precludes the use of this approach as a routine screening test for HFCS in honey. Concanavalin A and the host of other plant lectins now available, each with its sugar binding specificity, are of increasing interest. The use of various lectins can provide convenient methods for qualitative analysis, such as described here, and also important tools to be used in both the isolation (affinity chromatography) and structural investigation of polysaccharides.

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Determination of Orthotoluenesulfonamide (OTS) in Soluble Saccharin

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An improved method has been developed to measure orthotoluenesulfonamide (OTS), especially trace amounts, in soluble saccharin using gas chromatography. It employs short metal columns, isothermal operation, no internal standard, and no derivatization. The lower limit of detection is estimated to be 0.05 parts per million (ppm) OTS.

Saccharin is produced by two major processes, the Remsen-Fahlberg (R-F) process which starts with toluene, and the Sherwin-Williams (formerly Maumee) process which starts with phthalic anhydride. A natural consequence of the R-F process is the formation of the intermediate orthotoluenesulfonamide (OTS), some of which may survive further reaction and appear as a contaminant of the finished product. Because of a totally different synthesis route at Sherwin-Williams, there is no opportunity for OTS formation and none has been found. Confirmation of the absence of OTS has been made using thin-layer, gas, and high-performance liquid chromatography.

Since most saccharin is produced by the R-F process, it is most likely to contain OTS. This has given rise to a number of analytical methods for its determination. The Battelle Memorial Laboratories (Columbus, Ohio) developed a gas chromatographic method which has been adopted by the Food Chemical Codex as well as by the U.S. Pharmacopeia and the National Formulary. Stavric et al. (1974) has described the isolation, identification, and quantitation of OTS in saccharin by a gas chromatographic technique while Jacin (1975) has developed methods to determine OTS in saccharin quantitatively using spectrophotometry and gas chromatography following silylation.

We are describing a method to measure OTS in saccharin which is especially suited for OTS levels of 1 ppm or less. All previously published methods appear inadequate to measure OTS in saccharin at this level.

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EXPERIMENTAL SECTION

Apparatus. In addition to conventional glassware such as separatory funnels, beakers, etc. this study employed a Hewlett-Packard Model 5750 gas chromatograph equipped with dual-flame ionization detectors and a Hewlett-Packard Model 3370B electronic integrator. A Hewlett-Packard Model 7127A recorder was found to be an asset during this work by giving a video presentation of the assay in progress.

The chromatographic column was 2 ft × 0.25 in. stainless steel packed with 20% SE-30 on Anakrom ABS 90/100 mesh. It was not found necessary to have a set of matched columns. Any well-conditioned column of approximately the same dimensions was found adequate to balance the analytical column.

Reagents. Reagent grade methylene chloride, obtained from Fisher Scientific Co. was redistilled in all-glass apparatus and stored in glass bottles in the dark until needed. Orthotoluenesulfonamide, mp 156 °C, was purchased from Pfaltz & Bauer and used as received. Gas chromatographic reference solutions containing 20 to 1000 ppm OTS were prepared by dissolving the requisite amount of OTS in redistilled methylene chloride.

Procedure. Up to 100 g of soluble saccharin (amount dependent on OTS level expected and sensitivity desired) was warmed with sufficient water to completely dissolve. It was then transferred to a separatory funnel and extracted four times with equal volumes of the redistilled methylene chloride. The extracts were combined and shaken with 20 mL of 5% sodium bicarbonate solution.

