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ATMOSPHERIC PRESSURE PHOTOIONIZATION MASS SPECTROMETRY

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Atmospheric pressure photoionization (APPI) is the last arrival in the family of atmospheric pressure ionization (API) methods to couple mass spectrometry (MS) to liquid-phase separation techniques. The basic idea was to further extend the fields of application of liquid chromatography (LC)–MS to those molecules that are not, or are poorly amenable, to electrospray (ESI) or APCI. The present review explores the literature. After a short introduction with an historical background and the premises for its development, we describe the technique, its physical principles, and the factors that affect its efficiency. The review also presents a survey of applications in different fields.
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I. INTRODUCTION AND HISTORICAL BACKGROUND

Atmospheric pressure photoionization (APPI) is the youngest among the methods of soft ionization in mass spectrometry (MS), and complements the other two

atmospheric pressure ionization (API) techniques: electrospray ionization (ESI) and atmospheric-pressure chemical ionization (APCI). Its recent presentation (Robb, Covey, & Bruins, 2000) implies a relatively low number of papers in the literature. For this reason, a good part of the material cited in the present review comes from communications presented at important meetings.

Photoionization (PI) is not a revolutionary technique. It was introduced approximately 30 years ago as a detection method (PID) in gas chromatography (Driscoll, 1976; Driscoll & Clarici, 1976; Driscoll & Spaziani, 1976; Driscoll, 1977), and nearly one decade later also in liquid chromatography (LC) (Locke, Dhingra, & Baker, 1982; Driscoll et al., 1984), although, to the best of our knowledge, only a few applications of the latter method have been described in the literature.

Some APPI sources have been developed to couple with ion-mobility spectrometry (IMS); that is a field strictly related to MS. They have been used for the detection and quantitation of those classes of chemical compounds that are ionizable by a suitable vacuum-UV radiation. Some reports describe applications and developments of such a technology (Baim, Eatherton, &Hill, 1983; Leasure et al., 1986; Spangler et al., 1994) that were carried out with and without the addition of a dopant.

As far as we know, only a few reports on the coupling of APPI with MS are present in the literature. Several describe earlier APPI–MS experiments (Revel'skii et al., 1985, 1991) performed with a mixture of hydrocarbons that were delivered to the ion source in the vapor phase by a carrier gas (helium) stream. Gas-discharge lamps, with UV radiation energy in the in range 9–11 eV, were used.

II. SHORT THEORETICAL OUTLINE

PI takes place in the condensed and gas phases; in the former, the ionization energy (IE) is usually much lower (De Wit & Jorgenson, 1987) because the ionization products are stabilized by solvation. This situation is the case with water, whose IE in the gas phase is 12.62 eV, whereas in the condensed phase it is 6.05 eV. Therefore, liquid water cannot be used in PI detection because it has an ionization threshold that is close to a large number of solutes.

Because of the limitations cited above, the PI in gas phase appears to be more attractive, although the PI step must be preceded by the vaporization of the LC eluent.

Three main steps are responsible for detection by PI in the vapor phase: vaporization of the eluent, production of the photoions by interaction between a photon emitted by a UV source and analytes, and detection (which could also be performed by a mass spectrometer).

A large number of reactions could be involved in the PI process. Some produce the expected ions, whereas some others produce non-desired species, as follows:

$$\text{photoexcitation}: \quad AB + h\nu \rightarrow AB^{*}, \tag{1}$$

$$MP + h\nu \rightarrow MP^{*} \tag{2}$$

where AB is an analyte molecule, MP is a mobile-phase molecule, and AB* and MP* are their excited species, respectively.

Other steps follow the photoexcitation for AB* and MP*. Such processes are described here for AB. The same, obviously, could be written for MP:

$$\text{photodissociation}: \quad AB^{*} \rightarrow A + B, \tag{3}$$

$$\text{radiative decay}: \quad AB^{*} \rightarrow AB + h\nu, \tag{4}$$

$$\text{collisional quenching}: \quad AB^{*} + MP \rightarrow AB + MP^{*}, \tag{5}$$

$$\text{collisional quenching}: \quad AB^{*} + \text{gas} \rightarrow AB + \text{gas}^{*}, \tag{6}$$

where "gas" is any gas in the source, which in the case of an APPI–MS source could be air (the ionization takes place at atmospheric pressure) or N_2.

When $h\nu \geq$ IE, one more reaction occurs:

$$\text{ionization}: \quad AB^{*} \rightarrow AB^{\bullet +} + e^{-}. \tag{7}$$

After the ion formation, more processes could take place:

$$\text{recombination}: \quad AB^{\bullet +} + \text{gas} + e^{-} \rightarrow AB + \text{gas}, \tag{8}$$

$$AB^{\bullet +} + MP + e^{-} \rightarrow AB + MP, \tag{9}$$

$$AB^{\bullet +} + e^{-} \rightarrow AB. \tag{10}$$

Discrimination in the ions formed between solvent and analyte is possible. In fact, the UV source could be selected such that the photon-emission energy is higher than the IEs of the target molecules and lower than the IEs of the constituents of air and the most common solvents. In such a way, reaction 7 (Eq. 7) involves only the compounds of interest.

Evaluated ionization energies of some common compounds are reported in Table 1 (Lias, 2003), together with the energies of some discharge lamps usually used in PI. The most suitable lamp listed is probably the Kr-filled one (10 eV), because it has a photon energy lower than the major components of air; i.e., N_2, H_2O, and O_2, and some of the most commonly used solvents. On the contrary, a large part of the molecules usually under investigation have IEs below 10 eV.

A. The Use of a Dopant

Direct ionization of an analyte molecule occurs with a low-statistical probability, in part because solvent (according to

TABLE 1. Evaluated Ionization Energies (Lias, 2003)

Lamp	Compound	IE (eV)
	Nitrogen	15.58
	Water	12.62
	Acetonitrile	12.20
	Oxygen	12.07
Ar: 11.2 eV		
	Methanol	10.84
	Isopropanol	10.17
	Hexane	10.13
Kr: 10.0 eV		
	Heptane	9.93
	Isooctane	9.80
	Acetone	9.70
	Pyridine	9.26
	Benzene	9.24
	Furan	8.88
	Toluene	8.83
Xe: 8.4 eV		
	Naphtalene	8.14
	Triethylamine	7.53

reactions 2–6 (Eqs. 2–6) depletes the photons emitted by the discharge lamp.

A suitable substance, added in relatively large amounts (compared to the analyte), could significantly increase the number of ions (Spangler et al., 1994; Doering et al., 1999). Such a substance, called a dopant, is effective if it is photoionizable and can function as an intermediate between the photons and the analytes by reacting with them by charge exchange or proton transfer (Harrison, 1983).

To work as a dopant, a substance must be selected with an effective IE lower then the photon energy of the emitted light, such that its photoions have a high recombination energy or low proton affinity (PA).

