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Differential Mobility Spectrometry of Paralytic Shellfish Toxins for Enhanced Selectivity of LC-MS Analysis

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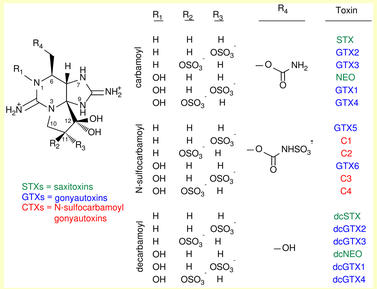
Introduction and Overview

- Paralytic Shellfish Toxins (PSTs) are a class of potent neurotoxin of algal origin that lead to intoxication and death annually as the result of consumption of contaminated shellfish.
- LC-MS is widely used for analysis of algal biotoxin, but challenges remain with small polar toxins like paralytic shellfish toxins (PSTs).
- PSTs exist as a mixture of isomers and analogues (Fig. 1) that must be separated prior to MS/MS analysis in complex biological and environmental samples prone to matrix interference.



	R1	R2	R3	R4	Toxin
carbamoyl	H	H	H		STX
	H	H	OSO_3^-		GTX2
	H	OSO_3^-	H		GTX3
	OH	H	H		NEO
	OH	H	OSO_3^-		GTX1
	OH	OSO_3^-	H		GTX4
N-sulfocarbamoyl	H	H	H		GTX5
	H	H	OSO_3^-		C1
	H	OSO_3^-	H		C2
	OH	H	H		GTX6
	OH	H	OSO_3^-		C3
	OH	OSO_3^-	H		C4
decarbamoyl	H	H	H		dcSTX
	H	H	OSO_3^-		dcGTX2
	H	OSO_3^-	H		dcGTX3
	OH	H	H		dcNEO
	OH	H	OSO_3^-		dcGTX1
	OH	OSO_3^-	H		dcGTX4

Figure 1. *Chemical structures of PST analogues*

- Recent work on the polar neurotoxin β-*N*-methylamino-L-alanine and its isomers showed the benefit to selectivity and limits of detection that could be achieved by using differential mobility spectrometry (DMS) in combination with LC and MS/MS detection.[1]
- Here, this approach is extended to PSTs with the goal of investigating their behaviour during DMS separation and developing a quantitative LC-DMS-MS/MS method.
- Preliminary using cylindrical FAIMS showed the promise of separating PSTs from one another using differential mobility.[2]
- Precise metering of low (< 1%) concentrations of solvent vapours as carrier gas modifiers has been critical to the optimization of DMS separations for small polar analytes.
- Insight is gained into the causes of in-source fragmentation of labile PSTs before, during and after DMS separation.

Experimental

- *SCIEX 5500 QTRAP operated in positive ionization mode*
- *NRC HILIC Method – Agilent 1260 LC*
  - 5 µm TSKgel Amide-80 column (250 x 2 mm ID, Toso Hass)
  - CH_3CN/H_2O gradient, 50 mM HCOOH + 2 mM NH_4OOCH
- *Routine UHPLC HILIC Method – Agilent 1290 LC*
  - 1.7 µm BEH Amide (100 mm x 2.1 mm ID, Waters)

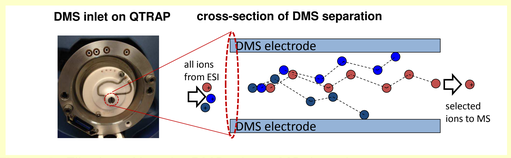


Figure 2. *Filtering of ions by DMS prior to MS detection*

- A SelexION DMS was modified for external metering of carrier gas modifier as described previously.[1,2,3]

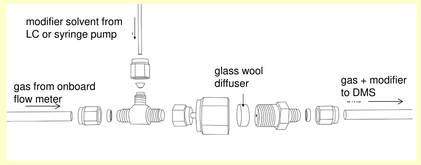


Figure 3. *Modified carrier gas modifier solvent delivery system*

Source Fragmentation of Labile PSTs

- Sulphated PSTs exist as pairs of extremely labile 11-α and stable 11-β epimers.
- For each source parameter, a compromise setting is needed that will preserve fragile PSTs but sensitively detect stable ones.

