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Determination of saccharin in shrimp by ion chromatography and capillary gas chromatography–mass spectrometry

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Abstract

A procedure is described for the detection, identification and determination of saccharin in shrimp. Undeclared use of this regulated substance has been noted. Shrimp is extracted with water, and the extract is treated with a C_{18} solid-phase extraction cartridge and a chloride removal cartridge. The method detection limit is 2 μg/g saccharin in shrimp. Recovery of a 16 μg/g saccharin spike averaged 91 ± 6%. The identity of saccharin is confirmed by gas chromatography–mass spectrometry of the methyl derivative which is prepared using an on-column methylating agent.

1. Introduction

The non-nutritive sweetener, saccharin, is subject to regulation and labeling constraints because of questions concerning possible adverse health effects. This additive is commonly used in beverages and prepared foods such as desserts, canned fruit and sauces. Analysis typically involves reversed-phase high-performance liquid chromatography [1–7]. Development of a method for the determination of saccharin in processed shrimp products was necessary because of the possibility of undeclared use of saccharin by some shrimp processors. Yip and Doucette [8] developed a method for saccharin, sodium benzoate, and potassium sorbate in preserved fish products because existing HPLC procedures for other food products were found to form troublesome emulsions. However, their method requires a relatively lengthy column chromatography sample preparation procedure.

Ion chromatography (IC) has been used to determine saccharin in gums, mints, candies, mouthwashes, cough syrups, and soft drinks [9,10]. Generally, minimal sample preparation has been necessary, often consisting of no more than filtration of an aqueous extract. In light of this, it was anticipated that IC might serve as the basis for a simpler analytical method than HPLC [8].

In circumstances where the illicit use of saccharin is suspected, gas chromatography with mass spectrometric detection (GC–MS) can provide confirmation of the presence of saccharin because of its increased power of identification. Saccharin has been determined in biological fluids by GC–MS after paired ion extraction and formation of the methyl derivative using a reaction with methyl iodide [11].

This paper describes a rapid, direct IC method for the quantitative determination of saccharin in

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shrimp. The separation used was adapted from a method for the determination of saccharin in soft drinks [10]. Confirmation for the presence of saccharin is provided via a GC–MS method which utilizes on-column derivatization.

2. Experimental

2.1. Apparatus

The IC instrument used consisted of a Waters Action analyzer (Millipore, Milford, MA, USA) with a Waters 700 Satellite WISP, 600E gradient module, 431 conductivity detector, and Maxima 820 software on a 386/25 microcomputer for data collection and calculation. The column used was a Dionex IonPac AS5, 250 × 4 mm with an IonPac AG5 guard column, 50 × 4 mm (Dionex, Sunnyvale, CA, USA). A Dionex Anion Micromembrane Suppressor II was also used.

The GC–MS instrument used consisted of a Hewlett-Packard (HP) (Palo Alto, CA, USA) 5917A mass-selective detector interfaced to an HP 5890 Series II gas chromatograph. The system is controlled and the data are collected and analyzed by HP G1034B software running on a HP Vectra 386/25 microcomputer. The analysis was performed on a narrow-bore methyl silicon capillary column (DB-1; 10 m × 0.18 mm I.D., 400 nm film thickness; J&W Scientific, Folsom, CA, USA).

2.2. IC Operating conditions

The IC method utilizes an isocratic eluent consisting of 33 m*M* sodium hydroxide, 7.7 m*M* sodium carbonate, 8 m*M* 4-cyanophenol and 2% (v/v) acetonitrile. The eluent flow-rate was 1.0 ml/min. The regenerant was 12.5 m*M* sulfuric acid at a flow-rate of 5 ml/min. The volume of sample extract injected onto the column was 100 μl. The base range of the conductivity detector was 50 μS.

2.3. GC–MS Operating conditions

The column head pressure was set to 5 kPa for a column flow-rate of 0.7 ml/min. A sample injection of 0.002 ml in the splitless mode was used with a split vent interval of 1.5 min and a split vent flow of about 30 ml/min.

The initial temperature of the oven was 50°C. After injection, this temperature was held for 2 min, and then increased to 100°C at a rate of 70°C/min followed by an increase to 250°C at a rate of 10°C/min.

The following detector conditions were used with the Hewlett-Packard 5971A. The instrument was autotuned for general scanning. A solvent delay of 5.00 min was set. The mass spectrum scan range was 30 to 330 a.m.u. with the threshold set at 500.

