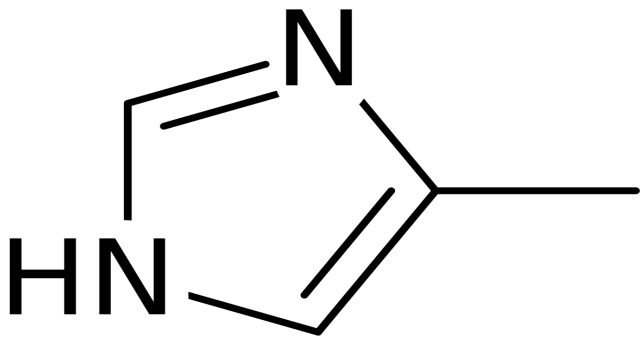
Simultaneous Determination of 2- and 4-methylimidazoles in Beverages using a Simple Filter and Shoot (FASt) Method



UCT Part Numbers

CSFAS203
Clean Screen FASt 200mg/3mL

Summary:

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, while 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 involve tedious ion-paring extraction and derivatization with GC or GC/MS detections, or solid phase extraction (SPE) with HPLC or LC/MS-MS detections. This application offers a simple, fast, and cost effective method to determine 2-MeI and 4-MeI inbeverages simultaneously.

Beverage samples were degassed, diluted by 10 times with acetonitrile (MeCN), and filtered through a SPE cartridge with 200 mg of the novel FASt sorbent. The undesired matrix components, such as sugars and organic acids were retained, resulting in clean samples 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.

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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 hr.

Filter and Shoot (FASt) procedure:

1) 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.
2) Attach the FASt cartridges (**CSFAS203**) to a glass block manifold or positive pressure manifold, insert test tubes or 2-mL auto-sampler vials into the manifold.
3) Transfer the diluted samples onto the cartridges, apply low vacuum or positive pressure and collect the filtrates.
4) The samples are ready for LC-MS/MS analysis.

LC-MS/MS Parameters	
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	0.400 mL/min
Injection Volume	10 µL
Auto-sampler Temperature	10 °C
Mobile Phase (Isocratic 8 min)	5% of 50 mM ammonium formate in water and 95% of MeCN
Divert Mobile Phase	To waste from 0 - 1 min to prevent ion source contamination

MS Parameters	
Polarity	ESI^+
Spray voltage V	5000 V
Vaporizer temperature	242 °C
Ion transfer capillary	398 °C
Sheath gas pressure	60 arbitrary units
Auxiliary gas pressure	20 arbitrary units
Q1 and Q3 peak width	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

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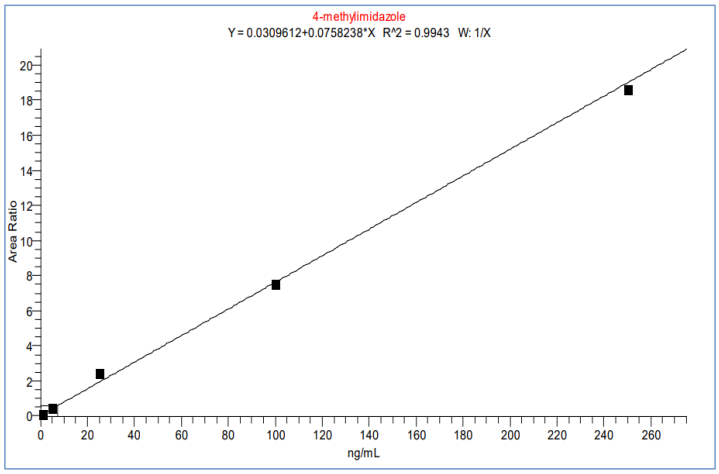
SRM Transitions							
Compound	Rt (min)	Precursor ion	Product ion 1	CE 1	Product ion 2	CE 2	S-lens (V)
Imidazole (IS)	1.96	69.07	42.01	21	28.08	74	65
4-Mel	3.18	83.08	56.05	17	42.00	27	45
2-Mel	5.72	83.07	42.04	20	56.05	19	48

