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Accurate Mass Quantification of Brominated Flame Retardants in Milk Based on Q-TOF

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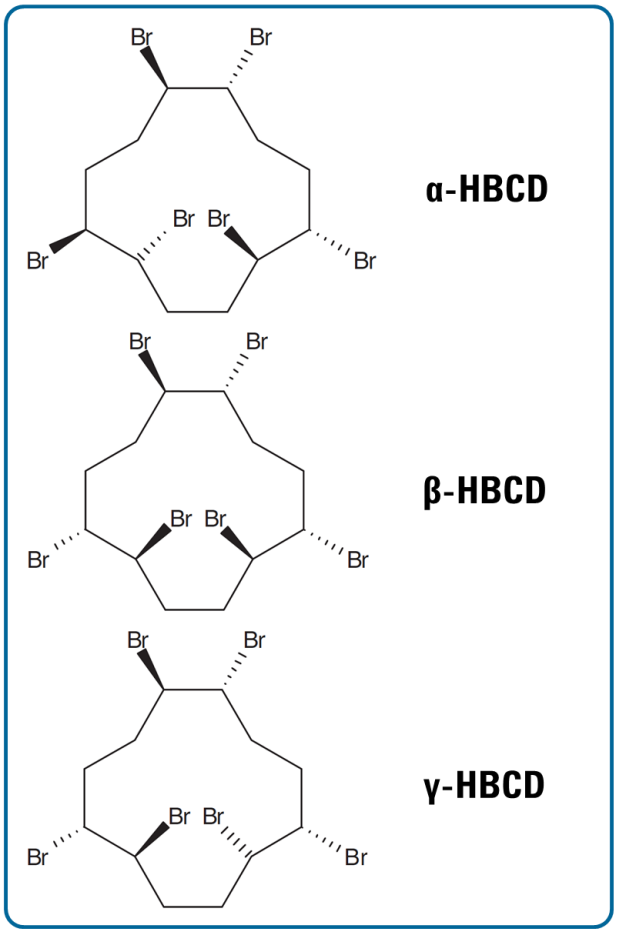
Introduction

Hexabromocyclododecane (HBCD) is a brominated flame retardant monitored as an environmental pollutant with α, β and γ diastereoisomers. Accurate quantification of these compounds in real samples plays a crucial role in the toxicological study. Quadrupole-time-of-flight (Q-TOF) for quantification is attractive because it can achieve accurate quantitation and qualitative analysis in a single run. The accurate mass based quantification efficiently eliminates interference with the same nominal mass as the analytes. In addition, it has no theoretical limit of the number of compounds for simultaneous quantification in the complex samples, which is especially critical for high-throughput of complicated samples. In this study, ultra high performance liquid chromatography tandem Q-TOF with isotopically labeled internal standard was used for quantifying α-, β- and γ-HBCD in milk.



Structure of HBCD diastereoisomers

Experimental

Reagents

The α-, β- and γ-HBCD and $^{13}C_{12}$ α-HBCD standards and samples were provided by Peking University. Methanol and acetonitrile were purchased from Merck Serono Co. Ltd.

Sample Preparation

To develop a method to quantifying HBCD in milk, both external standard method and internal standard method were employed for comparison. The internal standard $^{13}C_{12}$ α-HBCD α-HBCD was added into milk for all the three diastereoisomers, based on the assumption that the loss in the sample preparation procedure is similar for the three diastereoisomers. HBCD is extracted from milk using hexane/acetone (3:1, v/v) and then concentrated using silica gel column.

HPLC Conditions

Agilent 1290 Infinity HPLC series binary pump, well plate, thermostatted column compartment
Column: Agilent Poroshell 120-EC C18 (3.0×100 mm, 2.7 µm)

Column Temperature:	40 °C
Injection Volume:	10 µL
Autosampler Temperature:	4 °C
Needle Wash:	Methanol 10 seconds
Mobile Phase A:	Water
Mobile Phase B:	50%Methanol:50%Acetonitrile
Flow Rate:	0.4 ml/min
Gradient:	0 min- 30%A:70%B
	0.5 min- 30%A:70%B
	3.0 min- 20%A:80%B
	4.0 min- 15%A:85%B
	5.0 min- 0%A:100%B
Run/Stop time:	7 min/3 min

