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Enhanced sensitivity in the analysis of trace organochlorine compounds by negative-ion mass spectrometry with ammonia as reagent gas

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Abstract

A comparison of the performances of ammonia and methane as reagent gases for the analysis of trace organochlorine compounds by gas chromatography coupled to negative-ion chemical ionization mass spectrometry is performed in the present study. Examples of standard mixtures and human blood samples analyzed with both reagents in scan and selected-ion monitoring mode are shown. Important advantages are observed as a consequence of the use of ammonia. These concern detection and quantitation limits and the lower dependence of sensitivity on the degree of chlorine substitution of the compounds.

Keywords: Mass spectrometry; Organochlorine compounds; Pesticides; Hexachlorocyclohexanes; Hexachlorobenzene; Polychlorinated biphenyls; DDT; DDE

1. Introduction

The analysis of organochlorine compounds, namely pentachlorobenzene, hexachlorobenzene, hexachlorocyclohexanes (HCHs), DDTs and polychlorobiphenyls (PCBs), at trace concentrations in environmental or biological matrices is still a challenging task. Gas chromatography (GC) coupled to electron-capture detection (ECD) is the current technique of choice [1,2] due to its specificity for electronegative molecules. However, this technique does not provide structural information of the chemical species. Likewise, it does not allow the selective analysis of GC coeluting compounds.

GC coupled to mass spectrometry (GC–MS) in the chemical ionization mode and negative-ion recording (GC–NICI-MS) takes advantage of the electronegative character of these compounds and it has often been used when structural information is needed for the analyses [3–9]. However, the high differences in sensitivity related to the degree of chlorination of the compounds [10] constitute one important difficulty of this application. This drawback is particularly important in environmental and public health studies when the purpose of the analyses is devoted to determine the whole composition of organochlorine species.

In the present paper, it is shown that GC–NICI-MS using ammonia as reagent gas provides a more even response among aliphatic and aromatic organochlorine compounds substituted by three–seven chlorine atoms. Furthermore, this reagent gas gives

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rise to better sensitivities for most of the species analyzed. The results are compared with those obtained with methane because this reagent gas is the most common ionising agent in general purpose NICI applications.

2. Experimental

2.1. Standards

All PCB congeners were purchased from Promochem (Wesel, Germany). γ-HCH and *p,p′*-DDE were from Aldrich-Chemie (Steinheim, Germany), α- and δ-HCHs were from Promochem (National Physical Laboratory, Teddington, UK) and β-HCH, *p,p′*-DDT and HCB were from Dr. Ehrenstorfer (Augsburg, Germany). All standard mixtures were prepared in isooctane.

2.2. Instrumental analysis

All GC–NICI-MS analyses were performed using a Fisons MD 800 instrument (quadrupole detector, Thermo Instruments, Manchester, UK). The gas chromatograph was equipped with a nonpolar fused-silica capillary column HP-5-MS (30 m×0.25 mm I.D., 0.25-μm film thickness). Helium was used as carrier gas (1.1 ml/min). The oven temperature was programmed from 80°C (1 min) to 120°C at 15°C/min and then to 300° at 4°C/min with a final holding time of 10 min. The samples were injected in split/splitless mode (48 s) at 280°C (hot needle technique) and data acquisition started after a solvent delay of 4 min. Ion source and transfer line temperatures were 150 and 280°C, respectively. Methane and ammonia were used as reagent gases. Ion source pressure (currently 1.6 Torr) was adjusted to maximize the perfluorotributylamine ions (*m/z* 312, 452, 633 and 671) (1 Torr=133.322 Pa). Ion repeller was 1.5 V. Data were scanned from *m/z* 50 to 450 at 1 s per decade. Data were also acquired in selected-ion monitoring mode with a dwell time and span of 0.06 s and 0.10 u, respectively. The ion windows used in this second case are described in Table 1.

