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Original article

Investigations on the degradation of aspartame using high-performance liquid chromatography/tandem mass spectrometry

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ABSTRACT

Aspartame is a widely used sweetener, the long-term safety of which has been controversial ever since it was accepted for human consumption. It is unstable and can produce some harmful degradation products under certain storage conditions. A high-performance liquid chromatography/tandem mass spectrometry method was developed for the simultaneous analysis of aspartame and its four degradation products, including aspartic acid, phenylalanine, aspartyl-phenylalanine and 5-benzyl-3,6-dioxo-2-piperazieacetic acid in water and in diet soft drinks. Aspartame and its four degradation products were quantified by a matrix matched external standard calibration curve with excellent correlation coefficients. The limits of detection were 0.16–5.8 μg/L, which exhibited higher sensitivity than common methods. This method was rapid, sensitive, specific and capable of eliminating matrix interferences. It was also applied to the study of the degradation of aspartame at various pH and temperatures. The results indicated that aspartame was partly degraded under strong acidic or basic conditions and the extent of degradation increased with increasing temperature.

1. Introduction

The artificial sweetener, aspartame (L-aspartyl-L-phenylalanine methyl ester, APM) has long been commonly used as a substitute of sugar in low-calorie meals, soft drinks and frozen desserts [1] due to a degree of sweetness 180 times higher than sucrose [2]. The Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives approved the use of APM in 1982, with a recommended acceptable daily intake of 0–40 mg/kg body weight [3]. Nevertheless, the issue of safety of APM has been controversial since it was discovered [4,5]. A number of studies claimed some negative health effects from APM ingestion. It was reported that APM can cause changes in behavior (such as depression and insomnia), alteration in vision, and mental retardation, especially in children [2]. Furthermore, APM was also suspected as carcinogenic [6]. Under this controversy about the safety of APM, the European Commission required the European Food Safety Authority (EFSA) to re-evaluate the security of APM in May 2011. In the assessment process, the expert group of EFSA found that the data of 5-benzyl-3,6-dioxo-2-piperazieacetic acid (DKP), which was the degradation product of APM, was lacking and needed further relevant research.

While APM is harmless itself, it has poor stability at various pH and temperatures, and thus has different degrees of degradation, which can produce aspartic acid (ASP), phenylalanine (PHE), aspartyl-phenylalanine (ASP-PHE) and DKP [7]. These substances, especially DKP, may be harmful to the metabolic processes in the human body [4,8]. What is worse, the diet soft drinks which contain APM at different pH, depending on the matrix, may also be stored under different temperatures. Therefore, it is important to establish a sensitive, rapid and accurate method for the simultaneously detection of APM and its four degradation products. Furthermore, exploring the degradation of APM at various pH and temperatures is of great significance. A variety of analytical methods, including liquid chromatography [1,8] and capillary zone electrophoresis [9], have been reported for the quantitative analysis of APM, its degradation products and the other sweeteners. However, the sensitivities of these methods are limited and serious matrix interferences can hardly be avoided. In addition, it is difficult to chromatographically separate the peak response of APM and its degradation products, as they have similar structures [10].

It has been reported that single quadrupole mass spectrometry (MS) [7,11] was used to quantify APM, its degradation products and the other sweeteners. Compared to MS, tandem mass

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spectrometry (MS/MS) possesses further sensitivity and stability [12]. What is more, with the improvement of chromatographic techniques, high-performance liquid chromatography (HPLC) has been developed to shorten the analysis time and increase the resolution, capacity and sensitivity, especially when it is coupled with MS/MS [13–15]. Recently, many reports were focused on the application of HPLC–MS/MS in complex samples analysis [16,17]. However, to the best of our knowledge, to date no systematic study on the degradation of APM by HPLC–MS/MS has been published.

