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A novel algorithm for automating fragment ion structure assignment using high mass accuracy MS/MS data

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Overview

- To help the interpretation of accurate mass MS/MS spectra and to provide an effective means of structurally verifying component identification a novel algorithm has been developed to automate fragment ion annotation.
- The algorithm has been applied to high resolution accurate mass (HRAM) fragment ion data for a panel of chemically diverse small molecules. This test panel generated a wide variety of fragmentation mechanisms including single bond heterolytic and homolytic cleavages, ring opening and cleavage, and intramolecular rearrangements resulting in neutral losses such as ammonia, carbon monoxide and hydrogen cyanide.

Introduction

High mass accuracy mass spectrometry platforms are powerful analytical tools generating accurate mass measurement information and product ion spectra applied to component identification and/or verification. However, a key challenge in process relates to interpreting product ion spectra and assigning chemical structures to specific fragment ions. For the computational prediction and annotation of product ion spectra a number of methods have been proposed including input–output kernel regression machine learning spectra and bond dissociation approaches combined with data base searches. In this paper, a novel algorithm has been developed to assign structures to fragment ions to help fragment interpretation and compound verification but also as a tool to construct accurate mass fragment ion data for quantitative and qualitative Q-TOF analysis.

Materials and Methods

A panel of small molecules was used to test the algorithm in this research application, including pesticides extracted from complex food matrices using a standard QuEChERS protocol, drugs of abuse spiked into plasma and plasma crash samples from a metabolomics study. Data was acquired with the LCMS-9030 QTOF system (Shimadzu Corporation). The acquisition method design was typically set-up with a MS and DIA-MS/MS. HRAM Q-TOF (LCMS-9030 Shimadzu Corporation) data acquired sequential mass scans with a cycle time less than 1 second (MS and subsequent DIA-MS/MS mass scans with an isolation width of *m/z* 20). In each MS/MS event a collision energy (CE) spread was applied to generate precursor MS and product ion MS/MS spectra.

The algorithm functions by identifying groups of atoms that could be involved in a fragmentation mechanism, then uses the fragment ion data to confirm each putative mechanism. The first step in the process is the consideration of tautomeric shifts within the ionized precursor molecule. Collections of ionized tautomer molecules are created as the starting point in the process. Each charged tautomer is then analyzed for the presence of groups of atoms that could be involved in a putative mechanism. Next the rearrangement of electrons is considered with various outcomes postulated, such as homolytic or heterolytic cleavage or the creation of new bonds that did not originally exist. Molecular charge and chemical constraints are then evaluated, and only valid hypotheses accepted. Finally, iterative combinations of the hypothetical fragmentation mechanisms are then considered to give fragment ions that are formed by multistage mechanisms. Using acquired LCMS-9030 Q-TOF data, product ion spectra were annotated and used to build accurate mass methods and verify compound identification. Figures 1-3 highlight suggested fragments based on original structures.

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Results

Targeted and untargeted analysis food safety

The algorithm has been applied to annotate fragment structures to verify component identification and distinguish isomers in complex matrices. In the example below the research algorithm has been applied to DIA-MS/MS spectra of ethiofencarb and methiocarb following HRAM analysis.

Assigning fragment structures to ethiofencarb and methiocarb in a complex food extract
QuEChERS extract spiked with over 200 target pesticides
Untargeted MS and DIA-MS/MS; QTOF Data Acquisition LCMS-9030
Cycle time 0.8 second for all MS and DIA-MS/MS mass scans
MS mass scan; mass range 140-900 Da (40 msecs)
MS/MS 39 sequential mass scans; mass range 65-900 Da (20 msecs for each mass scan)
Precursor isolation width 20 m/z; CE spread 0-30V
External mass calibration

