Comparison of Highly-Fluorinated Chloroformates as Direct Aqueous Sample Derivatizing Agents for Hydrophilic Analytes and Drinking-Water Disinfection By-Products

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Four highly-fluorinated alkyl and aryl chloroformates, including 2,2,3,3,4,4,5,5-octafluoro-1-pentyl chloroformate (OFPCF), 2,3,4,5,6-pentafluorobenzyl chloroformate (PFBCF), 3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluoro-1-octyl chloroformate (TDFOCF), and 2-(2,3,4,5,6-pentafluorophenoxy)-ethyl chloroformate (PFPECF), were synthesized and tested as reagents for the direct water derivatization of polar and hydrophilic analytes. The goal of this research was to develop an optimal derivatizing agent to aid in the identification of highly polar ozonation drinking water disinfection by-products (DBPs) that are believed to be missed with current analytical procedures. The chemical properties (reactivity, selectivity, derivatization products, and their chromatographic and spectral features) for the four chloroformates were investigated using a set of highly polar standard analytes, including malic and tartaric acids, hydroxylamine, valine, 2-aminoethanol, resorcinol, 1,3,5-trihydroxybenzene, and 2,4-dihydroxybenzoic acid. Upon derivatization, the analytes were extracted from the aqueous solvent and analyzed by gas chromatography (GC)-mass spectrometry (MS) in the electron capture negative ionization (ECNI) mode. Positive chemical ionization (PCI)-MS was used for confirmation of molecular ions that were weak or absent in ECNI mass spectra. Of the four derivatizing reagents tested, OFPCF showed the best performance, with good reaction efficiency, good chromatographic and spectroscopic properties, low detection limits (10–100 fmol), and a linear response more than two orders of magnitude. Further, the entire procedure from raw aqueous sample to ready-to-inject hexane solutions of the derivatives requires less than 10 min. PFBCF showed ideal applicability for derivatizing aminoalcohols and aminoacids. The two chloroformates with the highest intrinsic stability (TDFOCF and PFPECF) failed to derivatize some of the analytes. Finally, the OFPCF derivatizing agent was tested with simulated ozonated drinking water (aqueous fulvic acid treated with ozone), and three highly polar reaction by-products were determined.   (J Am Soc Mass Spectrom 2005, 16, 803–813) © 2005 American Society for Mass Spectrometry

Both environmental and clinical analyses commonly use mass spectrometry (MS) to detect organic compounds at trace level in aqueous matrices. However, the identification and detection of the smallest and most hydrophilic organic analytes presents a difficult task, as these substances cannot be extracted into an organic solvent nor concentrated [1]. Moreover, their direct detection in the original matrix by liquid chromatography (LC)-MS can be difficult, because of the high background chemical noise in the low m/z region of the spectrum.

Small, highly hydrophilic organic disinfection by-products (DBPs) are produced in drinking water treatment by the reaction of disinfectants with the natural organic matter (e.g., humic and fulvic acids) [2–4] and anthropogenic substances released from wastewaters. To date, more than 500 DBPs have been identified [2, 5–8], yet approximately 63% of the assimilable organic carbon (AOC) produced in ozonated drinking water is not accounted for [1]. Particularly for ozone, it is believed that many of DBPs are highly hydrophilic and

are not being detected with current analytical techniques. DBPs of this kind are likely to be polyacids, hydroxyacids, ketoacids, glyoxals, hydroxylamines, aminoacids, aminoalcohols, and glycols [9].

Direct sample derivatization in water reverses the more typical sequence of derivatization after extraction, by converting the analyte's polar groups to more hydrophobic ones, enabling their extraction into organic solvents. However, most derivatizing agents undergo immediate hydrolysis as soon as they are introduced into water. Alkyl chloroformates are among the few derivatizing agents that tolerate water [10], and *n*-hexyl chloroformate has been successfully demonstrated in previous reports [11–15].

Despite the high derivatization efficiency of *n*-hexyl chloroformate with carboxylic, hydroxylic, and aminic compounds, polysubstituted products obtained from tri- and tetra-functional analytes (e.g., tartaric acid, dihydroxybenzoic acid) are not volatile and cannot be easily eluted from a GC column. To overcome this drawback and improve sensitivity, we set out to synthesize highly-fluorinated alkyl and aryl chloroformates [16, 17] for the derivatization and detection (by ECNI-MS) of a large variety of highly polar compounds.

Trifluoroethyl chloroformate [18] and pentafluorobenzyl chloroformate (PFBCF) [19, 20] were previously synthesized and used by other groups for the direct derivatization of aminoacids in human plasma and whole blood, followed by ECNI-MS detection [21]. We recently developed a rapid, safe, and efficient procedure to synthesize highly-fluorinated chloroformates on a small scale needed for analytical applications [17]. In the present study, we compared the analytical performance of four highly-fluorinated chloroformate derivatizing agents: (1) PFBCF; (2) 2,2,3,3,4,4,5,5-octafluoropentyl chloroformate (OFPCF); (3) 3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl chloroformate (TDFOCF); and (4) 2-(2,3,4,5,6-pentafluorophenoxy)ethyl chloroformate (PFBECF). The structures of these reagents are reported in Scheme **1**. OFPCF and PFBCF had been successfully used in the past [16, 19, 20], while TDFOCF and PFPECF were selected in the present study since the longer hydrocarbon spacer located between their reactive chloroformate group and the fluorinated moiety

(Scheme **1**) was expected to increase their intrinsic stability. Finally, the applicability of one of these reagents (OFPCF) was demonstrated by identifying ozone DBPs in simulated drinking water.

