SIMULTANEOUS DETERMINATION OF 2- AND 4-METHYLIMIDAZOLES IN BEVERAGES USING A FAST FILTER AND SHOOT METHOD

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OVERVIEW

- Extraction and analysis of 2-methylimidazole (2-MeI) and 4-methylimidazole (4-MeI) in caramel colored beverages
- Novel and simple filter and shoot technology employed in sample preparation
- Analysis performed by LC-MS/MS using a HILIC method
- Baseline separation of the 2 isomers achieved with excellent sensitivity and linearity
- Continued presence of 4-MeI at varied levels detected in caramel colored beverages

INTRODUCTION

2-methylimidazole (2-MeI) and 4-methylimidazole (4-MeI) are byproducts generated from the manufacture of caramel color additives used in beverages, soy sauces, baked foods, etc. The International Agency of Research on Cancer classifies these two compounds as "possibly carcinogenic to humans", and proposed the no significant risk level (NSRL) to be 29 µg/day for 4-MeI. California lists 4-MeI as a probable carcinogen and proposed a 16 µg/day NSRL. Meanwhile, the European Food Safety Authority (EFSA) considers 4-MeI safe and established a maximum level of 250 mg/kg in caramels.

Traditional analytical methods for 2-MeI and 4-MeI follow several pathways. One option involves tedious ion-pairing extraction, and derivatization paired with GC or GC/MS analysis. A second option uses solid phase extraction (SPE) combined with HPLC or LC/MS-MS detection. This application offers a simple, fast, and cost effective method to simultaneously determine 2-MeI and 4-MeI in beverages.

Beverage samples were degassed, diluted 10 fold with acetonitrile (MeCN), and filtered through a SPE cartridge with 200 mg of the novel FASt® sorbent. Undesired matrix components, such as sugars and organic acids were retained on the cartridge sorbent bed. The result was a clean filtrate ready for LC/MS-MS analysis. 2-MeI and 4-MeI are isomers with identical MS/MS transitions, making the separation and quantification of such compounds very difficult. A new HILIC HPLC method has been developed with baseline separation achieved in an 8-min run.

METHOD

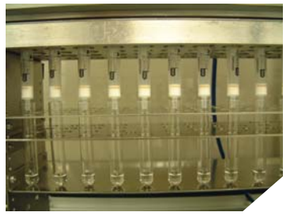
3.1 Experimental

Sample pretreatment

Pour the entire bottle or can of beverage samples into 500-mL beakers, and degas the samples by stirring at high speed for 2 hours.

Filter and Shoot (FASt®) procedure

a. Transfer 0.1 mL of the degassed samples into test tubes or glass vials, dilute with 0.9 mL of MeCN. Add 10 µL of a 10-ppm imidazole solution as internal standard (IS), and appropriate amounts of target analytes to fortified samples. Vortex for 10 sec.
b. Attach the FASt® cartridges (CSFAS203) to a glass block or positive pressure manifold, insert clean test tubes or 2-mL auto-sampler vials beneath each cartridge in the manifold.
c. Transfer the diluted samples into the cartridges, apply low vacuum or positive pressure and collect the filtrates.
d. The samples are ready for LC-MS/MS analysis.



Photograph of FASt® set-up using a positive pressure manifold system. The beverage samples were passed through the FASt® cartridges, with unwanted matrix components retained onto the sorbents (yellowish color), while the cleaned samples were collected in the test tubes

3.2 LC-MS/MS method

Instrumental Conditions	
HPLC:	Thermo Scientific, Dionex UltiMate 3000® LC System
Column:	Thermo Scientific, Accucore HILIC, 100 x 2.1 mm, 2.6 µm
Guard Column:	Thermo Scientific, Accucore HILIC, 10 x 2.1 mm, 2.6 µm
Column Temperature:	40 °C
Column Flow Rate:	0.4 mL/min
Auto-sampler Temperature	10 °C
Injection Volume:	10 µL
Mobile Phase (Isocratic):	50 mM ammonium formate in water : MeCN (5:95)
Run Time:	8 min
Divert mobile phase to waste from 0 - 1 min to prevent ion source contamination.	

