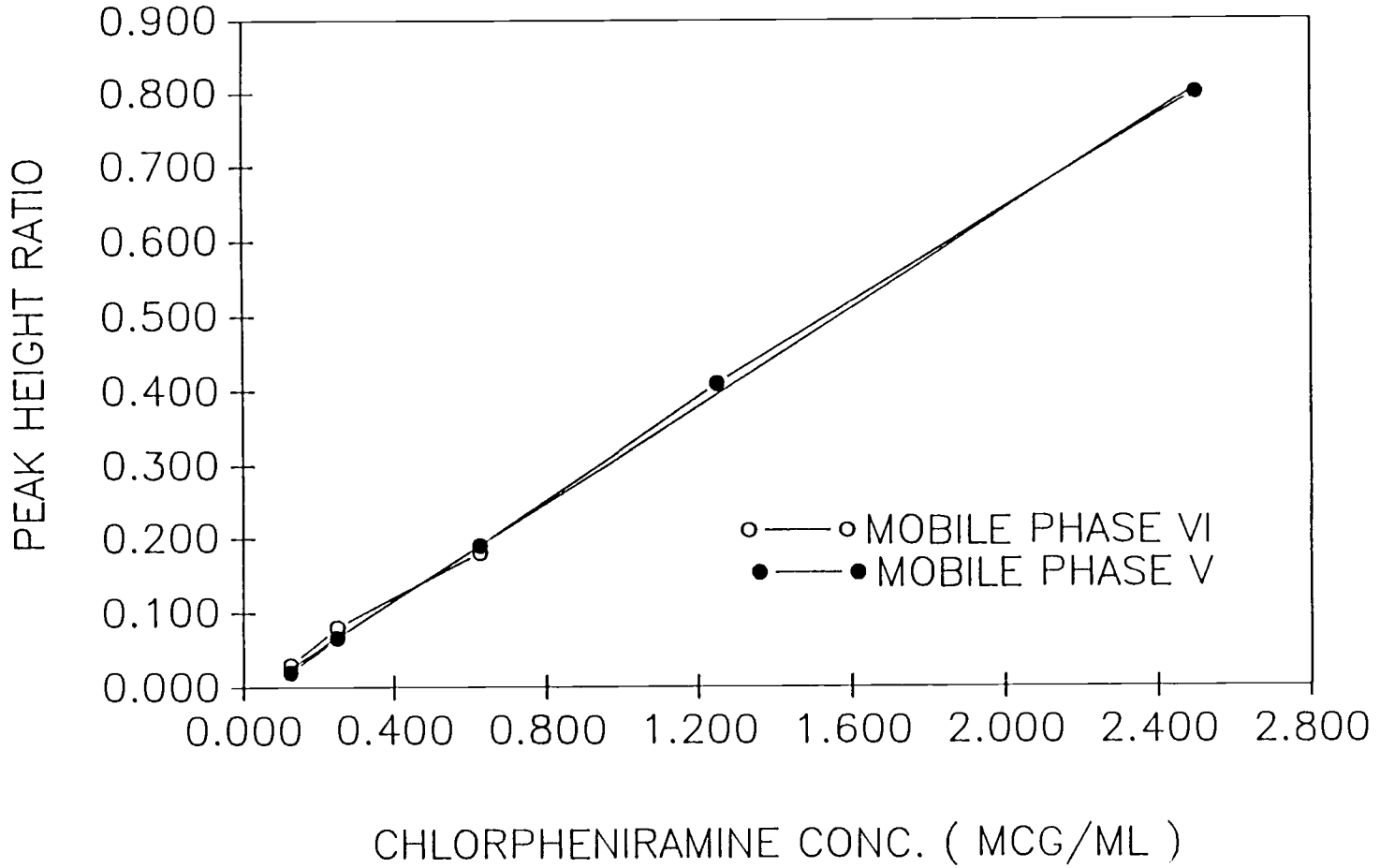


Figure I.16 Standard Curves of Chlorpheniramine Within the Range of 0.125-2.5 mcg/ml Using Mobile Phases (V) and (VI).

Table I.9a Bioavailability Results in Saliva samples of Two Normal Subjects.

(Injection Volume: 0.02 ml)

Time (hour)	Drug Peak Height (cm)	
	Subject 1	Subject 2
0	-	-
0.75	0.2 (Fig I.17b)	1.0 (Fig I.17b)
1.75	0.2	-
3	0.2	-
4.5	0.1	-

Table I.9b. Bioavailability Results in Urine Samples of Two Normal Subjects.

(Injection Volume: 0.02 ml)

Time (hour)	Drug Peak Height (cm)	
	Subject 1	Subject 2
0	-	-
0.75	-	-
1.75	1.1 (Fig I.18a)	0.8
3	1.3	1.0
4.5	0.6	1.2
8	0.5	2.8 (Fig I.18b)
14	-	-

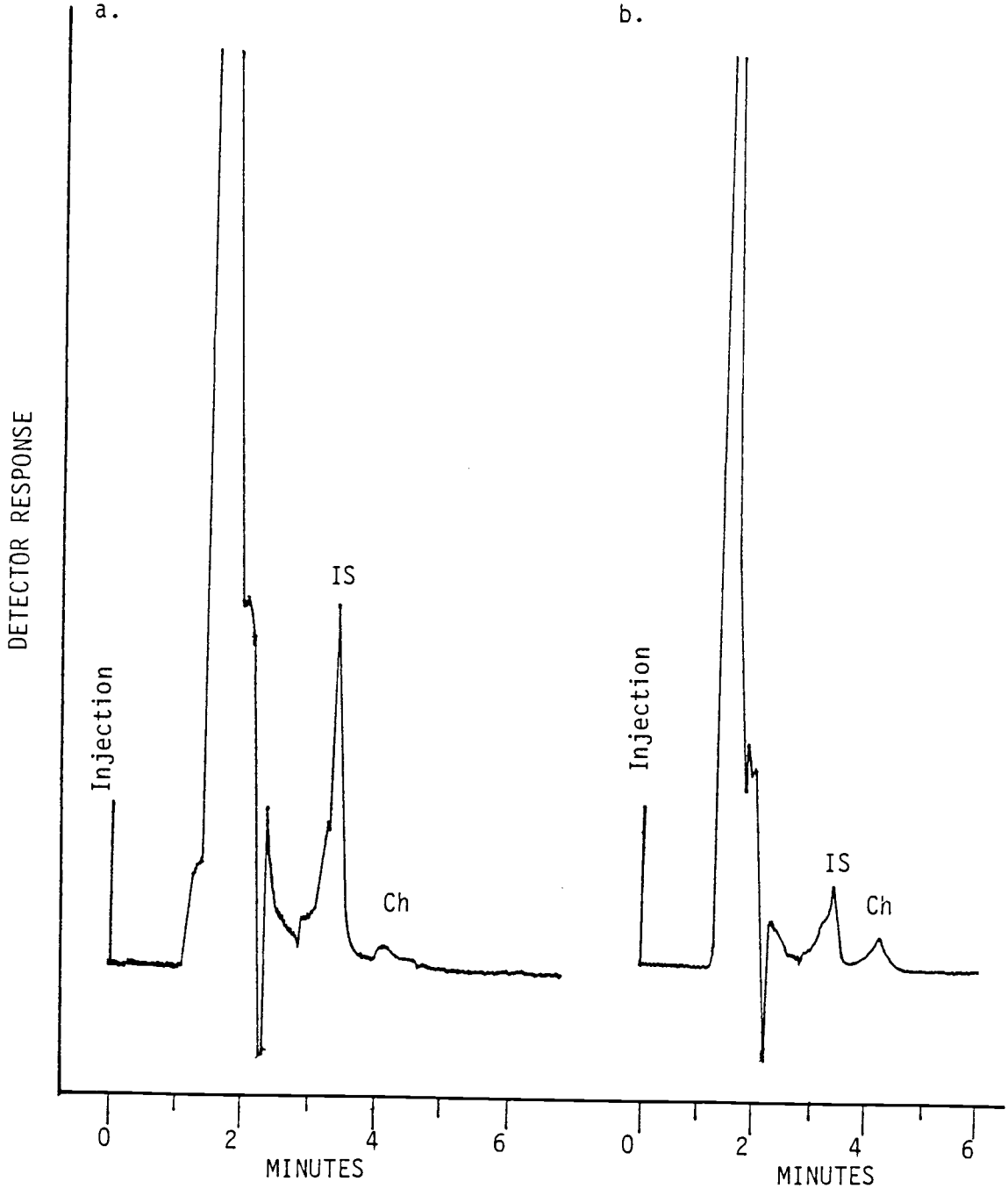


Figure I.17 Chromatogram from an Ether Extract of Saliva Sample Collected 0.75 h After an Oral Dose of 4 mg Chlorpheniramine Maleate to (a) Subject 1. Recorder Attenuation: 8. (b) Subject 2. Recorder Attenuation: 16.

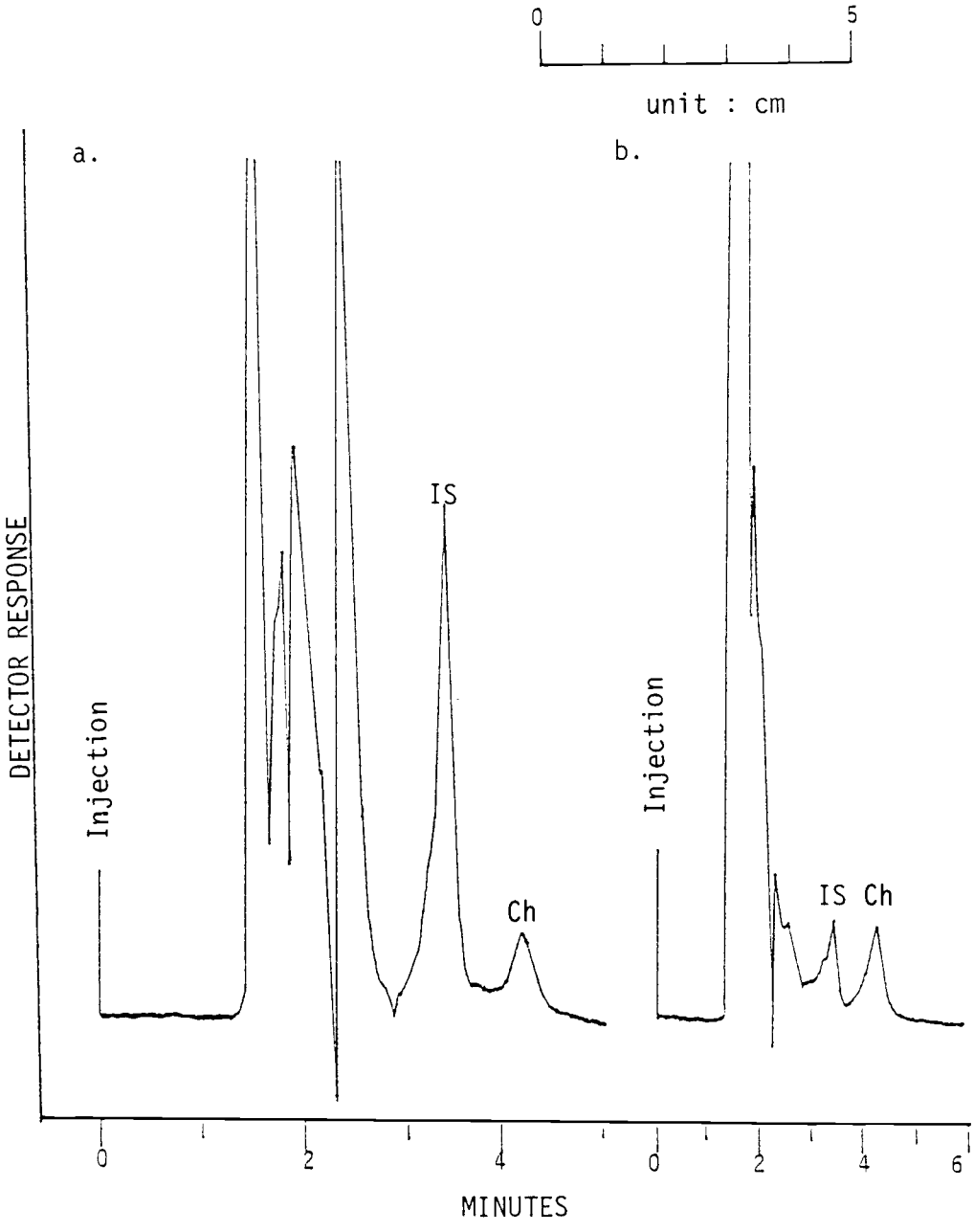


Figure I.18 Chromatogram from an Ether Extract of Urine Sample Collected 1.75 h After an Oral Dose of 4 mg Chlropheniramine Maleate to (a) Subject 1. Recorder Attenuation: 8. (b) 8 h After an Oral Dose of 4 mg Chlorpheniramine Maleate to Subject 2. Recorder Attenuation: 16.

CONCLUSIONS

It was obvious from the chromatograms obtained by different mobile phases that when the mobile phase was acidified, chlorpheniramine was eluted in a much smaller retention volume, as expected. Using nonacidified mobile phase, the retention time is very long, resulting in asymmetric and broad chlorpheniramine peaks. This result is consistent with theory and published literature (7). By using mobile phases (IV) and (V), chlorpheniramine concentrations in saliva and urine samples can be determined. However, with chlorpheniramine concentrations lower than 50 ng/ml, the developed HPLC method can not insure accurate and precise quantitative determination of chlorpheniramine. More sensitive analytical instruments or other methods need to be developed in order to accurately quantitate very small amounts of chlorpheniramine in saliva samples.

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CHAPTER II

IN VITRO DISSOLUTION OF COMMERCIAL COLD-COUGH PRODUCTS.

INTRODUCTION

Pharmacokinetic and pharmacodynamic studies are important to the development of rational drug therapy. Although commercially available antihistamine products have been extensively used for the relief of symptoms of cough and colds for more than 3 decades, pharmacokinetic and pharmacodynamic profiles appear not to have been fully evaluated.

There are many cold-cough products on the market. Most of these products contain a nasal decongestant such as pseudoephedrine hydrochloride or phenylpropanolamine hydrochloride, and an antihistamine such as chlorpheniramine maleate. The different dosage forms marketed include regular (or immediate) release and controlled (or sustained) release products. Little comparative information is currently available concerning dissolution and bioavailability of immediate-release versus sustained-release products, or a comparison among various sustained-release products themselves.

Sustained-action dosage forms often are administered to provide greater patient convenience and compliance and to minimize the occurrence of adverse effects caused by high plasma concentrations. Ideally, a sustained-action dosage form should release its contents at a constant rate, regardless of changes in the GI pH (1). Such a dosage form should avoid dose dumping and prevent large

fluctuations in plasma concentrations.

Dissolution analysis of pharmaceutical solid dosage forms has emerged as a very important test of quality of products. In several instances, dissolution results have been correlated with bioavailability, in which case the dissolution test also correlates with bioavailability of the product between batches that meet or fail dissolution criteria. Dissolution tests are compendial standards for content release and uniformity of many prescription drugs but only minimal data and no official dissolution specifications are available for non-prescription products. The probability of immediate release known as "dose dumping" of a large amount of drug in commercial products is low, but requires verification. Therefore, before conducting bioavailability studies, dissolution tests were carried out on several brands of sustained release and immediate release products of the same or different strengths.

MATERIALS AND METHODS

Dosage Forms:

All dosage forms studied were purchased commercially: Product A_1, Contac caplet (chlorpheniramine maleate 12 mg and phenylpropanolamine HCl 75 mg), lot #1136238 from SmithKline Beckman Company; Product A_2, Contac capsule (chlorpheniramine maleate 8 mg and phenylpropanolamine HCl 75 mg), lot #X956236; Product B_1, Allerest caplet (chlorpheniramine maleate 12 mg, phenylpropanolamine HCl 75 mg), lot #31907 from Pennwalt Corp; Product B_2, Allerest tablet (chlorpheniramine maleate 2 mg and phenylpropanolamine HCl 18.7 mg), lot #30910; Product C_1, Teldrin capsule (chlorpheniramine maleate, 12 mg), lot #X1046924 from SmithKline Beckman Company; Product C_2, Teldrin tablet (chlorpheniramine maleate 4 mg), lot #X16923; Product D, Afrinol Repetab (pseudoephedrine sulfate 120 mg), lot #6CRD3 from Schering Corporation; Product E_1, Sinutab Maximum Strength KAPSEAL capsule (chlorpheniramine maleate 2 mg, pseudoephedrine HCl 30 mg and acetaminophen 500 mg), lot #02476VD from Warner Lambert; Product E_2, Sinutab Maximum Strength Tablet , the ingredients are the same as product E_1, Lot #04386VD; Product E_3, Sinutab II Maximum Strength KAPSEAL Capsule (Pseudoephedrine HCl 30 mg and acetaminophen 500 mg), lot #02686VD; Product E_4 Sinutab II Maximum Strength Tablet, the ingredients are the same as product E_3,

Lot #01996VN. Products A_1, A_2, B_1 and C_1 are sustained-release dosage forms. Products E_{1-4}, B_2 and C_2 are immediate-release dosage forms.

Dissolution Apparatus:

The United States Pharmacopeia XX rotating-basket dissolution apparatus was used in all experiments. Studies were carried out in simulated enzyme-free gastric or intestinal fluid. Simulated gastric fluid was prepared by dissolving 6 g of NaCl in 21 ml of concentrated HCl and sufficient deaerated, deionized water to make 3L; the pH of this solution was adjusted to 1.4 ± 0.1 with hydrochloric acid. Simulated enzyme-free intestinal fluid was prepared by dissolving 20.4 g of potassium phosphate monobasic (EM Industries, Inc., Gibbstown, N.J.) in 750 ml of deaerated, deionized water. To this was added 570 ml of 0.2 N NaOH (8g/L) and 1.2 L of water. The solution was thoroughly mixed, and pH was adjusted to 7.4 ± 0.1 with 2 N NaOH before the volume was make up to 3L with water.

Procedure:

In each study, six of the same dosage forms were used for each dissolution experiment. The dosage form was not added until 900 ml of gastric fluid had reached 37 ± 0.5°C in a water bath. This temperature was maintained throughout the study. The baskets were rotated at 150 rpm.

All sustained-release dosage forms were tested first in simulated gastric fluid and then changed to simulated intestinal fluid after 2 hours to simulate passage of the residual dosage form from the stomach into the upper small intestine. The intestinal fluid was prepared fresh and brought to 37°C just prior to the actual dissolution test. Transfer of dosage forms from gastric to intestinal fluid was carried out by carefully removing the basket from the shaft, filtering the gastric fluid to recover any particles that may have come out of the basket, and then placing 900 ml of simulated intestinal fluid into the vessel, along with any recovered particles on the filter paper. The basket was then replaced onto the end of the shaft and rotation was resumed for the duration of the test. This "switching" procedure took 10 to 15 minutes.

Three ml samples were collected with a continuous flow (5-10 ml/min) eight channel peristaltic pump (Rainin minipuls 2, Rainin instrument Co. Inc., Woburn, MA.) fitted with stainless steel 20-30 μm in-line filters. Sampling times were 30 minutes, and 1, 2, 3, 4, 6, 8, 10, and 12 hours for sustained release dosage forms. Aliquots of 3 ml of the appropriate replacement medium were added to the dissolution flask immediately after withdrawal of each sample. All samples were refrigerated until active ingredients concentrations were determined by a HPLC procedure.

HPLC Instrumentation:

An HPLC solvent delivery pump (Model M-6000A), a sample injector (Model U6K), a 30 cm x 3.9 mm ID μBondapak C-18 column, and a UV detector (Model 480), all from Waters Association (Milford, Mass., U.S.A.), were used in this study.

Assay:

Chlorpheniramine, phenylpropanolamine and pseudoephedrine concentrations were determined by a modification of a HPLC procedure reported previously (2,3). Mobile phase was prepared by mixing acetonitrile and 0.01 M ammonium dihydrogenphosphate solution (35 : 65, by volume) to which 0.005 N octanesulfonic acid and 0.002N potassium nitrate was added. The final pH value of this mobile phase was adjusted to 3.0 ± 0.1 with phosphoric acid. The solvent was degassed prior to use by sonication under vacuum. The flow rate was set at 1.5 ml/min. The effluent from the column was minitored by UV absorption at 254 nm with a 0.005 a.u.f.s. sensitivity setting. The chart speed of the recorder was set at 8 inches/hour. Peak height ratios were used for quantitation based on standard curves established on the same day. All chromatographic separations were carried out at ambient temperature.

Preparation of Standard Solutions:

A. Products A and B

Stock solutions of chlorpheniramine and phenylpropanolamine were prepared in water at concentrations of 1, 2, 4, 6, 8, 16 mcg/ml and 10, 20, 40, 60, 80, 100 mcg/ml, respectively. 0.475 ml of chlorpheniramine stock solution and 0.475 ml of phenylpropanolamine stock solution were mixed and vortexed with 0.05 ml of internal standard from which 0.025 ml was injected into the HPLC system.

B. Products C and D

Stock solutions of chlorpheniramine and pseudoephedrine were prepared in water at concentrations of 1, 2, 3, 4, 5, 6 mcg/ml and 5, 10, 20, 30, 40, 50 mcg/ml, respectively. 0.95 ml of chlorpheniramine or pseudoephedrine stock solution was mixed and vortexed with 0.05 ml of internal standard from which 0.025 ml was injected into the HPLC system.

C. Products E

Stock solutions of acetaminophen were prepared in water at concentrations of 1.3, 1.2, 1, 0.8, 0.6, and 0.5 mcg/ml. 0.9 ml of acetaminophen stock solution was mixed and vortex with 0.1 ml of internal standard from which 0.015 ml was injected into HPLC system.

Internal Standard (IS) Solution:

Lidocaine was used as internal standard which is a stable compound (4). A stock solution of lidocaine was prepared at 1 mg/ml in 0.01 N hydrochloric acid. A concentration of 0.6 mg/ml was used.

Sample Preparation:

A. Chlorpheniramine, Phenylpropanolamine and Pseudoephedrine Quantitation

0.95 ml of dissolution sample and 0.05 ml of internal standard was mixed and vortexed from which 0.025 ml was injected into the HPLC system.

B. Acetaminophen Quantitation

The acetaminophen content in dissolution fluids samples was determined by diluting the sample 500 times with water. 0.9 ml of diluted sample was mixed and vortexed with 0.1 ml IS from which 0.015 ml was injected into HPLC system.

RESULTS AND DISCUSSION

The HPLC method with the ion-pairing agent was used to detect both phenylpropanolamine and chlorpheniramine or pseudoephedrine and chlorpheniramine simultaneously within 10 minutes. Typical chromatograms of the contents of some tested products are shown in Figures II.1, II.2 and II.3. Within the concentration range of 1-10 mcg/ml for chlorpheniramine and 5-100 mcg/ml for both phenylpropanolamine and pseudoephedrine, the drug-internal standard peak height ratios were found to be linear (Figures II.4a, II.4b, II.4c and II.4d). The coefficient of variation (CV) was below 8 %. Nevertheless, calibration curves were determined daily for each set of analyses.

Percentages of chlorpheniramine dissolved at various times from products A_1, A_2, B_1 and C_1 are present in Table II.1 and Figure II.5. Only the differences D_2 (the percent dissolved in 2 hours) between product A_2 and B_1 or A_1 and C_1 along with D_8 (the percent dissolved in 8 hour) between product A_2 and C_1 were not significant ($p=0.005$). A marked difference in dissolution profiles of these products was observed both in terms of rate and extent of dissolution (Figure II.5). After the initial release of drug through the first 0.5-1.0 hr, all of these four products displayed some type of sustained-release characteristics (Figure II.5). However, the variability

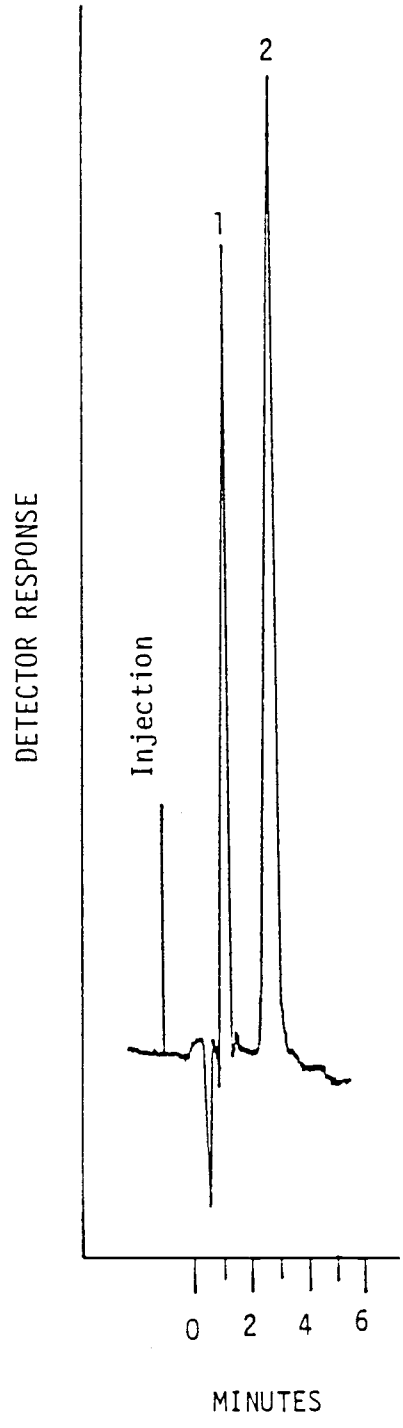


Figure II.1 Chromatograms Showing 5 Minute Dissolution Sample for Product E_4. Peaks: 1, Acetaminophen; 2, Lidocaine. Retention Times for 1 and 2 were 2.5 and 4.4 Minutes, Respectively.

