

Separation and simultaneous determination of four artificial sweeteners in food and beverages by ion chromatography

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Abstract

In this paper, the separation and determination of four artificial sweeteners (aspartame, sodium cyclamate, acesulfame-K and sodium saccharin) by ion chromatography coupled with suppressed conductivity detector is reported. The four artificial sweeteners were separated using KOH eluent generator. Due to the use of eluent generator, very low conductance background conductivity can be obtained and sensitivity of sweeteners has been greatly improved. Under the experimental condition, several inorganic anions, such as F^- , Cl^- , NO_3^- , NO_2^- , Br^- , SO_4^{2-} , PO_4^{3-} and some organic acid such as formate, acetate, benzoate, and citrate did not interfere with the determination. With this method, good linear relationship, sensitivity and reproducibility were obtained. Detection limits of aspartame, sodium cyclamate, acesulfame-K, sodium saccharin were 0.87, 0.032, 0.019, 0.045 mg/L, respectively. Rate of recovery were between 98.23 and 105.42%, 99.48 and 103.57%, 97.96 and 103.23%, 98.46 and 102.40%, respectively. The method has successfully applied to the determination of the four sweeteners in drinks and preserved fruits.

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1. Introduction

Food additives play a vital role in the modern food industry, and are generally used for maintaining food quality and characteristics as well as food safety. Among them, artificial sweeteners are widely used throughout the world. Artificial sweeteners are commonly used in the food, beverage, confectionary and pharmaceutical industries.

The most common artificial sweeteners include aspartame, sodium cyclamate, acesulfame-K, and sodium saccharin, which are all permitted in about 90 countries. They provide benefits to consumers by improving food choice, particularly for those demanding such products in order to reduce energy or sugar intakes for health reasons and to the food industry by increasing shelf life of some products compared with the sugar sweetened ones. Sodium saccharin is the oldest artificial sweetener, but it has been the center of controversy during the last few decades due to its possible

carcinogenic effects. The strictest restriction is placed on its usage owing to its potential toxicity and its acceptable daily intake (ADI) value formulated by the World Health Organization (WHO) is the lowest among the four sweeteners [1]. In China, the concentration in food is 0.15–5.0 g/kg BW [2]. Sodium cyclamate is banned in USA, while its usage is permitted in Europe and China [1], in most countries which are using sodium cyclamate, the ADI value is 0–11 mg/kg BW [3]. Because a small group of people who have the hereditary disease phenylketonuria are sensitive to phenylalanine, one of the metabolites of aspartame, all products containing ASP must be labeled for phenylalanine in many countries [4]. It has been used in about 100 countries, the ADI value is 40 mg/kg [3]. Acesulfame-K also has been used in about 100 countries, according to the FAQ/WHO, the ADI value is 0–15 mg/kg in China, the maximum amount of acesulfame-K in lacti-beverage is 0.3 mg/kg [3].

High-performance liquid chromatography (HPLC) has been the most popular choice for the determination of the four sweeteners. But only a few are suitable for the simultaneous determination of several sweeteners [5,6], and most HPLC

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procedures were based on isocratic or gradient reverse-phase (RP) chromatographic separation with acetonitrile, methanol or isopropanol in phosphate buffer or acetic acid solution as mobile phase and ultraviolet (UV) absorbance detection [7–10]. RP ion-pair chromatography [11] was also used for the simultaneous determination of sodium saccharin and aspartame.

In addition, ion chromatography (IC) offered an attractive alternative to traditional HPLC methods. Sodium saccharin, aspartame and acesulfame-K in foods could be separated on an anion-exchange separation column. Qu et al. [13] proposed the determination method for separation and determination of the sweeteners aspartame by ion chromatography coupled with electrochemical amperometric detection. Biemer [14] proposed two determination methods, one for sodium cyclamate separately and another for sodium saccharin along with acesulfame-K simultaneously. Chen simultaneously separated three artificial sweeteners and two preservatives caffeine, theobromine and theophylline on an anion-exchange analytical column by an isocratic elution and with wavelength-switching ultraviolet absorbance detection [15]. Chen et al. [12] used anion-exchange gradient program and ultraviolet absorbance detector for the separation of four artificial sweeteners and citric acid. To our knowledge, the method using eluent generator ion chromatography and couple with interference experiment has not been reported.

