

Method Development for the Analysis of 1,4-Dioxane in Drinking Water Using Solid-Phase Extraction and Gas Chromatography–Mass Spectrometry

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Abstract

1,4-Dioxane has been identified as a probable human carcinogen and an emerging contaminant in drinking water. The United States Environmental Protection Agency's (U.S. EPA) National Exposure Research Laboratory (NERL) has developed a method for the analysis of 1,4-dioxane in drinking water at ng/L concentrations. The method consists of an activated carbon solid-phase extraction of 500-mL or 100-mL water samples using dichloromethane as the elution solvent. The extracts are analyzed by gas chromatography–mass spectrometry (GC–MS) in selected ion monitoring (SIM) mode. In the NERL laboratory, recovery of 1,4-dioxane ranged from 94–110% in fortified laboratory reagent water and recoveries of 96–102% were demonstrated for fortified drinking water samples. The relative standard deviations for replicate analyses were less than 6% at concentrations exceeding the minimum reporting level.

Introduction

In February 2008, the U.S. EPA announced its latest draft Drinking Water Contaminant Candidate List (CCL3) (1). This list includes 93 chemical contaminants that are known or anticipated to occur in public water systems, and may require regulation under the Safe Drinking Water Act. The solvent stabilizer 1,4-dioxane, a probable human carcinogen, has been included on this list. Although the U.S. EPA has not established a Maximum Contaminant Level (MCL) for 1,4-dioxane in drinking water, it has previously set MCLs for carcinogens at the one in 10^6 lifetime cancer risk concentration. The one in 10^6 lifetime cancer risk concentration published by U.S. EPA for 1,4-dioxane is 3 $\mu\text{g/L}$ (2); however, this value is currently under review and may be revised. Because 1,4-dioxane has been identified in U.S. groundwaters and drinking water wells (3,4), some states have set notification levels and maximum standard levels ranging from 3–85 $\mu\text{g/L}$ (5–12).

The use of 1,4-dioxane as a stabilizer for chlorinated solvents accounts for the major source of its environmental presence.

1,1,1-Trichloroethane (TCA)-based solvents may contain up to 8% 1,4-dioxane. The improper disposal of chlorinated solvents from degreasing operations has contributed to the presence of 1,4-dioxane in groundwater and some surface waters. U.S. EPA's Toxic Chemical Release Inventory reported that nearly one million pounds of 1,4-dioxane were released into the environment in the U.S. in 1996 (12). Due to the relative difference in boiling points, waste solvents from degreasing operations have been shown to contain higher levels of the stabilizers than the original solvent. Isaacson et al. reported concentrations of 1,4-dioxane up to three times higher than the associated TCA concentrations in contaminated groundwater samples (13). The persistence of 1,4-dioxane in soil and groundwaters is of concern because its resistance to chemical and biological degradation minimizes natural attenuation. A by-product of ethoxylated detergents and surfactants, 1,4-dioxane may also be entering municipal waste-water streams through the use of consumer products (12). Concentrations of 1,4-dioxane as high as 200,000 $\mu\text{g/L}$ have been reported in contaminated groundwaters, and as high as 2100 $\mu\text{g/L}$ in drinking water (4).

In order for U.S. EPA to make a regulatory determination concerning 1,4-dioxane, nationwide occurrence data using a standardized method is required. While the method must be sensitive and highly specific to minimize false positives, it must also be cost effective and simple enough for commercial laboratory settings. To facilitate a large scale monitoring effort, and minimize analytical costs for both drinking water utilities and U.S. EPA, the ideal method should utilize instrumentation typically found in environmental laboratories and materials commercially available from multiple vendors and easily accessible in the U.S. The detection and quantitation limits should be as low as possible in the event that the risk assessment values for the carcinogenicity of 1,4-dioxane are revised to lower values.

Historically, the analysis of 1,4-dioxane in water has proved challenging. Direct aqueous injection methods have been attempted (14), and while avoiding extraction altogether, this technique suffers from high detection limits (mg/L). Direct inject techniques were recently reevaluated by Draper et al., but detection levels were not significantly improved (15).

Several extraction and concentration techniques have been

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attempted, with varying degrees of sensitivity, accuracy, and precision. Purge and trap methods yielded poor purging efficiency, with recoveries of less than 1% using U.S. EPA's standard purge and trap technology (16), because 1,4-dioxane has a high water solubility. Researchers have heated the sample and/or added large amounts of salt to the sample to increase purging efficiencies, but those techniques potentially expose the instrumentation to salt and water vapor, affecting precision and accuracy as well as instrument down-time (3).

Solid-phase microextraction (SPME) coupled with gas chromatography–mass spectroscopy (GC–MS) has been demonstrated to be effective for extraction of 1,4-dioxane in water by Shirey and Linton (17). Using a carboxen–polydimethylsiloxane (PDMS) fiber with a 20 min heated headspace exposure, the researchers' procedure was reported to be accurate and precise down to a limit of quantitation (LOQ) of 2.5 µg/L without background subtraction. When background subtraction was incorporated, the LOQ cited was 0.5 µg/L. However, background subtraction is not considered an acceptable practice in U.S. EPA drinking water methods. Nakamura et al. (18) reported a method detection limit (MDL) of 1.17 µg/L using a 100-µm PDMS fiber with a 30 min exposure at 60°C. In addition to SPME's relative lack of sensitivity, this technique is limited by the fact that it requires specialized instrumentation not typically available in environmental laboratories, and it is relatively expensive to automate.

Various procedures for liquid–liquid extraction (LLE) have also been used to isolate and concentrate 1,4-dioxane. Recoveries ranging from 5–80% have been reported for traditional separatory funnel and continuous LLE, depending on sample size, extracting solvent, and salt concentration added (15,19,20). In general, LLE methods can be time consuming and costly, due to the large amounts of organic solvents required. In addition, the volume and toxicity of the wastes produced by these techniques pose a serious disposal issue.

Solid-phase extraction (SPE) is a preferred extraction technique for many applications because it is faster, less expensive, and requires far less solvent than LLE methods. Although SPE is normally used for hydrophobic non-polar analytes, recent applications for hydrophilic, volatile compounds, such as *N*-nitrosamines (21,22,23), indicate that SPE using carbon-based sorbents to extract 1,4-dioxane may be a worthwhile pursuit. Kawata and colleagues (22) extracted 1,4-dioxane at 100% efficiency from 500-mL water samples using 0.5 g of carbon fiber felt, and eluted it with 5 mL of acetone. Kawata et al. also reported similar results from a commercially available Sep-Pak cartridge (24).

Separation, identification, and quantitation of 1,4-dioxane in solvent extracts is often accomplished by GC–MS (13,15,17–20,22–25). Traditional GC detectors, such as flame ionization detection and photoionization detection, lack sufficient specificity and sensitivity. Past experimental work performed by Yoo et al. (19) indicates that a GC column with a large film thickness and used at low initial oven temperatures will aid in the process of separating low molecular weight (MW) compounds such as 1,4-dioxane (MW 88) from the extraction solvent and potential co-extracted compounds. MS detection, in both full scan and selected ion monitoring (SIM) modes, is used almost exclusively

for the detection of 1,4-dioxane because of its relatively low MW and boiling point.

