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Improved procedure for *n*-hexyl chloroformate-mediated derivatization of highly hydrophilic substances directly in water: hydroxyaminic compounds

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Abstract

A fast derivatization procedure, carried out directly on aqueous solutions of highly hydrophilic substances, has been modified and improved in order to determine amino alcohols, hydroxylamines, aminophenols and other aminic substances in water. Aqueous samples were mixed with *n*-hexyl chloroformate under sonication and subsequently added to the catalyst, a saturated pyridine solution of dicyclohexylcarbodiimide. The *n*-hexane extracts were analyzed by GC–MS in the positive chemical ionization mode. Detection limits on the non-concentrated extracts (1:1, v/v vs. the sample) were in the 10–100 μg/l range, depending on the analyte. © 1998 Elsevier Science B.V.

Keywords: Derivatization, GC; Hydroxyaminic compounds; Hexyl chloroformate; Amines

1. Introduction

The analytical determination of small and highly hydrophilic compounds in water is usually difficult, since they generally do not contain chromophores for direct HPLC–photometric or fluorimetric detection, they can not easily be extracted from the aqueous matrix and their direct derivatization in water is prevented by fast hydrolysis of most derivatizing agents. Typical examples of such molecules are hydroxylamine, hydrazine, ethylene glycol and malic acid.

Derivatization with alkyl chloroformates of alkyl carboxylic acids [1,2], 2-hydroxy-alkyl carboxylic acids [3–5], phenolic acids [6] proved to be efficient even in the presence of moderate amounts of water in the reaction solvent. More recently, we demonstrated [7] that the direct derivatization of (poly)hydroxy-(poly)carboxylic acids, glycols and polyhydroxybenzenes is achievable in pure aqueous solutions, provided that an hydrophobic chloroformate is used, such as *n*-hexyl chloroformate, whose hydrolysis kinetics is slowed by the poor solubility of the derivatizing agent in water. The derivatization with *n*-hexyl chloroformate allowed us to detect a large variety of highly hydrophilic substances even at trace levels and in complex matrices such as wine [8], salty water [7] and river water [9]. All the derivatives could be extracted in *n*-hexane and determined by gas chromatography–mass spectrometry (GC–MS) in the chemical ionization (CI) mode (positive ions) at the 100 μg/l level.

Besides hydroxylic and carboxylic derivatives, the amine group also proved to be susceptible to de-

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rivatization by alkyl chloroformates [10–13]. Actually, most of the analytical applications of derivatizations with alkyl chloroformates, for GC or GC–MS detection, have been devoted to the determination of amino acids [14–18]. More recently, many analytical determinations of amino acids and amines in a variety of matrices are routinely carried out by derivatization with 9-fluorenylmethyl chloroformate or 1-(9-fluorenyl)ethyl chloroformate, followed by HPLC analysis with fluorimetric detection [19–28]. Occasionally, derivatization of amines and amino acids has been accomplished by using trichloroethyl [29], 2,2,2-trifluoroethyl [30] and pentafluorobenzyl [31,32] chloroformates. We also had preliminary evidence that the amine group could be derivatized by *n*-hexyl chloroformate when we determined two hydroxycarbamates in aqueous matrices [9].

In the present work, we subjected a number of aminophenols, amino alcohols, hydroxylamines and some other small amines (aniline, hydrazine) to the derivatizing procedure with *n*-hexyl chloroformate formerly described [7]. As the reaction yields appeared to be quite low for some of the compounds tested, and a few of them did not undergo derivatization at all, we reconsidered the procedure steps and the experimental parameters involved, and introduced two fundamental modifications to the method, that substantially increased the derivatization yields (by factors of 10 to 100), allowing the determination of almost all the compounds tested at the 100 μg/l level or below. The modifications of the method proved to be beneficial also for the determination of the analytes previously considered [7] (especially for glycols and polyhydroxybenzenes) whose detection limits could be substantially reduced.

2. Experimental

2.1. Materials

n-Hexyl chloroformate (97%), pyridine, 2-aminoethanol, 3-aminopropanol, aniline, *o*-, *m*- and *p*-aminophenols, hydrazine, N-methyl- and O-methylhydroxylamine, N,N-dimethylhydroxylamine, N-phenylhydroxylamine, acetohydroxamic acid, ethyl carbamate, hydroxyurethane, *n*-hexane, di- and triethylene glycols and methoxyethoxyethanol were purchased from Aldrich (Steinheim, Germany). Hydroquinone and hydroxylamine were from Merck (Darmstadt, Germany). Ethylene glycol was from Carlo Erba (Milan, Italy). N,N-Dicyclohexylcarbodiimide was from Fluka (Buchs, Switzerland).

