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Strategies for Detection and Quantification of Metabolites in SWATH® Acquisition Analysis

SCIEX

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ABSTRACT

SWATH® acquisition has recently emerged as a powerful data independent acquisition technique for next-generation omics analysis. The richness of SWATH acquisition data allows various ways of data extraction approaches including detection of analytes by prediction, mass defect filtering, and quantification from HRMS or HRMS/MS chromatograms. Processing of SWATH acquisition data for metabolomics is believed to be complex and does add some complications with current approaches due to many metabolites having related precursors, derivatives, degradation products, potentially more compounds per SWATH acquisition isolation window and overlapping fragment ion. Here we present ongoing work which is understanding the ionization behavior of different metabolites and also developing novel approaches for the extraction of metabolites and integrate into single processing pipeline. A single standard compounds analyzed by LC-MS often produced multiple peaks along with M+H/M-H due to adducts [Na, K, Li, NH4, Acetate, Formate, Cl etc.],multiply charged, dimers and in-source fragments. These multiple peaks for the same compound show same elution profile (retention times). These additional peaks represent also the actual compounds though they are generated during process of sampling and analysis. Advantages of these additional peaks is that they can be used for better peak group identification through better scoring algorithm and quantification of compounds to reduced false positive and also compounds with similar structural groups could be retrieved in rich SWATH acquisition data.

INTRODUCTION

Current approaches in mass spectrometry technologies are still unable to map whole metabolome. SWATH acquisition provides comprehensive untargeted data collection of molecular data. Processing of this rich and complex SWATH acquisition data is possible with known spectral data and predict spectra of similar/modified compounds for identification and quantification. But still there are many unknowns in data that could be other metabolites, breakdown products, artifacts (observed due to sample preparation or instrument data acquisition). Here we present ongoing work which is understanding the ionization behavior of different metabolites and their observed different features (Figure-1) per metabolite, which facilitates accurate identification and quantification of metabolites.

MATERIALS AND METHODS

Mass Spectrometry Metabolite Library (MSMLS) kit was purchased from Sigma-Aldrich(Now Merck). MSMLS kit contains 600 unique metabolites with 5 ug (dried weight) per well in 96 well format
Standard Plate preparation protocol was followed according to vendor suggestions.

Plates 1-5: Add 5% of final volume (up to 20 µL) of high purity methanol (MeOH) to every well of every plate. Add ultrapure water to make up the desired volume. The addition of water ensures the solubilization of the more polar compounds. A final 5% methanol solution is suggested. Pipette liquid up and down in the well 2-3 times to facilitate solubilization.

Plates 6 and 7: These plates contain primarily lipid-like compounds (with the exception of the water soluble sugar compounds in plate 6). It is recommended to solubilize these compounds in a solvent with a 1:1:0.3 ratio of chloroform:methanol:water.

LC/MS Conditions:

A Agilent 1290 Infinity II LC system with an Agilent BC-Poroshell HPH-C18,2.1x50mm at 40° C with a gradient of eluent A Water + 10mM ammonium formate and eluent B Methanol/Acetonitrile/Isopropanol (50/45/5) + 10mM ammonium formate was used, flow rate 400µl/min gradient: 0min %B 5, 0.5min %B 5, 2.2min %B 90, 2.5min %B 99, 2.51 min %B 5 and 3.5min %B 5.

MS conditions:

The SCIEX TripleTOF® 6600 System was used to acquire data in both positive and negative ion mode using IDA (information dependent acquisition) and SWATH acquisition mode [parameters CE was 30 and CES 15].

