Infect. Dis., **128S**, 347 (1973).
(2) C. Regamey, R. C. Gordon, and W. Kirby, *Arch. Intern. Med.*, **133**, 407 (1974).
(3) M. G. Bergeron, J. L. Brusch, M. Barza, and L. Weinstein, *Antimicrob. Agents Chemother.*, **4**, 396 (1973).
(4) W. M. M. Kirby and C. R. Regamey, *J. Infect. Dis.*, **128S**, 341 (1973).
(5) A. W. Czerwinski, J. A. Pederson, and J. P. Barry, *J. Clin. Pharmacol.*, **14**, 560 (1974).
(6) W. A. Craig, M. A. Evenson, K. P. Sarver, and J. P. Wagnild, *J. Lab. Clin. Med.*, **87**, 637 (1976).
(7) D. C. Grove and W. A. Randall, "Assay Methods of Antibiotics: A Laboratory Manual," Medical Encyclopedia, New York, N.Y., 1955, pp. 7–14.
(8) W. A. Craig, *Infection*, **4**, S137 (1976).
(9) C. M. Kunin, *Ann. N.Y. Acad. Sci.*, **145**, 282 (1967).
(10) W. F. Verwey and H. R. Williams, *Antimicrob. Agents Chemother.*, **1962**, 476 (1963).

Douglas S. Greene ˣ
College of Pharmacy
University of Rhode Island
Kingston, RI 02881

Alan D. Tice
Veterans Administration Hospital
Brown University
Providence, RI 02908

Received June 27, 1977.
Accepted for publication August 5, 1977.
The authors thank Victoria Hittinger for assaying the ultrafiltrate samples.
ˣ To whom inquiries should be directed.

Use of Trimethylanilinium Hydroxide–Tetramethylammonium Hydroxide as On-Column Methylating Agent for GLC Analysis of Phenytoin

Keyphrases □ Phenytoin—GLC analysis in plasma □ GLC—analysis, phenytoin in plasma □ Anticonvulsants—phenytoin, GLC analysis in plasma

To the Editor:

Numerous procedures for the GLC analysis of plasma phenytoin have been reported (1–7). One promising procedure was that of Orme *et al.* (7), who were able to analyze phenytoin from 100-μl plasma samples with a lower limit of sensitivity of 0.5 μg/ml. When I attempted to use this procedure, a peak for the on-column methylating agent, trimethylanilinium hydroxide, interfered with the peak for the internal standard, 5-(4-methylphenyl)-5-phenylhydantoin. This interfering peak was not an extracted plasma component or an impurity from the extracting solvent, toluene, since it was seen when just the methanolic trimethylanilinium hydroxide was injected on column as well as when the extractions were performed with water blanks.

Orme *et al.*'s procedure for phenytoin involved the extraction of phenytoin from a 100-μl plasma sample into toluene containing the internal standard. The toluene was then extracted with 25 μl of a 0.5 *M* trimethylanilinium hydroxide–50% (v/v) methanol–water mixture. One microliter of the lower aqueous layer, after aspiration of the upper toluene layer, was injected into a gas chromatograph and analyzed *via* a flame-ionization detector.

Attempts to overcome the peak interference in using Orme *et al.*'s procedure by lowering the trimethylanilinium hydroxide concentration to 0.1 *M* (concentration below 0.1 *M* led to problems with the final extraction step) and altering column conditions proved unsuccessful. The use of commercial as well as fresh batches of trimethylanilinium hydroxide, synthesized by the method of Brochmann–Hanssen and Oke (8) from trimethylanilinium chloride or iodide, was similarly unsuccessful in removing or lessening the interfering peak.

