Application Note

Food Testing & Agriculture

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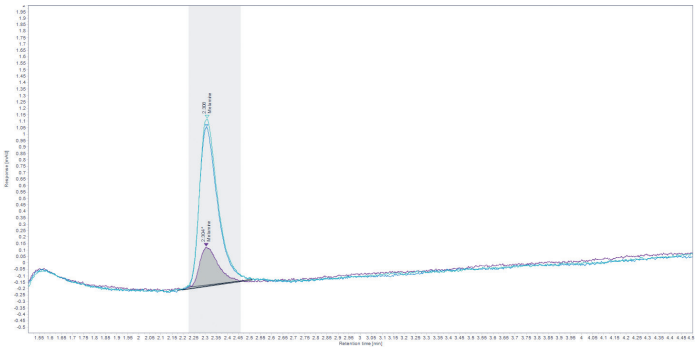
Does your Bamboo Coffee Cup Give off Melamine?

Analyzing melamine migration from bamboo kitchenware using the Agilent 1260 Infinity II Prime LC



Author

Sonja Schipperges
Agilent Technologies, Inc.

Abstract

Recently, the European Rapid Alert System for Food and Feed (RASFF) has stated numerous cases of melamine migration from bamboo kitchenware exceeding the specific migration limit (SML). Excessive melamine migration has occurred in items such as bamboo coffee-to-go cups. This application note demonstrates the analysis of melamine migration from two bamboo coffee-to-go cups and one bamboo cup for children using hydrophilic interaction liquid chromatography (HILIC) on the Agilent 1260 Infinity II Prime LC System. The Agilent 1260 Infinity II Diode Array Detector HS with the high sensitivity Agilent InfinityLab Max-Light cartridge cell provides high confidence in the determination of melamine migration below the SML due to the superior sensitivity. The analysis reveals that two of the three tested bamboo cups exceed the SML for melamine.

Introduction

In recent years, reusable coffee-to-go cups sold and marketed as made from bamboo are increasingly being used. These cups, however, are typically made from melamine-formaldehyde resin next to bamboo fibers, which are used as a filler material.

Melamine-formaldehyde resin is widely used in kitchenware. Upon contact with food, migration of melamine and formaldehyde into food may occur.[1,2] The regular intake of large amounts of melamine (2,4,6-triamine-1,3,5-triazine) over time may lead to the formation of stones in the urinary system and may induce kidney damage.[3]

In Commission Regulation (EU) No. 10/2011 on plastic materials and articles intended to come into contact with food, European legislation establishes a specific migration limit (SML) of 2.5 mg/kg for melamine.[4] The German Federal Institute for Risk Assessment (BfR) recently published a statement[3] concerning the migration of melamine and formaldehyde from coffee-to-go cups made from melamine-formaldehyde resin and bamboo fibers. 35% of tested coffee-to-go cups made from melamine-formaldehyde resin and bamboo fibers exceeded the SML of 2.5 mg/kg for melamine.[3] This percentage was even higher compared to tested coffee-to-go cups made solely from melamine-formaldehyde resin.[3] Accordingly, RASFF has cited numerous cases of increased melamine migration from bamboo kitchenware.[5]

This application note shows the analysis of melamine migration from cups made of melamine-formaldehyde resin and bamboo fibers using HILIC on the 1260 Infinity II Prime LC System.

Experimental

Equipment

The Agilent 1260 Infinity II Prime LC System comprised the following modules:

- Agilent 1260 Infinity II Flexible Pump (G7104C)
- Agilent 1260 Infinity II Vialsampler (G7129C) with sample thermostat (option 101)
- Agilent 1260 Infinity II Multicolumn Thermostat (G7116A)
- Agilent 1260 Infinity II Diode Array Detector HS (G7117C) with the high sensitivity InfinityLab Max-Light cartridge cell, 60 mm (G4212-60007)

Software

Agilent OpenLab CDS Version 2.4.

