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LIQUID CHROMATOGRAPHY–CHLORIDE-ATTACHMENT NEGATIVE CHEMICAL IONIZATION MASS SPECTROMETRY

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SUMMARY

Chloride-attachment negative chemical ionization mass spectra can be generated by the addition of chlorinated solvent modifiers to the high-performance liquid chromatographic mobile phase in liquid chromatography–mass spectrometry. The observed mass spectra of several organophosphorus pesticides are similar to those reported for direct probe analysis with methylene chloride as the reagent gas. For certain classes of compounds, this mode of ionization yields more structural information than does conventional negative chemical ionization mass spectrometry. The addition of chlorinated solvent modifiers to the high-performance liquid chromatographic mobile phase in liquid chromatography–mass spectrometry allows for the analysis of mixtures by chloride-attachment negative chemical ionization.

INTRODUCTION

It has been demonstrated that successful chloride-attachment negative chemical ionization (NCI) mass spectrometry, developed by Dougherty and co-workers[1–10], can be adapted to various environmentally and biologically important substances. While chloride-attachment NCI mass spectra have been induced by a variety of chlorinated reagent gases in direct probe analysis, the application to sophisticated microanalytical techniques such as combined liquid chromatography–mass spectrometry (LC–MS) has not yet been performed. It was our hypothesis that chloride-attachment NCI mass spectra could be obtained in direct liquid introduction (DLI) LC–MS analysis through the addition of a chlorinated solvent modifier.

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The formation of $(M + Cl)^-$ ions was first observed by Dougherty *et al.*[1] in the isobutane mass spectra of aromatic chlorinated pesticides. The source of the Cl^- was the pesticide molecule itself, by dissociative electron capture. Subsequently, Dougherty and his research group[2] studied chloride-attachment NCI using methylene chloride as the source of Cl^-. Strong $(M + Cl)^-$ peaks were observed for a variety of organic compounds, including aromatic and aliphatic carboxylic acids, amides, amino acids, and aromatic amines and phenols. Other classes of compounds, such as aliphatic hydrocarbons and lipids, were transparent to chloride-attachment NCI and it was postulated that in chloride-attachment NCI, sensitivity was highest for compounds with acidic protons[2,3]. The chloride-attachment technique was also used by Bose *et al.*[11] for compounds of biomedical interest, such as bile acids and bile-acid conjugates.

Earlier NCI work with organophosphorus pesticides[12,13] has shown that, while electron-capture NCI is a very sensitive method for the analysis of these compounds, there is often very little structural information contained in these spectra. The concept described in this paper is the application of chloride-attachment NCI to DLI LC–MS analysis, for the purpose of obtaining more structural information from the mass spectra obtained in an LC–MS analysis. The compounds selected for this study were organophosphorus pesticides, since their LC–chloride-attachment NCI spectra could be compared with those obtained by Dougherty and Wander using direct probe analysis[9].

MATERIALS AND METHODS

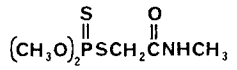
Equipment

The HPLC system used consisted of two Waters 6000A pumps (Waters Assoc., Milford, MA, U.S.A.) and an ISCO V4 UV–Vis detector (ISCO, Lincoln, NE, U.S.A.) operated at 254 nm. The column used was a DuPont Zorbax C_8 HPLC column (25 cm × 4.3 mm I.D.) (DuPont, Wilmington, DE, U.S.A.).

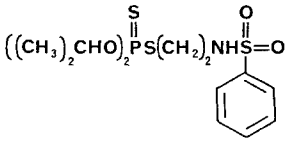
The LC–MS interface was an unmodified Hewlett-Packard Direct Liquid Insertion Probe[16] (Hewlett-Packard, Palo Alto, CA, U.S.A.).

The mass spectrometer used for the LC–MS study was a Finnigan 3300 chemical ionization mass spectrometer (Finnigan-MAT, San Jose, CA, U.S.A.) previously modified for NCI operation[14]. The mass spectrometer was interfaced to a Finnigan--Incos 2300 data system. The mass spectrometer was scanned at a rate of 4 sec per scan. Modifications to the inlet system and the source to allow use of the DLI probe have been described previously[13,15].