In this paper, a new method for simultaneous determination of four artificial sweeteners including aspartame, sodium cyclamate, acesulfame-K, sodium saccharin by using eluent generator ion chromatography has been described. Due to the use of eluent generator, very low conductance background and very sensitivity of sweeteners can be obtained, there is no need to use a UV detector at the same time. This method can be used for the routine analysis of these artificial sweeteners in drinks and preserved fruits.

2. Experimental

2.1. Reagents

Aspartame, sodium cyclamate, acesulfame-K, sodium saccharin were purchased from Sigma (St. Louis, MO, USA). The other reagents were of analytical reagent grade or higher purity. Distilled deionized water of 18.3 M Ω cm was used throughout. The stock solutions were prepared separately by dissolving appropriate amounts of these compounds in deionized water.

2.2. Instrumentation

A Dionex Model ICS-2000 ion chromatograph equipped with a 25 μ l sample loop. A Dionex Ionpac AG11 guard column (50 mm \times 2 mm i.d.) and a Dionex Ionpac AS11 Separation column (250 mm \times 2 mm i.d.) were used throughout. The eluent was achieved by a KOH eluent generator. The

suppression was achieved by a Dionex ASRS UL TRAI (2 mm) self-regenerating suppressor (recycle mode). The column temperature was 30 $^{\circ}$ C and the working electric current was 40 mA. The eluent flow rate is 0.25 ml/min. All the instrument control and data collection were performed by a Dionex Chromeleon 6.5. The injection volume is 25 μ l.

2.3. Preparation of real samples

All samples, including two carbonated cola drinks, (samples A and B), one fruit juice drink (sample C), one preserved fruit (sample D), were purchased from local market. They were pretreated by the relevant procedures as follows, the final solutions were filtered through 0.45 μ m Nylon filters before the analysis.

2.3.1. Carbonated cola drinks

The two carbonated cola drinks were degassed for 5 min in an ultrasonic bath before diluted by water. A 1-ml volume of cola drinks (A and B) were directly diluted by deionized water to a 50-ml volumetric flask.

2.3.2. Fruit juice drink

A 1-ml volume of the fruit juice drink (C) was directly diluted by deionized water to a 100-ml volumetric flask.

2.3.3. Preserved fruit

An amount of ca. 5 g of preserved fruit (D) was weighed and then be chopped into pieces into a 50-ml volumetric flask, 30 ml water was added, the flask was extracted ultrasonically for 15 min, and then the solution was diluted to 50 ml. A 1-ml of the diluted solution got from above process was more diluted by deionized water to 100 ml.

3. Results and discussion

3.1. Condition of separation

There is phenylalanine in aspartame, which is hard to be separated from aspartame, so we have to use a gradient program to separate phenylalanine from aspartame, and to improve the separation performance. The principle of the choice of the concentration is to improve the separation as well as shorting the analysis time. Using the choiced gradient program, phenylalanine can be well separated from aspartame, and another three sweeteners can be baseline separated, the resolution between peaks were 3.02, 5.85, 13.42 and 3.91, respectively, the analysis time is also short. Table 1 shows the gradient program.

