

Laser-Induced Electron Capture Mass Spectrometry

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Two techniques are reported for detection of electrophore-derivatized compounds by laser-induced electron capture time-of-flight mass spectrometry (LI-EC-TOF-MS). In both cases, a nitrogen laser is used to induce the electron capture. The analyte is deposited in a matrix consisting of a compound with a low ionization potential such as benzo[ghi]perylene in the first technique, where the electron for electron capture apparently comes from this matrix. In the second technique, the analyte is deposited on a silver surface in the absence of matrix. It seems that “monoenergetic” ions instantly desorb from the target surface in the latter case, since the peak width in the continuous extraction mode essentially matches the pulse width of the laser (4 ns). Ten picomoles of 3-O-(pentafluorobenzyl)- α -estradiol were detected at a S/N \geq 50, where the spot size of the laser was \sim 0.25% of the sample spot. It is attractive that simple conditions can enable sensitive detection of electrophores on routine TOF-MS equipment. The technique can be anticipated to broaden the range of analytes in both polarity and size that can be detected by EC-MS relative to the range for GC/EC-MS.

In electron capture mass spectrometry (EC-MS), analytes acting as electrophores are ionized by combining with a low-energy electron. From neutral analytes, this yields anionic products (as parent anion radicals or fragment anions) for detection. Most commonly the technique is practiced as gas chromatography electron capture mass spectrometry (GC/EC-MS), where the low-energy electrons usually are furnished by bombarding a dilute gas (e.g., 1 Torr of CH_4) with energetic electrons from a filament. Relying on the selected ion monitoring mode, one can routinely detect low-attomole amounts of strong electrophores as standards by GC/EC-MS with sector or quadrupole analyzers.¹

Polanyi and co-workers² introduced a technique in 1991 that we will refer to as “laser-induced electron capture mass spectrometry” (LI-EC-MS). In their method, which they studied additionally,³ chloromethanes deposited on a cold (near 100 K), polished silver (111) surface were subjected to laser desorption, yielding Cl^- ions that were detected by time-of-flight (TOF)-MS. Although

the resolution shown was low (peak width \sim 10 μs ; 193 nm laser), submonolayer detection was achieved. Remarkably, photons could be used with energies below the measured work function of silver. The authors concluded that electron capture took place by photoinduced, charge transfer of an electron from the silver to the adsorbed compound.

More recently, Reilly and co-workers,⁴ using “photoelectron capture negative ion mass spectrometry”, detected octafluoronaphthalene or *o*-dichlorobenzene deposited as a GC peak on a stainless steel surface. An argon fluoride laser (193 nm) was employed, and the resulting ions (mostly F^- and Cl^- , respectively) were detected by TOF-MS. Detection of 10 ng of the latter analyte was demonstrated. Kelly and co-workers⁵ detected electron capture product ions by subjecting nitrated polycyclic aromatic hydrocarbons (PAHs) to laser desorption (266 or 213 nm) TOF-MS, reporting a detection limit at the picomole level, with sensitivity dependent on the sample. Ehring et al.⁶ detected 7,7,8,8-tetracyanoquinodimethane as a molecular anion radical species by TOF-MS during the evaluation of this compound as a matrix material for matrix-assisted laser desorption/ionization (MALDI). Hurst and co-workers⁷ attempted, without success, to detect electrophore-labeled oligonucleotides by EC-MS in a MALDI-TOF-MS experiment using conventional matrixes.

Here we demonstrate that LI-EC-TOF-MS with ordinary equipment can provide sensitive, high-resolution detection of electrophore-derivatized analytes, including one that is at the edge or outside the range of GC/EC-MS.

EXPERIMENTAL SECTION

Materials. 4-[(4'-Methoxy-2,3,5,6-tetrafluorobenzyl)oxy]acetophenone was synthesized as described.⁸ 3-O-(Pentafluorobenzyl)- α -estradiol was prepared by vortexing a mixture of α -estradiol (0.1 mmol), K_2CO_3 (0.2 mmol), and tetrabutylammonium bromide (1 mg) in 1 mL of dimethylformamide for 1 h and then adding pentafluorobenzyl bromide (30 μL) and 3 drops of water. After

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stirring for 8 h at 60 °C, product (85% yield) was obtained by flash chromatography on silica with 1:3 ethyl acetate/hexane. Benzo[ghi]perylene and benzo[b]fluoranthene were from Aldrich. A PAH mixture (17 components, Z-014GR, 2 mg/mL in CH₂Cl₂:benzene) was obtained from AccuStandard (New Haven, CT).