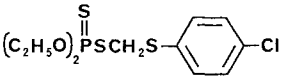
The collision-induced dissociation–mass-analyzed ion kinetic energy (CID–MIKE) experiment was performed on a ZAB-2F mass spectrometer (VG Analytical, Altrincham, U.K.) equipped with a Finnigan-Incos 2300 data system (Finnigan-MAT). Methylene chloride (Fisher Scientific, Fairlawn, NJ, U.S.A.) was admitted through the septum inlet reservoir to give a source pressure of *ca.* 0.05 Torr (in the source block). The source temperature was *ca.* 220°C, with 7 kV accelerating voltage, 70 eV electron energy, and 0.5 mA emission current. Helium was used as the collision gas, at a pressure sufficient to reduce the main beam intensity by *ca.* 50%.



DIMETHOATE MW 229



BENSULIDE MW 397



CARBOPHENOTHION MW 342

Fig. 1. Structures of dimethoate, bensulide, and carbophenothion.

Samples and solvents

Samples of the organophosphorus pesticides used were obtained from the U.S. Environmental Protection Agency. Stated purities of dimethoate, bensulide, and carbophenothion were 95, 95 and 98%, respectively. Structures of these compounds are shown in Fig. 1. High-performance liquid chromatographic (HPLC) analyses were consistent with these values.

The solvents used in this study were HPLC-grade acetonitrile (Fisher) and HPLC-grade water from a Millipore (Bedford, MA, U.S.A.) filtration system. The acetonitrile was filtered through a Millipore 0.5-μm FHUP filter; the water was filtered through a 0.45-μm Millipore HA filter. Chloroacetonitrile was obtained from Eastman Kodak (Rochester, NY, U.S.A.).

RESULTS AND DISCUSSION

LC solvent/reagent gas spectra

The reagent ion spectrum for 85% acetonitrile in water showed a base peak at m/z 26, corresponding to CN^-, and a peak at m/z 40 from CH_2CN^-. Adding 1% chloroacetonitrile to the acetonitrile leads to a spectrum dominated by ions from the chloroacetonitrile. The ions which carry almost all of the ion current were at m/z 35 and 37, corresponding to Cl^-. Other cluster ions and chloride-attachment ions occurred at m/z 53, 76, 93, 110 and 128, probably corresponding to the elements of

