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Note

Analysis of saccharin, acesulfame-K and sodium cyclamate by high-performance ion chromatography

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Artificial sweeteners are widely used in the food, beverage, confectionary and pharmaceutical industries throughout the world. Some examples of their application include soft drinks, candies, mints, gums, mouthwashes and pharmaceutical dosage forms such as cough syrups. The diversity of products containing artificial sweeteners is therefore quite large, and the sample matrices from which they are to be assayed may be complex. Additionally, the sweeteners may be used singly or in combination with other sweeteners.

Methods have been reported in the literature for the analysis of artificial sweeteners in various sample matrices. The most recent procedures for saccharin and acesulfame K, for example, are represented by high-performance liquid chromatography (HPLC) methods run in reversed-phase mode or reversed-phase mode with an ion pair reagent and using UV detection[1–3].

Analysis of sodium cyclamate is more difficult owing to its poor UV absorbing characteristics. Some of the reported methods for analyzing this sweetener include gas chromatography and colorimetric spectrophotometry[4,5]. These procedures can be time consuming since the compound must be first derivatized prior to analysis. More recently, cylcamate was determined using HPLC with indirect photometric detection[6]. In this technique, the compound's elution is monitored against a UV absorbing mobile phase component.

High-performance ion chromatography offers an attractive alternative to these more traditional methods of analysis. In contrast to organic solvent mediated separations, ion chromatography separations are performed using aqueous buffers. The buffers typically are prepared from inexpensive salts as compared to the expensive organic solvents required for HPLC. In addition, the effluent is usually innocuous since it is comprised of buffer salts and usually can be disposed down laboratory sinks as it is generated. Hydroorganic solvents used in reversed-phase chromatography require costly special handling and disposal.

Typically in ion chromatography, electrochemical detection is employed such as an amperometric or conductivity detector. Compounds having poor UV absorption may be ideal candidates for conductivity detection as in the case of sodium cyclamate, particularly if they readily ionize in solution. On the other hand, UV absorbing excipients such as flavors and dyes, may not give any electrochemical response and can therefore be eliminated as an interferent in quantitation of the

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analyte. Thus ion chromatography offers the opportunity to streamline methods development and increase sample throughput.

This paper describes the development of ion chromatography methods for the analysis of saccharin (or sodium saccharin), acesulfame K and sodium cyclamate. The methods have been tested on various products including gums, mints, boiled and soft confectionary candy, mouthwashes, and cough syrups. The methods have been used as described for the analysis of the sweeteners without any modification of the analytical conditions. It appears that little if any method development work would be required to use the methods on other similar types of products.

EXPERIMENTAL

Apparatus

The entire ion chromatographic system was the Dionex 4000i advanced chromatography module containing the pump, automatic injector and a Model CDM1 conductivity detector (Dionex, Sunnyvale, CA, U.S.A.). The signal from the conductivity detector was passed to a Heath d.c. offset module model EU-200-02 (Heath Schumberger, Benton Harbor, MI, U.S.A.) to reduce the 100-mV baseline signal to 1 mV and be compatible with the Sigma 10 data station (Perkin-Elmer, Norwalk, CT, U.S.A.) used to collect the peak areas. A spectrum 921 high-frequency module set at 0.01 (Spectrum, Newark, DE, U.S.A.) removed any stray high-frequency noise. A Dionex AS4A anion separator column and a AS4G guard column were used at room temperature. The micro membrane suppressor was the Dionex Model AMMS-1.

Reagents

All the reagents used were analytical grade. Water used in the preparation of the mobile phases and regenerate solutions was purified by passing through a Milli-RO4 system (Millipore, Bedford, MA, U.S.A.) and had a resistivity of 16 Ω or greater.

Chromatography conditions

System 1. The mobile phase for analysis of saccharin and acesulfame K consisted of 300 mg sodium carbonate dissolved in 1 l Milli-Q water. The solution was filtered through a 0.45-μm Nylon 66 membrane (Rainin, Woburn, MA, U.S.A.) and degassed using sonication and vacuum prior to use. The conditions used were as follows: flow-rate, 2.0 ml/min; back pressure, *ca.*1000 p.s.i.; injection volume, 50 μl; detector sensitivity, 30 μs; detector compensation temperature, 1.7°C; chart speed, 5 mm/min. The regenerate solution was prepared by diluting 2.4 ml sulfuric acid in 2 l Milli-Q water to give a 0.025 *M* solution.

