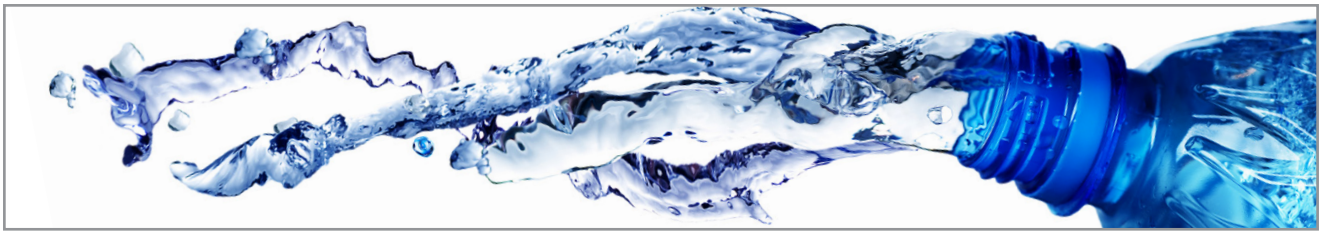

Combined Determination of 1,4-Dioxane and Nitrosamine Contaminants in Drinking Water

Using a Single SPE Cartridge and Concurrent Solvent Recondensation–Large Volume Splitless Injection (CSR-LVSI) With EI GC-MS

By Chris Rattray and Jack Cochran

Abstract

Global concern over the carcinogenic potential of 1,4-dioxane and several nitrosamines has resulted in increased interest in the development of more efficient testing methods for these contaminants in drinking waters. In the U.S., the current methodologies recommended for the analysis of 1,4-dioxane and nitrosamines in drinking waters are Environmental Protection Agency (EPA) Methods 522 and 521, respectively. EPA Method 522 is a relatively simple gas chromatography–mass spectrometry (GC-MS) method using electron ionization (EI), while Method 521 requires positive chemical ionization (PCI) using liquid methanol or acetonitrile reagent gas, along with tandem mass spectrometry (GC-MS/MS).

The method described here uses the same coconut charcoal sorbent solid phase extraction (SPE) cartridges and dichloromethane eluent recommended in EPA Methods 522 and 521 to concentrate 0.50 L water samples to 10 mL extracts. However, both the extraction and instrumental analysis portions of EPA Methods 522 and 521 have been combined by analyzing a single quantitatively collected SPE tube extract for both 1,4-dioxane and nitrosamines during a single chromatographic run. The benefits of the current combination method include fewer samples to collect, ship, and extract; a reduction in solvent use; and higher sample throughput. Because the final SPE extract cannot be concentrated via evaporation due to volatile compound loss, we employed concurrent solvent recondensation–large volume splitless injection (CSR-LVSI), which uses a standard splitless injector to deliver 50 µL injections of extract to a pre-column connected to a typical GC column for separation followed by EI MS analysis. When combined with selected ion monitoring (SIM), this large volume injection allows for practical quantitation limits (PQLs) as low as 10 ng/L for 1,4-dioxane and 0.5–2.0 ng/L for the nitrosamines.

Introduction

1,4-Dioxane is a highly water-soluble synthetic organic solvent used to stabilize chlorinated solvents; 1,1,1-trichloroethane (TCA), for example, may contain up to 8% 1,4-dioxane. Improper disposal of chlorinated solvents can lead to the accumulation of 1,4-dioxane in ground and surface waters used as drinking water sources [1]. Global concern over the carcinogenic potential of 1,4-dioxane, along with its identification as a Group 2B compound by the World Health Organization's (WHO) International Agency for Research on Cancer (IARC), has led to increased regulatory interest in this compound. For example, as a part of Unregulated Contaminant Monitoring Rule 3 (UCMR3), the U.S. EPA is requiring that all municipalities serving drinking water to more than 10,000 people monitor 1,4-dioxane levels for 12 consecutive months between 2013 and 2015. The mandated method for 1,4-dioxane analysis is EPA Method 522, which was developed for part-per-trillion (ppt) analysis of drinking waters using solid phase extraction (SPE) cartridges and gas chromatography–mass spectrometry (GC-MS) in selected ion monitoring (SIM) mode. The $1x10^{-6}$ cancer risk assessment level for 1,4-dioxane is 0.35 µg/L and, as a result, the proposed minimum reporting level (MRL) for 1,4-dioxane as part of UCMR3 is 70 ng/L (70 ppt) [2].

Tetrahydrofuran (THF) is another widely used volatile ether with a structure similar to that of 1,4-dioxane. We chose to include THF in the work here because the chemical similarities indicated that it would be well suited to the carbon SPE extraction method. Also, its high volatility makes it an ideal compound to highlight the benefits of CSR-LVSI when performing trace analysis of volatile compounds. THF is widely used as a solvent in polymerization reactions and as a starting material for elastomeric polyurethane fibers. THF dissolves polyvinyl chloride (PVC) polymers and is a major ingredient in PVC cement, making it a likely contaminant to find in certain residential settings. With an LD50 similar to that of acetone, its presence in drinking water is monitored, but not strictly regulated, with neither a maximum contaminant level nor a target maximum level published.

Nitrosamines are an emerging class of drinking water contaminants that can enter the drinking water system through multiple wastewater disinfection processes such as chlorination [3-6], chloramination [3-5], and chlorine dioxide treatment [3], as well as ozone treatment of high total organic content (TOC) wastewaters followed by chlorination [3]. Nitrosamine formation has also been shown to occur through the process of nitrogen fixation on the surface of the activated carbon sorbents used both for water treatment and quantitative analysis [7]. In addition to their creation as disinfection byproducts, nitrosamines can enter the environment through manufacturing processes where they are used as starting materials and formed as intermediates and byproducts during a variety of synthetic chemical processes [8, 9], especially in pesticide and pharmaceutical manufacturing.

N-nitrosodimethylamine (NDMA) is the primary nitrosamine of concern, like 1,4-dioxane and THF, its high water solubility limits the efficiency of purge-and-trap and liquid-liquid extraction when determining low ppt concentrations. The disinfection treatment study by Zhao et al. showed that NDMA is detected at the highest concentration and with the most frequency compared to other nitrosamines [3]. They suggest that NDMA can serve as a surrogate for nitrosamine exposure assessment, similar to the way benzo[a] pyrene has been used for polycyclic aromatic hydrocarbon (PAH) exposure assessment. NDMA is also one of the 13 suspected carcinogens listed in 29 CFR 1910.1003, requiring special precautions when handling concentrated solutions or neat material [10].

