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Discrimination of structural isomers of chlorinated phenols in waters using gas chromatography–mass spectrometry in the negative chemical ionization mode

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Abstract

The analysis and identification of structural isomers of mono-, di- and trichlorophenols is reported. The fragmentation of the phenols was examined by GC–MS in both electron impact (EI) and negative chemical ionization (NCI) modes, using methane as reagent gas. The ability of NCI to discriminate these isomeric compounds from differences in relative intensities for selected peaks is demonstrated. 3- and 4-chlorophenols have similar retention times; however, they can still be discriminated because their negative mass spectra are rather different. In dichlorophenols, the presence of one chlorine atom in the *ortho* position decreases their retention time and the relative intensity of the fragment ion at m/z 140. The NCI mass spectra for trichlorophenols are different from the rest, particularly for the m/z value corresponding to the chlorine atom. Tetra- and pentachlorophenols were also studied and sequential losses of Cl observed. An automatic solid-phase extraction system can optionally be used to preconcentrate chlorophenols in waters prior to determination at legally established toxic levels.

Keywords: Water analysis; Positional isomers; Chlorophenols; Phenols

1. Introduction

Research into the environmental fate of halogenated compounds has so far largely focussed on brominated and chlorinated compounds. The presence of phenol compounds in the environment is of great concern because they occur both as natural constituents and as the products of many industrial processes. Among them, chlorophenols are specially toxic and potentially carcinogenic. For this reason, most of them are considered high-priority pollutants in water. The European Union and the United States Environmental Protection Agency (EPA) have established their maximum admissible concentration in drinking water at 0.5 ng/ml [1,2]. Accurate, sensitive methods for reliable environmental control analyses for these compounds are thus needed. Gas chromatography (GC) is commonly used in the determination of phenols in water [3–5], soil [6] and air [7]. Because the phenols are usually present at low concentrations, sample preconcentration is usually recommended [3–7]; however, large volumes of samples contain also large numbers of impurities that call for highly selective detection methods such as those of the electron-capture (ECD) [4,6] or mass spectrometric (MS) [3,5,7] types.

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Discrimination between structural isomers is a major analytical challenge. Most references found in the literature are mainly concerned with chromatographic enantiomer separations by use of chiral stationary phases (preferentially in HPLC) or a derivatization reaction involving a chiral reagent; by contrast, available information on structural isomer differentiation is much scantier. In general, aromatic positional isomers can be separated in terms of their different retention times (dependent on their different polarities) on an appropriate chromatographic system. There are several references to the identification of structural isomers of environmentally interesting compounds using GC–Fourier transform (FT) IR [8–10]. The use of sophisticated, and expensive equipment, such as an MS–MS system allows the error-free identification of these substances provided the fragmentation is properly adjusted. Unfortunately, the electron impact (EI) mass spectra for structural isomers are almost always indistinguishable (even when reference spectra exist in the database); alternative ionization strategies have been proposed to circumvent this shortcoming. Thus, positive and negative chemical ionization have been used to obtain complementary information with a view to discriminating isomeric polycyclic aromatic hydrocarbons (PAHs) by using different reagent gases [11,12]. Recently, a method for the determination and identification of structural isomers of polychlorinated phenols was reported [10,13] that is based on the acetylation of the analytes following concentration on graphitized carbon cartridges; it uses GC coupled with FT-IR spectroscopy for the separation and detection of the derivatized chlorophenols. Although 2,4- and 2,5-dichlorophenols cannot be separated under the proposed chromatographic conditions, they can be identified by using a chemometric technique. Thus, GC–FT-IR and GC–MS–MS have enabled the resolution and quantitation of chlorophenol isomers at levels below 0.5 ng/ml in sample volumes of 1 l.

The purpose of this work was to examine the ability of negative chemical ionization to distinguish between structural isomers of chlorophenols. The study led to the construction of a library containing the mass spectra for various chlorophenols that allows their unequivocal identification; this is utterly impossible with the currently available electron impact libraries owing to the similarity of the mass spectra for structural isomers. By using a continuous preconcentration module, chlorophenols can be determined at the ng/ml level in waters.

2. Experimental

2.1. Chemicals

2-Chlorophenol (2-CP), 3-chlorophenol (3-CP), 4-chlorophenol (4-CP), 2,3-dichlorophenol (2,3-DCP), 2,4-dichlorophenol (2,4-DCP), 2,5-dichlorophenol (2,5-DCP), 2,6-dichlorophenol (2,6-DCP), 3,4-dichlorophenol (3,4-DCP), 3,5-dichlorophenol (3,5-DCP), 2,4,6-trichlorophenol (2,4,6-TCP), 2,3,6-trichlorophenol (2,3,6-TCP), 2,3,5-trichlorophenol (2,3,5-TCP), 2,4,5-trichlorophenol (2,4,5-TCP) 2,3,4,6-tetrachlorophenol (2,3,4,6-TeCP) and pentachlorophenol (PCP) were obtained from Aldrich-Chemie (Madrid, Spain). Ethyl acetate, ethanol and acetone, all in HPLC grade, were purchased from Romil Chemical (Loughborough, UK). XAD-2 (styrene–divinylbenzene), 50–100 μm, was supplied by Serva (Heidelberg, Germany).

