ION MOBILITY-ENABLED METABOLITE IDENTIFICATION OF TIENILIC ACID AND TIENILIC ACID ISOMER

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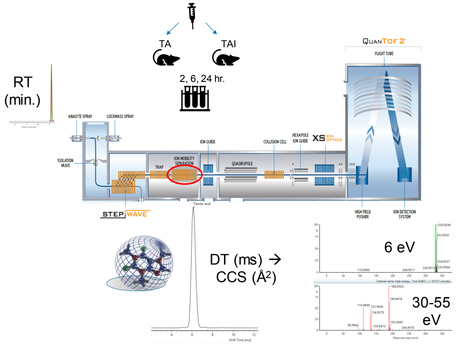
INTRODUCTION

- Tienilic acid (TA) is a uricosuric diuretic found to induce immune-mediated hepatotoxicity in patients, while its 3-thiophene isomer (TAI) exhibits direct hepatotoxic effects, and differential metabolism has been reported for these two compounds [1,2].
- The aim of this study is to demonstrate the use of high resolution mass spectrometry (HRMS) coupled with ion mobility separation (IMS) in the elucidation of metabolites from rat urine samples obtained over a course of treatment with TA or TAI.
- Specifically, IMS-derived collision-cross section (CCS) experimental values for proposed metabolites are compared with predicted values obtained through machine-learning models, providing an avenue for further metabolite structural identification support.



METHODS

SAMPLE INFORMATION:

Male Sprague-Dawley rats were dosed intravenously with 250 mg/kg TA or TAI. Urine was collected at the 2, 6 and 24 hr. time points following dosing. Blank vehicle dosed rat urine was also collected. Prior to LC-MS analysis the samples were diluted 9:1 (v/v) with LC-MS grade water. Additional sample information can be found in [2] and [3].

LC CONDITIONS:

LC System:	ACQUITY UPLC® I-Class
Column:	ACQUITY UPLC HSS T3 1.8 µm, 2.1 x 100 mm
Temp:	30 °C
Sample Temp:	10 °C
Injection Volume:	2 µL
Mobile Phases:	A: 0.1% formic acid in water B: 0.1% formic acid in acetonitrile

Gradient:

Time	Flow rate	% MP A	% MP B	Curve
Initial	0.5	99	1	Initial
1.00	0.5	99	1	6
3.00	0.5	85	15	6
9.00	0.5	50	50	6
10.50	0.5	5	95	1
12.00	0.5	99	1	1

MS CONDITIONS:

Instrument :	Vion IMS QTof
Ionization Mode:	ESI+
Collision Energy (LE):	6 eV
Collision Energy (HE ramp):	35-55 eV
Scan Time:	0.10 sec
Acquisition Range:	50-1200 m/z
Drift Gas:	N_2
IMS Wave Velocity:	250 m/s
IMS Wave Height (ramp):	20-55 V
Capillary:	1.0 kV
Source Temperature:	120°C
Desolvation Temperature:	500°C
Cone Gas Flow:	50 L/hr
Desolvation Gas Flow:	800 L/hr
Lockmass:	Leucine Enkephalin (556.2766 m/z)

INFORMATICS:

Instrument control and data acquisition was performed using UNIFI Scientific Information System (Waters Corporation). Data was processed using UNIFI and exported via API to WebMetabase (Lead Molecular Discovery, Ltd.) for further metabolite identification. Regression analysis of CCS values were performed using Spotfire (TIBCO Software Inc.)

References

1. Bonierbale E. et al. (1999) *Chem. Res. Toxicol.* 286-296
2. Coen M. et al. (2012) *Chem. Res. Toxicol.* 2412-2422
3. King A. et al. (2018) *J. Chrom. B* 142-148
4. Grant I. (2016) Thesis, Computational and Systems Medicine Imperial College London
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RESULTS AND DISCUSSION

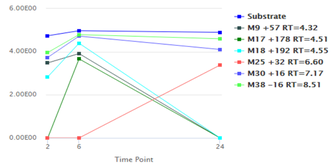
A) METABOLITE IDENTIFICATION

1 Summary of identified metabolites in TA/TAI treated rat urine samples.

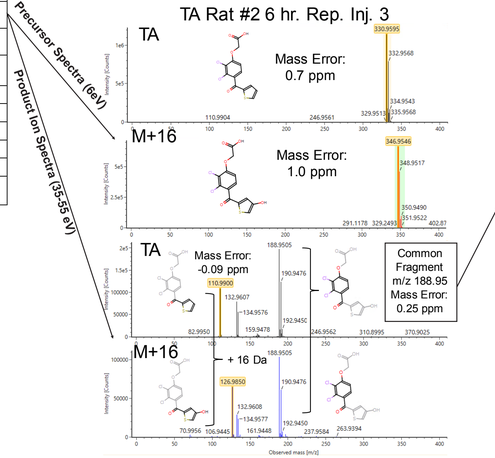
Metabolite	Mass Shift	Ion Formula	m/z	RT (min.)
Tienilic acid	N/A	$[C_{13}H_8Cl_2O_3S+H]^+$	330.96	9.09
Tienilic acid isomer	N/A	$[C_{13}H_8Cl_2O_3S+H]^+$	330.96	9.09
Hydroxylation	M+16	$[C_{13}H_8Cl_2O_4S+H]^+$	346.95	7.18 (TA); 8.67
O-Dealkylation and acetylation	M-16	$[C_{13}H_8Cl_2O_3S+H]^+$	314.96	8.51 (TA); 8.46
Hydroxylation and thiophene oxidation	M+32	$[C_{13}H_8Cl_2O_5S+H]^+$	362.95	6.60
Glycine conjugation	M+57	$[C_{15}H_{11}Cl_2NO_3S+H]^+$	387.98	4.34
Hydroxylation and acetylation	M+58	$[C_{15}H_{10}Cl_2O_4S+H]^+$	388.97	6.32
Reduction and glucuronidation	M+178	$[C_{19}H_{18}Cl_2O_{10}S+H]^+$	509.00	4.52
Hydroxylation and glucuronidation	M+192	$[C_{19}H_{16}Cl_2O_{11}S+H]^+$	522.99	4.55
Hydroxylation and methylation	M+30	$[C_{14}H_{10}Cl_2O_4S+H]^+$	360.97	7.32
Aromatic hydroxylation and glutamine conjugation	M+144	$[C_{18}H_{15}Cl_2N_2O_7S+H]^+$	475.01	6.39

