

# DETERMINATION OF 4-METHYLIMIDAZOLE AND 2-ACETYL-4-TETRAHYDROXY-BUTYLIMIDAZOLE IN CARAMEL COLOR AND PRODUCTS BY LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY

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## Abstract

The simultaneous determination of 4-MEI and THI in caramel and products by LC-MS/MS was developed with several advantages of simplicity, selectivity, and high sensitivity. Chromatographic separation was performed using a C18 column (100 × 2.1 mm, 1.7 μm), with a mobile phase consisting of ammonium formate pH = 4 and acetonitrile containing 0.1% formic acid employing a gradient elution mode. The analytes were detected using an MS/MS double mass spectrometer with an ESI+ ionization source. Samples were cleaned and enriched on a SPE MCX column. Method validation resulted in a linear range of 5.0 - 500 ppb for 4-MEI, and 1.0 - 500 for THI, with LODs of 0.30 g/kg for THI and 1.5 μg/kg for 4-MEI, and LOQs of 1 μg/kg for THI and 5 μg/kg for 4-MEI. Repeatability and reproducibility for 4-MEI ranged from 2.9% to 9.1% and 2.9% to 11.8%, respectively, and for THI from 3.5% to 9.4% and 4.8% to 11.5%, respectively. Recovery rates for with 4-MEI and THI met AOAC's requirements, ranging from 82.7% to 106.4% and 87.2% to 109.7%, respectively. Application of the analytical method for 24 market products, including caramel, beverages, condiments, and cakes, showed that the 4-MEI and THI concentrations in consumer products were within the permissible limits, while pure caramel samples exceeded the permissible limits for THI concentrations.

**Keywords:** LC-MS/MS; caramel; 4-MEI; THI.

## 1. Introduction

Coloring additives are widely used in the food industry to enhance visual appeal. Among these, caramel color stands out as one of the oldest additives used. During caramel production, low-molecular-weight compounds such as 4-methylimidazole (4-MEI; present in III and IV) and 2-acetyl-4-tetrahydroxy-butylimidazole (THI; present in III only) are generated [1].

The presence of 4-MEI and THI by-products in caramel color can pose hazards to human health due to their toxicity, with 4-MEI being neurotoxic and THI reported as an immunosuppressive compound [2]. Consequently, the European Union (EU) and the World

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Health Organization (WHO) have established maximum allowable level for 4-MEI as 200 mg/kg in E150c and 250 mg/kg in E150d, while the maximum allowable level for THI is 10 mg/kg in E150c [1, 3]. Monitoring the content of by-products like THI and 4-MEI in foods is essential to ensure their levels remain within acceptable limits and do not compromise user health.

Various techniques exist for determining the content of THI and MEI in food colors such as capillary electrophoresis [4], cation exchange chromatography [5], LC-MS/MS [3, 6, 7]. The simultaneous quantification of 4-MEI and THI by LC-MS/MS method offers advantages such as sensitivity, high selectivity, high accuracy, and the ability to identify substances with differing molecular weights. In this study, the LC-MS/MS method was utilized to determine 4-MEI, THI in caramel color, beverages, condiments, and cakes. The structural formulas of the studied compounds are illustrated in Fig. 1.

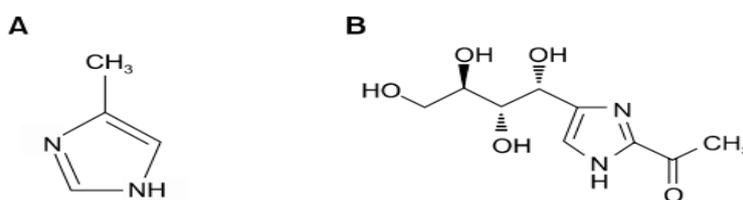


Fig. 1. Structure of 4-MEI (A) and THI (B).

## 2. Materials and methods

### 2.1. Chemical and Reagents

Standards, internal standards, chemicals: standards used in the study 4-methylimidazole (4-MEI) from Sigma-Aldrich, 2-acetyl-4-tetrahydroxy-butylimidazole (THI) from ChemCruz; internal standard 4-methylimidazole- $d_6$  (4-MEI- $d_6$ ), (R, S, R)-2-acetyl-4-tetrahydroxy-butylimidazole -  $^{13}C_6$  (IS-THI) from TRC - Canada; chemicals Methanol, Acetonitrile, formic acid, hydrochloric acid 37%, ammonia (25%), ammonium formate from Merck. Standards and internal standards were prepared in ACN/H<sub>2</sub>O (80:20, v:v). Other chemicals used in double distilled water were of analytical grade.

### 2.2. LC-MS/MS Conditions

Research used UPLC liquid chromatography system and Xevo TQ-XS mass spectrometry system of Waters company with ESI source. Use Waters Acquity BEH C18 column (100 × 2.1 mm, 1.7 μm (Waters), CORTECS HILIC column (2.1 mm × 100 mm; 1.6 μm) from Waters. Solid phase extraction column MCX Cartridge (Waters), solid phase extractor with vacuum, vortex shaker, and other laboratory equipment.