This manuscript describes the development of a method for the measurement of 1,4-dioxane at ng/L concentrations in drinking water. The method performance exceeds the sensitivity, accuracy, and precision required to meet U.S. EPA goals for its potential use in a future nationwide monitoring program to measure the occurrence of 1,4-dioxane in public drinking water systems. The final method uses a combination of activated carbon SPE and GC–MS–SIM to meet these performance goals.

Experimental

Chemicals, standards, and SPE media

Laboratory reagent water (LRW): Prepared from tap water using reverse osmosis followed by a Millipore (Billerica, MA) Milli-Q Ultrapure Gradient A-10 polishing unit.

Methanol, Purge and Trap grade: B&J Brand, Honeywell Burdick & Jackson (Muskegon, MI). Dichloromethane (DCM), Absolv grade: Tedia Company (Fairfield, OH).

Sodium sulfate (anhydrous), ACS grade: Thermo Fisher Scientific (Waltham, MA). The sodium sulfate was baked for 4 h at 400°C and packed into 3-mL Varian (Palo Alto, CA) polypropylene Bond Elut SPE column reservoirs or 6-mL Supelco (St. Louis, MO) glass columns with 20 µm polyethylene frits for removal of water from final extracts.

Ammonium chloride, ACS grade: Sigma-Aldrich (St. Louis, MO). Copper sulfate (CuSO₄·5H₂O), ACS grade: Thermo Fisher Scientific. Trizma pre-set crystals, pH 7.0: Sigma-Aldrich.

Sodium bisulfate (anhydrous), technical grade: Thermo Fisher Scientific. Sodium sulfite (anhydrous), ACS grade: Thermo Fisher Scientific.

Deuterated standards: 1,4-dioxane-*d*₈ surrogate standard and tetrahydrofuran-*d*₈ (THF-*d*₈) internal standard (Absolute Standards, Hamden, CT).

Analyte standards: 1,4-dioxane, Absolute Standards and SPEX Certiprep (Metuchen, NJ).

1,1,1-Trichloroethane, ReagentPlus grade: Sigma-Aldrich.

SPE columns/cartridges, activated carbon sorbent: Resprep EPA Method 521 2000-mg/6-mL columns (Restek, Bellefonte, PA), Enviro-Clean 521 EPA Method 2000-mg/6-mL columns (United Chemical Technologies, Bristol, PA), and Sep-Pak Plus AC-2 400-mg cartridges (Waters Corp., Milford, MA). Resprep and Enviro-Clean 2-g columns demonstrated comparable performance and were used interchangeably.

Glass sample bottles (125-mL and 1-L volume), with polytetrafluoroethylene (PTFE) lined caps, were washed with detergent and tap water, rinsed with tap water, followed by reagent water. Bottles were then heated in a muffle furnace at 400°C for 2 h.

Glass solvent collection vials (15-mL volume centrifuge and 2-mL volumetric), 1 mL glass volumetric flasks, and glass autosampler vials with PTFE-lined caps.

SPE procedure and apparatus

SPE column extraction (large scale)

Experiments utilizing 6-mL SPE columns containing 2 g activated carbon were performed using a Supelco Visiprep 24-posi-

tion vacuum manifold (Sigma-Aldrich, St. Louis, MO). Once inserted onto the manifold, SPE columns were conditioned with 3 mL DCM, followed by 2 × 3 mL methanol, and rinsed with 5 × 3 mL LRW. The sorbent bed was kept wet after the addition of methanol. Samples (500 mL) were fortified with the surrogate analyte, 1,4-dioxane-*d*₈ (2.5 µL of 2000 µg/mL stock), and passed through the SPE column under vacuum (−50 kPa) at a flow rate of 5–10 mL/min using PTFE Visiprep Large Volume Samplers (Sigma-Aldrich). Sample bottles were not rinsed with solvent because the hydrophilic nature of 1,4-dioxane made it unlikely that measurable amounts of 1,4-dioxane would remain on the glassware after sample loading. Once samples were passed through the SPE column, a 10-min drying time was employed under full vacuum. Columns were eluted under partial vacuum (−30 kPa) with ~9 mL DCM, collected in 15-mL centrifuge vials, and adjusted to 10-mL with DCM. For experiments requiring concentration, extracts were dried by passing through 6-mL glass columns packed with sodium sulfate (5 g) and collected in 15-mL centrifuge vials, which were then concentrated by nitrogen evaporation (N-EVAP, Organomation Assoc.; Berlin, MA) at room temperature to a volume of slightly less than 1 mL. Extracts were then transferred to 1-mL volumetric tubes, followed by the addition of internal standard (5.0 µL of 1000 µg/mL stock), and brought to final volume with DCM. Finally, extracts were transferred to GC autosampler vials and analyzed by GC–MS (full scan). For experiments requiring no extract concentration, samples were dried by adding sodium sulfate (2 g) directly to the 10-mL extract, mixed on a vortex mixer, followed by the addition of internal standard. Sample extracts were then transferred to GC autosampler vials and analyzed by GC–MS–SIM. Fortified LRW samples and fortified field samples were handled as described earlier, except that they were fortified with 1,4-dioxane just prior to extraction.

SPE column extraction (small scale)

Experiments utilizing Sep-Pak Plus AC 2 SPE cartridges (Waters Corp.) were performed using a Supelco Visiprep 24-position vacuum manifold (Sigma-Aldrich). Once inserted onto the manifold, Sep-Pak cartridges were conditioned with 1 mL DCM, followed by 2 × 1 mL methanol, and rinsed with 3 × 1 mL LRW. An empty 75-mL SPE column (Varian) was used as a sample reservoir. The sorbent bed was kept wet after the addition of methanol. LRW samples (100 mL) were fortified with the analyte and surrogate (5.0 µL of 200 µg/mL solution), and passed through the cartridge under vacuum (−50 kPa) at 5–10 mL/min. Sample bottles were not rinsed, consistent with the large-scale

extraction procedure. Once samples were passed through the column, a 10 min drying time was employed under full vacuum. Columns were eluted under partial vacuum (−30 kPa) with ~1.5 mL DCM and collected in 2-mL volumetric tubes. The extracts were spiked with internal standard (10 µL of 100 µg/mL solution), and then brought to final volume with DCM. Small-scale experiments required no concentration of the extract. The 2-mL extracts were then passed through a 3-mL polypropylene column with sodium sulfate (0.4 g) and collected directly into GC autosampler vials. Sample extracts were then analyzed by GC–MS–SIM.