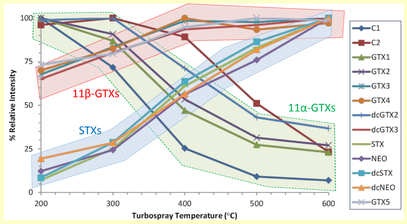


Figure 4. *Optimization of electrospray temperature for different classes of PSTs*

- Similar trends are observed for energies, temperatures and gas flows elsewhere in the MS source, during CID and for DMS optimization.

DMS Optimization

- The DMS carrier gas modifier delivery system was modified to allow for external metering of modifier solvent.
  - allowed for a full range of modifier concentrations to be investigated
  - improved signal stability and DMS peak shape
- Improved selectivity was observed at high dispersion voltage at the cost of sensitivity, especially for labile PSTs.

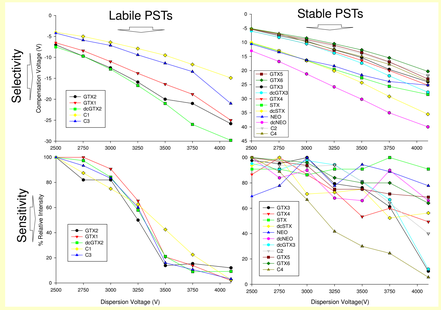


Figure 5. *Impacts of varying dispersion voltage on CV of transmission and sensitivity of PSTs analyzed by ESI-DMS-MS. (0.4 % MeCN modifier)*

- Improved selectivity was observed at low carrier gas concentration (~ 0.25 %), but higher concentrations (~ 1%) showed a protective effect on labile PSTs.

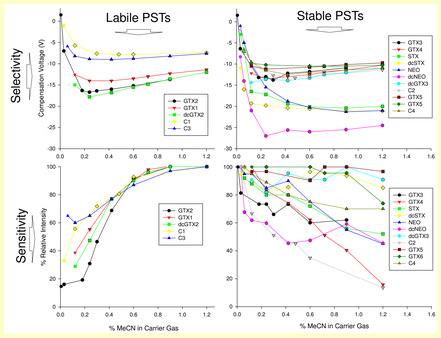


Figure 6. *Impacts of varying acetonitrile concentration in carrier gas on CV of transmission and sensitivity of PSTs analyzed by ESI-DMS-MS. (DV = 3250 V)*

Fragmentation of Labile PSTs in DMS

- Fragmentation before or during DMS led to detection of $[M+H-SO_3]^+$ product at a higher CV than the $[M+H]^+$ precursor.
- Products formed after DMS are detected at the CV of the precursor.

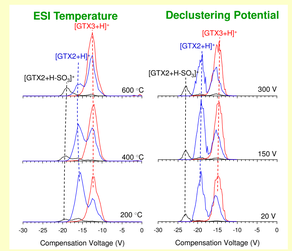


Figure 7 *Impact of ESI temperature and declustering potential on detection of labile (GTX2) and stable (GTX3) PST epimers by ESI-DMS-MS.*

- DMS parameters also cause fragmentation, but have an additional impact of changing the CV of precursor and product ions.

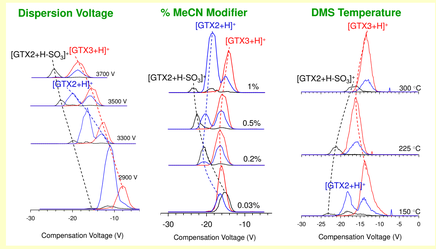


Figure 8. *Impact of DMS parameters on detection of labile (GTX2) and stable (GTX3) PST epimers by ESI-DMS-MS.*

