Bruker Daltonics

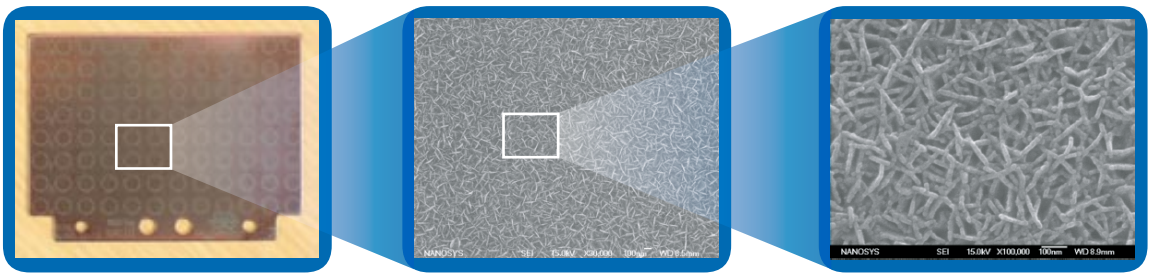

Fig. 1: NALDI target plate. The figure shows the NALDI target plate (left) and increasing magnification SEM images (center and right) of the nanostructured coating that provides the active surface for desorption/ionization of deposited analytes.

Technical Note # 22

Analyzing Small Molecule Drug Compounds by NALDI™ Mass Spectrometry

A novel nanostructured target plate removes the need for adding chemical matrices for the analysis of small molecules. This technology opens the door to routine analysis of pharmaceutical compounds by MALDI-TOF and TOF/TOF instrumentation reaping the benefits of speed and sensitivity offered by these analytical instruments. This note describes the ease of use of these new target plates and demonstrates the sensitivity that can be achieved in MS or MS/MS mode on Bruker Daltonics MALDI-TOF instruments.

Introduction

Matrix-free LDI-MS analysis of small molecules has not been widely adopted due to the non-optimal performance of surfaces tested to date. We have developed a novel matrix free target substrate which has exceptional performance termed Nano-Assisted Laser Desorption/Ionization (NALDI™) targets (Fig. 1) ref (1)(2). We have demonstrated sensitive detection of small molecules in the MS and MS/MS modes on Bruker Daltonics MALDI-TOF instruments using purified drug molecules or sample mixes that were deposited onto the sample plate and analyzed by MALDI-TOF and TOF-TOF instruments.

Mass spectral data have been obtained for a variety of different small molecules both alone and when mixed at concentrations as low as 100 femtogram/µL. Quantitation to <1pg sensitivity using MS and 1 pg in MS/MS modes has been demonstrated. Dramatically reduced background in the low mass region is observed, especially when compared to the background obtained from matrix in a MALDI-MS analysis. Stability studies show that the performance of this substrate is not significantly altered after at least 12-weeks

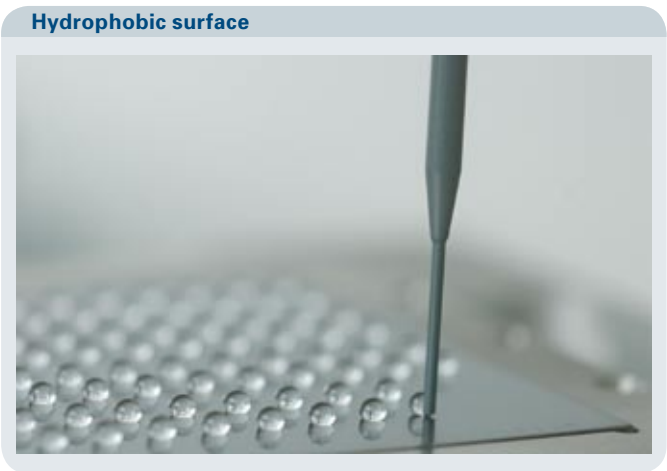

Fig. 2: The hydrophobic nature of the nanostructured NALDI surface allows sample solutions to be placed easily within the marked circles and allows concentrated drying of the sample into the center of the spot.

