StayClean™ QSight LC-MS/MS for Quantitation of Microcystins and Nodularin in Drinking Water According to EPA Method 544

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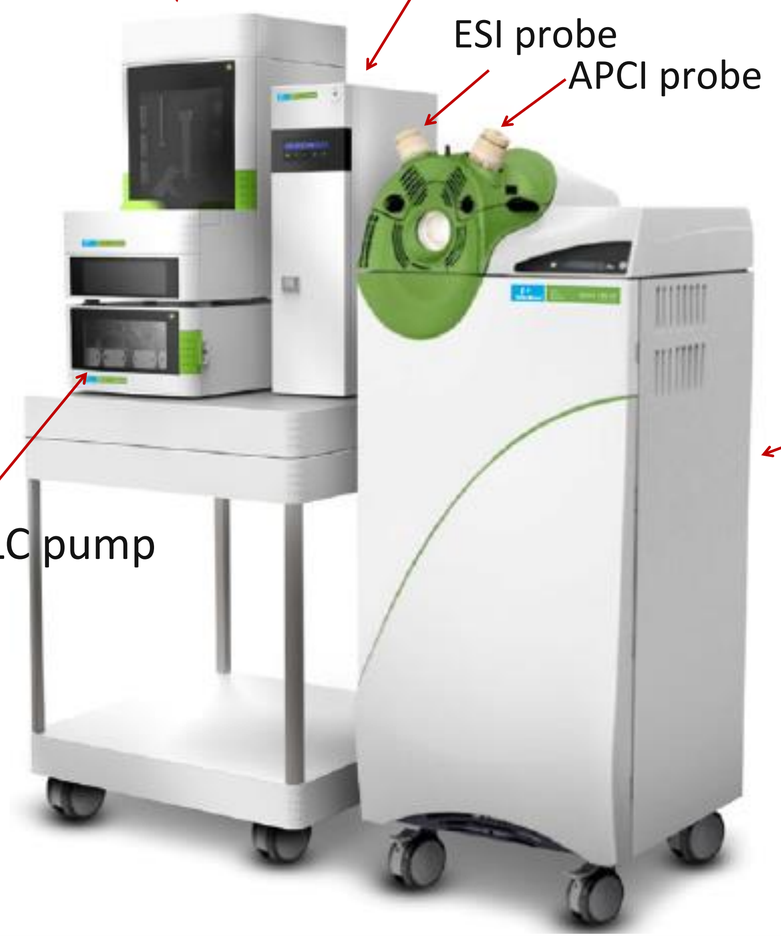
StayClean™ QSight LC-MS/MS

- Dual Binary LC Pump
  - Two Sets of Mobile Phases
    - A1 and B1 for ESI
    - A2 and B2 for APCI
- Dual ESI/APCI Source
  - ESI for Polars
  - APCI for Non-polars



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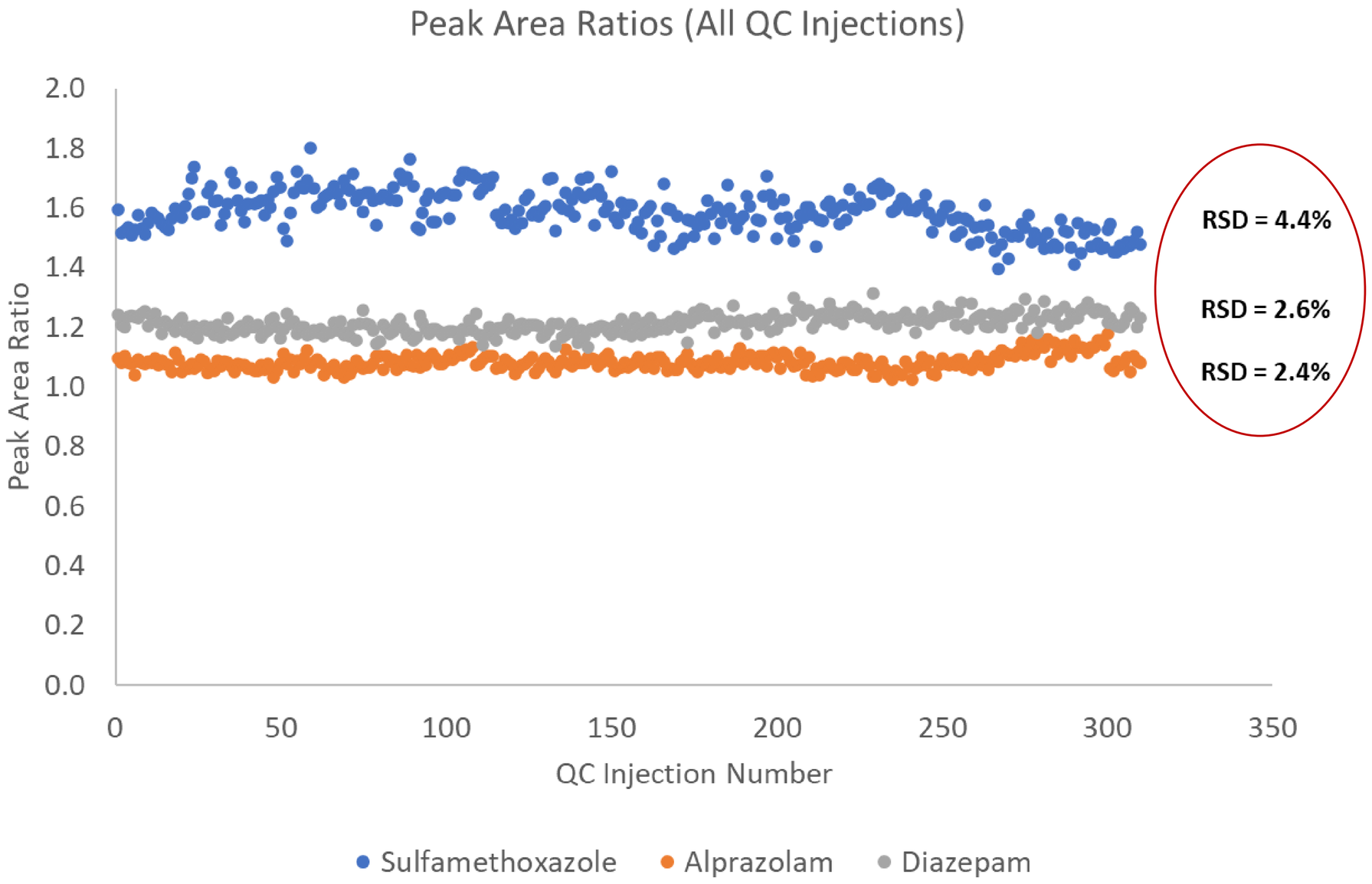
Robustness: 25,500 Continuous Injections of Fetal Bovine Serum



- Protein precipitation of FBS with methanol followed by a 50% dilution with water prior to injecting 5 µL for analysis
- Monitoring 3 analytes and their deuterated standards (1 ng/mL)
- Instrument robustness evaluated by intermittently monitoring SST samples between large blocks of FBS matrix injections