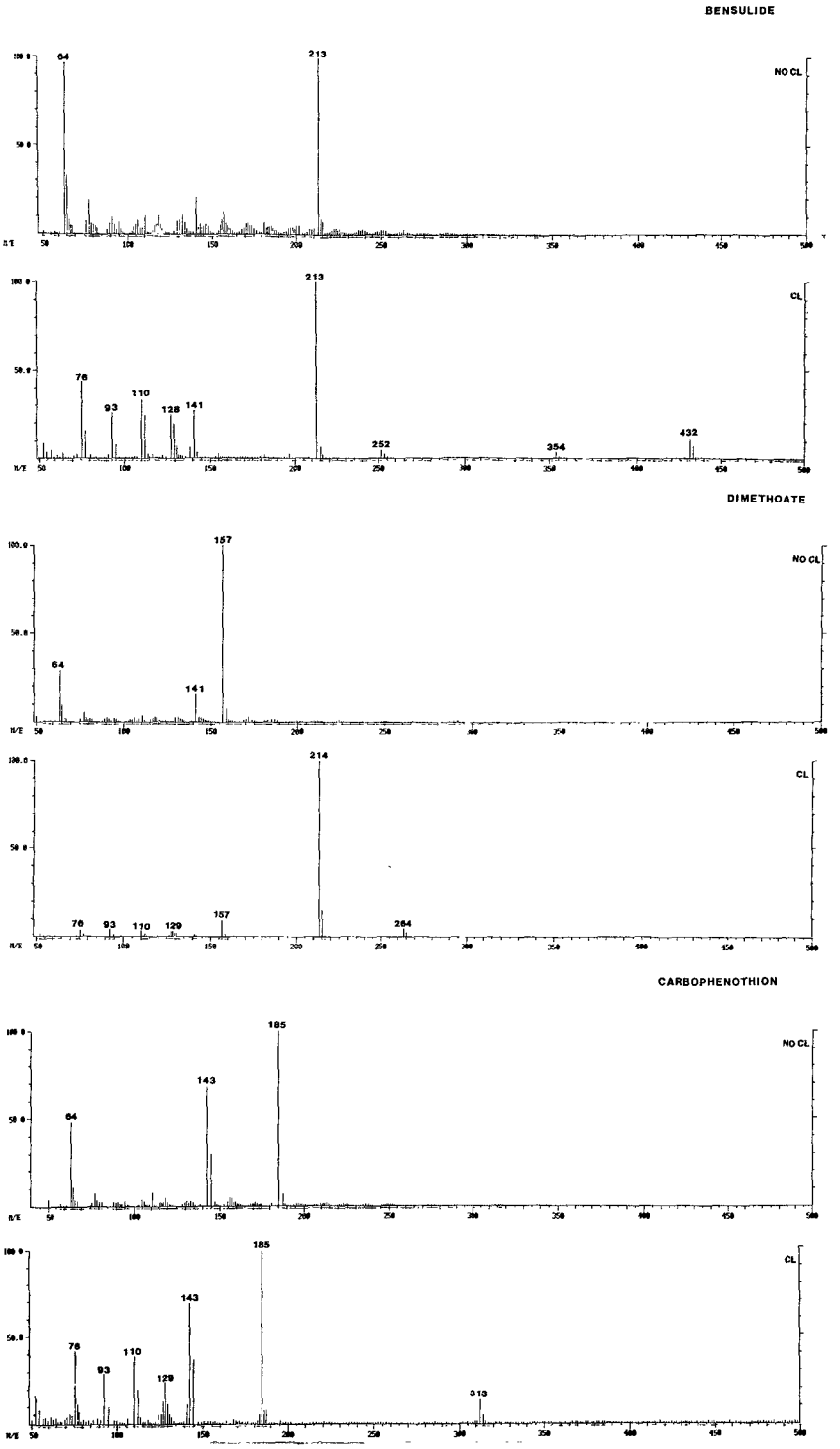


Fig. 2. LC–NCI mass spectra and LC–chloride-attachment NCI mass spectra of dimethoate, bensulide, and carbophenothion.

H_2OCl^-, $C_2H_3NCl^-$, $C_2H_2NCl_2^-$, and $C_2H_4Cl_2NO^-$, respectively. These are apparently cluster ions of acetonitrile, chloroacetonitrile, Cl^-, and water.

The "saturation" effect of chloroacetonitrile addition was demonstrated in a gradient analysis of a mixture of 1 mg/ml dimethoate in 80% acetonitrile in water (solvent A) and 80% (1% chloroacetonitrile in acetonitrile) in water (B). In this experiment, the amount of dimethoate remained constant, while the chloroacetonitrile concentration was varied from 0 to 0.8% over a 15-min. gradient. A sharp onset of chloride-attachment was observed when the chloroacetonitrile reached the source. No gradual increase in the intensity of the peak resulting from the chloride-attachment process was observed with increasing chloroacetonitrile concentrations; similar chloride-attachment spectra were observed with 1% and 0.1% chloroacetonitrile solutions.

NCI and chloride-attachment NCI mass spectra

LC–NCI and LC–chloride-attachment NCI mass spectra, obtained at a source temperature of 140°C, of dimethoate, bensulide, and carbophenothion are shown in Fig. 2. As can be seen, changes in the mass spectra can be induced by the addition of chlorinated solvent modifiers. Mass spectra of each pesticide are discussed below.

Dimethoate. The NCI mass spectrum of dimethoate (mol.wt. 229) gives mainly the *m/z* 157 ion $[(CH_3O)_2PS_2^-]$, which indicates that it is a dimethoxyphosphorodithioate, and a smaller oxygen-exchange peak at *m/z* 141, corresponding to $[(CH_3O)_2PSO^-]$, but does not give much structural information. From the NCI mass spectrum, it would be difficult to distinguish this compound from another dimethoxyphosphorodithioate, for example, malathion[12,13].

More information is obtained from the LC chloride-attachment spectra, *i.e.* a base peak corresponding in mass to $(M-15)^-$ and peaks at *m/z* 264 and 266 corresponding to $(M+Cl)^-$. The peak at *m/z* 157 is still present.

