Revised method for analyzing 2-acetyl-4-tetrahydroxybutylimidazole in caramel III

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Abstract

Caramel III, a food-coloring additive, is tested in Japan for the presence of the impurity, 2-acetyl-4-tetrahydroxybutylimidazole (THI), using an official HPLC method. In this HPLC method, THI is derivatized with 2,4-dinitrophenylhydrazine and then separated using octyl column. Improvement of the analytical conditions was attempted because contaminants can often compromise this test. Isolation of the analyte was improved when 0.1 mol/L phosphoric acid/methanol mixed solution (70:30) was used as the mobile phase. The revised method gave higher analyte concentrations compared to the standard method. The quantitative values obtained by LC/MS were equivalent to those obtained using the revised method, demonstrating the superiority of the revised method to the standard method.

I Introduction

Caramel III, a food-coloring additive, is tested in Japan for the presence of the impurity, 2-acetyl-4-tetrahydroxybutylimidazole (THI), using an official HPLC method. This method requires the derivatization of THI with 2,4-dinitrophenylhydrazine (DNPH, Fig. 1)[1-3]. THI is reported to have immunotoxicity, such as a lymphopenic effect in rats.[4, 5] The standard method is based on the method established by Kröplien et al.[6] Similar methods are defined under standards of JECFA, EU, and FCC[7, 8].

The method comprises purifying THI from caramel III on a column containing two kinds of cation exchange resins, reacting THI with DNPH to derivatize it to hydrazone (THI-DNPH, Fig. 1), and then using an octyl column to isolate and quantify THI-DNPH by HPLC using 0.1 mol/L phosphoric acid/methanol (50:50, v/v) as the mobile phase. However, using the official method, the separation of THI from contaminants is often poor, and the reliability of the quantitative values has been proven problematic. Also, although the JECFA standard recommends an HPLC octyl column with 10 μm particle size,
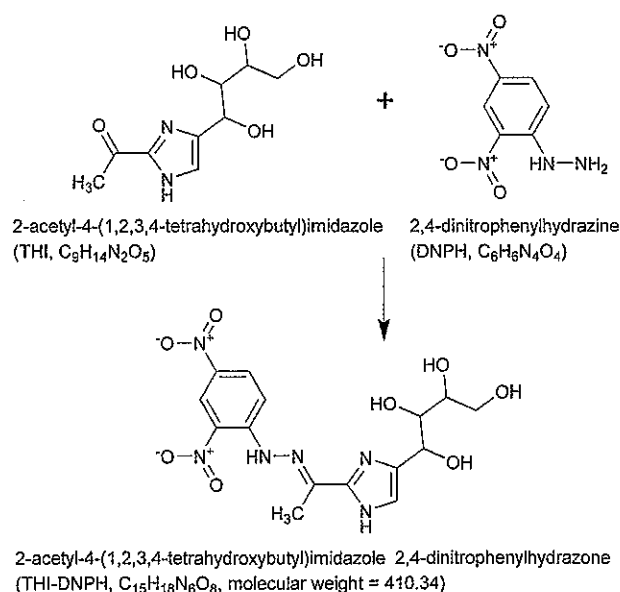

2-acetyl-4-(1,2,3,4-tetrahydroxybutyl)imidazole (THI, $C_9H_{14}N_2O_5$)

2,4-dinitrophenylhydrazine (DNPH, $C_6H_6N_4O_4$)

2-acetyl-4-(1,2,3,4-tetrahydroxybutyl)imidazole 2,4-dinitrophenylhydrazone (THI-DNPH, $C_{15}H_{18}N_6O_8$, molecular weight = 410.34)

Fig. 1.   Structures of 2-acetyl-4-(1,2,3,4-tetrahydroxybutyl) imidazole (THI) and its derivative, 2-acetyl-4-(1,2,3,4-tetrahydroxybutyl)imidazole 2,4-dinitrophenylhydrazone (THI-DNPH).

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