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Short communication

# Determination of aspartame by ion chromatography with electrochemical integrated amperometric detection

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**Abstract**

In this paper, the separation and determination of the sweetener aspartame by ion chromatography coupled with electrochemical amperometric detection is reported. Sodium saccharin, acesulfame-K and aspartame were separated using 27.5 mmol/l NaOH isocratic elution on a Dionex IonPac AS4A-SC separation column. Aspartame can be determined by integrated amperometric detection without interference from the other two sweeteners. The method can be applied to the determination of aspartame in powered tabletop, fruit juice and carbonated beverage samples, and the results obtained by integrated amperometry were in agreement with those obtained using a UV detection method. A method for determining analytes with an $NH_2$ group by ion chromatography with integrated amperometry was developed. 

*Keywords:* Electrochemical detection; Integrated amperometric detection; Detection, LC; Food analysis; Aspartame; Artificial sweeteners

## 1. Introduction

Aspartame, a low-calorie artificial sweetener with a sweetening power 180 times that of sucrose [1], is currently used as the safest additive and sugar substitute in food and beverages and in the pharmaceutical industry throughout the world. High concentrations of aspartame may cause illness, so the content is controlled by the health authorities in many countries. To monitor the concentration of aspartame, several analytical methods have been reported, including spectrophotometry [2,3], enzymatic analysis [4–6], gas chromatography [7] and the most frequently used method, high-performance liquid chromatographic separation with UV [8–17] and spectrofluorimetric detection [18,19].

Ion chromatography offers an attractive alternative to the HPLC method. In contrast to organic solvent-mediated separations, ion chromatographic separations are performed using an innocuous and inexpensive salt solution as the eluent. Recently, an ion-exchange method with UV and conductivity detectors for the simultaneous determination of four artificial sweeteners and citric acid was reported by our research group [20]. In the present method, the experimental conditions for separating aspartame, sodium saccharin and acesulfame-K were improved by using NaOH isocratic elution instead of $Na_2CO_3$ gradient elution.

Electrochemical amperometric detection is a useful and important means of determining carbohydrates, amines, sulfur compounds etc. However, few applications have been developed using integrated amperometric detection except for pulsed amperometric detection in carbohydrate analysis. In this

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paper, a method is reported for the determination of aspartame using ion chromatography with electrochemical integrated amperometric detection. The results of sample analysis using this detection method were consistent with those obtained using UV detection. Commonly used sweeteners, such as sodium saccharin, acesulfame-K and sodium cyclamate, give no electrochemical signal at all, and can be eliminated as interference. This method can be used for the analysis of aspartame in drinks and in powdered forms without interference from other commonly used sweeteners.

## 2. Experimental

### *2.1. Apparatus*

A Dionex Model DX-500 ion chromatograph (Sunnyvale, CA, USA) equipped with a 25-μl sample loop, a Dionex IonPac AS4A-SC guard column and an AS4A-SC separation column were used throughout. A Dionex AD20 absorbance detector and a Dionex ED40 electrochemical detector in the electrochemical integrated amperometric detection mode were used. A gold (Au) electrode was used as the work electrode, and a Ag/AgCl electrode was used as the reference electrode. The eluent flow-rate was 1.0 ml/min. All instrument control and data collection were performed by a Dionex PeakNet chromatography workstation.

### *2.2. Reagents*

Acesulfame-K was purchased from Hoechst (Frankfurt, Germany) and other sweeteners were from Sigma (St. Louis, MO, USA). The other reagents were of analytical reagent grade. Distilled deionized water was used throughout. Stock solutions of all of the analytes (1 mg/ml) were kept in a refrigerator at 4°C.

## 3. Results

### *3.1. Electrochemical detection*

Integrated amperometric detection with a Au electrode is capable of producing sensitive and reproducible detection responses for amines with at least one pair of non-bonded electrons on nitrogen. The electrode current was integrated while the potential was swept across the metal oxide formation wave and the oxide reduction wave throughout a rapid cyclic scan. The potential scan proceeds into positive scan and back out of the negative scan, the region of the oxide-catalyzed reaction for detection. Without the presence of an electrochemically active analyte, the net charge is approximately zero.

