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Application News

No. L551

High Performance Liquid Chromatography

Analysis of 4-Methylimidazole in Caramel Color and Study of High-Speed Analysis

Caramel color is a type of food additive that is used in a variety of food products to give those products a brown coloration. Caramel color is manufactured by heating saccharides such as sugar or glucose, but it also contains 4-methylimidazole as a byproduct formed in the manufacturing process. Animal experiments carried out by the National Toxicology Program (NTP) in the United States have revealed that 4-methylimidazole is highly carcinogenic, and based on that result, the International Agency for Research on Cancer (IARC) has classified the compound as a carcinogenic hazard in an IARC Monograph.[1] Against this backdrop, the EU and United States have established regulations on the allowable content of 4-methylimidazole in caramel color, and the State of California has set a limit on its daily intake.[2]

In this article, we introduce an example of an analysis of 4-methylimidazole in cola by using the Shimadzu Nexera-i™ MT.

K. Nakajima

Analysis of 4-Methylimidazole by Using HPLC Flow Line of Nexera-i MT

Table 1 shows the analytical conditions. Fig. 2 shows the chromatogram obtained by an analysis under these conditions using samples prepared by adding 50 µg/L of 4-methylimidazole to a cola stock solution. It was found that the elution peak of 4-methylimidazole could be analyzed under these conditions in Table 1 with no effect of impurities contained in the cola.

Next, a 5 mg/L standard solution was prepared by dissolving a 4-methylimidazole reference standard in ultrapure water. Standard solutions with concentrations of 5 mg/L, 1 mg/L, 500 µg/L, 100 µg/L, and 50 µg/L were then prepared by diluting the original standard solution with the mobile phase, and calibration curve was prepared by analyzing these diluted solutions (Fig. 3). Excellent linearity of the calibration curve was confirmed, including the low concentration range, as the contribution ratio $R^2 > 0.9999$. Table 2 shows the repeatability of the peak area and the result of a spike and recovery test with the same sample for the cola stock solution with 50 µg/L addition of 4-methylimidazole.

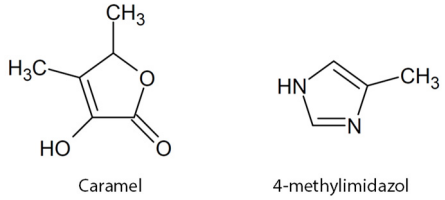

Fig. 1 Structures of Representative Caramel Color and 4-Methylimidazole

Table 1 Analytical Conditions (General Analysis)

System	:	Nexera-i MT
Column	:	Shim-pack™ VP-ODS (250 mmL. × 4.6 mmI.D., 5 µm)
Mobile phase	:	A: methanol B: water containing 25 mmol/L (sodium) phosphate pH=2.8 25 mmol/L sodium 1-octanesulfonate A/B=1/9
Flow rate	:	1 mL/min
Column temp.	:	40°C
Injection vol.	:	20 µL
Detection	:	PDA (214 nm)

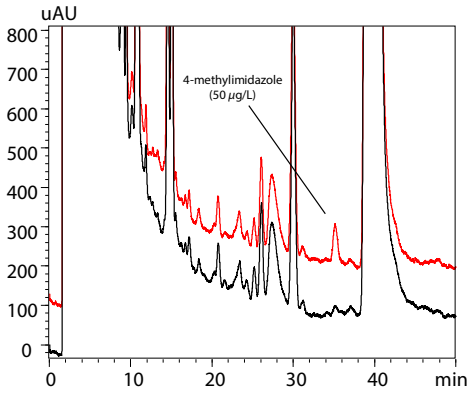

Fig. 2 Chromatogram of 4-Methylimidazole in Cola (Black: Cola Stock Solution, Red: Sample Prepared by Adding 4-Methylimidazole to Cola)

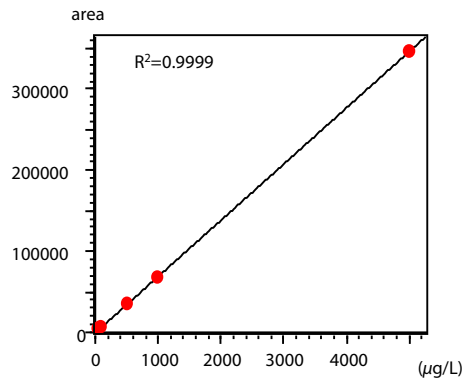

Fig. 3 Calibration Curve of 4-Methylimidazole (General Analysis) (5 mg/L, 1 mg/L, 500 µg/L, 100 µg/L, 50 µg/L)

Table 2 Results of Analysis of 4-Methylimidazole under General Analysis Conditions

	4-methylimidazole (50 µg/L in cola)
%RSD (peak area, n=6)	3.61%
Spike and Recovery Test	106.8%

Creation of Conditions for High Speed Analysis of 4-Methylimidazole by Method Transfer

The Shimadzu Nexera-i MT has both an HPLC flow line and a UHPLC flow line in the same system, enabling transfer from general conditions to high speed conditions with one instrument. Using this function, we studied the conditions for high speed analysis of the 4-methylimidazole in cola shown in Table 1. In addition, in order to achieve high speed in an analysis with an HPLC system by using a UHPLC system, it is necessary to transfer the analytical conditions. In this study, a method supporting high speed analysis was created by utilizing the Method Transfer function included in LabSolutions™ (Fig. 4).