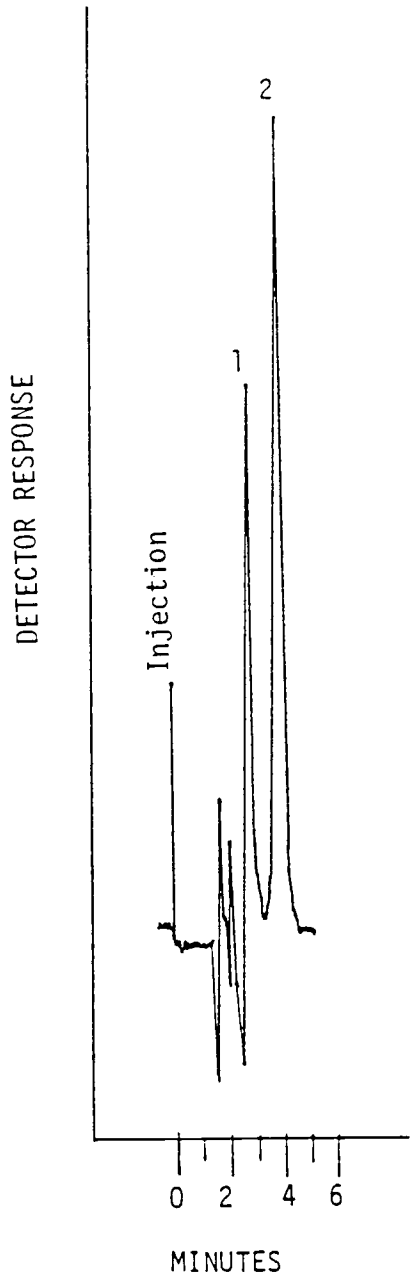


Figure II.2 Chromatograms Showing 8 hr Dissolution Sample for Product D. Peaks: 1, Pseudoephedrine; 2, Lidocaine. Retention Times for 1 and 2 were 3.2 and 4.4 Minutes, Respectively.

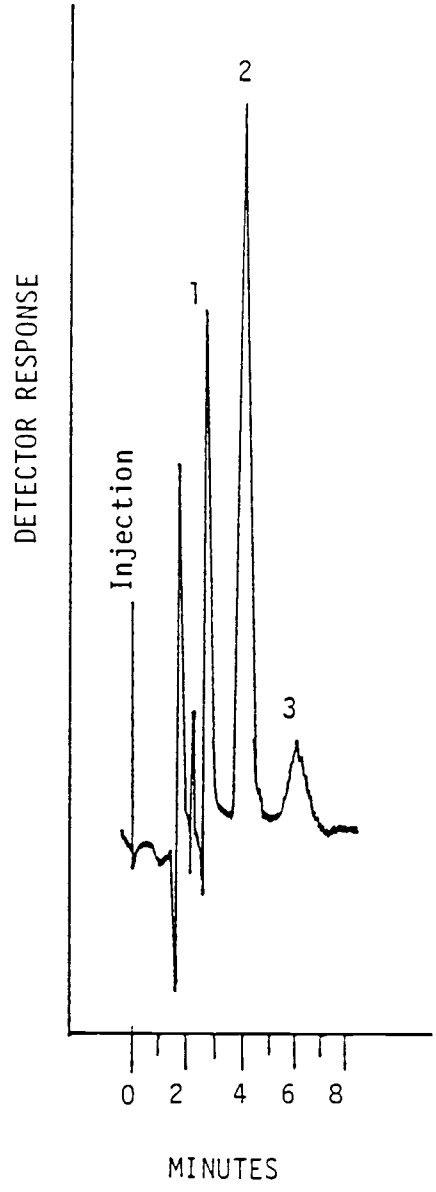


Figure II.3 Chromatogram Showing 12 hour Dissolution Sample for Product B_1. Peaks: 1, Phenylpropanolamine; 2, Lidocaine; 3, Chlorpheniramine. Retention Times For 1,2 and 3 were 3.0, 4.4 and 6.5 Minutes, Respectively.

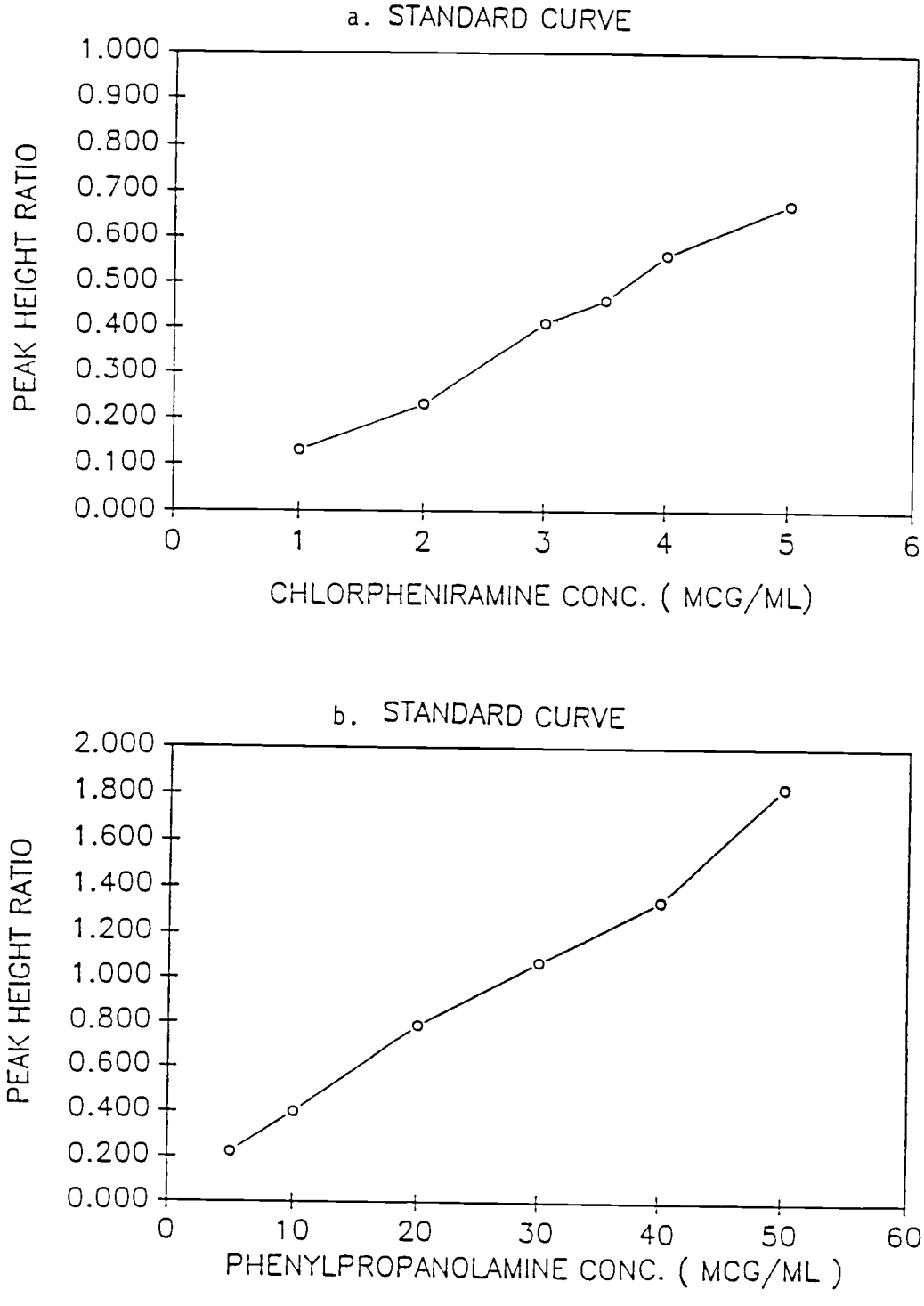
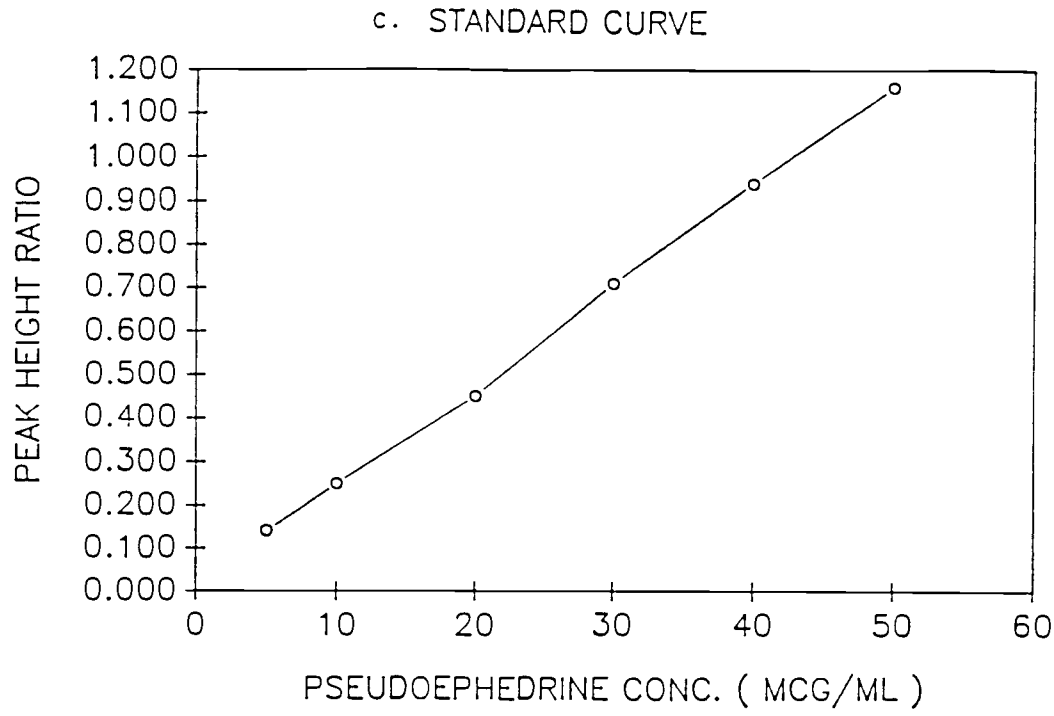


Figure II.4 Standard Curves of (a) Chlropheniramine Within the Concentration Range of 1-5 mcg/ml. (b) Phenylpropanolamine Within the Concentration Range of 5-50 mcg/ml.



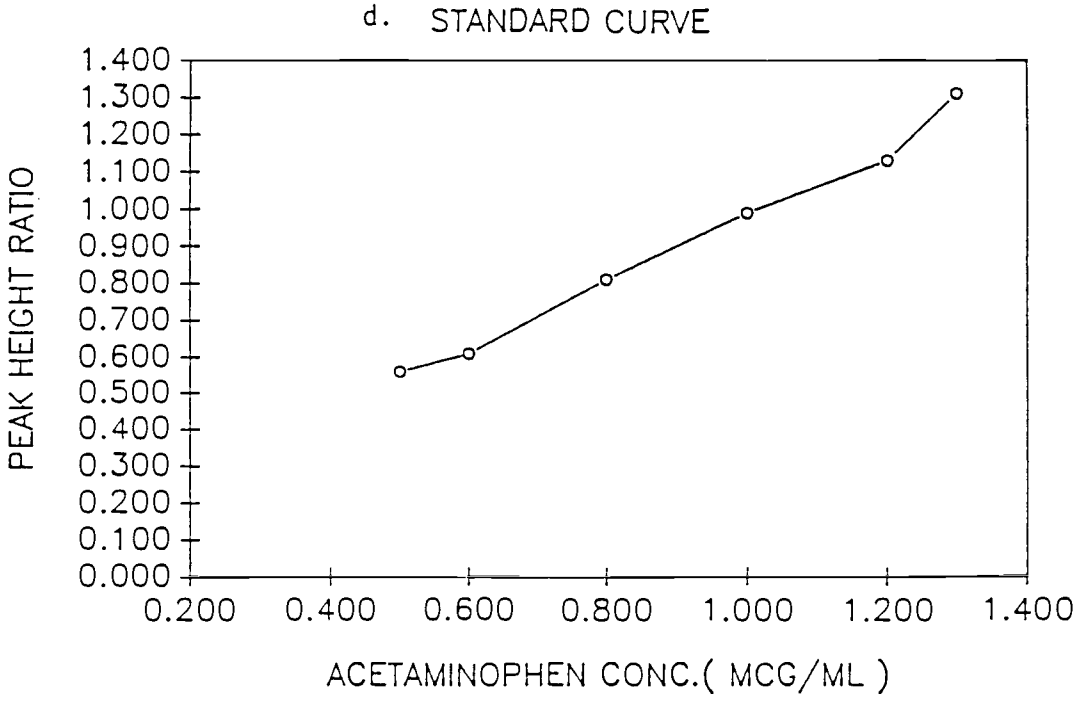


Figure II.4 Standard Curves of (c) Pseudoephedrine Within the Concentration Range of 5-50 mcg/ml. (d) Acetaminophen Within the Concentration Range of 0.5-1.5 mcg/ml.

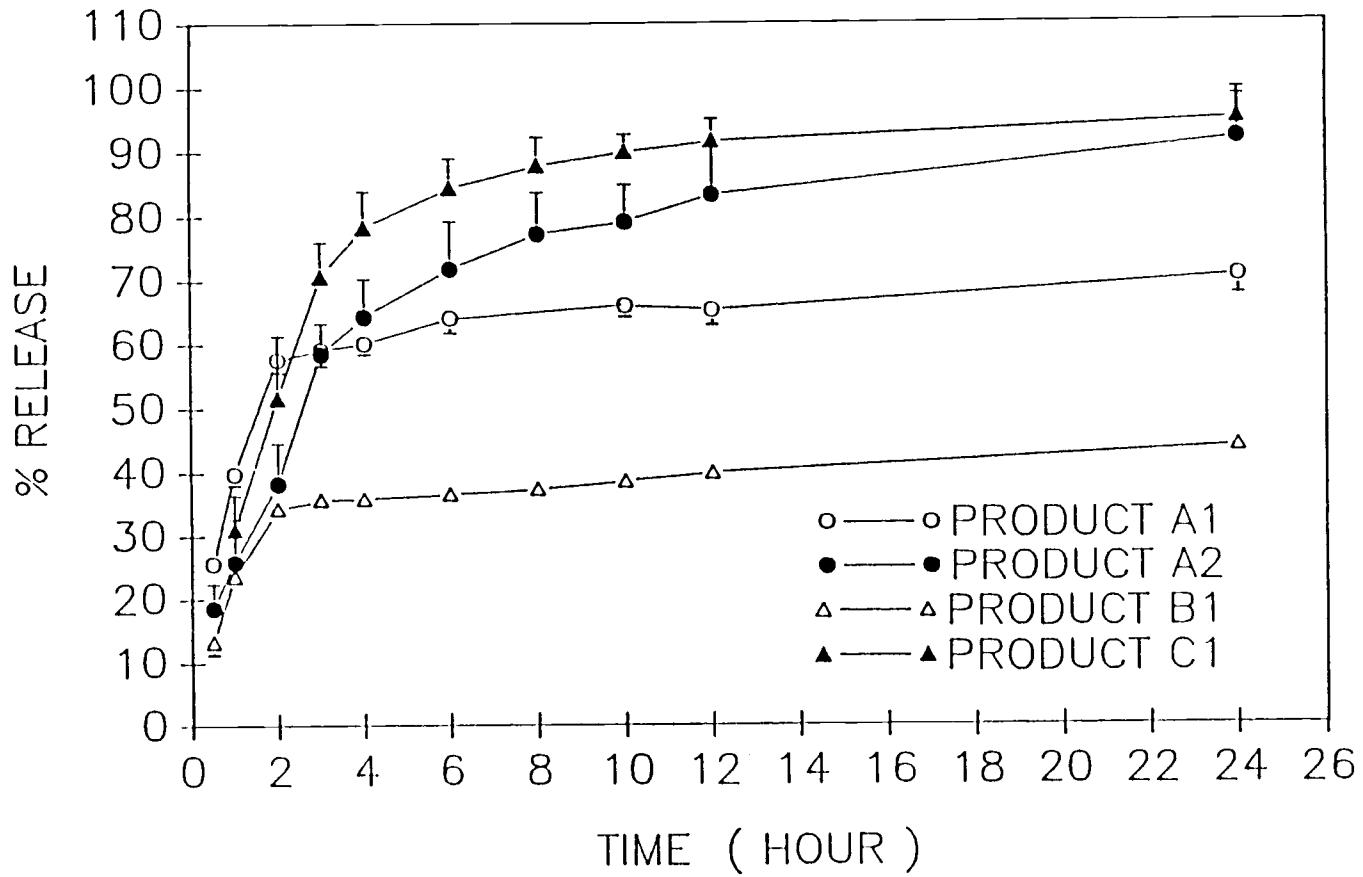


Figure II.5 Percent Chlorpheniramine Dissolved in Simulated Gastric and Intestinal Fluid Versus Time From Products A_1, A_2, B_1 and C_1.

Table II.1 Dissolution Profile of Commercial Available Cold-Cough Products in Simulated Gastric, Intestinal Fluids at 37°C Using Basket Method.

	Chlorpheniramine Dissolved, %[a]				
Product	0.5h	1h	2h	3h	4h
A_1	25.5 (1.4)	39.8 (1.8)	57.5 (2.0)	59.1 (2.6)	60.1 (1.8)
A_2	18.5 (3.9)	25.8 (6.9)	38.3 (6.4)	58.4 (4.9)	64.3 (6.0)
B_1	13.2 (2.0)	23.5 (1.0)	34.2 (0.7)	35.6 (1.0)	35.7 (1.0)
C_1	-[b] -	30.9 (5.5)	51.5 (9.8)	70.6 (5.4)	78.2 (5.7)
Product	6h	8h	10h	12h	24h
A_1	64.1 (2.4)	62.9 (1.5)	66.0 (1.8)	65.3 (2.5)	70.7 (3.1)
A_2	71.8 (7.4)	77.2 (6.5)	79.1 (5.8)	83.3 (7.4)	92.0 (6.6)
B_1	36.5 (1.2)	37.3 (0.6)	38.6 (0.7)	39.9 (0.9)	44.0 (1.4)
C_1	84.3 (4.7)	87.8 (4.5)	89.9 (2.8)	91.6 (3.5)	95.1 (4.6)

a. Mean values; Standard deviation in parentheses.
b. Samples not collected.

in dissolution among products suggests that these products are not readily interchangeable in terms of anticipated physiological effect. Dissolution studies in simulated gastric fluid were terminated after 2hr. At that time, the mean percentage of chlorpheniramine released ranged from 34.2 ± 0.7 to 57.5 ± 2.0 % (Table II.1). An interesting result is that the standard deviation (SD) of the percent release from caplets is smaller than from capsules. Most SD values of percent released from products A_1 and B_1 are smaller than 2 but are larger than 5 for products A_2 and C_1.

Of greater concern is the differences in the rate of release of active ingredients from different dosage forms of the same manufacturer. Products A_1 and A_2 are both sustain-released products manufactured by SmithKline Beckman company. Product A_1 is a caplet form containing 12 mg of chlorpheniramine maleate and 75 mg of phenylpropanolamine HCl. Product A_2 is barrier coated-beads in a capsule containing 8 mg of chlorpheniramine maleate and 75 mg phenylpropanolamine HCl. Product A_1 only released 70.7 ± 3.1 % while product A_2 released 92.0 ± 6.6 % of chlorpheniramine after 24 hr (Table II.1). After 72 hours of dissolution, product A_1 did not disintegrate and still only 70 % of the labeled amount of chloprhiniramine had been released. The caplet was then ground in a mortar and pestle and dissolved in gastric fluid. The intact caplet contain 31.0 ± 5.1 % of labeled

chlorpheniramine content which did not dissolved after 72 hours of the dissolution test. These in vitro studies may not reflect the in vivo situation. Bioavailability studies need to be performed.

Products A_1 and B_1 which contain the same amount of active ingredients, but are manufactured by different companies show great product variability in the dissolution results (Figure II.5). Product B_1 only released 44.0 ± 1.4 % of labeled chlorpheniramine content at 24 hours. The caplet was still intact after 24 hours of dissolution and assay by grinding the remaining intact caplet in simulated gastric fluid indicated it contained only 9.5 ± 1.4 % of chlorpheniramine labeled content. This result suggested that only about half of the labeled content of chlorpheniramine was contained in each caplet. When two caplets of the same lot were ground and allowed to dissolve in 3N hydrochloric acid (pH = 0.6) separately, assay results also indicated each caplet only contained about 6 mg of chlorpheniramine maleate. However, grinding and dissolution in 0.5 % phosphoric acid (pH = 1.5) showed the caplets contained 90 % of the labeled chlorpheniramine content. The failure to dissolve in HCl is surprising and no explanation can be offered. However, these data suggest that bioavailability may be incomplete.

Percent phenylpropanolamine dissolved data at various times are presented in Table II.2, and the percent dissolved versus time plot is shown in Figure II.6.

Table II.2 Percent Phenylpropanolamine Dissolved in Simulated Gastric and Intestinal Fluids at Various Times.

	Phenylpropanolamine Dissolved, %				
Product	0.5h	1h	2h	3h	4h
A_1	20.9 (1.2)	29.7 (1.6)	39.8 (1.6)	49.9 (1.3)	56.1 (3.2)
A_2	7.1 (2.9)	12.9 (3.3)	19.2 (3.9)	32.1 (3.5)	38.5 (4.0)
B_1	18.5 (1.1)	26.3 (1.3)	35.2 (1.7)	42.8 (1.7)	46.7 (3.0)
Product	6h	8h	10h	12h	24h
A_1	66.0 (3.5)	62.3 (3.1)	71.6 (1.7)	75.2 (1.7)	81.3 (2.8)
A_2	46.6 (5.5)	53.4 (4.1)	55.8 (4.4)	60.1 (5.7)	71.6 (9.1)
B_1	55.2 (2.6)	61.1 (3.0)	66.6 (2.2)	69.5 (3.1)	79.1 (5.7)

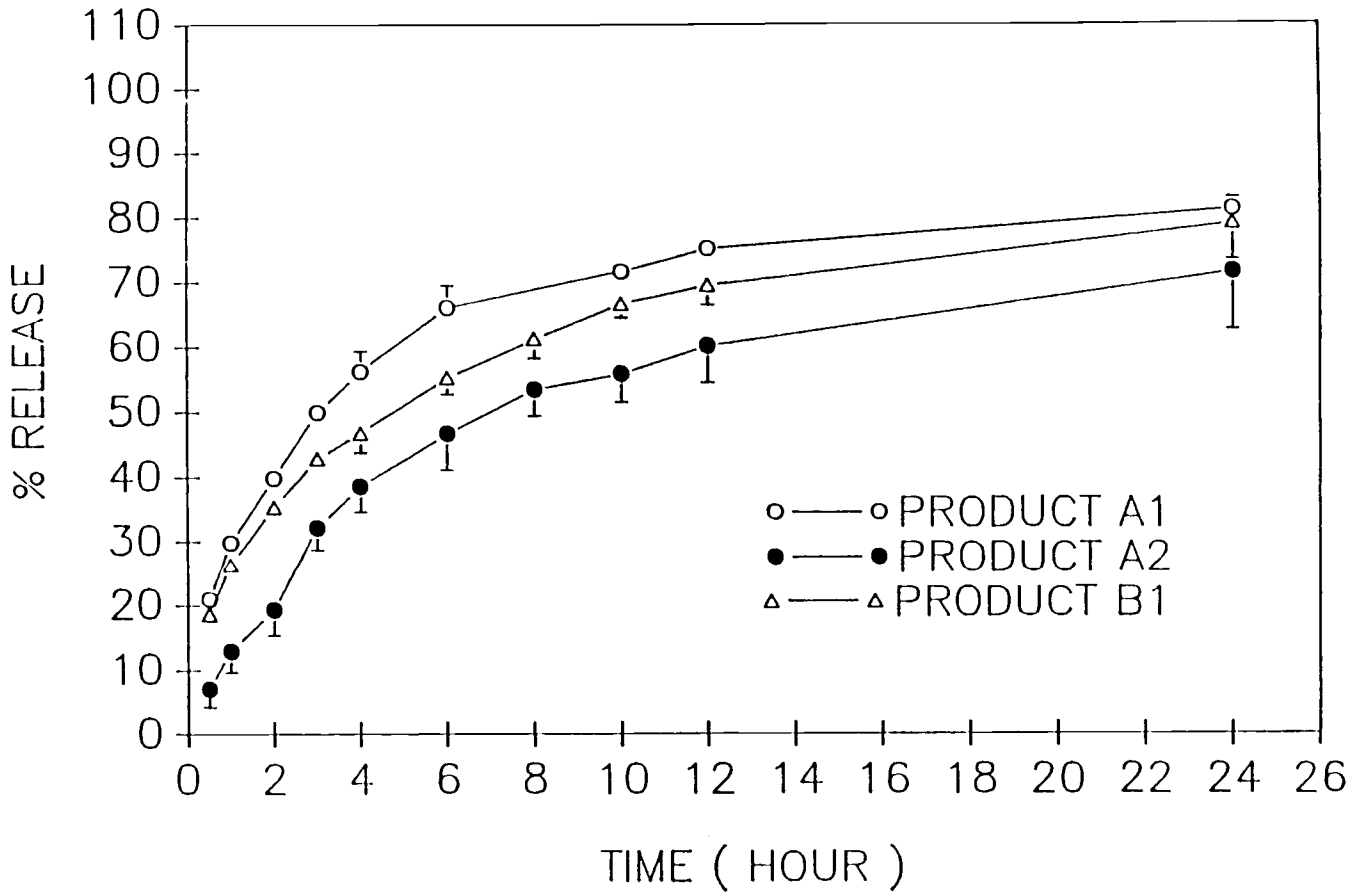


Figure II.6 Percent Phenylpropanolamine Dissolved in Simulated Gastric and Intestinal Fluid Versus Time From Products A_1, A_2 and B_1.