Table 1
Mass-ion programme for the chemical ionization mass spectral quantitation of organochlorine compounds with methane and ammonia

Time (min)	Compound	Mass ions *m/z*
4–7	Pentachlorobenzene	250
7–11	Hexachlorocyclohexanes	71, 255[a]
	Hexachlorobenzene	284
11–14	PCB No. 28	221, 255
	PCB No. 52	291, 255
14–18.5	*p,p′*-DDE	318
	PCB No. 101	326
18.5–20.5	PCB No. 118	326
	PCB No. 153	360
20.5–25.5	*p,p′*-DDT	71, 283
	PCB No. 138	360
	PCB No.180	394

[a] When two ions are indicated, the first was used for quantitation.

3. Results and discussion

3.1. Mass spectra

Several representative spectra of NICI-MS using ammonia as reagent gas are shown in Fig. 1. The spectra displayed in this figure are dominated by the molecular mass fragment resulting from the incorporation of thermal electrons produced by the moderating effect of ammonia. The stability of the molecular ion depends on the amount of chlorine atoms bound to aromatic carbons. Thus, the NICI spectra of the tetra-, penta-, hexa- and heptachlorobiphenyl compounds considered in this study (PCB Nos. 52, 101, 118, 138, 153 and 180) are dominated by the molecular ion. In the lower substituted biphenyls, e.g. PCB No. 28, the spectra is dominated by other fragments, e.g. *m/z* 221 (Table 1).

Comparison of the hexachlorobenzene and PCB mass spectra displayed in Fig. 1 with those obtained with methane as reagent gas [11–13] shows higher fragmentation with ammonia. Thus, the relative abundances of the [M-Cl+H] ions (ca. 25–85%) are considerably higher than those reported with methane (5–16%; [11–13]), even those obtained at high ion source temperatures, e.g. 250°C [13]. These differences are consistently observed despite the differences in fragmentation associated with the use

