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)

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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 quantitation 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-μm 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).9 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-μm particle size C8 HPLC column, which is currently not commercially available and generally provides poorer separation capacity than 5-μm 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...
commercially available 5-µm particle size C8 column.⁹ We investigated the effects of the mobile phase and reported that separation of the THI-DNPH peak is improved when 0.1 mol/L phosphoric acid/methanol (70:30) is used as the mobile phase. Compared with the JECFA standard method, the revised method indicated higher levels of THI in a caramel III commercial product. In order to verify the accuracy of the quantitative values obtained using the revised method, THI-DNPH in a product sample was also analyzed by LC/MS. The value obtained in the LC/MS analysis was equivalent to that obtained using the revised method. These results demonstrated the superiority of the revised method compared with the JECFA standard method. However, obtaining a true value for the THI content of a sample remains questionable due to several factors. First, no absolutely pure THI analytical standard is currently available on the reagent market. In addition, methods involving DNPH derivatization are based on the assumption that the THI-DNPH reaction yield from the THI analytical standard is identical to that from trace levels of THI as a byproduct in caramel III commercial products.

Several analytical methods for the determination of THI and other imidazole derivatives in foods or caramel III products have recently been published, including methods for the rapid and high-sensitivity analysis of THI and the imidazole derivative 2-methylimidazole (2-MI) using LC/MS¹⁰, simultaneous analysis of THI, 2-MI, 4-methylimidazole (4-MI), and 5-hydroxymethylfurfural (HMF) in caramel colors and beverages using LC/MS/MS¹¹,¹², solid-phase extraction (SPE)-LC/MS¹³, and SPE-UHPLC/MS/MS.¹⁴ Hydrophilic interaction chromatography (HILIC) effectively separates and retains polar analytes, even though reverse-phase chromatography with C18 column (C18RPC) is generally inappropriate for the analysis of polar analytes due to the basic principles of the separation.¹⁵ The feature of HILIC has been incorporated into a method for the simultaneous analysis of THI and 4-MI in coffee using supercritical fluid extraction (SFE)-LC/MS.¹⁶

A quantitative nuclear magnetic resonance (quantitative NMR: qNMR) technique has also been developed and is considered to be a primary ratio method.¹⁷,¹⁸ In qNMR, the purity or content of an analyte can be determined based on the ratio of the integral values of the specific signal of the analyte to that of an internal standard (IS), because the integral values of the analyte and IS are directly proportional to the number of protons per resonance line multiplied by the molar concentration of the analyte and the IS. Therefore, when a certified reference material (CRM) is used as the IS for qNMR, the quantitative value and/or absolute purity of the analyte can be determined with metrological traceability to the International System of Units (SI).

Under properly optimized analytical conditions, LC/MS or LC-MS/MS presents the potential for direct quantification of THI in caramel III without the need for sample pretreatment. The absolute purity of a THI analytical standard can be determined using qNMR. In this study, these advanced analytical techniques were combined to accurately determine the content of THI in caramel III commercial products. The objectives of the study were to develop a reliable analytical method for THI in caramel III commercial products, to obtain more reliable SI-traceable analytical data, and to propose an alternative to the JECFA standard method. The THI content in six samples of caramel III commercial products was directly determined using our proposed HILIC-LC/MS method and a THI analytical standard, the absolute purity of which was determined beforehand by qNMR. In addition, we compared the THI content in a sample of caramel III determined using four
II Materials and Methods

