DIRECT-EI IN LC–MS: TOWARDS A UNIVERSAL DETECTOR FOR SMALL-MOLECULE APPLICATIONS

Achille Cappiello,* Giorgio Famigli, Pierangela Palma, Elisabetta Pierini, Veronica Termopoli, and Helga Trufelli
DiSTeVA, Università degli Studi di Urbino “Carlo Bo,”
Piazza Rinascimento, 6, 61029 Urbino, Italy

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This review article will give an up-to-date and exhaustive overview on the efficient use of electron ionization (EI) to couple liquid chromatography and mass spectrometry (LC–MS) with an innovative interface called Direct-EI. EI is based on the gas-phase ionization of the analytes, and it is suitable for many applications in a wide range of LC-amenable compounds. In addition, thanks to its operating principles, it prevents unwelcome matrix effects (ME). In fact, although atmospheric pressure ionization (API) methodologies have boosted the use of LC–MS, the related analytical methods are sometime affected by inaccurate quantitative results, due to unavoidable and unpredictable ME. In addition, API’s soft ionization spectra always demand for costly and complex tandem mass spectrometry (MS/MS) instruments, which are essential to acquire an “information-rich” spectrum and to obtain accurate quantitative information. In EI a one-stage analyzer is sufficient for a qualitative investigation and MS/MS detection is only used to improve sensitivity and to cut chemical noise. The technology illustrated here provides a robust and straightforward access to classical, well-characterized EI data for a variety of LC applications, and readily interpretable spectra for a wide range of areas of research. The Direct-EI interface can represent the basis for a forthcoming universal LC–MS detector for small molecules. © 2011 Wiley Periodicals, Inc., Mass Spec Rev

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I. INTRODUCTION

Approximately two decades ago, the introduction of soft-ionization techniques, namely electrospray (ESI) and atmospheric pressure chemical ionization (APCI), projected mass spectrometry towards ambitious and previously unexplored applications, and evolved LC–MS as a mature and reliable instrumentation. Although soft-ionization techniques represent the heart and soul of modern LC–MS, their performance is still impaired by a few shortcomings that can be summarized as follows:

signal response can be influenced by the polarity of the analytes, and affected by ME (Cech & Enke, 2001; Taylor, 2005; Niessen, Manini, & Andreoli, 2006);

In ESI conditions, ionization takes place in the liquid phase through a chain of complex chemical reactions, in which acidic-base equilibria predominate, between the target compound and the surrounding matrix, whose composition is the result of sample preparation and chromatographic conditions (Kebarle, 2000; Cech & Enke, 2001). Depending on the experimental conditions involved in a specific application, positive or negative ions can be generated by proton or adduct additions and subtractions. The low vibrational energy involved in the chemical process inhibits fragmentation, and preserves the molecular structure for molecular weight measurements. Because chemical reactions take place at room temperature, all soft-ionization techniques are particularly suitable for large molecules, and require additional stages of fragmentation for structure characterization. API sources are also appropriate for small molecules, but, because of limited fragmentation, only partial information is obtained and tandem mass spectrometry (MSMS) is needed.

In quantitative analysis with ESI–MS, the actual ion abundance in the mass spectrum is the net result of the release of ions from the aerosol droplets in the gas-phase before they enter the mass analyzer. This process is influenced by several factors such as charge transfer and/or neutralization reactions, access to the droplet surface, changes in viscosity and surface tension of the droplets, and formation of solid precipitates due to the presence of non-volatile components (King et al., 2000; Cech & Enke, 2001; Zhou & Cook, 2001). It is intuitive that even subtle variations in matrix composition, sample preparation methodology, or chromatographic eluents in some cases can produce an unpredictable signal enhancement or suppression that leads to ME (Antignac et al., 2005; Taylor, 2005; Niessen, Manini, & Andreoli, 2006; Van Eechhout et al., 2009). The vast literature on this subject has proposed, so far, only improvements in the steps before final MS detection but, as a matter of fact, has accepted the existence of ME as a "side effect" of the technique that can be, in some cases, minimized but rarely eliminated (Antignac et al., 2005; Van Eechhout et al., 2009).