Instrumental analysis

Full Scan GC–MS

A Varian Saturn 2200 Ion Trap GC–MS with a CP-8400 autosampler was used for full scan GC–MS (electron ionization) analysis of 1,4-dioxane during the method development. Injections were made in the splitless mode. The GC oven was equipped with a CP-Select 624 CB (6% cyanopropyl-phenyl, 94% dimethylsiloxane phase) 30 m × 0.25 mm × 1.4 µm column from Varian. The injector temperature was 200°C. The initial oven temperature of 30°C was held for 1 min, programmed to 90°C at 8°C/min, then further programmed to 200°C at 20°C/min, and held at 200°C for 4 min. The carrier gas (helium) was set at a constant flow of 1 mL/min. The ion trap, manifold, and transfer line temperatures were set to 150°C, 40°C, and 250°C, respectively. The mass spectrometer was scanned from *m/z* 40–200 at a rate of 0.61 s/scan, starting 6 min after injection. The emission current was set to 25 µA, prescan ionization time to 100 µs, and background mass to *m/z* 44. Quantitative analysis of 1,4-dioxane was performed using internal standard quantitation, with THF-*d*₈ used as the internal standard at a concentration of 5 µg/mL. A nine-point linear calibration curve (0.1–10 µg/mL) was used, plotting the ratio of the peak area of 1,4-dioxane to the peak area of the internal standard versus the ratio of the amount of 1,4-dioxane to the amount of the internal standard. Recoveries of the surrogate analyte, 1,4-dioxane-*d*₈, were calculated in similar fashion.

GC–MS–SIM

A Thermo Finnigan Trace DSQ Quadrupole GC–MS with an AS2000 autosampler was used in SIM mode for the GC–MS analysis (electron ionization) of 1,4-dioxane during method development. Injections were made in the splitless mode. The GC oven was equipped with a CP-Select 624 CB (6% cyanopropyl-phenyl, 94% dimethylsiloxane phase) 30 m × 0.25 mm × 1.4 µm column from Varian. The injector temperature was 200°C. The initial oven temperature of 30°C was held for 1 min, programmed to 90°C at 7°C/min, then further programmed to 200°C at 20°C/min and held at 200°C for 3 min. The carrier gas (helium) was set at a constant flow of 1 mL/min. The mass spectrometer was scanned for *m/z* 46, 78, 80 (THF-*d*₈) at a rate of 0.36 s/scan in segment 1 (6–8 min from injection). Segment 2 was set to scan for *m/z* 58 and 88 (1,4-dioxane), as well as *m/z* 62, 64, and 96 (1,4-dioxane-*d*₈) at 0.60 s/scan from 8 min to the end of the chromatographic run. Each ion was assigned a dwell time of 100 µs. The ion source temperature was set to 200°C and emission current was set to 100 µA. Quantitative analysis of 1,4-dioxane

Table 1. Retention Times and Product Ions for Quantitation of Analytes using GC–MS–SIM*

Compound	Retention time (min) [†]	SIM ions (<i>m/z</i>)
1,4-dioxane	8.864	58, 88
1,4-dioxane- <i>d</i> ₈ (SUR)	8.786	62, 64, 96
THF- <i>d</i> ₈ (IS)	6.722	46 , 78, 80

* Quantitation ions used are in bold.
[†] Retention times obtained with the GC column and conditions listed for GC–MS–SIM.

was performed using internal standard quantitation, as described in the “Full scan GC–MS” section, with the exception that the internal standard concentration was 0.5 $\mu\text{g/mL}$ and the calibration curve ranged from 0.002–1.0 $\mu\text{g/mL}$. Specific retention times, along with SIM ions, are available in Table I.

Results and Discussion

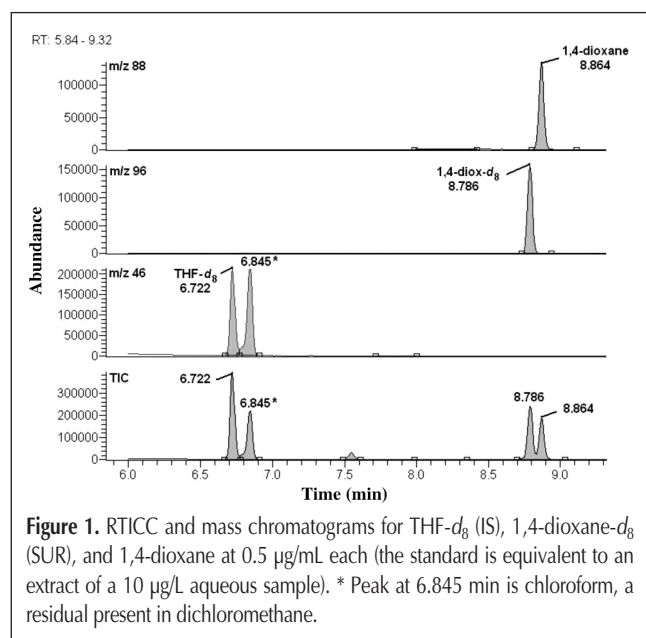
Preliminary experiments to evaluate analyte recovery through the SPE process and potential method sensitivity were first performed using the large scale SPE procedure, without extract concentration, and the full scan GC–MS analytical parameters described earlier (at the time of this initial work, the Sep-Pak Plus AC-2 SPE cartridges were not available in the U.S.). Although adequate performance was observed in fortified LRW samples at a concentration of 1 $\mu\text{g/L}$ using these procedures, accurate results for authentic drinking water samples were anticipated to be marginal, due to potential competition from other organic material in field sample matrices. To enhance sensitivity, extracts were concentrated to achieve a lower detection and quantitation level. Concentration was performed using gentle nitrogen stream evaporation in a water bath at ambient temperature (*N*-EVAP). Results were inconsistent ($> 15\%$ RSD) with analyte loss up to 30%, probably due to the volatility of 1,4-dioxane. Multiple literature references referring to improved target recovery using isotope dilution correction have been found, indicating that other researchers had also observed losses in the concentration process (14,25). Because the project objective was to develop a method suitable for nationwide monitoring, which would require strict adherence to analyte recovery criteria and consistent minimum reporting levels, it was decided to avoid an isotope dilution correction approach and to explore alternate ways to obtain the necessary sensitivity without extract concentration. Initial experiments in SIM mode predicted instrumental limits for fortified LRW to be 50–100 ng/L , well below the one in

10^6 cancer risk level (3 $\mu\text{g/L}$). Full scan data, along with library spectra, were used to select ions for identification and quantitation under GC–MS–SIM conditions. Secondary ions, and in some cases tertiary ions, necessary for monitoring proper abundance ratios for compound confirmation, were also selected. Retention time data were used to determine proper SIM time segments. GC–MS–SIM analyses were performed using a quadruple GC–MS, as described in the “Experimental” section. An example reconstructed total ion current chromatogram and mass chromatograms are displayed in Figure 1. The mass chromatograms of the quantitation ions for 1,4-dioxane and 1,4-dioxane- d_8 , m/z 88 and 96, respectively, show that baseline resolution was achieved, allowing accurate integration of both compounds. Near baseline resolution was achieved between the internal standard THF- d_8 and the chloroform interference from the DCM solvent, as shown in the mass chromatogram for m/z 46.

