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A static headspace GC-MS/MS method for the determination of ethanol, iso-butanol, and *n*-butanol at nanomolar concentrations in aqueous environmental samples

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Abstract

The increased use of small molecular weight alcohols such as ethanol and potentially butanol isomers as biofuels has raised questions about the fate of these compounds in the environment once emitted. In order to address these questions, a method for the simultaneous determination of nanomolar concentrations of ethanol, *n*-butanol, and iso-butanol in aqueous environmental matrices is presented. The method consists of static headspace gas chromatograph-mass spectrometer/mass spectrometer (GC-MS/MS) analysis with detection limits of 28 nM for ethanol and 9 nM for each butanol isomer. Accuracy of the new method was verified by comparing ethanol concentrations in authentic environmental samples by an independent technique utilizing solid phase microextraction (SPME). Results of an intercomparison study between the static headspace GC-MS/MS and SPME analyses produced a trend line with a slope of unity demonstrating that the methods produced statistically equivalent ethanol concentrations. Spiked additions of each alcohol in a variety of aqueous environmental matrices gave recoveries greater than 90% validating the accuracy of the headspace GC-MS/MS analysis. The new static headspace GC-MS/MS analysis represents the first methodology available with demonstrated accuracy in an array of environmental matrices with the requisite limit of detection capable of quantifying several alcohols in one analysis with minimal sample preparation and sample size requirement.

Energy security and climate change have been two topics that have led to the intense interest in biological based fuels (biofuels) for transportation in the U.S. Biofuels can be blended with diesel and gasoline with the percentage based upon the Renewable Fuel Standard established by the Energy Independence and Security Act in 2007. This act mandates the use of 36 billion gallons of renewable fuel by 2022 corresponding to roughly 17% of the projected transportation fuel for light duty vehicles (Anderson et al. 2009). Ethanol produced from renewable resources has been the primary focus of production and blending with gasoline with approximately 14 billion gallons produced in 2014 (www.eia.gov).

Butanol also qualifies as a renewable fuel dependent on feedstock for production. Butanol has four isomers but the majority of commercial processes focus on blending iso-butanol with gasoline. Butanol has distinct advantages over ethanol with higher energy content, lower vapor pressure, and generally fewer exhaust emissions compared to ethanol blended fuels (Karabektas and Hosoz 2009; Jin et al. 2011; Elfasakhany 2014). However, there are still challenges in butanol production before it can be used as a large-scale biofuel such as improving the butanol yield during the fermentation process (Jin et al. 2011; Su et al. 2015).

Biofuel production and consumption represent introduction avenues to the environment (Poulopoulos et al. 2001; Avery et al. 2016). Modeling efforts have identified and estimated ethanol emissions from a variety of sources but there remains considerable uncertainty in the data (Kirstine and Galbally 2012). The increase use of biofuels impacts a variety of fundamentally important atmospheric processes. Ethanol is a precursor to acetaldehyde and upon further oxidation in the presence of nitrogen dioxide (NO_2 forms peroxyacetyl nitrate, Tanner et al. 1988; Millet et al. 2010). These reactions have impacts on atmospheric oxidation chemistry that in turn have implications for atmospheric composition, air pollution, aerosol formation, greenhouse radiative forcing, and stratospheric ozone depletion (Thompson 1992). The atmospheric half-life in days of ethanol, *n*-butanol, and iso-butanol are 2.4, 0.96, and 1.0, respectively (Grosjean 1997); however, there are significant uncertainties in these lifetimes. Given the high water solubility of these alcohols in atmospheric waters, rainwater can act as a valuable tool to track the

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Table 1. Comparison of analytical methods for the analysis of ethanol in aqueous environmental samples. Limits of detection were the lowest given in the publication and measured range is for the concentration range of samples analyzed where applicable.