2.2. Derivatization procedure

Concentrated aqueous solutions (1 g/l) of each standard analyte were prepared using bidistilled water. Analytical solutions with concentrations ranging from 300 ng/l to 30 mg/l were freshly prepared before the experiment by progressive dilution with bidistilled water of the concentrated solutions.

A 300-μl volume of analytical aqueous solution was introduced into a 1-ml conical flask and transferred into an ultrasonic bath (Branson, model 2200) maintained at 30°C. *n*-Hexyl chloroformate (15 μl) was added during sonication. Then, 30 μl of a pyridine solution of N,N-dicyclohexylcarbodiimide (400 g/l) were added with a microsyringe, while maintaining the mixture under ultrasonic shaking. The reaction was allowed to proceed for 60 s and then 300 μl of *n*-hexane were added. The ultrasonic bath was switched off after a further 60 s, and the two phases were allowed to separate. One μl of the hexane layer was immediately injected into the GC–MS instrument for the analysis. In a few experiments, the hexane extract was separated and concentrated up to 30 times in a conical vial under a stream of nitrogen, leaving a pyridine solution.

2.3. Instrumentation and analysis

Two different GC–MS instruments were utilized. A Finnigan-MAT 95Q (Bremen, Germany) hybrid mass spectrometer, with BEQ geometry, was equipped with a Varian 3400 gas chromatograph (Palo Alto, CA, USA). The ions were collected at the first dynode-electron multiplier detector, located after the magnetic and electrostatic analyzers. The magnetic mass analyzer was utilized under continuous scanning, but the mass range and the scan speed were regulated according to the aim of the analysis. Qualitative investigations were typically run using a large mass range, while for target quantitative determinations short and frequent scans around the

ion-mass of interest were utilized (for example, 50–200 u in 0.5 s).

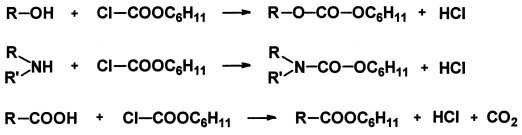
Positive chemical ionization (CI) conditions were established by admitting isobutane in the ion source, as the reagent gas, at a pressure of 50 Pa (0.5 mbar). With this pressure, the ratio between m/z 57 and m/z 43 peaks was 10:1. The electron energy was set to 200 eV and the electron current to 0.2 mA. The resolution was set to $m/\Delta m$ 1000 (5% valley) and the ion source temperature to 220°C. The Varian GC system was equipped with a bonded-phase DB-5-MS (5% diphenyl dimethyl siloxane) capillary column (J&W, Folsom, CA, USA), 30 m×0.25 mm I.D., 0.25 μm film thickness. A 1-μl volume of sample was introduced in the split/splitless injector, kept at 300°C and working in the splitless mode for 30 s. The carrier gas (helium) inlet pressure was maintained constant at 13 p.s.i. (≈90 000 Pa) The oven temperature was programmed as follows: isothermal at 50°C for 3 min, from 50°C to 300°C at 20°C/min and held at 300°C for 10 min.

The second GC–MS instrument used was a Hewlett-Packard HP 5972 quadrupole mass detector, with a HP 5890 Series II Plus gas chromatograph. This instrument was used for quantitative determinations only and, therefore was programmed in the selected ion monitoring mode, using each time the mass signals typical for the specific analyte tested. Positive CI was achieved using again isobutane as the reagent gas. Since actual pressure reading was not available, ionization conditions identical to those of the magnetic instrument, were obtained by adjusting the isobutane pressure so as to get a 10:1 abundance ratio between m/z 57 and m/z 43 ions. The electron energy was set at 70 eV and the electron current at 0.05 mA. An HP5-MS capillary column was used, having features similar to those of the J&W column previously described, except for the internal diameter, which was 0.21 mm. Also the oven temperature programming was the same as described above, but not the injection and carrier gas pressure conditions. Splitless (60 s) injection was performed under pressure pulse programming, by keeping the carrier gas inlet pressure at 30 p.s.i during the splitless time. Subsequently, the pressure was lowered to about 10 p.s.i. and then gradually increased, so as to maintain a constant gas flow (1.0 ml/min) during the entire oven temperature programming. These conditions assured more efficient sample injection into the GC column and produced slightly shorter retention times.

