Fig. 1: Omni Spray on the Bruker micrOTOF-Q.

Technical Note # 21

Omni Spray on Bruker Daltonics API Mass Spectrometers for Neat Sample Analysis

Desorption Electrospray Ionization (DESI) has been shown repeatedly in the literature to be applicable to the analysis of a wide variety of compound types with limited or no sample preparation [1,2]. In this note, we apply the Omni Spray™ DESI source to Bruker Daltonics API Mass Spectrometers for the neat analysis of several small molecules. Either through inherent sample complexity or chemical background, such neat analysis can lead to complex spectra. However, especially at low mass, high performance instruments can resolve the species in complex spectra and determine ion masses with high accuracy. Together with TIP™ (True Isotopic Pattern with SigmaFit™ algorithm), species of interest can be identified with a high degree of confidence.

Introduction

Since its introduction in 2004 [1], DESI has been used for the analysis of a wide variety of samples including explosives, drugs, material on or in plant and animal tissues, peptides, proteins, and metabolites. Especially interesting is DESI's capability to analyze samples neat – i.e. without preparation – at atmospheric pressure. However, implicit in a neat analysis, is a higher complexity of sample composition at the point of mass analysis. This necessarily leads to more complex spectra containing more peaks. In the analysis of such samples, higher performing mass spectrometers with the ability to resolve complex spectra and ultimately identify species of interest is critical.

Methods

An Omni Spray DESI source from Prosolia (Indianapolis, IN) was mounted on Bruker Daltonics API mass spectrometers. Shown in figures 1, 5, and 10, is the Omni Spray source mounted on a Bruker micrOTOF-Q™, an HCT™ ion trap, and an Apex Qe™ FTMS respectively. Samples of PEG (average MW 200Da), amitriptyline, and peptides were obtained from Sigma Aldrich (St. Louis, MO). Solvents were obtained from Burdick & Jackson (Morristown, NJ).PEG, amitriptyline, and the peptide mix were prepared in water and spotted onto the hydrophobic spots of an Omni Slide target from Prosolia. Tablets were mounted on Omni slides surface with double sided tape.

PEG was used as an external calibrant deposited on the same slide as the tablets. All tablets were analyzed neat. The ibuprofen and "pain away" tablets were obtained from Xpect (Manson, OH). The "cat's claw" tablet was obtained from Source Naturals, Inc. (Scotts Valley, CA). The DESI sprayer was positioned at a 70° angle, 2-4 mm from the sample. The sample was held 2-4 mm from the source capillary inlet. The capillary inlet was held at -2kV (for positive ion experiments), whereas the sprayer was held at ground potential. A pneumatic gas pressure of 110 psi was used to spray a solvent of 50:50 acetonitrile:water with 1% formic acid.

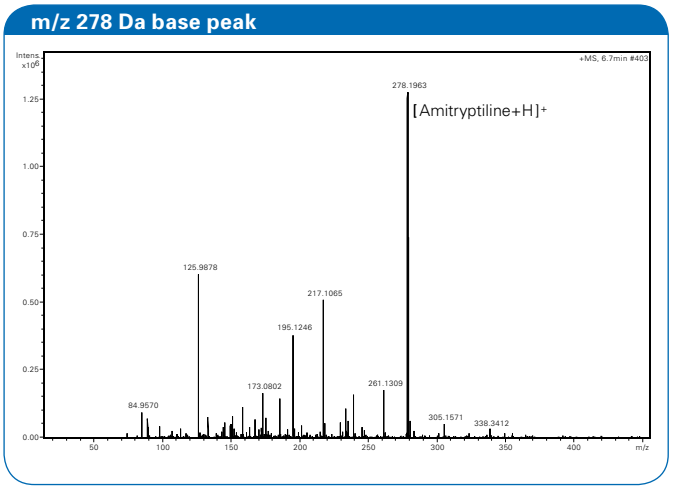


Fig. 2: Amitriptyline by DESI on micrOTOF-Q.

