

Algal toxins by LC-MS/MS

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NEMC 2018
New Orleans

Background

Algae



Algae

Huge diversity

Giant kelp to microalgae

Toxin-producing algae

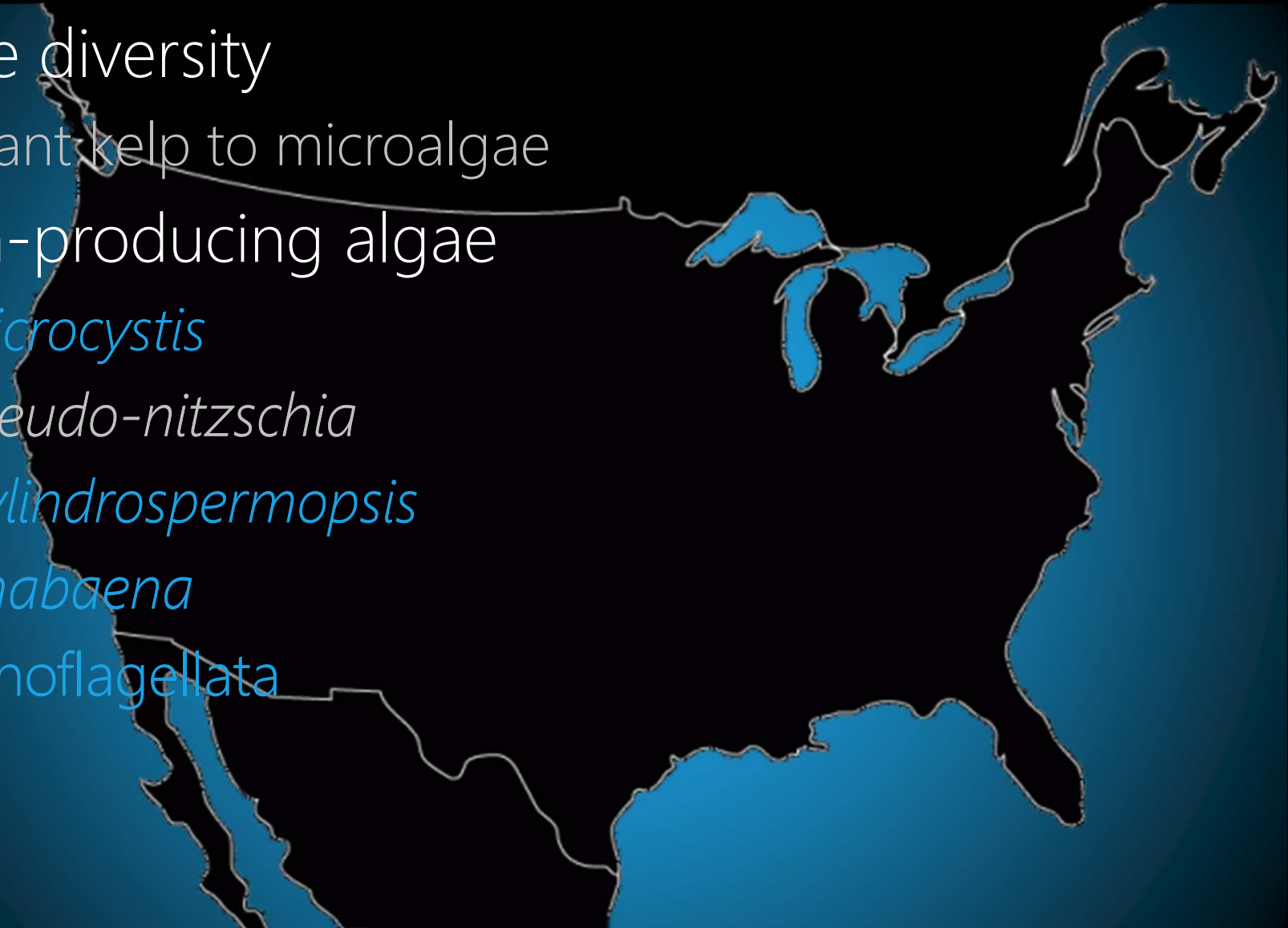
Microcystis

Pseudo-nitzschia

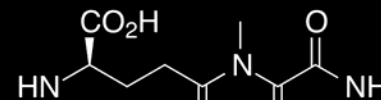
Cylindrospermopsis

Anabaena

Dinoflagellata







"It could be
the most
terrifying
motion
picture
I have
ever made!"