Stock standard solutions containing 2 mg/ml of each chlorophenol were prepared in 99.9% acetone and stored in glass-stoppered bottles at 4°C in the dark. Dilute aqueous solutions were prepared as required by diluting appropriate microvolumes of these stock solutions in 0.1 mol/l nitric acid.

2.2. GC–MS conditions

Analyses were carried out on an HRGC 8000/MD800 gas chromatograph–mass spectrometer from Thermo (Madrid, Spain). The instrument can be operated in both the electron impact and the chemical ionization (positive and negative) mode. The GC column was a 30 m×0.25 mm I.D. (5% phenyl)-methylpolysiloxane (HP-5MS) fused-silica capillary column (thickness 0.25 μm) from Hewlett-Packard (Madrid, Spain). Helium (6.0 grade; Air Liquide, Seville, Spain) at a constant pressure of 50 kPa regulated by a digital pressure and flow controller, was used as carrier gas. The optimal parameter values for the separation and identification of chloro-

Table 1
Flow injection variables for preconcentration and GC–MS conditions for the separation/fragmentation of chlorophenols

	Selected value
Flow injection variables	
Sample volume	25 ml in 0.1 mol/l HNO_3
Sample flow-rate	4 ml/min
Sorbent	XAD-2, 50 mg
Eluent	Ethyl acetate, 125 μl
Chromatographic conditions	
Injection port temperature	250°C
Injection volume	1 μl
Injection mode	Split (1:20)
Column flow-rate, He	0.9 ml/min
Transfer line temperature	200°C
Electron impact	
Source temperature	200°C
Scan range	*m/z* 50–450
Electron energy	70 eV
Negative chemical ionization	
Source temperature	150°C
Scan range	*m/z* 20–450
Electron energy	70 eV
Reagent gas	Methane, 5.5 grade

phenols are given in Table 1. The GC oven temperature program was as follows: 0–3 min 60°C, then to 86°C at 6°C/min, hold 6 min, then from 86 to 160°C at 10°C/min, then from 160 to 200°C at 12°C/min, hold 2 min. Chemical ionization mass spectra were obtained by using methane (5.5 grade; Air Liquide) as reagent gas. Ionization was carried out at 70 eV in the EI and CI modes; the ionization chamber was used as supplied by the manufacturer, with no alteration intended to increase the NCI sensitivity. The optimal conditions for fragmentation of the chlorophenols are also shown in Table 1.

3. Results and discussion

Polychlorinated phenols, including mono-, di-, tri-, tetra- and pentachlorophenol were studied; 2,3,4- and 3,4,5-trichlorophenol, and 2,3,4,5-tetrachlorophenol, were excluded because they were unavailable. The most relevant features of the resolution of these compounds using the EI and NCI ionization modes in GC–MS are described below.

3.1. Gas chromatographic behaviour

For this study, aqueous samples containing the analytes at concentrations of 10 ng/ml in 0.1 mol/l HNO_3 were preconcentrated by a solid-phase extraction system as previously described [14]. The optimal values of the chemical and flow variables are given in Table 1. When the EI mode was employed, the chromatogram for the 15 chlorophenols studied (Fig. 1) allowed the tentative identification of all compounds except 3-CP and 4-CP, which were overlapped, based on their retention times. With NCI, retention times obviously coincided; however, the sensitivity was up to 50% lower. The EI mass spectra for mono-, di- and trichlorophenol isomers are very difficult to distinguish even if compared with those available in the NIST library.

On the other hand, the retention time can be used to distinguish polychlorophenols because it increases with increasing number of chlorine substituents in the phenol (see Fig. 1). As can also be seen from Fig. 1, chlorophenols with a chlorine atom in the *ortho* position are eluted first in each group of isomers; in addition, 2-dichlorophenols and 2-trichlorophenols are eluted before mono- and dichlorophenols (possessing no chlorine atom in the *ortho* position), respectively. The retention times for chlorophenols containing a chlorine atom in *ortho* are decreased by the formation of a hydrogen bond between this atom and the hydroxyl group that decreases the interaction with the stationary phase of the chromatographic column. The ratio of the molecular ion in the isotopic zone of the mass spectrum allows one to distinguish between compounds containing a different number of chlorine atoms but not between structural isomers. In this respect, the chemical ionization technique produces fewer fragments and involves molecular combinations leading to adducts, so it may provide better discrimination. We chose to use NCI owing to the electronegative character of the analytes.