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Source Images

View from Source Window

Sample Cone

Plenum Chamber

After 25,500 Continuous Injections of Fetal Bovine Serum

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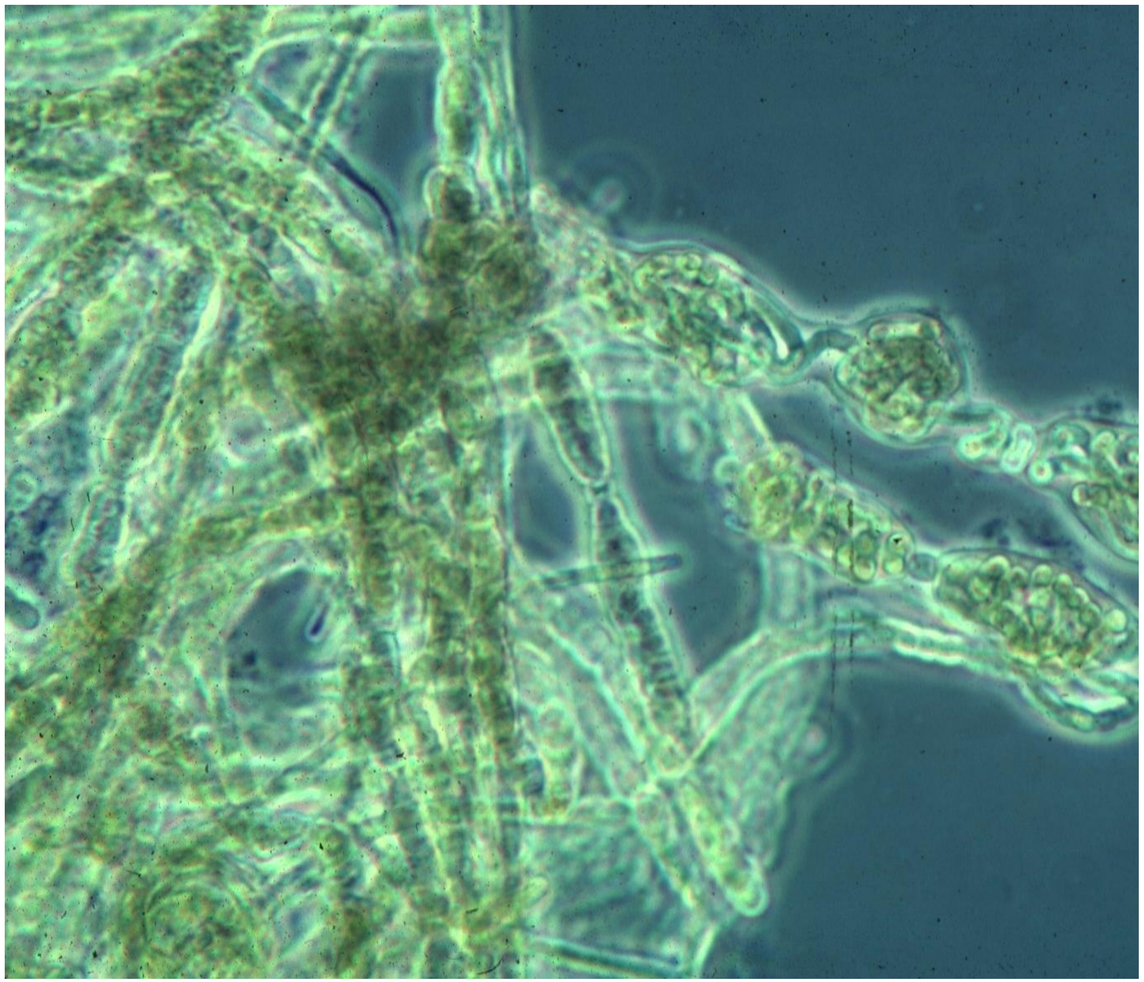
Method Background

- Cyanobacteria, aka blue-green algae, can produce high levels of toxins called microcystins and nodularin
- The US EPA has a health advisory for maximum levels of microcystins in drinking water
  - 0.3 ppb for young children and infants
  - 1.6 ppb for school aged children and adults
- WHO recommends keeping microcystin levels below 1 ppb
- US EPA method 544 analyzes 6 microcystins and nodularin in 500 mL drinking water samples using SPE-LC/MS/MS analysis

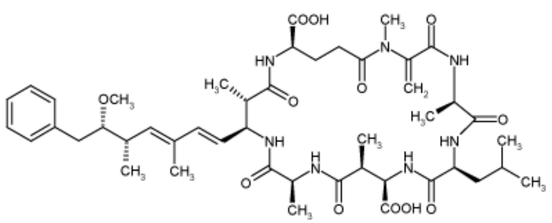


Objectives

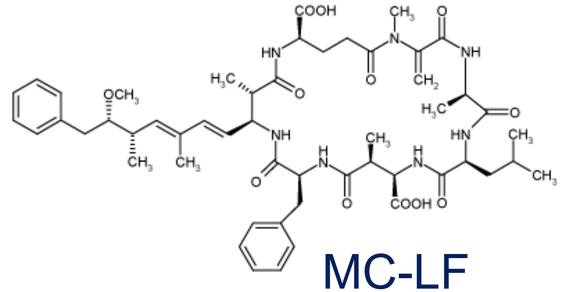
- Optimize LC and MS conditions on PerkinElmer QSight 220 LC-MS/MS
- Define minimum reporting levels (MRL) of method
- Determine precision and accuracy (P&A) of method
- Analyze unknown drinking water samples

Target Analytes

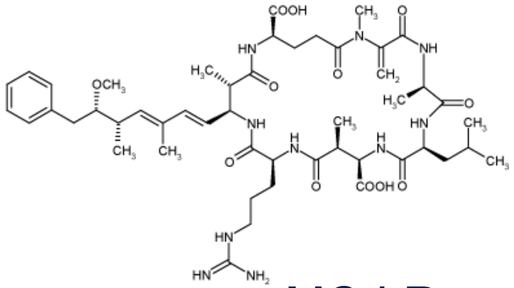
Cyanotoxin	Acronym
Microcystin-LA	MC-LA
Microcystin-LF	MC-LF
Microcystin-LR	MC-LR
Microcystin-LY	MC-LY
Microcystin-RR	MC-RR
Microcystin-YR	MC-YR
Nodularin	NOD
Ethylated Microcystin-LR	C_2D_5-MC-LR



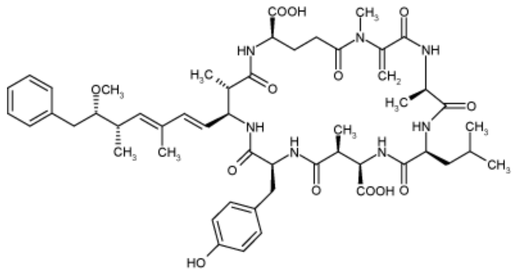
MC-LA



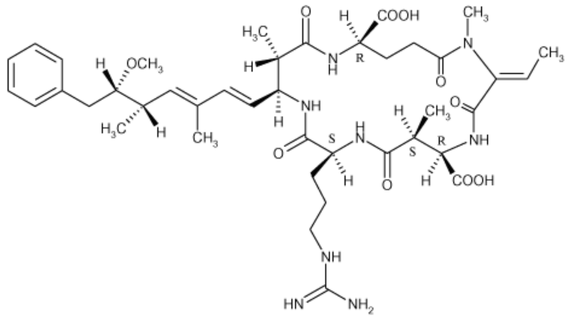
MC-LF



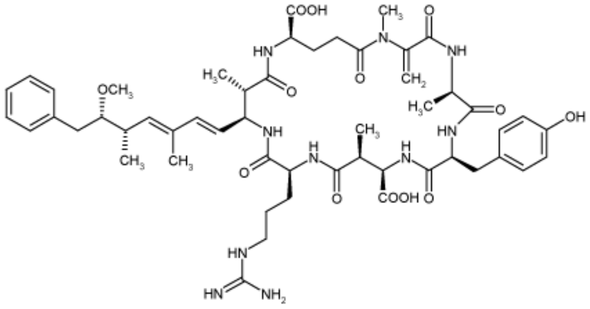
MC-LR



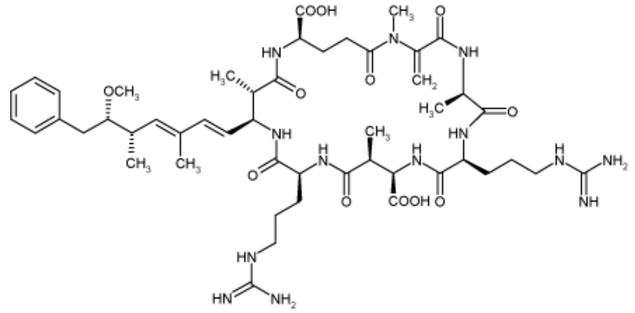
MC-LY



NOD



MC-YR



MC-RR

LC Conditions

LC Condition	QSight LX50
Column	Brownlee SPP, 2.7µm, C-18, 2.1 x 100mm
Flow Rate	0.600 mL/min
Injection Volume	10 µL
Mobile Phase A	0.1% FA in H_2O
Mobile Phase B	0.1% FA in ACN

Optimized LC Gradient:

	Time (min)	Flow (mL/min)	%A	%B	Curve
1	Initial	0.600	95	5	
2	4.0	0.600	95	5	Linear
3	9.0	0.600	5	95	Linear
4	10.00	0.600	5	95	Linear
5	10.10	0.600	95	5	Linear
6	13.0	0.600	95	5	Linear

- EPA Method 544 uses methanol as mobile phase B
- Shorten runtime by 50%, from 26 min. to 13 min. by increasing flow rate, etc.

