Table I—Binding of Acetazolamide to Human Erythrocytes *In Vitro*

		k_d, μg/ml	nM, μg/ml
1[a]	Erythrocytes in buffer, pH 7.4	0.50	21
2	Whole blood	0.23	27
	Erythrocytes in buffer, pH 7.4	0.23	28
3	Whole blood	0.41	29
	Erythrocytes in buffer, pH 7.4	0.24	27
4	Whole blood	0.43	32
	Erythrocytes in buffer, pH 7.4	0.40	31
	Mean	0.35	28
	SD	0.11	3.6

[a] Numbers indicate experiments performed with different units of whole blood.

still provide an estimate of the safe therapeutic limit for the plasma concentration of the drug.

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Direct Analysis of Salicylic Acid in Keratolytic Plaster by Gas–Solid Chromatography

PAK H. KO

Abstract □ A rapid gas–solid chromatographic method is reported for the direct analysis of salicylic acid in keratolytic plaster. The method requires no preseparation or derivatization and takes about 14 min. Since it requires only 2–7 mg of sample, it can determine the salicylic acid concentration in a particular area of plaster. The analyzing column is packed with porous polymer beads of 2,6-diphenyl-1,4-phenylene oxide. An automatic solid sampling system is used in connection with the gas chromatograph employing dual flame-ionization detectors. The average recovery of salicylic acid in synthetic samples containing 400–2700 μg was 100.6 ± 1.6%. The average correlation coefficient of the peak area against the standard was +0.9995 ± 0.0003 for samples in the 300–3000-μg range.

Keyphrases □ Salicylic acid—gas–solid chromatographic analysis, commercial plaster pads □ Gas–solid chromatography—analysis, salicylic acid in commercial plaster pads □ Keratolytics—salicylic acid, gas–solid chromatographic analysis in commercial plaster pads

The 40% salicylic acid (I) keratolytic plaster is a uniform mixture of I in a suitable base spread on a backing material such as paper or cloth. The USP (1) requires the plaster mass to contain not less than 90.0% and not more than 110.0% of the labeled amount of I.

To check for uniform distribution of I and for specification compliance, a specific method that does not require a fairly large sample is needed. Since the USP method (1) requires more than 1 g of the plaster base, excluding the backing material, it lacks the sensitivity desired for the type of product[1] with considerably less than 10 mg of the plaster base in a small disk. Furthermore, the USP method is very lengthy.

BACKGROUND

Literature methods for the determination of I that are based on measurement of UV absorption (2–4), color intensity of its ferric chelate (5, 6), fluorescence (7, 8), and alkalimetric titration (9) either required extensive separation manipulations or gave unsatisfactory results due to interference by the excipients in the plaster base.

Several investigators reported the use of GLC for the determination of I in pharmaceuticals and biological media (10–14). However, the methyl ester and methyl ether derivatives (11, 12) or the trimethylsilyl derivative (13, 14) of I had to be prepared before GLC analysis for sufficient volatility; otherwise, severe tailing occurred (10).

Because of the low solubility of the plaster base in most common solvents, methods requiring additional steps to extract I prior to GLC determination or other functional group analyses are less desirable than a direct method by which I in a plaster base can be analyzed without preseparation.

A direct method by gas–solid chromatography (GSC) for determining underivatized I with three other ingredients in a mixture was reported previously (15). This paper reports a rapid and specific GSC method using the same column (15) for the direct determination of I in the plaster base of callus pads without preliminary separation or derivatization.

[1] Zino Corn Pads, Scholl, Inc., Chicago, IL 60610.

Table I—Recovery of Salicylic Acid Added to Placebo Plaster Base

Ratio of Salicylic Acid–Placebo Plaster Base	Salicylic Acid Added, μg	Salicylic Acid Found, μg	Recovery, %
0.31	408	400	98.0
0.24	517	517	100.0
0.26	598	605	101.2
0.31	651	660	101.4
0.27	739	750	101.5
0.26	785	797	101.5
0.64	854	850	99.5
0.37	969	946	97.6
0.45	1011	1035	102.4
0.37	1050	1040	99.0
0.50	1197	1176	98.2
0.80	1237	1256	101.5
0.85	1494	1533	102.6
0.39	1514	1506	99.5
0.61	1605	1621	101.0
0.43	2089	2136	102.2
0.41	2713	2783	102.6
			100.6 ± 1.6

EXPERIMENTAL

Standard—USP grade salicylic acid[2] was used.

GSC Column—A pair of 1.8-m × 0.32-cm o.d. stainless steel columns were packed with 60–80-mesh porous polymer beads of 2,6-diphenyl-1,4-phenylene oxide[3]. Newly packed columns were conditioned at 260° with helium carrier gas flow for 1 hr before use.