3.2. Interference experiment

In general, some inorganic anion such as Cl^- , NO_3^- , SO_4^{2-} , PO_4^{3-} , some organic acid such as formate, acetate are

Table 1
Gradient condition for separation

Gradient program	
Time (min)	The concentration of KOH ($\times 10^{-3}$ mol/L)
0	1
7	15
30	15
30.01	1

in the water and some organic acid such as benzoate, citrate are naturally present or intentionally added in soft drinks, which may interfere the detection of the four sweeteners. Under the experimental conditions, the common seven organic anions F^- , Cl^- , NO_3^- , NO_2^- , Br^- , SO_4^{2-} , PO_4^{3-} and some organic acid including formate, acetate, benzoate, and citrate did not interfere with the four sweeteners' detection. The retention time of F^- , Cl^- , NO_3^- , NO_2^- , Br^- , SO_4^{2-} , and PO_4^{3-} were 2.477, 3.756, 4.003, 4.860, 6.147, 6.520, and 8.850 min, respectively, the retention time of formate, acetate, benzoate and citrate were 2.847, 2.627, 5.603, and 3.687 min, respectively. Compare to the retention time of the aspartame, sodium cyclamate, acesulfame-K, sodium saccharin were 3.583, 4.412, 8.597, and 10.460 min, respectively.

3.3. Linearity, precision and detection limits

Under the optimum experiment conditions, the analytes all showed good linear relationship, sensitivity and reproducibility. Repeating ten times, the relative standard deviation (RSD) of the retention time, peak area, peak height are shown in Table 2. And in this mode, the correlation coefficients of height for Aspartame, sodium cyclamate, acesulfame-K, and sodium saccharin were 0.9992, 0.9997, 0.9990, and 0.9994, respectively; the correlation coefficients of area were 0.9996, 0.9998, 1, and 0.9998, respectively. For the KOH eluent generator and self-regenerating suppressed conductivity are used, the background conductance is less than $0.2 \mu S$, and detection limits are greatly improved compare with former IC methods. The detection limits (signal-to-noise ratio 3:1) for aspartame, sodium cyclamate, acesulfame-K, and sodium

Table 2
The linearity and detection limits of four sweeteners (Y_1 the equation of height, Y_2 the equation of area)

Sweeteners	Regression equation	r	Detection limit (mg/L)
Aspartame	$Y_1 = 0.0119x - 0.0012$	0.9996	0.87
	$Y_2 = 0.0575x + 0.0439$	0.9992	
Sodium cyclamate	$Y_1 = 0.1825x - 0.0029$	0.9998	0.032
	$Y_2 = 1.2237x + 0.1272$	0.9997	
Acesulfame-K	$Y_1 = 0.2062x - 0.0222$	0.9999	0.019
	$Y_2 = 0.7855x + 0.3044$	1	
Sodium saccharin	$Y_1 = 0.169x - 0.0160$	0.9998	0.045
	$Y_2 = 0.3807x + 0.4938$	0.9994	

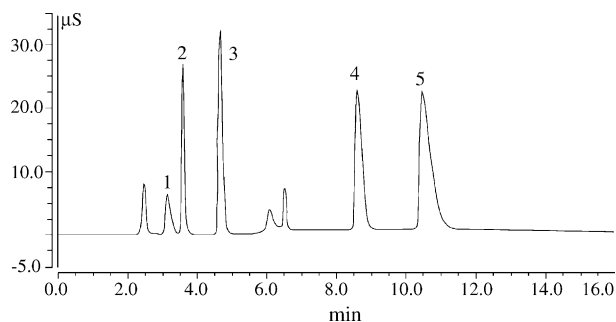


Fig. 1. The chromatogram of standard solution: (1) phenylalanine; (2) aspartame (20 mg/L); (3) sodium cyclamate (5 mg/L); (4) acesulfame-K (5 mg/L); (5) sodium saccharin (10 mg/L).