After each run, the column temperature was maintained at 250°C while two injections of MethElute reagent were made to clean the injection liner and to prevent carryover between runs. A 10-min interval was allowed for the material to exit from the column before starting the next run.

2.4. Reagents and standards

Distilled deionized water (DDW) was used in these studies (Milli-Q system, Millipore, Bedford, MA, USA). Saccharin reagent was obtained from Aldrich (98+%; Milwaukee, WI, USA). All other IC reagents were of the highest available purity. A 100 μg/ml IC stock standard of saccharin was prepared by dilution with DDW. Working IC standards were prepared at the 0.1, 0.5, 5.0 and 25 μg/ml levels.

A solution of 0.2 *M* trimethylanilinium hydroxide (TMAH) in methanol (MethElute Reagent; Pierce, Rockford, IL, USA) was used for the GC–MS on-column methylation reaction. All other GC–MS reagents were of the highest available purity.

2.5. IC Sample preparation

Frozen shrimp were thawed and the shells and tails removed. Approximately 15–30 shrimp were composited using a food processor. An accurately weighed 1-g portion of the composite was diluted with 80 ml of DDW in a 125-ml high-density polyethylene bottle. The solution was mixed for 30 min using a mechanical shaker

and centrifuged to remove heavier particulate from the solution. Approximately 8–10 ml of the extract were filtered through a 0.2-μm filter, an activated 300 mg C_{18} sample preparation cartridge (Maxiclean, Alltech) and a silver sample pretreatment cartridge (OnGuard Ag, Dionex) in series at a rate of approximately 1 ml/min. The filtrate served as the analytical sample.

2.6. GC–MS Sample preparation

A 2-g portion of thawed, composited shrimp was placed in a 20-ml glass vial and shaken with 10 ml of DDW. This mixture was centrifuged for 5 min at 2000 *g*. The aqueous supernatant was transferred to another vial and washed with 10 ml of diethyl ether. The ether wash should be discarded. Centrifugation was sometimes required to separate the organic and aqueous layers.

To the aqueous layer, 1.5 g of sodium chloride and 0.2 ml of hydrochloric acid were added and then thoroughly mixed by shaking. A 10-ml volume of diethyl ether was used to extract the saccharin from the acidified aqueous solution. The mixture was centrifuged (5 min, 2000 *g*) to provide two clear layers with a thin white band of solid material at the interface. The extraction was repeated and the ether extracts combined and dried over 1.5 g of sodium sulfate. The ether was evaporated just to dryness and the extract was reconstituted in 0.25 ml of TMAH for analysis by GC–MS.

3. Results and discussion

3.1. Determination of saccharin using IC

Fig. 1 shows a typical chromatogram of a 0.1 μg/ml saccharin standard. The saccharin peak elutes at a retention time of approximately 5.0 min. Peak area responses were used to quantitate saccharin throughout this work. A relative standard deviation (R.S.D.) of 1.2% was obtained for ten replicate injections of a 1 μg/ml saccharin standard. Peak area response was found to be linear with saccharin concentration over the range 0.1 to 25 μg/ml with a correlation

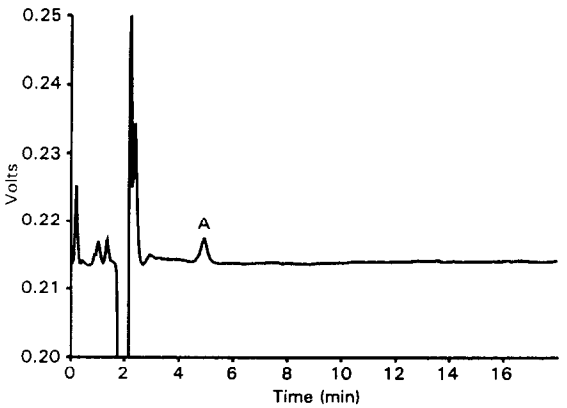

Fig. 1. Ion chromatogram obtained for a 0.1 μg/ml saccharin standard. Peak A = saccharin.

coefficient of 0.9996. The slope of the calibration curve was 297208 area counts/ppm and the *y*-intercept was −21022 area counts. The instrumental detection limit (IDL) for saccharin was found to be 0.02 μg/ml. IDL was defined as three times the standard deviation of blank baseline signal divided by the slope of the calibration curve.

