



## **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)

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### **Summary:**

2-methylimidazole (2-Mel) and 4-methylimidazole (4-Mel) 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-Mel, while California lists 4-Mel as a probable carcinogen and proposed a 16 µg/day NSRL. Meanwhile, the European Food Safety Authority (EFSA) considers 4-Mel safe and established a maximum level of 250 mg/kg in caramels.

Traditional analytical methods for 2-Mel and 4-Mel involve tedious ion-pairing 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-Mel and 4-Mel in beverages 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-Mel and 4-Mel 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.

## 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  $\mu$ L 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 method:

<b>HPLC:</b> Thermo Scientific, Dionex UltiMate 3000 <sup>®</sup> LC System
<b>Column:</b> Thermo Scientific, Accucore HILIC, 100 x 2.1 mm, 2.6 $\mu$ m
<b>Guard Column:</b> Thermo Scientific, Accucore HILIC, 10 x 2.1 mm, 2.6 $\mu$ m
<b>Column Temperature:</b> 40 °C
<b>Column Flow Rate:</b> 0.400 mL/min
<b>Auto-sampler Temperature:</b> 10 °C
<b>Injection Volume:</b> 10 $\mu$ L
<b>Mobile Phase (Isocratic 8 min):</b> 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 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

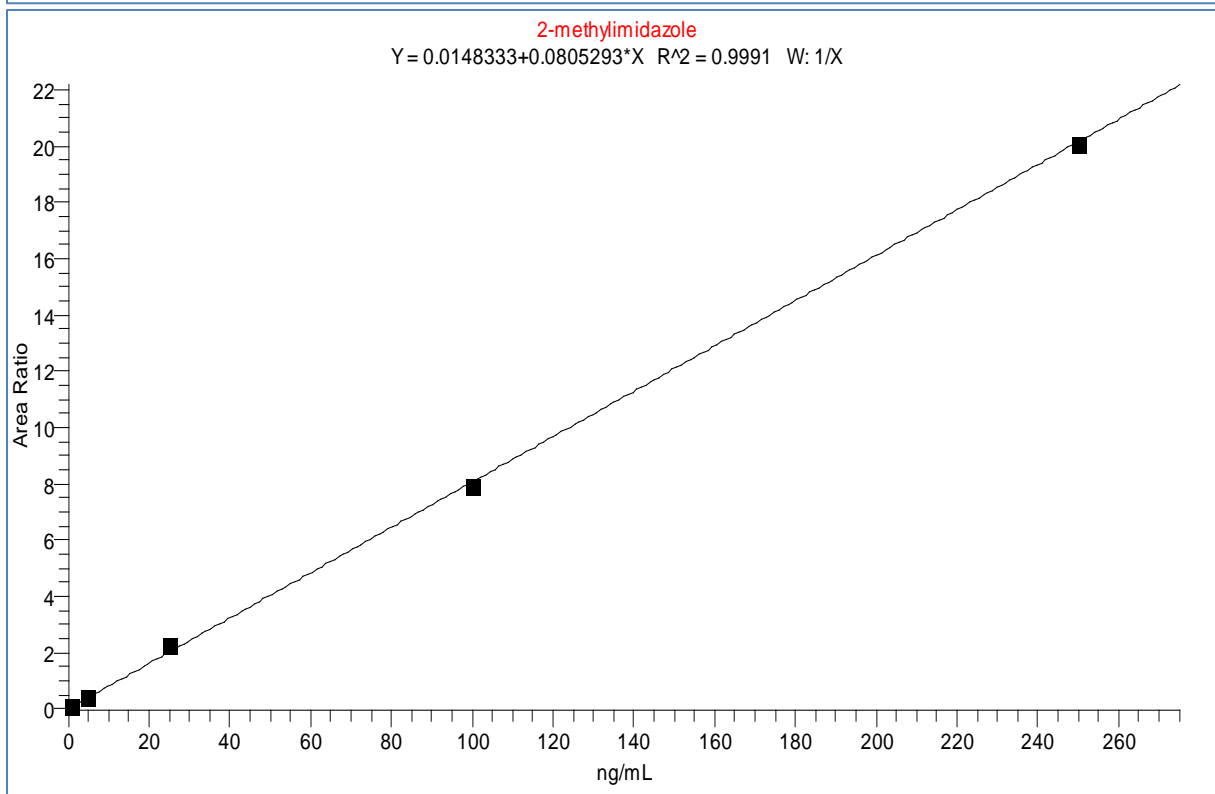
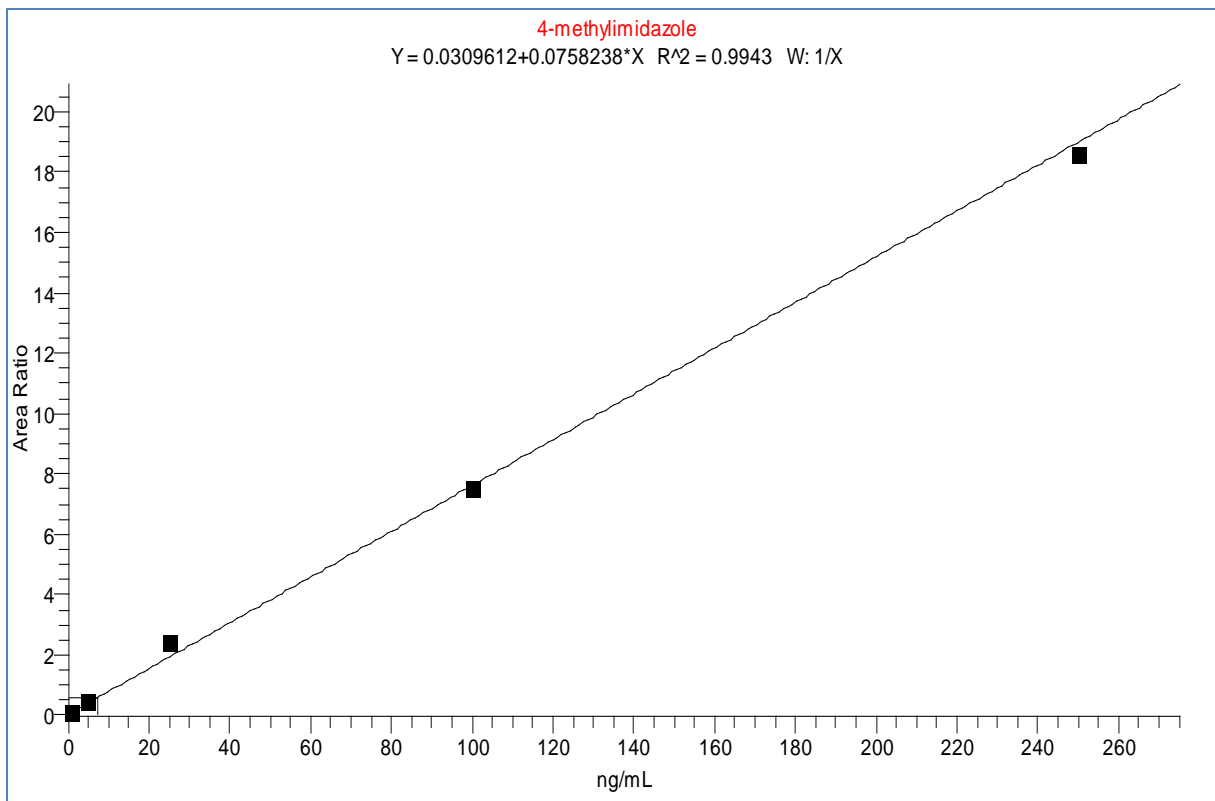
SRM transitions							
Compound	Rt (min)	Precursor ion	Product ion 1	CE 1	Product ion 2	CE 2	S-lens (V)
Imidazole (IS)	1.96	69.07	42.01	21	28.08	74	65
4-Mel	3.18	83.08	56.05	17	42.00	27	45
2-Mel	5.72	83.07	42.04	20	56.05	19	48