Matrix	Instrument	LOD (nM)	Measured range	Reference
Seawater	P/T-GC-FID	2	<LOD-34 nM	Beale et al. (2010)
Rainwater	P/T-GC/MS	20	1–5 μM	Monod et al.(2003)
Various	SPME GC/FID	19	89–8540 nM	Kieber et al. (2013)
Various	Static GC/FID	410	0.41–14.8 μM	Giubbina et al. (2017)
Groundwater	SPME GC/MS	326	<LOD	Cassada et al. (2000)
Freshwaters	Enzyme HPLC	10	89–8540 nM	Kieber et al. (2013)
Various	*HS-GC/MS*	*28*	*<LOD-2500 nM*	*This study*

GC/MS, gas chromatograph mass spectrometer; HS, headspace; LOD, limit of detection; P/T, purge and trap; SPME, solid phase microextraction.

increasing use of biofuels. Ethanol has already been detected in precipitation and natural waters from nM to μM levels (Kieber et al. 2014; Avery et al. 2016; Roebuck et al. 2016).

Quantifying these relatively small alcohols in aqueous samples is a challenge given their high polarity and high water solubility. Current analytical techniques employ a combination of gas chromatograph (GC) and high performance liquid chromatography (HPLC) methodologies with a variety of sample preparation techniques and detection methods. In an earlier study, purge and trap gas chromatograph-flame ionization detection (GC-FID) was used to measure ethanol in oceanic waters (Beale et al. 2010). The analysis had a very low limit of detection (LOD; 2 nM), however, it was only applied to seawater. In a subsequent study, enzymatic oxidation and derivatization followed by HPLC was used to quantify the ethanol in a variety of natural waters (Kieber et al. 2013). The accuracy of the Kieber et al. study was demonstrated in a two method intercomparison study however this latter technique was not optimized for other alcohols and is not suitable in all environmental matrices because the enzyme readily denatures. In a similar study, ethanol was quantified in freshwater lakes, marine water, and rainwater with a LOD of 410 nM (Giubbina et al. 2017) but did not measure *n*-butanol and iso-butanol.

The goal of the current study was to develop the first rapid method for the quantification of ethanol, *n*-butanol, and iso-butanol concentrations in a wide variety of aqueous environmental matrices, which include precipitation, fresh and marine waters, using static headspace GC/mass spectrometer (MS). When compared to existing techniques (Table 1), the new method is the only one that can determine both ethanol and butanol isomers in natural waters. In addition, it does not require expensive extraction materials and provides accurate analyte detection with a very low LOD suitable for a wide variety of environmental applications.

Materials and procedures

Sample collection and preparation

Rainwater was collected on the campus of the University of North Carolina Wilmington (UNCW; 34.2325 N 77.8778 W, 8.5 km from the Atlantic Ocean). All rainwater event samples were collected using an Aerochem Metrics Model 301 Automatic Sensing Wet/Dry Precipitation Collector that housed a 4-liter glass beaker placed within a high-density polyethylene (HDPE) plastic bucket. Atmospheric condensate samples were collected on the campus of UNCW using a method adapted from Farmer and Dawson (1982) (Farmer and Dawson 1982; Avery et al. 2016). Condensate collectors consisted of HDPE 5-gallon buckets with fitted lids containing twenty 3.5 cm-diameter holes. Each lid had six fitted holes designed to vertically hold a 30 cm long digestion tube. These digestion tubes were then filled with ice in order to condense atmospheric water on the outside surface of the tubes. Condensate was collected under each tube through a glass funnel into a 20 mL threaded borosilicate vial. Natural water samples, which included fresh, estuarine, and marine waters, were collected at Kerr Lake, North Carolina (36.4472°N, 78.3710°W), Snow's Cut, North Carolina (34.05517°N, 77.9010°W), Bradley Creek, North Carolina (34.2140°N, 77.8341°W), and Wrightsville Beach, North Carolina (34.2085°N, 77.7964°W). These samples were collected in combusted amber 1-liter glass bottles by hand from a small boat.

All glasswares, including rain collection beakers, were combusted at 450°C in a muffle furnace for a minimum of 4 h to remove organics prior to use. All samples were collected and immediately transported back to the laboratory at UNCW and passed through a 0.2 μm polysulfone filter and collected into a 20 mL borosilicate glass vial. The other natural waters were not filtered prior to analysis. The samples were preserved with mercury chloride ($HgCl_2$) to a final concentration of 100 mg L^{-1} to eliminate biological activity, including microbial degradation of the alcohols, and stored at 4°C until analysis. Previous work has shown the stability of ethanol preserved with $HgCl_2$ over several weeks (Kieber et al. 2013). It is recommended though laboratories employing this method should perform their own preservation experiments with their individual sample matrix as good laboratory practice.

