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A rapid method for the estimation of impurities in saccharin and sodium saccharin

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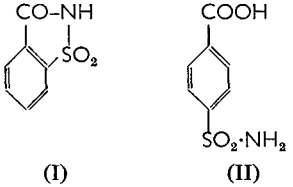
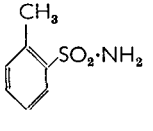
A procedure is described for the detection and estimation of *o*-toluenesulphonamide, saccharin-*o*-toluenesulphonylimide, toluene-2,4-disulphonamide, saccharin-4-sulphonamide, *o*-sulphamoylbenzoic acid, *p*-sulphamoylbenzoic acid and benzoic acid in saccharin and sodium saccharin using thin-layer chromatography on Kieselgel GF_{254}. The solvent system used is chloroform–methanol–strong ammonia solution (100:50:11·5). Compounds containing a free sulphonamide group are detected by an "*N*-chloro" reaction, while the other compounds are detected by examining the chromatogram in ultraviolet light (253·7 mμ). Impurities in commercial saccharin and sodium saccharin are estimated by comparison on the chromatogram with standards containing purified saccharin or sodium saccharin and suitable amounts of the impurities.

OF the impurities likely to be present in saccharin (I), *p*-sulphamoylbenzoic acid (*p*-acid, II) has received the most attention; the method of the British Pharmacopoeia (1963) for limiting the content of this impurity is a modification of the method suggested by Proctor (1905). It depends on the difference between the titration of total acidity and the titration of ammonia after hydrolysis. Since the method is a difference between two determinations, the results are subject to the errors of both; in addition it is not specific for *p*-acid. Other pharmacopoeias specify a method in which (II), if present, is precipitated when saccharin or sodium saccharin is suitably acidified. This method is not very sensitive.

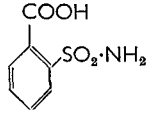


(I)

(II)

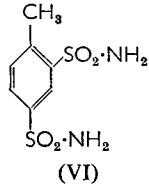


(III)

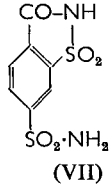


(IV)

(V)



(VI)



(VII)

As most saccharin is manufactured by oxidising *o*-toluenesulphonamide (*o*-amide, III), this is a likely impurity. Richmond & Hill (1919) have suggested an assay procedure in which the saccharin is dissolved in sodium

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bicarbonate solution and (III) extracted with ethyl acetate. The procedure is lengthy and is not specific since other weakly acidic and non-acidic impurities are also extracted by this solvent.

o-Sulphamoylbenzoic acid (*o*-acid, IV) is a less likely impurity: it is hydrolysed by acids to give the ammonium salt of *o*-sulphobenzoic acid and, therefore, if present, it will interfere with the assay procedure of the British Pharmacopoeia (1963). A complex procedure for determining (IV) (Richmond & Hill, 1919) depends on the fact that its solubility in water is relatively high compared with that of saccharin and *p*-acid (II). Although recoveries are reasonable at a 10% level of *o*-acid (IV), the method is quite inadequate for the levels of (IV) normally found in saccharin.

Other impurities have been isolated from saccharin or from the mother liquors during the manufacture of saccharin. These include: saccharin-*o*-toluenesulphonylimide (saccharin-*o*-imide, V) (Klages, 1927) and toluene-2,4-disulphonamide (VI) and saccharin-4-sulphonamide (VII) (Herzog, 1926). No methods have been offered whereby they might be determined in saccharin.

For benzoic and salicylic acids, several pharmacopoeias specify the well known reactions with ferric chloride. Although this reagent is sensitive for salicylic acid, it is not for benzoic acid.

One previous application of chromatography to the determination of *p*-acid (II) and *o*-amide (III) has been described by Franc (1959). This consists of an elaborate method whereby (III) and (II) are nitrated and the nitro-compounds separated by paper chromatography: they are then eluted and determined polarographically.

We have investigated the *direct* separation and estimation of the impurities using thin-layer chromatography.

Experimental

On a thin layer of silica gel using the solvent system chloroform–methanol–strong ammonia solution (100 : 50 : 11·5), saccharin is separated from the impurities benzoic acid and (II) to (VII), but not from salicylic acid. All the impurities were separated from one another, with the exception of *o*-acid (IV) and saccharin-4-sulphonamide (VII).

Small amounts of compounds containing a free sulphonamide group may be detected by a procedure similar to that used by Pan & Dutcher (1956) for the *N*-acetyl derivatives of the neomycin. This involves the formation of *N*-chloro-compounds by spraying with sodium hypochlorite solution, removing the excess hypochlorite by spraying with dextrose solution and finally detecting the *N*-chloro compounds by spraying with a solution of starch and potassium iodide. Saccharin and saccharin-*o*-imide, which do not contain a free sulphonamide group, give a weaker response in this procedure; they and benzoic acid may be detected, however, in ultraviolet light (253·7 mμ) if a suitable phosphor is incorporated in the silica gel. Although *o*-acid (IV) and saccharin-4-amide (VII) have the same Rf value and both are detected by the "*N*-chloro" reaction, only saccharin-4-sulphonamide (VII) is detected in ultraviolet light.

