Journal of Chromatography, 394 (1987) 51–64
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 19 422

HALOGENATED MOBILE PHASE ADDITIVES FOR IMPROVED DETECTION PERFORMANCE IN LIQUID CHROMATOGRAPHY–NEGATIVE CHEMICAL IONIZATION MASS SPECTROMETRY

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SUMMARY

The addition of 0.1–1% of chloroacetonitrile to an acetonitrile–water eluent can significantly improve the detection performance in column liquid chromatography–negative chemical ionization mass spectrometry (LC–NCI MS) using a direct liquid introduction interface. The addition of the chlorine-containing modifier does not change the retention times of the chlorophenols used as test compounds. However, the sensitivity for the lower chlorinated phenols is enhanced up to 30-fold. In addition, fragmentation is suppressed and the mass spectra are often characterized by only one main cluster over the whole 170–330°C temperature range studied. With most test compounds, so-called chloride attachment also occurs to some extent, especially at relatively low ion source temperatures. However, for phenol and mono- and dichlorophenol fairly intense $[M + Cl]^-$ clusters are even observed at source temperatures of 300°C.

For a further evaluation two phenoxyacetic acids, lindane, L-leucine and L-ascorbic acid were studied, and the utilization of other chlorine-, bromine- and fluorine-containing additives was explored.

INTRODUCTION

In the past decade, column liquid chromatography with on-line mass spectrometric detection (LC–MS) has emerged as a highly promising, but also slightly capricious analytical tool. The excellent performance of the mass spectrometer as a sensitive and selective detector is well known from the field of gas chromatography–mass spectrometry (GC–MS) and need not be discussed here. However, in LC–MS interfacing creates many more problems than it does in GC–MS, and none of the interfacing techniques used today can be said to provide a universal solution to all problems.

In our laboratory, a laboratory-made direct-liquid introduction (DLI) inter-

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0021-9673/87/$03.50

face[1] is employed for the on-line coupling of LC and MS. It uses a jet of helium gas to aid the nebulization of the LC effluent, which is vaporized by heat transfer from the MS ion source. In this, as in most other DLI-type interfaces, (one of) the constituents of the LC eluent mixture serve(s) as the chemical ionization (CI) reagent gas(es). Consequently, optimization of the CI MS detector performance is partly determined by the selection of suitable chromatographic conditions. So far, CI MS detector optimization by means of LC eluent variation has not attracted much attention and it must be admitted here that the available data do not indicate that major changes can easily be effected in this way. Still, in a study on the use of an extraction module inserted on-line between the LC column and the DLI interface, Apffel *et al.*[2] demonstrated that including an extraction process enables one to adjust the detection conditions more or less independently of the LC conditions: selection of suitable water-immiscible organic solvents was shown to effect, apart from a high percentage extraction, a serious reduction of the baseline noise and the occurrence of negative CI (NCI) spectra for selected analytes.

Under NCI conditions, negative ion spectra can be produced by electron-capture and by reactant-ion NCI. In the former instance, negative ions are formed by the capture of low-energy electrons from an inert enhancement gas. In the latter instance, the negative ions are formed by ion–molecule reactions using a reactive reagent gas[3,4]. Several years ago, Dougherty and co-workers[5–7], using 10–100 μg amounts of, *e.g.*, polycyclic chlorinated insecticide analytes, introduced into the mass spectrometer by a direct probe technique, observed NCI spectra generated under electron-capture conditions with methane as enhancement gas. These were, however, characterized by ion–molecule attachment species, principally $[M + Cl]^-$; the chlorine-containing analyte turned out to be the chloride source. These results led to the use of reagent gases such as dichloromethane and also Freon 12 (CF_2Cl_2) for successful chloride attachment[3,8]. In reversed-phase LC–MS, chloride ions will almost always be absent from the eluent, and an external chloride source has to be found[9]. Recently, Parker *et al.*[10] showed that LC-chloride attachment NCl MS using a DLI interface can be carried out successfully if chloroacetonitrile is added to the LC eluent. Several organophosphorus pesticides were used as test compounds. The percentage of chloride attachment was lower than in the direct-probe introduction study of Dougherty and Wander[11]; in addition, more fragmentation was observed.

In this work, three different aspects were studied. Firstly, the dependence of mass fragmentation and chloride attachment on the MS ion source temperature and the chloroacetonitrile concentration of the mobile phase was studied with several chlorophenols as test compounds. After the analytical usefulness of the addition of chloroacetonitrile for LC–NCI MS had been demonstrated, the study was extendend in two directions. On the one hand, the potential of fluorine- and bromine-containing mobile phase additives was evaluated, and on the other, the potential of chloroacetonitrile as an additive for a number of aromatic and aliphatic test compounds was briefly explored.

EXPERIMENTAL

Materials

Acetonitrile and water were of HPLC grade (Baker, Deventer, The Nether-

lands) and were passed through a filter of 0.45 μm pore diameter before use. Analytical-reagent grade 2-chloroethanol, dichloroacetic acid and 1-fluoroethyl acetate were obtained from Baker and analytical-reagent grade bromoacetonitrile and trifluoroacetic acid, chloroacetonitrile and 2,2,2-trifluoroethanol were obtained from Janssen Chimica (Beerse, Belgium), Fluka (Buchs, Switzerland) and Merck (Darmstadt, F.R.G.), respectively.