MS Conditions

Agilent 6530 Q-TOF Mass Spectrometer
Ion mode: Agilent Jet Stream Negative Mode

Gas Temperature:	325 °C
Gas Flow:	7 L/min
Nebulizer:	40 psi
Sheath Gas Temperature:	370 °C
Sheath Gas Flow:	11 L/min
Capillary Voltage:	3000V
Nozzle Voltage:	2000V
Mass Range:	100-1000
Acquisition Rate:	1.5 Hz
Fragmentor:	130V

Results and Discussion

HPLC Separation and Accurate Mass Quantification

The three diastereoisomers are base line separated under the HPLC condition employed. The high resolution mass spectra obtained showed isotope pattern in agreement with the brominated compounds, which confirmed the element components of the HBCD and $^{13}C_{12}$ HBCD. The extracted ion chromatogram peak area of the compounds are used for quantification.

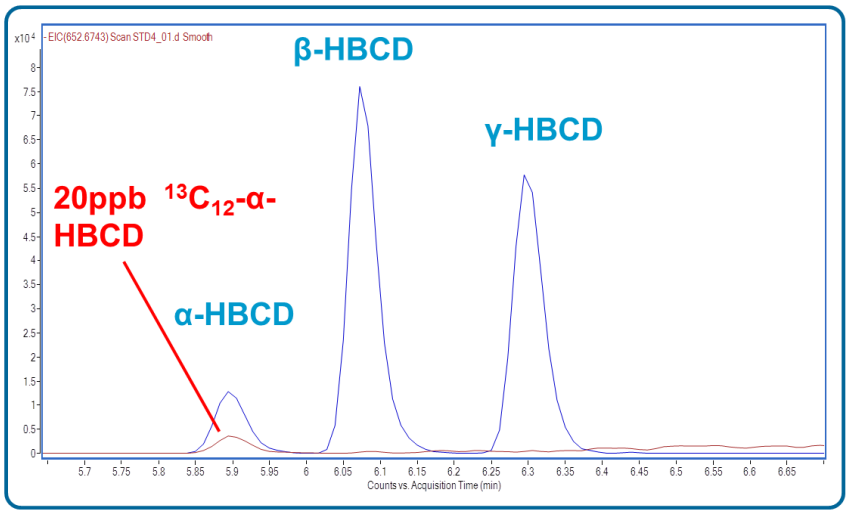


Figure 1. Extracted ion chromatogram of HBCD diastereoisomers and $^{13}C_{12}$ HBCD. The m/z 640.6416 and 652.6743 (tolerance window <10ppm) were used to extract the HBCD and $^{13}C_{12}$ HBCD respectively because they are the most abundant isotope peak of each compound.

Linearity

The linearity of external standard method and internal standard methods are different. Figure 1 shows the calibration curves of α-HBCD as an example. The external standard method shows two different linear relationship in the 40-200ppb and 200-2000ppb ranges. In contrast, the internal standard methods shows good linearity (R^2>0.997) in the concentration range (40-2000ppb, HBCD/ $^{13}C_{12}$ HBCD 2-100) in this experiment. The β- and γ-HBCD shows similar trends.