Mass Spectrometry. The LI-EC-TOF experiments were carried out on a Bruker Daltonics Proflex laser desorption TOF-MS instrument in the linear negative mode, using a power density at the sample in the range of 10⁶–10⁷ W/cm² (attenuation setting 50; 4 ns pulse width). Operation parameters were as follows: accelerating voltage 20 kV; grid voltage 18.8 kV with delay time setting “short” (~100 ns) when matrix was used or 15.6 kV in the absence of matrix with no delay time. The CCA matrix stock was a saturated solution of α -cyano-4-hydroxycinnamic acid in 1:1 acetonitrile/water with 0.5% trifluoroacetic acid overall. Benzo[ghi]perylene or benzo[b]fluoranthene was dissolved in toluene to give a 0.01 M stock matrix solution. Analytes in ethyl acetate or acetonitrile (or the PAH mixture as supplied) were mixed with matrix (1:1 ratio) before loading onto the target and evaporation. Silver foil (0.125 mm, Aldrich) was cleaned by sonication in 10 mL of 5% ammonia for 1 min, soaking in distilled water (2× briefly), and sonication for 1 min in 10 mL of methanol followed by 10 mL of ethyl acetate. This removes [AgCl]_nCl[−] background ions seen by LI-EC-TOF-MS without such cleaning. The foil was attached onto the regular stainless steel target of the TOF-MS with Scotch double-stick tape (<0.1-mm thickness, No. 136, 3M Co.). It was always easy to find a “sweet spot” (<5 random locations tested routinely), and the signal intensity among the spots sampled usually varied <10-fold. One-microliter samples in acetonitrile or ethyl acetate (without matrix) were loaded onto the silver surface. All samples spread to a diameter of ~2 mm on the metal target.

RESULTS AND DISCUSSION

This project began with our observation that MALDI-TOF-MS of a standard mixture of PAHs in a conventional matrix (CCA) yielded two, even-mass peaks corresponding to molecular cation radicals for at least two of the compounds. These peaks and the PAHs apparently responsible for them were at *m/z* 276 (benzo[ghi]perylene) and 252 (three possible origins: benzo[a]pyrene, benzo[b]fluoranthene, and benzo[k]fluoranthene). We obtained pure samples of benzo[ghi]perylene and benzo[b]fluoranthene and confirmed that these two compounds yielded molecular cation radicals under these conditions. This meant that electrons were released from these compounds during the MALDI step, raising the possibility that the compounds themselves might be useful as matrixes for ionization of electrophore analytes by electron capture.

PAHs have been studied previously in laser desorption and MALDI mass spectrometry experiments, and cation radical species have been observed or postulated. Hercules and co-workers observed cation radicals when samples of PAHs were subjected to laser desorption (LD)-TOF-MS using 266-nm photons for the LD step.⁹ Juhasz and Costello observed cation radicals from polymetalocene analytes in the presence of matrixes comprising anthracene derivatives in a MALDI-TOF-MS experiment and

