

The Determination of *ortho*- and *para*-Toluenesulphonamides in Saccharin Preparations

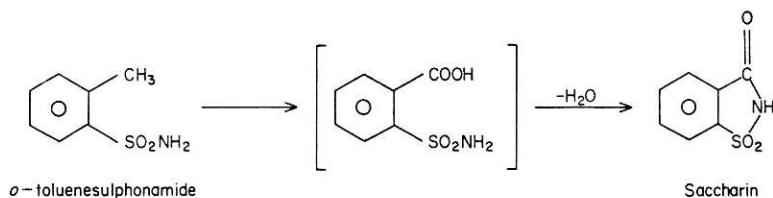
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A method is described for the determination of *ortho*-toluenesulphonamide (*o*-TS) and *para*-toluenesulphonamide (*p*-TS) in saccharin. The method is based on GLC and uses methyl stearate as internal standard. Recoveries of *o*-TS and *p*-TS added to saccharin preparations ranged from 96–104 per cent. Values are given for the contents of these impurities in some products currently available on the British market. The levels were considerably lower than in similar products tested about 2 years earlier.

Saccharin (1,2-benzisothiazol-3-one 1,1-dioxide) and its soluble sodium and calcium salts, have been used as non-nutritive sweetening agents for many years. Since the cyclamates were removed from the United Kingdom market in 1970 because of their possible carcinogenicity¹, saccharin and its salts have been the only permitted artificial sweetening agents in foods. Some doubts have been expressed on the safety in use of saccharin and it has been suggested that further studies should be considered before any final conclusion is made².

Although there are two major synthetic processes for the manufacture of saccharin, the Remsen–Fahlberg process is still the most widely used. This process involves the production of *o*-toluenesulphonamide from toluene followed by oxidation with aqueous permanganate to saccharin.



o-Toluenesulphonamide (*o*-TS) and *o*- and *p*-sulphamoylbenzoic acids are the major impurities in commercial saccharin and these compounds have structural similarities to known carcinogens². Until recently, the amount of *o*-TS in saccharin preparations has not been strictly controlled and amounts up to 5000 mg/kg have been reported from Canada³, whilst we found 1600 mg/kg in one sample of a three-year-old product in Britain. The alternative method of manufacture is reported to produce a product containing much lower levels of *o*-TS.

More recently, the following limits for *o*-TS in saccharin have been published:

* Now at the Public Analyst's Laboratory, 30 Park Place, Leeds 1.

British Pharmacopoeia, 1973:	1 per cent.
Food Chemicals Codex, 2nd Ed., 1st supplement:	100 mg/kg
W.H.O. Food Additives Series, No. 7:	100 mg/kg
U.S. Pharmacopoeia:	500 mg/kg

Previous methods for *o*-TS in saccharin have been based on TLC and GLC^{3,4}. This paper describes an evaluation of the GLC technique and reports results obtained on a few locally purchased samples of saccharin.

Experimental

When the GLC method⁴ given in the first supplement to the second edition of the Food Chemicals Codex was used on samples purchased in this country it was found that an impurity peak, other than *o*-TS or *p*-TS, overlapped the peak of the *n*-tricosane used as the internal standard and prevented its satisfactory measurement. An alternative internal standard, methyl stearate, was chosen such that its retention time, although not very different from that of *n*-tricosane was sufficiently far removed to ensure that there was no overlapping by other impurities in the samples being examined. Typical chromatograms showing the use of *n*-tricosane and methyl stearate as internal standards are given in Figure 1. The method given here for the determination of *o*-TS and

