ENVIRONMENTAL ANALYSIS

Synthesis of the *O*-(2,3,4,5,6-Pentafluorobenzyl)Hydroxylamine Oximes of Selected Carbonyl Compounds and Their Determination by Liquid Chromatography with Ultraviolet Detection

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The aims were to develop a liquid chromatographic (LC) method with ultraviolet detection (UVD) for *O*-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride (PFBHA) *O*-oximes of common aldehydes and ketones, and to define the steric limits of the synthetic reaction used to make the PFBHA *O*-oxime standards for gas chromatographic (GC) and LC methods. Ten new *O*-oximes were synthesized with the new optimized method, and their purities were demonstrated by GC/electron-capture detection (ECD), GC/mass spectrometry (MS), ultraviolet spectroscopy, infrared spectroscopy, and proton and ^{13}C-nuclear magnetic resonance spectroscopy. Ketones substituted at both β-carbons relative to the carbonyl carbon, like diisobutyl ketone and 2,4-hexanedione, showed lower synthetic yields by wet chemistry methods. A new C_{18} reverse-phase LC method with UVD at 200 nm and acetonitrile–water in both the isocratic and gradient-elution modes was then developed to sensitively resolve a mixture of 13 pure PFBHA *O*-oximes. The detection limit was near 100 ng *O*-oxime/mL or about 14–50 ng aldehyde/mL and the least quantifiable limits were near 500 ng/mL or about 70–250 ng aldehyde/mL, with lower limits for glyoxal, methylglyoxal, benzaldehyde, and acetophenone. Carbonyl compounds in 500 mL water samples were then determined in distilled water and tap water by gradient elution. Vapors of *n*-valeraldehyde and acrolein generated in gas bags at concentrations near occupational guidelines were also sampled, desorbed, and then determined by either isocratic or gradient elution at 200 or 254 nm within 30–45 min.

Liquid chromatography (LC) with ultraviolet detection (UVD) is the major analytical technique used for the determination of aldehydes and ketones. This technique determines 2,4-dinitrophenylhydrazones formed by the reaction between 2,4-dinitrophenylhydrazine (DNPH) and the carbonyl group of aldehydes and ketones and has been used for determination of aldehydes and ketones in air (1–3), water (4), urine (5, 6), breath (7), blood (8, 9), tobacco smoke (10), food (11), silage (12), and cosmetics (13). Only the formaldehyde DNPH derivative has been used for gas chromatography (GC; 14, 15) because most of the other 2,4-dinitrophenylhydrazones decompose on-column at high temperatures. DNPH does not react quantitatively with conjugated aliphatic carbonyl compounds (16), and recoveries may be variable from reactions with less-reactive carbonyl compounds and at different pH values (17). Some DNPH derivatives are light sensitive (16), and the reaction is affected by ozone (18). Therefore, other analytical methods have been tested.

The major alternative reagent is *O*-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride (PFBHA). Its applications in water and biological analyses have been reviewed (19). PFBHA has also been used in air sampling methods (20, 21). The *O*-oximes of PFBHA have been determined by GC/electron-capture detection (ECD) and GC/mass spectrometry (MS) at picogram sensitivity (19). The less-sensitive LC/UVD method for DNPH derivatives can compete only by analysis of a large original sample, with injection of a larger volume on-column at the expense of resolution, or total sample desorption/injection at the expense of precision assessment through multiple injections.

However, LC determination of carbonyl compounds of lower molecular weight might be favored for many analyses: workplace airborne aldehydes and ketones near the high hygienic air thresholds when the exposing aldehyde is known to be present (22); ozonolysis disinfection by-products in water samples (23); carbonyl compounds in aqueous biological

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samples, in foods, and in commercial aqueous solutions used as preservatives, embalming fluids, cleaners, and disinfectants; and in taste and odor research (19). LC methods are also needed to complement GC methods with the same standards when resolution, nonvolatility, or GC interference is a problem. In addition, methods using LC/UVD are less expensive than those using GC/ECD, and particularly those using GC/MS. Most laboratories in underdeveloped countries have basic GC/flame ionization detection (FID) and LC/UVD capabilities, even though they may not be able to afford GC/MS or even GC/ECD. Moreover, GC/MS operators need special expertise to run their instruments effectively.

The aims of the present study were to develop a sensitive and selective LC method for typical aldehydes and ketones of low molecular weight, to define the LC/UVD conditions of use, and to assess the generality of the reaction synthesizing the essential PFBHA *O*-oxime derivatives for both the GC and the LC methods. The most demanding analyses relative to sensitivity involve environmental samples of tap water containing ozonolysis by-products and samples of distilled water, because of the presence of aldehydes at nanogram-per-milliliter levels. The least demanding environmental samples relative to sensitivity are aldehyde vapors of hygienic concentrations in air. Selectivity requirements for matrix effects are also minimal for these samples. This work is the first reported determination of PFBHA *O*-oximes by LC, and it allows parallel quantitation for GC-based methods with the same standards using acetonitrile as the solvent.

Experimental

Chemicals

The following were from Aldrich (Milwaukee, WI): acetaldehyde (99.5%), acetone (99.5%), acetophenone (99%), acrolein (90%), benzaldehyde (99.5%), *n*-butyraldehyde (99.5%), *n*-crotonaldehyde (99%), decafluorobiphenyl (99%), *n*-decyl aldehyde or *n*-decanal (95%), formaldehyde as 37% formalin, glyoxal as a 40% aqueous solution, *n*-heptaldehyde or *n*-heptanal (95%), 2-heptanone (99%), pentafluorobenzaldehyde (98%), pentafluorobenzyl alcohol (98%), pyruvic aldehyde (methylglyoxal) as a 40% aqueous solution, and *n*-valeraldehyde (97%). Fisher Scientific (Tustin, CA) supplied optima grades of acetonitrile and hexanes, and reagent grade carbon tetrachloride. Isobutyraldehyde (99%), 2,6-dimethyl-4-heptanone or diisobutyl ketone (99%), 2-methylcyclohexanone (99%), and 2,4-hexanedione (99%) were from Kodak (Rochester, NY). Deuterated chloroform was from MSD Isotopes (St Louis, MO). PFBHA in the hydrochloride salt form was from Lancaster Laboratories (Lancaster, PA).

Acetonitrile LC solvent was filtered through a 0.45 μm Teflon filter (MSI, Westboro, MA). Distilled water was passed through a Millipore Super-Q water deionizing filter system (Millipore, Marlborough, MA), and further filtered through a 0.45 μm nylon filter (MSI). ASTM II grade Millipore Super-Q deionized water was produced after further distillation from overnight-refluxed potassium permanganate at pH 2. Helium (99.999%) from Alphagaz (Walnut Creek, CA) degassed the LC solvents, and methane–argon (5 + 95) and nitrogen of the same purity were also from Alphagaz. Tenax TA (80–100 mesh) for air sampling came from Alltech Associates (Deerfield, IL).