All samples for GC/MS analysis were placed in 20 mL glass headspace vials with reagent grade sodium chloride and a

magnetic stir bar. The salt was added to decrease hydrogen bonding of the soluble alcohols with water and hence decrease the alcohol aqueous solubility causing degassing (salting-out effect) (Endo et al. 2012). Vials were capped with 20 mm diameter aluminum crimp-top caps with a polytetrafluoroethylene/silicone septum liner immediately after sample addition. Vials were placed on a stir plate with a spin rate of 500 rpm for 5 min prior to analysis. All reagents and solvents were purchased from Fisher Scientific. The reagents were American Chemical Society (ACS) grade while all solvents used were ultra trace pesticide grade.

Instrument conditions

All alcohol analyses were performed on a Bruker static headspace sampler (SHS-40), with separation and detection on Bruker Scion 456 GC and triple-quad MS, respectively. The SHS-40 unit was controlled independently of the GC and MS, and was used to equilibrate, pressurize, and sample each vial prior to injection on the sample loop. Each vial was taken into the headspace instrument from a rotating carousel. The optimized headspace method utilizes an oven temperature of 95°C, valve and transfer line temperatures of 120°C, and a vial pressurization of 500 mbar above atmospheric with ultrahigh purity grade helium and a 1 mL sample loop. Gas chromatographic separation was performed on a Restek-624 30-meter fused silica GC column with 0.25 mm internal diameter and 1.4 μm film thickness. The GC injector port was maintained at 200°C with a split ratio of 1 : 10 and a deactivated injector sleeve with dimensions of 74 mm $\times$ 6.35 mm $\times$ 0.75 mm. The GC oven was held isothermal at 30°C for 5.5 min then ramped to 150°C at a rate of 30°C min^{-1} and held for 1 min. Ultra high purity helium was used as the mobile phase with a flow rate of 1 mL min^{-1}.

The mass spectrometer was operated in multiple reaction-monitoring mode (MRM) with an electron ionization source at 70 eV for each alcohol. Ethanol precursor mass of 46 m/z was isolated in quadrupole 1 (Q1) to give the quantifier ion of 45 m/z and qualifier ion of 31 m/z detected in quadrupole 3 (Q3) with a dwell time of 100 ms. Argon was used as collision gas in quadrupole 2 with a pressure of 0.10 mTorr, energy of 10 V. The *n*-butanol precursor mass of 56 m/z was isolated in Q1 with two transitions monitored with the quantifier transition of 56 m/z to 56 m/z and qualifier 56 m/z to 41 m/z with a dwell time of 50 ms for both transitions detected in Q3. The iso-butanol precursor mass was 43 m/z isolated in Q1. The 43 m/z to 43 m/z transition was used for quantification while the qualifier ion of 43 m/z to 41 m/z with both transitions having a dwell time of 50 ms. Each transition for *n*-butanol and iso-butanol had a collision energy optimized at 10 V and collision gas pressure of 0.10 Torr. All quantifications for each alcohol were performed using an external calibration curve typically ranging from 100 nM to 5 μM. The calibration points were made in deionized water and analyzed under the same conditions as a sample.

The LOD for each alcohol were determined using three times the standard deviation of the integrated noise of ten blanks divided by the slope of a triplicate six-point calibration curve at the appropriate retention time for each alcohol (Skoog et al. 2007). The LOD value is considered the minimum concentration that can be reliably differentiated from the background level. Additionally, all peak integrations required a minimum signal-to-noise ratio of 3 under the specified MRM parameters determined using optimized headspace analyzer and GC-MS/MS conditions. The LOD for ethanol was 28 nM and for *n*-butanol and iso-butanol was 9 nM each. Blanks were analyzed routinely which included vials that were empty and vials that contained de-ionized water. All blanks for each alcohol were below the LOD.

