Application Note

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Trace-level Quantification of Multiclass Emerging Disinfection Byproducts (DBPs) in Wastewater-Impacted Waters

Agilent triple quadrupole GC/MS with MRM optimization software

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Abstract

With increased infiltration of wastewater into surface water, along with increased need for water reuse to augment potable water supplies, understanding and quantifying the formation of emerging toxic DBPs during further treatment of secondary wastewater effluents is of growing interest. This application note demonstrates the suitability of the Agilent 7890B gas chromatograph (GC) system with a multimode inlet (MMI) coupled to the Agilent 7000C/D triple quadrupole GC/MS (GC/TQ) for the quantitation of more than 25 compounds, covering five emerging classes of DBPs in wastewater-impacted waters at parts-per-trillion levels. The solution provided good chromatographic performance, linearity, precision, and reproducibility, as well as excellent method detection limits of between 2.0 to 68.9 ng/L for ultrapure and wastewater-impacted samples. The sensitivity of the GC/TQ technology reduced sample concentration such that only 30 minutes of extraction time and 10 mL of sample were required. Due to the varying chemical properties of the different classes of DBPs, recoveries using one extraction method were variable, but predominantly acceptable.

Introduction

Disinfection byproducts (DBPs) are formed when organic matter in water reacts with disinfectants used to kill microbes during water treatment. Research shows that DBPs could possibly be carcinogenic and cause adverse birth outcomes in humans.[1,2] At the same time, rising demand for potable water is increasing the need to treat and reuse wastewaters. Therefore, understanding and quantifying the formation of emerging toxic DBPs during additional treatment of secondary wastewater effluents for reuse is an imperative.

DBPs consist of a large variety of mainly halogenated compounds. Common DBP classes include trihalomethanes (THMs) and haloacetic acids (HAAs). A subset, four THMS and five HAAs, are regulated in drinking water around the world. However, the vast majority of DBPs—many of which are more toxic than HAAs and THMs, and commonly detected in drinking water—remain unregulated. More than 700 DBPs have been identified in surface or groundwater disinfected with chlorine, chloramines, ozone, and chlorine dioxide.[3] Other DBP classes include haloacetonitriles, halonitromethanes, haloacetaldehydes, haloketones, haloacetamides, and haloacids.

Quantification of multiclass DBPs in wastewater-impacted water matrices represents a challenge because it is difficult to incorporate all the different chemical classes into one method. Existing multi-analyte methods are designed for less complex drinking water matrices. In addition to dealing with potential matrix effects, methods with higher sensitivity are needed to address the formation of unregulated DBPs from disinfection of pharmaceuticals, personal care products, and other environmental pollutants that are commonly present in wastewater at parts-per-trillion levels.

To meet these analytical challenges, Ortega-Hernandez *et al.* developed a highly sensitive GC/triple quadrupole (TQ) MS method that can quantify DBPs in wastewater-impacted waters at parts-per-trillion levels.[4] This method enabled the first research that comprehensively evaluated DBP formation potential from chlorination and chloramination for a full-scale water reuse facility.

This application note describes the suitability of the Agilent 7890B gas chromatograph (GC) system with a multimode inlet (MMI) coupled to the Agilent 7000C/D triple quadrupole GC/MS (GC/TQ) for the Ortega-Hernandez *et al.* method. Agilent MassHunter software was used to facilitate method optimization and data processing. The Agilent solution was evaluated in terms of chromatographic performance, linearity, method detection limits, recovery, and precision and reproducibility for ultrapure and wastewater-impacted samples.

Experimental

Sample preparation

The individual DBP reference standards listed in Table 1 were obtained from various sources and were weighed and diluted in anhydrous acetonitrile to make 4,000 mg/L stock solutions. One 100 mg/L sub-stock solution was prepared for each of the five classes of DBPs by mixing its individual components in anhydrous acetonitrile. As listed in Table 1, the DBP chemical classes were haloacetonitriles (HANs), haloketones (HKTs), haloaldehydes (HALDs), halonitromethanes (HANs), and iodo-trihalomethanes (I-THMs). Two master stocks were prepared by combining each DBP class to make 100 and 5 µg/L solutions. These master stocks were then used to prepare neat standards in acetonitrile (for percent recoveries) and calibration standards by spiking ultrapure water samples.