Different from API, hard-ionization techniques, such as EI, operate in a high-vacuum, high-temperature environment, that use a gas-phase, physical mechanism. The positive-radical ion produced by the interaction with high-energy electrons leads to reproducible, highly informative mass spectra, which are electronically comparable in reliable, commercially available libraries. Of course, these conditions are ideal for the detection of
a large number of gas chromatography (GC)-amenable compounds, but, with an appropriate combination of strategic adaptations, they can become available for any small molecule—even those carried by a liquid-phase after a conventional LC separation. Because of the low-pressure, a gas-phase ionization, such as EI, is not affected by ion–ion or ion–molecule reactions induced by co-eluted interfering substances; thus ME are reduced or totally eliminated (Cappiello et al., 2008). In addition, EI is obtained for any molecule intercepted by the electron beam, independent from its polarity or structure. The development of an efficient LC–MS interface based on a gas-phase, high-energy, EI technique overcomes most drawbacks of previous attempts (e.g., clogging of the nebulizer capillary, limited range of flow rates, better sensitivity for medium-molecular weight compounds), and offers a more-universal approach to the analysis of small molecules (Cappiello, Famiglini, & Palma, 2003; Cappiello et al., 2007a). This benefit can be observed for a wide range of compounds of different polarities and chemical properties, as well as in many biological applications, where conventional LC detection falls short. The possibility to use EI in LC will also provide legally defensible, reproducible, and easy-to-interpret mass spectra for the unambiguous identification of unknown substances, and for a wide number of low-medium molecular weight compounds that cannot be analyzed with GC.

On the other hand, molecular ion information is the Achilles’ heel for many applications where molecular ion abundance in the spectrum is negligible or not present. Although the presence of the molecular ion represents a point of strength of any soft ionization, including all API techniques, in EI its absence can be compensated with chemical ionization that, in our case, can be easily induced by a suitable reagent gas.

Background information, state-of-the-art of the research, and new applications of an EI-based LC–MS interface will be described in this review.

II. ELECTRON IONIZATION AND LIQUID CHROMATOGRAPHY

EI, formerly called electron-impact ionization, has been around for quite a long time. It was developed by Dempster (1921) at the beginning of the last century, and was improved by Bleakney (1929) and Nier (1947) many years later. Molecules are ionized exclusively in the gas phase through an interaction with high-energy electrons, which made EI the perfect “companion” for GC-amenable compounds. Electrons are usually emitted by an electrically heated filament under high-vacuum conditions, and are accelerated with an electric field. Before capture with an anode, electrons might intercept the gas-phase molecules producing an excited, positive radical ion, M+. Electron energies of 10–70 eV ionize all organic species. Ionization does not occur < 10 eV, because this limit is the least amount of energy required for primary ionization and varies as a function of the molecular structure. The probability for a successful ionization event increases with electron energy up to ~70 eV. Greater than this value, the cross-section of the ionization process retreats, and electron–molecule interactions become weaker and unproductive. In optimal conditions, the ionization efficiency is quite low, around 0.1%, but still sufficient to detect compounds at ultra-trace concentrations (<1 ppb). The high internal-energy increment transferred from the “impacting” electron, and not directly involved in the ionization process nor in the kinetic energy of the ejected electron, is responsible for an extensive fragmentation of the molecule, so that the molecular–ion is not always detected. However, fragments, molecular–ion abundance, and their distribution in the mass spectrum are unique to each molecule, because they are the result of rapid vibrational energy redistribution within the molecular structure. Each molecule responds in a distinctive, predictable fashion that leads to the formation of new cations through a chain of molecular rearrangements. The rarefied and low-density environment in which the ionization event takes place makes ion–molecule reactions very unlikely, and the reproducibility of the fragmentation process is preserved. Without external variables, the fragmentation mechanism is reproducible, reliable, and unique, and permits thousands of reference spectra of different analytes to be collected in electronic libraries for an easier compound identification. Figure 1 shows the sequence of events that lead to fragmentation. For a 100 U molecular ion accelerated with a 1,000 V potential toward the analyzer, the average time spent in the ion source depends on its speed and ranges between 10^-8 and 10^-7 sec. The interaction between an electron and a gas-phase molecule is a very high-speed process; the electron can cover a distance of 1.88 Å across a molecule in 10^-10 sec, during which the unimolecular interaction can be established and ionization might occur. For a 70 eV electron, the excess energy released to the molecule can be several eV (remember that the ionization energy for most organic molecules is about 10–15 eV, and that the process takes place under high-vacuum conditions). Under these conditions, the energy cannot be released by vibrational relaxation through the collision with other molecules; thus the only other possibility is photon emission. UV or visible-range radiation typically occurs in 10^-8 sec. If the energy is distributed among the different parts of a molecule, then a fragmentation is observed before photon emission. After 10^-8 sec, the whole process is complete, but the ions are still inside the ion source. This process illustrates why, even with different instruments and different times of flight, through changes in the accelerating potential (a few V for a quadrupole, kV for a sector instrument) the spectrum profile does not substantially change. EI spectra are unique because they are the result of only intra-molecular reactions, and are not influenced by other complex analytical parameters used in other forms of ionization.

