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Application News

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MALDI-TOF Mass Spectrometry

High-speed characterization of candle waxes using SALDI-MS with etched silver foil as substrates

Matrix-Assisted Laser Desorption/Ionization Time-of-flight Mass Spectrometry (MALDI-TOF MS) facilitates a simple and quick analysis to obtain molecular weight information on diverse types of samples from small to macromolecules. Thus, there is a wide application range for MALDI-TOF MS instruments in R&D and quality control of synthetics and biological molecules.

In conventional MALDI, the sample is co-crystallized with a matrix. For different analyte classes various matrices are available to support an efficient desorption and ionization process.

Here, we show a slightly modified method that goes without solvation step and therefore is even quicker compared to conventional MALDI. The matrix is replaced with an etched silver surface and the name of the technique is referred to as Surface-Assisted Laser Desorption/Ionization (SALDI)-TOF MS although the instrument is still a MALDI-TOF. In addition to a decreased sample preparation time, further analyte classes can be obtained with this method. As an example of complex lipid mixtures we analyzed different candle waxes.

The content of beeswax in candles is an important quality criterium often used in marketing. Due to the high price of beeswax, it is often replaced by cheaper alternatives like paraffin wax. Stearin is seen as renewable and therefore green alternative to paraffin as it is made of plant material and not of petroleum. To check marketing slogans, these main components can be detected due to the characteristic paraffin wax compounds produced by bees or plants.

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Sample preparation

For etched silver substrates, silver foil was cut, washed with pentane, acetone, methanol and water, flattened and etched in nitric acid (23 %) at 50 °C, until the surface appearance changes to grey. Finally, substrates were washed with water and stuck with conductive tape on a MALDI target.[1,2]

Wax was directly stuck from the candle on the silver substrate and analyzed with the MALDI-8020 benchtop linear MALDI-TOF mass spectrometer. To characterize the method, standards and dissolved candle samples were also analyzed.

With the statistical software eMSTAT Solution™ main components of the candle were determined and visualized in a score plot. The complete workflow is shown in figure 1.

Mass spectra of different lipid classes

The compounds of candles are detected as Ag^+-adducts. The range of lipid classes that can be observed by this method covers even alkanes although these fully saturated hydrocarbons do not possess any functional group. Their detection is only limited by the vapor pressure of these volatile compounds meaning that alkanes with 23 or more carbon atoms can be analyzed. Even shorter chain-lengths can be seen in some spectra, if the sample is analyzed directly after introduction into the mass spectrometer (figure 2).

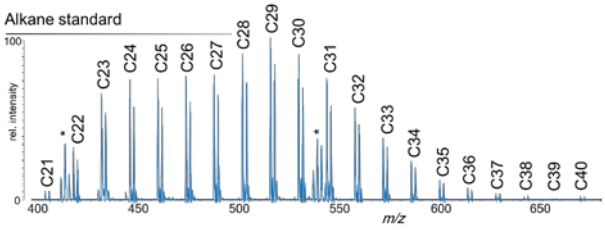

Fig. 2 Alkane mixture (GC-standard, same mass of each component)
Asterisks (*) indicate silver clusters.

The natural origin of beeswax can be verified by the characteristic profile that are exclusively produced by these insects (figure 3). It consists essentially of wax esters with an overall even number of carbon atoms[1,3] and of saturated or monounsaturated hydrocarbons with odd number of carbon atoms.[1,4]

Fig. 1 Workflow: Stuck wax directly from candle → Analyze in MALDI mass spectrometer → Match in statistical software

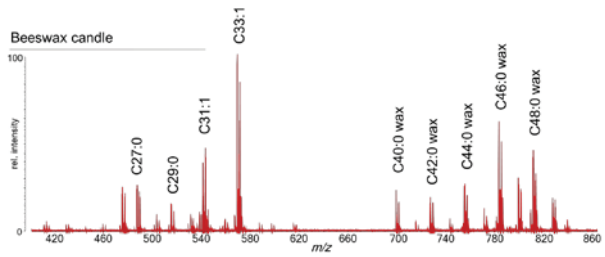

Fig. 3 Beeswax candle (already described[1,3,4] components annotated)

Stearin candles are made from vegetable or animal fat after saponification. Therefore they are seen as renewable – green alternative to petroleum. Stearin candles show a characteristic profile dominated by two free fatty acids (FFA): Palmitic and Stearic acid.