Columns

Agilent InfinityLab Poroshell 120 HILIC-Z, 2.1 × 100 mm, 2.7 µm (p/n 685775-924)

Chemicals

All solvents were LC grade. Acetonitrile was purchased from Merck (Darmstadt, Germany). Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with a 0.22 µm membrane point-of-use cartridge (Millipak, EMD Millipore, Billerica, MA, USA). Melamine was purchased from Sigma-Aldrich (Steinheim, Germany). Ammonium formate, formic acid, and acetic acid were obtained from VWR (Darmstadt, Germany).

Standards

A melamine stock solution was prepared at a concentration of 1.03 mg/mL by dissolution of melamine in water containing 3% acetic acid. From the stock solution, melamine standard solutions were prepared at concentrations of 10.3, 30.9, 61.8, 103, 309, 618, 1,030, and 3,090 ng/mL in acetonitrile/water containing 3% acetic acid (90/10, v/v). Calibration was performed in the range of 61.8 to 3090 ng/mL. Calibration standards were analyzed in triplicate. The lower concentrations of standard solutions were used for estimation of the limit of detection (LOD) and limit of quantification (LOQ) based on the signal-to-noise ratio (S/N).

A melamine control sample was prepared at a concentration of 289 ng/mL in acetonitrile/water containing 3% acetic acid (90/10, v/v). The concentration was chosen close to the expected melamine concentration for samples with melamine migration corresponding to the specific migration limit. The control sample was used to determine precision.

Samples and sample preparation

Two bamboo coffee-to-go cups and one bamboo cup for children were obtained from an online store. Investigation of melamine migration from the bamboo cups was performed in accordance with Commission Regulation (EU) No 10/2011.[4] Water with 3% acetic acid was used as a simulant and the conditions for articles intended for a hot-fill were used. Briefly, the bamboo cups were filled to approximately 0.5 cm below the rim with water containing 3% acetic acid preheated to 70 °C. The filled cups were covered with aluminum foil to limit evaporation. The cups were placed

in a water bath and kept at 70 °C for two hours. The volume of simulant was recorded for each cup. A 1 mL sample of the simulant was diluted to 10 mL with acetonitrile for HILIC analysis. The HILIC analysis was performed in triplicate. The migration experiment was repeated three times for each cup to simulate repeated use.

Results and discussion

Melamine (2,4,6-triamine-1,3,5-triazine, Figure 1) is a very polar compound, therefore, HILIC is ideally suited for its analysis. Figure 2 shows the analysis of a melamine standard, indicating sufficient retention as well as good peak shape.

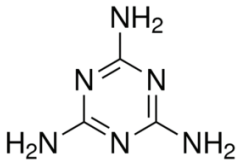


Figure 1. Melamine.

Table 1. HILIC method for melamine analysis.

Parameter	Value
Column	Agilent InfinityLab Poroshell 120 HILIC-Z, 2.1 × 100 mm, 2.7 µm
Solvent	A) 10 mM Ammonium formate in water pH 3 B) Acetonitrile/200 mM ammonium formate in water pH 3 (95/5, v/v)
Gradient	0.00 min – 100% B 3.00 min – 100% B 4.00 min – 60% B Stop time: 5 min Post time: 10 min
Flow Rate	0.500 mL/min
Temperature	30 °C
Detection	240 nm/4 nm, reference 360 nm/100 nm 20 Hz
Injection	Injection volume: 2 µL Sample temperature: 8 °C Needle wash: 3 s in water/acetonitrile (50/50)