1. Chemicals and Samples

THI was used as an analytical standard in this study and was obtained from the Japan Caramel Industrial Association (JCIA). Six samples of caramel III (ammonia caramel) commercial products (samples 1, 3, and 5 [powder type] and samples 2, 4, and 6 [liquid type; dry solid content of 74.5, 60.4, and 61.9%, respectively]) were obtained from the JCIA and were used as test samples. HPLC-grade acetonitrile and methanol were purchased from Kanto Chemical Co., Inc. (Tokyo, Japan), and 1 mol/L ammonium formate aqueous solution, trifluoroacetic acid (TFA), and 3-(trimethylsilyl)-1-propanesulfonic acid-d6 sodium salt (DSS-d6) reference material (certified purity 92.3±0.8% [w/w]) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Deuterium oxide (D2O) was obtained from ISOTEC (Miamisburg, OH, USA). Potassium hydrogen phthalate (PHP) (NMI CRM 3001-a, certified purity 100±0.027% [w/w]), which served as a CRM, was obtained from the National Metrology Institute of Japan–National Institute of Advanced Industrial Science and Technology (NMIJ-AIST). Milli-Q water (Millipore, Bedford, MA, USA) was used for analyses.

2. Instruments

qNMR spectra were recorded using a JNM ECA 600 MHz spectrometer (JEOL Ltd., Tokyo, Japan). An ACQUITY UPLC/ SQD system (Waters, Milford, MA, USA) was used for LC/MS analyses. An XPS2 ultramicrobalance (Mettler Toledo International Inc., Greifensee, Switzerland) and an Multipette Xstream electric auto-pipettor (Eppendorf AG, Hamburg, Germany) were used for accurate measurement of weight and volume.

3. Determination of THI absolute purity using qNMR

The qNMR reference solution was prepared by dissolving 5.0 mg of DSS-d6 in 0.5% TFA D2O. Calibration of the DSS-d6 concentration in the qNMR reference solution was carried out as follows. First, precisely 3.0 mg of PHP was dissolved in 1.0 mL of qNMR reference solution, and 0.6 mL of the resulting solution was transferred to an NMR test tube (φ 5 mm × 8 in.: Wako Pure Chemical Industries, Ltd.). The concentration of DSS-d6 in the qNMR reference solution was calculated using the ratio of the signal integral at δH 0 ppm (nine protons of DSS-d6) to that at δH 7.69 and 7.82 ppm (four protons of PHP). The DSS-d6 concentration was calculated as 0.09166 mg/mL according to the following equation (equation 1):

\[ C_{\text{DSS}} \text{ (mg/mL)} = \frac{(M_{\text{DSS}} - M_{\text{PHP}})W_{\text{PHP}}P_{\text{PHP}}}{(M_{\text{PHP}}P_{\text{PHP}})(V_{\text{DSS}} \times 100)} \]  

In equations 1 and 2 (presented below), C represents the concentration, M represents the molecular weight, I represents the signal area, H represents the number of protons associated with the signal, W represents the weight, V represents the volume of the solution, and P represents purity. Subscripts denote the particular substances analyzed.

Next, 1.5 mg of THI was accurately weighed and dissolved in 1.0 mL of qNMR reference solution. The solution was then subjected to qNMR analysis, and the purity of THI was calculated using the ratio of the signal at δH 0 ppm (nine protons of DSS-d6) to that at δH 7.65 ppm (one proton of THI). The purity of THI was calculated as 85.1% (w/w) according to the following equation:

\[ P_{\text{THI}} \%\text{w/w} = \frac{(M_{\text{THI}} - M_{\text{THI}} - M_{\text{DSS}} - M_{\text{DSS}})(V_{\text{DSS}} \times 100)}{(M_{\text{DSS}} + M_{\text{DSS}})H_{\text{THI}}(W_{\text{THI}})} \]  

qNMR analyses were conducted using optimized quantitative acquisition parameters: 5-mm broadband auto-tune probe; probe temperature around 23°C, spectral width of −5 to 15 ppm; auto-filter, 8 times; spectrum data points, 64 K; spectral resolution, 0.25 Hz; number of scans, 16; no spinning; pulse angle, 90°; relaxation delay, 60 s; multi-pulse decoupling with phase and frequency switching (MFP-8) 13C decoupling. The data were processed using Alice 2 for qNMR software (JEOL). The signal integrals were used for quantitation. The chemical shifts were referenced to DSS-d6 at 0 ppm.