The percent released for products A_1, A_2 and B_1 is not significantly different at 24 hr (pH = 0.005). However, variability and incompleteness of total phenylpropanolamine release from products A_1, A_2 and B_1 were found. With the paucity of information available in the literature, it was not possible to compare previously reported results with the results from the present study. A stability study (5) of a decongestant syrup formulation containing phenylpropanolamine hydrochloride in a sugar vehicle indicated a loss of phenylpropanolamine. It may be because of an interaction of phenylpropanolamine hydrochloride with aldose or ketose sugars. An in vivo test also showed that degraded phenylpropanolamine was not converted in vivo to phenylpropanolamine (5). In most of the products studied, sucrose or lactose was contained in the dosage forms as inactive ingredients. Could it be that phenylpropanolamine also degraded in these solid dosage forms? Alternatively, could phenylpropanolamine degrade during the dissolution test, so the dissolution test showed only about 75 % labeled content of phenylpropanolamine in products A_1, A_2 and B_1? It has been reported that identical dissolution profiles were observed for chlorpheniramine and pseudoephedrine at pH 1.5, 4.5 and 7.5 (6). Therefore, no changes in the release rate of phenylpropanolamine are expected in the GI tract with respect to changes in pH. However other factors such as the presence or absence of enzymes or

physical conditions may influence the dissolution rate of drugs. Further, in vitro results, may not mirror the in vivo situation.

Relatively slow release of phenylpropanolamine from product A_2 compare to products A_1 and B_1 during the initial stages of dissolution was surprising (Table II.2). The time for 50 % phenylpropanolamine release varied between 3 and 7 hours (Table II.3). The relationship between active ingredients release in the same product is shown in Figure II.7. The rate of percent release of chlorpheniramine is greater than that of phenylpropanolamine during the first two hours for products A_1 and B_1. After the second hour, the rate of percent release of phenylpropanolamine became much greater than that of chlorpheniramine. Similar results were found in product A_2 before and after the third hour of dissolution. However, the difference of rate of percent release for these two ingredients is not significant after the third hour in product A_2.

Product D is a 120 mg pseudoephedrine sulfate repeat action tablet. It is designed such that " half the dose (60 mg) is released after the tablet is swallowed and the other half is released hours later; continuous relief is provided for up to 12 hours " (7). The dissolution profile is presented in Figure II.8 and Table II.4 . The dissolution testing illustrated that 50 % of pseudoephedrine was released within 0.5 hours and the

Table II.3 The Time for 50 % Active Ingredients Release in Different Cold-Cough Commercial Products.

	$t_{50\%}$, hour[a]	
Product	Chlorpheniramine	Phenylpropanolamine
A_1	1.6	3.0
A_2	2.6	7.0
B_1	-[b]	4.8
C_1	1.9	-[c]

a. Time needed for 50 % of label content to dissolve. (From percent dissolved - time data).
b. Product B_1 only release 44.0 ± 1.4 % label content of chlorpheniramine at 24 hour.
c. Product C_1 does not contain phenylpropanolamine.

Table II.4 Percent Pseudoephedrine Sulfate Dissolved in Simulated Gastric and Intestinal Fluids at Various Times.

	Pseudoephedrine Dissolved, %				
Product	0.5h	1h	2h	3h	4h
D	53.8 (2.3)	54.1 (2.3)	55.4 (3.0)	58.4 (4.0)	61.8 (4.2)
	5h	6h	8h	10h	12h
	69.3 (7.2)	74.6 (9.7)	86.2 (12.8)	93.2 (11.9)	100.7 (9.2)

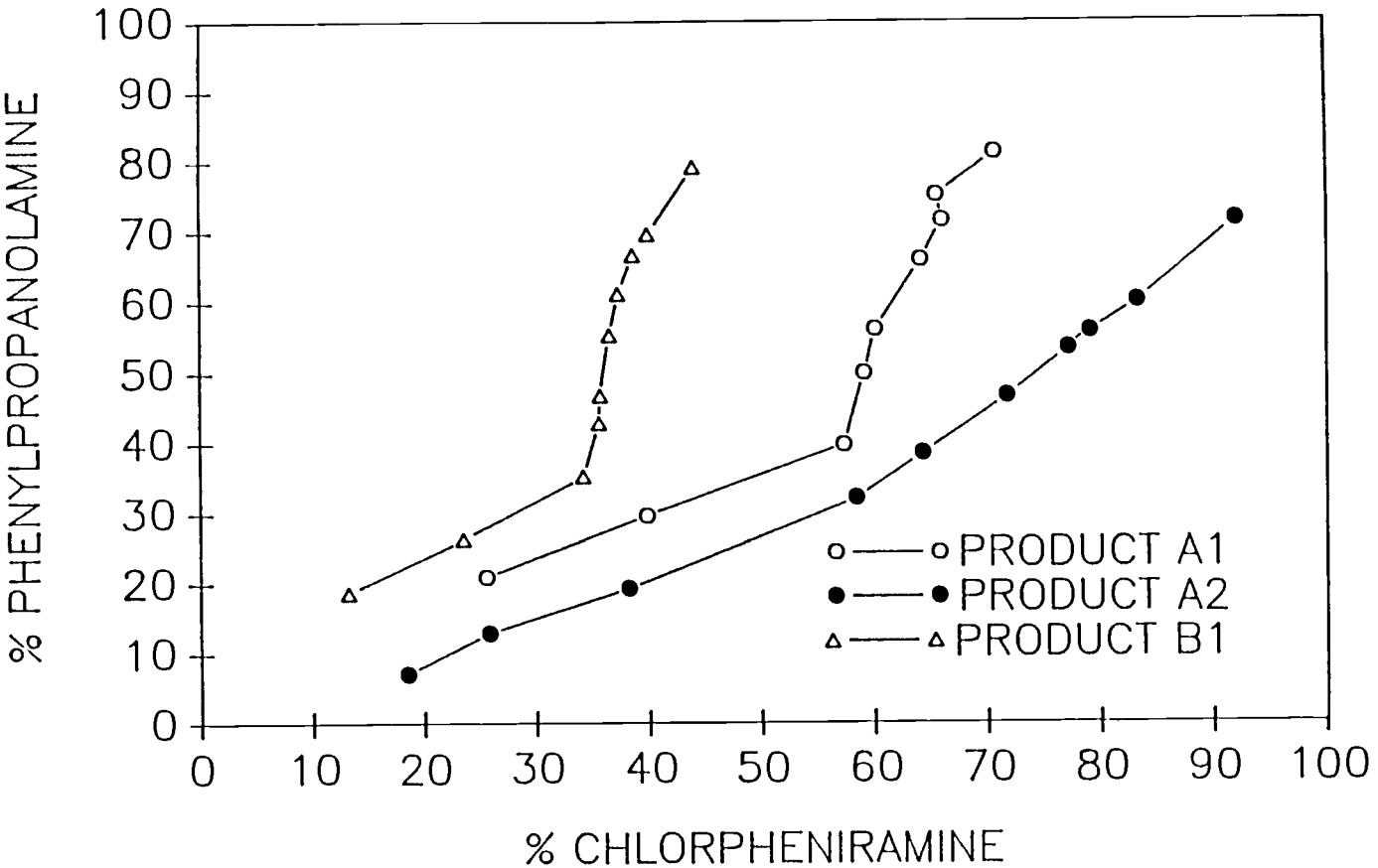


Figure II.7 Percent Dissolved of Chlorpheniramine versus Pseudoephedrine From the Same Formulation. (Products A_1, A_2 and B_1)

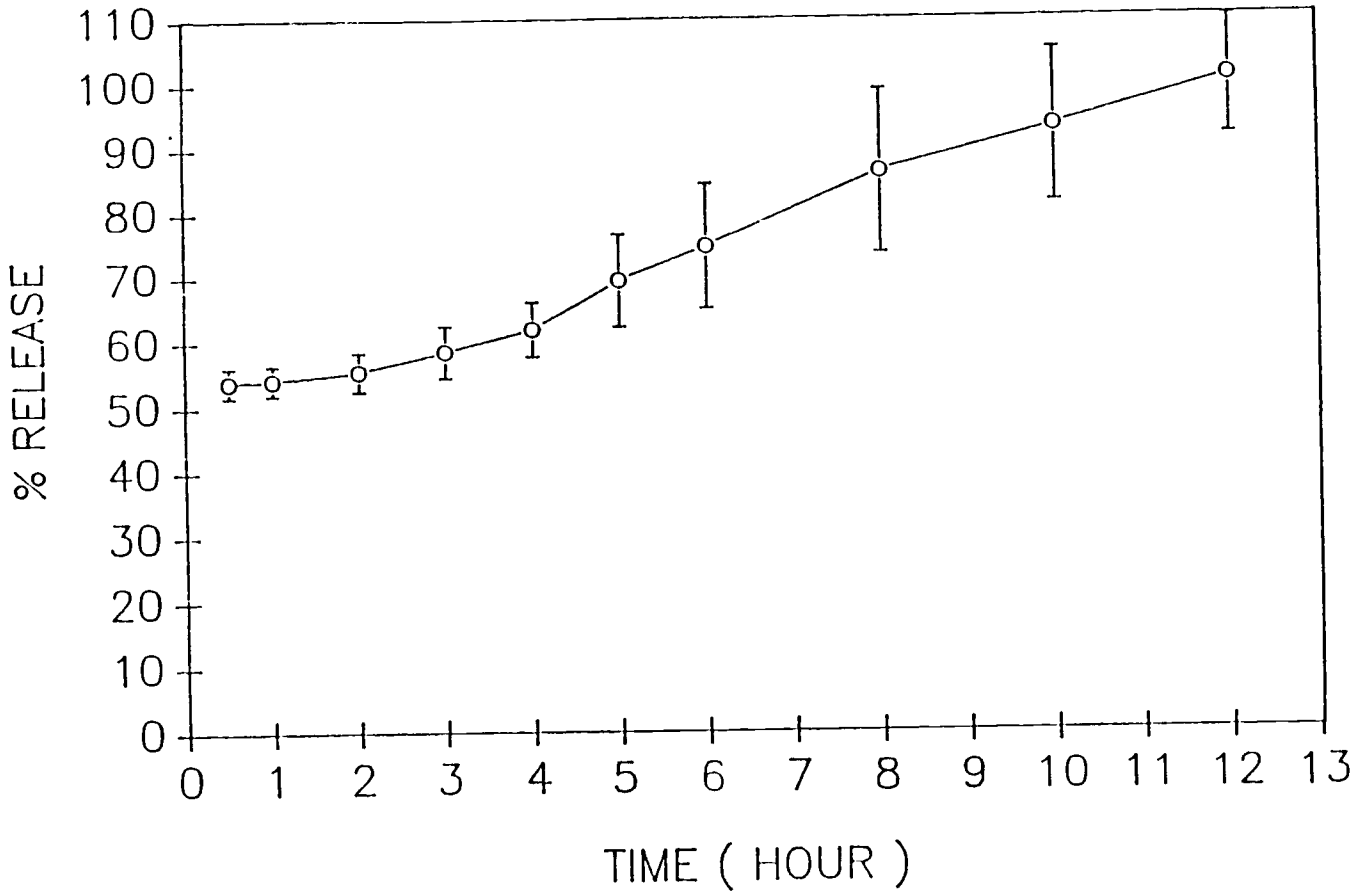


Figure II.8 Dissolution Profiles for Product D; Mean Values (n=6) in Simulated Gastric and Intestinal Fluid.

remaining 50 % was slowly released from 4 hr to 12 hr.

Immediate release dosage forms evaluated were products B_2, C_2 and E_{1-4}. Products B_2 and C_2 dissolved rapidly and achieved 90 % release in 1 hour (Table II.5). The dissolution pattern of immediate and sustained release products are compared in Figures II.9 and II.10.

Products E_1 and E_2 contain pseudoephedrine HCl, acetaminophen and chlorpheniramine maleate. The simultaneous chromatographic analysis of these three ingredients is difficult for two reasons. First, chlorpheniramine maleate and pseudoephedrine HCl are basic while acetaminophen is neutral. Secondly, the amount of drug ranges from 2 mg/dose to 500 mg/dose. However, the same mobile phase was used to detect both chlorpheniramine and acetaminophen. The dissolution samples for detecting acetaminophen concentration need to be diluted 500 times before injection on the HPLC (Appendix A for calculation). It was tedious to separate pseudoephedrine from the peak of acetaminophen. Many different mobile phase were tried. In each case pseudoephedrine did not separate from acetaminophen, i.e., acetaminophen started eluting before the pseudoephedrine had eluted. It is necessary to increase the retention time of acetaminophen and decrease that of pseudoephedrine.

Acetaminophen dissolution behavior of products E_{1-4} is shown in Figure II.11 and Table II.6. Chlorpheniramine dissolution profile of products E_1 and E_2 is shown in

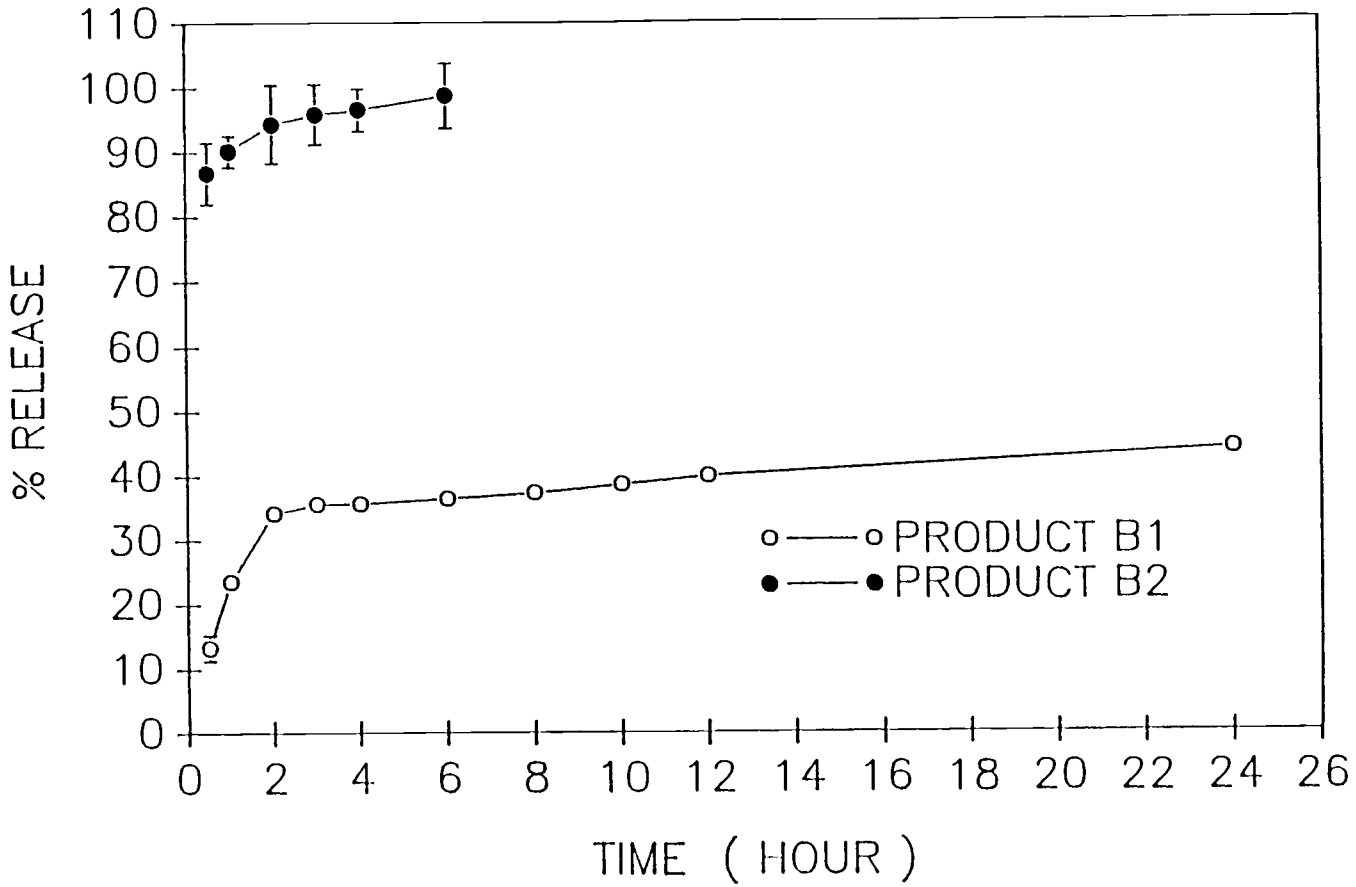


Figure II.9 Percent of Chlorpheniramine Dissolved as a Function of Time From Immediate and Sustained Release Dosage Form. (Products B_1 and B_2)

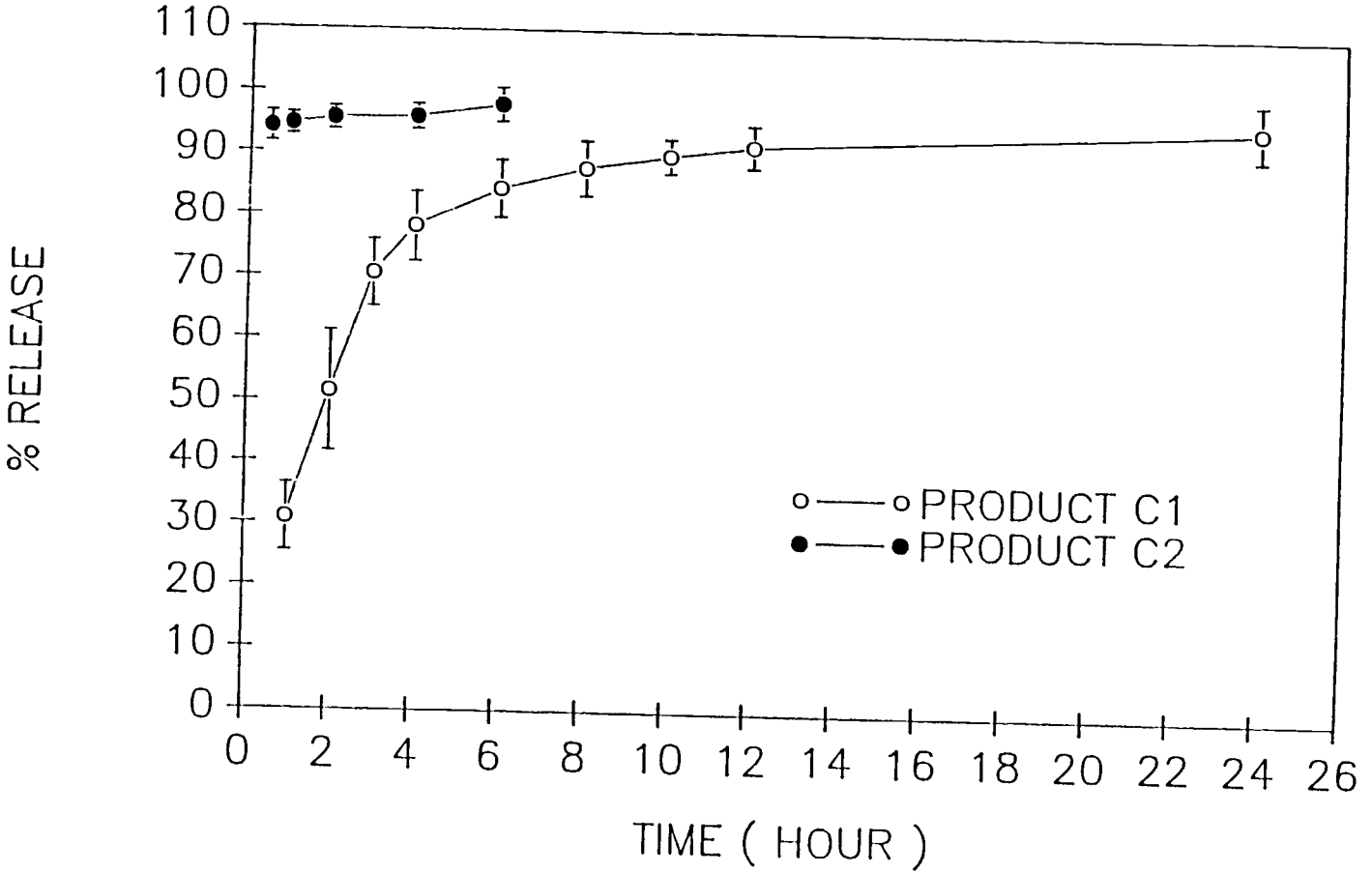


Figure II.10 Percent of Chlorpheniramine Dissolved as a Function of Time From Immediate and Sustained Release Dosage Forms. (Products C_1 and C_2)

Table II. 5 Percent Chlorpheniramine Dissolved in Simulated Gastric Fluid at Various Times for Products B_2 and C_2.

	Chlorpheniramine Dissolved, %[a]					
Product	0.5h	1h	2h	3h	4h	6h
B_2	86.7 (4.8)	90.1 (2.5)	94.3 (6.1)	95.8 (4.7)	96.5 (3.3)	98.7 (5.1)
C_2	94.1 (2.5)	94.6 (1.8)	95.6 (1.9)	-[a] -	95.9 (2.1)	98.0 (2.8)

a. Sample was not collected.

Figure II.12 and Table II.7. The relationship between chlorpheniramine and acetaminophen release in the same product is shown in Figure II.13. Chlorpheniramine released faster than acetaminophen after 5 minutes dissolution from product E_1, while for product E_2 both ingredients were released at similar speeds.

Are the controlled-release dosage forms successful? From the dissolution test results, some products demonstrate very poor drug dissolution in simulated gastric and intestinal fluids. From a pharmacokinetic view, the most successful controlled release dosage form should produce the same effective concentration at the same rate but extended, as an immediate release drug product. The dissolution pattern comparisons among several commercially available products provide information as to the product content, uniformity, rate and extent of drug release. It also provides preliminary data before conducting any bioavailability study.

Table II.6 Percent Acetaminophen Dissolved in Simulated Gastric Fluid at Various Times for Products E_{1-4}.

	Acetaminophen Dissolved, %					
Product	5 min	15 min	30 min	45 min	1 hr	2 hr
E_1	48.4 (7.3)	67.7 (5.1)	75.6 (5.7)	77.8 (4.5)	84.2 (3.1)	92.1 (7.4)
E_2	16.7 (2.5)	69.5 (6.2)	90.8 (2.5)	93.3 (1.8)	96.6 (3.0)	97.4 (2.0)
E_3	76.8 (9.6)	90.2 (8.8)	96.6 (5.9)	96.2 (8.1)	97.7 (3.5)	101.2 (5.6)
E_4	84.7 (9.4)	96.9 (4.5)	101.0 (3.0)	-a -	101.5 (2.5)	102.2 (2.8)

a. Sample was not collected.

Table II. 7 Percent Chlorpheniramine Dissolved in Simulated Gastric Fluid at Various Times for Products E_1 and E_2.

	Chlorpheniramine Dissolved %			
Product	5 min	15 min	30 min	45 min
E_1	32.5 (9.5)	93.3 (8.)	101.2 (2.3)	102.7 (3.9)
E_2	7.3 (4.0)	76.6 (9.5)	98.3 (2.8)	101.2 (2.3)

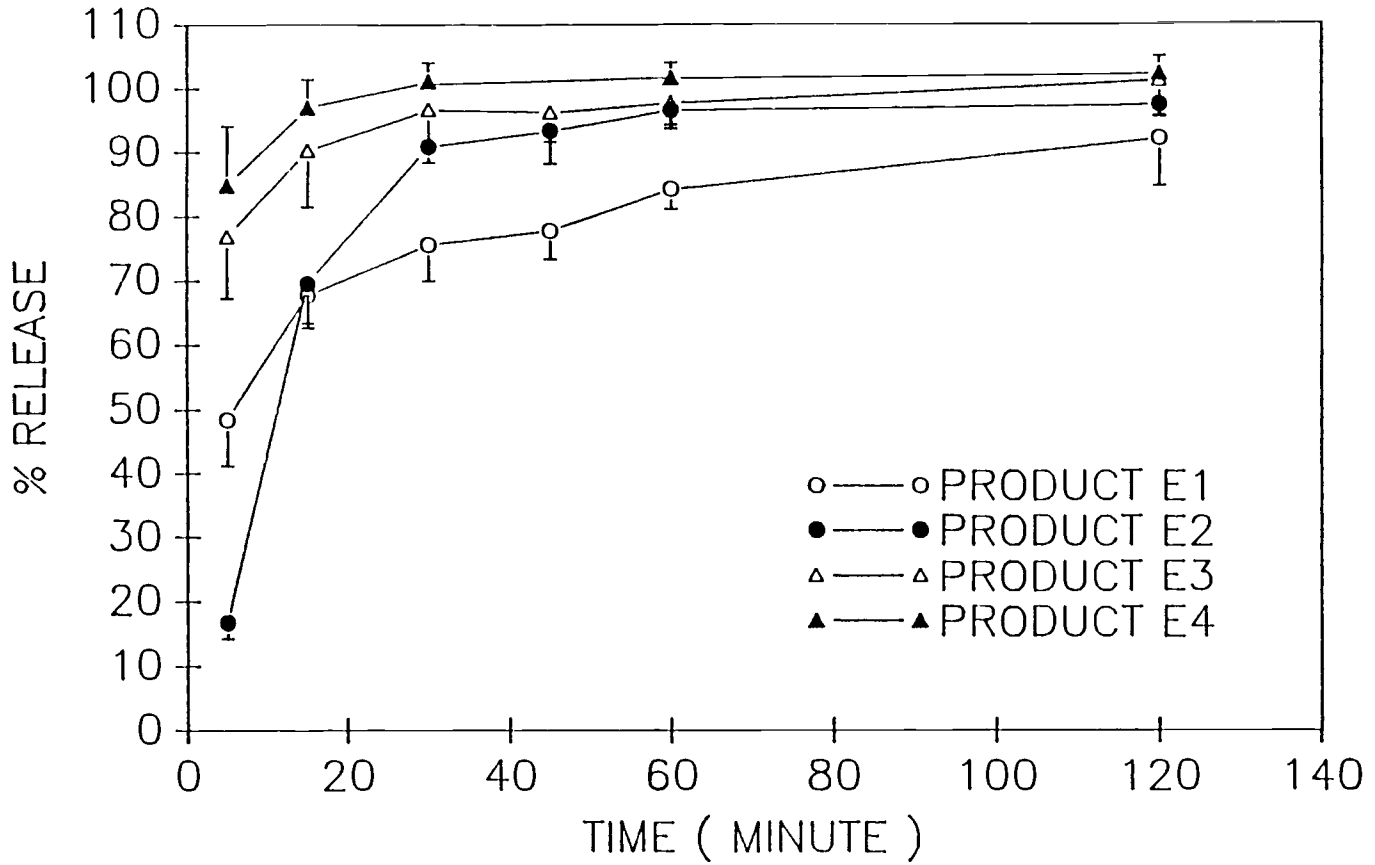


Figure II.11 Percent Acetaminophen Dissolved in Simulated Gastric Fluid Versus Time From Products E_1, E_2, E_3 and E_4.