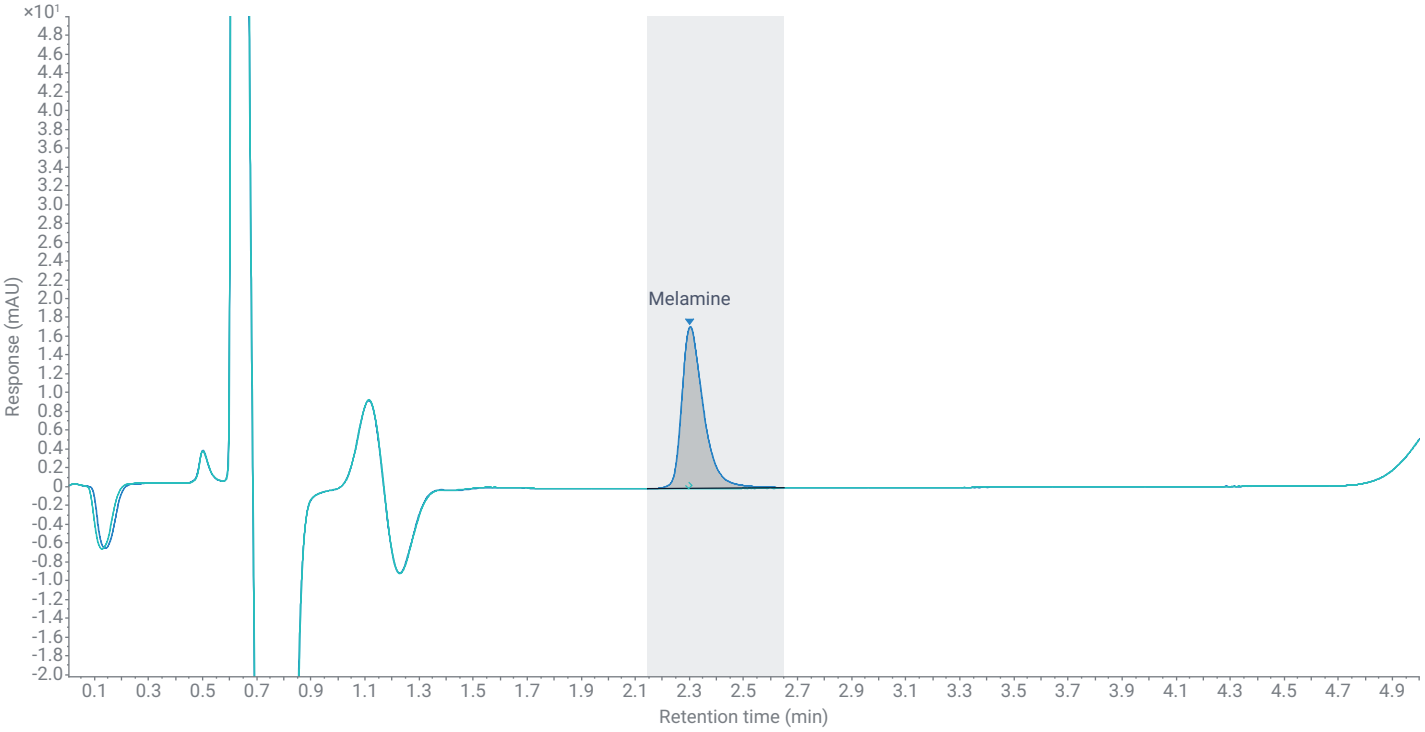