GC/MS/MS analysis and instrumentation

GC/MS/MS analysis of the DBPs was carried out using a 7890B gas chromatograph coupled with a 7000C GC/TQ. The 7890B GC was equipped with a multimode inlet (MMI) and an Agilent VF-200ms column (30 m × 0.25 mm, 0.25 µm, part number CP8858) containing an inert mid-polarity cross bond trifluoropropylmethyl polysiloxane stationary phase. The GC/TQ system was equipped with an EI source and operated in the multiple reaction monitoring (MRM) mode using the MRM parameters shown in Table 1. The GC and TQ instrument parameters are provided in Table 2. Sample data were acquired, processed, and quantified using Agilent MassHunter software.

MRM method optimization

The MRM method was developed and optimized for the DBP standards. The optimized parameters are listed in Table 1. First, the standards were analyzed in full-scan mode to identify retention time and precursor ions based on the base peak or the second most abundant peak in the spectra. After precursor ion selection, a product scan was carried out to select the two most abundant ions as the quantification (Quant) and qualification (Qual) ions.

Once the product ions were selected, the collision energies, dwell times, and time segments were optimized to maximize the signal for each transition. Optimization of these parameters can be a time-consuming multistep process. The MassHunter MRM transition optimizer was used to automate determination of the best CE values, saving substantial time. The optimizer varied the CE in increments of 2 eV between 0 to 60 eV, and then identified the CE value that provided the highest abundance for each transition.

Four retention time segments were applied: 0.00 to 5.20, 5.20 to 8.20, 8.20 to 10.20, and 10.20 to 47.60 minutes. Each time segment included six, seven, eight, and four DBPs, respectively. The time segments served to increase sensitivity by reducing the number of chemical transitions scanned per segment. Dwell time for each analyte was optimized to provide 15 data points across each peak.

Calibration and method detection limits

Calibration curve and method detection limits (MDLs) were determined using ultrapure water solutions spiked with the master DBP stocks. A 1,2-dibromopropane internal standard (IS) was added to the final extracts. Calibration solutions of 0.001, 0.005, 0.01, 0.025, 0.05, 0.10, 0.25, 0.50, 1, 5, 10, and 25 µg/L were prepared. To ensure linearity, two-part calibration curves were developed: the calibration curve between 0.001 to 0.50 µg/L was used for low-level compounds, while the other compounds were quantified using the curve ranging from 0.50 to 25 µg/L. All calibration curves had a coefficient of determination (R^2) greater than 0.99 and were linear over three orders of magnitude.

Table 1. DBPs analyzed with retention time and ions monitored.

DBP Class	DBP (Abbreviation)	Retention Time (min)	Precursor (*m/z*)	Quant Ion *m/z*	CE (eV)	Qual Ion	CE (eV)	Dwell Time (ms)
HAN	Chloroacetonitrile (CAN)	4.14	75	48	5	40.1	15	21.1
	Bromoacetonitrile (BAN)	6.69	120.9	40.1	10	41.1	10	18.3
	Iodoacetonitrile (IAN)	9.64	166.9	40.1	21	41.1	42	12.5
	Dichloroacetonitrile (DCAN)	3.86	73.9	47	21	40.1	32	22.2
	Dibromoacetonitrile (BCAN)	8.9	117.9	90.9	21	40.1	35	14.6
	Bromochloroacetonitrile	6.46	73.9	47	21	40.1	32	18.3
	Trichloroacetonitrile (TCAN)	2.95	107.8	72.9	29	47	60	21.7
HNM	Dichloronitromethane (DCNM)	4.85	82.9	48	52	47	55	27.8
	Dibromonitromethane (DBNM)	9.29	172.8	91.9	59	93.9	59	16.7
	Bromochloronitromethane (BCNM)	7.29	128.9	48	50	47	50	19.8
HAL	Bromodichloroacetaldehyde (BDCAld)	5.5	82.9	47	34	48	48	18.8
	Dibromochloroacetaldehyde (DBCAld)	7.91	128.9	48	48	47	50	18.8
	Tribromoacetaldehyde (TBAld)	9.94	172.8	91.9	59	93.9	58	20.8
HKT	1,1-Dichloropropanone (11DCP)	4.69	82.9	47	43	48	43	23.3
	1,3-Dichloropropanone (13DCP)	9.76	77	49	9	48	43	12.5
	1,1,1-Trichloropropanone (111TCP)	7.64	124.9	97	9	82.9	9	18.8
	1,1,3-Trichloropropanone (113TCP)	10.87	77	49	10	47	46	40.8
	1-Bromo-1,1-dichloropropanone (1B11DCP)	9.68	124.9	97	2	43.1	22	20.8
	1,1,3,3-Tetrachloropropanone (1133TeCP)	11.74	82.9	47	43	48	34	33.3
I-THMs	Dichloroiodomethane (DCIM)	4.12	209.9	82.9	1	84.9	12	22.2
	Bromochloroiodomethane (BCIM)	6.4	255.9	128.8	2	130.8	11	15.4
	Dibromoiodomethane (DBIM)	8.52	172.8	91.9	57	93.9	57	18.8
	Chlorodiiodomethane (CDIM)	9.02	174.9	48	53	47	60	20.8
	Bromodiiodomethane (BDIM)	10.8	218.8	91.9	60	140	60	45.8
	Iodoform (TIM)	12.74	266.8	140	60	127	60	50.0
I.S.	1,2-Dibromopropane (IS)	6.31	120.9	92.9	30	41.1	10	16.3

