REFERENCES

(1) S. M. Kupchan, B. Dasgupta, E. Jujita, and M. L. King, *Tetrahedron*, **19**, 227 (1963).

(2) S. G. Zelenski, Ph.D. thesis, University of Rhode Island, Kingston, R.I., 1973.

(3) J. Kunitomo, Y. Nagai, Y. Okamoto, and H. Furukawa, *J. Pharm. Soc. Jpn.*, **90** (9), 165 (1970).

(4) A. W. Duggan, D. Lodge, T. J. Bixcoe, and P. M. Headley, *Arch. Int. Pharmacodyn. Ther.*, **204** (1), 147 (1973).

(5) R. N. Icke and B. B. Wisegarver, "Organic Synthesis," coll. vol. III, Wiley, New York, N.Y., 1955, p. 723.

(6) M. Tomita, Y. Watanabe, M. Tomita, and H. Furukawa, *J. Pharm. Soc. Jpn.*, **81**, 469 (1961).

ACKNOWLEDGMENTS AND ADDRESSES

Received August 25, 1976, from the *School of Pharmacy, Northeast Louisiana University, Monroe, LA 71203.*

Accepted for publication January 31, 1977.

The author thanks Dr. Jun-ichi Kunitomo for samples of roemerine, *O*-nornuciferine, anonaine, nuciferine, and armepavine.

GLC Microanalyses of Phenacetin and Acetaminophen Plasma Levels

M. A. EVANS and R. D. HARBISON ˣ

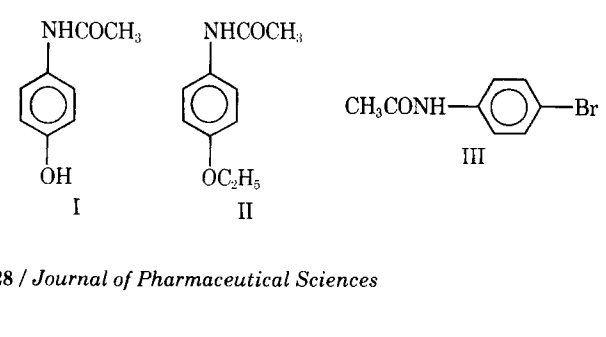
Abstract □ A GLC method utilizing a flame-ionization detector is described for the simultaneous analysis of acetaminophen and phenacetin in plasma. *p*-Bromoacetanilide is used as an internal standard. The drugs are extracted with ether from plasma diluted with 1 *M* phosphate buffer (pH 7.4). The ether extract is evaporated to dryness under nitrogen, and the residue is dissolved in 300 μl of ethyl acetate. The ethyl acetate is transferred to a microcentrifuge tube (0.4 ml), and the sample is evaporated in a vacuum centrifuge. Then the residue is redissolved in 0.2 *M* trimethylanilinium hydroxide in methanol for GLC analysis. Extraction efficiency of added phenacetin and acetaminophen in plasma at concentrations of 1–10 μg/ml was complete, and the limit of detection in plasma was less than 0.1 μg.

Keyphrases □ Phenacetin—GLC analysis in presence of acetaminophen, plasma □ Acetaminophen—GLC analysis in presence of phenacetin, plasma □ GLC—simultaneous analyses, phenacetin and acetaminophen in plasma □ Analgesics—phenacetin and acetaminophen, simultaneous GLC analyses in plasma

Acetaminophen (I) and phenacetin (II) are commonly used analgesics available without prescription. Acute overdose with either compound can produce a dose-dependent, potentially fatal hepatic necrosis (1). Renal tubular necrosis and hypoglycemic coma also can occur (2). Chronic overdosage of I or II has been reported to produce blood dyscrasia including methemoglobinemia, hemolytic anemia, and thrombocytopenia (3). Thus, toxicity coupled with widespread use necessitates specific and rapid detection of the compounds in biological specimens.

A sensitive and specific method for the estimations of I and II in plasma and urine by a GLC technique using trimethylsilyl derivatives was reported recently (4). However, it has a significant time requisite and absolute dryness is required to achieve effective silylating reactions. Furthermore, the continued use of silylating agents leads to rapid detector contamination. A GLC method for I based on sequential alkylation, followed by on-column derivatization with trimethylanilinium hydroxide, also was demonstrated (5). This method is time consuming and specific only for I (5).

Other reported methods for the estimation of I or II in biological specimens are nonspecific, lack sensitivity, or require a large sample volume (6–9). Therefore, a GLC method was developed for the rapid analysis of small amounts of I and II in plasma using an internal standard for quantitation. The sensitivity of this method was greater than 0.1 μg/ml of plasma for both compounds, and no interference was observed with peak identification.