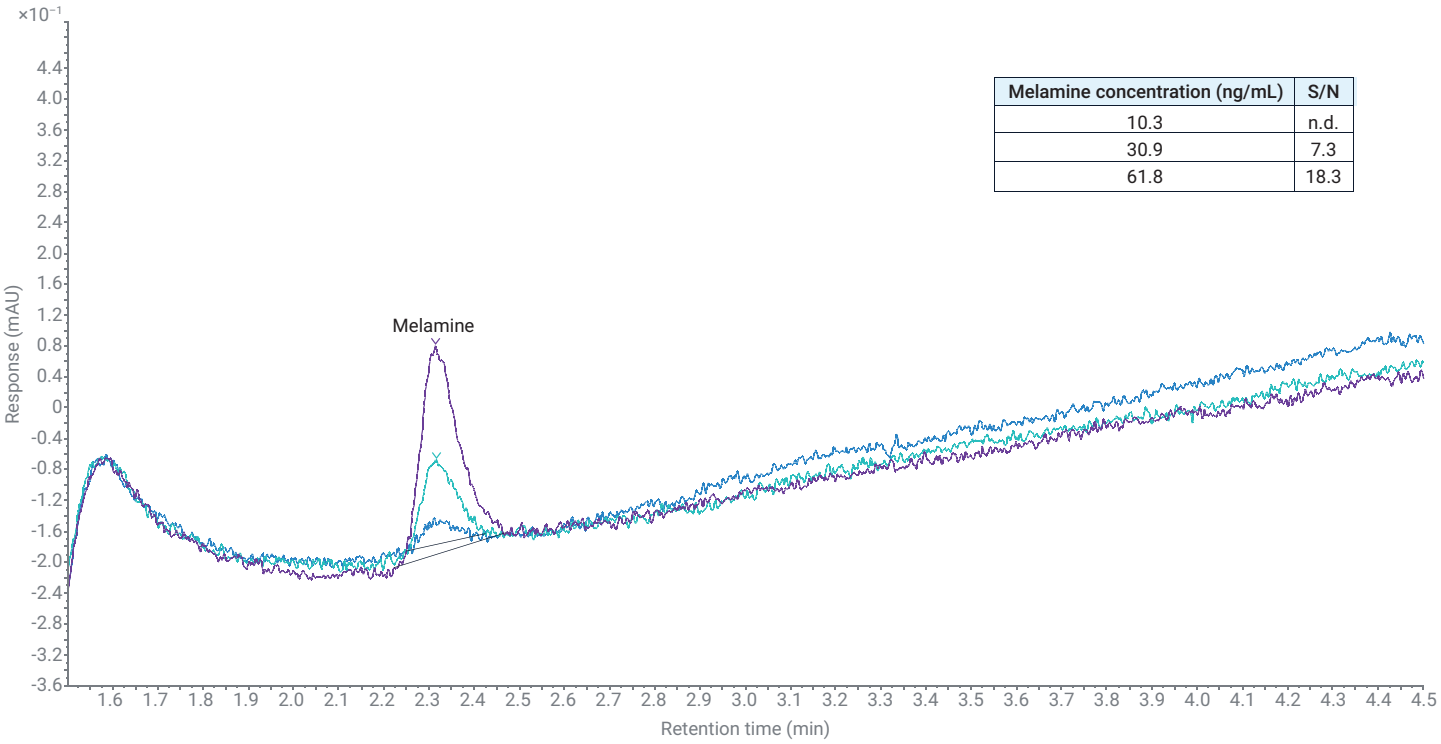


