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# SIMULTANEOUS DETERMINATION OF CODEINE AND CHLORPHENIRAMINE IN HUMAN PLASMA BY CAPILLARY COLUMN GAS CHROMATOGRAPHY

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## SUMMARY

A specific and highly sensitive capillary column gas chromatographic method was developed for the simultaneous determination of codeine and chlorpheniramine in human plasma. The method involves a solvent extraction and analysis by capillary column gas chromatography on a cross-linked 50% phenylmethyl silicone fused-silica capillary column with flame thermionic detection. A 10% solution of *n*-butanol in toluene was used as extraction medium and pyrilamine was used as internal standard. Reproducibility, linearity of calibration curves and specificity were all satisfactory with both drugs. The plasma concentration of codeine and chlorpheniramine could be measured at levels down to 0.9 ng/ml as codeine phosphate and 0.4 ng/ml as chlorpheniramine maleate, respectively. The method was applied to plasma samples from normal volunteers, and was confirmed to be adequate for biopharmaceutical and pharmacokinetic studies.

## INTRODUCTION

A combination of codeine phosphate (CDP) with chlorpheniramine maleate (CPM) is a widely prescribed antitussive. A specific and highly sensitive analytical method is required for the determination of codeine (CD) and chlorpheniramine (CP) in human plasma following oral administration of therapeutic doses of CDP and CPM. Various analytical methods for the determination of CD [1—4] or CP [5—7] and for simultaneous determination of CD and CP [8] have been reported.

Gas chromatography (GC) with nitrogen—phosphorus detection [3] and high-performance liquid chromatography (HPLC) [1, 2, 4] were employed for

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the determination of CD. For the determination of CP, GC with flame ionization detection [5, 6] and HPLC [7] were included. On the other hand, thin-layer chromatography (TLC) was developed for the simultaneous determination of CD and CP [8]. However, these GC, HPLC and TLC methods are not sensitive and reproducible enough for measuring low plasma levels (1—20 ng/ml) of CD and CP. In addition, the TLC method is complicated and time-consuming.

In order to investigate the bioavailability of lower doses of CDP and CPM, which are formulated in proprietary antitussive drugs, a more simple, specific and sensitive analytical method is required.

This paper describes a capillary GC method with flame thermionic detection (nitrogen–phosphorus-selective detection), which can determine simultaneously subnanogram quantities of CD and CP in human plasma. In addition, by this method the behaviour of CD and CP in human plasma after oral administration of CDP and CPM in low doses could be determined.

## EXPERIMENTAL

### *Reagents and chemicals*

Codeine phosphate, morphine hydrochloride and chlorpheniramine maleate were Japanese Pharmacopoeia standards. Pyrilamine maleate was obtained from Sigma (St. Louis, MO, U.S.A.). Toluene and *n*-butanol (Japanese Industrial Standards, guaranteed reagent) were obtained from Wako (Osaka, Japan). Monodesmethylchlorpheniramine and didesmethylchlorpheniramine were generously supplied by Pennwalt (Rochester, NY, U.S.A.). Norcodeine was synthesized in our laboratory by a modification of the method described by DeGraw et al. [9]. All other chemicals used here were of analytical-reagent grade.

### *Apparatus*

A Shimadzu GC-9A computer-controlled gas chromatograph (Shimadzu, Kyoto, Japan) equipped with a flame thermionic detector (an electrically heated alkali-bead nitrogen—phosphorus-selective detector) was used. The GC separation was achieved using a 5 m × 0.32 mm I.D., 0.25-$\mu$m cross-linked 50% phenylmethyl silicone fused-silica capillary column (J & W Scientific, Rancho Cordova, CA, U.S.A.) and helium was used as the carrier and make-up gas. The injection mode was splitless (Shimadzu SPL-G9 splitless injector).

The gas chromatograph was also equipped with an automatic injection unit, a Shimadzu AOC-9 autosampler. The peak heights were evaluated by a Shimadzu C-R3A integrator.