### *3.2. Choice of separation system*

In this work, attention was paid mainly to the electrochemical detection of aspartame. However, considering possible interference from other sweeteners often used in food and drinks, the separation of sodium saccharin, acesulfame-K and sodium cyclamate was still considered and UV detection was used to confirm the separation. These sweeteners all exist as anionic forms in basic aqueous solutions, and can be separated by an anion-exchange mechanism. We have reported their separation using $Na_2CO_3$ gradient elution [20]. Since isocratic elution is simpler and can keep the baseline stable without drift, isocratic NaOH was used as the eluent. Moreover, strongly basic NaOH can provide a high pH, which is needed in amperometric detection using a Au electrode [21,22]. Baseline separation between sodium saccharin, acesulfame-K, aspartame and decomposed product of aspartame, aspartic acid, could be achieved when 27.5 mmol/l NaOH was used as the eluent. Fig. 1 shows the chromatogram of a standard solution detected by integrated amperometry and UV detection. With the exception of aspartame, the other two sweeteners did not give any electrochemical response and could be eliminated as possible sources of interference. The only possible source of interference to the determination of aspartame should come from its decomposed products, aspartic acid and phenylalanine, which can be oxidized due to the existence of $NH_2$ groups in both of their molecular structures. However, under our experimental conditions, aspartic acid eluted earlier and could be separated from aspartame. Phenylalanine was retained weakly and co-eluted with the water peak. Sodium cyclamate

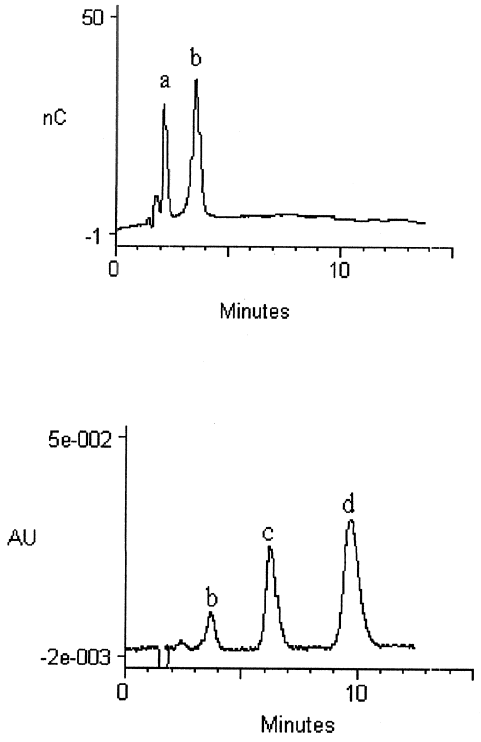


Fig. 1. Chromatogram of a standard solution detected by integrated amperometry and UV detection. (a) Aspartic acid; (b) aspartame; (c) acesulfame-K and (d) sodium saccharin.

does not have either a UV response or an electrochemical signal. No peak appeared in the chromatogram. Using this method, aspartame can be analyzed in less than 5 min.

### *3.3. Linearity, precision and detection limits*

Under the experimental conditions, in which 27.5 mmol/l NaOH was used as the eluent, the peak area response for aspartame was found to be linear in the range 0.1–10 μg/ml. For seven consecutive injections of a standard solution with a concentration of 5 μg/ml, the relative standard deviation (RSD) was 1.29% and the detection limit (signal-to-noise ratio of 3:1) was 0.031 μg/ml.

### *3.4. Analysis of samples*

All seven samples were purchased from a local market. They included two powdered tabletop sweeteners (samples A and B), two fruit juice drinks (samples C and D) and three carbonated drinks (samples E, F and G). A 50-mg amount of the solid samples was dissolved in 25 ml of deionized water, then diluted 17-fold. The fruit juice samples C and D were diluted tenfold. The carbonated beverage samples E, F and G were degassed in an ultrasonic water bath for 10 min at room temperature, to remove carbon dioxide gas, before being diluted. Sample E was diluted tenfold and F and G were diluted 50-fold. Aliquots of all of the diluted samples (25 μl) were injected after filtering them through 0.45-μm membrane filters. Table 1 shows the analytical results obtained for aspartame in four of seven samples using integrated amperometry; the results are consistent with those obtained using UV detection. The recoveries for samples ranged from 77.4 to 94.5%.