The determination of saccharin in shrimp was complicated by a high level of chloride in the samples. The samples of interest had also been processed with sodium chloride. Excessive tailing of the chloride peak often obscured the saccharin peak. This problem was minimized by passing the samples through Ag sample pretreatment cartridges. These cartridges contain an Ag cation-exchange resin which will remove much of the chloride from the sample. Experimentally it was found that Ag cartridges with a capacity of at least 1.8 mequiv./cartridge provided more consistent removal of chloride and thus better reproducibility of the saccharin peak than cartridges with smaller capacities.

After reduction of chloride in the samples through the use of the Ag cartridges, an orthophosphate peak is resolved from the chloride peak. The orthophosphate peak is easily lost in the large chloride signal in samples which have not been treated with the Ag cartridge. The orthophosphate peak tails significantly, and the saccharin peak elutes in the tail. Fig. 2 shows the chromatogram obtained from a processed shrimp sample after treatment with the Ag cartridge.

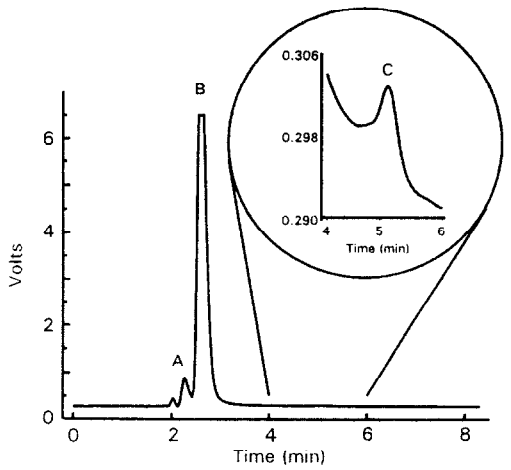

Fig. 2. Ion chromatogram obtained for a sample of shrimp containing saccharin. Peaks: A = chloride; B = orthophosphate; C = saccharin at a concentration of 0.31 μg/ml which corresponds to 25 μg/g in shrimp.

A C_{18} sample preparation cartridge was used to help remove organic materials prior to injection onto the IC column. Proteins and other organics can be retained on the IC column causing irreversible damage. Saccharin standards which were passed through both silver and C_{18} cartridges were not retained on the cartridges. Saccharin was spiked into an aliquot of unprocessed shrimp matrix at the level of 16 μg/g and taken through the extraction procedure. The percent recovery through the method averaged 91 ± 6% ($n = 5$). The reproducibility of saccharin peak area response for ten replicate injections of a processed shrimp sample with an average concentration of 25 μg/g was 3.2% R.S.D.

A method detection limit (MDL) was estimated at 2 μg/g in shrimp. An unprocessed shrimp sample was prepared as discussed previously and orthophosphate was added to the sample solution at approximately the same level (50 μg/ml) as found in the sample chromatogram shown in Fig. 2. The method detection limit was defined as three times the standard deviation of ten replicate analyses of an 8 μg/g saccharin spike to this sample.

Saccharin was detected in both cooked and uncooked processed shrimp samples. Uncooked shrimp samples were boiled for 5 min to imitate the cooking process. Saccharin concentrations did not significantly change after boiling whole shrimp in water for up to 5 min. Therefore, any saccharin added to raw shrimp prior to cooking should still be detected in the analysis of cooked product.

A single sample of processed shrimp was analyzed on three occasions over a six-week period. Concentrations of 26 ($n = 2$), 20 ($n = 5$) and 23 ($n = 3$) μg/g saccharin were obtained on the 1st, 35th and 43rd days, respectively. The average and 95% confidence limit of all values was determined to be 22 ± 2.1 μg/g saccharin. Clearly, the method provides consistent results over the 6-week period.

Earlier IC work utilized a 1:40 dilution of the shrimp matrix. Recoveries were somewhat lower in the less dilute solution, usually averaging approximately 75%. Lower recoveries were presumably the result of the larger amount of processed shrimp matrix (which includes added salt) involved. Several sample types have been analyzed using the 1:40 dilution. No saccharin was detected in three samples of raw unprocessed shrimp. Five samples of "in-process" shrimp and their associated brines were found to contain concentrations of saccharin ranging from 12–35 μg/g. Saccharin was also detected in five of six finished products at concentrations ranging from 8 to 40 μg/g. The presence of saccharin in the five finished products was confirmed qualitatively using GC–MS as discussed in the next section.

