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A simultaneous determination of acetylsalicylic acid, salicylic acid and salicylamide in plasma by gas liquid chromatography

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A novel method for the simultaneous determination of acetylsalicylic acid, salicylic acid and salicylamide in biological fluids by gas liquid chromatography is described. The assay has been used to determine the plasma concentration of salicylates in 10 volunteers after oral ingestion of three commercially available aspirin-containing formulations. No difficulty was encountered in determining low concentrations of acetylsalicylic acid in the presence of higher concentrations of salicylic acid. The *in vivo* plasma half life of acetylsalicylic acid in man was found to be 15·5 min.

Standard fluorometric and colorimetric methods of salicylate assay, when applied to the simultaneous determination of salicylic acid (SA) and acetylsalicylic acid (ASA), suffer from the disadvantage that ASA can only be measured by difference after hydrolysis to SA (Morgan & Truitt, 1965; Leonards, 1962a, b; Harris & Riegelman, 1967). As a result, it is difficult to obtain accurate values for low concentrations of ASA in the presence of an excess of SA. Lange & Bell (1966) used a chromatographic separation technique in an attempt to overcome this difficulty and Routh, Shane & others (1967) have reported a simultaneous assay for ASA and SA by differential spectrophotometry. Some commercially available aspirin preparations contain salicylamide and the presence of this compound or its metabolites in plasma creates further difficulties in the application of relatively unspecific techniques to the determination of salicylate mixtures in biological fluids. As a consequence of these problems, reports of plasma concentrations of ASA after oral administration of the drug are sparse and many studies give results only in terms of total salicylate levels.

Gas liquid chromatography (g.l.c.) methods for the simultaneous assay of SA and ASA in solid dosage forms have been described (Nikelly, 1964; Watson, Crescuolo & Matsui, 1971; Patel, Perrin & Windheuser, 1972). Rowland & Riegelman (1967) reported a method for the g.l.c. determination of ASA but used a fluorometric method to measure SA concentrations. Morris, Christian & others (1970) have described the g.l.c. properties of salicylate metabolites. A single report of the use of a g.l.c. method to determine ASA and SA simultaneously in biological fluids has recently been published (Thomas, Solomonraj & Coldwell, 1973).

This paper describes an extension of the g.l.c. method of salicylate assay to yield a simple, reproducible method for the simultaneous determination of ASA, SA and salicylamide in plasma as their trimethylsilyl (TMS) derivatives. The assay has been used to determine the plasma concentrations of salicylates in healthy human volunteers after oral administration of three different aspirin-containing formulations.

MATERIALS AND METHODS

Reagents and chemicals

Bis-(trimethylsilyl)-trifluoracetamide (BSTFA) was obtained from Fluorochem Ltd. Salicylic acid, acetylsalicylic acid and salicylamide used as standards were of B.P. purity. *m*-Toluic acid (BDH Ltd.) was LR grade. Diethyl ether was glass distilled before use. Phosphate buffer (0·2M) pH 7·4 was used throughout.

Aspirin formulations

Three commercial aspirin-containing preparations were used: a soluble preparation [1](A), a typical preparation containing salicylamide [2](B), and aspirin B.P.[3](C).

Chromatographic conditions

A Hewlett Packard 5700A gas chromatograph equipped with dual flame ionization detectors and temperature programmer was used. The chromatograph was fitted with two 1·8 m × 4 mm i.d. glass columns containing 5% OV17 on CQ (80–100 mesh) (JJ's Chromatography Ltd.) preheated at 230° for 24 h before use. The detector oven was heated at 250° and the column oven programmed from 160 to 200° at 2° min^{-1}. Nitrogen was used as the carrier gas at a flow rate of 50 ml min^{-1}. Hydrogen and air flow rates were 60 and 250 ml min^{-1} respectively. Injections were made manually and peak areas were measured by a Hewlett Packard 3370B integrator. Mass spectra of the derivatives were obtained using an LKB 2091 gas chromatography-mass spectrometry (g.c.-m.s.) instrument.

Assay of plasma samples

Plasma (1·0 ml) and *m*-toluic acid (10 μg in 100 μl phosphate buffer) as internal standard, were added to a 12 ml glass-stoppered centrifuge tube containing 5% potassium bisulphate solution (1·0 ml). Glass distilled ether (7 ml) was added and the tube contents mixed thoroughly. The organic layer was removed and dried over anhydrous sodium sulphate. The ether was then transferred to a clean tube and the sodium sulphate washed twice with aliquots (1 ml) of ether. The washings were combined with the ether extract and evaporated to dryness in a stream of nitrogen. BSTFA (40 μl) was added to the glass-stoppered tube containing the residue, which was then placed in a water bath at 50° for 1 h. An aliquot (1 μl) of this solution was injected into the chromatograph.