3. Results and discussion

3.1. Applicability of the method with the improved procedure

In our previous papers [7–9] the derivatization with *n*-hexyl chloroformate was directly applied to aqueous solutions of (poly)hydroxy-(poly)carboxylic acids, polyhydroxybenzenes, glycols, and two carbamates. Hydroxylic and aminic groups react with *n*-hexyl chloroformate by eliminating one molecule of HCl, yielding carbonates and carbamates, respectively. In the derivatization of the carboxylic group *n*-hexyl esters are produced, either by elimination of CO_2 from an *n*-hexyl anhydride intermediate or, more likely [17], by reaction of the same intermediate with *n*-hexyl alcohol (formed from the hydrolysis of chloroformate). The three overall reactions are schematized as follows:



Unlike shorter alkyl chloroformates, *n*-hexyl chloroformate derivatized compounds containing a combination of the preceding functional groups in a purely aqueous medium. Even though the unequivocal reasons for this different behavior have not been demonstrated, it can be advanced that the kinetics for the hydrolysis of the *n*-hexyl chloroformate are substantially slower than that for the other chloroformates, given its low solubility in water. This provides more time for the analytes to react at the interface between the two separated phases (water and *n*-hexyl chloroformate, the latter acting also as an extraction medium for the derivative). In any case, *n*-hexyl chloroformate is finally hydrolyzed itself, yielding di-*n*-hexyl carbonate as the main reaction byproduct, as verified by GC–MS. The derivatization yields for the various analytes are likely to depend on the relative kinetics for the two competing reactions, namely the derivatization of the

analyte and the hydrolysis of the chloroformate, as proved by Ahnfelt and Hertvig [33], even though in the absence of the pyridine catalyst.

While the applicability of the derivatization procedure to a variety of highly hydrophilic compounds carrying a combination of hydroxylic and carboxylic functional groups has been considered, in the present study we explored the applicability of the same procedure to substances carrying one or more aminic groups in combination with other substituents. To this purpose, we determined several aminophenols, amino alcohols, hydroxylamines and some other functionalized amines, listed in Table 1.

Standard solutions of each of the substances listed were derivatized according to the procedure described in Ref. [7]. While some of the aminic compounds considered appeared to react very efficiently producing a high abundance of the derivatization product (as determined by GC–MS in positive ion chemical ionization), others failed to exhibit in the chromatographic profile any peak which could be positively attributed to a derivatization product. Thus, the influence of a change in the experimental parameters (temperature, pH, presence of co-solvents or co-catalysts, amount of the catalyst and the derivatizing agent) was re-evaluated using the unreactive compounds together with other compounds, on which experience was gained in the course of the preceding studies [7–9]. Details of this study are reported elsewhere [34].

The exact reaction yields cannot be determined, since authentic standards for the derivatization products are not available. However, approximate reaction yields could be estimated by the size of the GC peak for the derivatization product relative to that of a structurally similar compound of known concentration [7]. The relative derivatization yields for isomeric analytes (i.e., *o*-, *m*- and *p*-aminophenol) are also easily obtained.

Among the experimental parameters which proved to have an unequivocal effect on the derivatization efficiency, only two of them provided a significant and generalized improvement of the reaction yield for all the analytes tested. These are (1) the addition to the pyridine of a small amount of dicyclohexylcarbodiimide (DCC), acting as co-catalyst for the reaction and (2) the inversion in the order of introduction of the reagents into the reaction flask.

DCC is a common dehydrating and coupling agent in organic synthesis, where it plays an active role in

Table 1
Analytes tested, grouped in four classes, and their chemical structures — useful derivatization products were obtained for all analytes, except ethyl carbamate

Analyte	Structure	Useful ions
Amino alcohols		
2-Aminoethanol	$NH_2–CH_2–CH_2–OH$	172 (318)
3-Aminopropanol	$NH_2–CH_2–CH_2–CH_2–OH$	186 (332)
Aromatic amines		
Aniline		222
o-Aminophenol		264, 494
m-Aminophenol		264 (366)
p-Aminophenol		264 (366)
Hydroxylamines and hydrazine		
Hydroxylamine	$NH_2–OH$	418
N-Methylhydroxylamine	$CH_3–NH–OH$	304
O-Methylhydroxylamine	$NH_2–O–CH_3$	176
N,N-Dimethylhydroxylamine	$(CH_3)_2N–OH$	190
N-Phenylhydroxylamine	Ph–NH–OH	264 (366)
Hydrazine	$NH_2–NH_2$	289
Carbamates and amides		
Acetohydroxamic acid	$HO–NH–CO–CH_3$	332, 204
Ethyl carbamate (urethane)	$NH_2–COO–C_2H_5$	
Hydroxyurethane	$HO–NH–COO–C_2H_5$	362