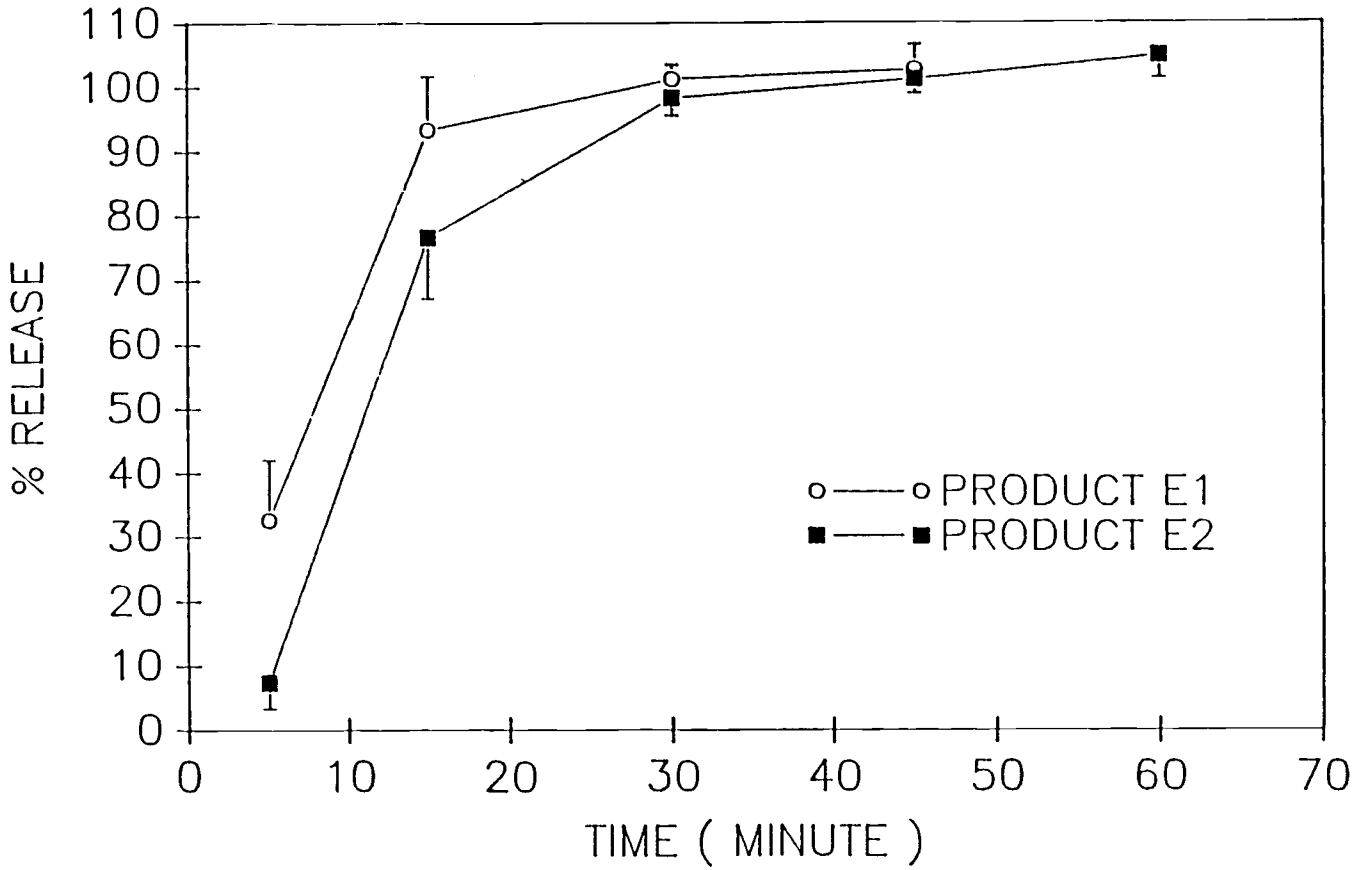


Figure II.12 Percent Chlorpheniramine Dissolved in Simulated Gastric Fluid Versus Time From Products E_1 and E_2.

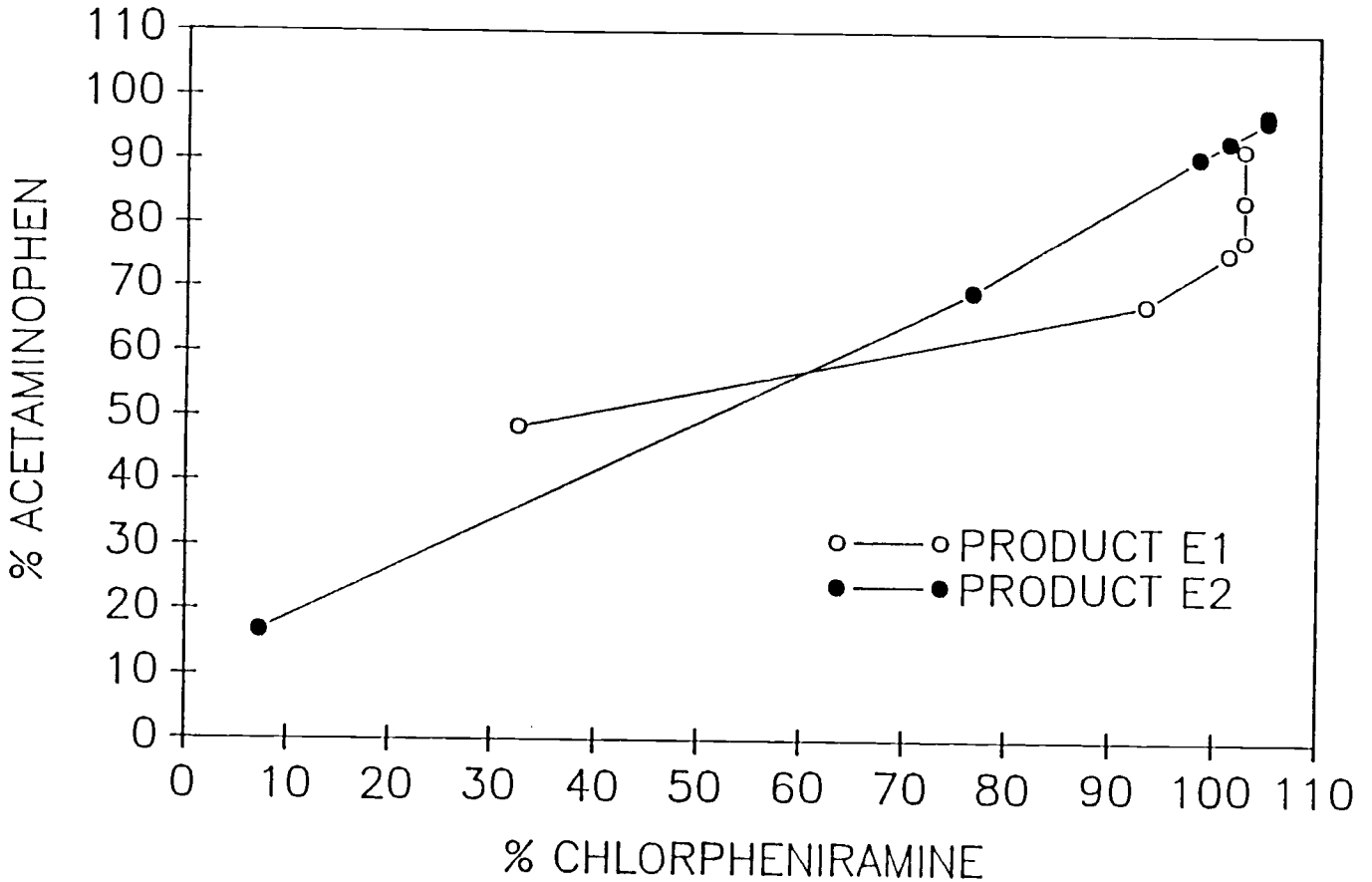


Figure II.13 Percent Dissolved of Chlorpheniramine Versus Acetaminophen From The Same Formulation. (Products E_1 and E_2)

CONCLUSIONS

The percentage of chlorpheniramine dissolved from sustained-release products show great product variability. Product C_1 which only contain chlorpheniramine release 95.1 % while products A_1 and B_1 only release 70.7 and 44.0 % at 24 hrs. From one brand of chlorpheniramine (two strength), also showed different dissolution pattern. These dissolution studies in simulated gastric and intestinal fluids show that products A_2 and C_1 (both are capsule form) are better formulations as sustained-release dosage forms, while product B_1 demonstrate very poor drug availability in dissolution fluids. However, these *in vitro* data may not mirror the *in vivo* situation. The percentage of phenylpropanolamine dissolved from these sustained-release products did not show much difference.

Dissolution results show that product D - a repeat action tablet is a successful dosage form which release 50 % of labeled pseudoephedrine content within 0.5 hr while the remaining 50 % was slowly released from 4 hr to 12 hr.

For all of the immediate release dosage forms almost all of the contents were released within 2 hours. These results indicate that all of the immediate-release dosage forms are good.

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CHAPTER III

BIOAVAILABILITY OF CHLORPHENIRAMINE AND ACETAMINOPHEN USING URINARY EXCRETION DATA.

INTRODUCTION

Chlorpheniramine maleate is commonly used in the treatment of various allergic conditions. It is available either alone or in combination with other drugs. The different dosage forms of chlorpheniramine maleate marketed include immediate and sustained release products. Recently, the development of sensitive, specific HPLC (1) and GC (2,3) assays have facilitated bioavailability studies (4,5). However, reported pharmacokinetic parameters from different studies varied considerably. For example, the reported elimination half lives of chlorpheniramine range from 17.6 (5) to 31.1 hours (6) after an 8 mg oral dose. Also only little comparative information is currently available (5) concerning the bioavailability of immediate release versus sustained release products or on comparisons among various sustained-release products themselves.

A GLC method was reported for the determination of chlorpheniramine in urine (7,8). In this study a specific high-pressure liquid chromatographic method based on former studies for the determination of chlorpheniramine in urine was developed and applied in a urinary excretion study of normal healthy subjects who received two different commercially available sustained-release cold-cough products and an immediate-release product as a reference. The advantage of using urinary excretion data

is that it is not as intrusive in nature as plasma sampling. It is also much more convenient to obtain a urine sample from someone than to withdraw blood periodically. Further, the assay does not require as great a sensitivity as drug is more concentrated in urine than saliva or plasma. A definite disadvantage is that chlorpheniramine is extensively metabolized (9-11) so only small fractions of the absorbed dose are excreted intact in the urine. The metabolites (9-11) (monodesmethyl and didesmethyl chlorpheniramine) are not available commercially and could not be obtained on request from either Schering Corporation or Warner-Lambert Corporation.

Relative bioavailability of acetaminophen contained in product E_1 was also studied in these four subjects by utilizing urinary excretion data and comparing these data with previous reports (14-18).

MATERIALS AND METHODS

Dosage Forms:

Two sustained release dosage forms - products A_1 and B_1 and one immediate-release dosage form - product E_1 were tested. Product A_1 was Contac caplet which contains 12 mg chlorpheniramine maleate and 75 mg phenylpropanolamine HCl (Lot #1136238 from SmithKline Beckman Company). Product B_1 was Allerest caplet which contains 12 mg chlorpheniramine maleate and 75 mg phenylpropanolamine HCl (Lot #31907 from Pennwalt Corp). Product E_1 was Sinutab Maximum Strength KAPSEAL capsule which contains 2 mg chlorpheniramine maleate, 30 mg pseudoephedrine HCl and 500 mg acetaminophen (Lot #02476VD from Warner-Lambert).

Subjects:

Four healthy subjects (1-4) (1 female and 3 males), age 27-45 years, body weight 45 - 77 kg participated in this study.

Study Design:

A single dose relative bioavailability study was conducted to compare three formulations. An experimental design with 4 subjects was used as shown in the following scheme.

	Order in which treatments were taken		
	First	Second	Third
Subject 1	A	B	C
Subject 2	C	A	B
Subject 3	B	C	A
Subject 4	A	C	B

Treatment definition:

Treatment A - A single dose of a caplet of commercial product: Contac (Product A_1) which contains 12 mg chlorpheniramine maleate and 75 mg phenylpropanolamine HCl.

Treatment B - A single dose of a caplet of commercial product : Allerest (Product B_1) which contains 12 mg chlorpheniramine maleate and 75 mg phenylpropanolamine HCl.

Treatment C - Three KAPSEAL capsules of commercial product : Sinutab (Product E_1) which contains 6 mg chlorpheniramine maleate, 90 mg pseudoephedrine HCl and 1.5 g acetaminophen in three capsules.

Subjects received the treatments after an overnight fast (over 12 hours), followed by an additional 3 hours fasting period. Eight ounces of water was taken half an hour before and with administration of the drug. Another eight ounces of water was received one and half hours after administering the drug. Urine pH and flow rate were

not controlled in this study. Urine samples were collected by completely emptying the bladder from -0.5 to 0 hr and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 15, 24, 30, 36, and 48 hours. The entire volume of urine voided during a sampling time interval was collected and mixed to insure a uniform specimen. The volume voided was measured in a graduated cylinder. Urine pH was determined at room temperature. An aliquot of 30 ml of the thoroughly mixed sample was withdrawn, centrifuged and stored at -20°C until assayed.

Assay:

The HPLC assay employed here is the same as described in chapter II for dissolution samples. Chlorpheniramine and acetaminophen analysis were performed by the same HPLC method at different times with no dilution for chlorpheniramine and dilution for acetaminophen. The limit of detection for chlorpheniramine was about 40 ng/ml. The mobile phase flow rate was set at 2 ml/min.

Internal Standard (IS) Solution:

For the assay of chlorpheniramine, dextromethorphan HBr was used as internal standard. A stock solution of dextromethorphan was prepared at 100 mcg/ml in water. For the assay of acetaminophen, lidocaine was used as internal standard. A concentration of 10 mg/ml was used.

Sample Preparation:

A. Chlorpheniramine

Two ml of urine sample was transferred into a 13x100 mm centrifuge tube (9). To the samples were added 0.2 ml of 10 % KOH solution and 0.2 ml of 100 mcg/ml dextromethorphan. Vortex for 30 seconds, then extract with 5 ml ether by vortex 1 min followed by centrifugation at 3000 rpm for 5 mins. The aqueous layer was frozen with the aid of a dry ice-alcohol bath and the ether decanted into a clean 13x100 mm centrifuge tube which contained 0.1 ml of 0.5 % phosphoric acid. The mixture was shaken for 1 min, centrifuged and refrozen. The ether layer was discarded, and the remaining aqueous portion was kept at room temperature in an evaporator (Lab-Line Instruments Inc., Melrose Park, Illinois.) at reduced pressure for 20 mins to remove traces of ether. 0.025 ml of aqueous solutions were taken into a syringe for chromatography (1,5,9,10).

B. Acetaminophen

0.25 ml samples of urine was taken for assay. To the samples were added 0.42 ml of 10mg/ml lidocaine HCl and 1.83 ml of water (Solution 1). Solution 1 was diluted 50 times with water (Solution 2). 0.02 ml of solution 2 was injected into HPLC.

Preparation of Standard Solutions:

A. Chlorpheniramine

Stock solutions of chlorpheniramine were prepared in water at concentrations of 1, 2, 5, 7.5, 10, and 20 mcg/ml. Take 0.1 ml of chlorpheniramine stock solution, add 1.9 ml of urine so the stock solution is diluted twenty times to the desired standard concentration. From this point, handle exactly as for the sample solution by adding potassium hydroxide, internal standard and extraction solvent.

B. Acetaminophen

Stock solutions of acetaminophen were prepared in water at concentrations of 250, 500, 1000, 1500, 2000 and 3000 mcg/ml. Take 0.025 ml of acetaminophen stock solution, add 0.225 ml of blank urine, so the stock solution is diluted 10 times to the desired standard solution. From this point, handle exactly as for sample solution by adding internal standard and water for dilution.

RESULTS AND DISCUSSION

A. CHLORPHENIRAMINE

Typical chromatograms of chlorpheniramine in urine are shown in Figure III.1. Calibration curves constructed by plotting the peak height ratio (PHR) of chlorpheniramine from urine to internal standard versus chlorpheniramine concentration were linear over the concentration range of 50-1000 ng/ml. Standard curves on different days prepared from urine sample are shown in Figures III.2a and III.2b. The use of dextromethorphan as an internal standard added at the beginning of the extraction procedure not only allows the accurate quantitation of chlorpheniramine but also assures that samples are extracted properly. The retention time of dextromethorphan is 8.0 min compare to 4.7 min for chlorpheniramine. The slopes and intercepts of the calibration curves did not vary significantly throughout the period of analysis of all samples from this study.

Urine collection intervals varied in reported studies, making it difficult to compare drug recovery among studies. Recovery of unchanged drug in 24 hour pooled urine samples was reported to be 4.5-11.5% (7), 5.2% (11), and 6.6-9.1% (9). Table III.1 shows the chlorpheniramine recovery in 24, 36, and 48 hrs for products A_1, B_1 and in 24 and 36 hrs for product E_1. The results are similar to reported literature data despite

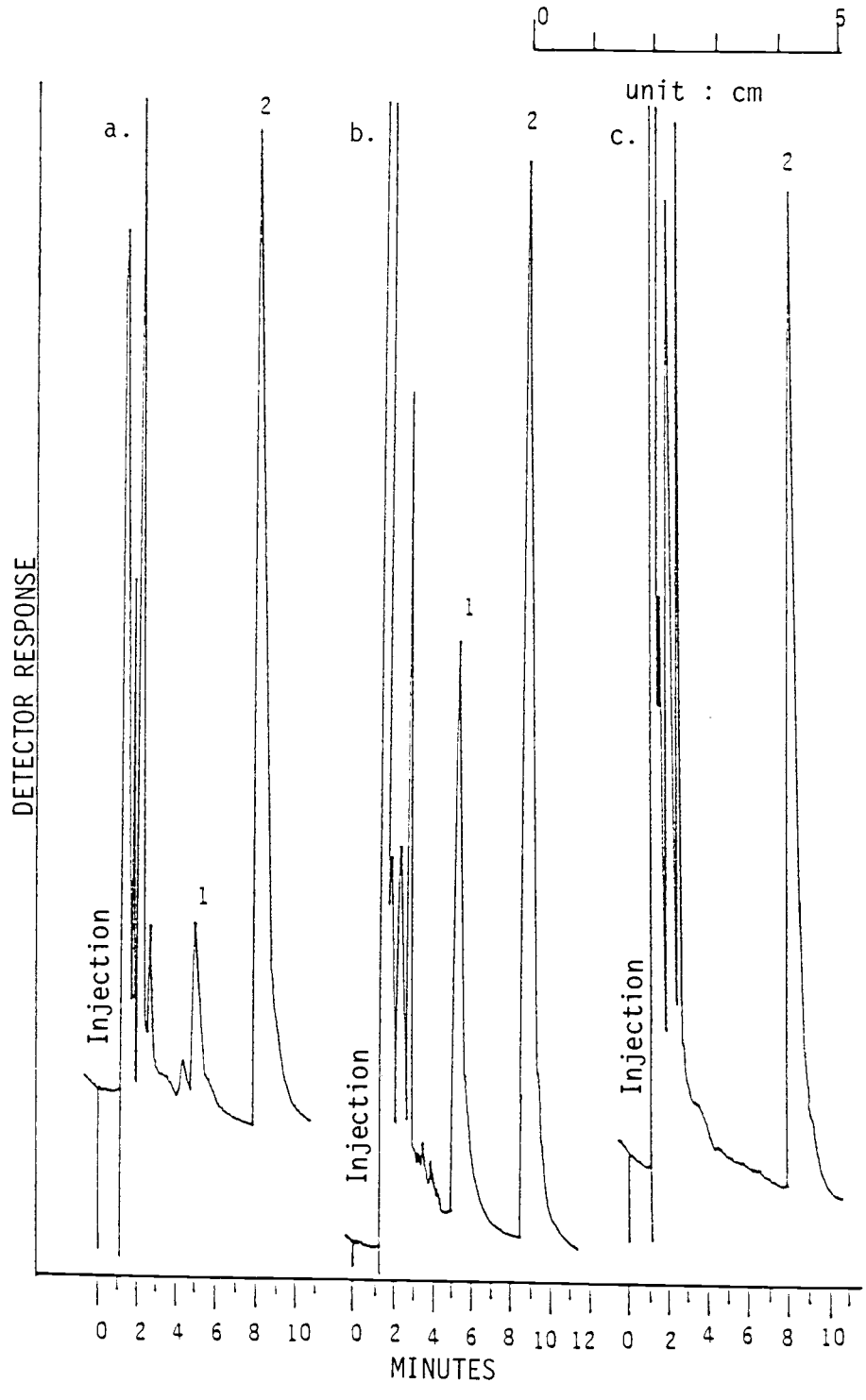

Figure III.1 Chromatograms of 1, Chlorpheniramine and 2, Dextromethorphan (IS) in (a) Subject Sample, (b) Standard, (c) Blank Control.

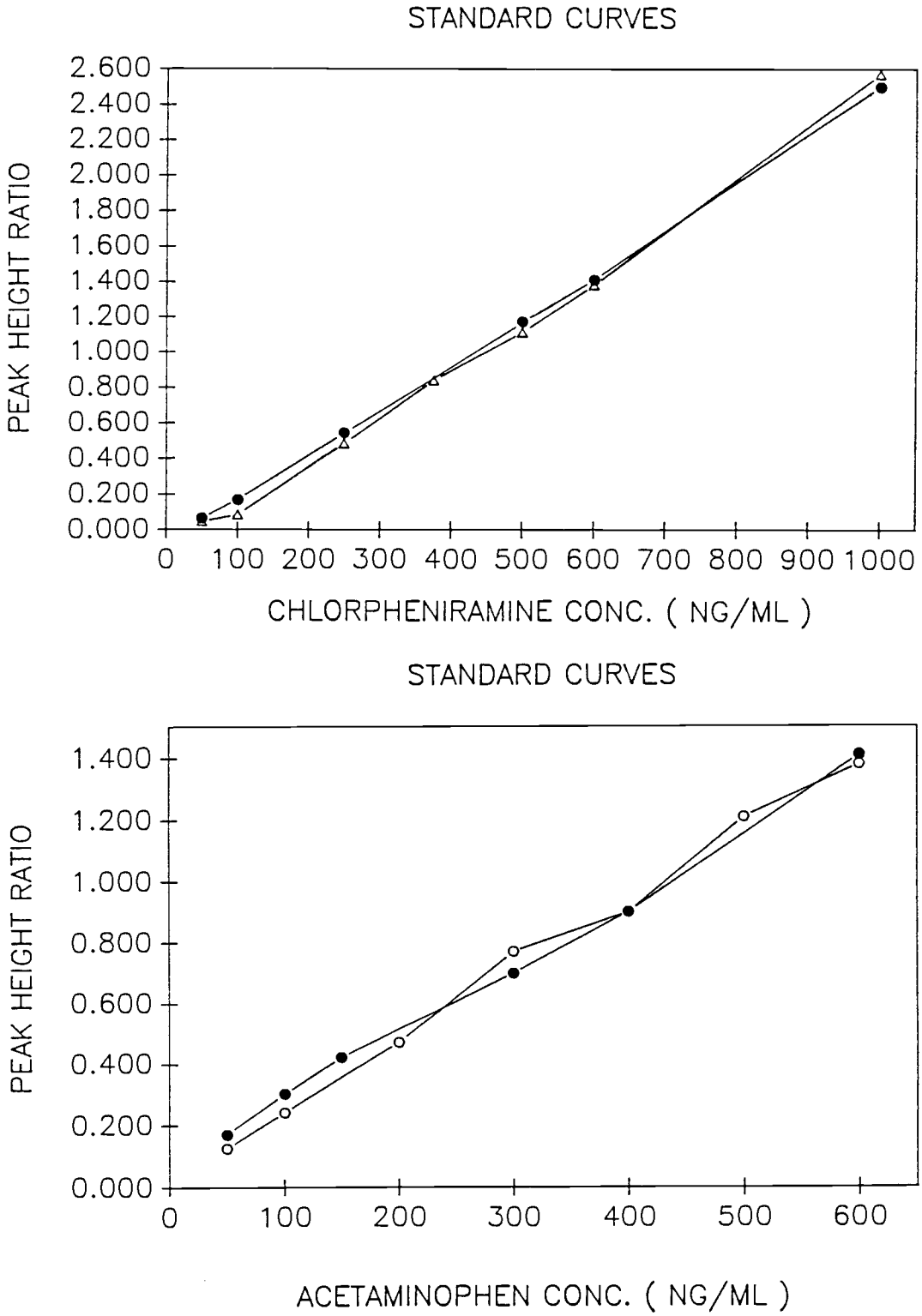

Figure III.2 Standard Curves of (a) Chlorpheniramine, (b) Acetaminophen on Different Days Prepared From Urine Samples.