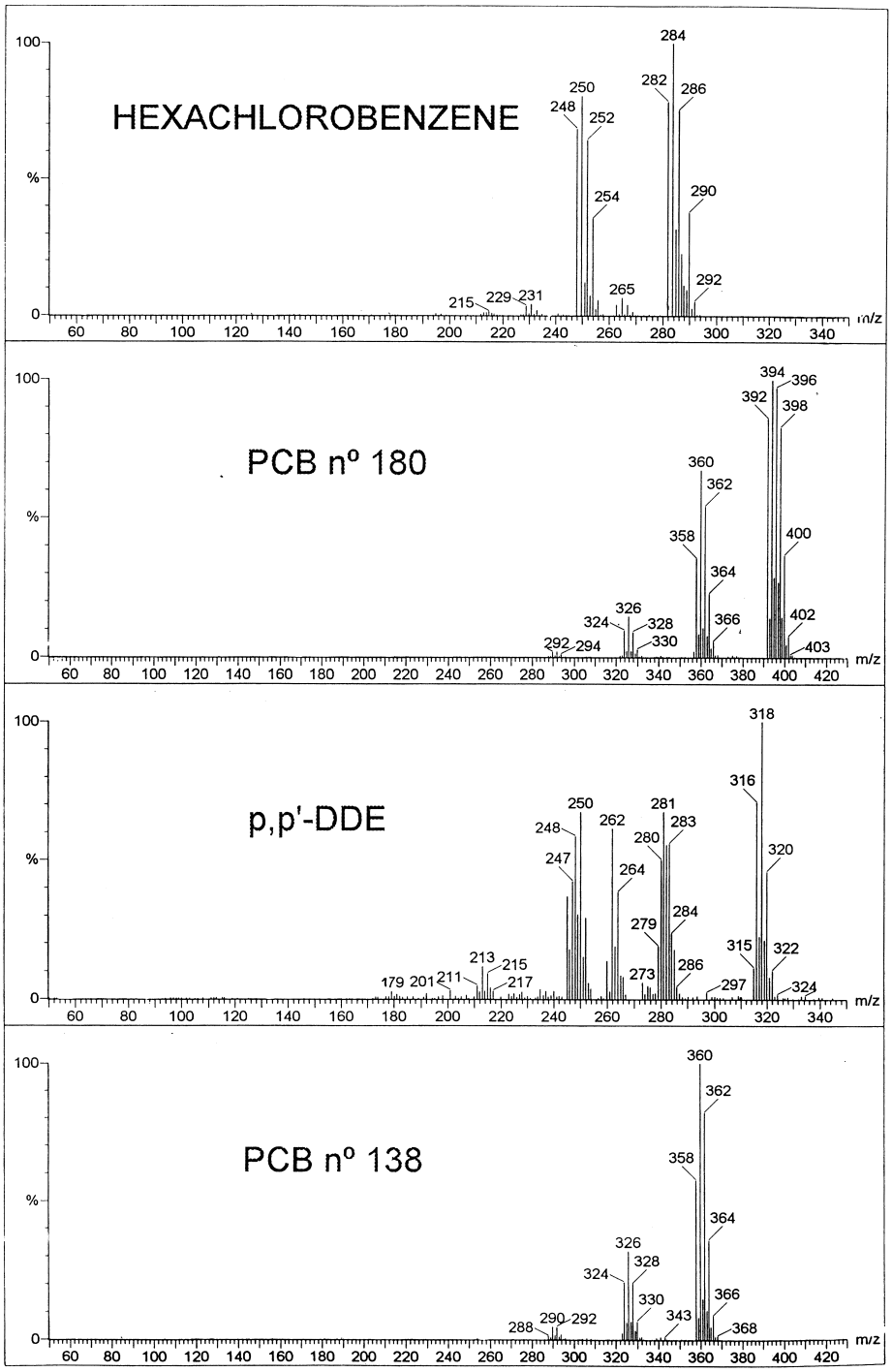

Fig. 1. Negative-ion chemical ionization mass spectra of hexachlorobenzene, *p,p′*-DDE and polychlorobiphenyl congeners Nos. 138 and 180 using ammonia as reagent gas.

of different instruments, that have been reported for methane NICI–MS [11].

A dominance of the molecular ion is also observed in the analysis of *p,p′*-DDE with ammonia NICl-MS (Table 1). In this molecule the double bond is conjugated with the benzene rings and all chlorine substituents are attached to 'aromatic' carbons. Again, this spectrum exhibits higher fragmentation than that obtained with methane NICl-MS. Whereas the *m/z* 262 and 281 fragments have a relative intensity of 80% in the former case (Fig. 1), the intensities of these fragments are 1 and 15%, respectively, with methane [12,13]. However, in this second case the fragmentation pattern has a high dependence on the ion source temperature and no molecular ion is observed at 250°C by methane NICI-MS.

Conversely, the NICI-MS of *p,p′*-DDT is dominated by the Cl_2 ion (Table 1). In this molecule, no conjugation is possible and only two of the five chlorine atoms are bound to aromatic carbons. The Cl_2 mass fragment also dominates the NICl-MS of hexachlorocyclohexanes (Table 1). In this case, all chlorine atoms are linked to aliphatic carbons.

Despite the differences in degree of fragmentation, the use of both gases give rise to the same mass ions. Thus, the use of one or the other gas for the analysis of organochlorine compounds does not require changes in the ions selected for identification or quantitation.