The reliability and robustness of EI has been the main reason for the worldwide success of GC–MS. In spite of a great research effort, the first attempts made to bring the same consistency into the LC–MS world were disappointing, and none of them was even close to the GC–MS performance (Scott & Lawrence, 1970; Scott & Kucera, 1973; Vestal et al., 1974; McFadden et al., 1979; Arpino et al., 1981; Tijssen et al., 1981; Bruins & Drenth, 1983; Arpino & Beaugrand, 1985; Kientz et al., 1996; Dijkstra et al., 1998; Scott, Little, & De la Pena, 2000; Scott, 2003). Only the particle beam (PB) interface, due to its brilliant and efficient interfacing mechanism, gave the possibility to collect EI spectra in an LC application. However, PB was soon abandoned for its disappointing response in several practical experimental conditions (Willoughby & Browner, 1984; Bellar & Budde, 1988; Bellar, Behymer, & Budde, 1990).

The sense of frustration prepared the ground for the revolution brought by ESI in which the gas-phase ionization was replaced by a liquid-phase, chemical-ionization process. The even-electron process is “softer” in terms of energy compared to EI, and produces a molecular ion without fragmentation. The
After 10−8 sec, the ion deactivates to the ground state if it does not fragment. This deactivation occurs well before the ion leaves the source, and it explains why EI is reproducible from one spectrometer to another.

Our approach requires a very simple apparatus, with a conventional EI source, but it requires nano-LC technology, and it provides typical EI spectra, such as those that are normally recorded with GC–MS. The interfacing process includes an aerosol production obtained at a nano-scale flow rate without the help of a make-up gas. It was called Direct-EI to emphasize a direct, simple connection between the column and the ion source (Cappiello et al., 2001, 2002, 2005, 2007a; Cappiello, Famiglietti, & Palma, 2003). The working mechanism is based on two distinct actions: a drastic reduction of the mobile-phase flow rate that enters the ion source, followed by a rapid vaporization of the analytes prior to ionization. The aerosol generated at low flow rate allows a faster solvent removal, and it produces smaller droplets. Thus, the vaporization of the analytes is eased by the fractionation of the solute mass into a large number of small aerosol droplets. The increase in the surface-to-mass ratio

