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TECHNIQUES FOR INCREASED USE OF THERMOSPRAY LIQUID CHROMATOGRAPHY–MASS SPECTROMETRY

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SUMMARY

Use of a thermospray source with buffer ionization usually provides a means of obtaining molecular adduct ions with little or no fragmentation. Although this process thus provides the very important datum of molecular weight, very little can be deduced regarding the identity of an unknown. At least four methods are available to provide or control formation of fragment ions; temperature variation of vaporizer and/or jet chamber (block), selection of filament or discharge ionization, collision-induced dissociation (CID) with the thermospray repeller, and CID with a triple stage quadrupole TSQ® or other MS–MS mass spectrometer. Typical examples are presented to demonstrate each of the above.

INTRODUCTION

Since the commercial introduction of thermospray, approximately three years ago, this technique has gained tremendous popularity both as a new ionization technique and as a versatile liquid chromatography–mass spectrometry (LC–MS) interface. A great deal of excellent work has been reported from many laboratories throughout the world[1]. Even with this degree of success, there are two denouncements that continually recur. The first of these is "Thermospray on my compounds gives mostly fragment ions and little or no molecular adduct ion". The second of these criticisms is "Thermospray on my compounds gives mostly molecular adduct ion and little or no fragment ions".

This apparent dichotomy is actually consistent with our present day expectations of a modern MS system. Traditionally, the organic chemist learned to translate electron-impact fragmentation patterns into chemical structures. As MS extended to regions of high molecular weight and high polarity, soft ionization processes were devised to effect ionization of the more difficult molecules, but these processes often produce only molecular ions or a molecular ion adduct. It thus became expedient to devise methods that produce fragment ions in conjunction with soft ionization processes.

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EXPERIMENTAL

A typical schematic diagram for thermospray experiments is shown in Fig. 1. Solvent flow from the chromatographic pump is directed through an injector to a selector valve that permits the operator to select a direct sample loop or to perform a chromatographic separation. The direct sample loop is extremely valuable for samples containing only a small number of components and is also used for a preliminary survey of complex mixtures prior to LC separation. From the loop or column, the sample passes through a protection valve and into the thermospray vaporizer. This vaporizer consists of a stainless-steel capillary with a nominal I.D. of 0.15 mm. In most configurations, the liquid is carefully heated by passing a current directly through the stainless-steel tubing so that the liquid emerges from the tip of the vaporizer as a fine spray, directed through the jet chamber or source block[2]. Because the droplets formed in this process are small, effective evaporation occurs with minimal thermal contact with the walls of the jet chamber.

Three processes are currently used for sample ionization; buffer ionization, filament ionization, and discharge ionization. The buffer ionzation mode requires that the chromatographic effluent contain a buffer salt, which is preferably volatile. Ammonium acetate has proven to be the most versatile buffer. It can be present during the chromatographic separations or, if desired, can be added post-column using an additional high-pressure pump and a zero-dead-volume tee. Many of the spray droplets emerging into the jet chamber will contain a net positive or negative charge, and as they evaporate in the vacuum, ions will be formed which are characteristic of the salt, the solvent, and any sample that is present in the effluent. The