Table III.1 Chlorpheniramine Recovery in 24, 36 and 48 hr for Products A_1, B_1 and in 24 and 36 hr for Product E_1.

	Product A_1			Product B_1			Product E_1			
	24h	36h	48h	24h	36h	48h	24h	36h	24h	36h
							a.		b.	
Subject 1	3.4%	5.1%	6.9%	6.3%	4.6%	5.1 %	6.4%	9.9%	4.9%	4.9%
Subject 2	5.4%	7.7%	8.7%	4.6%	7.4%	8.3%	9.7%	11.4%	4.3%	4.3%
Subject 3	3.0%	3.6%	4.1%	2.0%	2.4%	2.7%	5.5%	6.7%	6.1%	6.1%
Subject 4	3.8%	4.4%	5.1%	1.2%	1.5%	1.6%	2.6%	3.0%	3.1%	3.1%
Mean	3.9%	5.2%	6.2%	3.5%	4.0%	4.4%	6.1%	7.8%	4.6%	4.6%
± SD	1.1%	1.8%	2.0%	2.4%	2.6%	3.0%	2.9%	3.7%	1.2%	1.2%

a. Chlorpheniramine. b. Acetaminophen.

differences in the collection intervals.

Figure III.3 shows the average time course of cumulative urinary excretion of chlorpheniramine in four subjects after treatments A, B, and C. Since the total amount of unchanged drug excreted in the urine is generally accepted as a measure of relative efficiency of absorption, these values were also calculated in this study. A summary of average cumulative urinary excretion is shown in Table III.2. The total amount of chlorpheniramine excreted in urine in 36 hrs after treatments A, B, and C (corrected for dose) were 437 mcg, 328 mcg and 643 mcg, respectively. The cumulative urinary excretion amount after treatment C was corrected for dose since it only contained 4.2 mg of chlorpheniramine compared to 8.4 mg in products A_1 and B_1.

The data of Table III.2 were subjected to a split plot ANOVA (12) as shown in Table III.3a. The log values of cumulative amount excreted at different intervals were used to calculate sum squares (SS) and mean squares (MS), since this analysis is based on constant standard deviation. The F-test for " Treatment x Time " interaction is equal to 2.58. This significant result suggests that treatment effect is dependent on time. As is usually the case for such interaction, this is most easily visualized by means of a plot. As shown in Figure III.3, the lack of " parallelism " is easily seen in the first two data points. Therefore, the same analysis was

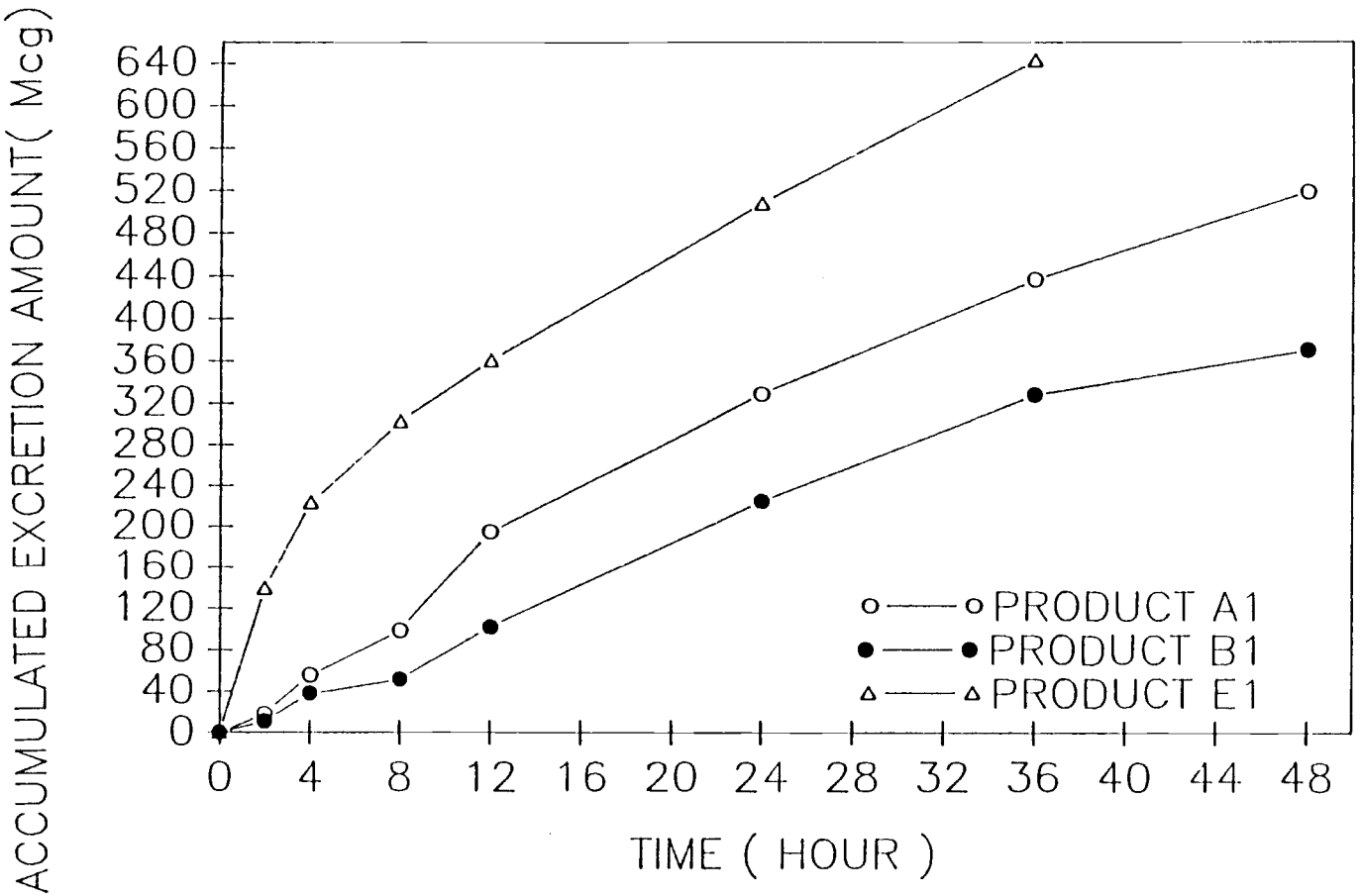

Figure III.3 Mean Cumulative Amount (mcg) of Chlorpheniramine Excreted for Four Subjects after Treatments A, B and C.

Table III.2 Summary of the Average Cumulative Amount of Chlorpheniramine and Acetaminophen Excreted Intact by Four Normal Subjects Following Treatments A, B and C.

	Cumulative Amount Excreted (mcg)		
Time Interval (hour)	Treatment A	Treatment B	Treatment C[a]
0-2	18.1 ± 14.4	11.0 ± 9.4	138.5 ± 186.8
0-4	55.2 ± 25.4	37.4 ± 21.3	222.6 ± 209.7
0-8	98.2 ± 31.8	73.5 ± 31.6	302.6 ± 244.4
0-12	194.6 ± 72.7	101.9 ± 41.3	360.7 ± 283.8
0-24	329.4 ± 89.5	224.8 ± 120	508.2 ± 245.2
0-36	436.7 ± 149.8	328.1 ± 215.1	643.3 ± 316.8
0-48	519.4 ± 170.6	370.9 ± 251.9	720[b]
Ratio at 36h[c]	67.9%	51.0%	100%
Ratio at 48h[c]	72.1%	51.5%	100%

a. The amounts have been doubled for treatment C to correct for dose.
b. This value was calculated from the linear regression line $y = 2.67-0.036x$ determined for the plot of average excretion rate versus midpoint of time for treatment C. Substitute midpoint of time (42 hrs) as x to solve y which gives a value of 1.16. Since ln 3.2 = 1.16, the excretion rate at 42 hrs is 3.2 mcg/ml and amount excreted from 36-48 hr was 76.8 mcg after correction for the dose.
c. This is the ratio of cumulative amount excreted for treatments A and B relative to treatment C.

done after separating the data into two groups. One group had only 2 and 4 hour data points, while the other group had 8, 12, 24 and 36 hour data points. The results are shown in Tables III.3b and III.3c.

The F test shows significant (at 1 % level) interaction of treatment and time at 2 and 4 hours while no significant interaction was found at 8, 12, 24 and 36 hours (Tables III.3b and III.3c). The F test for treatment differences is equal 5.8. The significant (at 5 % level) result indicates that the cumulative amount excreted of chlorpheniramine after treatments A, B and C (corrected for dose) were different.

Inspection of Tables III.1, III.2, III.3 and Figure III.3 suggests that a higher percentage recovery and cumulative amount of chlorpheniramine was excreted for the 4 subjects following treatment A than treatment B. Since some subjects had half-lives as long as 20 hours, the 36 hour recovery did not account for all drug absorbed and excreted in the urine. The urine concentrations of drug for the immediate release product (product E_1) and sustained release products (products A_1, B_1) were detectable at 0.5 hr and 1.5 hr, respectively. Products A_1 and B_1 have higher urine excretion rates at 36 hr than product E_1. A semilogarithmic plot of the mean urinary excretion rate versus time curves after treatments A, B and C is shown in Figure III.4.

Table III.3a Split-Plot ANOVA for the Cumulative Amount of Chlorpheniramine Excreted Intact in The Four Normal Subjects Following Treatments A, B and C.

Source	df	SS	MS	F
Total	71			
Subject	3	2.26		
Treatment (A)	2	3.84	1.92	6.62
Error (A)	6	1.75	0.29	
Time (B)	5	14.21	2.84	
Treatment x Time	10	1.15	0.115	2.58[a]
Error (AB)	45	2.0	0.044	

a.Significant at the 5 % level.

Table III.3b Split-Plot ANOVA for the Cumulative Amount of Chlorpheniramine Excreted Intact at 2 and 4 hours after Treatments A, B and C.

Source	df	SS	MS	F
Total	23	8.57		
Subject	3	2.3		
Treatment (A)	2	3.1		
Error (A)	6	0.98		
Time (B)	1	1.99		
Treatment x Time	2	0.17	0.085	25.5
Error (AB)	9	0.03	0.0033	

Table III.3c Split-Plot ANOVA for the Cumulative Amount of Chlorpheniramine Excreted Intact at 8, 12, 24 and 36 hours after Treatments A, B and C.

Source	df	SS	MS	F
Total	47	6.67		
Subject	3	0.9		
Treatment (A)	2	1.5	0.75	5.8[ab]
Error (A)	6	0.78	0.13	
Time (B)	3	2.2		
Treatment x Time	6	0.08	0.013	0.29[b]
Error (AB)	27	1.23	0.046	

a. Significant at the 5 % level.
b. Not Significant at the 1 % level.

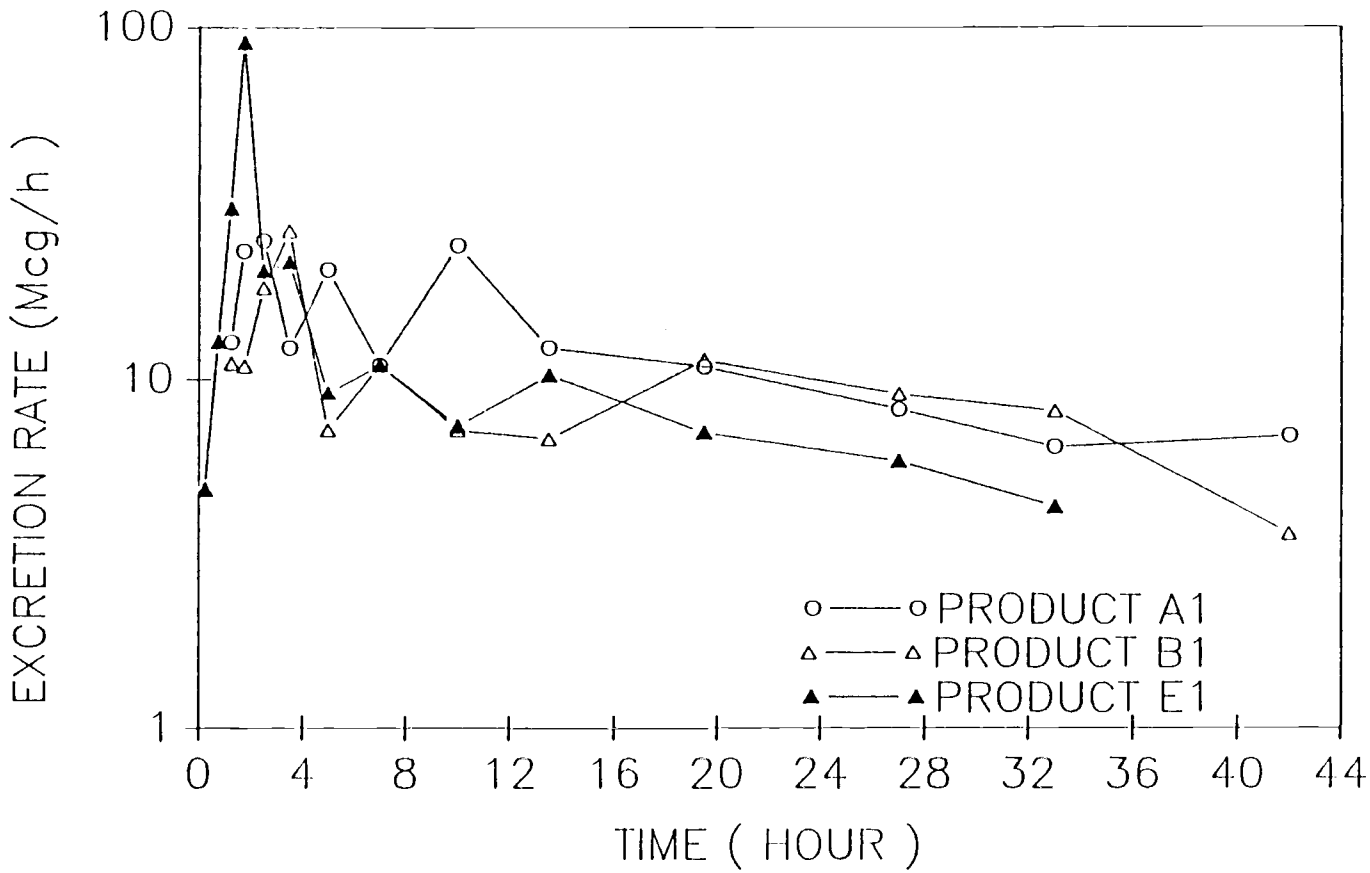

Figure III.4 A Semilogarithmic Plot of Mean Chlorpheniramine Urinary Excretion Rate Versus Midpoint Time after Treatments A, B and C.

A wide range of half-lives of chlorpheniramine have been reported in adults following oral (4,5,6) and intravenous (3,12) studies. The half-life in adults of four studies were 22.13 ± 5.92 h (4), 17.6 ± 4.4 h (5), 31.08 ± 8.3 h (6) and 24.4 ± 6.6 h (13). The half-life in this study was calculated from least-squares fits of the terminal slope of the log urinary excretion rate versus time curve. The elimination half-lives in the four subjects after treatment B were calculated in three different ways:

(1) Four points at 19.5, 27, 33, and 42 hrs were used to find the linear regression line.

(2) Five points at 13.5, 19.5, 27, 33 and 42 hrs were used to find the linear regression line.

(3) Three points at 19.5, 27, and 42 hrs were used to find the linear regression line.

The results are shown in Table III.4.

Table III.5 shows mean values of half-lives, elimination rate constants and AUC after different treatments in the four subjects. Comparison of half-lives after treatment B to treatments A and C show quite different results no matter which data points were used to estimate the half-life after treatment B. However, more dramatic changes in pH and urine flow rates in the four subjects after taking treatment B were also found in this study (see Appendix B), which makes it more difficult to estimate half-life.

Table III.4 Three Ways to Estimate Half-Life of Chlorpheniramine After Treatment B.

Parameters	(1)	(2)	(3)
Regression line	y=3.51-0.05x	y=2.64-0.024x	y=3.52-0.053x
$^{a}r^2$	0.89	0.39	0.98
K_{el}	0.05	0.024	0.053
$t_{1/2}$	13.9h	28.9h	13.1h

a. Coefficient of determination.

Table III.5 Pharmacokinetic Parameters in The Four Subjects After Oral Dosing.

Parameters	^{a}Treatment A	Treatment B	^{b}Treatment C
$t_{1/2}$, hr	20.4	^{c}13.1	19.3
$AUC_{0-\infty}{}^{d}$, ng/ml/h	611.5	405.7	^{e}912.4
K_{el}, hr^{-1}	0.034	0.053	0.036
Relative area	67%	44.5%	100%
r^2	0.98	0.98	0.96

a. The half-life after treatment A was estimated by using the points at 13.5, 19.5, 27,and 33 hrs.
b. The half-life after treatment C was estimated by using the point at 19.5, 27 and 33 hrs.
c. This half-life was chosen since the r^2 is closer to 1.
d. The area under the urinary excretion curve data were calculated using the linear trapezoidal method. The last urinary excretion rate at 19.5 hrs (c) was divided by the least-squares slope value for the postabsorption phase.
e. Corrected for the dose.

The 17.6 hr average half-life (using 13.1 hr for treatment B) for all dosage forms studied is comparable to the reported 15-30 hr values (4-6).

The area under the excretion rate versus time curve of products A_1 and B_1 was not equal to two times the AUC of product E_1. The amount of actual drug present in products A_1 and B_1 is twice the amount in product E_1. It is difficult to obtain an exact AUC by using urinary data since urinary excretion rate is quite variable with urine flow rate and urine pH. The AUC for product A_1 was 67 % of E_1. The data are very consistent with the ratios of intact drug recovered in the urine for these treatments (Table III.2). The AUC for product B_1 was 66.3 % of that for the product A_1. In vitro dissolution at 24 hr was only 44.0 % of the drug from product B_1 and 70.7 % from product A_1. Hence, product B_1 which had a lower AUC value and a lower fraction of dose recovered, gave a consistent performance with respect to dissolution data. The urine flow rate, pH and excretion rate of each subject are shown in Appendix B. The effect of urine pH and flow rate on the excretion of chlorpheniramine has been reported following single and multiple-dose studies (7,9,11). Beckett and Wilkinson (7) found that when the urine was maintained acidic (pH 5.0 ± 0.5) by the administration of ammonium chloride, 20.0 to 26.5 % of the dose was excreted, whereas when the urine was maintained alkaline (pH 8.0 ± 0.5) by the administration of sodium

bicarbonate, only 0.3 to 0.4 % of the dose was excreted. They also found that when urinary pH was maintained in the acidic range the urinary excretion rate appeared to be related to urine flow rate; a high flow rate resulted in a high excretion rate.

Urine flow rates and pH were not controlled in this study. These values are variable in different subjects after different treatments. Representative plots of urine flow rate, pH and excretion rate of chlorpheniramine versus time are shown in Figures III.5a and III.5b. Figure III.5a shows that at 13.5 and 19.5 hrs, the pH decreased slightly (from 5.9 to 5.6) while the excretion rate increased dramatically (from 8.4 to 26.2 mcg/ml). Figure III.5b shows urine flow rate, pH and excretion rate in subject 1 after treatment A. The results confirm that chlorpheniramine excretion rate is dependent on urine flow rate and pH. This effect can be explained by assuming that the tubular epithelium of the distal convoluted kidney tubule is selectively permeable to the unionized base (9-11). The concentration of unionized base in the tubules can be altered by a change in the pH of the tubular fluid, as the pK_a of chlorpheniramine is 9.3. In more acidic tubular fluid, chlorpheniramine is more ionized, therefore less reabsorption occurs which results in more chlorpheniramine excreted in urine.

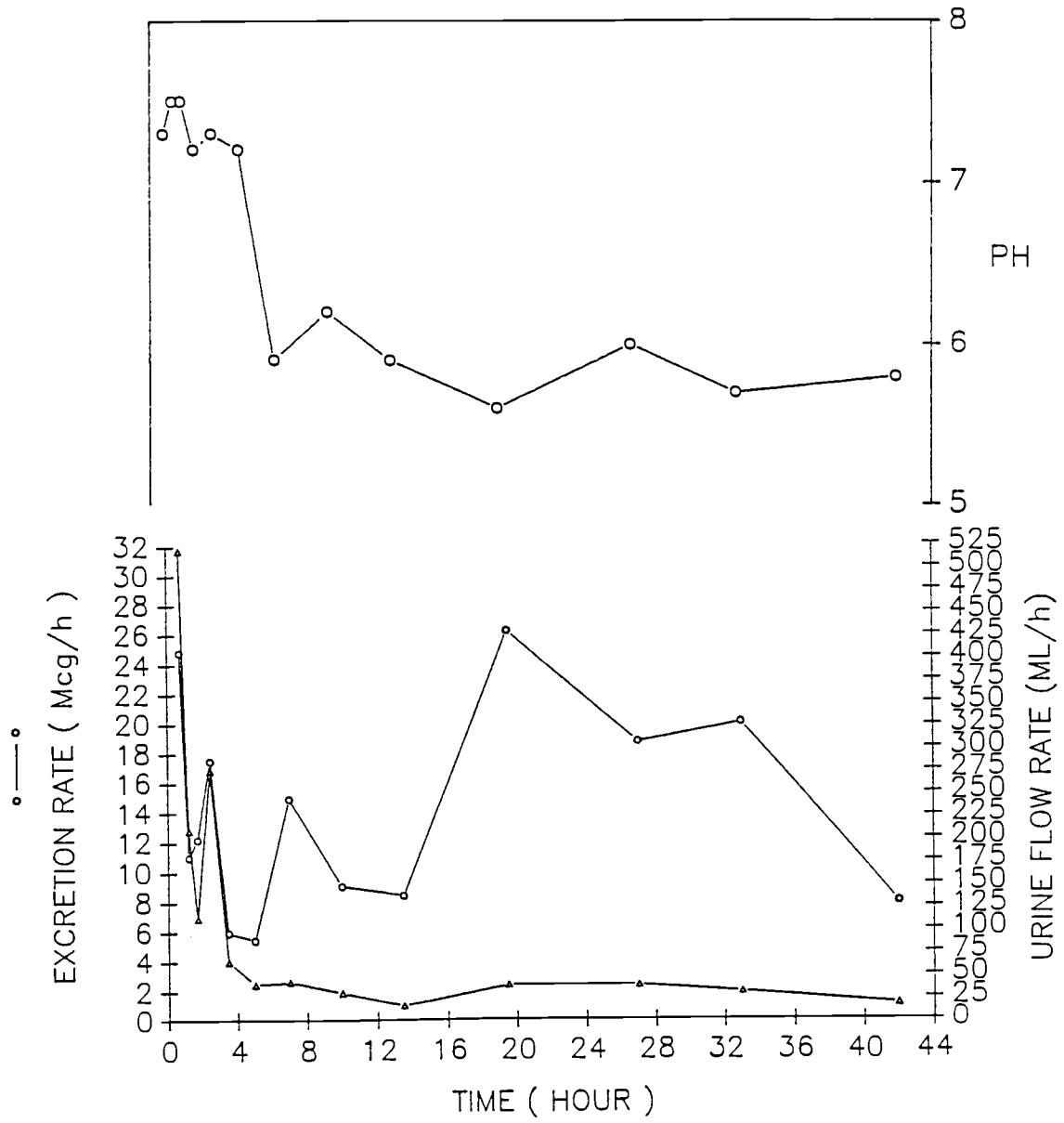

Figure III.5a Urinary Excretion Rate (mcg/h) of Chlorpheniramine, Urine Flow Rate (ml/h) and Urine pH Plotted Versus Midpoint Time in Subject 2 Following Treatment B.