FIGURE 1. Time sequence during EI. The energy is redistributed through a single vibration of the ion. After 10−8 sec, the ion deactivates to the ground state if it does not fragment. This deactivation occurs well before the ion leaves the source, and it explains why EI is reproducible from one spectrometer to another.
promotes solute exposure to the source heat, and limits thermal decomposition by speeding up the conversion into the gas-phase. The interface is entirely contained in the EI source (Fig. 3). The ion volume is that of a GC–MS source, with a distance between the spray tip and the opposite vaporization surface of 0.6 cm. The ion-source temperature can be increased up to 350°C for the highest-boiling compounds. The hardware consists of a spray tip, and a suitable vaporization surface incorporated into a conventional EI source. The reduced flow rate of nano-LC columns contributes to a rapid solvent evaporation and removal so that mass spectral results are not influenced by chemical interactions with residue of solvent vapors in the ion volume. If the use of nano-LC columns can be perceived by someone as a sort of limitation of this interface, then it is worthwhile to observe that today’s nano-columns can offer high-performance, and are successfully employed for all those applications where a high-sensitive LC–MS detection is required. For instance, a 200–400 nL/min flow rate, typical of a 75 μm i.d. LC column, generates a gas-phase flow comparable with that of a GC capillary column, with only a higher noise in the low-mass region of the spectrum. The noise is due to solvent molecules’ ionization and fragmentation, and it covers the mass spectrum up to m/z values corresponding to the molecular ions of each solvent component. As stated before, the mass spectrum is not influenced by solvent composition and flow rate. However, the shield effect of solvent vapors reduces electron flow and decreases ionization efficiency, so that the response is inversely proportional to the mobile-phase flow rate. An example is reported in Figure 4a, where the caffeine signal intensity versus mobile-phase composition and flow rate is reported. As clearly reported in the plots, the signal response rapidly decreases with the flow rate for any mobile-phase composition, and this behavior can be observed for any test compound. Of course, very low flow rates, such as those <100 nL/min, impose severe practical restrictions in the chromatographic process that can invalidate possible mass spectral benefits. Flow rates between 200 and 400 nL/min represent a valid compromise, and allow LODs comparable with those obtained in GC–MS with the same ion source (Cappiello et al., 2007a). The solvent choice can also influence the signal intensity, as shown in Figure 4b, where the effects of methanol–water and acetonitrile–water mixtures on caffeine response are reported. Differences in terms of surface tension and volatility of solvents can affect evaporation and aerosol quality, which are both fundamental for the interface performance. However, in general, the system is highly tolerant for different mobile-phase compositions, and no major constraint so far has been reported. A correct aerosol formation is mandatory for an accurate reproduction of the chromatographic profile. Because of the high-vacuum, high-temperature conditions of the EI source, the spray device should accomplish the following two criteria: a small orifice to increase the liquid impedance in a high-vacuum environment, and thermal insulation to contrast premature, in-tubing gas-phase conversion (thermospray effect). In Figure 5, two cases of nebulization are reported: the first one (a) shows the effect of a poor thermal insulation of the nebulizer. The mobile-phase, which evaporates before reaching the ion source, forms vapor bubbles that lead to an uneven nebulization, as observed in the related, sputtered chromatographic peak. In the other case, (b) the insulation reduces radiative heat transmission from the hot ion source to the incoming eluate, and maintains the eluate in the liquid state. Nebulization is smooth, and the related peak is visibly improved. Vaporization of solvents before spray formation compromises the correct aerosol generation, and causes in many cases, the precipitation of the solutes in the connecting capillary. Once the eluate is emitted from the spray tip in the form of aerosol droplets, the source heat promotes vaporization of the low-boiling fractions that become promptly available for EI. An example on how ion source temperature affects the signal intensities of testosterone, azinphos-ethyl, ibuprofen, and bisphenol A is reported in Figure 6. High-boiling compounds generally require a high-source temperature (>300°C), and/or the presence of a suitable vaporization surface on which the residues of unvaporized solute particles can be conveniently brought to the gas-phase, before removal with the pumping system. Different surface materials and aerosol conditions can optimize the liquid–gas phase transition. Some preliminary
results indicate that alternative materials, such as Teflon® PTFE (polytetrafluoro-ethylene), can provide superior performance for the vaporization of high-boiling compounds (Cappiello & Famigli, 1995). That research demonstrated that a suitable surface can improve sensitivity for selected analytes, compared to a stainless-steel surface. In fact, conventional EI source materials are normally tested for GC-amenable compounds already in the gas-phase, where interactions with the surface are minimal, and do not interfere with the analyte response nor with the mass spectral results. Larger, LC-amenable molecules might impact the surface while still in the solid state, so that the contact with a potentially active, hot surface can promote stronger interactions that lead to thermal decomposition or slower vaporization. On the contrary, inert materials involve weaker interactions with the analytes and can improve the response. In terms of performance, Direct-EI does not substantially differ from that typical of GC–MS. An example is given by atrazine, which can be analyzed with GC–MS and LC–MS to provide a nice and rich EI spectrum. The subtracted average spectra, and the results of a National Institute of Standards and Technology (NIST) library search for LC–EI–MS and GC–MS experiments, are reported in Figure 7a,b, respectively. The library search results of matching qualities of 91% for LC–EI–MS, and 89% for GC–MS supported the statement of high-spectral accuracy in the LC–EI–MS mode. Detection in the low-picogram level is achievable for many compounds in the SIM mode. In the full-scan mode, the response is slightly affected at low m/z by the presence of solvent residues in the ion source. Therefore, in general, to achieve an interpretable mass spectrum, nanomoles of material are required. Depending on the fragmentation and on the background chemical noise, low-mass noise rarely influences the matching quality during NIST library searching, and high-confidence identifications are normally achieved, as demonstrated by the Direct-EI–LC–MS spectrum of heptachlor in Figure 8. To increase sensitivity in practical applications, injection volumes of 0.5–1 μL can be easily performed in gradient analysis to achieve sub-ppb method LODs. Larger volumes for very dilute samples can be obtained with a sample-enrichment system.

The stability of response is an important feature of this system because compound identification can be achieved at any concentration within a range that is linear up to four orders of magnitude. Linearity was demonstrated for many substances with different chemical properties, and, for all of them, values of $r^2$ very close to 1.0 were reported (Cappiello et al., 2007a). Spectral quality is excellent even at very low concentrations, with an interpretable spectrum and a high reproducibility of the typical ion abundances. At the typical mobile-phase flow rate that, for this apparatus, does not generally exceed 500 nL/min, chemical ionization interferences, in the form of (M+H)$^+$ ions, were never observed, so that accurate isotopic intensities can be recorded and collected for molecular formula confirmation. The extreme simplicity of the interfacing mechanism provides a stable response within a working day, and for longer periods, without any further optimization or tuning procedures. One hundred seventy four injections of caffeine performed within 6 days, without any instrument modification, gave a standard deviation for the peak areas $\leq \pm 15\%$. The versatility of the interface can be evaluated through its capability to detect compounds with different polarities, stability, and structure types. This aspect is demonstrated in Figure 9 that summarizes the response of 101 compounds within a wide range of molecular weight and polarity. The polarity is expressed as octanol/water partition coefficient (Kow), which is defined as the ratio of the concentrations of a compound in octanol and in water in a two-phase octanol/water system. As shown in the figure, a Direct-EI–LC–MS interface can be used from totally non-polar compounds, such as n-alkanes, to high-polarity species, such as sugars. It is worthwhile to point out that all the compounds analyzed generated interpretable, library-matchable mass spectra. If compared to other API techniques, then Direct-EI offers a wider range of LC–MS detection for small-medium molecules. Another important aspect offered by the gas-phase ionization is that it is not prone to ME that extensively limit accuracy in ESI. The absence of ME improves the precision and the accuracy of the Direct-EI interface, factors which are needed in quantitative applications. A more-extensive discussion on this aspect is presented in the following chapter.
IV. EVALUATION OF MATRIX EFFECTS