The human health impact of nitrosamines has been a growing concern, with NDMA the focus of a 2006 WHO drinking water study [11]. The latest edition of the Report on Carcinogens (RoC) published by the National Toxicology program lists 15 nitrosamines as likely human carcinogens (N-methyl-*n*'-nitro-*n*-nitrosoguanidine, N-nitrosodi-*n*-butylamine, N-nitrosodiethanolamine, N-nitrosodiethylamine, N-nitrosodimethylamine, N-nitrosodi-*n*-propylamine, N-nitroso-*n*-ethylurea, 4-(N-nitrosomethylamino)-1-(3-pyridyl)-1-butanone, N-nitroso-*n*-methylurea, N-nitrosomethylvinylamine, N-nitrosomorpholine, N-nitrosonornicotine, N-nitrosopiperidine, N-nitrosopyrrolidine, N-nitrososarcosine) [9]. Table I lists the eight nitrosamines covered in this work, and all eight are included in the RoC report and the U.S. EPA's Resource Conservation and Recovery Act (RCRA) groundwater testing list [12]: seven alkyl-nitrosamines and one dioxane analog (N-nitrosomorpholine). Table I also lists the EPA's Integrated Risk Information System (IRIS) one in a million cancer risk assessment values (based on consumption of two liters of contaminated water a day for a lifetime [8]), which range from the low to sub nanogram per liter level for the nitrosamines in question [13].

Table I: Method Analytes

Analyte	CAS Registry Number	Boiling Point (°C)	Molecular Mass (AMU)	Vapor Pressure (mm Hg 20 °C)	IRIS $1x10^{-6}$ Cancer Concentration (ng/L) [13]	UCMR Study Number
Tetrahydrofuran (THF)	109-99-9	66	72	143	-	-
1,4-Dioxane	123-91-1	101.1	88	30	350	3
N-nitrosodi-methylamine (NDMA)	62-75-9	153	74	2.7	0.7	2
N-nitrosomethyl-ethylamine (NMEA)	10595-95-6	154.4	88	1.1	2.0	2
N-nitrosodi-ethylamine (NDEA)	55-18-5	173.9	102	0.86	0.2	2
N-nitrosodi-*n*-propylamine (NDPA)	621-64-7	206	130	0.086	5.0	2
N-nitrosodi-*n*-butylamine (NDBA)	924-16-3	116 (a)	158	0.05 (b)	6.0	2
N-nitroso-pyrrolidine (NPYR)	930-55-2	214	100	0.06	20	2
N-nitroso-piperidine (NPIP)	100-75-4	219	114	0.092	-	-
N-nitroso-morpholine (NMOR)	59-89-2	224	116	0.036	-	-

a. 14 mm Hg
b. 25 °C

Reliably quantitating down to low and sub ppt levels using standard laboratory equipment is a challenge. Several methods have been published recently which combine positive chemical ionization (PCI) with high resolution MS (HRMS) [14], tandem MS [8, 15, 16], and single quadrupole MS [17]. The benefits of PCI are numerous. The "softer" ionization preserves the molecular ion (plus adduct) at a much higher ratio than standard electron ionization (EI) [8, 16]. Also, using ammonia as the reagent gas enhances method selectivity and analyte signal-to-noise (relative to methanol). Similar proton affinities to ammonia cause amine and nitroso adducts to be favored, reducing background noise levels [16]. Ammonia is not without drawbacks, however. It is highly corrosive and not all chemical ionization sources are compatible; in addition, condensation is a concern, and leaks will quickly clear the lab.

The U.S. EPA method for analyzing nitrosamines in drinking water (Method 521) requires liquid chemical ionization (methanol or acetonitrile) [15] which is compatible with limited instrumentation (namely, the ion source in the Varian® Saturn 4 and Saturn 2000 instruments). An alternative single quadrupole MS method published by Agilent uses 20% methane as the ionization gas, which is inexpensive, readily available, and a common PCI gas [17]. The method we developed here is subject to the reduced sensitivity inherent to EI (i.e., relative to PCI). HRMS could make up for some of the reduced sensitivity, but HRMS instruments are much more expensive and difficult to operate than their low resolution counterparts. Instead, we used large volume injection (LVI) to overcome the relative sensitivity deficiency of EI; in fact, EPA Method 521 uses a large volume injection in concert with a programmed temperature vaporization (PTV) injector [8, 15].

Using large volume splitless injection in GC-MS is advantageous when trying to analyze trace-level contaminants in clean matrices like drinking water because greater levels of target compounds are introduced onto the analytical column resulting in better detectability. Generally, a special injection port such as a PTV injector is required for LVI [1]. With the PTV inlet temperature set near the boiling point of the solvent, the sample is introduced at a high split ratio and as the solvent evaporates the analytes of interest are concentrated in the inlet. After a predetermined time, the split valve is closed and the inlet temperature is increased to transfer the concentrated sample and remaining solvent onto the column. This solvent-venting, analyte-concentrating step requires a relatively large difference in boiling points between solvent and solute, more than 100 °C, in order to prevent loss of analytes of interest to the split vent [18]. This rules out using LVI with a PTV type injection port for the analyte list covered here due to inadequate differences in boiling points. The SPE elution solvent used for the work presented here is dichloromethane (DCM), which has a boiling point of 40 °C. THF and THF-d8 have boiling points around 66 °C, while 1,4-dioxane and its deuterated counterpart 1,4-dioxane-d8 both boil around 100 °C.

This lab has successfully demonstrated that concurrent solvent recondensation–large volume splitless injection (CSR-LVSI), a technique described by Magni and Porzano [19, 20], can be used without any modification to an Agilent®-style splitless injection port for a variety of analyses including polycyclic aromatic hydrocarbons (PAHs), total petroleum hydrocarbons (TPH), EPA Method 8270 semivolatiles [21], and brominated flame retardants [22], as well as many organochlorine, organonitrogen, and organophosphorus pesticides. Application chemists at Thermo Scientific have successfully applied a CSR-LVSI technique to drugs of abuse, pesticides, and polychlorinated biphenyls by injecting 20–35 µL and significantly improving limits of detection [23-25]. The CSR-LVSI technique can overcome the aforementioned LVI boiling point obstacle and was, therefore, adopted in the current study for analyzing trace levels of 1,4-dioxane and nitrosamines in drinking water. For this application, we used an Rxi® pre-column (10 m x 0.53 mm) press-fitted to a Rxi®-5Sil MS analytical column (30 m x 0.25 mm ID x 1.0 µm) and a starting GC oven temperature below the boiling point of the solvent. A fast autosampler injection with liquid band formation of the injected sample into a liner containing glass wool eliminates backflash in the hot injection port [26]. Most recently, a detection limit of 5 ng/L 1,4-dioxane in drinking water was demonstrated using a 10 µL CSR-LVSI combined with a 1 L solid phase extraction [27].

Experimental

Solid Phase Extraction (SPE)

Since a single SPE cartridge was to be utilized for the combined sampling of 1,4-dioxane, THF, and nitrosamines, it was imperative to evaluate method performance across a variety of concentrations. Therefore, three fortified samples were prepared at three different concentrations and used to evaluate recoveries. The deuterated 1,4-dioxane surrogate was added at 4,000 ng/L with an expected final extract concentration of 200 ng/mL. The deuterated N-nitrosodimethylamine surrogate was added at 400 ng/L so that the extracts would have a final surrogate concentration of 20 ng/mL. The target reporting limits for 1,4-dioxane and THF are ten times higher than most of the nitrosamines, so calibration standards, matrix fortification levels, and surrogate levels all reflect this difference. The bottled waters were fortified while still in their plastic bottles, recapped, mixed by inversion, and allowed to sit for several hours to ensure homogeneous samples. See Table II for compound-specific fortification levels.