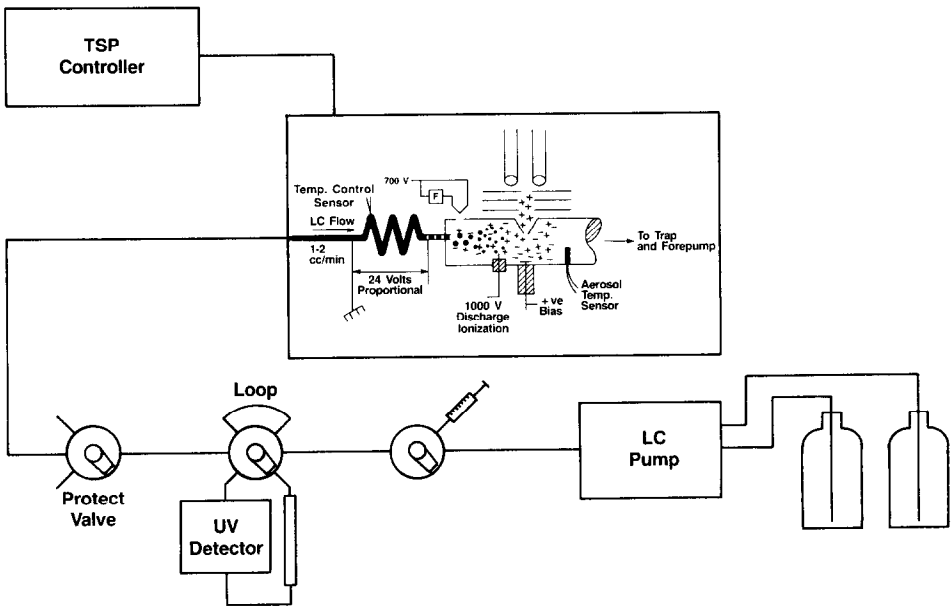

Fig. 1. Schematic diagram of thermospray LC–MS system.

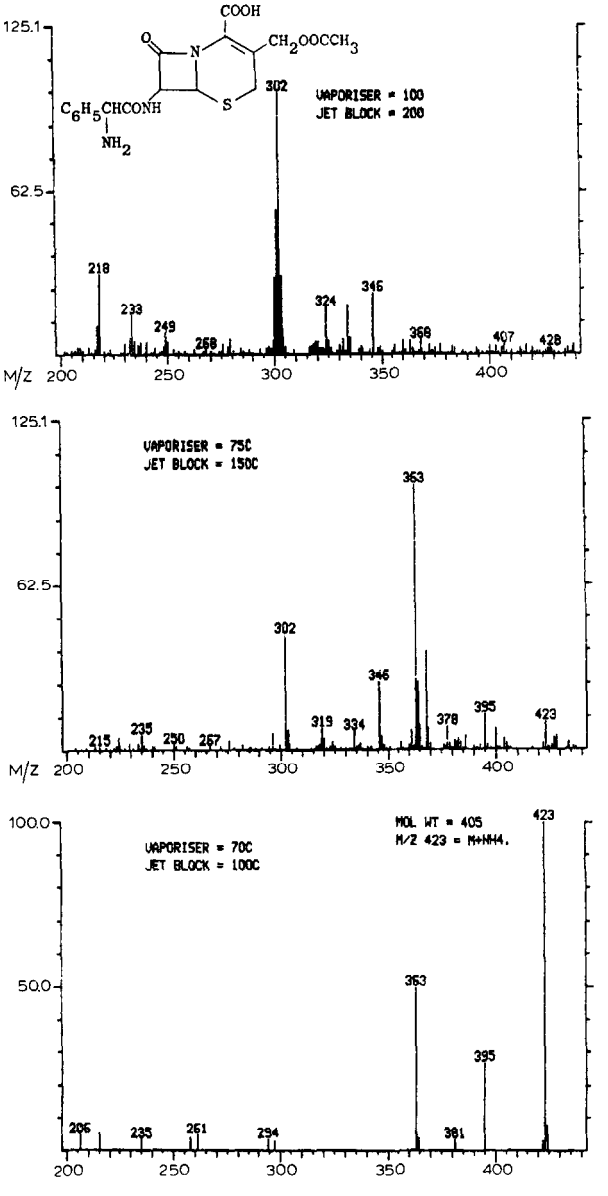

Fig. 2. Thermospray spectra of cephaloglycin, obtained at three different temperature conditions (buffer ionization, loop injections, positive-ion mode).

ions are transmitted into the mass analyzer by lateral expansion of the jet stream, aided by a repeller electrode opposite the sampling cone. Sample ions formed in this process are usually molecular adduct ions (*e.g.*, MH^+, MNH_4^+, etc.), and fragmentation is observed only for very sensitive compounds.