Figure 2. HILIC analysis of melamine showing an overlay of a 3,090 ng/mL melamine standard (blue) and a blank run (turquoise).

To verify that the method provides sufficient sensitivity for melamine detection and quantification below the SML of 2.5 mg/kg, the LOD and LOQ were determined. Figure 3 shows the analysis of melamine standards in the concentration range of 10.3 to 61.8 ng/mL and the resulting S/N values. The S/N was calculated using the peak-to-peak noise determination in OpenLab CDS. Based on these results, the LOD (S/N = 3) and the LOQ (S/N = 10) can be estimated as 13 ng/mL and 42 ng/mL, respectively. Considering that simulants from migration experiments need to be diluted tenfold with acetonitrile for successful HILIC analysis, an LOQ <250 ng/mL is required. This LOQ is achieved by the used method.

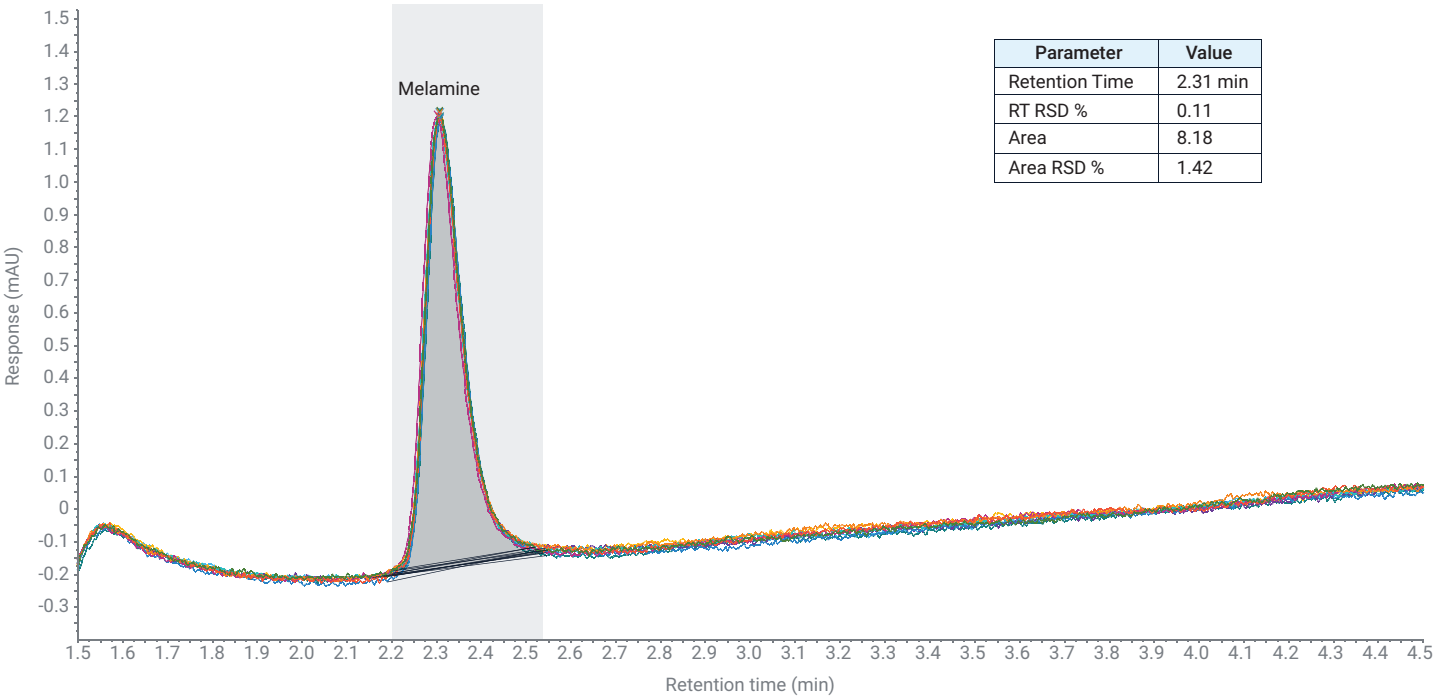
Precision of the HILIC analysis of melamine was investigated by tenfold analysis of the melamine control sample. Excellent retention time and peak area precision were obtained (Figure 4).

Figures 5 and 6 show the analysis of the melamine calibration standards in the concentration range of 61.6 to 3,090 ng/mL and the resulting calibration curve. Excellent linearity of melamine calibration with a correlation coefficient >0.9999 is observed.



Melamine concentration (ng/mL)	S/N
10.3	n.d.
30.9	7.3
61.8	18.3

Figure 3. HILIC analysis of 10.3 ng/mL (blue), 30.9 ng/mL (turquoise) and 61.8 ng/mL (purple) melamine standards and the corresponding S/N values.



Parameter	Value
Retention Time	2.31 min
RT RSD %	0.11
Area	8.18
Area RSD %	1.42

Figure 4. Precision of HILIC analysis of melamine investigated by tenfold analysis of the 289 ng/mL melamine control sample.