Each sample was extracted using a single Resprep® activated coconut charcoal SPE cartridge (cat.# 26032) following the procedure described in section 11.4 (SPE Procedure Option 1; Extraction of 500-mL Samples) of EPA Method 522. Immediately after solvent elution, the extracts were fortified with 50 µL of internal standard mix and brought up to 10 mL final volume resulting in a concentration of 10 ng/mL or 100 ng/mL in the extracts. The extracts were then transferred to a large storage vial and dried with anhydrous sodium sulfate.

Table II: Laboratory Fortified Blank (LFB) Concentrations (ng/L) (Extract Concentrations in Parenthesis [ng/mL])

Analyte	Blank	Low	Mid	High
THF	0.0 (0.0)	10 (0.50)	50 (2.5)	500 (25)
1,4-Dioxane-d8 (SS)	4,000 (200)	4,000 (200)	4,000 (200)	4,000 (200)
1,4-Dioxane	0.0 (0.0)	10 (0.50)	50 (2.5)	500 (25)
N-nitrosodimethylamine-d6 (SS)	400 (20)	400 (20)	400 (20)	400 (20)
N-nitrosodimethylamine	0.0 (0.0)	0.50 (0.025)	2.5 (0.13)	25 (1.3)
N-nitrosomethylethylamine	0.0 (0.0)	1.0 (0.050)	5.0 (0.25)	50 (2.5)
N-nitrosodiethylamine	0.0 (0.0)	1.0 (0.050)	5.0 (0.25)	50 (2.5)
N-nitrosopyrrolidine	0.0 (0.0)	1.0 (0.050)	5.0 (0.25)	50 (2.5)
N-nitrosodi-*n*-propylamine	0.0 (0.0)	1.0 (0.050)	5.0 (0.25)	50 (2.5)
N-nitrosomorpholine	0.0 (0.0)	0.50 (0.025)	2.5 (0.13)	25 (1.3)
N-nitrosopiperidine	0.0 (0.0)	1.0 (0.050)	5.0 (0.25)	50 (2.5)
N-nitrosodi-*n*-butylamine	0.0 (0.0)	1.0 (0.050)	5.0 (0.25)	50 (2.5)

GC-MS Conditions

Figure 1 illustrates the setup used for CSR-LVSI. Instrument conditions are presented in Table III.

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Figure 1: CSR-LVSI setup: 1. Custom single taper Restek Premium inlet liner with 15 mg of wool at the bottom, 2. 10 m x 0.53 mm ID Rxi® deactivated guard column (cat.# 10073), 3. SGE® 0.4–0.8 mm OD µ-union, 4. 30 m x 0.25 mm ID x 1.0 µm df Rxi®-5Sil MS analytical column (cat.# 13653), 5. 5975 MSD.

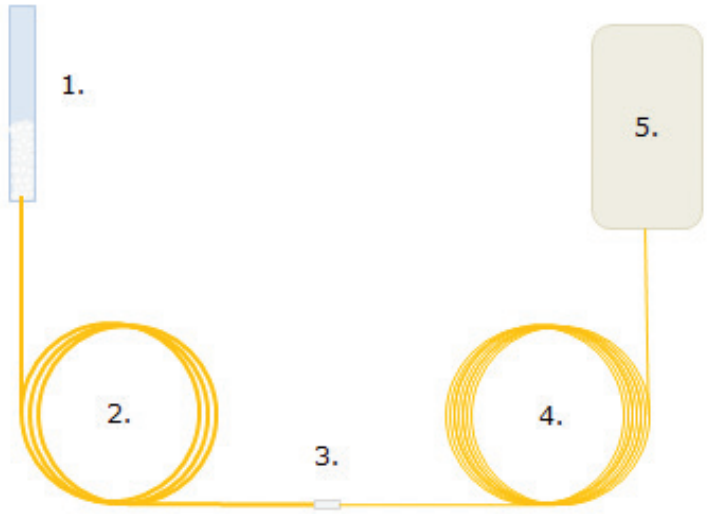


Table III: Agilent® 7890A-5975C GC-MS Parameters

7693 Automatic Liquid Sampler (ALS) Parameters			
Syringe	SGE® 100 µL gas tight syringe with fixed 26/23 gauge needle (cat.# 005668)		
Injection Volume	50 µL		
Injection Speed	4,000 µL/min		
Split/Splitless GC Inlet Parameters			
Inlet Mode	Splitless for 1.5 min, then split 100 mL/min		
Temperature	275 °C		
Liner	Custom Restek Premium 4 mm ID single taper liner with ~15 mg quartz wool		
GC Parameters			
Flow Program	5.08 mL/min (hold 8.9 min) to 2.0 mL/min at 1.0 mL/min/min		
Oven Temperature Program	35 °C (hold 1.5 min) to 50 °C at 50 °C/min (hold 7.1 min) to 320 °C at 11.12 °C/min (hold 1.5 min)		
Pre-Column	0.53 mm ID x 10 m Rxi® guard column (cat.# 10073)		
Analytical Column	30 m x 0.25 mm ID x 1.0 µm df Rxi®-5Sil MS (cat.# 13653)		
Column Union	SGE® µ-Union 0.8 to 0.4 (cat.# 073562)		
Carrier Gas	He, constant flow		
MS Parameters			
Transfer Line Temp.	320 °C		
Source Temp.	230 °C		
Quad Temp.	150 °C		
Electron Energy	70 eV		
Solvent Delay Time	9.90 min		
Tune Type	BFB		
Ionization Mode	EI		
SIM Program			
Group	**Start Time (min)**	**Ions (m/z)**	**Dwell (ms)**
1	9.90	42, 46, 71, 72, 78, 80	20
2	10.50	58, 62, 64, 88, 96	20
3	11.20	42, 43, 46, 48, 74, 80	20
4	12.00	43, 56, 88, 102	30
5	15.50	58, 68, 70, 78, 86, 100, 116, 130, 144	20
6	17.40	84, 99, 114, 116, 141, 158	20

Calibration Curve

To evaluate the viability of a CSR-LVSI approach to meeting the IRIS $1x10^{-6}$ cancer risk levels, a calibration curve was prepared using 1,4-dioxane (cat.# 30287); nitrosamine calibration mix, Method 521 (cat.# 31898); 8270 Appendix IX mix #1, revised (cat.# 32459); and tetrahydrofuran (cat.# 30414). The surrogates 1,4-dioxane-d8 (cat.# 30614) and N-nitrosodimethylamine-d6 (cat.# 33910) and internal standards THF-d8 (cat.# 30112) and N-nitrosodipropylamine-d14 (cat.# 33911) were added at constant concentrations to each level in the calibration curve (Table IV).

