Analysis of structural isomers of polychlorinated phenols in water by liquid-nitrogen-trapping gas chromatography–Fourier transform infrared spectroscopy

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Abstract

A procedure is described for the identification and determination of structural isomers of polychlorinated phenols in drinking water. First, their acetylation and concentration on graphitized carbon cartridges are carried out. Detection is accomplished by gas chromatography (GC) coupled with Fourier transform infrared spectroscopy (FTIR) using a direct deposition (DD) interface. In this way it is possible to accurately identify and differentiate variably substituted isomers of chlorophenols, which is very difficult or even impossible to do by means of the widespread gas chromatography–mass spectrometry (GC–MS) instrumentation. Chlorophenols occurring in water samples have been identified according to their IR spectra and additionally the identification was confirmed by means of the MS–MS spectra obtained with a GC–MS–MS tandem instrument.

Keywords: Water analysis; Environmental analysis; Infrared spectroscopy; Detectors, GC; Chlorophenols.

1. Introduction

Phenols and in particular chlorophenols are found in aqueous media as by-products of the carbon and oil industry [1]. The formation of chlorophenols can take place as a result of the degradation of insecticides and weed-killing agents in soils [2] as well as from unchlorinated phenols present in water submitted to a chlorination process.

The U.S. Environmental Protection Agency (EPA) [3] has elaborated a list of 11 phenols considered as high-priority pollutants in aquatic media, of which chlorophenols are the most toxic

and carcinogenic. The European Union (EU) [4] legislation also established the admissible maximum concentration of phenols present in drinking water at 0.5 ng/ml.

Since low levels of phenols are to be determined in aqueous samples, a previous concentration step is required. For this purpose, liquid–liquid extraction systems have been used [5–7], as well as solid-phase adsorbents such as C_{18} [8,9] and graphitized carbon [10–13].

Nowadays, the most widely used analytical technique for the determination of phenols is gas chromatography. However, phenols tend to produce broad peaks (often with tails) due to their high polarity and low vapour pressure, which increase with ageing of the column [14].

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To avoid this disadvantage, the derivatization of the phenols is carried out to give less polar compounds with better chromatographic characteristics, and to facilitate extraction or concentration procedures [15]. Among the derivatization procedures used, one of the simplest and most common is direct acetylation with acetic anhydride in the presence of carbonate or bicarbonate [16,17].

In the case of matrices like water, when large sample volumes (1 l or more) are concentrated, the chromatograms obtained show such a high number of peaks that it is difficult to reliably identify and determine the phenols. Nevertheless, there are two possible alternatives to overcome this problem. The first one consists of using highly selective detectors that only monitor the analytes of interest; an example of this is GC–microwave induced plasma atomic emission detection (MIP-AED) coupling, which permits a highly selective determination of chlorophenols in water samples, when the emission line of chlorine is monitored [13]. The second alternative is based on utilizing gas chromatography–mass spectrometry (GC–MS) or gas chromatography–Fourier transform infrared spectroscopy (GC–FTIR) couplings. In this case, although they are considered universal detectors, the corresponding spectra to each peak are obtained. This ensures the reliable identification of phenols, provided that a suitable spectral library is available.

The GC–MS coupling is widely used for peak characterization in chromatography due to its high sensitivity and rapidity. However, it cannot differentiate structural isomers [18]. GC–FTIR coupling, on the other hand, permits — in principle — a solution to this problem [19,20]. Several applications are found in the literature using the combination of these two techniques for the quantitation and differentiation of structural isomers of polychlorinated biphenyls (PCBs) [21] and other environmentally interesting compounds [22,23].

The sensitivity obtained by GC–FTIR depends heavily on the interface used between the chromatographic column and the spectroscopic device. The most common interfaces in the GC–FTIR coupling are described below.

In flow-cell interfaces (light pipe) the eluent of the column flows through a cell with windows which are transparent to IR radiation. In this way transmittance spectra are obtained as the analytes leave the column. In this sort of interface the key factor is volume, which ranges between 50 and 100% of the volume of carrier gas corresponding to the peak width at half its maximum height [24]. A smaller volume implies a loss of sensitivity, whereas an excessively large volume results in a degradation of the chromatographic resolution. This interface has been used in the study of environmental samples, but the identification limits are found in the range 20–50 ng for species with strong absorbent groups in the IR region [23]; therefore, it is often necessary to resort to techniques which permit injection of large sample volumes (higher sensitivity [25]). Another alternative may be to preconcentrate the samples with high enrichment factors.

In matrix isolated interfaces (GC–MI–FTIR) the eluates of the column together with an argon matrix are deposited on a mobile metal disk placed in a vacuum chamber at 12 K. Helium is used as the carrier gas in the chromatograph, since it does not condense on the disk at this temperature, while the molecules of analyte and argon do.

Once chromatographic separation is over, absorption spectra are obtained by reflection from each of the deposits trapped on the disk. According to the literature [26,27], this interface is about 50 times more sensitive than those based on the flow-cell principle.

With interfaces of direct deposition (GC–DD–FTIR) the analytes eluting from the column are deposited on a mobile plate of ZnSe (which is transparent to infrared radiation) located in a vacuum chamber cooled by liquid nitrogen. The chromatograms are obtained continuously, since a few seconds after each condensed drop of the eluate is deposited on the window of zinc selenide, its spectrum is shown. The transmitted radiation is focused by means of a series of lenses and the objective of a microscope on an MCT-type detector (mercury, cadmium, tellurium). The software of the equipment stores the position of each component (chromatographic peak) on the slide; thus, when the chromatographic

separation ends we can again focus the radiation of the source on the corresponding deposit of a given component and obtain its spectrum with a better signal-to-noise ratio. This is achieved through continuous scans and signal averaging. According to the literature [28–31], this sort of interface permits detection limits of about 300 pg for substances acting as strong absorbents in the IR region. Furthermore, this limit can be improved by signal averaging once the chromatograms are finished.

