ANALYSIS FOR ERINACINES AND HERICENONES IN LION'S MANE MUSHROOMS USING HRMS AND ION MOBILITY

Gordon T. Fujimoto, Sarah E. Dowd, Lindsay E. Hatch, Emily R. Britton
Waters Corporation, Milford, MA 01757 USA

CONNECTING SCIENCE To Solve Problems That Matter

Waters™

INTRODUCTION

Mushroom products are of interest to consumers for their potential health benefits. In particular Lion's Mane mushrooms (Hericium erinaceus) have been reported to be a source of natural products, erinacines and hericenones (Figs 3 & 6), which have been associated with a variety of health benefits including cognitive function[1]. Characterization of these natural products were done with ion mobility (IMS) and high resolution mass spectrometry (HRMS). The presence of erinacines and hericenones in a selection of Lion's Mane mushroom products were measured.

Figure 1: Lion's Mane Mushroom in Congaree National Park, Hopkins, South Carolina, USA

SAMPLE PREPARATION

Samples of powder extracts, tablets, and liquid drops, all advertised as containing Lion's Mane mushroom, as well as a package of dried mushrooms were obtained from online sources.

- Tablets were ground using a mortar and pestle; dried mushrooms were cut into smaller pieces (approximately 0.5 to 1 cm); powders were extracted as received.
- Based on packaging information, samples were extracted based on a ratio of 10 mL of organic solvent per one gram of Lion's Mane Mushroom.
- Samples were extracted by sonicating in ethanol for 30 minutes, followed by centrifugation (13,000 g for 30 min) and filtration (0.22 µm PTFE filter) before analysis.

METHODS

LC method:

Mobile Phase A: Water, 8 mM NH4OH

Mobile Phase B: Acetonitrile

Column: ACQUITY™ UPLC™ BEH™ C18 Column, 130Å, 1.7 µm, 2.1 mm X 100 mm

Injection Volume: : 5 µL

LC Gradient: 0 min 4% B
14 min 70% B
16 min 99% B
19 min 99% B
19.25 min 4% B
21.75 min 4% B

MS Method:

Instrument: Xevo™ G2-XS QTOF

Ionization: ESI$^-$

Capillary Voltage: 2 kV

Cone Voltage: 25 V

Desolvation Temperature: 450 °C

Desolvation Gas Flow: 800 L/hr

Waters SELECT SERIES™ Cyclic™ IMS

- Single pass resolution: R = 65 Ω/ΔΩ
- Multi-pass separation increases the IM resolution as the square root of the number of passes. Up to 6 passes (IMS resolution ~159 Ω/ΔΩ) was explored.
- Data were mined using MassLynx™ v4.2 and a DriftScope™ v3.0

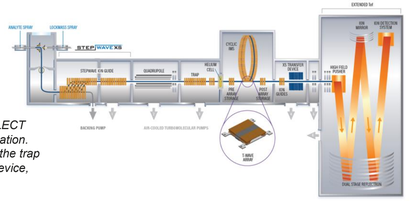


Figure 2. Schematic of the SELECT SERIES Cyclic IMS Instrumentation. It contains three main regions: the trap region, the cyclic ion mobility device, and the transfer region.

RESULTS: ERINACINE ISOMERS

Erinacines A, B, E, F are cyathin diterpinoids that all share the same chemical formula of $C_{25}H_{36}O_6$ (Figure 3). Example HRMS XIC's of erinacine isomers are shown in Figure 4.

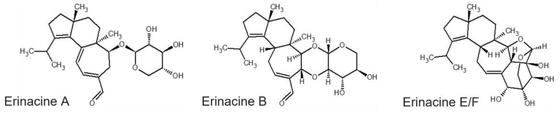


Figure 3: Erinacine A,B, E and F are geometric isomers have the molecular formula $C_{25}H_{36}O_6$. Erinacine F is similar in structure to erinacine E, but not all stereocenters have been defined.

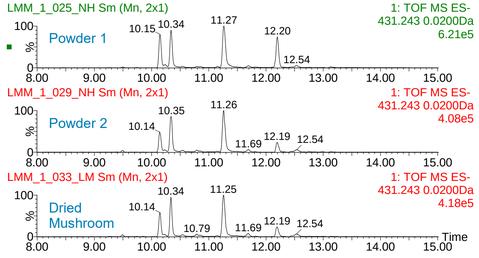
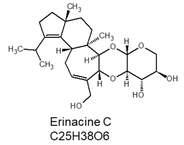


Figure 4: Extracted ion chromatograms from the negative ion analysis of selected mushroom samples for m/z=431.243 which corresponds to the M-H m/z for Erinacine A, B, E & F.

As commercial standards for the erinacine isomers are not available, more information was needed to identify specific isomers, which is why ion mobility in combination with chromatography was explored. The isomers are structurally very similar and high resolving power in the ion mobility dimension was needed to differentiate the individual erinacine isomers.