Equipment

A Hewlett-Packard 1090 Series L liquid chromatograph (Hewlett-Packard, Palo Alto, CA) preceded a Hewlett-Packard 1050 variable-wavelength diode-array detector with output displayed by a Hewlett-Packard 3396 controller/integrator. A Rheodyne (Rohnert Park, CA) 7012 manual injector with a 10 μL injection loop was loaded with 100 μL sample. The 250 × 4 mm stainless steel column contained BioSil ODS-5S C_{18}-reversed-phase with 5 μm film thickness (Bio-Rad, Richmond, CA).

The gas chromatograph/electron-capture detector (GC/ECD) was a Hewlett-Packard 5890 with a splitless 30 m × 0.25 mm DB-1701 (1 μm film) chemically bonded, fused silica capillary column (J&W Scientific, Folsom, CA) with a constant-current pulse-modulated ^{63}Ni-ECD, whose signal was displayed on a Hewlett-Packard 3396 controller/integrator. The temperature of the injector and detector was 250°C. The column temperature program was as follows: hold at 50°C for 1 min, 50–250°C at 5°/min, and hold at 250°C for 4 min. The total run time was 45 min, with an additional 10 min required for thermal reequilibrium to 50°C. The column flow of methane–argon (5 + 95) carrier was 2.5 ± 0.2 mL/min. The flows for the septum purge, makeup gas, and anode purge were 2.5 ± 0.2, 40 ± 3, and 4.0 ± 0.3 mL/min, respectively.

The same gas chromatograph and column were also interfaced with a Hewlett-Packard 5988A mass spectrometer instead of the electron-capture detector. The mass spectrometer was a quadrupole with electron-multiplier detector operated over the *m/z* range 50–500 for total ion current (TIC) analyses, and at *m/z* 181 for selected-ion monitoring (SIM) analyses. The temperature of the injector, transfer line, and 70 eV electron-impact ion source was 250°C. The column temperature program was the same as that used for GC/ECD. The flow of helium carrier was 3.0 ± 0.3 mL/min. The purge delay was 1 min. A 10 μL syringe was used to introduce 2 μL injections into the gas chromatograph (for GC/ECD and GC/MS).

Measurements by ^{13}C- and ^{1}H-nuclear magnetic resonance (NMR) spectroscopy were made at room temperature with a Bruker AF200/WB Fourier transform spectrometer (Bruker Instruments, Billerica, MA) at spectral widths of 25 000 and 10 000 Hz, respectively. A frequency of 17 Hz was used to decouple the ^{13}C-NMR resonances with a relaxation delay of 20 s. Solutions with concentrations of >10mM in deuterated chloroform were contained in 5 mm unfrosted quartz NMR tubes (Aldrich, Milwaukee, MI). Tetramethylsilane (TMS) at a 1% concentration was the internal reference compound. All spectra were corrected for the method blank.

Infrared (IR) spectra were obtained with a Perkin-Elmer 710B single-beam spectrophotometer (Perkin-Elmer, Norwalk, CT) by using sodium chloride 1 mm cells for liq-

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uids, with *O*-oximes dissolved in carbon tetrachloride. Scanning was from 600 to 4000 cm^{-1} at 4 cm^{-1} resolution.

Ultraviolet (UV) spectra were obtained with a Hewlett-Packard 8415A diode-array single-beam spectrophotometer at 2 mm bandwidth from 190 to 820 nm. *O*-oximes were dissolved in acetonitrile in 1 cm Suprasil cells. Beer's Law plots were prepared at wavelength maxima and at 254 nm.

General laboratory glassware was from Fisher Scientific. All glassware items, including solvent reservoirs, were scrubbed in detergent and water, rinsed, soaked in 10% nitric acid overnight, rinsed again in distilled water, and dried in a dustless oven.

Tedlar gas bags (SKC, Eighty Four, PA) were used to contain static air samples. A 10 μL syringe (Fisher Scientific) was used to inject liquid carbonyl compounds, and gas-tight syringes were used to transfer concentrated vapors. Compressed air (99.999% purity; Alphagaz) was also used. Personal sampling pumps (Model P30A) used to sample the gas-bag vapors were from DuPont (Wilmington, DE). Dry vapors were sampled by using 200 mg Tenax TA coated with 20% PFBHA in 7 cm × 5 mm id Pyrex tubes contained between two 5 mm glass wool plugs, as described elsewhere (20).

Synthesis and Purity of O-Oximes

The major carbonyl compounds selected for study were the following: those detected in surface water; ozonolysis disinfection by-products in drinking water (23); 2 common aldehydes monitored in workplace air (*n*-valeraldehyde and acrolein; 20); and selected industrial chemicals (20, 24), including the carcinogens formaldehyde, acetaldehyde, and crotonaldehyde, and the mutagens glyoxal and methylglyoxal.

The PFBHA *O*-oximes of aldehydes are not commercially available in the United States; therefore, they had to be synthesized. Hayashi Pure Chemical Industries Ltd. (Osaka, Japan) now apparently sells the PFBHA *O*-oximes of C_1–C_8 and C_{10} linear aldehydes, of C_4 and C_5 branched-chain aldehydes, and of glyoxal and methylglyoxal. The syntheses and purities of the derivatives of formaldehyde, acetaldehyde, *n*-heptanal, *n*-decanal, and glyoxal are detailed elsewhere (25), as are those for acrolein, crotonaldehyde, and *n*-valeraldehyde (20). New derivatives were synthesized for acetone, acetophenone, benzaldehyde, *n*-butanal, and methylglyoxal and studied for LC purposes. The other carbonyl compounds not studied for LC (2-heptanone, isobutyraldehyde, diisobutyl ketone, 2-methylcyclohexanone, and 2,4-hexanedione) were used to assess the influence of steric hindrance on the synthetic reaction. The syntheses of their PFBHA *O*-oximes are reported, for the first time. The mass spectra of most of these *O*-oxime derivatives are available elsewhere (24). The pure PFBHA *O*-oximes were used as LC and GC standards.