In this work a procedure is described for the determination of structural isomers of chlorophenols in samples of water. It is based on their direct acetylation in the original samples with alkaline acetic anhydride, followed by solid–liquid extraction of the derivatized compounds on graphitized carbon cartridges and determination of these derivatives by GC–DD–FTIR, GC–MS and GC–MS–MS. The purpose of this work is to compare the advantages and drawbacks of these techniques in the identification and differentiation of the structural isomers of chlorophenols, as well as to evaluate the usefulness of the GC–DD–FTIR techniques for the quantitative determination of such substances present under the levels permitted by international legislation [3,4].

2. Experimental

2.1. Reagents

Methanol, hexane, potassium carbonate and acetic anhydride of maximum purity were supplied by Merck (Darmstadt, Germany). Tetramethylammonium hydroxide (TMAOH) was supplied by Aldrich (Milwaukee, WI, USA).

The standards of 2-chlorophenol (2CP), 3-chlorophenol (3CP), 4-chlorophenol (4CP), 2,6-dichlorophenol (26DCP), 2,5-dichlorophenol (25DCP), 2,4-dichlorophenol (24DCP), 3,5-dichlorophenol (3,5DCP), 2,3-dichlorophenol (23DCP), 3,4-dichlorophenol (34DCP), 2,4,6-trichlorophenol (246TCP), 2,3,6-trichlorophenol (236TCP), 2,3,5-trichlorophenol (235TCP) 2,4,5-trichlorophenol (245TCP), 2,3,4-trichlorophenol (234TCP), 2,3,5,6-tetrachlorophenol (2356TCP), 2,3,4,6-tetrachlorophenol (2346TCP) and penta-

chlorophenol (PCP) were supplied by Aldrich and Merck.

Stock solutions of 4.0 mg/ml were prepared for each of the standards in methanol. These solutions were stored at 4°C, and light protected. Working solutions were prepared by mixture and appropriate dilution from the individual stocks.

Tap water samples were preconcentrated by means of graphitized carbon black cartridges, Supelclean, ENVI-Carb SPE (Supelco, Bellefonte, PA, USA) of 0.25 and 0.50 g.

2.2. Equipment

Gas chromatography–FTIR spectroscopy

A Hewlett-Packard (HP) (Avondale, PA, USA) Model 5890 Series II plus gas chromatograph, equipped with electronic pressure control (EPC) and an on-column injector, was connected to the FTIR instrument. The chromatographic column was a BP-5 (Scientific Glass Engineering, Ringwood, Australia) (30 m × 0.32 mm I.D.) methylphenylsilicone capillary column, 0.25 μm film thickness. Helium (99.999%) was used as carrier gas. Optimal parameters have been summarized in Table 1.

Table 1
GC–DD–FTIR conditions in the separation of chlorophenols (as acetates)

Chromatographic parameters	
Injection port	On column
Injection port temperature	250°C
Injection volume	1.5 μl
Column flow	0.9 ml/min
Oven program	
Initial temperature	60°C
Initial time	1 min
Rate	15°C/min
Temperature	115°C
Time	5 min
Rate	3°C/min
Temperature	175°C
Rate	30°C
Temperature	250°C
Final Time	10 min
Tracer conditions	
Transfer line temperature	250°C
Tip temperature	250°C

Gram–Schmidt reconstructed chromatograms (GSCs), functional group chromatograms (FGCs) and IR spectra were recorded using a Bio-Rad Digilab (Cambridge, MA, USA), FTS 45A spectrometer equipped with a Digilab Tracer direct deposition interface and an MCT detector that is able to record IR spectra between 4000 and 700 cm^{-1}. The spectroscopic system is controlled by a Bio-Rad data station SPC 3200. Spectra were collected by the addition of four scans at a resolution of 8 cm^{-1}.

Gas chromatography–mass spectrometry

A Varian Saturn 4 system (Walnut Creek, CA, USA) consisting of a Varian Star 3400 CX gas chromatograph connected to an ion trap mass spectrometer (ITD Varian Saturn 4). The injec-

tor was a septum-equipped programmable injector (SPI, Varian 1093). The chromatographic column was a DB-5 (J&W Scientific, Folsom, CA, USA) (30 m × 0.25 mm I.D.) methylphenylsilicone capillary column, 0.25 μm film thickness, using helium (99.999%) as carrier gas.

Mass spectra were recorded and processed by means of the Saturn software v. 5.0., running on a 486/50 computer. Table 2 summarizes the operational parameters used in GC–MS and GC–MS–MS analyses.

