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Analysis of cooking spices in natural waters

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Abstract

An isolation and quantification technique is presented for the analysis of semi-volatile spices and scents in aquatic waters including thymol, eugenol, limonene, carvacrol, vanillin, and cinnamaldehyde. Compounds are isolated from natural waters by solid phase extraction onto Waters Oasis HLB columns and eluted into ethyl acetate. Recovery efficiencies are >70% for most target compounds. Comparison with liquid-liquid extraction and C-18 solid phase extraction indicates that HLB cartridges are more robust and provide equal or better recoveries for target compounds relative to other approaches. Because the compounds are semi-volatile and prone to losses during solvent evaporation, we do not dry the samples completely before quantification by gas chromatography mass spectrometry. Compounds are analyzed using selected ion monitoring with a sensitivity ~1 pg compound per injection. This equates to a sensitivity of about 2–200 ng L^{-1} in natural waters, or part-per-billion levels. Column loading and matrix effects are minimal and are accounted for by careful monitoring of volumes and by standard additions during GC analysis. Environmental samples (treated sewage, lake, stream, and fjord samples) show a wide range of compounds and quantities, most in the 10–10,000 ng L^{-1} range. The artificial spice ethylvanillin, which is commonly used as an internal standard in analysis of woody lignin samples, is found in some natural waters. This is likely a result of the industrial-scale production of this compound for culinary and pharmaceutical use.

Introduction

Most common culinary spices are the products of plants. Flora typically produce spices and scents either to attract insects or animals for dispersion of pollen, or to chemically protect against predators (Goyret et al. 2008; Raguso 2008). Humans use spices to enhance the flavor of food and mask bitter flavors in medicines (Dignum et al. 2001). Because many spices have antibacterial or medicinal properties (Alpers 2009; Liu et al. 2007; Thangapazham et al. 2006), they are often used in homeopathic treatments. Demand for spices often exceeds their availability from natural sources (Dignum et al. 2001), and thus many common cooking spices are also produced synthetically (e.g., vanilla, cinnamon, limonene). Because of their dual sources (natural and petroleum) and because of their frequent use by humankind, spices can be a tracer of both natural and anthropogenic processes affecting a watershed or aquatic system. For example, because many spices are produced during angiosperm flowering, they represent a potential chemical link between watershed greening and within-water processes. Alternatively, the presence of artificial spices within a watershed clearly identifies an anthropogenic source for the material.

Many culinary spices have ringed chemical structures that make them amenable to gas chromatography. There are a number of long established techniques to measure macromolecular plant-derived phenols such as lignin phenols released after oxidation of solid samples (Hedges and Mann 1979a), and these techniques have been extended to investigate dissolved (<0.2 μm) organic matter (Louchouarn et al. 2000). There is currently no technique specifically aimed at evaluating the natural abundance of spice monomers in aquatic systems. Based loosely on the approach of Louchouarn et al. (2000), we developed a method for the extraction and analysis of spice monomers in both fresh and salt water. Our goal was to develop a method robust to varying salt content and environmental matrices and that was capable of detecting common spices over a wide concentration range (ng-μg L^{-1}) because we wish to evaluate a variety of fresh and salt water sample types from pristine waters to treated sewage effluent.

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Materials and procedures

Materials—All compounds and analytical chemicals were purchased from Sigma-Aldrich. Stock solutions of spices and scents (25–100 mM depending on solubility) were prepared separately for each compound by dissolving a known mass of the analyte in ethyl acetate or pyridine. Mixed standard solutions were made by combining individual standards and diluting to yield 10 mM concentrations for each compound. This primary standard solution was then diluted to a working standard of 0.25 mM (25–50 mg L^{-1}) and stored in amber glass vials with Teflon lids at –20°C until use.

All expendable glass and plastic ware was either muffled (400°C, 4 h) or 10% HCl-washed prior to use. Sampling containers were low-density polyethylene cubitainers. Solid phase extraction columns were Waters Oasis HLB, 200 mg sorbent. The gas chromatography system was an Agilent 6890N gas chromatograph fitted to a 5975 series mass selective detector (MSD). Peaks were identified using Agilent's MSD Productivity Workstation enhanced with the deconvolution reporting software. Some method development was conducted using an Agilent 6890 GC with flame ionization detection, but all quantifications of natural samples are currently made by mass spectrometry. The analytical column was either a J&W DB-5MS or a J&W DB-1 column (each 60 m length × 320 µm internal diameter, 0.25 µm film thickness).