Assessment and discussion

Optimization

Headspace gas chromatographic analysis is an analytical technique in which the aqueous-gas equilibrium of a volatile or semi-volatile component is physically manipulated to maximize partitioning of the analyte between the two phases. The parameters optimized in this study that can be adjusted to enhance signal response include temperature, equilibration time, ionic strength, sample volume, and agitation. The first parameter investigated was the equilibration temperature (EQ) needed to maximize partitioning of ethanol into the gas phase because gases are less soluble as temperature increases in aqueous solutions. Previous research has illustrated specifically how raising the sample incubation temperature significantly increases gas-phase equilibrium concentrations (Hu et al. 2015). The effect of sample temperature during a 5 min EQ defined period was investigated by comparing peak responses with an EQ temperature of 65°C, 75°C, 85°C, and 95°C for a 1μM ethanol sample (Fig. 1A). There was a linear increase in peak area between the four points with an average change of 11,000 counts per 10°C temperature rise. There was an overall 374% response increase between the lowest (65°C) and highest (95°C) temperature points. Based upon the results presented in Fig. 1A, 95°C was chosen as the optimum temperature of equilibration. The equilibration temperature was not raised higher than 95°C to prevent sample boiling.

Equilibration time was varied next to optimize signal response of a 1 μM solution of ethanol with a 95°C EQ temperature. Times of 1 min, 5 min, 10 min, and 20 min were analyzed. There was large (160%) increase in signal counts from 1 min to 5 min with minimal change between EQ times longer than 5 min (Fig. 1B). There was no statistical difference in peak response between 5 min and 10 min (two-tailed *t*-test, $p > 0.1$), which implies that after 5 min' liquid-gas equilibrium has been achieved. Based upon these results,