### 2.3. Analytical samples

Sample subjects included 4 caramel samples, 8 beverage samples, 10 condiment samples and 2 cake samples. All samples were taken randomly in Hanoi.

## 3. Results and discussion

### 3.1. LC-MS/MS conditions

By referencing several documents [3, 6-9] and the compound structures, this study investigates mass spectrometry conditions, chromatographic conditions, including the examination of 2 types of columns HILIC columns and C<sub>18</sub> columns, and the evaluation of 3 mobile phases HCOONH<sub>4</sub> 10 mM (pH = 4) - channel A, ACN (0.1% HCOOH) - channel B; HCOONH<sub>4</sub> 10 mM in ACN/H<sub>2</sub>O (95:5, v:v) - channel A, HCOONH<sub>4</sub> 30 mM in ACN/H<sub>2</sub>O (85:15, v:v) - channel B; both channels are acidified with 0.1% HCOOH and 5 mM NH<sub>3</sub> - channel A, ACN - channel B.

#### 3.1.1. Investigation of mass spectrometry conditions

By referencing several documents [3, 8] and the compound structure, this study conducted a survey to determine 4-MEI and THI simultaneously by electron spray ionization technique ESI with bombardment mode positive ion (ESI+). To optimize mass spectrometry conditions, inject 5 µL of 4-MEI and THI standard solutions using a single standard solution of 200 ppb concentration directly (without the separation column) into the mass spectrometer with automatic survey mode for each substance 4-MEI and THI to select parent ions and daughter ions for quantitative and qualitative determination of each substance. The results were presented in Table 1.

Table 1. Fragmentation condition optimization parameters

Compound	Ionization mode	Molecular Mass	Mother ion (m/z)	Product ion (m/z)	Cone voltage (V)	Collision Energy (Ev)
4-MEI	ESI+	82.10	82.88	42.3	31	16
				56.11*	6	14
4-MEI-d <sub>6</sub>	ESI+	88.14	87.77	61.00*	48	16
				57.22	48	54
THI	ESI+	230.21	231.12	195.11*	14	12
				153.10	14	18
IS-THI	ESI+	236.17	237.07	157.10	10	16
				201.13*	10	10

Note: flakes (\*) are quantitative pieces

#### 3.1.2. Investigation of liquid chromatographic conditions

##### 3.1.2.1. Static phase column survey

Based on the sample's nature and composition as well as the characteristics of the components, the appropriate separation column type, stationary phase, column size, and

column length should be selected. This study reviewed several documents [3, 7, 8] and surveyed 2 column types: the Acquity BEH C18 column of Waters brand (100 mm × 2.1 mm; 1.7 μm) and CORTECS UPLC HILIC column by Waters with column parameters. (2.1 mm × 100 mm; 1.6 μm). Proceed with a mixed solution of 4-MEI and THI standards 200 ppb and in the presence of two internal standards: 200 μL IS-THI 1 ppm and 400 μL 4-MEI-d<sub>6</sub> 1 ppm. The results are shown in Fig. 2.

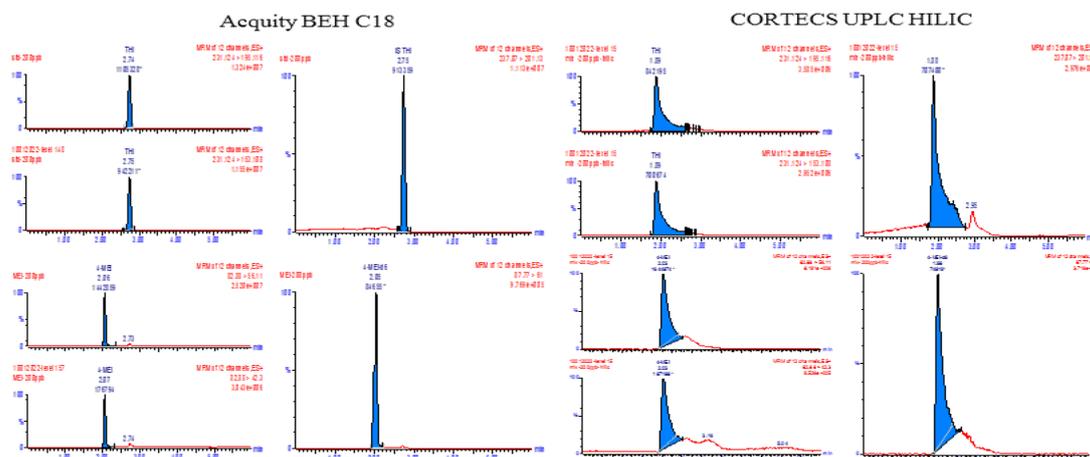


Fig. 2. Chromatography of THI and 4-MEI on 2 stationary phase columns.

