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Ionization Techniques

# A Universal Ionization Label for the APLI-(TOF)MS Analysis of Small Molecules and Polymers**

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The drastically increased demand for qualitative and quantitative determinations of increasingly complex samples represents a tremendous challenge to modern instrumental analysis. Currently, for complex organic samples only chromatographic or electrophoretic separation with subsequent mass spectrometric detection (MS) fulfills these requirements. For MS analysis coupled with separation by gas chromatography (GC), liquid chromatography (LC), or capillary electrophoresis (CE), the ionization of the separated analyte molecules must be as quantitative as possible. Different ionization methods have to be used for analytes of different molecular weight and polarity.[1]

For polar compounds, electrospray ionization (ESI) is the gold standard with respect to mass spectrometric analysis of small molecules and, because of multiple charging, of large biomolecules, as well.[2] Analytes of up to several thousand daltons (kDa) and of moderate polarity are favorably ionized by atmospheric-pressure chemical ionization (APCI),[3] while atmospheric-pressure photo-ionization (APPI) and dopant-assisted (DA)-APPI are usually applied for the ionization of non-polar substances.[4] Atmospheric-pressure laser ionization (APLI), recently developed by our groups, shows an outstanding sensitivity for moderately to non-polar aromatic compounds.[5] This selectivity towards aromatic compounds arises from the ionization mechanism of APLI: Multiphoton excitation of matrix compounds is minimized by adjusting the laser power density close to the threshold of resonantly enhanced $(1+1)$ multiphoton excitation, in other words, to roughly 1 MW cm$^{-2}$. Linear absorption of most matrix compounds becomes negligible when photons with a wavelength of 248 nm are used for excitation. Readily available small-footprint excimer lasers are sufficiently powerful light sources. With respect to efficient resonant two-photon ionization, the spectroscopic features of aromatic hydrocarbons are rather unique: They display strong linear absorption cross sections at 248 nm, long-lived intermediate electronic states, and highly vertical ionization transitions. Since hardly any other compound class exhibits such features, APLI is specific for arenes. On the other hand, this specificity may limit analytical applications. However, MS analysis of complex samples would largely benefit from selective ionization of analytes since ion suppression and the resulting failure in the quantitative determination is considerably reduced.[6] Currently this is only possible either with a high-performance preseparation by means of hyphenated techniques, such as GCxGC, LCxGC, and LCxLC,[7] which ensure a baseline separation of all matrix components, or with stable-isotope-labeled standards (SILS).[8] Hyphenated techniques, though, are expensive, time-consuming, and maintenance-intensive, while SILS are expensive and available only for a rather limited number of analytes.

In this work we present a derivatization strategy that facilitates selective ionization of polar and non-polar compounds in complex matrices without hyphenated techniques or SILS. With this procedure the analytes are detected by the mass analyzer without noticeable interference from the matrix. For analytical applications, derivatizations are widely used. As an example, methylation and silylation reagents are used to facilitate GC analysis of organic acids and alcohols.[9] In LC and CE, fluorescence markers are often applied to label analyte molecules in complex samples to realize a selective and also highly sensitive detection.[10] However, when several analytes must be derivatized with a single fluorescence marker, quantitative analysis requires correction factors to take into account the negative influence of the analytes on the fluorescent properties of the marker (fluorescence quenching).[10]

To our knowledge, with the exception of applications in REMPI-MS,[11] derivatizations are used in MS analysis only 1) to form GC-accessible analytes and 2) to increase the ionization efficiency.[9] In each case there is a drawback: the requirement of a time-intensive calibration or the addition of a SILS for a quantitative determination. Here, we show an alternative approach to circumvent this problem. In analogy to fluorescence markers, APLI markers (anthracene-9-yl-methoxyacetic acid (**1**) and anthracene-9-ylmethanol (**2**)) are used to derivatize alcohols, amines, and organic acids (Scheme 1) for analysis by APLI as described above. Starting with the commercially available alcohol **2**, carboxylic acid **1** was synthesized by reaction with bromoacetic acid.[12]