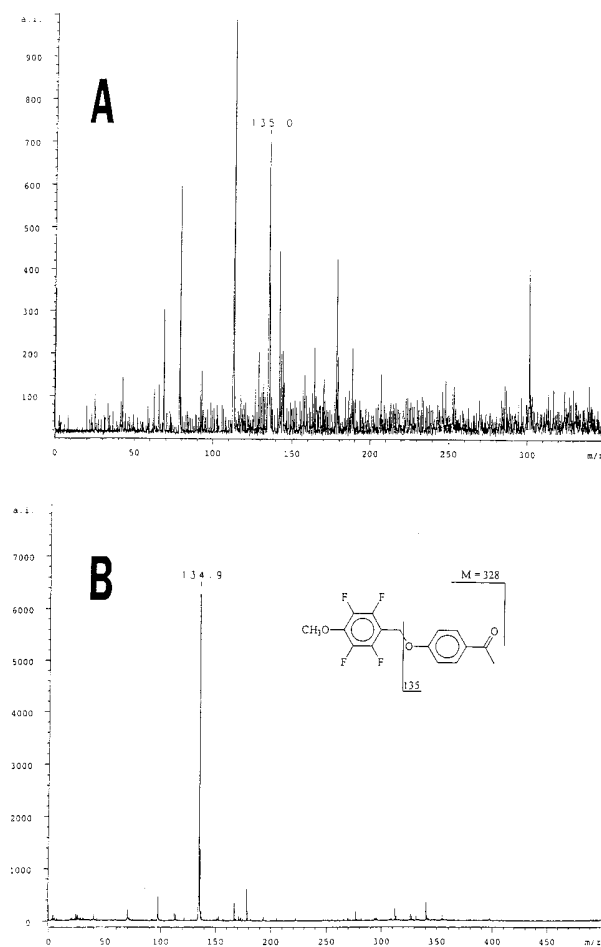


Figure 1. Detection of 4-[(4'-methoxy-2,3,5,6-tetrafluorobenzyl)oxy]acetophenone by LI-EC(matrix)-TOF-MS, with 20 laser shots. Sample deposition: 1 nmol in 1 μ L of ethyl acetate containing benzo[ghi]perylene matrix deposited onto a (A) stainless steel and (B) gold surface as a 2-mm spot size. Laser spot size 100 μ m.

considered that PAH cation radicals might be involved.¹⁰ Limbach and co-workers extended these observations to metallocene analytes with terthiophene and anthracene as matrixes and considered that primary cation radicals of these PAHs charge-exchanged with the metallocenes as the pathway to the metallocene cation radicals that were detected.¹¹

We tested 4-[(4'-methoxy-2,3,5,6-tetrafluorobenzyl)oxy]acetophenone (**1**) a ketone electrophore of interest in our laboratory,⁸ by MALDI-TOF-MS in both benzo[ghi]perylene (BGP) and benzo[b]fluoranthene as matrixes. The sample (7.1 nmol) was deposited as a 2-mm spot on a stainless steel target of the TOF-MS and subjected to 20 superimposed shots from a nitrogen laser, 100 μ m in diameter, corresponding to ~0.25% of the sample area. (Unless indicated otherwise, this spot size and laser beam diameter were used for all of the experiments described here.) We observed a peak for 4-acetylphenolate at *m/z* 135, as shown in Figure 1A, which is the same peak that dominates when this compound is subjected to GC/EC-MS. It seemed that the signal

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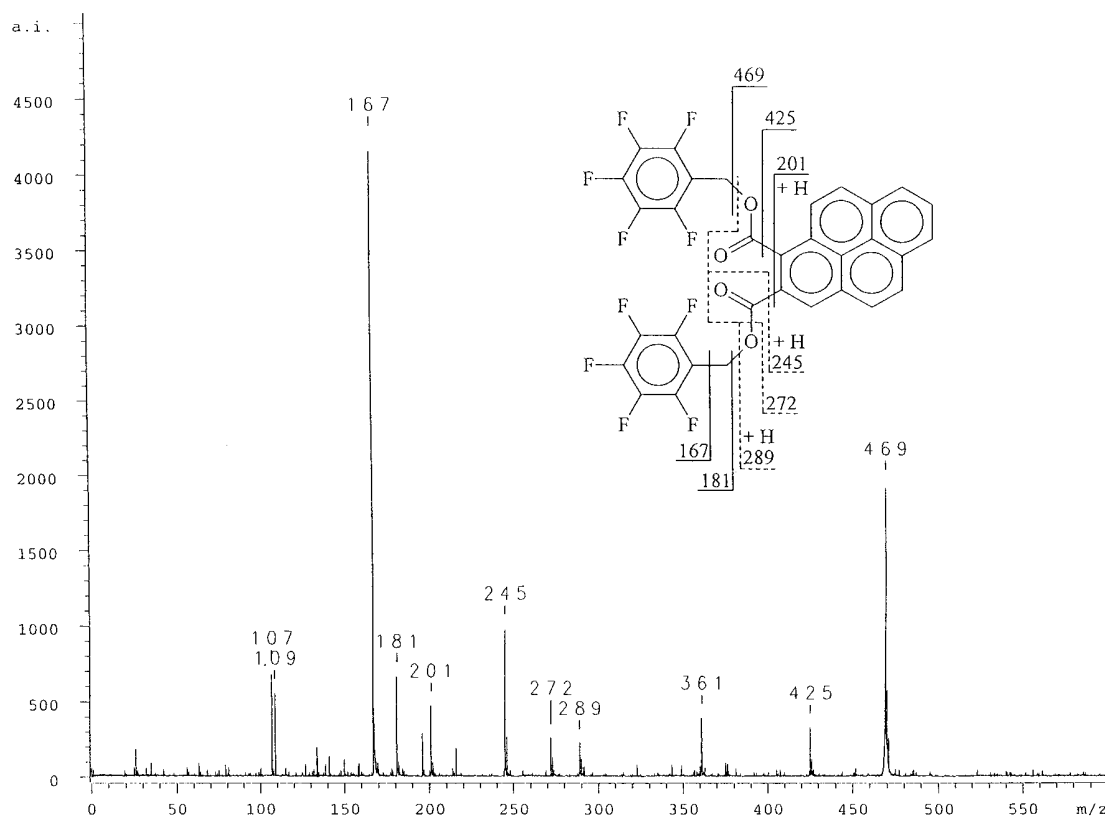


