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Note

High-performance liquid chromatographic determination of impurities in commercial saccharin

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Low calorie soft drinks are probably the most frequently consumed saccharin-containing products of the British diet. Most commercial saccharin is manufactured by the Remsen–Fahlberg method, which oxidises toluene 2-sulphonamide (*o*TS) to saccharin. Food-grade saccharin, therefore, often contains various amounts of this substance as an impurity. The other most frequently occurring impurity is 4-sulphamoylbenzoic acid (*p*SBA), which is the oxidation product of toluene 4-sulphonamide (*p*TS).

Until recently the *British Pharmacopoeia* (B.P.) set the upper limit of *o*TS and *p*SBA as 1% each in food-grade saccharin. The toxicological reports of recent years[1–4], which involved both saccharin and the *o*TS, have initiated the imposition of much stricter control over these impurities. As a result B.P. issued an addendum in December 1978 limiting the permissible level of *o*TS to 200 mg/kg. More recently the third supplement to the *Food Chemicals Codex*, 2nd edition, issued by the US National Academy of Science, reduced the presence of *o*TS from 100 mg/kg to not more than 25 mg/kg in all types of saccharin products. West Germany imposed the stringent limit of 10 mg/kg *o*TS in commercial saccharin[5].

In parallel with the toxicological studies the analytical aspects have also received worldwide attention in recent years. The analytical method described in the B.P. is based on the thin-layer chromatographic method (TLC) developed by King and Wragg[6]. The TLC method, although it can detect a number of impurities in saccharin, is time consuming and only semi-quantitative for both *o*TS and *p*SBA.

For the quantitation of the *o*TS several gas–liquid chromatographic (GLC) methods have been developed in recent years[7–12]. The GLC methods are sensitive and can resolve *o*TS from its isomer *p*TS after a suitable clean-up of the sample. The clean-up procedure usually involves solvent extraction prior to chromatography.

The sulphamoylbenzoic acids in commercial saccharin may be determined by various wet-chemical means[13–15]. These methods, however, are not very sensitive. More sensitive and specific is the recently developed high-performance liquid chromatography (HPLC) method which employs an ion-exchange column and aqueous sodium borate solution as mobile phase[16]. A sufficiently selective method suitable for the rapid determination of amides and acids has not been found in the literature.

The reversed-phase HPLC method presented here can separate both acid and amide isomers and after the removal of saccharin offers the basis of quantitative analysis.

EXPERIMENTAL

Chemicals

Toluene 2-sulphonamide and 4-sulphamoylbenzoic acid reference substances were purchased from the British Pharmacopoeia Commission (BPC) (Stanmore, Great Britain). Toluene 4-sulphonamide was obtained from Koch–Light Labs. (Colnbrook, Great Britain).

2-Sulphamoylbenzoic acid (*o*SBA) was prepared by alkaline hydrolysis of saccharin. The crude acid was purified by a two step solvent extraction with chloroform and with ethylacetate. The acid was finally repeatedly recrystallised from ethanol (m.p. 153°).

All reagents used were of Analar grade.

High-performance liquid chromatography

A Model 750/03 high-pressure pump of Applied Chromatography Systems was coupled with a Cecil 212 UV detector and a Perkin-Elmer 056 recorder. The analytical column used was a 25 cm × 4.6 mm Zorbax CN supplied by DuPont (Hitchin, Great Britain). The mobile phase was 5% (v/v) aqueous acetic acid.

The extraction procedure used for *o*TS was adapted from an earlier paper[17]. Sodium saccharin (1–5 g) was dissolved in 20 ml of a 1% disodium phosphate solution and extracted with 3 × 15 ml of methylene chloride. The combined extracts were evaporated to dryness under vacuum in a rotary evaporator. After the addition of the internal standard, the residue was dissolved in 1 ml absolute ethanol. A 20-μl volume of the ethanolic solution was chromatographed in duplicates.

The separation of the acid impurities is best achieved by methanolic extraction of saccharin[16]. The methanolic solution can be directly chromatographed after the addition of the internal standard.

RESULTS

The optimal HPLC separation of *o*TS and other impurities from saccharin was achieved under the conditions given in Fig. 1. 4-Hydroxybenzoic acid elutes between the acids and the amides, and therefore, it is highly suitable as internal standard. The elution order, retention times and the capacity ratios for each compound obtained under this condition are shown in Table I.

The quantitative analysis was based on the evaluation of peak-height ratios. Calibration was carried out in the range of 0–80 ppm (w/v) using ethanolic solutions of BPC reference substances. Standard solutions of 10, 20, 40 and 80 ppm (w/v) of both compounds were prepared with 5 ppm 4-hydroxybenzoic acid as internal standard. The correlation coefficients (r) were found to be 0.9986 and 0.9918 for the *o*TS and *p*SBA respectively by linear regression. The limit of detection is approximately 10 ng for both compounds. The range of recoveries was found to be 96–110% for the *o*TS and 92–110% for the *p*SBA. These results are demonstrated in Table II.

