Evaluation of Sustained-Action Chlorpheniramine–Pseudoephedrine Dosage Form in Humans

AVRAHAM YACOBI ×, ROGER G. STOLL, GEORGE C. CHAO, JAMES E. CARTER, DAVID M. BAASKE, BURDE L. KAMATH, ANTON H. AMANN, and CHII-MING LAI

Received November 5, 1979, from the *Department of Pharmaceutical Development, Research and Development, American Critical Care, McGaw Park, IL 60085.* Accepted for publication January 28, 1980.

Abstract □ This investigation compared the bioavailability of chlorpheniramine and pseudoephedrine from a sustained-action capsule and a combination of two reference standard tablets in 24 normal human subjects. The capsule contained 8 mg of chlorpheniramine maleate and 120 mg of pseudoephedrine hydrochloride, and the tablets each contained half of the amount of the chlorpheniramine or pseudoephedrine in the capsule. Because the capsule was a combination product, a new study design had to be developed to accommodate steady-state conditions for both drugs. Each subject received the capsule (every 12 hr) and the combination of the reference tablets (every 6 hr) for 8 days according to a two-way crossover design. Serial blood and urine samples were taken during the entire study. Plasma and urine samples were assayed for chlorpheniramine and pseudoephedrine by sensitive and specific high-pressure liquid chromatographic or GLC methods. There were no significant differences in the plasma concentration profiles of chlorpheniramine and pseudoephedrine at all times, except when the capsule developed peaks or the tablets developed nadirs. The highest mean peak plasma concentrations for the capsule and the tablets were 38.7 and 32.9 ng of chlorpheniramine/ml and 525 and 515 ng of pseudoephedrine/ml, respectively. The mean biological half-lives of chlorpheniramine and pseudoephedrine were 21.6 and 8.0 hr, respectively. The *AUC* and unchanged drug excreted in urine, after a single dose and at steady state, showed that the sustained-action capsule (given every 12 hr) and the reference standard tablets (given every 6 hr) were bioequivalent.

Keyphrases □ Chlorpheniramine—bioavailability in sustained-release dosage form with pseudoephedrine □ Pseudoephedrine—bioavailability in sustained-release dosage form with chlorpheniramine □ Dosage forms, sustained release—bioavailability of chlorpheniramine and pseudoephedrine in combination product □ Bioavailability—chlorpheniramine and pseudoephedrine in sustained-release dosage form

Sustained-action dosage forms often are administered to assure a uniform blood concentration and to provide greater patient convenience and compliance. This dosage form minimizes the occurrence of adverse reactions caused by high plasma concentrations and assures clinical efficacy over the entire regimen. Drugs that are used in sustained-action dosage forms usually possess short half-lives, and their absorption and elimination are affected by physiological factors such as variable urinary pH, enzyme induction or inhibition upon multiple dosing, and variable GI motility.

The physical characteristics of a sustained-release dosage form are such that absorption of the drug is prolonged. The possibility of immediate release of a large amount of drug (dose dumping) is unlikely but requires verification. By considering such factors, a bioavailability study involving multiple doses to achieve steady state will provide a reproducibility test for the *in vivo* performance of a sustained-action dosage form. Pharmacokinetically, such a test will be characterized by more reliable parameters and less variability due to physiological changes.

Ideally, a sustained-action dosage form should release its contents at a constant rate, regardless of changes in the GI pH. Such a dosage form should avoid dose dumping and prevent large fluctuation in plasma concentrations. The objective of this study was to test the bioavailability of chlorpheniramine and pseudoephedrine from a newly developed sustained-action capsule dosage form containing chlorpheniramine and pseudoephedrine by comparison of the plasma concentration *versus* time profile of the drugs following administration of single and multiple doses.

EXPERIMENTAL

In a two-way crossover design study, 24 healthy, nonobese male subjects, 19–41 years old and 62–99 kg (mean 73 kg), were selected randomly. They did not receive any drugs, including enzyme-inducing agents and monoamine oxidase inhibitors, 1 month before and during the study. Eleven subjects were smokers. Alcoholic beverages and those containing caffeine also were withheld during the study.

Each subject received either one sustained-action capsule[1], containing 8 mg of chlorpheniramine maleate and 120 mg of pseudoephedrine hydrochloride, or the combination of one 4-mg chlorpheniramine maleate[2] tablet and one 60-mg pseudoephedrine hydrochloride[3] tablet with 200 ml of water. Either one capsule was given every 12 hr (twice per day) or the combination of the chlorpheniramine and pseudoephedrine tablets was given every 6 hr (four times per day) for 8 days. On Days 1 and 8, only a single capsule or two doses of the combination of the tablets was administered. The subjects were not allowed food overnight, from 10 hr before dosing to 4 hr after administration of the first capsule or first tablet on Days 1 and 8 of the study. A 5-day washout period was allowed between the two treatments.

