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# Atmospheric Pressure Photoionization. 1. General Properties for LC/MS

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**In this work, we describe the performance of an atmospheric pressure photoionization (APPI) source for sampling liquid flows. The results presented here primarily focus on the mechanism of direct photoionization (PI), as compared to the dopant mechanism of PI. Measured detection limits for direct APPI were comparable to atmospheric pressure chemical ionization (APCI; e.g., 1 pg for reserpine). The ion signal is linear up to 10 ng injected quantity, with a useful dynamic range exceeding 100 ng. Evidence is presented indicating that APPI achieves significantly better sensitivity than APCI at flow rates below 200 μL/min, making it a useful source for capillary liquid chromatography and capillary electrophoresis. Results are presented indicating that APPI is less susceptible to ion suppression and salt buffer effects than APCI and electrospray ionization (ESI). The principal benefit of APPI, as compared to other ionization sources, is in efficiently ionizing broad classes of nonpolar compounds. Thus, APPI is an important complement to ESI and APCI by expanding the range and classes of compounds that can be analyzed. In this paper, we also discuss the role of direct APPI vs PI-induced APCI using dopants.**

LC/MS is one of the fastest growing segments of analytical instrumentation, and this growth is attributable almost solely to new applications in life sciences and biopharmaceuticals. An ionization source that can ionize a large variety of compounds and that can handle a wide range of LC conditions, such as modifiers and flow rates, would be highly desirable. Electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) are powerful methods within their limits of operation. APCI and ESI sources are inefficient at ionizing nonpolar compounds and are susceptible to ion suppression due to competition for charge from other analytes or additives. This is particularly problematic for ESI. APCI is a more predictable and robust ion source than ESI for small compounds that are thermally stabile and volatile. Positive mode APCI forms almost exclusively $[M + H]^+$ ions, whereas ESI produces not only protonated ions but also cation-adducted ions, such as sodium and potassium. APCI is more compatible with high HPLC flow rates than ESI. APCI is preferred for high flow rate, low-molecular-weight compound analysis. ESI sensitivity is greatest at low flow rates but can suffer from unreliable detection efficiency for small drug compounds.

Photoionization (PI) is an alternative ionization source that is showing evidence of being relatively nondiscriminate of nonpolar compounds and reasonably tolerant of matrix additives that can interfere with the mechanism of ESI and APCI. In this paper, we deal with atmospheric pressure photoionization (APPI)[1−3] for LC/MS applications, as contrasted to the method of low-pressure photoionization (LPPI)[2−4] that has also recently been implemented to mass spectrometry. APPI is a rapidly growing ionization method, and review articles are now appearing that describe these recent developments.[5,6] The results presented here focus on the direct mechanism of APPI, which is desirable for minimizing ion−molecule chemistry that may compete for charge and deplete the abundance of desired analyte ions. Bruins and co-workers have advanced an APPI method that is optimized for use with dopants to enhance signal level.[1] In this paper, we compare the two methods but emphasize the properties of direct photoionization. Both methods are growing rapidly in use, and studies are being reported to better understand APPI ionization efficiency, such as the work by Kostiainen and co-workers on the dependence on solvent and eluent conditions.[7,8] APPI is being reported to have sufficiently advantageous properties in key performance areas to emerge as a preferred method for many applications, such as detection of drug samples in biological matrixes[9,10] and characterization of hydrophobic peptides.[11] APPI is also being incorporated into multiple ion sources, such as APPI/APCI and APPI/ESI, which is expected to lead to interesting new applications.[12]

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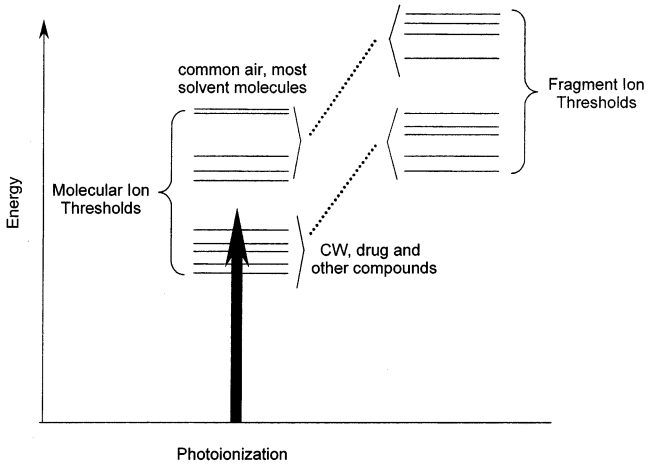


Figure 1. Schematic energy diagram showing the general property of threshold photoionization to ionize larger molecules of interest, while minimizing ionization of small molecules, such as air and many solvents and minimizing ion fragmentation.

Photoionization has a long history of use in mass spectrometry experiments, though mostly for research purposes and not for sensitive analytical applications. Pulsed lasers have been used for nonresonant multiphoton ionization (MPI),[13] resonance-enhanced MPI using tunable wavelengths,[14] and single-photon ionization using sum frequency generation in nonlinear media (usually gas cells).[15] Nonlaser sources of photoionization include discharge lamps and synchrotron radiation.[16] The former sources were not adaptable to high-sensitivity analytical applications because of low spectral brightness in the former case and large "facility-size" in the latter case. Meanwhile, photoionization has been used for GC detection[17] and as a source for ion mobility spectrometry[18] for many years, suggesting the potential for use in mass spectrometry. However, only in the past few years has a reliable, compact PI source been interfaced to MS for analytical purposes.

Figure 1 illustrates the principle of PIMS. The mechanism of PI is absorption of a photon by molecule M, followed by ejection of an electron to form the molecular radical ion $M^{\bullet+}$ (henceforth denoted $M^+$). This condition is met if the irradiating photon energy exceeds the ionization potential (IP) of molecule M. This is generally (and almost universally) true for larger analyte molecules. Small molecules, such as all major constituents of air and most common solvents (e.g., $H_2O$, $CH_3OH$, $CH_3CN$, halogenated solvents, etc.) have higher IPs that exceed the energy of the standard PI photon energy used. Because molecular ions $M^+$ are radicals having an unpaired electron, they have a propensity to undergo further reactions in collisional environments, particularly at atmospheric pressure. These reactions are usually innocuous, such as the abstraction of a hydrogen atom to form $MH^+$; such an event changes the appearance of the mass spectrum but not the total abundance of analyte ions. However, parasitic reactions may also occur that can remove the charge from $M^+$ by proton transfer or electron attachment.

