

Determination of 1, 4-Dioxane Using Automated Solid Phase Extraction (SPE), Compliant with US EPA 522 for Drinking Water

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Introduction

1, 4-dioxane is a compound that has become known as an emerging contaminant which may cause negative health effects in humans. The US Agency for Toxic Substances and Disease Registry (ATSDR) states that exposure to 1, 4-dioxane at high levels may cause liver and kidney damage. 1,4-dioxane is also reasonably anticipated to be a human carcinogen based on sufficient evidence of carcinogenicity in animals.¹ The US EPA has also classified 1,4-dioxane as “likely to be carcinogenic to humans” by all routes of exposure.² Recent research has evaluated exposure through drinking water and food, generating a comprehensive picture of possible carcinogenicity.

1,4-dioxane exposure occurs from a variety of sources, it's used as a stabilizer in certain chlorinated solvents, therefore it can be found in many products that are known to use chlorinated solvents such as; paint strippers, dyes, greases, anti-freeze and aircraft deicing fluids. Dioxane was also used as a solvent to facilitate SN₂ reactions in chemical synthesis because of its polar aprotic nature. Dioxane is also a by-product of ethoxylation reactions, many of which are carried out on a regular basis in cosmetic products that contain sodium laureth sulfate.² This reagent is so common among cosmetic products that detectable amounts of 1,4-dioxane can be found in nearly 57% of baby shampoos and 97% of hair relaxers. The FDA and the EU Scientific Committee on Consumer Safety, working on the advice of the International Cooperation on Cosmetics Regulation (ICCR), recommended the limit for 1, 4-dioxane in finished cosmetic products be less than 10 ppm.³



Since the main source of 1,4-dioxane is currently cosmetic products, it is no surprise that it can be found in both drinking water and ground water tables. Japan has observed levels in surface water up to 42.8 µg/L and found up to 79 µg/L in groundwater samples. In this case, a high correlation was observed with the presence of 1,1,1-trichloroethane. 1,4-Dioxane was found at a concentration of 0.2–1.5 µg/L in tap water samples from six cities in Kanagawa, Japan, in 1995–1996.⁴ In the US, 1, 4-dioxane was included in the third Unregulated Contaminant Monitoring Rule (UCMR-3), a list of candidate contaminant compounds based on toxicity and occurrence. The list of 30 compounds is monitored in large public water supplies and selected small water supplies to better understand the occurrence and magnitude in drinking water to aid in deciding if regulation is warranted.⁵ The results of UCMR-3 have not resulted in a regulated maximum contaminant level of 1, 4-dioxane, but some states are beginning to set regulations. These regulations can be observed in Table 1² The EPA risk assessments indicate that the drinking water concentration representing a 1×10^{-6} cancer risk level for 1,4-dioxane is 0.35 µg/L.⁶

Table 1. State Regulations for 1,4-dioxane concentration in various water sources (2017).

State	Guideline (µg/L)	Source
Alaska	77	AL DEC 2016
California	1.0	Cal/EPA 2011
Colorado	0.35	CDPHE 2017
Connecticut	3.0	CTDPH 2013
Delaware	6.0	DE DNR 1999
Florida	3.2	FDEP 2005
Indiana	7.8	IDEM 2015
Maine	4.0	MEDEP 2016
Massachusetts	0.3	MADEP 2004
Mississippi	6.09	MS DEQ 2002
New Hampshire	0.25	NH DES 2011
New Jersey	0.4	NJDEP 2015
North Carolina	3.0	NCDENR 2015
Pennsylvania	6.4	PADEP 2011
Texas	9.1	TCEQ 2016
Vermont	3.0	VTDEP 2016
Washington	0.438	WA ECY 2015
West Virginia	6.1	WV DEP 2009

Several concerns have arisen about measurement of 1, 4- dioxane in water samples due to dioxane's high affinity for water. The compound is completely miscible in water and although it is volatile, it is difficult to purge from water. Evaluation of 1,4-dioxane can be done by a number of existing US EPA methods employing liquid-liquid extraction or purging to remove 1, 4-dioxane from water for GC/MS measurement but these methods have proved to have worse detection limits than desired. US EPA method 522 from the drinking water program specifies solid phase extraction (SPE) and GC/MS analysis using single ion monitoring (SIM) and is the most successful method to date.²

This application note will evaluate the performance of the Biotage® Horizon 5000 automated solid phase extraction system in conjunction with US EPA Method 522.