Heart rate and blood pressure were measured daily during the entire study. Serial blood samples during Day 1 (for 24 hr), Days 2–7 (one sample at the minimum plasma concentration), and Day 8 (for 48 hr) were collected and the plasma was separated. Urine samples were collected during Day 1, at steady state, and for 48 hr after administration of the last capsule and tablet dose. All plasma and urine samples were frozen immediately and were kept frozen until they were assayed.

The plasma chlorpheniramine concentration was determined by a specific high-pressure liquid chromatographic (HPLC) method (1). The recovery and reproducibility of standard samples over the concentration range of 2.5–100 ng/ml were 96.6 and 95.4%, respectively. The plasma pseudoephedrine concentration was determined by GLC with electron-capture detection (2, 3). The recovery and reproducibility of standard samples over the concentration range of 10–700 ng/ml were 99.0 and 93.4%, respectively. Chlorpheniramine and pseudoephedrine concentrations in urine were determined simultaneously by a specific HPLC method (4, 5). The recovery and reproducibility of standard samples were 97.3 and 96.8%, respectively, for chlorpheniramine (0.12–3 μg/ml) and 89.7 and 96.8%, respectively, for pseudoephedrine (3–75 μg/ml). All samples were assayed in duplicate. Samples that showed more than a 15% difference between duplicate assays were reassayed in duplicate, when possible, and the two closest values were taken for final data analysis.

Dissolution testing of the capsule was carried out by a modification of the NF time-release procedure (6). The dissolution fluid consisted of 0.05 *M* potassium phosphate buffer (pH 4.5). There was no variation in the pH during the 7-hr dissolution procedure. The tablet dissolution testing was carried out using the USP XIX rotating-basket procedure. The concentrations of chlorpheniramine and pseudoephedrine in the dissolution fluids were determined by stability-indicating GLC proce-

[1] Isoclor Timesule, American Critical Care, McGaw Park, Ill.
[2] Chlor-Trimeton, Schering Corp., Kenilworth, N.J.
[3] Sudafed, Burroughs Wellcome Co., Research Triangle Park, N.C.

0022-3549/80/0900-1077$01.00/0

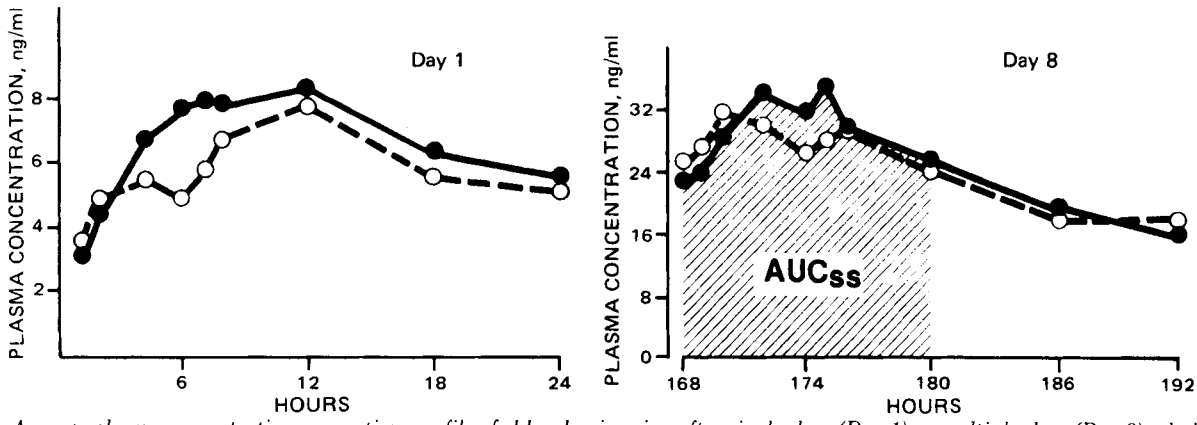

Figure 1—*Average plasma concentration* versus *time profile of chlorpheniramine after single-dose (Day 1) or multiple-dose (Day 8) administration of the sustained-release capsule* (●) *and the reference tablet* (○) *in 24 normal subjects.*