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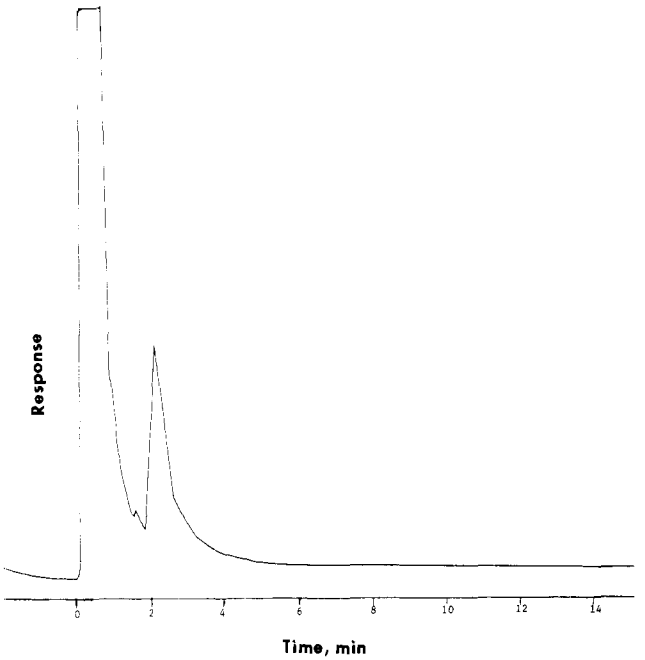

Figure 1. A gas chromatogram of Sherwin Williams saccharin to which had been added 0.1 ppm OTS. The peak at 2.5 min is OTS.

The organic phase was separated and concentrated to a volume of less than 1 mL by evaporation on a warm surface. Then, the extract was transferred to a 1-mL volumetric flask and made to volume with methylene chloride. A 2.5-μL sample was injected into the gas chromatograph operated with the following parameters: column temperature, 225 °C; injection port temperature, 300 °C; carrier gas, helium, ca. 85 mL/min, 80 psig; detector temperature, 330 °C; fuel, hydrogen, ca. 50 mL/min, 30 psig; support, air ca. 400 mL/min, 40 psig. The helium flow through the reference side was adjusted so as to maintain balance. These parameters were found to be optimum for resolution and quantitation. Under these conditions OTS has a retention time of about 2.5 min.

The area of the sample peak was compared to the area of one of the reference solutions nearest in area when chromatographed in the same manner. By suitable calculations the OTS level in the original sample was determined.

RESULTS AND DISCUSSION

Figure 1 shows a typical chromatogram of Sherwin-Williams soluble saccharin doped with 0.1 ppm OTS. The peak appearing at about 2.5 min is due to OTS.

We searched unsuccessfully for a suitable internal standard, but as Table I demonstrates an internal standard really was unnecessary. This table shows the reproducibility of areas of reference solutions. Relative standard deviations range from 7.5% for the 20 ppm reference to 4.5% for the 100 ppm reference. At 1 ppm the standard deviation is about 10% and above 100 ppm it levels out to about 4%.

Table II demonstrates the recovery of added OTS to Sherwin-Williams soluble saccharin.

Table I. Reproducibility of Gas Chromatographic Areas of Reference Solutions

Trial	20 ppm	50 ppm	100 ppm
1	311	628	2160
2	304	656	2286
3	278	630	2417
4	334	614	2308
5	274	628	2276
6	274	689	2338
7	282	668	2165
8	277	705	2123
Av	292	652	2259
Rel SD %	7.5	5.1	4.5

Table II. Recovery of Added OTS

Added, ppm	Found, ppm	Added, ppm	Found, ppm
0	0	10.0	9.6
0.1	0.09	10.0	9.9
0.1	0.11	50.0	49.8
0.1	0.12	50.0	50.7
1.0	1.2	50.0	51.0
1.0	1.2	750	768
10.0	10.1	750	770

Toluenesulfonamides are moderately soluble in methylene chloride whereas sodium saccharin is not, thus making liquid–liquid extractions practical. However, because of the possibility of trace contaminants in the solvent, redistillation is recommended when searching for trace levels.

For the determination of toluenesulfonamides in the acid form of saccharin the use of a stoichiometric amount of sodium hydroxide to dissolve the sample instead of plain water is the only change. If this modification is used, the saccharin solution must be cool prior to the extraction with methylene chloride. Over 100 samples of sodium saccharin produced by the R-F process have been examined, and OTS has been found in every sample. The amounts varied from 0.2 to 5000 ppm. Positive identification was made by UV spectrophotometry alone or thin-layer chromatography, followed by UV spectrophotometry.

Although isothermal operation was used for this study, column temperature programming may be used. It was found advantageous after several sample runs to elevate the column temperature to near 300 °C and allow the column to rid itself of high-boiling components that tend to accumulate.

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