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- The TriVersa NanoMate is the latest in chip-based electrospray ionization technology from Advion. It combines the benefits of liquid chromatography, mass spectrometry,chip-based infusion, fraction collection, and direct surface analysis into one integrated system. It allows analysts to obtain more information from complex samples than with LC/MS alone.

Application Note

Lipidomics

Evaluating Liquid Extraction Surface Analysis (LESA) for Comparative Lipidomics Profiling

Introduction

Professor Manuel Mayr and his group at the King's British Heart Foundation Center of King's College London evaluated liquid extraction surface analysis (LESA) compared to previously published tissue extraction techniques. The TriVersa NanoMate LESA capability was employed to provide automated and efficient sampling. In addition, it allowed for easy analysis of one sample to act as its own control. The group was able to evaluate the ionization efficiency in both negative and positive mode, thereby increasing the amount of information obtained.

By comparing LESA to previously published tissue extraction technique of shotgun lipidomics, the group was able to show comparable and often times better data without sample preparation, thereby saving time and money.

Application

Lipids of atherosclerotic plaques have been analyzed; however targeted measurements of individual lipid classes are insufficient to identify global lipid differences in atherosclerosis. Characterization that includes lipid species as well as lipid classes may provide better classification resulting in a better understanding of the biology of artherosclerotic lesions as well as identify potential biomarkers. This plays an important role in understanding the biological connection between atherosclerotic lesions and genotype-phenotype relationships, as well as leading to risk prediction, diagnosis, and personalized treatments.

Materials and Methods

Differentially Expressed Lipids Across Classes

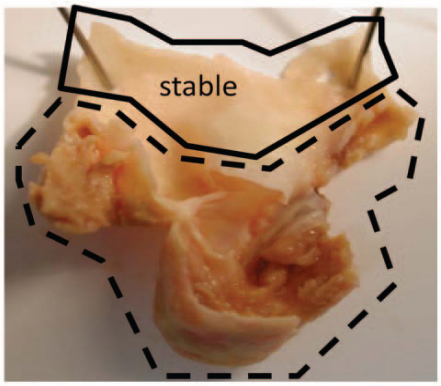

Figure 1: The 10 most differentially exressed species from 4 different lipid classes were sufficient to separate stable and unstable areas within the same lesion in PCA.

TriVersa NanoMate Settings for Comparative Lipidomics Profiling

Mass Spectrometer	TSQ Vantage, Thermo
Solvent	1.5 µL (chloroform:methanol: isopropanol1:2:4 containing 7.5 mmol/L ammonium acetate)
ESI Chip	4.1 µm nozzle
Ionization Voltage	1.2 kV
Gas Pressure	0.3 psi
Collission Gas	Argon, 1.0 mTorr
Temperature	150 °C

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Results

Figure 2: 10 min averaged MS data of 3.5 µM RNA-SL4 alone, Mw=6476.9 (inlet shows MaxEnt1 deconvolution, Mw=6476)

RNA SL4 shows strong charge envelope between m/z 1000 and m/z 1800, most prominent signal representing -5 charge state at m/z 1300

Figure 3: 3.5 µM RNA-SL4 incubated with 5 µM Tobramycin (Mw=467.5, blue trace) and 0.5 µM Neomycin B (Mw=614.6, green trace). Inserts show respective MaxEnt1 deconvolutions.

Ligand screening shows binding of both Neomycin B and Tobramycin, mass shifts in agreement with calc. masses
(calc.: 6476.9)

Figure 4: Titration of 3.5 µM RNA-SL4 with increasing concentrations of Neomycin B (black trace: 0.1 µM < 10% bound, blue trace: 0.5 µM >90% bound).

Kd estimate of 2 µM (Lit.: 1.0 ± 0.2 [Turner et al. 2006]

Conclusions

- Liquid extraction surface analysis (LESA) is a rapid approach to tissue analysis without sample preparation time and effort.
- Shotgun lipidomics provides valuable insights into the relationship within artherosclerotic plaques.
- LESA and other advanced mass spectrometry techniques can be employed for diagnostic and drug screening applications due to their complete profiling of lipid species and classes.