APLI is a soft ionization method. Therefore, the spectra in Figure 1 always show the signals of the radical cations as the base peak of the derivatization products of **1** or **2** with ethanol, cholesterol, dodecyl amine, and oleic acid. There are

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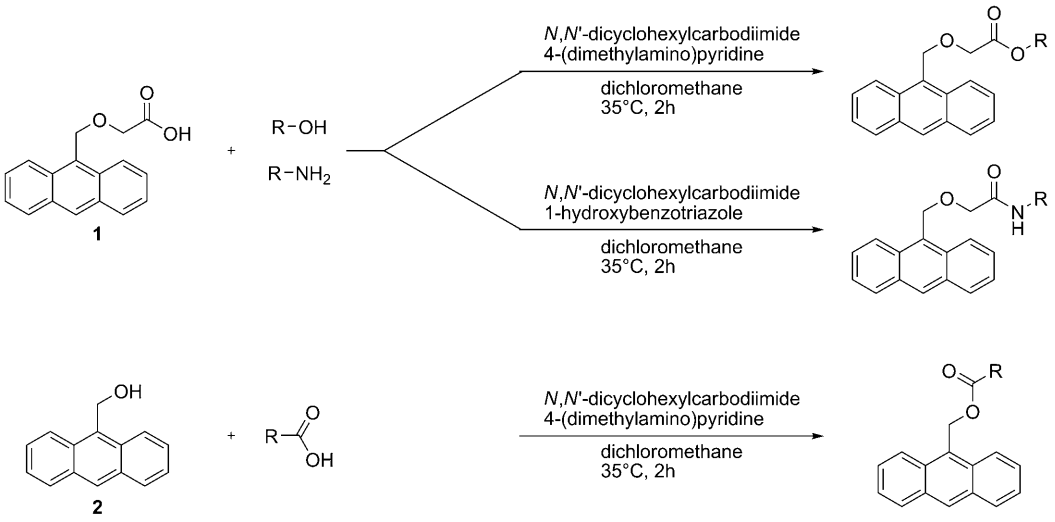



***Scheme 1.*** Derivatization of acids, amines, and alcohols with the APLI ionization labels.

only minor signals that correspond to impurities and a few fragments (e.g. *m*/*z* 191).

To assess the spectroscopic properties of selected compounds, atmospheric-pressure (AP)-REMPI spectra (Figure 2) were recorded for anthracene, **1**, and the derivatives listed in Figure 1 a–c. The tunable light source was a ns-Nd:YAG laser pumped, frequency-doubled optical parametric oscillator with a spectral resolution of 0.04 nm at 248 nm. The envelopes of the REMPI spectra of the derivatized analytes differ only marginally from the spectrum of the ionization label **1**. The comparison of the spectrum of anthracene with spectra of **1** and labeled analytes suggests that the presence of a C atom next to the ring system shifts the spectrum to the red, whereas further distant groups do not significantly affect the absorption features. It thus follows that the ionization yield for labeled analytes is virtually identical to that of the ionization marker. Naturally, the derivatization yield can be different for different analytes, but instead of expensive SILS, one or more homologous compounds can be added to the sample in a known amount, because similar derivatization yields are usually achieved in a homologous series. This was verified by the analysis of three fatty acids, palmitic, heptadecanoic, and stearic acid. Equimolar amounts of each acid were derivatized with **2** and analyzed with APLI-(TOF)MS. The detected counts of these acids were 155513, 150620, and 153296, respectively, which results in an average of $153343 \pm 2493$ (1.6% standard deviation). The derivatization yield for these compounds was 67%.