The general method of optimal synthesis of about 200 mg PFBHA *O*-oxime for both aldehydes and ketones was to use 1.15:1 molar ratios of PFBHA:monocarbonyl compound or 2.30:1 molar ratios of PFBHA:dicarbonyl compound. Pure PFBHA was dissolved in 10 mL ASTM II water in a 16 × 150 mm, Teflon-lined, screw-cap, Kimax culture tube (Fisher Scientific). If water soluble, the carbonyl compound was dissolved similarly in 2 mL ASTM II water; a methanolic aqueous solution was used for water-insoluble carbonyl compounds. For the latter, the minimum amount of methanol was added in 10 μL portions to 2 mL aqueous solution to promote water solubility on shaking. The volume of methanol added to the carbonyl compound was then added in 5 portions to the PFBHA solution. The aldehyde solution was added drop-wise to the PFBHA solution and manually agitated. After all of the aldehyde solution was added and the tube was capped, the PFBHA/aldehyde mixture was Vortex mixed. Any pressure was relieved by loosening the cap. The agitated turbid solution was then immediately heated at the lowest power setting of a commercial 850W, 2450 MHz microwave oven (Sharp Model R-3A85, Sharp Electronics Corp., Mahwah, NJ) until the first bubble appeared (ca 80–85°C water temperature after ca 10 s of heating). The tube was allowed to remain in the oven for 1 min (cap on) before it was removed to cool to room temperature. The tube was then immersed in an ice bath for 30 min. If a precipitate or a second layer appeared, the top (aqueous) layer was removed by Pasteur pipet and transferred to another screw-cap tube for further extraction (3 times, each with 1 mL hexane). The combined extracts were returned to the residual solid or liquid from the synthesis. If a precipitate or a second layer still remained after the latter mixture was vortexed, more hexane was added in 0.5 mL portions until dissolution was obtained with manual shaking. After centrifugation in a bench centrifuge for 2 min at 900 × *g*, the top (hexane) layer was removed by Pasteur pipet, and any residual liquid was washed 2 more times with 0.5 mL portions of hexane. The extracts were combined in a V-vial of appropriate volume, and the hexane was evaporated under nitrogen passed through a 20–40 mesh, 100–200 mg, large charcoal tube (SKC) until the container was no longer cool. The vial was then placed in a vacuum desiccator containing indicator Drierite desiccant until constant mass was achieved. The yields were calculated, and a known mass was taken for purity estimation through UV, Fourier-transform IR, and ^{13}C- and ^{1}H-NMR spectroscopies, and GC/MS. In GC/MS and GC/ECD runs, the purity was calculated by correcting for the content of unreacted PFBHA, pentafluorobenzyl alcohol, pentafluorobenzyl aldehyde, any other peaks that corresponded to other carbonyl compounds, and peaks that did not result from the reagents and solvents (25).

Standard curves of detector response versus mass injected for the GC methods were prepared by using 5 concentrations for each *O*-oxime in hexane or acetonitrile and 1 μL injections in triplicate (inter-run analysis); each tube contained decafluorobiphenyl internal standard at 400 ng/mL (*m/z* 334 in SIM GC/MS). One of the triplicate samples was also injected 3 times to define intra-run precision. All *O*-oximes were also combined in hexane or acetonitrile. The slopes (response factors) were determined by linear regression after the linear regions were defined. The formaldehyde *O*-oxime eluted at 4.59 min, and the last *O*-oxime to elute, the derivative of *n*-decanal, eluted at 32.85 min. One GC cycle took 55 min.

Essentially the same procedure using the pure PFBHA *O*-oximes was performed for LC standardizations with the

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acetonitrile solutions, but without the internal standard. The molar response factors and the molar relative response factors (RRFs) were also determined at the optimized isocratic and gradient-elution conditions. This step provided absolute PFBHA *O*-oxime determinations independent of reaction efficiency in the water sample and the factors that affected PFBHA *O*-oxime recoveries.

LC Method Quality Control

To prepare a stock solution of a known high concentration, a known weight of each pure *O*-oxime (ca 7–40 mg) was dissolved in acetonitrile, and the solution was diluted to 10 mL in a volumetric flask. All solutions were ultrasonicated at 50°C for 1 min. Subdilutions of a 50 μg/mL substock solution were prepared in 25 mL volumetric flasks to generate 5 concentrations ranging from 0.1 to 10 μg/mL for each *O*-oxime, and for the mixture. Intra-run (triplicate injections of the same solution) and inter-run (analysis of the triplicate samples) precisions were calculated. After use, all samples were stored at –20°C. The same concentrations were obtained on thawing to room temperature, and then ultrasonicating at 50°C for 1 min. With this procedure, standard solutions on dilution were reproducible for ≥6 months.

LC Method Development

The next objective after obtaining pure PFBHA derivatives was to select the appropriate LC column. The initial column was a 250 × 6.2 mm Supelcosil (Supelco, Bellefonte, PA) LC-1 reversed-phase column of 5 μm particle size, used originally to separate toluene, xylenes, and ethylbenzene, all nonpolar aromatic hydrocarbons. Resolution of the *O*-oximes was poor, and the long-chain oximes never eluted, even in 100% acetonitrile. The Biosil ODS-5S C_{18} reversed-phase column had been used to separate benzyl alcohol, benzaldehyde, and the ester, methyl benzoate. These more polar aromatic compounds proved to be closer in polarity to the *O*-oximes than the aromatic hydrocarbons.

After column selection, the water-miscible organic solvents acetonitrile, methanol, and tetrahydrofuran were evaluated in various aqueous solvent compositions (0, 25, 50, 75, and 100%, v/v) under isocratic conditions at different wavelengths, flow rates, and column temperatures. Gradient-elution conditions were then optimized by using the isocratic run data as a guide. The linear dynamic range at the optimum gradient elution and isocratic conditions at 200 nm was then determined for 10 μL loop injections of solutions containing 0, 0.1, 0.5, 0.75, 1.0, 2.4, 5.0, 7.5, 10, 25, and 50 μg *O*-oxime/mL acetonitrile. Some experiments were also performed at 254 nm for concentrations that were 25 times higher.

Analysis of Los Angeles Tap Water and Millipore Deionized Water

In addition to reagent blanks, 500 mL samples of Los Angeles tap water and deionized super Milli-Q water were analyzed in triplicate with the optimized gradient-elution method. Selection of the 500 mL volume was based on the lowest quantifiable level of the least-sensitive aldehyde at 200 nm.

A 10.0 mL portion of 0.1N sodium thiosulfate was added to the 500 mL tap water sample to reduce residual hypochlorite and ozone from the ozonation and chlorination disinfection of the Los Angeles tap water. A 50 mL portion of Millipore deionized water containing PFBHA at 10 mg/mL was then added. This step was the starting point for analysis of the 500 mL deionized water sample. The pH was adjusted to about 2.0 (range 0.9–2.0) by adding 10% HCl in 25 μL portions with stirring. The sample was heated in a microwave oven until the first bubbles appeared. After the sample cooled to room temperature, 5 mL 18N sulfuric acid was added with stirring to minimize excess PFBHA. Each sample was placed in a 1 L separatory funnel with a Teflon stopcock and stopper and extracted with three 10 mL portions of hexane. The hexane extracts for each sample were combined in individual 50 mL round-bottom flasks. Each hexane solution was evaporated to about 3 mL by rotary evaporation at 45°C. Each 3 mL sample was transferred to a preweighed V-vial and evaporated to constant residue weight under a gentle stream of nitrogen. After reweighing, the residue was redissolved in 0.600 mL acetonitrile, constituting an 833.3-fold increase over the original concentration. Distilled water treated in parallel served as the reagent blank to correct for background contamination. Reagent blanks were also analyzed in parallel, as were positive controls containing aldehyde mixtures at known parts-per-billion levels in 500 mL distilled water for determination of formaldehyde at 10 ng/mL, acetaldehyde at 10 ng/mL, *n*-butanal at 3.0 ng/mL, *n*-heptanal at 2.0 ng/mL, *n*-decanal at 1.0 ng/mL, which were the concentrations determined by Glaze et al. (23, 26).