The high-selectivity and sensitivity of modern MSMS have led to the development of high-throughput methods that entail little or no sample preparation, and simple chromatographic separations (Reemtsma, 2003; Prakash, Shaffer, & Nedderman, 2007; Xu et al., 2007). Thanks to these important aspects, LC–ESI–MSMS has become a well-established, standard analytical tool in different areas in which trace amounts of analytes in complex mixtures must be detected, characterized, and quantified. However, in the past few years the common perception that the high-selectivity of MSMS can be used to reduce sample-preparation procedures, and to guarantee against the effects of co-eluted sample impurities has been seriously challenged. In fact, many authors report that the presence of the co-extracted matrix can severely affect the quantification procedures based on ESI and APCI LC–MS methods. This phenomenon, called ME, is considered to be an unexpected either suppression or enhancement of the analyte response, induced by co-eluting components of the matrix. ME can heavily affect the reproducibility, linearity, and accuracy of the method (Reemtsma, 2003; Mallet et al., 2004; Antignac et al., 2005; Taylor, 2005). The relevance of ME as a major threat in a successful quantitative analysis in LC–ESI–MS has been widely recognized. ME are the result of several interactions of different strengths that might interfere during the liquid-phase, chemical ionization of ESI and APCI. ME represent a challenging problem for the user because they are compound-, matrix-, method-, and instrument-specific, and, in many cases, also sample- and lot-specific; thus, they affect also precision, and they are particularly difficult to eliminate (Bonfiglio et al., 1999; Matuszewsky, Constanzer, & Chavez-Eng, 2003). The several operational strategies that have been suggested to minimize ME generally include extensive clean-up procedures, more-efficient chromatographic separations and, more important, the use of stable isotope-labeled-internal standards (SIL-IS) (Antignac et al., 2005; Taylor, 2005; Niessen, Manini, & Andreoli, 2006; Van Eeckhaut et al., 2009). These strategies could help to reduce and control the extent of these effects, but they are laborious, time-consuming, and expensive. Nevertheless, it is worthwhile to notice that SIL-ISs are not always available; therefore, method development is particularly challenging.

The Direct-EI interface, thanks to the use of a gas-phase ionization, can overcome most of the ME observed with ESI (Cappiello et al., 2008). In fact, co-eluted compounds are simultaneously vaporized and subsequently ionized by a multitude of independent, single molecule–electron interactions. The positive radical ions generated from these events will remain isolated during the fragmentation processes. Single-ion signals will be dependent only on the mass spectrum and on the concentration of each component without any mutual, adverse interactions. To demonstrate this hypothesis, ME was assessed with Direct-EI and ESI–MSMS with pesticides and pharmaceutical target compounds on matrices of biological and environmental interest (human plasma, river and sea water) (Cappiello et al., 2007a,b, 2008; Famiglini et al., 2008). The analytes were extracted from the matrix with two different extraction methods; namely, liquid–liquid extraction (LLE), and solid-phase extraction (SPE). ME were assessed with post-column infusion and post-extraction addition experiments (Bonfiglio et al., 1999; Matuszewsky, Constanzer, & Chavez-Eng, 2003). The results obtained on the investigated drugs and pesticides demonstrated that Direct-EI–MS allows the analysis of the target molecules regardless of the presence of co-eluted interferences; conversely, ME was always observed for the same compounds with LC–ESI–MS, as illustrated in Table 1. The absolute ME was calculated in percent as the ratio between the average peak area of the sample spiked after extraction (n = 3), and the average peak area of the neat standard solution (n = 3), multiplied by 100. A value >100% signifies ionization enhancement, whereas a value <100% ionization suppression. As it can be clearly seen in the table, only in two cases ESI–MSMS response was not affected by ME. All the other results were affected by either signal enhancement or suppression. On the contrary, the data obtained with Direct-EI–MS analysis were not affected by ME, regardless of the matrix and the extraction method.

