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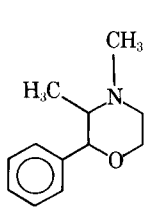
GLC Determination of Phendimetrazine in Serum

H. K. L. HUNDTˣ, E. C. CLARK, and F. O. MÜLLER

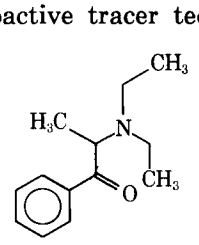
Abstract □ A sensitive, specific, and quantitative GLC method for the determination of phendimetrazine in serum is described. The procedure involves the addition of an internal standard to serum samples, followed by extraction at pH 13 into toluene. The extracted bases are back-extracted into 1 ml of 4 *M* hydrochloric acid and again into 100 μl of chloroform after making the 4 *M* hydrochloric acid extract basic with 1.5 ml of 4 *M* sodium hydroxide. The sensitivity of the method is such that 25 ng of material can be detected in 5 ml of serum

Keyphrases □ Phendimetrazine—GLC analysis in human serum □ GLC—analysis, phendimetrazine in human serum

Phendimetrazine (I) is an anorexigenic agent marketed in South Africa in the form of a standard formulation tablet. To satisfy the requirements of the local Drugs Control Council, the introduction of a timed-release preparation of I on the South African market necessitated the development of a sensitive quantitative method for the detection of I so that serum levels attained after administration of a single timed-release tablet (containing 105 mg of phendimetrazine bitartrate) could be compared with the levels attained after administration of a single tablet of the standard formulation (containing 35 mg of phendimetrazine bitartrate). Although various articles refer to the clinical and toxicological aspects (1–7) of I and its qualitative identification by TLC (8) and GC (9), only one reference dealing with the quantitative determination of phendimetrazine in plasma utilizing a ^{14}C-radioactive tracer technique (10) and one dealing with *in vitro* release studies of timed-release phendimetrazine (11) could be found.



I



II

This report describes a sensitive GLC method for I determinations, under conditions where only I was administered, using diethylpropion (II) as an internal standard.

Table I—Recovery of Phendimetrazine from Serum

Amount Added, ng/ml	Amount Recovered, ng/ml				Mean ± *SD*, ng/ml
20	20.2	20.7	20.9	19.4	20.30 ± 0.67
40	38.9	38.9	37.7	37.9	38.35 ± 0.64
60	59.0	61.2	61.8	62.3	61.08 ± 1.45
80	79.6	80.7	79.5	79.0	79.70 ± 0.71

EXPERIMENTAL

Reagents and Chemicals—The chemicals and reagents used were: 2 *M* sodium hydroxide[1], 4 *M* sodium hydroxide, 4 *M* hydrochloric acid[1], toluene[1], chloroform[1], phendimetrazine bitartrate[2], and diethylpropion hydrochloride[3].

Instrumentation—A gas chromatograph[4] equipped with a flame-ionization detector and a 183-cm × 6-mm glass column containing 3% SE-30 on Chromosorb W (AW HMDS), 80–100 mesh, was used. The injection port and detector were kept at 250°. The column temperature was kept at 140° for 8 min after injection and then programmed at 32°/min to 250°, where it was kept for 8 min. Nitrogen was used as the carrier gas at a flow rate of 80 ml/min. Quantitation was achieved by measuring peak heights. (Peak area calculation by a digital integrator was found to be less reliable.)

Measurement of I in Serum—To 5 ml of serum contained in a 20-ml glass-stoppered centrifuge tube were added 0.5 μg of internal standard (in 50 μl of water), 1 ml of 2 *M* sodium hydroxide, and 5 ml of toluene. The tube was shaken for 2 min (vortex) and centrifuged, and as much as possible of the organic phase was transferred to a 10-ml, glass-stoppered, tapered centrifuge tube contain-

[1] E. Merck, Darmstadt. Pro analysi.
[2] Supplied by Rio Ethicals (Pty.) Ltd.
[3] Supplied by Mer-National Laboratories.
[4] Hewlett-Packard model 5700 A.

