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THE DETERMINATION OF PHENDIMETRAZINE AND PHENMETRAZINE IN BIOLOGICAL FLUIDS AND IN DOSAGE FORMS *

by

G. Long and J. McHan

Biopharmaceutics, Inc, 1231 West Peachtree, Atlanta, Ga.

and

S.Z. Masih, M.K. Masih, and K.C. Jacob

Reid-Provident Laboratory, 5th Street, Atlanta, Ga.

ABSTRACT

A highly sensitive gas chromatographic assay was developed for the simultaneous determination of phendimetrazine and its N-demethylated metabolite, phenmetrazine. The analytical procedure proved adaptable for the determination of these drugs in dosage formulations as well as biological fluids. The procedure involves the

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addition of Isoxsuprine as the internal standard and extraction into diethyl ether. The two drugs along with the internal standard were well resolved on an SP-2250 column using helium as the carrier gas.

INTRODUCTION

Phendimetrazine and phenmetrazine have each been separately quantitated in both urine and serum by several gas chromatographic techniques. The techniques often require derivitization and an exhaustive clean-up procedure before chromatographing. Techniques for measuring phendimetrazine also exclude the metabolite phenmetrazine which would reduce the utility of the method in pharmacokinetic studies of phendimetrazine. This report describes a simple, rapid and reliable method for the simultaneous determination of phendimetrazine and phenmetrazine in both serum and urine. The method was also easily adaptable for the measurement of phendimetrazine in pharmaceuticals. Therapeutic and toxic levels for both drugs were within the linear range of the method.

EXPERIMENTAL

Material and Reagents-- Phendimetrazine hydrochloride was purchased from Applied Science; Phenmetrazine hydrochloride was furnished by Geigy and Isoxsuprine was furnished by Mead Johnson. Benzene (gas chromatographic grade), anhydrous diethyl other (peroxide free), isopropanol (HPLC grade) and

potassium carbonate (anhydrous certified ACS) were purchased from Fisher Scientific. Concentrated hydrochloric acid was received from J.T. Baker Chemical Company.

Standard Solutions-- A stock standard solution of phendimetrazine hydrochloride and phenmetrazine hydrochloride was prepared by dissolving 25 mg of each into 100 ml of isopropanol to obtain a concentration of 250 mg/L each. The stock standard was appropriately diluted with water to make working standards ranging from one to 500 ug/L.

Internal Standard--A stock solution of Isoxsuprine was prepared by dissolving 100 mg in 100 ml of isopropanol to achieve a concentration of 1000 mg/L. The stock solution was diluted 1:25 with isopropanol to give a working internal standard of 40 mg/L.

Extraction Methods-- Into separate 40 ml extraction vials was added 3 ml of standard solution, 3 ml of Quality Control, or 3 ml of patient plasma. One ml of 10% potassium carbonate was added to each tube followed by 50 ul of working internal standard. Twenty ml of anhydrous diethyl ether was then added to each vial. The vials were capped and gently shaken for 5 minutes on a horizontal shaker and then centrifuged for 5 min. at 2000 rpm. The lower aqueous layer was carefully aspirated and discarded. The remaining ether layer was transferred to a clean 40 ml vial. Three ml of 0.5 N HCl was added to each tube. The tubes were capped and vigorously shaken for 5 minutes and again centrifuged for 5 minutes at 2000 rpm.