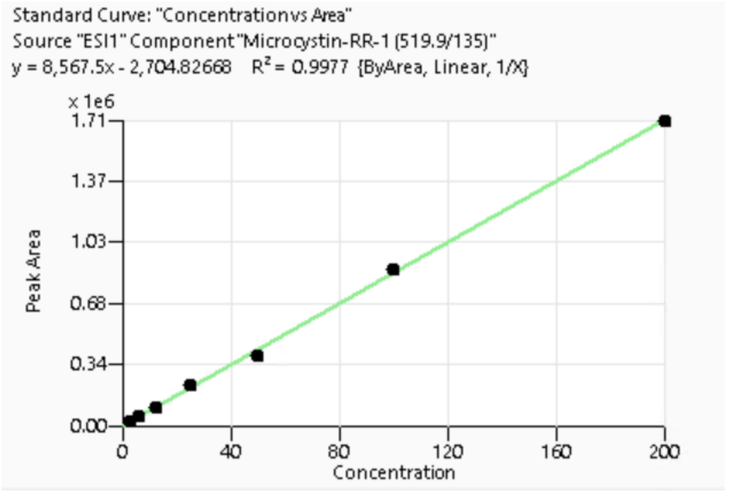
Optimized MS/MS Parameters

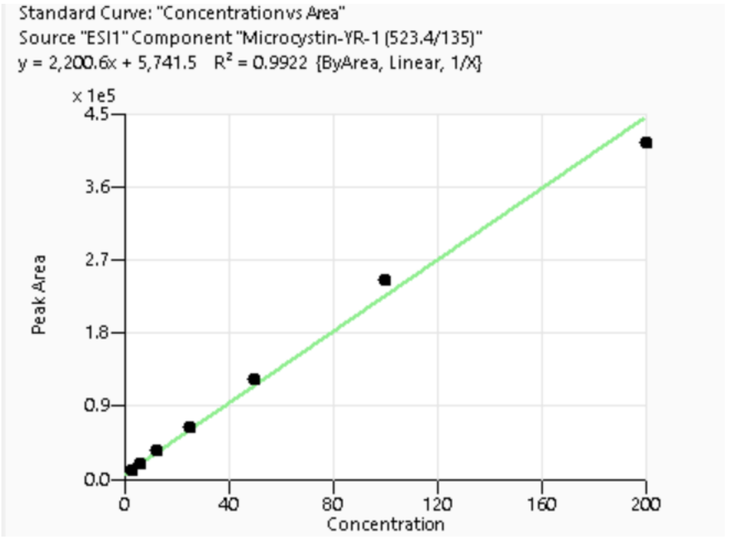
- Source parameters:
  - Ionization mode: ESI+
  - Drying gas: 120
  - HSID temperature: 250°C
  - Nebulizer gas: 350
  - Electrospray voltage: 5100 V
  - Source temperature: 315°C

Analyte	Parent Ion	Q1 Mass	Q2 Mass	Type	CE	EV	CCL2
MC-LR	$[M+2H]^{2+}$	498	135	Quantifier	-24	30	-65
MC-LR	$[M+2H]^{2+}$	498	482.3	Qualifier	-12	15	-80
NOD	$[M+H]^{+}$	825.4	135	Quantifier	-74	10	-205
NOD	$[M+H]^{+}$	825.4	103	Qualifier	-138	15	-195
MC-LA	$[M+H]^{+}$	910.4	135	Quantifier	-88	55	-180
MC-LA	$[M+H]^{+}$	910.4	106.9	Qualifier	-114	30	-185
MC-YR	$[M+2H]^{2+}$	523.4	135	Quantifier	-22	30	-85
MC-YR	$[M+2H]^{2+}$	523.4	91.1	Qualifier	-116	30	-145
MC-LF	$[M+2H]^{2+}$	493.9	135	Quantifier	-50	15	-90
MC-LF	$[M+2H]^{2+}$	493.9	102.9	Qualifier	-94	30	-100
MC-LY	$[M+H]^{+}$	1002.4	135	Quantifier	-82	20	-175
MC-LY	$[M+H]^{+}$	1002.4	374.9	Qualifier	-46	20	-185
MC-RR	$[M+2H]^{2+}$	519.9	135	Quantifier	-38	5	-130
MC-RR	$[M+2H]^{2+}$	519.9	103	Qualifier	-90	15	-140
C_2D_5-MC-LR	$[M+2H]^{2+}$	515	103.1	IS	-95	12	-110

Calibration Curve

Analyte	Cal 1 (µg/L)	Cal 2 (µg/L)	Cal 3 (µg/L)	Cal 4 (µg/L)	Cal 5 (µg/L)	Cal 6 (µg/L)	Cal 7 (µg/L)
MC-LF	3.125	6.25	12.5	25	50	100	200
MC-LR	3.125	6.25	12.5	25	50	100	200
MC-RR	3.125	6.25	12.5	25	50	100	200
MC-YR	3.125	6.25	12.5	25	50	100	200
NOD	3.125	6.25	12.5	25	50	100	200
MC-LA	6.25	12.5	25	50	100	200	400
MC-LY	6.25	12.5	25	50	100	200	400





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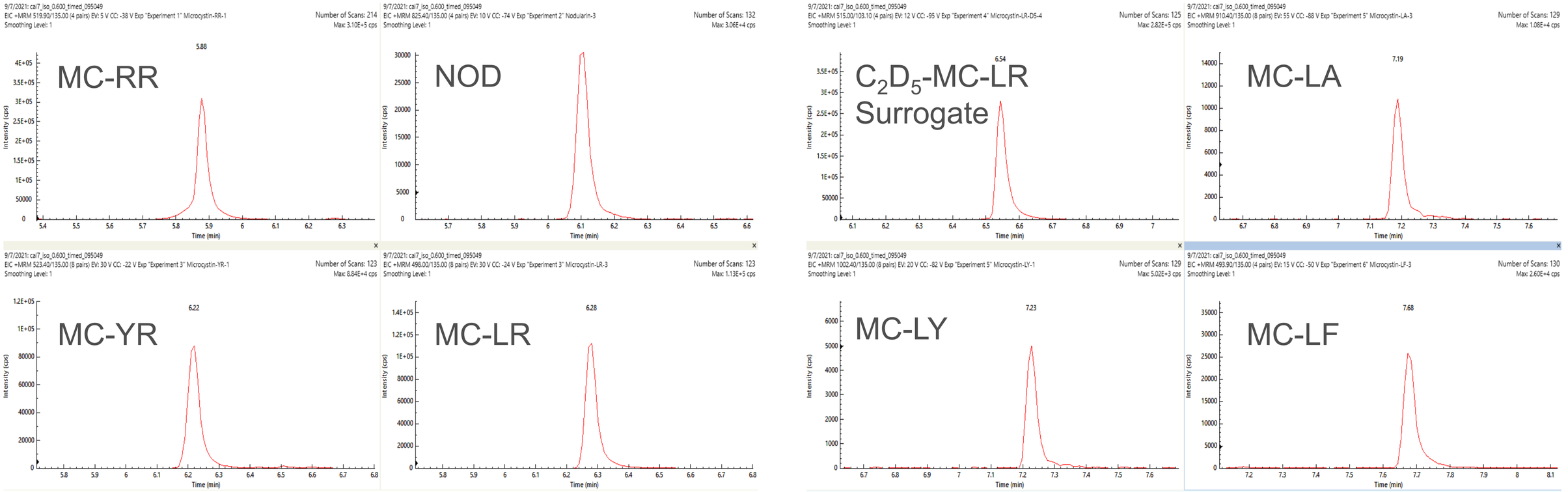
Chromatogram of Analytes