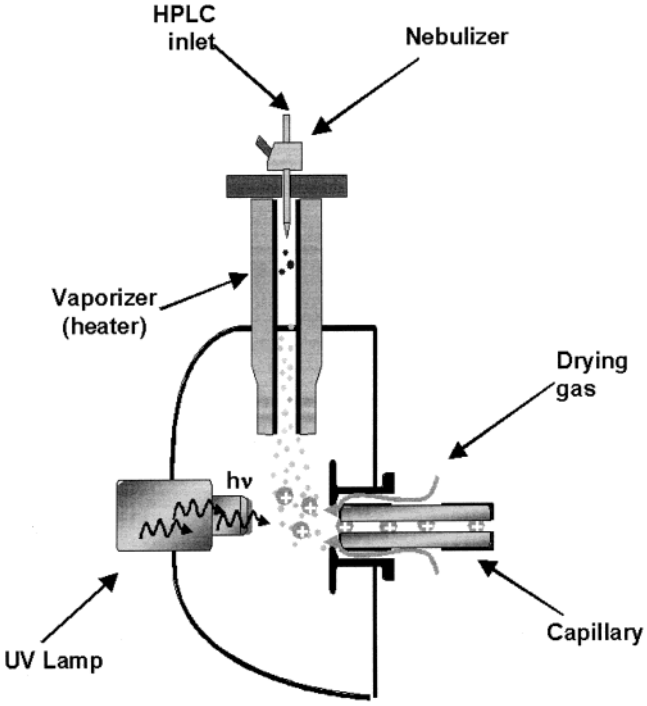


Figure 2. APPI source configuration employing a VUV light source and orthogonal spray geometry.

The PI light source uses a high-output gas discharge tube with a $MgF_2$ window for transmission of vacuum ultraviolet light into the ionization region. The fill gas is generally a rare gas, and the choice of gases, such as Kr, depends on the wavelength range of interest. Narrow-band vacuum ultraviolet (VUV) energy is available from ~8 to 12 eV, with 10 eV being the preferred energy, because most analytes of interest are ionized by it but common solvents and permanent gases are not.

## EXPERIMENTAL SECTION

All experiments were performed using an Agilent 1100 Series LC/MS system consisting of a binary pump, vacuum degasser, autosampler, thermostated column compartment, and diode-array detector, with either an LC/MSD trap SL or LC/MSD SL mass spectrometer. A quaternary pump was used for the addition of dopant, and the dopant stream was added postcolumn via a low volume tee (Part No. 0100-0969). Complete system control and data evaluation were carried out using Agilent LC/MSD software. Experimental conditions are detailed in the figure captions and text to follow.

A PhotoMate orthogonal APPI spray source (Syagen Technology) was installed on the mass spectrometers. The configuration of the APPI source relative to the vaporizer and the mass spectrometer inlet is illustrated in Figure 2. The APPI source is based on a radio frequency (RF) discharge of a gas mixture consisting primarily of Kr and operates on the atomic emission lines at 10.0 and 10.6 eV. The gas composition and pressure were

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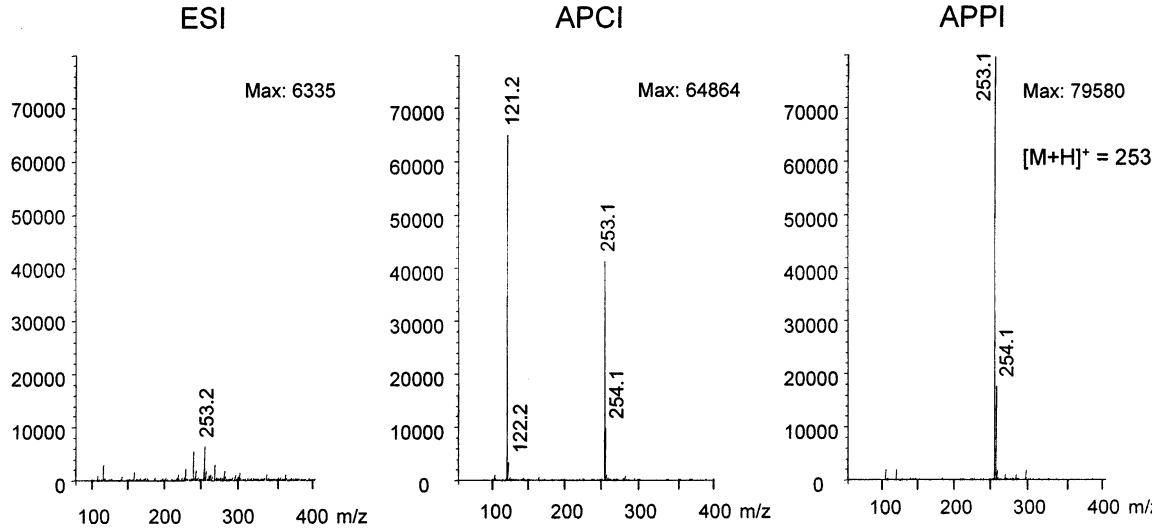


Figure 3. Comparison of ESI, APCI, and APPI for benzo[*a*]pyrene showing improved sensitivity for nonpolar compounds by APPI. LC conditions: flow injection analysis, flow rate 0.4 mL/min, 50% water, 50% THF. APPI source conditions: $V_{cap}$ 1500, drying gas temperature 350 °C at 5 L/min, vaporizer temperature 250 °C, nebulizer pressure 60 psig. APCI source conditions: $V_{cap}$ 4000, corona current 4 $\mu$A, drying gas temperature 350 °C at 5 L/min, vaporizer temperature 450 °C, nebulizer pressure 60 psig. ESI source conditions: $V_{cap}$ 4000, drying gas temperature 350 °C at 13 L/min, nebulizer pressure 25 psig.

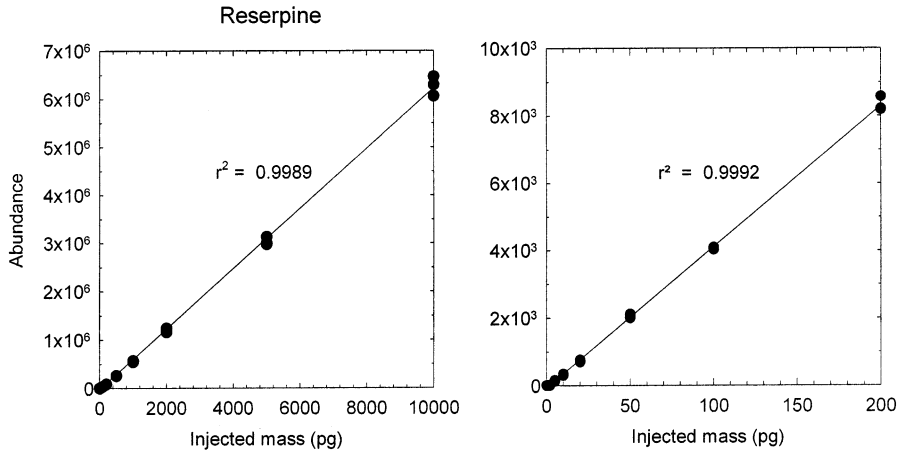


Figure 4. APPI linearity measurements. Flow rate 0.2 mL/min; mobile phase 25% water, 75% methanol, and 0.1% formic acid; column Zorbax SB-C18 rapid resolution cartridge 2.1 × 30 mm, 3.5 $\mu$m, injection size 1 $\mu$L.

optimized for maximum radiant output. The RF driver and coil were designed and optimized for maximum and most efficient coupling of power into the plasma.

Unless otherwise noted, all results in this paper were obtained by direct APPI and not by dopant APPI.

