Application Note

Food testing

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Analysis of 4-Methylimidazole Using an Agilent InfinityLab Poroshell 120 HILIC-OH5 Column

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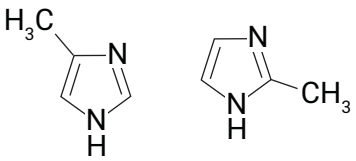
Abstract

4-Methylimidazole (4-MEI) is a chemical compound that is not directly added to food; rather, it is formed as a by-product in some foods and beverages during the normal cooking process. This compound also forms as a trace impurity during the manufacture of certain types of caramel coloring, which are used to color cola-type beverages and other foods. Hydrophilic interaction chromatography (HILIC) is a convenient and fast method for separating the polar molecule and its isomer 2-methylimidazole.

Introduction

4-methylimidazole and 2-methylimidazole are both polar compounds. Reversed-phased LC does not retain these compounds well, and HILIC mode could be a more effective way to retain and separate them. This Application Note shows how 4-methylimidazole and 2-methylimidazole are separated from a cola-type sample using the Agilent InfinityLab Poroshell 120 HILIC-OH5, 2.7 µm column. Both the InfinityLab Poroshell 120 HILIC-OH5 and InfinityLab Poroshell 120 HILIC-Z were screened for the separation of the two compounds. An Agilent 6460 triple quadrupole LC/MS was used for trace level detection of the sample.



4-Methylimidazole 2-Methylimidazole

Figure 1. Structures of 4-methylimidazole and 2-methylimidazole.

Experimental

Reagents and chemicals

All reagents were HPLC grade or higher. HPLC grade acetonitrile was from J. T. Baker (Center Valley, PA, U.S.A.). Water was purified using an ELGA PURELAB Chorus system (High Wycombe, UK). Ammonium acetate was from J&K Scientific (Beijing, China). 4-Methylimidazole and 2-methylimidazole were from Anpel (Shanghai, China).

Equipment and materials

- Agilent InfinityLab fittings
- Column inlet: Agilent InfinityLab Quick Connect fitting (p/n 50675965)
- Column outlet: Agilent InfinityLab Quick Turn fitting (p/n 5067-5966)
- Agilent Captiva Econofilter, PTFE membrane, 13 mm diameter, 0.2 µm pore size (p/n 5190-5265)
- Agilent vial, screw top, amber, writeon spot, certified, 2 mL, 100/pk (p/n 5182-0716)
- Agilent bonded screw cap, PTFE/red silicone septa (p/n 5190-7024)
- Agilent InfinityLab solvent bottle, amber, 1,000 mL (p/n 9301-6526)
- Agilent InfinityLab Stay Safe cap, GL45, three ports, one vent valve (p/n 5043-1219)
- Eppendorf pipettes and repeater
- Sonicator (VWR, Radnor, PA, USA)

Instrumentation

- Agilent 1290 Infinity II high speed pump (G7120A)
- Agilent 1290 Infinity II multisampler (G7167B)
- Agilent 1290 Infinity II multicolumn thermostat (G7116B)
- Agilent Agilent 6460 triple quadrupole LC/MS
- Agilent MassHunter workstation software LC/MS data acquisition, Version. B08.00
- Agilent MassHunter workstation software qualitative analysis, Version, B07.00

Sample preparation

Measure 3.0 mL of cola into a 5-mL volumetric flask and adjust the pH to approximately 7.0 with 0.1 mL of ammonium hydroxide. Next, add water to the scale and blend using sonication. Then, measure 1.0 mL of this mixture into a 10-mL volumetric flask with 8 mL acetonitrile, and blend using sonication before adding acetonitrile to the scale. Finally, take the upper level of acetonitrile and filter with an Agilent Captiva Econofilter PTFE membrane into sample vials.

Spiked samples were produced by adding standards into the cola sample before treatment following the same protocol.

Mobile phase preparation

Ammonium acetate was weighed and diluted to a concentration of 10 mM in water. Buffers were prepared 1 L at a time and replaced regularly to prevent degradation and microbial growth.