Experimental

Derivatization Procedure

Highly-fluorinated chloroformates were synthesized from the corresponding alcohols (all commercially available from Aldrich, Milan, Italy) using either acetone or *n*-pentane as the reaction solvent [17]. Acetone was preferred for most analytical applications. All details on the specific syntheses (reaction yields, purities, etc.) are provided elsewhere [17]. The resulting chloroformate solutions proved stable for at least two months, if properly sealed.

Aqueous samples (2 mL for all the experiments of the present work) were basified with 100 μL of 3 M NaOH. Then, 150 μL of chloroformate solution was added (2 μmol of chloroformate in acetone), while keeping the reaction tube under ultrasonic mixing at optimized power and distance from the emitting tip. Immediately, 3 μL of a saturated (400 g/L) dicyclohexylcarbodiimide (DCC) solution in pyridine was added. The reaction was allowed to proceed for 5–10 min under sonication. The reaction products were extracted in *n*-hexane (600 μL) over 1 min. The organic layer was separated and analyzed by GC-MS. All the derivatization products proved stable for at least several hours. WARNING: Ears should be protected with appropriate earplugs during sonication. The reported experimental conditions result from extensive optimization of the following parameters: amount of derivatizing agent, amount of catalyst, sonication time, and amount of base added.

Pyridine is used as a catalyst, while DCC is a coupling agent that increases derivatization yields and repeatability. Crude chloroformate solutions in acetone, used in the derivatization, still contain small amounts of HCl and phosgene that are converted into inert NaCl and NaCO$_3$, upon NaOH addition.

With respect to the derivatization procedure previ-

Scheme 1

ously published [16], the reaction media contains about 7% of organic solvent, either pentane or acetone. While the latter has virtually no impact on the derivatization efficiency, the presence of *n*-pentane proved detrimental for the most polar analytes (i.e., hydroxylamine, tartaric acid, 2-aminoethanol), in terms of detection limits (up to 15 times lower) and reproducibility (the standard deviation of repeated derivatizations could exceed 40%). These effects are attributed to the presence of two phases, preventing an effective interaction between analytes and the derivatizing agent.

Instrumentation and Analysis

A Branson Sonifier II W-450 (Danbury, CT) sonicator, with variable emission power, was used to enable the derivatizations with ultrasonic mixing. To assure optimal and reproducible mixing, a reaction bath was built from high-vacuum spare parts. However, similar results could be obtained using an ordinary water bath, but mechanical stirring should be avoided. The derivatizations were performed in a glass test tube maintained in a fixed vertical position by a rubber o-ring and a guide in the stainless steel reversed-T structure of the bath (Figure 1). The sonifier tip was screwed horizontally into the water bath from an opening on the lateral closure disk so as to produce maximum emission power over a 1 to 2 cm^2 area in the center of the bath. The ultrasonic emission contacted the reaction vessel perpendicularly from a fixed 1 cm distance at which the radiation was homogeneously distributed in the reaction volume. The emission power was optimized by a series of derivatizations in which the reaction yields were evaluated as a function of the sonication power. The mixing efficiency proved to increase with the emission power up to 160 W and then leveled off. This corresponds to an effective power of about 90 W released to the medium.

A benchtop Perkin Elmer TurboMass (Norwalk, CT) spectrometer equipped with an AutoSystem XL gas chromatograph was utilized for most analyses. The quadrupole analyzer had a *m/z* range of 1200. A chemical ionization source was used to acquire both PCI and ECNI mass spectra. Isobutane was employed as the reagent gas for both positive and negative ion experiments, at a pressure of 50 Pa. The ion source was maintained at the lowest temperature (150 °C), compatible with prevention of analyte condensation.
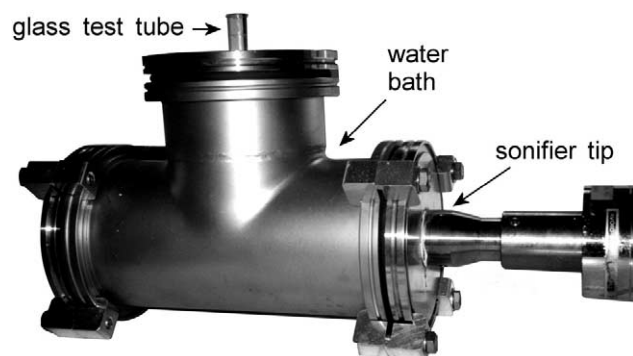
A DB-5MS (5% diphenyl dimethyl siloxane) capillary column (30 m long, 0.25 mm i.d., 0.25 μm film thickness, Agilent, Folsom, CA) was utilized. The samples were injected by an AutoSystem XL autosampler in the splitless mode at a temperature of 270 °C. The carrier gas (helium) was maintained constant at 1 mL/min. The oven temperature was programmed as follows: isothermal at 50 °C for 3 min, from 50 to 300 °C at 15 °C/min, isothermal at 300 °C for 10 min. The transfer line was maintained at 250 °C.

For occasional confirmation and mass assignment in the range above the quadrupole limit, a Finnigan-MAT 95Q (Bremen, Germany) magnetic sector mass spectrometer (*m/z* limit of 3500) was utilized under similar conditions, as described in detail elsewhere [16]. Scan ranges varied depending on the derivatives to be determined; typical ranges were *m/z* 250–920 or 300–1300, scanned at a rate of 1 s/decade.

