

Development of HILIC-LC/MS method for direct quantitation of 2-acetyl-4-tetrahydroxybutylimidazole in caramel III with the qNMR certified standard

(Received April 1, 2015)

(Accepted June 2, 2015)

Hiromi Kawasaki ^{a)}, Takumi Akiyama ^{a)}, Atsuko Tada ^{a)}, Wakana Sekiguchi ^{a)},
Yuzo Nishizaki ^{a)}, Yusai Ito ^{b)}, Naoki Sugimoto ^{a)}, Hiroshi Akiyama ^{a)}

a) National Institute of Health Sciences

b) Kyoritsu Women's University

Abstract

A method LC/MS with HILIC column (HILIC-LC/MS) was developed for direct quantitation of 2-acetyl-4-tetrahydroxybutylimidazole (THI), an undesired polar byproduct in caramel III colorants. To verify the reliability of the proposed analytical method for the quantification of THI in caramel III commercial products, we determined the absolute purity of a THI analytical standard using quantitative NMR (qNMR) and then performed absolute calibration and standard addition procedures using the analytical standard. The correlation coefficients were >0.99 and >0.97 for the absolute calibration and standard addition procedures, indicating satisfactory linearity of the respective calibration curves. The procedures also returned identical quantitation values in a sample. The THI content in six samples of caramel III commercial products in Japan was determined using the HILIC-LC/MS method. The THI content in each of these samples was lower than officially stipulated limits. The current JECFA standard method for determination of THI in caramel III by HPLC/UV using a 10- μ m particle size C8 column with derivatization of THI-2,4-dinitrophenylhydrazone gave lower THI values than the proposed HILIC-LC/MS method due to sub-optimal peak separation by the column recommended in the JECFA standard method. Our data suggest that the analytical conditions of the current JECFA standard method should be improved.

Keywords : HILIC, LC/MS, qNMR, caramel, 2-acetyl-4-tetrahydroxybutylimidazole

I Introduction

Caramel III (caramel class III), ammonia caramel, is a widely used coloring additive in foods and beverages. Caramel III may contain small amounts of the undesired byproduct 2-acetyl-4-tetrahydroxybutylimidazole (THI). Because THI is reportedly immunotoxic,¹⁻³⁾ limits of THI content in caramel III and analytical methods for its determination have been specified by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), the European Union (EU), the Food Chemicals Codex (FCC), and in Japanese regulations for food additives.⁴⁻⁷⁾ All current THI standard analytical methods are based on the method established by Kröplien et al.⁸⁾

The standard analytical method recommended by the JECFA involves purification of THI from caramel III via

cation exchange chromatography using two different resins, derivatization of THI with 2,4-dinitrophenylhydrazine (DNPH) to its hydrazone (THI-DNPH), and finally detection of THI-DNPH using HPLC/UV (Fig. 1).⁶⁾ Determination of THI in caramel III through DNPH derivatization has the significant advantage of requiring only classical HPLC equipped with a UV detector. However, the JECFA standard method defines for the use of a 10- μ m particle size C8 HPLC column, which is currently not commercially available and generally provides poorer separation capacity than 5- μ m particle size C8 columns. This leads to unsatisfactory separation of THI-DNPH from other interfering substances and a consequent reduction in the reliability of quantitation values. In a previous study, we therefore attempted to establish a more reliable method for analyzing THI-DNPH using a