Procedures—Samples are filtered (GF/F) under <15 mmHg, and a known volume (typically 1-2 L) of filtrate is dosed with the internal standard 3,4 dihydroxybenzoic acid to a final concentration of 2.6 µg L^{-1}. The sample is then acidified to a pH <3 using HPLC-grade hydrochloric acid and stored at 8°–10°C until further processing (within 3 d). For solid phase extraction, 200 mg Waters Oasis HLB cartridges are preconditioned sequentially with 1 mL methanol, 1 mL ethyl acetate, and 1 mL acidified (pH 2) MilliQ water. The acidified sample is then slowly (3 mL min^{-1}) passed through the cartridge, where the compounds are bound and removed from solution. It is necessary to know the exact volume of water extracted, so we weigh the cubitainer before and after extraction and calculate volumes from differences in mass (correcting for salinity). We use an Alltech vacuum manifold that accommodates up to sixteen samples simultaneously. Despite information from Waters stating that the cartridges can be allowed to dry, we have found that vacuum drying the cartridges decreases our yield and precision. This was determined by evaluating recoveries (quantified by GC-FID) with and without drying the columns in between extraction and elution. Thus, we store cartridges moist by running 1–2 mL acidified water through the cartridge at the end of a sample, turning off the vacuum after the last drops have come out of the cartridge. If necessary, we have found that cartridges can be stored at –20°C for as long as 1 month in individual reclosable plastic bags prior to elution. Elution is performed by attaching a Na_2SO_4 drying column to the bottom of the HLB column and flushing 5 mL ethyl acetate through the cartridges at a rate of about 1 drop per second. Extracted samples are collected in combusted 10 mL disposable glass test tubes. Bringing samples to complete dryness leads to significant and selective losses of aldehydes and ketones. Thus, we concentrate the sample under low heat (40°C) and a slow stream of nitrogen (using a Zymark TurboVap) to approximately 0.7 mL. The sample is then transferred with a glass pipette to a 1 mL glass volumetric flask and then brought volumetrically to 1 mL. The ethyl acetate that is used to adjust the volume is first swirled, in 100 µL increments, in the 10 mL disposable glass test tubes to solublize any residue adhering to the sides of the tube. The solution is transferred to a 2 mL amber glass vial, capped, and stored at –20°C until further analysis.

Samples are prepared for gas chromatographic analysis by combining 50 µL ethyl acetate extract with 50 µL BSTFA:TMCS (99:1) in autosampler GC vials sealed with Teflon-lined caps. During some early method development, samples were derivatized in pyridine. Prior to injection, samples are heated on a dry-block hot plate at 60°C for 10 min to silylate any exchangeable hydrogens present in the extract. GC-MS analysis is performed on an Agilent 6890N GC coupled to 5975 MSD fitted with a J&W DB-5MS column. The oven temperature is programmed at 2°C min^{-1} from 60–250°C then ramped to 320 at 25°C min^{-1} followed by a 15 min hold. Helium is used as a carrier gas at a constant flow of 1.5 mL min^{-1}. Samples are injected in splitless mode, with an injector temperature of 220°C and an injection volume of 1 µL. All samples are run in both full-scan (60–380 amu) and SIM mode (ions for each compound as in Table 1; chromatograph in Fig. 1). Peaks are identified by a combination of selected ions and quantified from ion-specific response factors determined from 3-point standard curves of the stock standard. All quantities are then corrected for recoveries (Table 2) relative to the internal standard 3,4 dihydroxybenzoic acid. Samples are run on the GC-MSD in batches of 20–30 with standards and blanks interspersed every five samples. Standard additions are conducted on approximately every fifth sample.

Assessment

Although no technique exists specifically for spices, there are many existing techniques with similar goals—the reproducible extraction of low-level organic chemicals from natural waters. Two popular approaches include liquid-liquid extraction (LLE) (Barber et al. 2000) and C-18 solid-phase extraction (Castillo et al. 1999). These are also the two techniques most commonly used to isolate individual lignin phenols after cupric oxide oxidation (Goni and Montgomery 2000; Louchouarn et al. 2000). Since several spices are liberated from woods after alkaline oxidation, and thus their extraction efficiencies by LLE or C18-SPE are well documented for aquatic samples, we compared the new technique to these two well-established techniques.