Figure 2. Detection of 2,3-bis(pentafluorobenzyl)pyrenedicarboxylate by LI-EC(Ag)-TOF-MS: 10 pmol on the target; 60 laser shots.

was slightly more reproducible and sensitive with BGP as a matrix, so we continued our work on matrix electron capture with this compound. We will designate this technique as "LI-EC(matrix)-TOF-MS."

Similar testing of **1** with BGP on a gold surface was done next. Relative to the highly polished stainless steel surface that we used initially, the unpolished gold was expected to be more absorptive and scattering for the photons, potentially releasing more electrons from the BGP. Further, the gold might act independently as a second donor of electrons. This experiment boosted the S/N by 100-fold, giving the mass spectrum shown in Figure 1B. We did not characterize this event in more detail.

We next tested **1** as an analyte on a silver surface based on the low work function of silver (4.26 eV). This technique, which we will designate as "LI-EC(Ag)-TOF-MS", also gave a strong response for the ion at m/z 135, yielding a spectrum (not shown) very similar to that shown in Figure 1B. Note that the photon energy of the nitrogen laser (3.68 eV) is significantly below the work function of silver. Subsequently we focused more on the EC(Ag) than the corresponding EC(matrix) technique, since the former precludes matrix ions. Nevertheless, in the long term, the analyte range of EC(matrix) might exceed that of the EC(Ag) technique, because of matrix-assisted desorption.

LI-EC(Ag)-TOF-MS of 10 pmol of 2,3-bis(pentafluorobenzyl)pyrene dicarboxylate (**2**), another electrophore of current interest in our laboratory,¹² gives the mass spectrum shown in Figure 2. As seen, considerable fragmentation takes place. The assignments of the masses are only tentative. This spectrum contrasts with

the simpler spectrum observed when **2** is tested by GC/EC-MS (data not shown). In the latter case, only three major ions are formed: m/z 196, 272, and 469. LI-EC(Ag)-TOF-MS spectra from single and multiple, superimposed laser pulses on **2** display the same fragmentation. Perhaps the more extensive fragmentation in the LI technique for this compound, relative to its behavior by GC/EC-MS, is a consequence of it undergoing both photon absorption and electron capture under the former conditions.

Can electrophores at the edge or outside of the range of GC/EC-MS be detected by LI-EC(Ag)-TOF-MS? A good response (S/N ≤ 50 for $\sim 0.25\%$ of the sample area in the laser beam) is observed from 10 pmol of a pentafluorobenzyl derivative of estradiol **3**, as shown in Figure 3. Because of the size and polarity of this compound, we would expect, at best, to detect it with poor sensitivity by GC/EC-MS, and only by using a new GC column (experiment not attempted). Consistent with our prior speculation that photon absorption in LI-EC-MS can promote fragmentation, only singular fragmentation is seen for **3**, a weak absorber at 337 nm.