Filament and discharge ionization are complementary ionization modes that are closely related to the chemical ionization process used with conventional ion

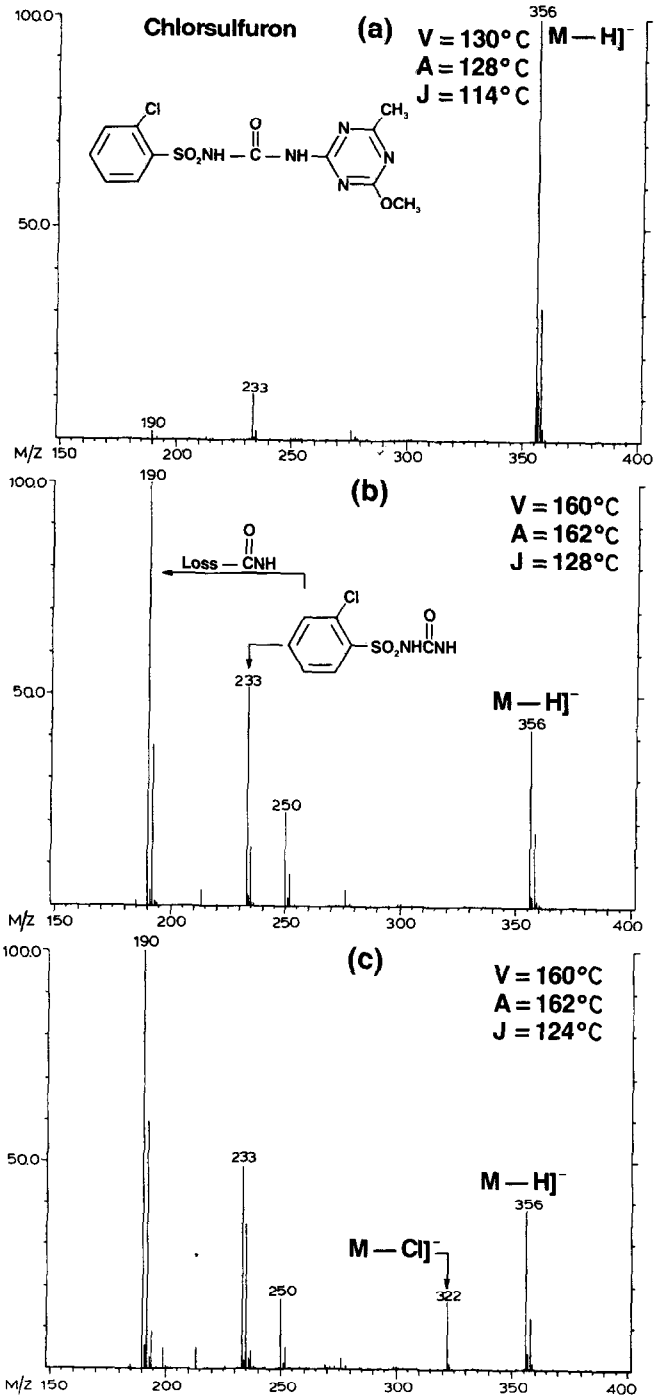

Fig. 3. Thermospray spectra of chlorsulfuron, obtained at two different temperature conditions (buffer ionization, loop injection, negative-ion mode; c has filament turned on).

sources. Filament ionization requires a rugged emitter (thoriated iridium is commonly used) and at least 600–800 V acceleration to obtain effective penetration of electrons into the jet chamber. Discharge ionization can be obtained with a high-voltage electrode which protudes slightly into the jet chamber. Because of the relatively high pressure in the thermospray jet chamber (about 5–15 torr), a stable discharge is obtained in a voltage range of 800–1400 V.

RESULTS AND DISCUSSION

The general uses of filament or discharge ionization are: (1) low-polarity solvents cannot dissolve a buffer salt; (2) presence of buffer may impair chromatographic separations; (3) some samples do not ionize efficiently by buffer ionization; (4) low-flow studies (*i.e.*, below 0.4 cm/min), where buffer ionization is ineffective; (5) some compounds that give only molecular adduct ions by buffer ionization may give fragment ions in filament-/ or buffer-ionization mode. These auxilliary modes of operation clearly extend the use and versastility of the thermospray system. For the most part, however, the following discussion will deal only with item 5, namely the ability to produce fragment ions in filament or discharge ionization mode. Four ways that ionic fragmentation can be obtained or controlled in thermospray experiments are: (1) control of vaporizer temperature; (2) selection of ionization mode; (filament or discharge ionization); (3) collision-induced dissociation (CID) with thermospray repeller[4,5]; (4) CID with triple-stage-quadrupole (or other MS–MS system)[6].