Sample extraction and recovery optimization

DBPs are small volatile molecules that are typically extracted from water matrices using liquid–liquid extraction (LLE). Though LLE methods are time-consuming, due to the sensitivity and selectivity of the GC/TQ, the sample extract concentration can be reduced, saving sample and solvent volume, reagents, and total time. The LLE extraction method used has been described by Cuthbertson *et al.*[5], then later optimized by Ortega-Hernandez to reduce extraction time from four hours to two. The factors optimized are listed in Table 3. Percent recoveries were calculated by comparing the area counts of a neat peak and an extracted peak.

Table 2. Optimized GC and TQ parameters.

Parameter	Value
Gas Chromatograph	
Model	Agilent 7890B gas chromatograph
Column	Agilent VF-200ms column, 30 m × 0.25 mm, 0.25 µm (p/n CP8858)
Column Pneumatics	Constant flow
Injector Mode	Pulsed-splitless
Injector Liner	Agilent 5190-4006; Lot R0700: 200 µL (Splitless, dimpled, Ultra Inert)
Injection Volume	1 µL
Inlet Temperature Program	35 °C to 170 °C at 360 °C/min, 720 °C/min to 250 °C
Injector Pulse Pressure	20 psi for 0.75 min followed by purge to split vent at 30 mL/min
Flow Rate	1.371 mL/min
Oven Temperature Program	35 °C for 5 min, 9 °C/min to 220 °C, 20 °C/min to 280 °C, hold 20 min
Total Run Time	47.6 min
Equilibration Time	0.5 min
Mass Spectrometer	
Model	Agilent 7000C GC/TQ
Ionization Mode	EI, 70 eV
Acquisition Mode	MRM
Filament Current	35 µA
Collision Gas	N_2 at 1.5 mL/min
Quench Gas	He at 2.25 mL/min
GC Interface/Transfer Line Temperature	250 °C
Ion Source Temperature	200 °C
Quadrupole 1 Temperature	150 °C
Quadrupole 2 Temperature	150 °C

Table 3. Extraction method parameters evaluated.

	Experiment 1	Experiment 2	Experiment 3	Experiment 4
Sample Volume (mL)	100	50	50	10
Organic Solvent (mL)	5 × 3	5 × 3	5 × 3	3 × 3
Sodium Sulfate (g)	30	15	15	3
Shake Time (min)	15	15	15	15
Rest Time (min)	15	15	15	15
Extract Volume (µL)	200	200	100	200

Based on the results of the optimization experiments (shown in Figure 1) the optimized extraction method selected used a 10 mL sample and a final extract volume of 200 µL. Sodium sulfate was adjusted to obtain a salt saturation of 0.3 g/mL. Solvent volume and shaking time were 3 mL × 3 and 10 minutes, respectively for a total of 30 minutes of total extraction time.

Precision and reproducibility determination

Precision was determined as percent relative standard deviation (%RSD) of the 25 DBPs using three concentrations (low 100 and mid 250 ng/L, and high 100 µg/L). RSD values were calculated from n = 7 injections from the same vial for each of the DBPs.

