GLC Determination of Chlorpheniramine in Human Plasma

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Abstract □ A specific and quantitative GLC method for the determination of chlorpheniramine in human plasma is described. The procedure involves the alkalinization of plasma samples followed by extraction with chloroform at pH 11. The sensitivity of the method is such that 0.1 μg of material can be detected in 1 ml of plasma. The method was used in a crossover study for comparative bioavailability of chlorpheniramine maleate from syrup formulations.

Keyphrases □ Chlorpheniramine—GLC determination in human plasma ■ GLC—analysis, chlorpheniramine in human plasma

Chlorpheniramine maleate is a widely used antihistaminic drug (1–3). Although it has been in use for many years, reports of its decomposition in animals (4), urinary excretion (5, 6), urinary metabolites (7), and blood levels in humans (8) have been appearing only recently.

Many sophisticated analytical methods have been published for the quantitation of the drug either *in vitro* from its pharmaceutical preparations or *in vivo* for its therapeutic and bioavailability evaluation. Among these are paper chromatography, TLC (8–10), spectrophotometry (5, 11), and fluorometry (8) procedures; however, these methods either lack the required specificity and sensitivity or are too elaborate and lengthy. Other GLC methods reported (12–14) were either not "adapted" for biological specimens or are time consuming.

This paper describes a sensitive and specific GLC method for chlorpheniramine determination in human plasma under conditions where only chlorpheniramine maleate was orally administered. The method was successfully applied in a crossover study

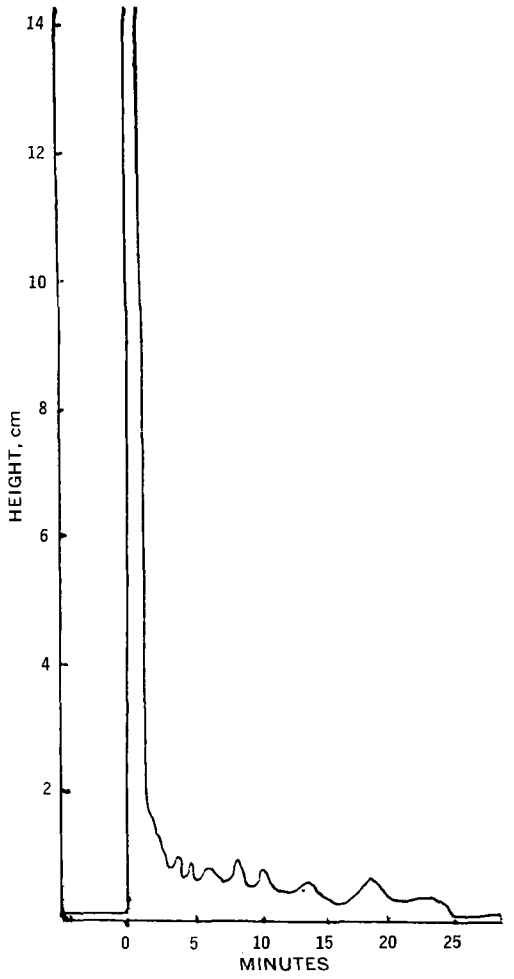

Figure 1—*Gas chromatogram showing plasma extract from Subject A before chlorpheniramine administration. Curve represents 2-μl injection of 50 μl of chloroform extract.*