Apparatus—The GSC analyses were performed on a gas chromatograph[4] equipped with dual flame-ionization detectors. An automatic capsule sampling system[5] was fitted over the head of the analyzing column. A digital integrator[6] was used to analyze the signals from the flame-ionization detector. The interfacing mechanism was modified to link the integrator, the chromatograph, and the programmer of the automatic capsule sampling system, which were made by different manufacturers. An electronic microbalance[7] for weighing standards and samples, special aluminum capsules[8] for holding standards and samples, and a capsule cold-welding accessory[9] for sealing aluminum capsules containing standards and samples were used.

The chromatography conditions were: injection port, 280°; interface, 290°; column, initially 180° and (after the capsule is punctured) immediately programmed at 16°/min to the upper limit of 245° and maintained at the upper limit for 8 min; helium gas flow, 30 ml/min; detector hydrogen, 40 ml/min; and detector air, 500 ml/min.

Calibration Curve—Standard I samples were weighed into tared aluminum capsules to prepare a calibration curve with the range of 300–3000 μg. The capsules were sealed immediately. The samples were analyzed according to the suggested experimental condition using the gas chromatograph and the automatic sampling system. Specific information pertaining to the use of the automatic sampling system was given elsewhere[10].

The area of the I peak by each standard, as printed out by the digital integrator, was used to establish the calibration curve of area *versus* mass of I for the day's work. The least-squares linear regression method was perferred for showing the calibration.

Analysis of Samples—Samples of plaster base containing I were scraped from the backing cloth with a thin spatula and weighed in tared capsules. The size of the sample was chosen depending on the concentration of the I in the product in order to conform to the range of the calibration curve. The samples were analyzed in the same manner as the standards. The sample's peak area counts were used directly to calculate the I content in the samples according to the slope and intercept obtained by the least-squares linear regression analysis of the calibration runs.

[2] Tenneco Chemicals, Heydon Division, New York, NY 10017.
[3] Tenax GC, Applied Science Labs., State College, PA 16801.
[4] Perkin-Elmer model 3920, Norwalk, CT 06856.
[5] Perkin-Elmer model AS41.
[6] Hewlett-Packard model 3380A.
[7] Perkin-Elmer model AD-1.
[8] Perkin-Elmer part No. 009-0709.
[9] Perkin-Elmer identification No. 043680.
[10] See the Perkin-Elmer Manual for Autosampler AS-41.

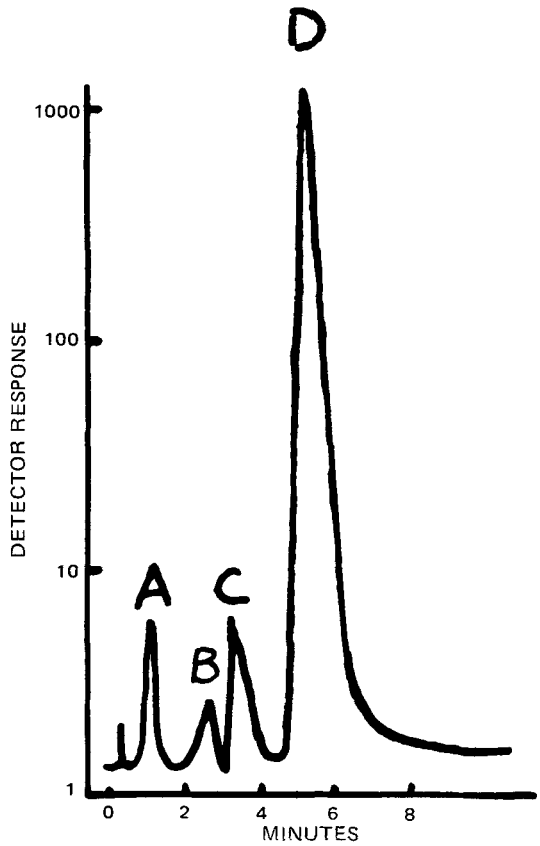

Figure 1—*Gas–solid chromatogram of keratolytic plaster. Key: A, B, and C, unidentified volatile ingredients; and D, salicylic acid.*

RESULTS AND DISCUSSION

Experimental results showed that underivatized solid I can be determined by this new method.

GSC Analysis Time Requirement—Complete elution of I was within 7 min from the time of injection, the time when the sample capsule was conveyed to the vaporizer port behind the trap door. A hollow thorn pierced the capsule, and the helium gas swept the sample vapors through the hollow thorn into the analyzing column. The complete cycle of temperature programming and cooling for the next sample required 14 min.

Calibration Curve Linearity and Sample Size Limitation—The correlation coefficients from various days were determined, and a mean value of +0.9995 was obtained with a standard deviation of 0.0003. Calibration routinely consisted of 15–29 individually weighed standards covering the range of 300–3000 μg of I, and the calibration curves obtained were linear.

