Novel real-time acquisition logic to prevent fragmentation of uninformative precursors

Heiko Neuweger[1], Nikolas Kessler[1], Christopher Clark[2], Roberto Lozano[1], Pelle Simpson[3]

[1] Bruker Daltonics GmbH & Co. KG, Fahrenheitstraße 4, 28359 Bremen, Germany

[2] Bruker Daltonics Inc., 40 Manning Road, Manning Park, Billerica, MA 01821, USA

[3] Enveda Biosciences, 1880 S Flatiron Court, Suite K, 80301 Boulder, CO

Introduction

Informative tandem mass spectrometry (MS/MS) data is crucial for the annotation of compounds. Modern instruments feature fast MS/MS data acquisition rates exceeding 120 Hz for unprecedented characterization of complex biological samples. However, the key metric of success is the number of informative MS/MS spectra obtained per unit time. Fragmentation of salt adducts, multimers or other artifactual signals may result in uninformative fragmentation spectra. By preventing these fragmentation events, more time can be spent on relevant precursors, thereby extending MS/MS coverage or alternatively allowing faster analysis while maintaining the same coverage. To achieve this, real-time and context sensitive control over the precursors to be targeted is needed to increase the information content of acquired MS/MS spectra.

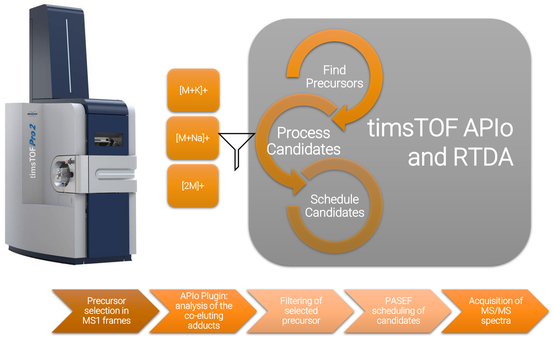

Figure 1. Real time API prototype to control PASEF precursor selection
All precursor candidates in every MS1 frame are subjected to a filter that identifies and masks co-eluting adducts and dimers based on their mass offsets. These precursor candidates are removed from the list subjected to the following Schedule Candidates step in the PASEF acquisition logic.

Methods

We implemented a novel real-time de-adducting (RTDA) plugin using the APIo interface of timsControl 4.1 running as the control software of a Bruker timsTOF Pro 2 instrument. We prioritize informative precursors by preventing fragmentation of sodium or potassium adducts or singly charged dimers. The logic for scanning the precursor candidates list in real-time was implemented in a Python plugin and embedded in a dedicated acquisition method.

The sample used for these experiments was a primary ethanolic extract of tobacco leaf. Tobacco leaves were dried in a dehydrator, ground to a powder and extracted in 100% ethanol for 4 hours. The extracts were cleared of debris twice by centrifugation at 21,000xg for 10 minutes and transfer of the supernatant to a pre-weighed vial. The supernatant was dried in a rotary evaporator (Genevac EZ-2, SP Scientific) and the resulting solid was reconstituted to 25 mg/mL in 80:20 methanol/water.

1µL of the reconstituted extract was injected on a Waters H-Class Bio UPLC with a 2.1 x 50 mm BEH C18 column, 1.7 µm particle size (Waters p/n 186009455). Solvents A and B were 0.1% FA in water and 0.1% FA in ACN, respectively. At a flow rate of 0.8 mL/min, solvent B was increased from 5% to 95% in 2.5 min, followed by a 0.25 min hold at 95% B and a return to 5% B over the next 0.25 min. The column was equilibrated for the last 0.5 min of the 3.5 min method at 5% B.

Nine technical replicates of these samples have been measured with the default PASEF and the RTDA method, respectively. Data processing was performed using the 4D workflow including 4D feature finding, de-adducting, de-isotoping and retention time alignment functionality provided by the Bruker MetaboScape software.

