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Laser Chemical Analysis

Richard N. Zare

Summary. Selected applications of laser methods to analytical problems are reviewed. Examples are chosen from multiphoton ionization and laser fluorescence analysis. Although efforts to carry out elemental analysis with laser techniques are probably the most advanced, studies suggest that the analysis of molecular species is also quite promising, particularly in regard to interfacing laser fluorimetric detection with high-performance liquid chromatography. Recent experiments indicate that analysts can expect to attain in a number of cases the ultimate limit of single-atom or single-molecule detection with laser-based methods.

The intimate association of chemistry and light ranges from fireworks displays and the color of solutions and precipitates to the spectroscopic analysis of new and unknown substances. The advent of the laser has only served to strengthen this natural bond, so that no major chemical research laboratory is without lasers today. Because of its high power, directionality, purity of color, and temporal coherence, the laser has become a highly versatile tool, first applied to the study of how chemical reactions occur, then to initiate chemical reactions upon irradiation, and finally as an extremely sensitive and selective means to analyze for the presence of chemical substances of interest. It is this last topic which is the subject of this brief selective review in which outstanding examples of recent advances in chemical analysis based on laser techniques are presented. Laser methodologies promise to improve dramatically the detection of trace substances embedded in "real" matrices, giving the analyst a most powerful means for determining the composition of materials.

Richard N. Zare is Shell Distinguished Professor of Chemistry in the Department of Chemistry, Stanford University, Stanford, California 94305.

Multiphoton Ionization

One of the most promising laser techniques is multiphoton ionization (MPI), in which an atom or molecule absorbs more than one photon to cause ejection of an electron (*1*, *2*). This nonlinear process is made possible by the high intensity of laser light sources. We first review how the MPI process works, then describe some recent applications.

Figure 1 compares multiphoton ionization to single-photon ionization. If the energy of the photon exceeds the ionization energy of the target atom or molecule, the target species will be ionized and can be detected by measuring the subsequent positively charged ion or negatively charged photoelectron (Fig. 1a). If, however, the energy of one photon lies below the ionization threshold, ionization can occur only by the simultaneous absorption of several photons whose energy sum exceeds the ionization potential (Fig. 1, b and c). Multiphoton processes may involve virtual levels (Fig. 1b) which are not eigenstates (real levels) of the isolated atom or molecule. The lifetime for such virtual levels is often on the order of 10^{-15} second. The MPI process is said to be resonant when the energy of an integral number, n, of photons approaches closely the energy of an n-photon–allowed transition. Because real levels have lifetimes typically of 10^{-9} to 10^{-6} second, the probability for absorbing subsequent photons is greatly increased (six orders of magnitude or more). Consequently, nonresonant multiphoton ionization usually requires laser powers of about 1 GW/cm^2, which can be achieved only by tightly focusing powerful pulsed lasers. In contrast, resonant enhanced multiphoton ionization (REMPI), also called resonance ionization spectroscopy (RIS), can be carried out with pulsed lasers of fairly modest intensity (1 MW/cm^2) and under favorable conditions even with continuous-wave laser sources. The

...wavelength gives REMPI its selectivity, and it is the availability of powerful tunable lasers that makes REMPI a nearly universal detector. One or more laser photons with the same or different frequencies may be employed, and variations include field ionization of high-lying Rydberg levels (*3–5*).

Because the laser intensity required for MPI is high, energy levels are often Stark-shifted. In practice, the laser intensity in the (focal) detection volume is uniform in neither space nor time, causing the target species to be subjected to a wide range of different intensities. This results in broadened line profiles of the resonant MPI process since different shifts occur in the interaction volume. Consequently, REMPI of atomic systems has poor selectivity with regard to different isotopes, although for molecular systems with much larger spectral isotope shifts REMPI can easily be made to be isotopically specific (*6*). In either case, isotopic analysis is usually realized by combining REMPI with mass analysis, using, for example, time of flight (TOF) (*7–9*), reflectron (*10*), quadrupole mass filter (*11, 12*), or ion cyclotron resonance (*13, 14*).

Bound-to-continuum transitions having cross sections typically less than 10^{-17} cm^2 are usually the rate-limiting step in REMPI. Whereas the behavior of the MPI rate near resonance may be rather intuitive, the MPI rate between resonances need not show a flat, monotonic behavior. Destructive interference effects, called anti-resonances, are possible and may lead to almost complete cancellation in the MPI rate (*2*).