Evaluation of commercially available SPE columns

The performance of SPE products from multiple vendors was evaluated for the ability to achieve accurate and precise data for 1,4-dioxane. Availability of multiple commercially available SPE products with acceptable method performance was an important consideration in developing a method for widespread use by multiple laboratories. Two 6-mL SPE columns, each containing 2 g of activated carbon (Resprep EPA Method 521 and Enviro-Clean 521), were initially evaluated for use with 500-mL LRW samples fortified at a concentration of 2 $\mu\text{g/L}$ of analyte. These products were selected because they have been shown to perform well for *N*-nitrosamines, which have water solubility characteristics similar to 1,4-dioxane (14). Mean recoveries of 1,4-dioxane for the Resprep column and the Enviro-Clean column were 87% and 92%, respectively, with RSD values of less than 3% ($n = 7$). Recoveries of the surrogate analyte, 1,4-dioxane- d_8 , were consistent with the target analyte values. Additional elution steps with volumes of DCM beyond 10 mL showed no significant improvement in recoveries.

During the course of the method development, the Sep-Pak Plus AC-2 SPE cartridge became available in the U.S. A procedure for smaller scale extractions was developed using this product. The Sep-Pak Plus AC-2 SPE cartridge was initially evaluated for use with 100-mL tap water samples fortified with 1,4-dioxane at a concentration of 1 $\mu\text{g/L}$. The mean recovery of 1,4-dioxane was 104%, with an RSD of 4% ($n = 4$). These results indicated the Sep-Pak cartridge procedure was a viable alternative to the larger scale extractions, providing similar performance while using less solvent and requiring less sample volume to obtain the same overall method sensitivity. These results also compare favorably to those of Kawata et al. (24), who previously described a method with the Sep-Pak Plus AC-2 SPE cartridge prior to the availability of the cartridge in the U.S. The Kawata procedure was somewhat more complicated than the NERL process, as it required elution of the carbon with 2 solvents (acetone and DCM) in the opposite direction of sample loading, followed by freezing the extract to remove excess water. Also, in the Kawata method, extracts were subsequently evaporated to 3 mL and analyzed by GC–MS–SIM using isotope dilution quantitation, presumably to increase sensitivity and possibly correct for evaporative loss of 1,4-dioxane.



Robustness testing of the SPE extraction procedure

Several extraction parameters were evaluated to optimize and evaluate the robustness of the SPE extraction procedure. The parameters included sample loading rate, sorbent drying time, and the amount of methanol used to perform fortification procedures. In addition, samples fortified with high concentrations of TCA were evaluated for potential interferences or low recoveries that could result from the competition of 1,4-dioxane and TCA on the SPE sorbent. All samples described in this section were fortified with 1.0 $\mu\text{g/L}$ of 1,4-dioxane and analyzed by GC-MS-SIM.

Sample loading rate

A direct comparison was performed between 500-mL LRW samples fortified with 1,4-dioxane loaded onto 2-g carbon columns at a rate of ~ 7 mL/min and samples loaded at ~ 20 mL/min. Another direct comparison of loading rates was repeated with Waters Sep-Pak cartridges using 100-mL LRW samples fortified with 1,4-dioxane. Data provided in Figure 2 show a recovery of 94% of 1,4-dioxane in 500-mL samples loaded at both rates, and a recovery of 101% at both rates when extracting 100-mL samples with Sep-Pak cartridges. Surrogate recoveries, also represented in Figure 2, were comparable to target levels in all cases. The results indicate that the method procedure is robust enough such that some variability in the sample-loading rate will not affect the accuracy of the results.

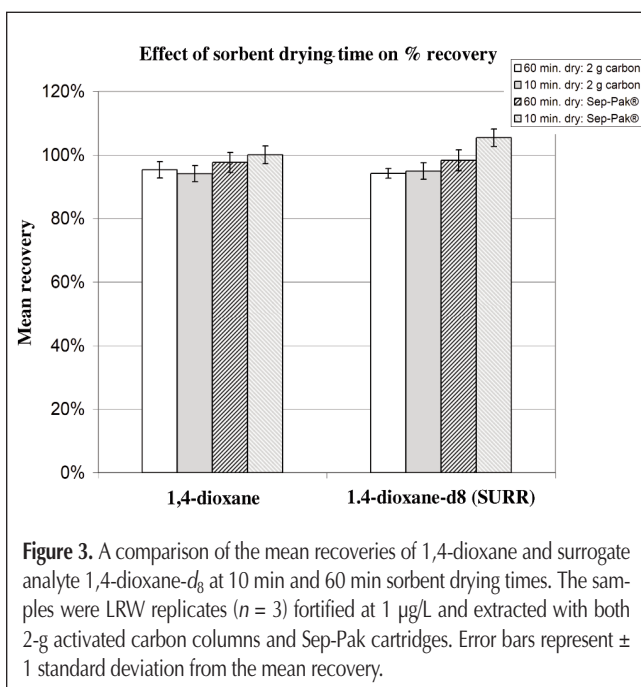
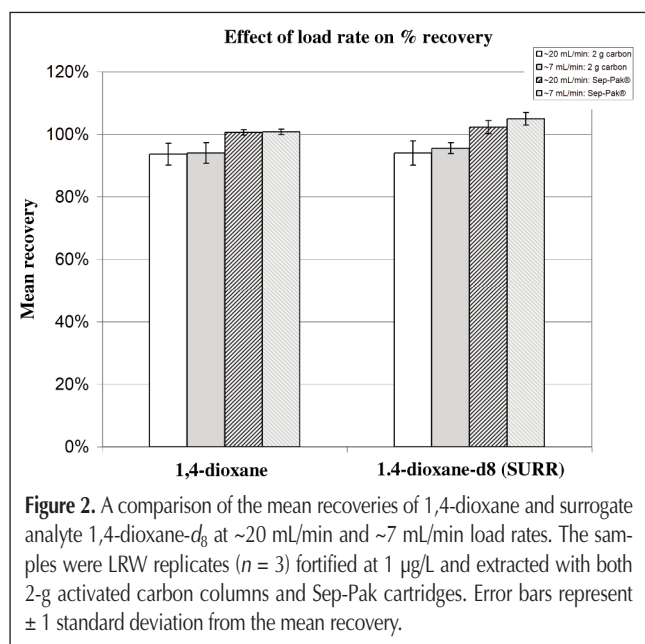
Sorbent drying time

Fortified LRW was extracted using both 500-mL samples collected on 2-g activated carbon columns and 100-mL samples collected on Sep-Pak cartridges, with sorbent drying times compared at 10 min versus 60 min. Data provided in Figure 3 show recoveries of 94–95% for 1,4-dioxane in 500-mL samples dried at both times, and recoveries of 98–105% at both drying times when extracting 100-mL samples with Sep-Pak cartridges. Surrogate recoveries, also represented in Figure 3, were comparable to target levels in all cases. The results indicate that the

method is robust enough that variability in the sorbent drying times of up to 1 h will yield acceptable recovery of 1,4-dioxane. It should be noted, however, that if the laboratory air is a source of 1,4-dioxane contamination, such as reported by Isaacson et al. (13), it would be important to minimize sorbent drying times and also keep them consistent so that method blanks will accurately portray any potential background contamination.