A 100 μL aliquot was taken to load the 10 μL injection loop for LC under isocratic or gradient-elution conditions. The optimum isocratic conditions were acetonitrile–water (57 + 43) at 39°C and a flow rate of 0.80 mL/min. The optimum solvent linear gradient relative to acetonitrile and water at 39°C and 0.80 mL/min was as follows: 47.5% acetonitrile at 0 min; 53% at 16.9 min; 60% at 22.5 min; 65% at 28 min; 73% at 36.70 min; 75% at 38 min; 80% at 45 min; 95% at 60 min; and (to clean the column before recycling) 100% at 70 min. As soon as the last analyte peak had eluted as evinced by integrator tick marks, the solvent for isocratic and gradient-elution runs was set at 100% acetonitrile to shorten the analyses and to clean the column, evinced by a flat baseline, before recycling to the initial conditions.

The PFBHA *O*-oximes were determined by the method of external standards using the pure PFBHA *O*-oximes produced during their syntheses. Acetonitrile was always the LC injection solvent. *O*-oxime concentrations in ng/mL were converted to carbonyl compound equivalents by multiplying by the molecular weight of the aldehyde divided by the molecular weight of the PFBHA *O*-oxime. Peaks and quantitative contributions due to the hexane solvent, reagents, and deionized water were assigned and corrected for. Distilled water provided the reagent blank data that were subtracted from the tap water data.

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Analysis of Gas-Bag Samples

The static air sample was generated by first half-filling the 10 L gas bag with pure air, and injecting the calculated mass of pure valeraldehyde liquid and acrolein in air (20), with a 10 μL syringe and a gas-tight syringe, respectively, that was equivalent to the 1997 threshold limit value (TLV; 22) over 8 h sampling (176 mg/m^3 for valeraldehyde and 0.23 mg/m^3 for acrolein). After adding another 5.0 L air, the bag was heated with a hair dryer. After cooling, the vapor was sampled at 10 mL/min by using 200 mg solid sorbent Tenax TA (80–100 mesh) coated with 20% PFBHA in a glass tube as described elsewhere (20). The solid sorbent was desorbed with 2 mL hexane in a small centrifuge tube for 2 h with shaking every 1/2 h as recommended (20). This technique allowed the validated air-sampling method to be used without revalidation for another desorbing solvent. The solution was centrifuged for 1 min on a desk centrifuge, and the supernatant was transferred to a V-vial. The hexane solvent was evaporated in a gentle stream of nitrogen by using a Pasteur pipet in a fume hood. A 2 mL portion of acetonitrile was added to the valeraldehyde tubes, and 1 mL was added to the acrolein tubes. Both sets of solutions were ultrasonicated for 1 min at 50°C. A 2 μL aliquot of the valeraldehyde PFBHA *O*-oxime solution was diluted to 1 mL with acetonitrile in a graduated 1 mL test tube, and LC analysis followed. The acrolein solution was injected directly without further dilution. All experiments were done in triplicate.

Results and Discussion

Table 1 shows the yields for all the PFBHA *O*-oximes of the LC study, some physical characteristics of the parent carbonyl compounds, the PFBHA *O*-oxime retention times for the optimized isocratic and gradient-elution LC and GC/ECD methods, the molar RRFs at 200 nm relative to the *O*-oxime of benzaldehyde as 100%, the intra- and inter-run precisions at 100 ng *O*-oxime/mL for the optimized gradient-elution LC method, and the equivalent concentrations of carbonyl compounds determined in 1 sample of Los Angeles tap water with the optimized method for distilled water.

Synthetic Yields of O-Oximes

Most PFBHA *O*-oxime synthetic products showed *E*- and *Z*-isomers in TIC GC/MS analysis, but all yields included both isomers.

All synthetic yields for compounds in Table 1 exceeded 84%. The yields for the 8 *O*-oximes already reported (20, 25) agreed with previously reported results at $P \leq 0.05$ (Student *t*-test). The yields for the 5 other carbonyl compounds in Table 1 (acetone, acetophenone, benzaldehyde, *n*-butanal, and methylglyoxal) are reported here for the first time.

Table 2 shows the yields for the other carbonyl compounds used to assess the dependence of the addition reaction on steric hindrance. These yields are also reported here for the first time. The lowest yields were for compounds whose steric

Table 1. Carbonyl compounds determined by LC and yields of the PFBHA *O*-oximes ($n = 3$)[a]

Compound	MW, g/mol	D, g/mL	Y (± SD), %	R_T GC, min	R_T LC_I, min	R_T LC_{GE}, min	CV, % Ia	CV, % Ir	RRF (SD)	TWC
Acetaldehyde	44.05	0.78	98.29 (0.23)	5.77/5.89		14.76/14.47	9.0	22	44.2 (0.7)	0.20
Acetone	68.03	0.79	100.00 (0.15)	6.51	14.67	18.64	18	29	55 (1)	1.7
Acetophenone	120.32	1.02	99.70 (0.11)		39.79	32.56/27.79	22	12	96 (2)	1.6
Acrolein	56.05	0.84	85.43 (0.36)	7.03	15.31	19.73	11	29	41.9 (0.9)	4.6[b]
Benzaldehyde	106.12	1.044	97.99 (0.22)	23.20	30.79	29.54/26.82	24	28	100 (2)	<0.04
n-Butanal	72.11	0.80	99.33 (0.20)	8.97	23.02	26.20	12	9.0	44 (1)	2.3
n-Crotonaldehyde	70.12	0.86	90.55 (0.21)			24.06/22.82	29	70	41 (1)	0.09
n-Decanal	156.27	0.83	99.54 (0.23)	32.85	112.57	51.78/52.11	10		35 (3)	2.5
Formaldehyde	30.03	0.82	99.64 (0.10)	4.59	9.24	11.83	13	13	35.0 (0.5)	5.5[b]
Glyoxal	58.04	1.14	84.45 (0.43)	23.58	67.71	37.40/36.86	24	18	52 (3)	1.1
n-Heptanal	114.19	0.818	99.27 (0.10)	14.52	75.52	38.79/38.07	12	13	54 (0.9)	14
Methylglyoxal	72.06	1.045	88.51 (0.22)	33.69	68.88[c]	41.93/41.32	3.0	23	50.2 (4)	1.3
n-Valeraldehyde	86.13	0.81	99.37 (0.31)	9.64/9.75		30.66	13	28	51.5 (0.8)	0.90

[a] MW, molecular weight; D, density; Y, yield corrected for purity; SD, standard deviation; R_T, retention time; GC, gas chromatography/electron capture detection; LC_I, liquid chromatography optimized isocratic method at 200 nm; LC_{GE}, liquid chromatography optimized gradient-elution method at 200 nm (more than one R_T signifies *E*- and *Z*-isomers with the peak having the largest area given first); CV, coefficient of variation for 100 ng/mL at 200 nm in the optimized gradient-elution LC method; Ia, intra-run; Ir, inter-run; RRF, relative response factor at 200 nm on a molar basis relative to benzaldehyde as 100% in the optimized gradient-elution LC method; and TWC, Los Angeles tap water concentration in ng carbonyl compound/mL tap water.