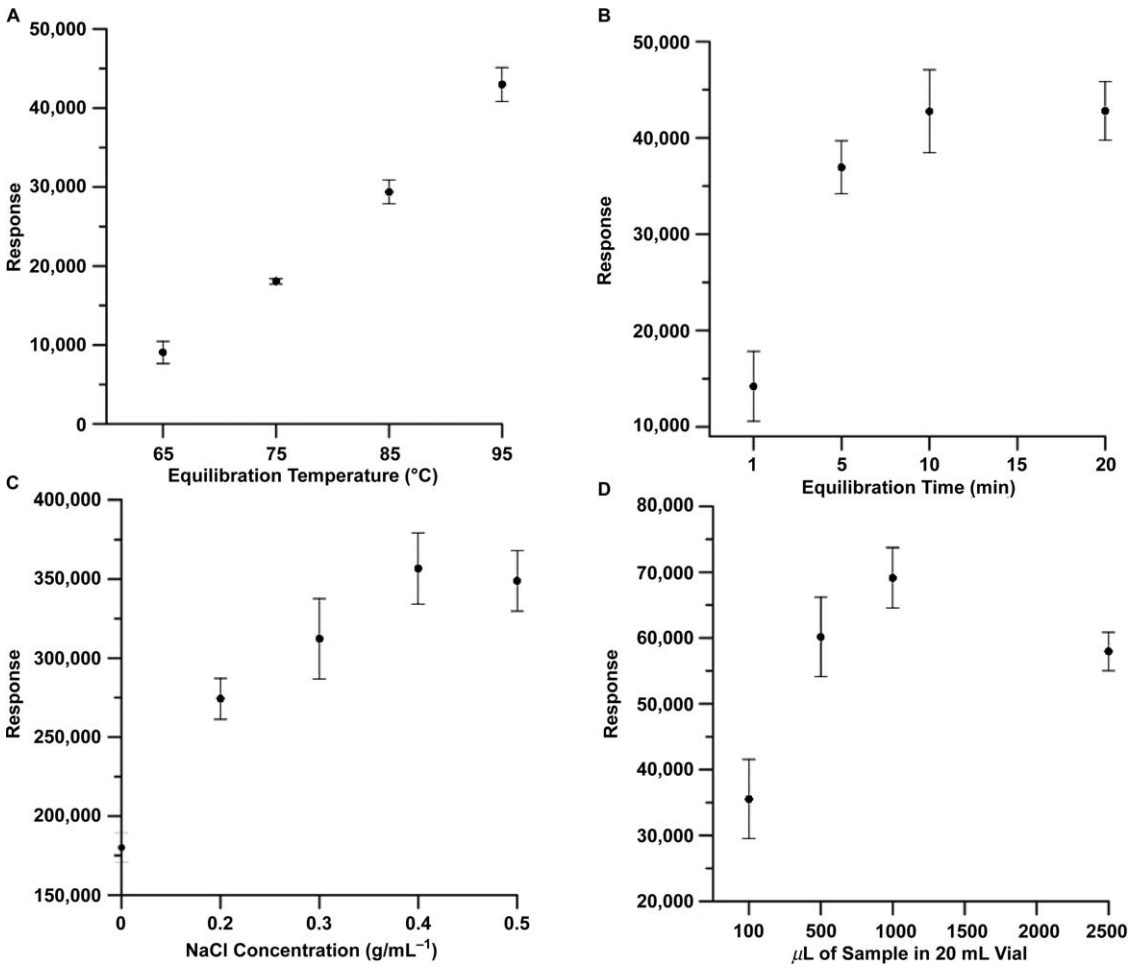

Fig. 1. Optimization of headspace variables using 1 μM ethanol in deionized water. GC-MS/MS was used for separation and detection as outlined in the experimental section. The error bars represent the standard deviation of three replicates. (**A**) Equilibration temperature (°C). (**B**) Equilibration time (min). (**C**) NaCl concentration (g mL^{-1}). (**D**) Sample volume (μL sample in 20 mL vial).

an equilibration time of 5 min at 95°C was chosen to maximize sample throughput.

The signal response of polar compounds can be increased in static headspace analysis by addition of salt to the aqueous sample. Addition of soluble ionic salts to a sample decreases the hydrogen bonding ability of the aqueous matrix thereby decreasing the solubility of other polar components including gases (Eisert and Levsen 1996; Tang and Isacsson 2008; Endo et al. 2012; Montesinos and Gallego 2012; Spietelun et al. 2013). Masses of 0.2 g, 0.3 g, 0.4 g, and 0.5 g NaCl were added to 1 mL of a 1 μM ethanol sample and analyzed with a 5 min equilibrium time at 95°C in order to determine the optimal amount of salt required (Fig. 1C). A 1 mL sample without any added salt was analyzed as well, representing the control. Results were in agreement with a previously published headspace optimization of NaCl in aqueous ethanol, where the largest increase in peak area from 0 g to 0.2 g dissolved salt (> 100% increase) followed by a leveling of peak response as the saturation point (0.39 mL^{-1} g at 95°C) (Tankeviciute et al. 2001) is approached (Fig. 1C). No statistical difference in peak response was observed above the solubility of NaCl (two-tailed *t*-test, $p > 0.1$) therefore 0.3 g NaCl mL^{-1} (5M) was used for subsequent analyses. The amount of salt in a seawater sample (approximately 0.5M NaCl or 0.03 g NaCl mL^{-1}) does not contribute enough salt to alter the analytical response, so the salt addition in this method does not have to be adjusted for seawater samples.

The ratio between aqueous sample and gaseous headspace volumes influences the static equilibrium between the two phases, because as the amount of liquid sample in the vial increases the volume of the gas phase decreases, causing the overall headspace concentration to increase (Kolb and Ettre 2006). However, for extremely soluble analytes with large water/air partition coefficient values such as ethanol, the effect of changing the aqueous phase and gas phase volumes causes minimal detectable changes in gas-phase concentrations (Kolb and Ettre 2006). To see how altering this ratio

affected method response, the sample volumes were varied and analyzed under optimized conditions (Fig. 1D). All samples were 1 μM ethanol with a 5 min/95°C EQ period and 30% by mass dissolved NaCl. Total volumes of 100 μL, 500 μL, 1000 μL, and 2500 μL were added to the headspace vial. The highest response was achieved with a sample volume of 1000 μL, a 94% increase from the peak area of the 100 μL sample. There was a slight statistically significant decrease (two-tailed *t*-test, $p < 0.05$) between the 1000 μL and 2500 μL samples, possibly because the 2500 μL sample had not yet reached equilibrium after the 5 min EQ time. A sample volume of 1000 μL was chosen for all subsequent analyses to maximize analytical response while keeping the 5 min EQ time for maximum throughput.

The headspace analyzer has an additional function that agitates the sample vial during the equilibration step. The sample vial is placed in a rotating vial rack for the duration of the equilibration time in order to shift equilibrium further to the gas phase. Triplicate analysis of a 1 μM ethanol sample prepared in deionized water exhibited a statistically significant increase (two-tailed *t*-test, $p < 0.05$) increase between agitation off and on. The headspace conditions used were an equilibrium time of 5 min at 95°C with 1 mL of sample and 30% by mass salt.