At low pressures (below 10^{-5} torr) each target species interacts essentially independently with the radiation field. In this case, the MPI signal is always proportional to the (partial) pressure of the species being detected. At high pressures (1 to 10 torr) collective (coherent) behavior becomes important, leading to such nonlinear processes as third harmonic generation. For intermediate pressures both types of nonlinear processes compete, and above 10^{-3} torr it appears that the MPI signal is often severely reduced because of third harmonic generation and the like (*15*). At high pressures there are the added problems of space charge, ion-electron recombination, ion cluster formation, and inability to use high-gain multipliers for the detection of charged particles. These considerations set a practical limit on the use of MPI as an analytical tool for gas samples and explain why MPI is usually used as a detector of rarefied gas samples, such as those found in evaporation from hot surfaces or filaments or in atomic or molecular beams.

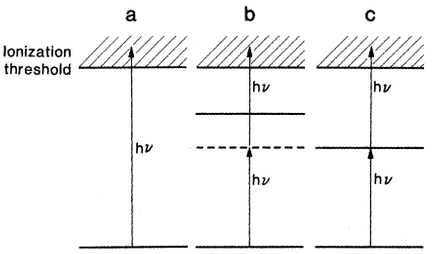

Fig. 1. Schematic energy diagram for (a) one-photon photoionization, (b) nonresonant multiphoton ionization, and (c) resonant multiphoton ionization. The solid horizontal lines represent real levels, the dashed horizontal lines virtual levels. In the multiphoton ionization process the photon frequencies may be the same as drawn (one-color experiment) or different (multicolor experiment).

Most analytical applications of REMPI involve elemental analysis or isotope ratio measurements, or both. Atoms of nearly every element can be detected by REMPI with pulsed tunable dye lasers (*16–18*). However, this procedure often has two drawbacks. First, the effective volume for detection is about 10^{-3} cm^3 or less because of the need to focus the laser output to obtain sufficiently high intensities to cause efficient ionization. Second, the pulsed nature of the high-intensity laser source implies a low duty cycle, often of only 5×10^{-6} or less. Both of these factors may cause the rate of material processing to be severely restricted.

Despite these limitations, some spectacular advances have already been achieved in chemical analysis with resonant and nonresonant MPI. One such example is surface analysis by multiphoton ionization, in which a minute fraction of a monolayer is desorbed, ablated, or sputtered from a surface exposed in a high-vacuum environment to a probe beam (ion beam, electron beam, or laser beam), and the resulting neutral component is ionized above the surface. Several groups (*19–28*) are pursuing the development of this method, which promises to permit trace analysis for some materials at concentrations below parts per billion (ppb) and in special cases parts per trillion (ppt). Immediate applications are in the analysis of semiconductor crystals, whose electrical properties are markedly altered by minute amounts of lattice impurities.

It is useful to make some comparisons with a well-established tool for surface analysis, secondary ion mass spectrometry (SIMS), which involves the analysis of the charged particles ejected from ion-bombarded surfaces (*29, 30*). The ~10 ppb detection limit in SIMS arises from three sources: (i) the fraction of ejected particles that are charged is often 10^{-3} or less; (ii) the sorting of the charged particles by mass using quadrupole or magnetic sectors involves ion transmissions in the range of 10^{-3} to 10^{-4}; and (iii) secondary ion formation is strongly influenced by the sample matrix, making quantification of measurements extremely difficult.

Surface analysis by MPI detection of the desorbed neutral components appears to be an improvement over the sensitivity and reliability of SIMS and at the same time can be applied to a wide range of surface materials, overlayers, and adsorbates. An important aspect of this laser surface analysis technique is that the desorption step is separated spatially and temporally from the ionization step, unlike the process in SIMS. This separation permits detection of the majority neutral fraction, greater control in the choice and type of the probe and ionizing beams with a resultant reduction in surface damage, a gain in quantitation of the ionization step, and avoidance of the large matrix effects seen when the ionization probability of the analyte varies strongly with the chemical environment. The laser ionization step may be resonantly enhanced or nonresonant. In the former case, as in recent studies of the sputtering of indium from an indium metal surface (*19–21*), mass analysis may be unnecessary, and there is a corresponding gain in sensitivity. Recent examples of this are the detection of as few as 10^{11} sodium atoms per cubic centimeter in laser ablation of single-crystal silicon (*22*) and the detection of iron at 50 parts per million (ppm) in iron-doped silicon targets (*25, 26*).