Whenever LI-EC-MS spectra were obtained in the continuous extraction mode (delayed extraction off), all of the peak widths (essentially 4 ns) matched that of the laser pulse (4 ns). This performance significantly exceeds the specification of this parameter on the instrument employed (Bruker Daltonics Proflex TOF-MS). To rationalize this unusual performance, we speculate that "monoenergetic" ions instantly desorb. The energy for desorption could have several origins, including a thermal component since the power density of the laser is high.

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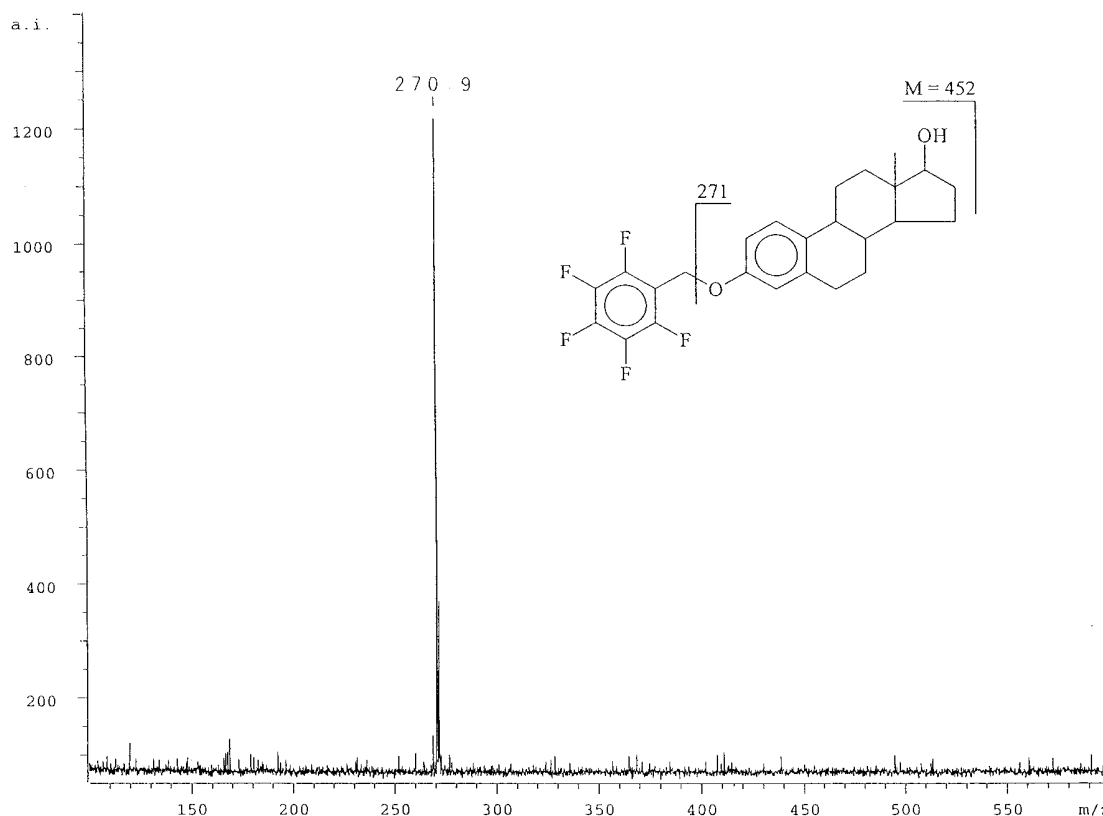


Figure 3. Detection of pentafluorobenzylated estradiol by LI-EC(Ag)-TOF-MS. Sample deposition: 10 pmol in 1 μ L of ethyl acetate giving a 2-mm spot. Laser spot size 100 μ m (0.25% of sample). Number of laser shots 50. S/N = 50

Although our experimental conditions are different from those used by Polanyi and co-workers² in their study of LI(Ag)-EC-MS, perhaps the mechanism they proposed takes place in our case as well: the adsorbed electrophore in LI-EC(Ag)-TOF-MS, when irradiated with a nitrogen laser, captures a photon-activated electron provided by the silver (and/or a contaminant thereon in our system), leading to an anion that desorbs. We cannot rule out a role for contaminants at this stage; it is difficult to remove contamination on Ag.¹³ Nevertheless, the lack (or near-lack) of a response when gold or polished stainless steel is substituted for silver (without matrix) argues against a critical, independent role for surface contamination. Also we cannot rule out thermal activation of the electrons.

