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REPELLER EFFECTS IN DISCHARGE IONIZATION IN LIQUID AND SUPERCRITICAL-FLUID CHROMATOGRAPHY–MASS SPECTROMETRY USING A THERMOSPRAY INTERFACE

II. CHANGES IN SOME ANALYTE SPECTRA

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SUMMARY

By changing the potential of the repeller electrode in a thermospray ion source operated under discharge-on conditions in liquid chromatography–mass spectrometry and supercritical-fluid chromatography–mass spectrometry the appearance of the mass spectra can be changed. Either abundant protonated molecules or considerable fragmentation can be obtained. The repeller-induced fragmentation can result in useful fragmentation. The selection of the most appropriate repeller potential is a delicate task because the effects appear to be compound-dependent. Compounds which are fragmented easily will generally not give useful spectra at very high repeller potentials. The results indicate that different mechanisms, *i.e.*, chemical ionization with different reagent gas compositions and collisionally induced dissociation, play important roles in fragmentation.

INTRODUCTION

Supercritical-fluid chromatography–mass spectrometry (SFC–MS) is an important technique. The field of application of SFC overlaps to some extent that of liquid chromatography (LC) and with that of gas chromatography (GC). Since SFC–MS is easier to accomplish than LC–MS, a shift from LC–MS to SFC–MS is expected, especially when with SFC–MS similar information can be obtained. The information content of SFC–MS largely depends on the type of ionization techniques that can be used.

SFC–MS has been investigated for several years, and an excellent review on the developments within this field has been written by Smith *et al.*¹ Interfacing between SFC and MS is based on either supersonic molecular beam systems^{2,3}, direct fluid introduction through a diaphragm⁴ or another type of restriction⁵, modified thermospray interfaces^{6,7} and moving belt interfaces⁸.

As usual with the moving belt, electron impact (EI) and chemical ionization (CI) are possible⁸, while in the direct introduction type of interfaces, filament-assisted ionization is performed in either the EI or the CI mode. With pure carbon dioxide charge exchange (CE) CI takes place, while a reagent gas can be added to obtain proton transfer CI. Ionization by means of a discharge instead of a filament in either a conventional CI source⁹ or a thermospray source⁷ has also been demonstrated. Recently, it was shown that in SFC-MS, similar to LC-MS, in the discharge-on mode the potential at the repeller electrode can be used to induce fragmentation^{10,11}. In LC-MS studies the repeller electrode in the discharge-on mode has been claimed to induce fragmentation, which has been explained in terms of collisionally induced dissociations (CIDs)^{12,13}. From our systematic studies on the repeller effects in the discharge-on mode in both LC-MS and SFC-MS it appears that CID of analyte ions is not the only process. In the first part of this series¹⁴ the effect of the repeller voltage on the reagent gas spectrum in the discharge-on mode with LC-MS and SFC-MS was discussed. In this second part the insights obtained are applied in the explanation of repeller-induced spectral changes in analyte mass spectra. Most emphasis is given to the SFC-MS results, although for some compounds a comparison is made with LC-MS data as well. The analytical potential of the repeller effects in both qualitative and quantitative analysis is discussed.

EXPERIMENTAL

(Tandem) MS was performed on a Finnigan MAT TSQ-70 instrument (Finnigan, San José, CA, U.S.A.), equipped with a Finnigan MAT thermospray interface. The discharge electrode was operated at potentials between 800 and 1200 V. MS-MS experiments were performed with air as a collision gas at a pressure of 0.05–0.15 Pa in the collision cell. The collision energy was optimized for each application.

The packed column SFC-MS experiments were performed on a laboratory-built SFC instrument consisting of slightly modified commercially available modules. The system has been described in detail elsewhere¹⁵. The mobile phase was carbon dioxide modified with 2–15% of methanol at a flow-rate of 2 ml/min. The laboratory-packed column (150 mm × 4.6 mm I.D.) was packed with either Nucleosil C₁₈ (5 μm; Macherey-Nagel, Düren, F.R.G.), or Rosil aminopropyl (7 μm; Alltech, Deerfield, IL, U.S.A.). The vaporizer capillary was used as the pressure restrictor by pinching the last part of the tube until stable back pressure of typically between 30 and 35 MPa was achieved. The block temperature was kept at 150°C and the vaporizer temperature was 50°C unless stated otherwise. The analytes were dissolved in methanol.

The LC-MS experiments were performed in the flow injection (FIA) mode with 20–80% of methanol in water at a flow-rate of 1.2 ml/min. The solvent was delivered with two Model 2150 LC pumps (LKB, Bromma, Sweden) controlled by an LKB Model 2152 LC controller. A block temperature of 200°C and a vaporizer temperature of 90–110°C were used. The samples were dissolved in the solvent used in that particular experiment.

RESULTS AND DISCUSSION

Polycyclic aromatic hydrocarbons

The first group of compounds for which the repeller effects in the discharge-on mode with SFC-MS were studied systematically, were some polycyclic aromatic hydrocarbons (PAHs). In Fig. 1 mass chromatograms are given for two successive injections at two different repeller potentials (20 and 120 V) of a standard mixture of anthracene (molecular weight, MW 128), phenanthrene (MW 178) and pyrene (MW 202). A mobile phase of 7% methanol in carbon dioxide was used. Signals from the molecular ions of the PAHs are given in the upper trace of Fig. 1 and signals from the protonated molecules in the lower trace. (The chromatograms at the right and at the left are normalized at the highest peak in the two; therefore no isotope peaks are detected in the lower trace.) With a repeller potential of 20 V, peaks from the protonated molecules of the PAHs are observed (Fig. 1A), while these peaks are not detected with a repeller potential of 120 V. At a repeller potential of 120 V, peaks from the molecular ions of the PAHs are observed (Fig. 1B), which are absent at a repeller potential of 20 V.