3.2. Identification of saccharin in shrimp using GC–MS

The extraction of saccharin from an acidified food matrix with diethyl ether has been described elsewhere [12]. In this work, shrimp samples were deproteinized by acidification with hydrochloric acid and saccharin was extracted with diethyl ether. The extract was taken to dryness and reconstituted in 0.2 *M* TMAH, a flash (or on-column) methylation reagent which has been used to derivatize a variety of drugs [13].

Fig. 3 shows a chromatogram obtained for a sample of shrimp which did not contain sac-

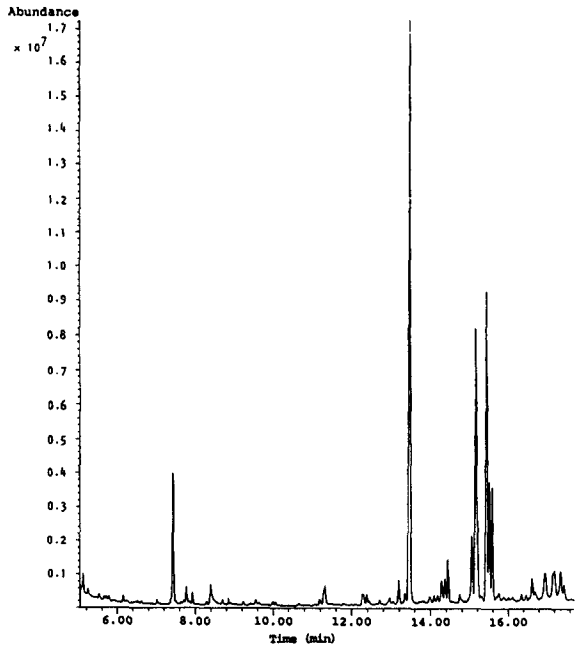

Fig. 3. GC–MS total ion current chromatogram of an untreated shrimp extract.

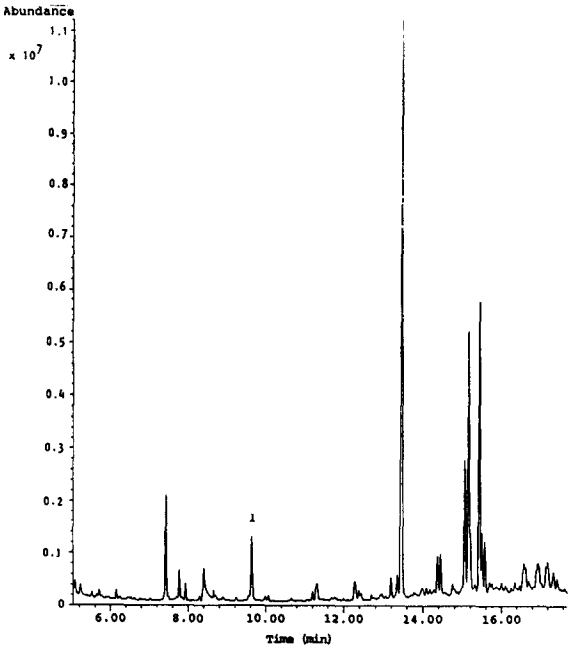

Fig. 4. GC–MS total ion current chromatogram of shrimp sample spiked with saccharin at 23 μg/g and taken through the method. Peak 1 = N-methylsaccharin.

charin. A number of prominent peaks are present with retention times as follows; 13.50, 15.07, 15.18, 15.46 and 15.60 min. Based upon a search of a commercially available database of mass spectra [14], these appear to be methyl esters of hexadecanoic acid, octadecanoic acid and octadecadienoic acids.

When saccharin was spiked into the shrimp matrix at a level of 23 μg/g, the chromatogram shown in Fig. 4 was obtained. A peak with the mass spectrum of the N-methyl derivative of saccharin [11] occurs at 9.65 min. This peak is cleanly resolved from the components of the matrix. The mass spectrum was matched [probability based matching (PBM)-reverse] against a commercially available database of mass spectra [14] and returned N-methylsaccharin with a match quality of 97 (Fig. 5).

Saccharin was spiked into an aliquot of shrimp matrix at the level of 23 μg/g and taken through the extraction procedure. The average percent recovery for two determinations was 36% (range = 8).

Although the recovery is somewhat disappointing, the method is useful for detecting and identifying saccharin at the 20 μg/g level. The mass spectrum of the methyl derivative shows a strong molecular ion ($m/z = 197$) and a number of other high mass ions which bodes well for the selectivity and robustness of the procedure across different matrices.