Brochmann–Hanssen and Oke (8) showed that trimethylanilinium hydroxide is a superior methylating agent compared to tetramethylammonium hydroxide and need only be present in reasonable excess to methylate barbiturates, *etc.*, quantitatively. For concentrations of up to 20 μg of phenytoin/ml in 100 μl of plasma and the internal standard used in Orme *et al.*'s procedure, the concentration of trimethylanilinium hydroxide in the final methanol–water mixture needed only to be slightly greater than 0.001 *M; i.e.*, for methylating purposes, the concentration of trimethylanilinium hydroxide of 0.5 *M* appeared to be unnecessary. Of course, the presence of trimethylanilinium hydroxide serves a secondary purpose in that the final methanol–water solution must be sufficiently alkaline to extract phenytoin and the internal standard efficiently from the toluene phase.

In the present study, sufficient alkalinity for the efficient extraction in the final extraction step and quantitative methylation of the extracted phenytoin and internal standard were achieved by the use of 0.1 *M* tetramethylammonium hydroxide–0.01 *M* trimethylanilinium hydroxide and a solvent of 50% (v/v) methanol–water. Tetramethylammonium hydroxide was present as the alkalizing agent and as a secondary methylating agent, while trimethylanilinium hydroxide was primarily present as the methylating agent.

Plasma samples, 100 μl, containing phenytoin were analyzed according to the procedure of Orme *et al.* (7) with the following modifications. To a 100-μl plasma sample was added 100 μl of freshly prepared 10% metaphosphoric acid. This addition was necessary because an interfering amine prodrug of phenytoin of low pKa was present in the plasma samples. Metaphosphoric acid also quenched the conversion of the prodrug to phenytoin. For normal samples, the addition of monobasic sodium phosphate, as suggested by Orme *et al.* (7), is sufficient.

Two milliliters of toluene containing 1.4 μg of 5-(4-methylphenyl)-5-phenylhydantoin was then added to the treated plasma sample, and the sample was vortexed vigorously for 1 min and then centrifuged. Approximately 1.8 ml of the toluene layer was removed by pipet. The toluene was then extracted with 25 μl of 50% (v/v) methanol–water that was 0.1 *M* in tetramethylammonium hydroxide and 0.01 *M* in trimethylanilinium hydroxide. This addition of the 25 μl of methanol–water *to* the toluene while vortexing was crucial for efficient extraction. After vortexing for 1 min and centrifuging, the upper toluene layer was removed by aspiration.

One microliter of the aqueous methanolic solution was then injected into a gas chromatograph[1] equipped with a flame-ionization detector. A 1.8-m glass column[2] (2 mm i.d.) was packed with 3% OV-17 on 100–120-mesh Gas Chrom Q[3]. The following conditions were used: injector temperature, 260°; column temperature, 210°; detector temperature, 250°; nitrogen flow, 35 ml/min; hydrogen flow, 50 ml/min; and compressed air (19–23% O_2 and 77–81% N_2) flow, 300 ml/min. The retention times for phenytoin and the internal standard were 4.1 and 5.8 min, respectively. With 0.5 *M* trimethylanilinium hydroxide in place of tetramethylammonium hydroxide (0.1 *M*)–trimethylanilinium hydroxide (0.01 *M*) and identical column conditions, the interfering peak had a retention time of 5.6 min.

Peak height ratios were used to prepare standard curves in the 1–20-μg/ml range. The lower limit of sensitivity was 0.5 μg/ml, and repeated injections showed assay reproducibility in the ±0.5-μg/ml range.

With the described procedure, a clean chromatogram that was easily quantitated was obtained. The phenytoin peak at the higher sensitivity settings, *i.e.*, low concentration range, did elute on the solvent front tailing. However, if analysis time is not critical, the column temperature can be lowered slightly, resulting in a slightly longer retention time but a better chromatogram for quantitation purposes.

Use of the mixed methylating agents apparently provides sufficient methylating and alkalizing properties while minimizing peak interferences resulting from high concentrations of trimethylanilinium hydroxide and the relatively poor methylating capability of tetramethylammonium hydroxide.

[1] Varian Aerograph model 2100, Walnut Creek, Calif.
[2] Supelcoport, Supelco, Inc., Supelco Park, Bellefonte, Pa.
[3] Applied Sciences Laboratories, State College, Pa.

