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Short communication

Simple and rapid high-performance liquid chromatographic method for the determination of aspartame and its metabolites in foods

Bernard F. Gibbs[a], Inteaz Alli[a], Catherine N. Mulligan[b,*]

[a]*Food Science and Agricultural Chemistry Department, McGill University, Macdonald Campus, P.O. Box 187, Ste. Anne de Bellevue, Que. H9X 3V9, Canada*

[b]*Bivan Consultants, Food Biotechnology Division, Suite 1415, 6550 Sherbrooke St. West, Montreal, Que. H4B 1N6, Canada*

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Abstract

A method for the determination of aspartame (N-L-α-aspartyl-L-phenylalanine methyl ester) and its metabolites, applicable on a routine quality assurance basis, is described. Liquid samples (diet Coke, 7-Up, Pepsi, etc.) were injected directly onto a mini-cartridge reversed-phase column on a high-performance liquid chromatographic system, whereas solid samples (Equal, hot chocolate powder, pudding, etc.) were extracted with water. Optimising chromatographic conditions resulted in resolved components of interest within 12 min. The by-products were confirmed by mass spectrometry. Although the method was developed on a two-pump HPLC system fitted with a diode-array detector, it is straightforward and can be transformed to the simplest HPLC configuration. Using a single-piston pump (with damper), a fixed-wavelength detector and a recorder/integrator, the degradation of products can be monitored as they decompose. The results obtained were in harmony with previously reported tedious methods. The method is simple, rapid, quantitative and does not involve complex, hazardous or toxic chemistry.

Keywords: Aspartame

1. Introduction

After the discovery of aspartame (N-L-α-aspartyl-L-phenylalanine methyl ester) in 1965 and its approval in dry food by the US Food and Drug Administration in 1981 for use as a low-calorie sweetener, the market has grown rapidly [1]. Sales have reached $ 150 million/year due to the trend towards 'light' foods. It has replaced saccharin and is found in numerous products such as diet 7-Up, Pepsi and Coke, iced tea, lemonade, breakfast cereals, chewing gum, the sweetener Equal and numerous other diet products. Aspartame is sold under the trade name NutraSweet by G.D. Searle. It is 180–200 times sweeter than sucrose, does not give the bitter aftertaste of saccharin and cyclamates and has not been implicated in causing cancer [2]. It has the same calorific value as sugar per gram but since considerably less is used, very few calories are added to the product.

Although it is relatively stable in its dry form, pH, temperature and time are very important factors affecting its stability in solution. Below pH 3, aspartame is unstable and hydrolyses to produce

*Corresponding author.

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aspartylphenylalanine and above pH 6, it cyclizes to form 5-benzyl-3,6 dioxo-2-piperazineacetic acid [3]. Both forms result in a loss of sweetness. It cannot be used in products that are baked or fried due to disintegration upon exposure to high temperatures.

Despite these restrictions, the growing use of aspartame warrants simple, reliable and rapid methods for its quantitation. Microbial and enzymatic methods have been examined. Generally, enzymatic assays involve tedious purification of enzymes before the initial cleavage of the aspartylphenylalanine bond. Several steps are required before the results are obtained. These methods cannot be used routinely because of their inherent complexities. Similar approaches including bienzymatic electrode [4] and microbial biosensors [5] have not been popular due to potential interferences from other constituents in foods.

Several HPLC methods have been reported for the quantitation of aspartame (see Ref. [6] and references therein). Most of them were developed on 250-mm columns with aspartame derivatized prior to injection. We have developed a simple rapid method where aspartame and all its breakdown products can be efficiently monitored using a 30-mm column with ultraviolet (UV) detection.

2. Experimental

2.1. Chemicals

Aspartame standard, aspartic acid, phenylalanine and aspartylphenylalanine dipeptide were purchased from Sigma (St. Louis, MO, USA). HPLC-grade acetonitrile and trifluoroacetic acid were obtained from Aldrich (MO, USA).

5-Benzyl-3,6-dioxo-2-piperazineacetic acid was synthesized by heating aspartame powder at 150°C for 20 h [7]. The conversion was 100%: 40% to the desired product and 60% to the dipeptide aspartylphenylalanine as confirmed by electrospray ionization mass spectrometry.

2.2. Standard and sample preparation

The standard contained 0.4 μg/μl of phenylalanine, aspartylphenylalanine, aspartame and 5-benzyl-3,6-dioxo-2-piperazineacetic acid and 20 μg/μl of aspartic acid, due to the enhanced absorptivity of phenylalanine (50 times greater) over aspartic acid at 210 nm. The beverages were degassed in an ultrasonic bath for 2 min before injection. 'Equal' tablets were crushed with a spoon. Approximately, 1 mg was dissolved in 1 ml of water. After vortex-mixing for 10 s, the samples were centrifuged (6000 *g*) for 3 min.