Mass spectra of phenanthrene, obtained at low and high repeller potentials, are given in Fig. 2. At a repeller potential of 20 V a protonated molecule is observed at $m/z = 179$ without any fragmentation, while at a repeller potential of 120 V a molecular ion is observed at $m/z = 178$, with considerable fragmentation. The type of fragmentation observed is well known from the EI spectra of phenanthrene.

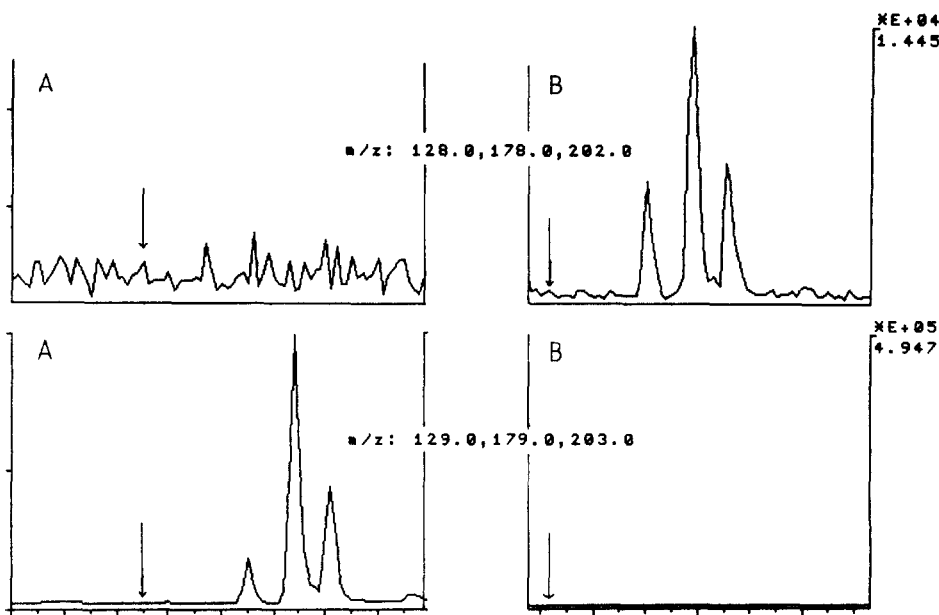


Fig. 1. Reconstructed mass chromatograms of a mixture of naphthalene (MW 128), phenanthrene (MW 178) and pyrene (MW 202) with a repeller potential of (A) 20 V and (B) 120 V. Conditions: SFC-MS with 7% of methanol in carbon dioxide and a C_{18} column. Other conditions: see text.

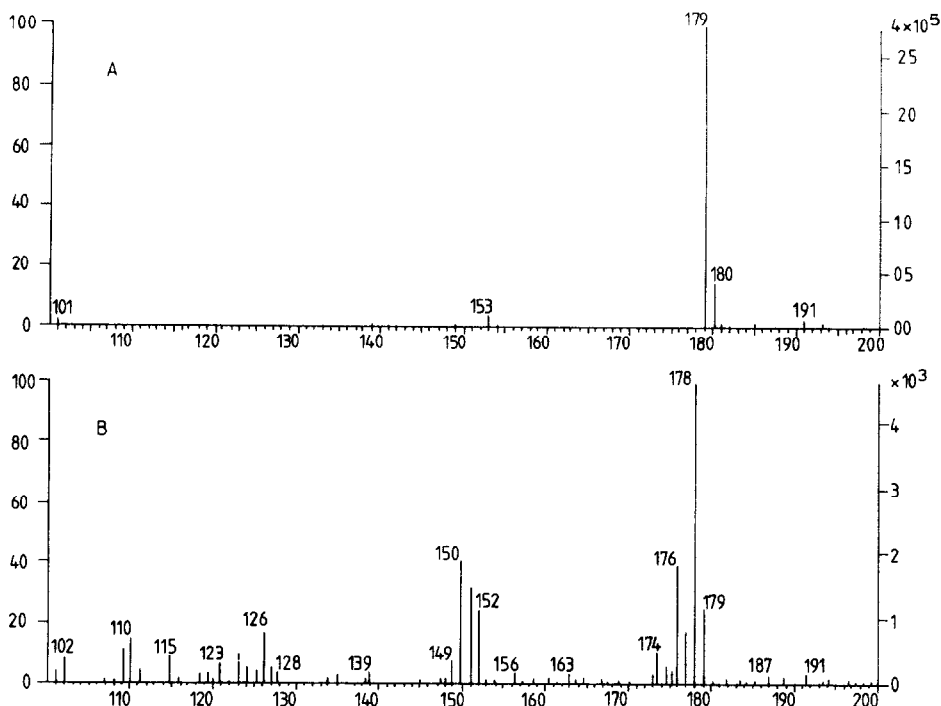


Fig. 2. Mass spectra of phenanthrene at a repeller potential of (A) 20 V and (B) 120 V. Conditions: see Fig. 1.

These results show that the change in the reagent gas spectrum, induced by the change of the repeller potential¹⁴, influence the analyte mass spectra as well. Proton transfer CI effects are observed at low repeller potentials, while charge exchange CI effects are observed at high repeller potentials. Protonation takes place by the protonated methanol (clusters), and perhaps to some extent by the protonated carbon dioxide, while the carbon dioxide molecular ion presumably is responsible for the charge exchange effects observed. This is in agreement with the changes in the reagent gas spectra reported previously¹⁴.