100 µg/L sample spiked with 129.8 µg/L surrogate

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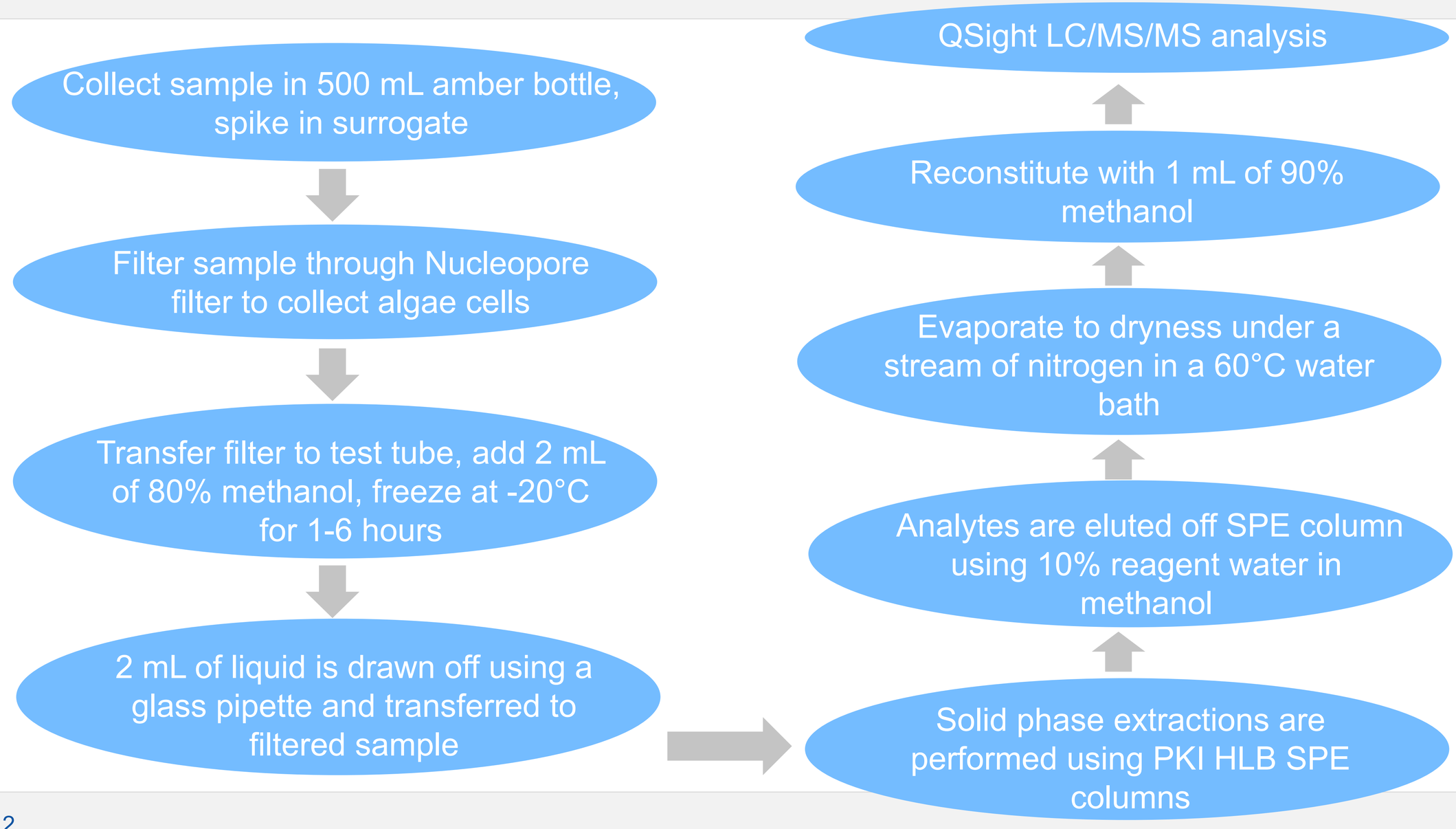
Sample Preparation Procedure



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Minimum Reporting Level (MRL) Confirmation

- The **minimum reporting level** (MRL) is the minimum concentration that can be reported as a quantitated value for a method analyte in a sample after analysis
  - **Section 9.2.4** in EPA Method 544 outlines the procedure for verifying the MRL
  - Fortify, extract, and analyze **7** laboratory fortified blanks at proposed MRL concentration

Analyte	Proposed MRL (ng/L)
MC-RR	24
Nodularin	20
MC-YR	16
MC-LR	16
MC-LA	80
MC-LY	80
MC-LF	60

Minimum Reporting Level Calculations

- Calculate mean and standard deviation of the 7 replicates
  - Determine half range for prediction interval of results (HR_{PIR}) using equation:
    - **HR_{PIR} = 3.963s**
    - where s = standard deviation and 3.963 = a constant value

- Confirm the upper and lower limits for the Prediction Interval of Result (PIR = Mean +/- HR_{PIR}) meet the upper recovery limit (≤150%) and the lower limit recovery (≥50%)

The Upper PIR Limit must be ≤ 150% recovery.

$$\frac{Mean + HR_{PIR}}{Fortified\ Concentrat\ ion} \times 100\% \leq 150\%$$

The Lower PIR Limit must be ≥ 50% recovery.

$$\frac{Mean - HR_{PIR}}{Fortified\ Concentrat\ ion} \times 100\% \geq 50\%$$

Minimum Reporting Level Data

Analyte	Fortified Concentration (ng/L)	LFB 1 (ng/L)	LFB 2 (ng/L)	LFB 3 (ng/L)	LFB 4 (ng/L)	LFB 5 (ng/L)	LFB 6 (ng/L)	LFB 7 (ng/L)	STDDEV	Mean	HR_{PIR}	Upper PIR	Lower PIR
MC-RR	24	22.7	21.7	18.0	18.6	18.3	19.1	21.4	1.9	20.0	7.5	114.6	52
Nodularin	20	18.4	16.9	18.7	15.9	17.8	15.9	17.4	1.1	17.3	4.5	108.7	64
MC-YR	16	18.4	14.0	15.7	14.7	14.1	15.7	13.2	1.7	15.1	6.7	136.6	52
MC-LR	16	14.8	12.8	13.4	13.9	13.6	15.0	15.0	0.9	14.1	3.4	109.3	67
MC-LA	80	84.5	80.7	86.4	93.1	97.8	94.2	81.7	6.7	88.4	26.4	143.5	77
MC-LY	80	80.4	86.9	80.3	82.1	97.4	87.5	68.0	9.0	83.2	35.5	148.4	60
MC-LF	60	66.3	66.8	61.8	62.0	62.5	62.5	66.2	2.3	64.0	9.0	121.8	92

Acceptance Criteria:

- Upper PIR must be ≤150%
- Lower PIR must be ≥50%

Precision and Accuracy (P&A) Studies

- Initial demonstration of P&A
  - Prepare, extract, and analyze 4 LFBs fortified near the midrange of the calibration curve

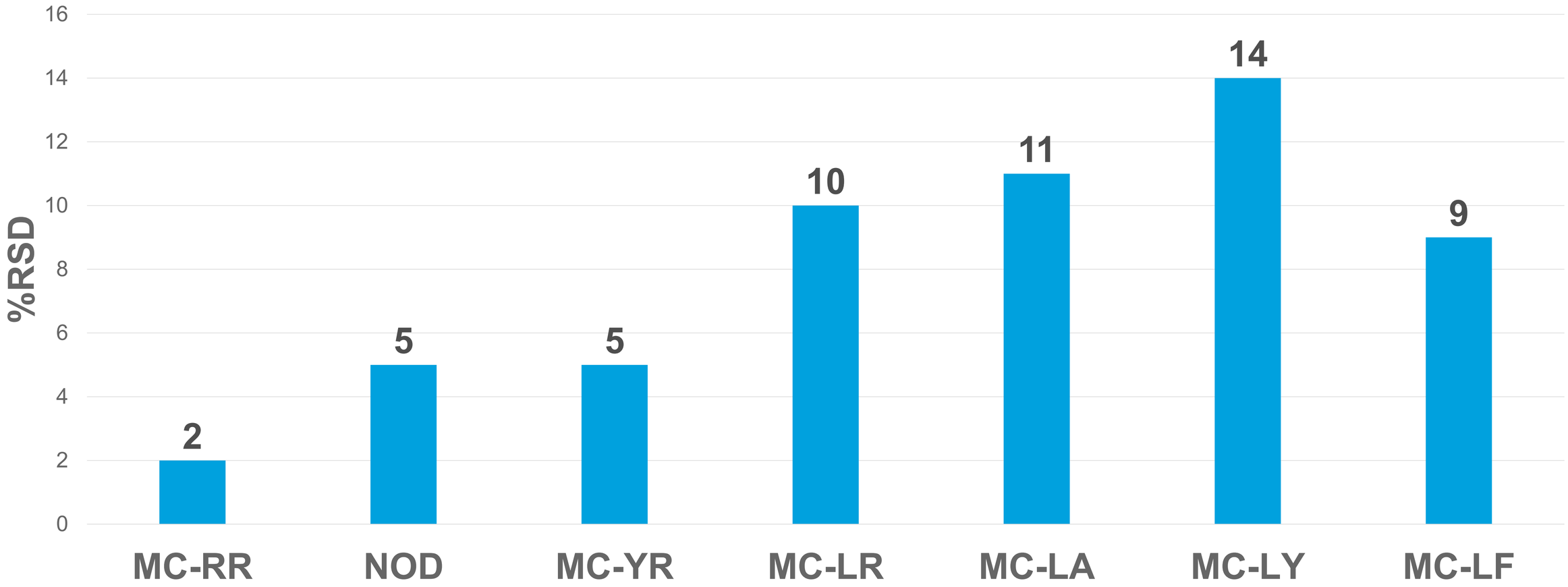
Analyte	Amount Spiked into 100 mL Samples
MC-RR	0.3 µg/L
Nodularin	0.3 µg/L
MC-YR	0.3 µg/L
MC-LR	0.3 µg/L
MC-LA	0.3 µg/L
MC-LY	0.3 µg/L
MC-LF	0.3 µg/L

  - For demonstration of precision, the %RSD of replicate analyses must be less than 30%
  - For demonstration of accuracy, the average recovery of replicate values must be +/-30% of the true value