To test the three techniques, we started with the "standard" suite of 14 lignin phenols (*see* Table 1). 20 mL aliquots of pH 2 MilliQ water were spiked with 24.0 µL of the 10 mM stock

Table 1. Target compounds (common names or odors/tastes in parentheses), molecular weights, major target ions, and qualifier ions for mass spectral identification and quantification, and the peak number for identification in Fig. 1.

	MW	Target ion	Qualifier ions		Peak nr
			Q1	Q2	
Acetosyringone	196.20	223	238	253	22
Acetovanillone (apocynin)	166.17	193	223	238	19
Benzaldehyde† (almond)	106.12	105	106	77	2
Benzyl acetate† (jasmine)	150.18	150	108	91	6
Benzyl propionate†,§ (banana)	164.20	164	108	91	8
Carvacrol (oregano)	150.22	222	207		11
S+ carvone† (caraway)	150.22	82	150	108	7
Cinnamaldehyde†§ (cinnamon)	132.16	132	131	103	8
trans-Cinnamic acid (cinnamon)	148.17	205	220	161	17
p-Coumaric acid (balsamic)	164.16	219	293	308	26
Cumene†	120.19	105	120		1
3.4-Dihydroxybenzoic acid*	154.12	193	370	355	24
Ethyl vanillin (artificial vanilla)	166.17	167	195	238	18
Eugenol (clove/nutmeg)	164.20	206	221	236	15
Ferulic acid	194.19	338	249	323	27
4′-Hydroxyacetophenone (sweet floral odor)	136.15	193	208	151	14
4-Hydroxybenzaldehyde	122.12	179	194	151	12
4-Hydroxybenzoic acid	138.12	267	223	193	20
Limonene† (oil of lemon)	136.24	68	93	136	3
Limonene oxide cis† (citrus)	152.23	137	109		4
Limonene oxide trans† (citrus)	152.23	108	137		5
Linalool‡ (coriander, lavender)	154.25	93	71	121	9
Syringaldehyde	182.17	224	239	254	21
Syringic acid	198.18	327	342	312	25
Thymol (thyme)	150.22	222	207		10
Vanillic acid	168.15	297	267	312	23
Vanillin (vanilla)	152.15	194	209	224	16
Veratraldehyde† (caramelized vanilla)	166.17	166	95		13

*Internal standard; †does not derivatize with BSTFA; ‡derivatizes in pyridine but not in ethyl acetate; §co-elute on DB-1 column but not on DB-5.

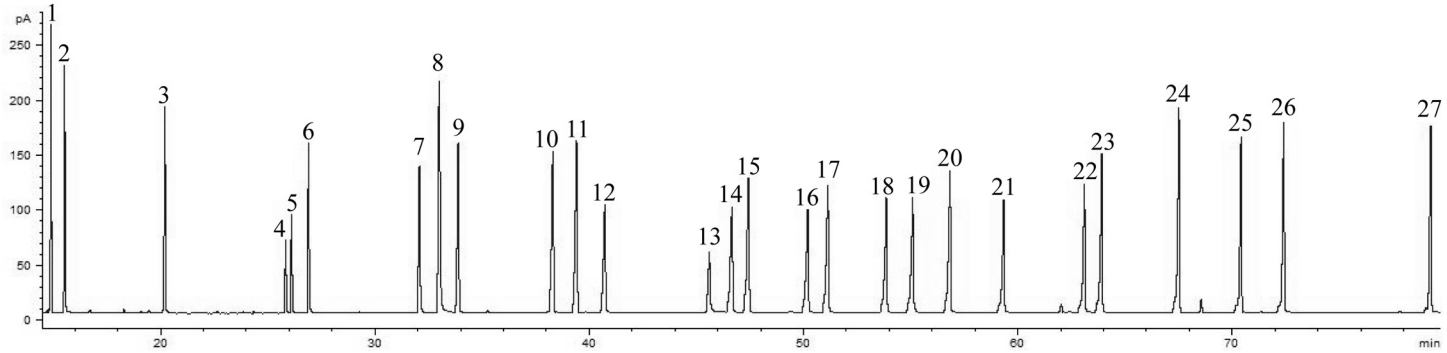

Fig. 1. Chromatogram of spice standards separated using a DB-1 column and the run conditions described in the text. Benzyl propionate and cinnamaldehyde co-elute using this column but do not co-elute using a DB-5 column. Peaks numbers as identified in Table 1.

