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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF TOLUENESULFONAMIDES AND POLAR IMPURITIES IN SACCHARIN AND SACCHARIN SODIUM

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SUMMARY

A reversed-phase high-performance liquid chromatographic procedure for the determination of *o*-toluenesulfonamide and *p*-toluenesulfonamide in saccharin and saccharin sodium is described. In a second step, polar byproducts of the synthesis and related polar substances are determined in a lower sensitivity range. Chromatography is performed on an octadecylsilane column using a mobile phase of water and tetrahydrofuran for the non-polar toluenesulfonamides and a solution of tetrabutylammonium phosphate in water–methanol for the related polar substances. For the non-polar impurities recovery and linearity were tested in the range 5–100 mg/kg, and the detection limit was 8 ng for *o*-toluenesulfonamide and 16 ng for *p*-toluenesulfonamide.

INTRODUCTION

In saccharin and saccharin sodium, usually synthesized by the Remsen–Fahlberg process, two main groups of impurities are of interest to analysts. The first consists of relatively non-polar substances, such as *o*-toluenesulfonamide (oTSA) and *p*-toluenesulfonamide (pTSA), resulting from the sulfochlorination of toluene and subsequent aminolysis. On the other hand some substances with polar properties may appear as impurities, such as benzoic acid 2-sulfonamide, benzoic acid 4-sulfonamide, 2-sulfobenzoic acid and 4-sulfobenzoic acid, which all are byproducts of the synthesis. In some rare cases substances added as fillers or blending agents, such as benzoic acid or salicylic acid, could be present[1]. In recent years regulations and compendia tended to stricter limitation of the toxic oTSA and pTSA. The *Food Chemical Codex*[2] and the *U.S. Pharmacopeia*[3] imposed a combined limit of oTSA and pTSA of 25 mg/kg (ppm). More strict is the Federal Republic of Germany in its *Regulation on Trade in Additives and Foodstuffs used as Additives*[4]: the limit for oTSA and pTSA was set at 10 mg/kg each. For the seventh edition of the *Swiss Pharmacopoea*[5] the introduction of a proportionately strict standardisation was imperative. However, the regulations in national compendia regarding the polar impurities are less strict and mostly limited in the percent range.

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Existing methods for the determination of the toluenesulfonamides employ thin-layer chromatography (TLC) or gas chromatography. The former (*e.g.* by the *British Pharmacopoeia*[6] lack in sensitivity, the latter (*e.g.* the U.S.P[3] method) need a large amount of sample. New methods depend on high-performance liquid chromatography (HPLC) on reversed-phase columns with octadecylsilane-modified silica[7,8] or propylnitrile-modified silica[9]. The first of these do not separate the toluenesulfonamide isomers, and in the second the separation is not satisfactory for good quantification.

For the new edition of the *Swiss Pharmacopeia* we have developed a simple and sensitive isocratic determination on commonly available reversed-phase columns, suitable for the quantification of oTSA and pTSA.

The polar impurities are usually determined by wet chemical methods[3,5,6] or TLC[6]. We have replaced the above time-consuming methods by a determination suitable for the detection range used in pharmacopeias and compendia and based on ion-pair HPLC. It would be possible to lower the detection range to the order of parts per million, as in a method published recently[10], or to determine the toluenesulfonamides simultaneously but then they would not be separated.

EXPERIMENTAL

Apparatus

A Perkin-Elmer Series 3 solvent delivery system (Norwalk, CT, U.S.A.), equipped with a Rheodyne Model 7105 injector (Cotati, CA, U.S.A.), a Perkin-Elmer variable-wavelength spectrophotometric detector Model LC 55, and a Hewlett-Packard reporting integrator Model 3390A (Avondale, PA, U.S.A.) were used.

Column

Best results were obtained with a self-packed column (125 × 4.6 mm I.D.) of Hypersil ODS (Shandon, Runcorn, Cheshire, U.K.).