All compounds used as test solutes were commerically available analytical-reagent or technical-grade products.

Methods

LC. The mobile phase for LC was delivered by a Gilson (Villiers-le-Bel, France) Model 302 pump capable of isocratic flow-rates from 0.5 to 5000 μl/min. A laboratory-made membrane pulse damper was used in conjunction with the Gilson pump. Samples were either introduced directly into the interface system (flow injection) or via the analytical column using a laboratory-made microinjection valve with a 0.05- or 0.5-μl internal loop volume. In this study, a laboratory-packed microbore column (200 × 0.7 mm I.D.) (glass-lined stainless-steel tubing; Scientific Glass Engineering, Melbourne, Australia) packed with 5-μm LiChrosorb RP-18 (Merck, Darmstadt, F.R.G.) was used.

MS. A Finnigan (Sunnyvale, CA, U.S.A.) Model 4021 quadrupole mass spectrometer, with Varian (Palo Alto, CA, U.S.A.) Model M4 and HS2 diffusion pumps for the vacuum system, was used. A Data General Nova 4 (Data General, Westboro, MA, U.S.A.) was employed for data acquisition and processing. The MS ion source temperature was varied between 170 and 330°C. Under the conditions used, the pressure in the ionization chamber was *ca.* 0.3 Torr and in the analyser *ca.* $6 \cdot 10^{-5}$ Torr. The electron multiplier was operated at 1250 V with a dynode voltage of 3 kV.

DLI interface. The laboratory-made interface has been described in detail previously[1]. It consists of a 35-cm long outer stainless-steel jacket which fits into the solid sample probe of the MS instrument. In our experiments, the LC effluent enters the MS source through a fused-silica capillary (0.16 mm O.D. × 0.05 mm I.D.). This capillary is coaxial with a stainless-steel capillary (1/16 in. O.D. × 0.3 mm I.D.), and helium (at an inlet pressure of 1–2 bar) flows between both capillaries and into the MS source.

When a new fused-silica capillary had to be installed, it was pre-washed with a few millilitres of 2 *M* nitric acid, water, methanol and toluene to prevent stability problems.

RESULTS AND DISCUSSION

In on-line DLI LC–MS, the nature of the reagent gas is determined by the composition of the LC eluent. The demand for a suitable eluent for good LC separation can seriously interfere with the optimization of the positive chemical ionization (PCI) or NCI MS detector performance. Obviously, the best solution is to use additives to the LC eluent that hardly influence LC analysis but (strongly) improve NCI MS detection. The idea of using chlorinated additives arose when we observed that, for our LC–NCI MS system, the mass spectra of chlorophenols mainly gave $[M - Cl]^-$ or even $[M - 2Cl]^-$ (*e.g.*, with pentachlorophenol) ions. As the spectra were also

dependent on the amount injected, with larger amounts favouring cluster ions of higher mass, *e.g.*, $[M - 1]^-$ or even $[M - 2Cl]$, we tried to suppress the dissociation of chloride ions from the chlorophenols by adding an abundance of chloride to the reagent gas. This approach follows the idea of Parker *et al.*[10], who added 1% of chloroacetonitrile to the eluent. A possible extra effect of the chloroacetonitrile addition is the occurrence of chloride attachment, also observed by Parker *et al.*[10]. In our study, we varied the percentage of chloroacetonitrile and the temperature of the ion source. In most DLI LC–MS work reported in the literature, ion source temperatures of 200–300°C were used, relatively high temperatures being preferred in order to ensure efficient solvent and analyte evaporation. Data[10,11] on chloride attachment NCI indicate, however, that lower temperatures improve the quality of the mass spectra, as fragmentation is reduced while the intensity of the base peak increases. Consequently, the detection sensitivity is improved. In the present project, we therefore studied a fairly wide temperature range, *i.e.*, from 170 to 330°C.

Reagent gas spectrum

The first experiments involved the flow injection analysis (FIA) of 3,4,5-trichlorophenol (3,4,5-TCP). The carrier stream was acetonitrile–water (70:30) in which 0, 0.1 or 1.0 % (v/v) of acetonitrile was replaced with chloroacetonitrile. The test compound was invariably injected as a solution in the carrier stream. This aspect is very important in FIA to avoid changes in the reagent gas during ionization of the test compound.

The reagent ion spectrum for acetonitrile–water (70:30) showed a base peak at *m/z* 26, corresponding to $[CN]^-$ and a peak at *m/z* 40 from $[CH_2CN]^-$. Some less intense ions were formed up to *m/z* 97. Addition of 1% of chloroacetonitrile to the eluent led to a spectrum dominated by ions from chloroacetonitrile. The ions which carried almost all of the ion current were at *m/z* 35 and 37, corresponding to $[Cl]^-$. Other ions were observed at *m/z* 53, 76 and 110, probably corresponding to $[H_2OCl]^-$, $[CH_3CNCl]^-$ and $[CH_2ClCNCl]^-$, respectively. A similar reagent ion spectrum was found by Parker *et al.*[10], although they also mentioned the presence of an ion at *m/z* 128 from $[H_2OCH_2ClCNCl]^-$. At 0.1% chloroacetonitrile the same reagent ions were formed, but their intensity was clearly lower. For obvious reasons, in all further work scanning was restricted to *m/z* values above 110. Increasing the level of chloroacetonitrile to several percent gave more intense reagent ions above *m/z* 100, *e.g.*, *m/z* 128. Levels of over 1% were therefore not used.

