CHARACTERIZATION OF DIFFERENTIAL METABOLITES OF TIENILIC ACID AND ITS 3-THIOPHENE ISOMER WITH ION MOBILITY ENABLED MASS SPECTROMETRY

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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 determination of common and differential metabolites from rat urine samples obtained over a course of treatment with TA or TAI.
- Specifically, IMS-derived collision-cross section (CCS) measurements for proposed metabolites are compared with predicted values obtained through a machine-learning model, providing an avenue for further metabolite structural identification support.

METHODS

SAMPLE INFORMATION:

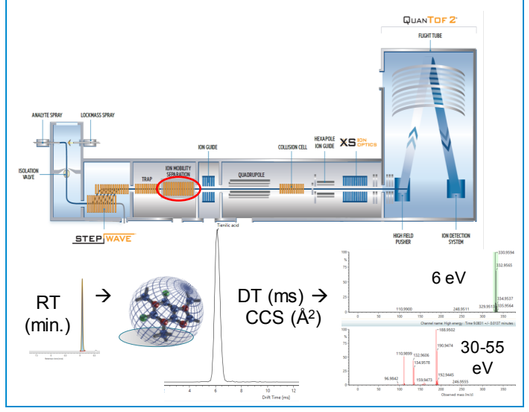
Male Sprague-Dawley rats were dosed intravenously with 250 mg/kg TA or TAI. Urine was collected at the 2, 6 and 24 hr. time points following dosing. Blank vehicle dosed rat urine was also collected. Prior to LC-MS analysis the samples were diluted 9:1 (v/v) with LC-MS grade water. Additional sample information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

LC CONDITIONS:

LC System:	ACQUITY UPLC® I-Class
Column:	ACQUITY UPLC HSS T3 1.8µm
Mobile Phase:	A: 0.1% formic acid in water B: 0.1% formic acid in acetonitrile
Run Time:	12 min.

MS CONDITIONS:

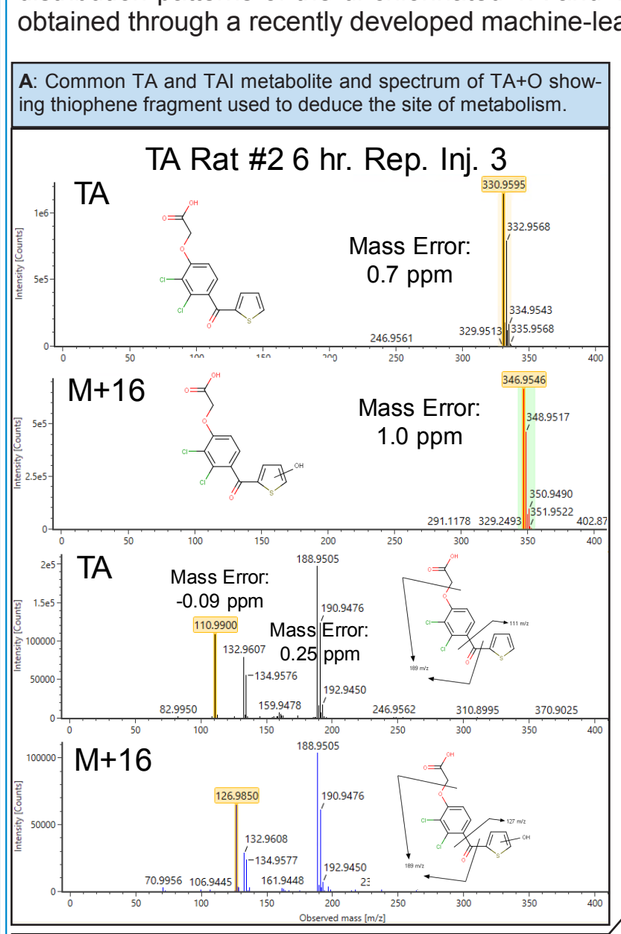
Instrument :	Vion IMS QTof
Ionization Mode:	ESI^+
Collision Energy (LE):	6 eV
Collision Energy (HE ramp):	35-55 eV
Scan Time:	0.10 sec
Acquisition Range:	50-1200 m/z
Drift Gas:	N_2
IMS Wave Velocity:	250 m/s
IMS Wave Height (ramp):	20-55 V
Lockmass:	Leu Enk (556.2766 m/z)