I II III

EXPERIMENTAL

Apparatus—A gas chromatograph[1] equipped with a flame-ionization detector was maintained with gas flows of 5, 30, and 150 ml/min for nitrogen, hydrogen, and air, respectively. A column oven temperature of 165° was used; the injection port and detector were maintained at 300°.

Column—A glass column, 2 m × 2 mm, was packed with 3% SP 2250 on 80–100-mesh Chromosorb W, AW/DMCS[2]. Before packing, the column was rinsed with methanol and acetone, dried, and conditioned 6 hr with a 10% solution of dimethyldichlorosilane[3] in toluene to silylate reactive sites. Following silylation, the column was again rinsed with acetone and dried.

Analytical Procedures—Plasma, 0.1–0.5 ml, was transferred to a 13-ml glass-stoppered centrifuge tube, mixed with the internal standard *p*-bromoacetanilide[4] (III) (10 μg/ml), and diluted with an equal volume of 1 *M* phosphate buffer (pH 7.4). The plasma was extracted with reagent grade ether (7 ml) by stirring on a vortex mixer (30 sec). The ether was transferred by disposable pipet to a dry, clean, 12-ml centrifuge tube and evaporated to dryness in a water bath (45°) using a nitrogen flow. Then the residue was dissolved in 300 μl of spectrophotometric grade ethyl acetate[5] and transferred to a microcentrifuge tube[6] (0.4 ml).

The sample was taken to dryness under vacuum in a rotary vacuum centrifuge[7] and redissolved in 4–8 μl of trimethylanilinium hydroxide (0.2 *M* in methanol)[8]. Samples of 1–3 μl were injected into the gas chro-

[1] Varian model 2100.
[2] Supelco, Bellefonte, Pa.
[3] Applied Science Laboratories, State College, Pa.
[4] Aldrich Chemicals, Atlanta, Ga.
[5] Mallinckrodt Chemicals, St. Louis, Mo.
[6] Brinkmann Instruments, Westbury, N.Y.
[7] Speed Vac concentrator, Savant Instruments, Hicksville, N.Y.
[8] Methulate, Pierce Chemicals, Rockford, Ill.

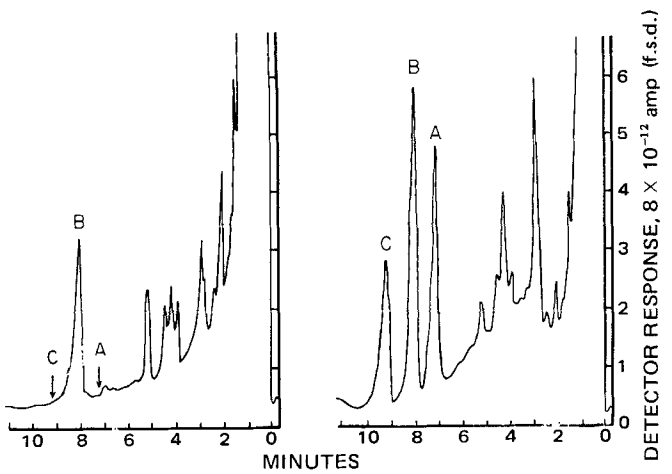


Figure 1—*Gas chromatograms of human plasma (0.5 ml). Key: left, control plasma containing 5 μg of internal standard (peak B); and right, plasma containing 2.5 μg of I (peak A) and II (peak C) with 5 μg of III. Compounds I–III were chromatographed as methylated derivatives.*

matograph for analysis. A total of 30 samples was analyzed for the determination of the standard curve. Extraction efficiency was determined by GLC peak height comparison of plasma extracts and standards prepared in ethyl acetate.

RESULTS AND DISCUSSION

The GLC retention times for the *N,O*-dimethyl derivative of I, the *N*-methyl derivative of III, and the *N*-methyl derivative of II were about 7.0, 8.0, and 9.0 min, respectively. A gas chromatogram from a control human plasma (0.5 ml) containing 10 μg of III/ml and plasma (0.5 ml) containing 5 μg of I–III/ml is shown in Fig. 1. Peaks for all three agents were symmetrical, so quantitation was made by comparison of peak heights. The detector response and calibration curve were linear for all three compounds over the 0.1–10-μg range. Blanks were prepared from plasma of drug-free subjects, and no peaks were observed that would interfere with the measurements of the compounds.