Calibration curves were prepared for each compound as follows. Various volumes of ether solutions (10 μg ml^{-1}) of ASA, SA and salicylamide were added to tubes containing *m*-toluic acid (10 μg) in ether (1·0 ml). The resulting mixture was evaporated to dryness in a stream of nitrogen and the TMS derivatives were prepared as above. Ratios of peak areas salicylate/*m*-toluic acid were plotted *vs* salicylate concentration.

Recovery was determined by the addition of known amounts (1·25–20 μg in phosphate buffer) of ASA, SA and salicylamide to plasma which was assayed as above.

Studies with volunteers

Plasma concentrations of ASA, SA and salicylamide (only after preparation B) were determined in ten healthy male volunteers(19–37 years; 57–92 kg). The three preparations were administered to each of the volunteers on separate occasions at intervals of

1. New Disprin, Reckitt and Colman Ltd. 300 mg ASA per tablet.
2. Anadin, International Chemical Co. 325 mg ASA + 65 mg salicylamide per tablet.
3. Boots Pure Drug Co. 300 mg ASA per tablet.

at least 14 days. The volunteers were fasted overnight before drug administration. No beverages were allowed until 90 min and no food until 4 h after dosing. The soluble aspirin preparation A (two tablets) was dissolved in water (50 ml) immediately before administration and the tablet formulations B and C (two tablets) were swallowed with water (50 ml). Blood samples (5 ml), taken by venipuncture at 0, 10, 20, 30, 45, 60, 90, 120, 240 and 420 min after dosing, were placed in chilled heparinized tubes (Turner-Stayne Laboratories) containing aqueous potassium fluoride solution (50 μl; 50% w/v) (Rowland & Riegelman, 1967). Blood samples, which were stored in an ice bath, were centrifuged and the plasma extracted within 60 min of sampling.

RESULTS

Fig. 1 presents a gas chromatogram of authentic *m*-toluic acid, ASA, SA and salicylamide as their trimethylsilyl derivatives.

A linear relation is observed when the ratio of peak areas (salicylate/internal standard) is plotted against the concentration of salicylate in all three cases. Gas chromatograms of control samples did not exhibit any peaks with the same retention time as any of the assayed derivatives. The recovery of the drugs from plasma is illustrated by the results shown in Table 1. The limit of detection for all three compounds was 0·2–0·5 μg ml^{-1}.

Some difficulties have previously been encountered due to the formation of multiple TMS derivatives of SA as both ester and ether derivation is possible (Rowland & Riegelman, 1967). Use of the forcing conditions of Thomas & others, (1973) resulted in a single symmetrical peak for SA (Fig. 1). Salicylamide, which also has two possible sites of reaction, gave one peak under the chromatographic conditions used.

Examination of the TMS derivatives of SA and salicylamide by g.c.-m.s. yielded mass spectra which were compatible in each case with a *bis*-TMS derivative. Each

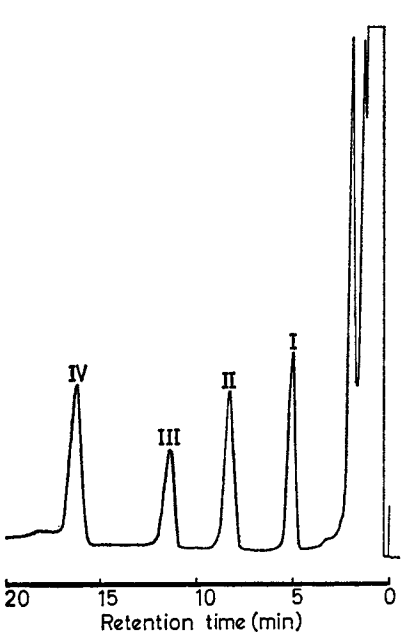

FIG. 1. Gas chromatogram of authentic salicylates as their trimethylsilyl derivatives. I-*m*-toluic acid (internal standard), II-SA, III-ASA, IV-salicylamide.