2.3. Acetylation of standards

The procedure followed for the acetylation of phenol standards used for calibration or making

Table 2
GC–MS conditions in the separation of chlorophenols (as acetates)

Chromatographic parameters	
Injection port	On column
Injection volume	1 μl
Column head pressure	8 psi
Transfer line temperature	260°C
Oven program	
As in Table 1	
Injector temperature program	
Initial temperature	60°C
Initial time	0.10 min
Rate	300°C/min
Final temperature	260°C
Final time	10 min
Mass spectrometry parameters (EI)	
Filament emission current	30 μA
Manifold temperature	170°C
Axial modulation	4.0 v
Maximum ionization time	25 ms
Scan range	55–300 m/z
Mass–mass spectrometry parameters	
Autogain control	On
Filament emission current	80 μA
Mass isolation window	3.0 m/z
Collision-induced dissociation (CID)	Waveform type: non-resonant
Excitation amplitude	Depending on ions (30–100 V)
Excitation storage level	Depending on ions (48–100 m/z)
Excitation time	20 ms

a library is based on that proposed by Renberg and Lindstrom [8], and recently optimized [32]. In it, 2 ml of a 5% K_2CO_3 solution and 2 ml of hexane containing 200 µl of acetic anhydride are added to 1 ml of a methanolic solution with different concentrations of the studied phenols. Then the mixture is stirred for 1 min and the organic phase separated. The aqueous phase (methanol–water) is extracted again with 1 ml of hexane (now without derivatizing agent). Both hexane phases are combined and dried with anhydrous sodium sulphate and later injected into the corresponding chromatographic system (GC–FTIR or GC–MS). In this way, by derivatizing different concentrations of phenols, we obtain calibration graphs in which we draw the concentration of each one of the phenols versus the peak height of their corresponding acetylated compounds. Calibration curves have been obtained in the range between 0.1 and 5 µg/ml, with seven levels of calibration in each one of them.

2.4. Sample treatment

A known amount of a standard of chlorophenols in methanol is added (in the case of spiked samples) to the sample of water (Milli-Q or tap water, previously filtered), and the pH is adjusted to 11–11.5 with K_2CO_3. Then acetic anhydride is added (5 ml/l water) and the mixture submitted to mechanical stirring for 15 min [33,34]. Finally, a small volume of methanol (around 2% of the volume of the sample) is added, and the solution passes through a previously conditioned carbon cartridge.

The conditioning of the cartridge is carried out as follows: Washing with 5 ml of methanol followed by activation of the cartridge with 5 ml of Milli-Q water at pH 2–3.

Once the samples are passed through the cartridges, they are dried for 20 min by means of an N_2 stream and further eluted with 3 ml of hexane containing 1% tetramethylammonium hydroxide (countercurrent with respect to sample flow). The final extract is concentrated by an N_2 stream of 55 kPa at room temperature to a final volume of 0.5 ml (using a Turbo Vap II Worksta-

tion; Zymark Corporation, Hopkinton, MA, USA). The final extract is then ready for injection in one of the chromatographic systems (GC–FTIR or GC–MS).

3. Results and discussion

One of the parameters that mostly affects the chromatographic resolution and the sensitivity of the GC–FTIR coupling is the interface. In the system studied, the resolution between chromatographic peaks is partly determined by the speed at which the slide of ZnSe is moving. The higher it is, the better the resolution will be. However, the thickness of the deposits collected on the slide will also be smaller, which implies a sensitivity decrease. Since the above-mentioned speed may be programmed by the user, it is possible — in each concrete case — to reach a compromise between resolution and sensitivity according to the objectives of the analysis.

Under the working conditions shown in Table 1, detection limits of 0.1 µg/ml for $S/N = 10$ have been obtained for each of the studied compounds when the chromatograms corresponding to the absorption in the region between 1750 and 1850 cm^{-1} were monitored (Fig. 1). Resolution appears adequate for quantitative
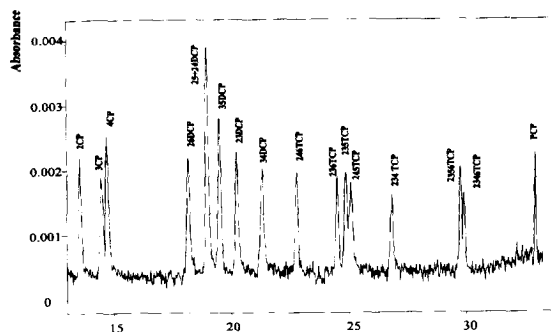

Fig. 1. Chromatogram for a standard of chlorophenols in the functional region 1750–1860 cm^{-1}. 2CP (0.18 µg/ml), 3CP (0.16 µg/ml), 4CP (0.19 µg/ml), 26DCP (0.18 µg/ml), 25DCP (0.19 µg/ml), 24DCP (0.19 µg/ml), 35DCP (0.21 µg/ml), 23DCP (0.19 µg/ml), 34DCP (0.18 µg/ml), 246TCP (0.16 µg/ml), 236TCP (0.18 µg/ml), 235TCP(0.18 µg/ml), 245TCP (0.18 µg/ml), 234TCP (0.15 µg/ml), 2356TCP (0.20 µg/ml), 2346TCP (0.18 µg/ml), PCP (0.19 µg/ml).

purposes, with the exception of 25DCP and 24DCP, which appear to overlap fully.

3.1. Qualitative analysis

GC–DD–FTIR system

The identification of the peaks corresponding to each of the individual chlorophenols was accomplished according to their corresponding IR spectra. We should take into account the fact that the derivatization of chlorophenols to obtain peaks with better chromatographic characteristics also modifies their IR spectra considerably. The characteristic band of the hydroxyl group above 3000 cm^{-1} disappears, and instead there is a sharp, narrow band corresponding to the carbonyl group that has been introduced into the molecule.

