Determination of Impurities in Phenacetin by Thin-Layer Chromatography

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A rapid and sensitive method has been devised to detect acetanilid, *p*-chloroacetanilid, and *p*-phenetidin contaminations in phenacetin by a single test, simplifying the U.S.P. technique which involves three separate procedures. The TLC method is not only faster, but also more sensitive than the official procedures.

PHENACETIN, one of the most popular constituents in analgesic combinations,[1] may contain impurities which increase toxicity. In U.S.P. XVII, requirements have been established for three of these impurities: acetanilid and *p*-phenetidin should be absent, and the *p*-chloroacetanilid content should not exceed 0.03%. The toxic properties of these contaminants are well described in the literature.

Several useful methods (1–5) have been published on the detection of the possible impurities in phenacetin. However, some of the methods do not consider all of the above-listed significant contaminants, and others apply laborious procedures and sophisticated equipment. The authors' objective was to develop a simple, rapid, and sensitive technique for the detection of the three impurities.

EXPERIMENTAL

Apparatus—Desaga-Brinkmann TLC equipment, 8 × 4 and 8 × 8 in. glass plates coated with a 250 μ layer of fluorescent Silica Gel (GF). Short-wave U.V. lamp, Mineralight,[2] model UVS-12. Long-wave U.V. lamp, Blak-Ray,[2] model UVL-22.

Reagents—Solvents (Table I): reagent grade. Phenacetin: U.S.P. grade. Acetanilid, *p*-chloroacetanilid, *p*-phenetidin: commercially available samples were used, without further purification.

Procedure—One gram of phenacetin was shaken with 5.0 ml. of ether for 5 min. in a small glass-stoppered flask. The suspension was allowed to settle, and 50 μl. of the supernatant was spotted on a Silica Gel GF plate. As a control, ethereal solutions of acetanilid, *p*-chloroacetanilid, and *p*-phenetidin were applied, containing 0.15, 0.30, and 3.0 mcg. of each. A partial list of solvents and solvent mixtures that were utilized to develop the chromatograms is included in Table I. The solvent front was allowed to ascend 10 to 15 cm. above the spot points.

The plate was then removed from the tank and dried in air. The dried plate was exposed to iodine vapor in an appropriate tank for 20 sec., and subsequently inspected under a short-wave (253.7 mμ) U.V. lamp. The substances appeared as dark spots against a bright green fluorescent background. (Fig. 1.)

As a corroborative estimation of the *p*-chloroacetanilid, the U.S.P. detection method was also applied (6). After exposing the plate to the short-wave U.V. radiation for about 5 min., the chromatogram was inspected under a long-wave (366.0 mμ) U.V. lamp: only the *p*-chloroacetanilid spots exhibited a bright blue fluoresence. This additional testing was more selective, but the range of sensitivity was the same as that of the first observation.

DISCUSSION AND RESULTS

In attempting to develop an improved procedure for detecting certain possible impurities in phenacetin, there were basically two problems to be solved: selecting the most suitable technique, and finding the optimal conditions for both the separation and identification of the substances.

Thin-layer chromatography was selected because of its easy availability, speed, and high degree of sensitivity. Unlike gas chromatography, the TLC method can be used without lengthy preliminary steps such as column preparation and equilibration. It facilitates the estimation of as little as 0.003% *p*-chloroacetanilid (the U.S.P. sets a limit of 0.03%). Furthermore, in proving the absence of acetanilid, it is much more accurate than the official method. Koshy and his co-workers (3) found that the U.S.P. test is sensitive only to concentrations greater than 1.2%. The same authors estimated that the sensitivity of the color reaction of iodine T.S. with *p*-phenetidin was in the range of 0.003%. It was found that by the described TLC method, a *p*-phenetidin contamination corresponding to a concentration of 0.0015% is safely detectable.

In selecting the optimal solvent system, it was obvious that the major difficulty was the separation of the acetanilid and *p*-chloroacetanilid, because the solubility properties of these compounds are very similar. Strongly polar solvents (methanol, acetone) moved all the compounds too fast and resulted in practically no separation. On the other hand, a solvent containing 90% petroleum ether left all substances at the starting line. It was found that ethyl ether mixed with a small volume (0.5–1.0%) of nonpolar solvent yielded a safe and reproducible separation of the four compounds. An ether–hexane (99:1) mixture proved suitable because of the comparatively speedy development (about 30 min.), and because of the availability of these solvents in reagent grade purity.