[b] Maximal because of interfering peaks.

[c] The monoderivative had a retention time of 14.67 min.

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Table 2. Steric dependence of selected carbonyl compounds relative to synthetic yield

Carbonyl compound	Yield corrected for purity/purity, %[a]
Diisobutyl ketone	0.2(0.2)/5(5)
2-Heptanone	85(4)/99.0(0.5)
2,4-Hexanedione	48(3)/68(2)
Isobutyraldehyde	75.4(0.6)/99.0(0.1)
2-Methylcyclohexanone	89.7(0.5)/98(1)

[a] $n = 3$. The quantities in parentheses are standard deviations.

hindrance was a factor to formation or further reaction of the tetrahedral addition intermediate. Yields decreased for ketones substituted with alkyl branches at both β-carbons relative to the carbonyl carbon. Thus, diisobutyl ketone had a final yield corrected for purity of <0.2%; 2,4-hexanedione had a corresponding yield of 48%. GC/MS TIC analyses revealed the presence of non-*O*-oxime impurities as the major problem for these 2 ketones. Yields for the straight-chain and the cyclohexyl ketones exceeded 80%. Thus, all aldehydes so far synthesized and straight-chain ketones that are monocarbonyls or diketones and not sterically hindered produced yields of ≥75%. GC/MS and GC/ECD showed the presence of the *E*- and *Z*-isomers of the *O*-oximes of asymmetric carbonyl compounds (24), although the optimized gradient-elution LC method resolved more isomers (Table 1 and Figure 1).

In the original synthesis method, the reaction was quenched immediately by using an ice bath for 30 min after the microwave step (20, 25). This method is still effective for straight-chain aliphatic aldehydes and glyoxal, but yields are much lower for methylglyoxal and ketones. The extra standing time of 1 min for the hot solution and the slow equilibration to room temperature allow the reaction to go to completion for ketones. Incomplete reaction for methylglyoxal was indicated by the presence of its monosubstituted *O*-oxime.

Spectroscopic Characterization

Mass spectra.—All *O*-oximes had *m/z* 181 as the base peak in their mass spectra; this peak facilitates sensitive and selective SIM for GC/MS (24). Observed linear ranges were generally 30–200 ng for TIC analyses and an order of magnitude lower for SIM analyses. The usual intra-run precision was <10% in the linear range. The usual inter-run precision was <17%.

UV spectroscopy.—Molar absorptivities (ε) at the long wavelength maximum of 264 nm varied between 491 and 667 L/cm·mol for PFBHA *O*-oximes of aliphatic monocarbonyl aldehydes with a midpoint of 579 L/cm·mol. In contrast, benzaldehyde PFBHA *O*-oxime had an ε of 17 260 ± 270 L/cm·mol. Methylglyoxal PFBHA *O*-oxime, a disubstituted derivative, had an ε of 8410 ± 100 L/cm·mol. The ε of the only other synthesized disubstituted PFBHA *O*-oxime, that for glyoxal, was 13 200 L/cm·mol at this wavelength (25). Thus, the benzaldehyde, methylglyoxal, and glyoxal PFBHA *O*-oximes can be detected, on average, with sensitivities 31, 15, and 24 times, respectively, those of aliphatic monocarbonyl PFBHA *O*-oximes at this wavelength.

At the short wavelength maximum of 200 nm, the ε varied from 10 200 to 15 000 L/cm·mol for the aliphatic monocarbonyl derivatives. The midpoint ε for aliphatic monocarbonyl PFBHA *O*-oximes was thus 12 600 L/cm·mol. The corresponding ε for benzaldehyde was 26 210 ± 500 L/cm·mol, and for methylglyoxal was 10 650 ± 580 L/cm·mol. The literature value for glyoxal was reported to be 13 600 ± 540 L/cm·mol (25). Thus, the benzaldehyde, methylglyoxal, and glyoxal PFBHA *O*-oximes can be detected on average with sensitivities 2.1, 0.85, and 1.1 times, respectively, those of aliphatic monocarbonyl PFBHA *O*-oximes at this wavelength. The ε values are far more uniform than at 264 nm at least for aliphatic mono- or dicarbonyl compounds, and are also high. For a 1 cm pathlength, an absorbance of 0.0010 is equivalent to 80nM or about 18 μg/L or 18 ppb formaldehyde PFBHA *O*-oxime.

At the second short wavelength maximum of 222 nm, PFBHA *O*-oximes of aliphatic monocarbonyl derivatives had ε values ranging from 2970 to 4480 L/cm·mol with a midpoint of 3730 L/cm·mol. Those for benzaldehyde and methylglyoxal were 8140 ± 200 and 13 300 ± 400 L/cm·mol, respectively. The corresponding ε for glyoxal was 10 900 ± 780 L/cm·mol (25). Thus, the benzaldehyde, methylglyoxal, and glyoxal PFBHA *O*-oximes can be detected on average with sensitivities 2.2, 3.6, and 2.9 times, respectively, those of aliphatic monocarbonyl PFBHA *O*-oximes at this wavelength. The aliphatic mono- and di-substituted PFBHA *O*-oximes can be distinguished from each other, unlike at 200 nm.

In general for the aliphatic monosubstituted PFBHA *O*-oximes at their 3 maxima, the UV sensitivity ratio of the ε midpoints for 200, 222, and 264 nm was 22:6.4:1.0, and should be diagnostic for other aliphatic monosubstituted PFBHA *O*-oximes also. In contrast, this ratio for the benzaldehyde derivative was 1.5:0.47:1.0, that for methylglyoxal was 1.3:1.6:1.0, and that for glyoxal was 1.0:0.83:1.0. These ratios can be used to confirm the suspected presence of these 3 derivatives during LC runs. The sensitivity at 200 nm relative to that at 254 nm, the most common fixed wavelength used in LC, was 30–40 times greater for aliphatic monosubstituted PFBHA *O*-oximes. Thus, 200 nm was the analytical wavelength of choice because it was the most sensitive, and because variation in ε and, thus, in the LC/UVD response factors was expected to be small.

IR spectroscopy.—The IR spectra were consistent with the presence of pure PFBHA *O*-oximes.