Since the IR spectra of the solid derivatized

species were not available (although, as a rule, the existing spectra in KBr pills are equivalent to those obtained by GC–DD–FTIR), an IR spectral library corresponding to the derivatized chlorophenols was developed. For this purpose, ca. 2 ng of each was injected and the spectrum recorded with a resolution of 8 cm^{-1}, four scans per spectrum being averaged in the region between 4000 and 700 cm^{-1}. The region of interest is found between 2000 and 700 cm^{-1}. As we are working with compounds of which many are structural isomers and others belong to a homologous series, the differences between their
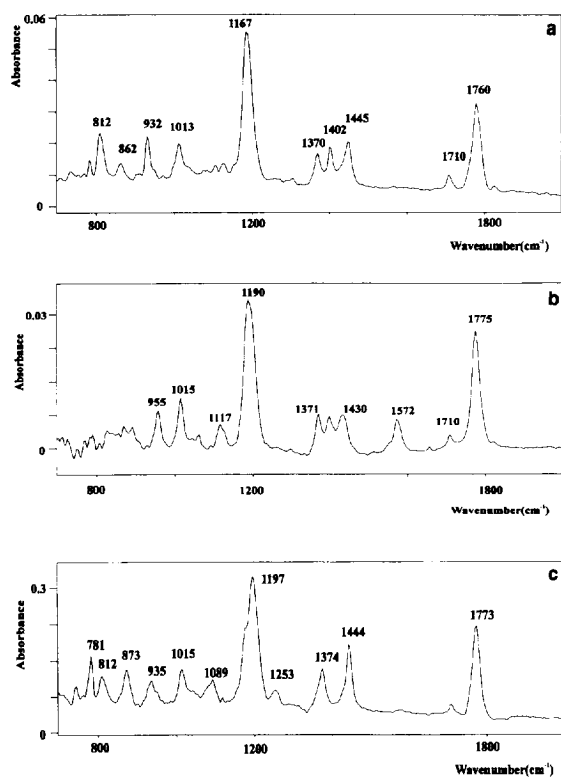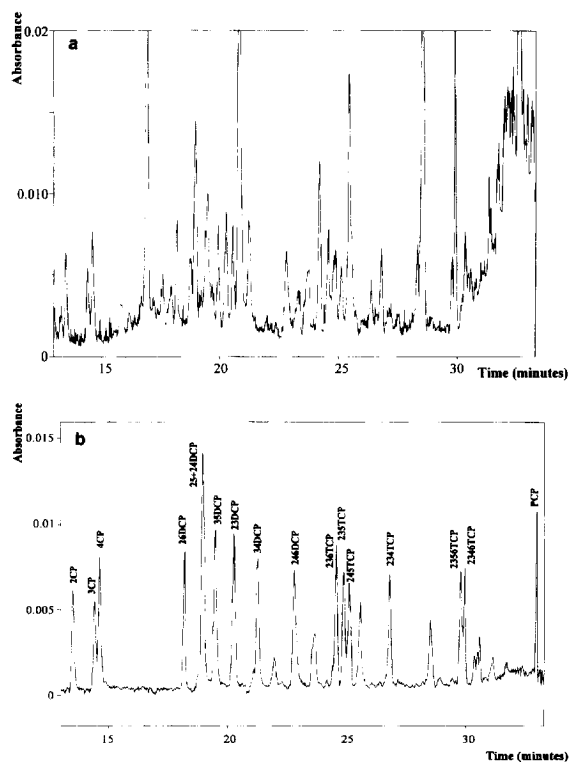

Fig. 3. Chromatogram for a sample of 1 l of spiked water at the following levels: 2CP (0.54 ng/ml), 3CP (0.48 ng/ml), 4CP (0.57 ng/ml), 26DCP (0.53 ng/ml), 25DCP (0.58 ng/ml), 24DCP (0.57 ng/ml), 35DCP (0.62 ng/ml), 23DCP (0.56 ng/ml), 34DCP (0.55 ng/ml), 246TCP (0.48 ng/ml), 236TCP (0.53 ng/ml), 235TCP (0.53 ng/ml), 245TCP (0.55 ng/ml), 234TCP (0.46 g/ml), 2356TCP (0.59 ng/ml), 2346TCP (0.55 ng/ml), PCP (0.56 ng/ml). (a) Gram–Schmidt reconstructed chromatogram; (b) functional group chromatogram (1750–1860 cm^{-1}).

Fig. 2. IR spectra for three isomeric chlorophenols (as acetyl derivatives): (a) 236TCP (2.13 ng), (b) 235TCP (2.36 ng), (c) 234TCP (1.84 ng).

spectra are not very obvious, although they are significant enough to permit their differentiation (Fig. 2).

The IR spectra obtained for water samples with or without the addition of phenols, after derivatization and concentration through carbon cartridges, were compared with those in the library. As shown in Fig. 3a, the chromatograms of the water samples show a great number of peaks; therefore, it is recommended to monitor the chromatograms corresponding to absorbance in concrete regions of the IR spectrum (Fig. 3b). Depending on the sample composition, monitoring in the 1150–1250 cm^{-1} or 1750–1860 cm^{-1} region could be advantageous, although both regions produce very similar chromatograms. In the region 1150–1250 cm^{-1} a reduced number of strange peaks appears; the region 1750–1860 cm^{-1} (Fig. 3b) exhibits better signal-to-noise ratios. In this way, much more selective records are obtained, from which it is easier to identify the chlorophenols.

As measure of the similarity (hit quality index, HQI) between the library spectra and those of the compounds whose identity is to be confirmed, the Euclidean distances have been utilized in such a way that HQI = 0 indicates that there is no difference between the spectra, whereas a value of 2 would correspond to the maximum difference. Values under 0.4 can be considered as valid identifications; even HQI values slightly exceeding 0.4 can correspond to valid identifications, provided that there is a difference of 0.15 or greater from the HQI of the following compound.