In the nonresonant case, it may be possible to carry out a simultaneous analysis of all of the neutral components evolving from the surface by using some form of TOF mass spectrometry, which permits simultaneous recording of all the ions. Quantitative analysis of relative amounts of desorbed species then requires knowledge of the relative nonresonant MPI efficiencies at the laser frequency employed. Figure 2 presents a portion of a TOF mass spectrum taken of the standard reference material NBS copper C1252 (*27, 28*). The room temperature sample was bombarded by a pulsed 1-μA, 2.7-keV argon ion beam, and the sputtered species were ionized by using pulsed (10-Hz) krypton fluoride (KrF) excimer laser radiation at 248 nm with a focused intensity of 7 GW/cm^2. The data collection time was 1 hour. For

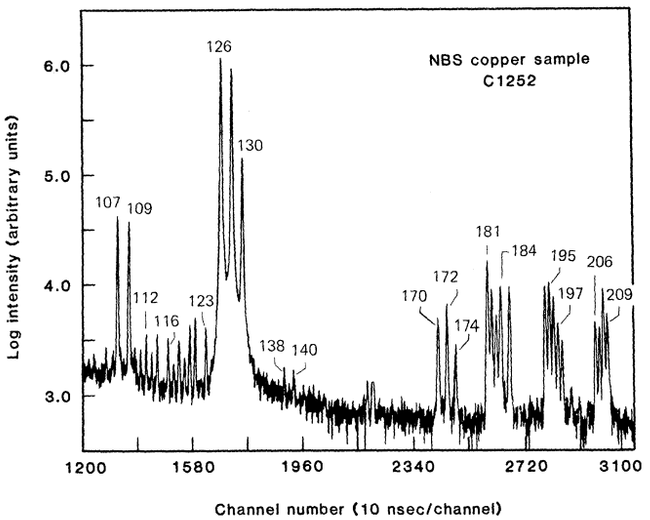

Fig. 2. Segment of a reflection time-of-flight mass spectrum obtained by Ar^+ bombardment of NBS reference material copper C1252 followed by nonresonant multiphoton ionization of the ejected neutral species near the surface with a pulsed KrF excimer laser. The number above a peak is its nominal mass; see the text for identification. This drawing is adapted from Becker and Gillen (*27*).

the mass range displayed in Fig. 2, the elements whose bulk atomic composition values were given by the National Bureau of Standards include ^{107}Ag (with a concentration of 51 ppm), ^{123}Sb (9 ppm), ^{197}Au (11 ppm), ^{206}Pb (4 ppm), and ^{209}Bi (6 ppm). The three largest peaks originate from Cu_2, and peaks at 170, 172, and 174 arise from AgCu dimers. Small amounts of other species (Cd, Sn, Ba, Ce, Hg, Pt, Ta, and W, the latter three as contaminants from the sample holder and the Ar^+ sputtering source) are also observed. It is estimated that a sensitivity of 1 ppm is obtained with only about 10^{-10} g of the sample removed (*27, 28*). This method appears to be well suited for studying the kinetics of surface segregation of impurities and differential evaporation.

Another striking application of laser multiphoton ionization to chemical analysis is the demonstration that individual atoms of nearly every element can be counted, one by one, in a gas sample or flow (*16, 31, 32*). All that is required to achieve this feat is to use the focused output of a pulsed laser having enough intensity that nearly every atom in the focal volume is ionized, that is, to saturate the REMPI process. While such capabilities far exceed the normal needs of elemental analysis, single-atom detection presents the opportunity to search for rare events of great interest. Examples include various nuclear reactions, such as neutrino detection by transmutation of elements, double beta decay events, and the production of short-lived superheavy elements or nuclear isomers. Moreover, the combination of resonant multiphoton ionization of atoms with nearly 100 percent efficiency and high-resolution mass analysis offers the possibility of dramatically improving on isotopic selectivity (*33*).

