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Determination of Brominated Vegetable Oil in Soft Drinks by UPC^2/MS

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Abstract

This application note demonstrates a rapid and simple analysis of BVO in soft drinks and beverages using UPC^2/MS. Convergence Chromatography (CC) coupled with mass spectrometry enables a rapid, simple, and direct analysis of BVO in beverages.

Benefits

- Direct analysis of representative BVO compounds
- No derivatization in sample preparation
- Short chromatography run time (9 min)
- High throughput analysis

Introduction

Brominated vegetable oil (BVO) is often used as a weighting agent, or as a solubility-transmitter for citrus oils and other lipophilic compounds[1] in soft drinks and beverages. Since lipophilic compounds are insoluble

in water and their densities are lower than 1 g/cm^3, they would gradually separate from the aqueous phase. BVO has a much higher density (1.33 g/cm^3). By blending BVO with the lipophilic ingredients, the density can be adjusted close enough to 1 g/cm^3, and a stable emulsion can be formed. The U.S. FDA has established a BVO limit at 15 ppm in finished beverages, while many countries in Europe, Asia, South America, and Australia, have banned its use in beverages.

BVO is generated by adding bromine to the double bonds of unsaturated triacylglycerides (TAGs) in vegetable oil (VO). The BVO's chemical structure or even composition is not clearly defined due to the non-uniform composition of the starting material, VO. Figure 1 shows the structure of a BVO compound. Analysis of BVO is rarely reported. Gas chromatography with mass spectrometry (GC-MS) has been proposed recently for the analysis of BVO in soft drinks and cocktail syrups.[2,3] This GC-MS method requires tedious derivatization (or saponification) of BVO, and has a long run time (about 50 min).

