Regular article

日本食品化学学会誌、Vol. 22(3), 170-174(2015)
Japanese Journal of Food Chemistry and Safety (JJFCS)

Determination method of 2-(5-benzyl-3,6-dioxopiperazin-2-yl)acetic acid in aspartame using high performance liquid chromatography

(Received May 27, 2015)
(Accepted July 31, 2015)

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Abstract

2-(5-Benzyl-3,6-dioxopiperazin-2-yl)acetic acid (DKP) is one of the degradation products of aspartame (APM). The Joint FAO/WHO Expert Committee on Food Additives (JECFA), USA, EU, and Japan has set 1.5 wt% as the maximum level of DKP in APM. In this study, we developed and validated a high-performance liquid chromatography (HPLC) method to determine the content of DKP in APM, based on the approach recommended in the Food Chemical Codex (FCC) from USA. For the separation of analyte, the column was investigated with 5 and 10 μm particle size. In the recovery test, the developed method gave satisfactory recoveries (99.1% and 99.5%) and repeatabilities (0.4%) from APM spiked with two concentrations (viz. 0.15 and 1.5 wt%). The limits of detection and quantification for DKP are estimated to be 0.0005 and 0.002 wt%, respectively. In the analyses of ten commercial APM samples, the DKP contents determined using the developed method are all in good agreement with those obtained using the conventional FCC method. This developed method is therefore applicable for the determination of DKP contents in commercial-grade APM.

Keywords : DKP, aspartame, HPLC

I Introduction

Aspartame (*N*-L-α-aspartyl-L-phenylalanine-1-methyl ester, APM, Fig. 1) is a high-intensity and non-caloric artificial sweetener approximately 200 times sweeter than sucrose,[1] and is used in more than 100 countries as a food additive.[2]

Aspartame is well-known to decompose under heat, moisture, and pH stress into its constituent amino acids and 2-(5-benzyl-3,6-dioxopiperazin-2-yl)acetic acid (DKP, Fig. 1).[3,4] Therefore, the maximum level of DKP in APM has been limited to 1.5 wt% by the Joint FAO/WHO Expert Committee on Food Additives (JECFA)[5], and similarly in the USA (Food Chemical Codex, FCC)[6], EU[7], and in Japan[8]. The JECFA specifications are particularly important because they are adopted in many countries to ensure the safety of food additives.

As specified by JECFA in 1981, the DKP content in APM should be measured via the silylated derivatives of the former using gas chromatography (GC) with a packed column. This method is also adapted to the APM specification of the eighth edition of the Japan's specifications and standards for food additives[8]. However, this approach is now more than thirty years old, and interference in the GC chromatograms from other substances make the precise quantification of DKP in APM difficult (Fig. 2). In addition, the silylation of DKP is complicated and time-consuming. In contrast, the FCC recommends the high performance liquid chromatography (HPLC) method using octadecyl silica (ODS) column with 10 μm of particle size[6]. Recently, the columns with smaller particles size, particularly 5 μm, are used to identify and quantitate the main components and impurities in food additives for improvement of the resolution and/or reduction of the mobile phase amount.

In this study, we developed and validated a HPLC method

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