According to reactions 1 and 7 (Eqs. 1 and 7), dopant (D) is ionized and then it acts as an intermediate:

$$D + h\nu \rightarrow D^{\bullet +}, \quad (11)$$

$$D^{\bullet +} + AB \rightarrow D + AB^{\bullet +}. \quad (12)$$

1. APPI–MS with a Dopant

Like all the techniques based on APPI, APPI–MS takes advantage of the use of a dopant. Several reports that explain the effects of the dopant describe the co-existence in the mass spectra of molecular ions ($M^{\bullet +}$) and non-expected protonated molecule ions ($[M + H]^+$). Koster & Bruins (2001) investigated the mechanism for the formation of $[M + H]^+$ ions, taking into account a possible involvement of the solvent. They carried out their experiments on a triple-quadrupole mass spectrometer equipped with an APPI source, using a toluene dopant and filling the collision cell (Q2, which works as a reaction chamber) with methanol or acetonitrile. In practice, the toluene molecular ion ($C_7H_8^{\bullet +}$, m/z 92) was selected in Q1 and sent to Q2; the reaction products were analyzed in Q3. The results suggested that, in the presence of toluene, those solvents act as intermediates between the ionized dopant and analyte, as follows (Eqs. 11, 13, and 14):

$$D^{\bullet +} + MP \rightarrow [D - H]^{\bullet} + [MP + H]^+, \quad (13)$$

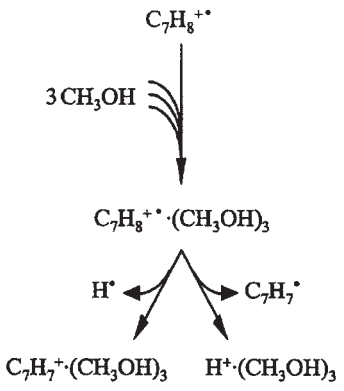
$$[MP + H]^+ + AB \rightarrow MP + [AB + H]^+. \quad (14)$$

When this process occurs, dopant ions are depleted and are no longer available for reaction 12 (Eq. 12).

Methanol interacts with the toluene molecular ion to form a cluster that contains three molecules of methanol. Such a cluster can rearrange and fragment either by the loss of a hydrogen or a benzyl radical, $C_7H_7^{\bullet}$ (see Scheme A); the latter produces a protonated solvent cluster, which participates in reaction 14 (Eq. 14). Also, acetonitrile forms clusters with $C_7H_8^{\bullet +}$: the mechanism is basically the same as methanol, but in this case only two molecules of solvent are involved.

In general, the proton transfer between toluene molecular ion and a solvent molecule takes place when the PA of the solvent is higher with respect to that of the benzyl radical. Thus, according to their PAs (see Table 2), proton transfer does not occur with water, hexane, and chloroform. However, proton transfer is thermodynamically possible when the solvent has a lower PA with respect to that of the benzyl radical, but forms clusters with higher PAs. This situation is the case with methanol and acetonitrile, which form solvent and solvent/water clusters (Meot-Ner, 1986) that have higher PAs than those of the individual monomer and $C_7H_7^{\bullet}$.

The mechanism reported here was confirmed by Kauppila et al. (2002), who investigated in-depth the ionization process and mechanism in the positive- and



SCHEME A.

TABLE 2. Proton Affinities of Some Solvents and Species (Hunter & Lias, 2003)

Compound	PA (kJ/mol)
Benzyl radical	831.4
Tetrahydrofuran	822.1
Isopropanol	793.0
Toluene	784.0
Acetonitrile	779.2
Methanol	754.3
Water	691.0

negative-ion modes by analyzing seven naphthalenes (1-naphthalenemethylamine, 2-acetonaphthone, 2-naphthol, 2-ethylnaphthalene, 2-naphthaleneethanol, 2-naphthylacetic acid, and 1,4-nophthoquinone) in 13 different solvent systems.

B. Further Parallel Reactions in APPI–MS

The mechanism of the APPI process is still not completely understood. Some parallel reactions take place because of the presence in the eluant of reactive organic species that interact with photons or with activate species. For example, some experiments carried out by Marotta et al. (2003) have shown that acetonitrile, used as a solvent, participates in the protonation of furocoumarins, even when no dopant is employed. That result is unexpected: because acetonitrile exhibits an IE (12.20 eV) that is higher than the photon energy of the (Kr)-filled lamp (10 eV) that was used for the study, no photoions of acetonitrile could be produced. ^{13}C- and ^{2}H-labeling experiments, semi-empirical calculations, and product-ion spectra suggested that acetonitrile, when photon-irradiated, could isomerize to produce some species (**1**, **2**, **3**, and **4** in Fig. 1). The calculated IEs of those compounds are close to, or lower than, the lamp photon-emission energy, so that they are possible precursors of ionic species that are generated by PI. According to energetic estimations, the ionic specie relative to compound **3** in Figure 1 provides the most stable molecular ion (**5**), and thus it can be assumed to be the most probable PI product of acetonitrile. The resulting compound **5** can react with a neutral acetonitrile molecule, as described in the reaction 15 (Eq. 15):

$$\begin{aligned} &H_2C{=}\underset{5}{C}{=}NH^{+\bullet} + H_3C{-}C{\equiv}N \\ &\rightarrow H_2C{=}\underset{6}{C}{=}\overset{+}{N}H_2 + H_2\overset{\bullet}{C}{-}C{\equiv}N. \end{aligned} \tag{15}$$

The even-electron ion **6** is particularly stable, and can ionize the analyte by proton transfer, therefore, rationalizing the formation of the protonated molecular ion of the analyte ($[M + H]^+$).

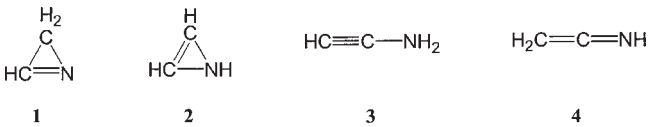


FIGURE 1. Some possible acetonitrile isomers generated by the photon irradiation of acetonitrile.

III. APPI SOURCES—OVERVIEW

A. In-Line Geometry Source

The source described in the present section (Robb, Covey, & Bruins, 2000) was originally designed for its coupling with a PE/Sciex (Concord, Ont., Canada) 300–3000 series triple-quadrupole mass spectrometer (Robb & Bruins, 2001). To facilitate its designing and engineering, it was strictly derived from the standard heated nebulizer (HN)-APCI source provided with those models of mass spectrometers. In practice, Bruins and co-workers thought that the necessary vaporization of the liquid eluent could have been obtained in the same way as for APCI, and thus they used the original PE/Sciex HN for that purpose. A discharge-lamp mounting bracket, placed straight on the stainless steel HN probe, replaced the discharge needle of the APCI source, and some simple modifications were made to the original APCI source housing to position an electrical connector for the high-voltage supply of the discharge lamp. A commercial power supply was employed for the ignition and the maintenance of the lamp discharge, which was filled with Kr and was equipped with a magnesium fluoride window (nominal photon energy at 10 eV, and real energy at 10.0 and 10.6 eV). An electrical potential was applied to the mounting bracket (offset potential) with a separate high-voltage power supply. The inventors demonstrated that its value has a huge influence on the sensitivity of the technique, and is also related to the distance between the end of the ion-guide tube, which protrudes from the mounting bracket, and the mass spectrometer's curtain plate. A dopant was used to increase the efficiency of ion formation. A syringe pump, connected to a thin fused-silica capillary inserted into the HN through the auxiliary gas line, supplied dopant. A schematic diagram of the APPI source is shown in Figure 2.