Table 1. *Recovery of salicylates from plasma.*

Salicylate	Plasma concentration (μg ml^{-1})	Concentration found (μg ml^{-1}) mean $\pm$ s.e. (n = 4)
Acetylsalicylic acid	1·25	1·2 $\pm$ 0·2
	2·5	2·3 $\pm$ 0·1
	5·0	5·2 $\pm$ 0·3
	10·0	10·0 $\pm$ 0·2
	20·0	20·7 $\pm$ 0·9
Salicylic acid	5·0	5·2 $\pm$ 0·4
	10·0	10·0 $\pm$ 0·7
	20·0	19·7 $\pm$ 1·2
Salicylamide	10·0	10·0 $\pm$ 0·2
	20·0	20·8 $\pm$ 0·5

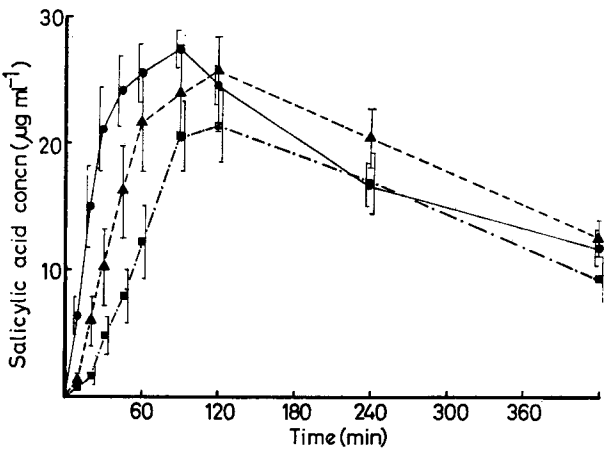

FIG. 2. Mean plasma concentrations of SA obtained in 10 volunteers after oral administration of preparation A (●), preparation B (■) and preparation C (▲). Bars represent s.e.

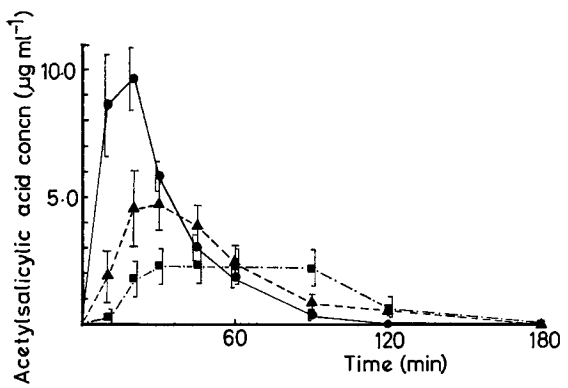

FIG. 3. Mean plasma concentrations of ASA obtained in 10 volunteers after oral administration of preparation A (●), preparation B (■) and preparation C (▲). Bars represent s.e.

showed a weak parent ion (*m/e* 282 and 281 respectively) with a strong peak at m-15 due to loss of a methyl group from the TMS function.

Figs 2 and 3 show the plasma concentration of SA and ASA respectively after volunteers had taken the three aspirin preparations.

No salicylamide was detected in the plasma of any volunteer after dosing with the preparation B.

DISCUSSION

Assay of standard plasma concentrations has shown the method to be capable of yielding reproducible and accurate results simultaneously for the three compounds.

To investigate the applicability of the method to the determination of salicylates *in vivo*, plasma concentrations of ASA, SA and salicylamide have been monitored in ten healthy volunteers after administration of three ASA containing preparations, one of which also contained salicylamide. The results obtained were compatible with those reported by Leonards (1962a, b) who used a colorimetric method of salicylate assay. In no case was any difficulty encountered in determining low concentrations of ASA even in the presence of much higher concentrations of SA.

Thus, using the g.l.c. methodology described above, information on plasma concentrations of ASA can be readily obtained. In the case of soluble preparation (A) the data can be plotted semilogarithmically to yield a value for the plasma half life of ASA *in vivo* of 15·5 min, which agrees well with that reported by Levy (1965) [from data obtained by Leonards (1962a)] (18 min) and with a value of 16 min determined by Sholkoff, Eyring & others (1967) in arthritic patients.

Levy (1965) has predicted on theoretical grounds that ASA preparations which release the drug at different rates will yield ASA plasma concentrations differing both in magnitude and time of occurrence. This is well illustrated in the present study in which the more rapidly absorbed soluble preparation gives both an earlier and a higher peak plasma concentration of ASA (Fig. 3).

Previous studies in animals and man involving oral dosing of salicylamide alone (Crampton & Voss, 1954; Weikel, 1958) have shown that very little of the free drug can be detected in plasma. This also appears to be the case with a mixed ASA/salicylamide preparation. At the dose used in this study (130 mg salicylamide, 650 mg ASA), the plasma concentration of unchanged salicylamide did not exceed 0·5 μg ml^{-1} in any volunteer at any time after dosing.

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