Diverse drug panel

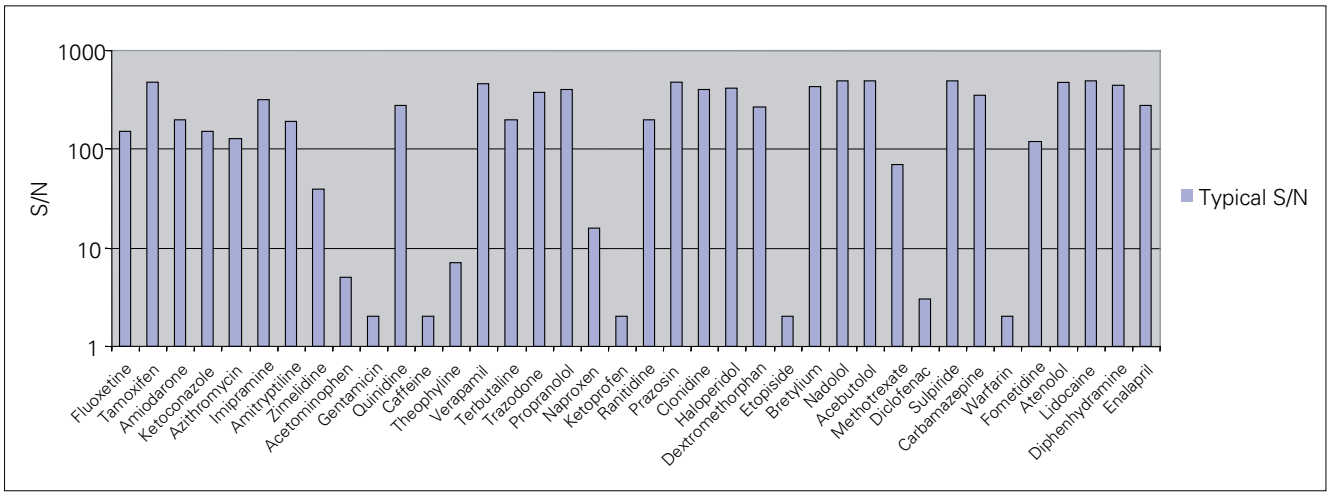

Fig. 3: 1ul of a 10pg/ul sample of each of the drug panel was spotted onto the NALDI target plate and analyzed. The bar graph shows the typical signal: noise opbtained for the peak corresponding to the molecule analyzed.

storage at 60ºC in a plastic box. No special preparation of the plate surface is required before or after sample deposition and multiple different solvents can be used for applying the sample either in purified form or by depositing from an LC-column.

Materials and methods

Spotting of drugs onto NALDI surface

In the data described in this note, unless stated otherwise, the small molecule analytes were diluted in 50% water/acetonitrile from a stock solution (500ug/ml in methanol). All small molecules were obtained from Sigma-Aldrich and all solvents used were HPLC grade. No organic matrix was added to the analyte solutions and no pretreatment of the NALDI target plate surface was required. In general, molecules were spotted at the indicated dilution in a total volume of 1ul which was applied directly to the center of a laser etched circle and allowed to dry. The surface of the NALDI target plates is hydrophobic and therefore it is easy to isolate volumes as large as 3ul within the laser spots (Fig 2).

Drug panel analysis

38 drugs selected for their variation in molecular weight and chemical structure were tested to determine any potential limitations of molecule desorption and ionization from NALDI surfaces. All molecules were diluted from 0.1 to 100pg/ul in 50% acetonitrile/water from a stock solution of 500ng/ul in methanol. 1 ul of each molecule was spotted onto a predefined circle on a NALDI plate and allowed to dry. Plates were then analyzed on a Bruker Ultraflex II or an Autoflex II in single MS mode at a laser frequency of up

Sub-femtomol detection of small drug molecules

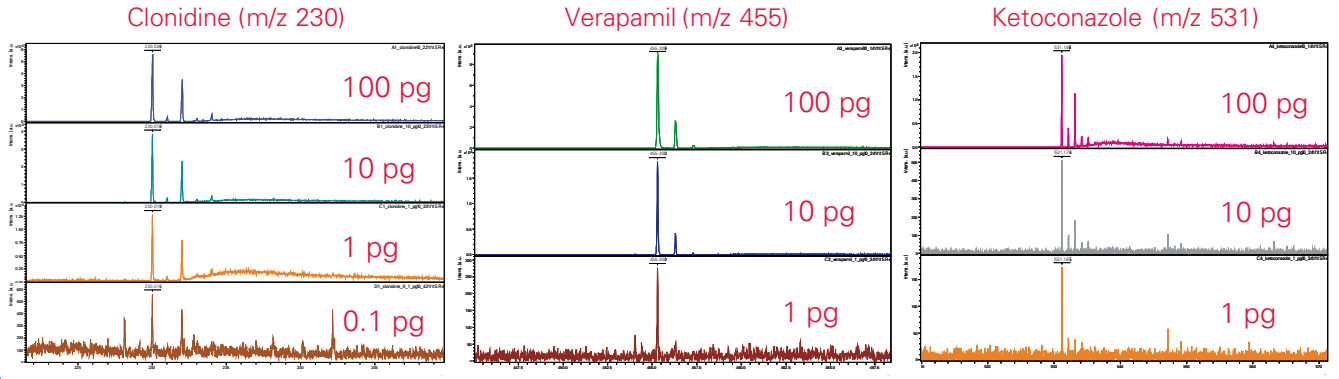

Fig. 4: These spectra demonstrate that NALDI target plates can detect small drug compounds at low and even sub-femtomolar concentrations.