3,4,5-Trichlorophenol

In Fig. 1, the relative intensity of the major fragments observed for 3,4,5-trichlorophenol (50 ng) is plotted as a function on the temperature of the ion source. Data are shown for 0, 0.1 and 1% of chloroacetonitrile. In the absence of chloroacetonitrile, the spectra are generally dominated by chloride abstraction, with $[M - HCl]^-$ ions giving the most intense cluster. Source temperatures below 200°C favoured the $[M]^-$ cluster. In addition, an intense $[M - Cl]^-$ cluster was obtained, its intensity slowly decreasing with increasing source temperature.

Manifest changes in the mass spectrum were observed on addition of 0.1 or 1.0% of chloroacetonitrile. The $[M - 1]^-$ peak, with a relative intensity of about 10% in the absence of the additive, now invariably was the base peak. Further, the inten-

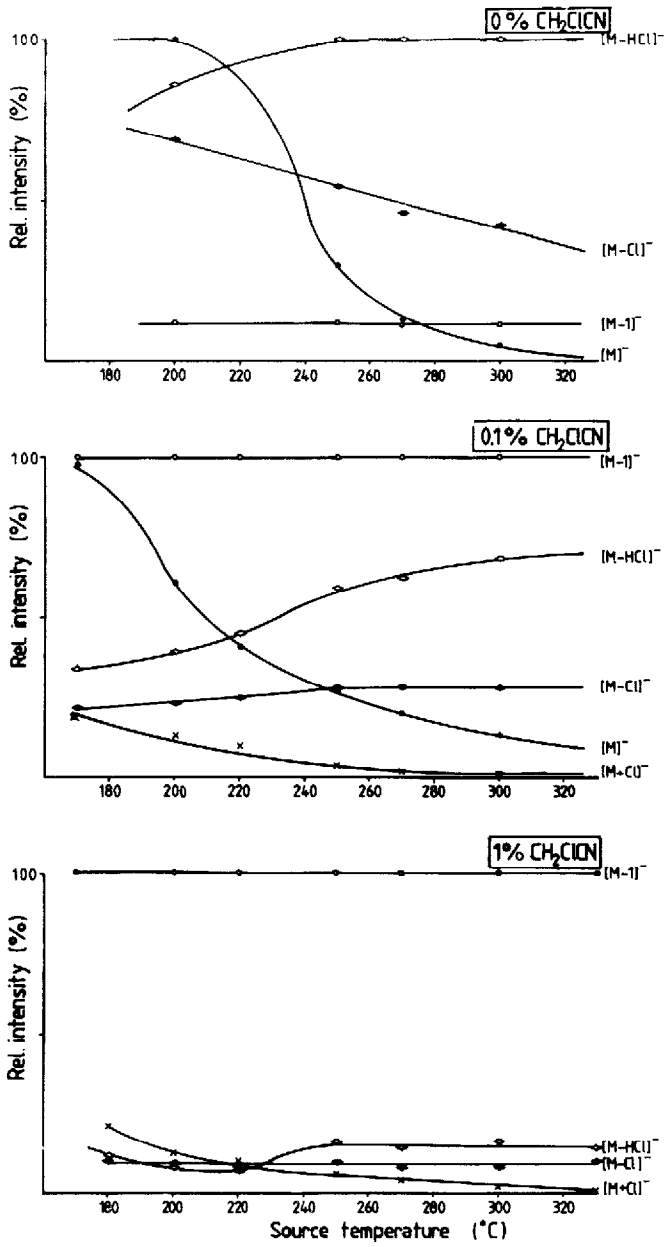

Fig. 1. Dependence of the relative intensity of the ion clusters in the NCI mass spectra of 3,4,5-trichlorophenol on the ion source temperature. LC eluent, acetonitrile–water (70:30) containing 0, 0.1 or 1% of chloroacetonitrile.

sity of $[M - HCl]^-$ and $[M - Cl]^-$ decreased rapidly on increasing the level of chloroacetonitrile from 0 via 0.1 to 1%. A striking difference between 0 and 0.1% compared with 1% of chloroacetonitrile is that in the former two instances, especially at

low temperatures, the $[M]^-$ cluster is very intense whereas it is totally absent with 1% of chloroacetonitrile. Finally, some chloride attachment was observed. It is a minor effect, which increased with decreasing source temperature; the relative intensity was about the same with both 0.1 and 1% of chloroacetonitrile. Parker *et al.*[10] also found that spectra with a similar degree of chloride attachment were observed with 1 and 0.1% of chloroacetonitrile. Our measurements with 3,4,5-trichlorophenol gave a significant difference in spectra between 0.1 and 1.0% of chloroacetonitrile for other mass fragments. This effect was not mentioned in ref. 10.

The spectra of 3,4,5-trichlorophenol obtained with 1% of chloroacetonitrile are simpler than those recorded under the other conditions. They are characterized by only one intense cluster, *i.e.*, $[M - 1]^-$, and do not change strongly on varying the temperature of the ion source. Further, it is interesting that the spectra were less dependent on the amount injected in the presence of 1% of chloroacetonitrile than in its absence. Further experiments on chlorophenols were restricted to eluents with 1% of and, for reference purposes, without chloroacetonitrile.