4. Preparation of standard and test solutions

The THI stock solution was prepared by placing 1 mg of THI into a 10-mL measuring flask and adding water to a final concentration of 100 μg/mL. The stock solution was then diluted to 0.02, 0.05, 0.10, 0.20, and 0.40 μg/mL with 0.01 mol/L ammonium formate/acetonitrile (5:95, v/v), and the standard solutions were used to determine the THI content in caramel III commercial products by absolute calibration and standard addition procedures.

The procedure for preparing the test solutions differed slightly for powder-type and liquid-type caramel III products. For powder-type samples, 100 mg of sample was accurately measured and placed into a 25-mL measuring flask and dissolved with methanol/water (90:10, v/v). The solution was then sonicated for 10 min and allowed to stand at room temperature to cool, after which the supernatant was filtered through a 0.45-μm Millex®-LH membrane filter (Merck KGaA, Darmstadt, Germany). The resulting filtrate served as the test solution. For liquid-type samples, 180 mg of sample
was accurately measured and transferred into a beaker. A small amount of methanol/water (90:10, v/v) was added to the flask and the sample was sonicated for about 2 min, after which the suspended solution was poured into a 25-mL measuring flask. This process was carried out three times until the entire sample was transferred to the measuring flask. Subsequent steps were identical to those for the preparation of powder-type products.

For absolute calibration procedure, a portion of each test solution was subjected to LC/MS analysis, and the THI content was determined based on an absolute calibration curve. In the standard addition procedure, 1.0 mL of the standard solution differed from the concentration of THI and the test solution were mixed, filtered through a 0.20-μm Millex-LG membrane filter (Merck KGaA), and subjected to LC/MS.

5. HiLIC-LC/MS analysis of the THI content in caramel III commercial products

Each test solution was subjected to LC/MS analysis using an Atlantis HiLIC silica column (2.1 mm i.d. × 150 mm; 5-μm particle size; Waters) under the following conditions:

LC: column temperature 40°C; mobile phase A = 0.01 mol/L ammonium formate, mobile phase B = acetonitrile; A/B = 5/95 (0-3 min) → 10/90 (15 min); flow rate, 0.2 mL/min; MS: capillary voltage, 3.0 kV; cone voltage, 30 V; source temperature, 110°C; desolvation temperature, 350°C; desolvation gas flow rate, 800 L/h; cone gas flow rate, 30 L/h; detection, ESI, selected ion recording (SIR) mode, THI for \(m/z\) 231.2 [M+H]+ and \(m/z\) 229.2 [M-H]-. The content of THI in caramel III commercial products was determined using the absolute calibration and standard addition procedures. The resulting experimentally determined values were finally corrected based on the purity of the THI analytical standard.

III Results and Discussion

1. Purity of the THI analytical standard

In order to accurately determine the THI content using the HPLC method, it would be necessary to use a THI CRM, which carries an assurance of absolute purity. However, no THI CRM is currently available on the reagent market. We were, however, able to obtain an analytical standard from a manufacturer of caramel colors. Because the absolute purity of the THI analytical standard provided by the JCIA for this study had not been determined, we determined its absolute purity using qNMR prior to the analysis of the THI content in caramel III commercial products.

We previously reported the development of a qNMR technique designated AQARI (accurate quantitative NMR with internal reference substance), which uses an SI-traceable reference material.\(^{19-21}\) qNMR is recognized as a promising analytical technique for determining the absolute purity or content of an organic substance because it does not require an identical standard material. The technique is utilized in a variety of fields, such as pharmaceutical sciences, environmental chemistry, and food chemistry.\(^{19, 20, 22, 23}\)

Using a previously reported procedure,\(^{21}\) we calibrated the concentration of DSS-d6 in the qNMR reference solution using PHP, which was one of the primary standard materials. The concentration of DSS-d6, which served as a secondary standard material, was determined as 0.09166 mg/mL in 0.5% TFA D2O. After a precise amount of THI analytical standard was dissolved in the qNMR reference solution, the absolute purity was calculated from the ratio of the signal area of DSS-d6 at δH = 0 ppm to that of the H5 position of THI at δH = 7.65 ppm. The proton signal of the H5 position on the imidazole ring of THI was suitable for THI quantification by qNMR, as it provided a single signal with no impurity signals in the integrated area. The absolute purity of THI was thus determined as 85.1% (w/w) (average of duplicate measurements, SD 0.05% [w/w]) and was indirectly SI-traceable through the PHP primary reference material.