Alfred Hitchcock



ALFRED HITCHCOCK'S "The Birds"

TECHNICOLOR



Based on Daphne Du Maurier's classic suspense story!

STARRING

ROD TAYLOR

JESSICA TANDY • SUZANNE PLESHETTE

and Introducing 'TIPPI' HEDREN



Screenplay by EVAN HUNTER • Directed by ALFRED HITCHCOCK



A UNIVERSAL-INTERNATIONAL RELEASE

PLEASE DO NOT SEE THE END FIRST!!! See it from the beginning.

Routes of exposure

Direct contact

Swimming

Drinking water

Toledo, OH

Food source

Fish

Filter feeders



Analytical methods and sample prep

Microcystins

EPA Method 546

ADDA specific ELISA

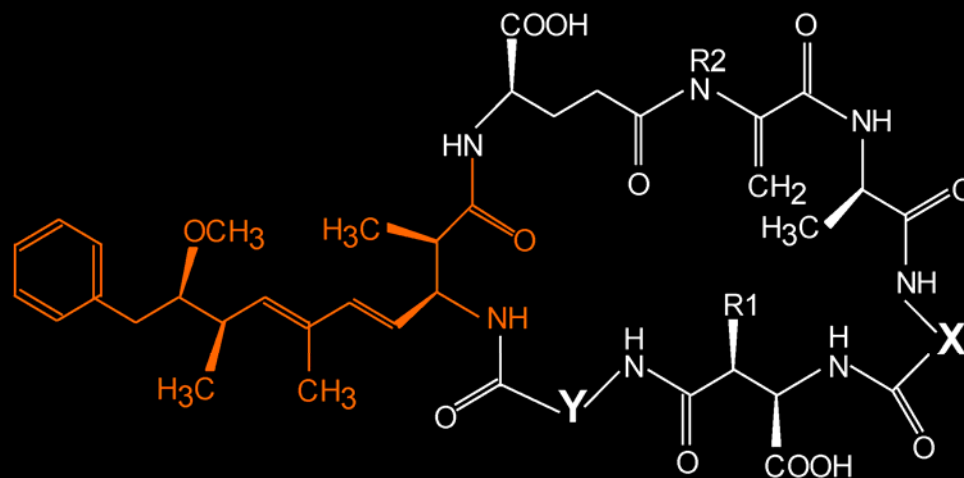
EPA Method 544

Offline SPE, LC-MS/MS

MC-LA, MC-LF, MC-LR

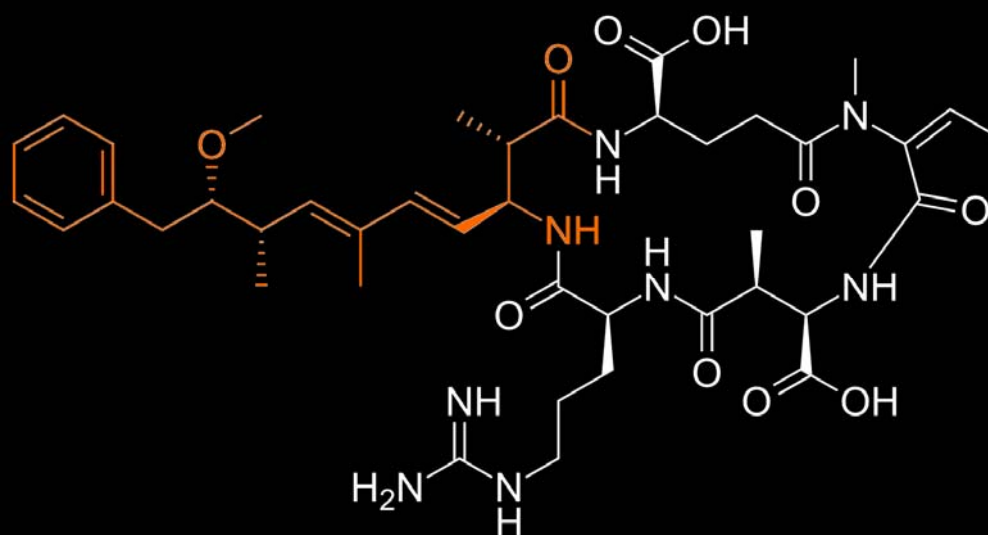
MC-LY, MC-RR, MC-LY

Nodularin-R

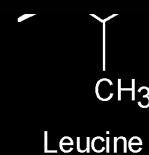


X=

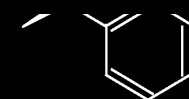
Y=



MC-LF