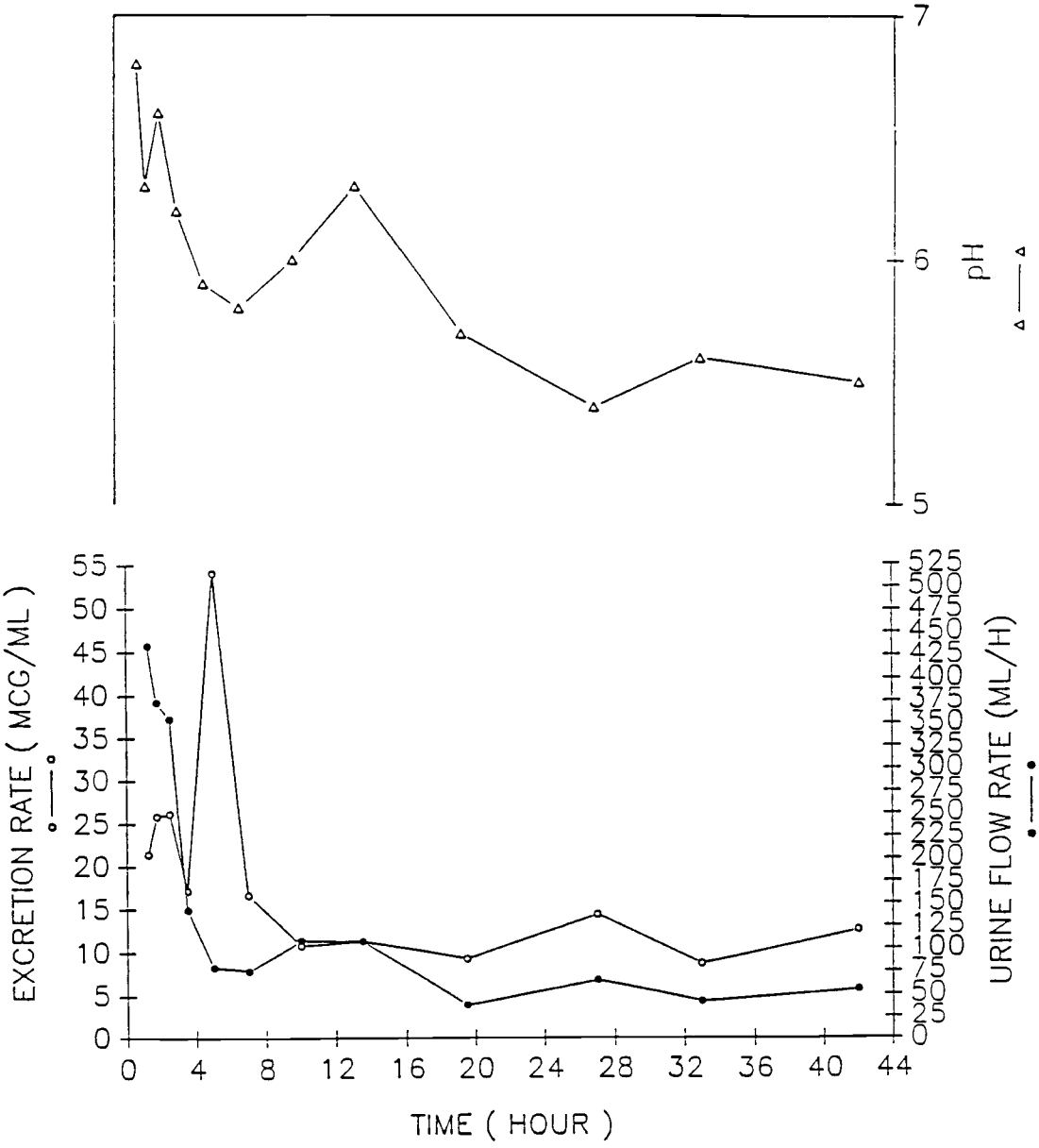

Figure III.5b Urinary Excretion Rate (mcg/h) of Chlorpheniramine, Urine Flow Rate (ml/h) and Urine pH Plotted Versus Midpoint Time in Subject 1 Following Treatment A.

B. ACETAMINOPHEN

Reasonable peak shapes for chlorpheniramine and acetaminophen were achieved during a single chromatographic analysis but, due to the relatively large amount of acetaminophen and urine solvent front, complete separation of these two components was not obtained. Therefore, direct injection of urine samples was not feasible. It was necessary to determine acetaminophen after dilution of samples with water, while chlorpheniramine was analyzed following an extraction procedure.

It has been reported that renal excretion of acetaminophen involves glomerular filtration and passive reabsorption (14). It is eliminated primary by formation of the glucuronide and sulfate conjugates (15,16,17). Cummings et al. (18) reported that 4 % of a 12 mg/kg dose was excreted as unchanged acetaminophen. Levy and Yamada (16) reported similar results for humans following 1 or 2 g doses, 3 % was excreted as acetaminophen. Consistent with previous reports, the present study shows 4.6 ± 1.2 % (Table III.1) of a 1.5g dose was excreted in unchanged form. A semilogarithmic plot of the urinary excretion rate verses time curve in four subjects is shown in Figure III.6. Subject 4 had a significantly lower urinary recovery and excretion rate in the absorption phase and also had a lower urine flow rate (Appendix B. and Figure III.6). It has been reported that renal clearance

of unmetabolized acetaminophen is positively correlated with urine flow rate (19,20).

The half-life of acetaminophen in this study was also calculated from least-squares fits of the terminal slope of the log urinary excretion rate versus time curve. The half-life in the first subject was found to be 4.3 h estimated by using the points at 5, 7, 10, and 14 hrs. The half-life of acetaminophen in this subject by using saliva data from an earlier study is 3.8 hr, which is close to the 4.3 hr obtained by using urine sample. The half-life in the second subject was 1.7 h by using the last three points. The half-life of acetaminophen in the third and the fourth subject were 4.6 h and 2.8 h by using the points at 7, 10, 19.5 and 7, 10, 13.5 hr, respectively. The mean half-life in the four subjects was 3.4 ± 1.4 h. Nelson and Morioka (21) reported a mean half-life of 1.95 ± 0.23 h, while Cummings et al.(18) and McGilveray et al.(22) reported values of 2.2 and 3.14 h, respectively.

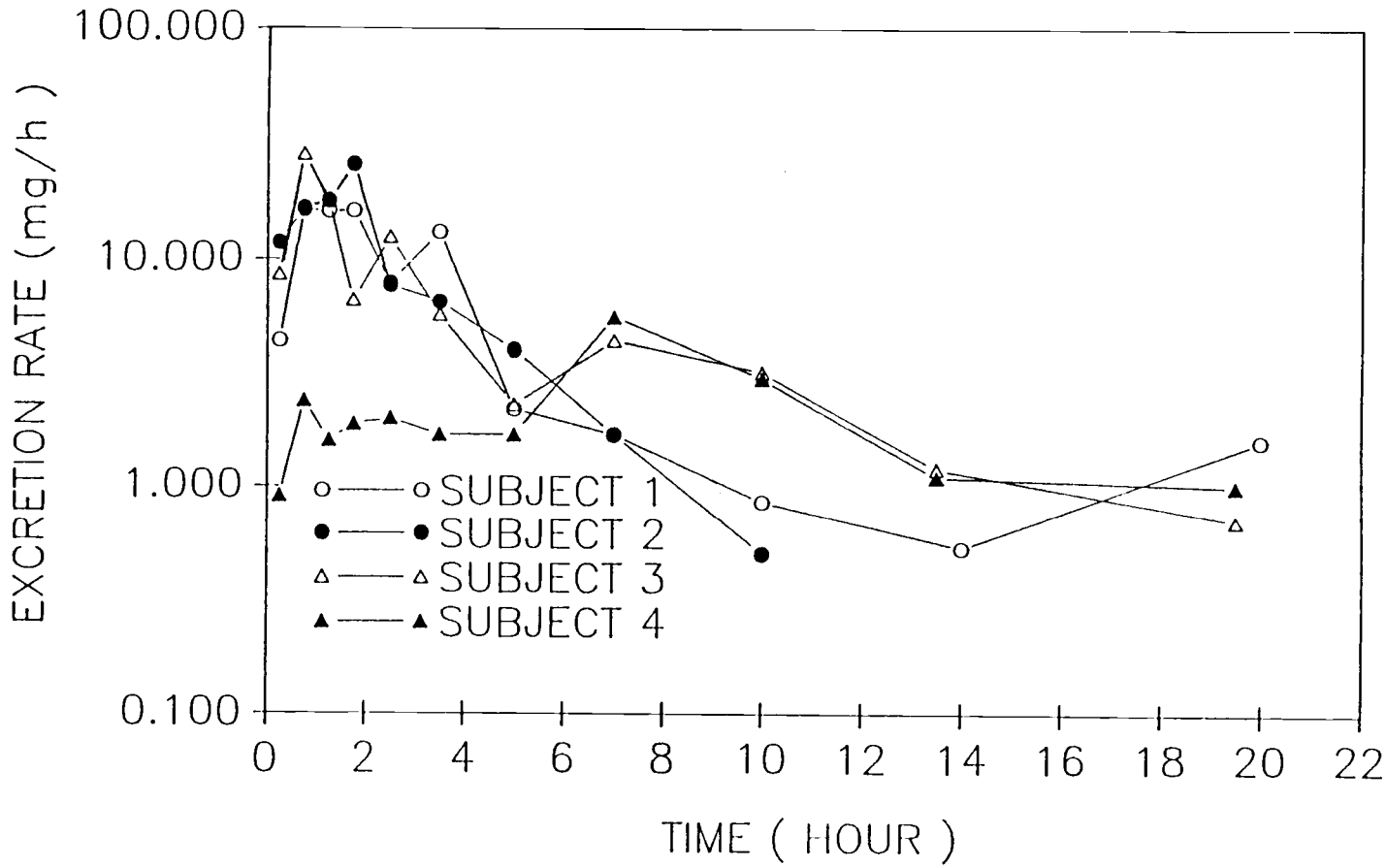

Figure III.6 A Semilogarithmic Plot of Acetaminophen Urinary Excretion Rate versus Midpoint Time in Four Subjects.

CONCLUSIONS

When the ratio of parameters characterizing the bioavailability of two drugs is one, the dosage forms are considered to be bioequivalent. With only urine data, the rate of drug absorption as given by the peak plasma concentration and time of peak can not be determined. However, the study described here has shown that following administration of equivalent doses of chlorpheniramine in sustained release caplets manufactured by different companies, urinary drug excretion varied to some degree. Products A_1 and B_1 are not bioequivalent in this small pilot study. The relative bioavailability results were consistent with dissolution results. The data also confirm that chlorpheniramine excretion rate is dependent on urine flow rate and pH, while acetaminophen excretion rate is dependent on flow rate.

Generally, drugs with relatively short half-lives (less than 6 hr) would be the most likely candidates for sustained delivery. Chlorpheniramine has a long half-life (more than 15 hr) and may not seem to be a good candidate to formulate as a sustained-release dosage form. However, there is still an advantage of chlorpheniramine sustained-release products: sustained-release dosage forms produce lower C_{max} (peak concentration in the plasma) than immediate release products which can reduce the side effects caused by chlorpheniramine such as drowsiness and

dryness of mouth.

In vitro studies showed products A_1 and B_1 dissolved more slowly than product E_1 which suggests the rate of absorption *in vivo* for these sustained release products would be slower than immediate release products, resulting in a lower peak concentration and less side effects. Consistent results were also found in this small study. The peak excretion rate for treatment C was four times that of treatments A and B (eight times when corrected for dose).

More valid conclusions regarding relative bioavailability could be made following both serum and urinary data in more subjects. The present study provides only preliminary information regarding the relative bioeqivalency of three products formulated by different company. This information should be considered in future pharmacokinetic studies of these drugs. The next step is to develop a sensitive assay for the drug and its metabolites and conduct a traditional bioavailability study with blood sampling and about 15 subjects.

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APPENDICES

APPENDIX A. DISSOLUTION DATA OF IMMEDIATE AND SUSTAINED RELEASE COLD AND COUGH DOSAGE FORMS.

Table A.1a Percentage Release of Chlorpheniramine from Product A1 (No.1)

Time(hr)	Conc. (mcg/ml)	Amount Release(mg)	Total Amount Release (mg)	Total % Release
[a]0.5	2.44	2.20	2.20	26.2
1	4.0	3.60	3.60	42.9
2	5.50	4.95	4.95	58.9
[b]3	0.02	0.02	4.97	59.2
4	0.21	0.19	5.14	61.2
6	0.75	0.67	5.62	66.9
10	0.66	0.56	5.51	65.6
12	0.86	0.78	5.73	68.2
24	1.49	1.34	6.29	74.9

a. Dosage forms were tested in simulated gastric fluid in the first two hours.
b. Dosage forms were tested in simulated intestinal fluid after two hours.

Table A.1b Percentage Release of Chlorpheniramine from Product A1 (No.2)

Time(hr)	Conc. (mcg/ml)	Amount Release(mg)	Total Amount Release (mg)	Total % release
[a]0.5	2.22	2.00	2.00	23.8
1	3.55	3.19	3.19	38.0
2	5.41	4.87	4.87	58.0
[b]3	0.21	0.19	5.06	60.3
4	0.19	0.17	5.04	60.0
6	0.33	0.29	5.16	61.5
10	0.76	0.68	5.55	66.1
12	0.59	0.53	5.40	64.3
24	1.22	1.10	5.97	71.0

a. Dosage forms were tested in simulated gastric fluid in the first two hours.
b. Dosage forms were tested in simulated intestinal fluid after two hours.

Table A.1c Percentage Release of Chlorpheniramine from Product A1 (No.3)

Time(hr)	Conc. (mcg/ml)	Amount Release(mg)	Total Amount Release (mg)	Total % Release
[a]0.5	2.36	2.13	2.13	25.3
1	3.67	3.30	3.30	39.3
2	5.42	4.88	4.88	58.1
[b]3	0.06	0.28	5.15	61.4
4	0.33	0.29	5.17	61.6
6	0.57	0.51	5.39	64.2
10	0.78	0.70	5.58	66.4
12	0.74	0.66	5.54	66.0
24	1.08	0.97	5.85	69.7

a. Dosage forms were tested in simulated gastric fluid in the first two hours.
b. Dosage forms were tested in simulated intestinal fluid after two hours.

Table A.1d Percentage Release of Chlorpheniramine from Product A1 (No.4)

Time(hr)	Conc. (mcg/ml)	Amount Release(mg)	Total Amount Release (mg)	Total % Release
[a]0.5	2.23	2.01	2.01	23.9
1	3.57	3.21	3.21	38.3
2	5.53	4.98	4.98	59.3
[b]3	0.023	0.02	5.00	59.5
4	0.22	0.20	5.18	61.6
6	0.68	0.61	5.59	66.6
10	0.78	0.70	5.68	67.6
12	0.75	0.67	5.65	67.2
24	1.31	1.18	6.16	73.3

a. Dosage forms were tested in simulated gastric fluid in the first two hours.
b. Dosage forms were tested in simulated intestinal fluid after two hours.

Table A.1e Percentage Release of Chlorpheniramine from Product A1 (No.5)

Time(hr)	Conc. (mcg/ml)	Amount Release(mg)	Total Amount Release (mg)	Total % Release
[a]0.5	2.55	2.30	2.30	27.3
1	3.77	3.39	3.39	40.3
2	5.34	4.80	4.80	57.2
[b]3	0.30	0.27	5.07	60.3
4	0.21	0.19	4.99	59.4
6	0.66	0.60	5.40	64.2
10	0.96	0.86	5.66	67.4
12	0.76	0.68	5.48	65.3
24	1.09	0.98	5.78	68.8

a. Dosage forms were tested in simulated gastric fluid in the first two hours.
b. Dosage forms were tested in simulated intestinal fluid after two hours.

Table A.1f Percentage Release of Chlorpheniramine from Product A1 (No.6)

Time(hr)	Conc. (mcg/ml)	Amount Release(mg)	Total Amount Release (mg)	Total % Release
[a]0.5	2.46	2.21	2.21	26.3
1	3.70	3.33	3.33	39.7
2	5.01	4.51	4.51	53.7
[b]3	0.034	0.031	4.54	54.1
4	0.30	0.27	4.78	56.9
6	0.70	0.63	5.14	61.2
10	0.81	0.73	5.27	62.7
12	0.68	0.62	5.12	61.0
24	1.18	1.06	5.57	66.3

a. Dosage forms were tested in simulated gastric fluid in the first two hours.
b. Dosage forms were tested in simulated intestual fluid after two hours.

Table A.2a Percentage Release of Phenylpropanolamine from Product A1 (No.1)

Time(hr)	Conc. (mcg/ml)	Amount Release(mg)	Total Amount Release (mg)	Total % Release
[a]0.5	14.77	13.29	13.29	22.0
1	21.67	19.50	19.50	32.3
2	26.60	23.94	23.94	39.6
[b]3	6.98	6.28	30.22	50.0
4	11.01	9.91	33.85	56.0
6	18.90	17.01	40.95	67.8
10	22.48	20.23	44.17	73.1
12	23.84	21.46	45.40	75.2
24	27.20	24.48	48.42	80.2

a. Dosage forms were tested in simulated gastric fluid in the first two hours.
b. Dosage forms were tested in simulated intestinal fluid after two hours.

Table A.2b Percentage Release of Phenylpropanolamine from Product A1 (No.2)

Time(hr)	Conc. (mcg/ml)	Amount Release(mg)	Total Amount Release (mg)	Total % Release
[a]0.5	14.84	13.36	13.36	22.1
1	20.57	18.51	18.51	30.6
2	25.79	23.21	23.21	38.4
[b]3	6.32	5.69	28.90	47.8
4	10.28	9.25	32.46	53.7
6	14.25	12.83	36.04	59.7
10	21.07	18.96	42.17	69.8
12	24.34	21.91	45.12	74.7
24	27.89	25.10	48.31	80.0

a. Dosage forms were tested in simulated gastric fluid in the first two hours.
b. Dosage forms were tested in simulated intestinal fluid after two hours.

Table A.2c Percentage Release of Phenylpropanolamine from Product A1 (No.3)

Time(hr)	Conc. (mcg/ml)	Amount Release(mg)	Total Amount Release (mg)	Total % Release
[a]0.5	14.25	12.82	12.82	21.2
1	19.37	17.43	17.43	28.9
2	26.80	24.14	24.14	40.0
[b]3	6.98	6.28	30.42	50.1
4	11.07	9.96	37.10	61.4
6	17.39	15.65	39.79	65.9
10	22.66	18.59	42.73	70.7
12	23.74	21.37	45.51	75.3
24	27.96	25.20	49.30	81.6

a. Dosage forms were tested in simulated gastric fluid in the first two hours.
b. Dosage forms were tested in simulated intestinal fluid after two hours.

Table A.2d Percentage Release of Phenylpropanolamine from Product A1 (No.4)

Time(hr)	Conc. (mcg/ml)	Amount Release(mg)	Total Amount Release (mg)	Total % Release
[a]0.5	12.80	11.52	11.52	19.1
1	18.58	16.37	16.37	27.7
2	25.38	22.84	22.84	37.8
[b]3	7.86	7.08	29.92	49.5
4	9.65	8.69	31.53	52.2
6	18.02	16.22	39.00	64.7
10	21.48	19.33	42.17	69.8
12	23.11	20.80	43.64	72.3
24	26.48	23.83	46.67	77.3

a. Dosage forms were tested in simulated gastric fluid in the first two hours.
b. Dosage forms were tested in simulated intestinal fluid after two hours.

Table A.2e Percentage Release of Phenylpropanolamine from Product A1 (No.5)

Time(hr)	Conc. (mcg/ml)	Amount Release(mg)	Total Amount Release (mg)	Total % Release
[a]0.5	13.96	12.57	12.57	20.8
1	19.65	17.69	17.69	29.3
2	27.89	25.10	25.10	41.6
[b]3	6.82	6.14	31.24	51.7
4	9.84	8.86	33.96	56.2
6	18.52	16.67	41.78	69.2
10	20.97	18.88	43.98	72.8
12	23.90	21.51	46.61	77.2
24	29.31	26.38	51.48	85.2

a. Dosage forms were tested in simulated gastric fluid in the first two hours.
b. Dosage forms were tested in simulated intestinal fluid after two hours.

Table A.2f Percentage Release of Phenylpropanolamine from Product A1 (No.6)

Time(hr)	Conc. (mcg/ml)	Amount Release(mg)	Total Amount Release (mg)	Total % Release
[a]0.5	13.55	12.20	12.20	19.9
1	19.62	17.66	17.66	29.2
2	27.86	25.08	25.08	41.5
[b]3	6.01	5.41	30.49	50.5
4	10.50	9.45	34.53	57.2
6	18.36	16.53	41.61	68.9
10	21.45	19.30	44.38	73.5
12	22.30	20.97	46.05	76.2
24	28.05	25.25	50.33	83.3

a. Dosage forms were tested in simulated gastric fluid in the first two hours.
b. Dosage forms were tested in simulated intestinal fluid after two hours.

Table A.3a Percentage Release of Chlorpheniramine from Product A2 (No.1)

Time(hr)	Conc. (mcg/ml)	Amount Release(mg)	Total Amount Release (mg)	Total % Release
[a]0.5	0.96	0.86	0.86	15.4
1	1.28	1.15	1.15	20.5
2	2.50	2.25	2.25	40.2
[b]3	1.20	1.08	3.33	59.5
4	1.61	1.45	3.70	66.1
6	2.24	2.01	4.26	76.1
8	2.70	2.34	4.68	83.6
10	2.69	2.42	4.67	83.4
12	3.28	2.95	5.20	92.9
24	3.90	3.51	5.76	102.0

a. Dosage forms were tested in simulated gastric fluid in the first two hours.
b. Dosage forms were tested in simulated intestinal fluid after two hours.

Table A.3b Percentage Release of Chlorpheniramine from Product A2 (No.2)

Time(hr)	Conc. (mcg/ml)	Amount Release(mg)	Total Amount Release (mg)	Total % Release
[a]0.5	1.19	1.07	1.07	19.1
1	1.96	1.76	1.76	31.4
2	2.31	2.08	2.08	37.1
[b]3	1.12	1.01	3.09	55.2
4	1.46	1.31	3.39	60.5
6	1.60	1.44	3.52	62.9
8	1.99	1.79	3.87	69.1
10	2.22	2.00	4.08	72.9
12	2.33	2.09	4.17	74.5
24	3.03	2.73	4.81	85.9

a. Dosage forms were tested in simulated gastric fluid in the first two hours.
b. Dosage forms were tested in simulated intestinal fluid after two hours.

Table A.3c Percentage Release of Chlorpheniramine from Product A2 (No.3)

Time(hr)	Conc. (mcg/ml)	Amount Release(mg)	Total Amount Release (mg)	Total % Release
[a]0.5	1.22	1.10	1.10	19.6
1	1.65	1.49	1.49	26.5
2	2.64	2.38	2.38	42.4
[b]3	1.37	1.23	3.61	64.5
4	1.94	1.75	4.12	73.6
6	2.34	2.11	4.49	80.2
8	2.47	2.22	4.60	82.1
10	2.80	2.52	4.90	87.4
12	3.03	2.73	5.11	91.3
24	3.46	3.11	5.49	98.0

a. Dosage forms were tested in simulated gastric fluid in the first two hours.
b. Dosage forms were tested in simulated intestinal fluid after two hours.

Table A.3d Percentage Release of Chlorpheniramine from Product A2 (No.4)

Time(hr)	Conc. (mcg/ml)	Amount Release(mg)	Total Amount Release (mg)	Total % Release
[a]0.5	1.28	1.15	1.15	20.6
1	1.63	1.47	1.47	26.2
2	2.64	2.38	2.38	42.4
[b]3	1.21	1.09	3.47	61.9
4	1.52	1.37	3.74	66.9
6	2.23	2.01	4.39	78.4
8	2.53	2.28	4.66	83.2
10	2.42	2.17	4.55	81.3
12	2.52	2.27	4.65	83.0
24	3.07	2.76	5.14	91.8

a. Dosage forms were tested in simulated gastric fluid in the first two hours.
b. Dosage forms were tested in simulated intestinal fluid after two hours.

Table A.3e Percentage Release of Chlorpheniramine from Product A2 (No.5)

Time(hr)	Conc. (mcg/ml)	Amount Release(mg)	Total Amount Release (mg)	Total % Release
[a]0.5	1.46	1.31	1316	23.5
1	2.15	1.94	1.94	34.6
2	2.60	2.34	2.34	41.8
[b]3	1.06	0.95	3.29	58.8
4	1.28	1.15	3.49	62.3
6	1.62	1.46	3.80	67.9
8	1.87	1.68	4.02	71.8
10	2.02	1.82	4.16	74.3
12	2.38	2.14	4.48	80.0
24	2.84	2.55	4.89	87.3

a. Dosage forms were tested in simulated gastric fluid in the first two hours.
b. Dosage forms were tested in simulated intestinal fluid after two hours.

Table A.3f Percentage Release of Chlorpheniramine from Product A2 (No.6)

Time(hr)	Conc. (mcg/ml)	Amount Release(mg)	Total Amount Release (mg)	Total % Release
[a]0.5	0.78	0.70	0.70	12.5
1	0.98	0.88	0.88	15.8
2	1.62	1.46	1.46	26.0
[b]3	1.54	1.39	2.85	50.7
4	1.87	1.68	3.14	56.1
6	2.43	2.19	3.65	65.2
8	2.96	2.66	4.12	73.6
10	3.06	2.75	4.21	75.2
12	3.22	2.90	4.36	77.9
24	3.80	3.42	4.88	87.1

a. Dosage forms were tested in simulated gastric fluid in the first two hours.
b. Dosage forms were tested in simulated intestinal fluid after two hours.

Table A.4a Percentage Release of Phenylpropanolamine from Product A2 (No.1)

Time(hr)	Conc. (mcg/ml)	Amount Release(mg)	Total Amount Release (mg)	Total % Release
[a]0.5	3.38	3.04	3.04	5.0
1	7.90	7.11	7.11	11.8
2	12.68	11.41	11.41	18.9
[b]3	8.89	8.00	19.41	32.1
4	15.19	13.67	25.08	41.5
6	20.38	18.34	29.75	49.3
8	26.65	23.98	35.39	58.6
10	25.48	22.93	34.34	56.9
12	33.35	30.02	41.43	68.6
24	47.35	42.61	54.02	89.4

a. Dosage forms were tested in simulated gastric fluid in the first two hours.
b. Dosage forms were tested in simulated intestinal fluid after two hours.

Table A.4b Percentage Release of Phenylpropanolamine from Product A2 (No.2)

Time(hr)	Conc. (mcg/ml)	Amount Release(mg)	Total Amount Release (mg)	Total % Release
[a]0.5	4.49	4.04	4.04	6.7
1	9.65	8.69	8.69	14.4
2	14.43	12.99	12.99	21.5
[b]3	6.76	6.08	19.07	31.6
4	9.71	8.74	21.73	36.0
6	15.54	13.99	26.98	44.7
8	19.07	17.16	30.15	49.9
10	21.40	19.26	32.25	53.4
12	23.15	20.83	33.82	56.0
24	31.90	28.71	41.70	69.0

a. Dosage forms were tested in simulated gastric fluid in the first two hours.
b. Dosage forms were tested in simulated intestinal fluid after two hours.