Experimental

The extraction was performed using the Biotage® Horizon 5000 automated solid phase extraction system, using the extraction program displayed in Table 2. A 500 mL water sample size was extracted at a neutral pH. To improve method performance, the consumable used for this application note was a 3-gram, 6 cc coconut charcoal cartridge. This change not only demonstrated optimal recovery rates but it also allowed the 5000 system to be operated at a sample loading speed of 3. This operational change allowed for the sample loading rate to be increased to approximately 25 mL/min from 10 mL/min and is method compliant due to the language in section 1.6 of EPA method 522. This saved up to approximately 20 minutes per sample. The analytical step was performed using GC/MS in the single ion mode (SIM) for the best sensitivity. The conditions for the Agilent 7890A GC coupled with the Agilent 5975C mass spectrometer are presented in Table 3.

Table 2. Extraction program used on the Biotage® Horizon 5000 system.

Step	Operation	Solvent	Solvent Volume (mL)	Vent Purge Time (s)	Vacuum Pump Rate (s)	Saturation Time (s)	Soak Time (s)	Drain Time (s)	Done Loading Sample Delay (s)	Dry Time (s)	N2 Blanket
1	Condition	Methylene chloride	5	30	3	4	10	60			
2	Condition	Methylene chloride	5	30	3	4	10	60			
3	Condition	Methanol	5	30	3	3	10	60			
4	Condition	Methanol	5	30	3	3	10	6			
5	Condition	Water	5	15	3	3	10	4			
6	Condition	Water	5	15	3	3	10	4			
7	Condition	Water	5	15	3	3	10	4			
8	Load sample				3				45		
9	Air dry disk timer				6					600	OFF
10	Elute sample container	Methylene chloride	3	15	3	3	120	60			OFF
11	Elute sample container	Methylene chloride	3	15	3	3	120	60			OFF
12	Elute sample container	Methylene chloride	3	15	3	3	120	90			OFF

Table 3. GC/MS parameters.

Injection	
Amount	1 µL
Inlet Temperature	280 °C
Mode	Splitless
Gas Type	Helium
Column Conditions	
Zebtron™ ZB-5 (Phenomenex), 30 m, 0.25 mm, 0.25 µm	
Mode	Consistent Flow
Oven Program	30 °C hold for 2 minutes Ramp 5 °C/min to 50 °C Ramp 50 °C/min to 200 °C Hold for 6 minutes
MS Ions Monitored	Tetrahydrofuran- <i>d</i> ₈ -46, 78, 80 1,4-dioxane- <i>d</i> ₈ - 62, 64, 96 1,4-dioxane - 58, 88

**Figure 1.** Two Biotage® Horizon 5000 extractors equipped with carbon cartridges for extraction. Both extractors are controlled using the PC in the middle of the image.**Table 6.** Initial demonstration of accuracy (IDA).

Analyte	Target Conc. (µg/L)	LFB 1 (µg/L)	LFB 2 (µg/L)	LFB 3 (µg/L)	LFB 4 (µg/L)	Mean Recovery (µg/L)	Average Recovery %
1,4-Dioxane	10.0	8.6	8.8	8.5	8.2	8.53	85.25
1,4-Dioxane-d8	500.0	420.0	430.0	410.0	395.0	413.8	82.75

Results and Discussion

Table 6 in EPA Method 522 lists the initial demonstration of capability (IDC) requirements as well as the quality control requirements for the analysis of 1,4-dioxane. Table 7 in Method 522 lists the ongoing quality control requirements that must continually be met.

The method states that a low background of the system and the reagents must be determined by examining a lab reagent blank (LRB). A surrogate is added to the reagent blank to ensure that the extraction was performed to the standard of the method. The 1, 4-dioxane and background interferences must be less than or equal to 1/3 of the MRL in order to continue with the IDC requirements. The results for one LRB sample are presented in Table 4.

Table 4. Method blank data.

Analyte	Target Conc. (µg/L)	Recovery (µg/L)	Recovery (%)
1,4-Dioxane	N/A	ND	ND
1,4-Dioxane-d8	500.0	515.7	103.1

A set of four laboratory fortified blanks (LFBs) was extracted on the Biotage® Horizon 5000 to determine the initial demonstration of precision (IDP). The precision (relative standard deviation (RSD)) of all four samples must be ≤20%. The precision results are presented in Table 5.

Table 5. Initial demonstration of precision (IDP).

Analyte	Target Conc. (µg/L)	LFB 1 (µg/L)	LFB 2 (µg/L)	LFB 3 (µg/L)	LFB 4 (µg/L)	RSD
1,4-Dioxane	10.0	8.6	8.8	8.5	8.2	2.93
1,4-Dioxane-d8	500.0	420.0	430.0	410.0	395.0	3.61

The initial demonstration of accuracy (IDA), presented in Table 6, uses the same four LFBs that were used for determining the IDP. The method specifies that in order to demonstrate accuracy, the mean recovery of the LFBs must be +/- 20% of the true value. The true value for each of the four samples was 10 µg/L.

