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Existing methods of mass spectrometry (MS) are not well suited for analyzing trace constituents in complex, multispecies mixtures in real time because of one or more of the following problems: fragmentation of ions that can overlap with trace compounds of interest, overwhelming signal from ionization of common air and solvent constituents, nonuniversal ionization efficiency, or ion signal suppression due to competition for charge. To achieve adequate detection accuracy, a front-end separation stage such as gas or liquid chromatography is usually employed. This paper describes photoionization mass spectrometry (PI MS) as a means that can overcome these problems so as to achieve accurate, real-time analysis. Most prior attempts to develop analytical PI MS systems have focused on the use of lasers.[1–3] In this work, a nonlaser PI source that is compact, robust, and sensitive is described.

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Figure 1 illustrates the principle of PI MS. The source shown (**Syagen Technology, Inc.**, Tustin, CA) imparts an energy that is higher than the ionization potentials (IPs) of typical target molecules, but lower than the IPs of virtually all constituents of air as well as most common solvents. These potential interferents are not ionized, enabling significantly greater dynamic range for detecting and measuring molecules at very low concentrations. Furthermore, because molecules of interest are ionized near their IP thresholds, there is minimal fragmentation to clutter a mass spectrum. These performance features provide tremendous benefits for analyzing mixtures and samples in complex matrices. The method of electron ionization (EI), by contrast, imparts energy that greatly exceeds the dissociation thresholds, leading to extensive fragmentation. The principle benefits of PI MS can be summarized as follows:

- Near-universal ionization efficiency for many classes of compounds (e.g., chemical weapons, drugs, aromatics, etc.)
- Minimal fragmentation and predominant molecular ion signal for most detected compounds
- Avoidance of unwanted signal from all common air molecules (e.g., N_2, O_2, H_2O, CO_2, CO, Ar, etc.) and many common solvents (CH_3OH, H_2O, CH_3CN, chloroalkanes, etc.)
- Linear relationship between signal and concentration and minimal charge-competition effects to enable accurate analysis.

Other methods of soft ionization are atmospheric pressure chemical ionization (APCI) and electrospray ionization (ESI). They are the two most commonly used methods for analyzing liquid samples such as drug compounds in solvents. APCI and ESI are affinity methods in which the analytes either protonate or form adducts for positive ion mode or deprotonate or electron at-

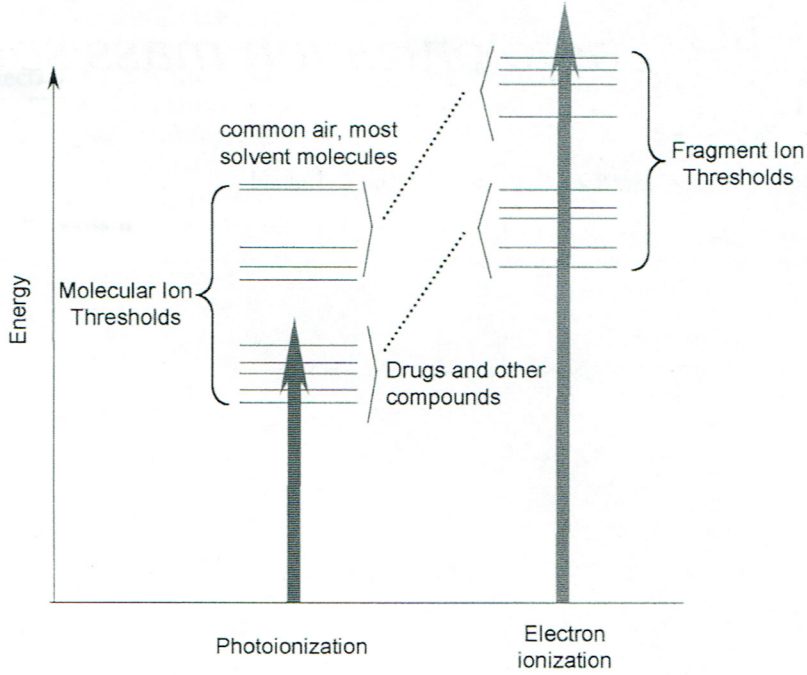

Figure 1 *Diagram showing the principle of PI for achieving threshold ionization of molecules. The process of EI is shown for comparison.*

tach for negative ion mode. These propensities are generally associated with polar compounds. PI is more universal across the polarity scale. Common problems for ion-molecule affinity ionization methods such as APCI and ESI that are largely overcome by PI include:

- Ionization efficiencies by APCI and ESI are very sensitive to charge affinity; many compounds are not detectable, including some classes of drug compounds
- Desired signal can be suppressed in APCI and ESI by compounds with higher charge affinities than the target compounds, a particular problem with mixtures
- Adduct formation with trace constituents (e.g., Na^+) is prevalent by ESI.