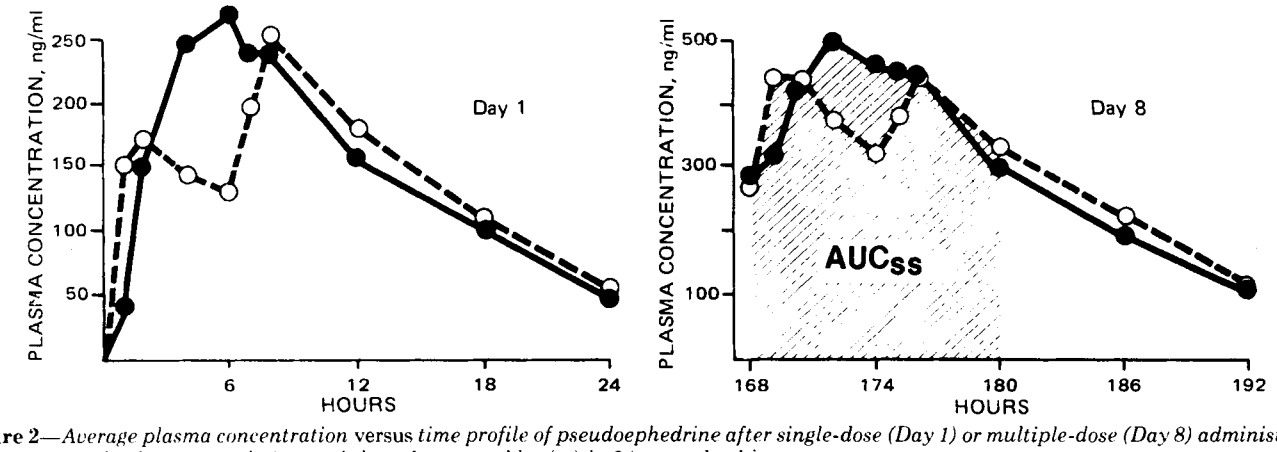

Figure 2—*Average plasma concentration* versus *time profile of pseudoephedrine after single-dose (Day 1) or multiple-dose (Day 8) administration of the sustained-release capsule* (●) *and the reference tablet* (○) *in 24 normal subjects.*

dures (7). This assay also was utilized to verify potency for each dosage form.

The biological half-life, $t_{1/2}$, of each drug in individual subjects was determined from the postabsorption-distribution phase after administration of the last capsule (168 hr). The ratio of the total body clearance, Cl, to the bioavailability factor, F, was determined by dividing the given dose by the area under the plasma concentration *versus* time profile, AUC, at steady state, AUC_{ss} (168–180 hr). The AUC from 0 to 24 hr or at steady state was determined by the trapezoidal method. The ratio of the volume of distribution, V_d, to F was determined from $Cl/\beta F$, where β is the disposition rate constant and is equal to $0.693/t_{1/2}$. The average plasma concentration at steady state, $\overline{C}$, was determined by dividing AUC_{ss} by the dosing interval, *i.e.*, 12 hr. The relative amount of drug absorbed was evaluated by comparing the AUC at steady state and the amount of drug excreted in the urine at steady state.

A nonparametric Wilcoxon's two-sample test (8) with its application to crossover design by Koch (9) was performed to compare formulations for plasma concentrations, urinary excretion and recovery of unchanged drug, and pharmacokinetic parameters of chlorpheniramine and pseudoephedrine following administration of each dosage form. The same test was used for comparison of the blood pressure and heart rate. The period, group, and formulation effects also were tested.

RESULTS

Figures 1 and 2 depict the average plasma concentration *versus* time profiles of chlorpheniramine and pseudoephedrine, respectively. Data after the first and last doses at steady state are shown. The plasma concentrations were significantly higher for the capsule only at 4, 6, and 7 hr after the single dose and at steady state after the last dose.

Table I summarizes the mean peak plasma concentration, peak time of chlorpheniramine and pseudoephedrine, and results of statistical analysis. The mean peak plasma concentrations of chlorpheniramine at steady state were 38.7 ng/ml (range[4] 17.3–75.8 ng/ml) after administration of the capsule and 32.9 ng/ml (range 17.7–79.5 ng/ml) after the tablet. Without the unusual chlorpheniramine data point[4], the mean peak plasma concentration becomes 35.5 ng/ml, which is not significantly different from the mean values obtained for the standard tablet. The mean peak plasma concentration times differed after the single dose ($p < 0.01$) and was the same after multiple dosing for both drugs.