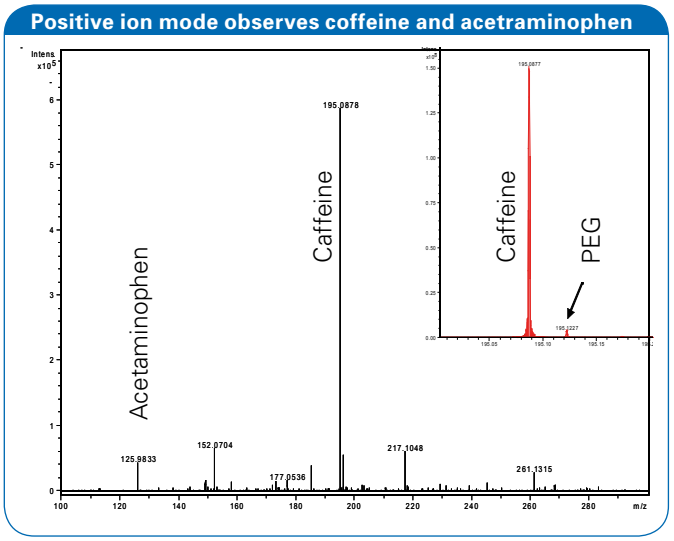


Fig. 3: DESI-OTOF and DESI-FTMS (insert) spectra of "pain away" tablet containing acetaminophen, aspirin, and caffeine.

Results

Figure 2 shows the result of the analysis of amitriptyline by DESI on a micrOTOF-Q. While the peak associated with the molecular ion at m/z 278Da is the base peak, many contaminant peaks appear in the spectrum. As the amount of amitriptyline deposited is decreased, such chemical background becomes more important.

The spectrum of figure 3 is the result of the analysis of "pain away" tablets with DESI on a micrOTOF-Q and an Apex-Qe. In these experiments, the spectra are calibrated externally using PEG having an average MW of 200 Da. The resolution and mass accuracy obtained via the micrOTOF-Q is, by itself, perhaps sufficient to identify the compounds of interest. The masses of the m/z 152 Da and 195 Da peaks are assigned to within nearly 1ppm of their theoretical value. However, in figure 4, TIP allows identification of the analyte with added confidence. Replacing the former approach of just using the measured mass for formula determination, the micrOTOF-Q adds a second dimension into the routine: Analysis of the isotope profile provides the so-called "True Isotopic Pattern" (TIP) which is matched against the measured spectrum – leading to results with significantly increased confidence. As one might expect, the best TIP fit for the m/z 152 Da and 195 Da peaks yields the molecular formula for acetaminophen and caffeine respectively.

As can be seen in the inset of figure 3, an Apex-Qe yields similar results as micrOTOF-Q but with higher resolution & accuracy. The higher resolution of FTMS allows, for example, the separation of analyte, caffeine, from a PEG contaminant peak.

Figure 5 shows the Omni Spray source on a Bruker Daltonics HCT ion trap. In Fig. 6 this setup was used to make a negative ion analysis of the "pain away" tablet. Notice, the "pain away" tablet contained 250 mg each of acetaminophen and aspirin and 65 mg of caffeine. Only in positive ion mode (Fig. 3) were caffeine and acetaminophen observed. Similarly, only in the negative ion spectrum of figure 6, was aspirin observed.

Similarly, in figure 7 ibuprofen was observed when an ibuprofen tablet was analyzed via negative ion mode using DESI. Fig. 8 is the result of the analysis of a "cat's claw" tablet. The cats claw tablet is an herbal drug derived from a plant of the same name found in the rain forests of Peru. This herb contains several biologically active compounds of purported benefit. Among these are rhynchophylline, and pteropodine and their isomers.

In a prior study performed with a Bruker esquire-LC electrospray ion trap, these compound were extracted from a cat's claw tablet prior to analysis via LC MS/MS [3]. In the prior study, an extract was prepared from 1 g of crushed cat's claw tablet by extracting at 25°C into 10 ml of ethyl acetate for two days.

In the present experiments, a tablet of cat's claw was analyzed neat by DESI on a Bruker HCT API ion trap. Results were comparable to those obtained in the previous study even though, in the present study, no extraction was performed.