Based on the chromatograms obtained with 2 types of columns, we see that the Acquity BEH C18 column gives a good signal, the peak shape is quite nice, and it was capable of separating 4-MEI and THI from the mixture when used with our equipment laboratory. Therefore, the study selected the column Acquity BEH C18 to conduct the analysis.

### 3.1.2.2. Investigation of mobile phase composition

The mobile phase in LC was the solvent system used to elute the solutes (analytes) from the separation column to perform the chromatographic process. The mobile phase not only affected the separation of the analytes but also influenced the ionization and signal of the analyte. By adding substances such as acetic acid, formic acid, ammonium acetate, the ionization process increases with the electron spray bombardment technique in positive ion mode. In our study, we investigated three mobile phase systems by referring to some documents [3, 6, 8, 9].

Procedure: Inject a mixture of 4-MEI and THI standards 200 ppb in the presence of two internal standards: 200 μL IS-THI 1 ppm and 400 μL 4-MEI-d<sub>6</sub> 1 ppm, run in isocratic, proportional mode A/B is 80/20, flow rate is 0.15 mL/min. The chromatogram results of 4-MEI and THI were shown in Figs. 3-5.

The mobile phase survey results were obtained in Figs. 3-5. Based on the chromatogram obtained during the survey, mobile phase 1 gave a beautiful peak shape,

the best analyte signal, and a low background noise signal, so in this study, mobile phase 1 was selected to conduct the investigation for further surveys.

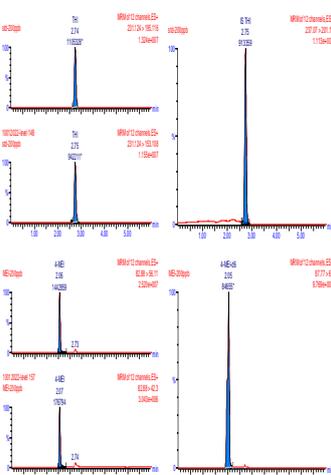


Fig. 3. Mobile phase 1.

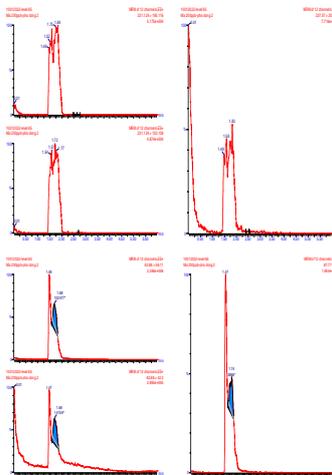


Fig. 4. Mobile phase 2.

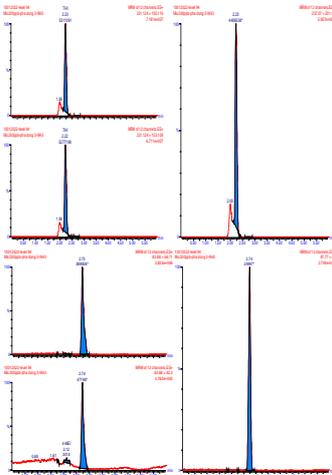


Fig. 5. Mobile phase 3.

### 3.1.2.3. Investigation of mobile phase concentration

After determining the mobile phase composition, survey the concentration of channel A and channel B.

- Change the concentration of channel A at concentrations of 5 mM, 10 mM, 15 mM, and 20 mM and keep channel B unchanged. Use a mixed solution of 4-MEI and THI standards 200 ppb and the presence of 2 contents standards: 200  $\mu$ L IS-THI 1 ppm and 400  $\mu$ L 4-MEI-d<sub>6</sub> 1 ppm for survey.

- After determining the appropriate concentration of channel A, survey channel B: Change the content of HCOOH in ACN at 0.0 levels; 0.05; 0.1; 0.15; 0.2 (%) and keep the selected channel A unchanged. Use a mixed solution of 4-MEI and THI standards of 200 ppb and the presence of two internal standards: 200  $\mu$ L IS-THI 1 ppm and 400  $\mu$ L 4-MEI-d<sub>6</sub> 1 ppm for investigation.

The survey results showed that at a concentration of 15 mM (channel A) and HCOOH content 0.1% (channel B). Hence, the signal was high, and met the necessary requirements, while also limiting contamination of the mass spectrometry system. In addition, high salt concentrations can cause ionic competition, resulting in pipe corrosion and compromising separation columns and mass spectrometry. By ensuring that the acid concentration is not too high, we can avoid these concerns and keep the system performing optimally.

### 3.1.2.4. pH investigation of mobile phase

Based on the ion fragmentation mechanism, it was found that the signals of 4-MEI

and THI were affected by the pH of the mobile phase. Conduct a pH survey of selected channel A at 3.0; 3.5; 4.0; 5.0 and 6.24 (initial pH) – adjust pH with 99% HCOOH solution. A mixed solution of 4-MEI and THI standards of 200 ppb and the presence of two internal standards: 200  $\mu$ L IS-THI 1 ppm and 400  $\mu$ L 4-MEI-d<sub>6</sub> 1 ppm were used for investigation. The results analyte signal was affected by the pH of channel A. When the pH decreased from 6.24 to 3.5, the analyte signal increased. On the other hand, when pH went down to level of 3, the analyte signal decreased. At pH 3.5 and 4.0, the analyte signal is similarly high and sensitive. In the study, pH = 4 was selected for further investigation to minimize the influence of the acidic environment on the mass spectrometer.