## RESULTS

APPI has shown a propensity to ionize nonpolar compounds relative to ESI and APCI. Figure 3 compares the APPI/MS spectra of benzo[*a*]pyrene by ESI, APCI, and APPI. The strongest signal is observed for APPI and is predominantly $MH^+$. APCI also gives a strong $MH^+$, whereas ESI is ineffective at ionizing the compound under these conditions.

**Sensitivity, Linearity, and Repeatability.** To be useful as a quantitation tool, an ion source must demonstrate high sensitivity, a large linear dynamic range, and give stable, repeatable measurements. Figure 4 shows a linearity plot for injections of a solution of reserpine onto a LC column. The 3$\sigma$ measured detection limit (MDL) was 1.2 pg in this case. A similar MDL was measured for reserpine using the Agilent APCI source. The linearity of the APPI source is excellent over the nearly 4 decades measured (up to 10 ng), as judged by the $R^2$ value of 0.999. On the basis of the results of triplicate measurements over an injected mass range of 1 pg to 10 ng, APPI also gives similar reproducibility to APCI. The quantitatable dynamic range measured for reserpine was ~1 pg to over 100 ng. The dynamic range of APPI appears to be significantly greater than that of ESI. This latter statement is also consistent with results for dopant-APPI.[19]

The APPI source exhibits relatively long-term stability. Figure 5 represents 600 measurements recorded at a rate of one injection every 1.5 min (40 injections/h for 15 h) for a 100-pg on-column injection. The results for each hour are overlayed to reveal any long-term drifts (top plot). There was no apparent change in signal level over the 15-h period of time. The RSD for the entire run was 2.9%. Individual LC traces are shown for the first hour of injections (bottom plot).

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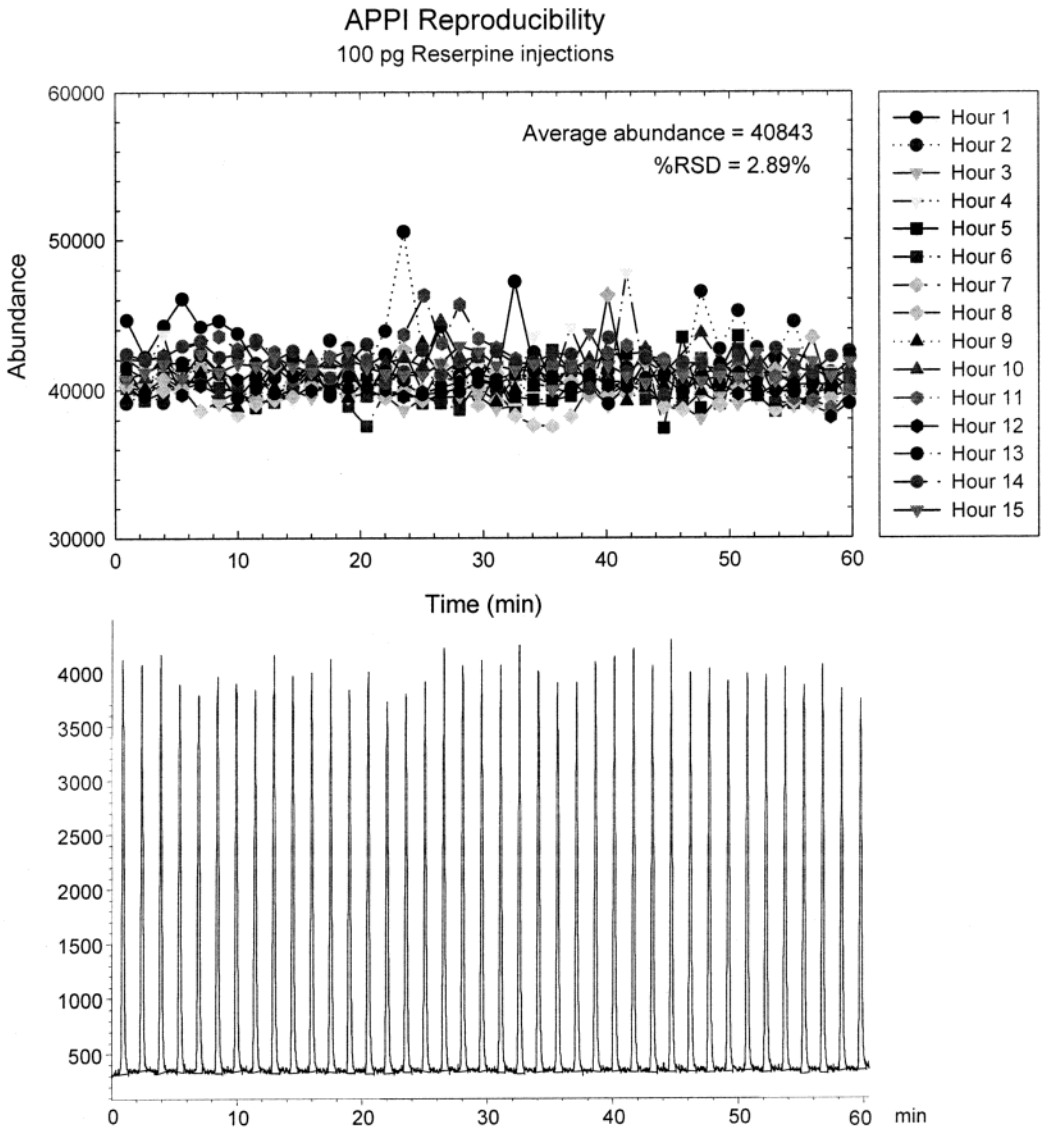


Figure 5. Recorded using APPI source on an 1100 LC/MSD. Concentration was 100 ng/mL reserpine, injection size was 1 $\mu$L, 100 pg reserpine on-column, 40 injections/hr, 15 h total run. LC conditions: Zorbax SB-C18, 2.1 $\times$ 30 mm, 3.5 $\mu$m, flow rate 0.4 mL/min, column temperature ambient. Solvent composition: 5 mM ammonium formate in 25% water and 75% methanol. APPI source conditions: $V_{cap}$ 1700, drying gas temperature 350 °C at 5 L/min, vaporizer temperature 250 °C, nebulizer pressure 60 psig, SIM ion 609.3.

**Signal Dependence on Flow Rate.** Sensitivity was also measured as a function of flow rate for a variety of analyte compounds, as shown in Figure 6. These measurements were made by varying the flow rate by continuous infusion to the ionizers. There are two notable results here: (1) the APPI source does considerably better than the APCI source at low flow rates (below 50 $\mu$L/min), and (2) it provides strong signal at higher flow rates (up to 1 mL/min), though it is not as sensitive as APCI. The rollover in signal intensity for the APPI source at higher flow rates is believed to be due to absorption of photons by solvent in the increasingly concentrated solvent vapor in the ion source. Below this region, the APPI signal is linear with the flow rate, which in turn is linear with the consumed analyte mass. This indicates that the direct PI process is first-order in analyte mass (i.e., a mass detector) and zero-order in solvent mass. This result is evidence that solvent is not involved in the PI process. APCI, on the other hand, is based on solvent as the charge carrier, and therefore, ion abundance is dependent on both solvent and analyte density. This explains the greater than linear falloff in the APCI signal with decreasing continuous infusion flow rate.