Experimental

Photoionization source

Some details of PI MS can be found in a previous publication.[4] The PI light source uses a high-output gas discharge tube with a MgF_2 window for transmission of ultraviolet light into the ionization region. The fill gas is generally a rare gas and the choice of gases depends on the wavelength range of interest. Narrow band energy is available from approx. 8 to 12 eV, although the authors usually operate at approx. 10 eV. The PI MS light sources are similar in principle to photoionization detectors (PIDs) for GC, except that the former have much greater radiant output and are optimized for coupling to the sample mixture. The authors have developed two types of PI sources:

- Low-pressure photoionization (LPPI) is used for air and liquid sampling of volatile and semivolatile compounds. The source operates at a pressure of less than 1 torr to minimize ion-molecule collisions that can lead to competition for charge and ion suppression effects. The air or volatilized liquid sample enters the source through a flow restrictor (e.g., a metering valve or a capillary). Ions that are formed are then focused using a series of ring electrodes onto an exit aperture where they pass through into the mass analyzer.
- Atmospheric pressure photoionization (APPI) is used for sampling from high-flow devices such as LC and for sampling high-molecular-weight compounds that require a nebulizer or other type of liquid spray nozzle.

The principle mechanism for photoionization of molecule M is photon absorption and electron

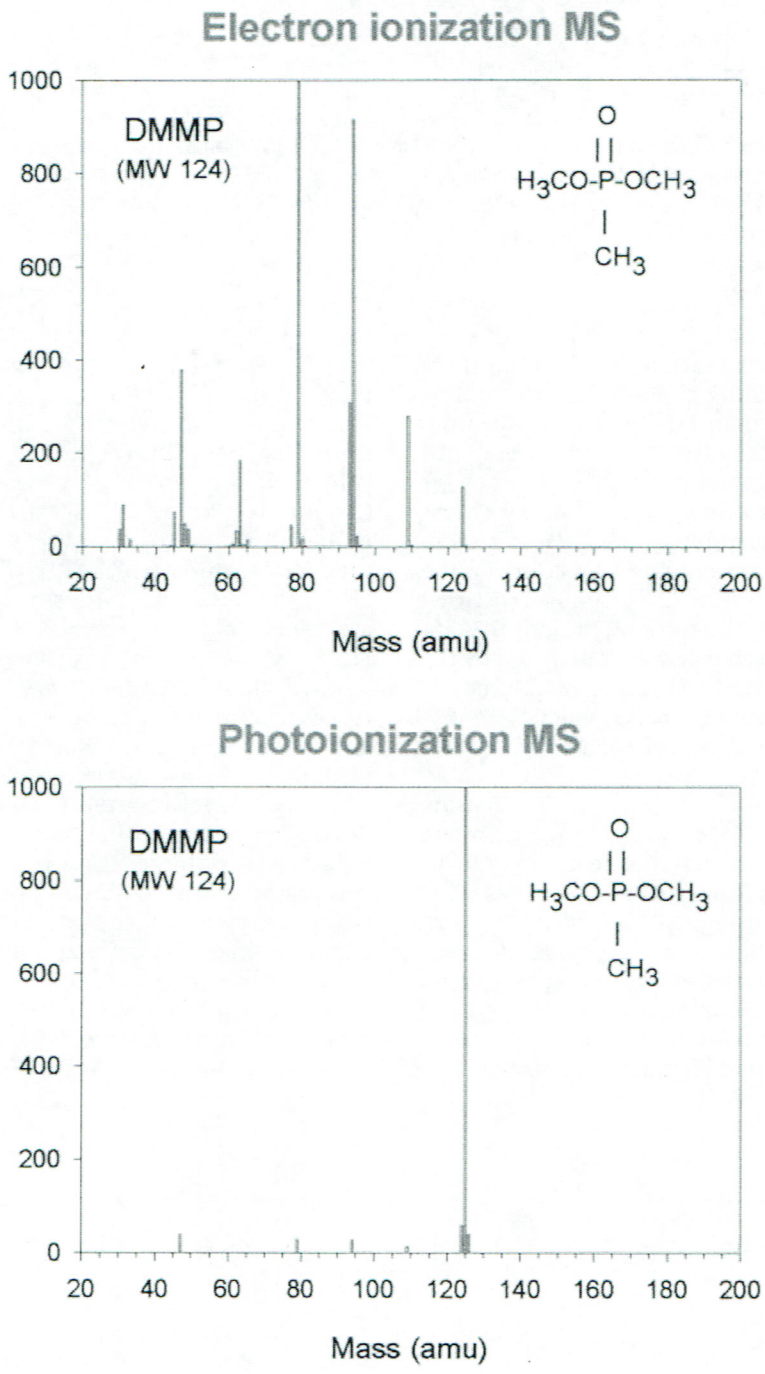

Figure 2 *Comparison of EI vs PI MS for a vapor sample of dimethyl methylphosphonate (DMMP).*