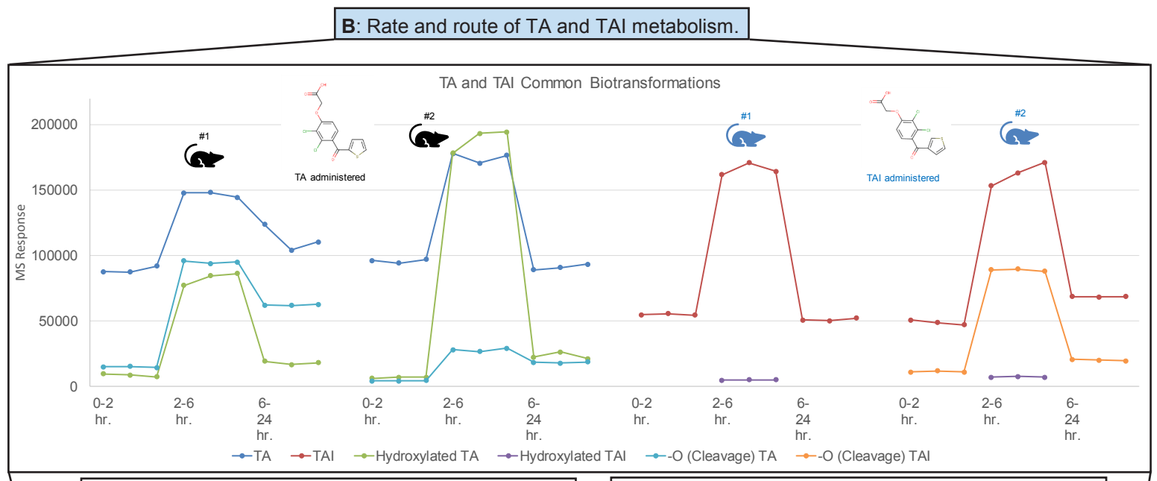
RESULTS AND DISCUSSION

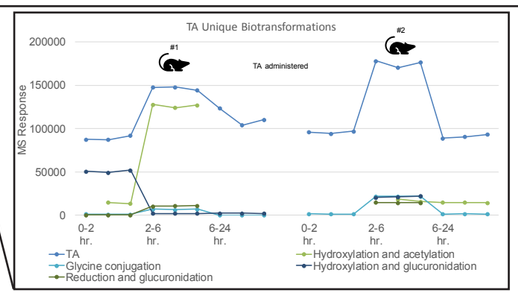
Metabolite identification in the urine samples indicated hydroxylation and other common biotransformations for both TA and TAI **(A)**. Additional major metabolites detected for TA and TAI are summarized in **(B)**. TAI treatment produced unique cysteine metabolites, indicative of the production of reactive species, not seen in TA treated animals. Identifications were supported by the presence of common fragments resulting from loss of the thiophene and carboxylic acid groups, low-collision energy fragments representing the cysteinyl group loss for the TAI metabolites (as described in [4]), and the expected isotope distribution patterns of the di-chlorinated TA and TAI molecules. Lastly, IMS-derived CCS experimental values were also compared with predicted values obtained through a recently developed machine-learning model [5], exhibiting a strong agreement between experimental and predicted values **(C).**

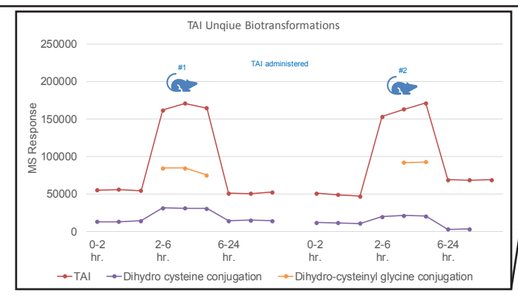
A: Common TA and TAI metabolite and spectrum of TA+O showing thiophene fragment used to deduce the site of metabolism.