### 3.1.2.5. Gradient survey

Four gradient elution procedures were tested to separate compounds while maintaining analytical time and solvents. The experiment used a mixed solution of 4-MEI and THI standards of 200 ppb, coupled with two internal standards: 200  $\mu$ L IS-THI 1 ppm and 400  $\mu$ L 4-MEI-d<sub>6</sub> 1 ppm. Figs. 6-9 show the analysis results.

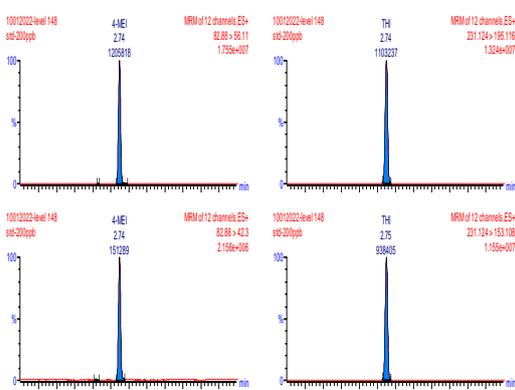


Fig. 6. Gradient 1.

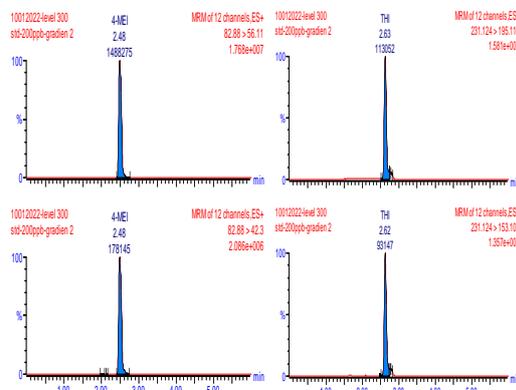


Fig. 7. Gradient 2.

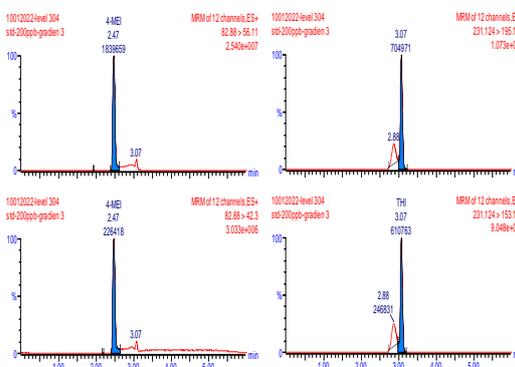


Fig. 8. Gradient 3.

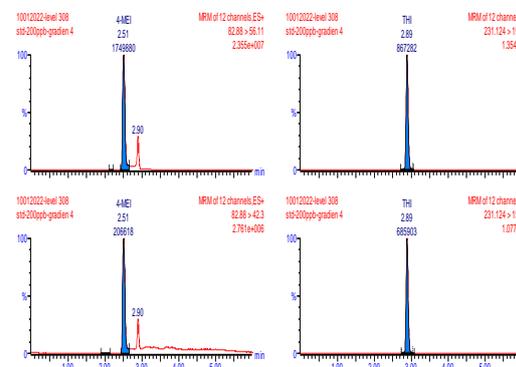


Fig. 9. Gradient 4.

Through the survey, it was found that the retention time of 4-MEI and THI ranges from 1.00 - 3.09 minutes. At gradient 1, the peaks of the analytes are clear, and

compact, with little split and no influence of background noise. Therefore, gradient 1 was chosen for the next study.

Through the survey processes, optimal parameters were obtained for the separation and quantification of 4-MEI and THI on LC-MS/MS equipment as shown in Table 2.

Table 2. Conditions for separation, quantification of 4-MEI and THI on LC-MS/MS equipment

No.	Condition	Parameter	Condition	Parameter
A. Liquid chromatography LC			B. Mass Spectrometry	
1	Split column	Column C <sub>18</sub> (100 × 2 mm; 1.7 μm)	Observation mode	MRM
2	Column temperature	40°C	Ionization technology	ESI +
3	Sample injection volume	5 μL	Ionization chamber temperature	500°C
4	Mobile phase	Channel A (HCOONH <sub>4</sub> 15 mM – pH = 4.0), channel B (ACN-0.1%HCOOH)	Air flow rate N <sub>2</sub>	10 L/min
5	Speed line	0.15 mL/min	Capillary voltage	5500 V (Negative)
6	Gradient program	0-0.99 minutes: 100%A 1-3.09 minutes: 40%A 3.10-6 minutes: 100%A	Nebulizer injection flow pressure	40 psi
<i>Note: ions (*) are quantitative ions</i>				

### 3.2. Survey of sample handling process

Referring to several published research [3, 7], the study surveyed the following samples: caramel, beverage samples, and caramel-containing condiments and cake samples. The sample processing procedure uses the SPE MCX column to extract the analyte from the sample matrix.