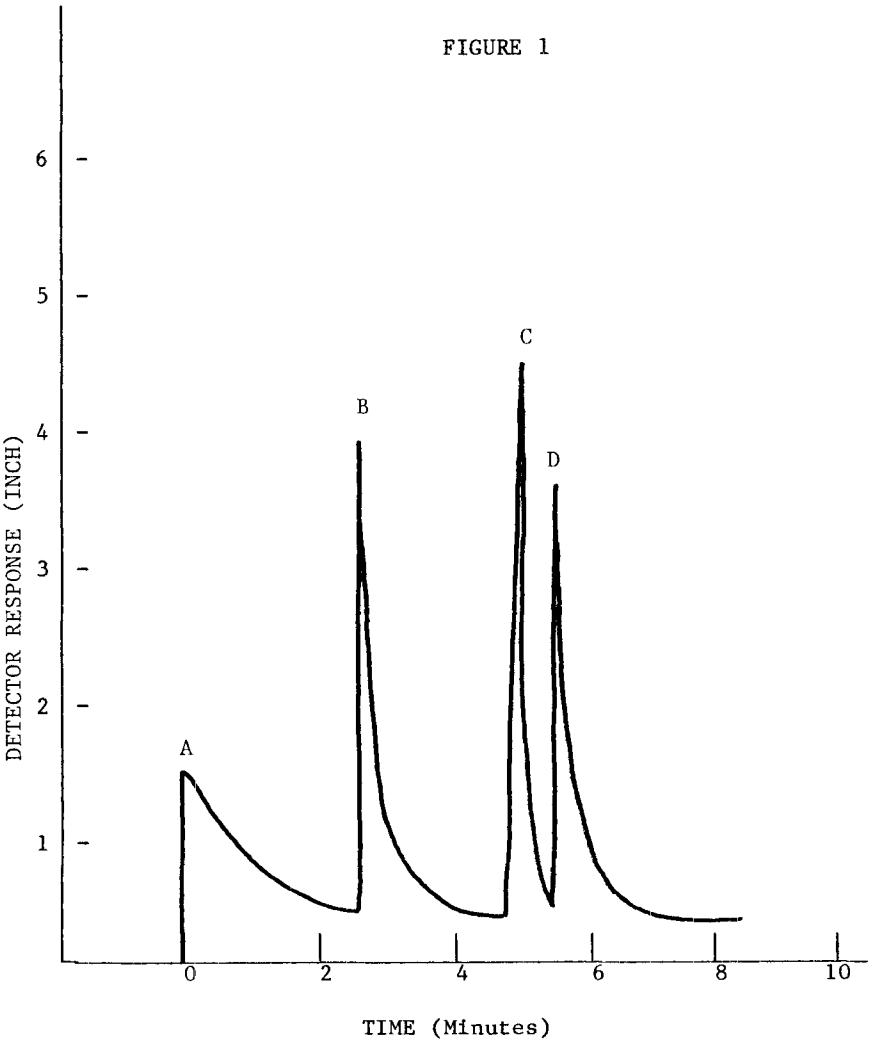
The upper ether layer was aspirated and discarded. Two ml of 10% potassium carbonate and 10 ml of anhydrous diethyl ether were added to the remaining aqueous phase. The samples were again capped, shaken and centrifuged as before. The ether layer was transferred to clean 15 ml test tubes (16 x 125 mm) and evaporated to dryness in a water bath at 56°C. Prior to injection into the Gas chromatograph each sample was reconstituted with 50 ul of benzene. The volume injected was 5 ul. The urine samples and the aqueous extracts from the dissolution studies were first diluted with water then subjected to the same procedure.

Gas Chromatograph-- A Hewlett Packard 5840A gas chromatograph equipped with NPD and a strip chart recorder was used. The column was 6 ft. X 2 mm ID packed with 3% SP-2250 on 100-120 supelcoport. Helium was used as the carrier gas with a flow rate of 30 ml/min. The column oven was operated isothermally at 155°C. Quantitation was based on peak height ratios. The injector and detector temperatures were 250°C and 350°C respectively.

Results and Discussion-- The internal standard, phendimetrazine and phemetrazine were well resolved using the described conditions. Figure 1 typifies the chromatogram of these after extracting from human serum.