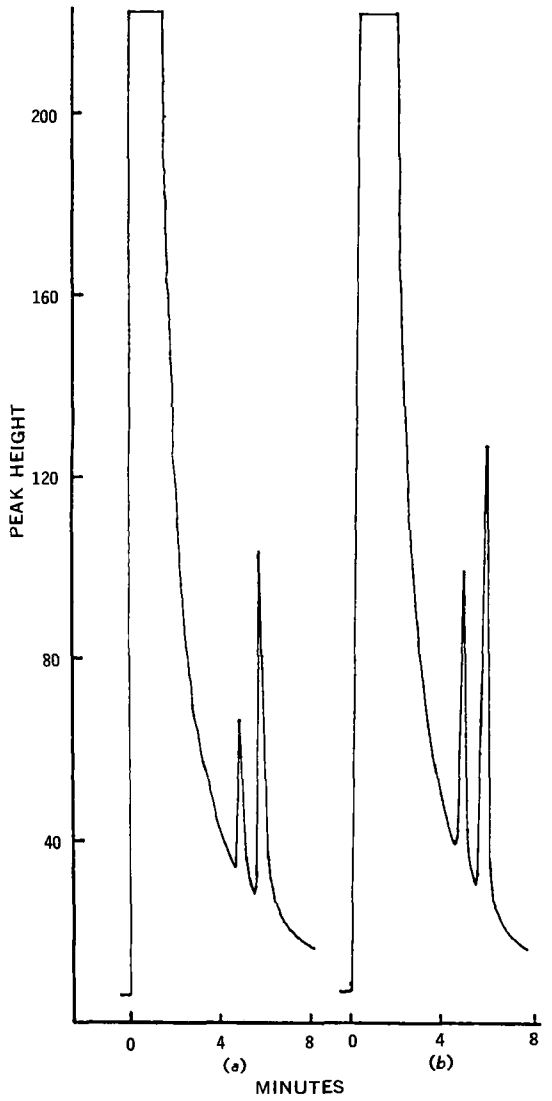


Figure 1—*Chromatograms of* (a) *I and II added to control serum and carried through extraction, and* (b) *material obtained from biological specimens after addition of II (internal standard).*

ing 1 ml of 4 *M* hydrochloric acid. After shaking for 2 min, the tube was centrifuged and the organic phase was removed by aspiration.

The aqueous phase was transferred to a special 5-ml glass-stoppered centrifuge tube equipped with a small nipple in which liquids denser than water can collect after centrifuging. The centrifuge tube was cooled for 2 min in an ice bath, the aqueous phase was made strongly alkaline by addition of 1.5 ml of 4 *M* sodium hydroxide, and the free bases were extracted into 0.1 ml of chloroform (vortex, 2 min). After brief centrifugation, 5 μl of the chloroform phase was injected into the gas chromatograph. The retention times of I and II were 292 and 345 sec, respectively.

Standard serum curves were constructed by plotting the peak height ratios (I/II) *versus* weight ratios (I/II) of standard samples which were run simultaneously with the unknown samples as previously described. The peak height ratio obtained from an unknown was then used to determine the amount of I present.

Absorption Studies—One tablet of the standard formulation was administered to each of five healthy human subjects who had fasted overnight. Blood was collected by venipuncture before administration of the drug and at intervals after administration. The serum was separated by centrifugation and frozen until assayed.

For assay, equal amounts of serum of each volunteer were pooled for each sample and the samples were analyzed in duplicate as previously described. After a washout period of 3 days, the same procedure was followed with the administration of one tablet of the timed-release preparation to the same volunteers.

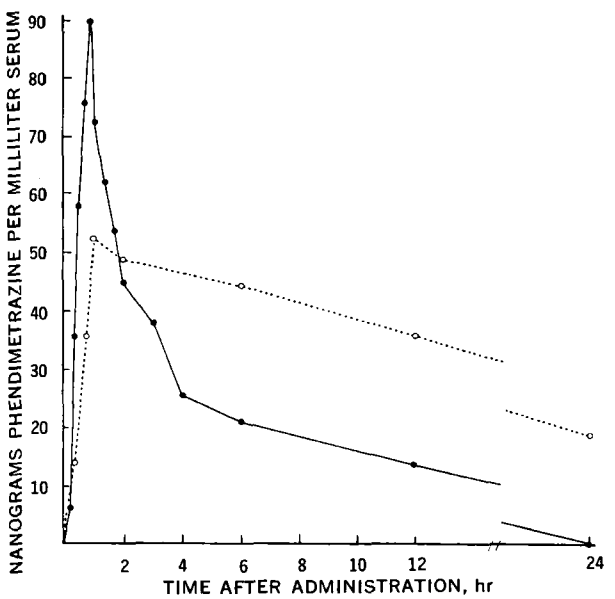


Figure 2—*Pooled serum levels of I after administration of standard formulation* (●) *and timed-release formulation* (○).

RESULTS AND DISCUSSION

Figure 1 represents the chromatogram of: *(a)* I and II added to control serum and carried through the extraction and GLC procedure, and *(b)* material obtained from biological specimens after the addition of II as the internal standard.

Figure 2 represents pooled serum levels of I after administration of the standard formulation and the timed-release formulation to the same five volunteers.

Gas chromatograms of control samples taken from the volunteers before any drug had been administered did not exhibit any peaks with retention similar to I or II. Possible interference by metabolites of I could not be checked because these metabolites were