An example of the power of this combination is the selective ionization of Lu in the presence of Yb (*34, 35*). These rare earth elements are notoriously difficult to separate chemically or by simple physical means. Of particular interest is the $^{173}Lu/^{175}Lu$ ratio in mixed lutetium/ytterbium samples in which isobaric interference from ^{173}Yb is severe. This is exacerbated by the fact that ytterbium is an order of magnitude more volatile than lutetium. By using REMPI with a continuous-wave laser scheme, discrimination factors approaching 10^6 have been measured against ^{173}Yb, a selectivity that is limited not by the REMPI process but by the resolution of the mass spectrometer, which allows tailing of the ^{175}Lu peak to lower mass. There are many other examples of the use of resonant multiphoton ionization to separate isobaric elements (*17, 18*). The precise measurement of the ratios of isotopes of a single element has many applications, including geochemical dating and the determination of patterns of air, water, and soil circulation.

Perhaps the most advanced application of REMPI for single-atom detection and isotopic ratio analysis is the very recent demonstration that 10^3 atoms of ^{81}Kr can be sorted out and counted in a sample along with 10^7 atoms of ^{80}Kr or ^{82}Kr plus 10^{12} other atoms or molecules, all in about an hour starting from a several liter gas sample (*36, 37*). Figure 3 is a diagram of the experimental setup, in which (i) a flash lamp–pumped dye laser is fired at krypton atoms condensed on a liquid helium–cooled finger to "bunch" them together in the ionization region (*38*); (ii) after a 7-μsec delay, the krypton atoms are resonantly ionized with a three-photon excitation scheme; (iii) the krypton ions are isotopically selected with a quadrupole mass filter of modest resolution; and (iv) the ions are accelerated to 10 kV onto a Be-Cu target, where they are implanted, while at the same time an electron multiplier with a gain of more than 10^6 counts each implanted ion by detecting the secondary electrons emitted by the target. Since the implanted krypton atoms remain "buried" in the Be-Cu target nearly indefinitely at room temperature, every istopically selected krypton atom will be counted once and only once (provided the sticking coefficient is unity). On the other hand, should a recount be desired, it is possible to expel all the inert gas atoms by heating an appropriately chosen target material under vacuum to an elevated temperature (*39*). This recycling technique may permit isotopic enrichment before final counting, if desired.

While past analytical applications of multiphoton ionization have concentrated almost exclusively on elemental analysis, there is no reason why the same principles will not permit single molecule detection. Already impressive advances have been made in detecting molecular hydrogen and its isotopic analogs (*40–42*), not only in identifying this species but also in determining the relative populations of individual quantum states. With regard to surface analysis, experiments have been reported on the REMPI detection of 10^{-8} to 10^{-10} of a monolayer of anthracene and naphthalene adsorbed on graphite (*23*). Once again it is possible to increase the sensitivity of the MPI detection scheme by accumulation of the molecules on a cooled substrate followed by pulsed desorption and photoionization above the surface (*43*). Extensive fragmentation of polyatomic ions is common in MPI (*1*), but often can be avoided by the choice of wavelengths and power levels. In either case, there is a great need to develop the necessary data base to allow these methods to become useful analytical tools. Perhaps it will also be possible to exploit the kinetic energy distribution of the photoelectrons, which carries a characteristic signature of the neutral species whose detection is sought.

Laser Fluorimetry

Multiphoton ionization rates are usually limited by the small cross sections (10^{-17} to 10^{-19} cm^2) for bound-to-continuum transitions. On the other hand, typical cross sections for strongly allowed

transitions are 10^{-11} to 10^{-14} cm^2. Thus, in cases where laser fluorescence is applicable, the effective detector volume often exceeds 10^3 times that for MPI, which may tip the balance in favor of laser fluorimetry for sensitive detection problems (*33*).

As in the case of MPI, maximum sensitivity is achieved by saturating the fluorescence transition. Such saturation effects may be particularly well suited for the detection of atomic species whose upper level reradiates nearly exclusively to the lower level being pumped. This may result in a "burst" of fluorescence photons when the atom passes through the laser beam (provided the radiative lifetime is short compared to the passage time). By recording such correlated fluorescence bursts a significant discrimination against background interference is achieved, allowing single-atom detection, particularly for single ions confined in a trap (*44–48*). However, saturated fluorescence may be less useful for the detection and quantitation of molecular species in which the upper level reradiates typically to many lower levels (*49*).

The detection of atoms by laser-induced fluorescence has been used to advantage in measuring atomic constituents, velocity distributions, and relative populations of excited state and fine structure levels of atoms sputtered from surface samples (*50*). Important applications of this work involve diagnostics for plasma devices and the determination of wall integrity of confined plasma sources.