standard solution and then either passed through the columns (HLB and C-18) or mixed with 6 mL ethyl acetate (LLE). The C-18 columns contained 1 g octadecyl carbon moieties (C18) chemically bonded to a silica support (C18-SPE Mega-Bond Elut; Varian). Cartridges were pretreated with 5 mL methanol and 5 mL acidified MilliQ water, ensuring the sorbent remained wet at all times. After passing the 20 mL standard solution through the sorbent, the C-18 cartridges were rinsed with 2 mL acidified

Table 2. Percent recoveries (± 1 standard deviation). Comparison of three extraction techniques, all using MilliQ water. Comparison of HLB recoveries for spices added to treated sewage effluent (West Point Facility, Seattle), lake water (Portage Bay, Lake Washington), and a marine fjord (Clayoquot Sound, British Columbia).

	MilliQ water			Effluent	Lake	Fjord
	LLE (n = 5)	C-18 (n = 4)	HLB (n = 4)	HLB (n = 3)	HLB (n = 3)	HLB (n = 3)
Acetosyringone	97.4 ± 1.7	101.6 ± 2.6	101.3 ± 2.3	89.8 ± 2.5	89.0 ± 2.3	93.1 ± 2.1
Acetovanillone	63.4 ± 9.7	81.0 ± 4.8	87.7 ± 6.5	71.2 ± 6.3	79.4 ± 3.8	71.9 ± 4.8
Benzaldehyde	ND	ND	70.8 ± 0.3	ND	ND	28.1 ± 2.2
Benzyl acetate	ND	ND	65.2 ± 1.6	ND	ND	72.7 ± 4.1
Benzyl propionate	ND	ND	86.0 ± 6.4	ND	ND	64.6 ± 2.6
Carvacrol	ND	ND	89.2 ± 1.2	ND	ND	73.0 ± 3.9
S+ Carvone	ND	ND	77.6 ± 0.4	ND	ND	80.9 ± 3.0
Cinnamaldehyde	ND	ND	86.0 ± 6.4	ND	ND	54.6 ± 2.6
trans-Cinnamic acid	74.1 ± 7.1	82.8 ± 2.3	86.4 ± 6.6	72.5 ± 6.0	82.5 ± 2.2	75.2 ± 3.1
p-Coumaric acid	99.1 ± 2.0	98.2 ± 2.3	96.3 ± 4.6	86.4 ± 4.7	82.9 ± 3.4	92.5 ± 4.1
Cumene	ND	ND	24.4 ± 0.7	ND	ND	65.0 ± 1.7
3,4 Dihydroxybenzoic acid	80.8 ± 2.3	15.1 ± 3.8	70.3 ± 2.9	78.2 ± 2.6	78.8 ± 2.6	87.0 ± 2.8
Ethyl vanillin	43.6 ± 13.2	64.8 ± 6.5	73.0 ± 5.6	57.1 ± 5.4	70.0 ± 1.8	59.9 ± 2.8
Eugenol	ND	ND	78.5 ± 0. 5	ND	ND	81.0 ± 1. 5
Ferulic acid	98.1 ± 1.7	97.5 ± 2.7	95.8 ± 4.8	87.9 ± 4.3	83.1 ± 2.3	92.1 ± 3.7
4′ Hydroxyacetophenone	84.3 ± 4.1	94.0 ± 2.7	94.6 ± 5.0	80.0 ± 6.2	86.3 ± 7.5	81.1 ± 6.2
4-Hydroxybenzaldehyde	61.3 ± 9.7	86.6 ± 2.6	81.8 ± 5.4	67.2 ± 4.1	79.7 ± 3.5	71.0 ± 4.7
4-Hydroxybenzoic acid	97.2 ± 1.9	4.9 ± 15.8	99.7 ± 3.6	87.0 ± 3.7	89.0 ± 2.4	92.4 ± 3.1
Limonene	ND	ND	30.5 ± 0.6	ND	ND	34.7 ± 4.2
Limonene oxide, Cis	ND	ND	35.6 ± 4.4	ND	ND	59.6 ± 4.4
Limonene oxide, trans	ND	ND	30.0 ± 14.4	ND	ND	29.6 ± 8.4
Linalool	ND	ND	76.2 ± 0.3	ND	ND	78.9 ± 3.3
Syringaldehyde	87.8 ± 1.7	92.9 ± 2.6	91.7 ± 2.6	83.2 ± 1.7	89.4 ± 1.7	87.9 ± 2.3
Syringic acid	96.1 ± 2.9	55.0 ± 10.2	99.6 ± 4.1	89.0 ± 4.1	87.8 ± 2.9	93.3 ± 3.6
Thymol	ND	ND	78.0 ± 2.2	ND	ND	79.2 ± 2.3
Vanillic acid	95.8 ± 1.8	19.6 ± 10.2	98.0 ± 4.5	87.4 ± 5.2	88.9 ± 2.9	90.2 ± 3.9
Vanillin	35.3 ± 13.8	67.4 ± 7.8	71.7 ± 8.2	56.2 ± 4.8	66.0 ± 3.6	58.6 ± 4.3
Veratraldehyde	ND	ND	40.0 ± 3.9	ND	ND	39.2 ± 3.9