the elimination of small molecules, such as water or HCl [35]. The similarity between some of these syntheses [35] and the present derivatization reactions suggested testing DCC as a co-catalyst, by dissolving it in the usual catalyst (pyridine). This innovation permitted the derivatization of analytes reluctant to react under the previous conditions (for example N,N-dimethylhydroxylamine) and produced a significant and generalized improvement of the derivatization yields. The extent of such improvement is evidenced in Table 2, where the increment of the analytical signal is calculated by the ratio of integrated GC peaks obtained by the new and the old procedures, in consecutive and replicated experiments.

In the published procedure [7], the chloroformate was introduced after the catalyst, under sonication, and just before the extraction solvent. The second innovation consisted of introducing into the reaction flask first the aqueous sample solution, secondly the chloroformate, and lastly (under sonication) the catalyst. Then, the reaction products were extracted in *n*-hexane. This inversion produced a drastic improvement in the derivatization yields and their increased reproducibility. It also demonstrated that neither the derivatization nor the hydrolysis of the chloroformate proceeds until the catalyst is added. The reason for this improvement is likely to be a closer and more homogeneous contact between the aqueous and the organic phases at the moment when the reaction takes place. This is evidently an important factor for a reaction occurring at the interface.

Table 2
Ratio ($R_{DCC\text{-}Py}/R_{Py}$) between the integrated GC peaks of the derivatives in consecutive experiments where, respectively, dicyclohexylcarbodiimide (DCC) was added or not added to the pyridine catalyst

Analyte	$R_{DCC\text{-}Py}/R_{Py}$
o-Aminophenol	5±1
m-Aminophenol	80±9
p-Aminophenol	32±7
Aniline	20±3
Hydroxylamine	35±5
N,N-Dimethylhydroxylamine	∞
Hydroquinone	4±1
Ethylene glycol	10±3
Diethylene glycol	5±1
Triethylene glycol	8±1
Methoxyethoxyethanol	120±23

For several analytes, the combined effect of the two innovations made the derivatization yields increase by two orders of magnitude, resulting in detection limits in the low μg/l range, after concentration of the extracts. Once implemented, the modified procedure was applied again to the complete range of compounds listed in Table 1, at different concentrations. It turned out that all aromatic amines, hydroxylamines and amino alcohols were efficiently derivatized and the reaction products could be easily detected in the GC trace even at low analyte concentration.

Among the remaining analytes tested, hydroxyurethane, acetohydroxamic acid and hydrazine underwent derivatization with high yields, while urethane failed to exhibit any useful derivatization product in the GC profile. The reactivity difference between urethane and hydroxyurethane, whose structures are very similar, is likely due to the electron-donor hydroxylic substituent which activates the derivatization of carbamic hydrogens.

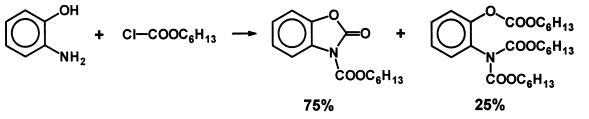
3.2. Mass spectra and ion chromatograms

It has been observed [7] that electron impact (EI) mass spectra are of little use in the identification and detection of the *n*-hexyl chloroformate derivatization products, since the presence of rather weak bonds in the structure of the derivatives generally brings about an extensive and unspecific fragmentation. Also flame ionization detection (FID) is not ideal, since a series of derivatization by-products $[(CO)_n(OC_6H_{13})_2]$ tend to make the GC profile rather complicated. In contrast, gas chromatography–(positive ion) chemical ionization mass spectrometry (GC–CI-MS) proved to be the detection method of choice for these compounds. The number and identity of the derivatives produced by each analyte were determined by varying the analyte concentration. All the GC peaks whose abundance varied proportionally to the concentration could be positively identified as derivatives. Even if most of the analytes were converted to only one product, in a few cases two derivatives with a different number of substituted hydrogens were formed. For example, this is the case for the amino alcohols (2-aminoethanol and 3-

aminopropanol), which produced both the mono- and the bi-derivatized products in approximate 40:60 yields (a constant ratio for the entire calibration range), as deduced by the intensity of the two GC peaks.