IMS RESULTS

Tentative identifications of the erinacine isomers were based on relative RT, MS/MS fragmentation and collisional cross section (CCS). The CCS values of the erinacine species separated in multi-pass experiments were calculated manually in the procedure described in McCullagh et al.[2] Calculated values were compared with predicted values from web-based tools AllCCS[3] and CCSOnDemand (Waters Corporation) to support identifications (Table 1).



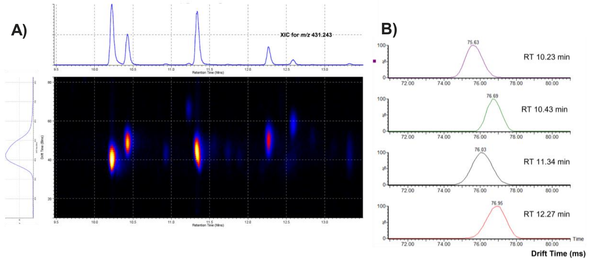


Figure 5. A) Plot of Drift time (bins) vs Retention time (min) for m/z 431.243 in a mushroom extract with ESI$^-$. Ion mobility separation was done with 6 passes (IMS resolution ~159 Ω/ΔΩ) in the cyclic IMS cell. B) Extracted arrival time distributions for the four chromatographically resolved peaks from the XIC for m/z 431.243 .

Table 1. Comparison of the predicted and calculated CCS for erinacine compounds found in mushroom extracts. Erinacines A,B, E, and F are geometric isomers and erinacine C is similar in structure to erinacine B.

Name	Formula	Ret Time (min)	All CCS $[M-H]^-$ CCS (Pred)	CCSOnDemand $[M-H]^-$ CCS (Pred)	$[M-H]^-$ CCS Calc 6 Pass ($Å^2$)
Erinacine A	C25H36O6	11.34	202.2	203.9	203.7
Erinacine B	C25H36O6	12.27	201.7	203.7	204.4
Erinacine C	C25H38O6	12.43	201.7	206.7	207.3
Erinacine E/F	C25H36O6	10.23	206.3	205.3	203.1
Erinacine E/F	C25H36O6	10.43	206.3	205.3	204.2

RESULTS: HRMS OF ERINACINES AND HERICENONES

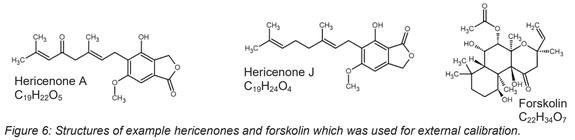


Figure 6: Structures of example hericenones and forskolin which was used for external calibration.

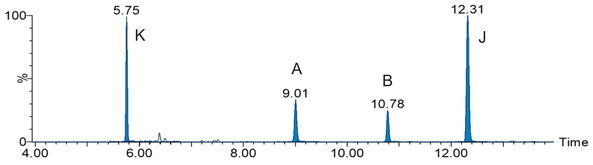


Figure 7: Extracted ion chromatograms for Hericenone A, B, J & K from the extraction of the dried mushroom sample. (A:m/z=329.1389; B:m/z=432.2175; J:m/z=315.1596; K:m/z=347.1495)

There was wide variation in the concentrations of erinacines and hericenones in the selection of online products assayed (Table 2). Standards of the compounds of interest were not readily available for this study. Relative quantitation was performed using forskolin $C_{22}H_{34}O_7$ (MW=410.5, M-H m/z=409.2) as an external calibration compound (Figure 6).

Table 2. Concentration of Erinacines & Hericenone in µg/g (LMM=Lions Mane Mushroom)

	Powder 1	Powder 2	Powder 3	Dried LMM	Tablet	Capsule
Erinacine ABEF	16	12	-	-	-	-
Erinacine C	57	49	-	-	-	-
Erinacine D	0.2	-	0.5	0.6	-	0.3
Erinacine H	4.0	-	0.2	0.2	-	0.2
Erinacine J	0.6	-	0.2	0.2	-	0.2
Erinacine KQ	58	100	0.1	0.3	0.2	0.4
Hericenone A	-	-	-	1.8	-	1.8
Hericenone B	-	-	-	1.3	-	0.5
Hericenone J	1.6	8.2	0.8	8.5	0.2	0.8
Hericenone K	0.4	0.7	1.3	3.2	0.3	0.6

CONCLUSIONS

HRMS is a useful tool to help identify natural products in foods as seen in this assay for erinacines and hericenones in Lion's mane mushrooms.

In cases where standards are not readily available, ion mobility can assist in the identification of natural products especially when some of the natural product compounds are present as geometric isomers of each other.

Future work will include expanding this work to cover other bioactive substances in other food products.

References

[1] https://www.healthline.com/nutrition/lions-mane-mushroom and reference there in.

[2] McCullagh, M., Goscinny, S., Palmer, M., Ujma, J. Investigations into pesticide charge site isomers using conventional IM and cIM systems. Talanta 234 (2021) 122604.

[3] Shanghai Institute of Organic Chemistry Chinese Academy of Sciences, Shanghai, China

TO DOWNLOAD A COPY OF THIS POSTER, VISIT WWW.WATERS.COM/POSTERS