### *Analytical conditions*

Flow-rates of the carrier gas and make-up gas were 4.2 and 50 ml/min, respectively. The column was maintained at 150°C for 1 min, then programmed up to 240°C at 10°C/min followed by isothermal operation for 1 min. The injector and the detector temperatures were maintained at 290°C. Aliquots of 2 $\mu$l of the samples were injected and the purge of excess solvent was carried out at a flow-rate of 60 ml/min from 1 min after sample injection to the end of the

*Assay procedure*

*Stock solutions.* Stock solutions of 1.08 μg/ml codeine phosphate, 0.48 μg/ml chlorpheniramine maleate and 0.48 μg/ml pyrilamine maleate in distilled water were prepared and stored at 4°C.

*Standard plasma samples for calibration curves.* Standard plasma samples 1—3 spiked with authentic drugs were prepared for the calibration curves. Codeine phosphate (25 μl) and chlorpheniramine maleate stock solution (25 μl) were added to 2.0 ml of blank plasma to yield standard plasma sample 1. Similar plasma samples were made up with 50 μl and 100 μl of these stock solutions to give standard plasma samples 2 and 3, respectively.

*Extraction procedure.* To 2.0 ml of plasma in a 10-ml test-tube, 50 μl of pyrilamine maleate stock solution, 0.4 ml of 1 *M* sodium hydroxide and 4.0 ml of 10% *n*-butanol in toluene were added. The tubes were shaken for 20 min (Iwaki, KM-Shaker) followed by centrifugation at 2000 *g* for 10 min (Kubota, KN-70 centrifuge). A 3.6-ml aliquot of the organic layer was transferred to the test-tube to which 0.4 ml of 0.05 *M* hydrochloric acid had been added. Then, the tubes were shaken for 20 min and centrifuged at 2000 *g* for 10 min. The upper organic layer was removed and the remaining organic solvent in the aqueous layer was flushed by a gentle stream of nitrogen. To these samples, 0.2 ml of 1 *M* sodium hydroxide and 0.2 ml of 10% *n*-butanol in toluene were added. After the tubes were shaken for 20 min, samples were transferred into disposable microcentrifuge tubes and centrifuged for 5 min (Eppendorf Model 5412 centrifuge). A 0.15-ml aliquot of the clear organic layer was transferred to an autosampler microvial and 2 μl were injected into the gas chromatograph.

*Quantitation.* Calibration curves were obtained by plotting the peak-height ratio of the authentic drug to the internal standard as a function of drug concentration in the standard plasma samples 1—3.

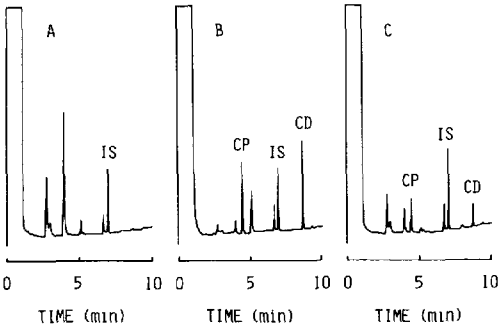
Quantitation of each drug in the plasma samples was made by reference to the standard calibration curve.

*Plasma samples*

Seven healthy male adult volunteers were fasted for 11 h before and 6 h after administration. Codeine phosphate (9 mg) and chlorpheniramine maleate (2 mg) in 10 ml of water were administered to each volunteer orally with 100 ml of distilled water. Each volunteer took the drugs three times every 4 h. Blood samples were collected in heparinized tubes by venipuncture at −1, 0.5, 1, 2, 4, 4.5, 5, 6, 8, 8.5, 9, 10, 12, 14, 24, 36 and 48 h after administration. Each plasma sample was separated by centrifugation immediately and was stored frozen at −40°C until analysis.