The following method was thus developed and used to estimate the impurities in saccharin and sodium saccharin.

REAGENTS AND MATERIALS

Chromatoplates. Spread a 0·25 mm layer of Kieselgel GF_{254} (Merck) on 20 × 20 cm glass plates, activate by heating for one hr at 110° and store over anhydrous silica gel.

Solvent system. Mix together chloroform (100 vol), methanol (50 vol) and strong ammonia solution B.P. (11·5 vol), all analytical reagent grade. One hr before introducing the chromatoplates, place in a tank lined with filter paper sufficient solvent mixture to form a layer 1·5 cm deep.

Saccharin was recrystallised from water until 750 μg applied to a 0·4 mm layer of Kieselgel GF_{254} showed no detectable impurities when examined by the procedure given below. M.p. 229°. *Sodium saccharin* was recrystallised from 90% v/v ethanol until 750 μg applied to a 0·4 mm layer of Kieselgel GF_{254} showed no detectable impurities when examined by the procedure given below.

o-*Toluenesulphonamide* (*III*) was recrystallised three times from water. M.p. 157·0°. p-*Sulphamoylbenzoic acid* (*II*) was recrystallised three times from water and then from 95% v/v ethanol. M.p. 278·8°. o-*Sulphamoylbenzoic acid* (IV) was made by the method of Richmond & Hill (1919). It had m.p. 153°. *Saccharin*-o-*imide* (*V*) (Klage, 1927) was recrystallised from water. M.p. 255·9°.

Toluene-2,4-*disulphonamide* (VI). Prepare toluene-2,4-disulphonic acid by treating toluene with oleum (20%) at 200–250°. Convert the acid to the dipotassium salt and reflux the salt (65·4 g) for 4 hr with phosphorus pentachloride (166 g) and phosphorus oxychloride (60 ml). Cool and filter off the potassium chloride and excess phosphorus pentachloride. Distil the toluene-2,4-disulphonylchloride at 8 mm Hg (b.p. 204). To the disulphonylchloride (20 g) add water (300 ml) and strong ammonia solution B.P. (25 ml) and stir for 4 hr at 60°. Neutralise with hydrochloric acid and reduce the volume to 150 ml. Filter off the toluene-2,4-disulphonamide and recrystallise from water. M.p. 190°.

Saccharin-4-*sulphonamide* (*VII*). Oxidise toluene-2,4-disulphonamide with alkaline potassium permanganate using the method of Vogel (1956) for the preparation of saccharin from *o*-toluenesulphonamide (III). Recrystallise from water. M.p. 301°.

Benzoic acid of analytical reagent grade.

Strong impurity solution. Dissolve *o*-amide (III) (40 mg), *p*-acid (II) (40 mg), *o*-acid (IV) (20 mg), toluene-2,4-disulphonamide (VI) (20 mg), saccharin-*o*-imide (V) (20 mg) and benzoic acid (100 mg) in methanol–acetone (4:1) (100 ml).

Standard impurity solutions. (*a*) For saccharin: to four 20 ml graduated flasks transfer, respectively, 5, 10, 15 and 20 ml of strong impurity solution. To each flask add 1·0 g purified saccharin, dilute to volume with methanol–acetone (4:1), mix to dissolve the saccharin and adjust to volume if necessary. (*b*) For sodium saccharin: to four 20 ml graduated flasks transfer, respectively, 3·1, 6·25, 9·4 and 12·5 ml of strong impurity solution.

To each flask add 1·25 g purified sodium saccharin, dilute to volume with methanol–acetone (4 : 1), mix to dissolve the sodium saccharin and adjust to volume if necessary.

Sodium hypochlorite solution. Dilute 2 ml of strong sodium hypochlorite solution (containing approximately 16% w/v available chlorine) to 70 ml with water. This solution should be freshly prepared.

Glucose solution. 5% w/v solution in water. This solution should be freshly prepared.

Starch and potassium iodide solution. Mix 50 ml freshly prepared 1% w/v aqueous starch solution with 50 ml 1% w/v aqueous potassium iodide solution and add 1 ml of glacial acetic acid.

PROCEDURE

Dissolve 1·0 g of commercial saccharin or 1·25 g of commercial sodium saccharin in methanol–acetone (4 : 1) and dilute to 20 ml. Apply three 1 μl amounts of the solution to a chromatoplate as a single spot, allowing the solvent to evaporate after each 1 μl application. The spot should be placed on a line approximately 2·5 cm from one edge of the chromatoplate. On the same line apply as single spots three 1 μl amounts of each of the four appropriate standard impurity solutions. The spots should be applied at least 2 cm from either side of the plate and not less than 1·5 cm apart. Score a line across the chromatoplate 10 cm from the line of spots, remove a narrow strip of adsorbent from the sides of the chromatoplate and place the plate in the tank so that the line of spots is a few mm above the solvent surface. When the solvent has ascended as far as the scored line, remove the chromatogram from the tank and dry for 10 min in a stream of warm air.