Direct-EI is very tolerant also to the so called “exogenous suppressors;” namely, different mobile-phase solvents and modifiers (Antignac et al., 2005). Different mobile-phase mixtures can change the aerosol properties; nevertheless, the Direct-EI interface is very tolerant to the use of any kind of buffer or modifier that is needed to improve the chromatographic separation. Different pH modifiers are routinely used to separate...
basic and acidic substances. It has been also demonstrated that even non-volatile buffers can be safely used without jeopardizing the source performance or the mass spectral quality; as briefly summarized in Table 2 (Cappiello et al., 2007a). A mobile-phase composed of a 1:1 v/v mixture of two buffers (A, H2O + 10 mM KH2PO4; B, 30% A-70% CH3CN) was continuously introduced into the system for 6 days (24 hr/day). A 10 ng/μL standard solution of caffeine was injected daily in the flow-injection analysis (FIA) mode. Mass spectra acquisition was carried out in the full scan and selected-ion monitoring (SIM) mode. The recorded peak areas in SIM, and the probability values from the NIST library comparison in scan, are reported in Table 2. The results clearly indicate that the buffer does not interfere with the interface performance. At low flow rates, the absolute amount of

□ testosterone; ▲ azinphos-ethyl; ◦ ibuprofen; ● bisphenol A

Area Counts
X 10^5

FIGURE 5. Effect of the capillary thermal insulation on the spray formation, and on the peak profile. a: unprotected nebulizer and related chromatographic peak, (b) insulated nebulizer and related chromatographic peak.

FIGURE 6. Relation between signal intensity and ion-source temperature.
FIGURE 7. a: Experimental LC–MS spectrum of 100 pg of atrazine recorded in FIA at a flow rate of 300 nL/min. Mobile-phase was water/acetonitrile (50:50, v/v). NIST library search is reported on the bottom of the figure. b: Experimental GC–MS spectrum of 100 pg of atrazine. NIST library search is reported on the bottom of the figure.
salts introduced into the system is extremely low, with a deposition rate of approximately 0.1 μg/min at 300 nL/min. The system was tested extensively with a 10 mM phosphate buffer solution introduced for five working days, and several parameters were continuously monitored for 40 hr of total acquisition time (Cappiello et al., 2005). Several critical parameters indicative of poor instrument performance, including the repeller potential, were never influenced by the long-term

![Heptachlor Mass Spectrum](image)

**FIGURE 8.** The Direct-EI mass spectrum of heptachlor in a tetrahydrofuran solution (6 mg/L) is shown in the upper trace, and is compared to the standard NIST EI library spectrum shown in the lower trace (matching factor 748, reversed matching factor 778, probability factor 95.8%).

ICA

![Diagram of Direct-EI–LC–MS Applications](image)

**FIGURE 9.** Schematic representation of different Direct-EI–LC–MS applications. The detected compounds are positioned on the basis of their polarity and molecular weight. The polarity is expressed as the octanol/water partition coefficient (K<sub>ow</sub>) Compound classes span from low-polarity hydrocarbons on the far right to high-polarity carbohydrate to the far left.
buffer intake; signal intensity and mass spectral quality were not affected. The last two factors are reported in Table 2. The buffer introduced into the system was hardly visible as a fine, even dispersion of white powder inside the ion source. This residue was mixed with other brownish, unvaporized material, and it was easily removed during a normal, scheduled cleaning procedure.

V. APPLICATIONS

The Direct-EI–LC–MS interface is particularly indicated in several small-molecule applications, which can benefit from an enhanced identification capability, and the absence of ME. An interesting topic regards the detection of compounds that give a poor or no signal with LC–API–MS. An example is the analysis of organochlorine pesticides (OCPs) in water samples. The contamination of water resources by OCPs is considered an analytical challenge, because these persistent and non-biodegradable pollutants are not amenable to LC–API–MS. This constraint is significant in the multiresidue analysis of real-world samples, when high-polarity, poorly volatile compounds are present as well (Famiglini et al., 2009). GC–MS provides an excellent response for OCPs, but it falls short when complex multiresidue analyses are required. For this reason, multi-step GC–MS and LC–API–MS methods are used together when multi-component methods (MCMs) that include trace analysis of OCPs and polar pesticides must be developed. In a recent publication, a Direct-EI–LC–MS method for the determination of 12 OCPs in river and sea-water samples was used (Famiglini et al., 2008). To date, it is the only LC–MS method for the