Laser fluorimetry is also well suited for trace analysis of complex organic mixtures in solution (*51*), where a sensitivity limit approaching that of single-molecule detection may be possible (*52, 53*). For volatile organics, gas chromatography in combination with such detectors as mass spectrometry is often the analytical method of choice, but for nonvolatile organics, a standard method of separation is high-performance liquid chromatography (HPLC) (*54*). Unfortunately, HPLC generally has poorer resolution than gas chromatography, and so far it has not been easy to combine it with powerful detectors such as mass spectrometers, although laser desorption mass spectrometry in combination with thermospray sample deposition appears to hold much promise for that purpose (*55*). The separation power of HPLC can be significantly increased by the use of microcolumns, which are generally of three types: narrow-bore packed columns, packed capillary columns, and open tubular capillaries (*56*). However, each of these requires analysis of minute quantities of effluent from the microcolumn. Indeed, the burden is placed on detector methods to compensate for the lack of chromatographic resolution of HPLC, and the development of sensitive and selective detectors for this purpose may be the gating factor in the use of microcolumn HPLC. It is in this area that laser methods have the potential for making an important advance (*57*).

Because of their coherence properties, lasers can deliver to a far-field target a highly collimated beam of light with power levels in excess of 10 mW and in some cases in excess of 10 W. In contrast, a sample exposed to the 254-nm output of a 100-W mercury-arc source receives only ~5 mW, and possibly even less if this radiation cannot be efficiently coupled into a small detection volume. However, laser power alone does not necessarily improve the limits of detection in laser fluorimetry, because these limits are usually set by background interference from the sample on which the fluorescence signal of interest is superimposed. This background interference arises from scattering of the laser light from cell walls (*58*), elastic (Rayleigh) scattering of the laser light, inelastic (Raman) scattering of the laser light, and fluorescence from other interfering species. A number of groups are actively seeking ways to overcome these limitations. Clearly, one of the easiest improvements is to employ (nonfluorescent) filters that transmit the fluorescence but reject scattered light at other wavelengths. Conventional fluorimetric systems have an excitation bandwidth on the order of 10 nm, so that the Rayleigh and Raman components of the scattered light are at least of comparable width. On the other hand, laser sources are typically narrower than 0.1 nm, and the possible reduction of the width of the Rayleigh and Raman components may aid in their rejection. Further discrimination may also be achieved by placing a polarization analyzer in front of the fluorescence detector, since Rayleigh scattering and the stronger Raman features are generally polarized. Beyond these improvements, a number of other options are available.

One approach is to eliminate interference arising from cell walls by using a "windowless" fluorescence cell in which the flowing sample forms a suspended droplet or a free-falling jet at the exit of the column (*59–61*). Vibrations and multiple reflection at the droplet-air or jet-air interface may adversely affect the performance of this system, but it has been possible to detect as little as 20 fg (2×10^{-14} g) of fluoranthene in 10 μl of hexane eluting from a microcolumn (*61*).

Another alternative is to couple the capillary tube to an optical fiber, which can be placed very close to the focused laser beam to achieve excellent collection efficiency (*62, 63*). Because optical fibers have a critical cone of acceptance,