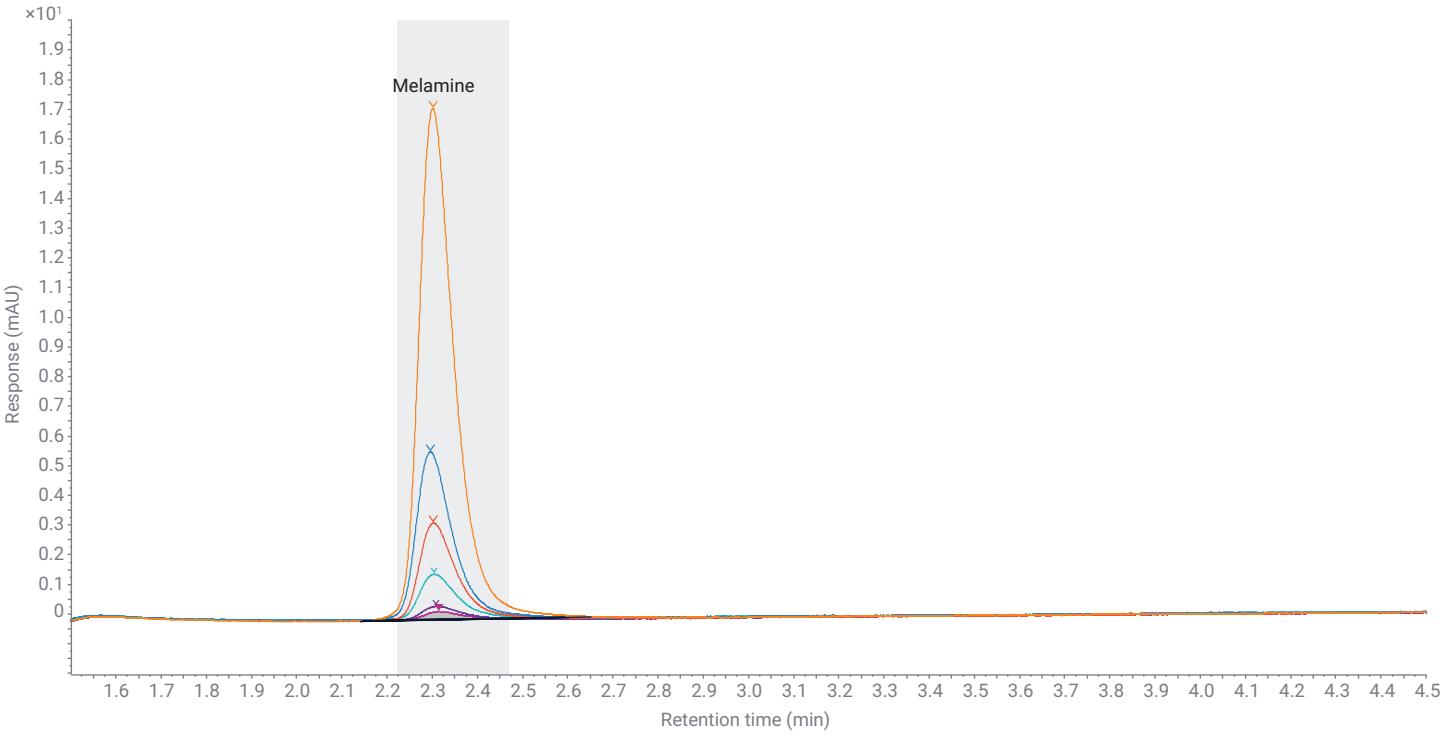


Figure 5. HILIC analysis of melamine calibration standards in the concentration range of 61.8 to 3,090 ng/mL.

The analysis of simulants obtained from migration experiments conducted in accordance with Commission Regulation (EU) No. 10/2011[4] for two bamboo coffee-to-go cups and one bamboo cup for children revealed migration of melamine for all samples. Figure 7 shows the analysis of simulants obtained from the third migration experiment for all samples. Melamine is clearly detected above the LOQ. Figure 8 shows the results from the quantification of melamine for all migration experiments.