Precision and Accuracy (P&A) Results

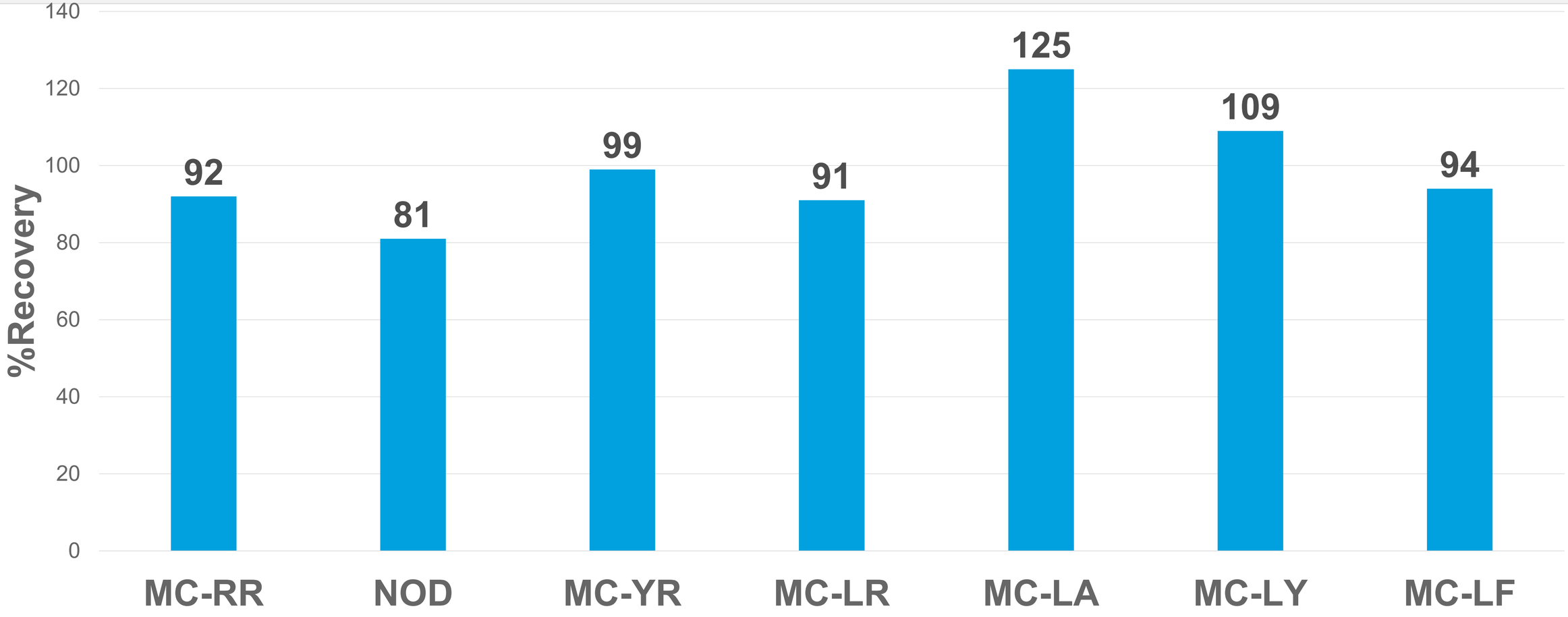
Analyte	Fortified Concentration (µg/L)	LFB 1 (µg/L)	LFB 2 (µg/L)	LFB 3 (µg/L)	LFB 4 (µg/L)	Mean (µg/L)
MC-RR	0.30	0.28	0.28	0.27	0.27	0.27
Nodularin	0.30	0.25	0.24	0.23	0.26	0.24
MC-YR	0.30	0.30	0.27	0.30	0.31	0.30
MC-LR	0.30	0.24	0.26	0.29	0.30	0.27
MC-LA	0.30	0.34	0.37	0.36	0.43	0.38
MC-LY	0.30	0.32	0.38	0.33	0.27	0.33
MC-LF	0.30	0.29	0.30	0.24	0.29	0.28

Demonstration of Precision (%RSD)



Acceptance Criteria: %RSD of replicate analyses must be < 30%

Demonstration of Accuracy (%Recovery)



Acceptance Criteria: Average spiked recovery must be within 70 to 130%

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Field Sample Batch Analysis

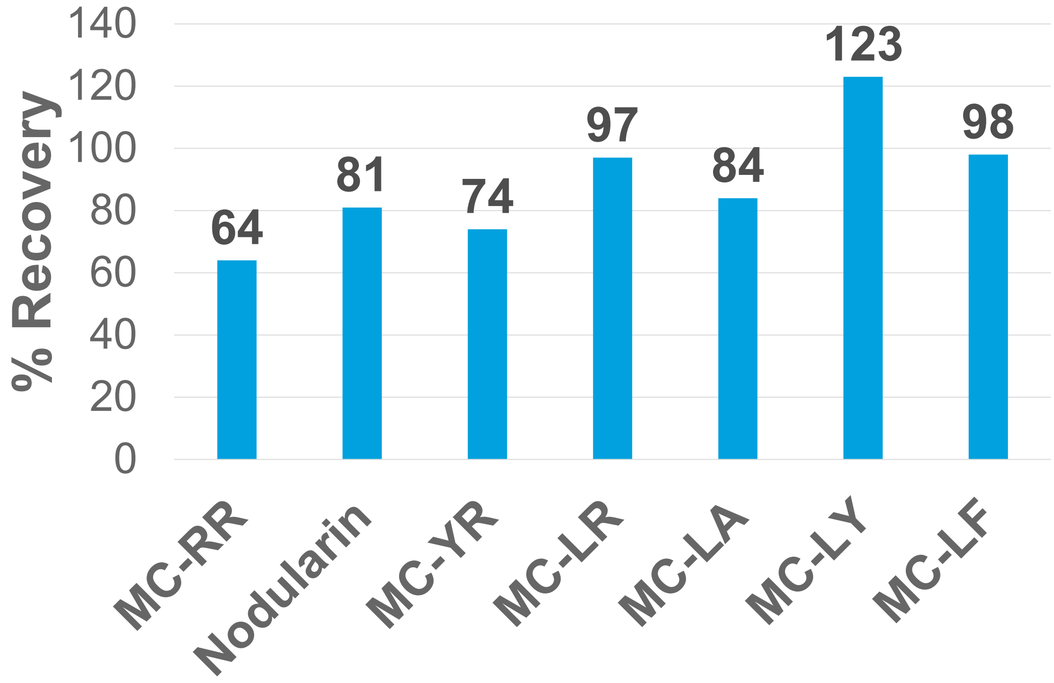
- Drinking water samples from Meriden, CT and Shelton, CT were collected in 500 mL amber glass bottles

- QC requirements (Method section 9.3):
  - Laboratory reagent blank (LRB)
  - Continuing calibration check (CCC)
  - Laboratory fortified blank (LFB)
  - Surrogate recovery (extracts: 60-130%, CCC: 70-130%)
  - Laboratory fortified sample matrix (LFSM) and duplicate (LFSMD)
  - Field sample duplicate (FD)

Laboratory Fortified Blank

Fortified at 2X MRL

Analyte	LFB Low, Fortified Concentration (ng/L)	LFB Low, Measured Concentration (ng/L)	% Recovery
MC-RR	48	30.5	64
Nodularin	40	32.4	81
MC-YR	32	23.6	74
MC-LR	32	31.0	97
MC-LA	160	135.0	84
MC-LY	160	196.3	123
MC-LF	120	117.8	98

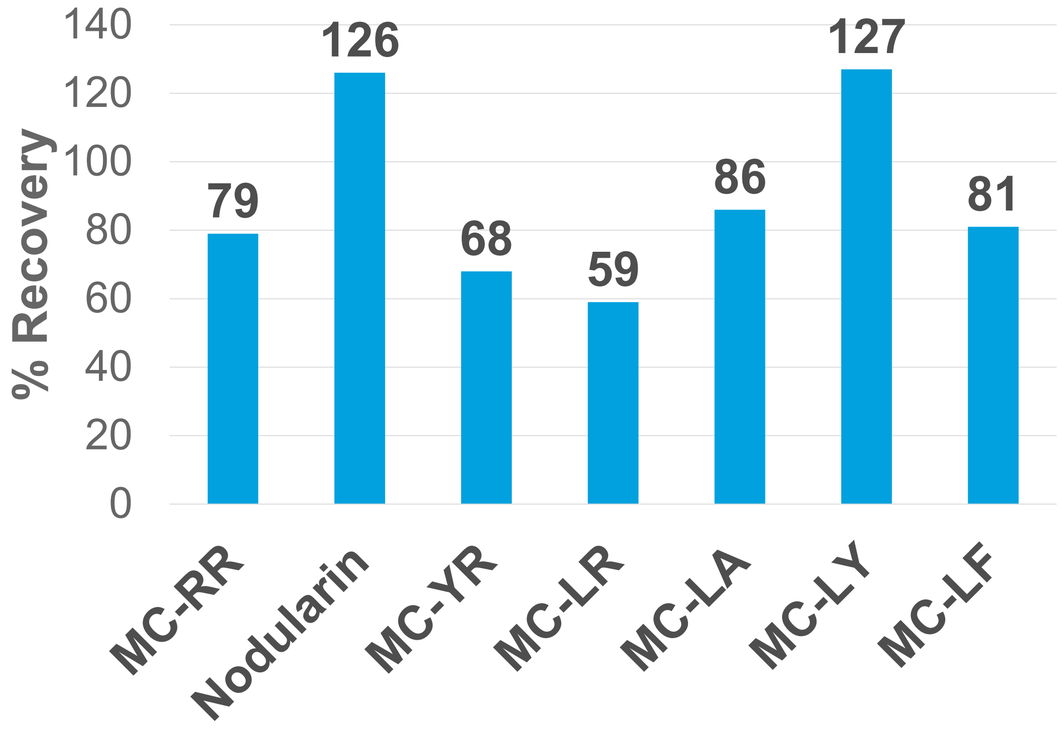


Acceptance Criteria: Average % recovery must be within 50 to 150%

Low Level Continuing Calibration Check (CCC)