Table IV: Calibration Curve (ng/mL)

Analyte	ICAL 1	ICAL 2	ICAL 3	ICAL 4	ICAL 5	ICAL 6	ICAL 7	ICAL 8
THF-d8 IS)	100	100	100	100	100	100	100	100
THF	0.10	0.20	0.50	1.0	2.5	5.0	25	50
1,4-Dioxane-d8 (SS)	200	200	200	200	200	200	200	200
1,4-Dioxane	0.10	0.20	0.50	1.0	2.5	5.0	25	50
N-nitrosodimethylamine-d6 (SS)	20	20	20	20	20	20	20	20
N-nitrosodimethylamine	0.005	0.01	0.025	0.050	0.13	0.25	1.3	2.5
N-nitrosomethylethylamine	0.010	0.020	0.050	0.10	0.25	0.50	2.5	5.0
N-nitrosodiethylamine	0.010	0.020	0.050	0.10	0.25	0.50	2.5	5.0
N-nitrosodi-*n*-propylamine-d14 (IS)	10	10	10	10	10	10	10	10
N-nitrosopyrrolidine	0.010	0.020	0.050	0.10	0.25	0.50	2.5	5.0
N-nitrosodi-*n*-propylamine	0.010	0.020	0.050	0.10	0.25	0.50	2.5	5.0
N-nitrosomorpholine	0.005	0.010	0.025	0.050	0.13	0.25	1.3	2.5
N-nitrosopiperidine	0.010	0.020	0.050	0.10	0.25	0.50	2.5	5.0
N-nitrosodi-*n*-butylamine	0.010	0.020	0.050	0.10	0.25	0.50	2.5	5.0

Results and Discussion

We set out with the goal of verifying the feasibility of 50 µL CSR-LVSI when combined with standard EI GC-MS equipment to meet the low levels of detection relevant to human health. Figure 2 is a demonstration of the viability of 50 µL CSR-LVSI. Using a high column flow (5 mL/min) during the solvent focusing step allowed us to shorten the analysis time while maintaining the resolution of 1,4-dioxane and THF from the solvent peak. In addition, the use of a high temperature stability Rxi®-5Sil MS column allowed us program the oven up to 330 °C at the end of each analysis to remove contaminants from the column and prevent carryover between injections (Figure 3). As shown in Table V, the combination of the CSR-LVSI method and single SPE cartridge approach offers considerable time savings. For example, a 20-sample batch can be extracted and analyzed in just under 18 hours using the combined method, compared to running both methods separately which was estimated to require 31.7 hours. In addition, the combined single SPE cartridge method resulted in a reduction in solvent consumption.

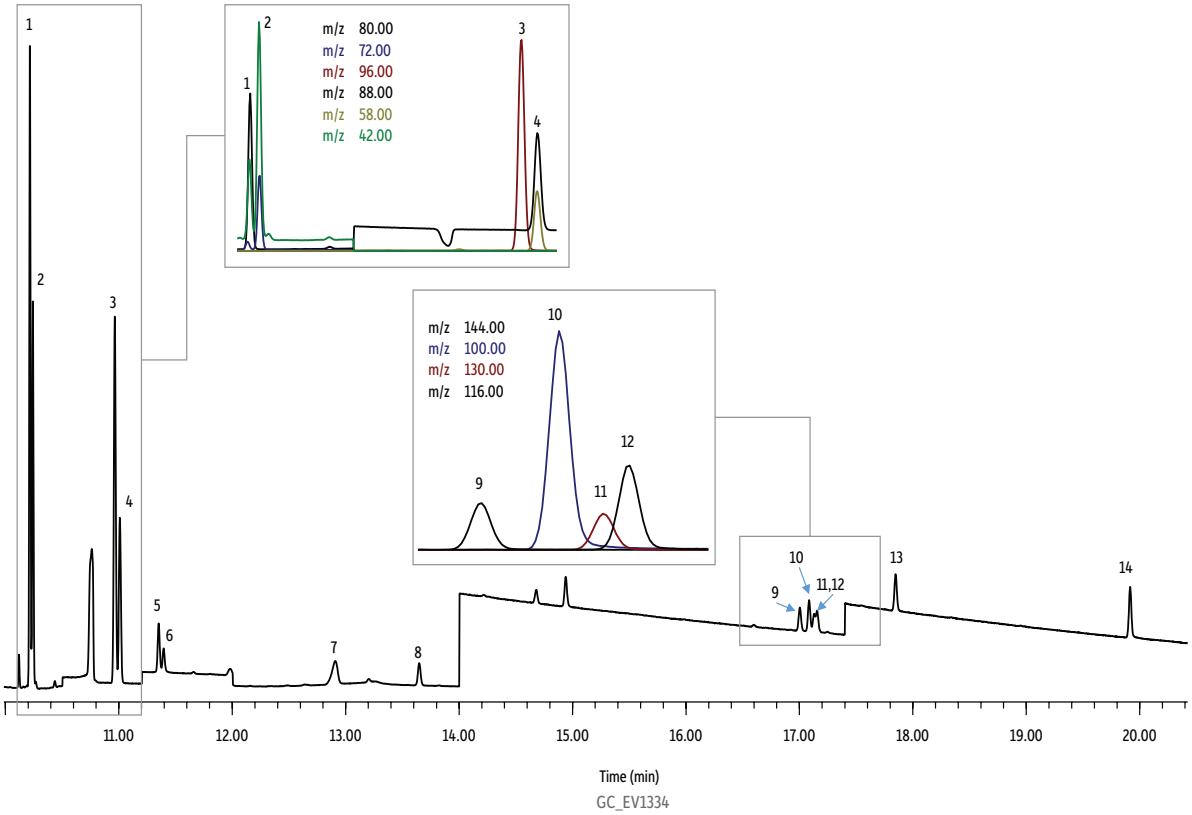
Table V: Combining the extraction and analysis of 1,4-dioxane (Method 522) and nitrosamines (Method 521) into a single method allows a time savings of approximately 14 hours per 20-sample batch, compared to running both methods separately.

	Hours Required for Extraction and Analysis of a 20-Sample Batch				
	Method 521	Method 522	Methods 521 and 522 Run Separately	Combined Method	Time Saved Using Combined Method
Sample Prep Time	8*	4	12*	4	8
GC Analysis Time	12.6	7.1	19.7	13.7	6
Total Time	20.6	11.1	31.7	17.7	14

Note: Sample preparation times were estimated. Analysis times were determined based on calculated oven program times listed in the methods. Oven cool down, equilibration, and injection sequence times were approximated based on comparison of the oven program times and actual times.
*Includes a required extract concentration step.