Method performance and application

Standards and samples were treated identically with the optimized method of 1 mL standard or sample volume, agitated, equilibrated at 95°C for 5 min with 0.3 g NaCl mL^{-1}. A calibration curve over the range of 100–5000 nM was produced utilizing the optimized headspace analyzer conditions determined for ethanol, highlighting the linearity and dynamic range of the method (Fig. 2A). The intra-day and inter-day relative standard deviations using the optimized conditions for ethanol were 0.2% and 0.1%, respectively. The instrumental conditions for ethanol were applied to *n*-butanol and iso-butanol as well with linear calibration over the range 50–1250 nM (Fig. 2B,C). The intra-day relative standard deviation for iso-butanol and *n*-butanol was 4% and 3%, respectively. The inter-day relative standard deviation for iso-butanol and *n*-butanol was 5% and 2%, respectively. A sample chromatogram of the alcohols analyzed in this study highlights the separation of each analyte (Fig. 3). The baseline shift is due to the different MRM transition energies and dwell times used for each alcohol. Of the three alcohols studied here, ethanol has been the focus of existing analytical methods having limits of detection in the order of tens of nM to μM concentrations (Table 1). These methods typically focus on only one type of environmental matrix or only use laboratory-purified water. The method described here provides a comparable LOD to other studies (28 nM) but is applicable to a much wider array of natural waters (e.g., rainwater and seawater). This is the first method

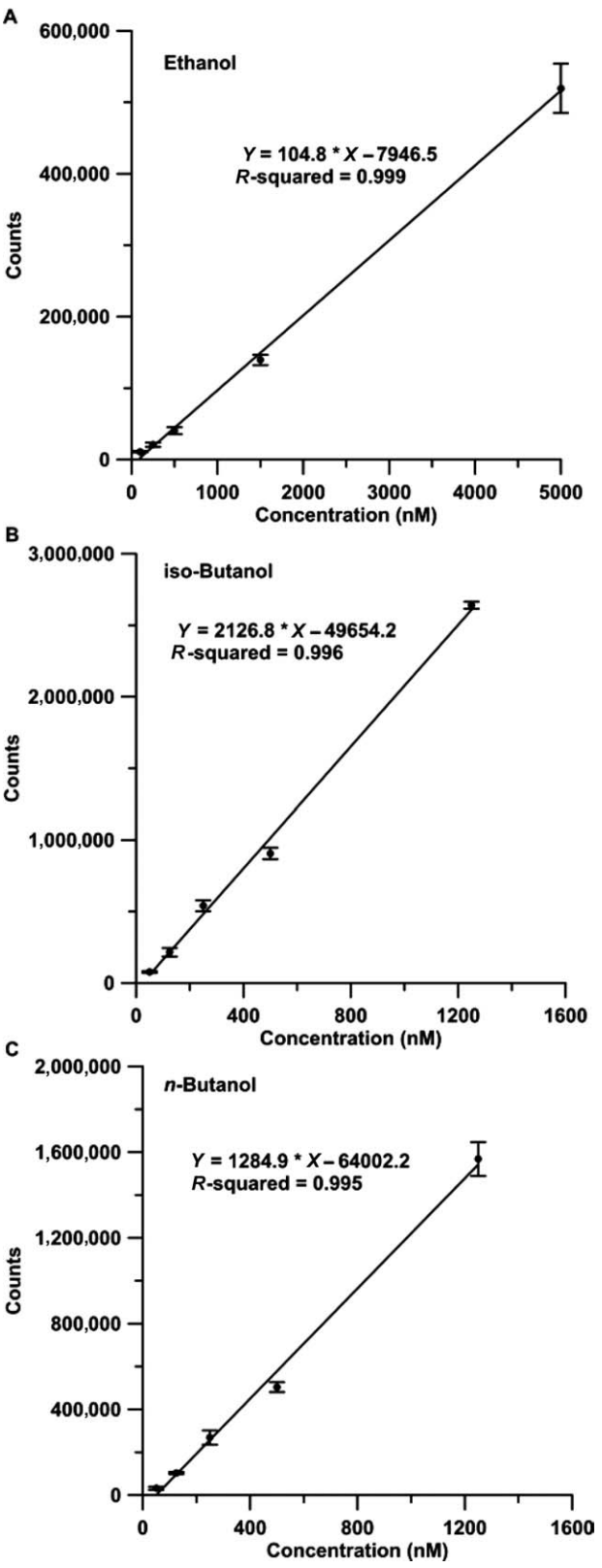

Fig. 2. Typical calibration curves of (**A**) ethanol, (**B**) iso-butanol, and (**C**) *n*-butanol. Error bars represent the standard deviation of three replicates.