Low level CCC – spiked at MRL

Analyte	CCC Low, Fortified Concentration (ng/L)	CCC Low, Measured Concentration (ng/L)
MC-RR	24	18.9
Nodularin	20	25.2
MC-YR	16	10.9
MC-LR	16	9.5
MC-LA	80	68.8
MC-LY	80	102.0
MC-LF	60	48.9



Acceptance Criteria: Average % recovery must be within 50 to 150%

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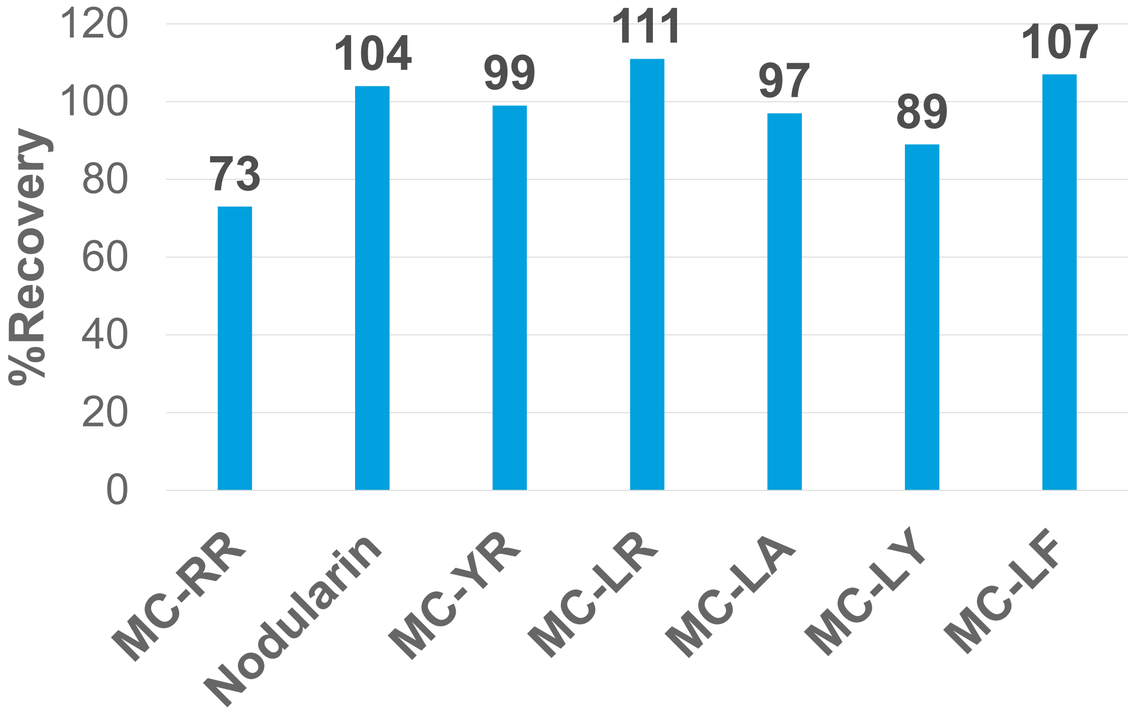
Mid Level Continuing Calibration Check (CCC)

Mid level CCC – Midrange of calibration curve

Analyte	CCC Mid, Fortified Concentration (ng/L)	CCC Mid, Measured Concentration (ng/L)
MC-RR	80	58.4
Nodularin	80	82.9
MC-YR	80	79.3
MC-LR	80	88.6
MC-LA	160	154.6
MC-LY	160	142.2
MC-LF	80	85.6



Acceptance Criteria: Average % recovery must be within 70 to 130%

Unknown Drinking Water Sample Results

500 mL samples were collected, extracted, and analyzed

Analyte	Meriden Result (ng/L)	Shelton Result (ng/L)
MC-RR	<MRL	<MRL
Nodularin	<MRL	<MRL
MC-YR	<MRL	<MRL
MC-LR	<MRL	<MRL
MC-LA	<MRL	<MRL
MC-LY	<MRL	<MRL
MC-LF	<MRL	<MRL

Summary

- QSight 220 LC-MS/MS is fit for purpose for EPA Method 544
- LC total runtime was reduced by 50% (26 min. vs. 13 min.)
- Minimum reporting levels, ranging from 16-80 ng/L, were well below EPA advisory limits and WHO guideline
- Good accuracy with all recoveries within 70-130% and good precision with all %RSD below 30
- Field drinking water samples were analyzed, and results were all below the MRL
- Instrument robustness with StayClean™ technology would allow injections of thousands of samples with no instrument cleaning and maintenance.

Thank you very much for attention! Any question?

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Innovative QSight Design

HSID Interface
Provides low background and reliable results day after day with equal response at any flow rate

Laminar Flow Ion Guide
Highly efficient field-free transmission

Collision Cell
Fast, efficient fragmentation (fast MRMs) shortens cycle time with zero crosstalk

Modular
Plug-and-play design for ease of service

Small Footprint, Vertical Design
Compact 50 cm x 50 cm x 115 cm (no benchtop needed)

Dual Source
Two independent probes provide true multiplexing flexibility

StayClean Source
Self-cleaning design delivers maximum sensitivity and exceptional uptime

Mass Filters
High-quality precision rods provide highly stable, precise mass filtering

UniField Detector
Patented technology counts positive and negative ions without high-voltage switching

First vertical MS system
- Dimensions: 50x50x110 cm
- Smallest footprint
- Saves customer lab space

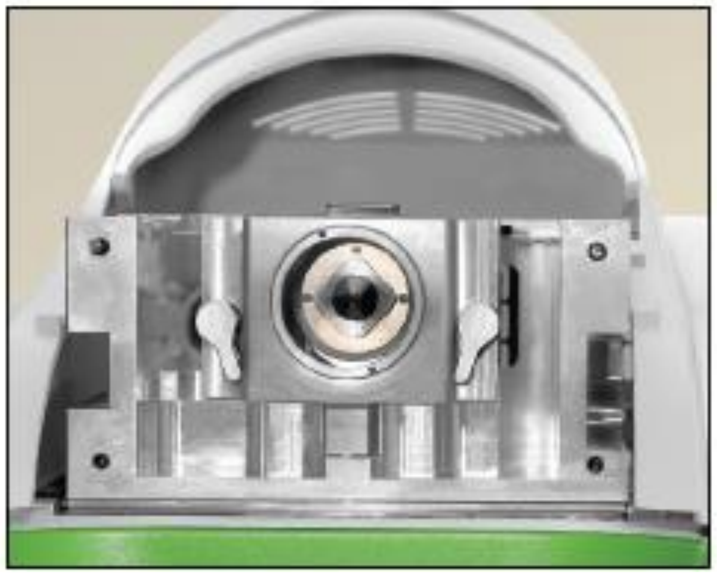
Modularized design
- Improved serviceability
- Fast recovery, low down time
- Quick replacement parts

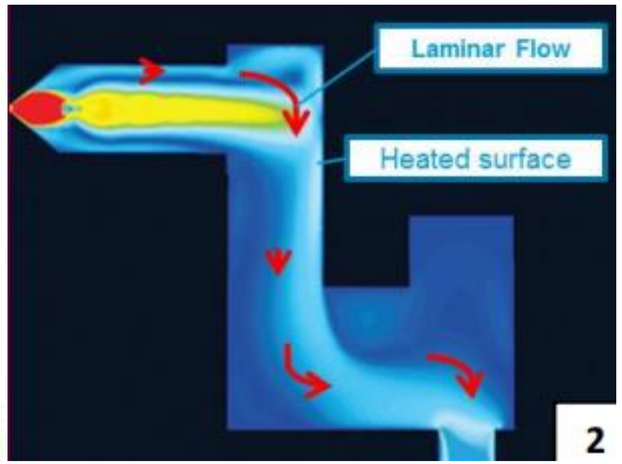
Smart
- Full diagnostics
- Remote controlling

Plug and play design
- Require minimum tooling
- Reduced cabling by 80%
- Self connecting modules

How StayClean™ Works?