## RESULTS AND DISCUSSION

Codeine is a fairly polar compound. Therefore, a polar solvent such as 10% *n*-butanol in toluene used here was desirable to extract codeine and chlorpheniramine simultaneously. The present procedure has an absolute extraction efficiency of 85% in the range 6.9–27.7 ng/ml codeine (equivalent to 9.2—36.8 ng/ml phosphate salt) in plasma by a single extraction. On the other hand, chlorpheniramine and pyrilamine were extracted completely in the range



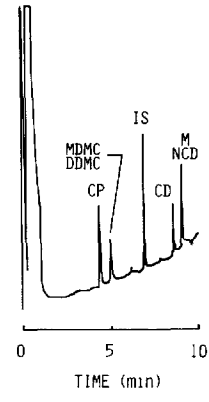




Fig. 1. Gas chromatograms of (A) blank plasma containing internal standard, pyrilamine; (B) plasma containing codeine, chlorpheniramine and internal standard; (C) real plasma of a volunteer (concentrations of codeine and chlorpheniramine are 6.5 and 6.3 ng/ml, respectively). Peaks: CD = codeine; CP = chlorpheniramine; IS = internal standard.

Fig. 2. Gas chromatogram of codeine, chlorpheniramine and their metabolites. Peaks: CD = codeine; CP = chlorpheniramine; M = morphine; NCD = norcodeine; MDMC = monodesmethylchlorpheniramine; DDMC = didesmethylchlorpheniramine; IS = internal standard.

3.0—11.9 ng/ml (equivalent to 4.2–16.9 ng/ml maleate salt) and 8.5 ng/ml (equivalent to 12 ng/ml maleate salt) in plasma, respectively.

Typical chromatograms obtained using the present procedure are shown in Fig. 1. There are no peaks in the blank plasma samples to interfere with the determination of codeine and chlorpheniramine.

The known metabolites of codeine in human plasma are morphine, norcodeine and their conjugates [10, 11]. It is also well known that there are two metabolites of chlorpheniramine. Morphine and the conjugates of morphine and norcodeine were not extracted by 10% *n*-butanol in toluene, so these compounds cannot interfere with the present assay. In addition, metabolites of chlorpheniramine, i.e. monodesmethylchlorpheniramine and didesmethylchlorpheniramine, have not been detected in plasma samples [6, 7]. Therefore, under the present analytical conditions, the interference due to these metabolites might not need to be taken into consideration. However, major metabolites of codeine and chlorpheniramine, i.e. morphine, norcodeine, monodesmethylchlorpheniramine and didesmethylchlorpheniramine, were analysed under the present analytical conditions to ensure that these metabolites would not interfere with the measurement of codeine and chlorpheniramine. A chromatogram of codeine and chlorpheniramine with their metabolites is shown in Fig. 2. As can be seen from Fig. 2, none of the peaks of the metabolites overlap with those of codeine, chlorpheniramine and the internal standard.

The peak-height ratios of replicate samples obtained from standard blank plasmas spiked with known amounts of codeine phosphate and chlorpheniramine maleate are summarized in Table I. The calibration curves for codeine and chlorpheniramine showed linear relationships between peak-height ratios and concentrations in the range 9.2—36.8 ng/ml as codeine phosphate ($r$ = 0.998), and 4.2—16.9 ng/ml as chlorpheniramine maleate ($r$ = 0.998), and

TABLE I

PRECISION AND ACCURACY OF THE ANALYTICAL METHOD FOR CODEINE AND CHLORPHENIRAMINE IN PLASMA

| Compound | Concentration added (ng/ml) | Peak-height ratio | |
|---|---|---|---|
| | | Mean value* | Coefficient of variation (%) |
| Codeine phosphate | 4.2 | 0.4160 | 7.0 |
| | 8.4 | 0.8586 | 3.7 |
| | 16.9 | 1.6815 | 4.8 |
| Chlorpheniramine maleate | 9.2 | 0.3499 | 8.7 |
| | 18.4 | 0.7329 | 0.7 |
| | 36.8 | 1.3480 | 2.4 |

*Each value represents the mean of three determinations.

intercepted near the origin (0.002 ± 0.042 and 0.016 ± 0.033), respectively. The coefficients of variation ranged from 3.7 to 7.0% for codeine and 0.7 to 8.7% for chlorpheniramine. It can be seen from these results that the present analytical method has good reproducibility.

