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High Resolution Quantitation of Microcystins with SCIEX X500R QTOF System

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Microcystins and Environmental Analysis

Why microcystins and nodularins?

- Toxic products of cyanobacteria and aquatic microfauna
- Microcystins represent an important contaminant class for environmental and drinking water analysis
- US EPA UCMR4 list, effective starting in 2018

LC-MS/MS Analysis

- Commonly conducted on triple quadrupole systems for sensitivity and robustness
- Environmental testing labs are frequently confronted with the need for more confirmatory information
  - Positive analyte identification in real world samples
  - False positives impact water supply
- Increased interest and adoption of high resolution/accurate mass (HRAM) systems
  - Specificity and qualitative confirmation of compound identification, while maintaining sensitive, robust, routine quantitation

Overview

Key Points Demonstrated

- Methods and data are presented which demonstrate use of HRAM for accurate and sensitive quantitation of microcystins and nodularin in water samples.
- Quantitation of a suite of eight microcystins (MC) and one nodularin (NOD) was achieved using high resolution accurate mass LC-MS/MS with the SCIEX X500R QTOF system.

SCIEX X500R QTOF System

Engineered for simplicity, functionality

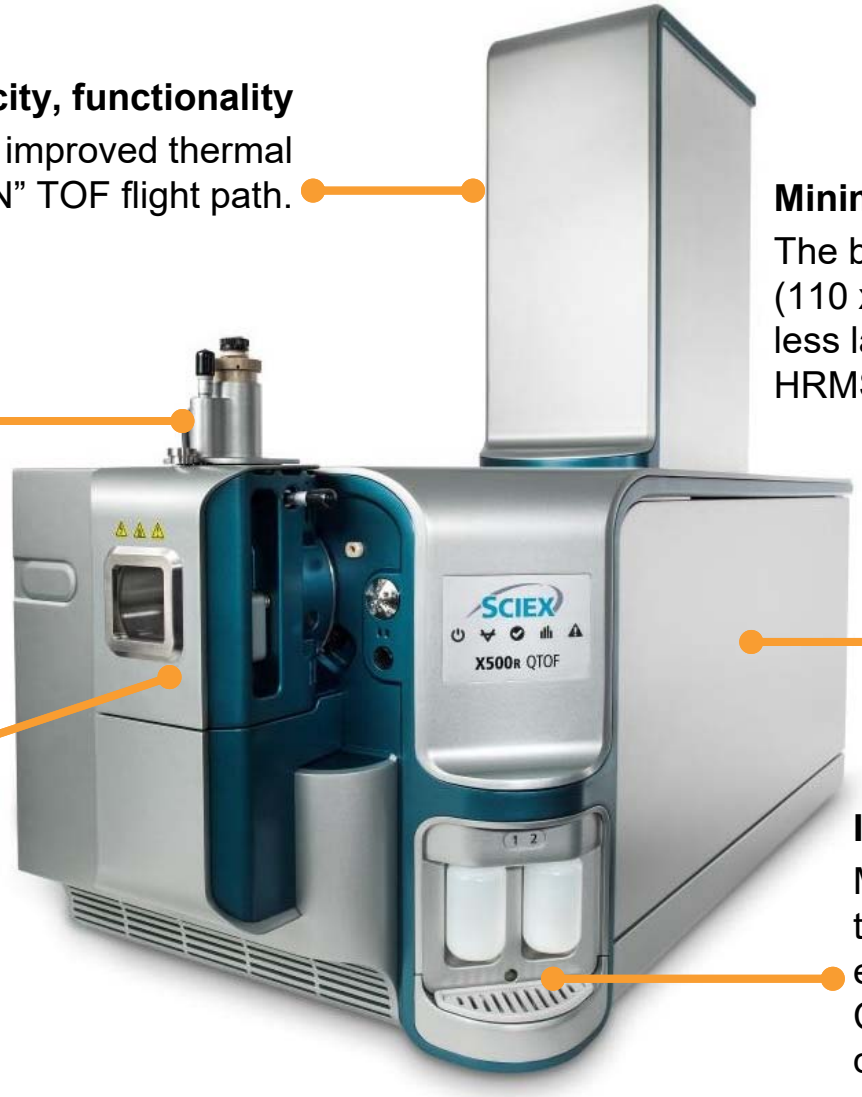
orter vertical profile and improved thermal insulation with “N” TOF flight path.