There can also be cases of high HQI values when the spectrum that is being compared with the library presents a low signal-to-noise ratio. In this case, due to the fact that the different substances separated by the column remain trapped on the ZnSe slide, we can focus the IR radiation on them and obtain their spectra by averaging a greater number of scans once the chromatographic separation is over. Noise then
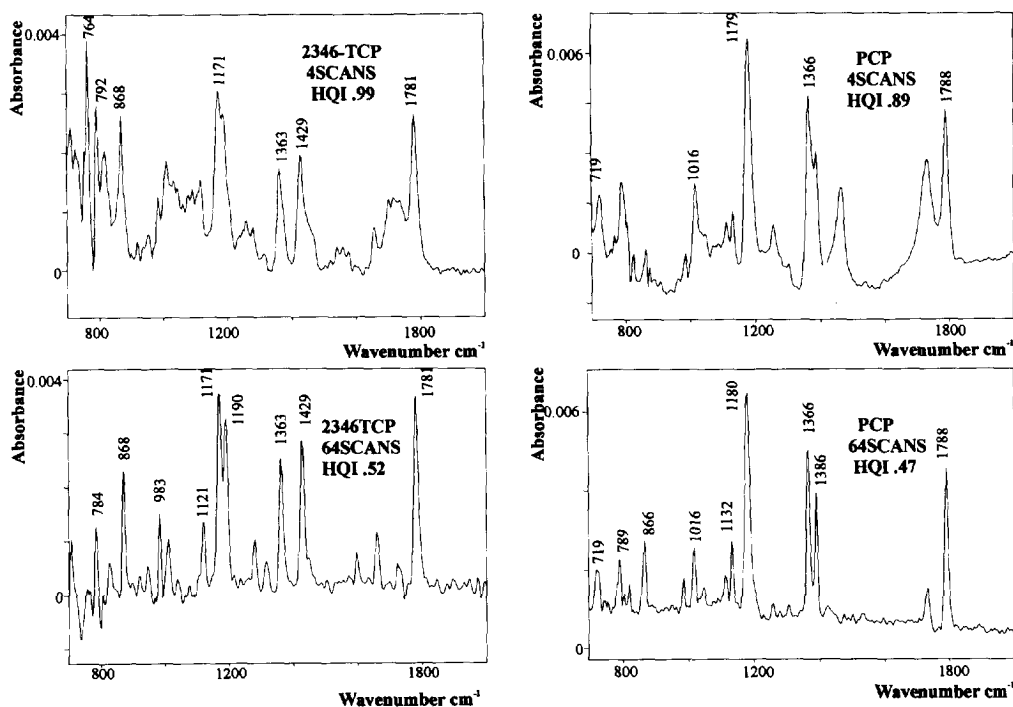

Fig. 4. Influence of the number of scans per spectrum on the signal-to-noise relationship of the IR spectra of two chlorophenols, from a sample of spiked water, 2346TCP (0.55 ng/ml) and PCP (0.56 ng/ml).

decreases and identifications improve (Fig. 4, Table 3).

Another factor affecting the identification of the compounds is the presence of water in the system. Because the slide is maintained at the temperature of liquid nitrogen, any trace of water arriving at the slide from the column, no matter how small it may be, is deposited as ice, along with the analytes, thus interfering with their IR spectra. Therefore, it is of vital importance that all the connections in the chromatographic system be free of leakage. Furthermore, the use of metal ferrules in the connections is required because graphite permits the passage of water by diffusion. These water interferences become increasingly important with increasing temperature in the chromatographic system and decreasing speed of the slide (Fig. 5).

We have not been able to separate 2,5- and 2,4-dichlorophenol with the chromatographic column used. Nevertheless, it is possible to identify 2,5-dichlorophenol at the beginning of the peak and 2,4-dichlorophenol in the tail provided that we obtain the IR spectra at the beginning and in the tail of the chromatographic peak corresponding to these two compounds with a good signal-to-noise relationship. At the maximum of the peak, we have an identical HQI for both of them (Table 3). In this case, it is possible to resort to a multivariate calibration through PLS2 (available in the software of the Bio-Rad data station associated with the instrument) to achieve the quantitation of both co-eluting species.

GC–MS system

In order to confirm the identification of chlorophenols in water samples through their IR spectra, similar samples were injected into the GC–MS system. The mass spectra obtained were compared with those in the NIST90 library. As found in the literature [18,35], it is impossible to

Table 3
Identification of chlorophenols in a spiked water sample, using an IR library made in the laboratory