Various chlorophenols

In order to demonstrate the advantage of using a chlorinated additive, a mixture of penta-, 2,3,4,5-tetra-, 2,4,6-tri-, 2,3,4-tri, 2,6-di and 4-chlorophenol and phenol was analysed in the system LiChrosorb RP-18/acetonitrile–water–acetic acid (70:30:0.2) with or without 1% of chloroacetonitrile in the eluent. The presence of acetic acid in the eluent gave rise to a fragment of *m/z* 119, due to $[(CH_3COO)_2H]^-$, in the reagent ion spectrum. However, the addition of an acid to the eluent was necessary to produce a reproducible separation and good peak shapes for the higher chlorinated phenols, which are slightly acidic.

The reconstructed ion current (RIC) chromatograms observed in LC–NCI MS in the absence and presence of chloroacetonitrile are shown in Fig. 2. Further information is given in Table I, which also includes data on 3,4,5-trichlorophenol to allow a comparison with the other trichlorophenols. As one can see from the two traces in Fig. 2, the addition of chloroacetonitrile does not affect either the retention times of the individual chlorophenols or the resolution. The concentrations of the chlorophenols were selected in such a way that in the lower trace all peak heights are in the same range. Scanning the mass spectra from *m/z* 120 upwards, which seemed to be a suitable threshold value, obviously does not register phenol in the absence of chloroacetonitrile. Under these conditions, *i.e.*, no chloroacetonitrile, the dominant ionization process that occurred with the higher chlorinated phenols was dissociative electron capture resulting in the loss of chlorine to give $[M - Cl]^-$ ions. The low-chlorinated phenols showed an electron-capture ionization process that resulted in $[M - 1]^-$ quasi-molecular ions.

The addition of 1% of chloroacetonitrile significantly changed the ionization process (see Table I). Reactant ion chemical ionization now was the dominant mode in most instances; for the low- and non-chlorinated phenols the base peak was the chloride adduct. In the RIC trace phenol can therefore now be detected. For the higher chlorinated phenols intense quasi-molecular $[M - 1]^-$ ions were obtained. As regards the three trichlorophenols, Table I shows that in LC–NCI MS without chloroacetonitrile, substitution *ortho* to the OH group favours $[M - Cl]^-$ as the base peak, compared with $[M - HCl]^-$ in the absence of *ortho* substitution. In the presence of chloroacetonitrile the spectra are very similar.

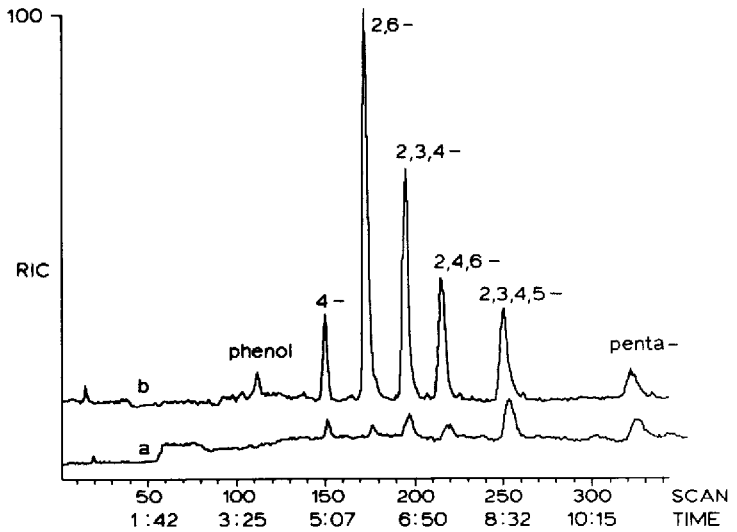

Fig. 2. Reconstructed ion current (RIC) mass chromatograms in LC–NCI MS for a mixture of seven (chloro)phenols with (a) 0 and (b) 1% of chloroacetonitrile in the eluent. Conditions: 200 × 0.7 mm I.D. column packed with 5-μm LiChrosorb RP-18; eluent, acetonitrile–water–acetic acid (70:30:0.2) or acetonitrile–water–chloroacetonitrile–acetic acid (69:30:1:0.2), flow-rate 20 μl/min; analyte concentrations, 120 μg/ml (4-monochlorophenol) to 6 μg/ml (pentachlorophenol); injection volume, 0.5 μl; mass range scanned, m/z 120–400; scan time, 2.0 s; ion source temperature, 300°C.

As can be seen in Fig. 2, on addition of 1% of chloroacetonitrile, the detection sensitivity for most chlorinated phenols increased significantly, especially (up to 30-fold) when the aromatic ring contained 1–3 chlorine atoms. Pentachlorophenol had a comparable sensitivity in both systems with minimum detectable amounts in the RIC mode of about 3 ng of injected analyte at a signal-to-noise ratio of 3:1. Table I shows data on the relative responses of the various chlorophenols, with pentachlorophenol as reference compound. It is surprising that in the presence of 1% of chloroacetonitrile, the responses of 2,6-dichlorophenol and the higher chlorinated phenols are about the same. The response of even phenol is only about one order of magnitude less than that of pentachlorophenol. Although the data in Table I indicate that 200°C is a better choice than 300°C as regards chloride attachment and the response of phenol and 4-chlorophenol relative to that of pentachlorophenol, one should realize that the absolute sensitivity is much lower at 200°C (the detection limit for pentachlorophenol is about 20 ng in the RIC mode), mainly because of instability of the reagent gas: the ion source heating also heats the tip of the interface, and the functioning of the interface deteriorates at lower source temperatures because of insufficient heat transport for the evaporation of the LC effluent.