A number of commercial saccharin samples from various sources have been analysed by the HPLC method. Some of these results are summarised in Table III. Typical HLPC chromatograms of a methylene chloride and of a methanolic extract is demonstrated in Fig. 2 and Fig. 3 respectively.

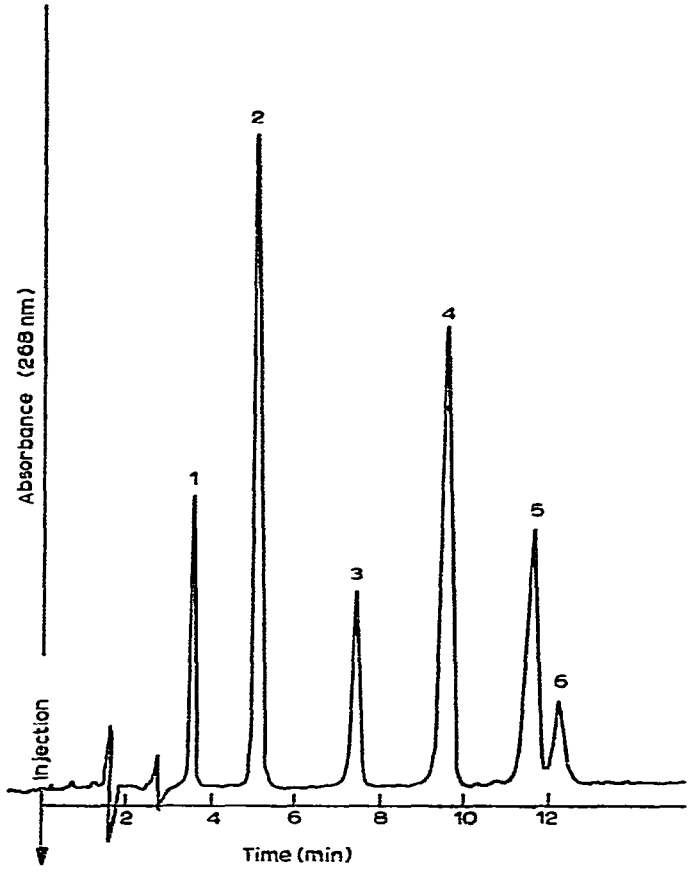

Fig. 1. HPLC chromatogram of saccharin and its impurities. Peaks: 1 = saccharin; 2 = *o*SBA; 3 = *p*SBA; 4 = 4-hydroxybenzoic acid (internal standards); 5 = *o*TS; 6 = *p*TS. Column: 25 cm × 4.6 mm Zorbax CN. Flow-rate: 1.7 ml/min. Detector: UV at 268 nm, 0.05 a.u.f.s. Chart speed: 1 cm/min.

TABLE I

HPLC CHROMATOGRAPHIC PARAMETERS OF SACCHARIN AND ITS IMPURITIES

Compound	*Retention time (min)*	*Capacity ratio k'*
Benzisothiazole-3-one-1,1-dioxide (saccharin)	3.6	1.2
2-Sulphamoylbenzoic acid (*o*SBA)	5.5	2.1
4-Sulphamoylbenzoic acid (*p*SBA)	7.6	3.5
4-Hydroxybenzoic acid	9.7	4.6
Toluene 2-sulphonamide (*o*TS)	11.4	5.7
Toluene 4-sulphonamide (*p*TS)	12.1	6.1

TABLE II

RECOVERIES OF *o*TS AND *p*SBA

oTS (ppm)		*Recovery (%)*	*pSBA (ppm)*		*Recovery (%)*
Added	*Found*		*Added*	*Found*	
25	26	104	10	11	110
25	27	108	10	10	100
50	55	110	25	23	92
50	55	110	25	25	100
100	96	96	30	29	97
100	97	97	30	31	103