For analytical purposes, the polysubstituted derivatives are more valuable because their higher molecular mass and GC retention time make them generally less subjected to interferences. The CI-MS mass spectrum of the bi-derivatized product of 3-aminopropanol is depicted in Fig. 1a. The protonated molecular ion is evident at m/z 332, but it is not the one exhibiting the largest abundance. Several fragment ions are present in the spectrum: the peak at m/z 230 corresponds to neutral loss of 1-hexanol from the protonated molecular ion; the one at m/z 186 (base peak) is likely to originate from m/z 230 by a loss of CO_2; the peak at m/z 102 corresponds to 1-hexanol and m/z 85 is the *n*-hexyl radical ion. This extensive fragmentation is relatively common for CI mass spectra of the *n*-hexyl chloroformate derivatives obtained by using isobutane as the reagent gas. The fragmentation can be drastically reduced by using ammonia as the reagent gas [8], but the sensitivity is generally lower than with isobutane.

The results obtained for the derivatization of the three aminophenol isomers are quite peculiar. While *m*- and *p*-aminophenols yielded the N,O-bisubstituted derivative as the main product and small amount of the mono-derivative, *o*-aminophenol gave some percentage (≈25%) of the tri-substituted derivative and large abundance of a more complex product, likely arising from the bi-derivative by the loss of a *n*-hexanol molecule. For *o*-aminophenol the following overall reaction can be proposed:



As the cyclic derivative is produced in constant and very high yield, it can be conveniently used for the analytical determinations. Low detection limits (see below) could be obtained, using its protonated molecular ion at m/z 264, almost a unique signal in the CI mass spectrum. The same ion also represents the base peak in the mass spectra of *m*-aminophenol,

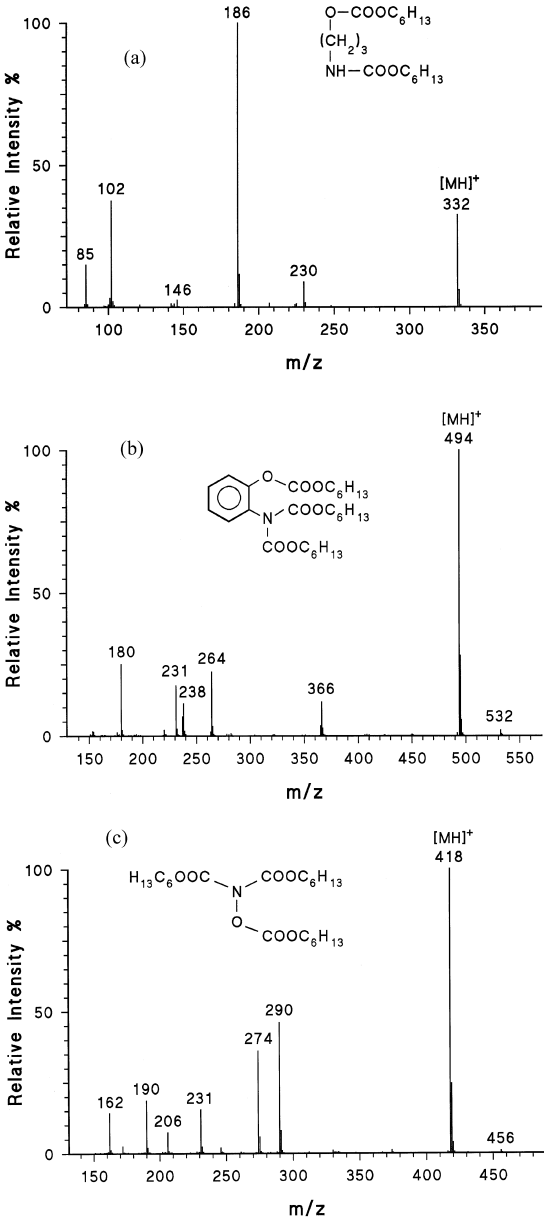


Fig. 1. Positive CI mass spectra (recorded by the magnetic instrument) of the derivatization products obtained from different analytes. (a) 3-Aminopropanol; (b) *o*-aminophenol; as the protonated molecular ion is an even-electron species, fragmentation takes place by loss of neutral molecules accompanied by transposition of one hydrogen atom, such as losses of $COOC_6H_{12}$ (128), $C_6H_{13}OH$ (102) and C_6H_{12} (84). (c) Hydroxylamine; protonation at the nitrogen atom activates the dissociation of both N–C and N–O bonds with transposition of one hydrogen atom, corresponding to neutral losses of $COOC_6H_{12}$ (m/z 290) and $OCOOC_6H_{12}$ (m/z 274), respectively.