Powdered Jello and viscous samples (approximately 1 mg each) were all dissolved in 1 ml water and treated in a similar fashion.

2.3. HPLC analysis

The analyses were performed on a Hewlett-Packard Model 1090 chromatograph fitted with a diode-array detector and Chem Workstation. The C_8 mini-column RP 300 (30×4.6 mm, 5 μm, Aquapore OD-300) was purchased from Applied Biosystems (CA, USA). The components were eluted isocratically with water–acetonitrile (90:10, v/v) containing 0.1% trifluoroacetic acid at a flow-rate of 0.3 ml/min. The elution was monitored at 210 nm. For beverages, 1–3 μl and for the water-extracted samples 4–10 μl were injected.

2.4. Ion-exchange chromatography (IEC)

The samples prepared for HPLC analysis were mixed with equal volumes of 10% trichloroacetic acid for deproteinization. The supernatants were diluted with 0.2 *M* sodium citrate buffer (pH 3) and injected in a high-performance amino acid analyser Beckman Model 6300 equipped with a strong cation-exchanger column in a procedure previously described [8].

2.5. Electrospray ionization mass spectrometry (ESI–MS)

Mass spectra were obtained in the positive-ion mode on a triple-stage mass spectrometer Model API-III (Sciex, Toronto, Canada). Briefly, the samples were infused through a stainless-steel capillary (100 μm I.D.). A stream of air (pneumatic nebulization) was introduced to assist in the formation of submicrometer droplets [9]. These droplets were

evaporated at the interface by nitrogen gas producing highly charged ions which were detected by the analyzer. The system's calibration was performed with the ammonium adduct ions of polypropylene glycol (PPG) with known mass-to-charge ratios throughout the range of the instrument (0–2470 amu). Instrument tuning, data acquisition and processing are controlled by a MacIntosh II computer with all software (no modifications necessary) provided by the instrument's manufacturer. Simple algorithms correlate the charges produced by these compounds to their molecular masses [10].

3. Results and discussion

The results for aspartame content are in agreement with the manufacturers' claims for a variety of products including diet Coke, Pepsi, 7-Up, Equal, hot chocolate powder and pudding (Table 1). The standards analysed by the mini-column system and the Beckman high-performance analyser are shown in Fig. 1. Fig. 2 shows a commercial product extracted when freshly received and after storage for one year at 25°C. The peaks were collected in 0.4-ml Eppendorf tubes, and infused into ESI–MS for confirmation of species.

5-Benzyl-3,6-dioxo-2-piperazineacetic acid (cyclized aspartame) does not form Ruhemann's purple with ninhydrin and therefore is not detected by this method. Fig. 3 shows the mass spectra of peaks 3, 4 and 5. The observed molecular masses were in harmony with the calculated masses.

Tsang et al. [6] used a regular analytical 250-mm column to resolve some of the aspartame by-products in soft drinks. They did not attempt to analyze other aspartame-containing foods such as powdered drinks and dessert mixes but reported only on diet colas and lemon–lime soft drinks. A 250-mm length column costs approximately eight times that of the 30-mm mini-cartridge – the latter which can be used for over 200 injections without any loss in resolution or increase in pressure. Since the column is shorter, analysis times can be reduced to less than 10 min. Solvent consumption is also considerably less. However, it is advisable to flush the cartridge with 70:30 acetonitrile–water (two column volumes, approximately 2 min duration at 0.3 ml/min) after every five runs. This will eliminate buildup of proteins which are present in some of the samples. We examined a variety of products to ensure that interfering substances were not problematic. Both methods were able to analyze aspartame and its degradation products at similar wavelengths and similar aspartame concentrations.

The concentration of aspartame in most beverages is in the millimolar range. Some of the solid samples, however, had a 100-fold greater concentration. A 2-μl beverage injection gives approximately 0.8 μg (2.9 nmol aspartame). The detection limit (signal-to-noise ratio of 2 to 1) was estimated at 400 ng (145 picomoles). The method was tested for accuracy by spiking water (as a control) with varying amounts of potential components in the dynamic working range. Recovery was always in excess of 88%. One of the drawbacks of rapid chromatographic methods is the lack of selectivity. Potential sources of interferences from dyes, flavors and other adjunct sweeteners (e.g.,

Table 1
Aspartame concentrations in dietary foods products

Products	Reported by manufacturer	Aspartame concentration[a]		
		HPLC with mini-cartridge	Beckman System 63001	Biosensor[b]
Diet Minute Maid	0.060	0.054±0.005[c]	0.052±0.006[c]	0.055
Diet Pepsi	0.055	0.055±0.005	0.053±0.005	0.058
Hot chocolate	0.62	0.61±0.08	0.67±0.07	0.85
Equal	3.9	4.0±0.21	3.9±0.19	4.1
Jello (raspberry)	3.8	4.0±0.24	4.3±0.21	3.5
Vanilla pudding	1.5	1.7±0.19	1.6±0.16	1.5

[a] mg Aspartame/100 mg product.
[b] Mulchandani et al. [8].
[c] Triplicate determinations.