Varying the vaporizer temperature is most commonly used to eliminate fragmentation but, when desired, can also be used to induce fragmentation. In general, the expected reduction of fragmentation is observed when temperatures are reduced[3], but when other effects such as wall adsorption occur, fragmentation may increase. The ability to produce a molecular adduct ion is a particularly important feature of soft ionization processes and is often considered a measure of the quality of the method. Fig. 2 shows three thermospray spectra of cephaloglycin, obtained at three different temperature conditions. In the top spectrum, the control temperature for the vaporizer was set at 100°C, and the jet chamber block was set at 200°C. No molecular adduct ion is observed, and it appears that the sample is suffering excessive thermal degradation. The other two spectra in Fig. 2 were obtained at lower vaporizer and jet block temperatures. It is observed that the amount of fragmentation has been significantly reduced, and in the bottom spectrum, the molecular adduct ion is the most prominent mass peak.

Another example of controlling fragmentation by varying operating temperatures is provided by data from the pesticide chlorsulfuron. In Fig. 3, negative ion spectra are shown for three operating conditions. In the top spectrum, the molecular adduct ion predominates, and minimal fragmentation is observed. The center spectrum, obtained at higher operating temperatures, show significant fragmentation indicative of structural features. In the bottom spectrum, the temperature conditions were not changed, but the filament was turned on while operating in the buffer ionization mode. Additional fragmentation is observed, providing further confirmation of the suggested structures. In this case, the filament mode provides only supplementary information, but there are many examples in which the filament or discharge ionization processes provide fragmentation not easily obtained by temperature variation.