Bensulide. The NCI mass spectrum of bensulide (mol.wt. 397) shows a base peak of *m/z* 213, corresponding to $[(i\text{-}PrO)_2PS_2]^-$, with a smaller unidentified fragment at *m/z* 141, possibly corresponding to $(C_6H_6SO_2)^-$.

The chloride-attachment mass spectrum of bensulide (mol.wt. 397) still shows as the base peak the fragment at *m/z* 213, but shows, in addition, a chloride-attachment peak at *m/z* 432, corresponding to $(M+Cl)^-$. Other small fragments probably correspond to $(SCH_2CH_2NHSO_2C_6H_5)^-$ at *m/z* 216, an unidentified ion at *m/z* 252, and an ion corresponding in mass to $(M-59)^-$ at *m/z* 354.

Carbophenothion. The NCI mass spectrum of carbophenothion (mol.wt. 342) shows a base peak at *m/z* 185, corresponding to the diethoxyphosphorodithioate ion, $[(CH_3CH_2O)_2PS_2]^-$, and peaks at *m/z* 143 and 145 corresponding to $[SC_6H_4Cl]^-$.

The chloride-attachment mass spectrum of carbophenothion also shows large peaks at *m/z* 185 and 143 and 145, as does the normal NCI mass spectrum. In addition, there are peaks at *m/z* 313, corresponding to $(M-29)$, and a low intensity peak at *m/z* 377, corresponding to the $(M+Cl)^-$ ion.

Temperature studies

In order to confirm that similar processes are occurring in both chloride-attachment NCI with methylene chloride as reagent gas and LC chloride-attachment with 80% (1% chloroacetonitrile in acetonitrile) in water (v/v) as reagent gas, tem-

