LC-MS/MS Analysis of Emerging Food Contaminants

Quantitation and Identification 4-Methylimidazole (4-MEI) in Beverages

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Introduction

4-Methylimidazole, also known as 4-MEI, is a by-product produced during the manufacturing of caramel coloring used to darken food products and can be found in carbonated beverages, such as cola, as well as in a variety of other products such as coffee, beer, soy sauce, and baked goods.

The International Agency for Research on Cancer (IARC) classified the 4-MEI as group 2B compound 'possibly carcinogenic to humans'.[1]

The use of caramel coloring in sodas, in particular, and the overall safety of 4-MEI has been the center of some recent controversy after the California Office of Environmental Health Hazard Assessment (OEHHA) included 4-MEI on its Proposition 65 list of potential human carcinogens on January 7, 2011 and proposed establishing a no significant risk level (NSRL) of 29 micrograms per day.[2-3] In response, large beverage producers have directed their caramel suppliers to modify their manufacturing processes to reduce the levels of 4-MEI.

The European Food Safety Authority (EFSA) considers 4-MEI as safe and established a maximum level for 4-MEI in caramel coloring (E 150c ammonia caramel and E 150d sulphite ammonia caramel) of 250 mg/kg.[4]

Different analytical techniques, including GC, GC-MS, and LC-UV are in use for the analysis of 4-MEI. The official method for caramel analysis written by the Joint FAO/WHO Expert Committee on Food Additives is based on a method of Wilks et al. using GC.[5] All of these method require long time for sample preparation and analysis. An online SPE-LC-UV method was developed by Moretton et al. which allows automation and accurate quantitation at low ppm levels.[6]

Here we present a new and improved method using LC-MS/MS to significantly simplify sample preparation, decrease chromatographic run time, and allow accurate and reproducible quantitation down to sub ng/mL (ppb) levels in beverages.

Experimental

Sample Preparation

The sensitivity and selectivity of the SCIEX QTRAP® 4500 System allows minimal sample preparation for this analysis. Beverage samples were centrifuged and diluted 10x with water before LC-MS/MS analysis.

LC

LC separation was achieved using the Shimadzu UFLC$_{XR}$ system with a Hypercarb 5 µm (100 x 2.1 mm) column with a gradient of water and methanol containing 0.1% formic acid at a flow rate of 0.5 mL/min. The injection volume was set to 20 µL.

MS/MS

The SCIEX QTRAP 4500 was operated in Multiple Reaction Monitoring (MRM) mode. The Turbo V™ source was used with an Electrospray Ionization (ESI) probe in positive polarity. Two selective MRM transitions were monitored for 4-MEI (83/56 and 83/42) using the ratio of quantifier and qualifier ion for compound identification (Table 1).

LC-MS/MS data was processed using the MultiQuant™ Software version 2.1.

Table 1. MS/MS Parameters for the detection of 4-MEI using the SCIEX QTRAP® 4500 system

MRM	Q1/Q3	DP (V)	CE (V)
4-MEI 1	83/56	26	25
4-MEI 2	83/42	26	35

Results and Discussion

First, limit of detection (LOD), limit of quantitation (LOQ), linearity, and reproducibility were evaluated using injections of 4-MEI ranging in concentration from 0.1 to 100 ng/mL.

Signal to noise (S/N) was calculated using the 3x standard deviation algorithm. By this approach, the LOD was determined to be 0.1 ng/mL (S/N > 3) and the LOQ, 0.5 ng/mL (S/N > 6). The MRM ratio was calculated using all standard injection resulting in an average of 0.193 (Figure 1).

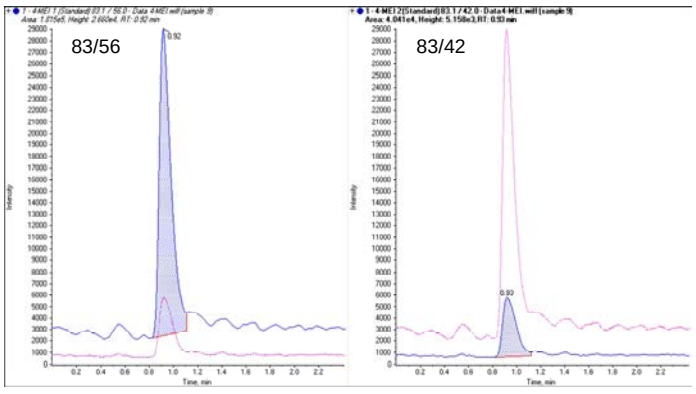

Figure 1. Injection of 1 ng/mL of 4-MEI, LOD was found at 0.1 ng/mL and LOQ at 0.5 ng/mL, the average ratio of quantifier and qualifier ion was 0.193

Linearity was observed from 0.1 to 100 ng/mL with accuracy values between 88.7 and 111.2% and a regression coefficient of 0.997 (Figure 2). Quality control samples at 1 ng/mL were injected 10 times resulting in a coefficient of variation (%CV) of 2.6%.

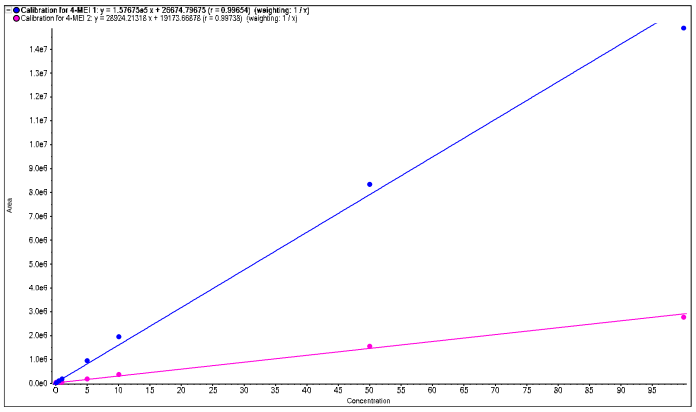

Figure 2. Linear range of the detection of 4-MEI from 0.1 to 100 ng/mL with an r of 0.997 for both MRM transitions

This level of sensitivity allows the direct injection of beverage samples without using time-consuming and extensive sample cleanup. Samples were injected directly after centrifugation and a simple 10x dilution to minimize possible matrix effects.

