Ion Mobility Separation Of Marine Natural Products: Characellides from the Deep-sea Sponge *Characella pachastrelloides*

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Introduction

***Characella pachastrelloides* was first described in 1876 as part of a collection of deep-sea sponges.[1] Deep-sea sponges are a source of unique natural products partly due to the extreme environment they inhabit. This study looks at a group of glycolipopeptides known as characellides that were isolated from *C. pachastrelloides* and found to be bioactive metabolites.[2,3] While HRMS can be used for some structural characterization, full elucidation of natural product structures remains challenging. The 'gold-standard' method for structural characterization of natural product compounds is nuclear magnetic resonance (NMR). However, certain compounds can be difficult to isolate or at concentrations too low for quality NMR analysis. This study investigates if ion mobility (IMS) can be another tool for structural assignments of these compounds.**

Sample Preparation

Figure 1. Illustration of the specimen of *C.pachastrelloides* photographed *in situ*
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- Samples of the deep-water sponge *Characella pachastrelloides* were collected from 809 m depth off the southwest coast of Ireland.
- Sponge biomass was powdered and extracted with MeOH −CH2Cl2 (1:1).
- Extract was fractioned using RP-C18 vacuum liquid chromatography(VLC), into five fractions: H2O, H2O; MeOH (1:1), H2O:MeOH (1:3), MeOH and MeOH:CH2Cl2 (1:1).
- Methanolic fraction was prepared to 0.2-2.0 mg/mL in H_2O.

LC-MS Experimental Parameters

LC: ACQUITY™ Premier Fixed Loop System

Column: ACQUITY Premier HSS T3,1.8 µm; 2.1mm x 100mm @ 35C

MP A: Water + 0.1% Formic Acid

MP B: Acetonitrile + 0.1% Formic Acid

Injection Volume: 5 µL

MS: SELECT SERIES™ Cyclic™ IMS System

Ionization: ESI^+

Capillary Voltage: +2 kV

Mass Range: *m/z* 50-1200

MS Analyzer Mode: V mode (Resolution ~60,000 FWHM)

Gradient:

Time (min)	Flow (mL/min)	%A	%B
Init.	0.5	90	10
1.5	0.5	70	30
9.0	0.5	40	60
10.0	0.5	0	100
13.0	0.5	0	100
13.1	0.5	90	10
16.0	0.5	90	10

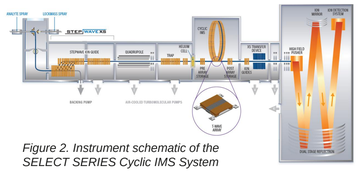
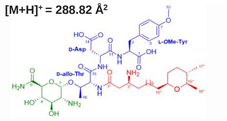


Figure 2. Instrument schematic of the SELECT SERIES Cyclic IMS System

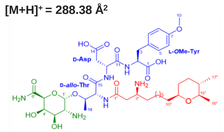
Results: Ion Mobility Separation of Characellide A and B

Characellide A and B are conformational isomers that share the same chemical formula of $C_{41}H_{66}N_6O_{14}$. These two compounds have been previously isolated and fully characterized with NMR.[1] the characellide isomers are structurally very similar and high resolving power in the ion mobility dimension was needed to differentiate the individual compounds. Predicted Collision Cross Section (CCS) values were generated with AllCCS.[4]

Characellide A
Predicted CCS
$[M+H]^+$ = 288.82 Å²



Characellide B
Predicted CCS
$[M+H]^+$ = 288.38 Å²



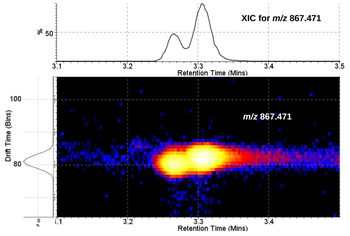


Figure 3. Plot of Drift time (bins) vs Retention time (min) for *m/z* 867.471 *in a sea sponge extract with ESI⁺. Ion mobility separation was done with a single pass (IMS resolution ~65 Ω/ΔΩ) in the cyclic IMS cell. While there is chromatographic separation of the isomers, there is very little separation in the IMS dimension.*

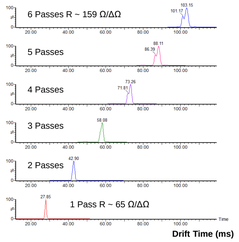


Figure 4. Extracted arrival time distribution (ATD) for m/z 867.471 in a sea sponge extract with multiple passes in the cyclic IMS Cell.

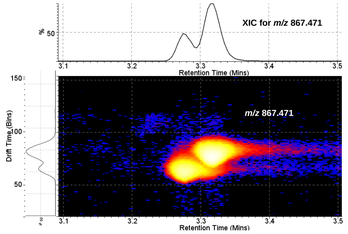


Figure 5. Plot of Drift time (bins) vs Retention time (min) for m/z 867.471 in a sea sponge extract with ESI⁺. Ion mobility separation was done with six passes (IMS resolution ~159 Ω/ΔΩ) in the cyclic IMS cell.

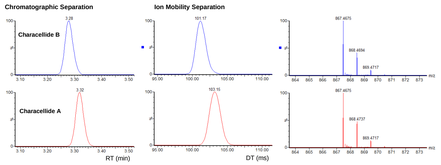
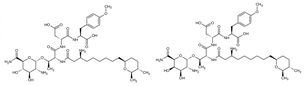


Figure 6. Separation of the characellide species by liquid chromatography (left) and ion mobility (middle). Based on the observed levels in previous studies, the peaks were tentatively identified. The right panel shows the mass spectra for the two compounds.