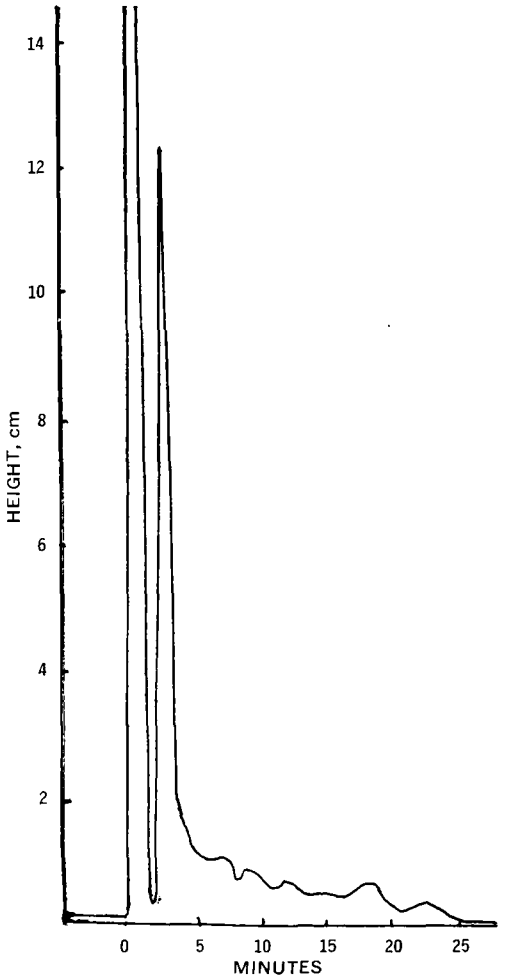

Figure 2—*Gas chromatogram showing plasma extract from Subject A plus 0.6 μg of chlorpheniramine maleate USP reference standard before chlorpheniramine administration. Curve represents 2-μl injection of 50 μl of chloroform extract.*