Figure 3 presents the analysis of an ethoxylated 1-octadecanol (Brij 72) with APLI and MALDI. For APLI the sample was simply dissolved in dichloromethane, derivatized with **1**, and injected with a syringe into the source without further treatment. The resulting spectrum (Figure 3 a) shows the signal pattern expected for this homologous polymer in the case of comparable derivatization yields; this distribution was verified by the MALDI experiment with 2,5-dihydroxybenzoic acid as the matrix and silver trifluoroacetate as the cationizing agent (Figure 3 b). As a further example we analyzed the free fatty acids in sunflower oil by dissolving an aliquot of the oil in dichloromethane and esterifying with **2** (Figure 4). Heptadecanoic acid was used as an internal standard, which allows the quantitative determination of free fatty acids in the sample. Table 1 lists the results in comparison with the total acid concentration determined by DIN EN ISO 660.

***Table 1:*** Analysis of free fatty acids in sunflower oil.

| Fatty acid | Content of free fatty acid [%] | |
|---|---|---|
| | APLI-(TOF) MS | DIN EN ISO 660 |
| palmitic acid | 0.14 | n.d.[a] |
| linoleic acid | 0.80 | n.d.[a] |
| oleic acid | 0.40 | n.d.[a] |
| stearic acid | 0.04 | n.d.[a] |
| total acid concentration | 1.38 | 1.47 |

[a] n.d.: not detectable.

Free fatty acids are frequently encountered as undesirable by-products of the synthesis and processing of fatty alcohols, and their total concentration is routinely determined by means of the acid number.[13] Often, however, knowledge of the concentration and identity of the individual free fatty acids is necessary to optimize the process. Unfortunately, fatty acids present in a fatty alcohol matrix cannot be derivatized with **2** by the synthetic route described since the fatty alcohols compete with the ionization marker. Therefore, we used 9-diazomethylanthracene (**3**), which is also known as a fluorescence marker for fatty acids,[14] in this work as an ionization label (Scheme 2). Under the conditions described in the literature, reaction with the fatty alcohols is not possible.[14] To determine the content of fatty acids in a fatty alcohol matrix of an industrial product, the sample was derivatized and analyzed twice by gas chromatography coupled to APLI-(TOF)MS. The source of the product was a coconut oil. Generally even-numbered chain lengths are found in plant-based products. Therefore, instead of an expensive SILS, a fatty acid with an odd-numbered chain length, heptadecanoic acid, was used as an internal standard. Table 2 shows the