No significant chromatographic peaks derived from impurities were observed in HPLC/UV analysis of the THI analytical standard, suggesting that the THI analytical standard was in the salt or hydrate form. This result showed that the absolute purity of the THI analytical standard used for accurate quantification by chromatography should be determined and that the bias reflected in the quantitative values should be corrected for based on the purity.

2. Direct analysis of THI content in caramel III using HiLIC-LC/MS

We evaluated both HPLC/UV and LC/MS in development of a method for direct determination of the THI content in caramel III commercial products. In evaluations of several HPLC column types with respect to retention and separation of THI, poor results were obtained with C18 reverse-phase columns. In contrast, HiLIC columns provided appropriate separation for the analysis of THI in caramel III products. Among several HiLIC column tested, the Atlantis HiLIC silica column provided the best separation and peak shape.

We further optimized the LC/MS conditions, such as the ratio of mobile phase solvents for peak separation and mass detector parameters for THI ionization. Under the optimized HiLIC-LC/MS conditions, THI produced a protonated molecule ([M+H]+ \(m/z\) 231.2) and a deprotonated molecule ([M-H]- \(m/z\) 229.2). A typical SIR chromatogram of a caramel III sample is shown in Fig. 2. The THI peak shows a retention time of 6.8 min. Negative-mode SIR detection was selected for quantification of THI because the baseline was flatter in
negative mode than in positive mode.

To validate the optimized HILIC-LC/MS method, we evaluated various experimental parameters, such as linearity, limit of detection (LOD), and limit of quantification (LOQ). The calibration curves were linear over the concentration range 0 to 0.4 µg/mL for the THI standard solutions, and the correlation coefficient ($r^2$) was >0.99 for the absolute calibration procedure and >0.97 for the standard addition procedure. The LOD was determined based on the lowest concentration with a signal to noise (S/N) ratio of >10, and the LOQ was defined as 2 times the LOD. The LOD and LOQ values were 0.29 µg/g and 0.57 µg/g for powder-type samples and 0.16 µg/g and 0.32 µg/g for liquid-type samples, respectively.

The THI content in six samples of caramel III commercial products was determined using the absolute calibration and standard addition procedures with the HILIC-LC/MS method and THI analytical standard, the absolute purity of which had been previously determined by qNMR. Test solutions were diluted and filtered without tedious pretreatment (e.g., SPE and DNPH derivatization). The THI content in each of the powder- and liquid-type samples is shown in Table 1. The THI content in the powder-type samples ranged from 20.2 to 29.4 µg/g as determined using the standard addition procedure and from 18.4 to 27.4 µg/g as determined using the absolute calibration procedure. The THI content of the liquid-type samples ranged from 6.3 to 21.6 µg/g as determined using the standard addition procedure and 3.6 to 21.0 µg/g as determined using the absolute calibration procedure. Analyses carried out using the absolute calibration procedure indicated a slightly lower THI content than those carried out using the standard addition procedure, suggesting that a matrix effect influences analyses using the absolute calibration procedure. All quantitation values were lower than the JECFA and Japanese specified limit for THI in caramel III, 40 µg/g.