Flow-rate-dependent measurements were also made by varying the LC column diameter and scaling the flow rate to the cross-sectional area in order to maintain a constant flow velocity through the column. The results show the general trends described in the above experiment. At high flow rates (4.6-mm-diam column, 1 mL/min flow rate), the ion signal by APCI exceeds that by APPI, whereas at lower flow rates, the reverse trend is observed.

**Ion Suppression Tests.** In this section, we present results that test the response of APPI to conditions that typically lead to ion suppression and chemical noise by APCI and ESI. These latter ionization processes are based on their affinity for acquiring a charged particle, such as a proton. Common problems with APCI and ESI include:

- Ionization efficiencies are very sensitive to charge affinity, and certain classes of compounds are not detectable (some steroids, agrochemicals, nonpolar protecting groups, etc.).

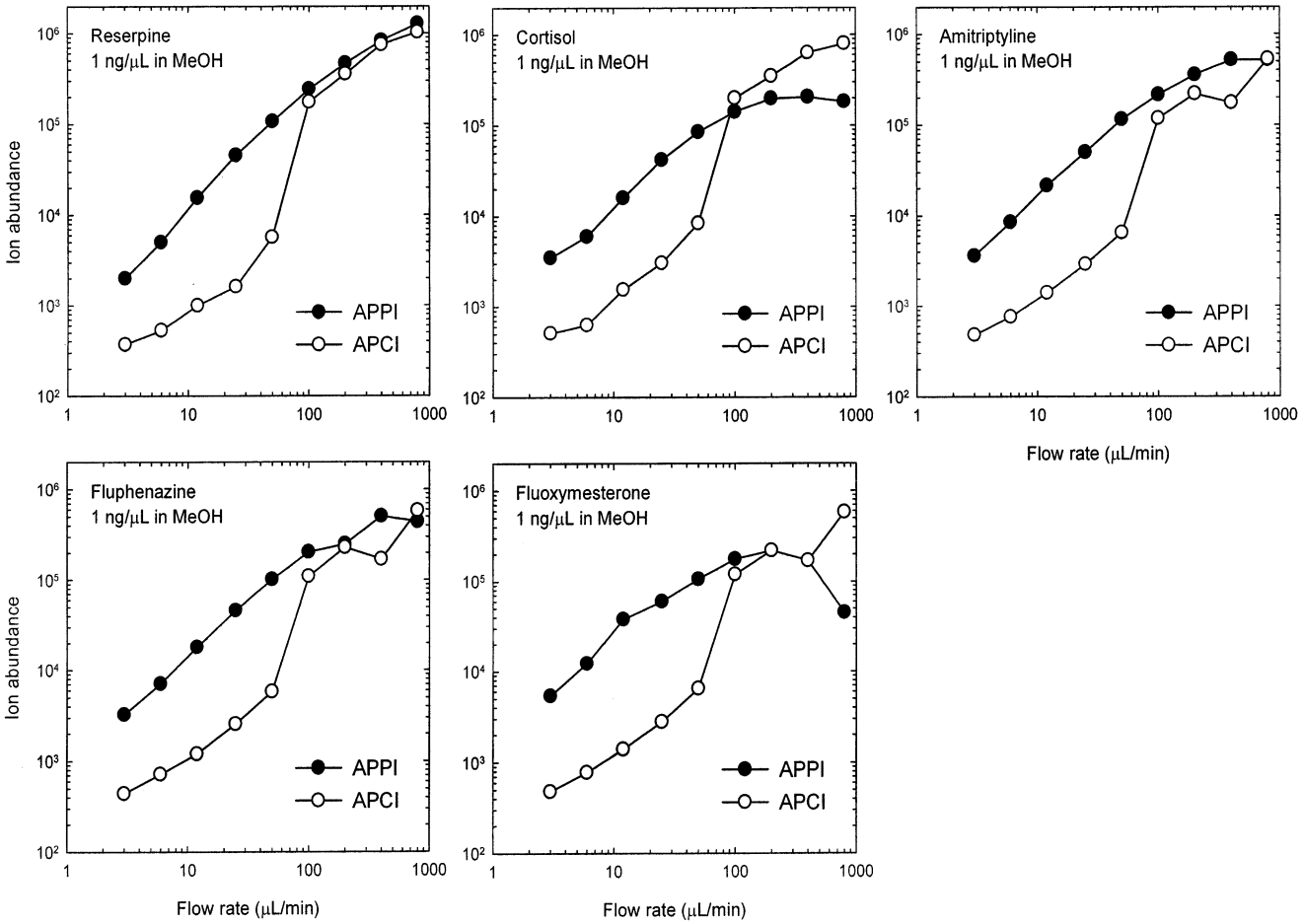


Figure 6. APPI versus APCI sensitivity as a function of flow rate. Sample introduction was by continuous infusion in methanol solvent.

• Target analyte ions can be suppressed by compounds that have higher charge affinities than the target compounds. In actuality, the target compounds are not suppressed, but rather, never get ionized due to competition for charge from the charge carriers.

• Adducts (e.g., $Na^+$) form readily with compounds during ESI, and charge-bearing salt complexes can contribute to chemical background.

APPI holds the potential to overcome or at least minimize some of these problems. Some indication of reduced susceptibility for APPI has been reported for detection of specific compounds. Yang and Henion compared dopant-APPI to APCI for quantitative analysis of idoxifene and some of its metabolites and observed that APPI tended to be less susceptible to ion suppression and chemical noise, as compared to APCI, particularly at lower flow rates.[20] Hsieh and co-workers also reported a minimal matrix ionization suppression effect for two test compounds by APPI, though a direct comparison to APCI or ESI was not made.[21]

A test of ion suppression was performed by introducing a continuous flow of a standard solution of fluphenazine (10 $\mu$g/mL in MeOH) after the HPLC column and noting the change in ion signal level due to LC elution of components from an injection of rat plasma. Figure 7 shows the total ion chromatogram (TIC)

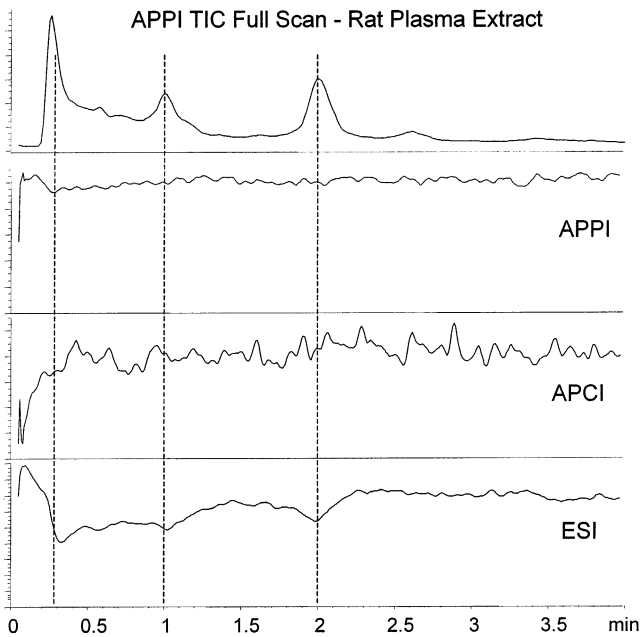


Figure 7. Determination of ion suppression susceptibility for APPI, APCI, and ESI by postcolumn addition and detection of fluphenazine while running an LC/MS chromatogram of rat plasma. Conditions were 2.5-$\mu$L injections of 1 ng/$\mu$L rat plasma; solvent composition 25% water, 75% methanol and 0.1% formic acid; and flow rate of 0.4 mL/min, 10 ng/$\mu$L fluphenazine infused postcolumn at 0.02 mL/min. Zorbax SB-C18, 2.1 × 30 mm, 3.5 mm, full scan *m/z* 100−650.