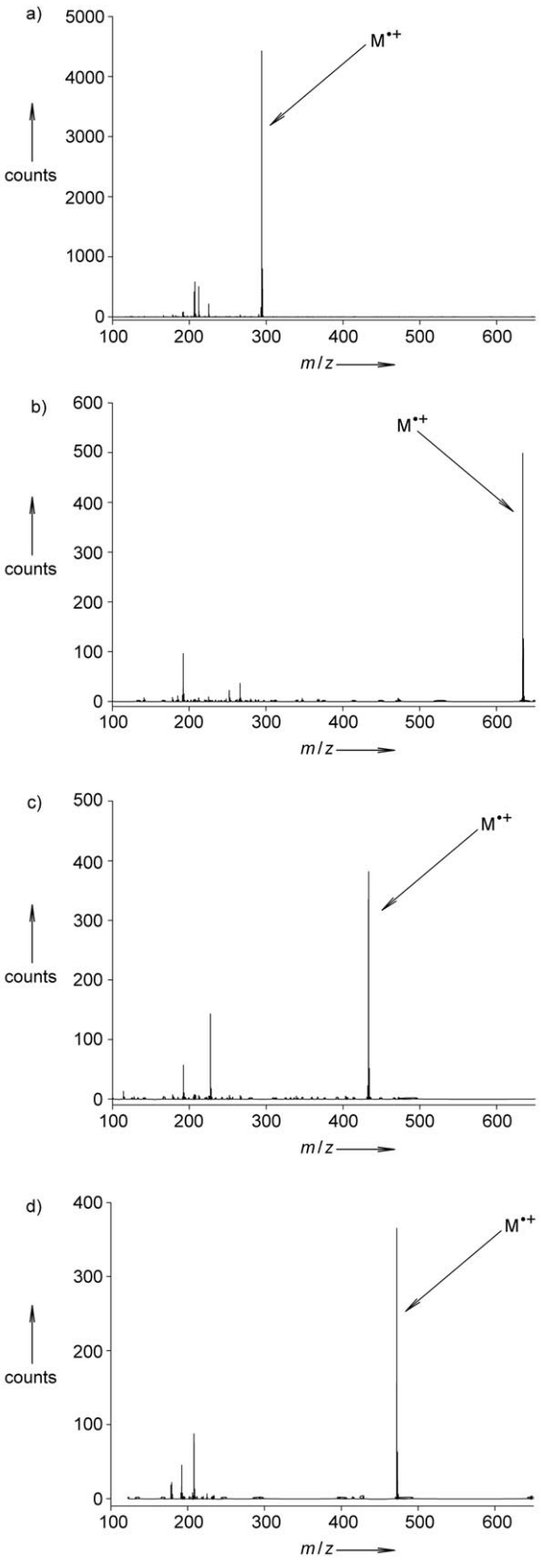



***Figure 1.*** APLI-(TOF)MS of a) ethanol, b) cholesterol, c) dodecyl amine, and d) oleic acid after derivatization with **1** or **2** and direct injection by syringe pump.

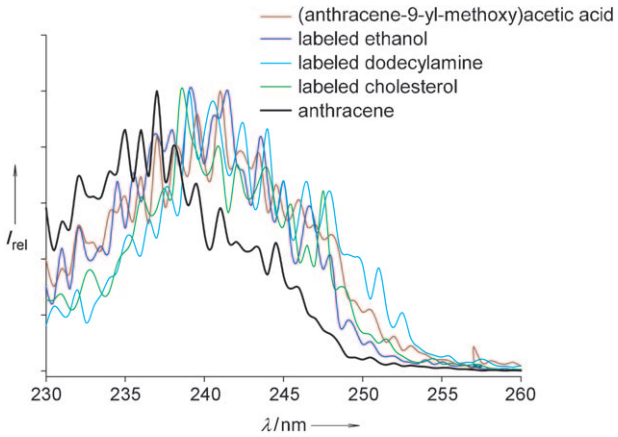



***Figure 2.*** Comparison of various AP-REMPI spectra.

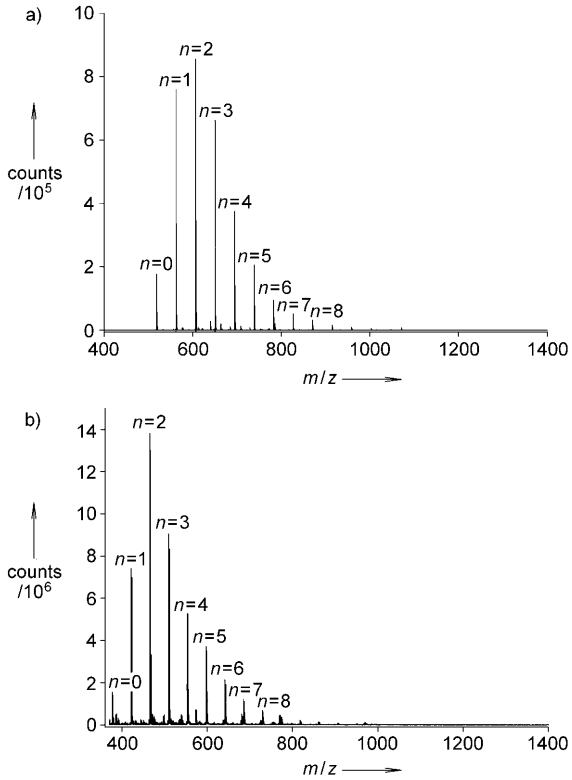



***Figure 3.*** Analysis of Brij 72 with a) APLI and b) MALDI with 2,5-dihydroxybenzoic acid as matrix and silver trifluoroacetate as cationizing agent. While the signals in APLI-(TOF)MS arose from the radical cations, in MALDI-(TOF)MS the silver adducts of the analytes were formed.

results ($n = 2$) and the total acid concentration specified by the company. Because of the low acid concentration a comparison with DIN EN ISO 660 was not possible.