![Fig. 2. HILIC-LC/MS chromatograms of the THI standard and test solutions.](image)

(A) THI standard (0.1 µg/mL) (B) Sample 4 (liquid type) (C) Sample 5 (powder type)

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Type</th>
<th>Content of THI (µg/g) a)</th>
<th>Negative mode [M-H]</th>
<th>Absolute calibration</th>
<th>Standard addition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>Powder</td>
<td>21.1 (0.9)</td>
<td>24.9 (1.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample 2</td>
<td>Liquid</td>
<td>3.6 (0.1)</td>
<td>6.3 (1.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample 3</td>
<td>Powder</td>
<td>18.4 (1.8)</td>
<td>20.2 (3.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample 4</td>
<td>Liquid</td>
<td>14.4 (0.3)</td>
<td>17.6 (1.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample 5</td>
<td>Powder</td>
<td>27.4 (2.2)</td>
<td>29.4 (3.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample 6</td>
<td>Liquid</td>
<td>21.0 (0.7)</td>
<td>21.6 (1.1)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Contents of THI in caramel III commercial products determined by HILIC-LC/MS

3. Comparison of THI content as determined by different methods

We previously reported a revised HPLC/UV method employing DNPH derivatization for the quantification of THI in caramel III commercial products and demonstrated the superiority of the revised method to the JECFA standard method. To evaluate the reliability of the method proposed in the present report, the THI content in sample 5 was quantified using the JECFA standard method, the previously reported revised method, the C18-LC/MS method, and the current HILIC-LC/MS method, and the results were compared. The experimental conditions are shown in Table 2.

As shown in Table 2, the JECFA standard method recommends the use of a classical 10-µm particle size C8 column (LiChrosorb RP-8, 4.6×250 mm, 10 µm) for analysis of the THI-DNPH derivative. The peak separation provided by the revised method using a modern 5-µm particle size C8 column was improved relative to that obtained using the
Table 2. Comparison of THI contents in sample-5 quantified by official, revised, C18-LC/MS and HILIC-LC/MS methods

<table>
<thead>
<tr>
<th>Method</th>
<th>Measurement target</th>
<th>Detection</th>
<th>Column</th>
<th>Quantitation procedure</th>
<th>THI content (μg/g) ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Official DNP derivative</td>
<td>DNP-HPLC/UV</td>
<td>UV</td>
<td>LiChrosorb RP-8</td>
<td>absolute calibration</td>
<td>24.9 ± 1.6</td>
</tr>
<tr>
<td>C8-HPLC/UV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Revised DNP derivative</td>
<td>DNP-HPLC/UV</td>
<td>UV</td>
<td>TSKgel Octyl-80T</td>
<td>absolute calibration</td>
<td>29.4 ± 2.3</td>
</tr>
<tr>
<td>C8-HPLC/UV</td>
<td>DNP-HPLC/UV</td>
<td>UV</td>
<td>Wakosil-II SC8 HG</td>
<td>absolute calibration</td>
<td>30.0 ± 1.7</td>
</tr>
<tr>
<td>C18-LC/MS</td>
<td>DNP-HPLC/UV</td>
<td>MS</td>
<td>L-column ODS</td>
<td>absolute calibration</td>
<td>30.1 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>DNP-HPLC/UV</td>
<td>MS</td>
<td>Inertsil ODS-4</td>
<td>absolute calibration</td>
<td>23.1 ± 1.6</td>
</tr>
<tr>
<td></td>
<td>DNP-HPLC/UV</td>
<td>MS</td>
<td>TSKgel ODS-100V</td>
<td>absolute calibration</td>
<td>31.9 ± 1.5</td>
</tr>
<tr>
<td>HILIC-LC/MS</td>
<td>THI</td>
<td>MS</td>
<td>Atlantis HILIC Silica</td>
<td>absolute calibration</td>
<td>27.4 ± 2.2</td>
</tr>
<tr>
<td></td>
<td>THI</td>
<td>MS</td>
<td>Atlantis HILIC Silica, 5 μm</td>
<td>standard addition</td>
<td>29.4 ± 3.0</td>
</tr>
</tbody>
</table>

Data are presented as mean ± standard deviation (n=3). The values were corrected by the absolute purity of THI analytical standard.