*ND, not determined.

MilliQ water and eluted with 5 mL methanol. HLB cartridges were activated and eluted as discussed previously. For LLE, the water-ethyl acetate sample was thoroughly shaken for 2 min, the two phases were allowed to separate, and the organic portion was transferred to a clean vial using a glass pipette. This process was repeated with an additional 6 mL ethyl acetate to maximize the recovery of the organic extract. Cleaned Na_2SO_4 was added to the ethyl acetate extract to absorb any excess water, and samples were dried down under low heat (40°C) and a slow stream of nitrogen gas. Immediately following evaporation, the samples were resuspended in 400 µL pyridine and subjected to GC-FID analysis. Percent recoveries were calculated by injecting the stock standard at a concentration of 0.3 mM, which is the concentration that a 24.0 µL spike of the 10 mM stock standard should yield given 100% recovery, and comparing the peak areas obtained in the samples to those given by the stock standard injection (Table 2).

In general, all three techniques gave adequate results for the majority of the test compounds. However, only the HLB column showed good (>70%) recovery for all compounds tested. The LLE technique had poor recoveries of vanillin (35.3%) and ethyl vanillin (43.6%), and 4 of 14 tested compounds had recoveries <70%. Using LLE, Goni and Montgomery (2000) reported slightly higher yields than we achieved for some compounds. The C-18 column had poor recovery of 4-hydroxybenzoic acid (4.9%), vanillic acid (19.6%), 3,4 dihydroxybenzoic acid (15.1%), and 6 of the 14 compounds had recoveries <70% (Table 2). For all three techniques, the reproducibility was within 5% to 10% for most compounds, but in addition to low recoveries for some compounds, both the LLE and C-18 techniques had reproducibility issues with select compounds. Thus, the HLB columns gave better overall performance than either the LLE or C-18 approaches.

In addition to showing reproducible yields, the technique needs to be robust for a variety of sample types. To evaluate this, we compared recoveries for treated sewage effluent (West Point treatment facility, Seattle, WA, USA), lake water (Portage Bay, WA, USA), and salt water (Tofino Inlet, BC, Canada, salinity 28). The West Point wastewater sample used for this analysis was collected on July 8, 2008 from the final effluent well after dechlorination, and the Tofino Inlet sample was collected in June 2007. To evaluate water type, 20 mL water samples from each location were spiked with 24.0 µL of the 10 mM stock standard solution, and processed in triplicate with HLB cartridges and analyzed as earlier described. Spiked samples were corrected for the "blank" (e.g., ambient concentration) prior to calculations. Compared to the recoveries for MilliQ water, all three natural waters had ~10% lower recoveries for the standards (Table 2). This could be due to competition for binding sites between the added standards and organic matter already present in the natural samples leading to inefficient extraction, or due to irreversible sorption on the columns. Interestingly, despite lower yields, reproducibility was the same, with most compounds having standard deviations of < 6% (Table 2). Using the HLB technique as developed; our lowest recoveries were for vanillin and ethyl vanillin, both of which were recovered with ~60% efficiency. There was no discernable salinity effect, as the recovery for salt water samples was not significantly different from that of the other two water types (ANOVA, $P < 0.001$) excepting ferulic acid, for which the recovery in sea water was slightly higher than for the other water types (Table 2).