3.2. Characteristics of the NCI mass spectra

One of the factors most strongly affecting NCI mass spectra is the source temperature [15]. This study was carried out under experimental conditions that favoured dissociative electron capture in order to

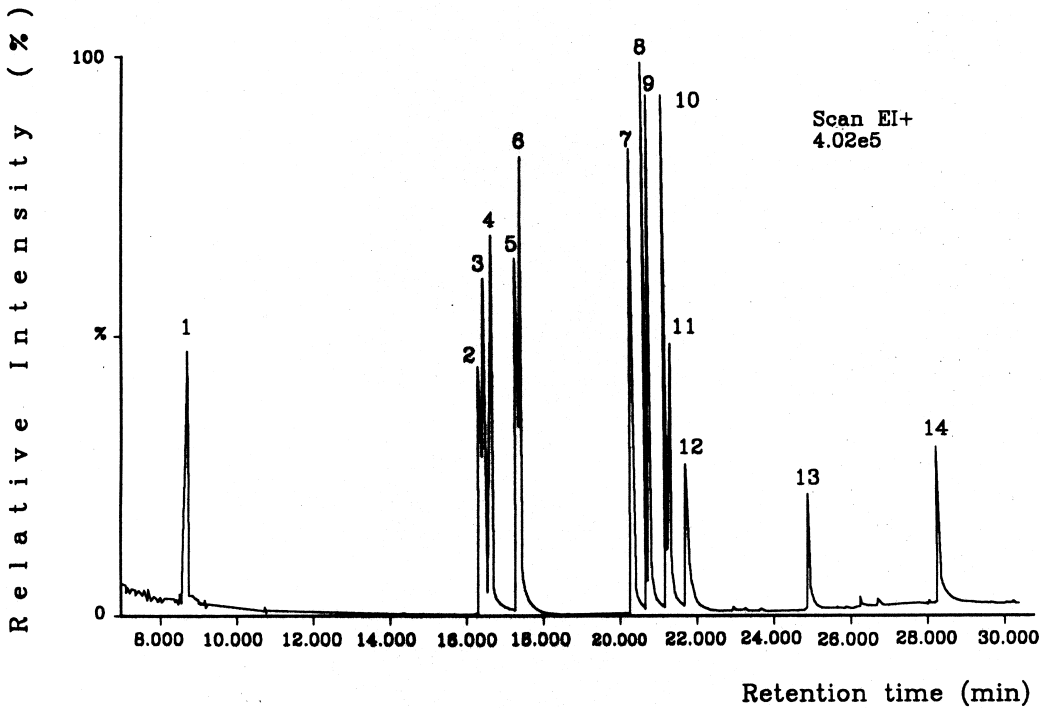

Fig. 1. Gas chromatogram for the 15 chlorophenols spiked (10 ng/ml) to a water sample, following preconcentration of a 25-ml sample volume in the flow system. (1) 2-CP, (2) 2,4-DCP, (3) 2,5-DCP, (4) 2,3-DCP, (5) 2,6-DCP, (6) 3-CP and 4-CP, (7) 2,3,6-TCP, (8) 2,4,6-TCP, (9) 2,4,5-TCP, (10) 2,3,5-TCP, (11) 3,5-DCP, (12) 3,4-DCP, (13) 2,3,4,6-TeCP and (14) PCP.

facilitate observation of the fragments sought (mainly those at m/z 35). Fragmentation was almost absent below 120°C; by contrast, above 180°C the fragment ion at m/z 35 was the base peak in the mass spectra for all the chlorophenols, which prevented isomer discrimination. The fragmentation pattern is similar over the temperature range 140–160°C; above 160°C, however, profound changes occur, particularly as the number of chlorine atoms increases. Therefore, an intermediate temperature of 150°C was thus selected as optimal source temperature as it resulted in significant differences in the mass spectra for structural isomers. Ionization was performed at 70 eV, the filament current set at 350 μA and the methane pressure optimized by using the ions at m/z 300, 452 and 633 from perfluoroter-tbutylamine.

Discrimination of structural isomers was based on the differences in relative intensity among the characteristic peaks in the NCI mass spectra.

3.2.1. Monochlorophenols

Distinguishing of 2-CP from 3-CP and 4-CP by GC–MS should pose no problem because they are well resolved by gas chromatography; on the other hand, 3-CP and 4-CP are very similar in terms of retention, so they are almost impossible to separate on non-polar columns unless a derivatization reaction is employed [10]; although their EI mass spectra are very similar (Fig. 2A,B), however, their NCI mass spectra are different enough to allow their differentiation (Fig. 2C,D). In this case, the fragment ions at m/z 35, 106 and 140 are indispensable for unambiguous identification of both isomers. 4-CP has an m/z 35 relative intensity of ca. 15% and an m/z 106 intensity lower than 5%, whereas 3-CP has a relative intensity of ca 35% in both cases. This difference can be ascribed to the strong resonance interaction between the oxygen and the chlorine atom in the *para* position in 4-CP [16]. 2-CP can also be distinguished as the relative intensity for the