As shown in figure 8 (top), while many other component peaks were observed, peaks corresponding to both pteropodine (m/z 369) and rhynchophylline (m/z 385) were present in the precursor ion spectrum. Performing MS/MS experiments on the m/z 369 peak (figure 8, middle spectrum) and on the m/z 385 peak (figure 8, bottom spectrum) yielded near identical fragmentation spectra as observed in the prior study.

Figure 10 shows the Omni Spray™ source on an Apex-Qe™ FTMS. This arrangement and that with the micrOTOF-Q

(see fig. 1) were used to produce the peptide mix spectrum of figure 9. As expected from an ESI-like ionization process, several multiply charge species are observed.

Analyte identification with added confidence

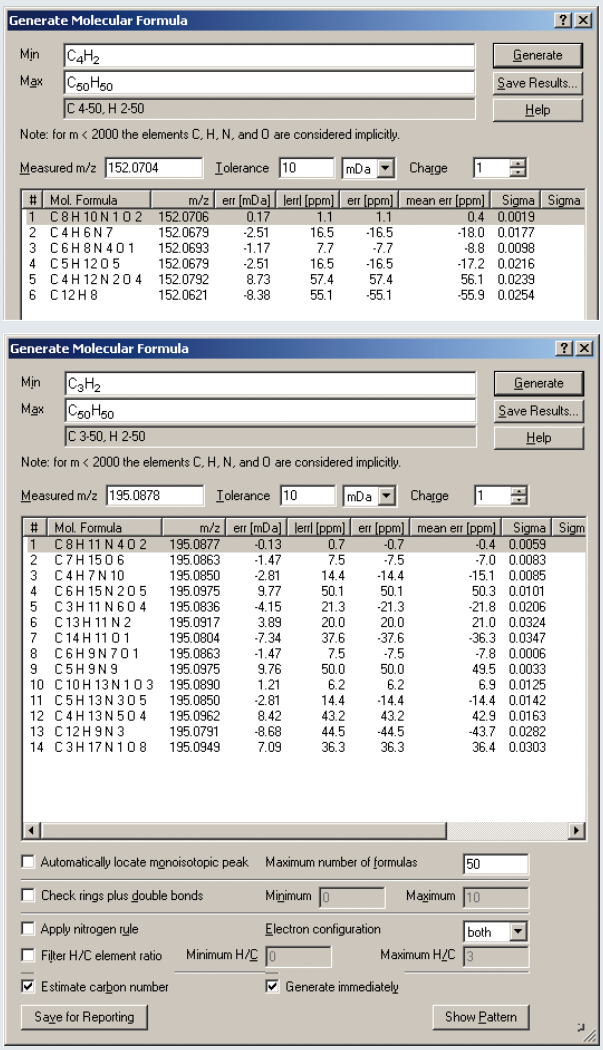


Fig. 4 SigmaFit analysis of m/z 152 (above) and 195 (below) peaks. The best fit in both cases yielded the correct molecular formula.

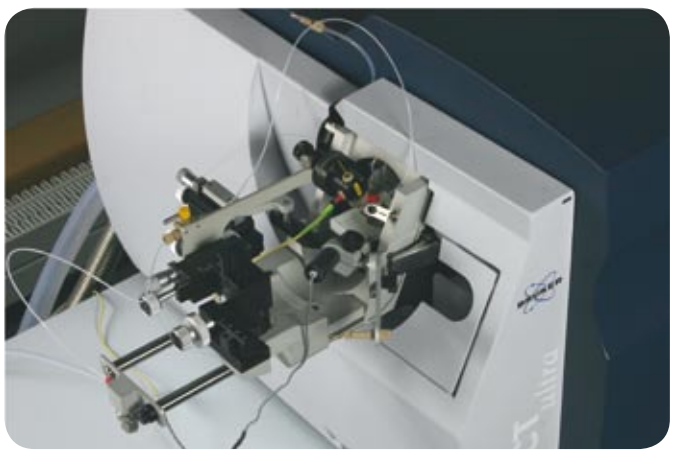


Fig. 5: Omni Spray™ on the Bruker HCT™ ion trap.