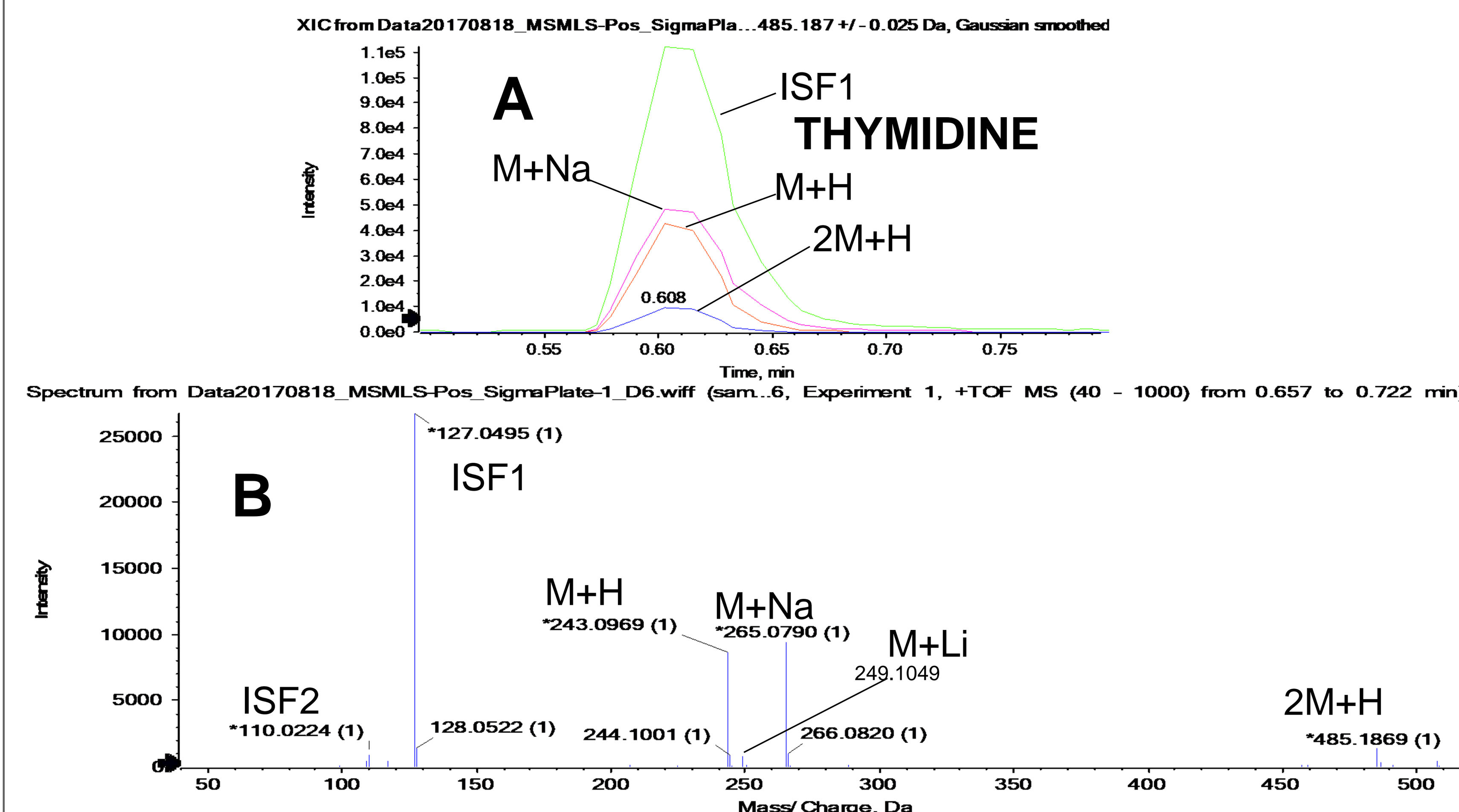


Figure 1. LCMS profile of THYMIDINE showing different Features
A. XIC profile of Thymidine standard with M+H,, M+Na, 2M+H, ISF1 [Insource Fragments]. B. MS profile of Thymidine with various features.

RESULTS

Each standard was injected at 500ng/ml concentration in batch of 7(96 samples per batch) with Adenosine as QC every 12th injection in both positive and negative mode. A single standard compounds analyzed by LC-MS often produced multiple peaks due to in-source fragmentation, adduct formation and dimers (Table-1 and Figure-1).