Seven LFBs were extracted to confirm the minimum reporting level (MRL) and determine the half range for the prediction interval of results (HR_{PIR}). This data set provides an RL for the Biotage® Horizon 5000 automated solid phase extraction instrument. The MRL and HR_{PIR} data is presented in Table 7. The equation for calculating HR_{PIR} is as follows:

$$HR_{PIR} = 3.963 S$$

Where S is the standard deviation and 3.963 is a constant value for seven replicates

Data from Table 7 was also used to confirm the upper and lower prediction interval of results (PIR). These two limits must be met in order to confirm that the MRL is valid. The upper PIR limit must be less than or equal to 150% while the lower PIR limit must be greater than or equal to 50%. The data for the upper and lower PIR limits is presented in Table 8. The equations for calculating Upper PIR and Lower PIR are as follows:

Upper:

$$(\text{Mean} + HR_{PIR} / \text{Fortified Concentration}) * 100$$

Lower:

$$(\text{Mean} - HR_{PIR} / \text{Fortified Concentration}) * 100$$

Table 7. MRL data for seven replicates used to calculate the HR_{PIR} . The reported values ($\mu\text{g/L}$) account for the 500 mL starting volume and the final extract volume of 10 mL.

Analyte	Target Conc. ($\mu\text{g/L}$)	MRL 1 ($\mu\text{g/L}$)	MRL 2 ($\mu\text{g/L}$)	MRL 3 ($\mu\text{g/L}$)	MRL 4 ($\mu\text{g/L}$)	MRL 5 ($\mu\text{g/L}$)	MRL 6 ($\mu\text{g/L}$)	MRL 7 ($\mu\text{g/L}$)	Mean ($\mu\text{g/L}$)	Std. Dev.	HR_{PIR}
1,4-Dioxane	0.150	0.155	0.153	0.137	0.150	0.144	0.140	0.137	0.145	0.008	0.032

Table 8. Upper and Lower PIR limit calculations.

Analyte	Target Conc. ($\mu\text{g/L}$)	Mean ($\mu\text{g/L}$)	HR_{PIR}	Upper PIR (%)	Lower PIR (%)
1,4-Dioxane	0.150	0.145	0.032	118%	75%

A method detection limit (MDL) (optional for an IDC) was calculated using the procedure in 40CFR, part 136 for an initial MDL. Eight LFBs were spiked at low concentration (0.15 $\mu\text{g/L}$) and extracted through the Biotage® Horizon 5000 over a period of one month. The standard deviation of the eight replicates was multiplied by the Student's T value of 2.998 to calculate the MDL. The results for the MDL study are presented in Table 9.

Table 9. MDL determination.

Analyte	Target Conc. ($\mu\text{g/L}$)	MDL 1 ($\mu\text{g/L}$)	MDL 2 ($\mu\text{g/L}$)	MDL 3 ($\mu\text{g/L}$)	MDL 4 ($\mu\text{g/L}$)	MDL 5 ($\mu\text{g/L}$)	MDL 6 ($\mu\text{g/L}$)	MDL 7 ($\mu\text{g/L}$)	MDL 8 ($\mu\text{g/L}$)	Std. Dev.	Calculated MDL ($\mu\text{g/L}$)
1,4-Dioxane	0.150	0.155	0.153	0.137	0.150	0.144	0.140	0.137	0.159	0.009	0.026

Table 10. Recovery values for Laboratory Fortified Blank samples.

LFB From Three Batches	Target Concentration ($\mu\text{g/L}$)	Measured 1,4-Dioxane ($\mu\text{g/L}$)	Recovery (%)
High LFB (05/22/2019)	10.0	8.70	87.0
Med LFB (05/23/2019)*	1.00	1.00	100.0
Low LFB (05/22/2019)	0.150	0.149	99.0

Method 522 specifies that an analytical batch containing between 10–20 samples must contain a laboratory fortified blank (LFB) as a quality control check. The concentration of the LFB should rotate between low, medium and high concentrations. The acceptance criteria for a low LFB is +/-50% of the true value while a medium and high LFB must fall within +/-30% of the true value. The data for three LFBs, one at each concentration, is presented in Table 10.

*Within 24 hours of the samples analyzed on 05/22/2019.