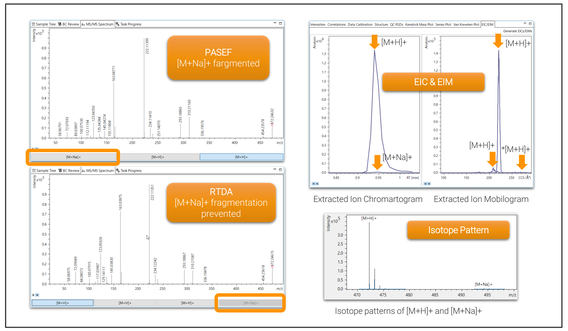

Figure 2. Positive effect of the RTDA plugin for a feature detected across all replicate measurements.
The PASEF and RTDA diagrams to the left depict the ions and MS/MS spectra of a feature detected with neutral mass *m/z* 471.238 eluting at 0.9 minutes. On top, the PASEF logic resulted in the fragmentation of the [M+Na]+ ion. Below with RTDA active, this ion was not targeted for fragmentation. To the right, the EIC and EIM traces and the Isotope Patterns of this compound are depicted. Intensity for [M+Na]+ was ~150 times lower than the [M+H]+. In addition, multiple [M+H]+ ion exhibiting different CCS values could be targeted.

Results

Type of acquisition	Total number of *K* and *Na* adducts	Assigned MS/MS	Ratio
PASEF	400	113	0.2825
RTDA	466	93	0.1996
Binomial test (lower) p-value		0.00003***	

Type of acquisition	Total number [M+H]+	Assigned MS/MS	Ratio
PASEF	5570	1700	0.3052
RTDA	5445	1983	0.3642
Binomial test (greater) p-value		$<10^{-16}$****	

Figure 3. Comparison of RTDA vs. PASEF
Table 1: total number of [M+K]+ and [M+Na]+ adducts and assigned MS/MS. Table 2: total number of [M+H]+ adducts and assigned MS/MS. We also tested the samples with a binomial test (lower or greater probability respectively).

The results of 4D data processing resulted in more than 5200 features managed in MetaboScape, respectively. Interactive analysis of features showed reproducibility and the positive effect of the RTDA plugin (Fig. 2). Using the MetaboScape REST API and Jupyter notebooks, we calculated for the complete datasets the absolute numbers and ratios of adducts and associated MS/MS spectra.

As a result, RTDA significantly reduces the ratio of [M+Na]+/[M+K]+ adducts with MS/MS from 0.28 to 0.20. Also, it improves the ratio of [M+H]+ with assigned MS/MS from 30.5% to 36.4%. (Fig. 3)

	Coef	Std. Err.	P>|z|
Intercept	0.7436	0.1595	$<10^{-16}$****
Ion H_2O^+	0.2929	0.1996	0.1424
Ion H^+	0.3956	0.1619	0.0145*
Ion K^+	0.0141	0.3512	0.9680
Ion Na^+	-0.1800	0.2003	0.3688

Figure 4. Coefficient and significance of the different adducts using a logistic regression between a binomial variable, informative or uninformative, and the respective adduct.

In this context, we also used logit regression to test our assumption about the adducts and we can see that the probability of obtaining an informative MS/MS with a precursor with a proton is increased. The odd ratios are 1.46 with potassium and 1.78 with sodium (Figure 4).

For the dimer analysis, we did not obtain statistically meaningful results. In PASEF, we detected a low number of 70 dimers, 31 with MS/MS, and 83 with activated RTDA, 41 with MS/MS.

Finally, both datasets have a consistent number of detected features (5387 for PASEF and 5283 for RTDA) and we obtained more unique annotation with RTDA (69) than with PASEF (61) using the same MS/MS spectral libraries.

Summary

- An APIo plugin for the timsControl software was developed and employed for controlling selection of precursors in real-time and preventing fragmentation of adducts that would result in uninformative MS/MS spectra.
- The implementation of the prototype plugin for the timsControl software is efficient and can handle PASEF acquisition rates without affecting duty cycle.
- MetaboScape based 4D feature finding and de-adducting allowed us to statistically assess the improvements in the number of informative MS/MS spectra obtained.
- The MetaboScape REST API was instrumental for statistical analysis via Jupyter notebooks; statistical tests confirmed the positive contribution of the RTDA.

Conclusion

- Real-time control over precursor selection can prevent fragmentation of uninformative sodium and potassium adducts.
- The RTDA plugin allows to obtain more informative MS/MS spectra per unit time supporting deeper analysis of compounds found in complex plant samples

Real-Time De-Adducting