JECFA standard method, resulting in higher quantitation with the revised method. The THI values determined using the revised method were similar to those obtained using the C18-LC/MS method (Table 2). These results were similar to those for direct analysis of THI in caramel III commercial products obtained using the HILIC-LC/MS method, indicating a THI content of around 30 μg/g, higher than the 24.9 μg/g value obtained using the JECFA standard method. The HILIC-LC/MS method allows for direct determination of THI content without the need for SPE or derivatization pretreatment. In addition, the absolute purity of the THI analytical standard was indirectly determined by qNMR with SI traceability through the CRM PHP. Therefore, we conclude that THI content data obtained using the HILIC-LC/MS method are more reliable than data obtained using the other methods examined in this study. Our data suggest that the previously reported revised method and the HILIC-LC/MS method described here provide more accurate data regarding the THI content of caramel III commercial products. Our findings also suggest that the JECFA standard method for analysis of THI in caramel III should be improved.

IV Conclusion

The JECFA standard method for determination of the THI content in caramel III commercial products does not require the use of expensive equipment, such as LC/MS instruments. However, the JECFA standard appears to provide less credible data, because the HPLC column recommended does not provide adequate separation of the THI peak from those of other constituents in caramel III. We previously reported a revised method using a modern C8 column that provides better separation for the determination of THI-DNPH. The improved peak separation afforded by the revised method yielded higher THI content values than the JECFA standard method. However, the complex pretreatment and derivatization steps required in the revised method raised concerns regarding the accuracy of results. In the present study, we therefore developed a simple analytical method using HILIC-LC/MS for the direct determination of THI. The proposed HILIC-LC/MS method can be used to directly determine the THI content in caramel III products through the use of a THI analytical standard, the absolute purity of which is determined beforehand with SI traceability by qNMR. Six samples of caramel III commercial products in Japan were analyzed in this study using the proposed HILIC-LC/MS method. The THI content of each of the samples was lower than the officially stipulated limit. Similar values for THI content (around 30 μg/g) were observed when comparing samples analyzed using the HILIC-LC/MS method, the previously reported revised method, and the C18-LC/MS method. This value was higher than the 24.9 μg/g determined using the JECFA method. We therefore conclude that the JECFA standard method should be improved in terms of the recommended analytical conditions. The proposed HILIC-LC/MS method and the previous revised method represent alternatives to the JECFA standard method for determining THI content.

V Acknowledgements

The authors thank the J CIA for providing THI analytical standard and commercial caramel III samples. This study was supported in part by a grant from the Japan Food Chemical Research Foundation and by a Health Labour Sciences Research Grant from the Ministry of Health, Labour and Welfare, Japan.
VI References


HILIC-LC/MSを用いたカラメルIII中の2-アセチル-4-テトラヒドロキシンプチルイミダゾールの直接定量分析法の開発
(2015年4月1日受付)
(2015年6月2日受理)

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キーワード：HILIC、LC/MS、qNMR、カラメル、2-アセチル-4-テトラヒドロキシンプチルイミダゾール

概要

親水性相互作用クロマトグラフィー（HILIC）カラムを用いたLC/MS（HILIC-LC/MS）によるカラメルIII中の不純物2-アセチル-4-テトラヒドロキシンプチルイミダゾール（THI）の直接定量分析法を開発した。本研究では、予め定量NMRにより絶対純度を求めた2-アセチル-4-テトラヒドロキシンプチルイミダゾールを定量用標準品とし、絶対検量線法及び標準添加法によりカラメルIII中のより信頼性の高い正確なTHI含量を求めた。現在、JECFAでは、カラメルIII中のTHIの定量分析法として、2,4-ジニトロフェニルヒドラジンにより誘導体化したTHIを粒子径10 μmのC8カラムを用いてHPLC/UVで分析する方法が設定されている。JECFA及び我々が開発したHILIC-LC/MSにより、同試料について求めた定量値を比較した結果、JECFAによる定量値は夾雑物との分離が不十分なため、HILIC-LC/MSによる定量値より低い値を示した。この結果は、JECFAの規定する分析条件には正確な定量値を得るための改良の余地があることを支持するものである。