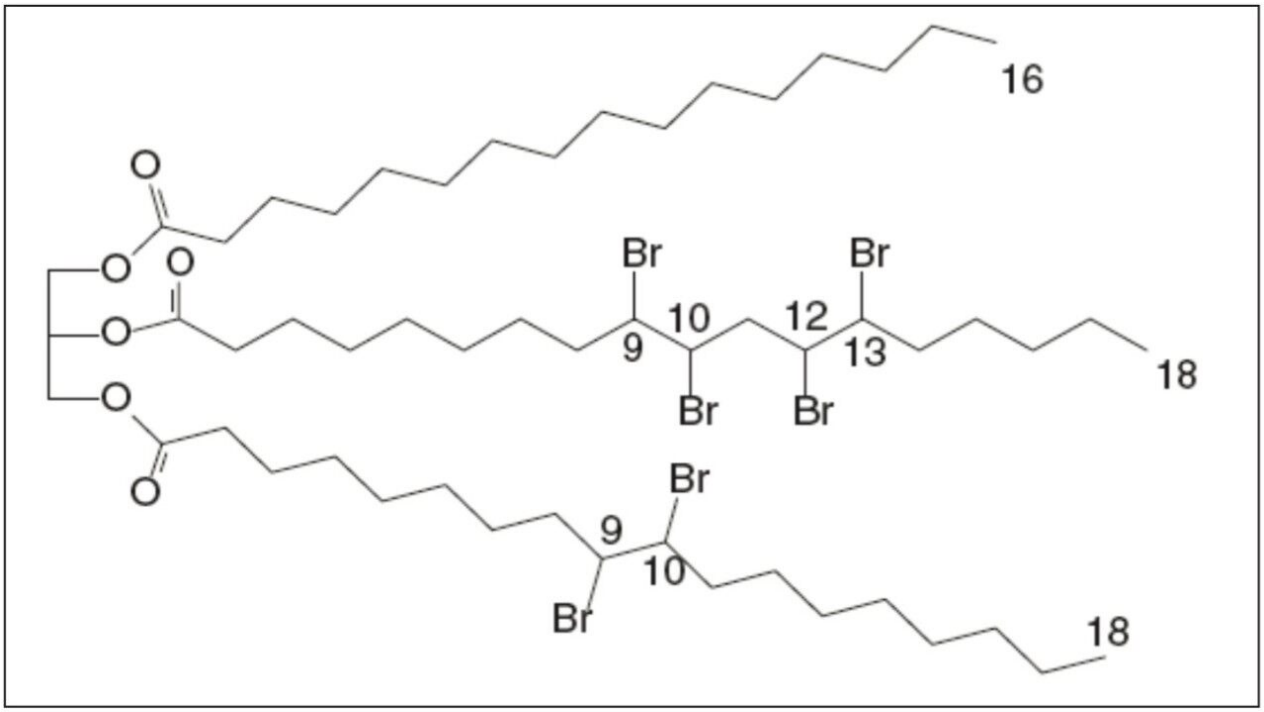

Figure 1. Structure of a brominated triacylglyceride composed of palmitic acid (16:0), threo-9,10-dibromooctadecanoic acid (Br_2-18:0) and threo,threo-9,10,12,13- tetrabromooctadecanoic acid (Br_4-18:0), which was obtained from a complete bromination of 1-palmitoyl-2-linoleyl-3-oleoylglycerol (PLO).

Waters UltraPerformance Convergence Chromatography (UPC^2) is a state-of-the-art supercritical fluid chromatography (SFC) technology that provides exceptional efficiency and speed of separation.[4] UPC^2 has been applied to a wide range of compounds, including VO, and has shown great benefits in selectivity,

throughput, and ease-of-use.[5] This application note demonstrates a rapid and simple analysis of BVO in soft drinks and beverages using UPC^2/MS. BVO was extracted and analyzed directly without any derivatization. The chromatography total run time was 9 min. The analytical method performance (limit of quantitation or LOQ, repeatability, linearity, and recovery), as well as the analysis of BVO in soft drinks and beverages are presented.

Experimental

Sample preparation

10.00 grams of decarbonated soft drinks and beverages that were purchased from a local grocery store were mixed with 10.0 mL (or 6.59 grams) hexanes, vortexed for 30 seconds, and centrifuged at 4000 rpm for 1 min. The upper layer (hexanes) was collected and weighed. The extract was dried under gentle nitrogen stream, and reconstituted with 1000 µL chloroform. A common BVO sample was obtained from a collaborator.

UPC^2 conditions

System:	ACQUITY UPC^2
Column:	ACQUITY UPC^2 BEH, 3 x 100 mm, 1.7 µm
Column temp.:	60 °C
Injection volume:	2 µL
Flow rate:	1.500 mL/min
Mobile phase A:	Compressed CO_2
Mobile phase B:	Isopropanol (IPA)
Gradient:	1% IPA for 2 min, linear ramp to 70% IPA in 3 min, and hold at 70% IPA for 1 min before

UPC^2 conditions	
	returning to the initial condition. Equilibrate for 3 min.

MS conditions

MS System:	Xevo TQ-S
Ionization mode:	ES-
Capillary voltage:	3.00 kV
Cone voltage:	20.00 V
Source temp.:	150 °C
Desolvation temp.:	500 °C
Cone gas flow:	150 L/hr
Desolvation gas flow:	1000 L/hr
Collision gas flow:	0.18 mL/min
Nebulizer gas flow:	7.00 Bar
Software:	MassLynx

Results and Discussion

The main challenge in the analysis of BVO is that it is comprised of a mixture of TAGs that differ in fatty acids

chain length, degree of unsaturation (number of double bonds), degree of bromination, and structural and spatial arrangement (isomerism). The different types of BVO compounds could number in the dozens or hundreds. In addition to that, the composition of BVO can differ from batch to batch due to variance in the VO source and the bromination process.

In this study, we employed UPC^2 with tandem mass spectrometry to analyze BVO. UPC^2 has been applied to the analysis of TAGs, and has shown exceptional efficiency and speed of separation.[6,7] Multiple reaction monitoring (MRM) transitions from molecular ions to bromide ions (Br-) can selectively detect brominated TAGs, and avoid potential interference from VO or other non-brominated compounds.