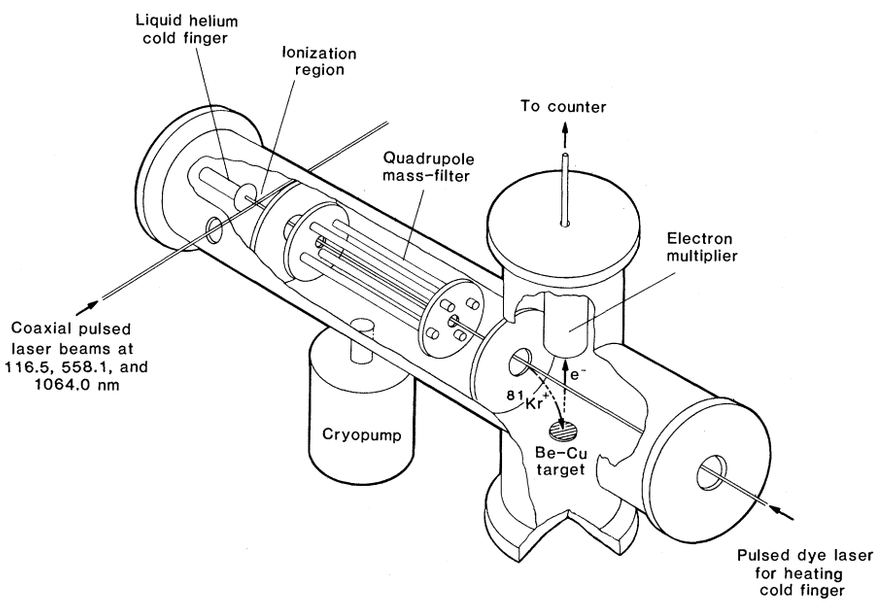

Fig. 3. Schematic diagram for the sorting and counting of ^{81}Kr atoms using the three-photon resonant multiphoton ionization scheme: Kr $4p^6$ + hν (116.5 nm) → $4p^5 5s\ [\frac{1}{2}]_1$ + hν (558.1 nm) → $4p^5 6p\ [\frac{1}{2}]_0$ + hν (1064.0 nm) → $Kr^+ + e^-$. This drawing is adapted from Chen *et al.* (*37*).

it is thus possible to reject scattering and fluorescence originating from the cell walls.

Yet another possibility is to use the hydrodynamic focusing technique employed in flow cytometry. Here the eluent is confined to the flow along the center of an ensheathing solvent stream under laminar flow conditions (*52, 53, 64, 65*). The laser excitation source is focused on the sample stream to provide a very small detection volume far removed from the cell walls. Because the sample stream is never in contact with the cell walls, molecular adsorption onto the walls is eliminated. By such means, as few as 22,000 dye molecules of aqueous rhodamine 6G have been detected in a probe volume of 11 pl during the 1-second time constant necessary to reduce the noise in the scattered light by signal averaging (*53*). At this detection limit, the probability of a single rhodamine 6G molecule being present in the probe volume is 0.6. It seems, then, that with further improvements, true single-molecule detection in condensed media is certainly within grasp.

Each of these flow cell designs has different advantages and drawbacks, and the actual detection limit depends, of course, on the species of interest, especially the relative and absolute spectral locations of the absorption and fluorescence "bands" and the nature of the background interference.

Two other laser schemes to overcome background interference may not be generally applicable, but where it has been possible to employ them the results have been extremely encouraging. One such scheme is two-photon fluorescence excitation, again made possible by the high powers available from pulsed lasers or tightly focused continuous-wave lasers (*66, 67*). This method provides additional spectral selectivity in HPLC detection based on the selection rules for two-photon transitions. In addition, excitation typically involves the use of visible lasers while fluorescence is observed in the ultraviolet, making the rejection of scattered light a very easy task. Another scheme is to employ time-resolved fluorescence detection, that is, to delay opening the detector until most of the scattered Rayleigh, Raman, and short-lived background fluorescence has passed by it (*68, 69*). Of course, this requires that (i) the analyte of interest have an intrinsically long-lived fluorescence or can be labeled with a long-lived fluorescent tag, and (ii) the excitation pulse be much shorter than the fluorescence lifetime, τ_f. Considerable success has been achieved. A detection limit of $1.8 \times 10^{-13} M$ for rubrene (τ_f = 17 nsec) has been obtained with a picosecond excitation source (*70*), and a detection limit of $2 \times 10^{-15} M$ was reported for the complex of europium (Eu^{3+}) with 1,1,1-trifluoro-4-(2-thienyl)-2,4-butanedione (τ_f = 420 μsec) with a 10-nsec pulsed nitrogen laser excitation source (*71*). Recently, time-resolved photon counting in conjunction with competitive binding fluorescence immunoassay has allowed human immunoglobulin G to be analyzed directly in serum-containing samples through the use of a long-lived fluorescence label (Tb^{3+}) attached to immunoglobulin G by a bifunctional chelating agent (*72*).

Application of laser fluorimetry to microbore HPLC is still far from routine but appears to hold much promise. This is illustrated in Fig. 4, in which 16 polynuclear aromatic hydrocarbons of low molecular weight are separated on a packed narrow-bore microcolumn (*73*). The upper trace was obtained with a 254-nm absorption detector, while the lower trace results from fluorescence excitation of the 5-nl volume with a 325-nm, 3-mW helium-cadmium laser viewed by a photomultiplier through a 430-nm interference filter (10 nm full width at half-maximum).

