icity was revealed through use of a vital staining technique to systems of replicating (L-cells) and nonreplicating (chick embryo cells) types.

Results of the tests demonstrated the usefulness of the techniques for rapid screening of all types of plastics for toxicity and further opened up an avenue for testing of liquid and solid extracts from plastic materials.

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Gas Chromatographic Analysis of Phenacetin and Probable Contaminants—Acetanilid, *p*-Chloroacetanilid, and *p*-Phenetidin

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Acetanilid, *p*-chloroacetanilid, and *p*-phenetidin are likely to be present as impurities in phenacetin. U.S.P. XVII describes qualitative tests for acetanilid and *p*-phenetidin and a semiquantitative paper chromatographic limit test for *p*-chloroacetanilid. A gas chromatographic procedure has been developed for the quantitative determination of these three impurities and of the phenacetin content of bulk material. The procedure utilizes Epon 1001 as the stationary phase on Chromosorb G in conjunction with a dual column instrument and a flame ionization detector. The proposed method is rapid and involves only a solvent extraction of the sample and no chemical modification prior to actual analysis. Impurities present in the raw materials in quantities as small as 10 p.p.m. can be estimated. If present in a proportion greater than 50 p.p.m., they may be determined quantitatively.

During the past few years, reports of toxicities associated with phenacetin have appeared in the medical literature. Some of the toxic symptoms have been attributed to impurities introduced during synthesis of the compound. Three impurities likely to be present are acetanilid, *p*-chloroacetanilid, and *p*-phenetidin. Gad (1) has reported that *p*-chloroacetanilid causes methaemoglobinemia. Hald (2) reported cyanosis in patients who had taken phenacetin which was subsequently shown to contain 18% *p*-chloroacetanilid. The 1958 edition of the "British Pharmacopoeia" allowed 0.17% *p*-chloroacetanilid in phenacetin, whereas the 1963 edition lowered this limit to 0.11%. This compendium also has a qualitative test for *p*-phenetidin. The sixteenth revision of the "United States Pharmacopeia" has qualitative tests for acetanilid only. The seventeenth revision has qualitative tests for *p*-phenetidin and acetanilid and allows a maximum of 0.03% of *p*-chloroacetanilid.

The B. P. test for *p*-chloroacetanilid is based on the procedure by Hald (3). This involves cleavage of the aromatic chloride bond with Raney nickel catalyst in the presence of sodium hydroxide, followed by visual estimation of the opalescence produced by the addition of silver nitrate solution. In the U.S.P. test, the *p*-chloroacetanilid is separated by reversed phase paper chromatography and the fluorescence produced on irradiation with ultraviolet light is estimated visually. These procedures are only semiquantitative. Jones and Page (4) have described a polarographic method for the determination of *p*-chloroacetanilid and have shown data for phenacetin which contained 0.1 to 0.34% of the impurity. Recently, Crummett *et al.* (5) reported a very sensitive procedure for *p*-chloroacetanilid. This involves hydrolysis with hydrobromic acid to *p*-chloroaniline and spectrophotometric determination following extraction from a basic medium. This procedure is subject to interference from acetanilid.

Both the B.P. and U.S.P. tests for *p*-phenetidin involve a color reaction with iodine T.S. The authors have found this reaction to be sensitive to concentrations as low as 0.003%. The U.S.P. test for acetanilid involves bromination of a saturated aqueous solution of phenacetin and visual observation of the turbidity due to the bromo derivative of any acetanilid present. This test was found to be sensitive only to concentrations greater than 1.2%. In addition, it can be misleading due to precipitation of some of the phenacetin from the saturated solution.

The purpose of this investigation was to develop a sensitive procedure for the quantitative determination of phenacetin and probable contaminants: acetanilid, *p*-chloroacetanilid, and *p*-phenetidin by gas-liquid chromatography. Such a procedure might aid toxicity evaluations especially in view of the recent FDA label warning required for phenacetin preparations.

EXPERIMENTAL

Apparatus and Materials.—F and M model 810 dual column gas chromatograph with a flame ionization detector and equipped with a Minneapolis Honeywell recorder and disk integrator was used.