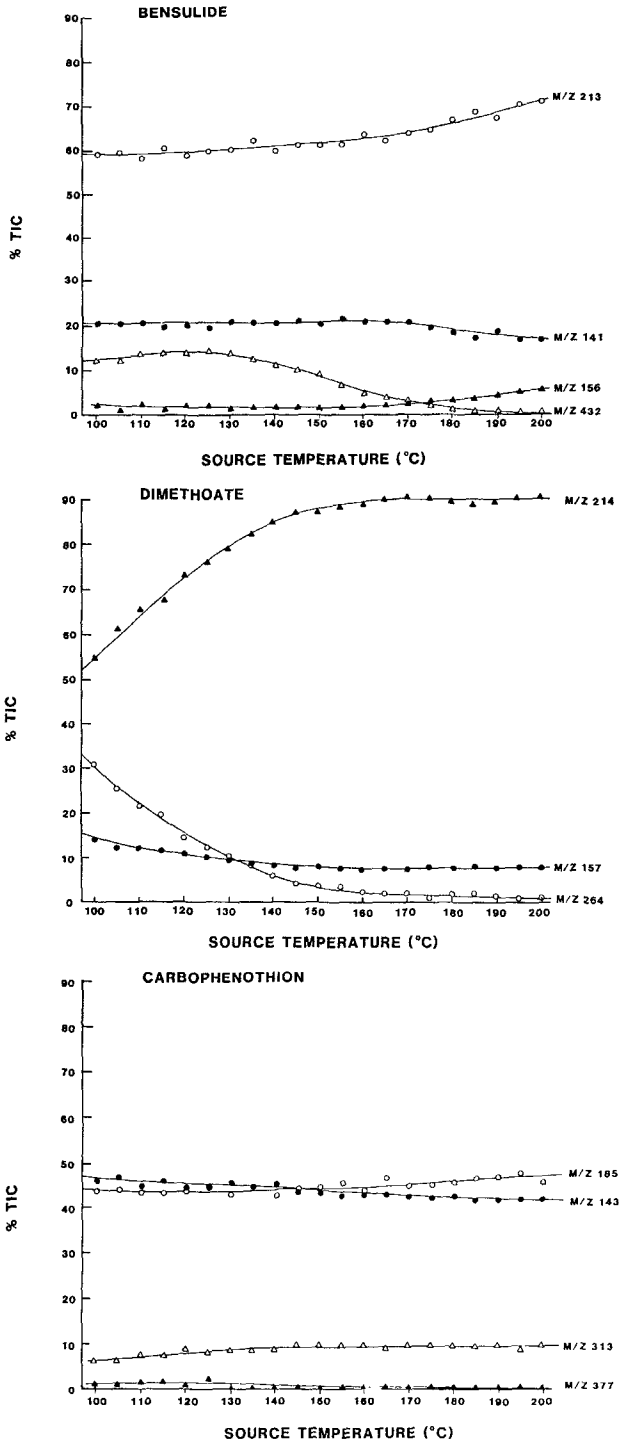


Fig. 3. Temperature study of selected ions from the LC–chloride-attachment mass spectra of dimethoate, bensulide, and carbophenothion.

perature studies were done on the three pesticides. A solution of *ca.* 0.8 mg/ml of each pesticide in 80% (1% chloroacetonitrile in acetonitrile in water (v/v)) was introduced into the mass spectrometer via the DLI interface. Source temperature was varied in 5°C increments over the range 100–200°C. Relative abundances of the major fragments from each pesticide are plotted in Fig. 3. As can be observed from this figure, the abundance of LC chloride-attachment peaks increases as the source temperature decreases, as has been observed in methylene chloride chloride-attachment NCI. It is also apparent from the figure that these three compounds behave differently with respect to temperature-induced changes in their spectra. This temperature effect was most pronounced for dimethoate. Spectra taken at 80°C and 130°C are shown in Fig. 4.

Changes in the mass spectra with temperature observed under LC–MS conditions were similar to changes in relative ion abundances observed by Dougherty and Wander[9] for four temperatures. Since chloride-attachment NCI and LC–chloride-attachment NCI mass spectra with chloroacetonitrile show such similar results, it is probable that the same processes are operating in both cases. The differing behavior of these three pesticides with changing source temperatures was attributed

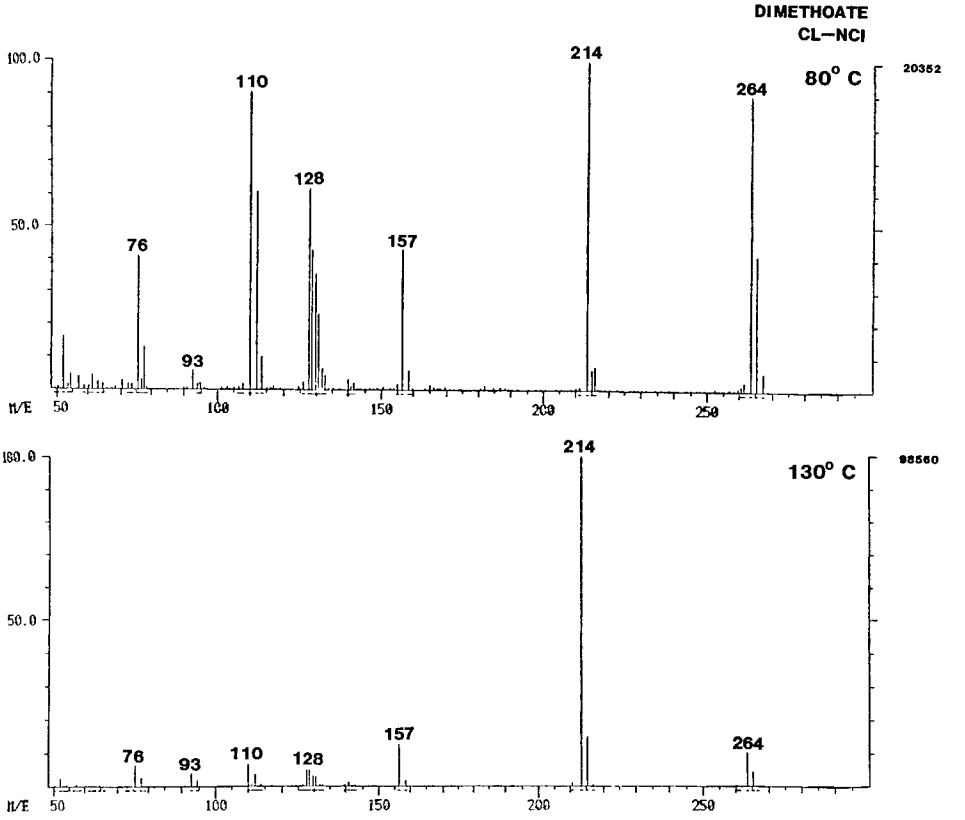


Fig. 4. LC–chloride-attachment NCI mass spectra of dimethoate at a source temperature of 80°C (A) and 130°C (B).