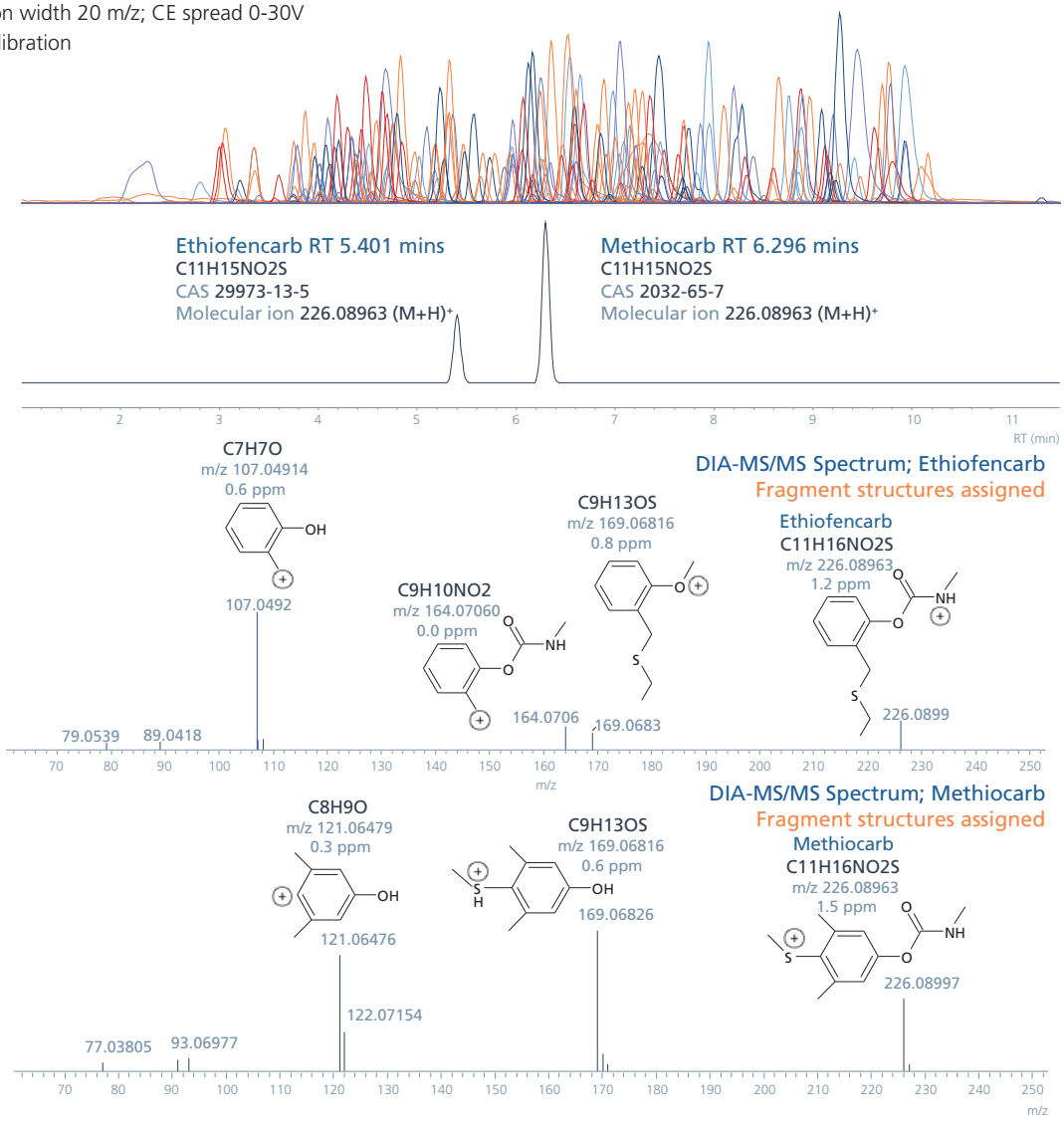

Figure 1. Automatic structural assignment for fragment ions of ethiofencarb and methiocarb. The DIA-MS/MS mass spectra differentiate the components based upon fragment ions at specific ions at m/z 121 for methiocarb and m/z 164 for ethiofencarb (ethiofencarb's loss of carbamate group leads to formation of ion at m/z 169 that subsequently leads to ion at m/z 121 after losing methanethiol; it is likely that the hydroxybenzyl cation at m/z 164 could rearrange to hydroxytropyllium ion (Nunez at al, Toxics 2018)).

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Fragment ion annotation in DoA screening

A panel of drugs of abuse (DoA) was spiked into human plasma and extracted using QuEChERS. As one example, to verify the identification of 2 selected drugs the algorithm was used to annotate the DIA-MS/MS spectra for fentanyl and cocaine in the test panel.