TwinSpray

An independent calibrant delivery path for auto-calibration that doesn’t interfere with heating within the source.

Turbo V Source

Proven ruggedness with capability for ESI and APCI configurations.



Minimized footprint

The benchtop stature (110 x 57 x 112 cm)* occupies less lab space than any other HRMS system on the market.

Integrated calibration

Maintains mass calibration through long runs without effect on sample flow. Completely integrated design eliminates external pumping systems.

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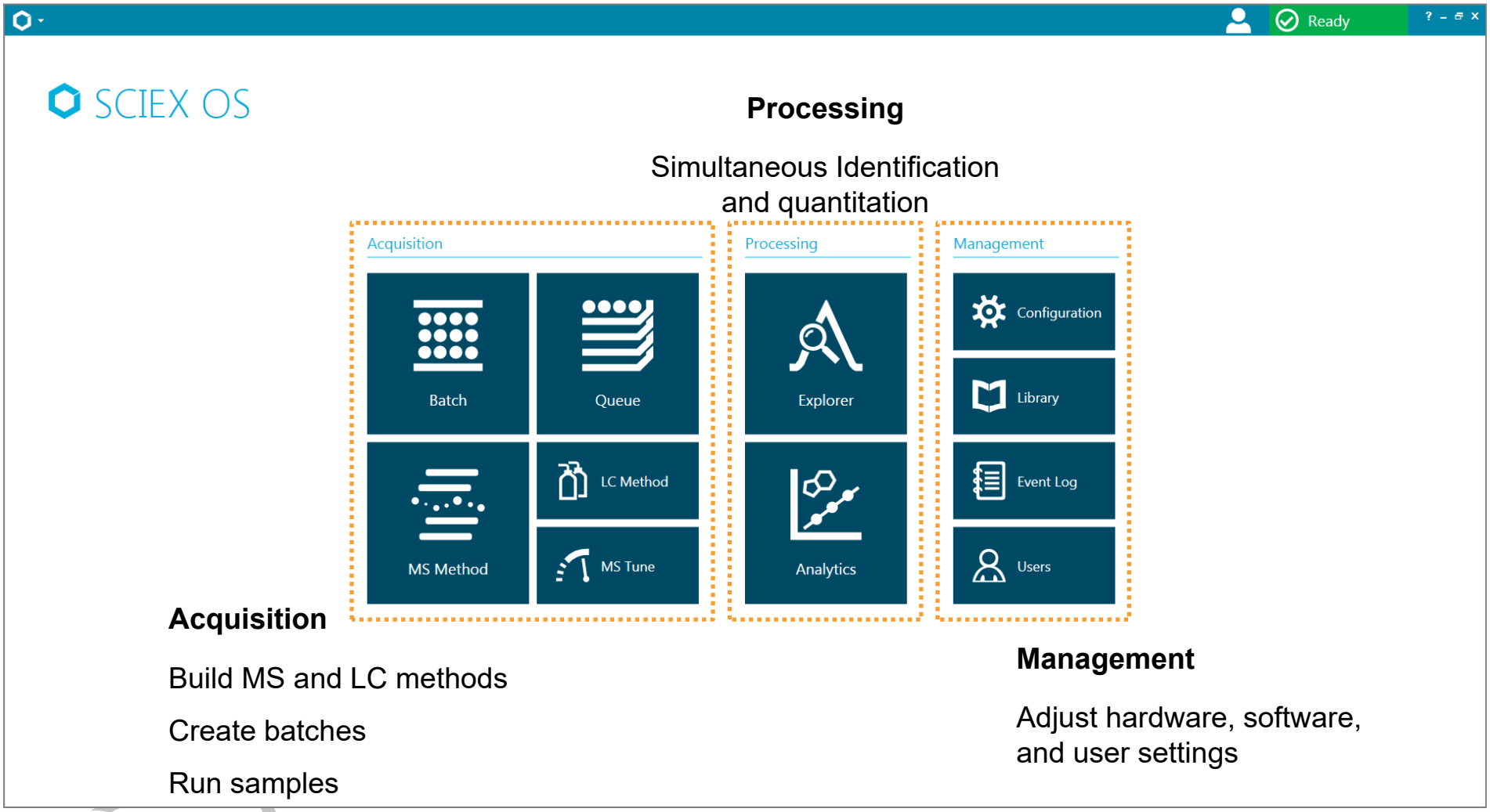
SCIEX OS Software – Home