## Results:

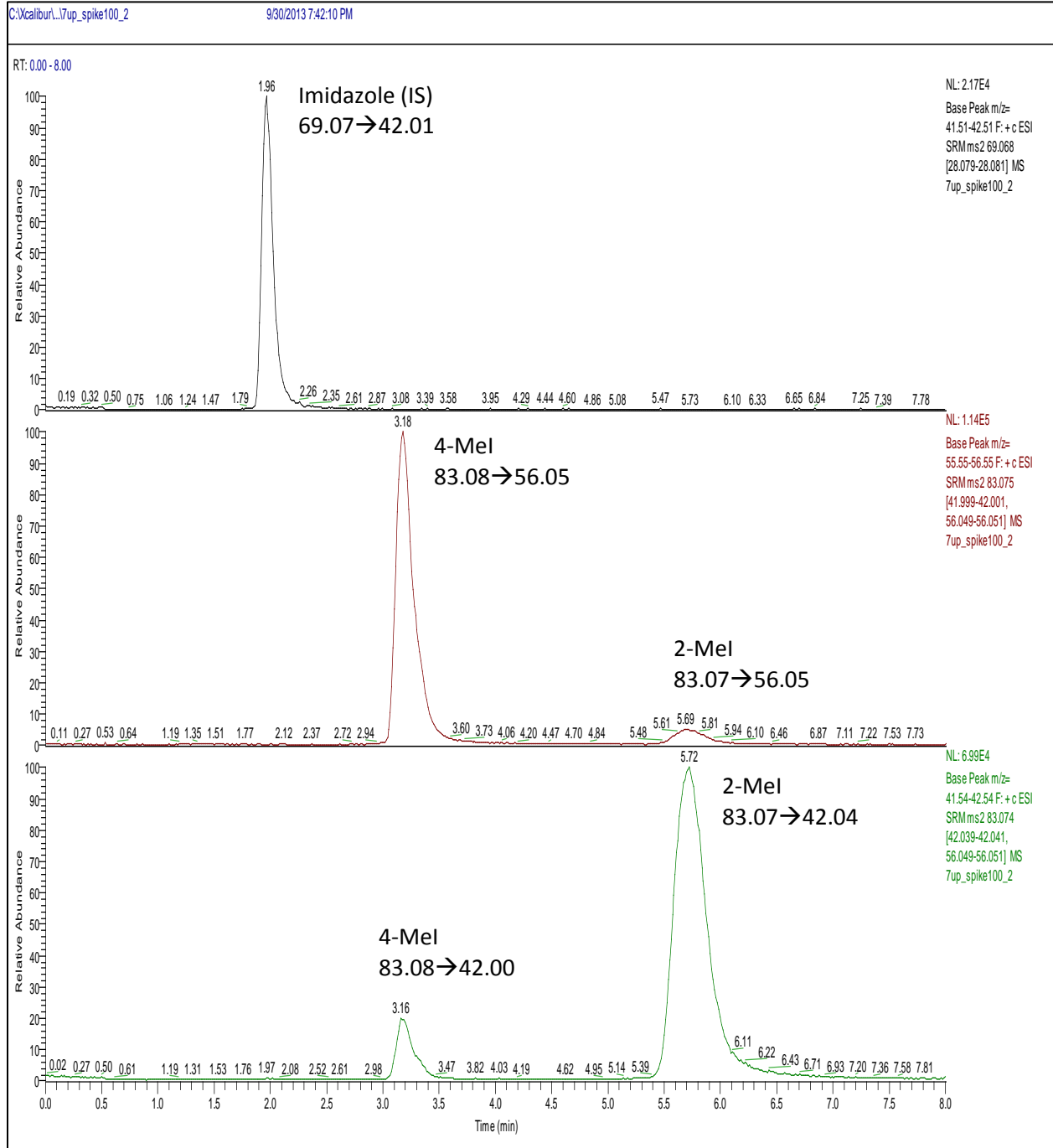
### Recovery and RSD Obtained from a Negative Beverage Sample Fortified with 100 ng/mL of 2-Mel and 4-Mel

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-Mel and 4-Mel



### Results of Real Sample Analysis

Beverages	Detected 4-Mel* (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-Mel was not detected in any samples tested in this study.