Detecting a mixture of compounds

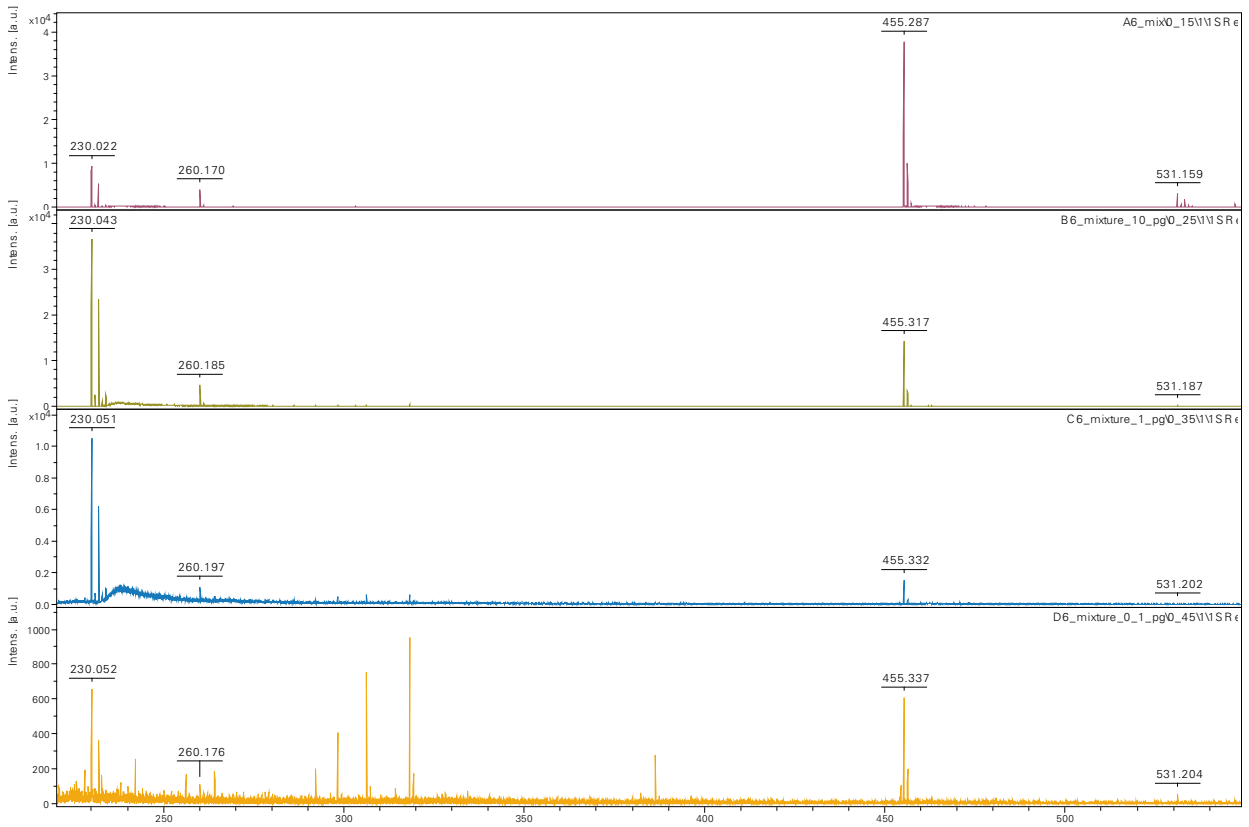

Fig. 5: These spectra show the detection of a mixture of 4 of our drug panel (Clonidine - m/z 230; Propranolol - m/z 260; Verapamil - m/z 455 and Ketoconazole - m/z 531) at various concentrations from 100-0.1pg/ul. 1ul of the mixture was spotted onto the NALDI target and as the spectra show all 4 molecules were detected at 1pg and 3 out of the 4 at 0.1pg.

to 200 Hz with the smartbeam™ laser. Laser power was adjusted to be slightly above laser threshold, and spectra were obtained by summing single shots which come from either one location, or a composite of multiple locations within the 1 ul deposited volume.