Linear regression analysis of the individual data points from the standards plotted as the ratio of peak height drug/peak height internal standard *versus* plasma drug level gave computed slopes of 0.274/μg of I and 0.172/μg of II. The correlation coefficient for I based on analysis of 30 samples was 0.990; for II, it was 0.998. The intercept for both compounds within the regression analysis was less than 0.02.

The extraction of both drugs from plasma was essentially complete within the 0.1–10-μg/ml range, and the reproducibility of the assay was good down to concentrations of less than 0.1 μg/ml of plasma. The mean recovery from 10 samples containing 0.1–10 μg of both compounds/ml was 97.8% (*SE* 4.2). No interference in peak height measurements was noted from plasma samples spiked with aspirin, caffeine, codeine, or barbiturates, all of which are likely to be in taken combination with I and II.

The use of the rotary vacuum centrifuge allows the efficient concentration of the sample. Previous use of this instrument was primarily restricted to a radioenzymatic assay for neurotransmitters where sample size was necessarily limited (10). Application to routine drug analysis by GLC allowed up to a 20-fold increase in sensitivity of the assay. Furthermore, the instrument is designed for routine, multisample use, and the total assay time from plasma extraction to GLC was less than 1.5 hr.

GLC analysis of I was reported previously (11), but in the present studies the drug could not be chromatographed directly in low concentrations without peak tailing and absorption losses. Flash heater methylation with trimethylanilinium hydroxide gave a complete reaction for the internal standard and both drugs under the described conditions without degradation peaks. On-column methylation to a less polar derivative results in a much more volatile compound suitable for quantitative GLC analysis in the submicrogram range (12). The use of trimethylanilinium hydroxide as a derivatizing agent also allows for rapid analysis without excessive detector contamination.

Maximum plasma concentrations for six normal adults following ingestion of 1 g of I ranged from 5.7 to 13.3 μg/ml. Acute ingestion of toxic amounts of I can produce a blood concentration above 100 μg/ml of plasma (13). Chronic intoxication with I and II, however, can produce intermediate plasma concentrations, necessitating specific quantitative analysis. Additionally, in the normal individual, 75–80% of administered II is rapidly metabolized to I, with peak plasma concentration of unchanged II occurring in about 1 hr and that of I occurring in 1–2 hr (1). This rapid method of analysis may be useful in correlating plasma levels with the prognosis of cases of intoxication and may be applied to the study of dosage regimens for both I and II.

REFERENCES

(1) "The Pharmacological Basis of Therapeutics," 5th ed., L. S. Goodman and A. Gilman, Eds., Macmillan, New York, N.Y., 1975, p. 345.

(2) G. E. Schreiner, *Ann. Intern. Med.*, **57**, 1047 (1902).

(3) C. H. Thienes and T. J. Hardy, "Clinical Toxicology," 5th ed., Lea and Febiger, Philadelphia, Pa., 1972, p. 80.

(4) L. F. Prescott, *J. Pharm. Pharmacol.*, **23**, 111 (1971).

(5) W. A. Dechliaruk, G. F. Johnson, and H. M. Soloman, *Clin. Chem.*, **22**, 879 (1976).

(6) H. Buch, K. Pflegel, and W. Rudigel, *Z. Klin. Chem.*, **5**, 110 (1967).

(7) A. J. Cummings, M. L. King, and B. K. Martin, *Br. J. Pharmacol. Chemother.*, **29**, 150 (1967).

(8) J. E. Wallace, J. D. Biggs, H. E. Hamilton, L. L. Foster, and K. Blum, *J. Pharm. Sci.*, **62**, 599 (1973).

(9) J. I. Routh, N. A. Shane, E. G. Arredongo, and W. D. Paul, *Clin. Chem.*, **14**, 882 (1968).

(10) B. P. Abramson, M. W. McCaman, and R. E. McCaman, *Anal. Biochem.*, **57**, (1974).

(11) A. Klutch and M. Bordun, *J. Pharm. Sci.*, **57**, 524 (1968).

(12) E. Brochmann-Hanssen and T. O. Oke, *ibid.*, **58**, 370 (1969).

(13) J. Grove, *J. Chromatogr.*, **58**, 289 (1971).

ACKNOWLEDGMENTS AND ADDRESSES

Received August 2, 1976, from the *Department of Pharmacology, Vanderbilt Medical Center, Nashville, TN 37232.*

Accepted for publication January 19, 1977.

Supported in part by U.S. Public Health Service Grants ES00267 and DA00141. M. A. Evans was supported by Research Development Award NS00346.

[x] To whom inquiries should be directed.