# prio-PASEF: Precision and Discovery in MetID Workflows

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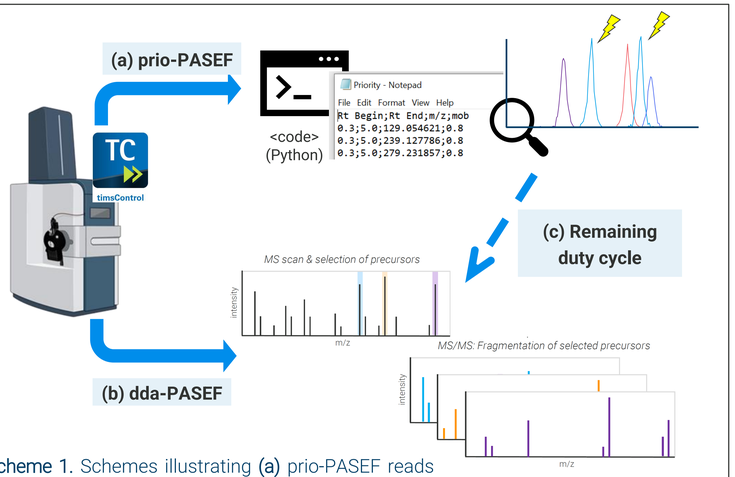
## Introduction

Assessing pharmacological and toxicological activity of biotransformation products ensures drug safety and efficacy. LC-HR-MS is crucial for characterizing these products, although some may be unanticipated and of low abundance. Fully characterizing both expected and unexpected products is challenging. Here we present prio-PASEF (**P**arallel **A**ccumulation **Se**rial **F**ragmentation), a novel timsTOF data acquisition mode that prioritizes precursor selection for predicted products while using the remaining duty cycle for untargeted data dependent acquisition (dda). Additionally, trapped ion mobility (TIMS) provides another dimension of separation for cleaner MS2 data and measures collisional cross section (CCS), a physicochemical property based on ion size and shape in the gas phase. This novel metID workflow ensures comprehensive coverage of both expected and unexpected metabolites.

## Methods

Cryopreserved human hepatocytes (Discovery Life Sciences) were incubated with testosterone at eight time points and three biological replicates. Quenched reactions were extracted for LC-TIMS-MS analysis using both standard 4D-Metabolomics instrument parameters with prio-PASEF and dda-PASEF.




Scheme 1. Schemes illustrating (a) prio-PASEF reads a priority list of compounds of interest implemented using Pythan script APIo and timsControl; (b) standard dda-PASEF; (c) standard dda-PASEF is performed in remaining duty cycle when no priority compounds scheduled

## Results

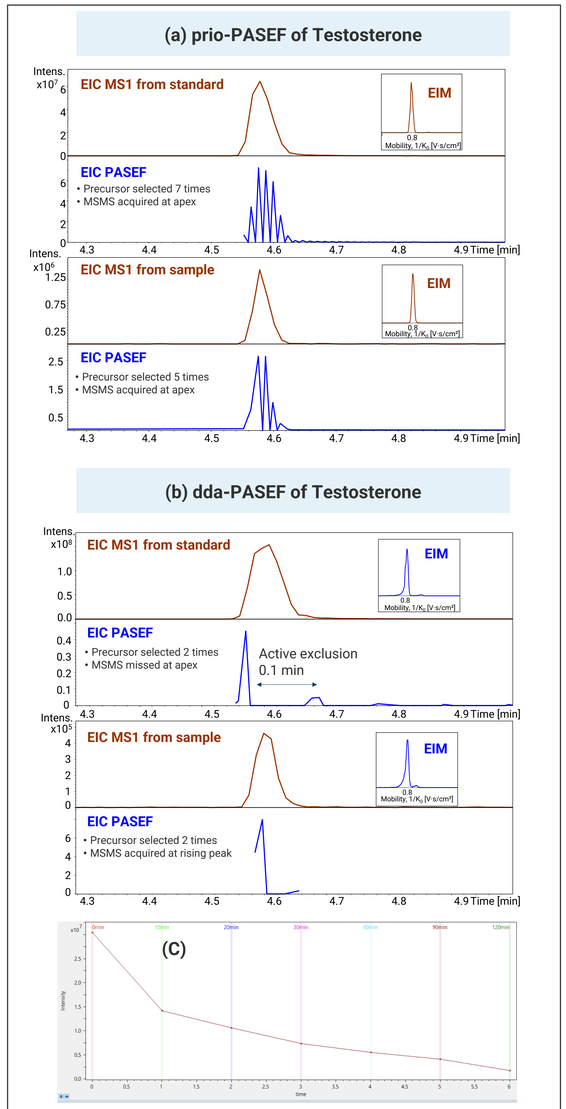



Figure 1. Extracted Ion Chromatogram (EIC) and Extracted Ion Mobilogram (EIM) of testosterone (*m/z* 289.2162) acquired using (a) prio-PASEF and (b) dda-PASEF. (c) Time series of testosterone intensity change over 120 min incubation time.