The higher concentrations of chlorpheniramine and pseudoephedrine attained in this study were not associated with an increase in the incidence or the type of adverse side effects.

Figure 3 depicts the mean minimum plasma concentration of chlorpheniramine and pseudoephedrine following multiple-dose administration of the sustained-release capsule and the reference tablets. There was essentially no significant difference between the values obtained for either dosage form. On the average, the steady-state minimum plasma concentration was achieved after 6 days for chlorpheniramine and 2 days for pseudoephedrine. The minimum plasma chlorpheniramine concentration was ~24 ng/ml and remained within a 10% range at Days 6–8; the minimum plasma concentration of pseudoephedrine increased from 260 ng/ml on Day 7 to ~315 ng/ml on Day 8.

Table II summarizes the mean AUC and amounts of unchanged chlorpheniramine and pseudoephedrine excreted in the urine. There was no significant difference between the two treatments. At steady state, the AUC and the urinary values were almost twice as high for chlorpheniramine and were ~27 and 12% higher for pseudoephedrine than those for the respective single doses. Additionally, at steady state, the amounts excreted in the urine for both drugs were higher at 156–168 hr than at 168–180 hr.

Table III lists the average pharmacokinetic parameters for chlorpheniramine and pseudoephedrine. These parameters were essentially identical for the capsule and reference tablets.

The relative amount of drugs absorbed was evaluated by comparing the AUC or the average steady-state plasma concentrations and the

[4] One subject showed an unusually high plasma concentration of 154 ng/ml 7 hr after the last dose; his second highest plasma concentration was 75.8 ng/ml 6 hr after the last dose.

Table I—Mean Peak Plasma Concentration and Peak Time of Chlorpheniramine and Pseudoephedrine and Results of Statistical Analysis

Parameter	Mean ± *SD*		Significance Level by Wilcoxon's Two-Sample Test		
	Capsules	Reference Tablets	Drug	Period	Group
		Chlorpheniramine			
Peak plasma concentration, ng/ml					
0–12 hr	10.6 ± 4.92	8.88 ± 2.47	NS[a]	NS	NS
168–180 hr	38.7[b] ± 27.1	32.9 ± 13.6	NS	NS	NS
Peak time[c], hr					
0–12 hr	7.88 ± 4.83	10.9 ± 4.90	0.01	NS	NS
168–180 hr	5.63 ± 2.90	4.54 ± 2.98	NS	NS	NS
		Pseudoephedrine			
Peak plasma concentration, ng/ml					
0–12 hr	282.7 ± 49.8	264.0 ± 59.6	NS	NS	NS
168–180 hr	525.4 ± 131.3	514.6 ± 98.0	NS	NS	NS
Peak time[c], hr					
0–12	5.83 ± 1.27	7.63 ± 2.55	0.01	NS	NS
168–180 hr	4.67 ± 1.66	4.25 ± 3.15	NS	NS	NS

[a] Not significant. [b] When an unusual plasma concentration of 154 ng/ml in one subject is eliminated, the mean value becomes 35.5 ng/ml, which does not change the statistical results. [c] Relative to administration of the capsule or the first tablet in each interval.

Table II—Mean Area under Plasma Concentration *versus* Time Profile and Unchanged Drug Excreted in Urine

Parameter	Chlorpheniramine[a]		Pseudoephedrine[a]	
	Capsules	Reference Tablets	Capsules	Reference Tablets
AUC, (ng hr)/ml				
Single dose, 0–24 hr	161.3 ± 73.2	140.7 ± 36.1	3620 ± 747	3456 ± 629
Steady state, 168–180 hr	351.8 ± 138.7	333.0 ± 147.1	4986 ± 1248	4647 ± 1123
Urinary excretion[b], mg				
Single dose, 0–24 hr	0.407 ± 0.302	0.424 ± 0.229	96.0 ± 11.3	92.6 ± 14.0
Steady state, 156–168 hr	1.06 ± 0.678	0.967 ± 0.512	109.9 ± 22.3	103.7 ± 30.7
Steady state, 168–180 hr	0.716 ± 0.748	0.667 ± 0.510	101.5 ± 22.7	92.6 ± 16.2
Washout, 168–216 hr	2.07 ± 1.42	1.91 ± 0.996	175.8 ± 31.8	165.1 ± 27.9

[a] Mean ± *SD*; there was no significant difference between treatments. [b] Amount given as chlorpheniramine maleate or pseudoephedrine hydrochloride.

urinary excretion of the drugs at steady state. Table IV summarizes the ratio of these parameters (capsule to reference tablet) with respect to either drug. The capsule showed slightly higher bioavailability than the reference tablet.