In summary, we have demonstrated that derivatization strategies significantly broaden the analytical applicability of APLI-MS. First, the range of ionizable analytes is not restricted by their spectroscopic features (and thus aromatic hydrocarbons) but solely by the presence of reactive anchor groups and the availability of suitable APLI labels. We are currently developing synthesis strategies for various APLI

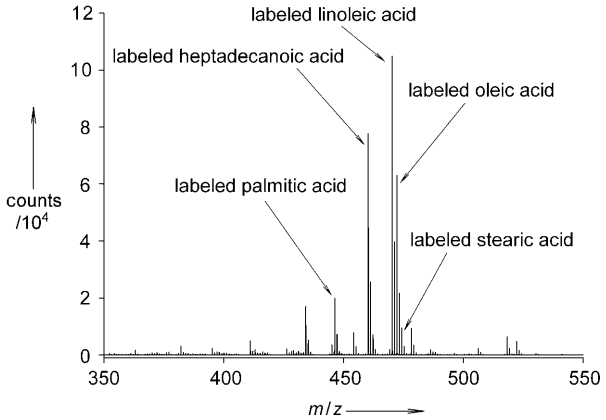
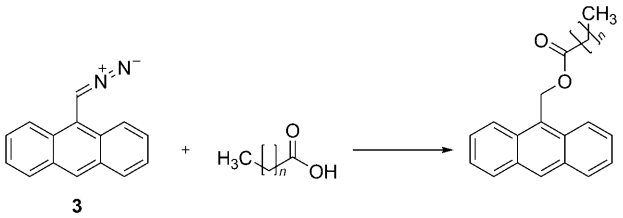



***Figure 4.*** Analysis of free fatty acids in sunflower oil after derivatization with **2** and direct injection by syringe pump.



**3**

***Scheme 2.*** Derivatization of fatty acids with 9-diazomethylanthracene.

***Table 2:*** GC-APLI-(TOF)MS of free fatty acids in an industrial product.

| Fatty acid | Content of free fatty acid [%] |
|---|---|
| dodecanoic acid | $1.05\times10^{-3}\pm2.3\times10^{-5}$ |
| tetradecanoic acid | $2.23\times10^{-4}\pm7.4\times10^{-5}$ |
| hexadecanoic acid | $1.86\times10^{-4}\pm2.2\times10^{-5}$ |
| octadecanoic acid | $2.78\times10^{-4}\pm2.8\times10^{-5}$ |
| total acid concentration | $1.73\times10^{-3}\pm4.6\times10^{-5}$ |
| specified value | $0–3.6\times10^{-2}$ |

labels that specifically target reactive analyte sites. Secondly, once tagged with a specific APLI active label, the complex exhibits spectroscopic features virtually identical to those of the label itself. This leads to the discussed advantages for the quantitative analysis of compounds in complex matrices. Research is currently underway to extend the derivatization strategy towards GC-APLI-MS.