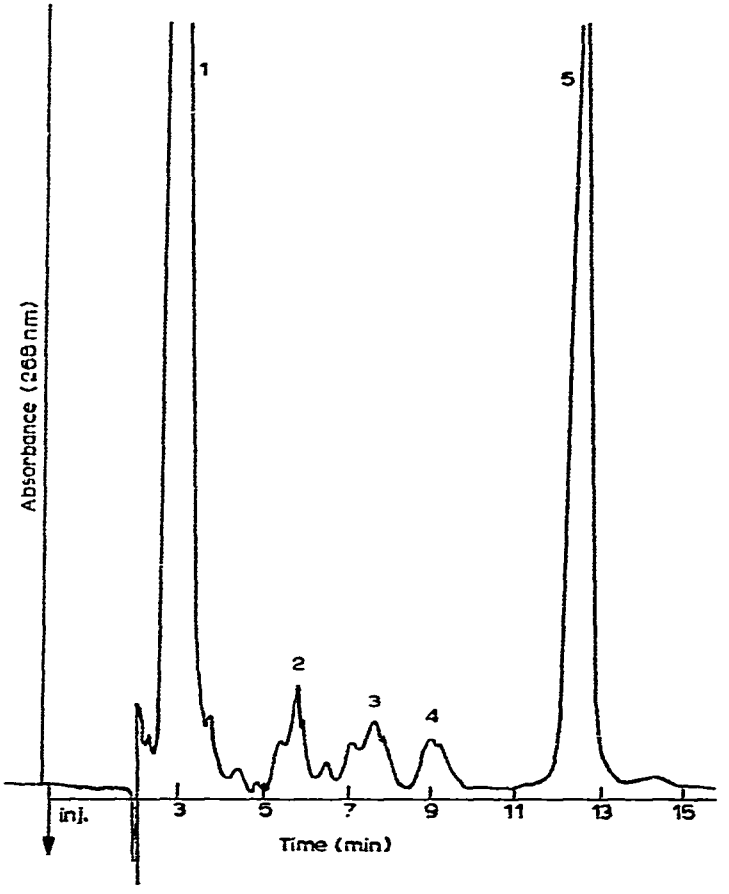

Fig. 2. Typical HPLC chromatogram of impurities in saccharin soluble in organic solvent. Peaks: 1 = saccharin; 2,3,4 = unidentified; 5 = *o*TS. Instrumental conditions as for Fig. 1. Detector sensitivity 0.02 a.u.f.s. Flow-rate, 1.5 ml/min.

TABLE III

*o*TS IN VARIOUS COMMERCIAL SACCHARIN SAMPLES

Origin of sample	*Year*	*oTS (mg/kg)*	*Found*	*pSBA (mg/kg)*	*Found*
Greece	1977	570	630	450	470
Greece	1977	680	730	420	410
India	1978	25	28	90	85
Korea	1977	24	23	105	100
Japan	1978	3.0	2.8	15	16
China	1978	10	12	not detected	
China	1979	2.2	1.7	not detected	
China	1979	3.4	3.1	not detected	

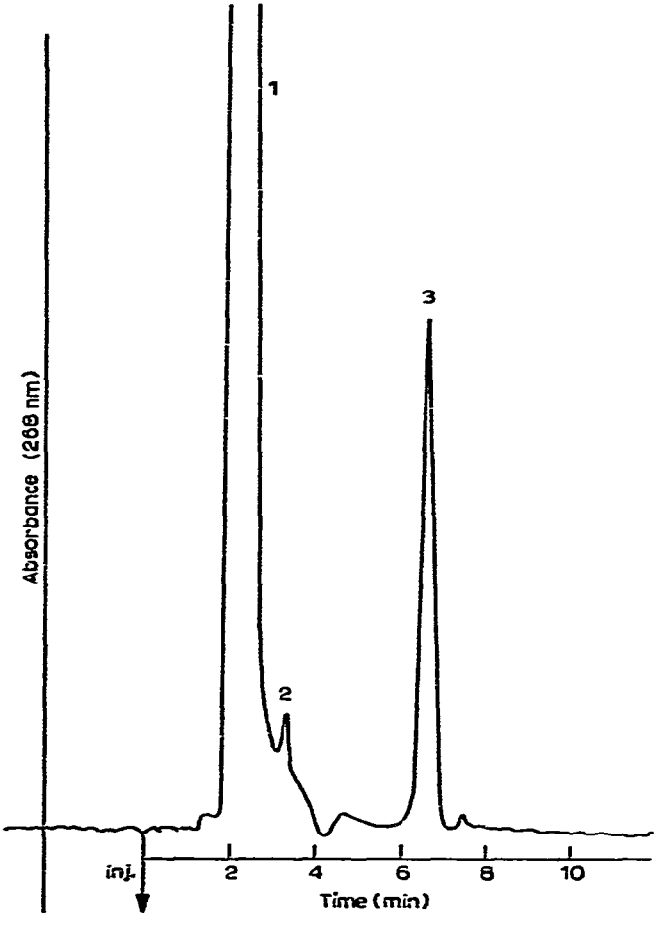

Fig. 3. HPLC chromatogram of methanol soluble impurities in saccharin. Peaks: 1 = saccharin; 2 = unidentified; 3 = *p*SBA. Conditions as for Fig. 1. Flow-rate, 2.0 ml/min.

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