Rapid Gas Chromatographic Determination of Serum Salicylates After Silylation

Keyphrases □ GLC analysis—Simultaneous determination of acetylsalicylic acid and salicylic acid in plasma, serum and whole blood, formation of trimethylsilyl derivatives

To the Editor:

Both acetylsalicylic acid and salicylic acid are pharmacologically active, but there are qualitative and quantitative differences in the analgesic and antiplatelet effects and in their inhibition of prostaglandin synthetase (1, 2). Gas–liquid chromatographic (GLC) methods for quantifying acetylsalicylic acid and salicylic acid simultaneously are time consuming, requiring extraction prior to the formation of the corresponding trimethylsilyl derivatives (3–5). Furthermore, the analyses are complicated by some hydrolysis of acetylsalicylic acid to salicylic acid during derivatization (6).

The GLC methods available for the quantitation of acetylsalicylic acid and salicylic acid require prior silylation at 50° for 60 min (3–5). Silylation of salicylic acid with hexamethyldisilazane in acetone gave two peaks with retention times of 1.5 and 2.2 min when chromatographed at 135° under our conditions. These peaks were considered to be the mono- and bis-trimethylsilyl derivatives of salicylic acid, respectively, since the intensity of the 1.5-min peak decreased with time with concomitant increase in the height of the 2.2-min peak (4). Therefore, a more appropriate system of derivatization was sought. Table I gives the effect of time on the silylation of acetylsalicylic acid, salicylic acid, and *p*-hydroxybenzoic acid at 23° and 60° with *N,O*-bis(trimethylsilyl)trifluoroacetamide in acetonitrile. *p*-Hydroxybenzoic acid was chosen as the internal standard since it readily forms a bis-trimethylsilyl derivative (7). Acetonitrile was chosen as the solvent, since it has been reported to be suitable for the silylation of catecholamines and related compounds (8). The results show that under these conditions of silylation[1] the maximum peak area ratio was obtained almost immediately at both temperatures and that the ratio was stable for at least 60 min, even at 60°. The mean peak area ratio obtained for salicylic acid at the various times after the addition of *N,O*-bis(trimethylsilyl)trifluoroacetamide was 0.96 ± 0.10 (*SD*) at 23° and 1.02 ± 0.05 (*SD*) at 60°, representing coefficients of variation of 10.4 and 5.0%, respectively. Similarly, the mean values of the peak area ratio for acetylsalicylic acid were 0.56 ± 0.01 (*SD*) at 23° and 0.55 ± 0.03 (*SD*) at 60°, giving coefficients of variation of 2.6 and 6.1%, respectively. The peak with a retention time of 1.5 min, considered to be the monosilylation product of salicylic acid, was not observed under these conditions. For rapid silylation of acetylsalicylic acid and salicylic acid, dry acetone can be substituted for acetonitrile. Also, the rate of silylation was faster when *N,O*-bis(trimethylsilyl)trifluoroacetamide was used instead of hexamethyldisilazane.

Table I—Effects of Time and Temperature on the Silylation of Acetylsalicylic Acid, Salicylic Acid, and *p*-Hydroxybenzoic Acid in Acetonitrile Using *N,O*-bis(trimethylsilyl)trifluoroacetamide as the Silylating Reagent [a]

	Peak Area Ratio of Disilylated Salicylic Acid or Monosilylated Acetylsalicylic Acid/Disilylated *p*-Hydroxybenzoic Acid			
	Acetylsalicylic Acid		Salicylic Acid	
Minutes [b]	23°	60°	23°	60°
0.2–1	0.57	—	1.08	—
10	0.56	0.60	1.04	1.07
20	0.56	0.56	0.90	1.02
30	0.56	0.56	0.87	1.06
40	0.54	0.57	0.83	0.94
50	0.59	0.49	1.03	0.98
60	0.56	0.54	1.00	1.03

[a] One ml of an ethereal solution containing 10 μg of each compound was evaporated to dryness; the residue was dissolved in 10 μl of acetonitrile and reacted with 5 μl of *N,O*-bis(trimethylsilyl)trifluoroacetamide. A 2–3-μl aliquot was injected into the gas chromatograph. [b] After addition of silylating reagent.