Ozonation of Fulvic Acids

Laboratory-scale batch ozonations were carried out at pH 7 by the addition of ozone (from a 30 mg/L freshly prepared, stock solution of ozone in water, at a dose of 2:1 ozone to dissolved organic carbon) to solutions of Suwannee River fulvic acid (SRFA, International Humic Substances Society, St. Paul, MN) in purified water (5 mg/L) containing 1 mM sodium bicarbonate buffer. SRFA was used as a natural organic matter constituent because it is a well-characterized organic matter, and fulvic acid has been previously shown to be a major precursor to the formation of DBPs [2]. Ozone stock solutions were prepared by sparging ozone from an Orec ozone generator (Osmonics, Phoenix, AZ) into purified water maintained close to 0 °C. Ozone concentrations were determined by measuring the UV absorbance (at 254 nm) of the stock solution and dividing by the molar absorptivity of ozone (2950 $M^{-1}cm^{-1}$) and the length of the light path through the sample [22]. A purified water blank (containing fulvic acid and buffer, but not ozonated) was also derivatized and analyzed as a control. Samples were collected after 3 h of reaction with ozone.

Results and Discussion

Reaction with chloroformates converts carboxylic acids into the corresponding esters, whereas alcohols and phenols form carbonates, and amines form carbamates (Scheme **2**) [10, 14]. For primary amines (R_2 = H in Scheme **2**), one or both aminic hydrogens undergo substitution, depending on the type of chloroformate used and the analyte reactivity [14, 16]. Reactivity differences among the four chloroformates were tested

Figure 1. Derivatization reaction bath, operating under ultrasonic mixing.
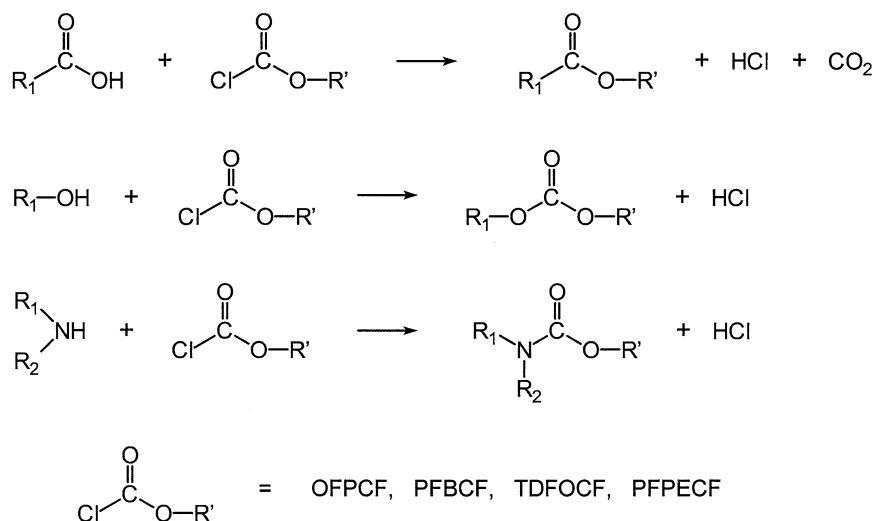
Scheme 2

on six candidate analytes representing five different classes of compounds (Table 1). OFPCF and PFBCF were successful in derivatizing all the analytes, whereas TDFOCF failed with both 3-aminopropanol and valine, and PFPECF failed with 3-aminopropanol, valine, and 2-aminoethanol (Table 1). The lower reactivity observed for TDFOCF and PFPECF is probably attributable to the reduced electron-withdrawing effect exerted by the perfluorinated moiety on the chloroformate group.

From Table 1, it is evident that OFPCF accomplishes the complete substitution of all active hydrogens, whereas PFBCF introduces only one substituent on the amine groups of valine, 2-aminoethanol and 3-aminopropanol, and two substituents on hydroxylamine. This was confirmed by PCI mass spectra. The lower degree of hydrogen substitution does not represent intrinsic evidence that PFBCF is less reactive than OFPCF toward the amine group, but indicates that the derivatization stops after the first substitution, as previously observed by Simpson and coworkers [20, 21].

In order to compare the reactivities of OFPCF and PFBCF further, a derivatization procedure for 2-aminoethanol, 3-aminopropanol, malic acid, and valine was performed using both derivatizing agents at once. Product distributions showed that 2-aminoethanol and 3-aminopropanol yielded both mixed products and derivatives containing only PFB terminals, (2-aminoethanol: OFP/PFB 37%, PFB/PFB 63%; 3-aminopropanol: OFP/PFB 21%, PFB/PFB 79%). Malic acid yielded only the OFPCF derivative (OFP/OFP 100%). Valine yielded multiple derivatives carrying all the combinations of the two terminal groups (OFP/OFP 12%, OFP/PFB 61%, PFB/PFB 27%). These data confirmed a slight preference of OFPCF for the carboxylic group and PFBCF for the aminic group.

The GC retention times of the OFPCF derivatives fall in the 13–15 min range (Table 1), corresponding to oven temperatures of 200 to 230 °C. This indicates that all OFPCF derivatives are relatively volatile because of the

presence of multiple highly-fluorinated substituents, despite the relatively high molecular weights. This volatility represents an ideal condition for wide applicability and nondiscriminating GC injection. PFBCF derivatives eluted at considerably higher retention times (Table 1), corresponding to oven temperatures of 250 to 290 °C. PFPECF produced even less volatile derivatives (for example, the resorcinol disubstituted derivative eluted at 20.31 min). This would restrict the applicability of PFPECF derivatization to the simplest analytes.

Mass Spectra of Highly-Fluorinated Derivatives

ECNI mass spectra of most investigated derivatives showed considerable complexity. Therefore, interpreting the observed fragmentation patterns is necessary in order to apply this method to the search and identification of new DBPs from ozonation water treatments.