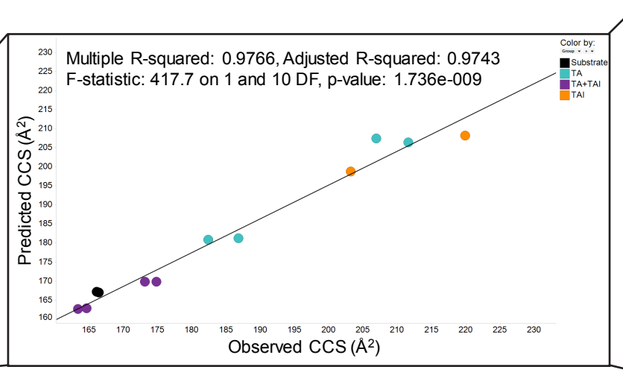
B: Rate and route of TA and TAI metabolism.







C: CCS (Å2) is a gas-phase measurement occurring after ionization, representing the average structural conformation of an ion as it moves through the mobility cell. Experimentally derived CCS values for all metabolites proposed above are compared to a predictive value obtained through a hybrid molecular modeling/machine-learning program (right). Use of predictive CCS is shown here to correspond within a mean error of 1.74% for the reported metabolites. Predictive values add further support to structural proposals by confirming its likely gas-phase behavior, and present an avenue for continued exploration.



Metabolite	Ion Formula	m/z	Observed CCS (Å²)	Hybrid Model predicted CCS
Tienilic acid	$[C_{13}H_8Cl_2O_4S + H]^+$	330.96	166.1	167.2
Tienilic acid isomer	$[C_{13}H_8Cl_2O_4S + H]^+$	330.96	166.4	167.0
Hydroxylation TA	$[C_{13}H_8Cl_2O_5S + H]^+$	346.95	173.1	169.9
Hydroxylation TAI	$[C_{13}H_8Cl_2O_5S + H]^+$	346.95	174.8	169.9
-O (Cleavage) TA	$[C_{13}H_7Cl_2O_3S + H]^+$	314.96	163.3	162.7
-O (Cleavage) TAI	$[C_{13}H_7Cl_2O_3S + H]^+$	314.96	164.6	162.9
Glycine conjugation	$[C_{15}H_{11}Cl_2NO_5S + H]^+$	387.98	186.74	181.3
Hydroxylation and acetylation	$[C_{15}H_{10}Cl_2O_6S + H]^+$	388.97	182.3	180.9
Reduction and glucuronidation	$[C_{19}H_{18}Cl_2O_{10}S + H]^+$	509.01	211.5	206.4
Hydroxylation and glucuronidation	$[C_{19}H_{16}Cl_2O_{11}S + H]^+$	522.99	206.86	207.5
Dihydro cysteine conjugation	$[C_{16}H_{15}Cl_2O_6S_2 + H]^+$	451.98	203.15	198.8
Dihydro-cysteinyl glycine conjugation	$C_{18}H_{18}Cl_2N_2O_7S_2 + H]$	509.00	219.85	208.2

CONCLUSIONS

- Elucidation of TA and TAI metabolites illustrates differential routes of metabolism
- By acquiring concurrent IMS with the DIA approach employed, CCS values for all analytes are obtained
- Following structural proposal, CCS predictions derived from a modelling program showed strong correlation with experimentally observed values, demonstrating the potential of predictive approaches in metabolite structural confirmation

References

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Acknowledgements

The authors wish to acknowledge Johannes PC Vissers of Waters Corporation and Robbin Bouwmeester of University of Ghent for creation and access to the hybrid CCS model program and Elisabeth Ortega-Carrasco from Lead Molecular Discovery for technical support.

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