The capsule potency was determined to be 101.2 and 104.0% of the labeled quantity of chlorpheniramine and pseudoephedrine, respectively. The chlorpheniramine and pseudoephedrine tablets contained 98.2 and 99.2%, respectively, of the labeled quantity. The dissolution testing indicated that the capsule released 84.5 and 89.0% of chlorpheniramine and pseudoephedrine, respectively, within 7 hr and that the tablets released 99.1 and 101.4% of chlorpheniramine and pseudoephedrine, respectively, within 15 min.

DISCUSSION

Because the sustained-action formulation tested was a combination product of chlorpheniramine and pseudoephedrine, a new study design had to be developed that would assure steady-state conditions for both drugs and use two reference standard tablets for the bioequivalency tests. A standard three-way crossover design would have made the study unduly

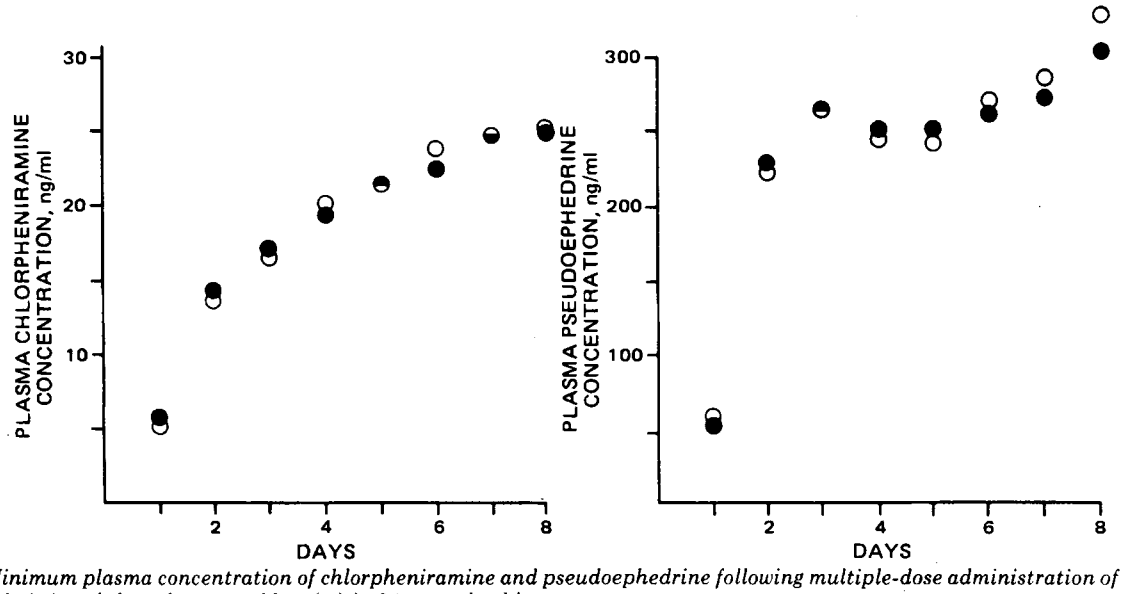

Figure 3—*Minimum plasma concentration of chlorpheniramine and pseudoephedrine following multiple-dose administration of the sustained-release capsule (●) and the reference tablets (○) in 24 normal subjects.*

Table III—Pharmacokinetic Parameters of Chlorpheniramine and Pseudoephedrine

Parameter	Sustained-Release Capsule[a] Mean ± SD	Sustained-Release Capsule[a] Range	Reference Tablets[a] Mean ± SD	Reference Tablets[a] Range
		Chlorpheniramine		
$\bar{C} = AUC_{ss}/12$, ng/ml	29.3 ± 11.6	14.0–63.5	27.8 ± 12.3	16.0–71.0
$t_{1/2}$, hr	22.13 ± 5.92	11.5–35.7	20.96 ± 4.94	13.9–33.0
V_d/F, liters/kg	7.51 ± 2.12	2.12–14.4	7.65 ± 2.06	3.13–11.3
Cl/F, ml/hr/kg	251.5 ± 93.2	111.8–456.8	264.1 ± 84.6	106.2–414.2
		Pseudoephedrine		
$\bar{C} = AUC_{ss}/12$, ng/ml	415.5 ± 104.0	255.0–609.0	387.2 ± 93.7	209.0–582.0
$t_{1/2}$, hr	8.23 ± 1.28	4.65–10.9	7.85 ± 1.63	5.02–10.9
V_d/F, liters/kg	3.40 ± 0.92	1.76–5.11	3.50 ± 1.12	1.46–5.73
Cl/F, ml/hr/kg	289.0 ± 74.6	164.7–434.1	309.8 ± 81.2	188.9–528.6

[a] Mean ± SD, $n = 24$; there was no significant difference between treatments.

cumbersome. The two-way crossover design allowed for simultaneous administration of the two reference standard tablets to one group and the sustained-action formulation to the other and provided the opportunity for a multiple-dose study within a short time period.