Figure 2 illustrates the type of ECNI mass spectra obtained using the four different derivatizing agents. In general, distinctive spectral patterns were present and structural features of the analytes could be deduced from the mass spectra. However, almost all derivatives showed extensive fragmentation, with molecular ions often absent in the mass spectra. PCI-MS was occasionally utilized for determining the molecular ions (and consequently the degree of hydrogen substitution in the derivatives), even if the sensitivity of PCI-MS was considerably lower (about two orders of magnitude) than ECNI-MS.

Certain spectral features are common to most derivatives. Both carbonate and carbamate groups contain weak chemical bonds and fragment on either side of the carbonyl group. For example, α-cleavage fragments [M-ROCO]⁻ are evident in the ECNI mass spectra of the resorcinol derivatives (at m/z 367 for OFPCF, m/z 333 for PFBCF, m/z 499 for TDFOCF, and m/z 363 for PFPECF, Figure 2). These processes occur in both ion polarities,

Table 1. Comparison of the derivatization products formed by reacting a series of highly-fluorinated chloroformates (OFPCF, PFBCF, TDFOCF, PFPECF) with a series of candidate analytes

Analyte	Number of Substituents				M.W. of the Derivatization Product				GC Retention Time			
	OFPCF	PFBCF	TDFOCF	PFPECF	OFPCF	PFBCF	TDFOCF	PFPECF	OFPCF	PFBCF	TDFOCF	PFPECF
Hydroxylamine	3	3	3	3	807	705	1203	795	13.12	17.85	14.34	18.02
Valine	3	2	-	-	847	521	-	-	13.60	16.73	-	-
Malic Acid	3	3	3	3	820	718	1216	808	13.70	18.05	15.37	19.22
2-Aminoethanol	3	2	2	-	835	509	841	-	13.70	17.58	13.74	-
3-Aminopropanol	3	2	-	-	849	523	-	-	13.88	18.21	-	-
Resorcinol	2	2	2	2	626	558	890	618	14.65	19.09	17.10	20.31

release the groups introduced in the derivatization, and may be followed by further fragmentation. For example, the OFPCF malic acid derivative consecutively releases one molecule of alcohol and one of CO_2 (m/z 544, Figure 3b). α-Cleavage fragments are prevalent also in the PCI mass spectra of the derivatives, but the molecular weight information is more easily obtained. For example, the protonated molecular ion (m/z 821) of the malic acid OFPCF derivative (Figure 3a) consecutively releases neutral molecules to give fragments at m/z 589 (-ROH), 545 (-ROH -CO_2), and 313 (-2 ROH -CO_2).

A slightly different fragmentation process is observed in the ECNI mass spectra of the aromatic analytes: in this case, a radical is released instead of a neutral molecule and the charge is sustained on the phenolic oxygen, yielding the ion [M-ROCO]$^-$ as the most abundant of the spectrum. This behavior is observed for the PFBCF derivarives of 2,4-dihydroxybenzoic acid, 1,3,5-trihydroxybenzene, m-aminophenol, and the OFPCF derivatives of resorcinol and m-aminophenol (Table 2).

The relative intensity of the most significant ions in ECNI mass spectra of OFPCF and PFBCF derivatives are reported in Table 2. Although the origin of these fragment ions is often not straightforward, a few considerations of general significance can be made.

1. The molecular ion is either missing or exhibits extremely low abundance.
2. Two analytes containing only carboxylic groups (methylmalonic and tricarballylic acids) exhibit rather unpredictable fragmentation patterns: cleavage of C—C bonds occurs for the PFBCF derivative of methylmalonic acid (m/z 253) and the OFPCF derivative of tricarballylic acid (m/z 544), but not for the other two derivatives.
3. OFPCF derivatives of aliphatic hydroxycarboxylic acids (malic, tartronic, tartaric) typically release the alcoholic oxygen upon fragmentation ([M -ROH -CO_2]$^-$), while this cleavage does not take place in PFBCF derivatives.
4. Aromatic amine and phenol derivatives typically fragment by α-clevage (the radical R-OCO$^\cdot$ is released, where R is either OFP- or PFB-). Subsequently a variety of consecutive dissociations take place (i.e., RO, CO_2, or HF are released). For example, the m-aminophenol OFP-derivative produces the ion at m/z 624 ([M -ROCO]$^-$) as the base peak and the ion at m/z 349 ([M -ROCO -RO -CO_2]$^-$) as the second most abundant in the ECNI mass spectrum. The corresponding PFB-derivative gives the primary fragment ion at m/z 556 ([M -ROCO]$^-$) which subsequently releases a CO_2 molecule (m/z 512).
5. OFPCF derivatives of hydroxylamine, 2-aminoethanol, 3-aminopropanol, and valine all produce the ion at m/z 532 as the base peak in the ECNI mass spectrum. This peak can be attributed to the [N(COOR)$_2$]$^-$ ion, which is common for all the analytes, since double substitution on the amine group takes place in the
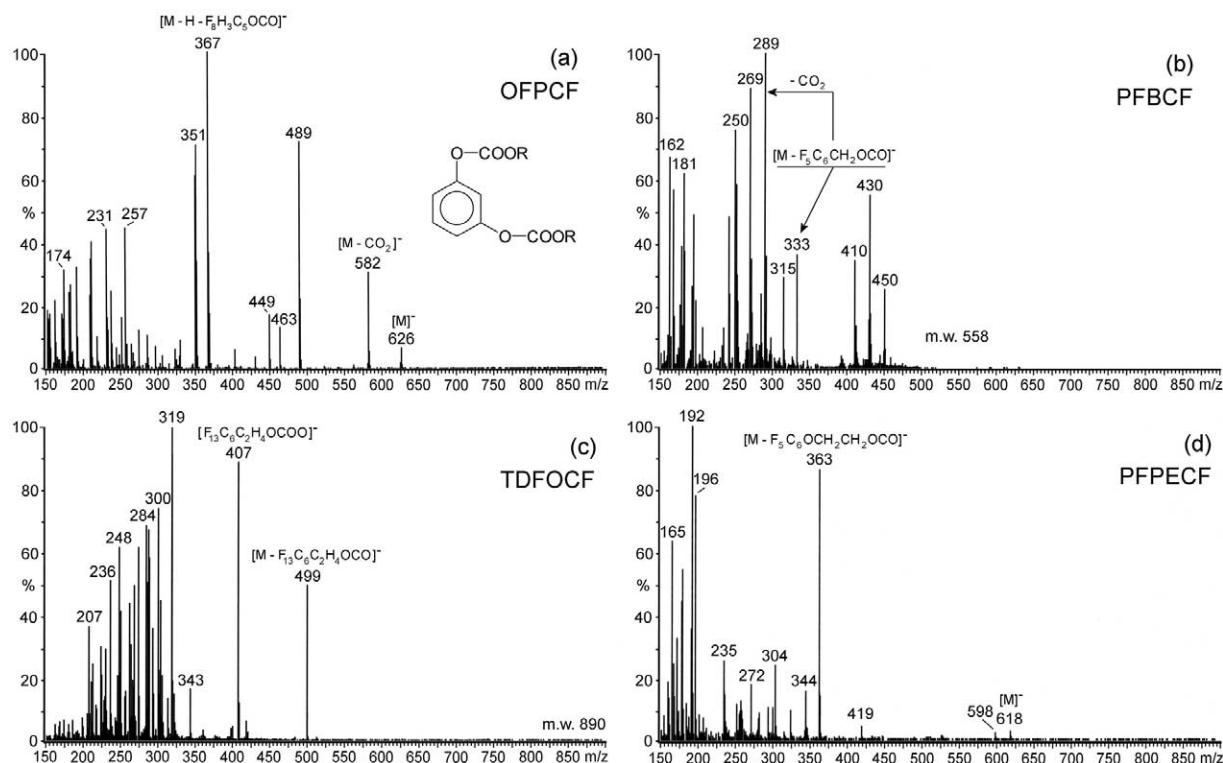
Figure 2. Electron capture negative ion mass spectra of resorcinol (1 mg/L) derivatized with: (**a**) OFPCF; (**b**) PFBCF; (**c**) TDFOCF; (**d**) PFPECF.