Figure 2: Combined SIM analysis of EPA Methods 522 (1,4-dioxane) and 521 (nitrosamines) compounds using CSR-LVSI. Analysis utilizes a 30 m x 0.25 mm x 1.0 µm Rxi®-5Sil MS analytical column and a 10 m x 0.53 mm ID Rxi® deactivated guard pre-column joined with an SGE® µ-union.

Peaks	Conc. (ng/mL)
1. Tetrahydrofuran-d8 (IS)	100
2. Tetrahydrofuran	50
3. 1,4-Dioxane-d8 (SS)	200
4. 1,4-Dioxane	50
5. N-Nitrosodimethylamine-d6 (SS)	20
6. N-Nitrosodimethylamine	2.5
7. N-Nitrosomethylethylamine	5.0
8. N-Nitrosodiethylamine	5.0
9. N-Nitrosodi-*n*-propylamine-d14 (IS)	10
10. N-Nitrosopyrrolidine	5.0
11. N-Nitrosodi-*n*-propylamine	5.0
12. N-Nitrosomorpholine	2.5
13. N-Nitrosopiperidine	5.0
14. N-Nitrosodi-*n*-butylamine	5.0



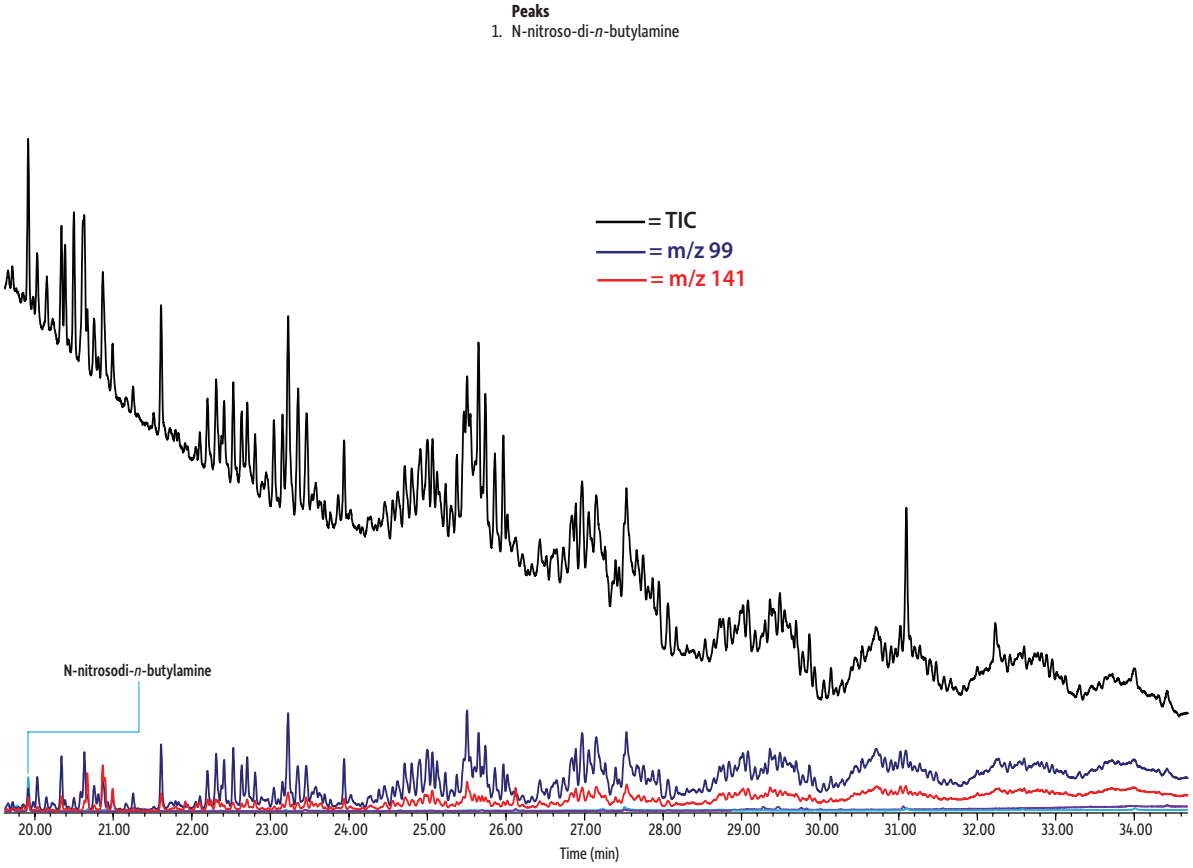
Column	Rxi®-5Sil MS, 30 m, 0.25 mm ID, 1.00 µm (cat.# 13653) using Rxi® guard column 10 m, 0.53 mm ID (cat.# 10073) with SGE® µ-union
Sample	N-Nitrosodimethylamine-d6 (cat.# 33910) 1,4-Dioxane-d8 (cat.# 30614) N-Nitrosodi-*n*-propylamine-d14 (cat.# 33911) Tetrahydrofuran-d8 (cat.# 30112) Nitrosamine calibration mix, Method 521 (cat.# 31898) Appendix IX mix #1, revised (cat.# 32459)
Diluent:	Dichloromethane
Injection	
Inj. Vol.:	50 µL splitless (hold 1.5 min)
Liner:	Custom single taper Restek Premium 4 mm ID liner with 15 mg quartz wool
Inj. Temp.:	275 °C
Purge Flow:	100 mL/min
Oven	
Oven Temp.:	35 °C (hold 1.5 min) to 50 °C at 50 °C/min (hold 7.1 min) to 320 °C at 11.12 °C/min (hold 1.5 min)
Carrier Gas	He, flow program
Flow Program:	5.08 mL/min (hold 8.9 min) to 2.0 mL/min at 1.0 mL/min/min

Detector	MS
Mode:	SIM
SIM Program:	

Group	Start Time (min)	Ion(s) (m/z)	Dwell (ms)
1	9.90	42, 46, 71, 72, 78, 80	20
2	10.50	58, 62, 64, 88, 96	20
3	11.20	42, 43, 46, 48, 74, 80	20
4	12.00	43, 56, 88, 102	30
5	15.50	58, 68, 70, 78, 86, 100, 116, 130, 144	20
6	17.40	84, 99, 114, 116, 141, 158	20

Transfer Line Temp.:	320 °C
Analyzer Type:	Quadrupole
Source Temp.:	230 °C
Quad Temp.:	150 °C
Solvent Delay Time:	9.9 min
Tune Type:	BFB
Ionization Mode:	EI
Instrument	Agilent 7890A GC & 5975C MSD

Figure 3: The laboratory fortified sample shown here illustrates the amount of co-extracted material eluted from the carbon cartridge. Selecting an Rxi®-5Sil MS column for this analysis allows us to program the oven up to 330 °C after each run, ensuring that column carryover is not a source of interference in subsequent analysis.