<p>| TABLE 1. Post-extraction addition ME evaluation on different matrices with ESI–MSMS and Direct-EI–LC–MS |
|---------------------------------------------------------|---------------------------------|---------------------------------|---------------------------------|</p>
<table>
<thead>
<tr>
<th><strong>Compound</strong></th>
<th><strong>Summary of the experimental conditions</strong></th>
<th><strong>ME ± RSD (%)</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Matrix</strong></td>
<td><strong>Extraction Method</strong></td>
<td><strong>ESI-MSMS</strong></td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>Human plasma</td>
<td>LL</td>
</tr>
<tr>
<td></td>
<td>Human plasma</td>
<td>SPE</td>
</tr>
<tr>
<td>Phenacetin</td>
<td>Human plasma</td>
<td>LL</td>
</tr>
<tr>
<td></td>
<td>Human plasma</td>
<td>SPE</td>
</tr>
<tr>
<td>Aldicarb</td>
<td>Fulvic acid artificial matrix</td>
<td>injected without extraction</td>
</tr>
<tr>
<td></td>
<td>River Water</td>
<td>SPE</td>
</tr>
<tr>
<td>Atrazine</td>
<td>Artificial matrix</td>
<td>injected without extraction</td>
</tr>
<tr>
<td></td>
<td>River Water</td>
<td>SPE</td>
</tr>
<tr>
<td>Methomyl</td>
<td>Artificial matrix</td>
<td>injected without extraction</td>
</tr>
<tr>
<td></td>
<td>River Water</td>
<td>SPE</td>
</tr>
<tr>
<td>Propazine</td>
<td>Artificial matrix</td>
<td>injected without extraction</td>
</tr>
<tr>
<td></td>
<td>River Water</td>
<td>SPE</td>
</tr>
</tbody>
</table>

| TABLE 2. Evaluation of the response of caffeine after the continuous introduction of phosphate buffer |
|----------------------------------------------------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| **Caffeine** | **Day 1** | **Day 2** | **Day 3** | **Day 4** | **Day 5** | **Day 6** | **Day 7** | **Mean** | **RSD%** |
| | **Morning** | **Afternoon** | **Morning** | **Afternoon** | **Morning** | **Afternoon** | **Morning** | **Afternoon** | **Morning** | **Afternoon** | **Mean** | **RSD%** |
| SIM (peak area count) | 410·10^3 | 460·10^3 | 422·10^3 | 427·10^3 | 477·10^3 | 439·10^3 | 468·10^3 | 495·10^3 | 470·10^3 | 450·10^3 | 6.6 |
| Scan (NIST probability value %) | 94.4 | 94.7 | 94.6 | 96.0 | 94.2 | 95.3 | 94.2 | 95.3 | 94.7 | 94.8 | 0.63 |
The selected pesticides at environmentally relevant concentrations were analyzed in river-water samples. The method was based on an off-line SPE and nano-flow injection analysis-Direct-EI (nano-FIA–Direct-EI–LC–MS).

Another topic that can benefit from the Direct-EI–LC–MS interface is the analysis of complex mixtures that contain a large number of compounds with a wide range of different physico-chemical properties. EI can offer a shortcut, a do-it-all solution when hard-to-detect substances are included, or when positive- and negative-ion modes analyses are required for a complete detection. This advantage can be exploited in the development of multi-component methods (MCMs); for instance, to environmental pollutants. In fact, all the published approaches to monitor a large number of environment-relevant analytes with LC–MS show some limitations because they cannot address the simultaneous characterization of polar and non-polar compounds (Alder et al., 2006; Niessen, Manini, & Andreoli, 2006; Famiglini et al., 2008). In a recent publication, a method to determine 29 endocrine-disrupting compounds (EDCs) in marine-water samples collected along the middle-western Adriatic Coast of Italy was presented (Famiglini et al., 2005). The target analytes belonged to the class of phenols, steroids, polycyclic aromatic hydrocarbons (PAHs), organochlorines, carbamates, triazines, phthalates, and alkaloids. The samples were SPE-treated and were analyzed with LC–Direct-EI–MS. High-quality EI spectra were collected for all the analytes, and a sensitive detection was obtained in a single chromatographic analysis. This advantage represents a step forward compared to ESI–MS methods, where, in the presence of a similar complex mixture, a double detection in the negative- and positive-ion modes is required (Benijts, Lamber, & De Leenheer, 2004). In addition, the chromatographic separation was performed with a mobile-phase without modifiers, which are commonly used in the LC–ESI–MS protocols. The Direct-EI capability to detect compounds with quite different polarities was further demonstrated in another environmental application: the simultaneous trace-level detection of OCs and phenoxy acid pesticides. Because of their high-polarity and low-volatility, phenoxy acid pesticides require derivatization prior to GC analysis; thus, LC–ESI–MS is the method of choice for the detection and quantification of these compounds in water (Famiglini et al., 2009). Phenoxy acid pesticides can be easily detected with Direct-EI–LC–MS, and a micro-particle beam interface, as demonstrated in publications (Cappiello, Famiglini, & Bruner, 1994; Cappiello & Famiglini, 1995). Based on those results, the EI response of seven phenoxy acids was carefully investigated using the Direct-EI interface. High-quality EI spectra were obtained that allowed the development of the first single-step method for the simultaneous analysis of phenoxy acids (LC-amenable compounds) and OCs (GC-amenable compounds). The approach was successfully applied to the analysis of 19 pesticides in river-water samples. The method LODs that span from 0.002 to 0.052 µg/L allowed the detection of the selected pesticides at environmentally relevant concentrations. The results of this work demonstrated the LC–Direct-EI–MS capability to identify compounds with quite different polarities and thermal stabilities in a single-step of analysis. This achievement represents a significant step forward in the development of a universal multi-residue screening programs, aimed at the rapid and sensitive identification of the main classes of pesticides in different water compartments (marine- and river-water).