| Compound	Conc. (ng/ml)	4 Scans per spectrum		64 Scans per spectrum	
		Euclidean distance	Right identification	Euclidean distance	Right identification
2CP	0.54	0.46	Yes	0.31	Yes
3CP	0.48	0.44	Yes	0.30	Yes
4CP	0.57	0.31	Yes	0.19	Yes
26DCP	0.53	0.52	Yes	0.43	Yes
25 + 24DCP	0.115				
Beginning		0.36 (25D)		0.24 (25D)	
		0.38 (24D)		0.32 (24D)	
Maximum		0.34 (24D)		0.27 (25D)	
		0.36 (25D)		0.27 (24D)	
Tail		0.28 (24D)		0.24 (24D)	
		0.35 (25D)		0.33 (25D)	
35DCP	0.62	0.28	Yes	0.26	Yes
23DCP	0.56	0.39	Yes	0.25	Yes
34DCP	0.55	0.45	Yes	0.32	Yes
246TCP	0.48	0.60	Yes	0.47	Yes
236TCP	0.53	0.45	Yes	0.36	Yes
235TCP	0.53	0.40	Yes	0.35	Yes
245TCP	0.55	0.64	Yes	0.37	Yes
234TCP	0.46	0.59	Yes	0.36	Yes
2356TCP	0.59	0.60	Yes	0.37	Yes
2346TCP	0.55	--	No	0.52	Yes
PCP	0.56	--	No	0.47	Yes
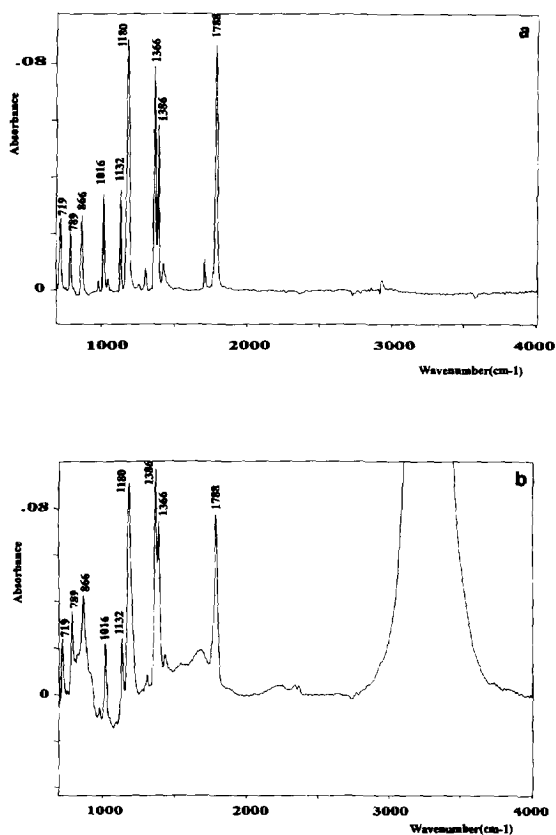
Fig. 5. Spectrum of a standard of PCP (2.98 μg/ml) at 8 cm⁻¹. (a) Normal slide speed, no ice interference; (b) slow slide speed, ice interference in the spectrum.

ions) were optimized for each group of isomers. This operation was carried out with the aim of obtaining spectra in which a high degree of fragmentation of the parent ion may be achieved. The purpose is to obtain characteristic, differentiating spectra for the structural isomers of chlorophenols (Fig. 6) which cannot be differentiated by their simple mass spectra. Table 4 shows the most important ions of the MS–MS spectra of the derivatized chlorophenols; the fragmentation conditions are indicated.

Following the same procedure as described above for the IR spectra, a library of MS–MS spectra of the chlorophenols was created by means of which the chlorophenols added to a Milli-Q water sample at levels of 0.5 ng/ml could be identified in a highly selective way. The exception was the region of monochlorophenols, where mass 100 corresponding to the fragmenta-

distinguish the structural isomers of chlorophenols with the same molecular mass.

An MS–MS system, however, allows for error-free identification of such substances if the fragmentation is properly adjusted. Therefore, the ions with the highest intensities in the electron-impact mass spectra are first submitted to isolation and then to fragmentation. In this way the mass–mass spectra of the derivatized chlorophenols are obtained. These ions correspond to the masses of the non-derivatized compounds (128 and 162 m/z, respectively) in the case of mono- and dichlorophenols and to those of a mass m + 2 of the non-derivatized compounds (198, 232, 266 m/z, respectively) for tri-, tetra- and pentachlorophenol. The fragmentation and storage conditions of the ions obtained (product
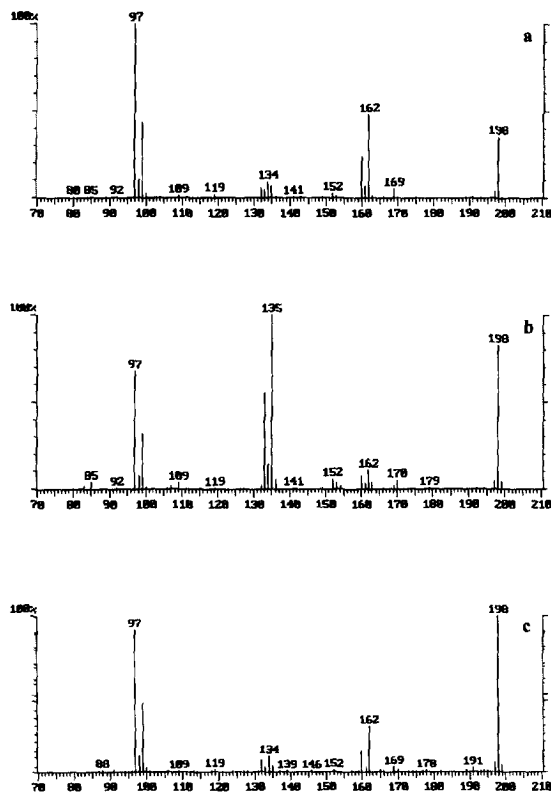

Fig. 6. MS–MS spectra for three isomeric chlorophenols: (a) 246TCP (16 pg), (b) 245TCP (18 pg), (c) 234TCP (15 pg).