The influence of the repeller potential on naphthalene spectra has been studied at 2, 7 and 10% of methanol in carbon dioxide. Similar effects of the repeller potential on the appearance of the mass spectra were found. However, the degree of fragmentation at a particular repeller potential is related to the methanol content of the mobile phase. At a particular repeller potential, more fragmentation of naphthalene is observed with 2% of methanol than with 10% of methanol. In general, higher repeller potentials are needed to induce fragmentation in a mobile phase with an high methanol content in carbon dioxide. As indicated in the first part of this series¹⁴ the reagent gas conditions at high repeller potentials become more dominated by methanol-related species when the methanol content in the mobile phase is increased. As a result of the decreasing intensity of the carbon dioxide molecular ion at higher percentages of methanol, the contribution of the CE effects at high repeller potentials is expected to diminish. This is indeed observed. It must be pointed out that the PAHs

are quite exceptional in that they, unlike more polar compounds, can be successfully analyzed in a wide range of methanol contents. With more polar compounds the percentage of methanol in the mobile phase cannot be chosen freely.

With 2% of methanol at very high repeller potentials considerably more fragmentation is observed in the mass spectra of the PAHs than in the corresponding spectra at 70 eV EI. Marked in this respect is the fact that the ion $[M - 2]^{++}$ is considerably more abundant in the spectra of phenanthrene and pyrene obtained under CE conditions than under EI conditions. Apparently, the appearance energy of that fragment ion can be reached readily in the more energetic CE process. As a result of these differences between CE and EI spectra, a computer library search of the CE spectra of the PAHs in the NBS EI library was not very successful.

One aspect, which is also important in judging the analytical usefulness of the repeller effects, is the sensitivity. At present, a considerable loss in intensity is observed, when comparing spectra at low and high repeller potentials. In the chromatogram in Fig. 1, for instance, the intensity of the phenanthrene peak at high repeller potential is 30 times lower than that at low repeller potential. This can be attributed only partially to the fragmentation induced. Up to a ten-fold loss in the reconstructed total ion current (RIC) has been observed for the PAHs. However, it is important to note that, by increasing the repeller potential, the intensity of the background above $m/z = 100$ and consequently the noise level is greatly reduced, which may result in similar or even better detection limits. This aspect is currently under investigation.

Diuron

The repeller effects on the mass spectra of the chlorinated herbicide diuron, N-(3,4-dichlorophenyl)-N',N'-dimethylurea (MW 232), have been investigated with both SFC-MS and LC-MS. SFC-MS spectra obtained with 2% of methanol in carbon dioxide at low, intermediate and high repeller potentials are given in Fig. 3. Fig. 4 shows the structure of diuron and tentative structures of some related compounds discussed below. At low repeller potential, proton transfer CI is observed, resulting in a peak from the protonated molecule at $m/z = 233$ (Fig. 3A), while at high repeller potential a peak from the molecular ion at $m/z = 232$ is observed, together with several fragment ions giving structural information (Fig. 3C). A thirty-fold decrease in RIC is observed when increasing the repeller potential from 20 to 180 V. The diuron spectrum obtained at high repeller potential closely resembles the EI spectrum of diuron; a computer library search in the NBS library was successful in this case (purity 600, fit 850).

In order to test whether an EI-like spectrum can be obtained as a result of CID effects, the protonated molecule of diuron was collisionally dissociated in a low energy MS-MS experiment with the triple quadrupole instrument. The only fragment observed is at $m/z = 72$, corresponding to $[(CH_3)_2N=C=O]^+$; it is found in the EI spectrum as well. Other EI fragments are not observed. From these results it appears that the diuron mass spectrum obtained at high repeller potential can be explained more easily by CE effects with the reagent gas ions present in the source than with CID effects. However, it must be pointed out that the fragment at $m/z = 72$ apparently has a low appearance energy. It is for instance also abundantly present in the diuron spectrum obtained at a repeller potential of 60 V (Fig. 3B), where protonated methanol is the most abundant reagent gas ion. Apparently, the protonated methanol