Assigning fragment structures to fentanyl and cocaine in a human plasma extract spiked with a panel of drugs of abuse (DoA) at 5 ng/mL (precursor mass chromatogram ±5ppm)
Untargeted MS and DIA-MS/MS; QTOF Data Acquisition LCMS-9030
Cycle time 0.4 second for all MS and DIA-MS/MS mass scans
MS mass scan; mass range 100-500 Da (20 msecs)
MS/MS 19 sequential mass scans; mass range 40-500 Da (20 msecs for each mass scan)
Precursor isolation width 20 m/z; CE spread 0-30V
External mass calibration

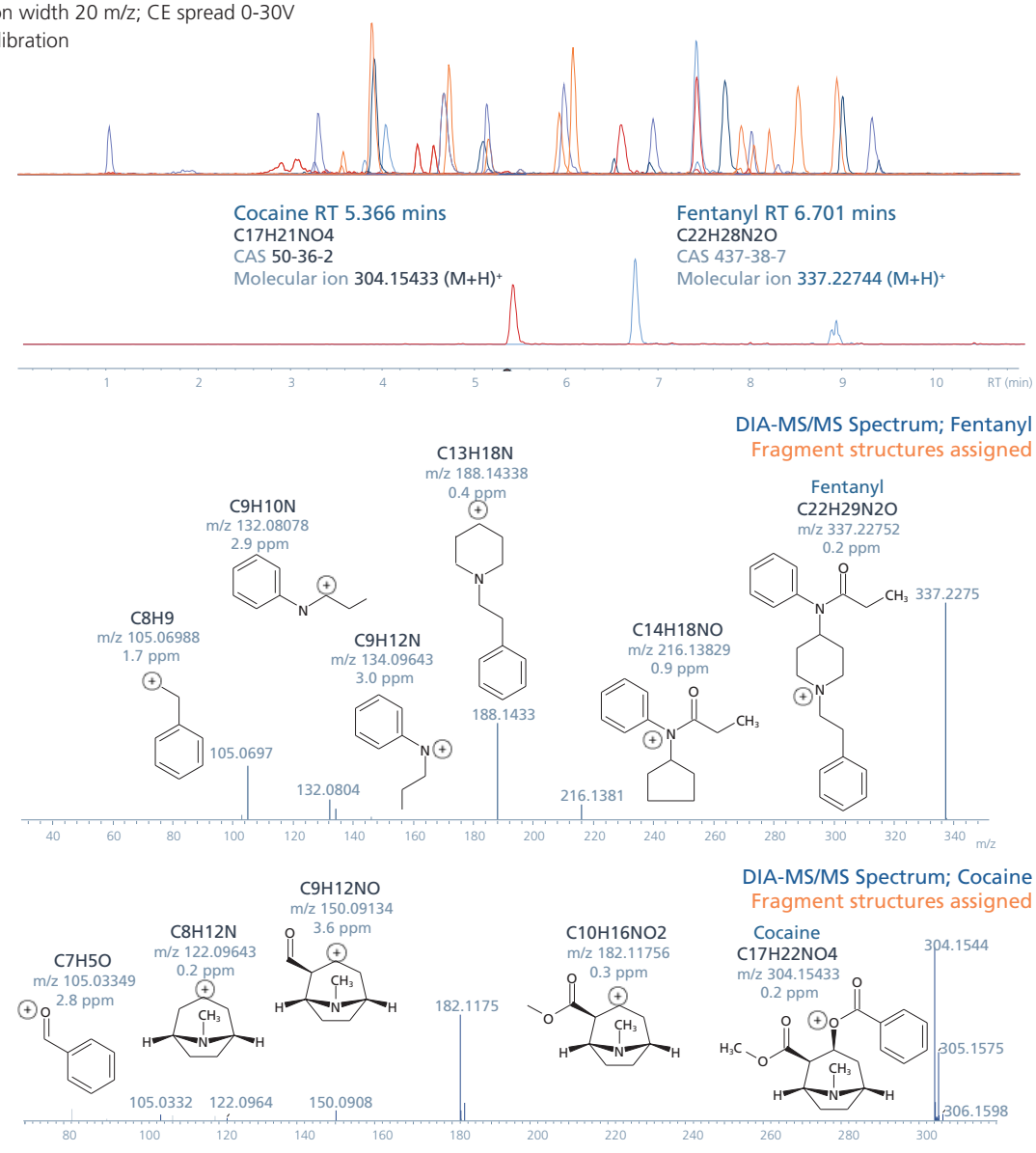

Figure 2. Assignment of DIA-MS/MS mass spectra for fentanyl and cocaine in a human plasma extract using a collision energy spread of 0-30V.

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Component verification in metabolomic studies

One of the key research areas for verifying component identification is in metabolomics and particularly with lipid species. The fragment ion annotation algorithm was applied to structural verification of lysophosphatidylcholine 16:0.

