Quantitative Determination of Benzoic Acid and Salicylic Acid in Ointments by High-Pressure Liquid Chromatography

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Abstract □ Benzoic acid and salicylic acid in ointments were analyzed quantitatively by reversed-phase high-pressure liquid chromatography. Both benzoic and salicylic acids can be assayed in combinations directly without interference from each other or the ointment bases. The method is accurate, simple, and rapid. Excellent results were obtained with three commercial bases.

Keyphrases □ Benzoic acid—reversed-phase high-pressure liquid chromatographic analysis, pharmaceutical formulations containing salicylic acid □ Salicylic acid—reversed-phase high-pressure liquid chromatographic analysis, pharmaceutical formulations containing benzoic acid □ High-pressure liquid chromatography, reversed phase—analysis, benzoic and salicylic acids, pharmaceutical formulations □ Antifungal agents—benzoic acid, reversed-phase high-pressure liquid chromatographic analysis, pharmaceutical formulations □ Keratolytic agents—salicylic acid, reversed-phase high-pressure liquid chromatographic analysis, pharmaceutical formulations

The quantitative determination of benzoic acid (I) and salicylic acid (II) in ointments was reported (1). That method involved the determination of II by its reaction with ferric nitrate. Benzoic acid was determined by difference from the total acidity reading. Any error in the determination of II is carried over to results on I. The purpose of these investigations was to develop a direct, simple, and accurate method for the quantitative determinations of I and II in combinations.

EXPERIMENTAL

Chemicals and Reagents—All chemicals and reagents were ACS, USP, or NF grade and were used without further purification.

Apparatus—A high-pressure liquid chromatograph[1] equipped with a UV detector (254 nm) and recorder[2] was used.

Column—A very nonpolar packing material[3] consisting of a monomolecular layer of octadecyltrichlorosilane, permanently bonded by silicon–carbon bonds, and a 30-cm long × 4-mm i.d. column were used.

Chromatographic Conditions—The chromatographic solvent was 0.02 *M* KH_2PO_4 in 10% (v/v) methanol in water (pH adjusted[4] to 6.2 with ~0.1 *N* NaOH). The temperature was ambient, and the solvent flow rate was 2.0 ml/min (at an inlet pressure of approximately 1100 psig). The detector was set at a sensitivity of 0.16 absorbance unit full-scale, and the chart speed was 30.5 cm (12 in.)/hr.

Solutions—A standard solution of I (100.0 mg/250 ml) or II (50.0 mg/250 ml) or a combination of I and II was prepared by heating the acid(s) in 15 ml of approximately 0.1 *N* NaOH and 60 ml of water to almost boiling. The mixture was then cooled and brought to volume (250.0 ml).

Preparation of Ointments—Three lots of ointments were prepared by the trituration process. Lot A contained 3.0% of II by weight in white petrolatum; Lot B contained 6.0% of I and 3.0% of II in a cold cream[5]; Lot C contained the same ingredients as Lot B in a different base[6] (2).

Assay Procedure for Ointments—Weigh accurately about 1.667 g of the ointment, transfer it to a 150-ml beaker, and add 15 ml of the

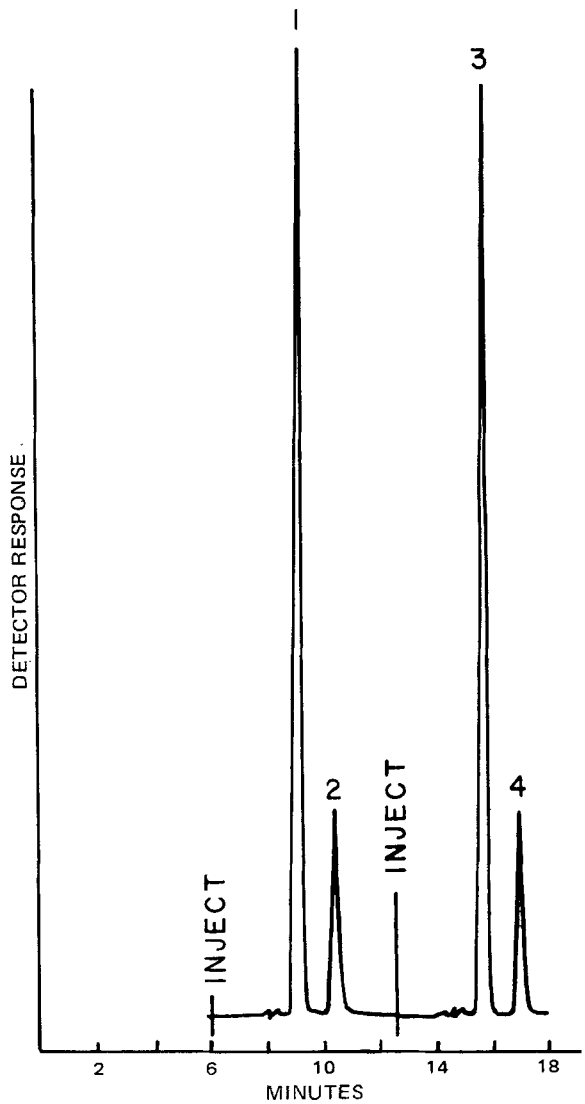

Figure 1—*Liquid chromatogram of I and II in combination (for conditions, see text). Peaks 1 and 2 are I and II from a standard solution, respectively; peaks 3 and 4 are I and II from an ointment sample, respectively.*

[1] Waters ALC 202 equipped with U6K universal chromatograph injector.
[2] Omniscribe 5213-12 equipped with an integrator.
[3] μBondapak-C18, Catalog No. 27324, Waters Associates.
[4] A Beckman Zeromatic SS-3 pH meter was used.
[5] The Upjohn Co., Kalamazoo, Mich. For ingredients, see Ref. 1.
[6] HEB base, Barnes-Hind Pharmaceuticals, Sunnyvale, Calif. For ingredients, see Ref. 2.