The IR spectra revealed strong C–O stretching at 1125–1141 cm^{-1}, moderate C–H stretches at 2825–3225 cm^{-1}, moderate C–C aromatic stretches at 1652–1675 cm^{-1}, and weak N–O stretches at 920–995 cm^{-1}. These observations agree with prior results (25). Carbon tetrachloride C–Cl absorption obscured the C–C stretch at 1500–1510 cm^{-1}, and the

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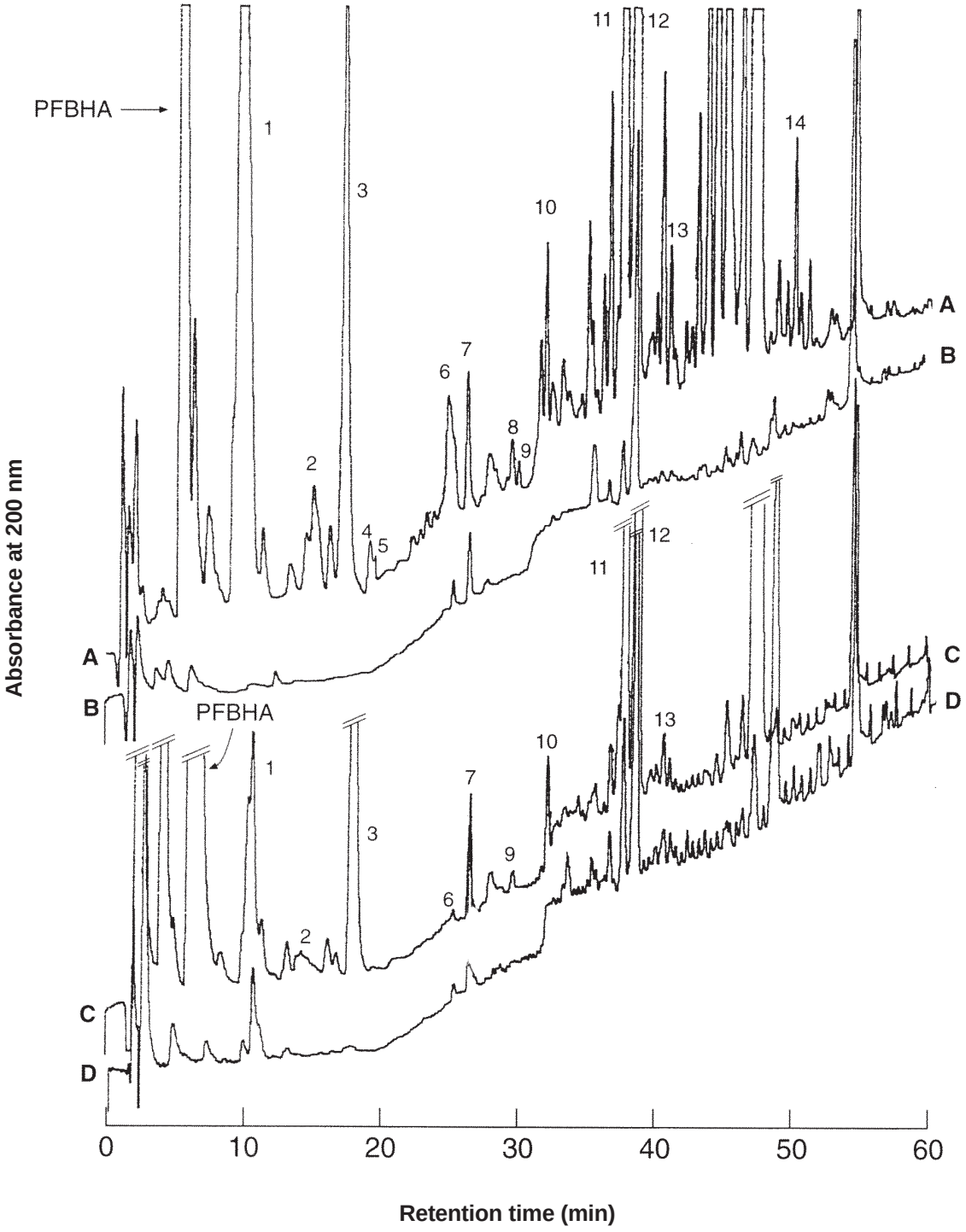

Figure 1. Chromatograms obtained by gradient-elution LC/UVD at the same linear solvent gradient and temperature conditions at 200 nm (acetonitrile–water at 39°C and 0.80 mL/min: 47.5% acetonitrile at 0 min; 53% at 16.9 min; 60% at 22.5 min; 65% at 28 min; 73% at 36.70 min; 75% at 38 min; 80% at 45 min; 95% at 60 min; and 100% at 70 min) for 500 mL water (10 mL 0.1N sodium thiosulfate added) with and without PFBHA at pH 2.0, followed by addition of 5 mL 18N sulfuric acid, extraction with hexane (3 × 10 mL), evaporation of the solvent, and solid residue reconstitution in 0.60 mL acetonitrile before a 10 μL injection loop was primed with 100 μL concentrate. A and B are tap water with and without PFBHA, respectively. C and D are distilled water with and without PFBHA, respectively. The identities of the PFBHA *O*-oxime peaks are provided for the original carbonyl compounds as follows: 1, formaldehyde; 2, acetaldehyde; 3, acetone; 4, acrolein; 5, *n*-crotonaldehyde; 6, *n*-butyraldehyde; 7, benzaldehyde; 8, *n*-valeraldehyde; 9, acetophenone; 10, glyoxal; 11, *n*-heptanal; 12, methylglyoxal; and 13, *n*-decanal.

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weak C=N stretch at 1400–1700 cm^{-1}. Analysis by GC/Fourier transform infrared spectroscopy (FTIR) is therefore recommended at 1125–1141 cm^{-1} in the fingerprint region.

NMR spectroscopy.—Both types of NMR spectra were consistent with the presence of pure PFBHA *O*-oximes.

The ^{1}H-NMR spectra showed the following respective *E*- and *Z*-isomer splittings downfield from TMS (in ppm): oxime protons, 6.47–6.78/7.07–7.82 with respective coupling constant J values of 5.50–7.49/5.99–7.43 Hz; ether protons, 5.14–5.33/5.09–5.27; and the protons on the α-carbon atom relative to the oxime carbon atom, 2.27–2.41/1.82–2.15 with respective J values of 5.15–5.68/6.57–7.28 Hz, except for 7.82 ppm for the benzaldehyde and acetophenone derivatives. The resonances of the protons in the methylene groups of long alkyl chains were superimposed between 1.06 and 1.44 ppm with multiplets. Terminal methyl groups resonated at 0.87–0.88 ppm (triplet). Benzaldehyde and acetophenone aromatic protons on carbons 2 and 6 resonated at 7.29 ppm, on carbons 3 and 5 at 7.41 ppm, and on carbon 5 at 7.57 ppm.