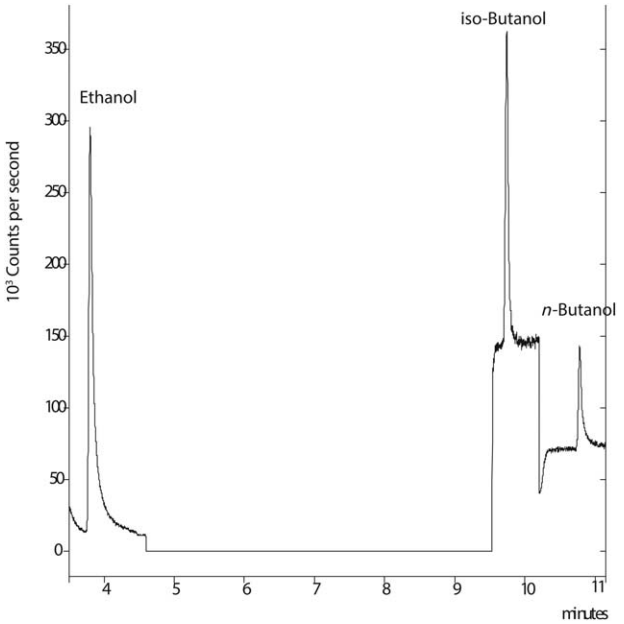

Fig. 3. A typical GC-MS/MS MRM chromatogram of all three alcohols in one analytical run. Instrumental conditions are explained in the methods section.

Table 2. Spike recovery data in various environmental matrices. The matrices are described in more detail in the methods section. Percent recoveries are based upon spiking levels of 1–4.1 μM for ethanol, 114–149 nM and 94–137 nM for *n*-butanol and iso-butanol, respectively. The number of samples analyzed is defined as *n*.

Matrix	*n*	% Recovery (avg ± SD)
Ethanol		
DI water	10	99 ± 6
Rainwater	10	102 ± 8
Condensate	4	101 ± 1
River water	8	86 ± 2
Seawater	8	96 ± 2
Iso-butanol		
Rainwater	9	90 ± 5
River water	3	90 ± 1
Seawater	9	91 ± 6
Butanol		
Rainwater	9	91 ± 7
River water	3	92 ± 3
Seawater	9	100 ± 5

DI, deionized water.

analyzing *n*-butanol and iso-butanol in aqueous environmental samples under ambient conditions.

The percent recovery of each alcohol in a wide variety of matrices was examined by spiking known concentrations of each analyte into deionized water, rainwater, river, lake, and ocean waters (Table 2). Recovery of ethanol was > 95% in all cases except for the Cape Fear River water where it was lower (86%). Percent recovery was determined by quantifying the concentration of ethanol before and after spiking to a final concentration that spanned 1–4.1 μM. The recovery of ethanol from the Cape Fear River water was the lowest, most likely due to the relatively high dissolved organic carbon concentration (ca. 1 μM) (Dixon et al. 2014). Decreased recoveries in matrix rich samples such as high DOC concentrations has been observed before for a variety of analytes utilizing headspace analysis (Sun et al. 2016). Even with the relatively lower recovery in the river water, the standard deviation was very small (2%) highlighting the precision of the method. *n*-Butanol and iso-butanol also exhibited > 90% recoveries in a wide variety of matrices with standard deviations typically less than 7%. Each sample was analyzed for ambient *n*-butanol and iso-butanol and there was none detected. The same samples were then spiked with *n*-butanol (114–149 nM) and iso-butanol (94–137 nM), quantified using an external calibration curve and the percent recovery calculated.

