GLC Determination of 1-(2,6-Dimethylphenoxy)-2-aminopropane in Urine

NAOMI SZINAI*, ROBERT J. PERCHALSKI*, RICHARD H. HAMMER†▲, B. JOE WILDER*, and RICHARD R. STREIFF*

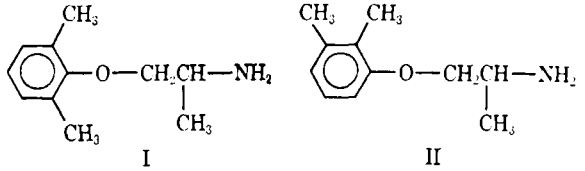
Abstract □ A rapid, sensitive, and accurate GLC method of analysis of a new anticonvulsant drug, 1-(2,6-dimethylphenoxy)-2-aminopropane, was developed, utilizing an acid hydrolysis procedure to increase its degree of recovery from urine and employing one of its isomers, 1-(2,3-dimethylphenoxy)-2-aminopropane, as an internal standard.

Keyphrases □ 1-(2,6-Dimethylphenoxy)-2-aminopropane—GLC analysis in urine □ GLC—analysis, 1-(2,6-dimethylphenoxy)-2-aminopropane in urine

A new anticonvulsant drug, 1-(2,6-dimethylphenoxy)-2-aminopropane[1] (I), is presently undergoing clinical testing and metabolic studies in humans (1, 2). Significant anticonvulsant activity has been demonstrated in patients with psychomotor seizures or temporal lobe epilepsy when receiving divided daily doses of 4–8 mg./kg. (1). To evaluate its rate of elimination and other kinetic parameters, a rapid and accurate method of analysis in urine of I was sought. Due to the structural similarity of I to amphetamine and its congeners, several GLC methods of analysis of amphetamine in biological fluids were evaluated from the literature (3–7). The method of Ramsey and Campbell (7) was found to be a simple, accurate, and rapid GLC method which was modified to accommodate the urinary analysis of I. This paper describes a fast (50 samples/day/technician), sensitive, and accurate GLC method of analysis for measuring urinary levels of I in humans.

EXPERIMENTAL

Extraction Procedure—Two milliliters of patient or standard urine was accurately measured and pipeted into a 15-ml. ground-glass conical centrifuge tube, and 1.0 ml. hydrochloric acid solution was added. The tube was stoppered and the mixture was heated in a constant-temperature bath at 100° for 1 hr. After cooling to room temperature, the pH was adjusted to 12 (Hydrion E paper) with a saturated solution of sodium hydroxide. To the alkaline solution, 0.5 ml. of internal standard (II)[2] solution (300 mcg./ml. water) and 0.1 ml. chloroform were accurately measured and added. The free bases were extracted into the chloroform layer by vigorous shaking for 1 min., followed by centrifugation for 4 min. After separation of the two layers, 1.0 μl. of the lower chloroform phase was injected into the gas chromatograph.



GLC—GLC experiments were performed on a dual-channel instrument[3] equipped with four hydrogen flame detectors. The 1.83-m. (6-ft.) long, U-shaped column (2-mm. i.d.) was packed with 3% OV-17 on Gas Chrom Q, 100–120 mesh. Operating temperatures were: injector port, 340°; column, 125° (isothermal); and detector, 260°. Flow rates (milliliters per minute) were: nitrogen, 60; air, 300; and hydrogen, 50.

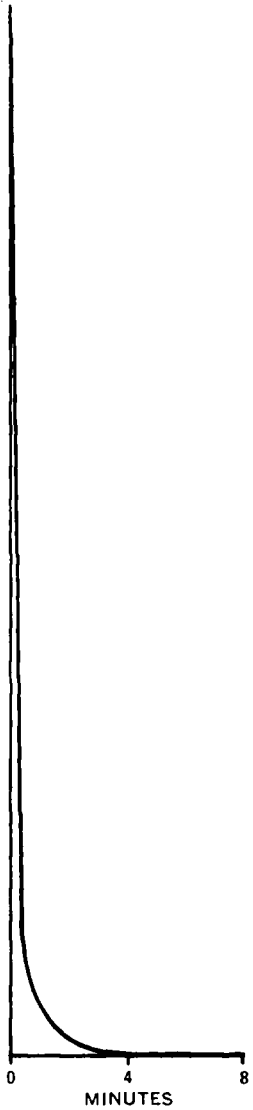


Figure 1—*Chromatogram of hydrolyzed normal urine.*

[1] Designated as KÖ 1173 and supplied as the hydrochloride salt by C. H. Boehringer Sohn, Ingelheim, Germany. Amounts and levels of KÖ 1173 and KÖ 1398 are designated throughout the paper in milligrams of free base rather than hydrochloride salt (1.0 g. KÖ 1173-HCl or KÖ 1398-HCl = 0.83 g. KÖ 1173 or KÖ 1398 free base, respectively).

[2] Designated as KÖ 1398 (II) [1-(2,3-dimethylphenoxy)-2-aminopropane] (isomer of KÖ 1173-I) and furnished as the hydrochloride salt by C. H. Boehringer Sohn, Ingelheim, Germany.