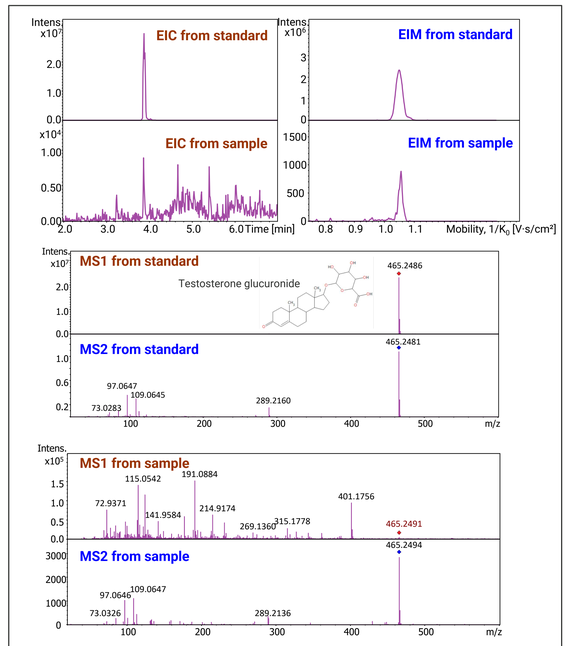



Figure 2. 4D prio-PASEF analysis of one metabolite of very low intensity, testosterone glucuronide, in chemical standard and in 60 min incubation sample. The metabolite can be confirmed by accurate mass, MS2 fragments, retention time, and ion mobility.

## Summary

1. prio-PASEF using APIo has been demonstrated to schedule PASEF events on molecules of interest from a priority list and picks precursors from optimal peak position (Fig.1).
2. When known metabolite target is present at very low abundance, prio-PASEF prioritized this precursor selection and provided quality MS2 spectrum (Fig. 2). 4D analysis provides high confidence of metabolite confirmation.
3. 4D feature finding and annotation in MetaboScape enables the identification of both expected and unexpected targets. Over 480 features containing MS/MS spectra were identified (310 unique) in an untargeted approach (Fig. 3).

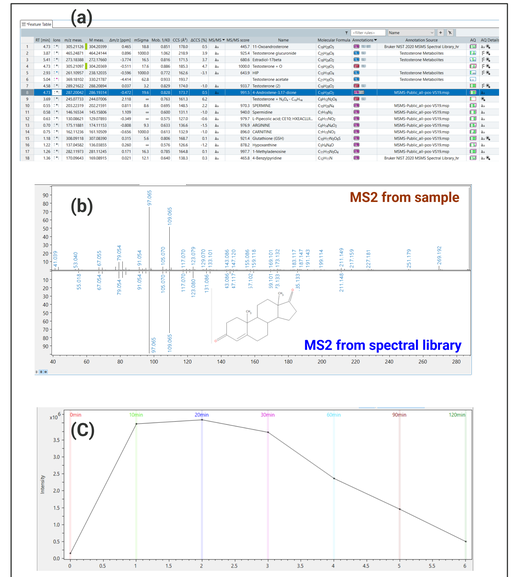



Figure 3. 4D feature finding and spectral library annotation was conducted to identify known targets without chemical standard, and unknown targets. (a) Screenshot of MetaboScape 2025 feature table for some of the annotated compounds. (b) Spectral library match of androstenedione with MS/MS score of 992. (c) Time series of androstenedione over 120 min.

B.W., X.P., E.M.F. and H.N. are employees of Bruker Corporation. Bruker manufactures and sells analytical instrumentation including mass spectrometers and software used in this study. J.R.P, R.G.P, K.P.M. E.S.P. and M.B. are employees of Discovery Life Sciences. Samples were acquired in association with a business opportunity.

## Conclusion

- prio-PASEF through timsControl using APIo provides a novel metID workflow
- Expected drug metabolites are prioritized for precursor selection even at low abundances
- Untargeted data dependent acquisition utilizing the remaining duty cycle provides feature identification in-depth for expected and unexpected metabolites

prio-PASEF on timsTOF