Table I—Assay Results[a] on Ointments

Ointment	Assay Results, % I	II
Lot A	0.0	99.6
Lot B	99.4	99.8
Lot C	97.9	99.2
Average deviation (based on six readings)	±2.1	±2.8

[a] Average of three.

0.1 *N* NaOH solution and 60 ml of water. Heat almost to boiling and cool until the base congeals at the top of the aqueous layer. Then decant and transfer the aqueous layer to a 250-ml volumetric flask, wash the beaker with small portions of water, and transfer the rinsings to a volumetric flask to bring the mixture to volume. Filter if necessary and inject 10–20 μl.

For comparison purposes, inject an identical volume of the appropriate standard solution after the assay sample is eluted.

Calculations—Since preliminary investigations indicated that the peak area of each ingredient was directly related to the concentration (range of 4–8 μg for I and of 2–4 μg for II), the results on I and II were calculated by direct comparison of the peak areas:

$$\frac{\text{corrected } A_a}{A_s} \times 100 = \text{percent of label claim} \qquad \text{(Eq. 1)}$$

where corrected A_a = peak area of the assay × (1.667/weight of sample in grams), and A_s = peak area of the standard solution.

A typical liquid chromatogram is shown in Fig. 1, and the assay results are presented in Table I.

DISCUSSION

The results (Table I) indicate that I and II in combinations can be assayed directly by high-pressure liquid chromatography. The separation of I from II is excellent (Fig. 1). The method is accurate, rapid, and simple. The three ointment bases used did not interfere with the assay; other bases may interfere. Any other base should be checked for interference by using the assay procedure on the plain base. It is necessary to run both the assay and the standard with the same lot of the chromatographic solvent, since slight differences in pH may change the area of the peak.

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Effects of Aspirin and Acetaminophen on Fetal and Placental Growth in Rats

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Abstract □ Aqueous suspensions of aspirin or acetaminophen (125 and 250 mg/kg/day) were administered orally to pregnant Sprague–Dawley rats on Days 8–19 of gestation. On gestation Day 20, each rat was sacrificed and the uterus was examined *in situ*. Each fetal–placental unit was resected and examined. Fetuses from rats given 125 or 250 mg/kg/day of aspirin were shorter and weighed less than those obtained from control rats. In animals receiving the higher dose of aspirin, the placentas were smaller and the number of fetal resorptions was increased. Acetaminophen (250 mg/kg/day) did not affect fetal length or weight or the incidence of resorptions. Acetaminophen interfered less with the normal growth of the rat fetus and placenta than did aspirin.

Keyphrases □ Aspirin—effects on fetal and placental growth, rats □ Acetaminophen—effects on fetal and placental growth, rats □ Fetal growth—effects of aspirin and acetaminophen, rats □ Placental growth—effects of aspirin and acetaminophen, rats

The antigonadal and antireproductive activities of the two most commonly used nonprescription analgesic drugs were reported previously. Chronic administration of acetaminophen to rats produced a decrease in testicular weight and impaired spermatogenesis (1). Also, chronic administration of acetaminophen significantly decreased fecundity in mice, but similar doses of aspirin were ineffective (2). Aspirin administration interfered with the rapid increase in ovarian size following hemicastration (3) and also caused embryotoxicity and teratogenecity in rats (4).

Many studies document the effects of salicylates on fetal growth and development (4–10); however, no studies are known that directly compare the effects of aspirin to those of acetaminophen. Since aspirin and acetaminophen may influence gonadal function, normal reproduction, and the growth of rapidly developing tissue, the effects of these widely used analgesics on fetal and placental growth were compared.

EXPERIMENTAL

Female Sprague–Dawley rats[1] were used. For gravid rats, the sperm-positive date indicated by the supplier was designated Day 0 of pregnancy. If allowed to progress to term, parturition occurred on Day 21. Animals were randomized, housed three or four to a wire mesh bottom cage, and fed rat chow[2] and water *ad libitum*.

From the 8th through the 19th day of gestation, groups of 21 sperm-positive rats were weighed and received aspirin[3] or acetaminophen[4] at 0, 125, or 250 mg/kg by gavage. Groups of nonmated female rats, comparable in age, were treated according to the same regimen. Each drug was suspended fresh daily in 0.5% methylcellulose with a homogenizer[5] at a concentration such that 1 ml was administered for every 200 g of body weight. Drugs were administered without anesthesia, since Kimmel *et al.* (5) observed abnormalities in offspring from rats anesthetized with ether.

[1] Holtzman Co., Madison, Wis.
[2] Purina.
[3] Merck and Co., Rahway, N.J.
[4] Provided by McNeil Laboratories, Fort Washington, Pa.
[5] Potter–Elvehjem.