[3] Varian 2100.

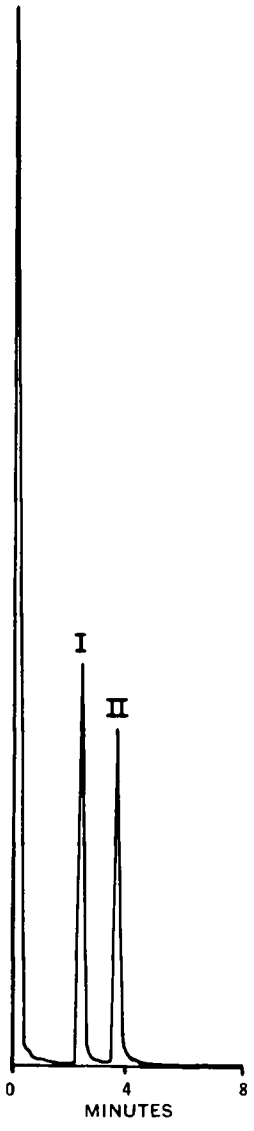


Figure 2—*Chromatogram of hydrolyzed normal urine with drug (I) and internal standard (II) added.*

The standard calibration curve was prepared by measuring the peak height at seven different concentration levels of I (5.06–150.90 mcg./ml. urine) and II (75 mcg./ml. urine) *via* GLC in normal urine after acid hydrolysis and extraction (Figs. 1–3). The mean of the peak height ratio of I:II of six determinations at each concentration level was plotted *versus* its respective concentration level of I (micrograms per milliliter urine). The least-squares method, employed to obtain the best fit for the seven concentration levels, indicated that the peak height ratios of I:II were linear with respect to the concentration of I (Fig. 4).

RESULTS AND DISCUSSION

GLC analysis of urine of epileptic patients who were taking I showed an increase in the chromatographic peak height of I when the urine was hydrolyzed in acid media *versus* the peak height of I measured in nonhydrolyzed urine. Apparently, I exists partly conjugated in urine and acid hydrolysis released the free drug, producing a larger peak height on the chromatogram. The optimum length of time for the urinary acid hydrolysis step was 1 hr. Additional hydrolysis time did not increase the amount of free unconjugated I in urine.

Amphetamine, 2,3-II, and 2,4- and 2,5-dimethyl isomers of I were evaluated as internal standards. Amphetamine had an undesirable short retention time, with its chromatographic peak occurring in front of I close to the solvent front. The most favorable

Table I—Typical Urinary Levels of I in Humans

Patient	Daily Dose, mg.	24-hr. Urine: Volume, ml.	24-hr. Urine: Concentration, mcg./ml.	24-hr. Urine: Total Amount Excreted, mg.
L.J.F.	100	3260	13.5	44.0
J.W.	100	1600	29.7	47.5
L.F.	200	1500	42.3	63.5
F.C.	200	1930	13.5	26.1
L.S.	300	1900	24.1	45.8
T.S.	300	1360	29.8	40.6

marker appeared to be II. It was completely separated from I and possessed a longer retention time than I. Both drugs (I and II) gave sharp, well-defined peaks with no tailing (Figs. 2 and 3). Initially, II was added as an internal standard before acid hydrolysis and then was hydrolyzed along with I for 1 hr. However, the results were erratic and inconsistent, and the standard calibration curve was not reproducible. GLC analysis of I added to standard urine before and after hydrolysis indicated that no acid degradation had taken place in either case. Therefore, the erratic quantitative results were not

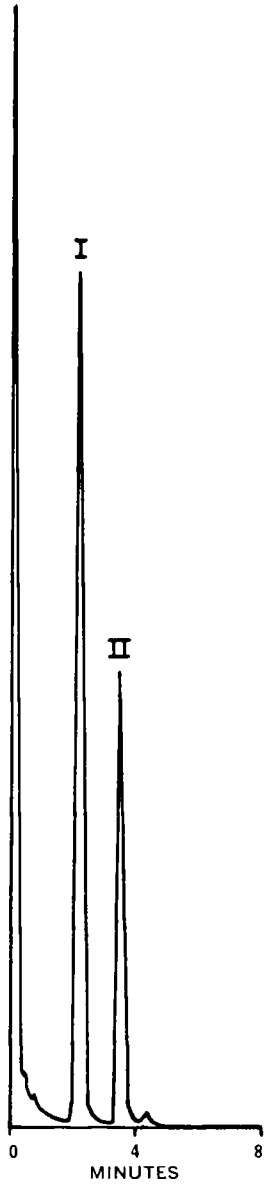


Figure 3—*Chromatogram of hydrolyzed patient urine with internal standard (II) added.*