The minimum concentrations of codeine and chlorpheniramine in plasma that could be determined by the present analytical method were 0.7 and 0.3 ng/ml, respectively. Among the analytical methods that have been reported for the determination of codeine or chlorpheniramine, some methods have fairly good sensitivity and accuracy. For example, Tsina et al. [1] and Visser et al. [2] reported that 10 ng/ml codeine in plasma could be quantitated, and Athanikar et al. [7] reported that 2 ng/ml chlorpheniramine in plasma could be quantitated. We also reported the bioavailability study of a new dosage form containing chlorpheniramine maleate [12]. In that study, the levels of chlorpheniramine in rabbit plasma could be determined by a modification of the HPLC method described by Athanikar et al. [7]. However, in the case of the bioavailability study using human volunteers, the plasma level of chlorpheniramine could not be determined accurately due to the low doses administered. Accordingly, to evaluate a newly developed proprietary drug delivery system containing lower doses of codeine or chlorpheniramine (commonly prescribed in proprietary drugs), a more highly sensitive analytical method was needed. The present analytical method fulfils the above needs.

In order to confirm the adaptability of the present analytical method for the bioavailability study, drug solutions containing 9 mg of codeine phosphate and 2 mg of chlorpheniramine maleate were administered to seven adult male volunteers three times every 4 h. Then, plasma samples were assayed with the present analytical method. The concentrations of both drugs administered in this study were widely prescribed in liquid antitussive dosage forms in the over-the-counter market. The results determined are shown in Fig. 3.

The maximum plasma concentration of codeine and chlorpheniramine occurred at 0.5—1.0 h and at 2.0—4.0 h after administration, respectively. At 36 and 48 h after administration, the concentration of codeine in the plasma was too low to be quantitated accurately. The biological half-life of

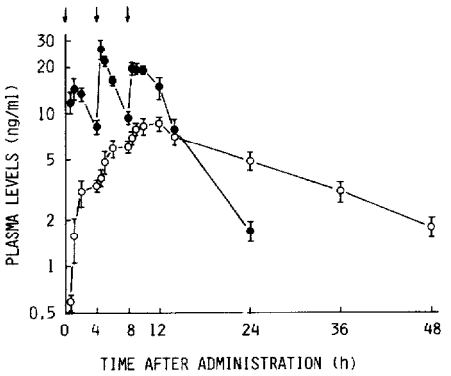



Fig. 3. Plasma levels of codeine (•) and chlorpheniramine (○) after oral administration of codeine phosphate and chlorpheniramine maleate. Codeine phoshate (9 mg) and chlorpheniramine maleate (2 mg) in aqueous solution were administered three times every 4 h. Each point represents the mean ± S.E. of seven volunteers. The arrows mark the times of administration.

codeine found in this study was 3.0—6.0 h and that of chlorpheniramine was 15.6—19.7 h.

Up to now, this method has been employed in analysing over 2000 plasma samples. In all the plasma samples, none of the peaks due to metabolites were found to interfere with determination of the drugs or the internal standard.

The plasma level of norcodeine has been reported to be very low even when 60 mg of codeine phosphate was administered [10]. In the literature, the metabolites of chlorpheniramine, i.e. monodesmethylchlorpheniramine and didesmethylchlorpheniramine, have not been observed in plasma [6, 7]. In the case of the lower dose administration in this study, it was accountable that metabolites of both drugs were not observed. Besides, as is already mentioned above, morphine was not extracted from plasma by the extraction solvent in this study, so this metabolite was not detected.

In conclusion, the GC method developed here was confirmed to be practically useful for biopharmaceutical study of low doses of drug containing codeine and/or chlorpheniramine.

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