#### 3.2.1. Investigate the processing conditions of caramel material samples

H<sub>2</sub>O:ACN was investigated in the ratio of 1:4 respectively; 2:3; 3:2; and 5:0 to completely dissolve the caramel sample. Each ratio will be surveyed 2 times. Survey results of the ratio of H<sub>2</sub>O:ACN dissolved caramel samples in Fig. 10.

The analytical results showed that the 4-MEI content was independent of the ratio of substances in the solvent to dissolve the sample. For THI, the highest analyte content was obtained in the ratio 3:2. Therefore, the study chose the ratio of H<sub>2</sub>O:ACN as 3:2 for the sample processing.

**Optimal procedure for handling caramel samples:** Accurately weigh approximately 0.1 g of caramel color sample into a centrifuge tube, add 1 mL IS-THI

10 ppm and 2 mL 4-MEI-d<sub>6</sub> 10 ppm, 30 mL H<sub>2</sub>O and 20 mL of ACN solution vortex for 1 min. Then centrifuge for 5 min at 6000 rpm. Take the supernatant and makeup to 50 mL with ACN in a volumetric flask. Filter the solution through a 0.2 μm syringe filter and analyze it on a UPLC-MS/MS system.

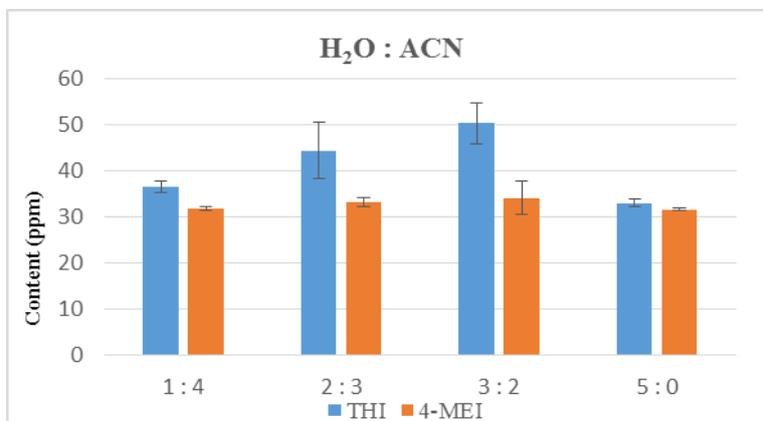


Fig. 10. Survey chart of ratio H<sub>2</sub>O:CAN.

### 3.2.2. Investigate the processing conditions of beverage samples

For the beverage template background. During sample processing, add 0.1M HCl in varying amounts of 20 μL; 40 μL; 60 μL; and 80 μL to acidify the sample. For each amount of HCl added, it will be examined twice. The survey results are shown in Fig. 11.

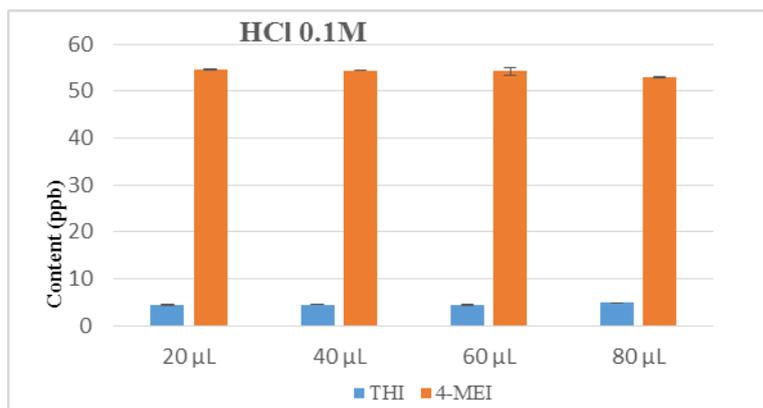


Fig. 11. Survey chart of HCl 0.1 M.

The analytical results in Fig. 11 show that, with different amounts of 0.1M HCl added, it does not affect the analyte content obtained, so it is not necessary to add HCl to the beverage sample treatment. From this, the optimal analytical procedure can be drawn.

**Optimal procedure for analyzing beverage samples:** After being opened, gently shaken, and sonicated for 30 minutes, take 2 mL of the sample into a 50 mL tube. Then add to the sample 200 μL IS-THI 1 ppm and 400 μL 4-MEI-d<sub>6</sub> 1 ppm. Add distilled water to about 5 mL. Homogenize and shake on a 1-minute vortex machine. Then

proceed to extract through the SPE MCX column. The column was first activated with 3 mL of MeOH of analytical grade, and 3 mL of distilled water, and then loaded with 5 mL of sample through the column, at a flow rate not exceeding 2 mL/min. When the entire sample has been loaded, remove impurities with 3 mL of HCOOH 2M and 3 mL of MeOH, respectively. Finally, elute with g 4 mL MeOH (5%NH<sub>4</sub> OH). The collected solution is blown dry with N<sub>2</sub> gas at 40°C. The collected residue is dissolved with 1 mL of ACN/H<sub>2</sub>O (80:20, v:v) for analysis on UPLC-MS/MS.