In principle, photoelectrons (assuming this mechanism) could arise from two-photon absorption in our experiments, but then we would expect to observe more fragmentation of **3**, analogous to that observed by Reilly and co-workers with 6.5-eV photons.⁴ This should be confirmed by testing linearity of response vs photon dose.

For LI-EC (matrix)-TOF-MS, we believe that it is important to employ a matrix with a low ionization potential. Of the PAHs that we tested originally by MALDI-TOF-MS in a CCA matrix, the ones with the lowest ionization potentials (to the degree that values could be found) formed cation radicals. Apparently the electron for electron capture in EC(matrix) comes from the matrix. With this technique the resolution is higher with delayed extraction

on, consistent with a mechanism more similar to that in a conventional MALDI experiment.

Where will the Ag and matrix techniques for LI-EC-TOF-MS take us in sensitivity? It is interesting that Polanyi and co-workers generally observed a maximum response for a monolayer of chloromethane analytes^{2,3} and observed submonolayer detection. (The exception was CCl₄, where the response peaked at two layers.) We have yet to examine this regime. If electron activation for EC(Ag) and EC(matrix) is as inefficient as the production of photoelectrons (10^{-3}),¹⁴ then a high laser fluence may be necessary to fully ionize an adsorbed sample. However, this may cause thermal degradation of the analyte and/or excessive fragmentation from 2-photon absorption. Further, it is difficult to control contamination at the monolayer level, which could compete with analyte for critical adsorption sites, if the latter exist. Thus, further study is needed to determine the ultimate sensitivity.

LI-EC-TOF-MS nicely complements GC/EC-MS. This topic already has been discussed in part by Reilly and co-workers.⁴ While the GC step in the latter technique contributes chromatographic resolution, there are associated costs and complexities of the GC column, gases (carrier and buffer), and ionization cell (hot filament and walls, slits, cleaning, reproducibility). Most GC/EC-MS equipment entails the use of a quadrupole or sector analyzer, necessitating selected ion monitoring for highest sensitivity. In contrast, LI-EC-TOF-MS eliminates many of the above costs and provides a full mass spectrum. Further, it appears that

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the range of electrophoretic analytes in size and polarity for LI-EC-TOF-MS will exceed that of GC/EC-MS, although the degree remains to be defined. This also means that less derivatization might be used, at least in some cases, to achieve detection based on electron capture.

CONCLUSION

Using simple conditions and standard TOF-MS equipment, detection of strong electrophores based on ionization by electron capture has been achieved, with good sensitivity and resolution. Many conditions such as laser wavelength, laser waist relative to sample spot diameter, surface structure and composition of the sample plate (e.g., silver foil is a rough, contamination-prone surface), and choice of matrix may be critical not only for maximizing the sensitivity of LI-EC-TOF-MS but also for controlling analyte fragmentation. Much about the mechanism and performance of LI-EC-TOF-MS remains to be defined, and the advantages along with routine availability of TOF-MS equipment add to the importance of studying this new method further.

ACKNOWLEDGMENT

Research described in this paper was conducted under contract to the Health Effects Institute (HEI), an organization jointly funded by the United States Environmental Protection Agency (EPA) (Assistance Agreement X-816285) and certain motor vehicle and engine manufacturers. The contents of this paper do not necessarily reflect the views of HEI, or its sponsors, nor do they necessarily reflect the views and policies of EPA or motor vehicle and engine manufacturers. The work was also funded in part by NIST award 70NANB5H1038. We thank Rong Jian Lu for synthesizing the estradiol derivative. Contribution No. 780 from the Barnett Institute.

Received for review September 20, 1999. Accepted December 2, 1999.

AC991091P