The phenacetin (Mallinckrodt Chemical Works) used in the preparation of the calibration curve and synthetic mixtures was recrystallized and shown

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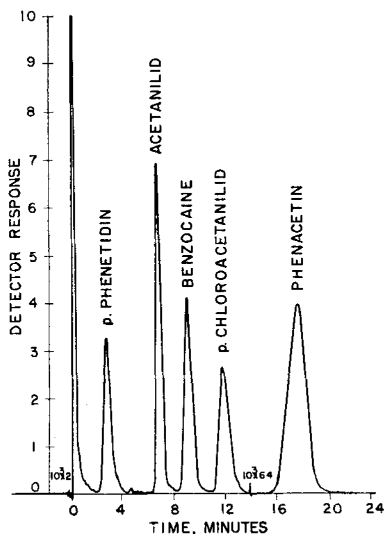

Fig 1.—Gas chromatogram of a chloroform solution of phenacetin and contaminants using benzocaine as internal standard.

chromatographically to be free of the three contaminants. *p*-Chloroacetanilid, acetanilid, and *p*-phenetidin were obtained from Eastman Organic Chemicals. The latter was distilled before use. Benzocaine was obtained from Abbott Laboratories and the reagent grade chloroform from Merck and Co.

Column Conditions.—The column was a 2 ft. × 1/4 in. aluminum tube containing silanized Chromosorb G[1] 70–80 mesh coated with 4% Epon 1001.[2] The column was conditioned at 200° for 48 hr. before use.

Other columns that were investigated but which were not selected because of incomplete resolution of peaks, tailing, or column instability were (A) 2 ft. × 1/4 in. Carbowax 1540,[3] 2% plus 2% KOH (B) 3 ft. × 1/4 in Apiezon L,[4] 10% plus 2% KOH (C) 3 ft. × 1/4 in. diisodecylphthalate, 10% and (D) 3 ft. × 1/4 in. Versamid 900,[5] 5%, all on silanized Chromosorb W,[1] 70–80 mesh.

The column temperature was varied depending on the nature of the analysis. For the analysis of phenacetin, acetanilid, and *p*-chloroacetanilid, the column was maintained at 188°. When analyzing for *p*-phenetidin also, the column was maintained at 145° for 3 min., then programmed to 180° at the rate of 15°/min. and maintained at 180° for 20 min. The injection port was at 260° and the detector at 240°.

Helium was the carrier gas and the flow rate was 100 ml./min. Hydrogen and air flow rates were 60 and 450 ml./min., respectively.

Calibration Curves

Phenacetin.—Benzocaine was used as an internal standard. Exactly 250 mg. of benzocaine was weighed into each of four 25-ml. volumetric flasks. Known amounts of phenacetin ranging from 125 to 500 mg. were added, dissolved, and brought to volume with chloroform. Five microliters of each solution was injected into the column. The ratios of the peak areas of phenacetin to benzocaine were plotted against the corresponding concentrations of phenacetin.

Acetanilid, p-Chloroacetanilid, and p-Phenetidin.—Exactly 2 mg. of benzocaine in chloroform was placed in each of four 10-ml. volumetric flasks. Chloroform solutions containing known amounts of the above compounds in the range of 0.5 to 3 mg. each were pipeted into each flask and brought to volume with chloroform. Five microliters of each solution was injected into the column. The ratios of the peak areas of each ingredient to that of benzocaine were plotted against the corresponding concentration.

Sample Preparation

For Phenacetin Determination.—Exactly 250 mg. of benzocaine and about 250 mg. of phenacetin sample were accurately weighed and transferred into a 25-ml. volumetric flask, dissolved, and brought to volume with chloroform. Five microliters was injected into the column.

For Acetanilid, p-Chloroacetanilid, and p-Phenetidin Determination.—Ten grams of sample was weighed into a glass-stoppered 50-ml. shaker tube. Smaller sample sizes were used for phenacetin containing larger quantities of the impurities. An aliquot of a chloroform solution containing exactly

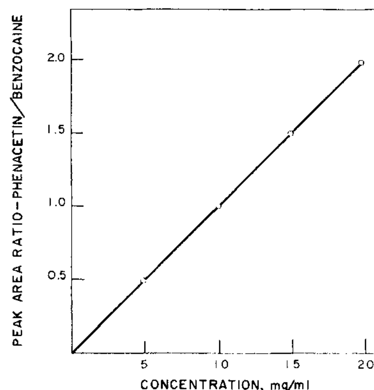

Fig. 2.—Calibration curve for phenacetin.

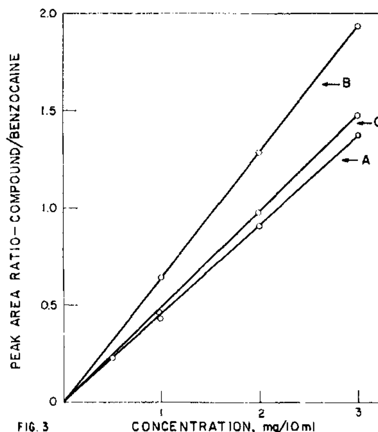

Fig. 3.—Calibration curves for (A) *p*-phenetidin, (B) acetanilid, and (C) *p*-chloroacetanilid.