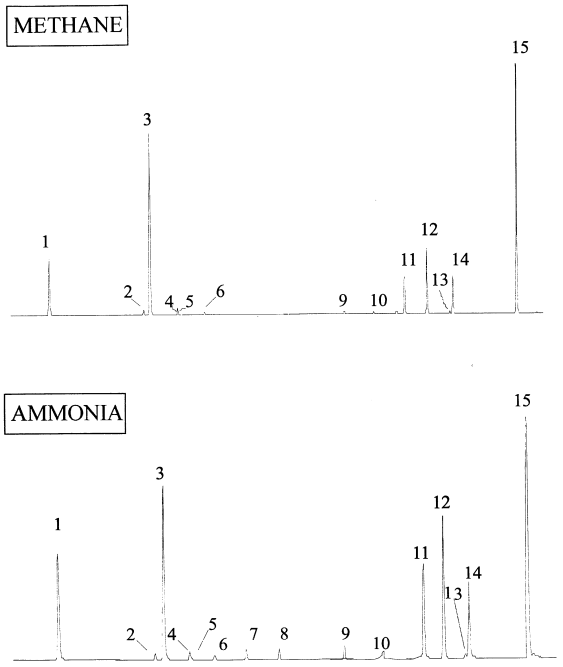

Fig. 2. Scan profiles (*m/z* 50–450 at 1 s) of a standard mixture of organochlorine compounds recorded by gas chromatography negative-ion chemical ionization mass spectrometry using methane and ammonia as reagent gases. (1) Pentachlorobenzene (160 pg), (2) α-HCH (190 pg), (3) hexachlorobenzene (160 pg), (4) β-HCH (310 pg), (5) γ-HCH (240 pg), (6) δ-HCH (220 pg), (7) PCB No. 28 (170 pg), (8) PCB No. 52 (160 pg), (9) PCB No. 101 (160 pg), (10) *p,p′*-DDE (260 pg), (11) PCB No. 118 (170 pg), (12) PCB No. 153 (210 pg), (13) *p,p′*-DDT (230 pg), (14) PCB No. 138 (190 pg), (15) PCB No. 180 (230 pg).

3.2. GC–MS profiles

The scan profiles (*m/z* 50–450 at 1 s) obtained by GC–NICl-MS analysis of a mixture of organochlorine standards with ammonia and methane as reagent gases are shown in Fig. 2. Consistently with previous studies [10–12], both profiles show a higher response for the compounds with higher degree of chlorination in aromatic rings. Thus, compounds containing five (pentachlorobenzene, PCB No. 118), six (hexachlorobenzene, PCB Nos. 138 and 153) or seven (PCB No. 180) chlorine substituents in benzene or biphenyls stand out in the mixture.

Nevertheless, the GC profile generated by ionization with ammonia exhibits a more uniform response. Thus, the relative proportion of the peaks corresponding to pentachlorobenzene and PCB Nos. 118, 153 and 138 is higher than in the methane profile which is essentially dominated by hexachlorobenzene and PCB No. 180. In addition to this difference, small chromatographic peaks corresponding to organochlorine compounds with a lower degree of chlorine substitution (e.g., PCB Nos. 28, 52 and 101 and *p,p′*-DDE) can be observed in the ammonia GC–NICl-MS record. These compounds are hardly detectable in the methane ionization profile. The use of ammonia as reagent gas gives rise to chromatographic traces with lower compound discrimination than chemical ionization with methane. Similar differences between the two gases are observed in selected-ion monitoring mode.

The difference in sensitivity between methane and ammonia is probably due to the buffer capacity of the two gases to decrease the energy of the electrons

emitted by the mass spectral filament to near-thermal values. Thus, experiments of electron decay in pulse irradiated mixtures of CCl_4 and diverse moderating gases showed that the CCl_4 electron attachment rate constant in the presence of ammonia, $5.9 \cdot 10^{-9}$ ml/s, was about seven times higher than in the presence of methane, $8.6 \cdot 10^{-10}$ ml/s [14]. Likewise, the unit pressure thermalization time of ammonia, $2.8 \cdot 10^{-8}$ s Torr, was about seven times shorter than that of methane, $2.0 \cdot 10^{-7}$ s Torr [14].

p,p′-DDT and HCHs show lower relative abundances when both methane and ammonia are used. The lack of stabilization of the molecular ion in the absence of a sufficient number of chlorine substituents to aromatic carbons is not only reflected in high fragmentation but also in low ion abundances. Thus, these compounds exhibit low relative responses even when they are analyzed by selected-ion monitoring (SIM) using the *m/z* fragment program of Table 1 that includes *m/z* 71 as their specific mass ion.