The complete APPI–LC–MS system used by Bruins and co-workers for the performance of their experiments is schematized in Figure 3. A variant of the present source is commercial, and Applied Biosystems/MDS Sciex sells it with the PhotoSpray™ trademark.

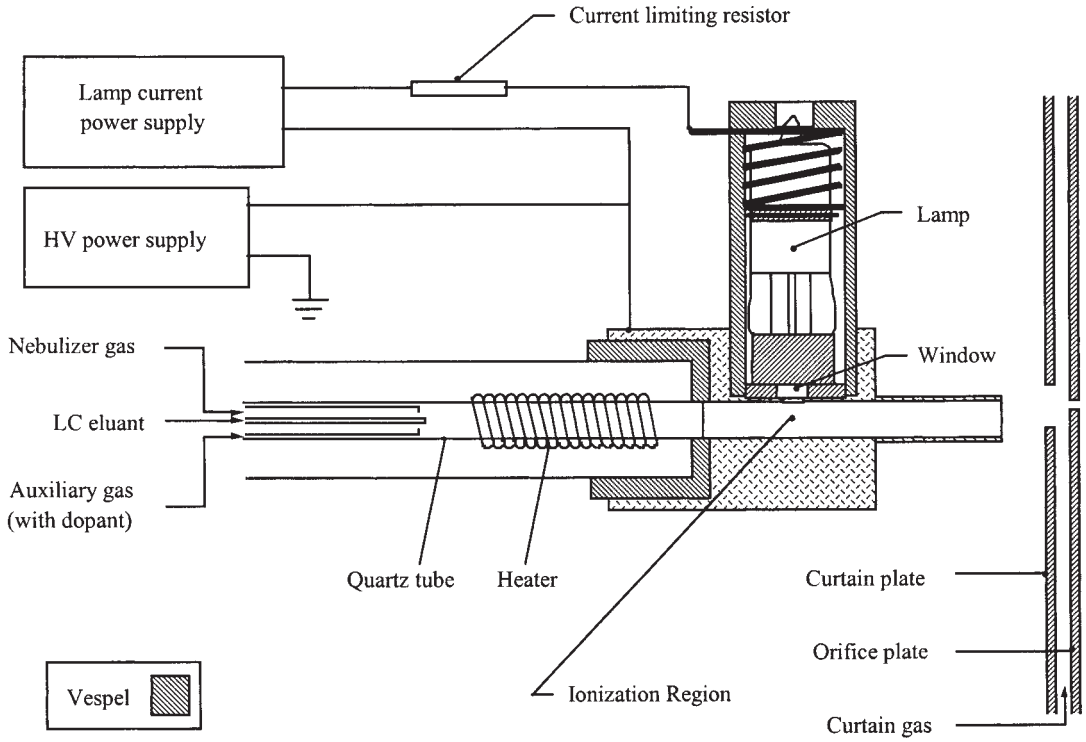


FIGURE 2. Schematic diagram of a complete atmospheric-pressure photoionization (APPI) in-line ion source [reproduced from Robb, Covey, & Bruins (2000) with the permission of the American Chemical Society © 2000].

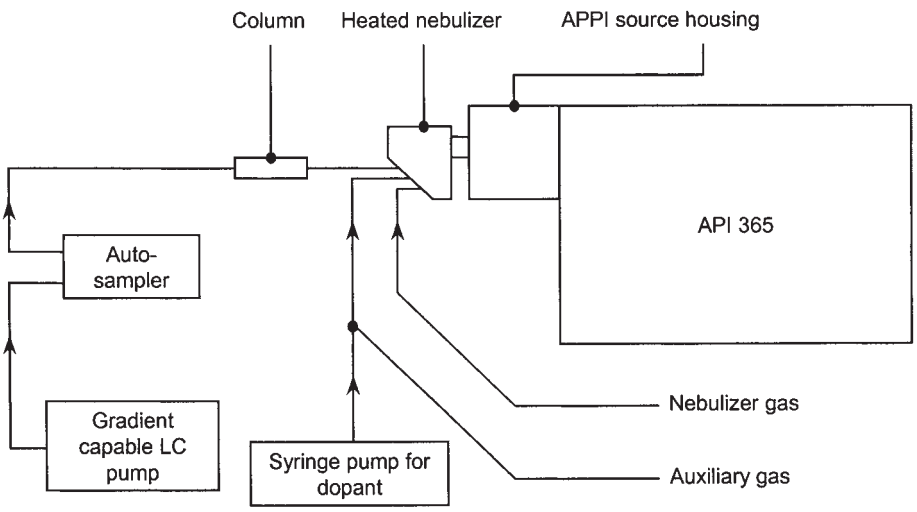


FIGURE 3. Schematic diagram of the complete APPI–liquid chromatography (LC)–mass spectrometry (MS) system [adapted from Robb, Covey, & Bruins (2000) with the permission of the American Chemical Society © 2000].

B. Orthogonal Geometry Source

An orthogonal APPI source was developed by Syagen Technology, Inc. (Tustin, CA), starting from the scheme of the Agilent Technologies (Palo Alto, CA) APCI source, and coupled to MSD 1100 mass spectrometer series (Syage et al., 2001) (Fig. 4). In the present design, the HN and the PI lamp are, respectively, perpendicular and in-line with respect to the mass spectrometer's ionic path, no ion-guide tube is used.

The commonly used discharge lamp also in this case is filled with Kr, and emits photons at 10.0 and 10.6 eV

Such a source was not designed specifically for use with a dopant, but several applications (Perkins, Miller, & Fischer, 2000; Cormia, Fischer, & Miller, 2001; Meng, 2002) made use of it to increase the sensitivity of the method (Fig. 5).

The commercial name of the source is PhotoMate®, and it is available from Agilent Technologies and ThermoFinnigan (San Jose, CA).

IV. FACTORS THAT INFLUENCE THE PERFORMANCE OF APPI

A. The Dopant

As already mentioned, in many cases a dopant gives advantages in terms of detection sensitivity of APPI–MS. From a theoretical point of view, a large number of substances may be used as a dopant, but at present only a few have been tested with APPI. However, the large part of the applications described in the literature makes use of toluene and acetone. Robb, Covey, & Bruins (2000) first described the effect of a suitable dopant on the sensitivity and selectivity of the method. In fact, they showed that, in selected-ion monitoring (SIM), the ion signal intensities of carbamazepine, acridine, naphthalene, and diphenyl sulfide increased from 25 through 100 when toluene is provided (compare Figs. 6 and 7). Such a result suggests that toluene enhances the sensitivity of APPI toward both compounds with low and high PA. On the contrary, acetone acts as a dopant only for those compounds that have high-PA (Fig. 7).