The aim of this paper is to develop a sensitive, rapid and accurate method using HPLC–MS/MS to simultaneously analyze APM and its four degradation products ASP, PHE, ASP-PHE and DKP. This method is also applied to the study of the degradation of APM at various pH and temperatures, which includes quantifying the main degradation products and exploring degraded pathways. The identification characteristics in the mass spectrum of the five analytes are also included.

2. Experimental

2.1. Materials

The organic reagents were of HPLC-grade and all other chemicals were of analytical reagent-grade. APM, ASP and ASP-PHE were purchased from J&K Chemical (Beijing, China). PHE and DKP were obtained from Sigma–Aldrich Chemistry (St. Louis, USA). Acetonitrile was purchased from Fisher Scientific (Hampton, USA). Formic acid was obtained from Fluka Analytical (Buchs, Switzerland). Ammonia solution was purchased from Xilong Chemical (Guangdong, China). Plastic bottled cola was obtained from a local supermarket.

2.2. Instrumentations

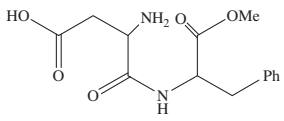
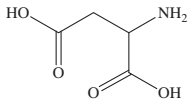
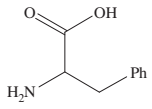
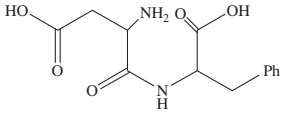
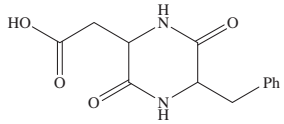
The HPLC–MS/MS instrumentations used in this paper were all obtained from Shimadzu (Kyoto, Japan). The HPLC was performed on a LC-20A system. The separation was carried out on the column VP-ODS (150 mm × 2 mm, 4.6 μm). Flow rate of the mobile phase was controlled by a LC-20AD Pump and CBM-20A Controller. The gradient solvent system consisted of solvent A (0.1% formic acid in water, v/v) and solvent B (acetonitrile). The content of solvent B in the mobile phase for the separation of the tested mixture increased from 10% to 45% in 5 min and was maintained at 45% for 5–7 min. The total time for one run was 7 min. The injection volume was 5 μL. The flow rate was set at 0.2 mL/min and the oven temperature was set at 30 °C.

The HPLC was coupled to a Shimadzu 8030 triple quadrupole mass spectrometer with an electrospray ionization interface. Nitrogen was supplied as the nebulizing gas and drying gas at the flow rates of 2.5 L/min and 15 L/min, respectively. The heat block temperature was set at 400 °C. For MS/MS measurements, the mass spectrometer was operated in the multiple reaction monitoring (MRM) mode. Data acquisition was controlled using Shimadzu LCMS Lab Solution software.

2.3. Standards

Aqueous standard solutions of APM, ASP, PHE, ASP-PHE and DKP were separately prepared by dissolving the standard materials in distilled water to give a final concentration of 1000 mg/L and then were stored at 4 °C in a refrigerator. The solutions used in the parameter optimization experiments were separately prepared

Table 1
Tandem mass spectrometry parameters of the five analytes.

Analyte	Structure	Retention time (min)	Molecular weight (g/mol)	Quantitative transition (*m*/*z*)	Qualitative transition (*m*/*z*)	Quadrupole 1 pre-bias (V)	Collision energy (V)	Quadrupole 3 pre-bias (V)
APM		6.2	294.15	295.15/120.20	295.15/120.20 295.15/180.30	−15 −15	−30 −15	−22 −11
ASP		2.1	133.10	134.10/74.00	134.10/74.00 134.10/87.90	−16 −16	−15 −15	−13 −17
PHE		3.3	165.10	166.10/120.05	166.10/103.10 166.10/120.05	−12 −12	−30 −15	−19 −24
ASP-PHE		5.1	280.05	281.05/166.25	281.05/166.25 281.05/235.25	−15 −15	−15 −15	−10 −15
DKP		6.3	262.10	263.10/91.10	263.10/91.10 263.10/245.15	−19 −19	−35 −15	−16 −15