Negative ion mode observes aspirin

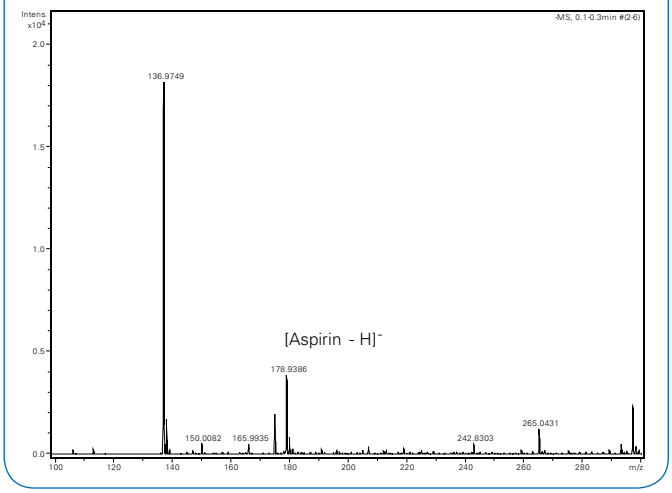


Fig. 6: HCT DESI negative ion spectrum of "pain away".

Negative ion mode observes ibuprofen

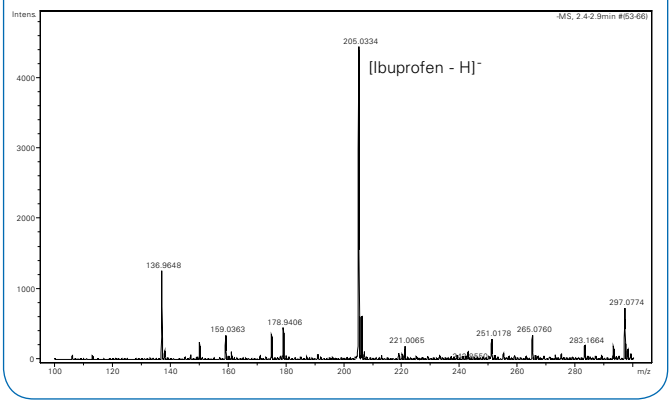
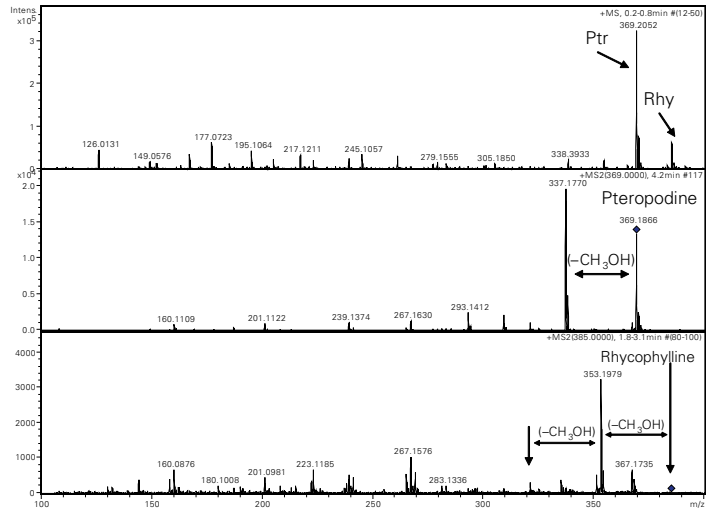


Fig. 7: HCT DESI negative ion spectrum of ibuprofen.

Conclusions

The Omni Spray DESI source clearly can be used to analyze a wide variety of compounds including drugs and peptides without prior sample preparation. Analyses which might previously have taken hours or days - as was the case in the "cat's claw" study – can now be performed in under an hour. High performance instruments like the micrOTOF-Q, HCT, and Apex-Qe and data analysis software like SigmaFit can be critical in the DESI analysis. Such high performance analyzers can resolve complex mixtures into discrete mass spectral peaks which then can be assigned an accurate mass. Additionally, the TIP™ algorithm provides further confidence in compound identification by using the entire isotopic pattern to determine a molecular formula.