ejection to form the molecular ion M^+. In the presence of water vapor or protic solvents, the molecular ion can extract H to form MH^+. This tends to occur if M has a high proton affinity. This does not affect quantitation accuracy as the sum of M^+ and MH^+ is constant. Drug compounds in protic solvents are usually observed as MH^+, whereas nonpolar compounds such as naphthalene usually form M^+.

Most applications the authors have addressed rely on minimizing ion-molecule effects. However, a number of photoinduced chemical ionization processes may be implemented. In one method, protic sources have been used as additives to convert M^+ to MH^+. This process may be controlled selectively depending on the proton affinity of M by choosing protic sources of varying ionic acidity. Another useful application for additives is to introduce an abundance of a compound that readily photoionizes that can act as a charge carrier for ionizing trace compounds by charge exchange. The authors have observed significantly increased ionization efficiency in their LPPI source by adding acetone as the additive. Unlike a photon that takes a straight path through the sample before absorbing on a surface, the charge carrier "persists" long enough to "find" an analyte molecule to ionize. Bruins and coworkers have demonstrated a similar ionization method for an APPI source by the addition of toluene and acetone.[5] It should be noted, however, that ionization by photo-induced charge carriers is another form of chemical ionization and may not be as broadly applicable to a wide range of compounds as is direct PI.

Mass analyzer

The mass analyzer is based on quadrupole ion trap, time-of-flight (QitTof) mass spectrometry and has the following characteristics: full mass spectra recorded at 60 Hz (>100 Hz possible), automated MS^n, mass range up to 3000 amu, and mass resolution $m/\Delta m$ of 3000 at fwhm. This technology is capable of performing reliable flow-injection analysis sampling of combinatorial libraries. The entire instrument is transportable and occupies a 5-ft^3 footprint.

Results and discussion

This section presents results that exemplify the benefits of PI that were enumerated previously. The next section shows examples of applications where these benefits are important.

Universality and minimum fragmentation

Minimal fragmentation was one of the benefits outlined previously. *Figure 2* shows a comparison of PI and EI mass spectra of the chemical agent surrogate dimethyl methylphosphonate (DMMP). These results show a considerable reduction in complexity of the ion signals by PI compared to EI.

The generality of the photoionization condition suggests the potential for near-universal soft ionization of a wide variety of compounds. A diverse set of over a hundred different compounds have been tested. Near universality (>90% detection rate) is observed for many general classes of compounds, such as aromatics and amines and specific classes of drug standards and chemical weapons related compounds. High detection efficiencies were also observed for validated combinatorial library samples for drug discovery.

Dynamic range and signal linearity

The dynamic range of a combined PI/QitTof instrument was measured for many classes of compounds. Signal level versus mass quantity of analyte is shown in *Figure 3* for naphthalene and imipramine for a fixed set of instrument conditions. These measurements were recorded using the LPPI source. The APPI source shows similar linearity and a detection limit of less than 10 pg. The results, which are generally observed for other classes of compounds, show a nearly four-decade