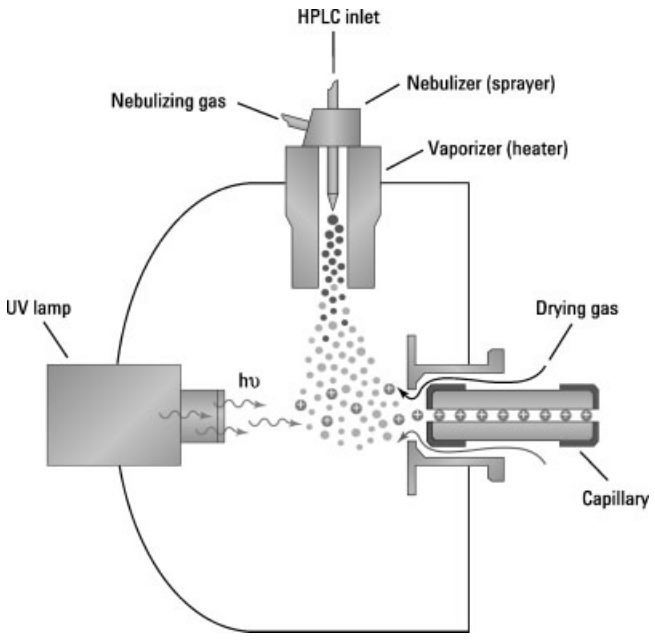


FIGURE 4. Schematic diagram of an orthogonal APPI source [© 2001 Agilent Technologies, Inc., reproduced with permission].

B. Mobile Phase Composition

A number of reports described the effect of the mobile phase composition on the APPI–LC–MS detection sensitivity (Robb, Covey, & Bruins, 2000; Cormia, Fischer, & Miller, 2001; Impey, Kieser, & Alary, 2001; Rauha, Vuorela, & Kostiainen, 2001; Alary et al., 2002; Kauppila et al., 2002; Meng, 2002; Yang & Henion, 2002). The influence of the water content of the eluent in reversed-phase HPLC has been described by Yang & Henion (2002), in a recently published paper. The authors tested three different mixtures of eluents (A: CH_3OH-H_2O (85:15, v/v) (1% HCOOH); B: CH_3OH with the addition of 1% HCOOH; C: pure CH_3OH) for the elution of idoxifene (a non-steroidal estrogen antagonist) and its metabolites SB245420 and SB245419 (the structures are shown in Fig. 8) contained in human plasma. The SIM APPI demonstrates that a reduction of the water content produced a general increment of sensitivity—particularly for SB245419, whose signal increases of a factor 15 (see Fig. 9).

Many applications in the HPLC–MS literature have been performed by reversed-phase chromatography, mainly because most of them make use of ESI. On the contrary, normal-phase chromatography can be coupled successfully with APCI and APPI to provide an improvement in the separation and a good response factor for some classes of compounds. As a general rule, in APPI–MS solvent must be selected carefully, because it could profoundly affect the limit of detection (LOD) of the compounds under investigation (Robb, Covey, & Bruins, 2000; Alary, 2001). An interesting report by Alary et al. (2002) compared the ion signals for testosterone and progesterone in five different solvents (isooctane, methylene chloride, 2-propanol, ethyl acetate, and acetonitrile), showing large differences. In particular, they demonstrated that ethyl acetate and acetonitrile greatly reduced the ion signal for both analytes, whereas isooctane and methylene chloride offer much better ionization conditions (see Fig. 10).

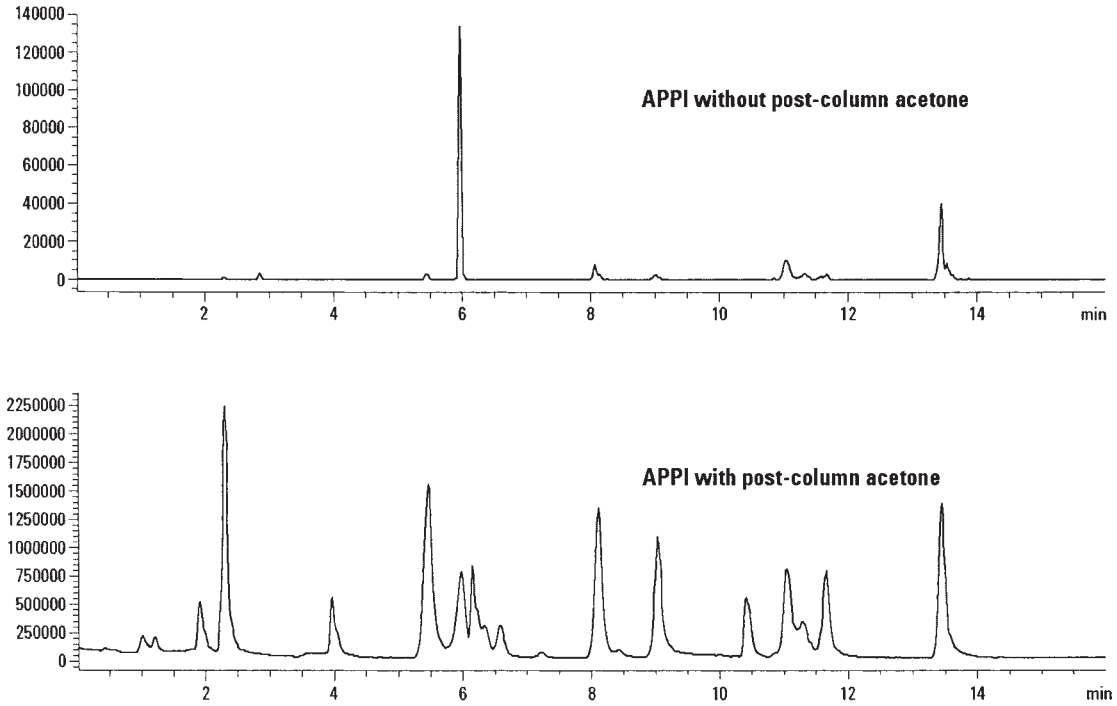


FIGURE 5. TIC shows the effect of adding post-column acetone to enhance the analyte signal in APPI. Methanol was used as solvent B for TICs [Meng, © 2002 Agilent Technologies, Inc., reproduced with permission].

V. APPLICATIONS

A. Pharmaceutical and Drug Metabolism

The determination of small molecules in biological matrixes has become an important part of drug discovery. Such research makes extensive use of LC coupled with API–MS, which probably is the preferred technique for a large number of drugs and drug metabolism studies in the pharmaceutical industry. The additional analytical capabilities offered by APPI–MS, with respect to ESI- and APCI–MS, have been successfully used to improve the detection limits of some classes of compounds.