derivatization. This ion is highly indicative of the presence of an amine group in the analyte, but provides little information for other structural features of the derivative. In contrast, PFB-derivatives (with single substitution on the amine group) retains the charge in the analyte hydrocarbon backbone during ECNI fragmentation, which primarily involves α-clevage.

6. Since α-cleavage is generally the expected major dissociation process, α-cleavage fragments, along

with the knowledge on the number of substituted hydrogens, serve as good indicators of the derivative structure.

The ECNI mass spectrum of the 2-aminoethanol TDFOCF derivative, depicted in Figure 4, represents a typical fragmentation pattern for TDFOCF derivatives, which, in general, are only scarcely informative. The higher m/z ion (m/z 431) corresponds to [M -ROCO -F]$^-$. Since a double radical loss is unlikely, the ion likely originates from the consecutive release of two (or three) neutral molecules, each requiring a hydrogen rearrangement, such as [M -(R-H) -CO_2 -HF]$^-$. The rest of the mass spectrum is unclear: a large number of peaks is evident in the m/z 200–320 range, most of which can be attributed to the fragmentation of the perfluorocarbon groups introduced by derivatization.

Analytical Performance

From the previous discussion, it appears that TD FOCF and PFPECF failed to react with some of the analytes tested. Moreover, their derivatives exhibit high GC retention times and scarcely informative ECNI mass spectra. Therefore, the subsequent quality assurance program was restricted to OFPCF and PFBCF. The overall analytical performance of the method was tested using the extended set of target

Figure 3. Positive and negative electron capture chemical ionization mass spectra of the malic acid (1 mg/L) OFPCF tri-derivative.

Table 2. ECNI mass spectra of OFPCF and PFBCF derivatives

Compound[a] m.w. (molecular weight); active hydrogens	OFPCF					PFBCF					
	Derivative M.W.	M	M-R-OCO	M-R-OCOO[b] (M-R-O)	Other Fragment Ions	Derivative M.W.	M	M-R	M-R-O	M-R-OCO	Other Fragment Ions
Resorcinol (m.w. 110; 2 active H)	626	(8)	367 (100)	351 (18)	582 (31)	558	(0)			333 (37)	289 (100) 269 (95)
1,3,5-Trihydroxybenzene (m.w. 126; 3 active H)	900	(0)	641 (4)		366 (100)	798	(0)			572 (100)	508 (56) 374 (16)
2,4-Dihydroxybenzoic acid (m.w. 154; 3 active H)	884	(0)	625 (32)		350 (100)	782	(1)			557 (100)	513 (98) 333 (22)
Methylmalonic acid (m.w. 118; 2 active H)	546	(13)		314 (100)[b]		478	(14)	297 (100)		253 (63)	477 (14)
Malic acid (m.w. 134; 3 active H)	820	(0)		544 (100)		718	(0)		521 (10)		377 (100) 333 (89)
Tartronic acid (m.w. 120; 3 active H)	806	(0)		531 (100)		704	(0)			477 (100)	457 (17)
Tricarballylic acid (m.w. 176; 3 active H)	818	(5)	558 (21)		544 (100) 372 (31)	716	(0)	535 (12)			371 (100) 337 (69)
Tartaric acid (m.w. 150; 4 active H)	1094	(0)			790 (100) 558 (19)	958	(0)				417 (49) 300 (100)
m-Aminophenol (m.w. 109; 3 active H)	883	(0)	624 (100)		349 (23)	781	(0)			556 (100)	512 (42) 312 (54)
m-Aminobenzoic acid (m.w. 137; 3 active H)	867	(0)	608 (8)		393 (44) 349 (100)	765	(0)	584 (61)		540 (92)	496 (100)
Hydroxylamine (m.w. 33; 3 active H)	807	(0)	548 (46)	532 (100)	301 (24)	705	(0)			480 (44)	329 (100)
2-Aminoethanol (m.w. 61; 3 or 2 active H)	835	(0)			532 (100)	509 (2H)	(0)	328 (4)		284 (100)	264 (98)
3-Aminopropanol (m.w. 75; 3 or 2 active H)	849	(0)			532 (100)	523 (2H)	(0)	342 (40)		298 (27)	278 (100) 479 (8)
Valine (m.w. 117; 3 or 2 active H)	847	(0)	588 (13)		532 (100) 356 (72)	521 (2H)	(1)	340 (100)	324 (13)		281 (60)