### 3.2.3. Investigate the processing conditions for condiments and cake samples

#### 3.2.3.1. Investigation of sample dissolution conditions

For the spice sample background, the study was carried out with the soy sauce sample. During the dissolution of the sample, survey the HCOOH content at concentrations of 0.5; 1.0; 1.5; and 2.0% to dissolve the sample. For each concentration of HCOOH in MeOH investigated, it was repeated 2 times. The survey results are shown in Fig. 12.

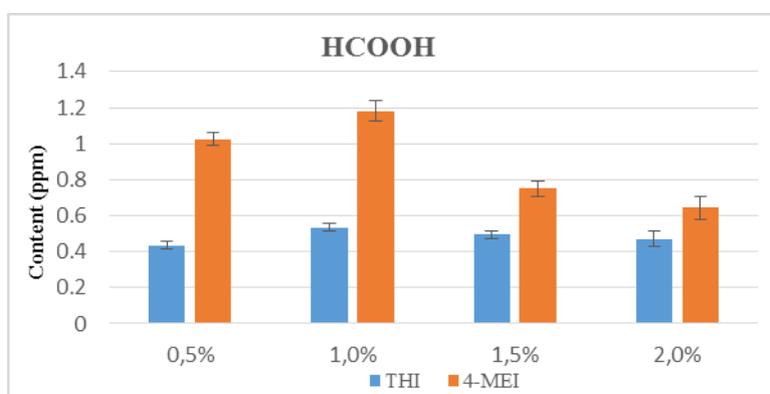


Fig. 12. Survey chart of HCOOH content in MeOH.

The analysis results in Fig. 12 showed that the THI content did not change significantly at different concentrations of HCOOH, however, the concentration of 4-MEI had a slight increase at the concentration of 0.5% to 1.0% and there was a sharp decrease from 1.0% to 1.5% and 2.0%. Therefore, the study selected the concentration of HCOOH in MeOH during the dissolution of the soy sauce sample to be 1.0% for the next study.

#### 3.2.3.2. Investigate the washing conditions for condiments and cake samples

After having obtained the optimal sample dissolution conditions, a survey on the washing conditions with HCOOH solution at concentrations of 1; 2; 3; and 4 (%). For each concentration of HCOOH in MeOH was repeated 2 times. The survey results are shown in Fig. 13.

The survey results in Fig. 13 show that the concentrations of 4-MEI and THI at 2% and 3% HCOOH concentrations are higher than 1% and 4% concentrations. Concentrations at 2% and 3% concentrations did not have a significant difference. However, in this study, a 2% concentration of HCOOH was selected to dissolve the residue to reduce the amount of acid to save and avoid wasting analytical chemicals.

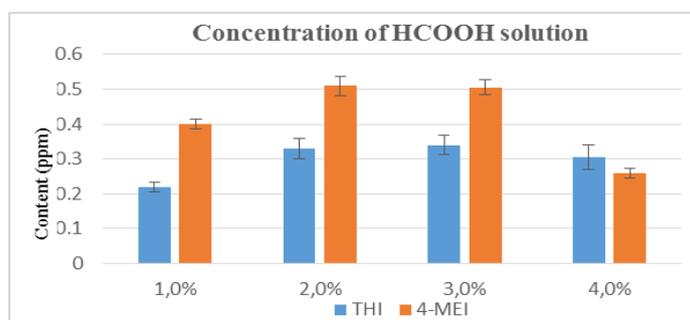


Fig. 13. Survey chart of cleaning solution.

After investigating the dissolution conditions and washing residues of the analyzed samples, *the process of processing condiment samples and cake samples was optimized* as follows: Cake samples/spice samples after being homogenized, weighed about 2g (cake sample)/0.2 g (spice sample) into a 50 mL centrifuge tube. Add to the sample 200  $\mu$ L IS-THI 1 ppm and 400  $\mu$ L 4-MEI-d<sub>6</sub> 1 ppm and 10 mL MeOH (1% HCOOH). Homogenize, shake on the vortex machine for 1 min and sonicate for 10 min. Then centrifuge at 6000 rpm for 5 minutes at room temperature. Take the solution and blow dry with N<sub>2</sub> gas at 40°C. Dissolve the residue obtained with 5 mL of 2% HCOOH solution. Then proceed to extract through the SPE MCX column similar to procedure 2. The obtained solution is blown dry with N<sub>2</sub> gas at 40°C. The resulting residue is dissolved with 1 mL of ACN/H<sub>2</sub>O (80:20, v:v) and then analyzed on UPLC-MS/MS.