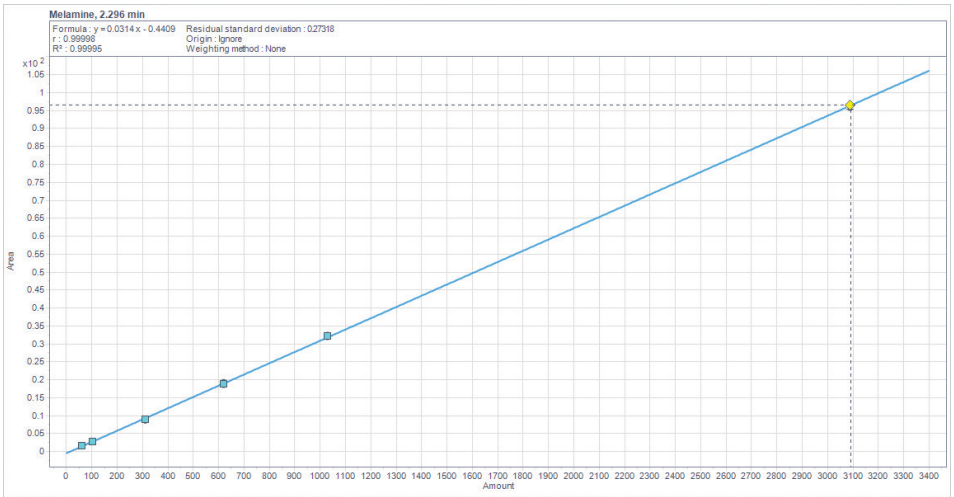


Figure 6. Melamine calibration curve in the concentration range of 61.8 to 3,090 ng/mL.