One of the most important, albeit challenging, aspects in the development of an analytical technique for the determination of low-level concentrations of small polar compounds in complex environmental matrices is the verification of analytical results. The accuracy of the static headspace GC/MS method was determined by comparison of analytical results with a completely independent method in which a wide variety of natural water samples were measured. Samples were split and analyzed by the headspace method and compared to a solid phase microextraction (SPME) method (Kieber et al. 2013). Briefly, SPME is a static headspace method that dissolved ethanol is allowed to equilibrate with a sorbent coated fiber that is held above the liquid in the gas phase. The SPME fiber is then retracted and analyzed by GC-FID. Eighteen natural water samples were collected and analyzed by the two independent methods (Fig. 4). The samples analyzed were a combination of authentic rainwater, coastal ocean, lake, and estuarine waters. The resulting intercomparison covered concentrations in the range of 107–2400 nM where the line of best fit had a slope of 1.02 with 3% error. The slope near unity confirms the accuracy of the new method when compared to a completely independent analysis. Interfering aqueous phase reactions are also a major concern with the determination of any low level analyte in complex mixtures. Data presented in Fig. 4 suggest that no such interfering reactions are present in the range of concentrations studied here. The randomness of data presented in Fig. 4 around the best-fit line between methods also suggests no large systematic interferences or errors were present in one analysis relative to the other.

Comments and recommendations

Results presented in this study demonstrate the utility of methodology for low-level determination of ethanol, *n*-

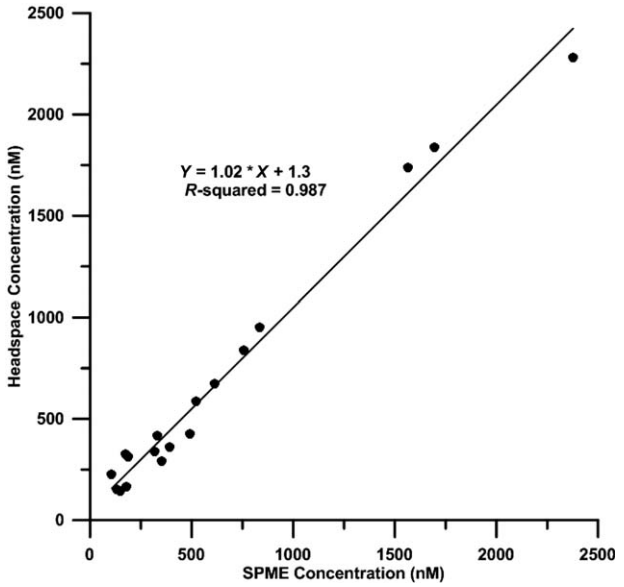

Fig. 4. Intercomparison of the headspace GC-MS/MS and SPME (Kieber et al. 2013) methods for the analysis of ethanol. Authentic natural waters ($n = 18$) were split and analyzed by both methods. The line is the regression through all of the data points, and the slope of unity indicates agreement between each method.

butanol, and iso-butanol in a wide variety of environmental matrices. This method can simultaneously detect and quantify low molecular weight alcohols in one analytical analysis in fresh and marine waters. This is a distinct advantage when compared to other published methods that have not reported such varied matrices (Table 1). In fact, the LOD for ethanol using the method in this study is lower than a majority of the methods presented in Table 1. A second advantage of this analysis is the minimal amount of sample and preparation required compared to previous methods. For example, 1 mL of sample and salt is required for the optimized method presented here while other methods typically need larger volumes, pH adjustment, and/or complicated sample pretreatment (Monod et al. 2003; Kieber et al. 2013). One of the most important characteristics of the headspace GC-MS/MS method that distinguishes it from existing analyses is validation of accuracy via independent intercomparison and spike recovery experiments which is not done in most earlier ethanol analyses (Monod et al. 2003; Beale et al. 2010). Furthermore, this method is the only one to date that includes *n*-butanol and iso-butanol in addition to ethanol, and it has great potential to be optimized for different analytes in addition to these three reported here. The quantification of ethanol in rainwater as well as other natural waters is important for the potential impact these alcohols may have biogeochemical processes (Kieber et al. 2014; Roebuck et al. 2016). For example, ethanol concentrations in rainwater collected from marine air mass is an order of magnitude lower than terrestrial derived storms (Felix et al. 2017). In fact, removal of ethanol by wet deposition accounts for 6–17% of the total ethanol emitted per year (Felix et al. 2017). Having a robust analytical technique that requires minimum sample volume will become more important in the future as the use of small alcohols as biofuels increases.

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Conflict of Interest

None declared.

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