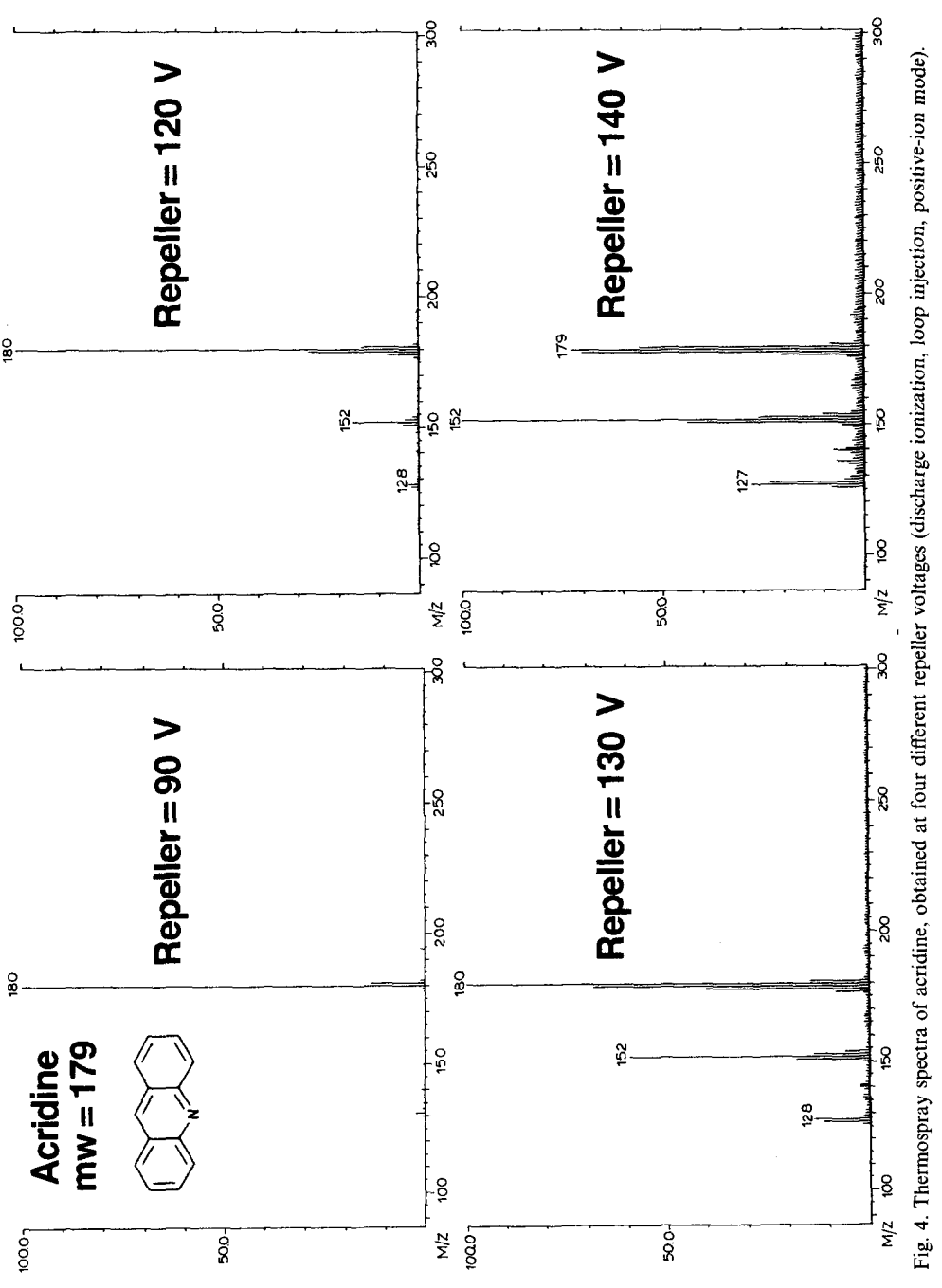

Fig. 4. Thermospray spectra of acridine, obtained at four different repeller voltages (discharge ionization, loop injection, positive-ion mode).

One of the more interesting operational variations now emerging is the use of the repeller electrode to effect collision-induced dissociation (CID)[4,5]. CID has become an indispensable partner for use with soft ionization processes. Several different methodologies have been developed for CID analyses for most types of commercial mass spectrometers, but the use of an ion acceleration electrode in a thermospray source is relatively new. The reactions are not fully understood at this time, and with the source described in this work, they occur only with discharge ionization. Nevertheless, preliminary results are of considerable interest and indicate the potential for this technique as it develops further.

The spectra shown in Fig. 4 provide a good example of thermospray repeller CID. Acridine is a stable polynuclear aromatic nitrogen compound that shows no fragmentation in any of the three common ionization modes under normal operating conditions. With discharge ionization, increased repeller voltage induces fragmentation and, as is seen in Fig. 4, the stable molecular ion is diminished, and fragment ions predominate in the spectrum. Another way of looking at these data is given in Fig. 5 which shows the ion profiles obtained for replicate loop injections of acridine with increasing repeller voltage from 90 to 160 V in increments of 10 V.

Use of the thermospray repeller to obtain CID in discharge-ionization mode provides a means of obtaining structural fragments without the more expensive MS–MS equipment. However, the data are limited in that there is no specificity of parent ion and no elimination of background ions. Thus, there is no assurance that the observed daughter fragments have come from dissociation of the desired parent

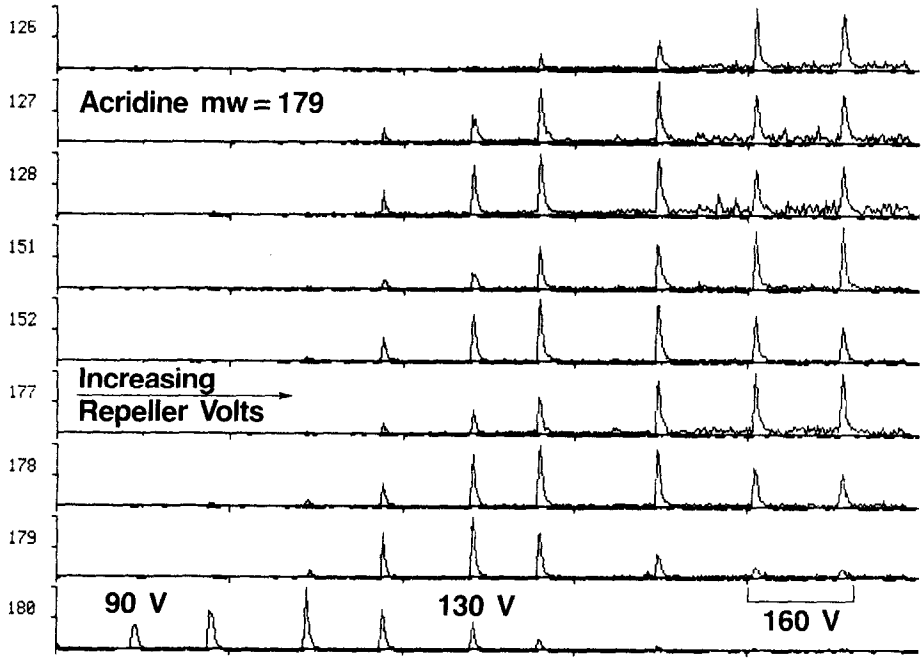

Fig. 5. Ion profiles, obtained from replicate loop injections of acridine. Repeller voltage increased by 10 V at each injection.