Matrix effects of secondary wastewater effluents

Extraction from matrices other than ultrapure water is expected to reduce recovery. To determine the impact of matrix, average percent recoveries were calculated for secondary wastewater effluent spiked to 5 µg/L. Wastewater effluents were obtained from Advancing Canadian Wastewater Assets (ACWA), a full-scale advanced tertiary wastewater treatment plan that treats secondary wastewater effluents with microfiltration (UF) membranes (pore size 0.02 mm), followed by reverse osmosis (RO) or ozone treatment (O_3).

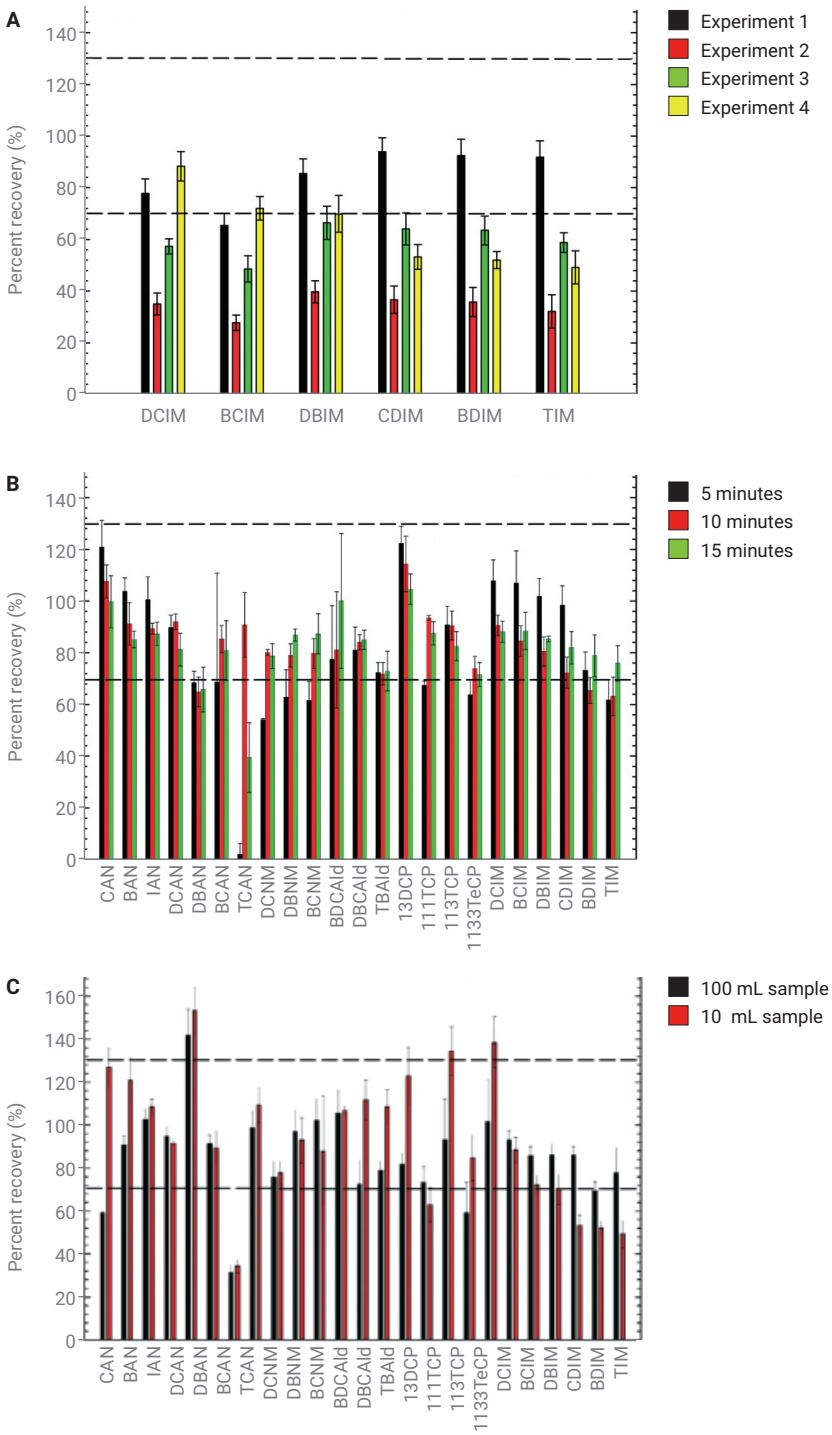

Figure 1. Average percent recoveries (n = 3) for (A) the sample volume optimization experiments and organic solvent volumes for I-THMs, (B) for three shake times with 3 mL × 3 of MTBE solvent extraction for all DBPs, and (C) for 100 ng/L standards spiked in ultrapure water for 100 and 10 mL sample volumes. Dashed lines represent the acceptable percent recovery range of 70 to 130%.