The detection of the phenacetin and its impurities can be achieved by alternate methods. Szász and his co-workers (1) exposed the plates to freshly generated nitrogen monoxide gas and subsequently sprayed the chromatogram with a 1% solution of β-naphthol in 2 *N* sodium hydroxide. The products (acetanilid was not included) appear in different colors; nevertheless, the limit of detection for *p*-chloroacetanilid is 0.1%. Other papers refer to the use of a spray reagent consisting of 3% potassium permanganate in strong sulfuric acid, or, in the

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[1] "American Drug Index," Wilson, C. O., and Jones, T. E., eds., J. B. Lippincott Co., Philadelphia, Pa., 1961, lists 119 phenacetin-containing combinations.

[2] Ultra-Violet Products, Inc., San Gabriel, Calif.

TABLE I—TLC OF PHENACETIN AND ITS IMPURITIES IN DIFFERENT SOLVENT SYSTEMS

Solvent System	*Rf* Phenacetin	*Rf* *p*-Phenetidin	*Rf* *p*-Chloroacetanilid	*Rf* Acetanilid
Pet. ether–chloroform, 9:1	0.00	0.00	0.00	0.00
Chloroform	0.05	0.23	0.09	0.09
Isopropyl ether	0.10	0.47	0.13	0.17
Ether–hexane, 90:10	0.20	0.47	0.23	0.27
Ether–pet. ether, 99:1	0.25	0.54	0.31	0.35
Ether–hexane, 99:1	0.27	0.53	0.32	0.36
Ether	0.30	0.67	0.37	0.37
Cyclohexane–acetone–diisobutylketone–methanol–water, 100:80:30:5:1[a]	0.40	0.55	0.45	0.49
Acetone	0.73	0.77	0.77	0.77
Methanol	0.75	0.70	0.80	0.80
Methanol–water, 3:1	0.90	0.90	0.90	0.90

[a] See *Reference 5*.

case of *p*-chloroacetanilid only, to the exposure to short-wave U.V. radiation followed by an examination under long-wave U.V. radiation (5, 6). It was found that by utilizing the fluorescent silica gel plate, no spray is necessary in order to detect the spots, and the use of the U.V. irradiation technique is not limited to the *p*-chloroacetanilid identification, but also facilitates the rapid and simultaneous examination of all four compounds. (The 20-sec. iodine exposure is necessary in order to make the *p*-phenetidin spot visible.)

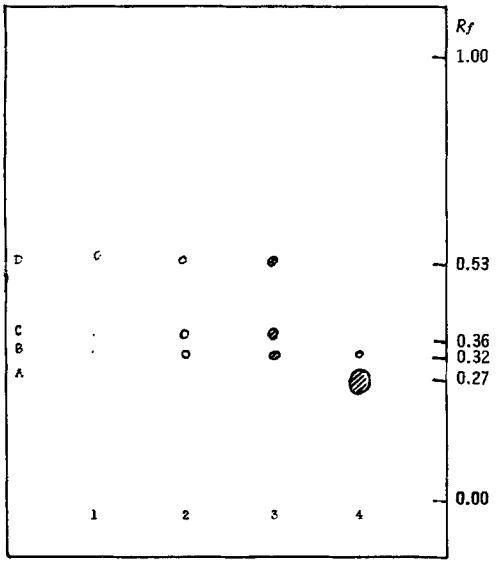

Fig. 1—Typical TLC of phenacetin (A), p-*chloroacetanilid* (B), *acetanilid* (C), *and* p-*phenetidin* (D). *Key:* 1—B, C, *and* D, *0.15 mcg. each;* 2—B, C, *and* D, *0.30 mcg. each;* 3—B, C, *and* D, *3.0 mcg. each;* 4—*phenacetin sample* (*containing 1.5 mcg. of* p-*chloroacetanilid in the spotted volume*).

SUMMARY

The separation, identification, and estimation of possible phenacetin impurities listed in the U.S.P. XVII monograph have been investigated.

A thin-layer chromatographic technique has been developed which offers a rapid identification of acetanilid, *p*-chloroacetanilid, and *p*-phenetidin impurities, and facilitates the near quantitative evaluation of their amounts. Limits of detection are 0.003% for acetanilid and *p*-chloroacetanilid, and 0.0015% for *p*-phenetidin.

The method presents a favorable alternative for the official limit test and two additional qualitative assays, by replacing them with a single, rapid, and highly sensitive procedure.

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