Results

General performance

Figure 3 shows the analysis of the diverse drug panel a a concentration of 10pg/ul with 1ul spotted onto the NALDI target plate.

The selected panel of small molecule analytes used to determine the limits of detection of our NALDI target plate system include prazosin, clonidine, verapamil, propranolol, haloperidol and ketoconazole. Figure 4 shows representative spectra from individual small molecule samples and figure 5 shows an analysis of multiple molecules mixed in a single sample at various concentrations.

Sample distribution

In order to demonstrate the uniform distribution of sample deposited onto the NALDI target substrate, we utilized the FlexImaging software which acquires data in a high density array within a chosen area on the target. After determining a pixal resolution across the desired surface, the software automatically measures the sample and saves individual spectra from each pixel. The acquired spectra are then processed to illustrate both the location and the intensity of a given mass by creating a representative pixel color gradient. As figure 6 shows the molecules distribute very evenly over the region they were deposited onto the target plate. This highlights another advantage of using NALDI technology over conventional matrix approaches where “hunting and pecking” for appropriate signals is required based on the formation of matrix crystals and speeds the analysis of multiple samples.

Conclusions

NALDI™ target plates in combination with Bruker Daltonics MALDI-TOF and TOF/TOF instrumentation provide a novel platform for the analysis of small drug compounds. The target plates are easy to use and allow the user to apply sample directly to the target plate surface without any requirement for an exogenous chemical matrix.

Keywords
Drug Analysis
Small molecules by MALDI-TOF

Instrumentation & Software
ultraflex
NALDI-targets

For research use only. Not for use in diagnostic procedures.

References

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Imaging of deposited analytes

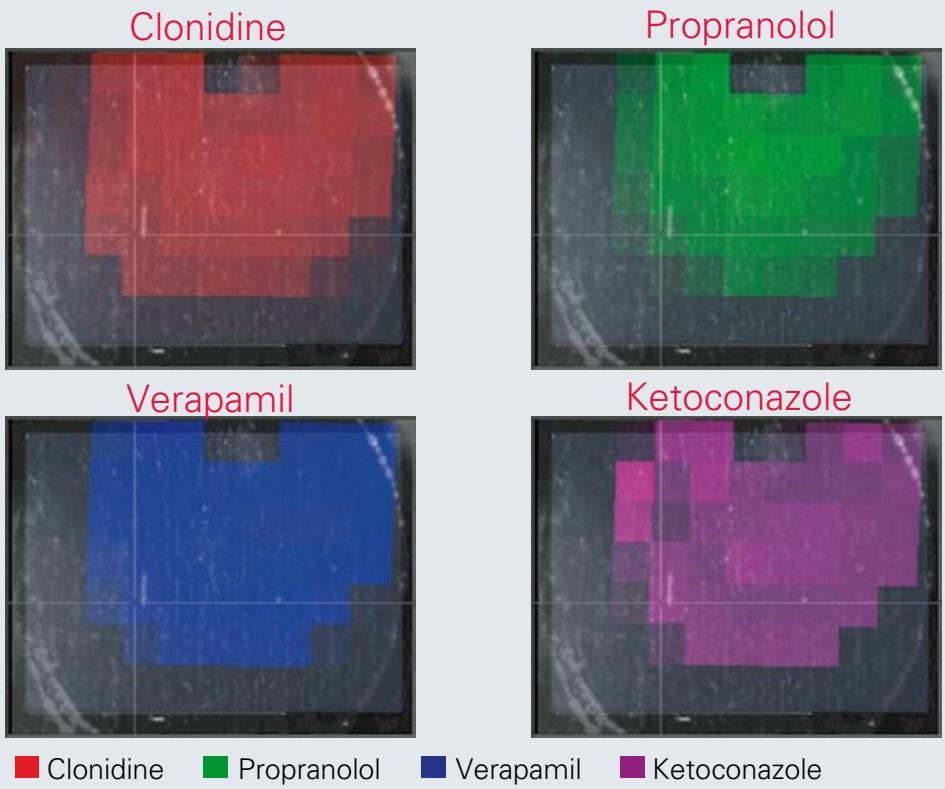

Fig. 6: A mixture of four of the drug panel was deposited onto a single NALDI spot at 100pg/ul. Note the even similar distribution of all four molecules over the region of deposition.

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