Overloading of HLB cartridges can lead to inefficiencies in extraction and underestimates of the compounds of interest. Two tests were performed to determine if the loading capacity of the 200 mg HLB cartridges was sufficient for natural samples. First, different concentrations of standards were dosed into the same volume of water (250 mL), and second, equal amounts of the standard were added to differing amounts of water. These tests were conducted using treated sewage effluent because it has the highest organic load of the water types that we are assessing, thus results from this test ought to be applicable to other water types. After accounting for recovery efficiencies, samples dosed with 3, 6, 9, and 12 µM additions of the fourteen spices showed no significant deviation from a linear trend and nearly 100% recovery (Fig. 2 illustrates four examples). Squared correlation coefficients (r^2) were above 0.99 for all fourteen compounds, and slopes ranged between 0.92 (ethyl vanillin) and 0.999 (3,4 dihydroxybenzoic acid). This indicates that the 200 mg HLB column is capable of quantitatively extracting spices at concentrations equal to or higher than that expected to be observed in nature (*see* later discussion).

The second test of adding the same quantity of spice standard to differing quantities of water assessed whether it was possible to overwhelm the HLB column with other compounds that compete for binding sites (Fig. 3). Of the fourteen spices evaluated, four showed significantly lower recovery from 2 L water relative to 1 L or less: p-coumaric acid, syringaldehyde, acetosyringone and ferulic acid. Yields of acetovanillone increased with larger volumes of water, suggesting that there was another compound co-eluting with acetovanillone that artificially enhanced apparent yields. The other nine compounds had linear recoveries at all volumes. Our interpretation of this

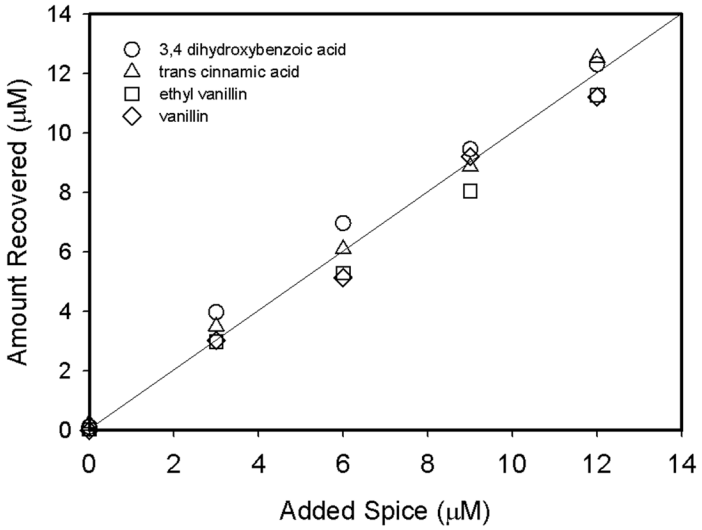

Fig. 2. Recovery of two acids and two aldehydes added to treated sewage effluent and extracted using HLB columns. All other compounds not shown for clarity and have similar responses. Quantification by GC-FID, data converted to µM after blank subtraction, and a correction for recoveries applied as per Table 2.

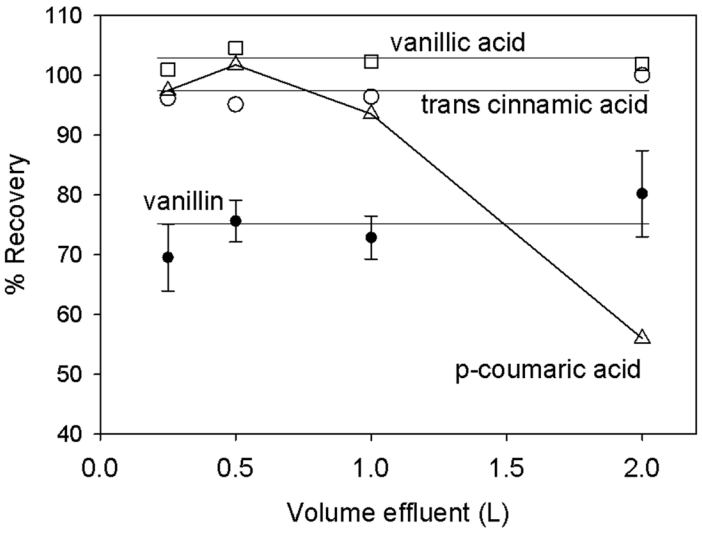

Fig. 3. Recovery of selected spices from different volumes of treated sewage effluent. Of the fourteen spices evaluated, four showed significantly lower recovery from 2 L water relative to 1 L or less: p-coumaric acid, syringaldehyde, acetosyringone, and ferulic acid. Acetovanillone (not shown) showed significantly higher yields, suggestive of coelution and peak mis-identification by GC-FID. The other nine showed linear recoveries at all volumes. Error bars on the vanillin data denote 1 standard deviation for triplicate analyses.

information is that, in general, for samples of 1 L or less, the extraction and recovery of spices does not change.