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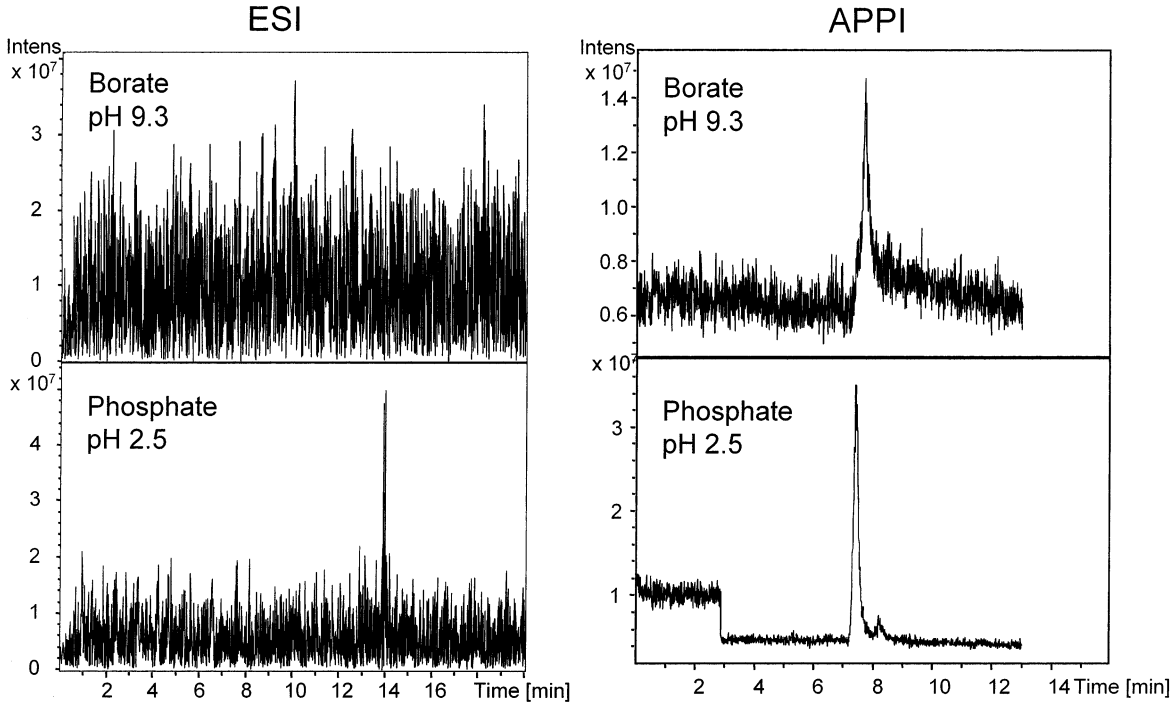


Figure 8. Comparison of ion abundance and signal-to-noise ratio for CE/MS analysis of labetalol, salbutamo, and terbutaline. Measurements were conducted for borate and phosphate buffer conditions for ESI and APPI detection. CE conditions: voltage 30 kV, capillary i.d. 50 $\mu$m × 300 mm, capillary temperature 25 °C; CZE buffer was 50 mM borate pH 9.3 or 50 mM phosphate pH 2.5; sheath flow 50 $\mu$L/min of 1% acetic acid, 49% water and 50% methanol. MS source conditions: $V_{cap}$ 1500, drying gas temperature 150 °C at 5 L/min, vaporizer temperature 275 °C, nebulizer pressure 60 psig.

for the total flow by APPI (top trace) and selected ion monitoring (SIM) chromatograms for the $[M + H]^+$ signal of fluphenazine by APPI, APCI, and ESI. The APPI signal is not suppressed by the eluting components of rat plasma. The APCI signal is likewise relatively unaffected by the rat plasma eluent, although in this example, the signal is less stable than the APPI signal. We did not examine this effect sufficiently to determine whether the signal instability was due to the effect of the eluting components. The ESI signal, on the other hand, was noticeably suppressed by the presence of the rat plasma eluents.

We also examined the response of APPI and ESI in the presence of the common capillary electrophoresis (CE) buffers sodium phosphate and sodium borate. The example in Figure 8 shows the TICs for labetalol, salbutamol, and terbutaline separated by capillary zone electrophoresis (CZE). The APPI source results in significantly greater ion abundance and signal-to-noise ratio for borate (pH 9.3) and phosphate (pH 2.5) buffers. The principal contributor to improved performance using APPI is reduction in noise relative to ESI. A similar comparison was reported by Nilsson and co-workers, also using an Agilent CE system coupled to a 1100 Series LC/MSD and orthogonal spray APPI source.[22] They reported lower chemical noise and signal suppression for APPI vs ESI, particularly for phosphate buffers, on the basis of measurements of 11 basic drug compounds.

## DISCUSSION AND APPLICATIONS

**Direct APPI vs Dopant-Assisted APPI.** Bruins and co-workers have reported an APPI method based on the addition of a large excess of a dopant such as toluene and acetone. The dopant is chosen to efficiently photoionize and to act as a charge carrier for ionizing trace levels of analyte by charge transfer. In this regard, the method is similar to APCI; however, it has led to better sensitivity than APCI in many cases. It still remains to be shown that this approach is as broadly applicable to a wide range of compounds as is direct APPI. The primary events leading to ionization by the two methods are summarized below. Once the primary events of ionization have occurred, secondary events, such as ion−molecule reactions, may occur to change the distribution of initially formed ions. In fact, dopant APPI relies on expedient ion−molecule reactions to form the desired ions. It may be viewed as photoionization-induced APCI. The double arrows leading to analyte ions represent possible multistep ion forming chemistry. Bruins and co-workers have obtained evidence that solvent complexation may assist in the charge-transfer process.[23] At atmospheric pressures, the molecular radical ion $M^+$ formed by PI can undergo further ion molecule reactions that can change the identity of $M^+$. The most common reaction is that which can

| | |
|---|---|
| direct APPI | |
| $M + h\nu \rightarrow M^+$ | analyte molecule M is ionized to a molecular radical ion $M^{\bullet\cdot +}$ (which we denote as $M^+$ for simplicity) |
| dopant APPI | |
| $D + h\nu \rightarrow D^+$ | a photoionizable dopant D is delivered in large concentration to yield many $D^+$ ions |
| $D^+ + M \rightarrow\rightarrow MH^+ + D[-H]$ | $D^+$ ionizes analyte M by proton transfer |
| $D^+ + M \rightarrow\rightarrow M^+ + D$ | $D^+$ ionizes analyte M by electron transfer |