When the I mass was less than 300 μg, the chromatographic response (peak area per unit weight of I) became slightly smaller. The cause for deviation has not been determined at this time. Therefore, sample sizes were adjusted to avoid having less than 300 μg of I. Samples with I content much higher than 3000 μg also were avoided because the volume in the aluminum capsule was limited. When the plaster base sample was too big and not enough empty space was available in the capsule, the gummy base material clogged the capillary of the thorn and blocked the carrier gas and the sample vapors from entering the column. Besides avoiding excessively large samples, a way to alleviate the clogging problem is to wrap the plaster base samples in small pieces of porous tissue paper before inserting samples into the capsules.

Continuous Calibration—Although experience showed no significant fluctuation of flame-ionization detector response during runs, slight changes of response were occasionally observed after shutdowns and startups of the instruments.

Since this method uses the I peak area for quantitation and the flame-ionization detector response is affected by the ratio of air and hydrogen, this laboratory routinely monitors the flame-ionization detector response during the span of analyses, usually for several days and nights, by intermittently analyzing known I standards along with unknown samples to ascertain the calibration validity.

Method Accuracy—Table I shows the amounts of I added and those recovered in the synthetic samples containing different ratios of placebo plaster base. The average recovery of I, covering the range of 400–2700 μg of I/sample, was 100.6 ± 1.6%. The accuracy of the method was maintained even when the I concentration was below the formula's theoretical level.

Advantages of Direct Solid Analysis—Because the plaster base samples were directly analyzed without preseparation such as extractions and filtrations, mechanical loss of I was avoided. The proposed method is superior to the methyl ester–methyl ether and trimethylsilyl GLC methods because multiple derivatives of I may be formed upon derivatization (11–14), which may lead to errors. With this method, interferences from solvents and derivatizing agents were totally eliminated.

Since all nonvolatile components in the plaster base were retained in the aluminum capsule and kept from contacting the column, there was essentially no contamination of the packing material. This undoubtedly contributed to the reproducible performance of the column, which has been used to analyze more than 1000 samples but shows no sign of deterioration.

No significant interference is caused by the other volatile ingredients for analysis of I using this method. Figure 1 shows a typical chromatogram (recorded in log scale of the digital integrator) for the analysis of I in a keratolytic plaster, illustrating the separation of I from the other impurities and the general elution characteristics of I.

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Stability of Phenylbutazone in Presence of Pharmaceutical Colors

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Abstract □ The degradation of phenylbutazone was studied in the presence of lakes suitable for coloring the sugar coats of phenylbutazone tablets. The drug was degraded, in light, in the presence of erythrosine sodium. The degradation probably proceeds *via* singlet oxygen generated by the light-excited dye. The degradation may be important in some quality control procedures and can lead, for example, to unusual results in dissolution rate testing.

Keyphrases □ Phenylbutazone—stability in presence of various pharmaceutical colors, effect of light □ Stability—phenylbutazone in presence of various pharmaceutical colors, effect of light □ Colors, various pharmaceutical—effect on stability of phenylbutazone, effect of light □ Antirheumatic agents—phenylbutazone, stability in presence of various pharmaceutical colors, effect of light

Colors are used in pharmaceutical dosage forms for their aesthetic appeal and as an aid to identification. Coated tablets are frequently colored, and lakes are becoming increasingly popular for this purpose because they offer advantages in the speed of application and cover uniformity. The stability of pharmaceutical dyes and lakes was assessed (1, 2), but little attention has been paid to the possible interactions between colors and drugs.

Amaranth (FD&C Red No. 2 lake), erythrosine sodium (FD&C Red No. 3 lake), tartrazine (FD&C Yellow No. 5 lake), and FD&C Yellow No. 6 lake may be used in combination to give a color suitable for incorporation in the sugar coats of phenylbutazone tablets. This report is concerned with the interaction between these lakes and phenylbutazone.

EXPERIMENTAL

Materials—Amaranth lake[1], erythrosine sodium lake[1], tartrazine lake[1], and FD&C Yellow No. 6 lake[1] (sunset yellow) were obtained as separate powders and as a mixture in syrup[1]. Phenylbutazone[2] was of BP quality, and soluble erythrosine sodium[3] and methylene blue[3] were of reagent grade.

Irradiation of Samples—Suspensions or solutions of the materials in phosphate buffer (pH 7.4) were prepared and stored in subdued light. The test mixtures were exposed, in 1-cm quartz cells, to unfiltered light from a 300-w projector bulb situated 50 cm from the cell. Measurements of the liquid temperature during runs indicated no heating effect by the light beam. Due to the fine particle size of the lakes, sedimentation problems did not arise during the experiment.

[1] Colorcon Ltd., Orpington, England.
[2] Thomas Kerfoot Ltd., Ashton under Lyne, England.
[3] B.D.H. Ltd., Poole, England.