MS parameters	
MS/MS	Thermo Scientific, TSQ Vantage™ tandem mass spectrometer
Polarity	ESI +
Spray voltage V	5000 V
Vaporizer Temperature	242 °C
Ion transfer capillary temperature	398 °C
Sheath gas pressure	60 arbitrary units
Auxiliary gas pressure	20 arbitrary units
Q1 and Q3 peak width (FWHM)	0.4 and 0.7 Da
Collision gas and pressure	Ar at 0.8 mTorr
Scan type	SRM
Cycle time	0.75 sec
Acquisition method	EZ Method

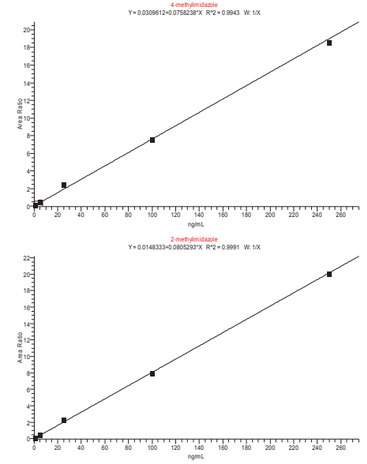
SRM transitions							
Compound	**Rt (min)**	**Precursor ion**	**Product ion 1**	**CE 1**	**Product ion 2**	**CE 2**	**S-lens (V)**
Imidazole (IS)	1.96	69.07	42.01	21	28.08	74	65
4-MeI	3.18	83.08	56.05	17	42.00	27	45
2-MeI	5.72	83.07	42.04	20	56.05	19	48

RESULTS

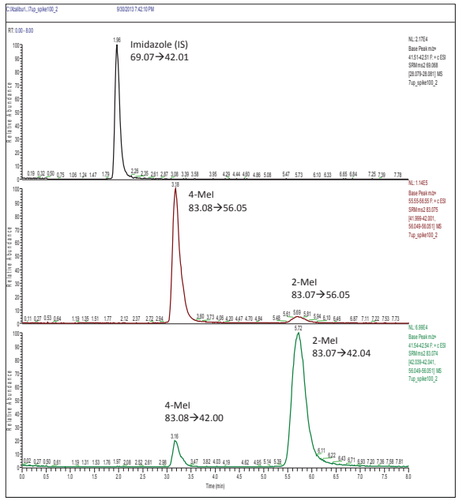
Precision and accuracy obtained from beverage samples fortified at 100 ng/mL

Compound	Recovery%	RSD% (n=6)
4-MeI	**103.6**	4.1
2-MeI	**102.9**	1.6

Matrix Matched Calibration Curves (Dynamic Linearity Range: 1 – 250 ng/mL)



Chromatogram of a Beverage Sample Fortified with 100 ng/mL 2-MeI and 4-MeI



Analysis of Real Samples

This efficient and reliable procedure has been applied to the analysis of 12 real world samples, including 1 colorless naturally flavored soda, 1 root beer, 1 sweet tea, and 9 colas of the same brand obtained from 9 different states (AZ, CO, LA, MD, MS, OR, TN, TX, and WA). 2-MeI was not detected in any of the samples, while 4-MeI was detected in 11 of the samples. The only exception was the colorless soda, which was thus used to prepare matrix matched calibration standards and fortified samples. The 9 cola samples were found to contain 4-MeI at varied levels. The lowest level of about 100 ng/mL was detected in the samples from WA and OR; while the highest level of 840 ng/mL was from AZ.

Beverages	Detected concentration (ng/mL)	
	4-MeI	2-MeI
Colorless soda	< 10	< 10
Root beer	74	< 10
Sweet tea	128	< 10
Cola_AZ	840	< 10
Cola _CO	283	< 10
Cola _LA	439	< 10
Cola _MD	464	< 10
Cola _MS	592	< 10
Cola _OR	107	< 10
Cola _TN	586	< 10
Cola _TX	592	< 10
Cola_WA	101	< 10

CONCLUSIONS

- Simple, fast, and cost-effective sample preparation based on Filter and Shoot technology
- Unwanted matrix components and particulates were trapped on the sorbent, resulting in purified samples for LC-MS/MS analysis
- Excellent recovery and reproducibility
- HILIC method with baseline separation for 2-MeI and 4-MeI isomers with identical MRM
- 4-MeI detected at varied levels in commercial caramel colored beverages

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