Fluorine- and bromine-containing reagent gases

Reagent gas ions. The behaviour of fluorine- and bromine-containing instead of chlorine-containing additives in the mobile phase was investigated. The possibility of halide attachment or exchange can partly be predicted by studying the reagent gas

TABLE I

RELATIVE INTENSITIES OF MASS FRAGMENTS OF CHLOROPHENOLS IN LC–NCI MS WITHOUT AND WITH THE ADDITION OF CHLOROACETONITRILE TO THE ELUENT

Conditions: C_{18}/acetonitrile–water (70:30); flow-rate, 20 μl/min.

Chlorophenol	*0% chloroacetonitrile; source temperature 300°C*				
	[M−HCl]⁻	*[M−Cl]⁻*	*[M−1]⁻*	*[M]⁻*	*Relative response**
Phenol	×**	×	×	×	
4-	×	×	100		0.05
2,6-		13	100		0.02
3,4,5-	100	42	11	5	
2,3,4-	11	100	10	9	0.25
2,4,6-		100	4	1	0.15
2,3,4,5-	20	100	1		1
Penta-		100			1
	1% chloroacetonitrile; source temperature 300°C				
	[M−HCl]⁻	*[M−Cl]⁻*	*[M−1]⁻*	*[M+Cl]⁻*	*Relative response**
Phenol	×	×	×	100	0.03
4-	×	×	65	100	0.15
2,6-			100	13	0.75
3,4,5-	16	8	100	2	
2,3,4-		8	100	1	2
2,4,6-		10	100		1
2,3,4,5-		27	100		2
Penta-		32	100		1
	1% chloroacetonitrile; source temperature 200°C				
	[M−HCl]⁻	*[M−Cl]⁻*	*[M−1]⁻*	*[M+Cl]⁻*	*Relative response**
Phenol	×	×	×	100	0.15
4-	×	×		100	0.6
2,6-			71	100	0.75
3,4,5-	8	9	100	13	
2,3,4-	1	3	100	19	2
2,4,6-		3	100	1	1
2,3,4,5-		31	100	3	2
Penta-		42	100		1

* Response relative to pentachlorophenol; for absolute values, see text.

** ×, m/z value does not exceed the threshold of 120.

ions formed. Table II reports the main ions formed with acetonitrile–water (70:30) containing eight different halogenated additives in the mobile phase. As fluoroacetonitrile was not commercially available, other fluorine-containing additives were used.

TABLE II

REAGENT GAS IONS (RELATIVE INTENSITY OVER 20%) WITH DIFFERENT ADDITIVES IN AN ACETONITRILE–WATER ELUENT

Conditions: acetonitrile–water (70:30), 20 μl/min; ion source temperature, 300°C; NCI mode.

Additive	*Reagent gas ions*		
–	100% $[CH_2CN]^-$	80% $[CN]^-$	
0.4% CH_3COOH	100% $[2CH_3COOH-1]^-$	60% $[CH_3COO]^-$	
0.1% CH_2ClCN	100% $[Cl]^-$		
1.0% CH_2ClCN	100% $[Cl]^-$		
0.5% $CHCl_2COOH$	100% $[CCl_2COO]^-$	20% $[CClCOO]^-$	20% $[Cl]^-$
1.0% CH_2ClCH_2OH	100% $[Cl]^-$		
0.3 *M* NH_4Cl	100% $[Cl]^-$		
0.1% CH_2BrCN	100% $[Br]^-$		
1.0% CH_2BrCN	100% $[Br]^-$		
0.3% CF_3COOH	100% $[CF_3COO]^-$	30% $[2CF_3COOH-1]^-$	
0.3% CF_3CH_2OH	100% $[CN]^-$	50% $[CF_3CH_2O]^-$	25% $[(CF_3CH_2C)_2H]^-$
1.0% CF_3CH_2OH	100% $[CF_3CH_2O]^-$	90% $[2CF_3CH_2OH-1]^-$	20% $[CN]^-$
1.0% $CH_2FCH_2OCOCH_3$	100% $[CH_2CN]^-$	40% $[CN]^-$	20% $[CH_2FCH_2OCOCH_2]^-$

Whereas most of the chlorine- and bromine-containing additives mainly generated $[Cl]^-$ and $[Br]^-$, none of the fluorine-containing modifiers generated any $[F]^-$. A disadvantage of bromine- over chlorine-containing additives is the relatively high mass of bromine. The formation of di-Br-containing mass fragments such as $[CH_2BrCNBr]^-$ with its *m/z* 198–200–202 cluster complicates scanning for masses with *m/z* values of less than 200. This limitation can be partly overcome by selected ion monitoring. The addition of halogenated acetic acids cannot be recommended because of the intense and stable $[M]^-$ and $[2M-1]^-$ mass fragments formed. Finally, it is interesting that the use of ammonium chloride also resulted in a reagent gas characterized by $[Cl]^-$.

For obvious reasons, bromoacetonitrile was used as additive in the subsequent experiments.

Bromoacetonitrile. For five di- to penta-substituted chlorophenols the main mass fragments obtained in the presence of 1% of bromoacetonitrile and at an ion source temperature of 200°C are presented in Table III. Scanning was generally carried out in the *m/z* range 205–400.