Due to the complex nature of BVO, it is challenging to detect all BVO components in any given sample. So, this study was designed to monitor a small, but representative, number of BVO compounds to quantify. Figure 2 shows the mass spectra of BVO and VO obtained by combining mass spectra across their chromatographic peaks. These were obtained on a UPC^2 BEH 1.7 µm, 3.0 x 100 mm Column under the gradient conditions described in the experimental section. 12 molecular ions across the mass range of BVO were selected for quantitation, shown in Figure 2. These ions were confirmed by the parent ion scan of the bromide ion (data not shown), and were absent in the VO mass spectrum. The purpose of selecting multiple ions across a wide range of *m/z* is to ensure a true representation of the BVO profile, and eliminate possible skewing of results due to batch to batch variation in the VO TAG composition and the bromination process. The extracted ion chromatogram (XIC) generated from these 12 MRM transitions was used for quantitation. Table 1 shows the MRM transition and the data acquisition conditions used in the study. No sample derivatization is needed in this approach.

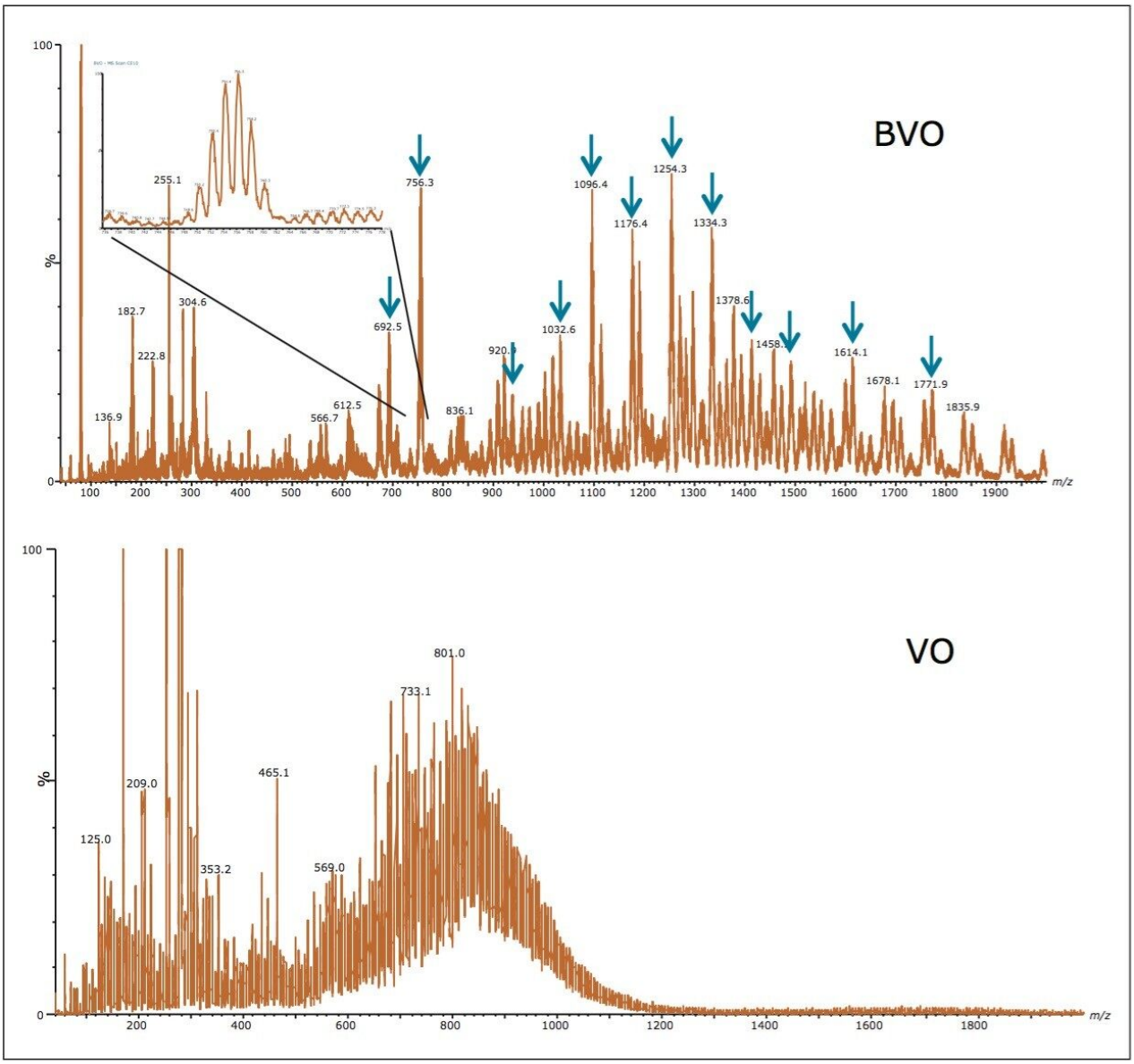

Figure 2. Comparison of BVO and VO mass spectra. 12 BVO molecular ions were selected for BVO quantitation. They are shown by arrows on the BVO mass spectrum. The characteristic isotope pattern of brominated compounds is shown in an enlarged portion of the spectrum.