Given these results, which were obtained using a GC-FID, we moved analyses to GC-MSD to evaluate matrix effects and co-elution of peaks. We also evaluated fourteen additional spices, of which eleven showed recoveries >60% and three of which showed low but reproducible recoveries (Table 2). Co-eluting compounds that originate from the original sample and are isolated along with compounds of interest can cause signal enhancement or suppression (Van De Steene et al. 2006). In this way, matrix effects can affect the reproducibility and accuracy of the method. We evaluated this by comparing yields of known amounts of spices added to lake water, sea water, and treated effluent. Matrix effects (ME) are defined as percentages above or below 100% when comparing standards added to samples versus added to clean water (Van De Steene et al. 2006). With the exception of ferulic acid, which had ME values >150%, matrix effects were determined to be within 20% of target values and were generally equal to or above 100% (data not shown). In GC-MS, matrix-induced response enhancement is the most common matrix effect negatively impacting quantitation accuracy (Hao et al. 2007). Our values are typical compared to other GC-MSD systems (Hao et al. 2007) and better than the values commonly observed for liquid chromatography systems (Van De Steene et al. 2006). To account for the small but detectable matrix effect, we run standard additions in every batch of samples, and run samples in water type-specific batches. We have not observed sample-to-sample variability in matrix effects that is larger than the analytical variability of the method, and thus do not run standard additions on every sample.

Brown (2008) suggests that detection limits be presented only for the mass detectable on the mass spectrometer, independent of prior analytical steps, but that the precision include all preparative steps. In our hands, the technique has a sensitivity on the GC-MSD of ~1 pg for each compound of interest. Given a typical sample size of 1 L processed through the HLB column and concentrated to 1 mL, a typical injection of a 1 µL sample diluted 1:1 with derivatizing agent, this equates to a detection limit of approximately 2–200 ng per liter depending on the compound (i.e., parts per billion; Table 3). Our precision varies slightly over time, influenced by things such as sample type (salt, fresh, etc.) and chromatographic conditions (GC column age, cleanliness of ion source, etc.). Generally, our precision is ±15% of the measured value, with precision decreasing as concentrations approach the detection limit (*see* data in Table 3).

Discussion

We used this method to analyze samples from a variety of locations to determine whether any of these compounds are routinely found in the aquatic environment. A broader sampling will be presented in greater detail elsewhere, but samples from four locations are presented here and illustrate that spice compounds are found at detectable levels in a variety of settings (Table 3). One of the more interesting results of our work to date is the observation that spice distributions include the synthetic compound ethylvanillin, indicative of an anthropogenic source for at least that single spice (Fig. 3). Also, a number of these spices are liberated from macromolecules during cupric oxide oxidation [the "lignin" technique; (Goni and Montgomery 2000)], prompting the question of whether the monomer compositions would mimic that of macromolecular substrates. Comparison of our SPE-extracted monomer concentrations with compound distributions resulting from the analysis of "Lake Washington Standard Mud" illustrate that the monomer compositions do not mimic that routinely observed for macromolecular lignin (data not shown). This is most likely due to the fact that, in addition to being incorporated into lignin, many phenols are also produced by plants specifically for their floral properties to be used in flowers, pollens, and barks (Raguso 2008). It has also been shown that "dissolved" lignin, which results from leaching, has a general composition that differs from macromolecular components (Benner et al. 1990; Hernes et al. 2007).

Gas chromatography is a common tool used to identify scents in forest ecology, and nearly two thousand compounds have been identified that are produced by plants and insects for chemical communication and quorum sensing (Raguso 2008). Because of their pleasant odors and tastes, many of these compounds are extracted in mass quantities or produced industrially and used in consumer products such as cleaners and perfumes (Dignum et al. 2001). Analysis of samples suggests that many spice compounds are found in natural waters, prompting questions regarding their sources and the information they may hold for evaluating coastal zone processes. Thus, a reproducible and sensitive method for their detection in natural waters has potential value.