Other research fields, such as lipidomics and metabolomics, can benefit from the use of Direct-EI. Preliminary results demonstrated that LC–Direct-EI–MS can be applied to the simultaneous profile non-esterified free fatty acids (NEFA) in biological matrices. The characterization of NEFA is of major interest in metabolomics, because of their prominent role in metabolic pathways. The current widespread methods to profile individual NEFA in complex samples (e.g., plasma, tissues) employ GC–MS after extraction, and derivatization of the lipid material. Although the GC–MS protocols provide satisfactory sensitivity and precision, the complex sample-preparation procedures make the determination of NEFA a time-consuming process. In the last few years, LC–API–MS has gained increased importance in the investigation of lipid metabolism. However, most of the current LC–API–MS methods do not represent a valid alternative to GC–MS (Johnson, 2005). In fact, derivatization is often required during sample preparation, and/or complex post-column pH adjustment after chromatography. In addition, quantitative results can be compromised by ion-suppression phenomena. The use of LC–EI–MS in the analysis of NEFA can offer several advantages in comparison with current GC–MS and LC–API–MS methods: the sample preparation procedure can be carried out without derivatization to reduce the analysis time; reproducible and high-quality NIST library-matchable EI spectra can be recorded to allow the accurate identification of the target compounds; the response is not influenced by ME. On this basis, LC–Direct-EI–MS was applied to analyze several saturated and unsaturated NEFA in plasma (Palma et al., 2010). The results are summarized in Figure 10, where the TIC chromatogram in scan mode of a NEFA standard mixture is reported. NEFA were analyzed in their intact form, without derivatization, and NIST probability factors higher than 80% were obtained during a library search report. These experiments demonstrated that lipidomics can represent a new research field in which the Direct-EI–LC–MS interface can play an important role.

VI. CONCLUSIONS

The Direct-EI–LC–MS interface mechanism was first presented in 2002. Since then, and especially in the last few years, it has been the object of constant technical developments that allowed new and challenging applications. The Direct-EI is now a modern approach for an almost universal detection of small molecules delivered through an LC separation. It is a robust and straightforward system that does not require any costly and complicated instrumentation. Several performance details, such as the absence of ME, and the tolerance for salts and other non-volatile buffers, are now well-recognized and readily available for method development. The number of detectable molecules has increased constantly, and include environmental, pharmaceutical, and biological applications. An improved interfacing process responsible for the detection of larger molecules, and for a wider range of compounds of different polarity makes the
Direct-EI one of the most flexible LC–MS interfaces. This factor is particularly evident when compared to ESI or APCI; each one characterized by a well-defined polarity window. Of course, the evolution of the Direct-EI–LC–MS interface continues in different directions, including sensitivity, ease-of-use, and new applications.

VII. NOMENCLATURE

APCI atmospheric-pressure chemical ionization
API atmospheric-pressure ionization
CID collision-induced dissociation
EDC endocrine-disrupting compound
EI electron ionization
ESI electrospray ionization
FIA flow-injection analysis
GC gas chromatography
IS internal standard
Kow partition-coefficient octanol/water
LC–API–MS liquid chromatography–atmospheric pressure ionization–mass spectrometry
LC–MS liquid chromatography–mass spectrometry
LC–MSMS liquid chromatography–tandem mass spectrometry
LC–UV liquid chromatography–ultraviolet detection
LLE liquid–liquid extraction
LOD limit of detection
LOQ limit of quantitation
LP liquid phase
ME matrix effects
MCM multi-component method
MSMS tandem mass spectrometry
NIST National Institute of Standards and Technology
NEFA non-esterified free fatty acids
OCP organochlorine pesticide
PAH polycyclic aromatic hydrocarbon
PB particle beam
PPB part-per-billion
SIL–IS stable isotope–labeled internal standard
SIM selected-ion monitoring
SMB supersonic molecular beam
SPE solid-phase extraction
SRM selected-reaction monitoring
Teflon® PTFE polytetrafluoro-ethylene
TFA trifluoroacetic acid
TIC total ion current

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