Leucine



Phenylalanine

Microcystins

EPA Method 546

ADDA specific ELISA

EPA Method 544

Offline LC-MS/MS

MC-LA, MC-LF, MC-LR

MC-LY, MC-RR, MC-LY

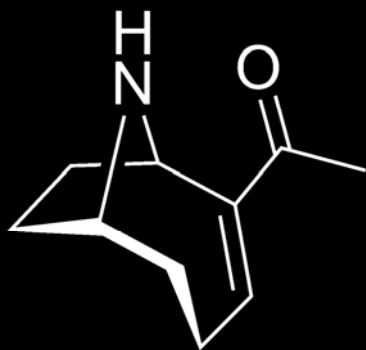
Nodularin-R



EPA 545

Direct-inject LCMS

Anatoxin-A

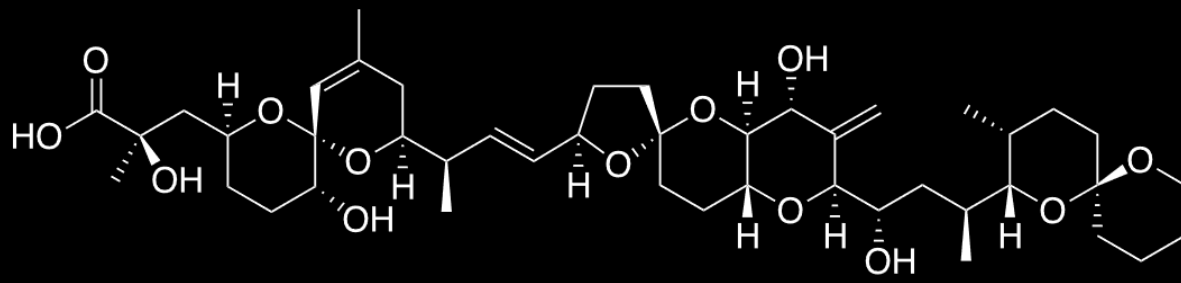


Cylindrospermopsin

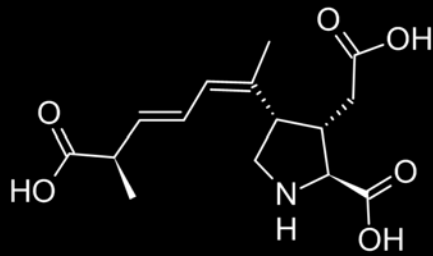


Method 545: Determination of Cylindrospermopsin and Anatoxin-a in Drinking Water by Liquid Chromatography Electrospray Ionization Tandem Mass Spectrometry (LC/ESI-MS/MS)

A close-up photograph of two mussels against a black background. The mussel in the foreground is open, revealing a bright orange, fleshy gill. The mussel behind it is closed, showing its dark, iridescent shell with a hint of purple and blue. The lighting highlights the textures of the shells and the vibrant color of the gill.