Among all HCHs, the β-isomer is the one exhibiting the lowest signal/response ratio with both gases. The low sensitivity of this HCH isomer on analysis by methane GC–NICl-MS or even electron-capture detection is known [15]. The different ionization rate of this isomer probably reflects its higher stability and lower dipole moment than the other HCHs.

The strong differences in relative area abundances shown in Fig. 2 are significant for the analysis of real samples such as human blood extracts (Fig. 3) which contain trace amounts of organochlorine compounds (in the order of a few ng/ml). These concentrations require high instrumental sensitivity since the current sample amounts available for analysis are in the order of 1 ml. Sample extraction and clean up procedures for the preparation of these extracts are described elsewhere [15].

At this low amount of organochlorine compounds, e.g. low pg range, only hexachlorobenzene and PCB No. 180 give detectable signals by methane GC–NICl-MS. Conversely, PCB congeners (Nos. 118, 153 and 138) and *p,p′*-DDE are recorded in addition to the methane-detected compounds when ammonia is used. The contrast between the two ionization agents is even higher than that observed with standard mixtures (Fig. 2) illustrating that the use of ammonia results in obvious advantages in the presence of interfering compounds such as those occurring in biological samples.

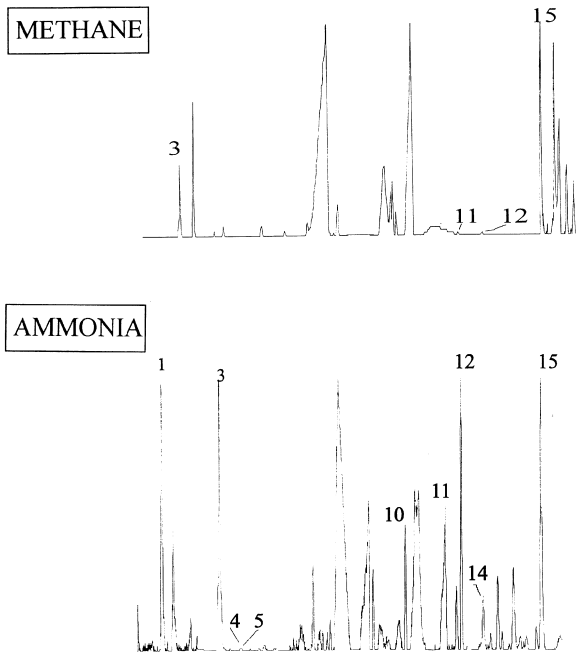

Fig. 3. Selected-ion monitoring profile of a human blood extract of organochlorine compounds recorded by gas chromatography–negative ion chemical ionization mass spectrometry using methane and ammonia as reagent gases (*m/z* ion-monitoring program as described in Table 1, the GC profiles are normalized at the maximum peak of each window). Peak identification as in Fig. 2.

3.3. Detection and quantitation limits

The detection and quantitation limits of the organochlorine compounds included in Figs. 2 and 3 are reported in Table 2. They have been obtained from the analysis of standard mixtures by selected-ion monitoring as described in Table 1. Several calibration curves were generated by progressive dilution of standard mixtures and subsequent instrumental analysis. The values reported in Table 2 correspond to those determined with the straight lines closer to the lowest detectable concentration range.

In nearly all cases, the limits of detection and quantitation are lower when ammonia is used as reagent gas. The contrast is particularly remarkable

Table 2
Detection and quantitation limits (in pg) of organochlorine compounds by chemical ionization mass spectral analysis using methane and ammonia as reagent gases