[a]methylmalonic acid = 2-methyl-1,3-propanedioic acid; malic acid = 2-hydroxy-1,4-butanedioic acid; tartronic acid = 2-hydroxy-1,3-propanedioic acid; tricarballylic acid = 3-carboxy-1,5-pentanedioic acid; tartaric acid = 2,3-dihydroxy-1,4-butanedioic acid
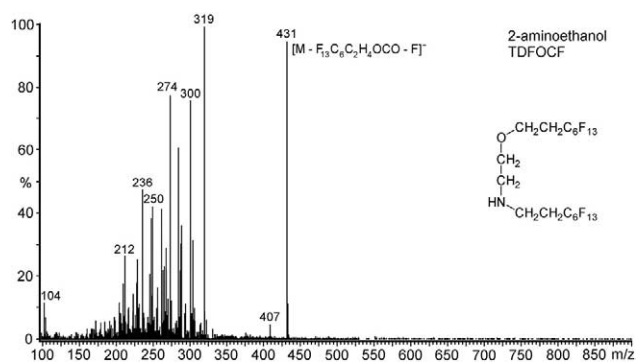[b]314 (100) cooresponds to the structure (M-R-O).

Figure 4. Electron capture negative ion mass spectrum of the 2-aminoethanol (1 mg/L) TDFOCF bi-derivative.

analytes listed in Table 2. The following analytical features were investigated: (1) repeatability of the derivatization; (2) repeatability of the instrumental

response; (3) detection limits for the various analytes; (4) linearity of the calibration curves. These results, summarized in Table 3, refer to the quantitative determination of target analytes.

The instrumental repeatability was tested by injecting five times each day the same derivatization mixtures arising from 100 μg/L aqueous analyte solutions, over five consecutive days. The standard deviations, expressed as percentage of the mean signal, ranged from 3 to 12%, demonstrating good stability of the derivatization products and acceptable reproducibility of the ECNI-MS conditions. Further confirmation of product ion stability was obtained by analyzing the same hexane solutions at regular time intervals over a month (stored at 4 °C between analyses).

The repeatability of the derivatization process was measured by treating five aliquots of 100 μg/L analyte solutions using the protocol described in the experi-

Table 3. Analytical features for the derivatizations (2 mL) performed with OFPCF and PFBCF, including (i) the percentage standard deviation for repeated analysis of the same sample (instrumental repeatability); (ii) the percentage standard deviation for repeated derivatization of the same analytical solution (derivatization repeatability); (iii) the observed detection limit (S/N > 5), expressed as the analyte concentration in the starting water solution; (iv) the linearity range of the calibration curve; (v) the angular coefficient ($\pm$ standard deviation) and (vi) the square correlation factor for the bilogarithmic calibration curves. All experiments were performed in the ECNI-MS-SIM mode, by selecting the ions listed in Table 2 within appropriate retention time windows

	OFPCF					
	Instr.Rep. (σ %)	Der.Rep. (σ %)	Det.Lim. (μg/L)	Cal.Range (μg/L)	log/log Ang.Coeff.	r^2
Resorcinol	3.0	5.5	0.1	0.3–300	1.01 $\pm$ 0.02	0.998
1,3,5-Trihydroxybenzene	7.8	8.3	0.3	0.3–500	1.18 $\pm$ 0.11	0.998
2,4-Dihydroxybenzoic acid	4.4	8.1	0.3	0.3–500	0.94 $\pm$ 0.02	0.997
Methylmalonic acid	5.4	7.4	3	5–500	1.00 $\pm$ 0.03	0.990
Malic acid	6.2	9.8	0.3	0.3–300	0.86 $\pm$ 0.02	0.992
Tartronic acid	3.7	7.4	0.3	1–500	0.89 $\pm$ 0.09	0.992
Tricarballylic acid	6.8	8.9	1	1–500	0.97 $\pm$ 0.08	0.980
Tartaric acid	8.5	11.7	0.3	0.3–500	0.86 $\pm$ 0.02	0.992
m-Aminophenol	5.7	19.1	1	1–500	0.98 $\pm$ 0.02	0.996
m-Aminobenzoic acid	7.1	15.3	5	5–500	1.15 $\pm$ 0.04	0.989
Hydroxylamine	6.3	13.9	0.3	0.3–300	0.85 $\pm$ 0.02	0.994
2-Aminoethanol	11.7	25.0	10	10–300	1.23 $\pm$ 0.12	0.979
3-Aminopropanol	10.6	22.1	10	10–300	1.26 $\pm$ 0.11	0.975
Valine	8.3	19.4	10	10–500	1.21 $\pm$ 0.04	0.982
	PFBCF					
	Instr.Rep. (σ %)	Der.Rep. (σ %)	Det.Lim. (μg/L)	Cal.Range (μg/L)	log/log Ang.Coeff.	r^2
Resorcinol	5.2	14.3	0.1	0.3–100	0.70 $\pm$ 0.09	0.986
1,3,5-Trihydroxybenzene	10.1	21.1	100	100–500	0.98 $\pm$ 0.07	0.980
2,4-Dihydroxybenzoic acid	9.0	16.6	30	30–500	1.28 $\pm$ 0.12	0.943
Methylmalonic acid	5.6	24.3	100	100–500	0.98 $\pm$ 0.09	0.970
Malic acid	6.8	25.6	10	10–500	1.08 $\pm$ 0.04	0.986
Tartronic acid	4.1	18.6	1	1–500	0.92 $\pm$ 0.05	0.969
Tricarballylic acid	7.5	21.6	5	5–500	1.11 $\pm$ 0.10	0.982
Tartaric acid	7.6	29.4	100	100–500	1.22 $\pm$ 0.12	0.966
m-Aminophenol	9.9	12.1	30	100–500	1.31 $\pm$ 0.15	0.953
m-Aminobenzoic acid	7.1	9.6	30	100–500	1.23 $\pm$ 0.14	0.989
Hydroxylamine	5.4	11.4	30	100–500	0.75 $\pm$ 0.14	0.980
2-Aminoethanol	10.4	10.3	3	5–500	1.11 $\pm$ 0.05	0.983
3-Aminopropanol	11.0	12.4	1	1–100	0.92 $\pm$ 0.09	0.972
Valine	9.3	15.3	10	10–500	1.03 $\pm$ 0.06	0.982