	MRM transition (*m/z*)	Dwell time (sec)	Cone voltage (V)	Collision energy (eV)
1	692.5 > 80.9	0.024	20	30
2	754.6 > 80.9	0.024	20	30
3	938.7> 80.9	0.024	20	30
4	1032.6 > 80.9	0.024	20	30
5	1096.4 > 80.9	0.024	20	30
6	1176.4 > 80.9	0.024	20	30
7	1254.3 > 80.9	0.024	20	30
8	1334.1 > 80.9	0.024	20	30
9	1428.0 > 80.9	0.024	20	30
10	1491.9 > 80.9	0.024	20	30
11	1614.1 > 80.9	0.024	20	30
12	1771.9 > 80.9	0.024	20	30

Table 1. 12 MRM transitions and their acquisition parameters for the quantitation of BVO in soft drink beverages.

Figure 3 shows an XIC of a BVO solvent standard, and the established calibration curve from concentration 10 to 500 µg/mL. Table 2 shows the corresponding calibration results. A calibration correlation coefficient (R^2) of 0.998 was obtained. The LOQ was estimated at 5.7 µg/mL, which corresponds to 0.6 ppm in finished beverages. Repeatability (RSDs in peak area) was <5% at three levels of standard concentrations, shown in Table 3.

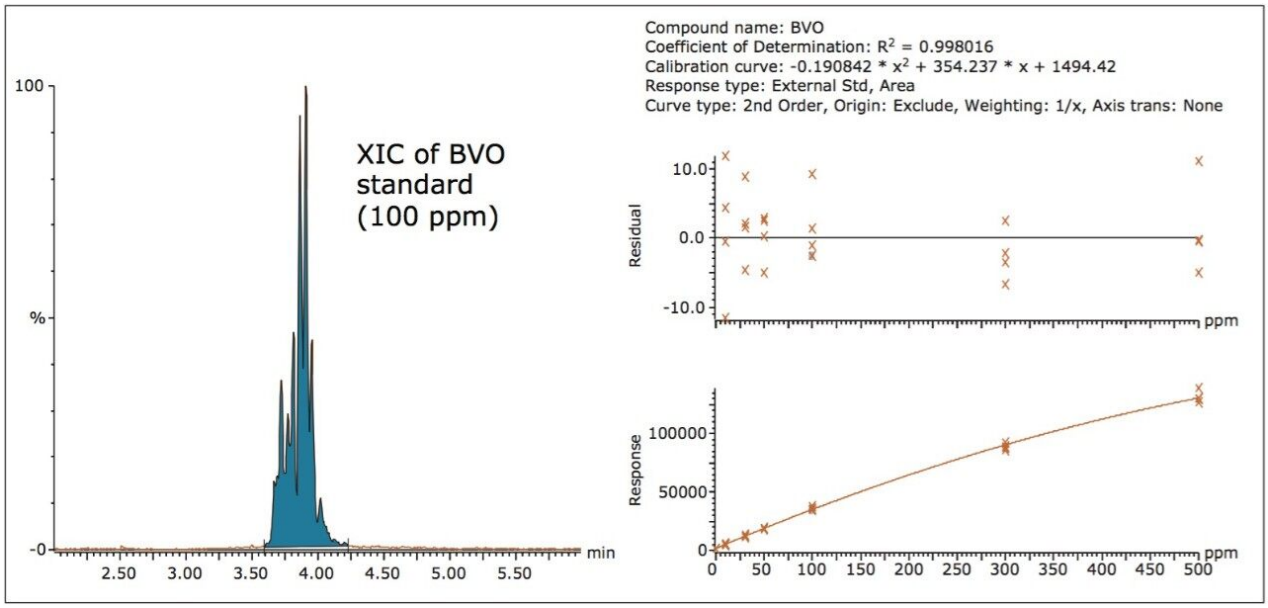

Figure 3. Extracted ion chromatogram of the BVO solvent standard at 100 ppm and the BVO calibration and residue plots.