Flow- ASE g



erie



Dilute and SPE procedure

Add 1.0 grams homogenized sample to 15 mL tube

Add 10 mL 80:20 MeOH:H₂O shake for 60 minutes at <6°C

Load supernatant onto an unconditioned reversed-phased flow-through SPE cartridge, collecting eluent

Wash the centrifuge tube with two 5 mL 80:20 MeOH:H₂O portions and load onto the SPE cartridge

Combine fractions, and raise up to 20 mL



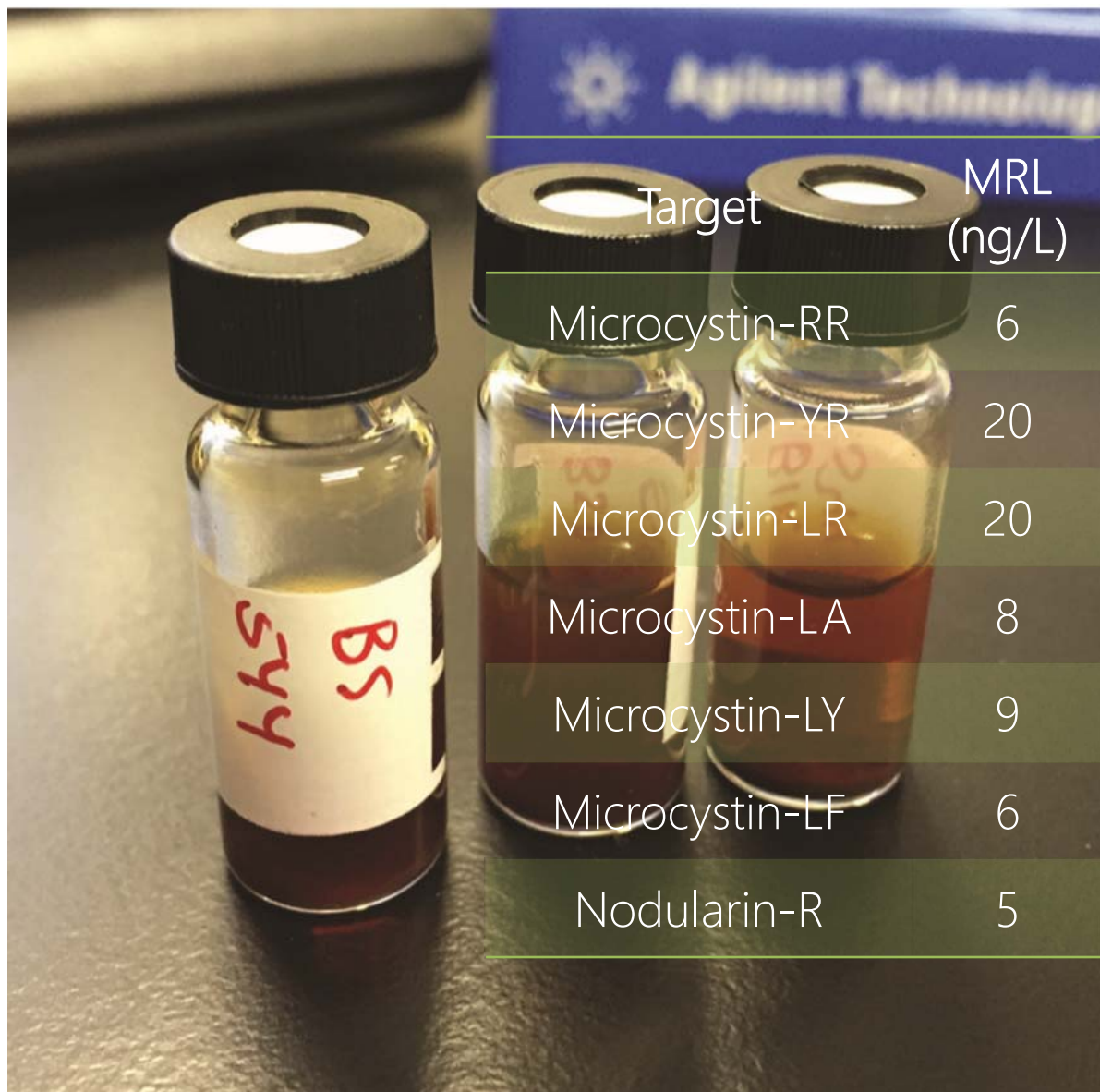
1.0 g into 20 mL for an initial 20x dilution