Figure 1 shows typical chromatograms of ethereal extracts of drug-free serum carried through the analytical procedure (A); of drug-free serum to which 15 μg of acetylsalicylic acid, salicylic acid, and the internal standard *p*-hydroxybenzoic acid were added (B); and of a mixture of acetanilide, methyl salicylate, salicylic acid, *p*-hydroxy-

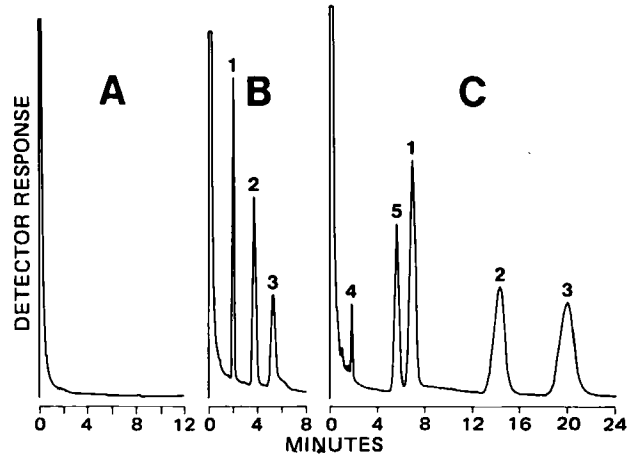

Figure 1—*Chromatograms of drug-free serum carried through the procedure (A), drug-free serum to which 15 μg of acetylsalicylic acid, salicylic acid, and p-hydroxybenzoic acid were added and analyzed, as described in the procedure (B), and of a mixture of acetanilide, methyl salicylate, salicylic acid, p-hydroxybenzoic acid, and acetylsalicylic acid analyzed at 110° (C). The derivatization was carried out in acetonitrile using* N,O-*bis(trimethylsilyl)trifluoroacetamide as silylating reagent. Key: (1) salicylic acid; (2)* p-*hydroxybenzoic acid; (3) acetylsalicylic acid; (4) acetanilide; (5) methyl salicylate.*

[1] Sample formulation: To each 0.10-ml aliquot of serum, plasma, or whole blood, 2 ml of 1 *N* HCl was added; *p*-hydroxybenzoic acid (15 μg in 1 ml of water) was added as the internal standard; and the mixture was extracted twice with 5 ml of freshly distilled ether. The organic phase was transferred to another tube and evaporated to dryness under a stream of nitrogen in a water bath at 42–44°. The residue was dissolved in 10 μl of acetonitrile and derivatized with 5 μl of *N,O*-bis(trimethylsilyl)trifluoroacetamide (Pierce Chemical Co.). A 2–3-μl sample was injected into a gas chromatograph (Hewlett-Packard model 5710A) equipped with a flame-ionization detector and a chart recorder-integrator (Hewlett-Packard model 3380s). The column was 1.2 m long ×4-mm i.d. glass tubing packed with 2% OV-225 on 80–100 mesh Gas Chrom W (Chromatographic Specialities, Ltd.) It was conditioned overnight at 225° and treated with hexamethyldisilazane before use. The operating conditions were: injection port temperature, 250°; oven temperature (isothermal), 135 or 110°; detector temperature, 300°. The nitrogen (carrier gas), air, and hydrogen flow rates were 60, 100, and 30 ml/min, respectively.

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benzoic acid, and acetylsalicylic acid (C). Parts A and B were determined at 135° with part C determined at 110° in order to have base line resolution of all the peaks. The ethereal extract of control serum sample (A) did not give any peak that interfered with the GLC analysis of acetylsalicylic acid, salicylic acid, and *p*-hydroxybenzoic acid. Similar results were obtained using samples of either rat or human plasma. The retention times of the trimethylsilyl derivatives of salicylic acid, *p*-hydroxybenzoic acid, and acetylsalicylic acid were 2.2, 4.4, and 5.4, respectively at 135°. Under these conditions, the retention times of the trimethylsilyl derivatives of methyl salicylate, ibuprofen, salicylamide, and acetaminophen were 1.9, 3.7, 10.8, and >30 min, respectively. Acetanilide and phenacetin did not form a trimethylsilyl derivative, but each compound produced a single peak with retention time values of 0.9 and 4.7 min, respectively, when chromatographed at 135°. Other nonsteroid anti-inflammatory drugs such as ketoprofen, indomethacin, and naproxen did not produce a peak when silylated under identical conditions. Complete separation between the peak of the trimethylsilyl derivatives of methylsalicylic acid and salicylic acid was obtained at 110° (Fig. 1C). Under these conditions, the retention time values of underivatized acetanilide and of the trimethylsilyl derivatives of methylsalicylic acid, salicylic acid, *p*-hydroxybenzoic acid, and acetylsalicylic acid were 2.0, 5.1, 7.1, 14.35, and 20.05 min, respectively. Salicyluric acid (2-hydroxyhippuric acid), the major metabolite of salicylic acid, did not give a peak under the analytical conditions used. The chromatographic conditions determined at 135° were suitable for the simultaneous analysis of acetylsalicylic acid and salicylic acid in serum samples. There was no interference from the commonly used analgesics often prescribed in combination with acetylsalicylic acid, except for phenacetin, which gave a peak of similar retention time value close to that of the trimethylsilyl derivative of *p*-hydroxybenzoic acid. In cases where phenacetin is also being administered, 4-chlorophenylacetic acid can be used as a reference standard since its trimethylsilyl derivative has a retention time value of 2.9 min.