The ^{13}C-NMR spectra showed resonances between 141.31 and 160.00 ppm for oxime group carbons, and between 60.00 and 65.11 ppm for ether carbons. The aromatic carbons of the fluorinated ring had the following resonances (in ppm): 138.71–145.98 for *ortho*, 142.91–151.89 for *meta*, and 146.98–154.49 for *para* positions. The *O*-oxime of *n*-butanal showed the following respective *E* and *Z* resonances downfield from TMS (in ppm): the oxime carbon, 152.4/153.5; the α-carbon relative to the oxime carbon, 27.52/31.4; and the β-carbon relative to the oxime carbon, 19.41/19.8. Isobutyraldehyde in contrast showed no *E*- and *Z*-isomers. The *E* and *Z* resonances for the acetaldehyde, *n*-heptanal, *n*-decanal, and glyoxal derivatives are provided elsewhere (25). The signals for both aromatic carbonyl and PFBHA aromatic rings (25) were superimposed for *ortho*, *meta*, and *para* carbons. The ring carbon of side-chain attachment had resonances at 113.50–114.49 ppm for the fluorinated ring, and 127.2 ppm for the aromatic carbonyl ring. The other aromatic carbonyl ring resonances (in ppm) were *ortho*, 128.76; *meta*, 131.03; and *para*, 130.3. The methyl group on methylglyoxal had an upfield signal of 9.64 ppm because of shielding of the 2 oxime carbons, compared with 13.66–17.07 ppm for unshielded terminal methyl groups of straight-chain compounds, and 19.78 ppm for the 2 equivalent methyl groups of isobutyraldehyde. The β-carbon relative to the oxime carbon in the long chain for isobutyraldehyde resonated at 29.35 ppm. Superposition occurred for unshielded methylene carbon atoms of long-chain aldehydes between 29.00 and 32.27 ppm. The NMR data were consistent with previously published data for other pure aldehyde PFBHA *O*-oximes (25).

LC Method Development

Isocratic conditions.—At a 1 mL/min flow rate at 39°C, pure methanol did not elute all the long-chain *O*-oximes listed in Table 1, which were injected as a 10 μg/mL mixture, within 1 h, whereas pure acetonitrile and tetrahydrofuran eluted all. All compounds eluted in 36 min in pure acetonitrile, with good peak shape and sensitivity, but poor resolution. Tetrahydrofuran did not provide sufficient resolution for the short-chain *O*-oximes when the water content of the eluent was increased. At 39°C, water produced good resolution but poor sensitivity and very broad peaks, and runs took hours. Partial resolution was obtained with acetonitrile–water mixtures. Acetonitrile concentrations of 25 and 50% still required very long runs of >120 min at 39°C. Because partial resolution was achieved with 75% acetonitrile, the optimum acetonitrile composition was between 50 and 75% at 39°C and 1.0 mL/min.

Complete resolution under isocratic conditions took about 115 min and was achieved with 57% acetonitrile at 39°C and a flow rate of 0.80 mL/min (Table 1). At 1.5 mL/min, acrolein, *n*-crotonaldehyde, and *n*-valeraldehyde were not resolved. At 0.4 mL/min, chromatographic runs lasted 160 min, and sensitivity decreased because of peak broadening, especially for the late-eluting compounds. The detection limits for injection loop volumes of 5, 10, and 200 μL were concentrations of 1000, 100, and 10 ng/mL of injected *O*-oximes in acetonitrile at optimal isocratic conditions. The 200 μL injection showed very poor resolution, and the 5 μL injection was not sensitive enough. The 10 μL injection-loop volume provided the best resolution and sensitivity, and, thus, was selected for all subsequent work.

The logarithm of the retention time, t_R, corrected for dead volume time, t_M, denoted as t_R' and plotted versus *O*-oxime molecular weight, *MW*, was linear for all monocarbonyl data including the monosubstituted methylglyoxal at the optimized isocratic conditions.

$$\log t_R' = 0.0091\ MW - 1.153$$
$$r = 0.9503 \text{ for } n = 9,\ P \le 0.05 \qquad (1)$$

A similar regression emerged for the same compounds when the capacity factor k' replaced t_R' (27):

$$\log k' = 0.0093\ MW - 1.021$$
$$r = 0.9523 \text{ for } n = 9,\ P \le 0.05 \qquad (2)$$

where

$$k' = (t_R/t_M - 1) = t_R'/t_M. \qquad (3)$$

The fully PFBHA-substituted dicarbonyl compounds did not obey regression equation 1 or 2.

Equations 1 and 2 allow t_R', t_R, and k' to be determined for any straight-chain aldehyde or ketone under the optimum isocratic conditions for PFBHA *O*-oxime congeners between and including carbonyl compounds with 1–10 carbon atoms and 1 carbonyl group. The model does not distinguish either skeletal or *E*- and *Z*-isomers.

The plate count *N* is dependent on t_R and the peak half-width $W_{0.5}$ through equation 4 (27):

$$N = 5.54\ (t_R/W_{0.5})^2 \qquad (4)$$

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Thus, equations 3 and 4 lead to equation 5:

$$\log t_R = 0.5 \log N - 0.3718 + (\log W_{0.5})/2.303 = \log [t_M(k' + 1)] \quad (5)$$

For the acetophenone derivative under isocratic conditions, t_R = 39.785 min (Table 1) and $W_{0.5}$ = 0.746 min, so that N is about 15 800. Similarly, the respective N values for the formaldehyde and *n*-decanal derivatives are 12 500 and 17 700. N is not constant because $W_{0.5}$ increases nonlinearly as t_R increases.

Gradient elution.—A complete gradient-elution LC cycle to resolve all 13 PFBHA *O*-oximes including column cleaning and recycling to the original conditions took 90 min. A complete GC cycle, in contrast, took 55 min.

Gradient elution provided better peak shapes and resolution, as well as shorter run times, than did isocratic conditions. The last *O*-oxime, i.e., for *n*-decanal, eluted between 51 and 53 min (Table 1, Figure 1), compared with 112 min at optimized isocratic conditions. Other components not related to PFBHA *O*-oximes had to be eluted to ensure that the column was clean, but they prolonged run times. Acetonitrile gave peaks at 1.7 min (large), 2.4 min (moderate), and 4.7 min (small). Other background peaks occurred at 55.5 min (large), as shown in Figure 1, 66.2 min (large), 72.0 min (moderate), and 77.5 min (small) for the acetonitrile mixture of *O*-oximes. That better resolution was attained under gradient-elution conditions was also demonstrated by the presence of more resolved *E*- and *Z*-isomers of the *O*-oximes than were found at isocratic conditions. Both peaks or 1 peak can be used for quantitation, depending on whether there are any interferences. One-peak analysis is less sensitive than 2-peak analysis. Nonpolar substituents absorbing at 200 nm and present in distilled water, tap water, hydrochloric acid, and hexane are coextracted with the PFBHA oximes after PFBHA is added and upon hexane extraction and concentration. Any interfering peak areas from these blanks were subtracted before PFBHA *O*-oxime concentrations were calculated. Such interference was minimal for tap water, but more important for distilled water (Figure 1). The corrected distilled water chromatogram is effectively the PFBHA reagent blank for the corrected chromatogram for tap water. Thus, further subtraction yielded the results for tap water in Table 1.

In gradient elution, the $W_{0.5}$ in equation 5 was kept as constant as possible by changing the Snyder solvent polarity index (27). Log t_R then varies almost linearly with log N.