System 2. The mobile phase for analysis of sodium cyclamate consisted of 140 mg sodium bicarbonate dissolved in 1 l Milli-Q water and filtered and degassed as above. The conditions for cyclamate analysis were as follows: flow-rate, 1.5 ml/min; back pressure, 800 p.s.i.; injection volume, 50 μl; detector sensitivity, 30 μS; detector compensation temperature, 1.7°C; chart speed, 5 mm/min. The regenerate solution was 0.012 *M* sulfuric acid prepared by diluting 1.2 ml sulfuric acid in 2 l Milli-Q water.

For both analyses, the regenerate flow was adjusted to give a background conductivity of 10–18 μS for baseline.

Standard preparation

Stock solutions of saccharin or acesulfame K were prepared by dissolving 100 mg saccharin or acesulfame K in 100 ml of sodium carbonate (300 mg/l) buffer. A 5-ml aliquot was taken into a 50-ml volumetric flask containing 2 ml of a 0.90-mg/ml sodium fumarate internal standard then diluted to volume with buffer. The working standard was prepared by making a final 1:10 dilution of this solution.

A stock solution of sodium cyclamate was prepared by dissolving 90 mg sodium cyclamate in 50 ml of a buffer solution containing 140 mg/l sodium bicarbonate. A 3-ml aliquot was then pipetted into a 50-ml volumetric flask containing 2 ml of potassium bromide (1.5 mg/ml) internal standard then diluted to volume with bicarbonate buffer. A 10-ml aliquot was then diluted to 100 ml to prepare the working standard.

Sample preparation

Gum samples were prepared by placing the sample in a 125-ml erlenmeyer flask and extracting with a mixture of 1 ml glacial acetic acid, 2 ml internal standard, 50 ml water, and 25 ml chloroform. Hard or soft candy samples were shaken with 2 ml internal standard and 50 ml of water until dissolved. An aliquot of the aqueous phase was then taken and diluted to a suitable volume using either the carbonate or bicarbonate buffer. Liquid samples were prepared by diluting aliquots of the sample to an appropriate volume using buffer solution. Samples and standards were filtered through a 0.45-μm Nylon 66 filter prior to injection in the ion chromatograph.

RESULTS AND DISCUSSION

Response was linear for each of the analytes in the following concentration ranges: sodium cyclamate, 0.0508–0.0010 mg/ml; sodium saccharin, 0.0262–0.0022 mg/ml; and acesulfame K, 0.0245–0.0041 mg/ml. The correlation coefficients for the three analytes were 0.994, 0.9998 and 0.9999 respectively.

Chromatographic reproducibility results were obtained by injecting spiked samples of a gum and hard candy eight times throughout the day using the analytical conditions described for each sweetener. The relative standard deviation of area counts for each analyte was: cyclamate, 0.5%; bromide, 1.16%; acesulfame-K, 0.9%; saccharin, 1.1%; and fumarate, 0.5%. Recovery studies were conducted for each analyte by spiking blank gum and candy samples. Recovery of sweeteners from spiked gum is presented in Table I. Similar recovery data was obtained for spiked candy samples.

Table II shows calculated values for plates counts (N), capacity factors (k'), and asymmetry factors (a) for the compounds studied. A typical chromatogram of extracted gum sample spiked with saccharin and acesulfame K is presented in Fig. 1. Fig. 2 shows a typical chromatogram of a gum sample containing sodium cyclamate.

To ascertain the overall usefulness of the procedures, several different types of sugar based fruit flavored candy, and cinnamon, spearmint and peppermint flavored confections were analyzed to ascertain if there were peaks interfering with quantita-