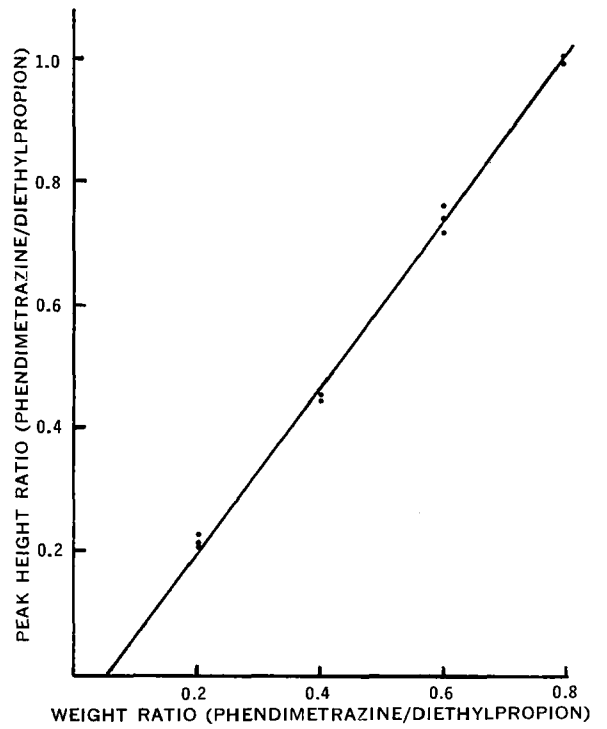


Figure 3—*Relationship between peak height ratio and weight ratio (phendimetrazine/diethylpropion). Five microliters was injected in each case out of 100 μl following extraction from serum.*

not available. However, a small peak with a retention time of 252 sec appeared occasionally in extracts of serum from volunteers but did not interfere with the assay; it was attributed to a metabolite.

A typical standard graph of peak height ratio (I/II) *versus* weight ratio (I/II) is presented in Fig. 3. A straight line with a slope varying between 1.31 and 1.35 and an intercept varying between 0.04 and 0.06 on the weight ratio axis was obtained by linear regression analysis over 1 month during which the analyses were performed. The intercept on the weight ratio axis corresponds to a loss of 0.8–1.2 ng of I on the GLC column during chromatography, thus imposing a lower limit of detection of I in serum by this method at about 5 ng/ml. A peak for I just begins to appear in the chromatogram at this concentration. A summary of the recovery results of I obtained with human serum when 20–80 ng/ml was added to the serum is presented in Table I.

This GLC method was applied to a large number of samples of human serum during a trial comparing serum levels attained after administration of a standard formulation with levels attained after administration of a timed-release formulation of I.

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Colorimetric Determination of Formaldehyde *via* Free Radical Formation

JAMES W. MUNSON [x] and THOMAS G. HODGKINS

Abstract □ Aliphatic aldehydes react with 2,3-dimethyl-2,3-bis(hydroxylamino)butane and sodium periodate to form colored free radicals. These radicals were stabilized with pyridine in aqueous solution. Low levels of formaldehyde in aqueous solutions were determined utilizing this reaction.

Keyphrases □ Formaldehyde—stabilized colorimetric analysis in aqueous solution *via* reaction with 2,3-dimethyl-2,3-bis(hydroxylamino)butane and sodium periodate □ 2,3-Dimethyl-2,3-bis(hydroxylamino)butane—colorimetric reagent for determination of formaldehyde in aqueous solution □ Colorimetry—analysis, formaldehyde in aqueous solution using 2,3-dimethyl-2,3-bis(hydroxylamino)butane

Aldehydes have been shown to form stable free radicals when reacted with 2,3-dimethyl-2,3-bis(hydroxylamino)butane (I) (1) (Scheme I). The anhydro product (II) can be isolated directly or may be converted to the free radical (III) by the action of lead dioxide or sodium periodate. The free radicals are red or blue in solution. The color of these free radicals suggests that this reaction may be suitable for colorimetric determination of aldehydes.

Preliminary data suggested that only aliphatic aldehydes and ketones react with I to form a colored product in aqueous solution. A spot test based on this reaction was developed that allows detection of as little as 0.2 μg of aliphatic aldehydes (2). Aliphatic ketones gave a much smaller response, with 100–200 μg being the minimum detectable quantity.

Initial attempts to utilize this reaction in a quantitative manner were plagued by the instability of the color of the free radical. The half-life for the disappearance of color was about 7 min at 25°. This paper reports the stabilization of the color and the adaptation of this reaction to the determination of formaldehyde at low concentrations in aqueous solution.

EXPERIMENTAL

Materials—Formaldehyde stock solutions were analyzed by peroxide oxidation (3). Acetaldehyde was redistilled and chilled. A portion of acetaldehyde was added to a tared flask, sealed, and reweighed. Water was then added to give a known concentration. All other aldehyde stock solutions were prepared by dissolving weighed amounts of the redistilled aldehyde in water. 2,3-Dimethyl-2,3-bis(hydroxylamino)butane sulfate[1] was recrystallized from a 2-propanol–water mixture, mp 185° dec.

Anal.—Calc. for $C_6H_{18}N_2O_6S$: C, 29.27; H, 7.32; N, 11.38. Found: C, 27.78; H, 7.54; N, 11.12.

Sodium periodate[2] and the organic solvents were reagent grade and were used directly.

Effect of Solvent on Stability of Color—An aqueous reaction mixture containing formaldehyde (1.34×10^{-3} *M*) and 2,3-dimethyl-2,3-bis(hydroxylamino)butane sulfate (Ia) (1.76×10^{-2} *M*) was prepared and allowed to react for 1 hr at 25°. One-milliliter aliquots of the reaction mixture were added to a tube containing 1 ml of the solvent being studied and 1 ml of sodium periodate (0.094 *M*).

[1] Eastman Organic Chemicals

[2] J. T. Baker Chemical Co.