Potential high-level TCA effects

Since a major use of 1,4-dioxane has been to stabilize TCA degreasing solvents, evaluating the efficiency of the extraction method with significant levels of background TCA was an important consideration. In the work of Isaacson et al. (80 mL samples extracted on a 25-mm carbon disk), spike-addition experiments were used to show reduced recoveries of both 1,4-dioxane and tetrahydrofuran in a contaminated groundwater as their concentrations increased (13). They proposed that this was the result of competitive adsorption of organic compounds with higher organic carbon adsorption coefficient (K_{oc}) values than their target analytes. The K_{oc} of TCA is nearly 100 times that of 1,4-dioxane. Competitive loss of 1,4-dioxane on the sorbent due to high TCA levels and other co-contaminants must be ruled out, and therefore, a set of experiments were conducted with 100-mL and 500-mL LRW samples and with drinking water samples naturally high in total organic carbon (TOC) (> 4 mg/L). The LRW and high TOC drinking water samples were fortified with 1,4-dioxane and up to 500 $\mu\text{g/L}$ TCA. Extraction of samples with and without TCA yielded mean recoveries in the range of 91–112% for target and surrogate compounds, with all TCA fortified samples having mean recoveries within 7% of their controls. These results indicate that co-contamination of TCA (up to 500 $\mu\text{g/L}$), even in a challenging sample with high TOC, will show no negative effects on 1,4-dioxane recovery. The loss demonstrated by Isaacson et al. may have been due to a lack of sorbent capacity on the 25-mm carbon disks.



Methanol in the sample matrix

Since fortified samples usually contain a small amount of solvent from the fortification stock standard, it was important to determine any potential effect that excess methanol (fortifying solution solvent) may have on the sorbent capacity. Methanol (50 μL) was added to a series of 500-mL and 100-mL fortified LRW samples and extracted under conditions described earlier. This was used to mimic a standard addition of 1,4-dioxane using 50 μL of methanol. Extraction of samples with extra methanol and those without methanol yielded similar target and surrogate recoveries (results not shown). Therefore, results indicate a spiking volume of up to 50 μL methanol is possible without negative effects on 1,4-dioxane recovery.

Drinking water sample preservation and storage

Although generally thought to be resistant to biodegradation, use of 1,4-dioxane as a carbon and energy source for microorganisms under aerobic conditions has been reported (13). Because the exact nature and extent of potential biodegradation is unknown, it was decided that it was prudent to include a mechanism in the method to preserve drinking water samples collected for 1,4-dioxane analysis. Preservation of drinking water samples between the time of collection and their analysis usually requires a microbial inhibitor and a dechlorinating agent (26). Commonly used microbial inhibitors in drinking water methods include acidification with mineral acids or organic acids, or use of a toxic metal such as copper in conjunction with a buffer to keep the metal in solution (26). For this method, organic acids were avoided as an option due to the potential of compromising the capacity of the carbon sorbent. Thus, initial experiments used copper sulfate (0.5 g/L) as the microbial inhibitor. It has been used successfully in this capacity in other U.S. EPA drinking water methods (26–28), specifically in conjunction with a pH 7 Trizma buffer to keep the copper sulfate in solution and to avoid the formation of a precipitate in hard water samples. In this method, both ammonium chloride (5.0 g/L) and Trizma buffer (pH 7.0) at 5.0 g/L were used with the copper sulfate for

the purposes of preventing precipitation. Sodium sulfite (50 mg/L) was used as the dechlorinating agent. Although the Trizma buffer and copper sulfate combination was successful in keeping precipitate from forming, when added to a surface water with a high TOC content, 1,4-dioxane extraction recoveries dropped to 80% (3% RSD, $n = 7$). It was postulated that Trizma, an organic buffer, when combined with a matrix with high carbon content, competed with the target analyte for retention on the sorbent. Experiments where ammonium chloride was substituted for the Trizma were promising with certain tap water sources, but other sources caused precipitate formation in the samples, restricting flow during the extraction. Recoveries exhibited a 10–20% loss when precipitate was formed. As a result, copper sulfate was not pursued further as a preservative.

Sodium bisulfate was investigated as a potential microbial inhibitor. Sodium bisulfate is an attractive acidifying agent because as a solid material, it is easier to handle in the field than liquid mineral acids for sample collection teams. In previous sample preservation experiments, Bassett et al. showed that water samples inoculated with a population of river water microbes and stored under refrigeration at pH 3.9 for 28 days had a heterotrophic plate count of < 10 colony forming units (CFUs), compared to 300,000 CFUs for identical samples stored at pH 7.9 (29). These data, in conjunction with the widely held principle that microbial viability is limited to a pH range of 4.5–9.0 (26,30,31), were used to determine the amount of sodium bisulfate to be added. A concentration of 1 g/L of sodium bisulfate reduced a variety of drinking water matrices to pH < 3, including hard groundwater (289 mg/L) and a high TOC surface water (5 mg/L). Experiments were then performed to assay for the efficacy of the dechlorinating agent, sodium sulfite, in the presence of the sodium bisulfate microbial inhibitor. Free chlorine analysis revealed incomplete dechlorination when the compounds were added together or if the antimicrobial was added prior to the dechlorinating agent. This may be due to partial breakdown of sodium sulfite in the presence of an acidifying compound. Therefore, it was determined that the dechlorinating agent would be added prior to acidification for effective preservation.

A holding time experiment was performed to test the chemical stability of 1,4-dioxane in the presence of the preservation agents during simulated shipping and a desired 28-day holding time. Replicate samples of a chlorinated tap water were collected, dechlorinated with sodium sulfite (50 mg/L), acidified with sodium bisulfate (1 g/L), and fortified to a concentration of

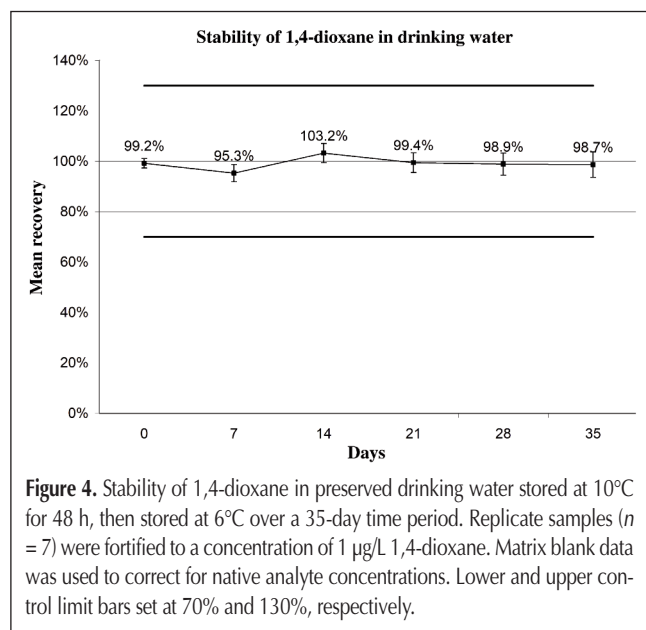


Table II. LCMRL and MDL Calculated for Each Extraction Option

Compound/extraction option	LCMRL ($\mu\text{g/L}$)	MDL ($\mu\text{g/L}$)
1,4-Dioxane (500 mL w/2 g activated carbon)	0.047	0.026*
1,4-Dioxane (100 mL w/ Waters Sep-Pak cartridge)	0.036	0.020†

* Calculated from LRW replicates fortified at 0.040 $\mu\text{g/L}$, $n = 7$.
† Calculated from LRW replicates fortified at 0.030 $\mu\text{g/L}$, $n = 8$.