mental section. An internal standard (perfluorotri-phenylphosphine) was added to each sample before GC-MS analysis. Standard deviations ranging from 5 to 12% were observed for polycarboxylic acids, polyhy-droxycarboxylic acids, and polyhydroxybenzenes deri-vatized with OFPCF. Derivatization of analytes with an amine group exhibited lower repeatability (14% < σ% < 25%). For derivatizations performed with PFPCF, the opposite was observed: variability was lower for ami-noalcohols and aminoacids (10% < σ% < 15%) than for hydroxy-carboxylic compounds (14% < σ% < 30%). These findings, combined with the results from com-petitive derivatization experiments (where OFPCF and PFBCF were mixed) suggest that the repeatability is correlated with the reaction kinetics. In fact, it appears that the faster the derivatization takes place, the more repeatable quantitative determinations are.

Detection limits and calibration ranges proved excel-lent for the derivatizations performed with OFPCF, with absolute detection limits in the low fmol range for hydroxycarboxylic acids, di- and trihydroxybenzenes, and hydroxylamine (Figure 5). For these compounds, further concentration of the final hexane solutions can yield detection limits in the tens of ppt (ng/L) range. Calibration curves were calculated in bilogarithmic scales, so that homogeneous distribution of data points

Figure 5. Selected ion mass chromatograms obtained from OFPCF derivatization of a 1 μg/L solution of (**a**) 1,3,5-trihydroxybenzene and (**b**) 2,4-dihydroxybenzoic acid. Selected ions are m/z 641 and 366 for 1,3,5-trihydroxybenzene and m/z 625 and 350 for 2,4-dihydroxybenzoic acid.

along the calibration range was obtained. Linear plots with satisfactory correlation factors were observed (r^2 = 0.975 to 0.998).

Considerably higher detection limits were observed for the derivatizations performed with PFBCF (typically hundreds of fmol), with the remarkable exception of aminoalcohols, which could be determined in the low fmol range. Also, the calibration curves for PFBCF derivatives exhibited more limited linearity ranges, with slopes deviating from unity and lower correlation factors than for OFPCF (r^2 > 0.943 for PFBCF deriva-tives; r^2 > 0.975 for OFPCF derivatives). It can be concluded that OFPCF is a better performing derivatiz-ing agent than PFBCF for most polar analytes, and its use is recommended for survey analyses of unknown analytes, such as the search for unidentified drinking water DBPs.

Further tests to validate our derivatization protocol were made to evaluate possible effects of an actual drinking water matrix. Since our effort focused on the detection of drinking water DBPs, comparative experi-ments were performed, using tap water instead of distilled water, to prepare standard and analytical so-lutions of the candidate analytes listed in Table 2. No differences were observed, and the overall variability of the experimental data matched that determined for distilled water solutions (Table 3). We also wanted to make sure that the presence of multiple polar compo-nents did not suppress their derivatization. Therefore, the derivatization procedure with OFPCF and PFBCF was performed on a mixture of eleven candidate ana-lytes. The GC profile obtained from one of these exper-iments is reported in Figure 6, and shows that the OFPCF derivatization occurred with the usual effi-ciency for all the analytes. Similar results were also observed for PFBCF derivatization.

Application to the Detection of Ozonation By-Products

In order to verify the derivatization procedure in an application of real interest, water solutions of Suwannee River Fulvic Acid (SRFA) were subjected to oxidative treatment with ozone for variable time intervals. The resulting solutions were subsequently derivatized with OFPCF, and the final hexane solutions were analyzed by GC-MS, under full-scan conditions, in order to identify any possible SRFA oxidation products. Blank samples of untreated SRFA solutions were derivatized and analyzed, to rule out the components already present in SRFA and the derivatization by-products.