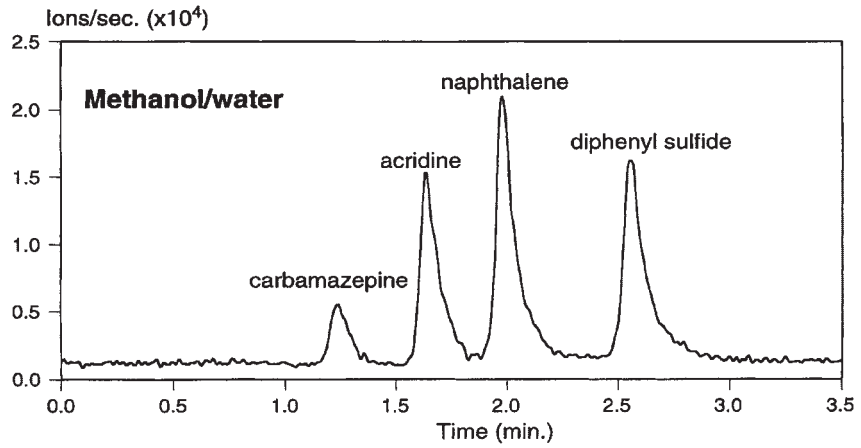


FIGURE 6. HPLC–MS chromatograms of carbamazepine (1 pmol), acridine (1 pmol), naphthalene (100 pmol), and diphenyl sulfide (100 pmol) obtained by SIM–APPI of *m/z* 237, 180, 128, and 186, respectively, without the use of a dopant, for methanol–water eluant [reproduced from Robb, Covey, & Bruins (2000) with the permission of the American Chemical Society © 2000].

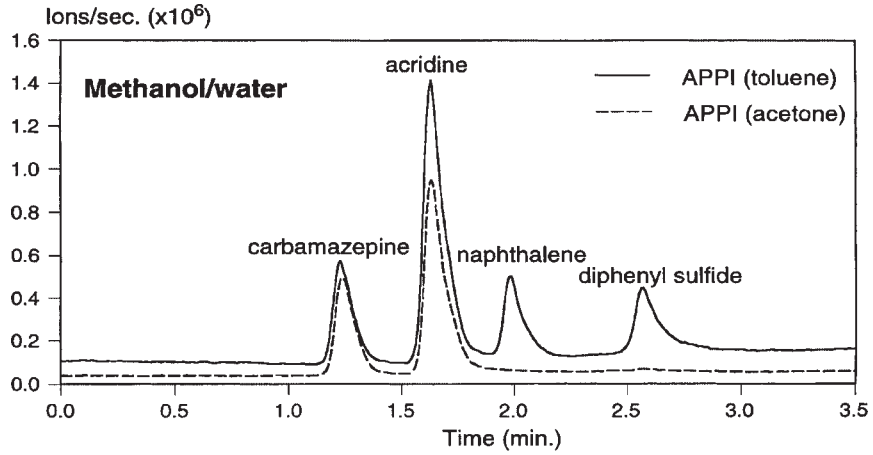


FIGURE 7. APPI–HPLC–MS chromatograms of carbamazepine (1 pmol), acridine (1 pmol), naphthalene (100 pmol), and diphenyl sulfide (100 pmol), obtained by SIM–APPI of *m/z* 237, 180, 128, and 186 respectively, by using toluene and acetone as dopants, and methanol–water as a mobile phase [reproduced from Robb, Covey, & Bruins (2000) with the permission of the American Chemical Society © 2000].

1. Non-Steroidal Compounds

The aforementioned paper by Yang & Henion (2002) on idoxifene and its metabolites in human plasma (see Mobile Phase Composition), makes an interesting comparison between APPI and APCI in terms of detection sensitivity. Basically, they demonstrated that the two techniques provide identical result in the full-scan mode as far as idoxifene and SB245419 are concerned. On the contrary, the mass spectrum of SB245420 could not be obtained in the APCI mode, because of the lack of sensitivity, whereas in APPI it was achieved by using a higher concentration of SB245420 (100-times the concentration of idoxifene or SB245419). More experiments, performed in the SIM mode, confirmed a general higher sensitivity for APPI for all of the compounds under investigation. However, the sensitivity increment was still not sufficient for the detection of SB245420 at clinically relevant levels in human plasma.

APPI has also been used to detect drug conjugates in biological samples. In particular, Keski-Hinnilä et al. (2002) tested the source in the determination of apomorphine, dobutamine, and entacapone phase II metabolites in rat urine and in *in vitro* incubation mixtures, and compared the results to those relative to APCI and ESI. In that kind of application, APPI appears to be less efficient with respect to ESI and quite similar to APCI. In particular, methyl conjugates were detected by all of the ionization techniques, only some glucuronides detected by ESI could be found by APPI and APCI, and sulfate conjugates were detected only by ESI. According to the authors, the reasons for the low efficiency of the APPI source, as well as for that of APCI, were the poor vaporization of charged glucuronide and sulfate conjugates and the thermal degradation of the glucuronides.

APPI–MS has also been coupled with capillary electrophoresis. To the best of our knowledge, Nilsson et al. (2002) first reported that coupling. They studied the

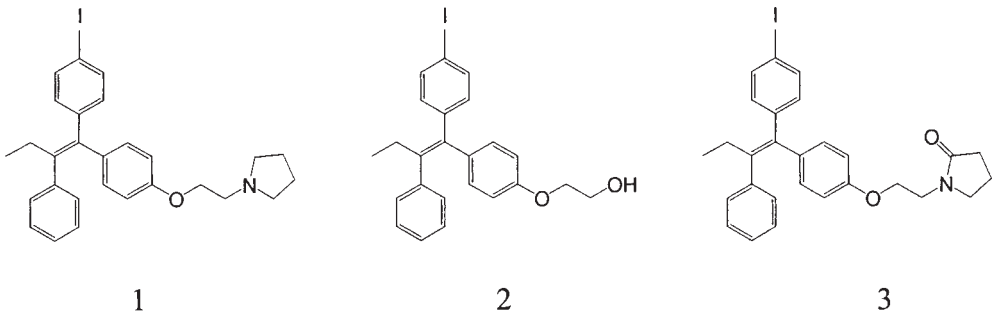


FIGURE 8. Structures of idoxifene (**1**), SB245420 (**2**), and SB245419 (**3**).