Several cola samples were purchased from a local store and analyzed by the method described. The MRM chromatograms of two samples are shown in Figure 3. When quantified and corrected for dilution, the samples were determined to contain 46.6 ng/mL and 78.2 ng/mL of 4-MEI, respectively.

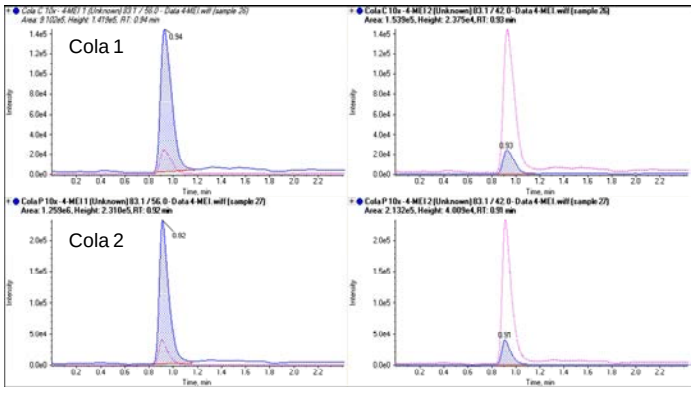

Figure 3. Quantitation and identification of 4-MEI in store bought cola

MRM ratios for compound identification were automatically calculated and compared in the MultiQuant™ Software. The MRM ratios of 0.169 and 0.166, respectively, were well in between the 20% tolerances of 0.154 and 0.232 clearly confirming the identity of 4-MEI.

Ideally, an isotopically labeled internal standard of 4-MEI should be used to eliminate matrix effects (ion suppression or ion enhancement) and to improve accuracy of detection in unknown samples, but such an internal standard was not available.

To evaluate matrix effects in this analysis, we performed standard additions to more accurately quantify 4-MEI in the matrix itself. For our standard addition analysis, we added defined concentrations of 4-MEI to aliquots of the unknown sample. These standards, along with an aliquot of the unknown sample which does not contain any added standard, were analyzed. The resulting calibration curve was extrapolated and the absolute value of the intercept with the concentration axis was used to determine the concentration of the target compound in the unknown sample.

Standard addition can be calculated automatically in the MultiQuant Software using the 'Standard Addition' query (Figure 4).

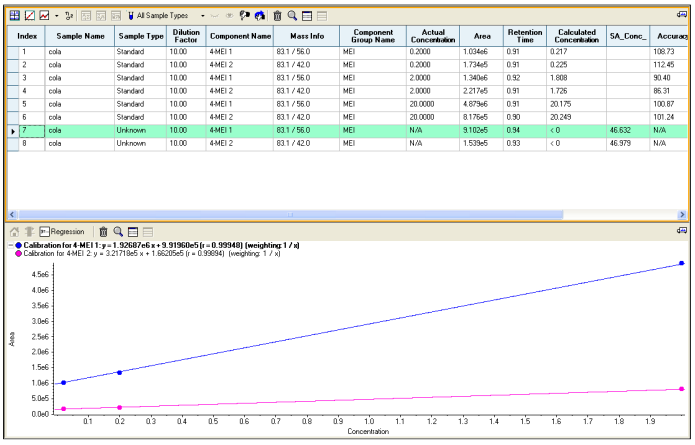

Figure 4. Automatic quantitation using the 'Standard Addition' query in the MultiQuant Software

Summary

The method and data presented here showcase the fast and accurate solution for the quantitation and identification of 4-MEI in beverage samples by LC-MS/MS. The SCIEX QTRAP® 4500 System provides excellent sensitivity and selectivity for this analysis, with minimal sample preparation allowing maximized throughput for the analysis of many samples in a short time period.

4-MEI was quantified in store bought cola samples. Automatic MRM ratio calculation in MultiQuant Software was used for compound identification.

Standard addition was also used to assess any possible quantitation errors caused by ion suppression or ion enhancement. Calculations were automatically performed using the 'Standard Addition' query.

References

1. Agents Classified by the IARC Monographs, Volumes 1-104 (last update March 27, 2012) http://monographs.iarc.fr/ENG/Classification/ClassificationsAlphaOrder.pdf
2. Proposition 65: 'Chemicals known to the state to cause cancer or reproductive toxicity' (last update March 16, 2012) http://oehha.ca.gov/prop65.html
3. Proposition 65: 'No significant risk level (NSRL) for the proposition 65 carcinogen 4-methylimidazole' (October 2011) http://oehha.ca.gov/prop65/law/pdf_zip/1007114MEI.pdf
4. EU Commission Directive 2008/128/EC 'Specific purity criteria concerning colours for use in foodstuffs' (December 22, 2008)
5. R. A. Wilks et al.: 'An Improved Method for the Determination of 4-Methylimidazole in Caramel Color' J. Agric. Food Chem. 25 (1977) 605-608
6. C. Moretton et al.: 'Quantification of 4-Methylimidazole in Class III and IV Caramel Colors: Validation of a New method Based on Heart-Cutting Two-Dimensional Liquid Chromatography (LC-LC)' J. Agric. Food Chem. 59 (2011) 3544-3550

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