Table 4
MS–MS spectra for chlorophenols

Compound	Excitation amplitude	Excitation storage level	Mass (intensity%)
2CP	68	60	128(100), 100(36), 92(29), 65(6), 126(3)
3CP	68	60	100(100), 128(69), 65(19), 92(7), 127(3)
4CP	68	60	128(100), 100(95), 65(16), 92(4), 73(3)
26DCP	81	75	162(100), 126(42), 99(23), 134(4), 98(2)
25 + 24DCP	81	75	162(100), 99(40), 126(20), 134(7), 98(3)
25DCP	81	75	162(100), 99(59), 126(13), 134(10)
24DCP	81	75	162(100), 126(24), 99(19), 134(4), 98(4)
35DCP	81	75	99(100), 162(73), 134(20), 126(7)
23DCP	81	75	126(100), 162(94), 98(22), 99(5), 91(3)
34DCP	81	75	162(100), 99(70), 134(18), 126(3)
246TCP	74	70	97(100), 162(48), 99(44), 198(35), 160(24)
236TCP	74	70	97(100), 99(47), 162(17), 198(11), 98(10)
235TCP	74	70	97(100), 99(48), 135(10), 98(10), 198(9)
245TCP	74	70	135(100), 198(83), 97(68), 133(55), 99(32)
234TCP	74	72	97(100), 99(45), 198(21), 162(12), 98(10)
2356TCP	82	80	133(100), 131(99), 232(42), 168(17), 196(13)
2346TCP	82	80	131(100), 134(95), 232(67), 196(25), 168(21)
PPCP	92	90	167(100), 165(60), 266(51), 230(17), 202(13)

tion of the parent ion must be monitored so that the chromatograms could be fully selective (Fig. 7).

3.2. Quantitative analysis by means of a GC–DD–FTIR system

Although the theoretically most interesting aspect of the GC–DD–FTIR combination is the identification of unknown substances through their IR spectra at concentration levels unreadable with flow-cell interfaces (light pipe), its

Fig. 7. MS–MS reconstructed chromatogram of 1 l of spiked water at the same levels as indicated in Fig. 3.

possible application in quantitative analysis has also been evaluated. For this purpose, chromatograms of the absorption in the IR region between 1750 and 1860 cm^{-1} have been chosen; this is the region corresponding to the vibration of the carbonyl group, which is a strong absorbent in the IR region.

In order to study the linearity of the system response as a function of the concentration, calibration curves were run for each of the derivatized chlorophenols, as stated in the Experimental section, in the range between 0.1 and 5 μg/ml. The calibration graph is linear throughout this concentration range (Table 5).

After obtaining the calibration graphs, the recoveries obtained on carbon cartridges for Milli-Q water samples were studied. The results are shown in Table 6. It should be noted that 2,5- and 2,4-dichlorophenols were determined through a multivariate calibration, since their chromatographic separation was not possible.
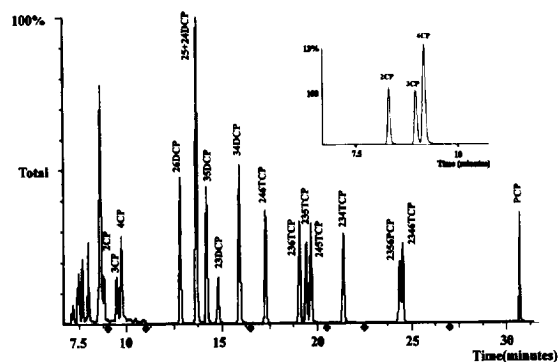
4. Conclusions

Both techniques (GC–DD–FTIR and GC–MS–MS) permit the differentiation of structural

Table 5
Study of the linearity of the response of the GC–DD–FTIR system

Compound	Slope ($\times10^3$)	Slope standard error ($\times10^3$)	Intercept ($\times10^3$)	Intercept standard error ($\times10^3$)	Correlation coeff. (R^2)
2CP	13.1	0.7	−6.1	2.1	0.992
3CP	15.1	0.4	−6.0	0.9	0.997
4CP	23.9	0.6	−11.3	1.8	0.997
26DCP	18.3	0.7	−7.4	2.0	0.992
25DCP + 24DCP	11.6	0.4	−6.8	2.2	0.995
35DCP	12.4	0.3	−5.2	1.1	0.996
23DCP	16.6	0.5	−6.7	1.5	0.995
34DCP	9.7	0.3	−3.3	1.0	0.994
246TCP	10.9	0.4	−3.0	0.9	0.995
236TCP	11.0	0.3	−3.4	0.7	0.997
235TCP	11.9	0.7	−2.5	1.8	0.985
245TCP	9.0	0.3	−3.0	0.9	0.994
234TCP	7.7	0.3	−2.1	0.6	0.994
2356TCP	8.4	0.3	−1.8	0.8	0.995
2346TCP	10.7	0.3	−2.9	0.8	0.997
PCP	15.3	0.8	−3.2	2.5	0.98

The range of concentration studied was between 0.1 and 5 μg/ml. Determinated at seven concentration levels. Each level was analyzed in duplicate.

Table 6
Recoveries from a sample of 500 ml Milli-Q water spiked with chlorophenols, derivatized and concentrated using a 0.25-g cartridge of graphitized carbon

Compound	Concentration (ng/ml)	Recovery (%)	S.D.
2CP	1.08	97.73	7.6
3CP	0.96	81.99	11.2
4CP	1.14	76.78	6.3
26DCP	1.07	83.47	7.4
25DCP	1.17	79.68	8.3
24DCP	1.13	79.30	8.7
35DCP	1.24	90.55	7.9
23DCP	1.12	85.29	4.4
34DCP	1.10	97.96	13.2
246TCP	0.95	88.53	5.7
236TCP	1.07	89.30	8.3
235TCP	1.06	76.51	5.6
245TCP	1.10	92.20	5.8
234TCP	0.92	95.15	11.3
2356TCP	1.18	83.81	13.7
2346TCP	1.10	74.50	8.5
PCP	1.12	71.89	6.9

Four samples measured in duplicate.

isomers of chlorophenols present in drinking water samples in quantities below the levels permitted by legislation (0.5 ng/ml). The GC–DD–FTIR system, although more sensitive than other GC–FTIR interfaces, is less sensitive than GC–MS–MS, which implies that scans must be averaged when levels near the detection limit are reached. This should be done once the chromatographic separation is over in order to obtain a better signal-to-noise ratio in the IR spectra and therefore a more reliable identification and quantification. The combination of this technique with appropriate preconcentration procedures allows very sensitive and accurate analyses. Even in cases where extensive peak overlapping occurs, compounds can be determined by resorting to PLS chemometric techniques.