GC_EV1355

Column	Rxi®-5Sil MS, 30 m, 0.25 mm ID, 1.00 µm (cat.# 13653) using Rxi® guard column 10 m, 0.53 mm ID (cat.# 10073) with SGE® µ-union
Sample	N-Nitrosodimethylamine-d6 (cat.# 33910) 1,4-Dioxane-d8 (cat.# 30614) N-Nitrosodi-*n*-propylamine-d14 (cat.# 33911) Tetrahydrofuran-d8 (cat.# 30112) Nitrosamine calibration mix, Method 521 (cat.# 31898) Appendix IX mix #1, revised (cat.# 32459)
Diluent:	Dichloromethane
Conc.:	50 ng/L (sample fortification level)
Injection	
Inj. Vol.:	50 µL splitless (hold 1.5 min)
Liner:	Custom single taper Restek Premium liner with 15 mg wool on bottom
Inj. Temp.:	275 °C
Purge Flow:	100 mL/min
Oven	
Oven Temp.:	35 °C (hold 1.5 min) to 50 °C at 50 °C/min (hold 7.1 min) to 320 °C at 11.12 °C/min (hold 1.5 min)
Carrier Gas	He, flow program
Flow Program:	5.08 mL/min (hold 8.9 min) to 2.0 mL/min at 1.0 mL/min/min
Detector	MS
Mode:	SIM

SIM Program:

Group	Start Time (min)	Ion(s) (m/z)	Dwell (ms)
1	9.90	42, 46, 71, 72, 78, 80	20
2	10.50	58, 62, 64, 88, 96	20
3	11.20	42, 43, 46, 48, 74, 80	20
4	12.00	43, 56, 88, 102	30
5	15.50	58, 68, 70, 78, 86, 100, 116, 130, 144	20
6	17.40	84, 99, 114, 116, 141, 158	20

Transfer Line Temp.:	320 °C
Analyzer Type:	Quadrupole
Source Type:	Inert
Drawout Plate:	6 mm ID
Source Temp.:	230 °C
Quad Temp.:	150 °C
Solvent Delay Time:	9.9 min
Tune Type:	BFB
Ionization Mode:	EI
Instrument	Agilent 7890A GC & 5975C MSD

Linearity

An eight-point calibration curve was generated by injecting 50 µL each of the calibration levels described in Table VI. Calibration linearity was evaluated using a 1/x weighted linear regression, with R values between 0.994 and 0.999. Surrogates were evaluated by the % relative standard deviation (RSD) of their relative response factors (RRF), and both showed less than 4.0% RSD across eight calibration levels. Note that while operating in selected ion mode, especially when dealing with low molecular weight ions, it is critical that the peaks of interest be separated from interferences. In fact, EPA Method 522 explicitly states that the analyst must verify the absence of interferences for 1,4-dioxane at both the quantitation ion (m/z 88) and the confirmation ion (m/z 58). We dropped the two lowest points of the 1,4-dioxane calibration because the quantitation ion could not be resolved from the residual dichloromethane background, even though the confirmation ion showed good recovery.

Table VI: Eight-point calibration curve levels. For each level, the split cells give the extract concentration on the left (ng/mL) and equivalent sample concentrations on the right (ng/L). 50 µL injections were used for low ppt-level SIM analysis.

	Level 1		Level 2		Level 3		Level 4		Level 5		Level 6		Level 7		Level 8		R
Analytes	**ICAL**	**Sample**	**ICAL**	**Sample**	**ICAL**	**Sample**	**ICAL**	**Sample**	**ICAL**	**Sample**	**ICAL**	**Sample**	**ICAL**	**Sample**	**ICAL**	**Sample**	
THF-d8 IS)	100	--	100	--	100	--	100	--	100	--	100	--	100	--	100	--	--
THF	0.10	2.0	0.20	4.0	0.50	10	1.0	20	2.5	50	5.0	100	25	500	50	1,000	0.998
1,4-Dioxane-d8 (SS)	200	4,000	200	4,000	200	4,000	200	4,000	200	4,000	200	4,000	200	4,000	200	4,000	1.90%*
1,4-Dioxane	--	--	--	--	0.50	10	1.0	20	2.5	50	5.0	100	25	500	50	1,000	0.999
N-nitrosodimethylamine-d6 (SS)	20	400	20	400	20	400	20	400	20	400	20	400	20	400	20	400	3.60%*
N-nitrosodimethylamine	--	--	--	--	0.025	0.50	0.050	1.0	0.13	2.5	0.25	5.0	1.3	25	2.5	50	0.998
N-nitrosomethylethylamine	--	--	--	--	--	--	0.10	2.0	0.25	5.0	0.50	10	2.5	50	5.0	100	0.998
N-nitrosodiethylamine	0.010	0.20	0.020	0.40	0.050	1.0	0.10	2.0	0.25	5.0	0.50	10	2.5	50	5.0	100	0.998
N-nitrosodi-*n*-propylamine-d14 (IS)	10	--	10	--	10	--	10	--	10	--	10	--	10	--	10	--	--
N-nitrosopyrrolidine	0.010	0.20	0.020	0.40	0.050	1.0	0.10	2.0	0.25	5.0	0.50	10	2.5	50	5.0	100	0.997
N-nitrosodi-*n*-propylamine	--	--	--	--	0.050	1.0	0.10	2.0	0.25	5.0	0.50	10	2.5	50	5.0	100	0.997
N-nitrosomorpholine	--	--	0.010	0.20	0.025	0.50	0.050	1.0	0.13	2.5	0.25	5.0	1.3	25	2.5	50	0.994
N-nitrosopiperidine	0.010	0.20	0.020	0.40	0.050	1.0	0.10	2.0	0.25	5.0	0.50	10	2.5	50	5.0	100	0.997
N-nitrosodi-*n*-butylamine	--	--	0.020	0.40	0.050	1.0	0.10	2.0	0.25	5.0	0.50	10	2.5	50	5.0	100	0.997

For surrogate standards, linearity was assessed based on the % RSD of their relative response factors across eight calibration levels.
Levels with "—" instead of a concentration were dropped from the calibration because of poor signal-to-noise ratios.
Internal standards were added to the extracts and have no equivalent sample concentration.

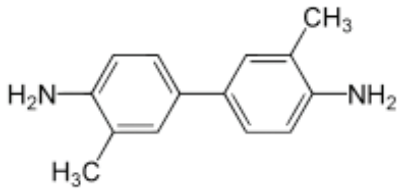
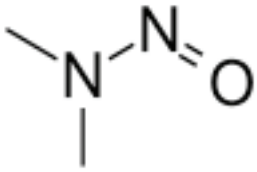
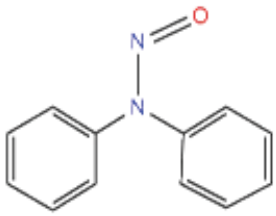
Practical Quantitation Limits

Unlike instrument detection limits (IDLs) and method detection limits (MDLs), practical quantitation limits (PQLs) are not statistically determined. Also, there is no regulation (outside of an individual laboratory's quality plan) governing the determination of a PQL, making its assignment somewhat arbitrary. Some laboratories have defined the PQL internally as the IDL × 10 or the MDL × 6, but most set the PQL for each compound as the lowest point in the calibration curve, and this is how we set the quantitation limits for this work. Using the lowest point in the calibration curve gives a higher confidence in the value at these low levels than a multiple of a statistically determined number.