Comments and recommendations

Our method uses the derivatizing agent N,O-bis-trimethylsilyl-trifluoroacetamide (BSTFA) which replaces trimethylsilyl groups in place of exchangeable hydrogens on polar molecules in order to increase their volatility. Of the twenty eight compounds we quantify, ten do not derivatize (Table 1) and are amenable to our technique without the derivatization step (Fig. 1). Thus these compounds are amenable to 14C analysis by preparative GC (Eglinton et al. 2006).

One obvious improvement to our technique would be the incorporation of isotopically labeled standards into our protocol (Van De Steene et al. 2006). Unfortunately, isotopically-labeled materials are not commercially available for the majority of compounds of interest. The most common alternative is the use of structural analogues. However, this is a difficult solution for the study of spices because virtually all analogous compounds are potentially found in natural samples. For example, ethyl vanillin, a synthetic analog of vanillin, is typically used as an internal standard for the analysis of lignin

Table 3. Concentrations (μg L^{-1}) within natural samples for spices and scents. Blank spaces denote concentrations lower than the detection limit. Effluent from West Point sewage treatment facility, after treatment and chlorination/dechlorination. Portage Bay is an arm of Lake Washington. Pipers Creek is an urban creek in Seattle. Clayoquot Sound, British Columbia, Tofino Inlet.

	Effluent	Portage Bay	Puget Sound	Pipers Creek	Clayoquot Sound
Acetosyringone	3.07 ± 0.31				
Acetovanillone	6.54 ± 0.95				9.31 ± 0.65
Benzaldehyde			0.55 ± 0.11	1.15 ± 0.12	
Benzyl acetate	1.12 ± 0.13				
Benzyl propionate			0.05 ± 0.05		
Carvacrol			0.07 ± 0.01		
S+ Carvone	0.15 ± 0.03				
Cinnamaldehyde	1.12 ± 0.16	0.41 ± 0.1	0.013 ± 0.06	0.57 ± 0.06	0.38 ± 0.08
trans-Cinnamic acid		1.14 ± 0.1	0.55 ± 0.21	1.25 ± 0.33	0.79 ± 0.28
p-Coumaric acid	8.64 ± 0.31				0.19 ± 0.55
Cumene			0.27 ± 0.2	0.38 ± 0.03	
3,4 Dihydroxybenzoic acid					
Ethyl vanillin	13.71 ± 0.57	0.14 ± 0.11	5.1 ± 0.30	0.11 ± 0.06	
Eugenol		2.4 ± 0.17	0.45 ± 0.19		
Ferulic acid	16.9 ± 0.93				
4 Hydroxyacetophenone	0.9 ± 0.11				
4 Hydroxybenzaldehyde	6.72 ± 0.37		1.28 ± 0.22		1.35 ± 0.27
4 Hydroxybenzoic acid	5.89 ± 0.33		2.05 ± 0.59		2.17 ± 0.40
Limonene		0.19 ± 0.08	0.68 ± 0.05	0.48 ± 0.11	
Limonene oxide, Cis			0.41 ± 0.08		
Limonene oxide, trans		0.27 ± 0.03			
Linalool	0.31 ± 0.06				
Syringaldehyde	0.29 ± 0.04		0.38 ± 0.06		
Syringic acid	0.14 ± 0.11				
Thymol			0.23 ± 0.10	0.01 ± 0.01	0.04 ± 0.02
Vanillic acid	0.34 ± 0.03	0.18 ± 0.06			0.87 ± 0.06
Vanillin	8.46 ± 0.27	0.68 ± 0.11	1.12 ± 0.31	0.32 ± 0.17	0.82 ± 0.27
Veratraldehyde	7.15 ± 0.32		0.09 ± 0.06		0.03 ± 0.02

phenols (Goni and Montgomery 2000; Hedges and Mann 1979b), but we observe this compound in effluent and in natural waters. Thus, our current approach is to use standard additions for quantification.

Another area for methodological improvement is the evaluation of alternative solid phase extraction columns that may have better recoveries or decreased matrix effects. In addition to HLB columns, a variety of new substrates including Phenyl and Strata X-polymeric SCX/RP sorbents have proven useful in isolating organic compounds from natural waters (Van De Steene et al. 2006). Finally, analytical advances (e.g., GC × GC, tandem mass spectrometry) allow for enhanced sensitivity and selectivity, which could be useful when tracing these compounds at sub-nanogram levels.

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