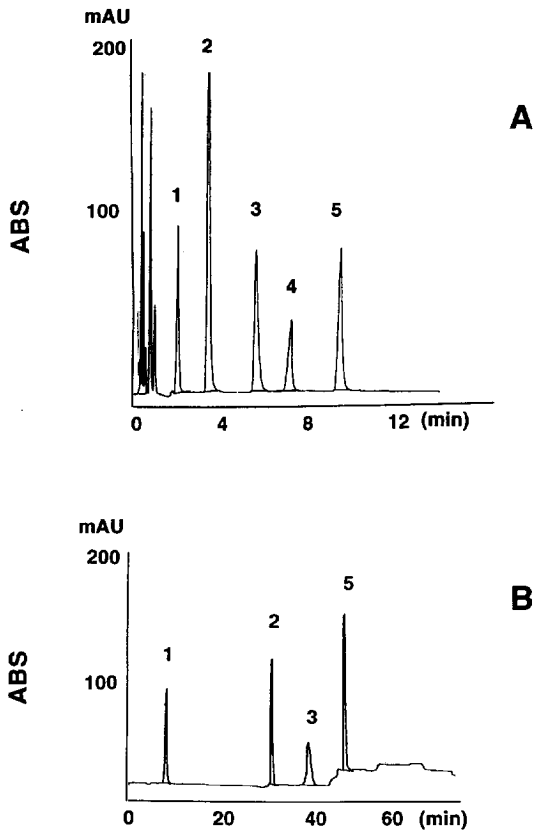

Fig. 1. Standard mixture of aspartame and its metabolites determined by (A) the HPLC mini-cartridge system and (B) the Beckman Model 6300 high-performance analyser. Peaks: 1= aspartic acid; 2=phenylalanine; 3=aspartylphenylalanine; 4=5-benzyl-3,6-dioxo-2-piperazineacetic acid; 5=aspartame. The concentrations were 0.1 m*M* for each component except for aspartic acid which was 1.0 m*M* in the reversed-phase column compared to 0.02 m*M* in the cation exchanger. Note the absence of cyclized aspartame in the Beckman chromatogram. This compound does not form a colour complex with ninhydrin reagent and hence it is undetectable.

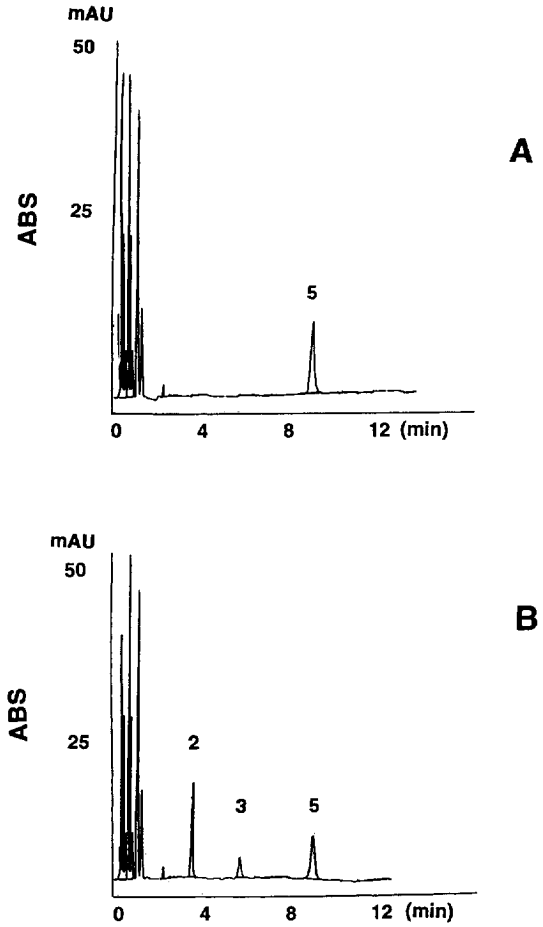

Fig. 2. Mini-cartridge system analysis of a diet sample (A) on receipt and (B) after storage at 25°C for one year.

saccharin) when used, should be taken into account. New products should be tested for interferences.