### 3.3. Method validation

The specificity/selectivity of the method by calculating the IP score (identification score). According to the Council of Europe (EU 2021/808) the IP score for the double liquid chromatography-mass spectrometry (LC-MS/MS) technique is 5, then it takes 1 parent ion to eject 2 daughter ions. The results are shown in Table 3.

Table 3. IP scores of standards and internal standards

Standard/internal standard	Mother ion	Sub ion 1	Sub-ions 2	IP Points
4-MEI	82.88	42.3	56.11	5
4-MEI-d <sub>6</sub>	87.77	57.22	61.00	5
THI	231.12	153.10	195.11	5
IS-THI	237.07	157.10	201.13	5

Based on the optimal conditions obtained, the 4-MEI analytical standard curve is linear in the concentration range from 5 to 500  $\mu\text{g}/\text{kg}$ , and the analytical standard curve for THI is linear in the concentration range from 1.00 to 500  $\mu\text{g}/\text{kg}$  with correlation coefficient  $> 0.99$ . Results of the standard curve equation, correlation coefficient, limit of detection (LOD) and limit of quantitation (LOQ), repeatability ( $\text{RSD}_r$ ), reproducibility ( $\text{RSD}_R$ ), and recovery (R) of the analytes are presented in Tables 4, 5.

Table 4. Standard curve equations and limits of detection, limits of quantification of 4-MEI, THI

Analytes	Linear equation	Correlation coefficient $R^2$	LOD	LOQ
4-MEI	$Y = 0.036869 X + 0.135502$	0.998	1.5 $\mu\text{g}/\text{kg}$	5 $\mu\text{g}/\text{kg}$
THI	$Y = 0.00124334 X + 0.000348471$	0.999	0.3 $\mu\text{g}/\text{kg}$	1 $\mu\text{g}/\text{kg}$

Table 5. Repeatability, reproducibility, and recovery of 4-MEI, THI

Samples	Analytes	$\text{RSD}_r$ (%)	$\text{RSD}_R$ (%)	R (%)
Caramel	4-MEI	4.2	4.1	90.2 - 106.4
	THI	4.9	5.0	92.5 - 106.1
Beverages	4-MEI	2.9	2.9	92.9 - 105.1
	THI	3.5	4.8	87.2 - 98.9
Condiments and cakes	4-MEI	9.1	11.8	82.7 - 101.6
	THI	9.4	11.5	92.4 - 109.7

The linear correlation coefficient ( $R^2$ ) was  $> 0.99$  for both 4-MEI and THI. The repeatability, reproducibility, and recovery of 4-MEI and THI in different samples were all within the acceptable limits of AOAC, indicating the high accuracy of the method. Therefore, this method can be fully applied to the analysis of 4-MEI and THI content in real samples.

### 3.4. Analysis of 4-MEI, THI content in some real samples

Conduct determination of 4-MEI and THI content on 24 actual samples. The results of the analysis conducted on 24 actual samples indicate that 23 of them contained 4-MEI, while 21 samples were found to contain THI. Caramel samples, 4-MEI content from 17.99 - 40.79  $\text{mg}/\text{kg}$ , THI from 72.20 - 135.76  $\text{mg}/\text{kg}$ . Beverages had very low concentrations of 4-MEI and THI, almost zero. Similarly, condiment and cake samples had 4-MEI concentrations from 0.25 - 1.87  $\text{mg}/\text{kg}$ , and THI from 1.32 - 3.11  $\text{mg}/\text{kg}$ . All analyzed samples had 4-MEI content within the allowable limits of TCVN and WHO [10-12]. However, THI content in caramel samples was found to be much higher than the allowable limit (TCVN was 25  $\text{mg}/\text{kg}$  and WHO was 10  $\text{mg}/\text{kg}$ ), and consumer product samples: beverages, ginseng juice, and Soy sauce all have THI

content within the allowable limit. Upon analyzing 20 samples of beverages, condiments and cakes, most of them had 4-MEI and THI concentrations much lower than those observed in caramel ones.

### 3.5. Comparison with other studies

The research results and comparison with other studies utilized the LC-MS method, similar to this one [13-16] as shown in Table 6 demonstrate that this method offers a rapid and straightforward analytical procedure, ensuring high sensitivity and accuracy for quantifying 4-MEI and THI levels in diverse sample matrices.