Processing

Simultaneous Identification and quantitation

Acquisition

Build MS and LC methods

Create batches

Run samples

Management

Adjust hardware, software, and user settings

Analytical Conditions

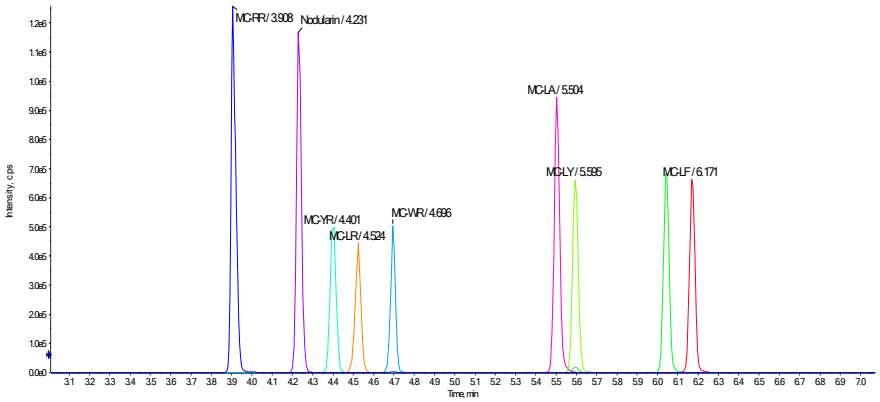
HPLC Conditions

- SCIEX ExionLC™ AD
- Chromatographic separation under gradient conditions using a Phenomenex Kinetex® C8 column (2.6 µm particle size, 100 x 3 mm)
- Flow rate of 0.500 mL/min.
- Column oven temperature was 40° C and a 20 µL injection
- Run time was 11 minutes for the full gradient

TOF MS Conditions

- SCIEX X500R QTOF system
- Turbo V™ source operated in positive mode electrospray ionization (ESI)
- TOF MS scan conducted from 500-1100 *m/z*
- MRM^{HR} experiment monitored 2 transitions for each analyte
  - Optimized compound-specific voltages were designated for maximum sensitivity and specificity
  - RT values were specified for each MRM^{HR} transition to optimize cycle time for best peak shape and quantitation

Notes on Chromatography



Chromatographic Separation of 8 Microcystins and 1 Nodularin. Separation was achieved using a SCIEX ExionLC AD system and a Phenomenex Kinetex C8 column over an 11-minute gradient with water and acetonitrile.

- The 11-minute chromatographic gradient with the Kinetex C8 column achieved baseline separation for all compounds.
- The gradient is **15 min shorter** than the program described in EPA Method 544, resulting in considerable time savings while maintaining separation and peak quality