Analysis

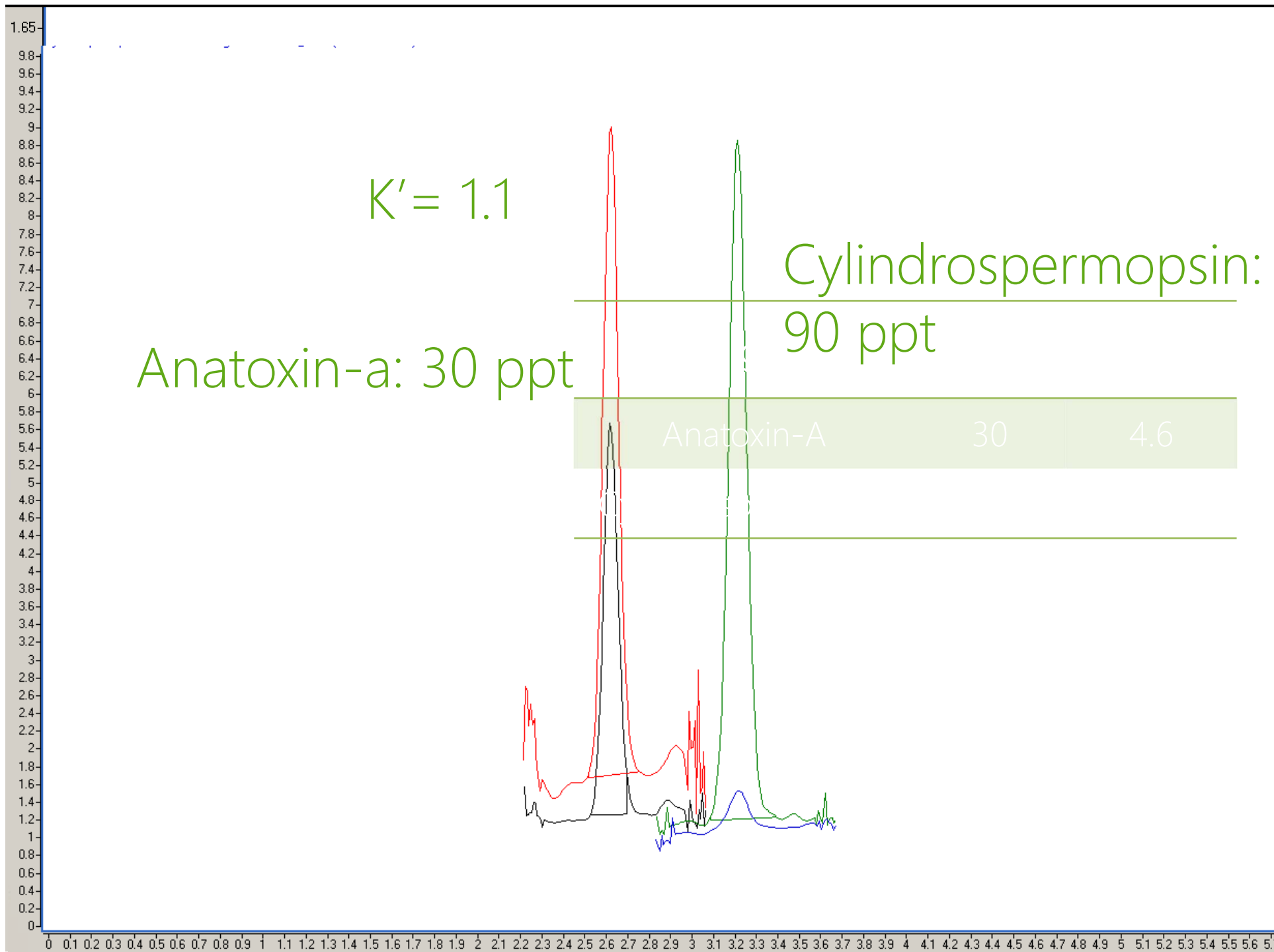
Instrumentation



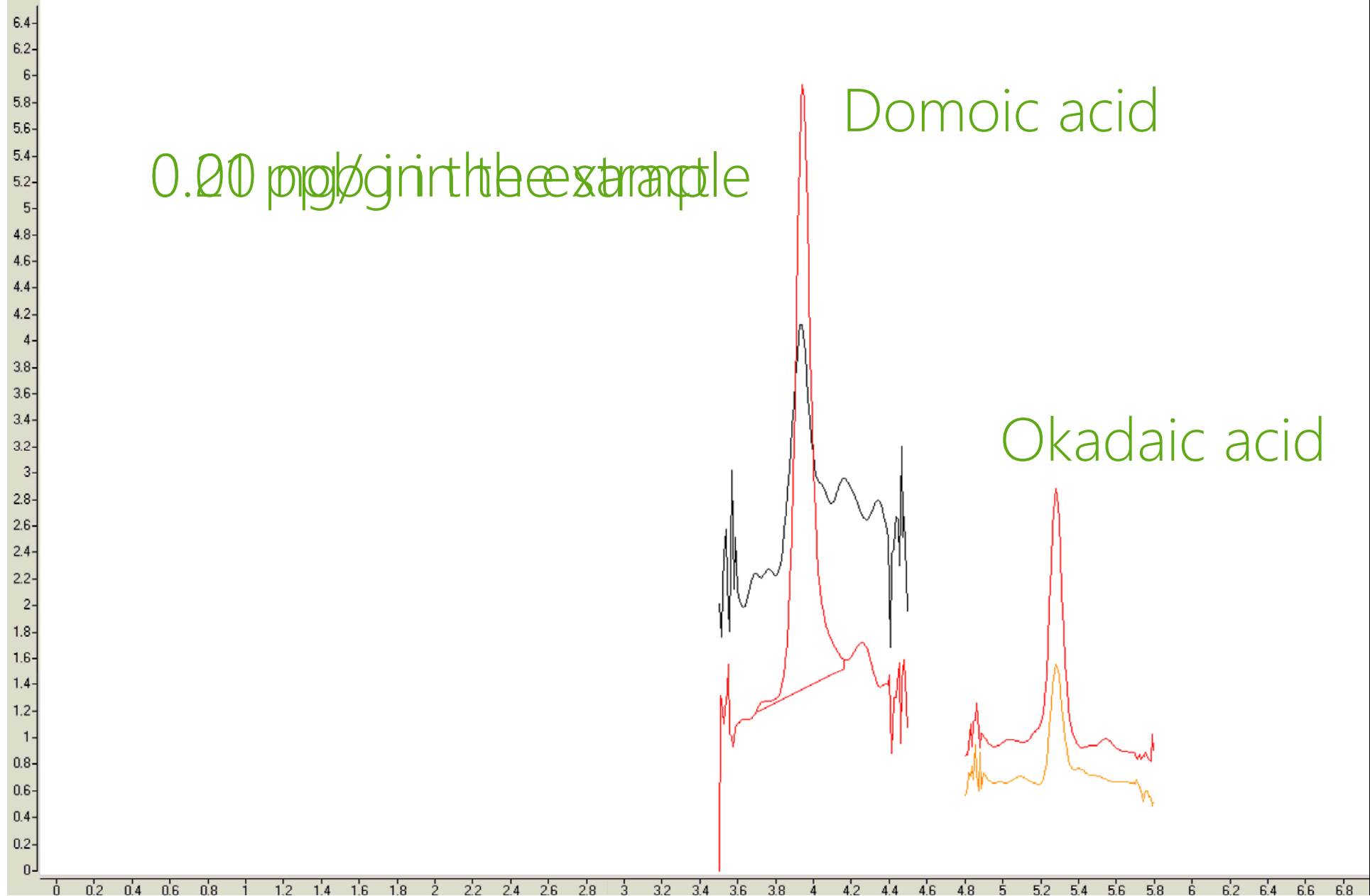
Lowest calibration point: 2.5 to 10 ppt



Target	MRL (ng/L)	
Microcystin-RR	6	1.3
Microcystin-YR	20	
Microcystin-LR	20	3.3
Microcystin-LA	8	
Microcystin-LY	9	1.8
Microcystin-LF	6	
Nodularin-R	5	1.0