The recovery can probably be improved by enhancements to the extraction procedure such as the use of solid phase extraction to collect saccharin from the deproteinized extract and subsequent elution with isopropanol which forms a useful binary azeotrope with water. Moreover, use of selected-ion monitoring coupled with an internal standard of suitable retention and fragmentation would improve the signal stability. Based upon a reference spectrum, such a standard may be 2,4-dinitromethylbenzoate.

On a practical note, one important aspect to the use of on-column methylation is that incompletely reacted material may deposit in the injection port liner or on the column. This could produce a significant blank in subsequent runs. It

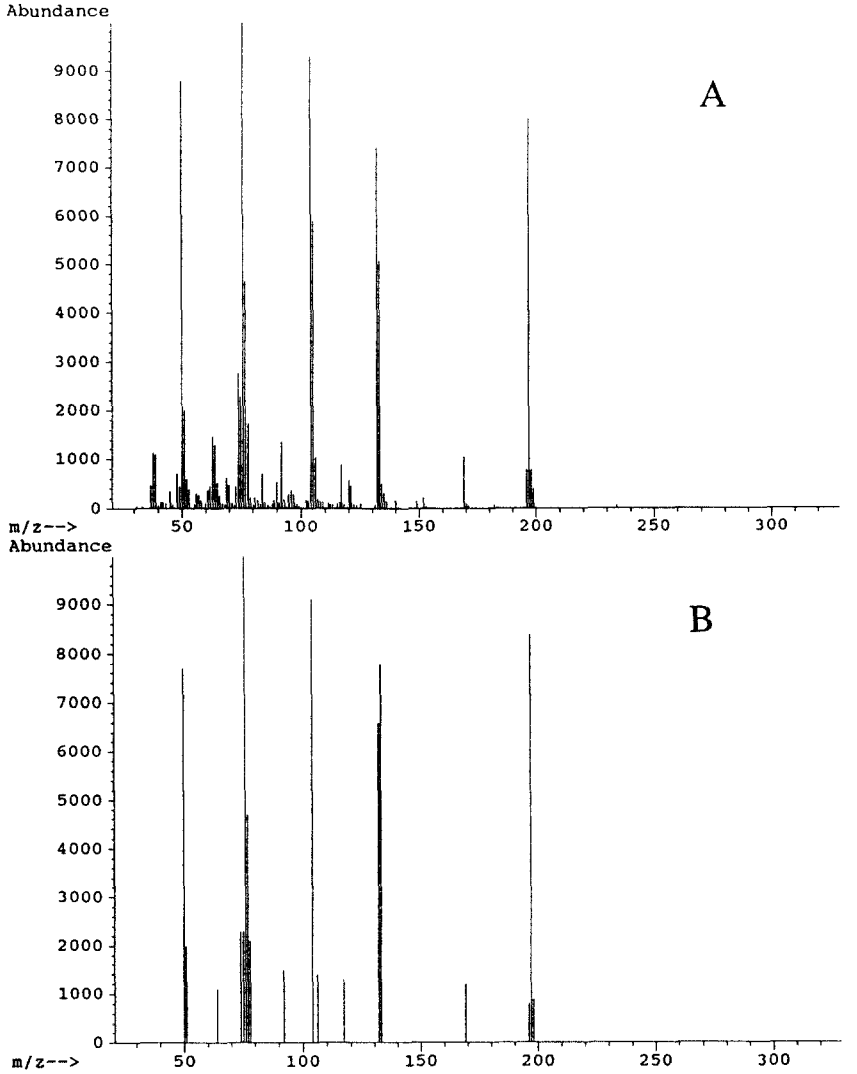

Fig. 5. Comparison of (A) the mass spectrum for the peak assigned to N-methylsaccharin in Fig. 4 and (B) a reference spectrum for N-methylsaccharin [14].

is important to flush the system as described earlier with blank injections of the methylating reagent to control this feature.

4. Conclusions

A rapid IC procedure for the determination of saccharin in shrimp has been developed. This approach is simpler than existing HPLC methodology. The method is capable of detecting saccharin in shrimp at the 2 μg/g level and has demonstrated recoveries of 91 ± 6% at 16 μg/g. Confirmation of the presence of saccharin can be effectively accomplished by capillary GC of the N-methyl derivative which is prepared "on-line" using a flash methylation reagent.

5. References

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