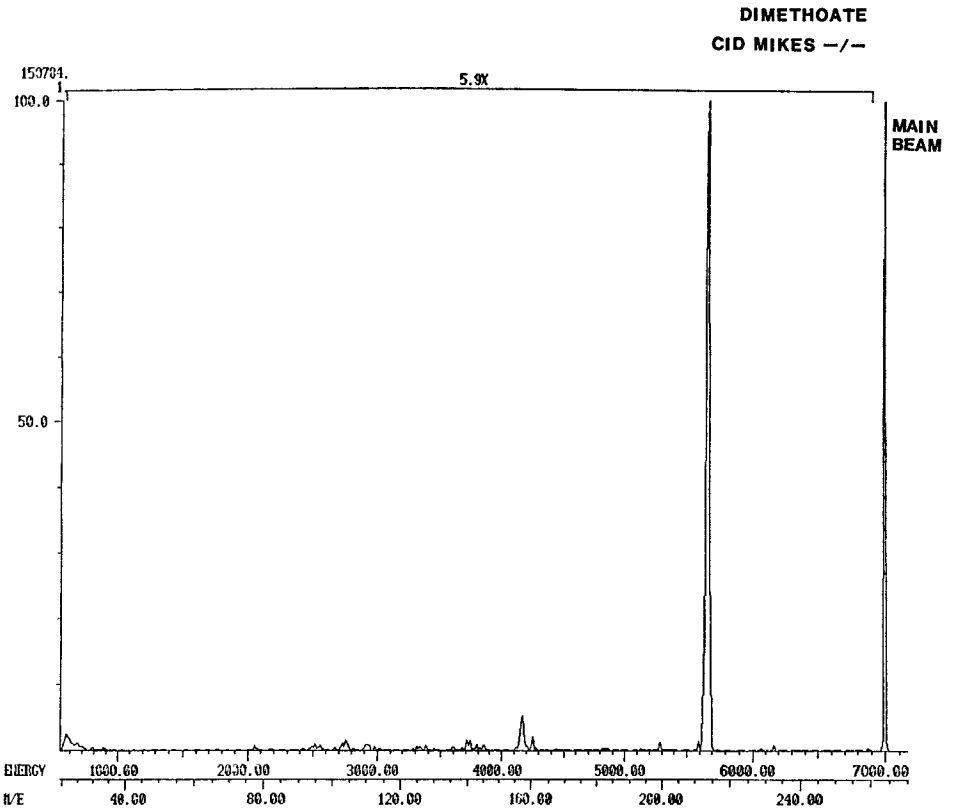


Fig. 5. CID–MIKE spectrum of dimethoate. Main beam, *m/z* 264.

by Dougherty and Wander[9] to varying combinations of dissociative electron capture, thermal activation of dissociative electron capture, and chloride attachment.

Interestingly, the ion assigned by Dougherty and Wander to $(M-R)^-$, *i.e.* $(M-CH_3)^-$, $(M-C_3H_7)^-$, and $(M-C_2H_5)^-$, and in dimethoate, bensulide, and carbophenothion, respectively, did not appear in the electron-capture NCI mass spectrum. These researchers assigned this ion to thermal activation of dissociative electron capture. Since in dimethoate the $(M-R)^-$ peak is the most intense peak in the chloride-attachment NCI spectrum, but was not observed in the electron-capture NCI mass spectra, this ion was selected for further studies. In a CID–MIKES experiment, the chloride-attachment peak $(M+Cl)^-$ at *m/z* 264 was selected as the main beam. The resulting spectrum is shown in Fig. 5. This experiment indicates that at least a portion of the *m/z* 214 ion is actually a loss of 50 amu from the $(M+Cl)^-$ ion, probably $(M+Cl-CH_3Cl)^-$. Since there was no M^- or $(M-1)^-$ ion in the spectrum, it was not possible to determine whether these ions could have also been precursors to the *m/z* 214 ion.