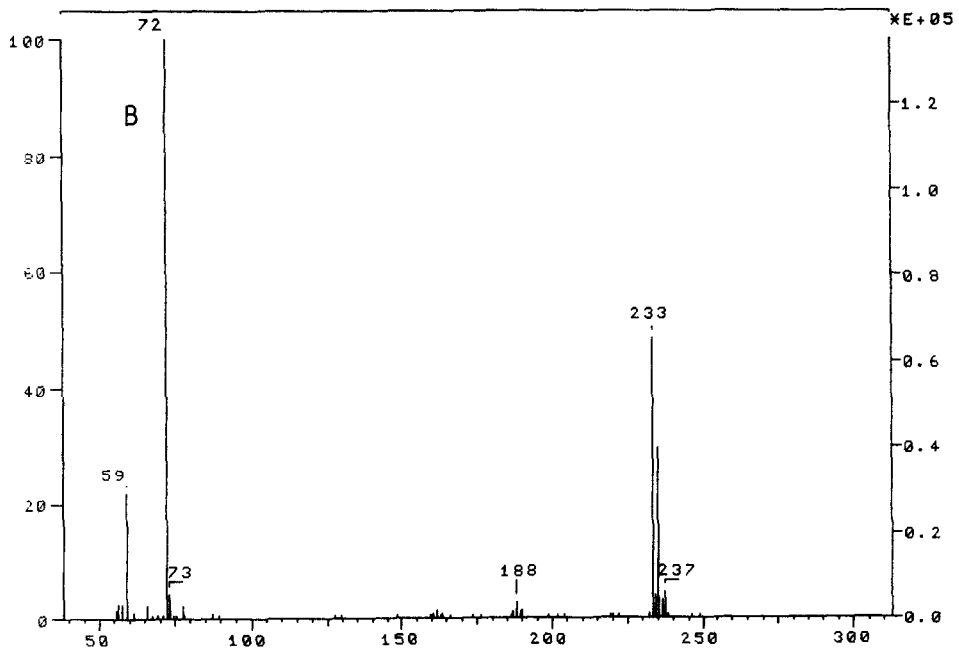
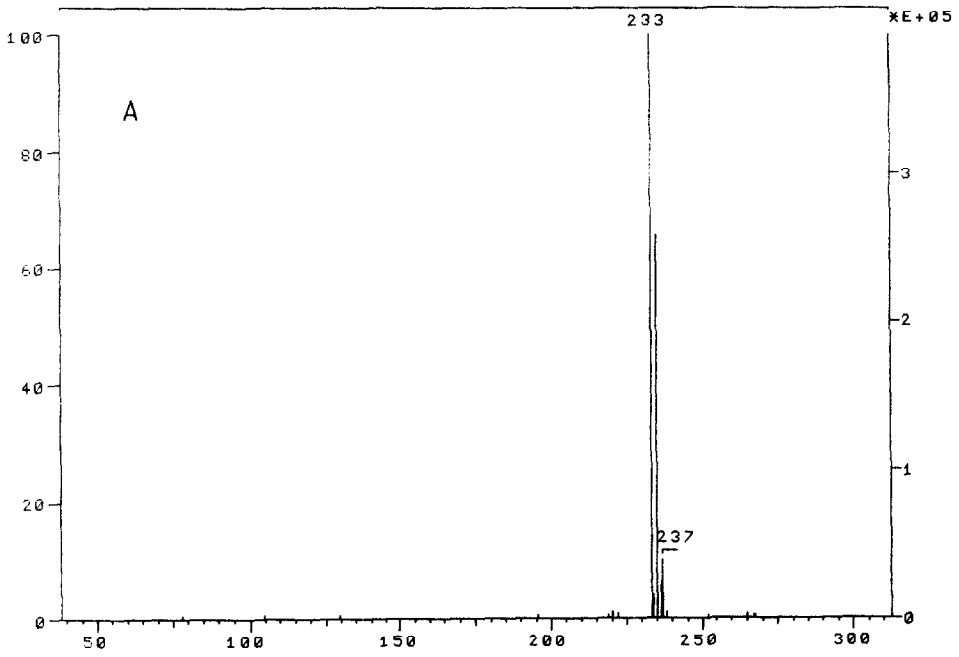


Fig. 3.

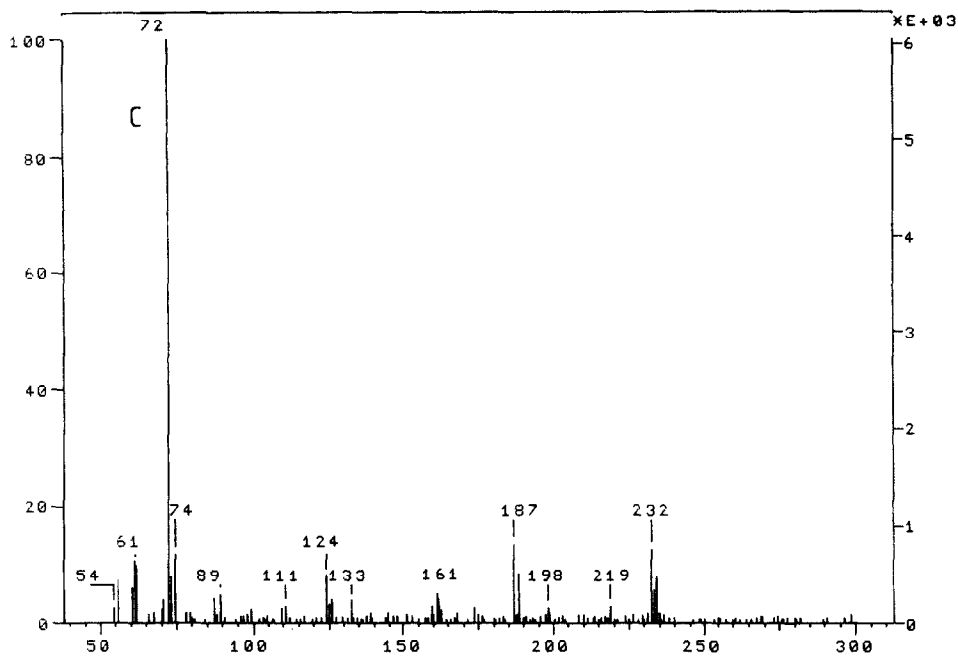


Fig. 3. Mass spectra of diuron at (A) low (20 V), (B) intermediate (60 V) and (C) high (180 V) repeller potentials. Conditions: SFC-MS with 2% of methanol in carbon dioxide and an aminopropyl column. Other conditions: see text.

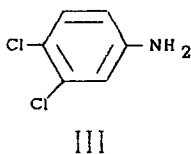
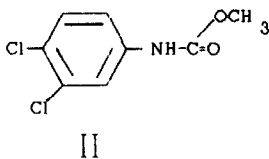
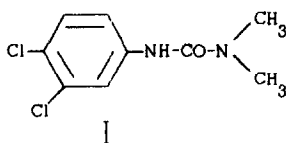


Fig. 4. Structure of diuron and tentative structures of some related compounds in SFC-MS and LC-MS. Explanation: see text.

clusters, the most abundant reagent gas ions at a repeller potential of 20 eV have slightly higher proton affinities than that of protonated methanol itself, resulting in a softer ionization with the clusters.