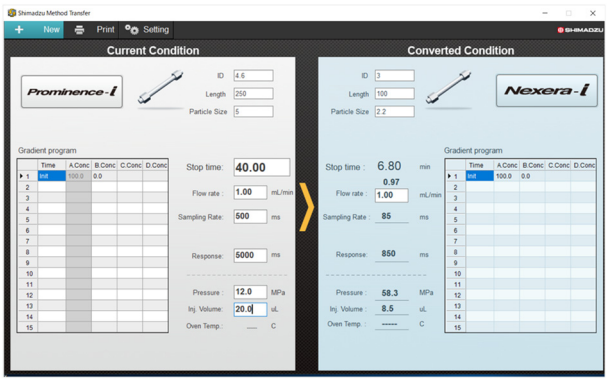

Fig. 4 High Speed Analysis Condition Creation Screen of Method Transfer

High Speed Analysis of 4-Methylimidazole Using UHPLC Flow Line of Nexera-i MT

Table 3 shows the analytical conditions created by using Method Transfer. Fig. 5 shows a chromatogram obtained by analysis of 4-methylimidazole added to cola under the conditions in Table 3. Fig. 6 shows the calibration curve under the same analytical conditions. An excellent contribution factor of R^2 > 0.9999 was obtained. Table 4 shows the repeatability and spike and recovery test results. The high speed analysis conditions created in this experiment showed the same quantitativity as the general analysis.

Table 3 Analytical Conditions (High Speed Analysis)

System	:	Nexera-i MT
Column	:	Shim-pack XR-ODS II (100 mmL. × 3 mmI.D., 2.2 μm)
Mobile phase	:	A: methanol B: water containing 25 mmol/L (sodium) phosphate pH=2.8 25 mmol/L sodium 1-octanesulfonate A/B=1/9
Flow rate	:	1 mL/min
Column temp.	:	40 °C
Injection vol.	:	5 μL
Detection	:	PDA (214 nm)

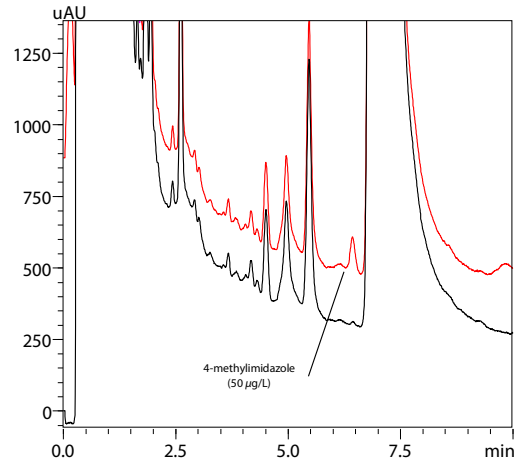

Fig. 5 Analysis Chromatogram of 4-Methylimidazole in Cola (Black: Cola Stock Solution, Red: Sample Prepared by Adding 4-Methylimidazole to Cola)

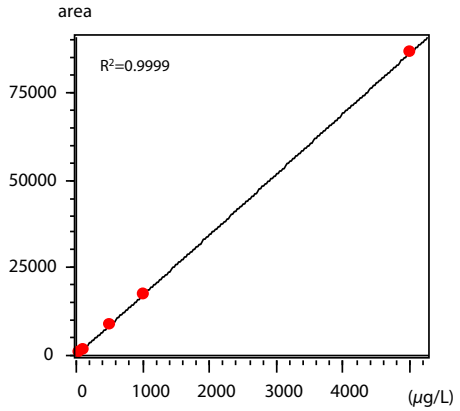

Fig. 6 Calibration Curve of 4-Methylimidazole (High Speed Analysis) (5 mg/L, 1 mg/L, 500 μg/L, 100 μg/L, 50 μg/L)

Table 4 Results of Analysis of 4-Methylimidazole under High Speed Analysis Conditions

	4-methylimidazole (50 μg/L in cola)
%RSD (peak area, n=6)	2.41%
Spike and Recovery Test	106.8%

Conclusion

An HPLC analysis of 4-methylimidazole in cola was conducted using the Shimadzu Nexera-i MT. The conditions for a high speed analysis using the UHPLC flow line were created in a simple operation by using the Method Transfer function of LabSolutions. Under the high speed conditions, the analysis time was shortened from 50 min to 10 min with one instrument, and the same quantitativity as in the HPLC analysis was confirmed.

(1) IARC Monographs on the Identification of Carcinogenic Hazards to Humans, Vol. 101
(2) California's Proposition 65 list

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