1 µg/L 1,4-dioxane. Matrix blanks were prepared in the same manner, but without target compound fortification. Samples were stored at 10°C for 48 h to simulate shipping conditions, and then stored at 6°C for the remainder of the period. Randomly selected fortified samples ($n = 7$) and one matrix blank were extracted and analyzed on Day 0, with the process repeated on Days 7, 14, 21, 28, and 35. Holding time stability data is represented in Figure 4. No loss of 1,4-dioxane was observed in the preserved and stored samples.

Sample extract preservation and storage

Extracts ($n = 7$) prepared on Day 0 of an extract holding time experiment were stored in amber vials at -5°C and reanalyzed at 14, 28, and 42 days. Extract stability data is represented in Figure 5. No loss of 1,4-dioxane was observed in the stored extracts.

Final method procedures

The final method procedures are outlined as follows. *Option 1:* (a) Collect a 500-mL water sample. Immediately after collection, dechlorinate with 25 mg sodium sulfite, and then acidify with 0.5 g sodium bisulfate. Add surrogate, 1,4-dioxane- d_8 , immediately before extraction. (b) Rinse and condition 2 g activated carbon SPE column with 3 mL DCM, 2 × 3 mL methanol, and 5 × 3 mL LRW. (c) Pass the 500-mL water sample through the SPE column at a rate of 10 mL/min. (d) Dry the SPE column sorbent under full vacuum for 10 min. (e) Elute compounds from the column with 9 mL DCM. (f) Add internal standard, THF- d_8 , and adjust to 10 mL with DCM. (g) Dry extract by directly adding 2 g sodium sulfate and vortex mixing. (h) Analyze the extract by GC-MS-SIM using parameters listed in the “Experimental” section

Option 2: (a) Collect a 100-mL water sample. Immediately after collection, dechlorinate with 5 mg sodium sulfite, and then acidify with 100 mg sodium bisulfate. Add surrogate, 1,4-dioxane- d_8 , immediately before extraction. (b) Rinse and condition Sep-Pak cartridge with 1 mL DCM, 2 mL methanol, and 3 mL LRW. (c) Pass the 100-mL water sample through the cartridge at a rate of 10 mL/min. (d) Dry the SPE cartridge sorbent under full vacuum for 10 min. (e) Elute compounds from the cartridge with 1.5 mL DCM. (f) Add internal standard, THF- d_8 , and adjust to 2 mL with DCM. (g) Dry extract by passing it through a 3 mL column containing 0.4 g sodium sulfate. (h) Analyze the extract by GC-MS-SIM using parameters listed in the “Experimental” section.

Method performance

Accuracy and precision data have been generated in reagent water, finished ground, and surface waters. The single laboratory lowest concentration minimum reporting level (LCMRL) has also been determined in reagent water. The LCMRL is the lowest true concentration for which the future recovery is predicted to fall, with high confidence (99%), between 50 and 150% recovery. The procedure used to determine the LCMRL is described

elsewhere (32,33). LCMRL values for both the 500-mL extraction and the 100-mL extraction are listed in Table II, along with MDL values calculated using methods described by Glaser et al. (34). Replicates of LRW samples spiked at low (0.030 and 0.040 µg/L), mid-range (1.0 µg/L), and high concentrations (10.0 µg/L) relative to the calibration curve were extracted and analyzed, with recoveries and precision data displayed in Table III.

Replicates ($n = 7$) of surface water samples, surface water samples high in TOC (5 mg/L TOC), and groundwater samples with significant mineral content (289 mg/L hardness) were extracted and analyzed using both extraction techniques listed earlier, with performance data listed in Table IV. The results indicate waters

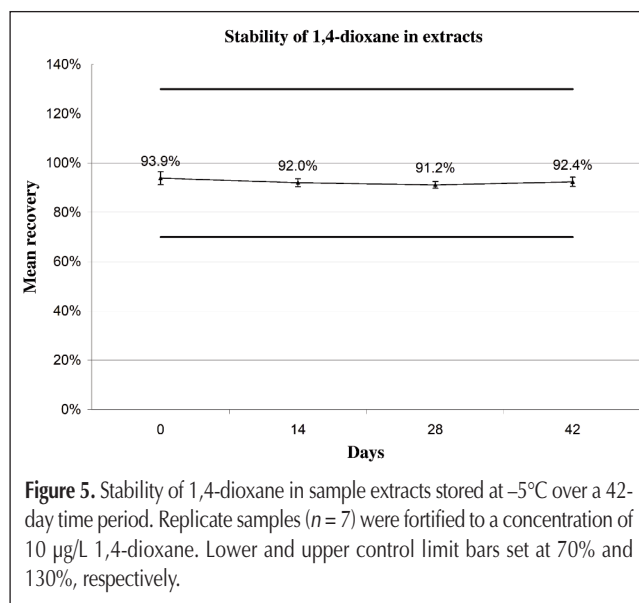


Figure 5. Stability of 1,4-dioxane in sample extracts stored at -5°C over a 42-day time period. Replicate samples ($n = 7$) were fortified to a concentration of 10 µg/L 1,4-dioxane. Lower and upper control limit bars set at 70% and 130%, respectively.

Table III. Demonstration of Method Performance in Laboratory Reagent Water Fortified with 1,4-Dioxane

Compound/extraction option reagent	Water fortified at 0.030 or 0.040 µg/L* ($n = 7$ or 8)	
	Mean % recovery	RSD (%)
1,4-dioxane (500 mL w/2 g activated carbon)	110	19
1,4-dioxane- d_8 (SUR) (500 mL w/2 g activated carbon)	92.0	3.5
1,4-dioxane (100 mL w/Waters Sep-Pak cartridge)	110	20
1,4-dioxane- d_8 (SUR) (100 mL w/Waters Sep-Pak cartridge)	102	3.8
Reagent water fortified at 1.0 µg/L ($n = 6$)		
	Mean % recovery	RSD (%)
1,4-dioxane (500 mL w/2 g activated carbon)	98.0	6.2
1,4-dioxane- d_8 (SUR) (500 mL w/2 g activated carbon)	97.1	5.2
1,4-dioxane (100 mL w/Waters Sep-Pak cartridge)	101	4.2
1,4-dioxane- d_8 (SUR) (100 mL w/Waters Sep-Pak cartridge)	105	4.8
Reagent water fortified at 10.0 µg/L ($n = 7$)		
	Mean % recovery	RSD (%)
1,4-dioxane (500 mL w/2 g activated carbon)	93.9	2.8
1,4-dioxane- d_8 (SUR) (500 mL w/2 g activated carbon)	90.1	2.8
1,4-dioxane (100 mL w/Waters Sep-Pak cartridge)	95.2	2.9
1,4-dioxane- d_8 (SUR) (100 mL w/Waters Sep-Pak cartridge)	99.3	3.0

* LRW (500-mL) extracted on 2 g activated carbon were fortified at 0.040 µg/L, $n = 7$. LRW (100-mL) extracted on Sep-Pak were fortified at 0.030 µg/L, $n = 8$

high in TOC or mineral content will not suffer from poor 1,4-dioxane recovery.