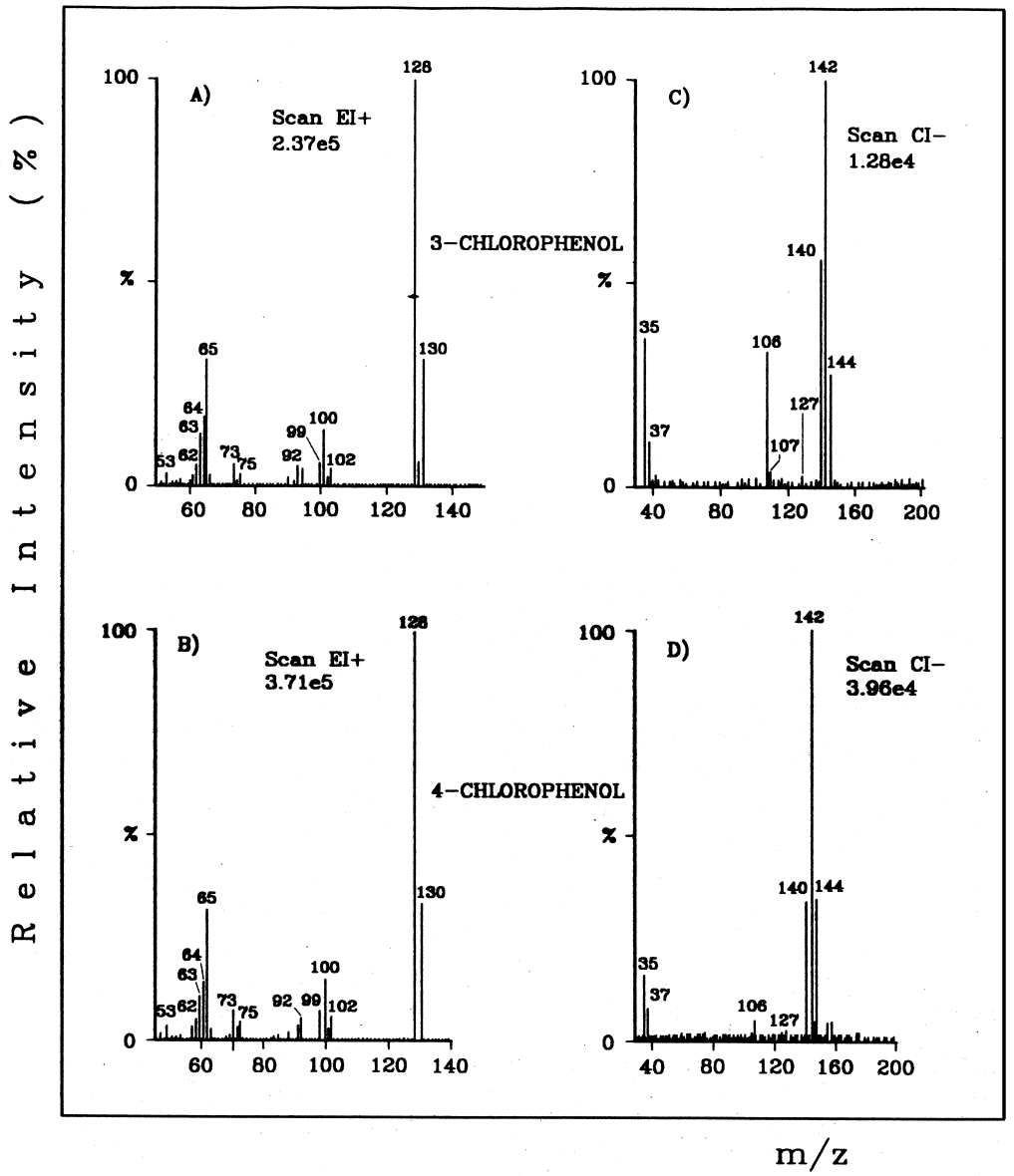

Fig. 2. Mass spectra for 3-chlorophenol and 4-chlorophenol obtained in the electron impact (A,B) and negative chemical ionization modes (C,D).

ion at m/z 106 $[M-HCl+CH_4]^-$ is higher than 60%. In summary, the relative intensity of the fragment ion corresponding to the loss of HCl from the adduct formed by reaction of the monochlorophenol with the reagent ions of methane decreases dramatically from the *ortho* to the *meta* and *para* positions and is thus the most characteristic parameter for their discrimination.