Table II gives the coefficient of variation and the recovery of different concentrations of acetylsalicylic acid and salicylic acid added to the control serum. The coefficients of variation of triplicate analysis varied from 0.0 to 6.9% for acetylsalicylic acid and from 1.1 to 6.4% for salicylic acid. The mean values for the coefficient of variation of the eight concentrations of acetylsalicylic acid and salicylic acid used were 2.6 ± 2.6 and 3.7 ± 1.8 (*SD*%) respectively. The recovery of these two compounds was essentially quantitative. The overall recovery for acetylsalicylic acid was 104.0 ± 8.4 (*SD*)%, while that of salicylic acid was 98.2 ± 5.3 (*SD*)%. Calibration curves were obtained for acetylsalicylic acid ($y = 0.03 + 0.0474x$ and $r = 0.996$) and for salicylic acid ($y = 0.20 + 0.064x$ and $r = 0.997$) in the range of concentrations of 2.5–25 μg/0.1 ml of serum, *i.e.*, within the concentrations determined in pharmacokinetic studies. It should be pointed out that the hydrolysis of acetylsalicylic acid to salicylic in blood samples at 37° is rapid (9–11) and, therefore, it is necessary to extract the acetylsalicylic acid as soon as possible to avoid its breakdown in storage. There was no breakdown of acetylsalicylic acid to salicylic acid during the extraction, evaporation, and derivatization processes described herein.

Table II—Recovery of Different Amounts of Acetylsalicylic Acid and Salicylic Acid Added to Control Serum

Amount Added, μg/0.1 ml	Acetylsalicylic Acid: Amount Recovered[a], μg/0.1 ml ± *SD*	*CV*, %[b]	Recovery, %	Salicylic Acid: Amount Recovered[a], μg/0.1 ml ± SD	CV, %[b]	Recovery, %
2.5	2.25 ± 0.13	5.8	90.0	2.7 ± 0.1	3.3	108.0
7.5	9.1 ± 0.6	6.9	121.0	7.4 ± 0.3	5.3	98.7
10.0	10.5 ± 0.0	0.0	105.0	9.7 ± 0.3	3.2	97.0
12.5	12.9 ± 0.2	1.6	103.2	11.7 ± 0.55	4.7	93.6
15.0	15.6 ± 0.2	1.3	104.0	13.8 ± 0.6	4.5	92.0
17.5	17.9 ± 0.7	4.1	102.3	16.6 ± 0.2	1.4	94.8
20.0	21.1 ± 0.0	0.0	105.5	19.7 ± 0.2	1.1	98.5
25.0	25.3 ± 0.2	0.8	101.2	25.8 ± 1.6	6.4	103.2

[a] Mean value ± *SD* of triplicate analyses. [b] Coefficient of variation = *SD*/mean × 100.

The proposed GLC assay of acetylsalicylic acid and salicylic acid in serum is simpler and faster than other methods that have been published for the same purpose.

(1) C. Patrono, G. Ciabattoni, F. Pugliese, E. Pinca, G. Castrucci, A. De Salvo, M. A. Satla, and M. Parachini, *Agents Actions*, **4** (Suppl.), 138 (1979).

(2) D. C. Atkinson and H. O. J. Collier, *Adv. Pharmacol. Chemother.*, **17**, 233 (1981).

(3) B. H. Thomas, G. Solomonraj, and B. B. Caldwell, *J. Pharm. Pharmacol.*, **25**, 201 (1973).

(4) L. J. Walter, D. F. Biggs, and R. T. Coutts, *J. Pharm. Sci.*, **63**, 1754 (1974).

(5) M. J. Rance, B. J. Jordan, and J. D. Nichols, *J. Pharm. Pharmacol.*, **27**, 425 (1975).

(6) S. L. Ali, *Chromatographia*, **8**, 33 (1975).

(7) C. E. Dalgliesh, E. C. Horning, M. G. Horning, K. L. Knox, and K. Yarger, *Biochem. J.*, **101**, 792 (1966).

(8) M. G. Horning, A. M. Moss, and E. C. Horning, *Biochim. Biophys. Acta*, **148**, 597 (1967).

(9) E. B. Truitt, Jr. and A. M. Morgan, *Arch. Int. Pharmacodyn. Ther.*, **135**, 105 (1962).

(10) P. A. Harris and S. Riegelman, *J. Pharm. Sci.*, **56**, 713 (1967).

(11) M. Rowland and S. Riegelman, *J. Pharm. Sci.*, **56**, 717 (1967).

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Is Aspirin Phenylalanine Ethyl Ester a Prodrug for Aspirin?

Keyphrases □ Aspirin—high-performance liquid chromatography, phenylalanine ethyl ester as prodrug

To the Editor:

It is known that oral administration of aspirin induces gastric irritation and bleeding because of local irritation