LC–MS traces

While the addition of a small amount of chloroacetonitrile to the LC solvent significantly affects the mass spectra, it does not appear to have a deleterious effect

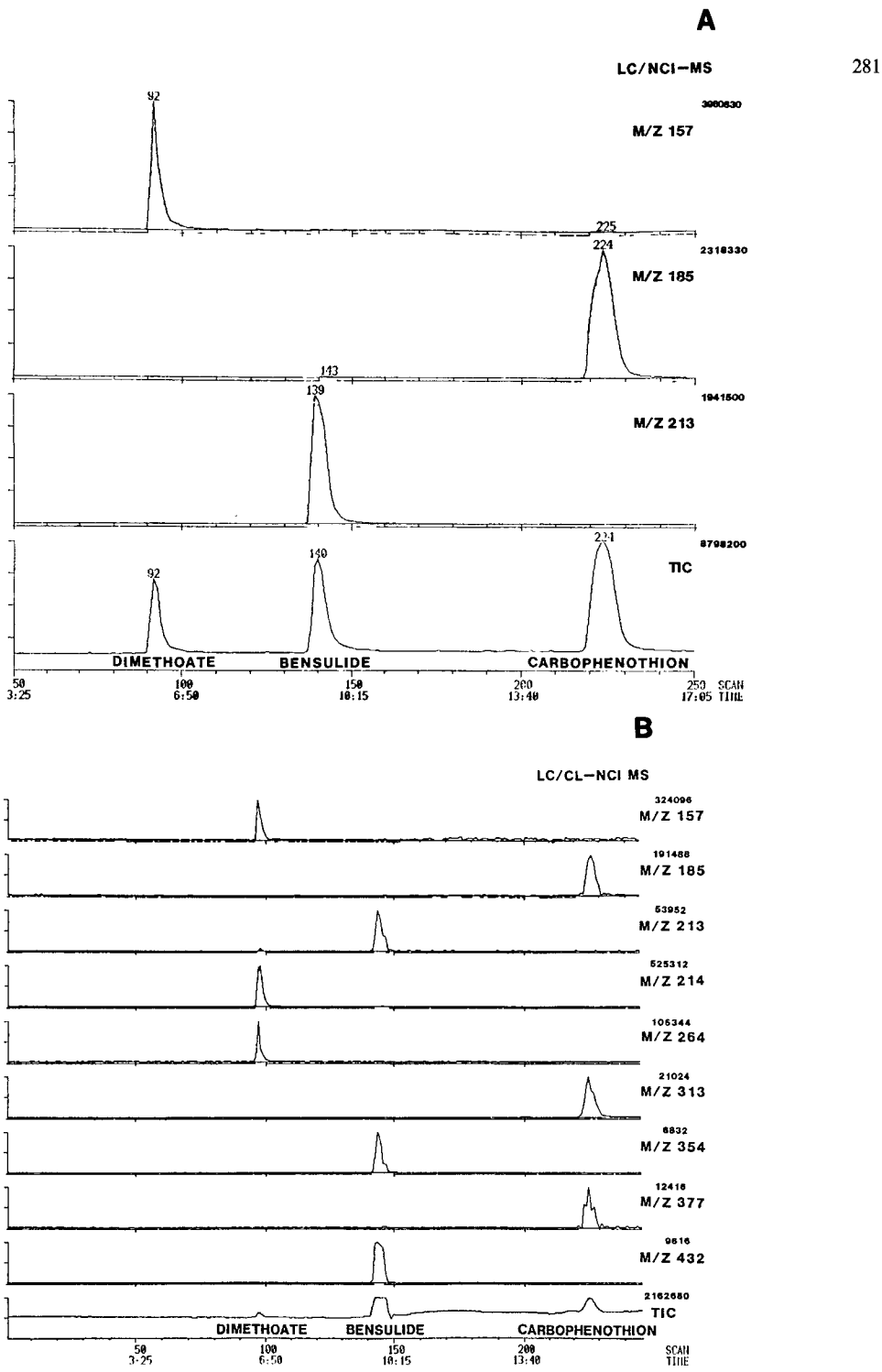


Fig. 6. LC–MS analysis of a mixture of the three pesticides. TIC and RIC traces for a mixture of dimethoate, bensulide, and carbophenothion: (A) LC–NCI-MS, (B) LC–chloride-attachment NCI-MS. Approximately 1.5 μg of each component; flow-rate, 500 μl/min, 80% (1% chloroacetonitrile in acetonitrile) in water, 25 cm × 4.3 mm I.D. Zorbax C-8 column.