3.2.2. Dichlorophenols

The study of dichlorophenols was somewhat more complex. In principle, they can be split according to

whether or not they possess a chlorine atom in *ortho*. Its presence not only decreases the retention time —through decreased interaction with the stationary phase— but also considerably alters the EI and NCI mass spectra, particularly the latter. By way of example, Fig. 3 shows the NCI mass spectra for 2,6-DCP and 3,5-DCP. As can be seen, the differences are obvious. In both cases, the base peak corresponds to the molecular ion (m/z 162); however, a comparison of the relative intensities of the three most characteristic ions for these DCPs (m/z 35, 126, 140) leads to the following conclusions: (a) the intensity of the fragment ion at m/z 140, which corresponds to $[M-HCl+CH_4]^-$, is low (ca 5%) for 2,6-DCP owing to the steric hindrance of the Cl atoms in *ortho*. On the other hand, the contribution of this fragment ion to the mass spectrum for 3,5-DCP is much more significant (ca 50%); and (b) the intensities of the peaks at m/z 35 $[Cl]^-$ and 126 $[M-HCl]^-$ reveal that, when the two chlorine atoms are in *meta*, the molecule is more extensively fragmented because the negative charge is not stabilized. For this reason, the peak at m/z 126 is more abundant for 3,5-DCP than for 2,6-DCP. Fig. 4 shows the differences between the relative intensities (average for $n=5$) of the peaks with the most characteristic m/z values for the DCPs (35, 126 and 140)—the base peak was excluded from the figure because it corresponded to the molecular ion in all instances. *ortho*-Dichlorophenols can be discriminated basically in terms of the fragment ion at m/z 35. The minimum value occurs when the second chlorine atom is in *para* position. This effect is also observed when the two *meta*-dichlorophenols (3,4-DCP and 3,5-DCP) are compared. The fragment ion at m/z 126 corresponding to the loss of HCl is scarcely abundant in 2,4-DCP, highly abundant in 3,5-DCP and similarly abundant in the other DCPs. This is a result of less extensive fragmentation in dichlorophenols where the second substituent is in

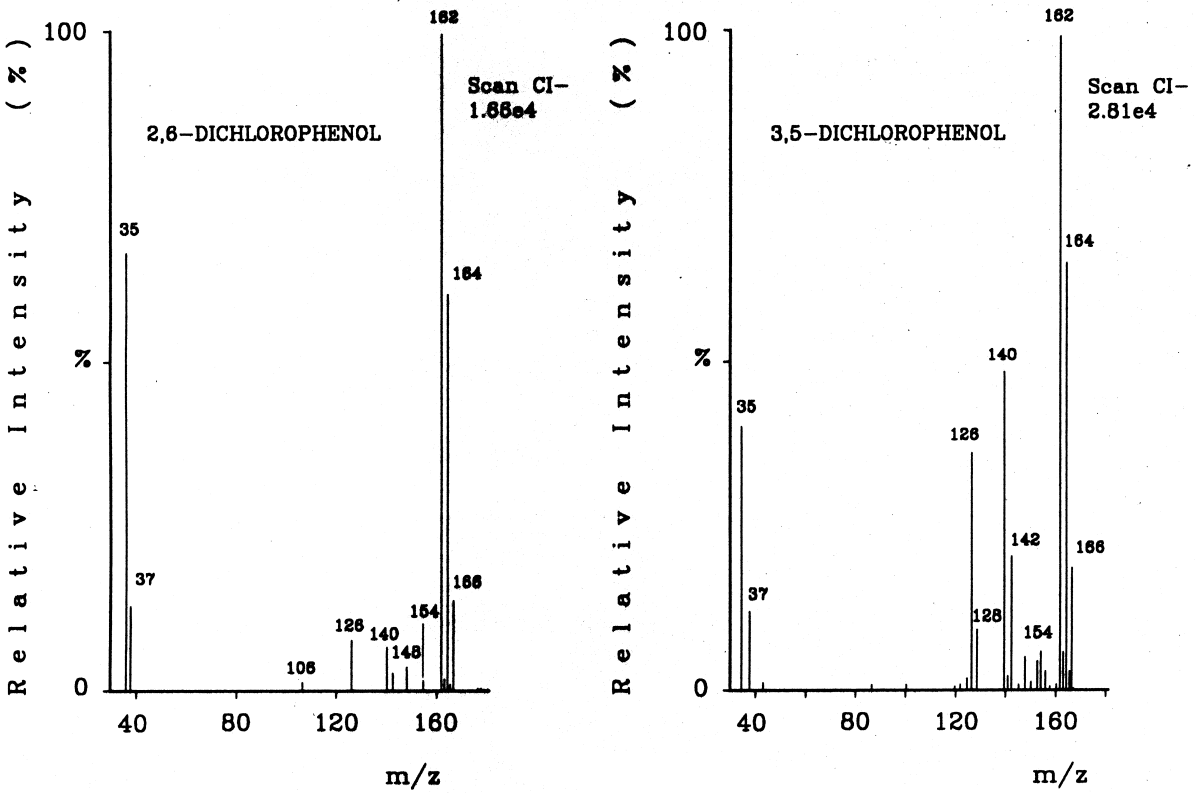

Fig. 3. Fragmentation and specific ions of 2,6-DCP and 3,5-DCP observed under NCI conditions.