Data Processing- Extracting Target Masses from TOFMS Data

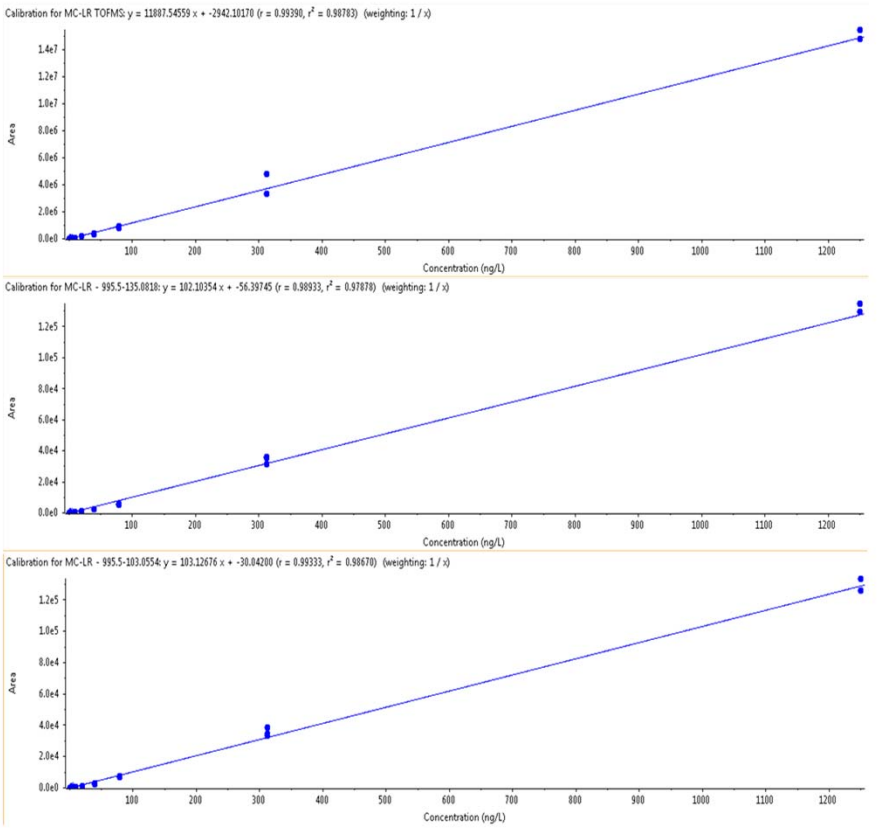
Accurate Masses for Microcystins and Nodularin. The formula and accurate monoisotopic *m/z* for each analyte studied is provided.			
Compound ID	Formula	Adduct	Monoisotopic m/z
MC-RR	$C_{49}H_{75}N_{13}O_{12}$	[M+2H]++	519.79018
Nodularin	$C_{41}H_{60}N_8O_{10}$	[M+H]+	825.45052
MC-LA	$C_{47}H_{68}N_6O_{12}$	[M+H]+	909.49680
MC-LF	$C_{52}H_{71}N_7O_{12}$	[M+H]+	986.52335
MC-LR	$C_{49}H_{74}N_{10}O_{12}$	[M+H]+	995.55604
MC-LY	$C_{52}H_{71}N_7O_{13}$	[M+H]+	1002.51826
MC-LW	$C_{54}H_{72}N_8O_{12}$	[M+H]+	1025.53425
MC-YR	$C_{52}H_{72}N_{10}O_{13}$	[M+H]+	1045.53531
MC-WR	$C_{54}H_{73}N_{11}O_{12}$	[M+H]+	1068.55129

Building a processing method for quantitation based on TOFMS data is as simple as entering the formula and expected adduct. The software calculates the monoisotopic mass and pulls the extracted peak from the TOF MS trace.

- Quantitation was achieved by utilizing high resolution TOF MS data of each precursor.
- MRM^{HR} transitions were acquired and used for quantitation and confirmation.
- Both scans occur within every cycle during an injection, and processing of the acquired data can utilize either or both.
- TOF MS data was processed using known analyte charged monoisotopic masses (*m/z*) and a mass extraction width (XIC width) of 0.02 Da

Data Analysis and Method Performance Evaluation

- Data acquisition and processing were performed using SCIEX OS software.
- Calibration curves over seven concentration levels from 0.12-312 µg/L
- Linear regression models with 1/x weighting to fit all calibration curves
- (LOD) was defined as the lowest calibrator S/N ≥3 and (LOQ) was defined as the lowest calibrator with S/N ≥10



Calibration Curves for MC-LR. Curves are shown for one of the target analytes, MC-LR, for three different acquisition channels. The isolation of the TOF MS accurate mass (top), as well as the two optimized MRM^{HR} transitions, can all be detected in calibration standards to produce a regression for quantitation.

Data Analysis and Method Performance Evaluation

Using the TOF MS for quantitation results in excellent sensitivity with sub-ppb detection limits and exceptional precision at low and mid range concentrations

Quantitative Analysis Method Performance. Detection and quantitation limits, linearity, and reproducibility for the microcystins suite. TOF MS data processed with a mass extraction width (XIC width) of 0.02 Da.