HLIC-DMS-MS/MS Method Development

- Two sets of DMS conditions investigated in HILIC-DMS-MS/MS:
  - Maximum selectivity at 0.35% MeCN modifier and DV = 3250
  - Improved duty cycle and detection of labile PSTs at 2% MeCN and DV = 3500
- Selected reaction monitoring (SRM) transitions become compound specific once a CV is assigned
- DMS CV switching time slow (msec) compared to SRM (µsec)

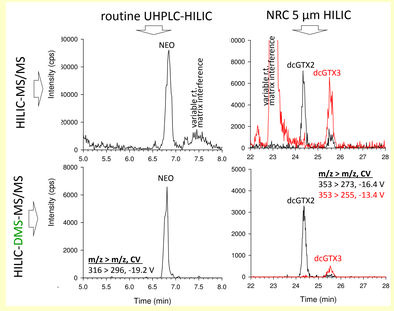


Figure 9. *Improvements in selectivity of PST detection using two different HILIC-DMS-MS/MS methods.*

- LOD proportional to the number of SRM transitions monitored.[1] Retention time scheduling used to maximize duty cycle in final method.

Quantitation of PSTs by HILIC-DMS-MS/MS

- Limits of detection estimated from S/N ratios of low level matrix matched standards.
- Differences in LOD between runs with and without DMS were highly analyte dependent.

LOD	STX	dcSTX	NEO	GTX1	GTX4	GTX2	GTX3	dcGTX2	dcGTX3	C2	C1	GTX5
HILIC-MS/MS (nM)	132	95	73	23	7.8	8.0	8.4	5.2	6.6	0.60	5.4	3.8
HILIC-DMS-MS/MS (nM)	23	48	22	2.8	36	5.0	3.0	1.5	1.6	1.5	0.62	5.4
HILIC-MS/MS (nmol/kg tissue)	661	476	364	116	39	40	42	26	33	3.0	27	19
HILIC-DMS- MS/MS (nmol/kg tissue)	114	240	111	14	181	25	15	7.4	8.0	7.6	3.1	27

- Quantitation of PSTs in three different shellfish tissue reference materials was compared by HILIC-MS/MS and HILIC-DMS-MS/MS.
- Good agreement observed with reference values and especially with HILIC-MS/MS results.

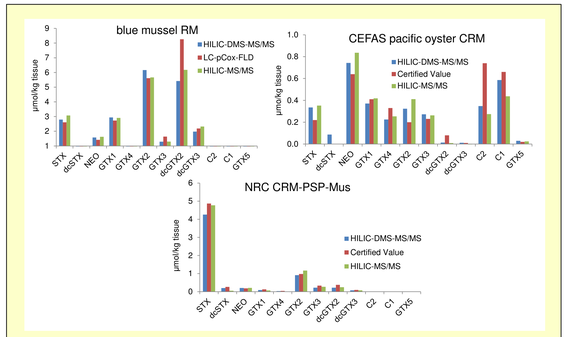


Figure 10 *Comparison of quantitative analysis by HILIC-MS/MS and HILIC-DMS-MS/MS to reference values for three shellfish tissue reference materials. PSTs were extracted using a dispersive single step extraction and quantitated using matrix matched calibration.*

Conclusions and Future Work

- DMS showed excellent selectivity for PSTs and was able to separate all isomers from one another.
- Labile analytes are prone to dissociation during DMS separation.
- DMS conditions with higher modifier concentrations and lower temperatures help preserve labile analytes.
- Multidimensional HLIC-DMS-MS/MS method showed improved selectivity compared to HILIC-MS/MS, but at the cost of sensitivity.
- Planar DMS showed similar ability to separate PST epimers to previous work using cylindrical FAIMS[3], both of which showed significantly higher resolution of epimers than linear traveling wave ion mobility spectrometry[4].
- Going forward, HILIC-DMS-MS/MS will be used for PST quantitation in cases where matrix interference is suspected.
- In the future, DMS could be used for direct screening by flow injection DMS-MS/MS, without the need for chromatography.
- The utility of DMS for other classes of algal biotoxins will continue to be investigated.

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