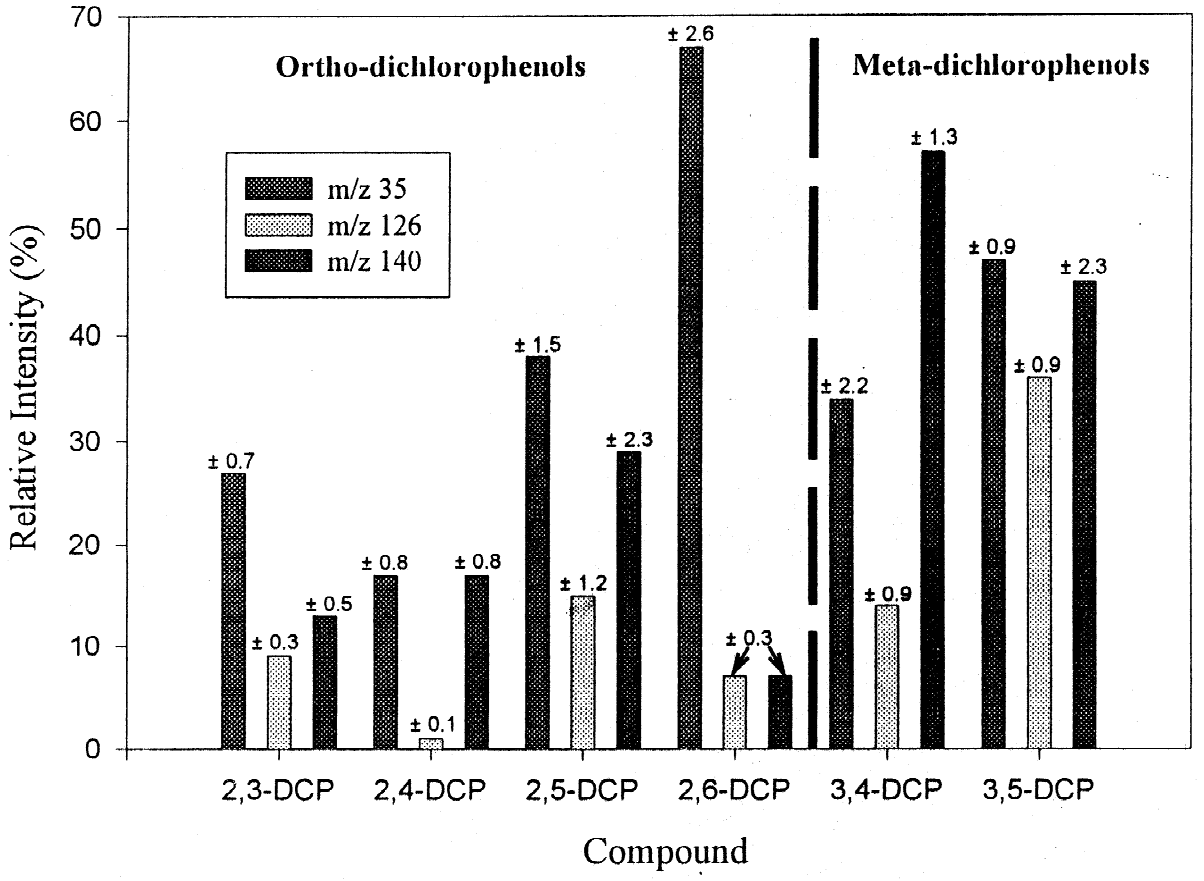

Fig. 4. Relative intensity of the most relevant fragment ions for dichlorophenols (m/z 35, 126 and 140). ± Value indicates the standard deviation (five replicates).

para. The relative intensities of the fragment ions at m/z 140 $[M-HCl+CH_4]^-$ are lowest with the two chlorine atoms in *ortho* (i.e., in 2,6-DCP) and highest in the *meta–para* isomer (i.e., 3,4-DCP). As can be seen, the differences are large enough to enable discrimination among DCPs on the basis of their mass spectra; the intensities of the three selected fragment ions are low for 2,4-DCP and high for 3,5-DCP.

3.2.3. Trichlorophenols

The mass spectra for the four TCPs available are shown in Fig. 5. As can be seen, there are significant differences among them that allow their unequivocal identification. When two chlorine atoms are in *ortho* position and the third is in *meta* (2,3,6-TCP) or *para* positions (2,4,6-TCP), the fragment ion at m/z 35 is stronger, similarly to the DCP; the stabilizing effect of the chlorine atom in *para* position reflects in the decreased intensity of the fragment, as can be seen by comparing the spectra for 2,3,5-TCP and 2,4,5-TCP. The differences in relative intensity of the fragment ion at m/z 160 $[M-HCl]^-$ are less useful for distinguishing these isomers owing to the low intensity of the fragment (particularly for 2,4,6-TCP).