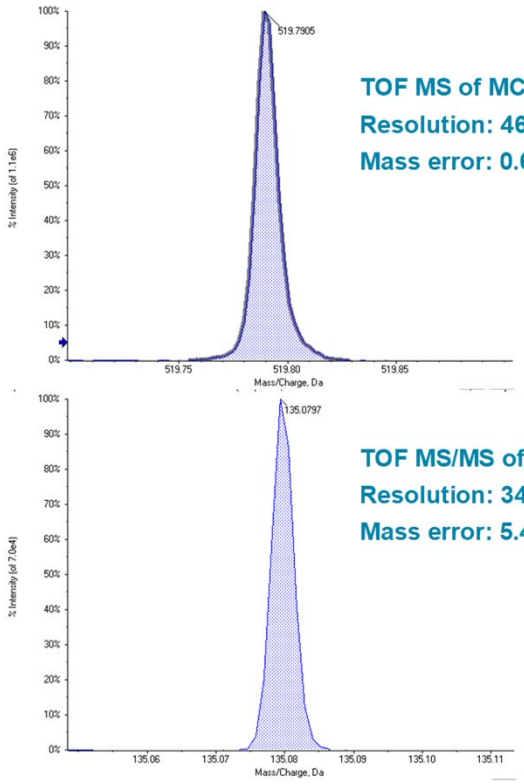
Compound ID	LOD (µg/L)	S/N at 0.12µg/L	%CV at 0.12µg/L	%CV at 4.88ppb	Cal Range (µg/L)	Curve fit, Weighting	Dynamic Range (log[ULOQ/LLOQ])
MC-RR	<0.12	91	7%	4%	0.12 – 312.5	Linear, 1/x	3.4
Nodularin	<0.12	60	2%	3%	0.12 – 312.5	Linear, 1/x	3.4
MC-LA	<0.12	39	2%	5%	0.12 – 312.5	Linear, 1/x	3.4
MC-LF	<0.12	36	2%	2%	0.12 – 312.5	Linear, 1/x	3.4
MC-LR	<0.12	27	2%	4%	0.12 – 78	Linear, 1/x	2.8
MC-LY	<0.12	32	2%	3%	0.12 – 78	Linear, 1/x	2.8
MC-LW	<0.12	31	2%	2%	0.12 – 78	Linear, 1/x	2.8
MC-YR	<0.12	17	2%	2%	0.12 – 312.5	Linear, 1/x	3.4
MC-WR	<0.12	15	2%	1%	0.12 – 39	Linear, 1/x	2.5

High Quality MS and MSMS Data for Confirmation

A key advantage of HRAM approach to quantitation is the ability to apply multiple points of confirmation to avoid reporting false positive detection The five points of confirmation applied in this method are:

1. Accurate mass of the target precursor
2. Confirmation with the MRM^{HR} transition and the accurate mass of the fragment ion
3. Isotope ratio matching to theoretical pattern based on target formula
4. Ion ratio matching between standard and unknown sample
5. Retention time matching between standard and sample