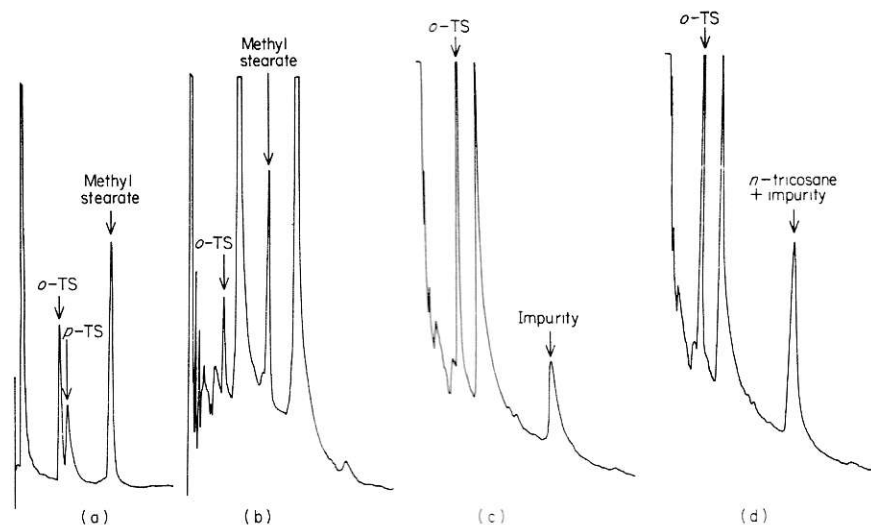


Fig. 1. Use of *n*-tricosane and methyl stearate as internal standards. (a) Mixture of *o*-TS, *p*-TS and methyl stearate alone. (b) Typical chromatogram showing *o*-TS and methyl stearate peaks interspersed with impurities. (c) Sample with added *o*-TS but no internal standard, showing peak at same retention time as *n*-tricosane. (d) Sample with *o*-TS spike and *n*-tricosane as internal standard. Shows *n*-tricosane and impurity peaks superimposed.

p-TS in saccharin is essentially that in the first supplement to the Food Chemicals Codex, 2nd edition⁴ but modified to deal adequately with products on the retail market in the United Kingdom.

APPARATUS

Chromatographic tubes (25 × 250 mm) with stopcocks and glass frits.
 Water bath.

Snyder columns⁵.

Gas Chromatograph, Becker, Model 409, connected to Honeywell Chart Recorder.

Column: glass (3 m $\times$ 4.0 mm i.d.) packed with 3 per cent. OV-17 on Gas Chrom. Q.

Operating conditions: column temperature = 180°C;
injection port temperature = 225°C;
detector temperature = 250°C.

Carrier gas flow rate = 30 ml of nitrogen/minute.

Detector: flame ionisation, with gas pressures set according to the manufacturer's operating instructions.

REAGENTS

1. *Methylene chloride* (A.R.).
2. *n-Heptane* (A.R.).
3. *o-Toluenesulphonamide* and *p-toluenesulphonamide* stock solution.
Dissolve 20.0 mg of each in methylene chloride and make up to 10 ml in a volumetric flask.
4. *Methyl stearate* internal standard solution. Dissolve 100.0 mg methyl stearate in 10 ml of heptane.
5. 5 per cent. w/v sodium bicarbonate solution.
6. Celite 545.

PROCEDURE

Preparation of Calibration Graph

Pipette 0.25, 0.50, 1.00 and 2.5 ml of the stock sulphonamide solution into four 10 ml volumetric flasks and add 0.25 ml of internal standard solution to each. Dilute to volume with methylene chloride and mix well. These solutions are equivalent to 50, 100, 200 and 500 $\mu\text{g/ml}$ of *o*-TS and *p*-TS respectively, plus 250 $\mu\text{g/ml}$ of methyl stearate. Inject 2.5 μl of the 200 $\mu\text{g/ml}$ standard into the gas chromatograph with range and attenuation set so that the toluenesulphonamides give a response between 40–80 per cent. of full-scale deflection. Repeat using the same injection volumes for the other standards and vary the attenuation as necessary. For each standard, inject at least twice to give replicate chromatograms and measure the areas of the *o*-TS, *p*-TS and methyl stearate peaks. Calculate the average ratio of each toluenesulphonamide area to that of the methyl stearate area for each standard, and construct calibration graphs by plotting the ratio for each standard against its concentration in $\mu\text{g/ml}$.

Treatment of Samples

Disperse 2.00 g of sample (more for liquids) completely in 8 ml of 5 per cent. sodium bicarbonate solution. Add 10 g of Celite 545 (or equivalent) and mix thoroughly. Transfer the mixture to a chromatographic tube and pack firmly by tapping the column gently on a soft surface. Tamp the column from the top to ensure there is no loose material. Pour into the tube 100 ml of methylene chloride, adjusting the stopcock so that 50 ml of eluate is collected

within 20–30 minutes. To this solution add 25 μ l of internal standard solution, mix well, then concentrate down to 1 ml in a graduated test tube fitted with a Snyder column, over a boiling water bath. The extract is now ready for injection. Inject 2.5 μ l of each sample extract into the gas chromatograph; make at least two injections for each sample. Measure the area of the peaks found at the appropriate retention times. Calculate for each sample, the ratio of *o*-TS peak area and *p*-TS peak area to that of the methyl stearate; hence use each calculated ratio to determine *o*-TS, *p*-TS concentration in μ g/ml from standard curves. Divide by 2 to convert to parts per million for a 2 g sample weight.

If the concentration of either toluenesulphonamide is greater than 500 p.p.m., crystallisation from the solvent may occur. This can be avoided by making a dilution with methylene chloride containing 250 μ g/ml methyl stearate (1:10 is satisfactory), and applying the dilution factor to the calculation.