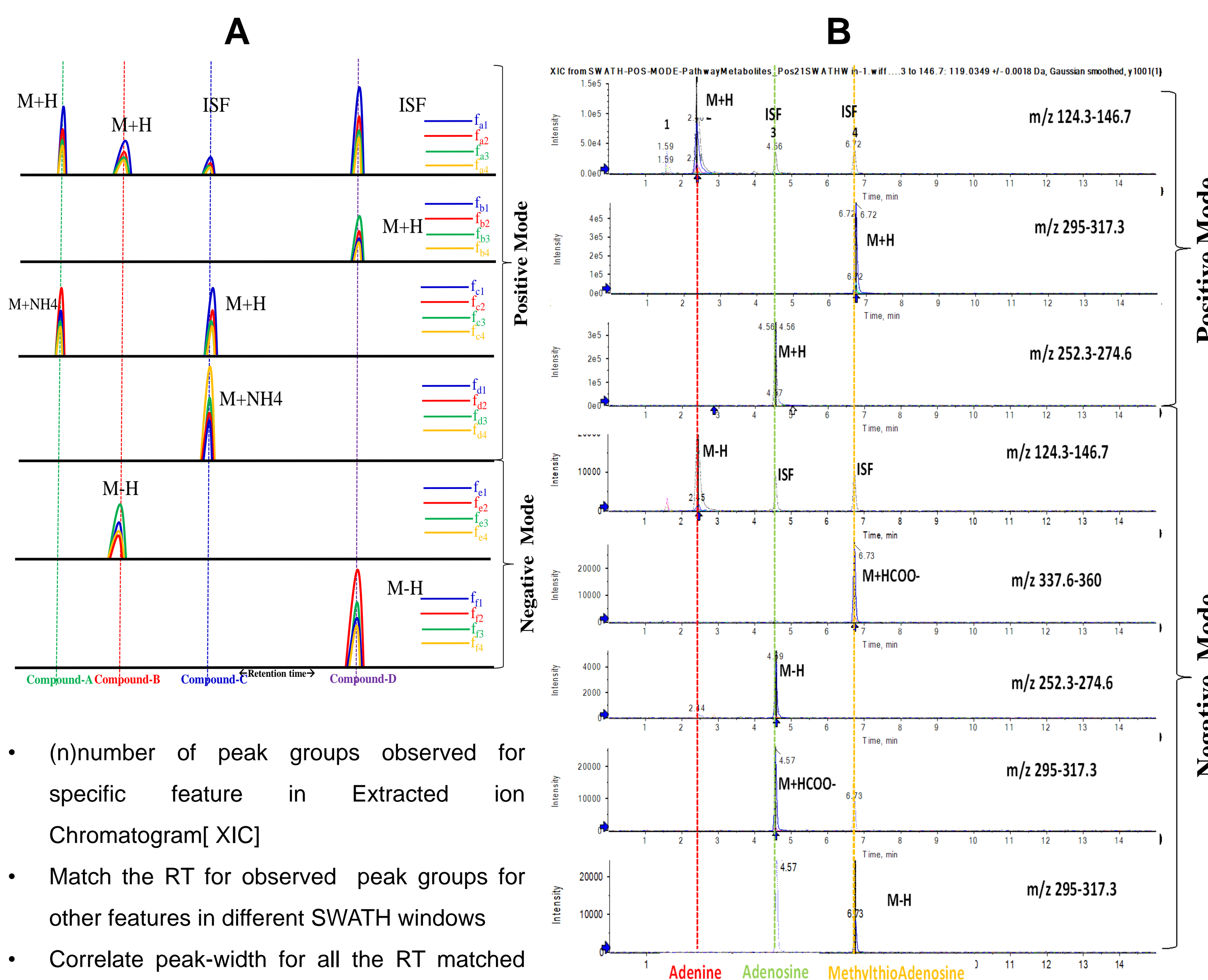
	Detected peaks	No. of Compounds	Relative %
Positive Mode	M+H/M+	354/4	27.70
	M+2H	6	0.46
	M+Na	212	16.36
	M+NH4	30	2.31
	M+K	82	6.33
	2M+H	33	2.55
	M+Li	31	2.39
	ISF1+	281	21.68
	ISF2+	172	13.27
	ISF3+	90	6.94
Negative Mode	M-H	394	53.03
	M-2H	5	0.67
	$[M+HCOO]^-$	34	4.58
	$[M+Na]^-$	61	8.21
	$[M+Cl]^-$	23	3.10
	2M+H	2	0.27
	ISF1-	166	22.34
	ISF2-	47	6.33
	ISF3-	11	1.48