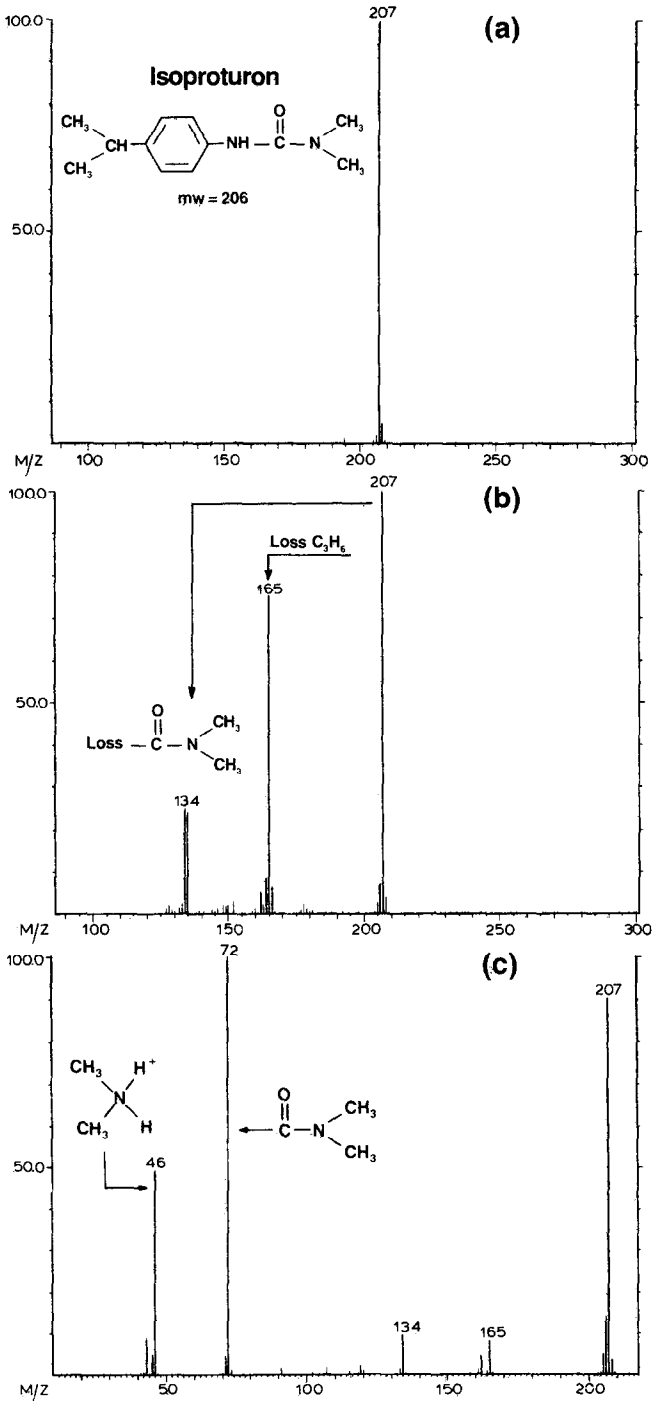

Fig. 6. Thermospray spectra of isoproturon. (a) Discharge ionization, repeller voltage = 70 V; (b) discharge ionization, repeller voltage = 110 V; (c) TSQ MS–MS mode; daughter of mass 207 by buffer ionization.