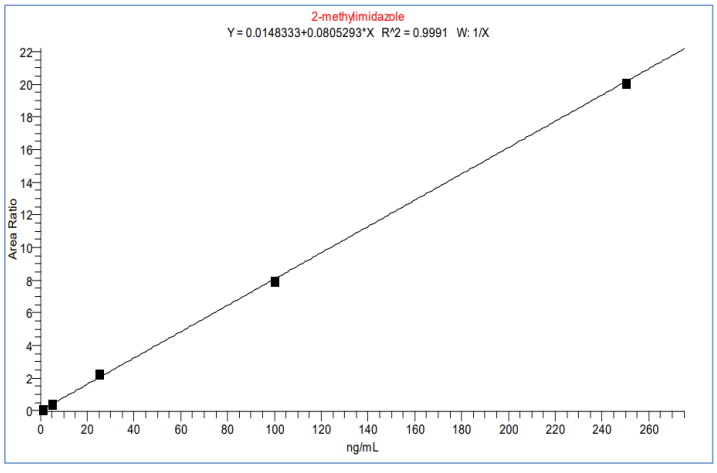
Results:

Recovery and RSD Obtained from a Negative Beverage Sample Fortified with 100 ng/mL of 2-Mel and 4-Mel

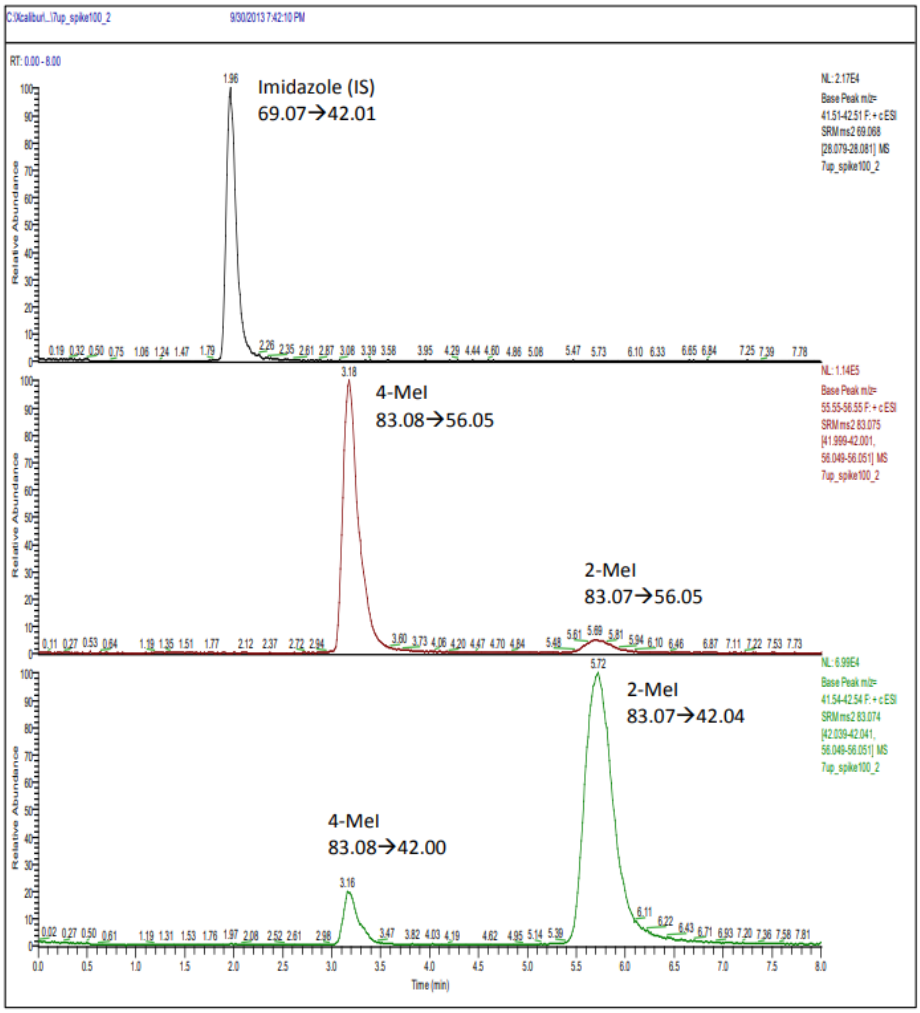
Compound	Recovery %	RSD % (n=6)
4-Mel	**103.6**	4.1
2-Mel	**102.9**	1.6

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





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



Results of Real Sample Analysis

Beverages	Detected 4-MeI* (ng/mL)	
	Conc. in the diluted sample	Conc. in the original sample
Colorless soda	< 1	**< 10**
Root beer	7.4	**74**
Sweet tea	12.8	**128**
Cola_AZ	84.0	**840**
Cola _CO	28.3	**283**
Cola _LA	43.9	**439**
Cola _MD	46.4	**464**
Cola _MS	59.2	**592**
Cola _OR	10.7	**107**
Cola _TN	58.6	**586**
Cola _TX	59.2	**592**
Cola_WA	10.1	**101**

*: 2-MeI was not detected in any samples tested in this study.

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