Diuron is a thermally labile compound. Therefore, the influence of the vaporizer temperature on the mass spectrum at low repeller voltage in SFC-MS has been investigated. It appears that the vaporizer temperature setting is very critical. At 60°C, instead of the usual 50°C, a peak at $m/z = 220$ is the most abundant; the isotope peaks present at $m/z = 222$ and 224 indicate the dichloro character of this thermal decomposition product. Daughter MS-MS spectra of $m/z = 220$ and 222 show a peak at $m/z = 59$ and peaks due to losses of 32 (probably methanol) and 60 mass units (probably methanol and CO). A tentative structure, II, for this compound is given in Fig. 4. At a vaporizer temperature of 100°C practically the only peak observed is at $m/z = 162$ with isotope peaks at $m/z = 164$ and 166, which is due to protonated dichloroaniline (structure III in Fig. 4), a well known thermal decomposition product of diuron and its analogues¹⁶.

Diuron has also been investigated in LC-MS under discharge-on conditions. In a mobile phase of 80% methanol in water at a vaporizer temperature of 90°C and a repeller potential of 20 V a strong peak of the protonated molecule is observed at $m/z = 233$ (Fig. 5A), as well as less intense adduct peaks at $m/z = 265$, due to $(M + \text{CH}_3\text{OH} + \text{H})^+$, and at $m/z = 278$ which will be explained below. Both ions show a dichloro character. The low-intensity peaks at $m/z = 199$ with monochloro character and at $m/z = 165$ without chloro addition indicate that the substitution of chlorine atoms by hydrogen, which has been observed under other CI conditions^{17,18}, takes place here as well. This effect is not observed in the SFC-MS experiments.

At the low mass end of the LC-MS spectrum peaks are detected at $m/z = 74$, 78 and 106. In the daughter MS-MS spectra of $m/z = 74$ and 106 the losses of one, and one and two methanol molecules, respectively, are observed, resulting in a fragment at $m/z = 42$, which might be protonated acetonitrile, but that is difficult to explain. From the daughter MS-MS spectrum of $m/z = 78$ it can be concluded that this peak is due to the methanol adduct of protonated dimethylamine (DMA). The peak of the dichloro compound at $m/z = 278$ is probably due to the DMA adduct of diuron itself, which explains the strong fragment at $m/z = 46$ in the daughter spectrum of $m/z = 278$. The presence of substantial amounts of DMA in the ion source is rather surprising, although it is formed via structure II in Fig. 4 and has been detected as one of the thermal decomposition product of diuron and its analogues as well¹⁶. In the reaction gas it is a compound with an higher proton affinity than that of diuron and the other reagent gas constituents.

At higher repeller potentials the intensity of the protonated molecule decreases, while a strong peak at $m/z = 72$ and some other minor fragments appear. The informative fragmentation, found at high repeller potential in SFC-MS, is not present in the LC-MS spectra. The RIC due to diuron first decreases with increasing repeller potential, but when the peak at $m/z = 72$ becomes the base peak the RIC starts to increase up to the original level. As it is less specific, the peak at $m/z = 72$ is of course less attractive for quantitation purposes than the protonated molecule cluster at $m/z = 233$ -237.

In a mobile phase of 20% methanol in water at a vaporizer temperature of 110°C and a repeller potential of 20 V, the peak due to the protonated dichloroaniline