Table 1
Analysis of samples by UV and integrated amperometric (IA) detection

| Samples | UV detection | IA detection | |
|---|---|---|---|
| | Content (mg/g, mg/ml) | Content (mg/g or mg/ml) | Recovery (%) |
| A | 30.71 | 31.67 | 94.5 |
| B | 29.77 | 30.84 | 91.5 |
| C | 0.0238 | 0.023 1 | 77.4 |
| E | 0.0103 | 0.0098 | 83.2 |

In aqueous solution, aspartame undergoes decomposition, which is closely related to the length of storage, temperature and pH of the diet foods and beverages. In dry powder, aspartame is more stable. Its stability is strongly dependent on the length of time it is stored for and the temperature during storage. To check the percentage of decomposition, the powered tabletop samples, A and B, which had been used two years ago and then stored at room temperature, were reanalyzed. Compared with the results reported by Chen et al. [20], the content of aspartame in samples A and B decreased by 16.66 and 13.11%, respectively. Fig. 2 shows the chromatogram of sample A.

## 4. Discussion

Amperometric detection mechanisms are believed

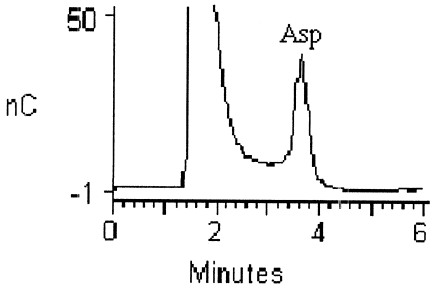


Fig. 2. Chromatogram of sample A. Asp=aspartame.

to involve prior adsorption of amines at the oxide-free electrode surface. Detection occurs simultaneously with the formation of gold electrode surface oxide and the anodic mechanism is believed to be catalyzed by the formation of surface oxide product (AuO) [21,22]. The advantage of integrated amperometry relates to maximization of the analyte signal and minimization of baseline magnitude and drift for oxide-catalyzed detection [21]. Positive and negative cleaning pulse potentials are added to keep the native activity of the 'cleaned' noble metal following the integration period.

Both aspartame (aspartyl-phenylalanine methyl ester) and the products of its decomposition, aspartic acid and phenylalanine, have a free $NH_2$-group in their molecular structure. By anion-exchange separation and integrated amperometric detection on a gold electrode, sensitive determination of aspartame was obtained. To find the optimized potentials in integrated amperometry, basic parameters offered for integrated amperometry detection have been discussed [21–23]. Fig. 3 shows the optimizing waveform and parameters for getting the larger peak area and lower baseline noise for the determination of aspartame.

Based on the detection mechanism of integrated amperometry, it is expected that sodium saccharin, acesulfame-K and sodium cyclamate should give electrochemical signals since they all have nitrogens with a pair of non-bonded electrons in their molecular structures. However, negative results were obtained in our experiment. Therefore, we propose that the conjugation and induction effect resulted in making the electrons on the nitrogen atom more stable and less easy to lose. Compared with them, aspartame, with an isolated $NH_2$- group in the molecular structure, can be oxidized easily.

## 5. Conclusion

Ion chromatographic separation with integrated amperometric detection can be used to determine aspartame in tabletop, fruit juice and carbonated beverage samples. This paper gives not only a method for the determination of aspartame by ion

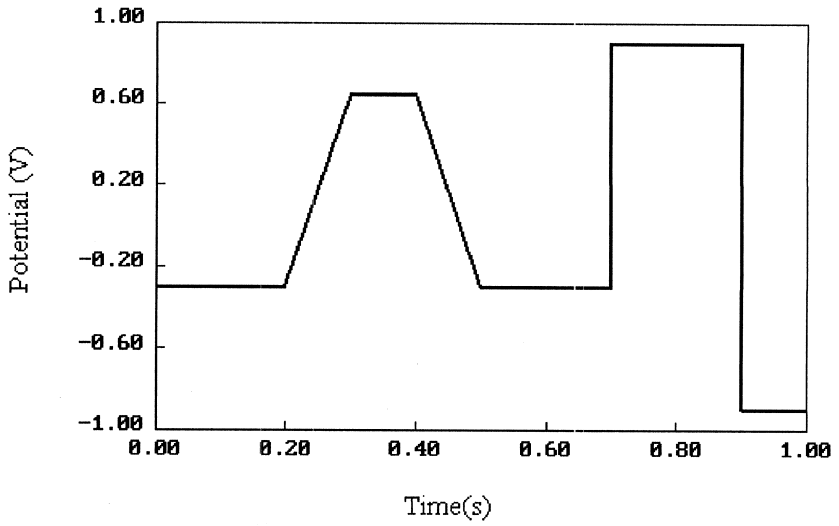


Fig. 3. Integrated amperometry waveform and parameters.

chromatography but, more important, it also gives a method to determine aliphatic amines, which, without an ultraviolet chromophore, cannot be detected by UV detection. Related work is in progress.

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