Chemicals

Chemicals used were benzoic acid (Art. 12349, Fluka, Buchs, Switzerland), benzoic acid-2-sulfonamide, prepared by hydrolysis of saccharin (m.p. 155–156°C), benzoic acid-4-sulfonamide (Art. 86050 Fluka), resorcinol (Art. 83600 Fluka), freshly sublimed, salicylic acid (Art. 84210 Fluka), 2-sulfobenzoic acid, prepared from the corresponding ammonium salt (Art. 821600 Merck), 4-sulfobenzoic acid, prepared from the corresponding monopotassium salt (Art. P 1647, Eastman-Kodak), toluene-2-sulfonamide (BP CRS, British Pharmacopoeia Commision, Stanmore, U.K.), toluene-4-sulfonamide (Art. 89749 Fluka), solution of tetrabutylammonium phosphate (PIC-Reagent "A", Waters Assoc., Milford, MA, U.S.A.). Solvents such as dichloromethane, methanol and tetrahydrofuran were of Merck LiChrosolv grade. Phosphate buffer solution (pH 4.5) was prepared by dissolving 13.661 g of potassium dihydrogen phosphate (Art. 4873 Merck) in 1 l of water.

Determination of o- and p-toluenesulfonamide

Operating conditions. The mobile phase was aqueous phosphate buffer solution (pH 4.5)–tetrahydrofuran (92:8, v/v), filtered through a 0.45 μm filter (Art. HVLP,

Millipore, Bedford, MA, U.S.A.), degassed and pumped at a rate of 1 ml/min. Detector wavelength was set at 269 nm. Column temperature was ambient.

Standard solutions. To prepare the *o*-toluenesulfonamide standard solution, *ca.* 6.0 mg of oTSA and 2.0 mg of resorcinol were dissolved in 2.0 ml of tetrahydrofuran. To prepare the *p*-toluenesulfonamide standard solution, *ca.* 12.0 mg of pTSA and 3.0 mg of resorcinol were dissolved in 2.0 ml of tetrahydrofuran. To prepare the stock solution, *ca.* 1.0 mg of resorcinol was dissolved in 50.0 ml of tetrahydrofuran.

Test solutions. For samples containing saccharin, 10.000 g of saccharin were dissolved in 15 ml of water containing 5.5–6 ml of 10 *N* sodium hydroxide; the pH was adjusted to *ca.* 7.0 to 7.2 with 1 *N* hydrochloric acid. The solution was extracted six times with 20 ml of dichloromethane, then dried over anhydrous sodium sulfate and filtered. The filter and the sodium sulfate were rinsed twice with 10 ml of dichloromethane. The extracts were evaporated to dryness and the residue was dissolved in 0.5 ml of stock solution.

For samples containing saccharin sodium, an aliquot amount of saccharin sodium was dissolved in 20 ml of water. The extraction and following procedure were carried out as above.

Prior to use, the test solutions were filtered through a 0.2 μm PTFE filter (Art. FG, Millipore).

Reference solution. oTSA standard solution (1 ml) and pTSA standard solution (1 ml) were mixed.

Column suitability test. A 1-μl volume of the reference solution was injected onto the column. The peaks of oTSA and pTSA should be separated to the baseline.

Determination of the reference factors R_{oTSA} and R_{pTSA}. A 1-μl volume of the oTSA standard solution or the pTSA standard solution was injected onto the column. The response factors were calculated as follows:

$$R_{TSA} = \frac{A_{TSA} \cdot m_{Res}}{A_{Res} \cdot m_{TSA}}$$

where R_{TSA} is the response factor for oTSA (or pTSA), A_{TSA} is the peak area of oTSA (or pTSA) in the chromatogram, A_{Res} is the peak area of resorcinol in the chromatogram, m_{TSA} is the weighed amount of oTSA (or pTSA) in milligrams and m_{Res} is the weighed amount of resorcinol in the standard solutions.