Stepwise dilutions showed that at 200 nm with the gradient-elution method, the concentration of PFBHA *O*-oxime at twice the noise level was about 100 ng/mL with intra-run coefficients of variation (CV) between 3.0 and 29% (Table 1). The inter-run CV was between 9.0 and 70% (Table 1). Above this concentration, intra-run CVs decreased to 2–15% at 0.5–1.0 μg/mL, and 0.1–7.0% at 2.5–10 μg/mL. Thus, although detection of the PFBHA *O*-oxime at 100 ng/mL is possible (or a minimum of about 14 ng/mL of formaldehyde equivalent), quantitation with acceptable precision occurs at >500 ng/mL (about 70 ng/mL of formaldehyde equivalent), and is the general least quantifiable limit (LQL). The linear dynamic range was from 0.100 to 25 μg/mL (about 0.014–4 μg/mL of formaldehyde equivalent), corresponding to an absorbance range of 0.001–0.010.

The LC relative response factors (RRFs) relative to the benzaldehyde *O*-oxime reference of 100% at 200 nm in Table 1 for the optimized gradient-elution method show a clear distinction between PFBHA *O*-oximes of the aromatic carbonyl compounds (96 and 100% for acetophenone and benzaldehyde, respectively) and those of other carbonyl compounds (35% for formaldehyde to 55% for acetone). RRFs increased from formaldehyde through *n*-heptanal but then decreased to *n*-decanal for the long-chain aliphatic aldehydes. Table 1 shows that the average RRF was 54 ± 21% with a CV of 39% for all compounds, with the 11 aliphatic *O*-oximes as a group having an average RRF of 46 ± 7% with a CV of 15%. The latter is consistent with uniform ε values at 200 nm for the aliphatic mono- and disubstituted carbonyl compounds.

Tap Water and Deionized Water Analyses

The only solid residue detected after evaporation of the hexane extract and before resolubilization in acetonitrile was 1.8 mg for tap water. Excess PFBHA eluted at 5.9–6.2 min, and was the highest peak (Figure 1). PFBHA was well-separated from the closest PFBHA *O*-oxime peak for formaldehyde. This separation is an advantage over the GC method, in which PFBHA elutes between the PFBHA *O*-oximes of formaldehyde and acetaldehyde, and the addition of sulfuric acid before hexane extraction is required to minimize potential PFBHA interference with these 2 PFBHA *O*-oximes. Elution of all compounds required 110 min. A small peak at t_R = 6.9 min was contributed by hexane. Peaks at 3.6 min (small), 10.8 min (small), 39.4 min (moderate), 44.1 min (small), 48.0 min (small), and 49.7 min (moderate) were noncarbonyl components contributed by the other reagents except sodium thiosulfate. Small noncarbonyl peaks from sodium thiosulfate were at t_R of 25.8, 36.3, and 38.5 min. The derivatization reaction preferentially selected out carbonyl compounds, and any interferences coextracted by hexane would have to be nonpolar and of large molecular weight (>200) to interfere with the resolution of the PFBHA *O*-oximes.

As expected, the concentrations of carbonyl compounds in the distilled deionized water corrected for the presence of reagents were low. The concentrations of carbonyl compounds (in ng/mL) were acetone, 2.5; acetophenone, 1.8; benzaldehyde, 6.8; crotonaldehyde, 0.024; formaldehyde, 0.13; *n*-valeraldehyde, 9.7; and *n*-heptanal, 11. The concentrations of the other aldehydes were below their LQLs.

The concentrations of carbonyl compounds in tap water were generally higher than in distilled water (Table 1). The concentration of crotonaldehyde is probably the most uncertain. The CVs are in the range of 10–17% for the inter-run replicates. Of the 36 ng aldehydes/mL detected, 39% was *n*-heptanal, 15% was formaldehyde, 13% was acrolein, 6.9% was *n*-decanal, and 6.4% was *n*-butanal, comprising 80% of the mass of aldehydes determined. These aldehydes are the classical ozonolysis by-products from bacterial precursors (23, 26).

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Glaze et al. (23, 26) found 3.8–25 ng/mL of formaldehyde, 9.5 ng/mL of acetaldehyde, 3.1 ng/mL of *n*-butanal, 2.0–2.3 ng/mL of *n*-heptanal, and 1.1 ng/mL of *n*-decanal in some finished waters. The recoveries with the PFBHA methods for carbonyl compounds in water exceeded 80% (23, 24, 26), and these results were also confirmed in the present study by the >80% recoveries of the components of a defined aldehyde mixture based on the data of Glaze et al.

The 19th Ed. of *Standard Methods for the Examination of Water and Wastewater* contained Method 6252 that used PFBHA in a liquid–liquid extraction mode. The latest edition published in 1998, the 20th, contained a slightly revised method (28). In the latter, ammonium chloride or ammonium sulfate was recommended as a reducing agent to ensure that hypochlorite ion did not interfere with the PFBHA derivatization reaction. The addition of sodium thiosulfate or potassium iodide was recommended to reduce excess ozone. EPA Method 556, published in 1998 (29), used the same method except that it warned that sodium thiosulfate could partially reduce unspecified organic material from microorganisms to aldehydes. Both of these standard methods differ from the technique used in the present work by using a potassium phthalate buffer solution for the PFBHA derivatization instead of a pH 2.0 PFBHA solution. Method 6252 used a pH 6.0 buffer, whereas the EPA method used a pH 4.0 buffer. No comparison of the 2 methods has been published.

Determination of Desorbed PFBHA O-Oximes of n-Valeraldehyde and Acrolein Vapors

The analysis for desorbed acrolein and *n*-valeraldehyde could be done either isocratically or by gradient elution; however, isocratic analysis was much quicker for acrolein, and gradient elution was faster for *n*-valeraldehyde (Table 1), with acetonitrile content being set at 100% as soon as the analyte eluted. The cycle time for analysis for acrolein was 30 min and that for *n*-valeraldehyde was 45 min. Both analyte peaks were well separated from the huge PFBHA peak.

A wavelength of 200 nm was satisfactory even for the acrolein PFBHA *O*-oxime, for which sensitivity requirements were the highest because acrolein has the lowest TLV–TWA of any aldehyde (22). For *n*-valeraldehyde, if a wavelength of 254 nm was used at gradient-elution conditions, only a 2-fold dilution was necessary for quantitation. The LC method has flexibility because the volume of acetonitrile added after the hexane evaporation and any subsequent dilution ratio can be varied, depending on the hygienic guidance value; in addition 200, 222, 254, and 264 nm can be used as analytical wavelengths, as discussed in the UV spectroscopy section. Thus, LC can provide fast, accurate, and selective determination of aldehyde vapors near their hygienic guidance levels, and there is no reason why the technique will not be effective for sterically unhindered ketones also, especially because their TLV–TWA values are larger than those of aldehydes having the same number of carbon atoms (22).

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