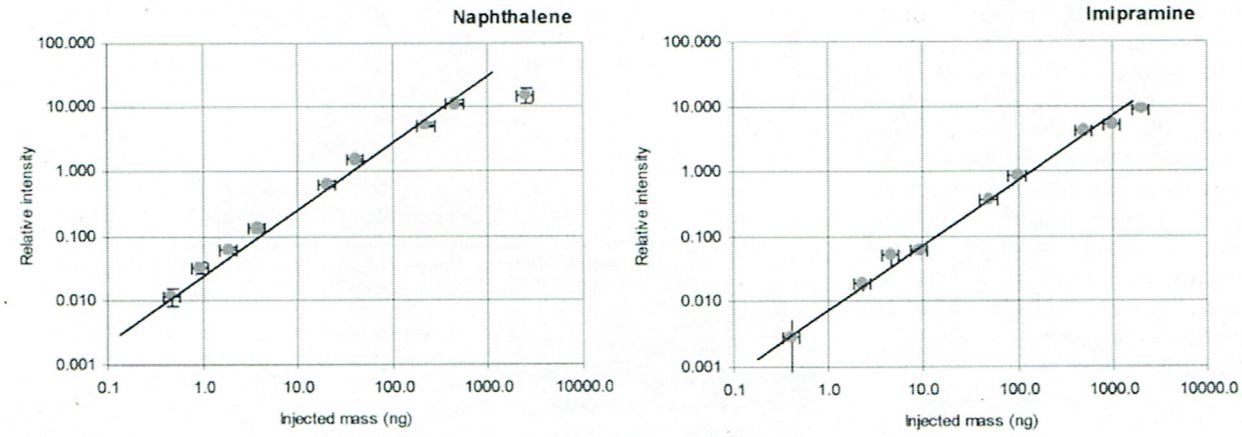

Figure 3 *Plot showing linearity of signal vs injected mass. Linearity also holds as a function of concentration (see text).*

dynamic range that is linear over more than three decades. The intrinsic dynamic range and linearity of the PI source itself has not yet been fully determined. The injected masses in Figure 3 were obtained by varying both sample concentration and injected volume. Linear relationships were also observed as a function of concentration (fixed injection volume) over a three–four decade range and as a function of injected volume (fixed concentration) over about a two-decade range (0.1–10 µL).

The linear dependence of the LPPI source is relatively insensitive to the presence of other compounds in a mixture. This property is useful for conducting quantitation of a target compound in mixtures, and becomes very useful for high-throughput applications because it minimizes the need to conduct chromatographic separation in some cases. Affinity-based ionization methods, such as APCI and ESI, are susceptible to competition for charge effects, which makes them less reliable for conducting quantitative measurements in the absence of a separation step.

Applications

The utility of PI MS for high-speed and high-throughput analysis is discussed for two applications that **Syagen** currently addresses.

Threat detection and treaty monitoring

The currently accepted method for validating the presence of alleged Chemical Weapons Convention (CWC) Treaty–scheduled compounds during an inspection is GC-MS. The slow speed of this method limits the number of samples that can be analyzed in a given inspection time period, potentially compromising the thoroughness of a challenge inspection. The throughput could be greatly increased if a large number of samples were rapidly screened for the presence of alleged compounds. Only samples that test positive for alleged compounds are then analyzed by GC-MS for confirmation. A fast molecular screening capability could also meet the requirements for real-time detection of chemical threats and other high-speed, high-throughput applications.

Syagen has developed a PI MS instrument that employs an integrated autosampler to enable reliable detection of targeted compounds in potentially complex mixtures. The instrument uses the LPPI source because of its suitability for volatile samples, its adaptability to a GC front end, and its minimization of ion-molecule chemistry that can lead to secondary ions and ion suppression effects at atmospheric pressure. Phosphonate compounds have been tested with an APPI and gave similar spectra to the LPPI.

The PI/QitTof was used to screen a 48-compound library of CWC Treaty–scheduled compounds; 45 yielded molecular ion signals typically with minimal fragmentation. The instrument achieved detection limits in the range of 5–100 ppb for direct air sampling (generally 5 sec sampling time). Liquid sampling detection limits were <1 µg/mL in a variety of solvents (e.g., methanol and water) using direct syringe injections (typically µL injections) or continuous capillary infusions. Live agent testing was conducted for nerve agents, mustards, and riot control compounds.

The PI MS system provides near-universal detection efficiency for a wide class of compounds including the following, which are important to threat detection and site inspection:

- VX, G-agents, other nerve agents, phosphonates, and phosphonic acids
- Mustards and sulfides
- Nearly all CWC-relevant classes of compounds.

Figure 4 shows the PI mass spectra of VX showing strong molecular ion and minimal fragmentation. With the QitTof mass analyzer, the molecular ion may then be selectively dissociated by resonance excitation in the ion trap to generate a confirmatory fragment ion for the positive identification of VX.