Parameters	BVO
Range (µg/mL)	10 to 500
Equation	Peak area=$(-0.1908)X^2$ +354.2X +1494.4
Weighing	1/x
Regression (R_2)	0.998
LOQ (µg/mL)	5.7*

Table 2. Calibration results and LOQ.

**This LOQ in solvent standards corresponds to approximately 0.6 mg/kg in finished beverage because of 10 fold of concentration in sample preparation.*

Cone level	RSD in peak area
50 ppm	3%
100 ppm	5%
500 ppm	4%

Table 3. Relative standard deviation in peak area in replicate measurement of BVO (n=4).

Figure 4 shows the XIC of a blank solvent sample and five soft drink samples. The soft drinks were products from major brands. Samples B, C, and E have BVO in their label claims, whereas samples A and D do not. Table 4 shows the analysis results for these five soft drinks and the recovery study results on spiked soft drink samples. The calibration curve from the solvent standards was used in the analysis and the recovery study. No BVO was detected in samples A and D, which is in agreement with their label claims. Samples B, C, and E contained 4.1 to 6.5 ppm BVO, which is within the U.S. FDA limit for finished beverages. The relative error in the duplicated measurement was <1.6% for the three samples that contained BVO. Sample D was used as a blank sample, into which BVO was spiked at 3 ppm and 30 ppm for recovery study. Recoveries of 105% and 82% were obtained at 3 ppm and 30 ppm, respectively.