Compound	Ammonia		Methane	
	Limit detection (pg)	Limit of quantitation (pg)	Limit of detection (pg)	Limit of quantitation (pg)
Pentachlorobenzene	0.08	0.27	–[a]	–[a]
Hexachlorobenzene	0.005	0.02	0.03	0.16
α-Hexachlorocyclohexane	0.15	0.80	0.3	1.2
β-Hexachlorocyclohexane	1.3	4.5	1.4	4.5
γ-Hexachlorocyclohexane	0.2	1.0	0.36	1.5
δ-Hexachlorocyclohexane	0.05	0.2	0.1	0.3
PCB congener No. 28	1.4	5.4	5.6	26
PCB congener No. 52	1.0	2.8	4.5	23
PCB congener No. 101	0.3	0.8	1.3	6.0
p,p′-DDE	3.4	11	11	53
PCB congener No. 118	0.2	0.6	0.40	1.6
PCB congener No. 153	0.1	0.4	0.55	2.2
PCB congener No. 138	0.08	0.30	0.36	1.4
PCB congener No. 180	0.04	0.14	0.4	1.6

[a] Not determined.

among the PCB congeners and *p,p′*-DDE (five to ten times) which, as outlined in Fig. 2, give rise to significant differences for the determination of organochlorine compounds in real samples.

In the case of HCHs, the differences are smaller although the limits are generally lower with ammonia than with methane. β-HCH does not exhibit significant differences from the use of one or the other reagent. Likely the use of ammonia does not involve any significant advantage for enhanced ionization in this compound.

4. Conclusions

The use of ammonia as reagent gas in GC–NICl-MS provides lower limits of detection and quantitation than methane for most organochlorine compounds. These lower limits allow the detection and quantitation of species with three and four chlorine substituents in aromatic rings which are difficult to determine at trace levels when methane is used as reagent gas. The use of ammonia as reagent gas also facilitates the quantitation of the DDT derivatives.

The mass spectra obtained with ammonia and methane correspond to the molecular incorporation of thermal electrons rather than true chemical ionization reactions. Despite the differences in fragmentation patterns associated to these two reagent gases, the use of one or the other does not require changes in the compound-specific ions selected for the SIM mode.

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References

[1] J.S. Waid, PCBs and the Environment, CRC Press, Boca Raton, FL, 1986.
[2] M.D. Erickson, Analytical Chemistry of PCBs, Lewis, Boca Raton, FL, 1991.

[3] J.R. Hass, M.D. Friesen, D.J. Harvan, C.E. Parker, Anal. Chem. 50 (1978) 1474.
[4] A. Cavallaro, G. Bandi, G. Invernizzi, L. Luciani, E. Mongini, G. Gorni, Environ. Sci. 5 (1982) 55.
[5] E. Lewis, W.D. Jamieson, Int. J. Mass Spectrom. Ion Phys. 48 (1983) 303.
[6] M. Oehme, P. Kirschmer, Anal. Chem. 56 (1984) 2754.
[7] J.-A.B. Campbell, D.A. Griffin, M.L. Deinzer, Org. Mass Spectrom. 20 (1985) 122.
[8] M. Oehme, D. Stöckl, H. Knöppel, Anal. Chem. 58 (1986) 554.
[9] L. Canton, J.O. Grimalt, Chemosphere 23 (1991) 327.
[10] R.B. Westerberg, S.L. Alibrando, F.J. van Lenten, J. Chromatogr. 284 (1984) 447.
[11] E.A. Stemmler, R.A. Hites, B. Arbogast, W.L. Budde, M.L. Deinzer, R.C. Dougherty, J.W. Eichelberger, R.L. Foltz, C. Grimm, E.P. Grimsrud, C. Sakashita, L.J. Sears, Anal. Chem. 60 (1988) 781.
[12] E.A. Stemmler, R.A. Hites, Biomed. Environ. Mass Spectrom. 17 (1988) 311.
[13] E.A. Stemmler, R.A. Hites, Electron Capture Negative-ion Mass Spectra of Environmental Contaminants and Related Compounds, VCH, New York, 1988, 390 p.
[14] J. Warman, M.R. Sauer Jr., J. Chem. Phys. 62 (1975) 1971.
[15] R. Otero, M. Santiago-Silva, J.O. Grimalt, J. Chromatogr. A 778 (1997) 87.