Legend: Substrate | TA/TAI common metabolite | TA only | TAI only

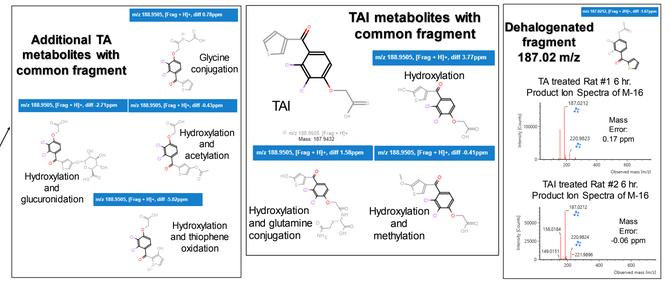
2 Metabolic clearance of TA and it's respective metabolites over three time points of urine collection.

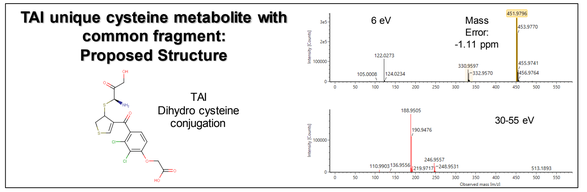


3 Structural confirmation of the site of metabolism for the hydroxylated metabolite was provided in the elevated collision energy spectrum through the presence of a +16 Da shift in the thiophene moiety product ion at 110.99 m/z .



4 The expected halogenated isotopic distribution pattern reflective of –Cl were used to support identifications. A di-chlorinated product ion at 188.9505 m/z was present in most metabolites, while the O-dealkylated and acetylated metabolite formed a non-halogenated product ion. Additional searching of the unassigned masses acquired was performed isolating analytes which contained a 2-Cl isotope signature as well as the common 188.95 m/z product ion resulted in a cysteine metabolite being identified, unique to TAI treatment. This finding was supported by available literature [4].





B) IMS-DERIVED EXPERIMENTAL VS. PREDICTED COLLISION CROSS SECTION VALUES

5 The ion mobility derived measurement collision cross section (CCS, measured in units of Å²) was recorded for all metabolites. Here, the observed CCS values for TA and TAI metabolites were

Metabolite	CCS (Å²)	SVR Model predicted CCS	Hybrid Model predicted CCS
Tienilic acid	166.1	183.7	167.2
Tienilic acid isomer	166.3	183.7	167.0
Hydroxylation	173.2	184.7	169.9
O-Dealkylation and acetylation	163.5	168.8	162.6
O-Dealkylation and acetylation	164.5	168.8	162.9
Hydroxylation and thiophene oxi-	177.4	--	173.6
Glycine conjugation	186.0	193.8	181.3
Hydroxylation and acetylation	182.5	198.1	180.9
Reduction and glucuronidation	212.1	223.1	206.4
Hydroxylation and glucuronidation	206.7	237.8	207.5
Hydroxylation and methylation	178.0	192.5	172.6
Aromatic hydroxylation and gluta-	197.9	214.6	200.3
Dihydro cysteine conjugation	203.6	209.8	198.8

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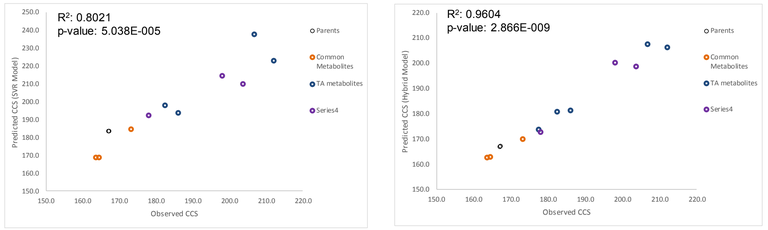

Correlation of modelled vs. observed CCS values were stronger when using the hybrid CCS model as opposed to the SVR method [5]. Both models were derived from machine learning approaches, while the hybrid model was specifically trained via a gradient boosting algorithm. Fidelity of CCS values in the hybrid model specifically provide an avenue for increasing structural identification confidence.

CONCLUSION

Differential metabolite identification of TA and TAI was greatly aided by the use of halogen isotope pattern and common structural product ion recognition. By acquiring concurrent IMS with the DIA approach employed, CCS values for all analytes are obtained. Following structural proposal, a hybrid CCS prediction model showed strong correlation with experimentally observed values, demonstrating the potential of predictive approaches in metabolite confirmation.

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