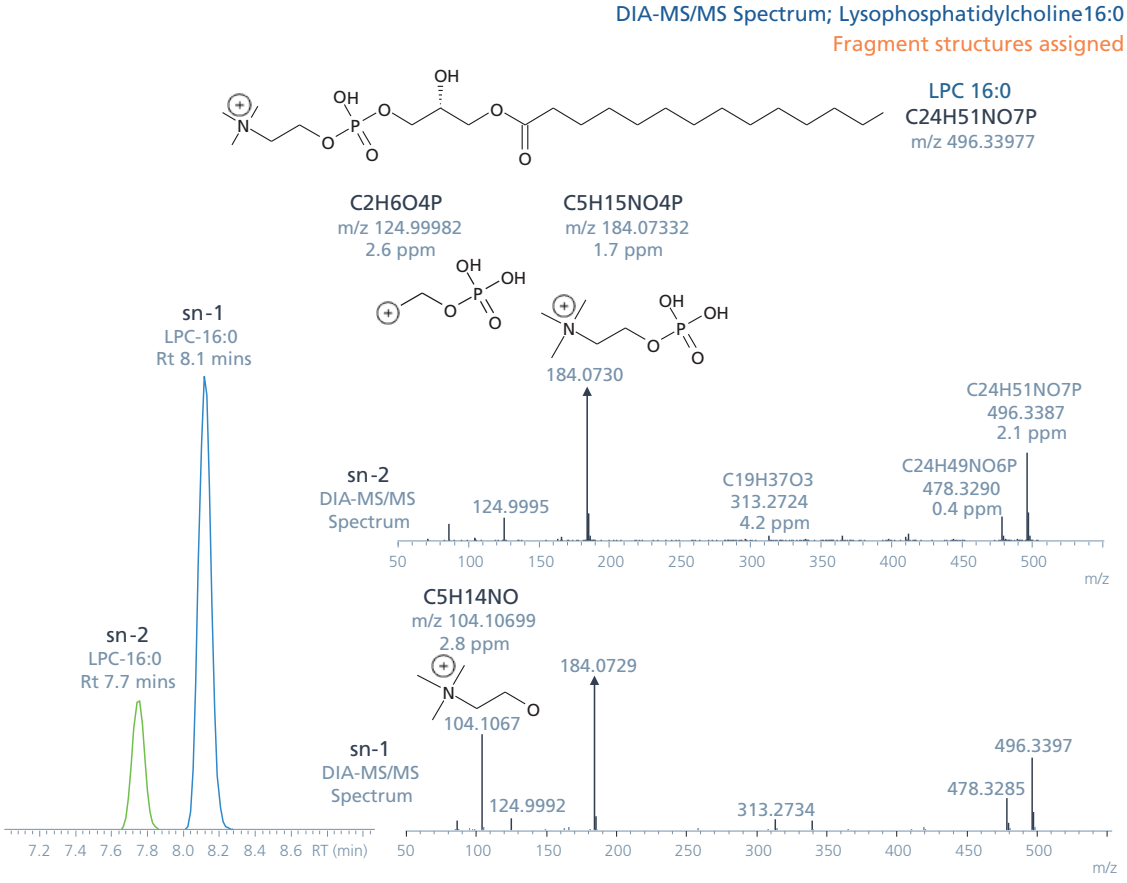

Figure 3. DIA-MS/MS mass spectrum annotation for lysophosphatidylcholine 16:0 including the characteristic product ion at *m/z* 104 to identify the *sn-1* isoform.

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Conclusions

- The fragment ion data for a panel of chemically diverse small molecules generated a wide variety of fragmentation mechanisms including single bond heterolytic and homolytic cleavages, ring opening and cleavage, and intramolecular rearrangements resulting in neutral losses such as ammonia, carbon monoxide and hydrogen cyanide. Using acquired LCMS-9030 Q-TOF data, product ion spectra were annotated and used to build accurate mass methods and verify compound identification.
- Automated correlation of experimental fragmentation spectra with hypothetical fragment ion structures.
- The research application was used to verify component identification in complex matrices using DIA-MS/MS mass spectra acquired using cycle times less than 1 second (typically for a mass range of 100-1000 Da, MS and 38 subsequent DIA-MS/MS mass scans were acquired with an isolation width of *m/z* 20). In each MS/MS event a collision energy spread of 0-30V was applied to generate product ions spectra.

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