Performance evaluation (PE) water sample concentrates in methanol (3 concentrations, $n = 5$ per concentration) containing 1,4-dioxane were obtained from an independent third party for blind analysis. Samples were diluted in LRW, extracted using the Sep-Pak cartridge method listed earlier (*Option 2*), then analyzed. Once results were reported, nominal value reports from the independent lab indicated mean recovery and precision of 103% ($\pm 1.7\%$), 102.6% ($\pm 2.2\%$), and 99.4% ($\pm 3.5\%$) for low

(0–10 $\mu\text{g/L}$), medium (10–20 $\mu\text{g/L}$), and high (> 20 $\mu\text{g/L}$) concentrations, respectively.

An interlaboratory comparison of sensitivity, accuracy, and precision was performed with results described in Table V. Results indicate the method is rugged, accurate, and precise, with LCMRL values well below the one in 10^6 lifetime cancer risk concentration of 3 $\mu\text{g/L}$ published by U.S. EPA and the drinking water guideline of 50 $\mu\text{g/L}$ set by the World Health Organization (35).

Table IV. Demonstration of Method Performance in Drinking Water Matrices Using Each of the SPE Options; Matrix Samples Fortified at 1.0 $\mu\text{g/L}$ ($n = 7$ for each matrix)

Compound/extraction option	Surface water	
	Mean % recovery	RSD (%)
1,4-dioxane (500 mL w/2 g activated carbon)	99.0*	4.9
1,4-dioxane- d_8 (SUR) (500 mL w/2 g activated carbon)	100	2.5
1,4-dioxane (100 mL w/Waters Sep-Pak cartridge)	97.0†	4.6
1,4-dioxane- d_8 (SUR) (100 mL w/Waters Sep-Pak cartridge)	98.5	2.5
	Surface water (high in TOC) [‡]	
	Mean % recovery	RSD (%)
1,4-dioxane (500 mL w/2 g activated carbon)	102	3.5
1,4-dioxane- d_8 (SUR) (500 mL w/2 g activated carbon)	99.8	2.4
1,4-dioxane (100 mL w/Waters Sep-Pak cartridge)	98.5	5.6
1,4-dioxane- d_8 (SUR) (100 mL w/Waters Sep-Pak cartridge)	101	4.2
	Groundwater (high in mineral content) [§]	
	Mean % recovery	RSD (%)
1,4-dioxane (500 mL w/2 g activated carbon)	95.9**	2.1
1,4-dioxane- d_8 (SUR) (500 mL w/2 g activated carbon)	98.2	2.5
1,4-dioxane (100 mL w/ Waters Sep-Pak cartridge)	101 ^{††}	3.3
1,4-dioxane- d_8 (SUR) (100 mL w/Waters Sep-Pak cartridge)	104	5.9

* Percent recovery after correction for matrix background of 0.66 $\mu\text{g/L}$. Samples collected at different times, thus the difference in background concentrations.
† Percent recovery after correction for matrix background of 0.42 $\mu\text{g/L}$. Samples collected at different times, thus the difference in background concentrations.
‡ Total organic carbon measured at 4.950 mg/L.
§ Hardness measured at 289 mg/L as calcium carbonate.
** Percent recovery after correction for matrix background of 0.068 $\mu\text{g/L}$.
†† Percent recovery after correction for matrix background of 0.077 $\mu\text{g/L}$.

Table V. Comparison of Method Sensitivity, Accuracy, and Precision in Three Laboratories (Small-Scale Extraction)

	LCMRL ($\mu\text{g/L}$)	MDL ($\mu\text{g/L}$)	% Recovery (RSD) tap water	% Recovery (RSD) reagent water
Lab 1	0.036	0.020	97 (4.6)*	110 (20.0)†
Lab 2	0.013	0.012	101 (2.3)‡	100 (10.1)§
Lab 3	0.080**	0.021	88 (6.0) ^{††}	99 (7.0) ^{††}

* Percent recovery based on fortified drinking water at 1.0 $\mu\text{g/L}$ ($n = 7$).
† Percent recovery based on fortified reagent water at 0.03 $\mu\text{g/L}$ ($n = 8$).
‡ Percent recovery based on fortified drinking water at 0.2 $\mu\text{g/L}$ ($n = 8$).
§ Percent recovery based on fortified reagent water at 0.04 $\mu\text{g/L}$ ($n = 8$).
** The calculated LCMRL was lower than the lowest concentration tested. Therefore, the LCMRL presented is defaulted to the value of the lowest fortified concentration.
†† Percent recovery based on fortified drinking water at 10.0 $\mu\text{g/L}$ ($n = 9$).
‡‡ Percent recovery based on fortified reagent water at 0.10 $\mu\text{g/L}$ ($n = 8$).

Conclusions

NERL has developed a method for the analysis of 1,4-dioxane in drinking water that is relatively simple, inexpensive, and demonstrates excellent accuracy, precision, and sensitivity (the mean LCMRL value is 70 times lower than the one in 10^6 lifetime cancer risk of 3 $\mu\text{g/L}$). Three different activated carbon SPE products were evaluated, and robustness testing revealed a highly reproducible analysis of surface waters and groundwaters, even at exaggerated loading rates, extended drying times, excessive organic fortification solvent, and with large amounts of TCA co-contamination. At NERL, trained laboratory personnel can extract an estimated 16 to 20 500-mL samples, or 24 to 30 100-mL samples, per day while using significantly less amounts of extraction solvents as compared to costly and time-consuming LLE methods.

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References

1. *Code of Federal Regulations* Volume 73, Number 35, Environmental Protection Agency, Drinking Water Contaminant