Determination of the amount of oTSA and pTSA. A 5-μl volume of the test solution was injected. The amount of oTSA (or pTSA) in mg/kg (ppm) was calculated from the following equation:

$$\frac{A_{TSA} \cdot m_{Res}}{A_{Res} \cdot R_{TSA} \cdot m_{Sacch}}$$

where A_{TSA}, A_{Res}, R_{TSA} and m_{Res} (in micrograms) are as above, and m_{Sacch} is the weighed amount of saccharin in the test solution, in grams.

Determination of the polar impurities

Operating conditions. The mobile phase was a 0.005 *M* solution of tetrabutyl-

ammonium phosphate in water–methanol (68:32, v/v), filtered through a 0.45 μm filter (Art. HVPL, Millipore), degassed and pumped at a rate of 1 ml/min. Detector wavelength was set at 271 nm. Column temperature was ambient.

Standard reference solutions. For samples containing saccharin, 0.1 g saccharin and 1 mg each of benzoic acid, benzoic acid 2-sulfonamide, benzoic acid 4-sulfonamide, 2-sulfobenzoic acid, 4-sulfobenzoic acid and salicylic acid were dissolved in *ca.* 5.5 ml of 0.1 *N* sodium hydroxide, the pH was adjusted to 7.0–7.3, and the volume was taken to 10 ml with water.

For samples containing saccharin sodium, 0.13 g of saccharin sodium and 1 mg each of benzoic acid, benzoic acid 2-sulphonamide, benzoic acid 4-sulphonamide, 2-sulfobenzoic acid, 4-sulfobenzoic acid, and salicylic acid were dissolved in 10 ml of the eluent.

Test solutions. For samples containing saccharin, 0.1 g of the sample was dissolved in *ca.* 5.5 ml of 0.1 *N* sodium hydroxide, the pH was adjusted to 7.0–7.3, and the solution was diluted with water to 10 ml.

For samples containing saccharin sodium, 0.13 g of the sample was dissolved in 10 ml of the eluent.

Column suitability test. A 5-μl volume of the standard reference solution was injected. The peaks of the substances added to saccharin should be separated to the baseline.

Limitations of the amount of polar impurities. A 5-μl volume of the test solution was injected. No area of a peak (excepting that which corresponds to saccharin) of which the retention time is shorter than or equal to the retention time of salicylic acid in the column suitability test chromatogram should be greater than 1% of the area of the salicylic acid peak.

RESULTS AND DISCUSSION

Since the prescribed maximum amounts of the non-polar impurities oTSA and pTSA are in a much lower detection range than the limits for the related polar substances, the determination has to be done in two steps, necessitating different sample preparations.

Determination of the toluenesulfonamides

In order to develop a simple, low cost, fast chromatographic procedure, an isocratic separation was developed on a commonly available C_{18} column with average separation power. Sample preparation could not be shortened using Extrelut® or other commercially available disposable clean-up columns. These were overloaded by the large amount of sample necessary to provide a reasonably precise determination. The resolution of oTSA and pTSA in the column suitability test was R_s = 1.7, and the capacity factors were k' = 13.8 for oTSA, k' = 12.5 for pTSA and k' = 9.5 for the internal standard. A typical chromatogram is shown in Fig. 1, and a chromatogram of a commercial product is shown in Fig. 2.

In order to determine the precision of the method, known amounts of oTSA (or pTSA, or both) were added to toluenesulfonamide-free saccharin prior to the extraction procedure. Recoveries are listed in Table I. The detection limit was *ca.* 8 ng for oTSA and 16 ng for pTSA at the maximum wavelengths of 269 nm and 263