HPLC Conditions	
Column	InfinityLab Poroshell 120 HILIC-Z, 2.1 × 100 mm (p/n 685775-924)
Mobile phase A	10 % 10 mM ammonium acetate
Mobile phase B	90 % Acetonitrile
Flow rate	0.30 mL/min
Column temperature	30 °C
Injection volume	5 µL
MS Conditions	
Ion mode	ESI/Jet Stream ESI, Positive
Drying gas temperature	210 °C
Drying gas flow	13 L/min
Nebulizer pressure	35 psi
Sheath gas temperature	400 °C
Sheath gas flow	12 L/min
Capillary voltage (+)	2,500 V
Nozzle voltage (+)	0 V
MRM condition	ΔEMV, 500 V

Results

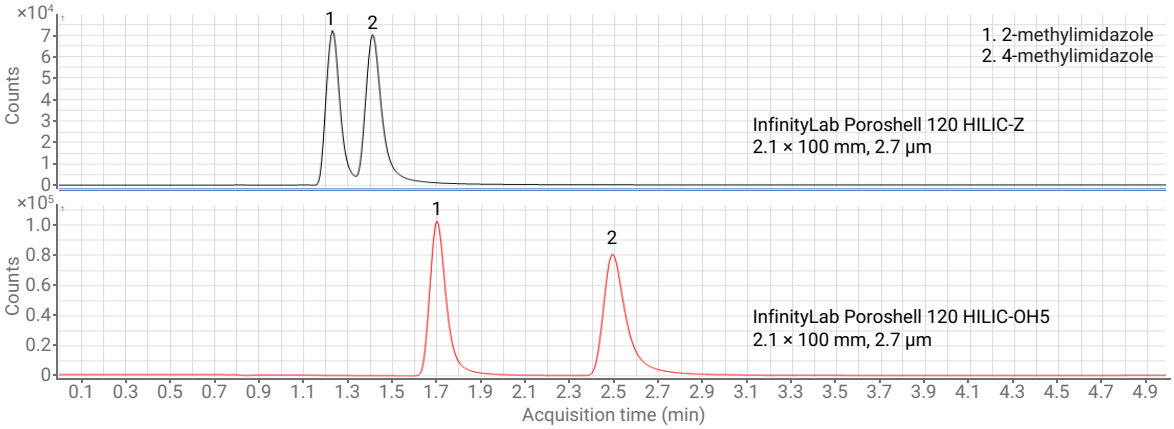


Figure 2. A comparison of the retention of 4-methylimidazole and 2-methylimidazole on an InfinityLab Poroshell 120 HILIC-Z and an InfinityLab Poroshell 120 HILIC-OH5 column with the same mobile phase.

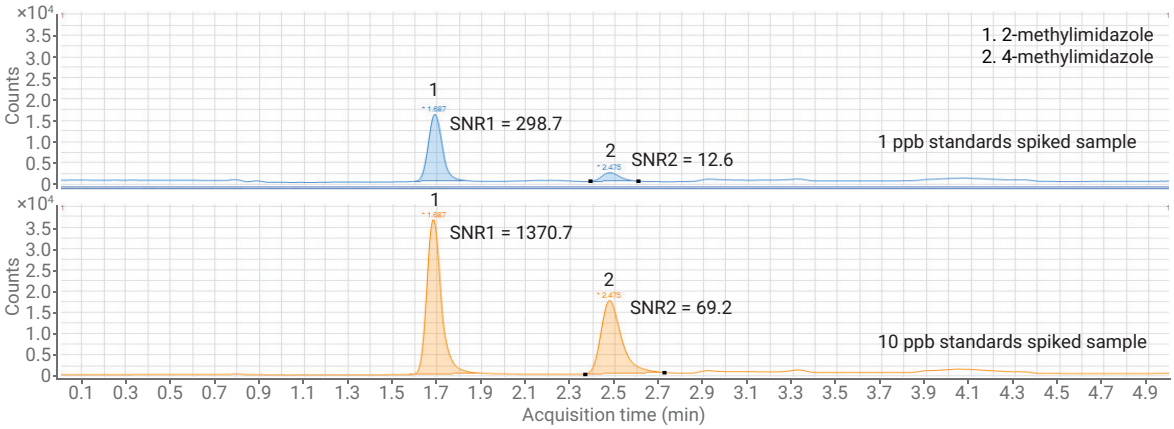


Figure 3. Chromatograms for 1 and 10 ppb standard spiked samples separated on an InfinityLab Poroshell 120 HILIC-OH5 column.

Conclusions

Better retention and successful separation of 4-methylimidazole and 2-methylimidazole was achieved using an InfinityLab Poroshell 120 HILIC-OH5 column. The limit of detection (LOD) at a signal-to-noise ratio (S/N) of 3 is below 1 ppb in the spiked sample. The sensitivity of the method was good enough to detect both compounds in cola-type beverages.

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