Comparison of the data in Tables I and III readily shows that the effects of chloroacetonitrile and bromoacetonitrile are notably different. Some examples will be briefly discussed. With 1% of bromoacetonitrile, the pentachlorophenol spectrum consisted of $[M-1]^-$ only, whereas, at the same ion source temperature, 1% of chloroacetonitrile gave a substantial (42%) $[M-Cl]^-$ peak next to the $[M-1]^-$ base peak. For 2,3,4,5-tetrachlorophenol and 2,4,6-trichlorophenol the spectra are dominated by $[M+Br]^-$, while attachment was virtually absent when using chloroacetonitrile. Surprisingly, the other trichlorophenol (2,3,4-) and 2,6-dichlorophenol did not give any bromide attachment but relatively intensive mass fragments formed by chloride–bromide exchange. Further, with chloroacetonitrile the spectra of 2,3,4-

TABLE III

RELATIVE INTENSITIES OF MASS FRAGMENTS OF CHLOROPHENOLS IN LC–NCI MS USING 1% OF BROMOACETONITRILE IN THE ELUENT

Conditions: C_{18}/acetonitrile–water–bromoacetonitrile–acetic acid (69:30:1:0.2); flow-rate, 20 μl/min; ion source temperature, 200°C.

Fragment ion	*Relative intensity (%) of fragments for chlorophenol*				
	2,6-Di-	*2,3,4-Tri-*	*2,4,6-Tri-*	*2,3,4,5-Tetra-*	*Penta-*
$[M - Cl]^-$	×*	10	–	–	–
$[M - I]^-$	–	–	30	50	100
$[M - Cl + HBr]^-$	100	100	–	–	–
$[M + Br]^-$	–	–	100	100	–
Relative response**	0.5	4	2	2	

* ×, *m/z* value does not exceed the threshold of 120.

** Response relative to pentachlorophenol; for absolute values, see text.

trichlorophenol and 2,4,6-trichlorophenol were very similar (see Table I), whereas with bromoacetonitrile the spectra were totally different. Finally, with bromoacetonitrile, 4-chlorophenol and also phenol could not be detected even when scanning from a mass of 150, *i.e.*, with these analytes there is neither bromide attachment nor halide exchange. Obviously, in comparison with chloroacetonitrile, which gave intense fragments due to chloride attachment for the lower and non-chlorinated phenol(s), bromoacetonitrile behaves completely differently.

The relative responses compared with pentachlorophenol (in the RIC mode) were about the same with bromoacetonitrile and chloroacetonitrile (*cf.*, Tables I and III). At an ion source temperature of 200°C and scanning from *m/z* 205 to 400, a detection limit for pentachlorophenol of about 10 ng was obtained.

Fluorine-containing additives. In accordance with the expectations based on the data in Table II, with all three fluorine-containing additives the spectra for the chlorophenols were the same as with acetonitrile–water–acetic acid as eluent, that is, there was no noticeable difference in either fragmentation pattern or halide attachment.

Further test compounds

Five test compounds were selected, *viz.*, L-leucine, L-ascorbic acid, the herbicides 2,4-dichloro- and 2,4,5-trichlorophenoxyacetic acid (2,4-D and 2,4,5-T, respectively) and lindane. Measurements were made by FIA of 0.5 mg/ml solutions using 0.5-μl injection plugs, and carrying out FIA–NCI MS and, occasionally, FIA–PCI MS. The measurements were made with source temperatures from 200 to 300°C and with 0, 0.1 and 1% of chloroacetonitrile in the carrier stream.

2,4-D and 2,4,5-T. Recently, Voyksner *et al.*[12] studied 2,4-D and 2,4,5-T using a Hewlett-Packard probe with a 1:100 splitting ratio and a source temperature of 180°C; the eluent was acetonitrile–water (60:40). In LC–PCI MS, the detection limits were poor owing to a lack of sensitivity, and most of the fragment ions observed could not be identified. As regards sensitivity, the LC–NCI MS results were one order of magnitude better, but again structural information was poor. Because of the ab-

TABLE IV

MASS FRAGMENTS OF 2,4-D AND 2,4,5-T IN FIA–NCI MS USING 0–1% OF CHLOROACETONITRILE IN THE CARRIER STREAM

Conditions: carrier stream, acetonitrile–water (70:30); ion source temperature, 200 or 300°C.

Compound	*m/z*	*Fragment ion*	*Relative intensity (%) at source temperature of:*					
			300°C			*200°C*		
			*0% CAN**	*0.1% CAN*	*1.0% CAN*	*0% CAN*	*0.1% CAN*	*1.0% CAN*
2,4-D	148	$[M-2HCl]^-$	55	11		49	13	
	161	$[M-CH_2COOH]^-$	100	15		90	20	
	184	$[M-HCl]^-$	49	11		100	28	
	219	$[M-1]^-$	43	100		30	100	
	255	$[M+Cl]^-$	3	2		4	18	
2,4,5-T	161	$[M-CHClCOOH]^-$	100	100	21	90	74	24
	182	$[M-2HCl]^-$	9	11	3	18	17	3
	195	$[M-CH_2COOH]^-$	15	35	20	12	28	33
	218	$[M-HCl]^-$	24	30	9	100	100	17
	253	$[M-1]^-$	4	60	100	3	48	100
	289	$[M+Cl]^-$	0	0	0	0	3	8

* Chloroacetonitrile.

sence of $[M]^-$ or $[M-1]^-$ ions, no molecular weight information was available; for both compounds the base peak was $[M-HCOOH]^-$.