TABLE I

RECOVERY OF ARTIFICIAL SWEETENERS FROM GUM

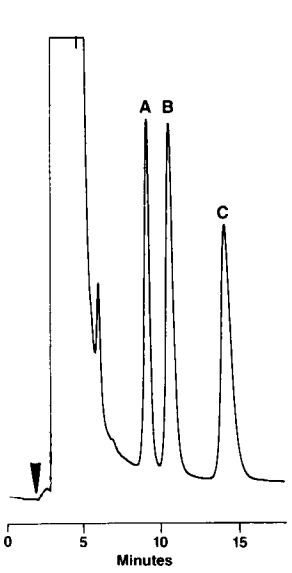
Added (mg)	*Found (mg)*	*Recovery (%)*
Cyclamate		
29.62	31.28	105.6
24.68	27.00	109.4
21.72	22.22	102.4
19.74	19.74	100.0
17.77	17.77	100.0
9.87	9.37	94.9
4.94	4.75	96.3
Saccharin		
6.55	6.72	102.5
5.46	5.36	98.2
4.32	4.38	100.3
3.28	3.34	101.8
2.18	2.17	99.8
Acesulfame K		
6.12	6.34	103.6
5.09	5.05	99.2
4.08	4.08	100.0
3.06	3.10	101.2
2.04	2.08	102.2

tion of the artificial sweeteners. None of the samples tested contained any extra excipient peaks eluting at the retention times of the sweeteners. Common inorganic anions such as fluoride, chloride, nitrite, phosphate or sulfate, for example, did not interfere in the analysis of saccharin or acesulfame K. The cyclamate analysis is also free from these interferences except for chloride. When analyzing samples containing chloride and cyclamates, the sodium bicarbonate should be reduced to *ca.* 50 mg/l in order to obtain adequate resolution between the chloride and cyclamate peaks. As with any sample, there is always the possibility of an excipient coeluting with the analyte since ingredients can vary from sample to sample and country of origin. One of the distinct advantages is the use of conductivity detection of the artificial sweeteners since exipients may not exhibit an electrochemical response hence not appear in the chromatogram. Additionally, use of ion chromatography can offer considerable savings in the purchase of HPLC grade solvents and subsequent disposal of spent solvents.

TABLE II

PLATE NUMBERS (N), CAPACITY FACTORS (k') AND ASYMETRY FACTORS (a) FOR THE COMPOUNDS STUDIED

	N	k'	a
Cyclamate	1495	4.10	1.17
Acesulfame K	1700	6.98	1.00
Saccharin	1615	10.45	1.23



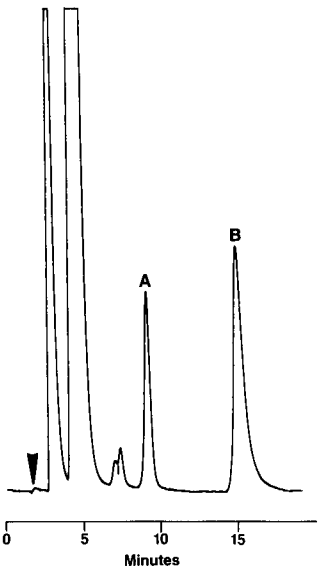


Fig. 1. Chromatogram of gum sample spiked with saccharin and acesulfame K. A = fumarate; B = acesulfame K; C = saccharin; conditions, system 1.

Fig. 2. Chromatogram of gum sample spiked with sodium cyclamate. A = cyclamate; B = bromide; conditions, system 2.

In the method for the analysis of cyclamate, some samples required use of a 'wash injection'. It was observed that after several injections, there was a noticeable decline in retention time for cyclamate and bromide. This situation would probably arise when analyzing products containing species such as phosphate, citrate, or other strongly retained compounds. In a short time, the column began to lose ion-exchange capacity as evidenced by the decreasing retention times of the peaks of interest. In these situations, use of a 0.2 *M* sodium carbonate wash injection during the analysis (*i.e.*, after 6–7 sample injections) helped elute the strongly retained components thus reactivating exchange sites in the resin and regenerating full ion-exchange capacity. The cyclamate analysis required a separate mobile phase because it is a weaker anion than either saccharin or acesulfame K. Therefore, a weak mobile phase was required to retain this compound on the column.

The internal standard methods described above are intended to simplify preparation of solid samples and are not indicative of any imprecision expected in the chromatography or injection of the sample. As a matter of choice, it appeared easier to place samples into wide mouth glassware and pipet small volumes of internal standard than attempting to quantitatively transfer bulky samples to volumetric glassware. In the case of liquid samples, aliquots were taken and diluted to a known volume without adding the internal standard. Using either procedure, the methods are accurate and yield excellent quantitative results.

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