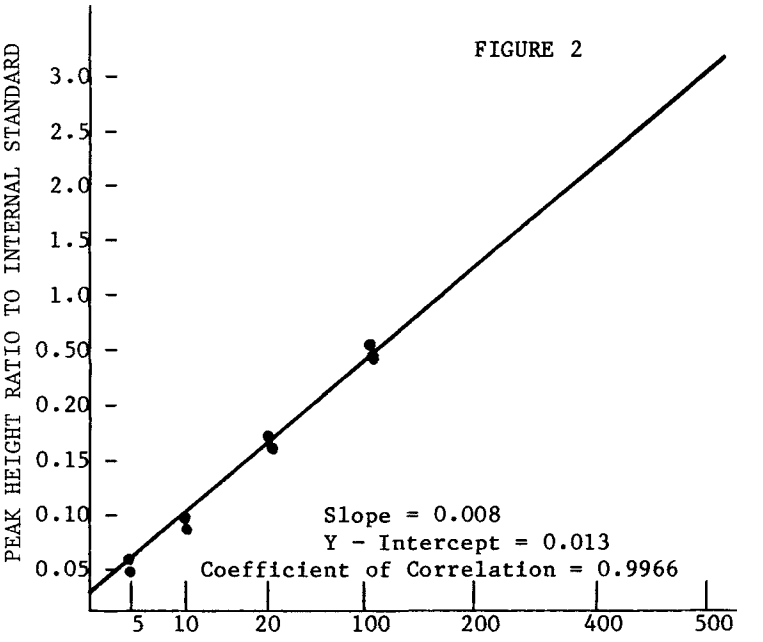
The linear range for phendimetrazine and phenmetrazine was 10 ug/L to 500 ug/L with a correlation coefficient of 0.9966 for phendimetrazine (Figure 2), -

FIGURE 1



Typical Gas Chromatogram of Phendimetrazine from Human Serum.

A = Benzene Solvent Peak; B = Isoxsuprine (internal standard); C = Phendimetrazine (parent drug); D = Phenmetrazine (metabolite)



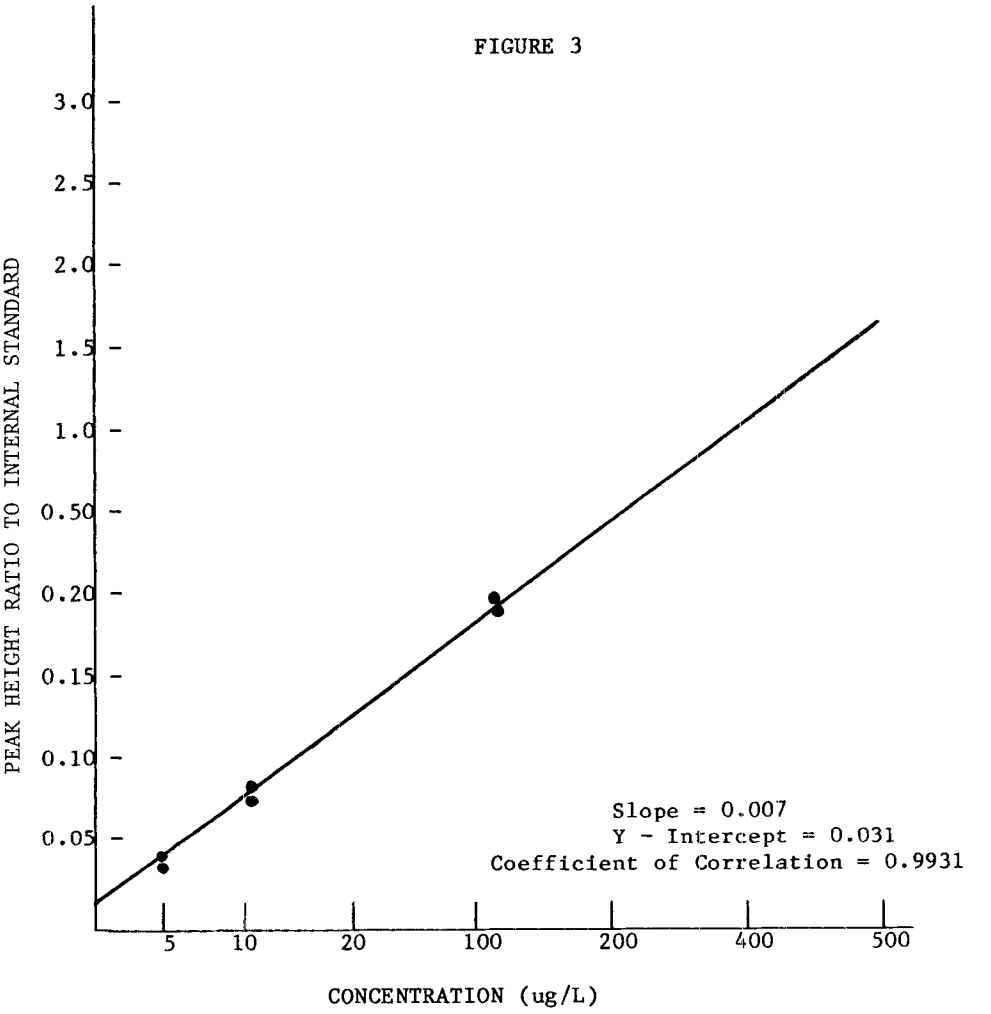
GLC Calibration Curve for Phendimetrazine in Plasma and Water