Table 6. Compare research results

	Author	Matrix	Analytes	R <sup>2</sup>	Recovery(%)	LOD/LOQ	Sample preparation method	Time (minute)
1	Phuong Thao Nguyen <i>et al.</i>	Caramel, beverages, condiments, and cakes	4-MEI THI	4-MEI: 0.9981 THI: 0.9997	4-MEI: 82.7-106.4 THI: 87.2-109.7	<b>LOD:</b> 4-MEI: 1.5 µg/kg; THI: 0.3 µg/kg <b>LOQ:</b> 4-MEI: 5.0 µg/kg; THI: 1.0 µg/kg	SPE	6
2	Yamaguchi <i>et al.</i> (2011) [13]	Soy sauce	4-MEI	0.990	94.3-104.5	<b>LOD:</b> 0.4 µg/L <b>LOQ:</b> 0.7 µg/L	SPE	36
3	Wu <i>et al.</i> (2019) [14]	Beverages	THI	0.9991	83.4-96.1	<b>LOQ:</b> 5 µg/L	D-µ-SPE	9
4	Schlee <i>et al.</i> (2013) [15]	Caramel and beverages	2-MEI, 4-MEI, THI	0.9998	4-MEI 84.0-102.0 THI 125.4-135.7	<b>LOD</b> 4-MEI: 2 µg/L; THI: 5 µg/L <b>LOQ</b> 4-MEI: 4 µg/L; THI: 9 µg/L	Dilute and filter sterilize using a 0.2 µm membrane filter	35
5	Kim <i>et al.</i> (2013) [7]	Caramel and foods	4-MEI, THI	4-MEI: 0.9980 THI: 0.9998	4-MEI 97.1-111.0 THI 83.6-106.4	<b>LOD</b> 4-MEI: 3 µg/kg; THI: 0.5 µg/kg <b>LOQ</b> 4-MEI: 5 µg/kg; THI: 2 µg/kg	Dissolve in acetonitrile, centrifuge, and filter	15

## 4. Conclusion

The study succeeded in the simultaneous determination of 4-MEI and THI by liquid chromatography coupled to mass spectrometry and applied the optimal analytical procedure to determine the 4-MEI and THI content in some product samples. The analytical procedure is fast and simple, and the method ensures sensitivity and accuracy for the determination of 4-MEI and THI concentrations in various matrix samples.

The method's LOQ was determined to be 1 µg/kg for THI and 5 µg/kg for 4-MEI, while the LOD was 0.30 µg/kg for THI and 1.5 µg/kg for 4-MEI. The repeatability was evaluated with 4-MEI (2.9 - 9.1%) and THI (3.5 - 9.4%), while the reproducibility was evaluated with 4-MEI (2.9 - 11.8%) and THI (4.8 - 11.5%). The accuracy was evaluated through recovery, which was 82.7 - 106.4% for 4-MEI and 87.2 - 109.7% for THI, meeting the AOAC requirements.

The analysis results of 24 real samples showed that 23 samples contained 4-MEI and 21 samples contained THI. The highest content of 4-MEI was 40.79 mg/kg and the highest content of THI was 135.76 mg/kg are all in the caramel sample. 4-MEI and THI were mainly present in caramel samples, while the contents in other food samples such as cakes, condiments, and beverages were very low and negligible.

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# NGHIÊN CỨU PHƯƠNG PHÁP XÁC ĐỊNH ĐỒNG THỜI 4-METHYLIMIDAZOLE VÀ 2-ACETYL-4-TETRAHYDROXY- BUTYLIMIDAZOLE TRONG MÀU CAMEL VÀ CÁC SẢN PHẨM BẰNG PHƯƠNG PHÁP SẮC KÝ LÔNG GHÉP NỐI KHỐI PHỔ

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**Tóm tắt:** Phát triển phương pháp xác định đồng thời 4-MEI và THI trong nền caramel và các sản phẩm chứa caramel bằng LC-MS/MS với nhiều ưu điểm đơn giản, độ chọn lọc và độ nhạy cao. Quá trình tách sắc ký sử dụng cột C18 (100 × 2,1 mm, 1,7 µm), pha động gồm amonifomat pH = 4 và acetonitril chứa 0,1% acid formic cùng chế độ rửa giải gradient. Chất phân tích được phát hiện trên hệ khối phổ hai lần MS/MS với nguồn ion hóa ESI+. Mẫu được làm sạch và làm giàu trên cột SPE MCX. Thẩm định phương pháp cho khoảng tuyến tính của 4-MEI trong khoảng 5,0 - 500 ppb, của THI trong khoảng 1,0 - 500 ppb; MDL của phương pháp là 0,30 µg/kg của THI và 1,5 µg/kg của 4-MEI; MQL là 1 µg/kg với THI và 5 µg/kg với 4-MEI, độ lặp lại với 4-MEI (2,9 - 9,1%), THI (3,5 - 9,4%); độ tái lập đối với 4-MEI (2,9 - 11,8%), THI (4,8 - 11,5%); độ thu hồi với 4-MEI (82,7 - 106,4%), THI (87,2 - 109,7%) đạt yêu cầu của AOAC. Ứng dụng phương pháp phân tích 24 sản phẩm trên thị trường (bao gồm caramel, đồ uống, gia vị và bánh ngọt) cho thấy hàm lượng 4-MEI và THI của các sản phẩm tiêu dùng đều nằm trong giới hạn cho phép, các mẫu caramel nguyên chất có hàm lượng THI vượt quá giới hạn cho phép.

**Từ khoá:** LC-MS/MS; caramel; 4-MEI; THI.

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