Apffel *et al.*[2] used an LC–MS system, interfaced via a post-column extraction system, to analyse 2,4-D and 2,4,5-T. A reversed-phase separation was carried out in a buffered solution at relatively low pH to suppres the ionization of the acids. After the column the compounds were extracted into 1,2-dichloroethane and directed to the mass spectrometer. The NCI spectra of 2,4-D in this instance was characterized by only $[M]^-$ and a considerable enhancement in sensitivity compared with PCI was obtained.

In this study, the herbicides were analysed with acetonitrile–water (70:30) as the carrier stream. Relevant FIA–NCI MS data obtained in the absence and presence of chloroacetonitrile are reported in Table IV. It is evident that these results differ considerably from those of Voyksner *et al.*[12]. For example, at 300°C and without chloroacetonitrile, FIA–NCI MS provides molecular weight information for 2,4-D (relative intensity *ca.* 40%), together with structural information due to the strong fragments $[M-HCl]^-$, $[M-2HCl]^-$ and $[M-CH_2COOH]^-$. With 2,4,5-T, the molecular weight information is poor (relative intensity 4%), but the strong fragments $[M-HCl]^-$ and $[M-CHClCOOH]^-$ are highly specific. For both phenoxyacetic acids, the *m/z* 161 fragment, *i.e.*, $[C_6H_3Cl_2CH_3]^-$, is very strong. Further, one should note that with 2,4-D abstraction of both chlorine atoms, as 2HCl, from the parent molecule takes place easily, whereas the abstraction of 2HCl was below 20% for 2,4,5-T.

On the introduction of 0.1 or 1% of chloroacetonitrile into the carrier stream,

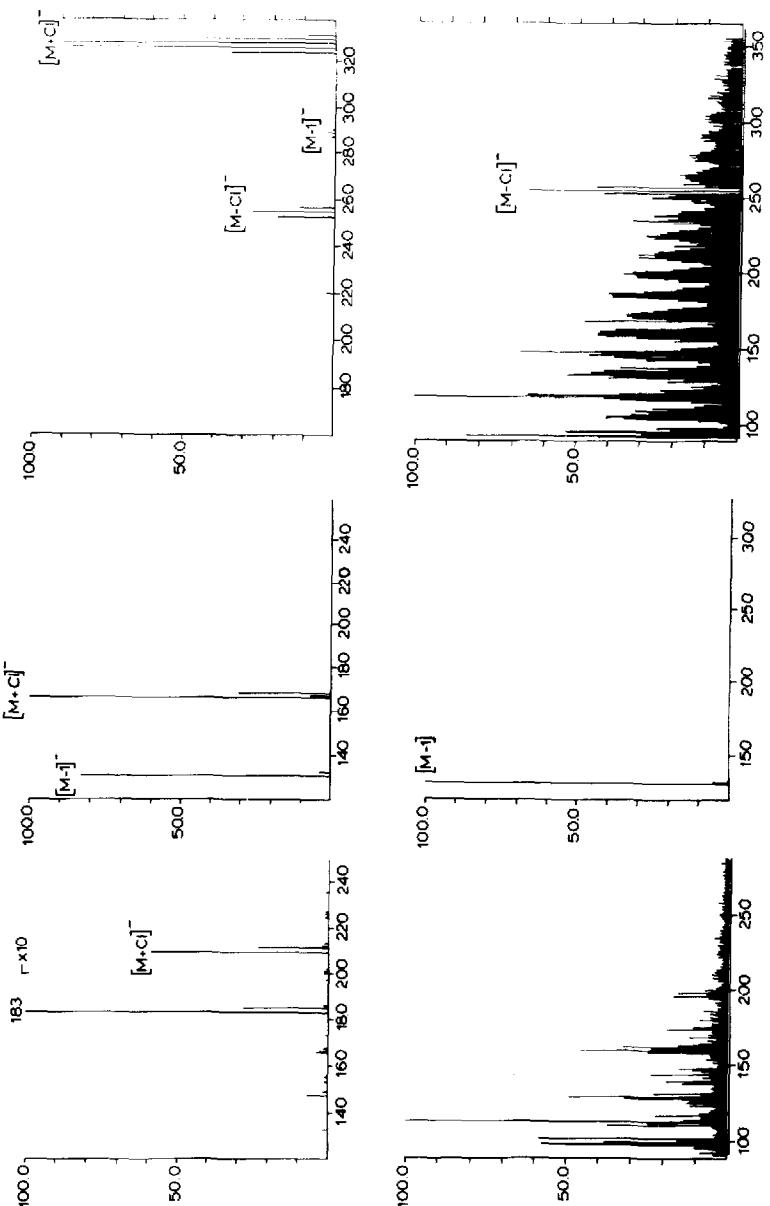

Fig. 3. FIA–NCI mass spectra of L-ascorbic acid, L-leucine and lindane with (above) 0% and (below) 1% of chloroacetonitrile in the carrier stream [acetonitrile–water (70:30)] at an ion source temperature of 250°C.

the NCI mass spectra started to change considerably. From Table IV, these changes are seen to be qualitatively similar for both test compounds, but a higher percentage of chlorinated additive has to be added with 2,4,5-T (1%) than 2,4-D (0.1%) in order to obtain $[M - 1]^-$ as the base peak. Under these conditions, the base peak for both acids is $[M - 1]$ over the temperature range tested. The $[M - HCl]$ and m/z 161 fragment peaks show a sharply decreased intensity.