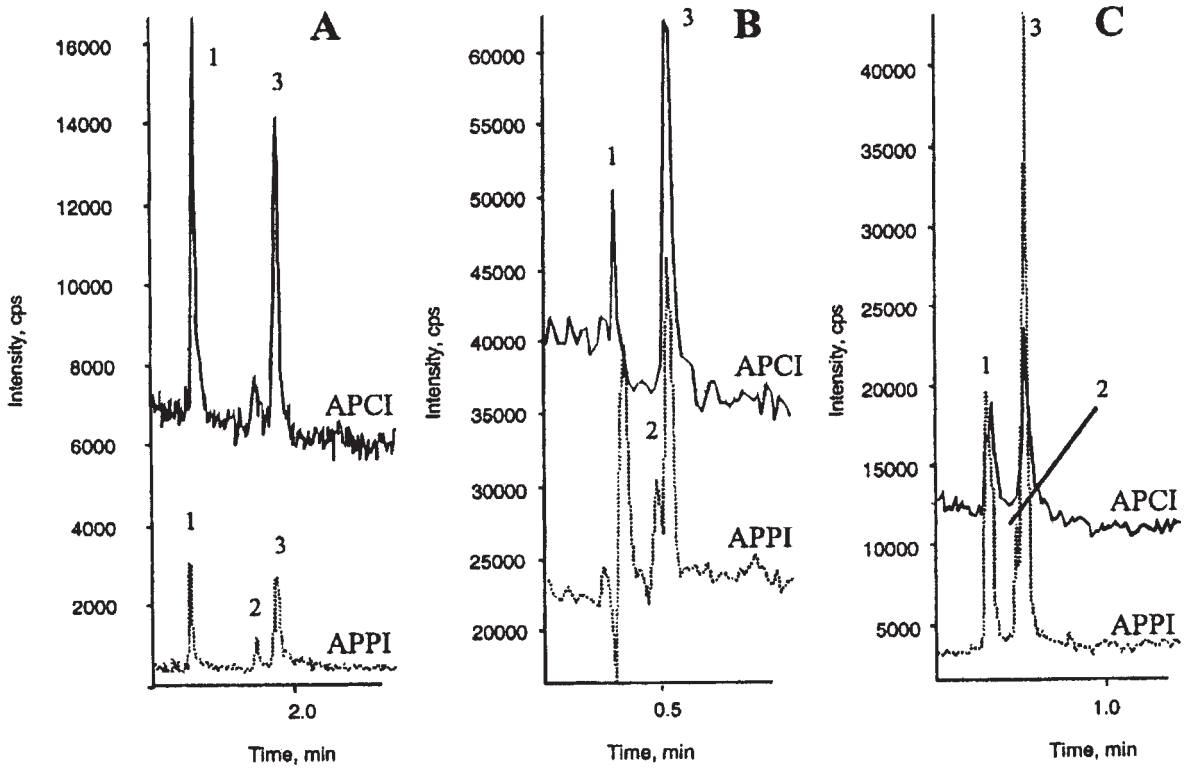


FIGURE 9. Comparison of SIM APCI–LC–MS obtained from three mobile phases: (**A**) MeOH-H_2O 85:15, (1% HCOOH); (**B**) MeOH 99% (1% HCOOH); (**C**) MeOH (100%). Other LC conditions: flow-rate of mobile phase 0.7 ml/min, 10 μL sample injection. Peak identities and sample concentration in mixture: (**1**) idoxifene (25 nM), (**2**) SB245420 (2.5 μM), (**3**) SB245419 (25 nM). The SIM selected ions were *m/z* 524, 471, and 538 for peaks **1**, **2**, and **3**, respectively [reproduced from Yang & Henion (2002) with the permission of the Elsevier Science B.V. © 2002].

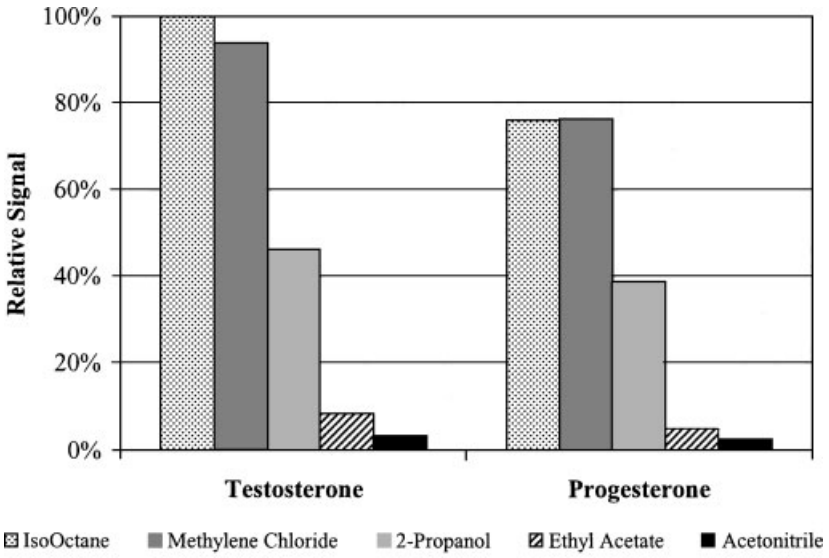


FIGURE 10. Influence of some solvents on the APPI–MS ion signal towards testosterone and progesterone [adapted from Alary et al. (2002)].

effect of non-volatile buffers on selectivity and sensitivity of CE-APPI–MS, using as test compounds some low molecular weight nitrogen-containing compounds of pharmaceutical interest. The experimental results confirmed that a phosphate buffer (pH 2.5, 50 mM) provided better results in terms of selectivity, separation, and peak shape with respect to ammonium formate/formic acid buffer (pH 2.7, 50 mM). In addition, a comparison between CE-APPI–MS and CE-ESI–MS (see Fig. 11) shows that, when a non-volatile buffer is used, APPI gives a lower background ion signal compared to ESI.

2. *Steroidal Compounds*

The determination of some anabolic androgenic steroids (AAS) have been carried out by LC–APPI–MS/MS (Leinonen, Kuuranne, & Kostiainen, 2002). Oxandrolone, *6β*-hydroxy-4-chlorodehydromethyltestosterone, and *3′*-hydroxystanozolol (compounds **1**, **2**, and **3** in Fig. 12, respectively) were selected as test compounds because they were considered to be representative of the main different structures of the AAS metabolite-free forms that can be found in human urine. All of them were detected by APPI assisted by toluene or acetone as a dopant. In every case, the ionization of the analytes occurred with the exclusive formation of protonated species, suggesting the involvement in the ionization process of the mechanism described in "APPI–MS with a dopant." The LOD's obtained in the multiple-reaction monitoring (MRM) mode are in the range 0.4–0.9 nmol/mL—higher with respect to those found by ESI (0.06–0.5 nmol/mL) and APCI (0.08-0.9 nmol/mL).

Alary et al. (personal communication) developed a method for the evaluation of five key metabolites of testosterone (*6β*-hydroxyandrostenediol, *7β*-hydroxyandrostenediol, *2α*-hydroxyandrostenediol, *16α*-hydroxyandrostenediol, and *5α*-androstene-*3α*,*17β*-diol) based on normal-phase HPLC coupled to APPI–MS. A normal-phase chromatographic method was preferred to a reversed-phase one for its better capability in the separation of the isomeric compounds under investigation. Moreover, as already illustrated, the eluent mixture, composed of isooctane and isopropyl alcohol (gradient: C_3H_7OH from 20% through 40%), also offers an advantage in terms of sensitivity of APPI towards the compounds of interest.