- Candidate List 3—Draft, Government Printing Office, Washington, DC, 2008, 9627–54.
2. USEPA, Integrated Risk Information System (IRIS), 1990. <http://www.epa.gov/iris/sust/0326.htm>.
 3. M.J. Zenker, R.C. Borden, and M.A. Barlaz. Occurrence and treatment of 1,4-dioxane in aqueous environments. *Environ. Eng. Sci.* **20**: 423–32 (2003).
 4. C.T. DeRosa, S. Wilbur, J. Holler, P. Richter, and Y. Stevens. Health evaluation of 1,4-dioxane. *Toxicol. Ind. Health* **12**: 1–43 (1996).
 5. California Dept. of Health & Human Services website (2006) <http://www.dhs.ca.gov/ps/ddwem/chemicals/AL/PDFs/notificationoverview.pdf>.
 6. Colorado Public Health & Environment website (2007) http://www.cdphe.state.co.us/regulations/wqccregs/31_0207ts.pdf.
 7. Michigan Dept. of Environmental Quality website (2004) www.deq.state.mi.us/documents/deq-rrd-GS-InformationBulletinMarch2004.pdf.
 8. North Carolina Dept. of Environment & Natural Resources website (2005) http://h2o.enr.state.nc.us/admin/rules/documents/15ANCAC2Lbooklet_000.pdf.
 9. State of Maine Dept. of Human Services website (2007). <http://www.maine.gov/dhhs/eohp/wells/documents/megtable.pdf>.
 10. State of Massachusetts website (2007) <http://www.mass.gov/dep/water/dwstand.pdf>.
 11. EPA, Region 1 website (2004) <http://www.epa.gov/region1/superfund/sites/durham/204850.pdf>.
 12. T.K.G. Mohr. *Solvent Stabilizers: White Paper*. Santa Clara Valley Water District, San Jose, CA, 2001.
 13. C. Isaacson, T.K.G. Mohr, and J.A. Field. Quantitative determination of 1,4-dioxane and tetrahydrofuran in groundwater by solid phase extraction GC/MS/MS. *Environ. Sci. Technol.* **40**: 7305–11 (2006).
 14. L.E. Harris, W.L. Budde, and J.W. Eichelberger. Direct analysis of water samples for organic pollutants with gas chromatography-mass spectrometry. *Anal. Chem.* **46**: 1912–17 (1974).
 15. W.M. Draper, J.S. Dhoot, J.W. Remoy, and S.K. Perera. Trace-level determination of 1,4-dioxane in water by isotopic dilution GC and GC-MS. *Analyst* **125**: 1403–1408 (2000).
 16. J.W. Munch and J.W. Eichelberger. Evaluation of 48 compounds for possible inclusion in the U.S. EPA Method 524.2, Revision 3.0: expansion of the method analyte list to a total of 83 compounds. *J. Chromatog. Sci.* **30**: 471–77 (1992).
 17. R.E. Shirey and C.M. Linton. The extraction and analysis of 1,4-dioxane from water using solid-phase microextraction coupled with gas chromatography and gas chromatography-mass spectrometry. *J. Chromatog. Sci.* **44**: 444–50 (2006).
 18. S. Nakamura and S. Daishima. Simultaneous determination of 22 volatile organic compounds, methyl-*tert*-butyl ether, 1,4-dioxane, 2-methylisoborneol and geosmin in water by headspace solid phase microextraction-gas chromatography-mass spectrometry. *Anal. Chim. Acta* **548**: 79–85 (2005).
 19. L.J. Yoo, S. Fitzsimmons, and M. Wehner. Simultaneous determination of 1,4-dioxane and *N*-nitrodimethylamine from drinking water by GC/MS using positive chemical ionization. *WQTC Proceedings*, American Water Works Association, Denver, CO, 2001.
 20. J. Grindstaff, J. Peterson, and R. Holden. "The analysis of 1,4-dioxane by GC/MS with large volume injection." Poster at *The Society of Environmental Toxicology and Chemistry 25th Annual Meeting*, Portland, OR, 2004.
 21. J.W. Munch and M.V. Bassett. *Method 521: Determination of Nitrosamines in Drinking Water by Solid Phase Extraction and Capillary Column Gas Chromatography with Large Volume Injection and Chemical Ionization Tandem Mass Spectrometry (MS/MS)*, National Exposure Research Laboratory, Office of Research and Development, U.S. EPA, Cincinnati, Ohio, 2004.
 22. K. Kawata, T. Ibaraki, A. Tanabe, H. Yagoh, A. Shinoda, H. Suzuki, and A. Yasuhara. Gas chromatographic-mass spectrometric determination of hydrophilic compounds in environmental water by solid-phase extraction with activated carbon fiber felt. *J. Chromatogr. A.* **911**: 75–83 (2001).
 23. A. Abe. Distribution of 1,4-dioxane in relation to possible sources in the water environment. *Sci. Total Environ.* **227**: 41–47 (1999).
 24. K. Kawata, T. Ibaraki, A. Tanabe, and A. Yasuhara. Distribution of 1,4-dioxane and *N,N*-dimethylformamide in river water from Niigata, Japan. *Bull. Environ. Contam. Toxicol.* **70**: 876–82 (2003).
 25. T. Nishimura, S. Iizuka, N. Kibune, and M. Ando. Study of 1,4-dioxane intake in the total diet using the market-basket Method. *J. Health Sci.* **50**: 101–107 (2004).
 26. S.D. Winslow, B.V. Pepich, M.V. Bassett, S.C. Wendelken, D.J. Munch, and J.L. Sinclair. Microbial inhibitors for U.S. EPA drinking water methods for the determination of organic compounds. *Environ. Sci. Technol.* **35**: 4103–10 (2001).
 27. M.V. Bassett, S.C. Wendelken, T.A. Dattilio, B.V. Pepich, and D.J. Munch. *Method 532: Determination of Phenylurea Compounds in Drinking Water by Solid Phase Extraction and High Performance Liquid Chromatography with UV Detection*. National Exposure Research Laboratory, Office of Research and Development, U.S. EPA, Cincinnati, Ohio, 2000.
 28. J.W. Munch. *Method 529: Determination of Explosives and Related Compounds in Drinking Water by Solid Phase Extraction and Capillary Column Gas Chromatography/Mass Spectrometry (GC/MS)*. National Exposure Research Laboratory, Office of Research and Development, U.S. EPA, Cincinnati, Ohio, 2002.
 29. M.V. Bassett, S.C. Wendelken, B.V. Pepich, and D.J. Munch. Improvements to EPA Method 531.1 for the analysis of carbamates that resulted in the Development of U.S. EPA Method 531.2. *J. Chromatog. Sci.* **41**: 100–106 (2003).
 30. S.S. Block. *Disinfection, Sterilization, and Preservation*, 4th ed. Lea and Febiger, Philadelphia, PA, 1991, p. 60.
 31. T.D. Brock. *Biology of Microorganisms*. Prentice-Hall, Inc., Englewood Cliffs, NJ, 1974, p. 309.
 32. *Statistical Protocol for the Determination of the Single-Laboratory Lowest Concentration Minimum Reporting Level (LCMRL) and Validation of the Minimum Reporting Level (MRL)*. (2005) Available at www.epa.gov/OGWDW/methods/sourcalt.html.
 33. S.D. Winslow, B.V. Pepich, J.J. Martin, G.R. Hallberg, D.J. Munch, C.P. Frebis, E.J. Hedrick, and R.A. Krop. Statistical procedures for determination and verification of minimum reporting levels for drinking water methods. *Environ. Sci. Technol.* **40**: 281–88 (2006).
 34. J.A. Glaser, D.L. Foerst, G.D. McKee, S.A. Quave, and W.L. Budde. Trace analyses for wastewaters. *Environ. Sci. Technol.* **15**: 1426–1435 (1981).
 35. World Health Organization; Guidelines for drinking-water quality: incorporating first addendum. Vol. 1, Recommendations.—3rd ed., WHO Press, Geneva, Switzerland, 2006, p. 188.

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