Neat Plant Herbal Drug Analysis on HCT Ion Trap



References

[1] Z. Takats, J.M. Wiseman, B. Gologan, R.G. Cooks, Science 306, 471(04).

[2] Z. Takats, J.M. Wiseman, R.G. Cooks, J Mass Spectrom 40, 1261(05).

[3] C. Stacey, R. Phillips, Proceedings of the 45th ASMS Conference on Mass Spectrometry and Allied Topics, 1997.

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Fig. 8: HCT DESI analysis of "cats claw" tablet showing (top) the precursor ion spectrum (middle) the m/z 369 fragment ion spectrum, and (bottom) the m/z 385 fragment ion spectrum.

Peptide Mix Spectrum

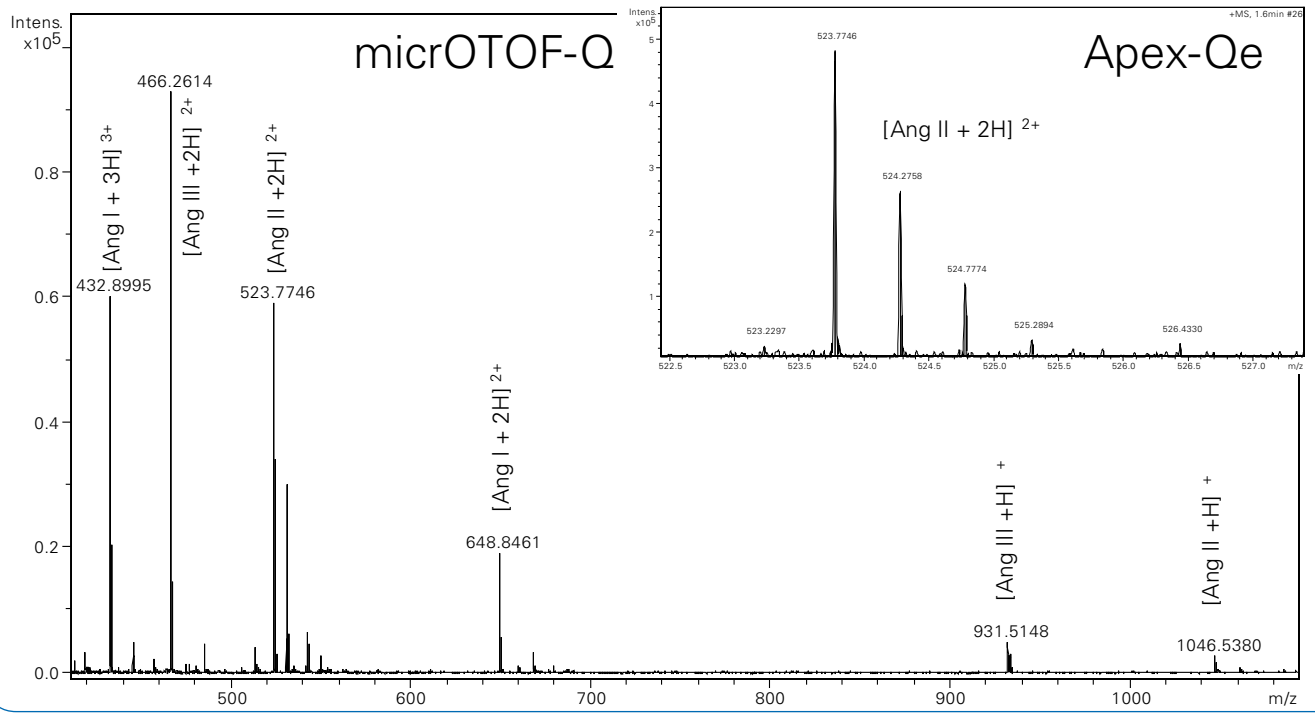

Fig. 9: Spectra of a peptide mix containing angiotensins I, II, and II, analyzed via micrOTOF and Apex-Qe FTMS (inset) with the Omni Spray source.

Fig. 10: Omni Spray™ on the Bruker Apex-Qe™.

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