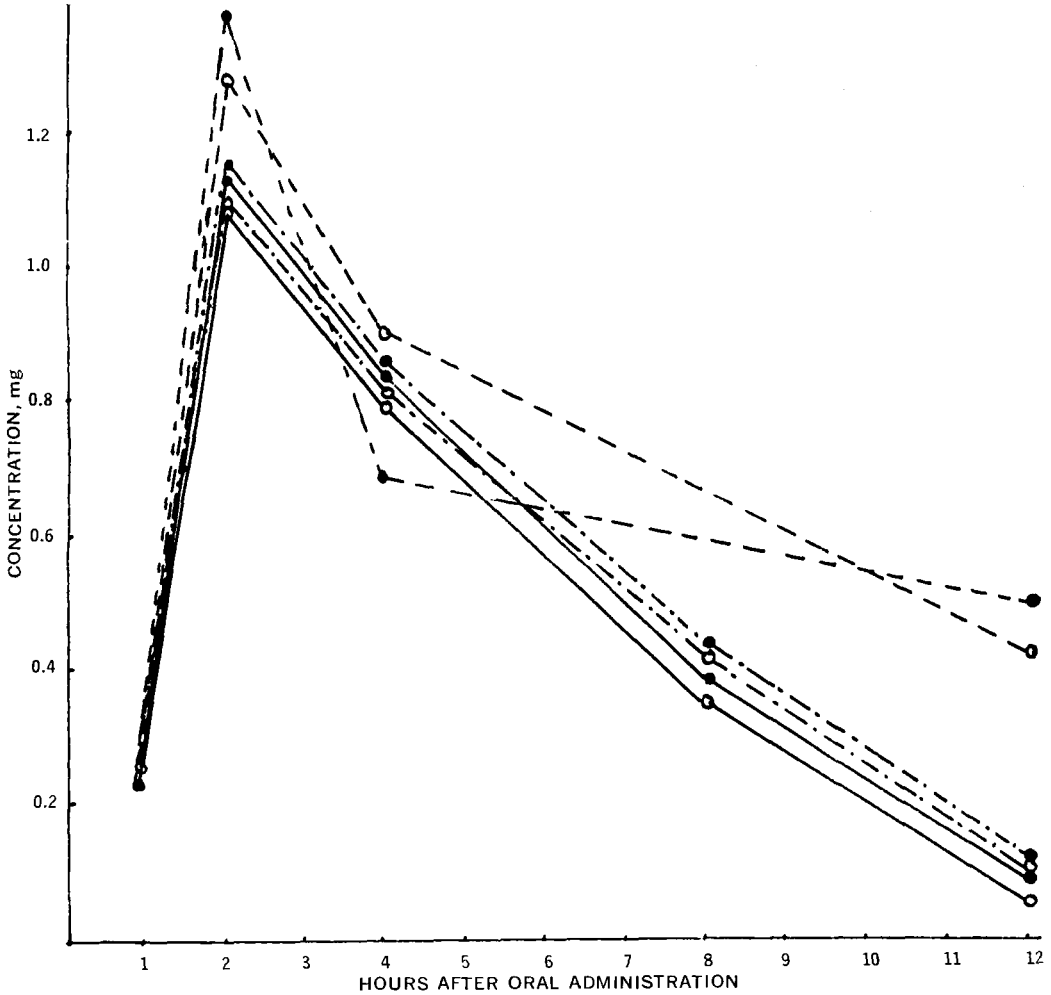

Figure 3—*Chlorpheniramine plasma levels after 4 mg oral administration. Key:* ○, *new formula, 4 mg chlorpheniramine maleate;* ●, *commercial product, 4 mg chlorpheniramine maleate;* ——, *Subject A;* – – –, *Subject B; and* ·–·–·, *Subject C.*

for comparative bioavailability of chlorpheniramine maleate from syrup formulations.

EXPERIMENTAL

Reagents and Chemicals—The reagents and chemicals used were: sodium hydroxide, chloroform, and sodium oxalate, all analyzed reagents[1]; SE-30[2], 60–80-mesh Chromosorb W DMCS[3]; and chlorpheniramine maleate USP reference standard.

Instrumentation—A gas chromatograph[4] equipped with dual hydrogen flame-ionization detectors was used for GLC analysis, using a 1.8 m (6 ft) long and 0.3 cm (0.125 in.) o.d. stainless steel column packed with 5% SE-30 on 60–80-mesh Chromosorb W DMCS. The following conditions were used: column temperature, 250°; injection port temperature, 270°; detector block temperature, 250°; hydrogen flow rate, 25 ml/min; air flow rate, 250 ml/min; and nitrogen carrier gas flow rate, 50 ml/min. Chromatograms were recorded on a 1-mv recorder[5].

Determination of Chlorpheniramine in Human Plasma—Three male Caucasian subjects, 27–38 years of age, in normal state of health as determined by prior physical and laboratory medical examination, were used. Each was administered an oral dosage of 8 ml of syrup (equivalent to 4 mg of chlorpheniramine maleate). At least 96 hr elapsed between the administration of the new formula and the commercial samples[6]. Two milliliters of blood was taken from each individual at 1, 2, 4, 8, and 12 hr after administration.

Prior to the administration of each syrup, an equal volume of blood was taken from each individual and extracted in the same way as the test samples. A portion of the chloroform extract of the normal plasma was used as a blank when injected alone into the gas chromatograph (Fig. 1), while the second portion was used for quantitative calculation. Two to three drops of 4% sodium oxalate aqueous solution were added to each blood sample and the plasma was immediately separated by centrifuging. The plasma was made alkaline (pH 11) by adding drops of 10% sodium hydroxide aqueous solution. The drug was extracted from the alkaline plasma with 2 × 15 ml of chloroform.

The chloroform extracts were pooled and evaporated to dryness under vacuum at 60° and the residue was dissolved in 50 μl of chloroform. Two microliters of this solution was injected into the gas chromatograph previously set up as mentioned. The injection technique of Rader and Aranda (15) was used to eliminate the need for an internal standard. Since the peaks obtained were sharp and narrow and their retention time was less than 5 min, calculations were based on peak heights.

The chlorpheniramine plasma level of the test blood samples was calculated by the volume of blood in the whole body to give a total plasma level of the body, using the equation of Wasserman *et al.* (16), for each determination.

The validity of this method was verified by adding various known amounts of chlorpheniramine maleate USP reference standard to the blank plasma. This plasma was extracted according to the described method, and 2 μl was injected into the gas chromatograph. A linear relationship was observed when peak heights were plotted *versus* amounts injected. The range of linearity was found to extend from 0.1 to 0.7 μg. Several samples of plasma taken at 1, 2, 4, 8, and 12 hr were pooled, extracted, and chromatographed.