- Heated Surface Induced Desolvation (HSID)
- Self Clean 24/7
  - Better desolvation
  - Less Maintenance
  - Increased uptime





Self-cleaning Heated Surface Induced Desolvation (HSID)

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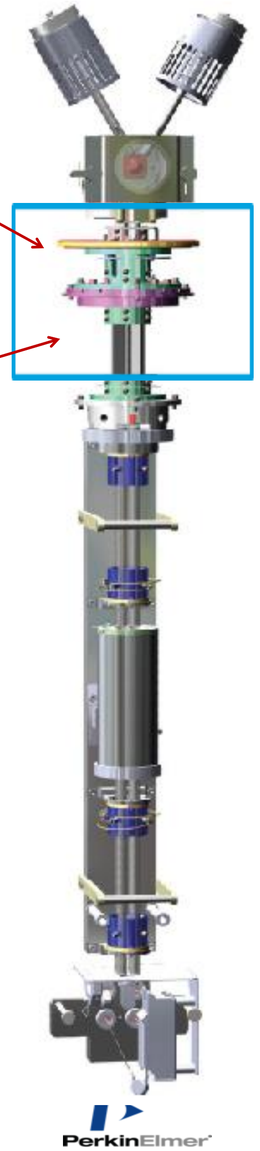
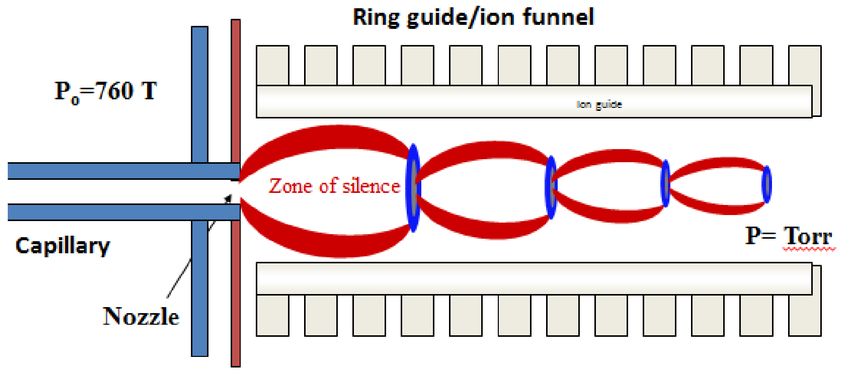
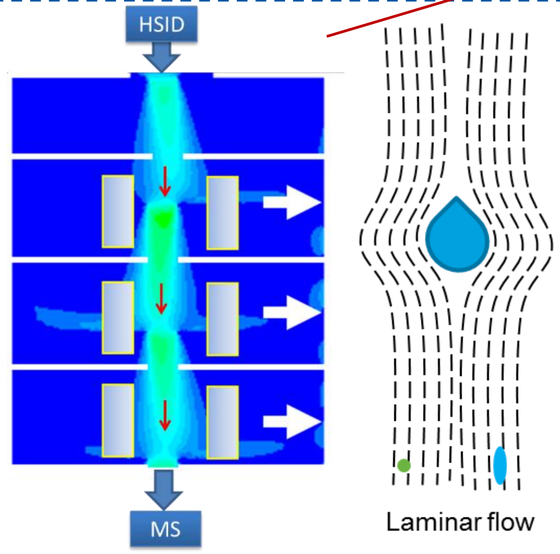
Laminar Flow Ion Guide (Patent Technology)

- No voltage needed to guild ions
- Ions go with the flow, no mass discrimination
- No voltage re-optimization needed (Never re-tune ion guide)
- ~98% ion transmission rate





Traditional Ion Guide



Laminar Flow Ion Guide

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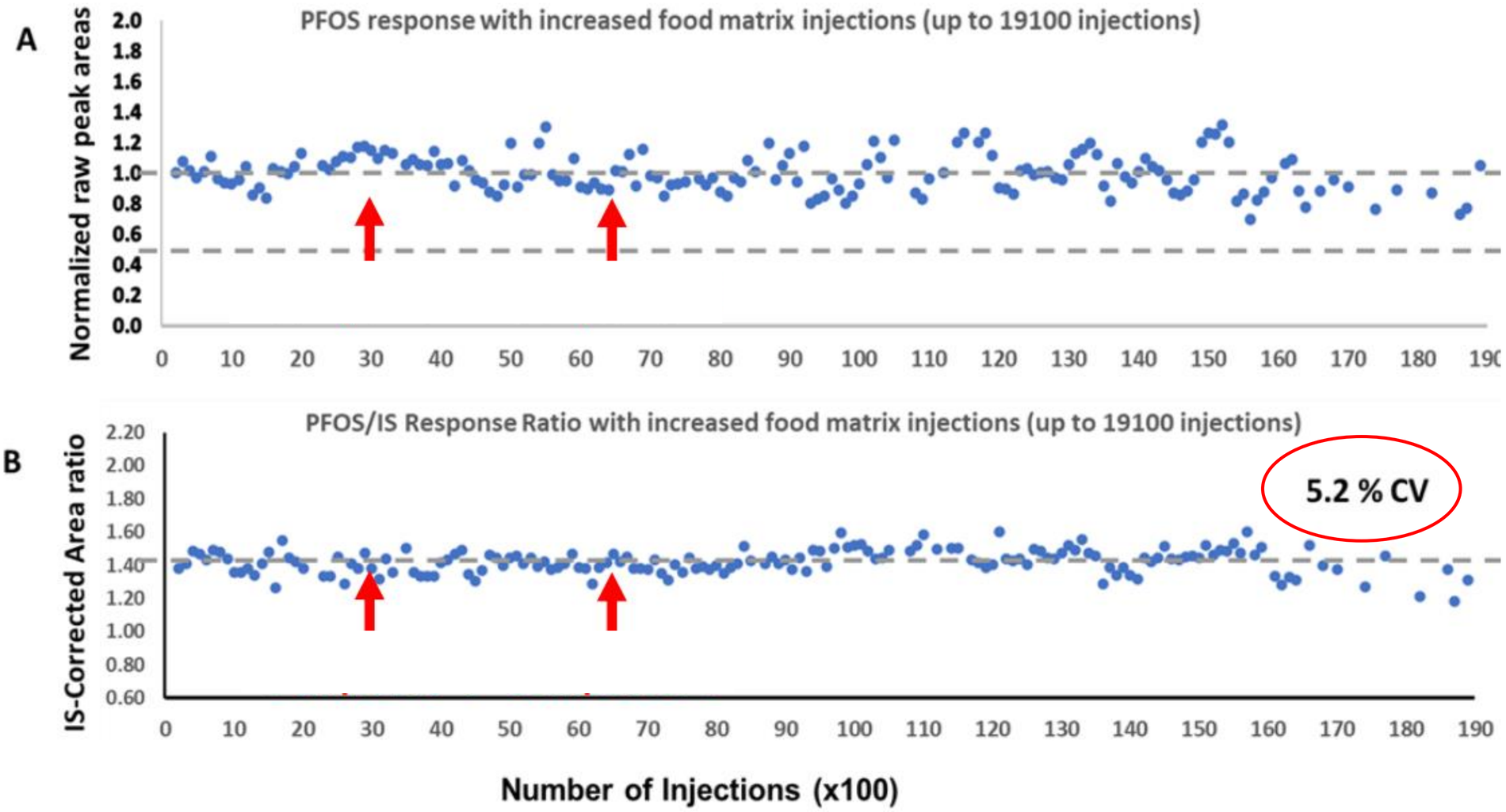
Robustness: PFAS in Food Matrices (19,100 Continuous Injections)