The sustained-action capsule showed gradual *in vivo* absorption and produced plasma concentrations virtually identical to those obtained after administration of the reference tablets. Statistical differences were noted when the capsule reached the peak level at approximately the same time as the every 6-hr regimen reached its nadir. There was no indication of dose dumping after capsule administration. This conclusion is substantiated by sustained plasma concentrations during one dosing interval at steady state, similarity of peak plasma concentrations, and similarity of the time of peak plasma concentrations of either dosage form at steady state.

For both dosage forms, the time to achieve the highest peak plasma concentration was examined. Since the tablets were given every 6 hr, it was expected that the peak after the second 6-hr dose (*i.e.*, the second tablet) would be the highest; this expectation was verified statistically. For both drugs and at steady state, every 6-hr administration of the reference tablets should produce virtually the same peak at the same relative time. This response is apparent in the data and also is the reason for having a shorter peak plasma concentration time at steady state than after the single-dose administration (*i.e.*, the first two doses of the every 6-hr regimen) of the reference tablets.

The gradual *in vivo* absorption of the sustained-action capsule was dictated by its *in vitro* dissolution characteristics (Fig. 4). Identical dissolution profiles were observed at pH 1.5, 4.5, and 7.5. Therefore, no changes in the release rate are expected in the GI tract with respect to changes in pH. Other factors such as the presence or absence of enzymes may influence the dissolution rate of the drugs. The pH-independent dissolution of chlorpheniramine and pseudoephedrine in the capsule assures complete release of the drugs and minimizes the chances for dose dumping, which can cause side effects.

The results showed minimum occurrence of side effects during the 8 days of the study, with no effect on the heart rate or blood pressure by either dosage form. The chlorpheniramine and pseudoephedrine doses used in this study are considered safe. In a more rigorous clinical study, Bye *et al.* (10) observed signs of insomnia which lasted for the first 3 days among subjects who received chronic dosing with a 180-mg sustained-action product of pseudoephedrine. In another study, Dickerson *et al.* (11) found that chronic dosing with 120- and 150-mg sustained-action products of pseudoephedrine increased the pulse rate but decreased blood pressure.

Consistent with the half-lives of chlorpheniramine (22 hr) and pseudoephedrine (8 hr), the minimum steady-state plasma concentrations were achieved in 6 and 2 days, respectively. Both chlorpheniramine and pseudoephedrine are weak bases, and their elimination in urine is pH dependent (4, 12, 13). Day-to-day fluctuations in the urine pH will result in a fluctuation in the plasma concentration as well as in the urinary excretion of unchanged drugs. In this study, the minimum steady-state plasma concentration of both drugs, particularly pseudoephedrine, increased during the 168–180-hr interval; the urinary excretion of unchanged drugs in the same interval diminished. This change occurred just after hospitalization of the subjects and standardization of the food, which may have directly affected the urinary pH. In fact, consistent with the decrease in the urinary excretion of the drugs, the average urinary pH values did increase significantly from 5.8 at the 156–168-hr interval to ~6.1 at the 168–180-hr collection interval in both treatments (Table V).

The steady-state approach allowed determination of the pharmacokinetic parameters and bioequivalency of chlorpheniramine and pseudoephedrine between the sustained-action capsule and the reference standard tablets. The single-dose data after 24-hr blood sampling and urinary collection resulted in smaller *AUC* and urinary excretion values of unchanged drugs for both chlorpheniramine (44 and 50%, respectively) and pseudoephedrine (73 and 88%, respectively). This finding warrants a longer washout period for both drugs after a single dose. However, for chlorpheniramine, the sensitivity of the assay also must be considered.