Table-1: Summary of analysis of peaks detected by LC-MS. There are multiple peaks observed for each standard represented as adducts, In-source fragments(ISF), complexes. I[SF- Only top 3 in-source fragments were selected based on intensity for data mining] .

All the resulting features were acquired in MS/MS mode for generating Accurate Mass Metabolite library

- MS/MS was acquired for total of 2038 features in targeted mode
- After screening for good quality spectra for ion library generation, managed to get 761 features [From 342 compounds (83 compounds are unique to Positive mode)] in Positive mode and 515 features [From 320 compounds(61 compounds are unique for Negative mode)] in Negative mode
- Finally Accurate mass metabolite library of 403 compounds with 1276 features was generated

These additional features are also the actual compounds though they are generated during process of sampling and analysis. Advantages of these additional features is that they can be used for better peak group identification and quantification of compounds to reduced false positive and also compounds with similar structural groups could be retrieved in rich SWATH acquisition data (Figure 2).



- (n)number of peak groups observed for specific feature in Extracted ion Chromatogram[XIC]
- Match the RT for observed peak groups for other features in different SWATH windows
- Correlate peak-width for all the RT matched peak groups

Figure 2. Peak group scoring methods for metabolomics A. Model to show how compounds with different features can be seen aligned at similar Retention times (RT) and strategies to improve peak group scoring.
B. Metabolite standard mix with 91 standards. Example: XIC [SWATH window 124.3-146.7] of Adenine (peak group 2) shows 4 peak groups. Peak groups 3,4 identified as insource fragments [ISF] from Adenosine and methylthioadenosine. Without RT information in library for the Adenine its impossible to identify the right peak group using single feature M+H or M-H. Here we show using other features of same compound can be used even without predefined RT in library to identify right compounds avoiding False positives.

CONCLUSIONS

Though there are several computational tools and resources to annotate data from untargeted metabolomics experiments which involves still manual curation due to rich and complex nature of metabolomics data. Here we show how to use additional features produced per compounds as additional specificity and specificity for peak group identification. Further more we are improving algorithms and strategies and then these tools will be validated in biological matrix like urine, plasma etc.

REFERENCES

1. Gillet LC, Navarro P, Tate S, Röst H, Selevek N, Reiter L, Bonner R, Aebersold R. *Mol Cell Proteomics* 2012 Jun; 11(6): O111.016717.
2. Tsugawa H, Cajka T, Kind T, Ma Y, Higgins B, Ikeda K, Kanazawa M, VanderGheynst J, Fiehn O, Arita M. *Nat Meth* 2015 Jun;12(6):523-6
3. Domingo-Almenara X, Montenegro-Burke JR, Benton HP, Siuzdak G. *Anal. Chem* 2018 Jan 2; 90(1): 480-489
4. Bonner R, Hopfgartner G. *Bioanalysis.* 2016 Aug;8(16):1735-50.
5. Mahieu NG, Patti GJ *Anal Chem* 2017 Oct 3;89(19):10397-10406
6. Baljit Ubhi . Untargeted Metabolomics by LCMS; 2013 presentation

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Document number: [RUO-MKT-10-7824]