High-throughput drug discovery

Developing a successful drug from inception typically takes

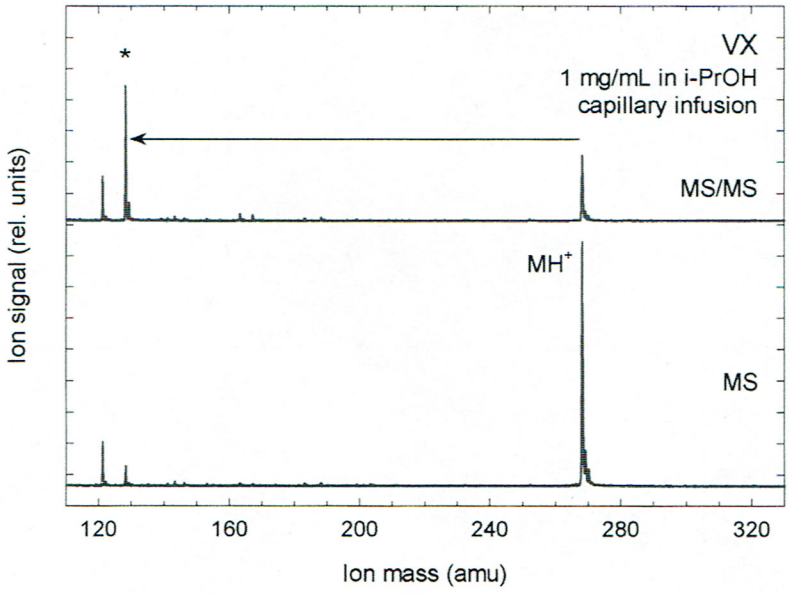

Figure 4 *PI MS spectra of the nerve agent VX in the signature region. The MS plot (bottom) shows a predominant molecular ion for VX. The MS-MS plot (top) shows mass-selective dissociation to give the confirmatory fragment signal at 128 amu (indicated by an asterisk).*

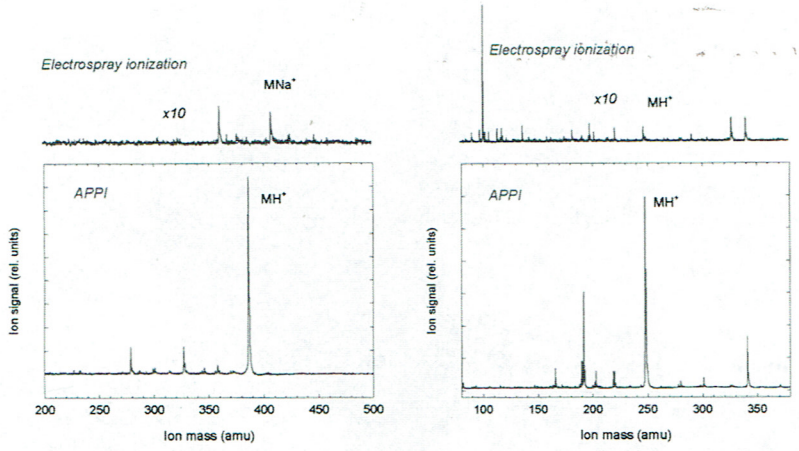

Figure 5 *Comparison of PI MS (APPI) and ESI MS for combinatorial library samples (provided by* ***DuPont/Combichem,*** *San Diego, CA) using a dual ESI and APPI source. The APPI pressure in this example was reduced to 200 torr.*

more than a decade and costs on the order of $500 million. The success rate for new compounds reaching Phase III clinical trials is less than 1 in 1000. Critical bottlenecks drive up the cost and lengthen the time to market for new drugs. Combinatorial chemistry and high-throughput biological screening are speeding up the process of drug discovery.[6] However, the full potential of this approach is seriously impeded by the limited throughput of existing technology for conducting molecular analysis for purity assessment. The most widely used method of analysis is LC-MS using ESI. Attempts to speed up the analysis have led to methods of direct flow injection analysis (FIA) MS using ESI. However, ESI is susceptible to ion suppression in unseparated mixtures that can cause compounds to go undetected. ESI is also not sufficiently quantitative to conduct purity assessment.

The benefits of PI compared to ESI are higher detection probability and better quantitation accuracy. Though PI may replace ESI for some key applications, it is more likely that PI will fulfill a complementary role as an alternative method for analyzing pharmaceutical samples. There are many examples where PI efficiently detects compounds that are not detectable by ESI or APCI. *Figure 5* shows examples of combinatorial library samples that were recorded simultaneously by ESI and PI, but that show reliable detection only by the latter method.

PI MS is ideally suited to conducting high-throughput molecular analysis of large libraries for compound confirmation and purity assessment. Other important combinatorial chemistry applications include mass-selected fractionation for purifying libraries and screening for preclinical type properties. The latter application is intended to weed out bad performers early so as to improve the success rate for bringing a drug candidate into clinical trials. In vitro tests in array plate format now exist for bioavailability/permeability testing as well as for other pharmacokinetic testing.

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