p-aminophenol and N-phenylhydroxylamine bi-substituted derivatives. Therefore, the same ion chromatogram (*m*/*z* 264) (Fig. 2) can be utilized for the detection of the all aminophenol isomers and N-phenylhydroxylamine, even if the more compact chemical structure makes the GC retention time for the *o*-aminophenol derivative ($t_R = 14.12$ min) considerably shorter than that for *m*- and *p*- aminophenol ($t_R = 18.10$ min and $t_R = 18.28$ min, respectively, under the present experimental conditions) and N-phenylhydroxylamine ($t_R = 16.38$ min) bi-substituted derivatives ($[MH]^+$ *m*/*z* 366). From Fig. 2 it is evident that, besides *m*/*z* 264, also the protonated molecular ion $[MH]^+$ (*m*/*z* 494) of *o*-aminophenol tri-substituted derivative can be utilized for the analytical determination of such compounds, despite the incomplete (25%) conversion yield. To compensate this effect, good sensitivity is assured by the small chance of chemical interferences from reaction byproducts in both the high mass and the high retention time, as is evident from Fig. 2. In particular, the mass spectrum of *o*- aminophenol tri-derivative (Fig. 1b) is characterized by an abundant protonated molecular ion and very little fragmentation, with no fragment peak exceeding 25% relative intensity.

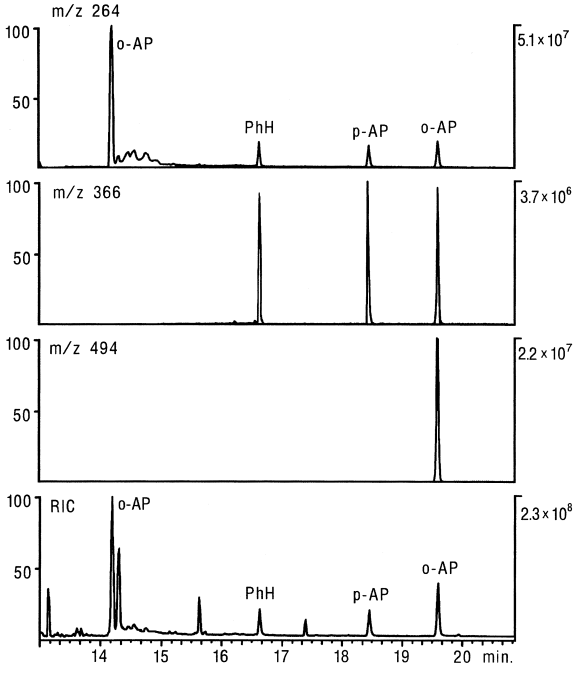


Fig. 2. Reconstructed total ion current (RIC) and reconstructed ion chromatograms (*m*/*z* 264, 366 and 494) obtained from an experiment where a 30 mg/l mixture of phenylhydroxylamine, *o*-aminophenol and *p*-aminophenol was derivatized. The magnetic mass analyzer was continuously scanned from *m*/*z* 150 to *m*/*z* 560. Besides the derivatization products, in the RIC trace are evident some peaks relative to chloroformate byproducts. The numbers on the right side of GC traces are ion current counts, an arbitrary scale proportional to the intensity of the signal.

All the active hydrogens of hydroxylamine and substituted hydroxylamines undergo derivatization by *n*-hexyl chloroformate. So, from hydroxylamine the tri-substituted derivative is obtained, while methyl- and phenyl-hydroxylamines yield the bi-substituted derivative and N,N-dimethylhydroxylamine the mono-derivatized product. The case of hydroxylamine is particularly interesting for the analytical applications. In fact, this is an extremely hydrophilic molecule, which cannot be extracted from the aqueous phase without modifying its structure by some form of derivatization. Moreover, hydroxylamine does not contain chromophores and its low molecular mass ($M_r = 33$) makes the determination by mass spectrometric methods difficult. The derivatization with *n*-hexyl chloroformate converts hydroxylamine into an extremely hydrophobic structure, which can be quantitatively extracted into *n*-hexane. With a single and rapid reaction, its molecular mass is increased from M_r 33 to M_r 417, and the derivative is eluted from the GC column at a convenient retention time ($t_R = 16.43$ min), yielding a narrow and symmetrical peak that can be determined with high sensitivity. The mass spectrum of the hydroxylamine derivative is shown in Fig. 1c. The protonated molecular ion $[MH]^+$, at *m*/*z* 418, provides the base peak of the spectrum and only little fragmentation (details are reported in figure) is observed. Thus, as for Fig. 1b, almost the entire analytical signal is concentrated in a single mass peak, located in the high mass range, where interferences are seldom encountered.