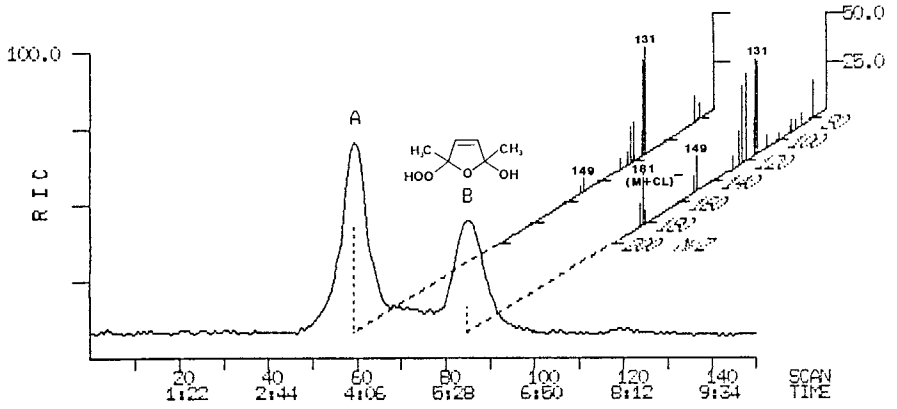


Fig. 7. Normal-phase LC–MS analysis of a substituted furan (peak B) and its decomposition product (peak A), 0.5 ml/min, acetonitrile–methylene chloride (1:3).

on the separation. Total ion current (TIC) chromatograms and reconstructed ion chromatograms (RIC) for selected ions for a mixture of the three pesticides with and without chloroacetonitrile are shown in Fig. 6. Source temperatures were *ca.* 145°C. Although it would have been desirable to work at lower source temperatures, where the intensities of the chloride-attachment peaks are higher, the lowest source temperature achievable on the Finnigan 3300 in continuous LC–MS operation is *ca.* 140–150°C, even with the source heaters off, due to heating from the filament. Lower source temperatures can be achieved (for short LC–MS runs) by leaving the filament off between analyses.

LC–chloride-attachment NCI-MS is not restricted to chloroacetonitrile. Similar mass spectral alterations have been induced by the additions of chloroacetic acid and other chlorinated solvents. It should be noted, however, that acidic solvent modifiers may change the elution order in certain separations. The molecular weight of the chlorinated solvent modifier must also be taken into account in LC–MS applications, since higher m/z fragments and cluster ions may interfere with the analysis of the sample.

Chloride-attachment is also not restricted to reversed-phase systems. Chloride-attachment mass spectra have been observed in this laboratory with normal-phase solvent systems such as methylene chloride–acetonitrile. Fig. 7 shows the analysis of 2,5-dimethyl-2-hydroxy-5-hydroperoxy-2,5-dimethylfuran (peak B). This compound is formed when superoxide is trapped with dimethylfuran in xanthine oxidase–acetaldehyde systems. Chloride-attachment NCI DLI LC–MS gave molecular weight information for peak B (peak A is a breakdown product of peak B).

CONCLUSION

In summary, chloride-attachment NCI mass spectra can be obtained by the addition of a small amount of chlorinated solvent modifier to a conventional reversed-phase solvent system. As has been previously noted for DLI LC–NCI-MS[13], more fragmentation is observed in LC–chloride-attachment NCI-MS than has been

reported for chloride-attachment NCI-MS with methylene chloride as the reagent gas, but the same mechanisms appear to be operating in both cases. Chloride-attachment can be thought of as analogous to a gas-phase derivatization technique. For compounds amenable to this technique, it provides an additional "fingerprint" of a compound, a supplement to the retention time and mass spectral information provided by conventional LC–PCI- and LC–NCI-MS.

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