The method described in this paper can monitor the breakdown products (aspartic acid, phenylalanine, the free dipeptide, methyl ester and cyclized peptide) as commercial products deteriorate with time on the store shelves and warehouses. A major advantage of this method is that few steps are involved in the analysis. At most, a centrifugation step is required to remove particles prior to injection onto the mini-cartridge. Otherwise, direct injection of totally soluble materials is possible. pH Adjustment of samples is also not required.

Another method [11] used precolumn derivatization to enhance the sensitivity of the monitoring system for aspartic acid and phenylalanine. However, to obtain the fluorogenic derivatives, a reaction of naphthalene-2,3-dicarboxaldehyde (NDA) in the presence of the cyanide ion is required. The cyanide ion is in the form of sodium cyanide which is a deadly toxic chemical and must be handled with extreme care. Exposure to light must also be avoided. The ability to detect 5-benzyl-3,6-dioxo-2-piperazineacetic acid was never demonstrated since its presence was ignored. Our HPLC method, although not as sensitive in the subpicomole range, is

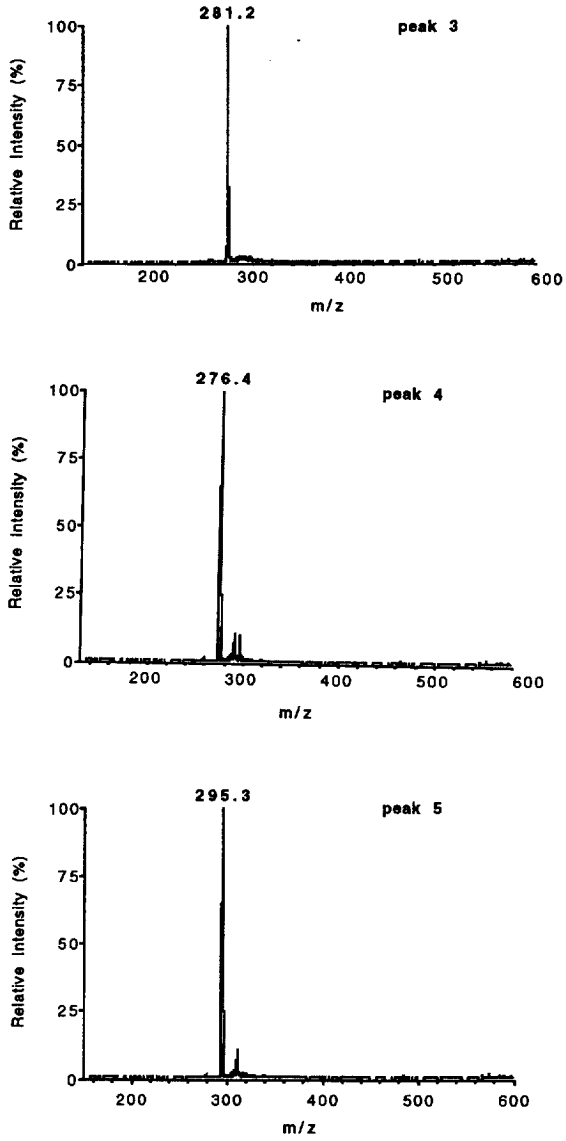

Fig. 3. ESI–MS of peaks 3, 4 and 5. The observed molecular masses (281.20, 276.30 and 295.30) agree with the calculated masses (281.20, 276.40 and 295.40), respectively.

much safer to use and is sufficiently sensitive for routine food analysis.

Most enzymatic assays developed for aspartame analysis have exhibited problems. *Bacillus subtilis* cells utilized with an oxygen electrode demonstrated glucose and amino acid interference [4]. When carboxypeptidase A and aspartase were used with an ammonia electrode [5], the presence of amines in the foods interfered with the analysis. Our method with the encased mini-column did not exhibit any of these problems in the wide variety of foods tested.

Although the method was developed on a two-pump HPLC system with a diode-array detector, it is straightforward and can be transformed to the simplest HPLC configuration using a single pump (with damper) and a fixed-wavelength detector with a recorder/integrator. Our method showed excellent repeatability and thus could be used for routine analysis in quality control of aspartame-containing products.

4. Conclusion

A simple and rapid HPLC method for quantitation of aspartame and its degradation products, aspartic acid, phenylalanine, 5-benzyl-3,6-dioxo-2-piperazineacetic acid and aspartylphenylalanine, has been described in this paper. This method can be used on a routine basis to monitor the deterioration of these commercial products particularly due to the influence of pH, moisture level and temperature on the stability of aspartame.

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