B. Environmental

APPI–MS has been applied to the determination of compounds of environmental concern, such as pesticides, and organic compounds in air. An HPLC–MS-based method for the analysis of phenyl ureas (a class of herbicides) and carbamates (a class of highly effective insecticides) in ground- and surface-water was developed by Meng (2002). In that study, the author compared the sensitivity of APPI towards the analytes to that of ESI and APCI, and demonstrated that, in general, carbamates give a better response in ESI than in APPI, and phenyl ureas give the opposite result. In addition, APPI usually provided

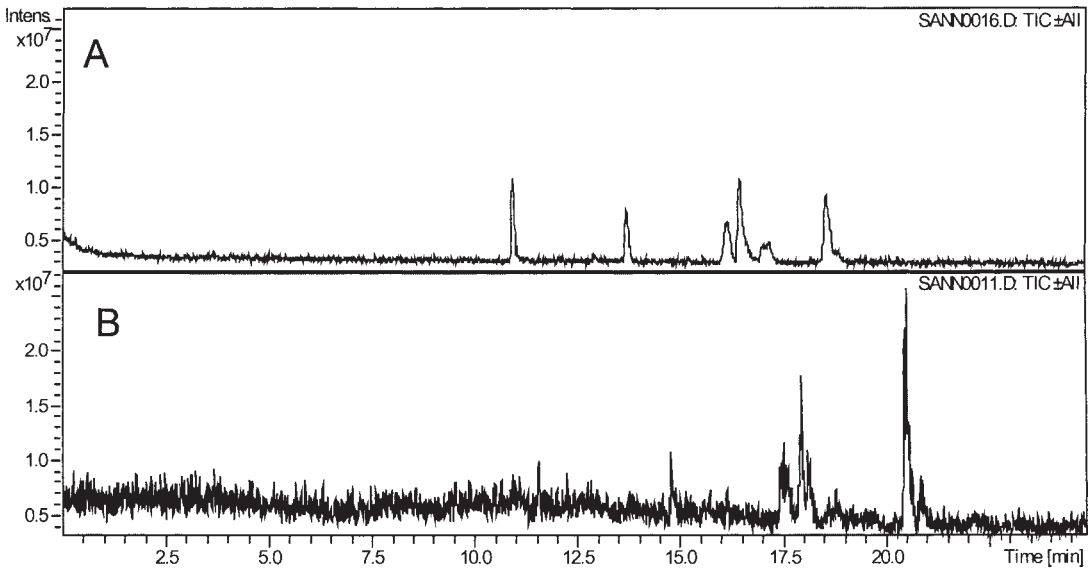
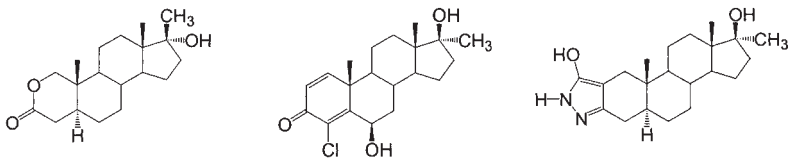


FIGURE 11. Total ion current diagrams relative to a sample that contained some nitrogen compounds, acquired by CE-APPI–MS (**A**) and CE-ESI–MS (**B**), using phosphate buffer [courtesy of Nilsson et al. (2002)].



1 2 3

FIGURE 12. Structures of oxandrolone (**1**), the 6β-hydroxy-4-chlorodehydromethyltestosterone (**2**), and the 3′-hydroxystanozolol (**3**).

higher signal-to-noise ratios with respect to APCI. PI can also be an attractive ionization method for on-line mass spectrometric measurements of gas phase organics. Using a custom-made orthogonal ion source in combination with a commercial mass spectrometer, Hoffmann et al. (2002) carried out experiments on a test gas mixture of aniline in a continuous flow of synthetic air, in the presence of acetone dopant. In practice, they demonstrated the potential of the method, which provided an estimated 80 ppt (v/v) LOD for aniline.

APPI–MS is a suitable technique also in the analysis of polycyclic aromatic hydrocarbons, PAHs (Robb, Covey, & Bruins, 2000). Before the marketing of APPI, in MS PAHs could be analyzed exclusively by GC–MS, because LC–MS was not a suitable technique for the low-ionization efficiencies of ESI and APCI towards this class of compounds. Nowadays, things are different. Cormia, Fischer, & Miller (2001) reported that APPI with toluene dopant provides good results in the analysis of such compounds, coupled to reversed-phase and to normal-phase chromatography. Reversed-phase HPLC–APPI–MS with methanol was demonstrated to be as sensitive as normal-phase HPLC–APPI–MS with hexane, except for naphthalene, whose signal is significantly higher in the latter. However, the better chromatographic separation offered by reversed-phase HPLC is very helpful when a single quadrupole mass spectrometer is used, as for the present research, because multiple groups of PAHs have the same mass. On the contrary, Impey, Kieser, & Alary (2001) confirmed that the availability of triple-quadrupole mass spectrometers allows the development of suitable MRM based methods that sacrifice the chromatographic separation for an increase in the sensitivity. Actually, they analyzed a test mixture that contained 16 of the most common PAHs, and found LODs 4–80 times lower in normal-phase chromatographic conditions (isooctane, isocratic mode) than in reversed-HPLC (acetonitrile–water, gradient mode).

C. Natural Products

The analysis of simple sugars by LC–MS usually requires a pre- or post-column derivatization (ESI) or the post-column addition of $CHCl_3$ or CH_2Cl_2 (APCI) to achieve high-sensitivity levels. Also, APPI gives excellent results with such a class of compounds. When used in the negative-ion mode, with acetone dopant and a small percentage of formic, acetic, or trifluoroacetic acid in the mobile phase (Perkins, Miller, & Fischer, 2002), it provided a response similar to that obtained by the APCI method, and approximately one order lower with respect to the ESI-based method, with no need for further additions. Under the conditions of the method, in fact, simple sugars form the acylate anion adducts (Fig. 13), which have a good response in MS.

A method for the analysis of flavonoids was developed by Rauha, Vuorela, & Kostiainen (2001). The authors found out that with a mobile phase composed by 40% organic solvent (CH_3OH and CH_3CN 3:4) and 60% water without any aqueous buffers, the best sensitivity is achieved in the positive- and negative-ion modes. However, a better HPLC resolution was obtained by adding some ammonium acetate to the eluent; for this reason, it was decided that a good compromise between chromatographic behavior and sensitivity could have been achieved by adding ammonium acetate to water at a concentration of 5–10 mM. The APPI spectra of flavonoids acquired in such conditions, in the positive- and negative-ion modes, show protonated and deprotonated molecules, respectively; those results suggested that the primary ionization mechanism is a proton transfer reaction. In conclusion, no significant differences in the ionization efficiencies between the positive- and negative-ion modes were noticed. In any events, the lower background ion signal in the negative-ion mode yielded higher LODs. In addition, a comparison with APCI and ESI proved that the LODs of all of the techniques are comparable, although the negative-ion mode ESI provided somewhat higher ones. Saba et al. (2002) carried out some more experiments on the use of APPI in the analysis of some food components. They developed methods for the determination of some phenolic antioxidants present in wine (catechin, quercetin, and resveratrol), some components of olive oil (α-, γ-, and δ-tocopherols), and some species from essential oils from citrus fruits, such as cumarins, psoralenes, and