Results and discussion

Chromatographic performance

The GC/TQ method produced good chromatographic separation and detection of the 25 target DBPs (Figure 2.)

Method detection limits

MDLs were determined as described by Ortega-Hernandez *et al.* using the equation:

$$MDL = t_{N-1,1-\alpha=0.99} CL \frac{SD_{peak\ area}}{AV_{peak\ area}}$$

All calibration curves had a coefficient of determination (R^2) greater than 0.99 and were linear over three orders of magnitude, providing confidence in the calculated MDLs. The method provided good sensitivity with exceptionally low MDLs between 2.0 to 68.9 ng/L (Table 4) while comprehensively analyzing all five classes of DBPs. The ability to analyze multiple DBP classes reduces the need to develop and carry out multiple analytical methods.

Method accuracy could be improved by using isotopically labeled internal standards, if commercially available or synthesized. Currently, these can be difficult to source for emerging DBPs.

The 7000D GC/TQ includes the EI Extractor Ion Source for confident trace analysis and the MassHunter MRM Optimizer tool for ultra-trace-level analysis of target compounds. The tool offers fully automated end-to-end MRM method development to meet experimental goals.[6] Alternatively, as was the case in this study, optimization of MRM parameters such as precursor ion identification, product ion selection, and CE optimization can be performed individually.

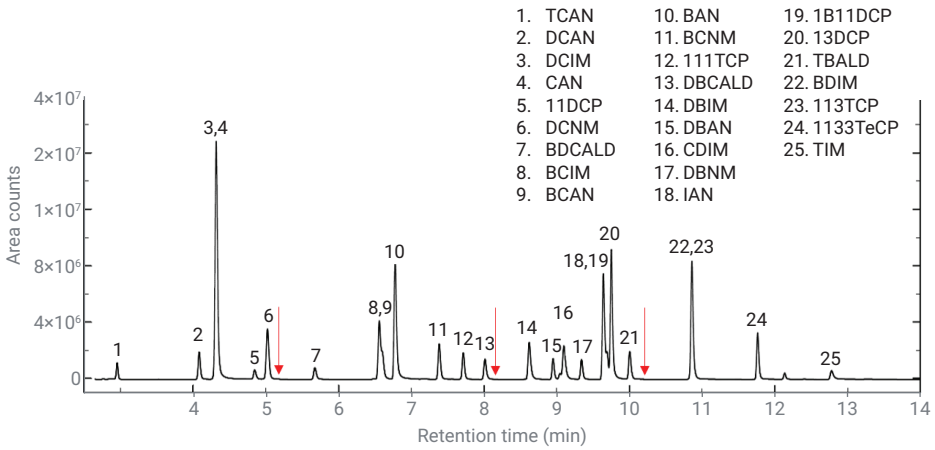

Figure 2. Good separation of the 25 DBPs at 1 mg/L was obtained for each of the four retention time segments delineated by the red arrows.

Table 4. Method recovery and precision (as percent relative standard deviation (RSD)) for 100 ng/L DBPs, and MDLs in ultrapure water.

DBP	Average Recovery (%)	RSD (%)	MDL (ng/L)
CAN	126.8	5.7	5.7
BAN	120.8	7	3.6
IAN	108.4	8.2	6.3
DCAN	91.4	4.6	3.2
DBAN	153.1	10.1	68.9
BCAN	89.2	8.8	3.7
TCAN	34.1	43.9	3.2
DCNM	109.2	6.5	4.1
DBNM	77.7	9.1	2.3
BCNM	92.9	8.8	4.1
BDCAld	87.7	MDL	50.0
DBCAld	106.6	9.6	11.9
TBAld	111.7	10.3	13.0
11DCP	108.5	MDL	25.7
13DCP	122.6	8.6	6.8
111TCP	62.9	10.6	30.6
113TCP	134.2	10.4	7.5
1B11DCP	84.7	7.3	56.2
1133TeCP	138.2	11.4	5.5
DCIM	88.3	6.4	5.7
BCIM	72.1	6.8	7.5
DBIM	69.9	8.2	2.0
CDIM	53.2	6.7	3.6
BDIM	52.0	9.9	5.6
TIM	49.2	8.7	3.1