Recoveries From Blanks and Fortified Samples

We expected that the coconut charcoal would strongly retain the planar molecules, and that the dichloromethane would only efficiently elute the alkyl nitrosamine compounds. Table VII gives examples of the planar geometries that would be strongly retained by the coconut charcoal, as well as the alkyl chemistries that would undergo near complete elution by dichloromethane. N-nitrosodiphenylamine was not expected to be fully eluted from the coconut SPE tube, therefore, it was not included in the method (although it is on the RCRA compound list and the analyte test list for both the wastewater and hazardous waste nitrosamines testing methods [Methods 607 and 8070A, respectively]). Munch and Bassett described detecting N-nitrosodiphenylamine in their blanks when using coconut charcoal SPE tubes [8]; we experienced the same phenomenon, indicating that this SPE technique is inappropriate for N-nitrosodiphenylamine. This may be an example of the mechanism for nitrosamine formation from secondary amines on the surface of activated carbon recently published by Padhye et al. [7].

Table VII: Planar compounds are expected to be retained by the charcoal SPE cartridges, whereas alkyl molecules should be nearly completely eluted from the cartridges with dichloromethane.

Planar Molecule	Non-Planar Molecule	Nitrosamine With Planar Ligands
		
o-Toluidine	N-nitrosodi-methylamine	N-nitrosodiphenylamine

A thorough evaluation of the method-specific Resprep® SPE cartridge for EPA 522 (cat.# 26032), which is a 6 mL cartridge with 2 g activated charcoal, was described in the papers on the development of EPA Methods 521 and 522 [1, 8]. The cartridge was demonstrated to be effective for samples ranging between 0.5 L and 1 L, though high levels of suspended solids may severely restrict flow or completely clog the cartridge before the full sample amount has passed. Nitrosamines were also demonstrated to show reduced recoveries when 1 L samples were analyzed, likely due to breakthrough. Average recoveries of 1,4-dioxane and nitrosamines were in the mid-80 to low-90 percentile with %RSDs less than 5 (n=7 for each matrix) when the activated carbon SPE cartridges were used to extract three different drinking water sources (surface water, high total organic carbon surface water, and high mineral content ground water)[1, 8]. In light of this work, our concern regarding the SPE cartridge was not extraction efficiency, but rather the consequences of injecting 50 times the normal amount of sample matrix into the GC.

Several of the higher molecular weight nitrosamines as well as 1,4-dioxane and tetrahydrofuran, were detected in the blank samples above the lowest calibration levels, which had been used as the practical quantitation limits (Table VIII). There are several possible reasons for this; lab contamination, especially with 1,4-dioxane and THF is a known problem. With this in mind, we used purchased bottled water for the blanks as well as the fortified samples. The most likely source of contamination is the finished drinking waters used for the blanks. We were surprised by the background level of THF found, given that we purchased what we expected to be "clean" filtered drinking waters from a major commercial supplier. Another possible source of low-level nitrosamine contamination may be reactivity between chloramines and the activated coconut charcoal in the extraction tube [7]. Also, at these extremely low levels, isobaric interferences cannot be discounted. The quant ions were changed for several of the nitrosamine compounds when it was discovered that the primary ions selected from NIST spectra showed interferences with co-extracted material in sample extracts. In order to report the analyte list at the trace levels detected, we modified the default reporting limit of any compound detected in the blank to twice the amount detected. These "blank adjusted" reporting limits are also presented in Table VIII along with the initial expected PQLs and the EPA Method 522/521 minimum reporting limits.

Analyte recoveries from laboratory fortified samples are presented in Table IX. The low-level fortified samples showed higher than expected recoveries for the same compounds that showed signs of contamination in the blank. NDMA, the nitrosamine of highest concern, was detected at sub-ppt levels, below its IRIS 10^{-6} cancer risk assessment level (0.7 ng/L) using this CSR-LVSI technique (Figure 4). NDEA, another nitrosamine of concern, showed an average recovery of 75% for waters spiked at 1.0 ng/L. While not quite the risk assessment level for NDEA (0.2 ng/L), this is well below the notification level of 10 ng/L used by the California EPA and other states.

The mid-level laboratory fortified samples showed much better percent recoveries for all but a few compounds (THF, 1,4-dioxane, and N-nitroso-di-*n*-butylamine) as shown in Table IX. This is most likely due to source water contamination. The high-level laboratory fortified samples showed recoveries that conformed to expectations better than the two previous levels, though THF and NDMA showed deviations higher than the rest of the compounds (Table IX). At 50 ppt, the high bias of N-nitroso-di-*n*-butylamine disappeared.

Table VIII: Laboratory Blank Recoveries and Adjusted Reporting Limits

	Limits			Blank		
Analyte	EPA Min Reporting Limit (ng/L)	Initial PQL (ng/L)	Blank Adjusted Reporting Limit (ng/L)	Avg. Recovery (ng/L) N = 3	Avg. % Recovery	% RSD
THF	NA	2.0	340	170	--	69
1,4-Dioxane-d8 (SS)	--	--	--	4,300	110	5.0
1,4-Dioxane	70	10	36	18	--	8.1
N-nitrosodimethylamine-d6 (SS)	--	--	--	350	88	6.4
N-nitrosodimethylamine	1.6	0.50	0.50	<0.50	--	--
N-nitrosomethylethylamine	1.5	2.0	2.0	<2.0	--	--
N-nitrosodiethylamine	2.1	0.20	0.56	0.28	--	--*
N-nitrosopyrrolidine	1.4	0.20	2.8	1.4	--	3.6
N-nitrosodi-*n*-propylamine	1.2	1.0	6.8	3.4	--	1.4
N-nitrosomorpholine	NA	0.20	1.1	0.56	--	0.21
N-nitrosopiperidine	1.4	0.20	1.4	0.68	--	44
N-nitrosodi-*n*-butylamine	1.4	0.40	14	7.0	--	16