Table A.4c Percentage Release of Phenylpropanolamine from Product A2 (No.3)

Time(hr)	Conc. (mcg/ml)	Amount Release(mg)	Total Amount Release (mg)	Total % Release
[a]0.5	4.75	4.28	4.28	7.1
1	8.37	7.53	7.53	12.5
2	13.97	12.57	12.57	20.8
[b]3	11.20	10.08	22.65	37.5
4	15.92	14.33	26.90	44.5
6	23.09	20.78	33.35	55.2
8	23.34	21.91	34.48	57.1
10	28.98	26.08	38.65	64.0
12	30.44	27.39	39.96	66.2
24	34.52	31.07	43.64	72.3

a. Dosage forms were tested in simulated gastric fluid in the first two hours.
b. Dosage forms were tested in simulated intestinal fluid after two hours.

Table A.4d Percentage Release of Phenylpropanolamine from Product A2 (No.4)

Time(hr)	Conc. (mcg/ml)	Amount Release(mg)	Total Amount Release (mg)	Total % Release
[a]0.5	6.50	5.85	5.85	9.7
1	9.80	8.82	8.82	14.6
2	13.97	12.57	12.57	20.8
[b]3	7.90	7.11	19.68	32.6
4	11.72	10.55	23.12	38.3
6	18.43	16.58	29.15	48.3
8	23.06	20.75	33.32	55.2
10	23.15	20.83	33.40	55.3
12	24.31	21.88	34.45	57.0
24	31.02	27.92	40.49	67.0

a. Dosage forms were tested in simulated gastric fluid in the first two hours.
b. Dosage forms were tested in simulated intestinal fluid after two hours.

Table A.4e Percentage Release of Phenylpropanolamine from Product A2 (No.5)

Time(hr)	Conc. (mcg/ml)	Amount Release(mg)	Total Amount Release (mg)	Total % Release
[a]0.5	7.30	6.57	6.57	10.88
1	11.25	10.13	10.13	11.8
2	14.66	13.19	13.19	21.8
[b]3	6.88	6.19	19.38	32.1
4	10.32	9.29	22.48	37.2
6	13.09	11.78	24.97	41.3
8	18.37	16.53	29.72	49.2
10	20.52	18.47	31.66	52.4
12	23.00	20.70	33.89	56.1
24	29.85	26.87	40.06	66.3

a. Dosage forms were tested in simulated gastric fluid in the first two hours.
b. Dosage forms were tested in simulated intestinal fluid after two hours.

Table A.4f Percentage Release of Phenylpropanolamine from Product A2 (No.6)

Time(hr)	Conc. (mcg/ml)	Amount Release(mg)	Total Amount Release (mg)	Total % Release
[a]0.5	2.10	1.89	1.89	3.1
1	4.84	4.36	4.36	7.2
2	7.70	6.93	6.93	11.5
[b]3	10.03	9.03	15.96	26.4
4	14.81	13.33	20.26	33.35
6	19.74	17.76	24.69	40.9
8	26.01	23.41	30.34	50.2
10	27.81	25.03	31.96	52.9
12	30.44	27.39	34.32	56.8
24	36.27	32.64	39.57	65.6

a. Dosage forms were tested in simulated gastric fluid in the first two hours.
b. Dosage forms were tested in simulated intestinal fluid after two hours.

Table A.5a Percentage Release of Chlorpheniramine from Product B1 (No.1)

Time(hr)	Conc. (mcg/ml)	Amount Release(mg)	Total Amount Release (mg)	Total % Release
[a]0.5	1.09	0.98	0.98	11.7
1	2.2	1.98	1.98	23.6
2	3.08	2.78	2.78	33.0
[b]3	0.11	0.098	2.87	34.2
4	0.1	0.093	2.87	34.2
6	0.19	0.18	2.95	35.1
8	0.32	0.28	3.06	36.4
10	0.41	0.37	3.14	37.4
12	0.50	0.45	3.23	38.4
24	0.87	0.78	3.56	42.4

a. Dosage forms were tested in simulated gastric fluid in the first two hours.
b. Dosage forms were tested in simulated intestinal fluid after two hours.

Table A.5b Percentage Release of Chlorpheniramine from Product B1 (No.2)

Time(hr)	Conc. (mcg/ml)	Amount Release(mg)	Total Amount Release (mg)	Total % Release
[a]0.5	1.4	1.26	1.26	15.0
1	2.21	2.0	2.0	23.6
2	3.21	2.89	2.89	34.4
[b]3	0.12	0.11	3.0	35.7
4	0.16	0.15	3.04	36.2
6	0.09	0.08	2.97	35.4
8	0.32	0.28	3.18	37.8
10	0.43	0.39	3.28	39.1
12	0.45	0.41	3.30	39.3
24	0.75	0.67	3.57	42.4

a. Dosage forms were tested in simulated gastric fluid in the first two hours.
b. Dosage forms were tested in simulated intestinal fluid after two hours.

Table A.5c Percentage Release of Chlorpheniramine from Product B1 (No.3)

Time(hr)	Conc. (mcg/ml)	Amount Release(mg)	Total Amount Release (mg)	Total % Release
[a]0.5	1.08	0.98	0.98	11.6
1	2.17	1.96	1.96	23.3
2	3.26	2.93	2.93	34.9
[b]3	0.17	0.15	3.08	36.7
4	0.17	0.15	3.08	36.7
6	0.27	0.24	3.18	37.8
8	0.22	0.20	3.13	37.3
10	0.40	0.36	3.29	39.2
12	0.51	0.46	3.39	40.4
24	0.85	0.77	3.70	44.0

a. Dosage forms were tested in simulated gastric fluid in the first two hours.
b. Dosage forms were tested in simulated intestinal fluid after two hours.

Table A.5d Percentage Release of Chlorpheniramine from Product B1 (No.4)

Time(hr)	Conc. (mcg/ml)	Amount Release(mg)	Total Amount Release (mg)	Total % Release
[a]0.5	1.2	1.08	1.08	12.9
1	2.34	2.1	2.1	25
2	3.21	2.89	2.89	34.4
[b]3	0.17	0.15	3.04	36.2
4	0.17	0.16	3.05	36.3
6	0.30	0.27	3.17	37.7
8	0.28	0.25	3.14	37.4
10	0.36	0.32	3.21	38.2
12	0.61	0.55	3.44	41.0
24	0.92	0.83	3.72	44.3

a. Dosage forms were tested in simulated gastric fluid in the first two hours.
b. Dosage forms were tested in simulated intestinal fluid after two hours.

Table A.5e Percentage Release of Chlorpheniramine from Product B1 (No.5)

Time(hr)	Conc. (mcg/ml)	Amount Release(mg)	Total Amount Release (mg)	Total % Release
[a]0.5	1.07	0.96	0.96	11.5
1	2.05	1.85	1.85	22.0
2	3.21	2.89	2.89	34.4
[b]3	0.17	0.15	3.04	36.2
4	0.17	0.16	3.05	36.3
6	0.27	0.24	3.13	37.3
8	0.33	0.30	3.19	38.0
10	0.45	0.41	3.30	39.3
12	0.56	0.51	3.40	40.4
24	1.03	0.93	3.8	45.5

a. Dosage forms were tested in simulated gastric fluid in the first two hours.
b. Dosage forms were tested in simulated intestinal fluid after two hours.

Table A.5f Percentage Release of Chlorpheniramine from Product B1 (No.6)

Time(hr)	Conc. (mcg/ml)	Amount Release(mg)	Total Amount Release (mg)	Total % Release
[a]0.5	1.52	1.37	1.37	16.3
1	2.2	1.98	1.98	23.6
2	3.15	2.83	2.83	33.7
[b]3	0.09	0.081	2.91	34.7
4	0.1	0.093	2.93	34.8
6	0.19	0.17	3.0	35.7
8	0.30	0.27	3.10	36.9
10	0.47	0.42	3.25	38.7
12	0.56	0.50	3.33	39.7
24	1.1	0.99	3.83	45.6

a. Dosage forms were tested in simulated gastric fluid in the first two hours.
b. Dosage forms were tested in simulated intestinal fluid after two hours.

Table A.6a Percentage Release of Phenylpropanolamine from Product B1 (No.1)

Time(hr)	Conc. (mcg/ml)	Amount Release(mg)	Total Amount Release (mg)	Total % Release
[a]0.5	11.0	9.9	9.9	16.4
1	17.13	15.41	15.41	25.5
2	21.5	19.35	19.35	32.0
[b]3	5.09	4.58	23.94	39.6
4	6.03	5.43	24.78	41.0
6	12.81	11.53	30.88	51.1
8	15.91	14.31	33.67	55.7
10	20.34	18.31	37.66	62.3
12	20.91	18.82	38.17	63.2
24	27.19	24.47	43.82	72.5

a. Dosage forms were tested in simulated gastric fluid in the first two hours.
b. Dosage forms were tested in simulated intestinal fluid after two hours.

Table A.6b Percentage Release of Phenylpropanolamine from Product B1 (No.2)

Time(hr)	Conc. (mcg/ml)	Amount Release(mg)	Total Amount Release (mg)	Total % Release
[a]0.5	12.88	11.59	11.59	19.2
1	18.84	16.96	16.96	28.1
2	23.72	21.35	21.35	35.3
[b]3	4.91	4.42	25.76	42.7
4	7.81	7.03	28.38	47.0
6	12.34	11.11	32.46	53.7
8	18.56	16.71	38.05	63.0
10	20.97	48.82	40.16	66.5
12	23.72	21.35	42.69	70.7
24	27.69	24.92	46.27	76.6

a. Dosage forms were tested in simulated gastric fluid in the first two hours.
b. Dosage forms were tested in simulated intestinal fluid after two hours.

Table A.6c Percentage Release of Phenylpropanolamine from Product B1 (No.3)

Time(hr)	Conc. (mcg/ml)	Amount Release(mg)	Total Amount Release (mg)	Total % Release
[a]0.5	12.56	11.31	11.31	18.7
1	16.47	14.82	14.82	24.5
2	24.81	22.33	22.33	37.0
[b]3	5.13	4.61	26.94	44.6
4	8.22	7.40	29.73	49.2
6	14.41	12.97	35.30	58.4
8	17.72	15.95	38.28	63.4
10	20.60	18.53	40.87	67.7
12	22.78	20.50	42.83	70.9
24	28.38	25.54	47.87	79.3

a. Dosage forms were tested in simulated gastric fluid in the first two hours.
b. Dosage forms were tested in simulated intestinal fluid after two hours.

Table A.6d Percentage Release of Phenylpropanolamine from Product B1 (No.4)

Time(hr)	Conc. (mcg/ml)	Amount Release(mg)	Total Amount Release (mg)	Total % Release
[a]0.5	13.03	11.73	11.73	19.4
1	17.94	16.14	16.14	26.7
2	23.53	21.18	21.18	35.1
[b]3	5.31	4.78	25.96	43.0
4	7.75	6.98	28.15	46.6
6	13.09	11.78	32.96	54.6
8	16.44	14.79	35.97	59.6
10	21.75	19.58	40.75	67.5
12	24.13	21.71	42.89	71.0
24	28.16	25.34	46.52	77.0

a. Dosage forms were tested in simulated gastric fluid in the first two hours.
b. Dosage forms were tested in simulated intestinal fluid after two hours.

Table A.6e Percentage Release of Phenylpropanolamine from Product B1 (No.5)

Time(hr)	Conc. (mcg/ml)	Amount Release(mg)	Total Amount Release (mg)	Total % Release
[a]0.5	12.81	11.53	11.53	19.1
1	18.16	16.34	16.34	27.1
2	24.16	21.74	21.74	36.0
[b]3	5.19	4.67	26.41	43.7
4	8.09	7.28	29.03	48.1
6	14.03	12.63	34.37	56.9
8	17.81	16.03	37.77	62.5
10	21.88	19.69	41.43	68.6
12	22.63	20.36	42.17	69.7
24	29.53	26.58	48.32	80.0

a. Dosage forms were tested in simulated gastric fluid in the first two hours.
b. Dosage forms were tested in simulated intestinal fluid after two hours.

Table A.6f Percentage Release of Phenylpropanolamine from Product B1 (No.6)

Time(hr)	Conc. (mcg/ml)	Amount Release(mg)	Total Amount Release (mg)	Total % Release
[a]0.5	12.58	11.14	11.14	18.4
1	17.28	15.55	15.55	25.8
2	23.84	21.46	21.46	35.5
[b]3	5.16	4.64	26.1	43.2
4	8.66	7.79	29.25	48.4
6	13.82	12.43	33.89	56.1
8	18.13	16.31	37.77	62.5
10	21.22	19.11	40.56	67.2
12	23.97	21.57	43.03	71.2
24	36.13	32.5	53.97	89.4

a. Dosage forms were tested in simulated gastric fluid in the first two hours.
b. Dosage forms were tested in simulated intestinal fluid after two hours.

Table A.7a Percentage Release of Chlorpheniramine from Product B2 (No.1)

Time(hr)	Conc.(mcg/ml)	Amount Release(mg)	% Release
0.5	1.25	1.12	79.7
1	1.45	1.31	92.9
2	1.50	1.35	95.8
3	1.39	1.25	89.2
4	1.55	1.39	99.1
6	1.52	1.37	97.5

Table A.7b Percentage Release of Chlorpheniramine from Product B2 (No.2)

Time(hr)	Conc.(mcg/ml)	Amount Release(mg)	% Release
0.5	1.45	1.31	93.0
1	1.36	1.22	87.1
2	1.51	1.36	99.6
3	1.51	1.36	99.6
4	1.50	1.35	96.2
6	1.54	1.39	98.7

Table A.7c Percentage Release of Chlorpheniramine from Product B2 (No.3)

Time(hr)	Conc.(mcg/ml)	Amount Release(mg)	% Release
0.5	1.39	1.25	89.2
1	1.37	1.24	88.0
2	1.41	1.27	90.4
3	1.54	1.39	98.7
4	1.55	1.39	99.1
6	1.66	1.49	106.1

Table A.7d Percentage Release of Chlorpheniramine from Product B2 (No.4)

Time(hr)	Conc.(mcg/ml)	Amount Release(mg)	% Release
0.5	1.40	1.26	89.6
1	1.45	1.31	92.9
2	1.52	1.37	97.5
3	1.41	1.27	90.4
4	1.44	1.30	92.5
6	1.60	1.44	102.4

Table A.7e Percentage Release of Chlorpheniramine from Product B2 (No.5)

Time(hr)	Conc.(mcg/ml)	Amount Release(mg)	% Release
0.5	1.33	1.20	85.1
1	1.41	1.27	90.4
2	1.31	1.18	83.8
3	1.54	1.38	98.3
4	1.44	1.30	92.5
6	1.50	1.35	96.2

Table A.7f Percentage Release of Chlorpheniramine from Product B2 (No.6)

Time(hr)	Conc.(mcg/ml)	Amount Release(mg)	% Release
0.5	1.31	1.18	83.8
1	1.39	1.25	89.2
2	1.54	1.39	98.7
3	1.54	1.39	98.7
4	1.55	1.40	99.6
6	1.42	1.28	91.3

Table A.8a Percentage Release of Phenylpropanolamine from Product B2 (No.1)

Time(hr)	Conc.(mcg/ml)	Amount Release(mg)	% Release
0.5	12.28	11.05	73.4
1	15.09	13.58	90.2
2	14.75	13.28	88.2
3	14.59	13.13	87.2
4	14.41	12.97	86.2
6	14.75	13.28	88.2

Table A.8b Percentage Release of Phenylpropanolamine from Product B2 (No.2)

Time(hr)	Conc.(mcg/ml)	Amount Release(mg)	% Release
0.5	12.53	11.28	74.89
1	13.22	11.9	79.0
2	15.28	13.75	91.3
3	14.75	13.28	88.2
4	14.91	13.42	89.1
6	14.62	13.16	87.4

Table A.8c Percentage Release of Phenylpropanolamine from Product B2 (No.3)

Time(hr)	Conc.(mcg/ml)	Amount Release(mg)	% Release
0.5	12.53	11.28	74.9
1	12.47	11.22	74.5
2	14.56	13.11	87.0
3	14.56	13.11	87.0
4	14.06	12.66	84.0
6	14.75	13.28	88.2

Table A.8d Percentage Release of Phenylpropanolamine from Product B2 (No.4)

Time(hr)	Conc.(mcg/ml)	Amount Release(mg)	% Release
0.5	13.88	12.49	82.92
1	14.41	12.97	86.09
2	13.72	12.35	81.98
3	14.06	12.66	84.04
4	14.72	13.25	87.96
6	14.47	13.02	86.47

Table A.8e Percentage Release of Phenylpropanolamine from Product B2 (No.5)

Time(hr)	Conc.(mcg/ml)	Amount Release(mg)	% Release
0.5	13.59	12.23	81.2
1	14.25	12.83	85.2
2	13.44	12.09	80.3
3	15.59	14.03	93.2
4	14.72	13.24	88.0
6	16.13	14.51	96.4

Table A.8f Percentage Release of Phenylpropanolamine from Product B2 (No.6)

Time(hr)	Conc.(mcg/ml)	Amount Release(mg)	% Release
0.5	13.75	12.38	82.2
1	15.09	13.58	90.2
2	15.9	14.32	95.1
3	14.56	13.11	87.0
4	15.94	14.34	95.2
6	15.97	14.37	95.4

Table A.9a Percentage Release of Chlorpheniramine from Product C1 (No.1)

Time(hr)	Conc. (mcg/ml)	Amount Release(mg)	Total Amount Release (mg)	Total % Release
[a]1	2.37	2.13	2.13	25.4
2	3.84	3.45	3.45	41.1
[b]3	2.42	2.18	5.63	67.0
4	3.32	2.99	6.44	76.7
6	3.91	3.52	6.97	83.0
8	3.87	3.48	6.93	82.5
10	4.55	4.10	7.55	89.9
12	4.72	4.25	7.70	91.6
24	5.20	4.68	8.13	96.8

a. Dosage forms were tested in simulated gastric fluid in the first two hours.
b. Dosage forms were tested in simulated intestinal fluid after two hours.

Table A.9b Percentage Release of Chlorpheniramine from Product C1 (No.2)

Time(hr)	Conc. (mcg/ml)	Amount Release(mg)	Total Amount Release (mg)	Total % Release
[a]1	3.52	3.16	3.16	37.7
2	5.68	5.11	5.11	60.8
[b]3	1.72	1.55	6.66	79.3
4	2.59	2.33	7.44	88.5
6	2.95	2.65	7.76	92.4
8	3.26	2.93	8.04	95.7
10	3.05	2.75	7.85	93.4
12	3.40	3.06	8.17	97.2
24	3.91	3.52	8.63	102.8

a. Dosage forms were tested in simulated gastric fluid in the first two hours.
b. Dosage forms were tested in simulated intestinal fluid after two hours.

Table A.9c Percentage Release of Chlorpheniramine from Product C1 (No.3)

Time(hr)	Conc. (mcg/ml)	Amount Release(mg)	Total Amount Release (mg)	Total % Release
[a]1	3.30	2.97	2.97	35.3
2	5.23	4.71	4.71	56.0
[b]3	1.35	1.21	5.92	70.5
4	1.89	1.70	6.41	76.3
6	2.53	2.28	6.99	83.2
8	2.91	2.62	7.33	87.3
10	3.16	2.84	7.55	89.9
12	3.18	2.87	7.58	90.2
24	3.51	3.16	7.87	93.6

a. Dosage forms were tested in simulated gastric fluid in the first two hours.
b. Dosage forms were tested in simulated intestinal fluid after two hours.

Table A.9d Percentage Release of Chlorpheniramine from Product C1 (No.4)

Time(hr)	Conc. (mcg/ml)	Amount Release(mg)	Total Amount Release (mg)	Total % Release
[a]1	3.08	2.78	2.78	33.1
2	5.09	4.58	4.58	54.6
[b]3	1.58	1.42	6.0	71.4
4	2.16	1.94	6.52	77.6
6	2.89	2.58	7.16	85.3
8	3.18	2.87	7.45	88.6
10	3.36	3.02	7.6	90.5
12	3.51	3.16	7.74	92.1
24	3.59	3.23	7.81	92.9

a. Dosage forms were tested in simulated gastric fluid in the first two hours.
b. Dosage forms were tested in simulated intestinal fluid after two hours.

Table A.9e Percentage Release of Chlorpheniramine from Product C1 (No.5)

Time(hr)	Conc. (mcg/ml)	Amount Release(mg)	Total Amount Release (mg)	Total % Release
[a]1	2.22	2.0	2.0	23.8
2	3.51	3.16	3.16	37.6
[b]3	2.41	2.17	5.33	63.4
4	3.12	2.81	5.97	71.1
6	3.78	3.40	6.56	78.1
8	4.38	3.94	7.10	84.5
10	4.40	3.96	7.12	84.7
12	4.54	4.09	7.25	86.3
24	4.79	4.31	7.47	88.9

a. Dosage forms were tested in simulated gastric fluid in the first two hours.
b. Dosage forms were tested in simulated intestinal fluid after two hours.

Table A.9f Percentage Release of Chlorpheniramine from Product C1 (No.6)

Time(hr)	Conc. (mcg/ml)	Amount Release(mg)	Total Amount Release (mg)	Total % Release
[a]1	2.78	2.50	2.50	29.8
2	5.52	4.97	4.97	59.1
[b]3	1.22	1.1	6.07	72.2
4	1.83	1.65	6.62	78.8
6	2.28	2.05	7.02	83.6
8	2.73	2.46	7.43	88.4
10	2.95	2.66	7.63	90.8
12	3.09	2.78	7.75	92.3
24	3.39	3.05	8.02	95.5

a. Dosage forms were tested in simulated gastric fluid in the first two hours.
b. Dosage forms were tested in simulated intestinal fluid after two hours.

Table A.10a Percentage Release of Chlorpheniramine from Product C2 (No.1)

Time(hr)	Conc.(mcg/ml)	Amount Release(mg)	% Release
0.5	2.99	2.69	96.2
1	2.88	2.59	92.4
2	2.89	2.60	92.8
4	2.93	2.64	94.1
6	2.93	2.64	94.1

Table A.10b Percentage Release of Chlorpheniramine from Product C2 (No.2)

Time(hr)	Conc.(mcg/ml)	Amount Release(mg)	% Release
0.5	2.86	2.58	92.1
1	2.99	2.69	96.2
2	2.99	2.69	96.2
4	2.93	2.64	94.1
6	3.13	2.81	100.5

Table A.10c Percentage Release of Chlorpheniramine from Product C2 (No.3)

Time(hr)	Conc.(mcg/ml)	Amount Release(mg)	% Release
0.5	3.03	2.72	97.3
1	2.96	2.66	95.2
2	3.06	2.75	98.3
4	3.06	2.75	98.3
6	3.13	2.81	100.5

Table A.10d Percentage Release of Chlorpheniramine from Product C2 (No.4)

Time(hr)	Conc.(mcg/ml)	Amount Release(mg)	% Release
0.5	2.93	2.64	94.1
1	2.96	2.66	95.2
2	2.93	2.64	94.1
4	2.93	2.64	94.1
6	3.13	2.81	100.5

Table A.10e Percentage Release of Chlorpheniramine from Product C2 (No.5)

Time(hr)	Conc.(mcg/ml)	Amount Release(mg)	% Release
0.5	2.82	2.54	90.7
1	2.86	2.58	92.1
2	2.99	2.69	96.2
4	2.99	2.69	96.2
6	2.99	2.69	96.2

Table A.10f Percentage Release of Chlorpheniramine from Product C2 (No.6)

Time(hr)	Conc.(mcg/ml)	Amount Release(mg)	% Release
0.5	2.93	2.64	94.1
1	2.99	2.69	96.2
2	2.99	2.69	96.2
4	3.06	2.75	98.3
6	2.99	2.69	96.2

Table A.11a Percentage Release of Pseudoephedrine from Product D (No.1)

Time(hr)	Conc. (mcg/ml)	Amount Release(mg)	Total Amount Release (mg)	Total % Release
[a]0.5	45.02	40.52	40.52	53.4
1	44.54	40.09	40.09	52.9
2	45.41	40.87	40.87	53.9
[b]3	2.18	1.96	42.84	56.4
4	4.76	4.28	45.15	59.5
5	22.44	20.2	61.07	80.5
6	32.01	28.83	69.68	91.8
8	45.85	41.39	82.14	108.2
10	46.55	41.96	82.76	109.0
12	46.56	41.98	82.76	109.0

a. Dosage forms were tested in simulated gastric fluid in the first two hours.
b. Dosage forms were tested in simulated intestinal fluid after two hours.

Table A.11b Percentage Release of Pseudoephedrine from Product D (No.2)

Time(hr)	Conc. (mcg/ml)	Amount Release(mg)	Total Amount Release (mg)	Total % Release
[a]0.5	48.03	43.23	43.23	57.0
1	48.38	43.54	43.54	57.4
2	48.86	43.98	43.98	57.9
[b]3	2.18	1.96	45.94	60.5
4	3.76	3.38	47.36	62.4
5	9.17	8.25	52.23	68.8
6	10.87	9.79	53.77	70.8
8	20.66	18.59	62.57	82.4
10	26.81	24.13	68.11	89.7
12	33.80	30.42	74.4	98.0

a. Dosage forms were tested in simulated gastric fluid in the first two hours.
b. Dosage forms were tested in simulated intestinal fluid after two hours.