Recoveries in ultrapure water and secondary wastewater effluent

The average percent recoveries (n = 3) for the optimized extraction procedure for 100 ng/L of DBPs in ultrapure water are listed in Table 4. The percent recoveries fell within the 70 to 130% target range except for DBAN, TCAN, 111TCP, 113TCP, 1133TeCP, TIM, BDIM, DBIM and, CDIM. TCAN had the lowest recovery, possibly due to its low boiling point (84 °C) and higher volatility compared to other HANs (boiling points ≥110 °C). During extraction, the solvent extracts are "blown down" under a slow nitrogen stream, which could have caused loss of TCAN. The percent recoveries at the higher spike level of 5 µg/L in ultrapure water ranged between 31 to 104% (Figure 3).

Figure 3 also presents the average percent recovery (n = 3) for each analyte at 5 µg/L from secondary wastewater effluent. The recoveries from secondary effluents were acceptable and ranged from 29 to 83%, which was only slightly lower than the recoveries obtained for ultrapure water. The differences between ultrapure and wastewater recoveries ranged from 0.5 to 30%, which was acceptable. The low matrix effects might be explained due to the low sample volume (10 mL) used to extract DBPs, which likely included a low number of interfering compounds that could be extracted along with the DBPs.

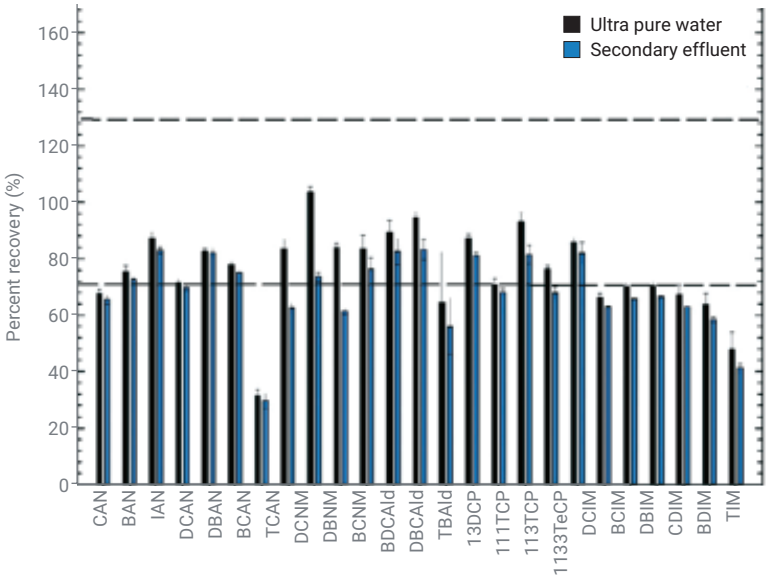

Figure 3. Average percent recoveries (n = 3) obtained from 5 µg/L standards spiked into ultrapure water (black bars) and secondary effluent (blue bars). Dashed lines represent the acceptable percent recovery range between 70 to 130%.

Reproducibility and precision

Precision is acceptable when %RSDs are approximately 10% or less. The majority of analytes displayed less than 3.1% RSD at the highest concentration of 100 µg/L. Table 4 shows %RSDs at the lowest concentration tested, 100 ng/L. Overall, TCAN displayed a high average %RSD of 60%.

Conclusion

The Agilent 7000C/D GC/TQ was determined to be well suited for comprehensive multiclass analysis of DBPs in wastewater-impacted matrices. The sensitivity of Agilent GC/TQ technology reduced sample concentration such that only 30 minutes of extraction time and 10 mL of sample were required. In addition, MDLs were between 2.0 to 68.9 ng/L—the lowest reported to date. Due to the varying chemical properties of the different classes of DBPs, recoveries using one extraction method were indeed variable, but predominantly fell in the acceptable range of 70 to 130%.

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