[1] Johns-Manville, New York, N. Y.
[2] Shell Chemical Co., New York, N. Y.
[3] Union Carbide Chemicals Co., New York, N. Y.
[4] Associated Electrical Industries Ltd., England.
[5] Analabs, Inc., Hamden, Conn.

TABLE I.—ANALYSIS OF SYNTHETIC MIXTURES OF CONTAMINANTS IN PHENACETIN[a]

	Found, p.p.m.		
Added, p.p.m.	*p*-Phenetidin	Acetanilid	*p*-Chloro-acetanilid
300	308	305	308
	297	298	297
	None added	300	294
200	202	194	197
	199	202	197
	198	211	203
100	96	100	102
	91	105	108
	None added	103	96
50	54	46	48
	50	49	50

[a] Results represent the average from two chromatograms for each sample.

TABLE II.—PHENACETIN ANALYSIS OF RAW MATERIALS[a]

Sample	Phenacetin Content, %
1	100.1
2	99.3
3	99.4
4	99.8

[a] Results are from a single chromatogram for each sample.

2 mg. of benzocaine was pipeted into the tube. Twenty-five milliliters of chloroform was added and the mixture shaken on a mechanical shaker for 10 min. The tube was cooled in an ice bath for 5 min. and the mixture filtered and the undissolved solid was washed with a few milliliters of cold chloroform. The filtrate and washings were combined and evaporated to a volume of about 3–4 ml., transferred quantitatively using chloroform into a 10-ml. volumetric flask and adjusted to volume with chloroform. Five microliters was injected into the column. For samples containing less than 50 p.p.m. of the impurities, the filtrate can be evaporated to dryness and redissolved in a smaller volume of chloroform.

RESULTS

A typical chromatogram of a mixture of *p*-phenetidin, acetanilid, *p*-chloroacetanilid, phenacetin, and benzocaine is shown in Fig. 1. This was obtained by programming the column temperature from 145 to 180° as described previously. Quantitative analysis of mixtures could be made from chromatograms of this type when the concentrations of the impurities were 0.01% or higher. With lower concentrations, the instrument had to be set at higher sensitivity, and base line drifts were noticed on programming the temperature. Consequently, the data presented in this report are from chromatograms obtained under two conditions: programmed temperature for the analysis of *p*-phenetidin and isothermal at 188° for the other components.

Calibration curves for phenacetin and for the various contaminants are given in Figs. 2 and 3, respectively. Synthetic mixtures made by adding known amounts of *p*-phenetidin, acetanilid, *p*-chloroacetanilid, and benzocaine to a pure sample of phenacetin were analyzed. Results presented in Table I clearly indicate quantitative recovery of the impurities in concentrations as low as 50 p.p.m.

TABLE III.—*p*-PHENETIDIN, ACETANILID, AND *p*-CHLOROACETANILID CONTENT OF PHENACETIN SAMPLES

Sample	*p*-Phenetidin, %	Acetanilid, %	*p*-Chloro-acetanilid, %
1	<0.001	<0.001	0.13
2	<0.001	<0.001	0.012
3	0	<0.001	0.14
4	0	<0.001	0.11
5	0	0	0.17
6	<0.001	<0.001	0.07
7	0	<0.001	0.04
8	0	0	0

Table II shows data on the determination of phenacetin in a few raw material samples. The results indicate the applicability of the method to the analysis of bulk material.

A few commercial samples of phenacetin were analyzed for *p*-phenetidin, acetanilid, and *p*-chloroacetanilid. These samples were obtained from various sources and were of unknown age. Results of the analysis are shown in Table III. All the eight samples contained 0–10 p.p.m. of *p*-phenetidin and acetanilid. However, only two contained less than 0.03% of *p*-chloroacetanilid which is the upper limit established by U.S.P. XVII. One contained as high as 0.17% of this impurity. It was interesting to note that two samples contained two and one contained three other unidentified impurities in trace amounts.

SUMMARY

A rapid and precise gas chromatographic procedure has been developed for the analysis of phenacetin and its probable contaminants: *p*-phenetidin, acetanilid, and *p*-chloroacetanilid. The procedure uses 4% Epon 1001 as the stationary phase, supported on Chromosorb G in a 2 ft. × 1/4 in. aluminum tube. Impurities at the concentration level of 50 p.p.m. or higher can be determined quantitatively and impurities as low as 10 p.p.m. can be estimated.

Of eight commercial samples analyzed, contamination by *p*-phenetidin and acetanilid was negligible. However, only two of these samples met U.S.P. XVII specifications for *p*-chloroacetanilid.

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