The material obtained in the chlorpheniramine peak was

[1] J. T. Baker Chemicals Co.
[2] Varian Aerograph.
[3] Johns-Manville.
[4] Varian Aerograph model 204-IC.
[5] Honeywell Electronik-16.
[6] Chlor-Trimeton Syrup, Schering Corp., Bloomfield, NJ 17003

trapped and analyzed with a mass spectrophotometer[7]. The mass spectrum of the collected chlorpheniramine peak was identical with that obtained from a USP reference standard. The absence of the two mass peaks at *m/e* 44 and 30 in the mass spectrum supported the absence of the two known chlorpheniramine metabolite structures, *N*-monodesmethyl and *N*-didesmethyl, from the collected peak of the gas chromatograms (7).

A summary of the recovery results of chlorpheniramine obtained with human plasma is presented in Table I. A typical chromatogram is shown in Fig. 2.

RESULTS AND DISCUSSION

The quantity of chlorpheniramine found in the plasma of the three subjects after oral administration is shown in Table II. The pattern of chlorpheniramine plasma level is shown in Fig. 3.

There was a sharp rise in chlorpheniramine plasma levels from zero to 2 hr after the administration of 4 mg of the drug of both comparative samples and then a smooth, gradual decline from 2 to 12 hr (Figs. 4 and 5). No plasma samples were taken after this 12-hr sample period.

The total percentage of recovery of chlorpheniramine in plasma of each individual after the oral dosage compared favorably for both the newly developed formula and the commercial product samples.

The standard deviations of the plasma recovery of chlorpheniramine in three subjects compared favorably with the two samples (Table II). The *t* value for the *t* test of significance between the two samples was calculated with the pooled variance and found to be 0.1221. The degree of freedom in the pooled standard deviation was 28; in the *t* table (17), for $p = 0.05$, $df = 28$, *t* was given as 2.048. The calculated *t* was much less than this value. Therefore, it is concluded that there is no significant difference between the newly developed formula and the commercial sample taken as a standard in this investigation.

Table I—Recovery of Chlorpheniramine after *In Vitro* Addition to Human Plasma

Added, μg/ml	Mean Recovery[a], μg/ml	± *SD*
1	1.10	±0.25
3	2.99	±0.248
5	4.99	±0.303
7	7.12	±0.286

[a] Average of five determinations.

CONCLUSIONS

A specific and sensitive GLC method for the determination of chlorpheniramine in human plasma was presented. When used in a two-way crossover bioavailability study on plasma samples of the subjects under test, the method showed that the drug was easily absorbed into the blood from its oral syrup formulations at a dose level of 4 mg. The difference between the bioavailability results of

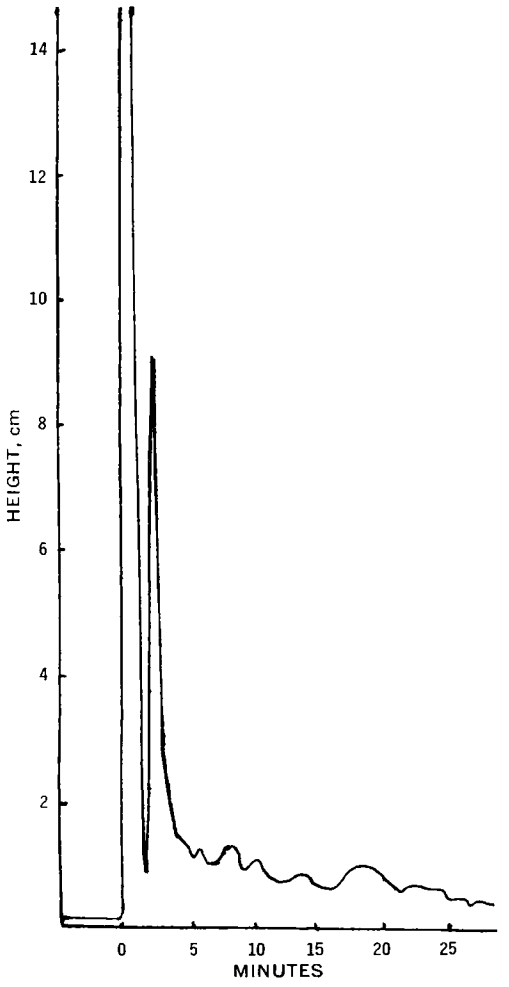

Figure 4—*Gas chromatogram showing plasma sample for Subject A 2 hr after oral administration of the new formula. Curve represents 2-μl injection of 50 μl of chloroform extract.*