- and a correlation coefficient of 0.9931 for phenmetrazine (See Figure 3).

A control of both phendimetrazine and phenmetrazine gave a CV of 4.0 for both drugs. A day to day CV was determined from data generated over a period of 4 months. (Table 1).

The method was relatively free of interference from other drugs in this class. Table 2 contains a list of drugs which were evaluated. Only diethylpropion interferred with the quantitation of phenmetrazine.

FIGURE 3



GLC Calibration Curve for Phenmetrazine
in Plasma and Water

TABLE 1 - ANALYSIS OF CONTROL SAMPLES

Amount of Phendimetrazine Added (ug/L)	Mean Recovered (ug/L)	Mean Recovery %	SD
50 (N = 20)	49.6	99.2	4.52
100 (N = 20)	98.2	98.2	3.76

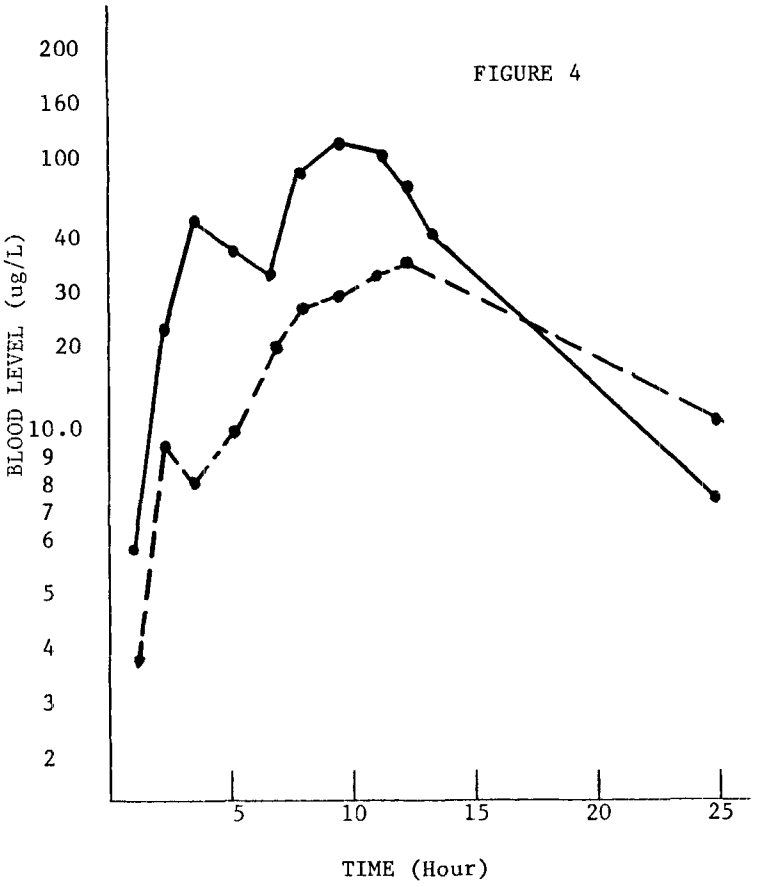
Amount of Phenmetrazine Added (ug/L)	Mean Recovered ug/L	Mean Recovery %	SD
50 (N = 20)	49.3	98.6	5.64
100 (N = 20)	100.3	100.3	5.63