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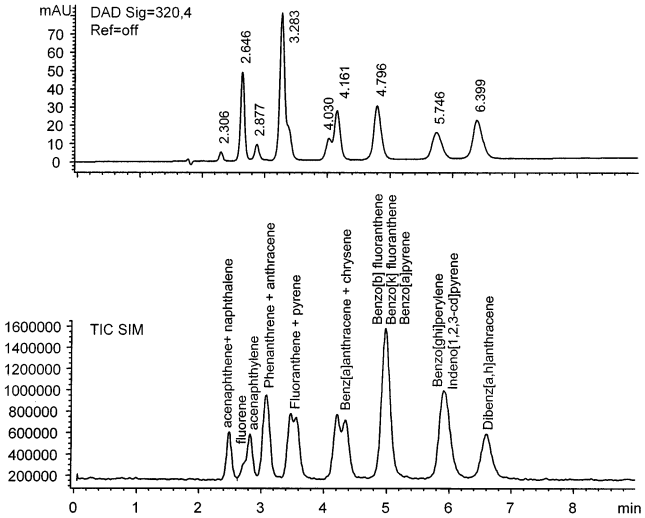


Figure 9. Comparison of UV and TIC LC/MS traces for a mixture of nonpolar aromatics. The APPI signal was recorded using acetone as dopant. LC conditions: two columns in series of 2.1 × 100 Hypersil APS, 5 $\mu$m, flow rate 0.4 mL/min hexane with 50 $\mu$L/min postcolumn addition of acetone, column temperature 10 °C. MS source conditions: drying gas temperature 350 °C at 5 L/min, $V_{cap}$ 3000 V, vaporizer temperature 350 °C, nebulizer pressure 60 psig.

lead to $MH^+$, presumably due to hydrogen atom abstraction from the abundant protic solvents generally used,[24] though other parallel mechanisms leading to $MH^+$ have also been proposed.[25] In many, if not most cases, $MH^+$ dominates over $M^+$. This is a relatively innocuous reaction in that the identity of the molecule is preserved. It is also possible for $M^+$ to react to form other ions or to lose its charge under the highly collisional environment of atmospheric pressure. We believe this is an important area of research to pursue.

Figure 9 shows the efficiency of ionizing nonpolar compounds. This example used acetone dopant, which can increase signal abundance for many compounds. Figure 10 shows examples of compounds that gave about order-of-magnitude increases in ion abundance using acetone and toluene dopant. However, it is also the case that signal-to-noise ratio does not generally improve significantly and can sometimes decrease, even for greater signal, particularly when using toluene dopant (presumed to be due to trace impurities in toluene). Figure 11 shows LC chromatograms for fat-soluble vitamins. In this example, dopant did not enhance signal and, in fact, generated increased noise. Acetone did not increase noise; however, the signal was attenuated relative to the dopant-free case. Similar to the linearity results presented in Figure 3, the vitamins give a high linear dynamic range. Figure 12 shows excellent linearity over a range of 1−1000 ppb concentration.

The magnitude of dopant enhancement may be a function of the brightness of the PI lamp. For high-output lamps, the ion density by direct PI may be approaching a saturable limit, at which further enhancement may be minimal. This effect is evident in work reported by Bruins et al.[1,26] For example, the dopant

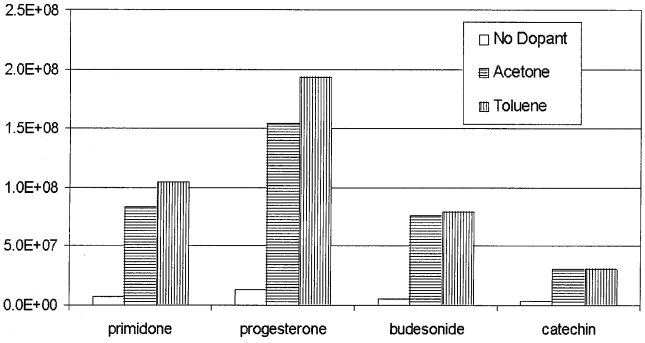


Figure 10. Effect of toluene and acetone dopant on $[M + H]^+$ APPI signal. LC conditions: flow injection analysis, flow rate 0.4 mL/min, 50% water and 50% methanol with 50 $\mu$L/min postcolumn addition of acetone or toluene for dopant data. APPI source conditions nondopant: $V_{cap}$ 1500, drying gas temperature 350 °C at 5 L/min, vaporizer temperature 250 °C, nebulizer pressure 60 psig. APPI source conditions with dopant: $V_{cap}$ 3000, drying gas temperature 350 °C at 5 L/min, vaporizer temperature 250 °C, nebulizer pressure 60 psig.

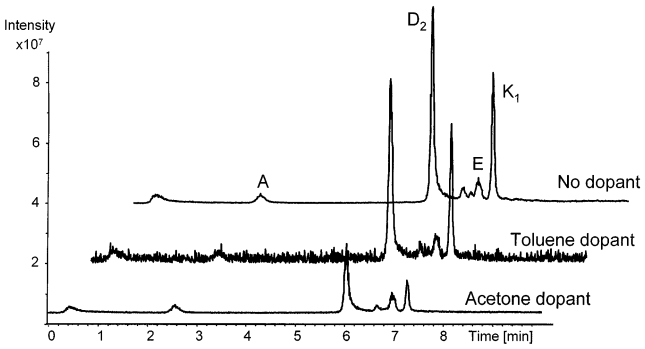


Figure 11. LC chromatograms showing effect of toluene and acetone dopant on the signal for fat-soluble vitamins. LC conditions: Zorbax XDB-C18, 2.1 × 50 mm, 5 $\mu$m, flow rate 0.4 mL/min with 50 $\mu$L/min postcolumn addition of acetone or toluene for dopant data, column temperature 25 °C, solvent A = water, solvent B = methanol. Gradient: time = 0 min, 80% B; time = 5 min, 100% B. Nondopant APPI source conditions: $V_{cap}$ 1700, drying gas temperature 350 °C at 5 L/min, vaporizer temperature 250 °C, nebulizer pressure 60 psig. Dopant APPI source conditions: $V_{cap}$ 3000, drying gas temperature 350 °C at 5 L/min, vaporizer temperature 250 °C, nebulizer pressure 60 psig.

enhancement for acridine was about a factor of 60 for the Sciex lamp source (toluene dopant and $MeOH/H_2O$ at 200 $\mu$L/min)[1] and only about a factor of 2 for the Agilent/Syagen source (AcCN at 200 $\mu$L/min).[26] The latter source is believed to have upward of an order of magnitude greater radiant output. Independent measurements conducted with different intensity PI sources support the conclusion that the dopant enhancement is greater for lower intensity sources. The interplay between direct and dopant PI and the dependence on light intensity is an important topic of study, but is beyond the scope of this work.

**Low-Flow Applications (Capillary LC and CE).** The results described in the section Signal Dependence on Flow Rate and presented in Figures 6 and 7 showing superior performance for APPI vs APCI at low flow rates suggest that APPI may be the

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(24) To be published.

(25) Marottaa, E.; Seraglia, R.; Fabris, F.; Traldi, P. *Int. J. Mass Spectrom.* **2003**, *228*, 841−849.