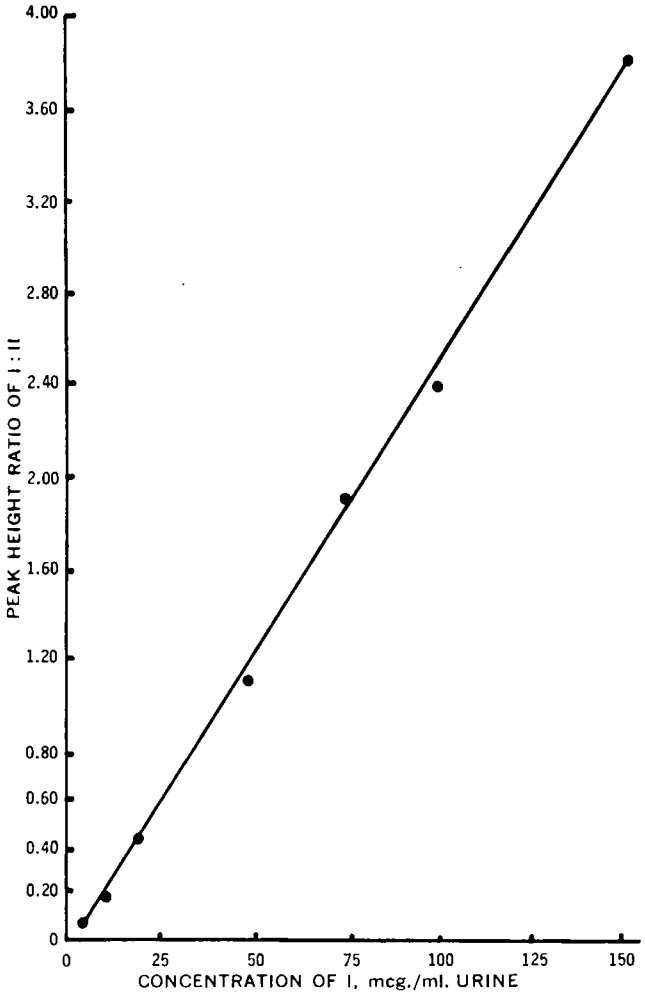


Figure 4—*Standard calibration curve.*

due to degradation of I. Similar studies of II revealed some acid degradation when it was added before hydrolysis. The problem of erratic quantitative results was, therefore, traced to acid degradation of II during the hydrolysis step.

One can postulate that I is stable to acid degradation through steric hindrance imposed by the 2,6-dimethyl groups shielding the ether oxygen to protonation. However, II, with its 2,3-dimethyl group substitution, cannot provide the same degree of steric hindrance as I and would be expected to degrade partially in acid media. In the case of amphetamine, the quantitative results were the same whether it was added before or after the acid hydrolysis, indicating acid stability. When II was added after the acid hydrolysis step, the results were consistent with the values obtained when using amphetamine either before or after hydrolysis. Therefore, the 2,3-dimethyl isomer (II) of I was chosen as the internal standard rather than amphetamine or one of the other isomers of I. The addition of II after the acid hydrolysis step led to reproducible and accurate results, as indicated by the linearity of the standard calibration curve (Fig. 4, mean standard deviation = ±5.9%).

The advantage in using small volumes of extracting solvent (*e.g.*, 0.1 ml. chloroform) *versus* larger volumes is in the reduction of impurities that would normally be extracted with larger volumes (Figs. 2 and 3; note absence of impurities). An additional advantage is that only one centrifuge tube is used in the extraction.

Several examples of urinary levels of I in humans are cited in Table I. The complete results of the clinical trial and kinetic studies in humans will be published at a later date.

REFERENCES

(1) B. J. Wilder, Y. Langlois, and R. H. Hammer, *Epilepsia*, in press.

(2) M. W. Couch, K. N. Scott, B. J. Wilder, and C. M. Williams, *Drug Metab. Disposition*, in press.

(3) G. F. Thompson and K. Smith, *Anal. Chem.*, **37**, 1591(1965).

(4) A. H. Beckett and M. Rowland, *J. Pharm. Pharmacol.*, **17**, 59 (1965).

(5) D. J. Blackmore and R. W. Jenkins, *J. Forensic Sci.*, **8**, 34 (1968).

(6) A. Anggard, L. M. Gunne, and F. Niklasson, *Scand. J. Clin. Lab. Invest.*, **26**, 137(1970).

(7) J. Ramsey and D. B. Campbell, *J. Chromatogr.*, **63**, 303 (1971).

ACKNOWLEDGMENTS AND ADDRESSES

Received December 18, 1972, from the **Veterans Administration Hospital and College of Medicine, University of Florida, Gainesville, FL 32601*, and the *†College of Pharmacy, Department of Pharmaceutical Chemistry, University of Florida, Gainesville, FL 32601*

Accepted for publication February 16, 1973.

Supported in part by grants from Pharma-Research Canada Ltd.

The authors are grateful to Dr. Y. Langlois of Pharma-Research Canada Ltd. and Dr. W. Pöllman of C. H. Boehringer Sohn for their helpful suggestions and for providing samples of KÖ 1173 and KÖ 1398. The authors are grateful to Mr. Tom Munyer and Miss Dora Mitchell for their excellent technical assistance.

▲ To whom inquiries should be directed.