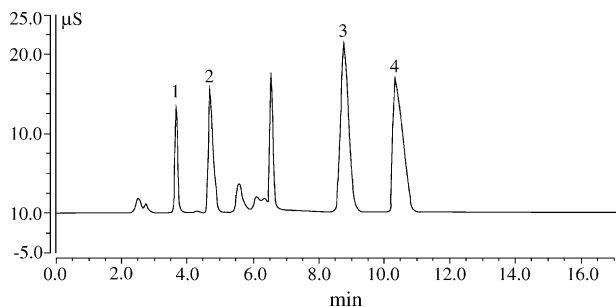


Fig. 2. The chromatogram of cola drinks B: (1) aspartame; (2) sodium cyclamate; (3) acesulfame-K; (4) sodium saccharin.

saccharin were 0.87, 0.032, 0.019, and 0.045 mg/L, respectively. Compare to the ADI of the four sweeteners, the detection limits were much lower. Table 2 shows the linearity and the detection limits of four sweeteners. Fig. 1 shows the chromatogram of standard solution. From the values of RSD and the detection limits we can find that the suppressed conductivity detection mode is effective in simultaneously separate and detect the four sweeteners. And also we can say that it is a very sensitive method for detection of the four sweeteners because of the very low detection limits of the four.

3.4. Analysis of real sample

All real samples were analyzed according to the experimental conditions. Figs. 2–4 show the chromatogram of samples. Table 3 shows the results of all the samples. Table 4

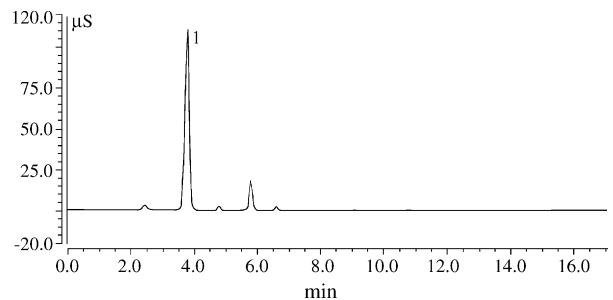


Fig. 3. The chromatogram of fruit juice drink: (1) aspartame.

Table 3
Analytical results and the recovery data of sweeteners in the samples

Samples	Aspartame (mg/L) or (g/g)	Sodium cyclamate (g/g) or (mg/L)	Acesulfame-K (mg/L) or (g/g)	Sodium saccharin (mg/L) or (g/g)
Sample A	7234.66	504.93	1105.37	576.17
Sample B	2826.33	744.84	1475.18	187.45
Sample C	317.20	7.1	4.5	2.4
Sample D	729.70	–	–	–

Table 4
The recovery datum of sweeteners in the samples

Samples	Aspartame (%)	Sodium cyclamate (%)	Acesulfame-K (%)	Sodium saccharin (%)
Sample A	105.42	100.24	103.23	99.27
Sample B	98.23	99.56	101.21	98.46
Sample C	99.45	103.57	97.96	102.40
Sample D	103.25	99.48	101.26	99.58

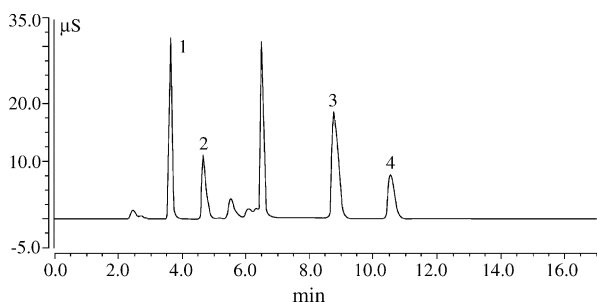


Fig. 4. The chromatogram of the chromatogram of cola drink A: (1) aspartame; (2) sodium cyclamate; (3) acesulfame-K; (4) sodium saccharin.

shows the recovery datum of the real samples. The datum were gained from the matrices tested spiked with the analytes of interest.

4. Conclusions

A high-performance anion-exchange chromatographic coupled with KOH eluent generator and suppressed conductivity detector for simultaneously separated and detected four sweeteners was developed. In this method, very low conductance background can be obtained and sensitivity of sweeteners has been greatly improved, and also it is a novel and very sensitive determination for simultaneous determination of four sweeteners assayed on real samples, the detection limits have down to 1 ppm for aspartame, 0.05 ppm for sodium cyclamate, acesulfame-K, and sodium saccharin. The results of real sample analysis are satisfactory.

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