The most serious problem of the GC–DD–FTIR coupling is the presence of water as an interfering agent in the spectra; any trace of water reaching the interface is deposited as ice on the ZnSe plate, thus interfering with the IR spectra of the analytes. This problem can be easily overcome by assuring leak-tight connections using metal ferrules.

References

[1] A.G. Calleley (Editor), Treatment of Industrial Effluents, Hodder and Stoughton, London, 1977, pp. 40–47.
[2] E.M. Lores, T.R. Edgerton and R.F. Moseman, J. Chromatogr. Sci., 19 (1977) 466.
[3] L.H. Keith (Editor), Compilation of Sampling Analysis Methods, U.S. Environmental Protection Agency, Boca Raton, FL, 1991, pp. 389–486.
[4] Directiva 80/77/CEE 15-7-1990; Diario Oficial de las Comunidades Europeas, 30-8-1990, European Community, Brussels, 1990.
[5] R.T. Coutts, E.E. Hargesheuner and F.M. Pasutto, J. Chromatogr., 179 (1979) 291–299.
[6] J. Knuutinen, J. Chromatogr., 209 (1981) 446–450.
[7] H.B. Lee, L.D. Weng and A.S.Y. Chau, J. Assoc. Off. Anal. Chem., 67 (1984) 789–794.
[8] L. Renberg and K. Lindstrom, J. Chromatogr., 214 (1981) 327–334.
[9] S.A. Schuette, R.E. Smith, L.R. Holdan and J.A. Graham, Anal. Chim. Acta, 236 (1990) 141–144.
[10] A. Di Corcia, S. Marchese, R. Samperi, G. Cecchini and L. Cirilli, J. Assoc. Off. Anal. Chem., 77 (1994) 446–453.
[11] C. Borra, A. Di Corcia, M. Marchetti and R. Samperi, Anal. Chem., 58 (1986) 2048–2052.
[12] A. Di Corcia, S. Marchese and R. Samperi, J. Chromatogr., 642 (1993) 163–174.
[13] M.I. Turnes, I. Rodriguez, M.C. Mejuto and R. Cela, J. Chromatogr. A, 683 (1994) 21–29.
[14] P. Mubmann, K. Levsen and W. Radeck, Fresenius J. Anal. Chem., 348 (1994) 654–659.
[15] V. Janda and H. van Langenhove, J. Chromatogr., 472 (1989) 327–330.
[16] H. Lee, R.L. Hong-You and A.S.Y. Chau, J. Assoc. Off. Anal. Chem., 67 (1984) 789–794.
[17] H. Lee, R.L. Hong-You and A.S.Y. Chau, J. Assoc. Off. Anal. Chem., 68 (1985) 422–426.
[18] P.R. Griffith., S.L. Pentoney and K.L. Shafer, Anal. Chem., 58 (1986) 1349–1364A.
[19] C. Fujimoto and K. Jinno, Anal. Chem., 64 (1992) 476–481A.
[20] P.R. Griffiths and K.L. Norton, Microchem. J., 46 (1992) 261–270.
[21] P. Doumenq, M. Guiliano and G. Mille, Talanta, 39 (1992) 149–154.
[22] D.F. Gurka, S.M. Pyle and I. Farnham, J. Chromatogr. Sci., 29 (1991) 339–344.
[23] D.F. Gurka and S.M. Pyle, Environ. Sci. Technol., 22 (1988) 963–967.
[24] P.R. Griffiths, Appl. Spectrosc., 31(1977) 284.
[25] J.H. Malissa, G. Szölgyenyi and G. Winsauer, Fresenius' Z. Anal. Chem., 321 (1985) 17–26.
[26] R.S. Brown and J.J. Lennon, Appl. Spectrosc., 45 (1991) 666–672.
[27] D.H. Hembree, A.A. Garrison, R.A. Crocombe, R.A. Yokley, E.L. Wehry and G. Mamatov, Anal. Chem., 53 (1981) 1783–1788.
[28] A.M. Haefner, K.L. Norton, P.R. Griffiths, S. Bourne and R. Curbelo, Anal. Chem., 60 (1988) 2441–2444.
[29] R.S. Brown and C.L. Wilkins, Anal. Chem., 60 (1988) 1483–1488.
[30] S. Bourne, A.M. Haefner, K.L. Norton and P.R. Griffiths, Anal. Chem., 62 (1990) 2448–2452.
[31] N.R. Smyrl, D.M. Hembree, W.E. Davis, D.M. Williams and J.C. Vance, Appl. Spectrosc., 46 (1992) 277–282
[32] I. Rodriguez, M.I. Turnes, M.C. Mejuto and R. Cela, J. Chromatogr. A, 721 (1996) 297.
[33] R.T. Coutts, E.E. Hargesheimer and F.T. Pasutto, J. Chromatogr., 179 (1979) 291–299.
[34] C. Schlett and B. Pfeifer, Vom Wasser, 79 (1992) 65–74.
[35] I.O.O. Korhonen and J. Kunutinen, J. Chromatogr., 256 (1983) 135–142.