(1) J. MacGee, *Anal. Chem.*, **42**, 421 (1970).
(2) R. H. Hammer, B. J. Wilder, R. R. Streiff, and A. Meyersdorf, *J. Pharm. Sci.*, **60**, 327 (1971).
(3) H. J. Kupferberg, *Clin. Chim. Acta*, **29**, 283 (1970).
(4) A. Estes and P. A. Dumont, *J. Chromatogr.*, **82**, 307 (1973).
(5) M. A. Evenson, P. Jones, and B. Darcey, *Clin. Chem.*, **16**, 107 (1970).
(6) K. K. Midha, I. J. McGilveray, and D. L. Wilson, *J. Pharm. Sci.*, **65**, 1240 (1976).
(7) M. Orme, O. Borgå, C. E. Cook, and F. Sjöquist, *Clin. Chem.*, **22**, 246 (1976).
(8) E. Brochmann-Hanssen and T. O. Oke, *J. Pharm. Sci.*, **58**, 370 (1969).

V. J. Stella

Department of Pharmaceutical Chemistry
University of Kansas
Lawrence, KS 66045

Received April 22, 1977.
Accepted for publication August 1, 1977.
Supported by a grant from INTERx Research Corp., Lawrence, KS 66044.
The technical assistance of Mr. Steve Schuller is gratefully acknowledged.

BOOKS

REVIEWS

Textbook of Organic Medicinal and Pharmaceutical Chemistry. 7th ed. Edited by CHARLES O. WILSON, OLE GISVOLD, and ROBERT F. DOERGE. Lippincott, East Washington Square, Philadelphia, PA 19105, 1977. 1085 pp. 18 × 26 cm. Price $33.00.

The seventh edition of this book represents a continued effort of the editors to provide a useful undergraduate pharmacy textbook. The book includes a discussion of all products described in USP XIX, NF XIV, and "Accepted Dental Remedies," as well as other important pharmaceuticals. The introductory material of a few chapters has been completely rewritten, but the overall format remains essentially unchanged.

Three major changes in style represent a departure from the older editions and will considerably improve the practical application of the textbook. One outstanding feature is the extensive use of tables to focus attention upon groups of therapeutic agents in common use (a combination of chemical and pharmacologic classification). The information provided by the tables includes the official title of the drug, proprietary name(s), official dosage forms, usual dose and route of administration, and usual dose range. For the appropriate drugs, the usual pediatric dose is given in terms of body weight and body surface.

A second outstanding feature of this edition is the addition of appendixes. Material relative to pharmaceutic aids and necessities has been extracted from the several chapters of the older editions and placed in one appendix. The title of another appendix, Amine Salts, is misleading, because it actually provides the USAN designations, chemical names, and structural formulas of the anions of the more common organic acids used to form salts with amines. A third appendix provides a referenced list of pKa values of several drugs and reference compounds. A fourth appendix provides an alphabetical list of official titles of chemical entities in the USP and NF. For each title, the Chemical Abstracts Service Index Name and Registry Number, IUPAC name, empirical formula, and molecular weight are given.

A third change from the previous editions is deletion of the material previously discussed in Chapters 4–8, thus reducing the number of chapters to 24. Much of the deleted material has been placed in other appropriate chapters and in an appendix.

All chapters now include literature citations and a selected reading list.

The titles of the remaining 24 chapters are the same as those of the sixth edition. But seven of the chapters were contributed by different authors, thus giving a different slant to the introductory material in each. In the seventh edition, the new authors are Robert F. Doerge, Sulfonamides and Sulfones with Antibacterial Action (Chapter 6) and Adrenergic Agents (Chapter 12); H. Wayne Schultz, Surfactants and Chelating Agents (Chapter 7); Arnold R. Martin, Antibiotics (Chapter 9); Dwight S. Fullerton, Steroids and Therapeutically Related Compounds; and Jaime N. Delgado, Carbohydrates (Chapter 21) and Amino Acids, Proteins, Enzymes, and Hormones with Protein-like Structure (Chapter 22).

The purchaser should be aware of several printing errors that appear in Chapter 20. If the Errata list mailed to schools of pharmacy in January