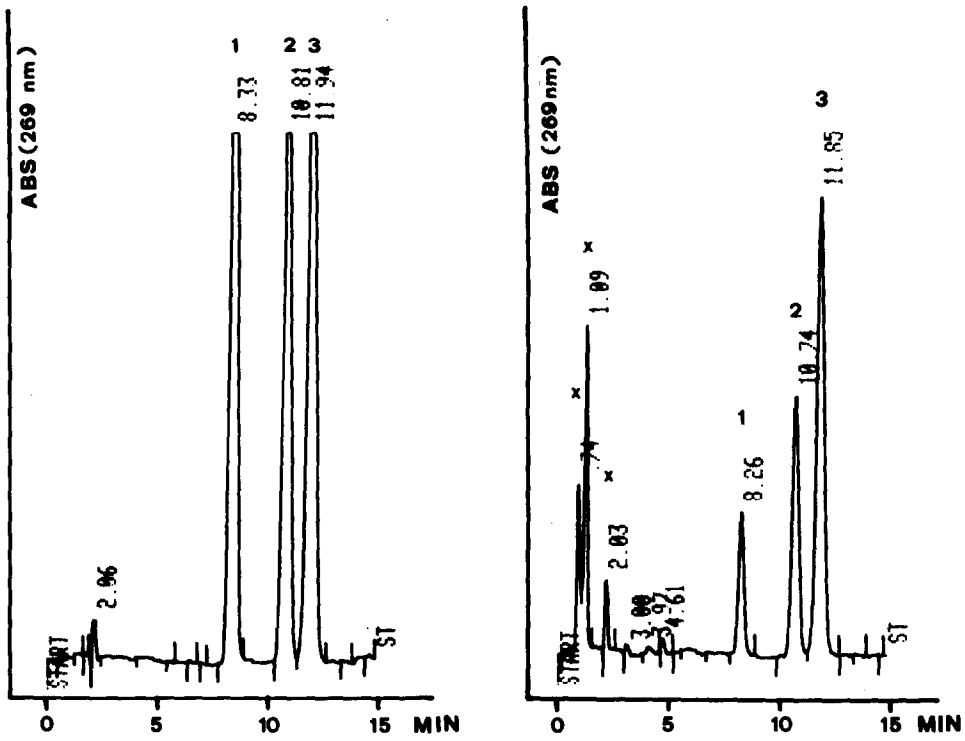

Fig. 1. HPLC chromatogram of a mixture of standard solutions: Peaks: 1 = resorcinol; 2 = pTSA; 3 = oTSA.

Fig. 2. HPLC chromatogram of a commercial sample of saccharin, spiked with 20 ppm oTSA and 20 ppm pTSA. Peaks: 1 = resorcinol; 2 = pTSA; 3 = oTSA; x = unknown.

nm, respectively. Typical response factors were $R_{oTSA} = 0.456$ and $R_{pTSA} = 0.122$ at the detection wavelength of 269 nm. The difference in response factors is due to different molar absorptivities at the detection wavelength. The linearity of the method was checked by plotting the ratios of peak area of toluenesulfonamides *versus* peak area of internal standard *versus* added contents of TSA. Curve shapes were linear in the range in question. Statistical analysis by a linear least-squares method indicated good linearity with a coefficient of correlation of 0.9999 for oTSA (0.9999 for pTSA),

TABLE I

RECOVERIES OF TOLUENESULFONAMIDES IN SACCHARIN SAMPLES

oTSA added (mg/kg)	*Recovery (%)*	*pTSA added (mg/kg)*	*Recovery (%)*
5	83–101	5	80–100
10	84–101	10	101–108
		25	84
50	82–83	50	97
		100	92

TABLE II

CONTENT OF TOLUENESULFONAMIDES IN SOME COMMERCIAL SAMPLES OF SACCHARIN

Sample		*oTSA (mg/kg)*	*pTSA (mg/kg)*
I	(1946, unknown supplier)	10,000	28
II	(1963, Swiss supplier)	11,000	20
III	(1963, British supplier)	8000	not determined
IV	(1978, German supplier)	1	–
V	(1976, Swiss supplier)	3000	not determined
VI	(1977, unknown supplier)	not determined	not determined
VII	(1983, Swiss supplier)	1	<0.05
VIII*	(1977, German supplier)	3	–

* Saccharin sodium.