3.2.4. 2,3,4,6-Tetrachlorophenol and pentachlorophenol

These two compounds emerge at the end of the chromatogram (Fig. 1) and can be distinguished by their retention times. TeCP is the sole chlorophenol (in the conditions fixed) whose base peak in the NCI mass spectrum (Fig. 6A) corresponds to the fragment

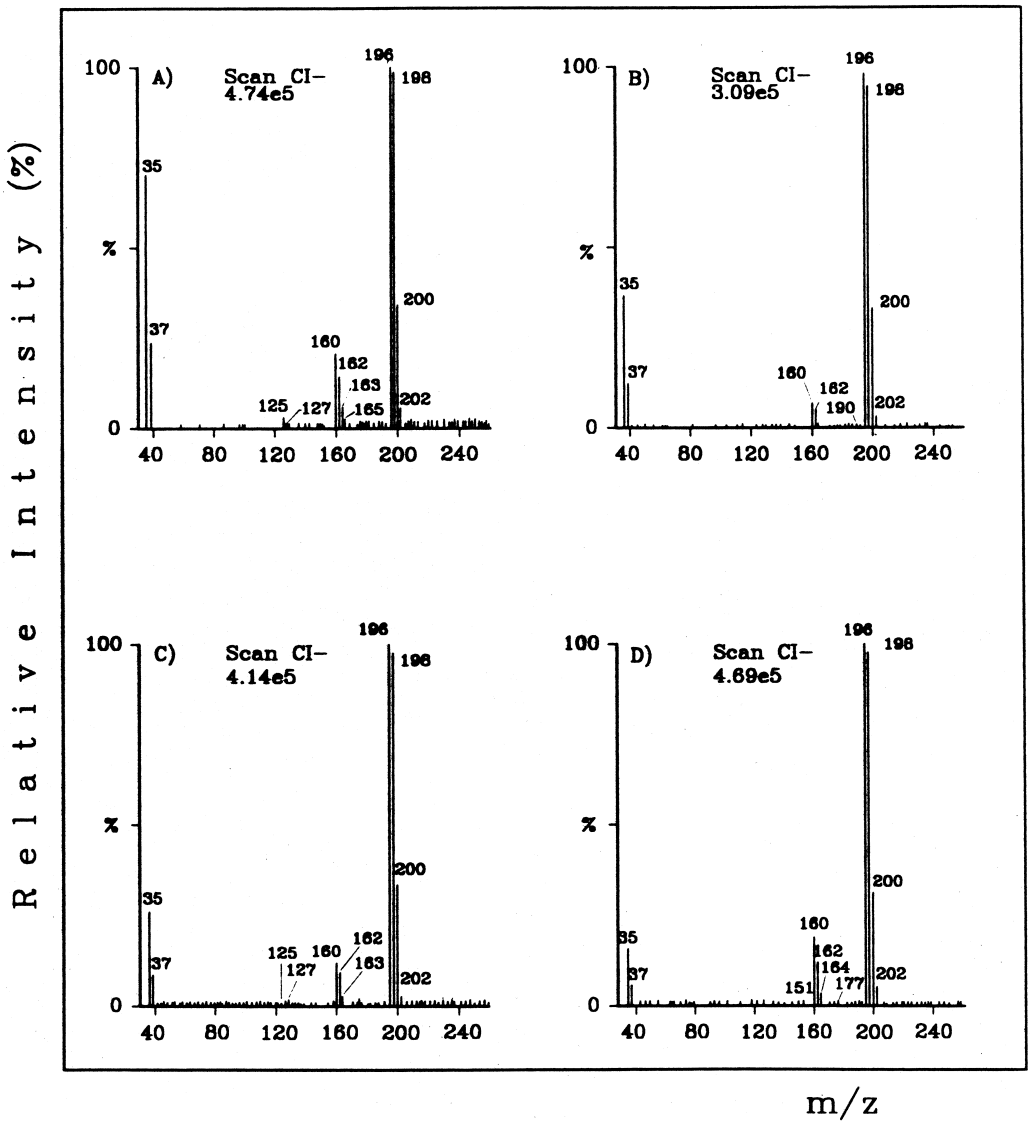

Fig. 5. Negative chemical mass spectra for trichlorophenols. 2,3,6-TCP (A), 2,4,6-TCP (B), 2,3,5-TCP (C) and 2,4,5-TCP (D).

ion at m/z 35 rather than to the molecular ion as in EI. The next NCI peak in decreasing order of intensity corresponds to the loss of an HCl molecule (m/z 194); notwithstanding the presence of such a strong fragment ion, the compound can never be mistaken with a trichlorophenol because the mass spectrum contains vestiges of the molecular ion (m/z 230) with its isotopic zone. The EI mass spectrum exhibits more extensive fragmentation that corresponds to the sequential loss of HCl (m/z 194), CO (m/z 166) and Cl (m/z 131).