Examine the chromatogram in ultraviolet light (253·7 mμ) and compare in size and intensity the standard saccharin-*o*-imide and benzoic acid spots with that of any spot from the sample having a similar Rf value.

Heat the chromatogram at 100° (5 min) and while it is still hot, spray with sodium hypochlorite solution until the adsorbent begins to show signs of dampness. Dry (2 min) in a stream of cold air and then spray with glucose solution until the odour of hypochlorous acid is no longer detectable. If the chromatogram has a damp appearance, dry again (2 min) in a stream of cold air. Spray with sufficient starch and potassium iodide solution to render the impurity spots visible. Compare in size and intensity the standard impurity spots with that of any spot from the sample having a corresponding Rf value.

If the amount of an impurity in a sample exceeds that of the highest standard, the procedure should be repeated, taking a reduced amount of sample and adding sufficient purified saccharin or purified sodium saccharin to make the total weight taken equal 1·0 g for saccharin or 1·25 g in the case of sodium saccharin.

If, when the chromatogram is examined in ultraviolet light, an impurity is detected with an Rf value corresponding to *o*-acid (IV), this is probably due to saccharin-4-sulphonamide (VII). Estimate the content of saccharin-4-sulphonamide by repeating the above procedure but replacing the

standards by ones containing saccharin-4-sulphonamide and purified saccharin or sodium saccharin. Estimate the content of *o*-acid by difference.

Results and discussion

The approximate Rf values of the impurities considered are given in Table 1, together with the limits of detection. The latter were determined by chromatographing standard mixtures of purified sodium saccharin with progressively smaller amounts of the impurities. (When purified saccharin was used in preparing the standard mixtures, the Rf values and limits of detection were similar.)

TABLE 1. APPROXIMATE Rf VALUES AND LIMITS OF DETECTION

Compound	Approximate Rf value*	Limits of detection (μg)*	
		"*N*-chloro" reaction	Ultraviolet light (253·7 mμ)
o-Toluenesulphonamide	0·9	0·04	—
Saccharin-*o*-toluenesulphonylimide	0·75	—	0·05
Toluene-2,4-disulphonamide	0·65	0·04	—
Saccharin	0·36–0·55†	—	—
Benzoic acid	0·34	—	1
Saccharin-4-sulphonamide	0·27	0·02	0·05
o-Sulphamoylbenzoic acid	0·27	0·02	—
p-Sulphamoylbenzoic acid	0·21	0·02	0·02

* The approximate Rf values and limits of detection of the impurities apply when the impurities are chromatographed in the presence of 187·5 μg of sodium saccharin.
† The Rf values quoted for saccharin are for the rear and front of the spot. All other Rf values are measured from the centre of the spot.

Using the specified standards, there is a good gradation in the size and intensity of the spots for each impurity. The corresponding impurity in a sample of commercial saccharin or sodium saccharin can be placed to the nearest 0·1% in the range 0·1–0·5% or to the nearest 0·2% in the range 0·5–1·0%.

TABLE 2. EXAMINATION OF COMMERCIAL SACCHARIN AND SODIUM SACCHARIN

Sample	% impurity (determined by the proposed chromatographic method)						
	o-Amide (III)	Saccharin-*o*-imide (V)	2,4-Di-amide (VI)	*o*-Acid (IV)	*p*-Acid (II)	Saccharin-4-sulphon-amide (VII)	Benzoic acid
Saccharin A	0·7	<0·1	None detected	<0·1	1·0	None detected	None detected
" B	0·8	0·6	"	<0·1	0·6	"	"
" C	0·8	<0·1	"	0·2	<0·2	"	"
" D	0·2	<0·1	"	<0·1	<0·2	"	"
" E	0·8	0·1	<0·1	<0·1	<0·2	"	"
" F	None detected	None detected	None detected	None detected	None detected	"	"
Sodium saccharin G	<0·1	"	"	<0·05	<0·1	"	"
" " H	0·1	"	"	<0·05	0·1	"	"
" " I	0·1	"	"	<0·05	0·1	"	"
" " J	0·1	"	"	<0·05	1·7	"	"
" " K	0·8	"	"	<0·05	0·2	"	"
" " L	0·3	"	"	0·2	<0·1	"	"

Although the benzoic acid is not detected at such low concentrations as are the other impurities, the above method is a considerable improvement on the pharmacopoeial test. The limit of detection of benzoic acid by the method of the United States Pharmacopeia (1965), for example, is 4%. Salicylic acid is not separated from saccharin by the chromatographic procedure and is not estimated. In this case, however, the method of the U.S.P. (1965) will detect as little as 0·05%.

A number of samples of commercial saccharin and sodium saccharin from various countries were examined by the proposed procedure and the results are shown in Table 2. These results suggest that *o*-amide (III) and *p*-acid (II) are the two impurities that occur in the largest proportion, and that the other impurities considered occur occasionally in small amounts or not at all.

Compared with previously suggested methods, which are often time-consuming or inaccurate, the suggested procedure using thin-layer chromatography offers a rapid and reasonably accurate means of estimating all the impurities considered except salicylic acid.

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