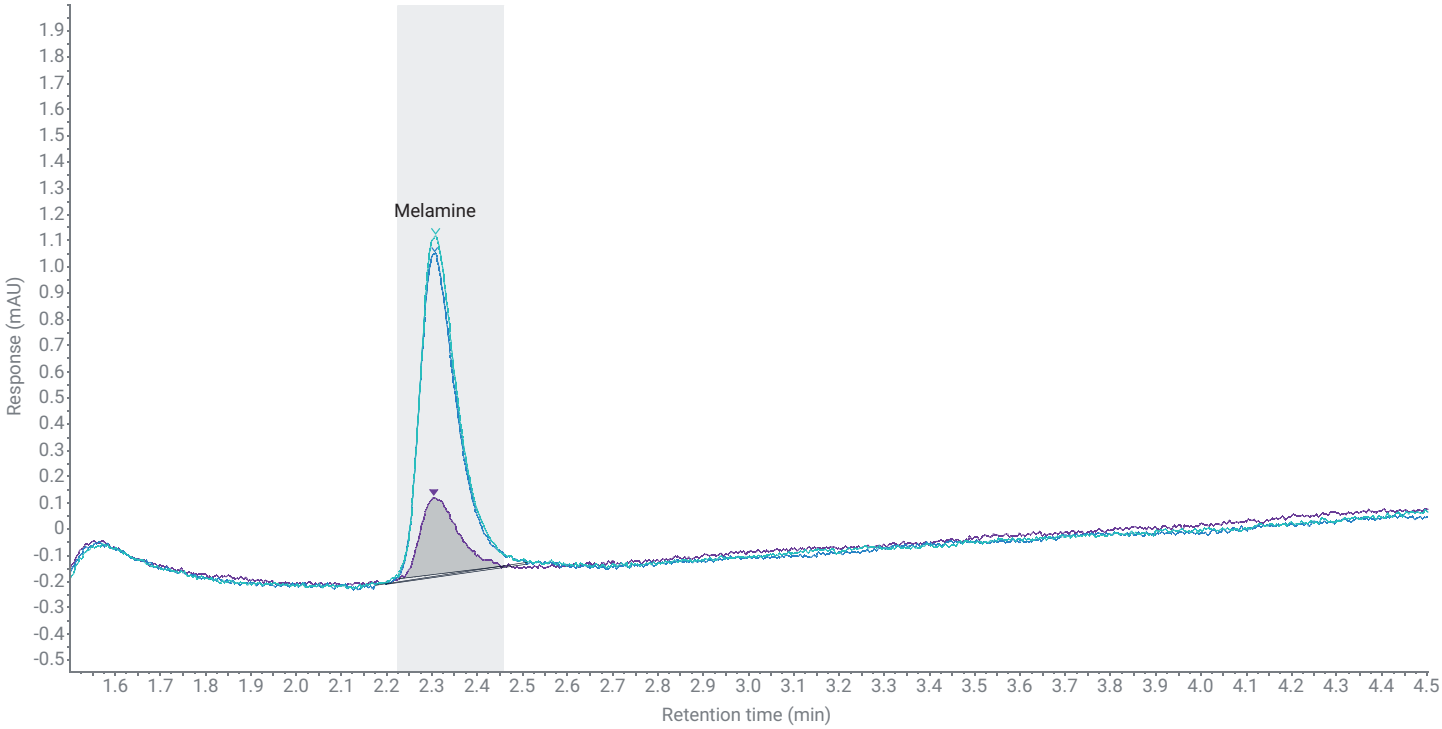


Figure 7. HILIC analysis of simulants obtained from the third migration experiment for bamboo coffee cup 1 (purple), bamboo coffee cup 2 (blue) and the bamboo cup for children (turquoise).

For repeated use articles, such as the tested bamboo cups, compliance should be checked against the level of migration found in the third migration experiment according to Commission Regulation (EU) No. 10/2011.[4] In the third migration experiment, one of the bamboo coffee-to-go cups as well as the bamboo cup for children exceeded the SML for melamine of 2.5 mg/kg.

According to the statement from the BfR,[3] repeated migration experiments showed an increase in the release of melamine from coffee-to-go cups made from melamine-formaldehyde resin and bamboo fibers. The assumed reason for this increasing release of melamine is that the material is affected by contact with hot liquid.[3] This finding leads to the expectation that melamine migration from the tested bamboo cups would increase in further migration experiments.

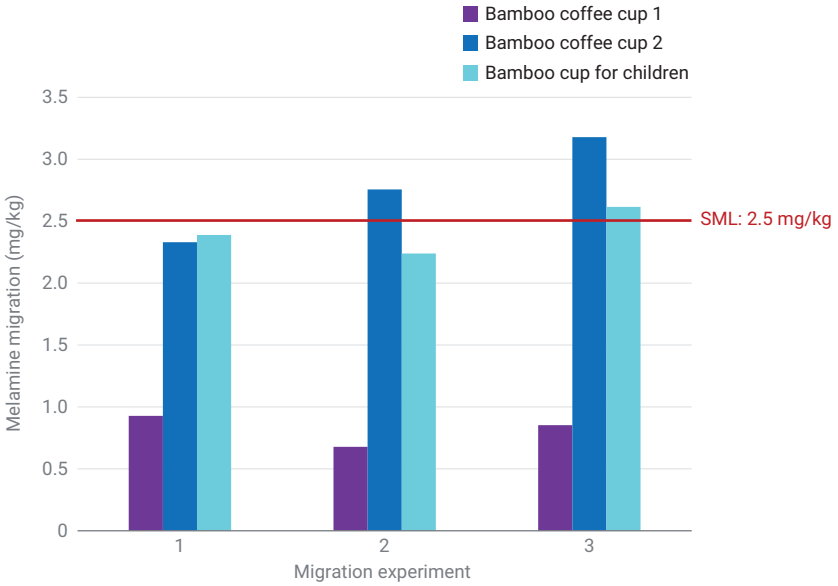


Figure 8. Melamine migration quantified for the three migration experiments performed for the bamboo coffee cups and the bamboo cup for children.

Conclusion

Bamboo coffee-to-go cups are typically made from melamine-formaldehyde resin and bamboo fibers, and can release melamine upon contact with food. The HILIC analysis of melamine using the 1260 Infinity II Prime LC System shows excellent precision and linearity. The 1260 Infinity II Diode Array Detector HS with the high sensitivity InfinityLab Max-Light cartridge cell offers superior sensitivity. This sensitivity allows higher confidence in the determination of melamine migration below the specific migration limit. The analysis of melamine migration from bamboo cups, according to Commission Regulation (EU) No. 10/2011,[4] revealed that two out of three tested bamboo cups exceeded the SML for melamine.

References

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