Mass Accuracy Match: TOF MS

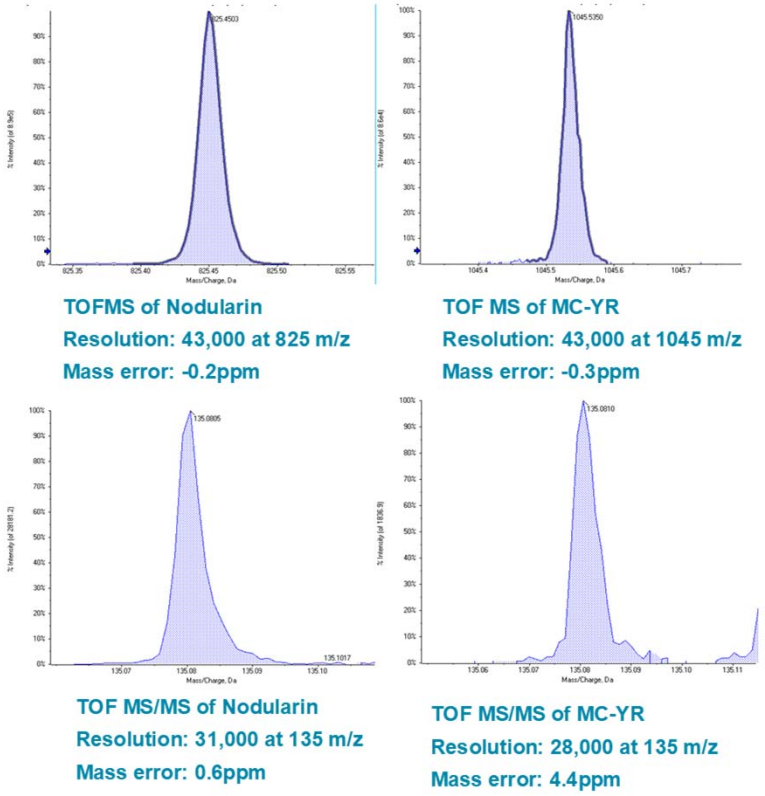


TOF MS of MC-RR
Resolution: 46,000 at 519 *m/z*
Mass error: 0.6ppm

TOF MS/MS of MC-RR
Resolution: 34,000 at 135 m/z
Mass error: 5.4ppm

HRAM TOF MS Analysis of Microcystin MC-RR. Mass spectral data shows high mass resolution and mass accuracy, providing specificity for the target analyte. Mass accuracy within 2ppm for the precursor for the TOF MS precursor reinforces confidence in the identification of the targets. MRM^{HR} fragments also demonstrate excellent specificity and confidence with resolution >28,000 and mass error <6ppm.

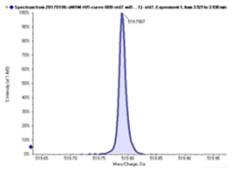
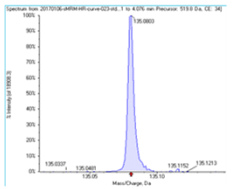
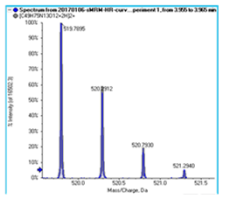
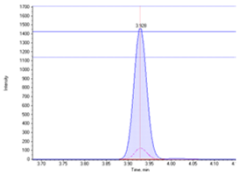
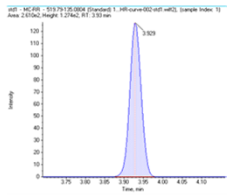
Mass Accuracy Match: TOF MS/MS



HRAM TOF MS Analysis of Microcystin MC-YR and Nodularin. Mass spectral data shows high mass resolution and mass accuracy, providing specificity for these target analytes. Mass accuracy within 2ppm for the precursor for the TOF MS precursor reinforces confidence in the identification of the targets. MRM^{HR} fragments also demonstrate excellent specificity and confidence with resolution >28,000 and mass error <5ppm.