a regression coefficient of 0.4445 for oTSA (0.1088 for pTSA) and an intercept of −0.0032 for oTSA (−0.0020 for pTSA). Before and after each set of determinations, the response factors of the toluenesulfonamides were checked. The method showed good day-to-day stability. Different samples of oTSA or pTSA showed very small deviations of their response factors. Table II shows some typical contents of toluenesulfonamides of commercial samples of saccharin. The older samples contained oTSA in the percent range and pTSA in the ppm range, but more recent samples

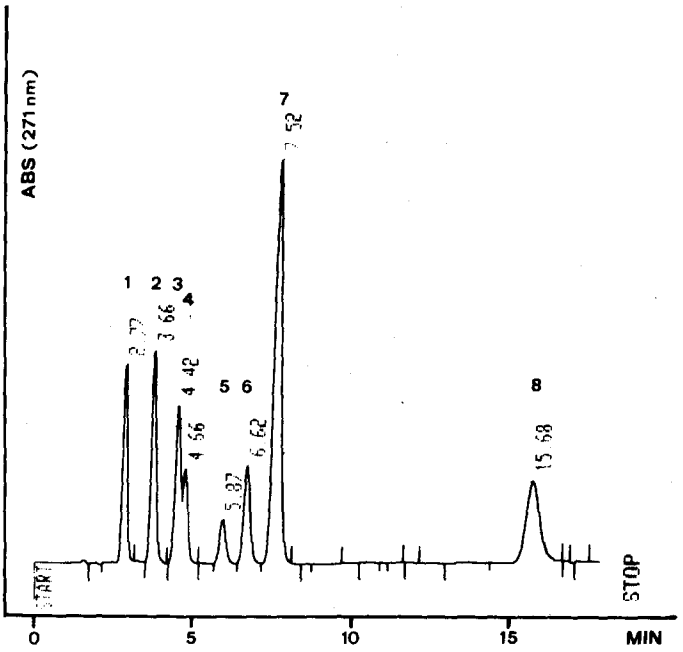

Fig. 3. HPLC chromatogram of polar impurities of saccharin. Peaks: 1 = benzoic acid 4-sulfonamide; 2 = benzoic acid 2-sulfonamide; 3 = 4-sulfobenzoic acid; 4 = 2-sulfobenzoic acid; 5 = oTSA and pTSA; 6 = benzoic acid; 7 = saccharin; 8 = salicylic acid.

TABLE III

CONTENT OF POLAR IMPURITIES OF SACCHARIN (IN AREA PERCENT)

*Sample**	*Benzoic acid 2-sulfonamide*	*Benzoic acid 4-sulfonamide*	*2- or 4-sulfobenzoic acid*	*Benzoic acid*	*Salicylic acid*	*Other impurities*
I	–	0.6	–	–	–	0.3
II	<0.1	<0.1	–	–	–	0.1
III	<0.1	<0.1	–	–	–	0.1
IV	–	–	–	–	–	0.3
V	–	–	–	–	–	0.2
VI	0.4	–	–	–	–	0.6
VII	1.3	–	–	–	–	0.1
VIII	–	–	–	–	–	0.4

* See Table II.

were far below the 10-ppm limits for oTSA and pTSA could not be detected, as expected.

Determination of the polar impurities

The polar impurities and related polar substances were detected in a satisfactory manner using ion-pair HPLC. A chromatogram of a column suitability test is shown in Fig. 3. The detector wavelength was set at 271 nm, where the common polar impurities show comparable molar absorptivities, so the limitation could be executed by simple normalization. Some of these chromatograms showed unspecific peaks in the region in question. In such cases it seems reasonable to include these substances in the limiting procedure for pharmacopeias or compendia. Table III lists the contents of polar impurities of some commercial samples of saccharin and saccharin sodium.

CONCLUSION

The above method works under simple chromatographic conditions with a sample pretreatment as simple as possible, and shows good accuracy and reproducibility in the range of the official prescriptions in question.

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