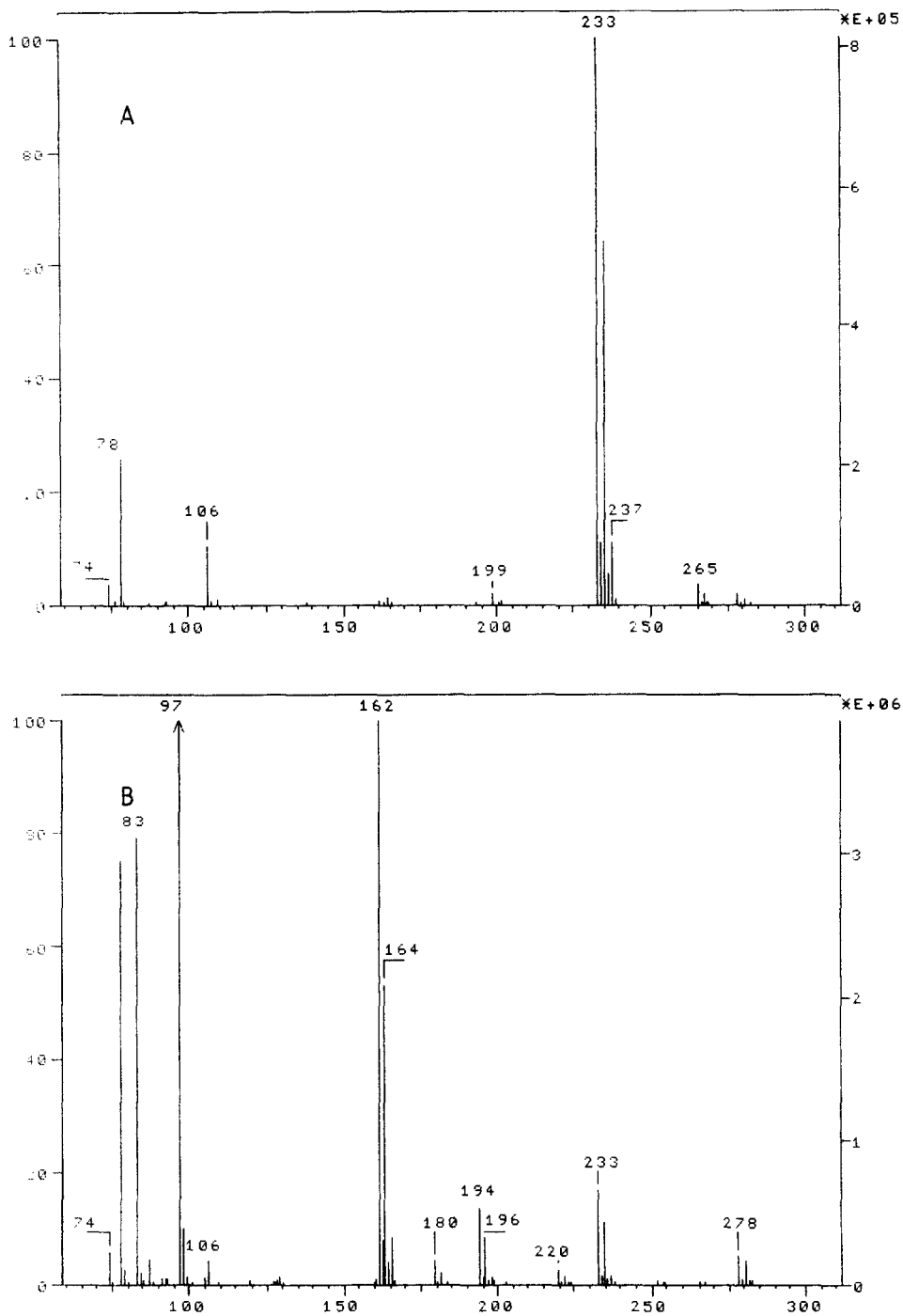


Fig. 5. Mass spectra of diuron at a repeller potential of 20 V in LC-MS, with (A) 80% methanol in water and vaporizer temperature 90°C, and (B) 20% methanol in water and vaporizer temperature 110°C. Other conditions: see text.

at $m/z = 162$ is the base peak, while an intense peak at $m/z = 78$ which has been explained above is also observed. The protonated molecule at $m/z = 233$ is 10–40% of the base peak depending on the condition of the vaporizer. Other peaks are solvent clusters, *e.g.*, $m/z = 83$, which is $[2\text{CH}_3\text{OH} + \text{H}_2\text{O} + \text{H}]^+$, $m/z = 97$, which is $[3\text{CH}_3\text{OH} + \text{H}]^+$, and $m/z = 129$, which is $[4\text{CH}_3\text{OH} + \text{H}]^{+14}$. The peaks at $m/z = 180$ and 194 , both showing a dichloro character, can be explained as water and methanol adducts of the protonated dichloroaniline, respectively. The DMA adduct ion at $m/z = 278$ is also observed.

The change in the spectrum by decreasing the methanol content of the solvent is somewhat surprising. Since in fact two parameters are changed at the same time, *i.e.*, the methanol content and the vaporizer temperature, spectra of diuron have also been obtained with a vaporizer temperature of 110°C and 80% methanol in water, and with a vaporizer temperature of 90°C and 20% methanol in water. In both cases the vaporization conditions are unfavourable: the vaporizer temperature is either too low for optimum stability or too high, resulting in a dry spray. However, essentially no spectral change is observed in those two cases; the relative abundance of the protonated diuron is somewhat higher at low vaporizer temperature with 20% methanol in water. Apparently, hydrolysis of diuron to dichloroaniline takes place in the highly aqueous environment more easily than in the other solvent.

At still higher vaporizer temperatures, thermal decomposition of diuron is observed in both solvents, starting at higher temperatures with 80% methanol in water than with 20% methanol in water. Under these conditions the peak at $m/z = 220$ with dichloro character, which has also been found in the SFC–MS experiments, appears in the spectrum as the second most intense cluster. The methanol adduct of this compound is also observed at $m/z = 252$. At still higher temperatures a peak due to a monochloro compound is observed at $m/z = 127$. The nature of this compound is unclear.

Phenacetin and caffeine

The repeller effects in the mass spectrum of phenacetin (see Fig. 6 for the structure) have been studied in SFC–MS with a mobile phase of 2% of methanol in carbon dioxide. The mass spectra at four different repeller potentials are given in Fig. 6. At low repeller voltage a strong protonated molecule is observed without any fragmentation. At repeller potentials where the protonated molecule of methanol has become the most abundant reagent gas ion some fragmentation is observed: loss of ethene resulting in $m/z = 152$, loss of 42 ($\text{H}_2\text{CC}=\text{O}$, formaldehyde) resulting in $m/z = 138$ and the loss of ethene and formaldehyde resulting in $m/z = 110$. At higher repeller potential the fragment peaks become more abundant than the protonated molecule. At still higher repeller potential the phenacetin molecular ion is observed, next to fragments at $m/z = 137$ and 109 . These peaks are also observed in the EI spectrum of phenacetin. The sensitivity decreases dramatically at higher repeller potentials.

The repeller effects in the mass spectrum of caffeine (MW 194) have been studied with SFC–MS as well as LC–MS. In SFC–MS with 2% methanol in carbon dioxide a strong protonated caffeine molecule is observed at $m/z = 195$ and a methanol adduct at $m/z = 227$ at low repeller potential. Comparable to the SFC–MS spectra of diuron and phenacetin, some fragmentation in the caffeine spectrum is