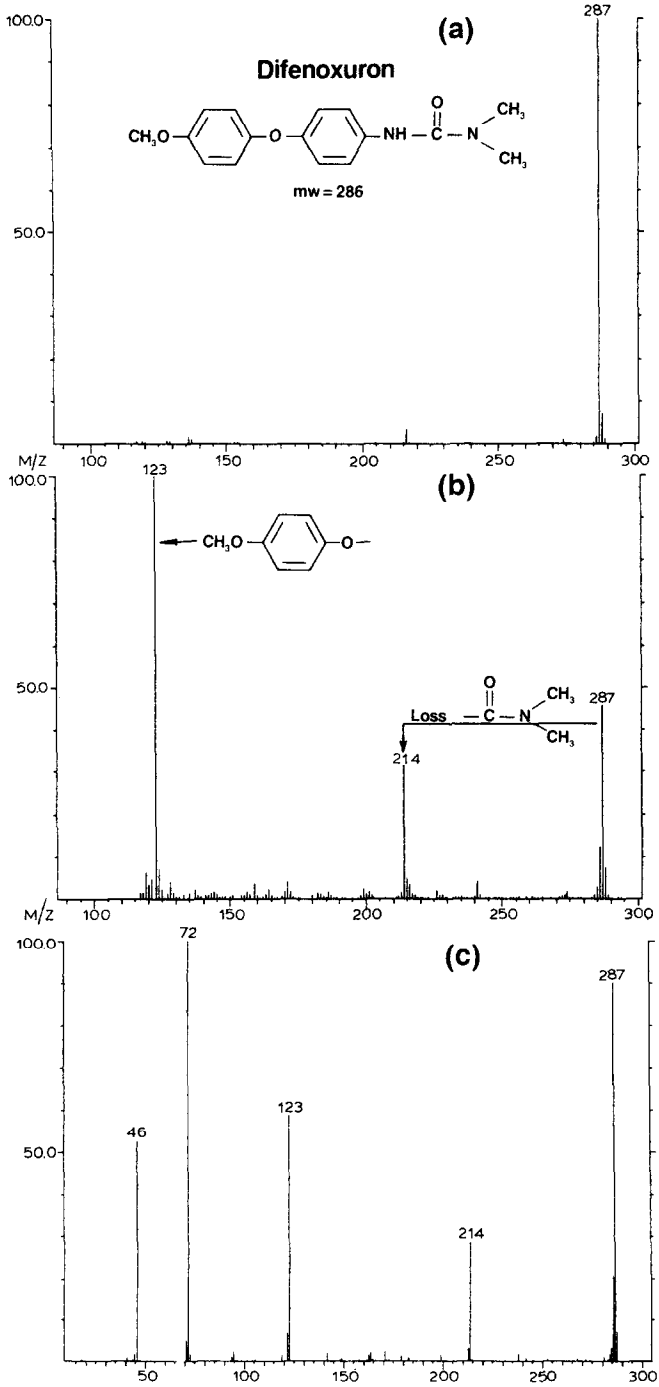

Fig. 7. Thermospray spectra of difenoxuron. (a) Discharge ionization, repeller voltage = 80 V; (b) discharge ionization, repeller voltage = 120 V; (c) TSQ MS–MS mode; daughters of mass 287 by buffer ionization.