Finally, it is interesting that, owing to intermolecular exchange, with 2,4-D some chloride attachment is observed even in the absence of chloroacetonitrile.

L-Ascorbic acid. Very poor results were obtained with L-ascorbic acid as test compound: neither in FIA–PCI MS nor in FIA–NCI MS (without chloroacetonitrile) was a chromatographic peak observed or could a spectrum be recorded. On addition of chloroacetonitrile to the carrier stream the situation changed considerably (see Fig. 3). Over the whole temperature range from 200 to 300°C, a distinct base peak was now observed at m/z 183 (fragment not identified), and a major peak due to chloride attachment also showed up.

With each of the three ionization modes tested, the fragments and intensities obtained under specified conditions were the same from run to run. However, they often varied considerably from day to day. This phenomenon probably reflects the ease of fragmentation of the analyte under the present conditions. For such a labile analyte, it can be expected that small changes in the experimental conditions (position of the tip of the fused-silica capillary; internal diameter of the capillary; flow-rate of the carrier stream) effect dramatic changes in the mass spectral intensity.

L-Leucine. L-Leucine showed up well in PCI MS; the only peak occurring was $[M + 1]^+$. As is demonstrated in Fig. 3, the mass spectrum was also very simple in NCI MS (without chloroacetonitrile; only $[M - 1]^-$ will show up), but this instance the sensitivity was less good. Experiments in the presence of chloroacetonitrile yielded a sensitivity comparable to that found with PCI MS. At temperatures below about 270°C the chloride-attachment fragment $[M + Cl]^-$ is the base peak, with a second large peak due to $[M - 1]^-$ showing up. The latter peak is the base peak at temperatures above 270°C.

Lindane. In FIA–PCI MS, a small positive peak was visible in the RIC chromatogram, but, owing to fragmentation, no fragments with m/z values of over 120 were observed in the mass spectrum. In FIA–NCI MS (without chloroacetonitrile) the mass spectra were also very poor (see Fig. 3), but now a small negative peak showed up in the RIC chromatogram. A similar phenomenon was observed with several brominated compounds, *e.g.*, tribromomethane and 4,4′-dibromobiphenyl.

Results in the presence of chloroacetonitrile showed the presence of two main clusters, one due to chloride abstraction, $[M - Cl]^-$, and one to chloride attachment, $[M + Cl]^-$. The latter cluster was invariably the base peak, irrespective of the ion source temperature, and the percentage of chloroacetonitrile. In the presence of chloroacetonitrile, a positive peak showed up in the RIC chromatogram.

CONCLUSIONS

The detection potential of on-line LC–NCI MS can easily be extended by adding a small amount of a halogenated modifier to the LC eluent. In this respect, the use of chloroacetonitrile can be recommended, because it is relatively inexpensive, does not change the chromatographic behaviour of the analytes and only yields reagent gas ions with low m/z values. Interesting changes occur, however, in the mass

spectra of the analytes when working the chloride-enhanced NCI instead of in the conventional NCI mode.

In this study, the addition of 0.1–1% of chloroacetonitrile to the LC eluent was shown to have a profound effect on the mass spectra of all chlorophenols tested. With phenol and the low-chlorinated phenols, the $[M + Cl]^-$ cluster became the dominant peak (instead of $[M - 1]^-$) while the detection sensitivity showed a considerable increase. With the tri- and higher chlorinated phenols, $[M - 1]^-$ invariably became the base peak (instead of $[M - Cl]^-$), while the detection sensitivity increased only modestly, if at all. Similar behaviour was observed for the phenoxyacetic acids 2,4-D and 2,4,5-T, with chloride attachment occurring more easily with the former compound.

Chloride attachment, which almost always increases with a decreasing ion source temperature, was also observed for L-leucine, L-ascorbic acid and lindane. In addition, for both these and the earlier test compounds, it was observed that the presence of chloroacetonitrile in the system generally simplifies the mass spectra and makes them easier to interpret.

The combined data, which support and considerably extend earlier findings reported by Parker *et al.*[10], illustrate the analytical usefulness of on-line LC with chloride-enhanced NCI MS detection. The general experience is, however, too limited and the number of test compounds is still too small to draw valid conclusions about the classes of compounds that will benefit most from the new detection technique. To quote one example, Dougherty and Wander[11] concluded that strong chloride attachment will take place for compounds containing an acidic group. In our work, this conclusion is borne out by the behaviour of L-leucine and L-ascorbic acid but not, however, of the phenoxyacetic acids tested.

Future work will be directed at the further evaluation of the analytical advantages of chloride-enhanced NCI MS detection. Special attention will be paid to various classes of organophosphorus pesticides, which frequently cause problems in trace-level determinations by means of gas chromatography. The study of these compounds will also allow a direct comparison with the work of Parker *et al.*[10]. This is of some interest, because our present results seem to indicate that chloride attachment can be observed at much higher source temperatures (200–300°C) than was reported by Parker *et al.* (80–200°C). Should the same conclusion be reached for pesticides such as dimethoate also studied by Parker *et al.*, then this will demonstrate a major role of the type of LC–MS interface used. In the opposite case, the relative importance of the class of test compounds selected will be indicated.

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