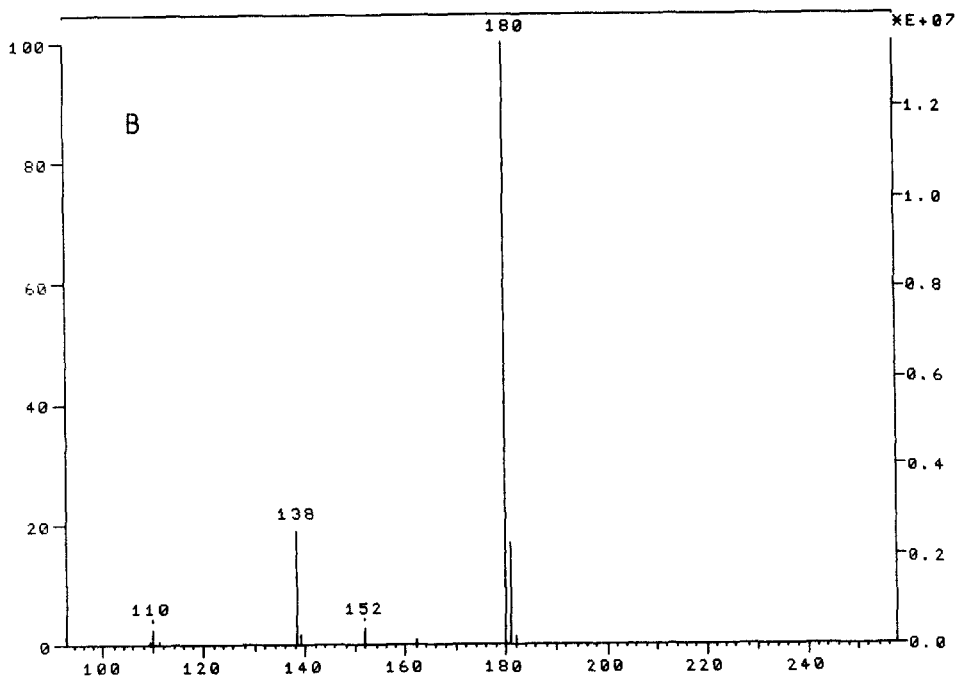
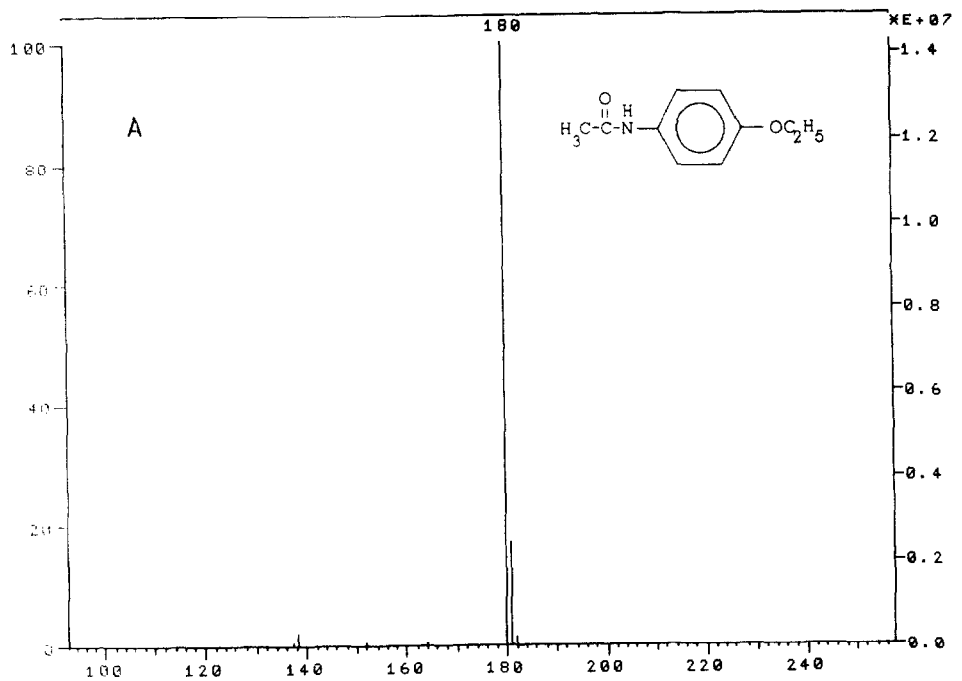


Fig. 6.