Qualitative Tools for Matching can be used Simultaneously

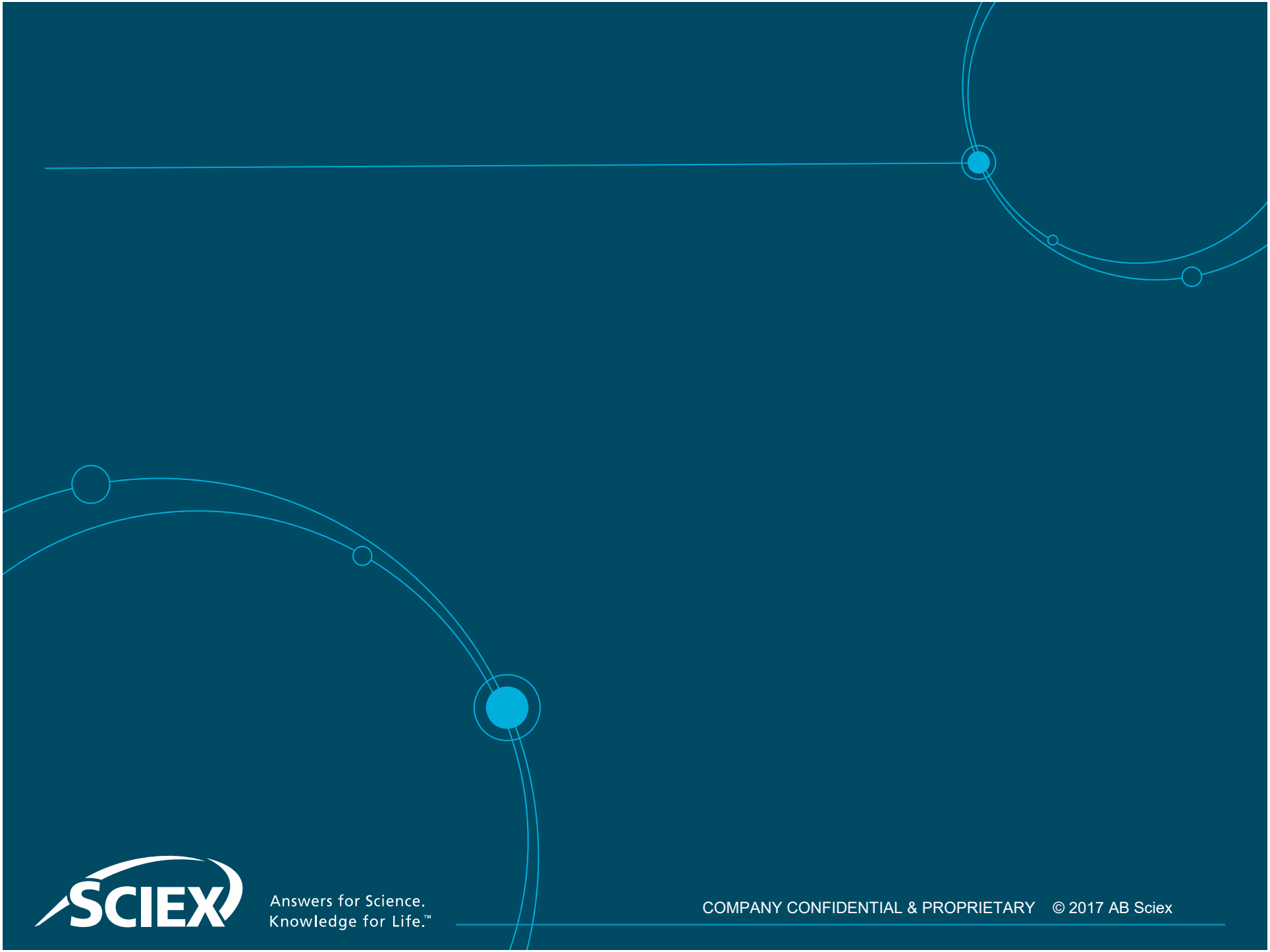
- Mass accuracy within 2ppm reinforces confidence in the identification of the targets
- High resolution fragments collected using MRM^{HR} also demonstrate excellent specificity and confidence
- TOF MS pattern of resolved MS isotopes compared to the theoretical isotope pattern provide additional confirmation and are displayed in the results
- Precursor XIC and MRM^{HR} transition data grouped together allows the software to automatically evaluate ion ratios as another metric for matching and confirmation

Accurate mass of target precursor		*TOFMS spectrum of MC-RR Resolution: 46,000 at 519.8 m/z Mass error: <1 ppm*	✔
MRM transition and accurate mass of fragment		*TOFMSMS spectrum of MC-RR Precursor: 519.8 m/z Fragment Mass error: < 1 ppm*	✔
Isotope matching to theoretical target formula		*Isotope ratio difference <4% MC-RR, $[C_{49}H_{75}N_{13}O_{12}+2H]^{+2}$*	✔
Ion ratio of TOF MS peak to MRM^{HR} peak match to standard		*Ion ratio percent difference <6%*	✔
Retention time match between standard and unknown		*Chromatographic peak eluting at 3.9min is <1% difference from expected RT*	✔

Target Identification Points of Confirmation. Identification and quantitation of microcystins in unknown samples can be achieved with high confidence using HRAM analysis due to multiple points of matching using accurate mass, MRM^{HR}, isotope pattern matching, ion ratio, and chromatographic retention time.

Summary

- Quantitation of a suite of microcystins and nodularin was demonstrated using LC-MS/MS on SCIEX X500R QTOF system.
  - Excellent quantitation results with sensitive detection (<0.1µg/L LOD) and exceptional reproducibility (%CV <7%) at low concentrations
  - If used with the EPA Method 544 suggested concentration factor of 500-fold, these detection limits are theoretically extended to 0.0002 µg/L
- Concurrent MRM^{HR} data allows for additional quantitative information and enhanced confidence in qualitative analysis without requiring additional injections or processing.
- HRAM approach provides confirmation of target identification using accurate precursor mass matching, isotope pattern matching, accurate fragment mass matching, ion ratio matching, and retention time matching
- Verifying across these five points reinforces the ability to confirm the presence of the analyte and helps safeguard against reporting false positives



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