(26) Kauppila, T. J.; Kostiainen, R.; Bruins, A. P. Effect of the Flow Rate on the Ionization Efficiency in Atmospheric Pressure Photoionization Mass Spectometry. Proceedings of the 51st ASMS Conference on Mass Spectrometry and Allied Topics, Montreal, June 8−12, 2003.

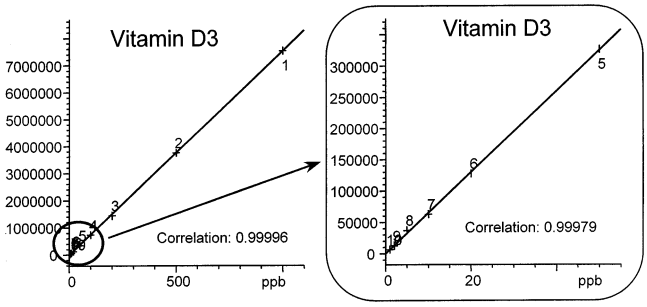


Figure 12. Linearity plots for vitamin D3. LC conditions: Zorbax Eclipse XDB-C18, 4.6 × 50 mm, 3.5 $\mu$m at 0.6 mL/min, column temperature 40 °C, solvent composition 10 mM ammonium acetate, 10% water and 90% acetonitrile. MS source conditions: drying gas temperature 350 °C at 5 L/min, $V_{cap}$ 1500 V, vaporizer temperature 350 °C, nebulizer pressure 60 psig.

preferred source for applications involving capillary LC or capillary electrophoresis (CE). The latter application involves high concentrations of buffers. Because APPI appears to be more immune to buffer conditions than ESI (Figure 8) and APCI, APPI may be significantly more suited to CE than either of these sources.

We conducted further tests of the APPI source with CE/MS. Figure 13 shows CE chromatograms for a drug mixture consisting of Terbutaline ($[M + H]^+$, $m/z$ 228), Salbutamol ($[M + H]^+$, $m/z$ 240), and Labetalol ($[M + H]^+$, $m/z$ 329). These results were recorded using phosphate buffer (pH 2.5). The CE interface used a liquid sheath flow to couple the CE capillary end to the standard APPI nebulizer/vaporizer unit. The sheath flow was operated at 50 $\mu$L/min. The sheath flow must be an electrolytic buffer solution to maintain electrical conductivity to the end of the CE capillary. The results in Figure 13 show that APPI gives significantly better detectability (e.g., signal-to-noise ratio) than ESI primarily due to reduced noise by the APPI source.

The results obtained in this work are in accord with those obtained by Nilsson and co-workers using a similar CE/APPI/MS system.[22] Phosphate buffer conditions gave optimal CE/MS results for basic drug compounds. Borate buffer gave acceptable APPI detectability (Figure 8), but leads to clustering that contributes to chemical noise.

**Negative Ion Detection.** The APPI source leads to efficient production of negative ions. This is achieved because the photon source is an excellent source of low-energy electrons. Electrons can be produced by at least two mechanisms. Metal surfaces abound in the ionization region. The electron binding energy to most metals, including stainless steel and aluminum, lies in the 3−5 eV range. Consequently, 10 eV photons are very efficient at liberating electrons when they strike a metal surface. Another effective method to produce low-energy electrons is to introduce a dopant, as is done for positive ion detection. Clearly, for every positive dopant ion that is used to chemically ionize analyte molecules, there is a corresponding electron that can lead to negative ion formation by the usual ionization mechanisms known for APCI (electron attachment, deprotonation, negative adduct attachment, etc.).

Figure 14 compares positive and negative ionization by APCI, ESI, and APPI for trifluorobenzoic acid. None of the sources is effective at producing positive ions for this compound. On the other hand, all the sources efficiently produce negative ions. In this example, APPI is no less efficient than the other sources. This is generally true for other compounds that typically form negative ions. Consequently, APPI may be used for a wide variety of applications requiring both positive and negative ion detection.

Figure 15 shows the dependence of signal level on the capillary entrance voltage ($V_{cap}$) for production of negative ions with and without dopant. Acetone was the preferred dopant in this work. There are two observations to be made from these results: (1) the negative ion abundance can be just as large without using dopant, and (2) the dopant-derived signal shows less dependence on $V_{cap}$ than the nondopant-derived signal. This may reflect the fact that electrons may be formed over a larger volume using dopants, as compared to photoemission of electrons from a surface. In the latter case, the electron volume and subsequent negative ionization volume may be more localized, leading to a narrower set of optimized $V_{cap}$ conditions. It should be noted that

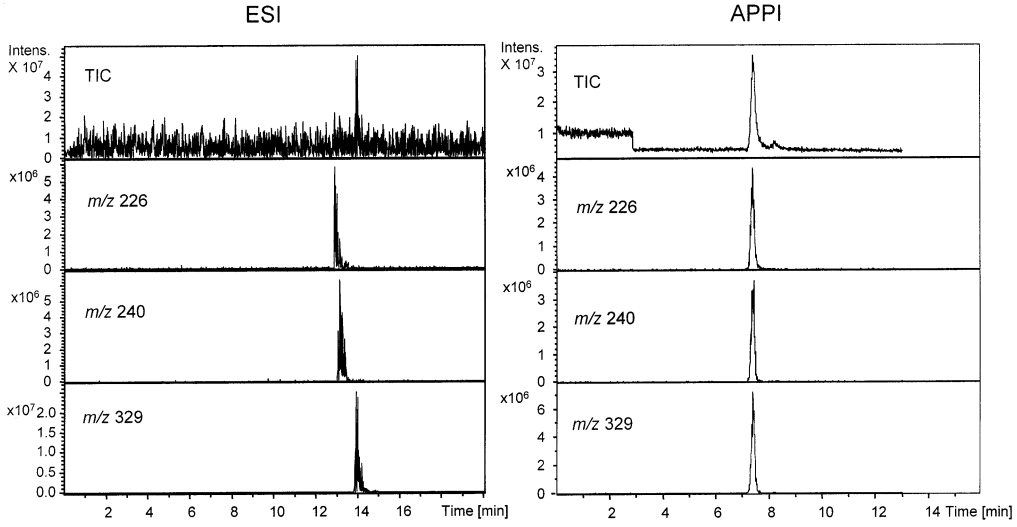


Figure 13. CE chromatograms using phosphate buffer for a three-drug mixture comparing signal response for ESI and APPI. CE conditions: capillary electrophoresis voltage 30 kV; capillary i.d. 50 $\mu$m × 300 mm; capillary temperature 25 °C; CZE buffer 50 mM borate pH 9.3 or 50 mM phosphate pH 2.5; sheath flow 50 $\mu$L/min of 1% acetic acid, 49% water and 50% methanol. MS source conditions: $V_{cap}$ 1500, drying gas temperature 150 °C at 5 L/min, vaporizer temperature 275 °C, nebulizer pressure 60 psig.