Table A.11c Percentage Release of Pseudoephedrine from Product D (No.3)

Time(hr)	Conc. (mcg/ml)	Amount Release(mg)	Total Amount Release (mg)	Total % Release
[a]0.5	45.02	40.52	40.52	53.4
1	45.02	40.52	40.52	53.4
2	47.20	42.48	42.48	56.0
[b]3	3.67	3.30	45.78	60.3
4	6.86	6.17	48.65	64.1
5	11.66	10.49	52.97	69.8
6	15.28	13.76	56.24	74.1
8	23.97	21.58	64.06	84.4
10	30.39	27.35	69.83	92.0
12	40.0	36.0	78.48	103.4

a. Dosage forms were tested in simulated gastric fluid in the first two hours.
b. Dosage forms were tested in simulated intestinal fluid after two hours.

Table A.11d Percentage Release of Pseudoephedrine from Product D (No.4)

Time(hr)	Conc. (mcg/ml)	Amount Release(mg)	Total Amount Release (mg)	Total % Release
[a]0.5	42.49	38.24	38.24	50.4
1	43.32	38.99	38.99	51.4
2	43.46	39.03	39.03	51.4
[b]3	1.67	1.49	40.52	53.4
4	4.37	3.93	42.96	56.6
5	6.86	6.17	45.20	59.6
6	10.61	9.55	48.58	64.0
8	17.42	15.68	54.71	72.1
10	23.32	20.99	60.02	79.1
12	32.10	28.89	67.92	89.5

a. Dosage forms were tested in simulated gastric fluid in the first two hours.
b. Dosage forms were tested in simulated intestinal fluid after two hours.

Table A.11e Percentage Release of Pseudoephedrine from Product D (No.5)

Time(hr)	Conc. (mcg/ml)	Amount Release(mg)	Total Amount Release (mg)	Total % Release
[a]0.5	44.58	40.13	40.13	52.9
1	44.63	40.16	40.16	52.9
2	45.06	40.56	40.56	53.4
[b]3	1.79	1.61	42.17	55.6
4	5.24	4.72	45.28	59.6
5	9.17	8.25	48.81	64.3
6	13.01	11.71	52.27	68.9
8	20.26	18.24	58.80	77.5
10	25.63	23.07	63.63	83.8
12	32.62	29.36	69.92	92.1

a. Dosage forms were tested in simulated gastric fluid in the first two hours.
b. Dosage forms were tested in simulated intestinal fluid after two hours.

Table A.11f Percentage Release of Pseudoephedrine from Product D (No.6)

Time(hr)	Conc. (mcg/ml)	Amount Release(mg)	Total Amount Release (mg)	Total % Release
[a]0.5	46.81	42.13	42.13	55.5
1	47.60	42.84	42.84	56.4
2	50.26	45.24	45.24	59.6
[b]3	3.97	3.58	48.82	64.3
4	7.60	6.84	52.08	68.6
5	11.80	10.06	55.37	72.9
6	15.63	14.07	59.31	78.1
8	27.95	25.15	70.40	92.7
10	38.86	34.98	85.30	105.7
12	44.50	40.05	90.40	112.4

a. Dosage forms were tested in simulated gastric fluid in the first two hours.
b. Dosage forms were tested in simulated intestinal fluid after two hours.

Table A.12a Percentage Release of Acetaminophen from Product E1 (No.1)

Time(min)	[a]Conc. (mcg/ml)	[b]Conc. (mcg/ml)	Amount Release(mg)	% Release
5	0.61	306.8	276.1	55.2
15	0.8	400.1	360.1	72.0
30	0.88	438.6	394.7	78.9
45	0.9	449.6	404.6	80.9
60	0.92	460.4	414.4	82.9
120	1.18	592.2	533.0	106.6

a. Concentration after dilution. (dilution factor: 500)
b. Concentration before dilution.

Table A.12b Percentage Release of Acetaminophen from Product E1 (No.2)

Time(min)	[a]Conc. (mcg/ml)	[b]Conc. (mcg/ml)	Amount Release(mg)	% Release
5	0.48	240.9	216.8	43.3
15	0.69	345.2	310.7	62.1
30	0.78	389.1	350.2	70.0
45	0.80	400.1	360.1	72.0
60	0.98	487.9	439.1	87.8
120	1.0	498.9	449.0	89.8

a. Concentration after dilution. (dilution factor: 500)
b. Concentration before dilution.

Table A.12c Percentage Release of Acetaminophen from Product E1 (No.3)

Time(min)	[a]Conc. (mcg/ml)	[b]Conc. (mcg/ml)	Amount Release(mg)	% Release
5	0.62	312.2	281.0	56.2
15	0.68	339.8	305.8	61.2
30	0.76	378.1	341.3	68.1
45	0.81	405.6	365.0	73.0
60	0.89	444.0	399.6	79.9
120	0.99	493.4	444.1	88.8

a. Concentration after dilution. (dilution factor: 500)
b. Concentration before dilution.

Table A.12d Percentage Release of Acetaminophen from Product E1 (No.4)

Time(min)	[a]Conc. (mcg/ml)	[b]Conc. (mcg/ml)	Amount Release(mg)	% Release
5	0.59	295.8	266.2	53.2
15	0.79	394.7	355.2	71.0
30	0.83	416.6	374.9	75.0
45	0.86	427.6	384.8	77.0
60	0.95	477.0	429.3	85.9
120	1.03	515.3	463.8	92.8

a. Concentration after dilution. (dilution factor: 500)
b. Concentration before dilution.

Table A.12e Percentage Release of Acetaminophen from Product E1 (No.5)

Time(min)	[a]Conc. (mcg/ml)	[b]Conc. (mcg/ml)	Amount Release(mg)	% Release
5	0.48	240.9	216.8	43.3
15	0.81	405.6	365.0	73.0
30	0.88	438.6	394.7	78.9
45	0.91	455.0	409.5	81.9
60	0.91	455.0	409.5	81.9
120	0.95	477.0	429.3	85.9

a. Concentration after dilution. (dilution factor: 500)
b. Concentration before dilution.

Table A.12f Percentage Release of Acetaminophen from Product E1 (No.6)

Time(min)	[a]Conc. (mcg/ml)	[b]Conc. (mcg/ml)	Amount Release(mg)	% Release
5	0.44	229.0	197.1	39.4
15	0.75	372.7	335.4	67.1
30	0.92	460.4	414.4	82.7
45	0.91	455	409.5	81.9
60	0.96	482.4	434.2	86.8
120	0.99	493.4	444.1	88.8

a. Concentration after dilution. (dilution factor: 500)
b. Concentration before dilution.

Table A.13a Percentage Release of Chlorpheniramine from Product E1 (No.1)

Time (min)	Conc.(mcg/ml)	Amount Release(mg)	% Release
5	0.33	0.29	20.9
15	1.54	1.38	98.3
30	1.54	1.38	98.3
45	1.54	1.38	98.3

Table A.13b Percentage Release of Chlorpheniramine from Product E1 (No.2)

Time (min)	Conc.(mcg/ml)	Amount Release(mg)	% Release
5	0.45	0.40	28.7
15	1.47	1.32	94.0
30	1.54	1.38	98.3
45	1.54	1.38	98.3

Table A.13c Percentage Release of Chlorpheniramine from Product E1 (No.3)

Time (min)	Conc.(mcg/ml)	Amount Release(mg)	% Release
5	0.45	0.40	28.7
15	1.27	1.14	80.9
30	1.61	1.44	102.7
45	1.67	1.51	107.0

Table A.13d Percentage Release of Chlorpheniramine from Product E1 (No.4)

Time (min)	Conc.(mcg/ml)	Amount Release(mg)	% Release
5	0.59	0.65	46.2
15	1.33	1.20	85.3
30	1.61	1.44	102.7
45	1.67	1.51	107.0

Table A.13e Percentage Release of Chlorpheniramine from Product E1 (No.5)

Time (min)	Conc.(mcg/ml)	Amount Release(mg)	% Release
5	0.45	0.40	28.7
15	1.54	1.38	98.3
30	1.61	1.44	102.7
45	1.61	1.44	102.7

Table A.13f Percentage Release of Chlorpheniramine from Product E1 (No.6)

Time (min)	Conc.(mcg/ml)	Amount Release(mg)	% Release
5	0.65	0.59	41.8
15	1.61	1.44	102.7
30	1.61	1.44	102.7
45	1.61	1.44	102.7

Table A.14a Percentage Release of Acetaminophen from Product E2 (No.1)

Time(min)	[a]Conc. (mcg/ml)	[b]Conc. (mcg/ml)	Amount Release(mg)	% Release
5	0.22	109.2	98.3	19.7
15	0.70	350.7	315.6	63.1
30	1.02	509.9	458.9	91.8
45	1.02	509.9	458.9	91.8
60	1.09	542.8	488.5	97.7
120	1.09	542.8	488.5	97.7

a. Concentration after dilution. (dilution factor: 500)
b. Concentration before dilution.

Table A.14b Percentage Release of Acetaminophen from Product E2 (No.2)

Time(min)	[a]Conc. (mcg/ml)	[b]Conc. (mcg/ml)	Amount Release(mg)	% Release
5	0.15	76.3	68.7	13.7
15	0.78	389.1	350.2	70.0
30	1.02	509.9	458.9	91.8
45	1.03	515.3	463.8	92.8
60	1.06	531.8	478.6	95.7
120	1.06	531.8	478.6	95.7

a. Concentration after dilution. (dilution factor: 500)
b. Concentration before dilution.

Table A.14c Percentage Release of Acetaminophen from Product E2 (No.3)

Time(min)	[a]Conc. (mcg/ml)	[b]Conc. (mcg/ml)	Amount Release(mg)	% Release
5	0.15	76.3	68.7	13.7
15	0.70	350.7	315.6	63.1
30	1.02	509.9	458.9	91.8
45	1.02	509.9	458.9	91.8
60	1.02	509.9	458.9	91.8
120	1.06	531.8	478.6	95.7

a. Concentration after dilution. (dilution factor: 500)
b. Concentration before dilution.

Table A.14d Percentage Release of Acetaminophen from Product E2 (No.4)

Time(min)	[a]Conc. (mcg/ml)	[b]Conc. (mcg/ml)	Amount Release(mg)	% Release
5	0.21	103.8	93.4	18.7
15	0.89	444.0	399.6	79.9
30	0.95	477.0	429.3	85.9
45	1.03	515.3	463.8	92.8
60	1.06	531.8	478.6	95.7
120	1.06	531.8	478.6	95.7

a. Concentration after dilution. (dilution factor: 500)
b. Concentration before dilution.

Table A.14e Percentage Release of Acetaminophen from Product E2 (No.5)

Time(min)	[a]Conc. (mcg/ml)	[b]Conc. (mcg/ml)	Amount Release(mg)	% Release
5	0.20	98.2	88.4	17.7
15	0.78	389.1	350.2	70.0
30	1.0	504.4	454.0	90.8
45	1.01	537.3	483.6	96.7
60	1.10	559.2	503.3	100.7
120	1.10	559.2	503.3	100.7

a. Concentration after dilution. (dilution factor: 500)
b. Concentration before dilution.

Table A.14f Percentage Release of Acetaminophen from Product E2 (No.6)

Time(min)	[a]Conc. (mcg/ml)	[b]Conc. (mcg/ml)	Amount Release(mg)	% Release
5	0.19	92.8	83.5	16.7
15	0.79	394.7	355.2	71.0
30	1.03	515.3	463.8	92.8
45	1.04	520.9	468.8	93.8
60	1.09	524.8	488.5	97.7
120	1.10	553.8	498.4	99.7

a. Concentration after dilution. (dilution factor: 500)
b. Concentration before dilution.

Table A.15a Percentage Release of Chlorpheniramine from Product E2 (No.1)

Time (min)	Conc.(mcg/ml)	Amount Release(mg)	% Release
5	0.21	0.19	13.5
15	1.20	1.08	96.6
30	1.61	1.44	102.7
45	1.61	1.44	102.7
60	1.61	1.44	102.7

Table A.15b Percentage Release of Chlorpheniramine from Product E2 (No.2)

Time (min)	Conc.(mcg/ml)	Amount Release(mg)	% Release
5	0.15	0.13	9.6
15	1.27	1.14	80.9
30	1.54	1.38	98.3
45	1.61	1.44	102.7
60	1.61	1.44	102.7

Table A.15c Percentage Release of Chlorpheniramine from Product E2 (No.3)

Time (min)	Conc.(mcg/ml)	Amount Release(mg)	% Release
5	0.095	0.086	6.1
15	0.99	0.89	63.5
30	1.47	1.32	94.0
45	1.61	1.44	102.7
60	1.74	1.57	111.3

Table A.15d Percentage Release of Chlorpheniramine from Product E2 (No.4)

Time (min)	Conc.(mcg/ml)	Amount Release(mg)	% Release
5	0.088	0.08	5.7
15	1.40	1.26	89.6
30	1.54	1.38	98.3
45	1.61	1.44	102.7
60	1.61	1.44	102.7

Table A.15e Percentage Release of Chlorpheniramine from Product E2 (No.5)

Time (min)	Conc.(mcg/ml)	Amount Release(mg)	% Release
5	0.11	0.098	7.0
15	1.06	0.96	67.9
30	1.54	1.38	98.3
45	1.54	1.38	98.3
60	1.61	1.44	102.7

Table A.15f Percentage Release of Chlorpheniramine from Product E2 (No.6)

Time (min)	Conc.(mcg/ml)	Amount Release(mg)	% Release
5	0.014	0.012	1.7
15	1.27	1.14	80.9
30	1.54	1.38	98.3
45	1.54	1.38	98.3
60	1.67	1.51	107.0

Table A.16a Percentage Release of Acetaminophen from Product E3 (No.1)

Time(min)	[a]Conc. (mcg/ml)	[b]Conc. (mcg/ml)	Amount Release(mg)	% Release
5	0.84	422.1	379.9	76.0
15	0.84	422.1	379.9	76.0
30	0.99	493.4	444.1	88.8
45	1.0	498.9	449.0	89.8
60	1.03	515.3	463.8	92.8
120	1.12	559.2	503.3	100.7

a. Concentration after dilution. (dilution factor: 500)
b. Concentration before dilution.

Table A.16b Percentage Release of Acetaminophen from Product E3 (No.2)

Time(min)	[a]Conc. (mcg/ml)	[b]Conc. (mcg/ml)	Amount Release(mg)	% Release
5	0.66	328.8	295.9	59.2
15	1.03	515.3	463.8	92.8
30	1.12	559.2	503.3	100.7
45	1.0	504.1	454.0	90.8
60	1.12	559.2	503.3	100.7
120	1.12	559.2	503.3	100.7

a. Concentration after dilution. (dilution factor: 500)
b. Concentration before dilution.

Table A.16c Percentage Release of Acetaminophen from Product E3 (No.3)

Time(min)	[a]Conc. (mcg/ml)	[b]Conc. (mcg/ml)	Amount Release(mg)	% Release
5	0.94	471.4	424.3	84.9
15	1.12	559.2	503.3	100.7
30	1.12	559.2	503.3	100.7
45	1.23	614.1	552.7	110.5
60	1.11	553.8	498.4	99.7
120	1.23	614.1	552.7	110.5

a. Concentration after dilution. (dilution factor: 500)
b. Concentration before dilution.

Table A.16d Percentage Release of Acetaminophen from Product E3 (No.4)

Time(min)	[a]Conc. (mcg/ml)	[b]Conc. (mcg/ml)	Amount Release(mg)	% Release
5	0.84	416.6	374.9	75.0
15	1.03	515.3	463.8	92.8
30	1.03	515.3	463.8	92.8
45	1.05	526.3	473.7	94.7
60	1.07	537.3	486.3	96.7
120	1.07	537.3	486.3	96.7

a. Concentration after dilution. (dilution factor: 500)
b. Concentration before dilution.

Table A.16e Percentage Release of Acetaminophen from Product E3 (No.5)

Time(min)	[a]Conc. (mcg/ml)	[b]Conc. (mcg/ml)	Amount Release(mg)	% Release
5	0.90	449.6	404.6	80.9
15	0.93	466.0	419.4	83.9
30	1.03	515.3	453.8	92.8
45	1.01	504.4	454.0	90.8
60	1.05	526.3	473.7	94.7
120	1.05	526.3	473.7	94.7

a. Concentration after dilution. (dilution factor: 500)
b. Concentration before dilution.

Table A.16f Percentage Release of Acetaminophen from Product E3 (No.6)

Time(min)	[a]Conc. (mcg/ml)	[b]Conc. (mcg/ml)	Amount Release(mg)	% Release
5	0.94	471.4	424.3	84.9
15	1.05	526.3	473.7	94.7
30	1.15	575.8	518.2	103.6
45	1.12	559.2	503.3	100.7
60	1.13	564.8	508.3	101.7
120	1.15	575.8	518.2	103.6

a. Concentration after dilution. (dilution factor: 500)
b. Concentration before dilution.

Table A.17a Percentage Release of Acetaminophen from Product E4 (No.1)

Time(min)	[a]Conc. (mcg/ml)	[b]Conc. (mcg/ml)	Amount Release(mg)	% Release
5	0.76	378.1	340.3	68.1
15	1.05	526.3	473.7	94.7
30	1.10	548.3	493.5	98.7
60	1.10	548.3	493.5	98.7
120	1.11	553.8	498.4	99.7

a.Concentration after dilution. (dilution factor: 500)
b. Concentration before dilution.

Table A.17b Percentage Release of Acetaminophen from Product E4 (No.2)

Time(min)	[a]Conc. (mcg/ml)	[b]Conc. (mcg/ml)	Amount Release(mg)	% Release
5	1.06	531.8	478.6	95.7
15	1.14	570.2	513.2	102.6
30	1.11	553.8	498.4	99.7
60	1.14	570.2	513.2	102.6
120	1.13	564.8	508.3	101.7

a. Concentration after dilution. (dilution factor: 500)
b. Concentration before dilution.

Table A.17c Percentage Release of Acetaminophen from Product E4 (No.3)

Time(min)	[a]Conc. (mcg/ml)	[b]Conc. (mcg/ml)	Amount Release(mg)	% Release
5	1.0	504.4	454.0	90.8
15	1.04	520.9	468.8	93.8
30	1.12	559.2	503.3	100.7
60	1.12	559.2	503.3	100.7
120	1.11	553.8	498.4	99.7

a. Concentration after dilution. (dilution factor: 500)
b. Concentration before dilution.

Table A.17d Percentage Release of Acetaminophen from Product E4 (No.4)

Time(min)	[a]Conc. (mcg/ml)	[b]Conc. (mcg/ml)	Amount Release(mg)	% Release
5	0.91	455.0	409.5	81.9
15	1.0	504.4	454.0	90.8
30	1.18	592.2	533.0	106.6
60	1.16	581.2	523.1	104.6
120	1.17	586.7	528.0	105.6

a. Concentration after dilution. (dilution factor: 500)
b. Concentration before dilution.

Table A.17e Percentage Release of Acetaminophen from Product E4 (No.5)

Time(min)	[a]Conc. (mcg/ml)	[b]Conc. (mcg/ml)	Amount Release(mg)	% Release
5	0.95	547.0	492.3	85.9
15	1.10	548.3	493.5	98.7
30	1.10	548.3	493.5	98.7
60	1.10	548.3	493.5	98.7
120	1.17	586.7	528.0	105.6

a. Concentration after dilution. (dilution factor: 500)
b. Concentration before dilution.

Table A.17f Percentage Release of Acetaminophen from Product E4 (No.6)

Time(min)	[a]Conc. (mcg/ml)	[b]Conc. (mcg/ml)	Amount Release(mg)	% Release
5	0.95	547.0	492.3	85.9
15	1.12	559.2	503.3	100.7
30	1.13	564.8	508.3	101.7
60	1.15	575.8	518.2	103.6
120	1.12	559.2	503.3	100.7

a. Concentration after dilution. (dilution factor: 500)
b. Concentration before dilution.

APPENDIX B. URINE FLOW RATE, pH AND EXCRETION RATES OF CHLORPHENIRAMINE AND ACETAMINOPHEN IN THE FOUR SUBJECTS AFTER TREATMENTS A, B AND C.

Table B.1. Urine Flow Rate, pH and Excretion Rates of Chlorpheniramine in Subjects Given Product A_1.

	Time (hour)													
	0.5	1	1.5	2	3	4	6	8	12	15	24	30	36	48
Subject 1.														
UFR[a]	260	500	436	374	355	142	79	75	109	108	38	65	42	55
pH	5.8	6.5	6.8	6.3	6.6	6.2	5.9	5.8	6.0	6.3	5.7	5.4	5.6	5.3
ER[b]	0	0	21.4	25.8	26.1	17.1	54	16.6	10.8	11.3	9.3	14.4	8.7	12.6
Subject 2.														
UFR	332	412	260	456	415	100	61	98	44	43	16	23	34	15
pH	6.5	6.7	6.9	7.3	7.2	7.8	7.4	7.0	5.2	6.1	5.6	5.7	5.2	5.5
ER	0	0	25.4	45.0	40.3	10.1	6.3	12.1	45.1	14	12.5	12.4	19.6	6.8
Subject 3.														
UFR	110	207	98	344	168	26	29	34	72	127	42	43	58	28
pH	7.3	7.3	7.4	7.4	7.3	6.6	7.2	7.9	6.5	7.0	6.6	6.7	7.0	6.5
ER	0	0	4.4	17.6	11.3	11.9	12	1.6	23.8	13.4	6.2	3.9	5.0	3.2
Subject 4.														
URF	60	60	78	102	397	54	33	27	38	33	22	42	25	33
pH	6.5	6.9	7.5	7.8	7.5	6.4	5.6	5.6	5.6	5.8	5.6	7.7	7.3	5.9
ER	0	0	0	4.8	21.9	10	10.1	13.7	16.8	10.5	15.6	2.1	5.5	5

a. UFR: urine flow rate, ml/hour
b. ER: excretion rate, mcg/hour

Table B.2. Urine Flow Rates, pH and Excretion Rates of Chlorpheniramine in Subjects Given Product B_1.

	Time (hour)													
	0.5	1	1.5	2	3	4	6	8	12	15	24	30	36	48
Subject 1.														
UFR	70	164	468	326	356	180	58	288	60	114	44	62	83	41
pH	5.5	6.1	6.4	6.5	6.7	6.7	5.9	6.3	5.9	6.3	5.8	5.8	6.2	6.5
ER	0	0	26.8	20.4	25.6	11.5	7	20.8	8.6	10.1	9.2	9.5	10.8	3.4
Subject 2.														
UFR	80	520	210	113	276	65	40	43	31	17	39	39	31	18
PH	7.4	7.3	7.5	7.5	7.2	7.3	7.8	5.9	6.2	5.9	5.6	6.0	5.7	5.8
ER	0	24.8	11	12.2	17.5	5.9	5.4	14.9	9	8.4	30.6	18.7	20	7.9
Subject 3.														
UFR	348	360	58	100	310	62	45	26	40	21	15	20	10	17
pH	7.2	7.0	7.1	7.2	7.4	6.9	7.3	7.0	6.3	6.7	5.7	5.9	6.4	6.1
ER	0	0	5.2	9.4	28.6	8.4	4.9	3.2	8.6	5.1	6.2	4.4	1.1	2.3
Subject 4.														
UFR	42	40	30	36	30	40	40	32	40	67	50	45	50	41
pH	5.8	6.0	6.0	6.3	6.5	5.7	5.5	5.7	6.1	6.3	5.8	5.3	5.5	5.8
ER	0	0	1.2	1.5	1.5	6.9	11.1	5.0	2.2	3.3	4.0	3.6	0.7	0.6

Table B.3. Urine Flow Rates, pH and Excretion Rates of Chlorpheniramin (I) and Acetaminophen (II) in subjects Given Product E_1.

	Time (hour)												
	0.5	1	1.5	2	3	4	6	8	12	15	24	30	36
Subject 1.													
UFR	46	110	250	330	154	315	56	118	44	89	25	149	38
pH	6.6	7.2	6.6	6.7	6.3	6.6	6.1	7.0	6.8	6.8	5.6	5.9	5.4
ER													
I. mcg/h	0	0	31	37.6	32.2	36.1	12.8	8.1	3.9	8.1	9.7	11.4	10.8
II.mg/h	4.4	16.4	16.2	16.2	7.9	13.2	2.2	1.7	0.9	0.5	1.6	0	0
Subject 2.													
UFR	190	234	250	516	45	61	53	27	26	-	11	13	25
pH	6.9	7.2	7.2	8.0	6.6	6.8	7.2	5.7	6.4	-	6.7	5.6	6.0
ER													
I. mcg/h	19	41.2	69.2	287.4	22.5	31.1	13.8	18.4	15	-	1.7	5.9	4.6
II.mg/h	11.9	16.6	17.9	25.7	7.7	6.5	4.0	1.7	0.5	-	0	0	0
Subject 3.													
UFR	58	127	108	254	98	20	35	41	69	11	38	28	8
pH	6.6	6.7	7.0	7.3	7.0	7.0	7.4	7.5	7.0	6.8	5.6	6.2	6.4
ER													
I. mcg/h	0	9.8	16	26.6	14.6	6.0	2.5	9.5	7.5	1.2	14.1	5.0	0.7
II.mg/h	8.6	28.4	17.8	6.6	12.5	5.7	2.3	4.4	3.2	1.2	0.7	0	0
Subject 4.													
UFR	21	14	12	12	20	40	30	34	68	61	53	25	41
pH	6.1	5.9	5.8	5.9	5.7	5.6	5.8	5.5	7.3	6.1	6.1	5.7	5.7
ER													
I. mcg/h	0	0.3	6.6	9.2	12.2	13.4	7.5	7.9	2.7	3.3	2.7	1.2	1.2
II.mg/h	0.9	2.4	1.6	1.9	2.0	1.7	1.7	5.6	3.0	1.1	1.0	-	-