Also the direct derivatization of N-phenylhydroxylamine (a relatively labile molecule) in water has interesting analytical applications. We were able to identify it, at tens of micrograms/litre concentration, as an unstable intermediate in the photocatalytic interconversion of nitrosobenzene and aniline in aqueous slurries [36].

With regard to the remaining analytes considered (Table 1), hydroxyurethane and acetohydroxamic acid have been considered in detail elsewhere [9]. Hydrazine undergoes double derivatization, under the present experimental conditions. It is not clear at the moment why complete (quadruple) derivatization is not achieved for this molecule, unlike hydroxylamine. Steric hindrance cannot be invoked as an explanation, since we obtained quadruple derivatization of hydrazine with even more bulky substituents [34]. However, the substitution of two active hydrogens with *n*-hexylcarboxylic groups proved sufficient to make the derivative easily extractable from the aqueous phase into *n*-hexane. The CI mass spectrum of such compound is similar to that of the hydroxylamine derivative, as it is characterized by an abundant protonated molecular ion $[MH]^+$, at m/z 289, and little fragmentation. Quite a large signal could be obtained in the m/z 289 ion chromatogram.

3.3. Analytical performance

The analytical performances of the new derivatization procedure have been tested by using various detection conditions, in order to ascertain the general applicability of the method. Two different mass spectrometers were utilized, namely a large magnetic instrument and a small benchtop quadrupole mass-detector. With the magnetic mass spectrometer, we operated in the scan mode, while the quadrupole mass-detector was mainly utilized in the selected ion monitoring mode, for quantitation purposes. Quite obviously, the overall sensitivity of the method depended not only on the intrinsic efficiency of the derivatization procedure, but also on the instrument and detection conditions chosen for the analysis. However, we did not notice significant differences in the ion chromatograms obtained by running the quadrupole mass-detector in the SIM mode and those reconstructed from mass spectra obtained by fast scanning the magnetic mass-analyser over small mass ranges (50–200 u). The detection limits discussed in the forthcoming paragraphs can be referred to any of these conditions.

The second element considerably affecting the sensitivity of the method is the concentration factor. In our standardized procedure, equal volumes of the aqueous sample solution and the extraction solvent were utilized. However, if the initial solution is quite clean, as in our case, the hexane solution to be analysed can be concentrated up to 30 times, resulting in much lower detection limits. As the practicality of the procedure has to be checked, we measured all detection limits without any concentration of the extracts. The detection limits for all the analytes tested are indicated in Table 3. For ethylene

Table 3
Detection limits (D.L., calculated on the basis of a 5:10 S/N ratio) and calibration curve parameters for ten analytes

Analyte	D.L. (μg/l)	$m \pm \Delta m$ (S.E.)	Δm (95% CI)	r
2-Aminoethanol	100	0.77±0.08	±0.26	0.988
3-Aminopropanol	100	0.83±0.03	±0.08	0.998
o-Aminophenol	10	0.83±0.07	±0.24	0.988
m-Aminophenol	100	0.81±0.07	±0.23	0.989
p-Aminophenol	30	1.11±0.06	±0.18	0.992
Aniline	30	1.27±0.07	±0.22	0.990
Hydroxylamine	10	0.90±0.08[a]	±0.27[a]	0.992[a]
N,N-Dimethylhydroxylamine	100	0.76±0.03	±0.09	0.998
Hydroxyurethane	100	1.15±0.04	±0.11	0.998
Acetohydroxamic acid	100	1.28±0.06	±0.17	0.992

[a] Calculated without considering the 30 mg/l point, for which saturation of the signal was observed.

Detection limits are obtained from 1 μl injection of non-concentrated extracts, at the same dilution as the starting aqueous solutions.

Angular (m) and correlation (r) coefficients refer to bilogarithmic plots of integrated selected ion current vs. concentration in the range 0.1–30 mg/l (6 points), unless specified otherwise.

The standard error Δm (S.E.), is expressed as the square root of the variance of the angular coefficient.

Δm (95% CI) is the confidence interval qualified by 95% confidence level. Calibration curves were obtained by operating the quadrupole mass-detector in SIM mode.

glycol and hydroquinone, the detection limits previously indicated [7] have been improved to 100 μg/l and 30 μg/l, respectively. Detection limits are different for the various compounds (Table 3), because they depend on (i) the derivatization yield, (ii) the response factor for the ion selected to represent the derivative, (iii) the interferences present in the ion-chromatogram at retention times close to that of the analyte. Fig. 3 reports the *m/z* 418 ion-chromatogram relative to the tri-derivative obtained from a 10 μg/l solution of hydroxylamine. The effect of a 30× concentration of the hexane extract is shown in Fig. 4, which reports the *m/z* 222+264 ion-chromatogram obtained from a 1 μg/l solution of aniline and 2-aminophenol. Upon 30× concentration of the extracts, detection limits of 300 ng/l were obtained for both 2-aminophenol and hydroxylamine.