Results and Discussion

The values obtained from the chromatograms of the calibration standards are given in Table I.

TABLE I
CALIBRATION GRAPH DATA

Standards, mg/kg		<i>o</i> -TS peak area	<i>p</i> -TS peak area	Methyl stearate peak area	Ratio <i>o</i> -TS	Ratio <i>p</i> -TS
					methyl stearate	methyl stearate
50	1	45	49	350	0.13	0.14
	2	41	45	346	0.12	0.13
	3	44	55	422	0.10	0.13
	4	46	59	430	0.11	0.14
100	1	103	130	412	0.25	0.32
	2	83	94	346	0.24	0.27
	3	85	109	410	0.21	0.27
	4	93	133	416	0.22	0.32
200	1	174	246	362	0.48	0.68
	2	194	267	401	0.48	0.67
	3	192	287	405	0.47	0.71
	4	194	260	409	0.47	0.64
500	1	466	624	394	1.18	1.58
	2	434	588	368	1.18	1.60
	3	518	640	454	1.14	1.41
	4	494	604	430	1.15	1.41

All injections: 2.5 μ l.

Retention times: *o*-TS about 4 min; *p*-TS about 4.5 min; methyl stearate about 8 min.

The saccharin preparations examined were in one of three forms: tablets, liquid drops and blends. They were bought in 1975 from retail outlets in London and the Home Counties. The results are shown in Table II. No *p*-TS was found in any of the samples and additionally no *o*-TS was detected in any of the four blends examined which consisted of mixtures of sugar and

saccharin. In all the preparations, the saccharin was present either as its sodium salt or as the compound itself. *o*-TS values, based on the whole product, ranged from 0 to 220 mg/kg for the recently-acquired sweetening products. In three products known to have been stored for at least two years in dry dark conditions the levels found were from 400 to 1600 mg/kg. Such high levels could have been caused by differences in the manufacturing processes being used at that time. Alternatively, there is the possibility, which is unlikely on theoretical grounds, that *o*-TS could be produced on storage by a reversal of the Remsen-Fahlberg synthesis.

A series of ageing tests, in which 2 samples (909 and 916) were irradiated with u.v. light for 24 hours, showed no change in their *o*-TS content. It would appear therefore that manufacturers in this country have reduced significantly the amount of *o*-TS remaining in their saccharin preparations during the last few years. No samples known to have been imported were available for test.

TABLE II
o-TS FOUND IN COMMERCIAL SACCHARIN PREPARATIONS

Sample		<i>o</i> -TS peak area	Methyl stearate peak area	Ratio <i>o</i> -TS	<i>o</i> -TS, mg/kg	Rounded mean, mg/kg
				methyl stearate		
909 (T)	1	174	394	0.44	93	95
	2	144	338	0.43	90	
	3	200	442	0.45	95	
	4	194	412	0.47	98	
910 (T)	1	464	414	1.12	235	220
	2	462	434	1.06	222	
	3	788	764	1.03	218	
	4	736	724	1.02	215	
913 (T)	1	213	367	0.58	123	125
	2	221	372	0.59	123	
	3	285	492	0.58	123	
	4	306	499	0.61	128	
911 (T)	1	134	528	0.25	53	60
	2	138	534	0.26	55	
	3	96	300	0.32	70	
	4	92	290	0.32	70	
912 (T)	1	158	490	0.32	67	65
	2	152	484	0.31	65	
	3	144	492	0.29	63	
	4	156	512	0.31	67	
914 (D)	1	157	412	0.38	21	20
	2	160	402	0.40	22	
	3	137	372	0.37	20	
	4	142	375	0.38	21	
995 (T)	1	268	360	0.74	160	160
	2	276	382	0.72	158	
	3	280	372	0.75	162	
	4	294	402	0.73	159	
916 (T)	1	28	412	0.07	15	20
	2	36	402	0.08	17	
	3	40	372	0.09	20	
	4	36	375	0.09	20	

All injections: 2.5 μ l.

T, tablets; D, liquid drops.

Several other impurities were seen in the gas-liquid chromatogram. No attempt was made to identify them. In only one case did the peak of a spurious compound overlap the *o*-TS peak rendering its measurement impossible. However, there may be other saccharin preparations available, resembling this particular product and if so, yet another internal standard would be required.

All samples were extracted in duplicate and from each extract at least two injections were made into the gas chromatograph. Recoveries for *o*-TS and *p*-TS and sweeteners spiked with *o*-TS ranged between 96 and 104 per cent.

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