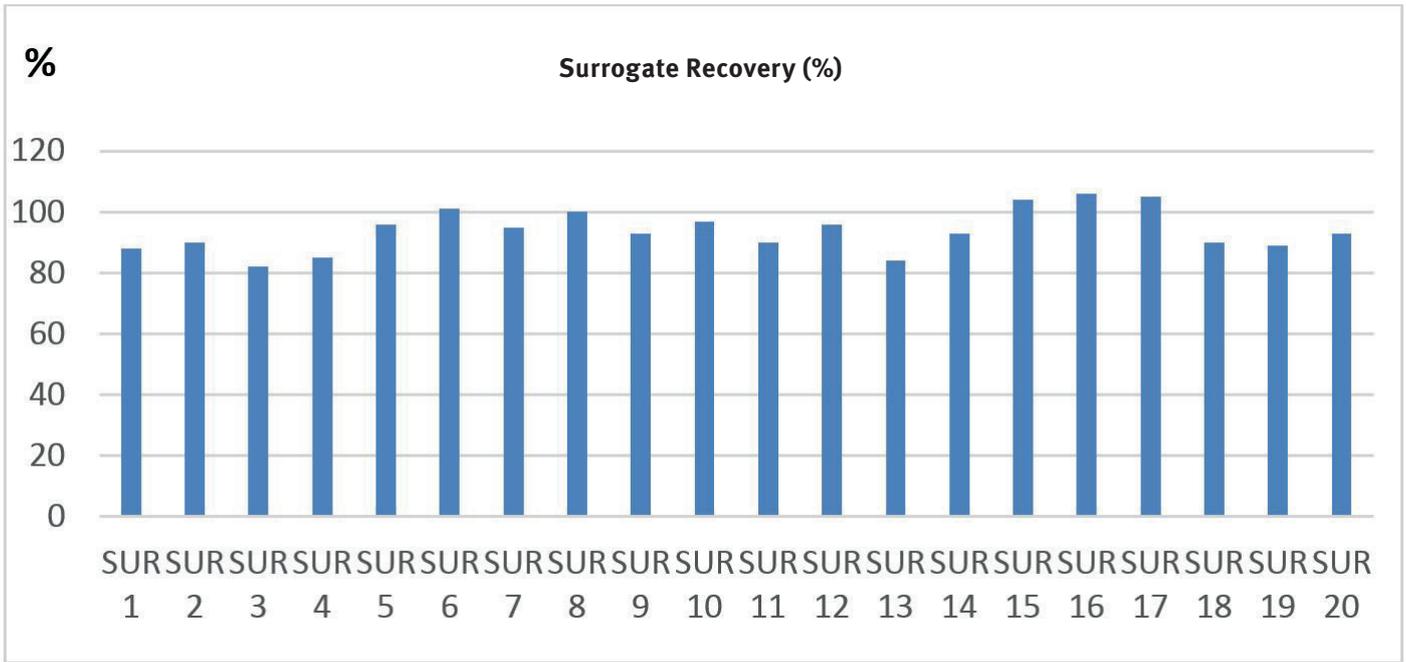


Figure 2. Recovery values for twenty samples spiked with surrogate.

The three-gram cartridges performed exceedingly well and passed all IDC and ongoing QC requirements. The three-gram cartridge allowed for faster sample processing because the larger sorbent volume allows the sample to be pulled through faster while preventing breakthrough. The measured recovery of twenty surrogates is presented in Figure 2.

A blind performance testing sample (PT) was analyzed in order to make sure that the extraction process, as well as the analytical method, are capable of quantitation. A sample was received from an accredited provider and extracted using the Biotage® Horizon 5000. The results as well as the acceptance criteria are presented in Table 11.

Table 11. Recovery values for the performance testing sample.

Sample	True Value (µg/L)	Measured Value (µg/L)	Recovery (%)	Acceptable Range (µg/L)
PT Sample 1	16.0	18.1	113.1	6.4–25.6

Conclusion

This application note proves that EPA method 522 can be successfully implemented in a laboratory using the Biotage® Horizon 5000 automated solid phase extraction system. Four LFB samples were analyzed for precision and accuracy, yielding an average recovery value of 85.25% with an RSD of 2.93%. Both values meet the acceptance criteria of the method. The batch to batch quality control requirements set by the EPA method are easily met using this extraction method. A blind performance test sample validated the accuracy of results obtained for drinking water. The automation of this method provides less analyst intervention which reduces any possible outside contamination. The 10 mL final extract volume eliminates any losses due to evaporation while the larger sorbent bed has allows for faster flow rate with better performance. All of these factors lead to an increase in productivity while easily meeting all of the quality control requirements for EPA Method 522.

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