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[1] H. Hayen, U. Karst, *J. Chromatogr. A* **2003**, *1000*, 549 – 565.
[2] R. B. Cole, *Electrospray Ionization Mass Spectrometry: Fundamentals, Instrumentation and Applications*, Wiley, New York, **1997**.
[3] W. M. A. Niessen, *Liquid Chromatography—Mass Spectrometry*, Marcel Dekker, New York, **1999**.
[4] a) J. A. Syage, M. D. Evans, K. A. Hanold, *Am. Lab.* **2000**, *32*, 24 – 29; b) J. A. Syage, M. D. Evans, *Spectroscopy* **2001**, *16*, 15 – 21; c) D. B. Robb, T. R. Covery, A. P. Bruins, *Anal. Chem.* **2000**, *72*, 3653 – 3659.
[5] a) M. Constapel, M. Schellenträger, O. J. Schmitz, S. Gäb, K. J. Brockmann, R. Giese, T. Benter, *Rapid Commun. Mass Spectrom.* **2005**, *19*, 326 – 336; b) S. Droste, M. Schellenträger, M. Constapel, S. Gäb, M. Lorenz, K. J. Brockmann, T. Benter, D. Lubda, O. J. Schmitz, *Electrophoresis* **2005**, *26*, 4098 – 4103; c) R. Schiewek, M. Schellenträger, R. Mönnikes, M. Lorenz, R. Giese, K. J. Brockmann, S. Gäb, T. Benter, O. J. Schmitz, *Anal. Chem.* **2007**, *79*, 4135 – 4140; d) M. Lorenz, R. Schiewek, K. J. Brockmann, O. J. Schmitz, S. Gäb, T. Benter, *J. Am. Soc. Mass Spectrom.* **2008**, *19*, 400 – 410; e) P. Schmitt-Kopplin, M. Englmann, R. Rossello-Mora, R. Schiewek, K. J. Brockmann, T. Benter, O. J. Schmitz, *Anal. Bioanal. Chem.* **2008**, *391*, 2803 – 2809; f) W. Schrader, S. K. Panda, K. J. Brockmann, T. Benter, *Analyst* **2008**, *133*, 867 – 869; g) R. Schiewek, M. Lorenz, R. Giese, K. J. Brockmann, T. Benter, S. Gäb, O. J. Schmitz, *Anal. Bioanal. Chem.* **2008**, *392*, 87 – 96.
[6] a) T. M. Annesley, *Clin. Chem.* **2003**, *49*, 1041 – 1044; b) K. Georgi, K. S. Boos, *Chromatographia* **2006**, *63*, 523 – 531; c) L. L. Jessome, D. A. Vomer, *LCGC North Am.* **2006**, *24*, 498 – 510.
[7] a) Z. Liu, J. B. Phillips, *J. Chromatogr. Sci.* **1991**, *29*, 227 – 231; b) T. Jiang, Y. Guan, *J. Chromatogr. Sci.* **1999**, *37*, 255 – 262; c) M. M. Bushey, J. W. Jorgenson, *Anal. Chem.* **1990**, *62*, 161 – 167.
[8] D. G. Burke, L. G. Mackay, *Anal. Chem.* **2008**, *80*, 5071 – 5078.
[9] a) K. Vosmann, E. Klein, N. Weber, *J. Chromatogr. A* **1997**, *773*, 239 – 247; b) M. Morvai-Vitányi, I. Molnanperl, D. Knausz, P. Sass, *Chromatographia* **1993**, *36*, 204 – 206.
[10] O. Schmitz, C. Wörth, D. Stach, M. Wießler, *Angew. Chem.* **2002**, *114*, 461 – 464; *Angew. Chem. Int. Ed.* **2002**, *41*, 445 – 448.
[11] a) M. Fernandes-Whaley, F. Mühlberger, A. Whaley, T. Adam, R. Zimmermann, E. Rohwer, A. Walte, *Anal. Chem.* **2005**, *77*, 1 – 10; b) J. R. Srinivasan, L. J. Romano, R. J. Levis, *J. Phys. Chem.* **1995**, *99*, 13272 – 13279.
[12] N. Ouwerkerk, J. H. van Boom, J. Lugtenburg, J. Raap, *Eur. J. Org. Chem.* **2000**, 861 – 866.
[13] S. Mahajan, S. K. Konar, D. G. B. Boocock, *J. Am. Oil Chem. Soc.* **2006**, *83*, 567 – 570.
[14] S. A. Barker, J. A. Monti, S. T. Christian, F. Benington, R. D. Morin, *Anal. Biochem.* **1980**, *107*, 116 – 123.

  