The fragmentation in the EI mode for PCP is similar to that for TeCP; the largest differences between the two compounds is observed under NCI conditions. Thus, the most intense fragment ions are in the m/z region from 228 to 232 and are due to the loss of the first chlorine atom—the molecular ion (m/z 264) is completely absent. However, the mass

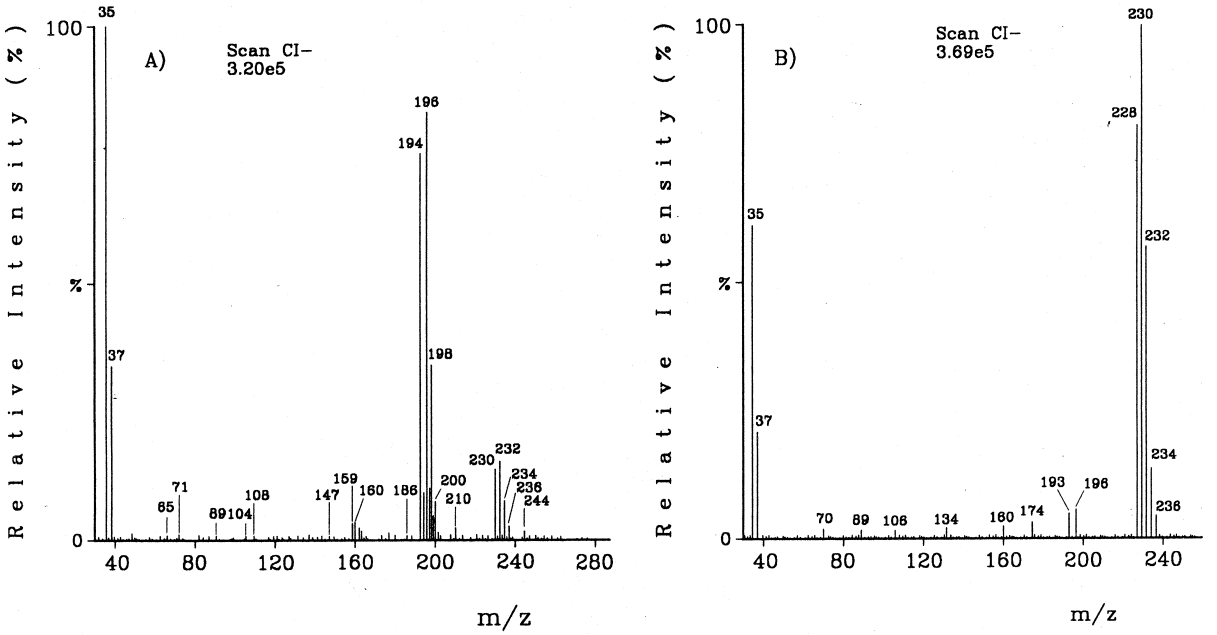

Fig. 6. Negative chemical ionization mass spectra for 2,3,4,6-tetrachlorophenol (A) and pentachlorophenol (B).

spectrum can never be confused with that for a TeCP because the most intense zone in the latter coincides with that for a TCP and the zone corresponding to the molecular ion persists (see Fig. 6B).

4. Conclusions

Chlorinated phenols can be separated and identified by GC–MS. However, electron impact mass spectra cannot discriminate between structural isomers owing to their similarity. An alternative ionization approach was used in this work to circumvent this limitation. Thus, negative chemical ionization with methane as reagent gas enabled the unequivocal identification of all chlorophenols present in the sample. Only 2,3,5-TCP and 2,4,5-TCP pose some problem in this respect because the differences between their mass spectra are very slight. From the results it follows that the fragment ion at m/z 35 (corresponding to the chlorine atom) characteristic of this ionization mode allows the discrimination of all the isomers; the ability to form fragment ions corresponding to the adduct, with loss of HCl, $[M-HCl+CH_4]^-$, only allows mono- and dichlorophenols to be distinguished as it is absent from the other chlorophenols. In addition, although tri-, tetra- and pentachlorophenols form no adducts, the fragment corresponding to the molecular ion with loss of HCl permits their identification. In any case, the user can create a customized library from mass spectra for standards of chlorophenols under the selected NCI conditions. The identification/confirmation of the analytes in the sample can thus be accomplished by comparison of their spectra with those in the library.

In conclusion, the approach used in this work provides two principal advantages, namely: the reagent gas, methane, is the most commonly used in NCI and sample manipulation is minimized by the use of a simple continuous system that requires no derivatization. Based on the results, GC–MS with NCI allows the discrimination of structural isomers as efficiently as other alternatives such as GC–FT-IR.

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