The average plasma concentrations of chlorpheniramine and pseudoephedrine were the same for both dosage forms. In a group of 16 subjects who received a regimen of pseudoephedrine, the estimated minimum steady-state plasma concentration was 447 ng/ml (11), which is consistent with the present finding. The biological half-life of chlorpheniramine was in agreement with values reported in a study that utilized an identical assay (14). The average biological half-life of pseudoephedrine found in the present study (8.0 hr) was higher than values reported previously (5.9 hr) for 16 subjects (11). However, the higher half-life may account for the higher V_d/F value found in this study (3.4 liters/kg) than in the same 16 subjects (2.6 liters/kg). However, these differences also may be due to the study design and methods for estimation of V_d. The reproducibility of the pharmacokinetic parameters is indicative of completeness of absorption of chlorpheniramine and pseudoephedrine from both dosage forms. The reproducibility of the minimum steady-state plasma concentration also suggests that there was no enzyme induction with respect to chlorpheniramine metabolism or

Table IV—Relative Bioavailability of Chlorpheniramine and Pseudoephedrine after the Sustained-Release Capsule

Parameter	Ratio of Parameters[a] of Capsule to Tablets: Chlorpheniramine	Ratio of Parameters[a] of Capsule to Tablets: Pseudoephedrine
Excretion in urine		
0–24 hr	0.96	1.04
156–168[b] hr	1.10	1.06
168–180[b] hr	1.06	1.06
168–216 hr	1.08	1.06
Area under plasma concentration *versus* time profile		
0–24 hr	1.15	1.05
168–180[b] hr	1.06	1.07

[a] Ratio of the mean parameters obtained after administration of the capsule and the combination of the tablets. Ratio of one indicates that two dosage forms are bioequivalent. [b] These parameters are determined at steady state and give the true bioequivalence data.

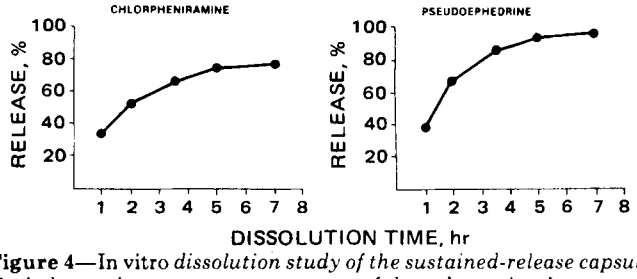

Figure 4—In vitro *dissolution study of the sustained-release capsule. Each data point represents an average of three determinations at each pH. Identical dissolution profiles were obtained at pH 1.5, 4.5, and 7.5.*

Table V—Mean Urine pH at Steady State and Results of Statistical Analysis

Urine Collection Interval, hr	Capsules pH[a]	Capsules p^b	Reference Tablets pH[a]	Reference Tablets p^b
156–168	5.78 ± 0.36		5.84 ± 0.51	
168–174	6.08 ± 0.65	<0.01	6.23 ± 0.68	<0.01
174–180	6.20 ± 0.67	<0.01	6.20 ± 6.16	<0.05
168–180[c]	6.14 ± 0.62	<0.01	6.21 ± 0.55	<0.01

[a] Mean ± *SD*, $n = 24$; there was no statistically significant difference between two treatments. [b] Statistically significant difference from 156–168-hr urine sample pH. [c] Average of 168–174- and 174–180-hr pH values.

changes in urinary excretion of either drug. While the urinary excretion of unchanged chlorpheniramine is a small fraction of the administered dose (4), the urinary excretion of unchanged pseudoephedrine is the major fraction of the administered dose and can be a reliable indicator for bioequivalency tests.

The bioequivalency test for sustained-release dosage forms can be best made at steady state when the amount excreted in urine reflects the amount absorbed. Table II substantiates this premise since the 0–24-hr *AUC* does not accurately reflect the amount of chlorpheniramine absorbed. At steady state, the *AUC* for a single dosing interval was more than twice as large as that found during the 0–24-hr interval following a single dose of the capsule. The slow absorption associated with this sustained-action dosage form makes it difficult to evaluate such systems using a single-dose study design. When the ratio of parameters characterizing the bioavailability of two drugs is one, the dosage forms are considered to be bioequivalent. In this study, the average ratio (capsule to reference tablet) was 1.06. Thus, both the rate of drug absorption as given by the peak plasma concentration and time of peak (Table I) and the extent of absorption (Tables II and IV) showed virtually identical bioavailability for the sustained-action capsule and reference standard tablets.

REFERENCES

(1) N. K. Athanikar, G. W. Pang, R. L. Nation, and W. L. Chiou, *J. Chromatogr.*, **162,** 367 (1979).