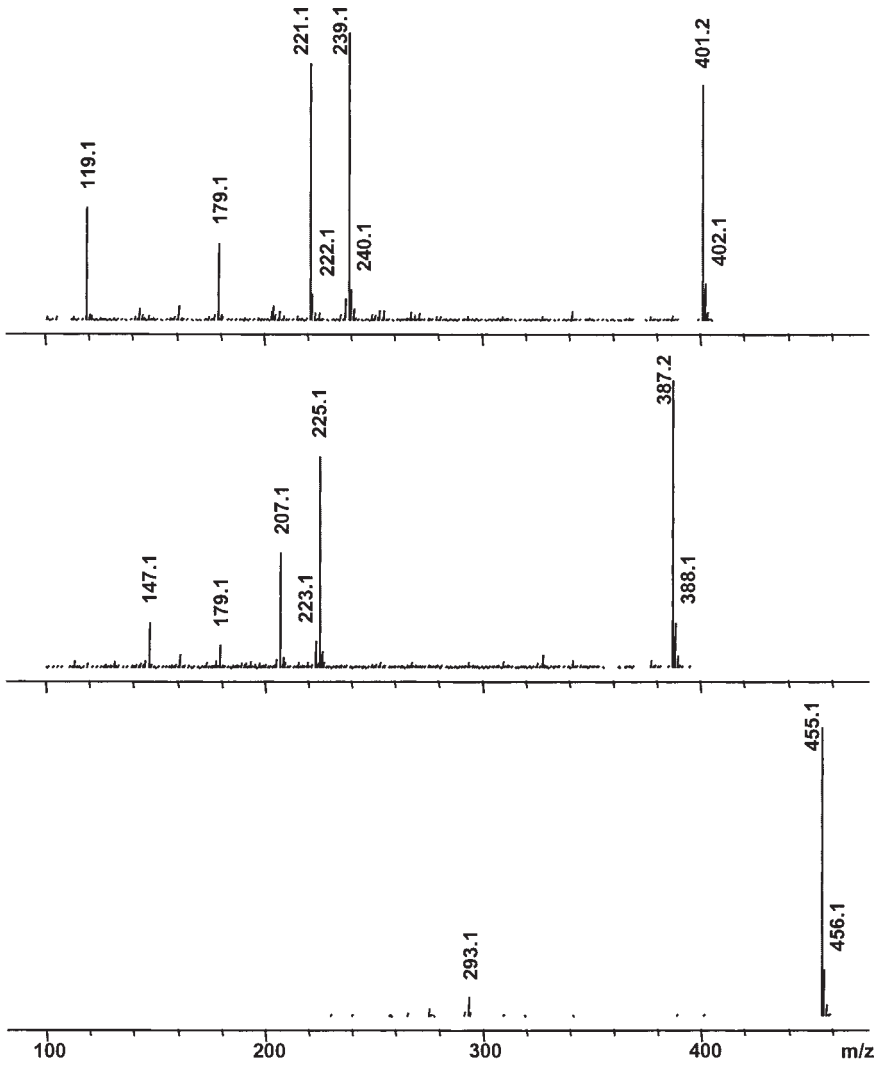


FIGURE 13. Negative-ion adduct of sucrose (MW 342) with acetate (**top**), formate (**middle**), and trifluoroacetate (**bottom**). Fragments in the spectra indicative of the monomeric subunits of sucrose are present [© 2002 Agilent Technologies, Inc., reproduced with permission.].

polymethoxyflavons. In particular, as far as the essential oils are concerned, the interest was focused on those compounds that did not respond either in ESI or in APCI; the results demonstrated that APPI can detect some of them in real samples.

D. Synthetic Organics

Kertesz & Van Berkel (2002) tested APPI as an interface for on-line electrochemistry mass spectrometry (EC/MS) experiments. During the course of investigating APPI for the study of the electrochemical formation of conductive polymers, the authors found that some of the species of interest were reduced in the ion source. In particular, they noticed a reduction of the oligomers formed from on-line electropolymerization of aniline, the compound *N*-phenyl-1,4-phenylenediimine, and the thiazine dye thionin. Such a reduction process probably involves hydrogen radicals formed from protic solvents in the API plasma.

This phenomenon was enhanced when an eluent mixture that contained water, aqueous methanol, and proton transfer modifiers was used, as well as low temperatures of the HN ($\leq$400°C), and a dirty quartz liner (present inside the PE/Sciex HN). The comparison with APCI showed that the two sources behave exactly in the same way. In contrast, no reduction takes place in EC/ESI–MS.

VI. CONCLUSIONS

APPI is the most recent acquisition in the field of soft ionization techniques for coupling MS to liquid-phase separation techniques. Its potentialities include the possibility to use LC–MS for analytes that are not completely amenable for ESI and APCI. It promises, in particular, to take care of the particle-beam orphan molecules, and to effectively extend the present range of application of this hyphenated technique. Of course, one should not think to completely substitute for GC–MS: for volatile, thermally stable molecules, the latter is surely the technique of choice.

At present, such potentialities have not yet been sufficiently explored, and much research remains to fully understand its features and application fields. In particular, more fundamental research is needed to understand the really important parameters and factors that affect the ionization efficiency. The ability, for instance, to direct the preferential ion formation towards one particular ion type ($M^{\bullet+}$, $[M+H]^+$, etc. in the positive-ion mode, $M^{\bullet-}$, $[M-H]^-$, and so on in the negative-ion mode) can be extremely useful for qualitative and quantitative determinations. For this purpose, a better insight in the processes involved in the ionization steps is strongly needed. The role of the different species present in the ionization plasma (e.g., solvents from the mobile phase, dopant, with electron- or proton-exchange capabilities, use of air or nitrogen, etc.) must be fully understood. In this respect, the chemical nature of the analyte, probably the most important parameter in the choice of the ionic (radical or even-electron) species must be taken into account in the development of the HPLC–MS methods.

It must be pointed out that, even if almost all the papers presented here deal with positive ions, APPI offers a good ionization efficiency in the negative-ion mode, too. As far as the ''softness'' of the technique is concerned, the research that has been developed and published so far clearly indicates that the behavior of this ionization technique is very close to that of APCI, also because the operative conditions are similar. This similarity means that the ionization process is quite soft, but that one can observe possible thermal degradation effects. At present, it is not easy to say which compounds are amenable to APPI instead of APCI or ESI, and it appears to be useful to test all three of them in all cases.

In this frame, it would be very nice to have the availability of instruments equipped with combined ion sources. The presence of ESI, APCI, and APPI in the same source, with the possibility to exchange them via software or even to operate them in the same run, could really open large perspectives in all the fields of the application of LC–MS.

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