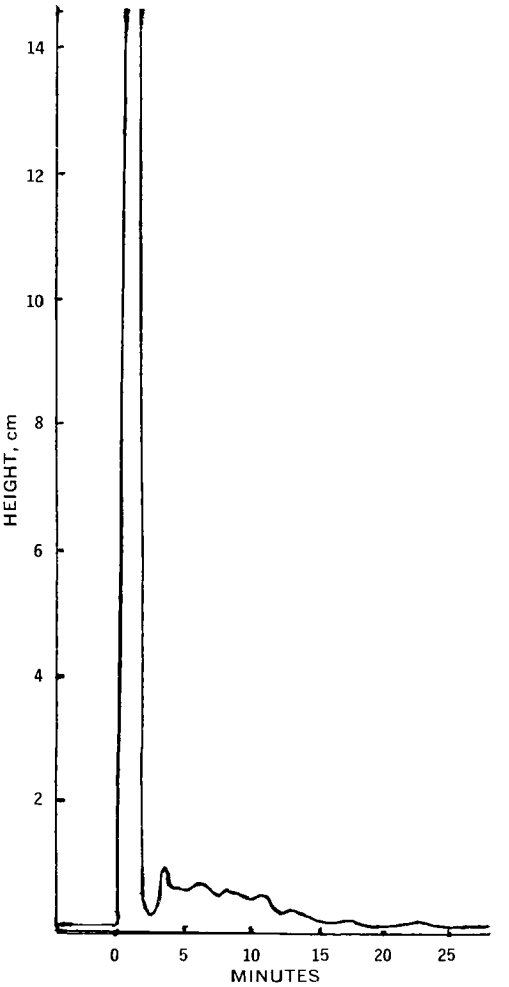

Figure 5—*Gas chromatogram showing plasma sample for Subject A 12 hr after oral administration of the commercial product. Curve represents 2-μl injection of 50 μl of chloroform extract.*

[7] Associated Electrical Industry mass spectrometer 902.

Table II—Chlorpheniramine Plasma Levels after 4 mg Oral Administration

Subject[a]	Sample[b]	Concentration, μg/2 μl[c] 1 hr	2 hr	4 hr	8 hr	12 hr	Amount, mg, in Whole Body Plasma in 12 hr[d]	Total Recovery, %	Standard Deviation of Three Subjects	t^e Value
A	a	0.1087	0.4414	0.3268	0.1400	0.0280	2.567	64.4	a ± 0.150	0.1221
38, 79	b	0.1129	0.4500	0.3354	0.1511	0.0425	2.688	67.2	b ± 0.153	0.1221
B	a	0.0780	0.3690	0.2565	—[f]	0.1185	2.836	70.9	a ± 0.150	0.1221
27, 110	b	0.0720	0.3948	0.1995	—[f]	0.1443	2.796	69.9	b ± 0.153	0.1221
C	a	0.1087	0.4421	0.3112	0.1575	0.0488	2.636	65.9	a ± 0.150	0.1221
33, 78	b	0.1125	0.4579	0.3431	0.1684	0.0521	2.792	69.8	b ± 0.153	0.1221

[a] The first number is for age (years), and the second number is for body weight (kilograms). [b] a = new formula, and b = commercial product. [c] Obtained by calculation from the reading values of that of USP reference standard. [d] Obtained by calculating the volume of blood in the whole body to give a total plasma level of body. [e] t Test of significance where $t = (\bar{x}_1 - \bar{x}_2)/S\sqrt{(n_1n_2/n_1 + n_2)}$; t value calculated with the pooled variance of the two syrup samples. [f] No blood sample was taken.

the newly developed formula and the commercial product taken as a standard in this investigation was insignificant.

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