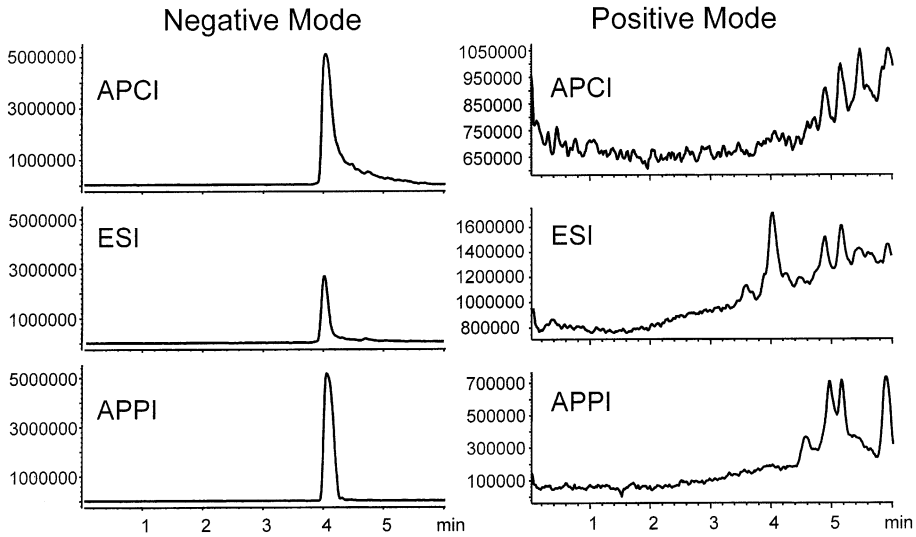


Figure 14. Comparison of LC/MS chromatograms for trifluorobenzoic acid in positive and negative ion detection by APCI, ESI, and APPI. LC conditions: Zorbax SB-C18, 2.1 × 30 mm, 3.5 $\mu$m, flow rate 0.4 mL/min with 50 $\mu$L/min postcolumn addition of acetone for negative ion APPI data, column temperature 30 °C, solvent A = 0.1% formic acid in water, solvent B = 0.1% formic acid in methanol. Gradient: time = 0 min, 5% B; time = 5 min, 90% B. APCI source conditions: $V_{cap}$ 4000, corona current 4 $\mu$A positive ion and 15 $\mu$A negative ion, drying gas temperature 350 °C at 5 L/min, vaporizer temperature 350 °C, nebulizer pressure 60 psig. ESI source conditions: $V_{cap}$ 4000, drying gas temperature 350 °C at 13 L/min, nebulizer pressure 25 psig. Nondopant APPI source conditions: $V_{cap}$ 1700, drying gas temperature 350 °C at 5 L/min, vaporizer temperature 250 °C, nebulizer pressure 60 psig. Dopant APPI source conditions: $V_{cap}$ 2500, drying gas temperature 350 °C at 5 L/min, vaporizer temperature 250 °C, nebulizer pressure 60 psig.

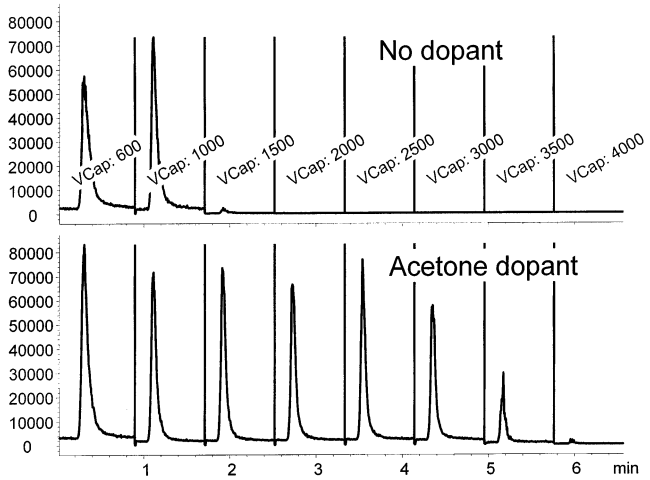


Figure 15. Dependence of APPI negative ion signal with and without acetone dopant as a function of the $V_{cap}$ capillary voltage on the Agilent MSD. LC conditions, flow injection analysis: flow rate 0.4 mL/min, 50% water and 50% methanol with 50 $\mu$L/min postcolumn addition of acetone. APPI nondopant source conditions: $V_{cap}$ variable, drying gas temperature 350 °C at 5 L/min, vaporizer temperature 250 °C, nebulizer pressure 60 psig. APPI dopant source conditions: $V_{cap}$ variable, drying gas temperature 350 °C at 5 L/min, vaporizer temperature 250 °C, nebulizer pressure 60 psig.

no attempt was made to optimize the photoemission efficiency by adjusting the position of metal surfaces or adding supplementary metal or other electron emitting material to the preferred ionization volume.

Takino and co-workers in a series of studies reported on the effect of APPI negative ion detection under dopant and nondopant conditions. Their results were also obtained on an Agilent 1100 LC/MSD system with the orthogonal-spray APPI source. Not surprisingly, they observed similar dependences as described above for dopant and nondopant signal intensity with $V_{cap}$.[27−29]

## SUMMARY AND CONCLUSIONS

The propensity of a molecule to photoionize is dependent only on the interaction of the molecule with a photon of sufficient energy. This is in contrast to APCI and ESI, which depend on ion−molecule chemistry to form ions of interest. These properties suggest that APPI has the potential to be a more general purpose ionizer than APCI. At the very least, APPI ionizes many types of compounds that are not efficiently ionized by ESI and APCI. PI can therefore serve as a complementary ionization source.

The results presented here lead to the following conclusions:

- APPI may be effective for detecting a broad range of compounds and is not strongly dependent on molecular polarity.
- Direct APPI has comparable sensitivity to APCI at high flow rates and superior sensitivity at low flow rates.
- APPI appears to be less susceptible to matrix-induced ion suppression and buffer-created chemical noise.
- APPI has a large dynamic range and excellent sensitivity extending down to low flow rates.

APPI has unique attributes for low-flow chromatographies, such as capillary and CE. The sensitivity of APPI significantly exceeds the sensitivity of APCI at low flow rates (<100 $\mu$L/min). Furthermore the strong buffer conditions of CE lead to significantly greater ion suppression for ESI than for APPI.

Considerable research still remains before the benefits of APPI and PI in general are fully realized. Although PI is a direct process that is independent of the surrounding mixture, the ions generated by PI can undergo further chemistry at atmospheric pressure that may deplete the yield of ions of interest. Furthermore, it should

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(28) Takino, M.; Daishima, S.; Nakahara, T. *Rapid Commun. Mass Spectrom.* **2003**, *17*, 383−390.
(29) Takino, M.; Daishima, S.; Nakahara, T. *J. Chromatogr., A* **2003**, *1011*, 67−75.

be realized that APPI properties may be very much dependent on the source properties. In particular, the dopant vs nondopant comparisons will be specific to the APPI source being used. Two different commercial source configurations are being used today: a closed, axial source designed to enhance dopant-assisted ion−molecule reactions (Sciex) and the open, orthogonal source designed to enhance the direct method of APPI (Agilent, Thermo Electron, Waters). Furthermore, radiant output can affect behavior as discussed in the section titled Direct APPI vs Dopant-Assisted APPI.

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