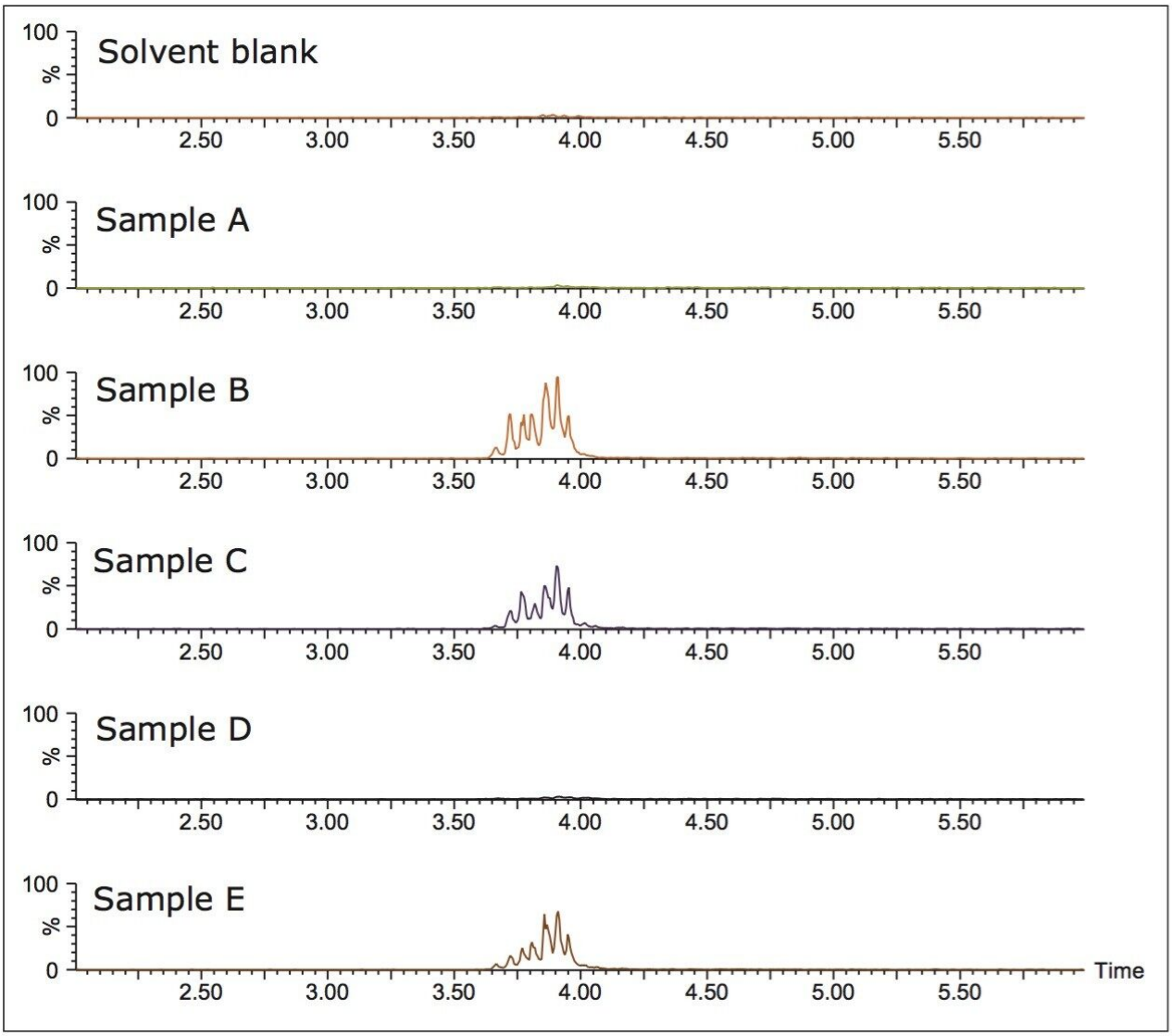

Figure 4. XIC of a solvent blank and five soft drink samples.

Sample	A	B	C	D	E	F*	G*
Mean (mg/kg)	Not detectable	6.5	4.5	Not detectable	4.1	3.2	24.7
Relative error (%)		0.5	1.6		1.0	1.7	0.2
Recovery (%)						105	82

*Table 4. Analysis results for five soft drinks and the recovery study results on spiked soft drink samples. *Sample F is Sample D spiked with BVO at 3 ppm level; Sample G is Sample D spiked with BVO at 30 ppm level.*

Conclusion

Convergence Chromatography (CC) coupled with mass spectrometry enables a rapid, simple, and direct analysis of BVO in beverages. Unlike the GC-MS method, there is no derivatization in this UPC^2/MS method. After a simple liquid-liquid extract, BVO can be analyzed directly by UPC^2/MS. The chromatographic run time is 9 min, which is at least five times faster than the GC-MS method. The UPC^2/MS method shows good accuracy and precision in the analysis of BVOs. It can potentially be used in food testing labs for routine BVO determination. For countries where BVO is banned for food additives, this method could be used for screening of BVO existence in beverages.

References

1. Chanamai R, McClements DJ. Impact of weighting agents and sucrose on gravitational separation of beverage emulsions. *J Agric. Food Chemistry*. 2000, 48(11): 5561-5565.
2. Bendig P, Maier L, Vetter W. Brominated vegetable oil in soft drinks - an underrated source of human organobromine intake. *Food Chemistry* 2012, 133: 678-682.
3. Bendig P, Maier L, Lehnert K, Knapp H, Vetter W. Mass spectra of methyl esters of brominated fatty acids and their presence in soft drinks and cocktail syrups. *Rapid Commun. Mass Spectrom*. 2013, 27:1083-1089.
4. Grand-Guillaume Perrenoud A, Veuthey JL, Guillarme D. Comparison of ultra-high performance supercritical fluid chromatography and ultra-high performance liquid chromatography for the analysis of pharmaceutical compounds. *J Chromatogra A.* 2012, 1266:158-67.
5. Please see website: www.waters.com/upc2 for a complete list of UPC^2 applications.
6. Yang J and Isaac G. Characterization of Triacylglycerols in Edible Oils Using the ACQUITY UPC^2 System and Mass Spectrometry. Waters Technology Brief No. 720004809en, October, 2013.
7. Zhou Q, Gao B, Zhang X, Xu Y, Shi H, Yu L. Chemical profiling of triacylglycerols and diacylglycerols in cow milk fat by ultra-performance convergence chromatography combined with a quadrupole time-of-flight mass spectrometry. *Food Chemistry*. 2014 Jan; 143:199-204.

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