Determination of Impurities in Phenacetin by Thin-Layer Chromatography II

Sir:

We reported earlier (1) that TLC can be applied to detect certain impurities in phenacetin. Additional experimentation in this laboratory prompted us to introduce a modification which, in addition to enhancing the extraction of the impurities, extends the upper limit of detection. The modified procedure is as follows:

(*a*) Control solution—Dissolve 10.0 mg. each of acetanilid, *p*-chloroacetanilid, and *p*-phenetidin in 100.0 ml. of absolute ether.

(*b*) Test and control preparations—Place 1.0 g. of finely powdered phenacetin into an 18- × 150-mm. test tube, add 3.0 ml. of absolute ether, and mark the volume. To a similar test tube, add 1.0 g. of finely powdered phenacetin reference standard and 3.0 ml. of the control solution, and mark the volume. To both test tubes, add 15 ml. of the ether and, by gently boiling on a steam bath, evaporate to the mark. Cool, adjust the volume to the mark with the ether, add 2.0 ml. of *n*-hexane, mix, and allow the particles to settle.

(*c*) Procedure—Perform TLC concurrently with the control preparation and the test preparation. Use a plate with a 0.25-mm. layer of Silica Gel GF,[1] a freshly prepared developing solvent consisting of 99 volumes of absolute ether and 1 volume of *n*-hexane, and a chromatographic chamber not lined with filter paper. Spot 50 µl. each of the supernatant obtained in the test preparation and in the control preparation, applying a continuous current of warm air in order to concentrate the spots and to completely evaporate the solvent. Allow the developing solvent to ascend at least 15 cm. above the spot points, remove the plate, allow it to air dry, and expose it to iodine vapor for 20 sec. Observe the chromatogram under short-wave UV light. (The spots from the control preparation appear in the following order above the principal spot of phenacetin: *p*-chloroacetanilid, acetanilid, and *p*-phenetidin.)

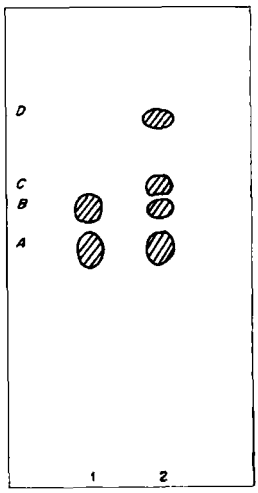

Fig. 1—TLC of: A, *phenacetin;* B, p-*chloroacetanilid;* C, *acetanilid;* D, p-*phenetidin. Key: 1* B, *30.0 mcg. (0.3%); 2* B, C, *and* D, *15.0 mcg. (0.15%) each.*

As a corroborative estimation of the *p*-chloroacetanilid, the plate should be exposed to short-wave UV radiation for about 5 min., and then inspected under a long-wave UV lamp: *p*-chloroacetanilid exhibits a bright blue fluorescence.

The significant differences between the original procedure and the modified procedure are as follows. (*a*) Original Procedure—Impurities are extracted by agitating 1 g. of phenacetin with 5.0 ml. of absolute ether for 5 min.; solvent in the final mixture is 5.0 ml. of ether; development is 10 to 15 cm. (*b*) Modified Procedure—Impurities are extracted by boiling 1 g. of phenacetin with an initial volume of 18 ml. of ether and continuing the warm extraction until the volume is reduced to 3.0 ml.; solvent in the final mixture is 3.0 ml. of absolute ether and 2.0 ml. of *n*-hexane; development is at least 15 cm.

The modifications were adapted basically for the following reasons.

1. The extraction technique provides for a more efficient dissolution of the impurities due to the initially larger volume of the solvent and to the applied heat.

2. Absolute ether–*n*-hexane (3:2) dissolves about 2.7 times less phenacetin than does absolute ether. Consequently, the area of the phenacetin spot becomes much smaller when the mixed solvent is applied. This reduces the pos-

[1] Among the adsorbents tested, Silica Gel GF_{254} (from E. Merck AG., Darmstadt, Germany) allowed for a relatively rapid development.

sible overlapping of the phenacetin and *p*-chloroacetanilid spots, and extends the upper limit of detection and estimation of the latter. A typical chromatogram is shown in Fig. 1.

3. By allowing the solvent front to ascend at least 15 cm. from the starting line, resolution of the spots is increased.

Summarizing our results, it was found that the proposed modified procedure provides an improved technique for identifying and estimating *p*-chloroacetanilid, acetanilid, and *p*-phenetidin in phenacetin with the aid of TLC.

(1) Turi, P., and Polesuk, J., *J. Pharm Sci.*, **56**, 1011(1967).

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Received August 15, 1968.
Accepted for publication September 27, 1968.

Keyphrases

Phenacetin—impurities determination
TLC—analysis, identity
UV light—spot fluorescence

Books

REVIEWS

Atomic Absorption Spectroscopy and Analysis by Atomic Absorption Flame Photometry. By JUAN RAMEREZ MUNOZ. American Elsevier Publishing Co. Inc., 52 Vanderbilt Avenue, New York, NY 10017, 1968. xii + 493 pp. 16 × 23.8 cm. Price $28.50.

This book is highly recommended for those interested in becoming familiar with this rapidly advancing technique and it will be of interest to the advanced student of atomic absorption. The book is divided into five sections. Part I covers the fundamentals and adequately covers the principals for a beginner. Part II on instrumentation is well written but slightly out of date which is to be expected in such a rapidly moving field. Part III covers the elements applicable to atomic absorption and the sensitivity for these elements whereas Parts IV and V cover methods and application. The book has an excellent bibliography and a very useful appendix.

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A Textbook of Pharmaceutical Analysis. By KENNETH A. CONNORS. John Wiley & Sons, Inc., 605 Third Ave., New York, NY 10016, 1967. xvii + 614 pp. 15.5 × 23.5 cm. Price $12.50.

This textbook for use in a course in pharmaceutical analysis has a somewhat new approach to the subject—it is not a commentary on the official compendia nor a catalog of assay methods for specific drugs. It is a presentation of basic concepts with experimentation only to provide the basis for these concepts, so that the reader will understand drug analyses in principle and many in detail. In the preface the author points out that his wish is to enable students to approach with understanding not only the official compendia, but also reference works in analytical chemistry and specialized monographs. He has presented the field of pharmaceutical analysis in five parts—fundamental titrimetric analysis, physical methods of analysis, separation techniques, elemental analysis, and functional group analysis. There are many experiments included and more than 200 problems. Important new subjects included are phase solubility analysis, enzymes as analytical reagents, and decisions an analyst must make when selecting an assay method and interpreting his data.

Staff review

Introduction to Chromatography. By JAMES M. BOBBITT, ARTHUR E. SCHWARTING, and ROY J. GRITTER. Reinhold Book Corporation, 430 Park Avenue, New York, NY 10022, 1968. xii + 160 pp. 15.5 × 23 cm. Price $3.95. Paperbound.

This is one of the books in the Reinhold Science Studies series, and although small it contains a good deal of information. As its title indicates, the book is meant only to introduce one to the subject and give a practical introduction to the more common techniques in this area. The authors have limited the book to discussions of only three chromatographic techniques: thin-layer, column, and gas. Not included is paper chromatography and although it is so well known the authors note that it is more likely that thin-layer will someday supplant