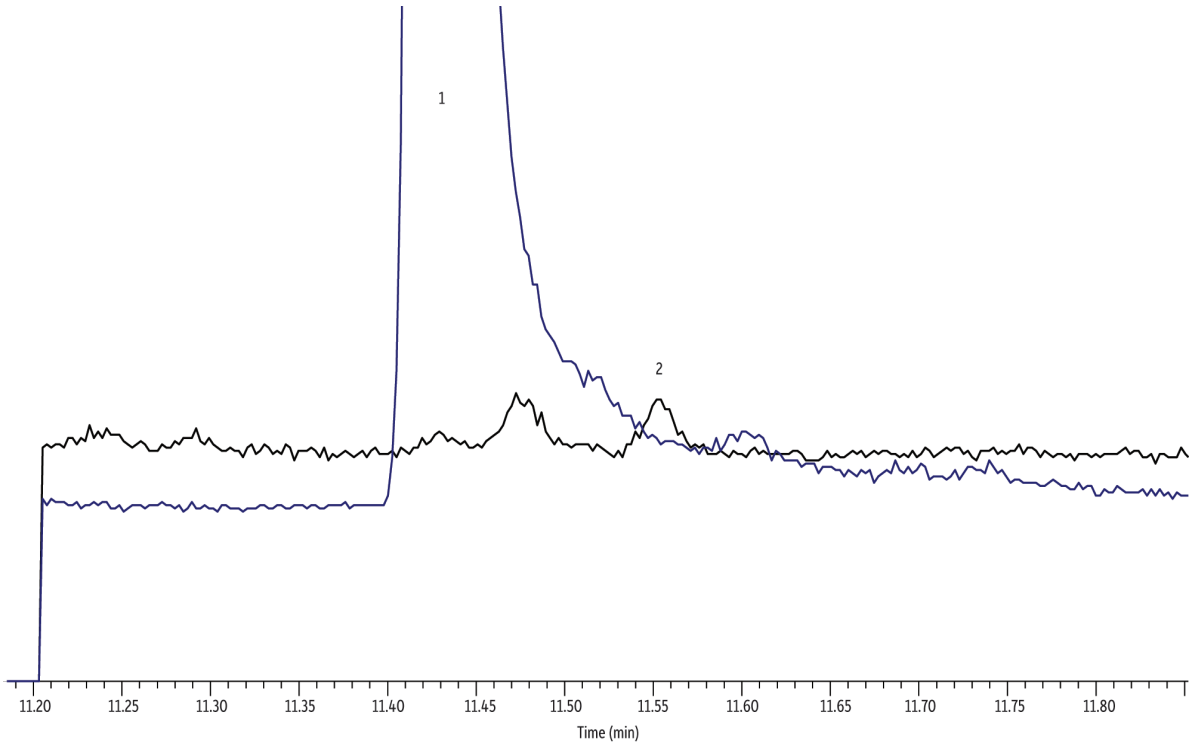
*N-nitrosodiethylamine was detected in only one of three blank samples.

Table IX: Analyte Recoveries From Laboratory Fortified Samples

			Low Level			Mid Level			High Level		
Analyte	Blank-Adjusted Reporting Limit (ng/L)	IRIS $1x10^{-6}$ Cancer Conc. (ng/L)	Avg. Recovery (ng/L) N = 3	Avg. % Recovery	% RSD	Avg. Recovery (ng/L) N = 3	Avg. % Recovery	% RSD	Avg. Recovery (ng/L) N = 3	Avg. % Recovery	% RSD
THF	340	--	<340	--	--	<340	--	--	610	120	20
1,4-Dioxane	36	350	<36	--	--	72	140	1.9	510	100	19
N-nitrosodimethylamine	0.50	0.7	0.63	130	14	2.3	92	9.3	17	68	26
N-nitrosomethylethylamine	2.0	2.0	<2.0	--	--	3.9	78	22	48	96	4.8
N-nitrosodiethylamine	0.56	0.2	0.75	75	18	4.7	94	4.7	48	96	1.2
N-nitrosopyrrolidine	2.8	20	<2.8	--	--	5.3	106	1.9	46	92	5.1
N-nitrosodi-*n*-propylamine	6.8	5.0	<6.8	--	--	<6.8	--	--	43	86	3.8
N-nitrosomorpholine	1.1	--	<1.1	--	--	2.5	100	3.6	23	92	4
N-nitrosopiperidine	1.4	--	<1.4	--	--	4.5	90	1.6	46	92	2.4
N-nitrosodi-*n*-butylamine	14	6.0	<14	--	--	<14	--	--	49	98	4.9

Figure 4: N-nitrosodimethylamine (NDMA) quantitation ion EIC for a low-level fortified sample (0.50 ng/L).

Peaks	Conc. (ng/mL)	m/z
1. N-Nitrosodimethylamine-d6	10	80
2. N-Nitrosodimethylamine	0.025	74



Column	Rxi®-5Sil MS, 30 m, 0.25 mm ID, 1.00 µm (cat.# 13653) using Rxi® guard column 10 m, 0.53 mm ID (cat.# 10073) with SGE µ-union
Sample	
Diluent:	Dichloromethane
Injection	
Inj. Vol.:	50 µL splitless (hold 1.5 min)
Liner:	Custom Restek Premium single taper liner with 15 mg quartz wool
Inj. Temp.:	275 °C
Purge Flow:	100 mL/min
Oven	
Oven Temp.:	35 °C (hold 1.5 min) to 50 °C at 50 °C/min (hold 7.1 min) to 320 °C at 11.12 °C/min (hold 1.5 min)
Carrier Gas	He, flow program
Flow Program:	5.08 mL/min (hold 8.9 min) to 2.0 mL/min at 1.0 mL/min/min
Detector	MS
Mode:	SIM
SIM Program:	42, 43, 46, 48, 74, 80 m/z
Transfer Line Temp.:	320 °C
Analyzer Type:	Quadrupole
Source Type:	Inert
Source Temp.:	230 °C
Quad Temp.:	150 °C
Solvent Delay Time:	9.9 min
Tune Type:	BFB
Ionization Mode:	EI
Instrument	Agilent 7890A GC & 5975C MSD

Conclusion

The preceding work was conducted to evaluate the effectiveness of combining concurrent solvent recondensation–large volume injection with an unmodified splitless inlet to lower the detection limits of standard GC-MS equipment operating in EI mode, while combining volatile and semivolatile analytes that use similar extraction procedures in order to minimize sample prep and analysis times.

Our anticipated reporting limits (PQLs), based on the lowest calibration point for each individual compound were well below the minimum reporting limits published in EPA Methods 522 and 521. However, the contamination of the blank water used for the laboratory fortified samples caused us to raise the minimum reporting limits used for this application. Unfortunately, some of these limits were raised above the levels published in the EPA methods.

When performing this work in the future, it would be prudent to further purify the reagent water used for laboratory control samples. Inert gas is commonly used to purge volatiles from blank reagent water, but the "volatile" impurities found in our blank water do not purge efficiently enough to expect the reagent water to test clean at the low ng/L levels we targeted in this work. One of the recommended uses for the carbon Empore® disk is cleaning up water for purge-and-trap applications. This type of activated carbon disk or cartridge cleanup should be applied to any reagent water before laboratory fortification.

With this combined approach, we estimate a 20-sample batch could be extracted and analyzed in less than 18 hours, which is 14 hours faster than when running the methods separately. In addition to providing a means of reducing sample prep costs and increasing sample throughput, this work offers a good starting point for modifying wastewater or hazardous waste methods to speed up sample prep and sample analysis. This 50 µL CSR-LVSI technique, when combined with SPE sample preparation, may reduce the necessary wastewater or hazardous waste sample from 500 mL to just tens of milliliters, significantly reducing sample handling costs.

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