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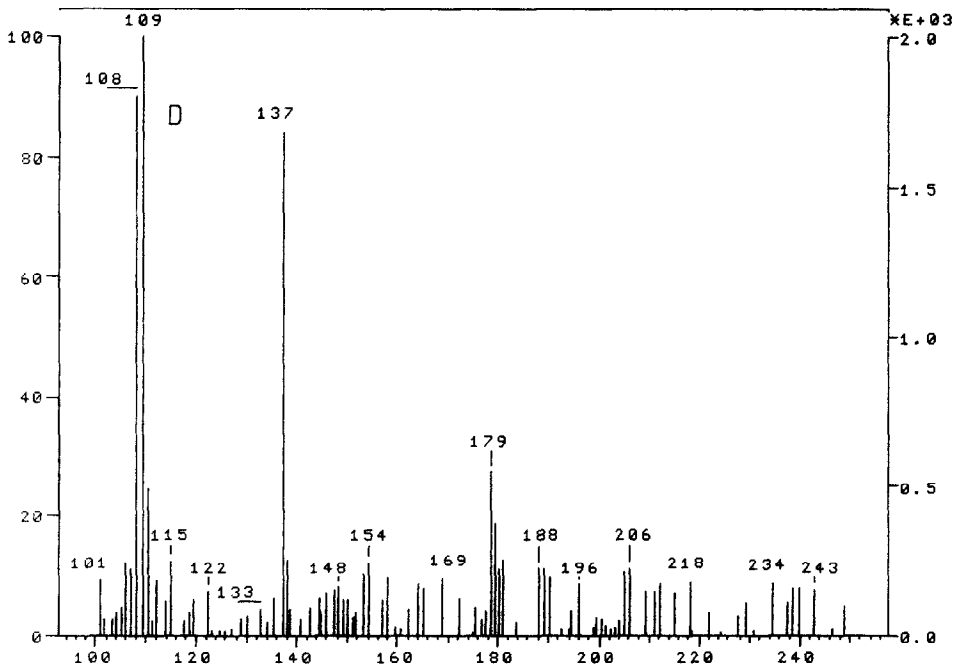
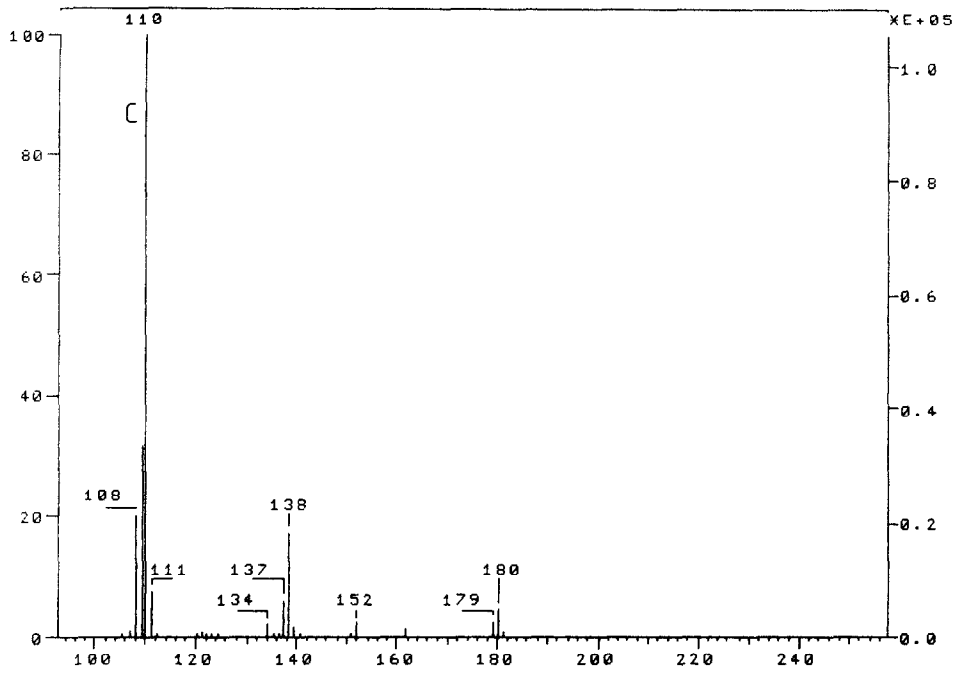


Fig. 6. Mass spectra and structure of phenacetin at repeller potentials of (A) 40, (B) 50, (C) 70 and (D) 150 V. Conditions: SFC-MS with 2% of methanol in carbon dioxide and a C_{18} column. Other conditions: see text.

present under conditions where protonated methanol is the most abundant reagent gas ion. The loss of $\text{CH}_3\text{-N}=\text{C}=\text{O}$ from the protonated molecule results in fragment peak at $m/z = 138$. At still higher repeller potential the fragment peak at $m/z = 138$ becomes the base peak, while several other fragment peaks are observed, for instance $m/z = 110$, resulting from the loss of CO from $m/z = 138$. A weak peak at $m/z = 194$ is also observed. As in the phenacetin spectrum, the information becomes less clear at higher repeller potential, as a result of the transition from proton-transfer CI to CE phenomena and the drop in sensitivity. The fragments found at intermediate repeller potentials are similar to those found in the MS-MS daughter spectrum of the protonated caffeine.

In LC-MS, caffeine has been studied with 80% methanol in water. Protonated caffeine ($m/z = 195$) is observed at all repeller potentials studied (0–180 V), while some fragmentation, similar to that described for SFC-MS, is found at repeller potentials above 100 V. While in SFC-MS a considerable loss in RIC is observed at higher potentials, in LC-MS the RIC is not influenced. The intensity of the protonated molecule decreases about twenty-fold when fragmentation is induced.

CONCLUSIONS

Induction of fragmentation at higher repeller potentials is observed as a general trend in the mass spectra of the analytes studied so far with SFC-MS in the discharge-on mode. At intermediate repeller potentials, where protonated methanol and protonated carbon dioxide are the most abundant species in the reagent gas, useful structural information is obtained without much loss in intensity. At high repeller potentials the CE with the carbon dioxide molecular ions results in extensive fragmentation and a significant loss in sensitivity. At high repeller potentials, useful fragmentation is obtained only in some cases. Sometimes the interpretation is also inhibited by the unfavourable signal-to-noise ratio of the spectra. Extensive fragmentation, which can differ from EI fragmentation, is observed; the ionization produces ions with higher internal energy than in the common EI spectra at 70 eV.

Many compounds have been investigated under discharge-on conditions with various mobile phases and at different repeller potentials in LC-MS. In general, it can be stated that useful structural information can be obtained in many cases under these conditions. The loss of sensitivity at high repeller potential is small or absent in LC-MS.

The mechanisms leading to the fragmentation at higher repeller potentials are not clear. The advent of fragmentation coincides with changes in the reagent gas spectrum, and some of the observed effects have also been predicted from those changes in the reagent gas composition. On the other hand, CID of protonated molecules in the high pressure ion source may play a significant role as well. Some changes in the reagent gas spectrum can be explained from CID processes of the reagent gas cluster ions, especially in LC-MS. However, significant differences in high repeller voltage spectra and CID spectra are also observed, especially in SFC-MS, which indicate different mechanisms for different compounds. Further investigations are being performed and will be reported in due course.

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