Calibration curves were calculated in bi-logarithmic plots, in order to have homogeneous distribution of data-points along the graph. The experimental angular coefficients are reported in Table 3 along with their errors. Linear relationship between the integrated peak in the ion-chromatogram and the analyte concentration were found for all compounds tested in the 100 μg/l–30 mg/l range. Most angular coefficients are close to one, as expected. Positive deviations are associated with partial loss of signal in the low concentration range, usually due to a noisy background. Negative deviations are possibly due to incomplete derivatization at the highest concentrations or to saturation effects. Intercepts are of little significance in bilogarithmic plots and, therefore, are not reported in Table 3. Repeatability is excellent under strictly controlled and constant operating conditions, during both the derivatization steps (times, volumes, etc.) and the analysis (pressure of the CI reagent gas, temperature of the ion-source, etc.).

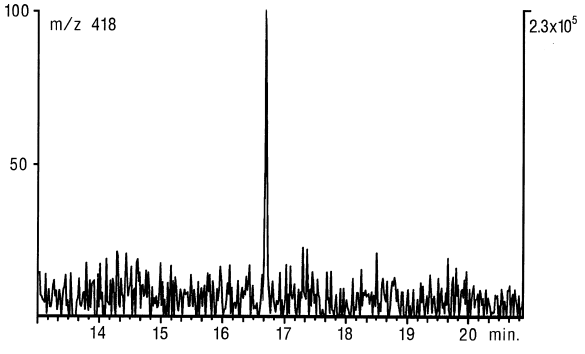


Fig. 3. Reconstructed ion-chromatogram of *m/z* 418, evidencing the GC peak relative to the tri-substituted derivative of hydroxylamine, obtained from a 10 μg/l water solution, without concentration of the extract. The magnetic instrument under continuous scanning was utilized.

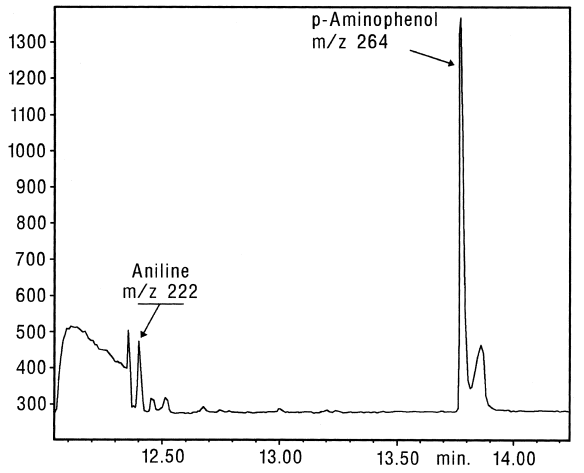


Fig. 4. Sum of the *m/z* 222 and *m/z* 264 ion-chromatograms, relative to the derivatization products of aniline and *o*-aminophenol. The injected sample was obtained from a 1 μg/l water solution of each analyte, which was derivatized, extracted in hexane and concentrated 30× before the analysis. The analysis was executed by the quadrupole mass detector under SIM conditions.

4. Conclusions

In the present study, it was demonstrated that the procedure, previously developed to derivatize polycarboxylic and polyhydroxylic substances directly in water, is effective also in the derivatization of amino alcohols, aminophenols and hydroxylamines. All these highly hydrophilic compounds could be converted into hydrophobic derivatives and then extracted from water and analyzed by a sequence of simple and very rapid steps (2–3 min, excluding the analysis time).

In order to increase the derivatization yields, important improvements have been introduced in the procedure with respect to the conditions previously

published. These involved a different sequence of the procedure steps and the introduction of dicyclohexylcarbodiimide as co-catalyst for the derivatization. These innovations also proved advantageous for the classes of compounds considered in the previous studies, resulting in lower detection limits and a wider applicability.

The general applicability of this procedure is unique, as compared to more conventional derivatizations, and allows direct treatment of the aqueous matrix, with good sensitivity. Even if we believe that the most effective employment of this method is for the quantitative determination of pre-defined analytes, interesting applications might also be found in the identification of unknown components of aqueous solutions, such as, for example, water treatment byproducts in potable water.

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