(2) L. M. Cummins and M. J. Fourier, *Anal. Lett.*, **2,** 403 (1969).

(3) E. T. Lin, D. C. Brater, and L. Z. Benet, *J. Chromatogr.*, **140,** 275 (1979).

(4) C. M. Lai, R. G. Stoll, Z. M. Look, and A. Yacobi, *J. Pharm. Sci.*, **68,** 1243 (1979).

(5) D. M. Baaske, C. M. Lai, L. Klein, Z. M. Look, and A. Yacobi, *ibid.*, **68,** 1472 (1979).

(6) "The National Formulary," 14th ed., Mack Publishing Co., Easton, Pa., 1975, p. 985.

(7) A. Yacobi, Z. M. Look, and C. M. Lai, *J. Pharm. Sci.*, **67,** 1668 (1978).

(8) M. Hollander and D. Wolfe, "Nonparametric Statistical Methods," Wiley, New York, N.Y., 1973, pp. 68–75, 185.

(9) G. G. Koch, *Biometrics*, **28,** 577 (1972).

(10) C. Bye, H. M. Hill, D. T. D. Hughes, and A. W. Peck, *Eur. J. Clin. Pharmacol.*, **8,** 47 (1975).

(11) J. Dickerson, D. Perrier, M. Mayersohn, and R. Bressler, *ibid.*, **14,** 253 (1978).

(12) G. R. Wilkinson and A. H. Beckett, *J. Pharm. Pharmacol.*, **11,** 256 (1965).

(13) R. G. Kuntzman, I. Tsai, L. Brand, and L. C. Mark, *Clin. Pharmacol. Ther.*, **12,** 62 (1971).

(14) W. L. Chiou, N. K. Athanikar, and S. M. Huang, *N. Engl. J. Med.*, **300,** 501 (1979).

High-Performance Liquid Chromatographic Assay of Codeine in Acetaminophen with Codeine Dosage Forms

C. Y. KO ˣ, F. C. MARZIANI, and C. A. JANICKI

Received March 4, 1980, from the *Pharmacy Research Department, McNeil Laboratories, Fort Washington, PA 19034.* Accepted for publication March 27, 1980.

Abstract □ An accurate, rapid, and specific high-performance liquid chromatographic (HPLC) assay was developed for codeine in acetaminophen with codeine combination products. The internal standard (chlorpheniramine maleate), codeine, acetaminophen, and several other test compounds or impurities were well separated. A complete analysis took <10 min. The relative standard deviations of the retention time, precision, and accuracy were 0.5, 0.4, and 0.5%, respectively. An excellent linear correlation was obtained between the HPLC and GLC methods.

Keyphrases □ Codeine—high-performance liquid chromatographic analysis of capsules, tablets, and elixirs with acetaminophen and codeine, comparison with GLC analysis □ Acetaminophen—high-performance liquid chromatographic analysis of codeine and acetaminophen in capsules, tablets, and elixirs, comparison with GLC analysis □ High-performance liquid chromatography—analysis, codeine and acetaminophen in capsules, tablets, and elixirs, comparison with GLC analysis

Various analytical methods (1–11) have been reported for codeine, a narcotic analgesic and antitussive drug. Sell and Rajzer (1) determined codeine in nonaqueous media using a differentiating potentiometric titration method. Mulé (2) reported UV, TLC, and GLC methods for the determination of narcotic analgesics in humans. GLC methods (3–5) also were used extensively to determine codeine in various media.

Steady progress in high-performance liquid chromatographic (HPLC) analyses of codeine has been reported (6–11). Following reports that the HPLC technique was useful for codeine analysis (6, 7, 11), reversed-phase and ion-pair HPLC techniques were developed (8, 9). Baker *et al.* (10) recently analyzed codeine using a reversed-phase HPLC system equipped with dual-wavelength UV detection. Gupta (12) reported the simultaneous separation of acetaminophen, aspirin, caffeine, codeine phosphate, phenacetin, and salicylamide using a reversed-phase chromatographic method.

The purpose of this study was to develop an HPLC method that could be used routinely to assay codeine in acetaminophen with codeine combination products. This method is fast, simple, specific, precise, and accurate.

EXPERIMENTAL

Reagents and Materials—The *n*-hexane used was distilled in glass. Ammonium hydroxide was ACS grade. Chloroform[1], methanol[1], and sodium hydroxide[1] were analytical reagent grade. The codeine phosphate,

[1] Mallinckrodt, St. Louis, MO 63147.

0022-3549/80/0900-1081$01.00/0