TABLE 2 - CNS STIMULANTS TESTED FOR INTERFERENCE

Amphetamine	Mephentermine
Methamphetamine	Phentermine
Benzphetamine	Propylhexadrine
Methylphenidate	Cyclopentamine
Ephedrine	Tuaminoheptane
Hydroxyamphetamine	Naphazoline
Phenylephrine	Tetrahydrozoline
Xylometazoline	Oxymetazoline
Nylidrin	Methoxyphenamine
Phenylpropanolamine	Fenfluramine
	Diethylpropion*

*Drug eluted at the same time as phenmetrazine

FIGURE 4

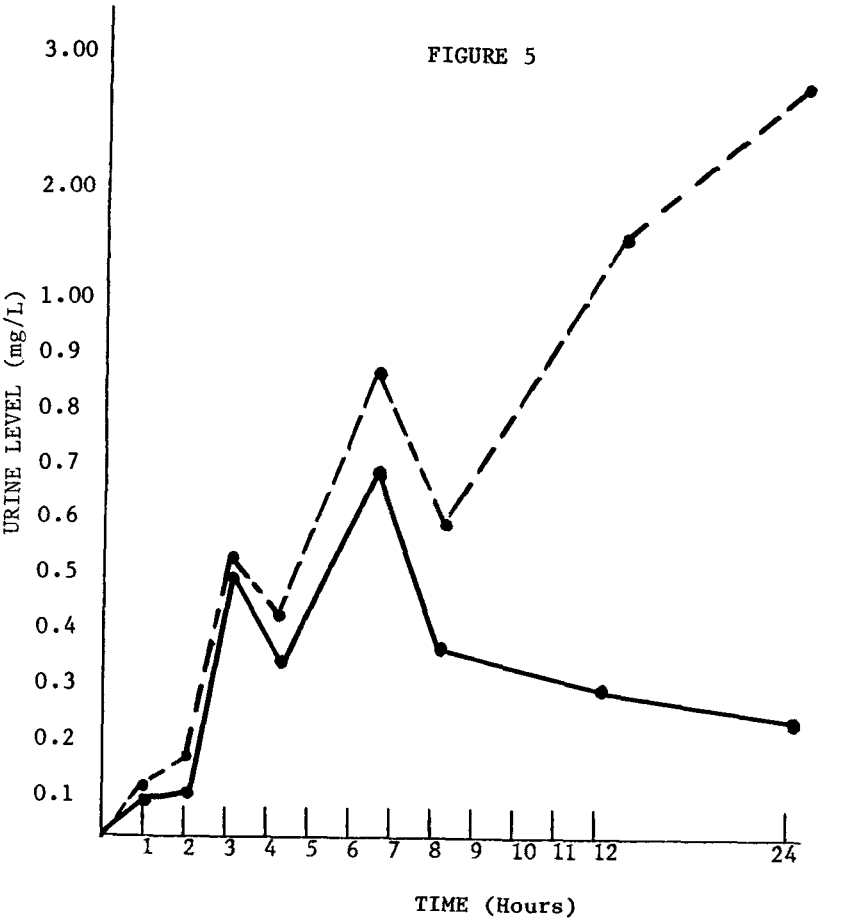


BLOOD LEVEL TIME PROFILE OF PHENDIMETRAZINE AND ITS METABOLITE PHENMETRAZINE FOLLOWING ORAL ADMINISTRATION OF 105 mg. SUSTAINED RELEASE CAPSULES ONCE A DAY TO HUMAN VOLUNTEERS SUBJECT - BP

(-) S-105 mg. SUSTAINED RELEASE CAPSULES
(--) S-105 METABOLITE

The method was evaluated in a pharmacokinetic study of phendimetrazine. The serum levels of phendimetrazine and phenmetrazine were easily estimated using this method (Figure 4).

FIGURE 5



URINARY EXCRETION FOLLOWING ORAL ADMINISTRATION OF A TIME-SUSTAINED PHENDIMETRAZINE FORMULATION.
SUBJECT - BP (AVERAGE URINE PH = 5.5

(-) S-105 mg. PHENDIMETRAZINE
(--) S-105 mg. PHENMETRAZINE METABOLITE

After the appropriate dilution of urine samples urine levels were easily estimated (Figure 5).

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3. A.H. Beckett and M.A. Salami, J. Pharm. Pharmac., 24, 900 (1972).