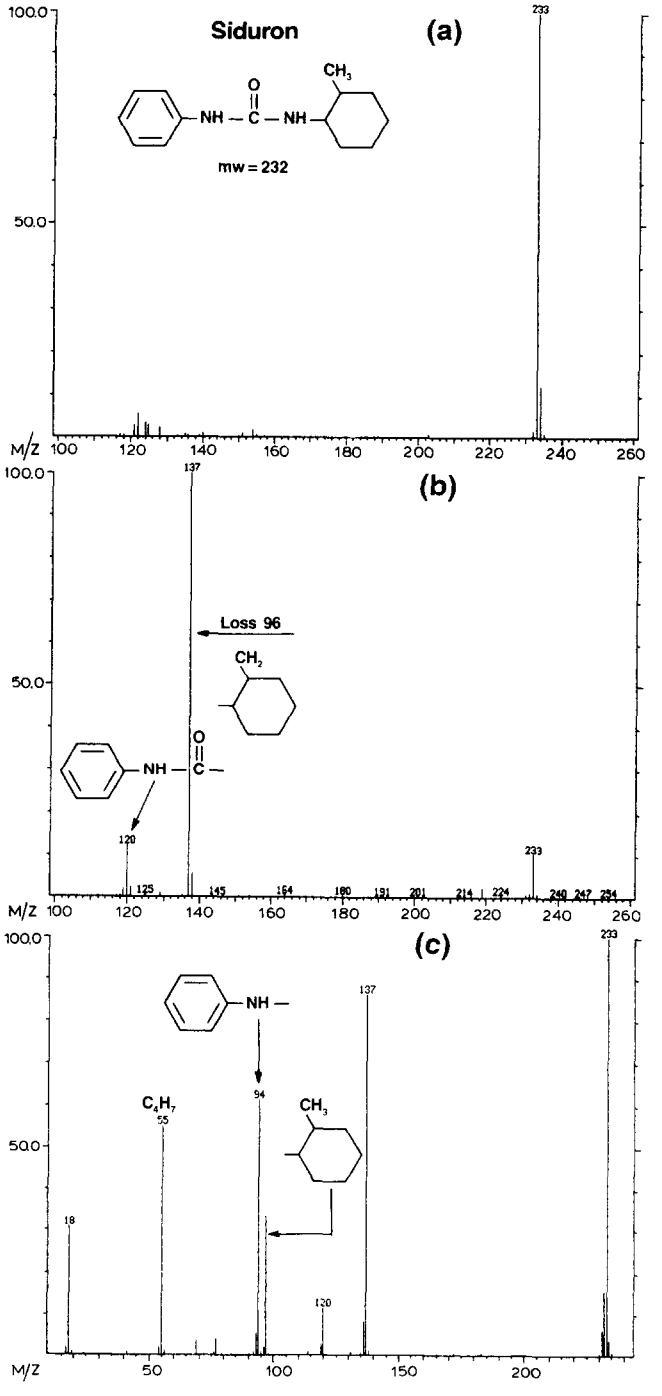

Fig. 8. Thermospray spectra of siduron. (a) Discharge ionization, repeller voltage = 70 V; (b) discharge ionization, repeller voltage = 120 V; (c) TSQ MS–MS mode; daughters of mass 233 by buffer ionization.

ion and, furthermore, it is seldom possible to scan daughter ions much below mass 120 without excessive interference from thermospray background ions.

The preferred method of obtaining CID data is to use a mass spectrometer specifically designed to perform MS–MS experiments[6]. The most popular instrument for this purpose is the triple-stage quadrupole, primarily because the computer control systems in current use provide relatively easy operation. In a triple quadrupole, the first quadrupole is used to select a desired mass. During the cycle time that this mass is selected, all other ionic masses are rejected, thus guaranteeing that all observed fragment ions came from the specified parent. The ion of the selected mass is accelerated into the second quadrupole region and undergoes energetic collisions with the collision gas (usually argon at approximately 1–10 mTorr). This collision quadrupole is operated in a radio frequency-only mode so that ions entering or created in the collision chamber are transmitted to the third quadrupole with high transmission efficiency. The resultant spectrum is completely free of interference from other ionic species, and the resultant daughter ions can be detected in the low-mass region (< 100 a.m.u.) with complete assurance that any ions observed are the result of fragmentation of the selected parent ion. Interference can occur only with isotopes, isomers, or isobars.

Figs. 6–8 present thermospray data obtained (a) without CID, (b) with CID and the thermospray repeller, and (c) with CID and a triple-stage quadrupole. When analyzed without CID, each of these pesticide samples show only the molecular adduct ion, regardless of which ionization mode is used. The center spectrum in each of these figures shows the fragmentation obtained by increasing the repeller voltage in discharge-ionization mode. The indicated fragment ions give significant information regarding the structure of the molecule. The bottom spectrum in each of these figures shows the pattern obtained by CID in MS–MS mode. Typically, the CID fragments are the same for each process. However, in MS–MS mode, thermospray background ions are eliminated by the complete mass selectivity occurring in the first MS stage. As a consequence, useful mass data are obtained down to low masses. The spectra of Figs. 6–8 show fragment peaks at masses 46 and 72, which are complementary to the higher mass fragments. Depending upon the chemical system, such data can provide increased specificity and increased sensitivity. For some samples, all fragmentation may occur below mass 100 and, hence, be unobservable, except in the MS–MS mode.

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