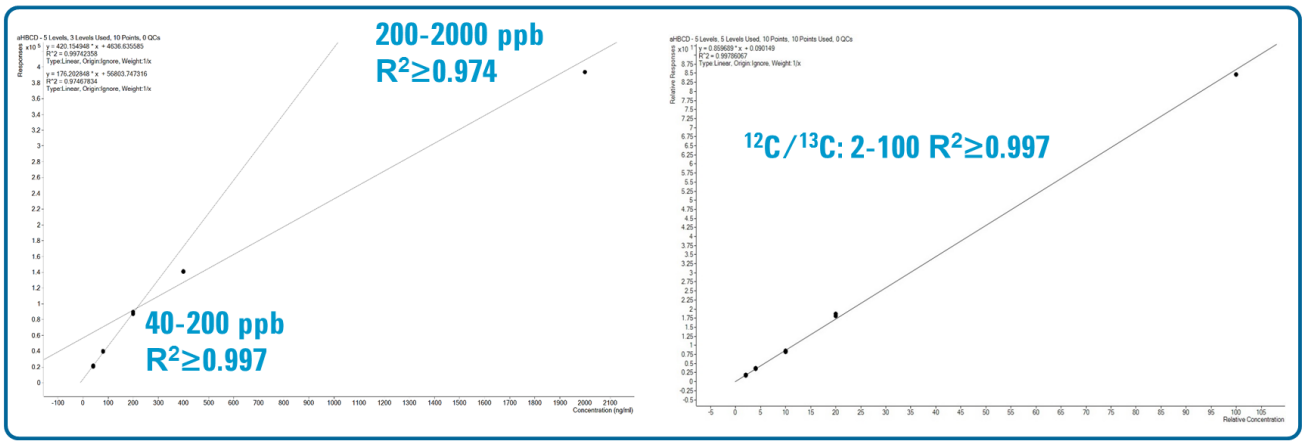


Figure 2. Calibration curve of α-HBCD using external standard method (left) and internal standard method (right).

Results and Discussion

Quantification

The external standard methods gives obviously lower concentration than those from internal standard methods, indicating the lose in the sample preparation method and suppression from matrix cannot be ignored. The established internal standard quantitative method demonstrates excellent repeatability (RSD of peak areas and retention times at 10 ng/ml is below 3.0% for all targeted compounds, n=5), good linearity (R > 0.995, 2-100ppb), and excellent sensitivity (LOQ>2 ng/ml for three analytes).

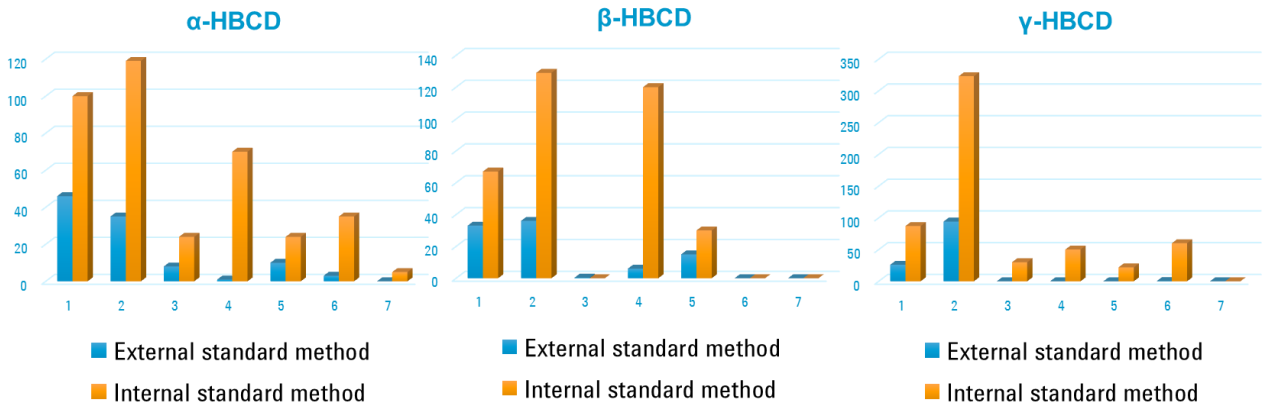


Figure 3. The α-, β- and γ-HBCD (left, middle and right, respectively) concentrations of 7 samples using external standard method and internal standard methods.

Table 3. The α-, β- and γ-HBCD concentrations of 7 samples.

#	Internal standard method/ppb			External standard method/ppb		
	α-HBCD	β-HBCD	γ-HBCD	α-HBCD	β-HBCD	γ-HBCD
1	100	67	87	46	33	26
2	119	129	323	35	36	94
3	24	0	30	8	0.3	0
4	70	120	50	0.9	6	0
5	24	30	22	10	15	0
6	35	0	60	3	0	0.2
7	5	0	0	0	0	0

Conclusions

Accurate mass quantification using Agilent 6530 Q-TOF mass spectrometry is used for HBCD determination successfully.

The established UHPLC-QTOF method provides a robust tool for simultaneously screening and determining α-, β- and γ-diastereoisomers in the milk samples with high sensitivity, high-throughput.

The internal standard methods gives better linearity and more accurate results than the external standard method.