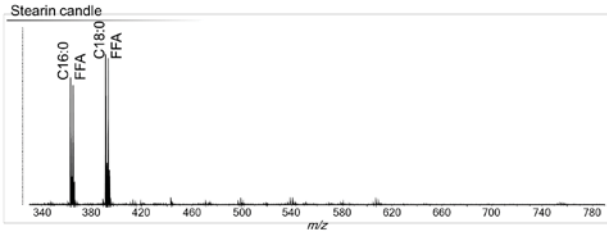

Fig. 4 Stearin candle (Palmitic acid (C16) and Stearic acid (C18))

Paraffin is the cheapest and therefore most common resource to produce candles. Due to their origin in petroleum, alkanes with all different chain lengths of the used fraction are present in the mass spectrum.

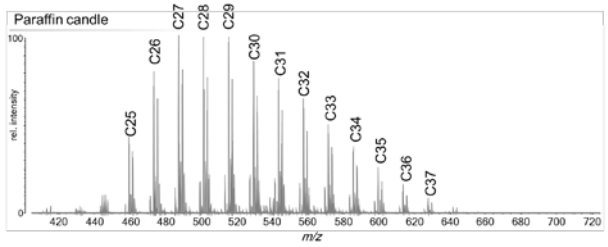

Fig. 5 Paraffin candle (alkanes from C25-C37).

These three examples show the large range of different lipid classes that can be analyzed with SALDI-MS using etched silver foil as substrates: (i) Alkanes without any functional group which are classically analyzed via GC and, (ii) more polar and less volatile lipids like FFA and wax esters that are commonly not suggested to be analyzed via GC without derivatization. As no chromatographic separation is necessary, this method is much faster.

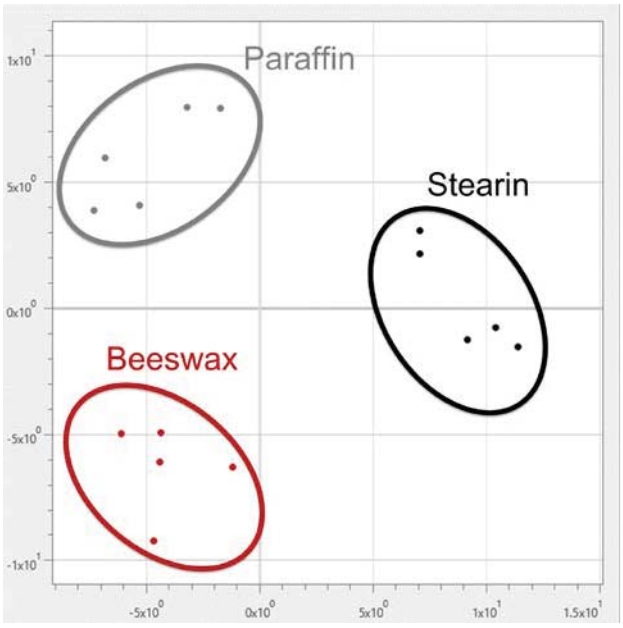

Fig. 6 Score plot in statistical software eMSTAT Solution

Main component determination with statistical software

With a statistical software like eMSTAT Solution, the main components can be determined. With multivariate analysis, the different raw materials in the candles can be separated (figure 6). Discriminate analysis gives the main component and rates the similarity with a score.

Conclusion

This application shows the potential of the method to analyze complex lipid mixtures that contain both hydrocarbons and less volatile compounds that are challenging to detect with GC-MS. Possible application fields are the analysis of technical waxes, petroleum, and insect pheromones.
The method is very fast because neither a chromatographic separation nor a derivatization is needed and it is not even necessary to dissolve the sample.
Statistical software as eMSTAT Solution can help to correlate sample spectra to reference spectra.

References

(1) Schnapp et al., *Methods*, 2016, 104, 194-203.
(2) Bien et al., *Anal Bioanal Chem*, 2019, 411 (13), 2981-2993.
(3) Fröhlich et al., *J Chem Ecol*, 2000, 26, 123-137.
(4) Ferreira-Caliman et al., *J Chem Ecol*, 2012, 38, 418-426.

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