Results: Ion Mobility Separation of Characellide C and D

Characellide C and D are smaller versions of A and B with one less CH_2 repeat in the alkyl chain. Characellide C and D were investigated but could not be fully isolated from one another for full structural elucidation by NMR.[1]



Characellide C **Characellide D**

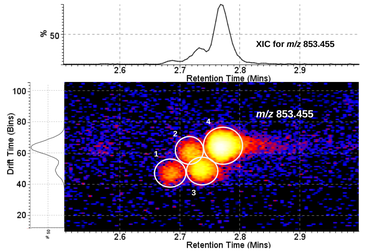


Figure 7: Plot of Drift time (bins) vs Retention time (min) for m/z 853.455 *in a sea sponge extract with ESI⁺. Ion mobility separation was done with 6 passes in the cyclic IMS cell.*

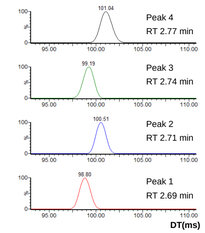


Figure 8: Extracted ATDs for the four peaks detected at m/z 853.455 from the sea sponge extract.

MS/MS Structural Elucidation

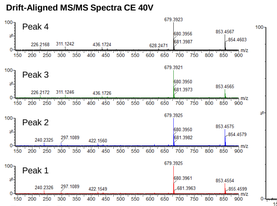


Figure 9. Retention and drift time-aligned fragmentation spectra for the four m/z 853.455 species. CID fragmentation was done in the transfer cell after IMS separation.

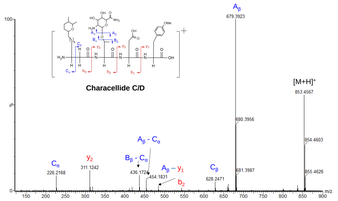


Figure 10. Annotated MS/MS spectrum for peak 4 RT 2.77 min with the structure for Characellide C/D.

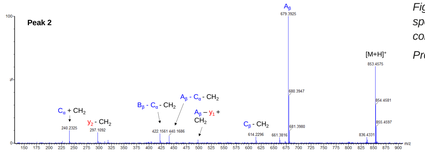
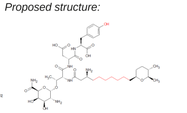


Figure 11. Annotated MS/MS spectrum for peak 2 RT 2.71 min compared with Characellide C/D.

Proposed structure:



Molecular Networking and CCS Measurement of Additional Characellides

A 0.2 mg/mL sample of the sea-sponge extract was analyzed with $HDMS^E$ data-independent acquisition and analyzed with feature based molecular networking (FBMN) utilizing the Global Natural Products Social (GNPS) Platform.[5] The molecular network can be visualized in Figure 13. The CCS of the detected characellides in multi-pass IMS acquisitions were manually calculated using the method described in McCullagh *et al.*[6] and presented in Table 1.

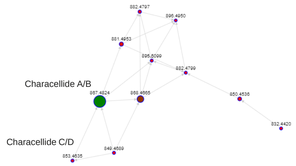


Figure 12: Feature based molecular network of methanol fraction of C. pachastrelloides focused on the characellides.

Table 1. Characellides identified in the extract and their calculated CCS values.

Name	Formula	Observed m/z	Ret. Time (min)	Calc CCS (Å²)
Characellide B	$C_{41}H_{66}N_6O_{14}$	867.468	3.26	286.84
Characellide A	$C_{41}H_{66}N_6O_{14}$	867.468	3.31	289.51
Characellide D	$C_{40}H_{64}N_6O_{14}$	853.451	2.74	284.35
Characellide C	$C_{40}H_{64}N_6O_{14}$	853.451	2.77	286.79
Characellide 849	$C_{41}H_{64}N_6O_{13}$	849.462	3.21	289.60
Characellide 849	$C_{41}H_{64}N_6O_{13}$	849.462	3.24	287.34
Characellide 853	$C_{40}H_{64}N_6O_{14}$	853.451	2.69	283.67
Characellide 853	$C_{40}H_{64}N_6O_{14}$	853.451	2.71	286.09
Characellide 854	$C_{40}H_{63}N_5O_{15}$	854.441	2.94	287.83
Characellide 868	$C_{41}H_{65}N_6O_{15}$	868.457	3.50	290.58
Characellide 881	$C_{42}H_{68}N_6O_{14}$	881.488	3.20	293.16
Characellide 881	$C_{42}H_{68}N_6O_{14}$	881.488	3.23	296.29
Characellide 881	$C_{42}H_{68}N_6O_{14}$	881.488	3.71	288.99
Characellide 881	$C_{42}H_{68}N_6O_{14}$	881.488	3.75	291.91

Conclusions

- **The structurally similar characellide isomers A and B were able to be separated in ion mobility space by utilizing multiple passes in the Cyclic IMS device.**
- **The added separation power of IMS led to the discovery of two new compounds with the same molecular formula as Characellides C and D.**
- **Additional related compounds were identified with molecular networking and their CCS values were calculated to give insights into the structural diversity of this unique set of compounds.**

References

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Conflict of Interest Disclosure: Sarah Dowd is an employee of Waters Corporation and is presenting the poster on behalf of the company.

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