From this comparative analysis, three SRFA ozona-tion by-products were identified, namely malic acid, tricarballylic acid, and 1,2,3-benzenetricarboxylic acid. To our knowledge, these have not been previously reported as ozonation by-products. A previous study did report two other benzenetricarboxylic acid DBPs in ozonated drinking water: the 1,2,4- and 1,3,5-isomers
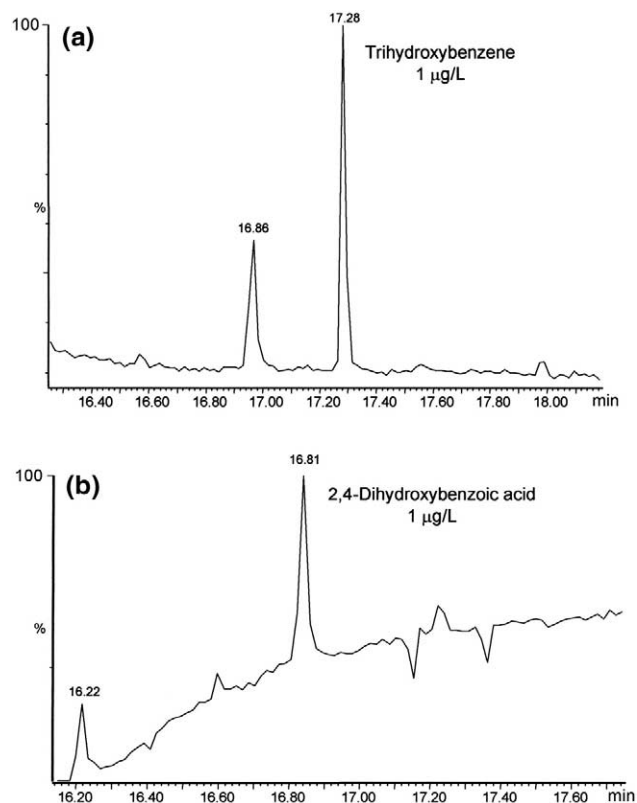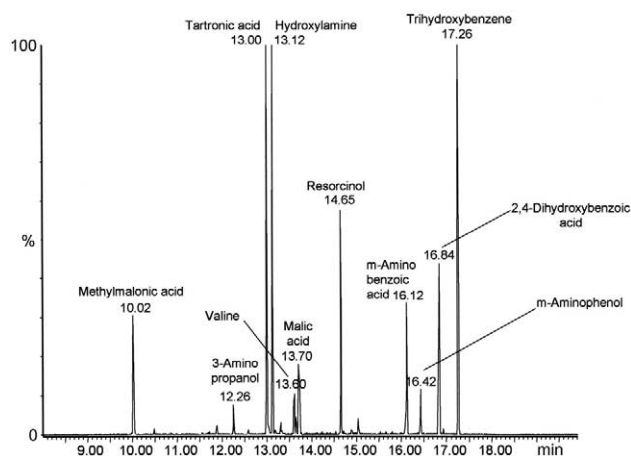
Figure 6. Selected ion mass chromatogram obtained from OFPCF derivatization of a eleven standard analyte solutions (500 μg/L each). Signals from all the selected ions are added together.

[23]. While determining the first two acids presented no difficulty as the mass spectra of their derivatives were already known, attributing the mass spectrum of the third component as 1,2,3-benzenetricarboxylic acid required mass spectral interpretation and subsequent confirmation with a pure standard. This ECNI spectrum presents a weak molecular ion at m/z 852 (1%), primary fragment ions at m/z 652 (2%, $[M-C_4F_8]^-$), m/z 635 (7%, $[M -H -CHF_2(CF_2)_3CH_2]^-$), m/z 231 (87%, $[CHF_2(CF_2)_3CH_2O]^-$), and a main secondary fragment ion at m/z 403 (100%, $[635 -CHF_2(CF_2)_3CH_2OH]^-$). In the positive ion mode, the DBPs were not present at high enough concentrations to enable full PCI mass spectra to be obtained, but SIM profiles matched those of the pure standards. Systematic investigation of ozonation by-products from humic and fulvic acid solutions, as well as from real drinking water from treatment plants, is underway.

Conclusions

Highly-fluorinated chloroformates proved to be powerful derivatizing agents for polar hydrophilic analytes, which react directly in aqueous solution, avoiding the risk of incomplete extraction and recovery of the polar analytes. Four different fluorinated chloroformates could be easily synthesized and all of them proved capable of carrying out direct aqueous sample derivatization. However, among them, OFPCF exhibited superior performance in terms of general applicability, sensitivity, and linearity of response, while PFBCF found specific application in the analysis of aminoalcohols. While most derivatizing agents undergo rapid decomposition in water, the insolubility of highly-fluorinated chloroformates considerably slows down their hydrolysis kinetics.

A unique feature of OFPCF and PFBCF derivatizations is their effectiveness in reacting with carboxylic, hydroxylic, and aminic groups at once, releasing

multiply-substituted derivatives that can be easily extracted and determined by GC-(ECNI)-MS. The entire procedure from raw aqueous sample to ready-to-inject hexane solution of the derivatives requires less than ten min. Another positive aspect of this procedure is that it produces stable derivatives, with optimal volatility for GC separation, and high electron-affinity, which allows their detection as negative ions at trace level.

We believe that the easy preparation of these derivatizing agents, combined with their wide applicability and unique analytical features, open up innovative and still unexplored avenues in several application fields, including clinical, environmental, and food chemistry. Promising results are expected in the determination of unknown water disinfection by-products for which only the hydrophobic and moderately hydrophilic fractions have been adequately characterized. While these fractions are prevalent in chlorinated drinking water, ozone is expected to produce a large variety of highly polar by-products, which remain to be identified. We are hopeful that the use of direct methods, such as the OFPCF and PFBCF derivatizations presented here, will enable their identification.

Acknowledgments

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