Salmon, Avocado, Tomato Mix, Spice Powder, Dog/Cat Feed

Food Matrices: salmon/avocado/tomato mix, spice powder, dog/cat feed

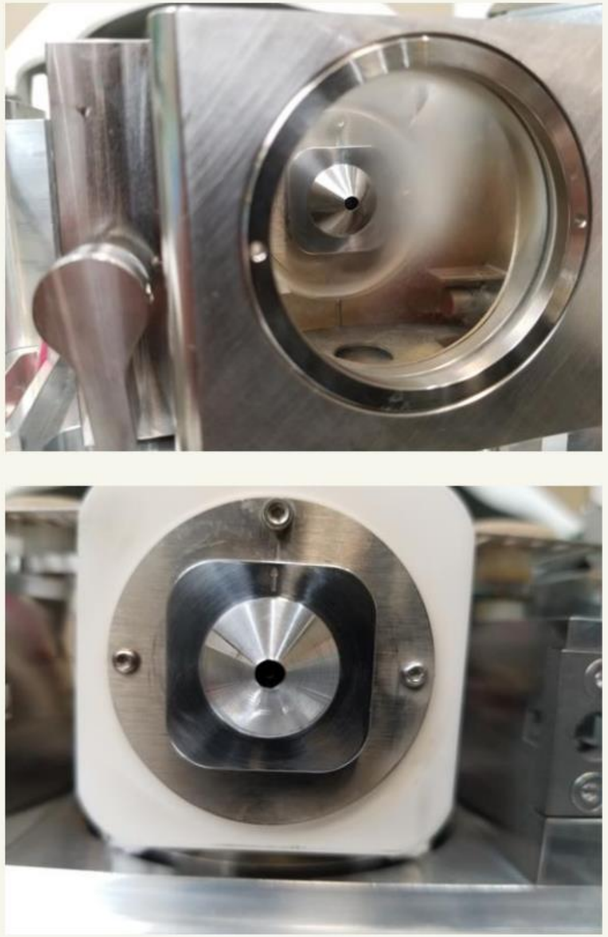
Preparation using QuEChERS[1] method developed for PFAS analysis in food, but the final SPE step was omitted to maximize sample matrix components in the final matrix extracts

Instrument robustness evaluated by intermittently monitoring solvent QC samples between large blocks of 100 consecutive matrix injections

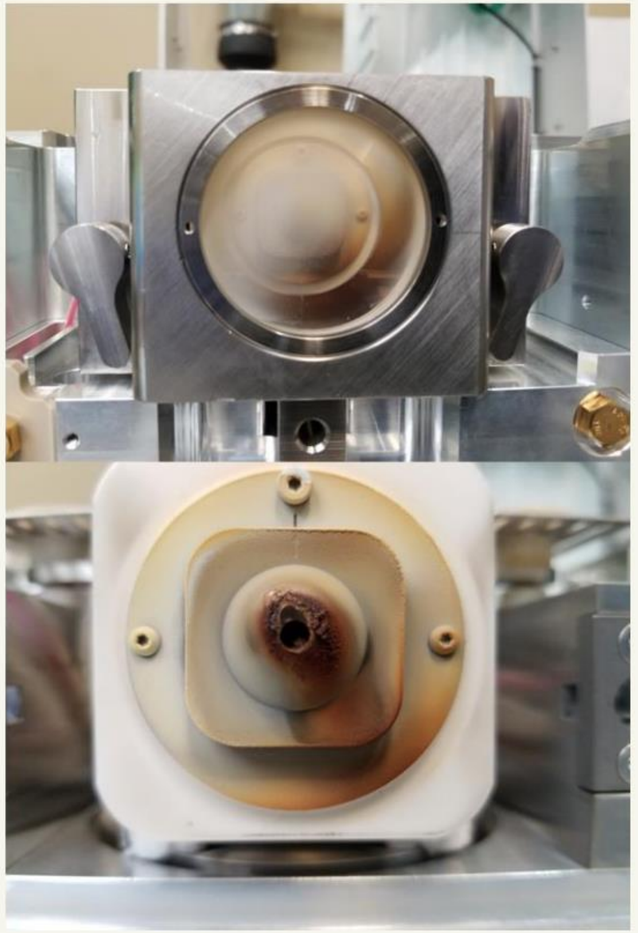
PerkinElmer

Source Images: PFAS in Food Matrices

Before

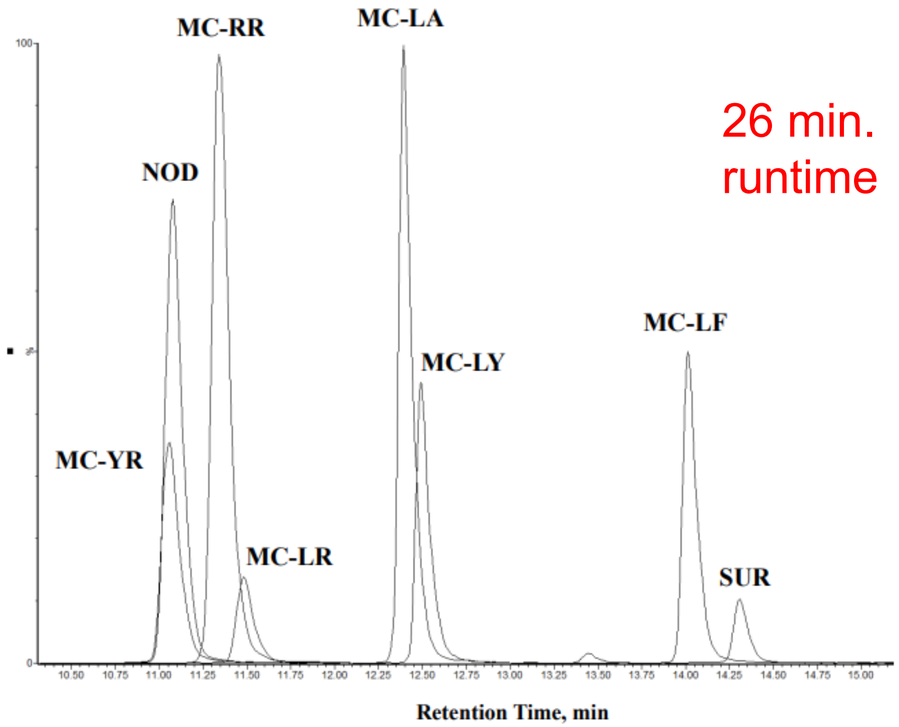


After

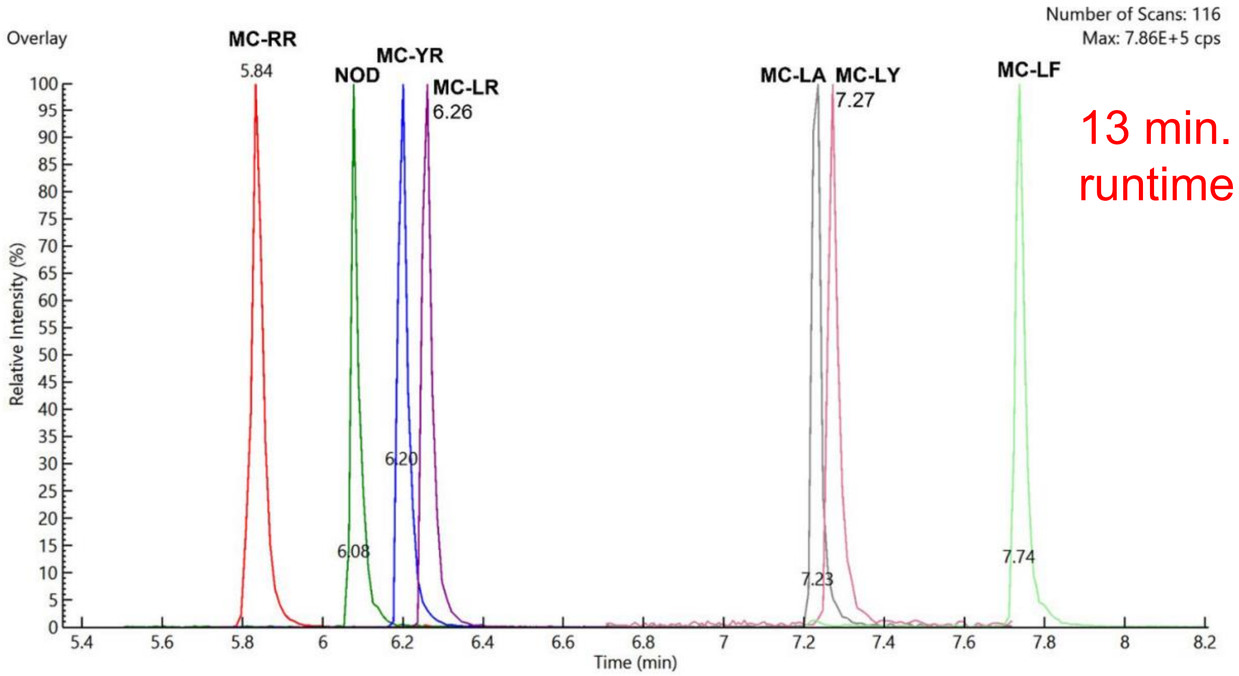


After 19,100 Continuous Injections of Salmon, Avocado, Tomato Mix, Spice Powder, Dog/Cat Feed

LC Separation (EPA vs. PerkinElmer)



26 min. runtime



13 min. runtime

- EPA method: 26 min total runtime
- LC column: Phenomenex Kinetex C8
- Mobile phase: MeOH/Water
- Flow rate: 0.3 mL/min

- PerkinElmer method: 13 min total runtime
- LC column: PerkinElmer SPP C18
- Mobile phase: ACN/Water
- Flow rate: 0.6 mL/min