This chromatogram illustrates several features of laser fluorimetry. First, not all compounds are naturally fluorescent. This allows selective detection but may require that the analyte of interest be derivatized to incorporate a fluorescent tag. Second, when laser fluorescence analysis is applicable, the signal-to-noise ratio is far superior to that in ultraviolet absorbance, and readily permits picogram detection levels. Third, the laser system and detection optics required for laser fluorimetric detection with microcolumn HPLC are rather simple and inexpensive—far from the state of the art—so that such instrumentation should be widely applicable. For example, this technique is already being applied to a variety of biomedical problems, including dansyl amino acids (*74*), derivatized fatty acids (*74*), bile acids (*75*), and other lipids (*75*).

The examples above give only a glimpse into the exciting possibilities laser methods are opening to the analyst. Many other laser-related techniques (*76*) have gone unmentioned here because of space constraints. Nevertheless, it would seem that as the separation and analysis of mixtures of ever increasing

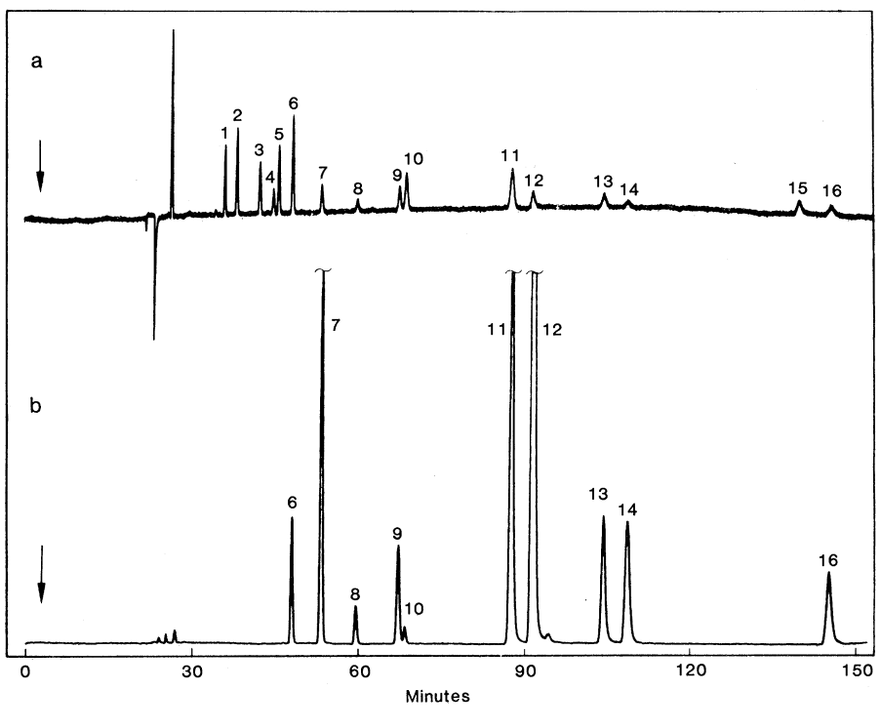

Fig. 4. Chromatograph (*73*) of polynuclear aromatic hydrocarbon standards obtained with a narrow-bore fused silica capillary (0.2 mm inner diameter, 1.33 m length) packed with Micropak-SP C18 (3 μm): (a) ultraviolet absorbance, and (b) laser fluorescence, 5 nl optical volume, excitation wavelength = 325 nm, fluorescence wavelength = 430 nm. The mobile phase is 92.5 percent aqueous acetonitrile at 1.2 μl/min. The solutes are: 1, naphthalene, 2.5 ng; 2, acenaphthylene, 5.5 ng; 3, acenaphthene, 2.5 ng; 4, fluorene, 0.5 ng; 5, phenanthrene, 0.2 ng; 6, anthracene, 0.2 ng; 7, fluoranthene, 0.5 ng; 8, pyrene, 0.2 ng; 9, benzo[*a*]anthracene, 0.2 ng; 10, chrysene, 0.2 ng; 11, benzo[*b*]fluoranthene, 0.5 ng; 12, benzo[*k*]fluoranthene, 0.2 ng; 13, benzo[*a*]pyrene, 0.2 ng; 14, dibenz[*a,h*]anthracene, 0.5 ng; 15, benzo[*ghi*]perylene, 0.5 ng; and 16, indeno(1,2,3-*cd*)pyrene, 0.2 ng.

complexity are demanded, laser techniques will continue to offer unique possibilities for achieving trace analysis with unprecedented sensitivity and selectivity. Moreover, laser techniques may permit ultratrace analysis to be carried out in practical situations in which matrix interference often proves to be the real limiting factor.

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