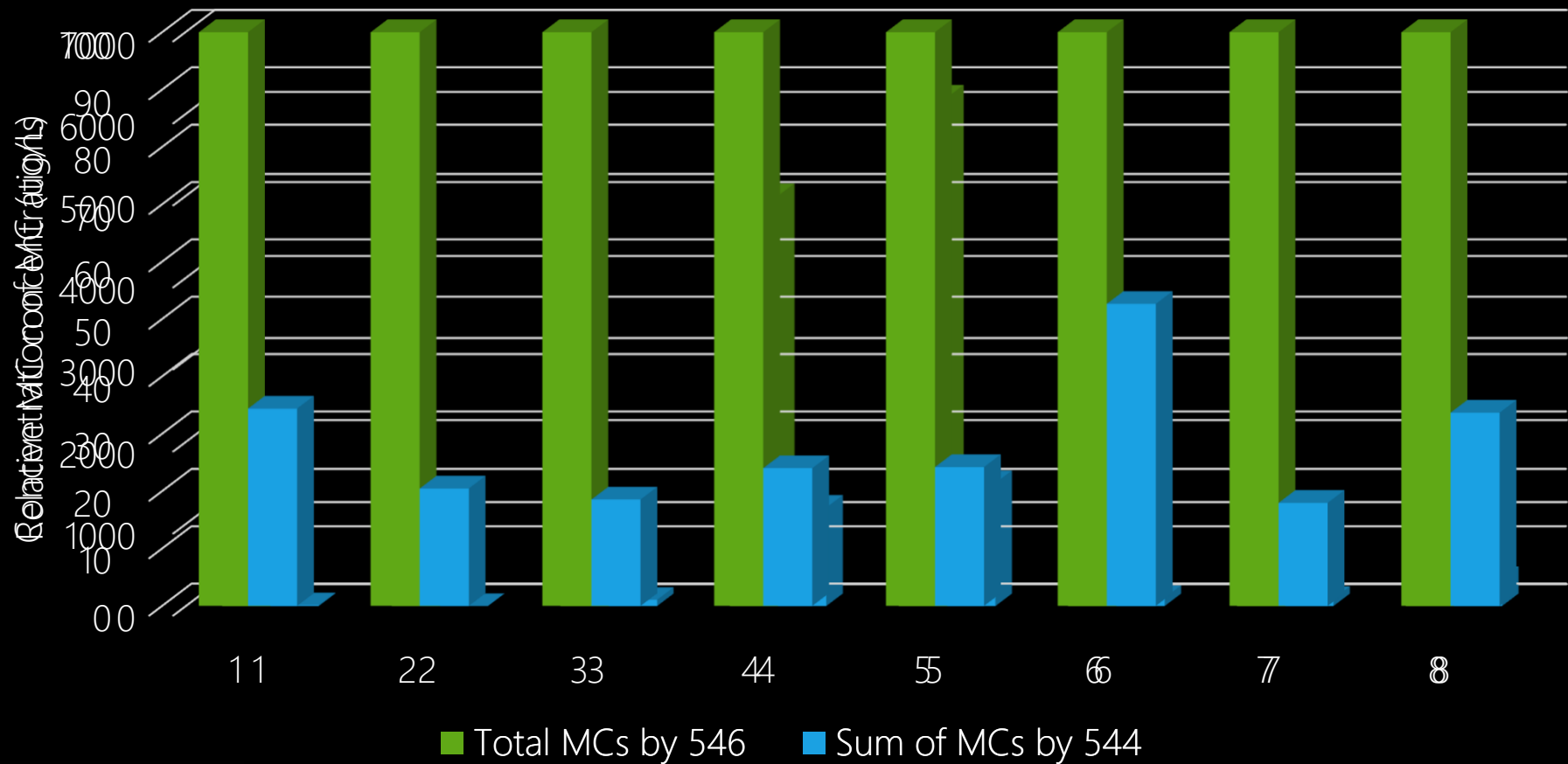
Domoic and okadaic acids



Data

Microcystin comparison

Comparing 544 and 546



n=8

Domoic and okadaic acid recoveries

Domoic Acid recoveries



Okadaic Acid recoveries



Mussels Sediment

n=8

Summary

EPA 544

EPA 54

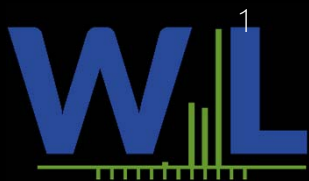
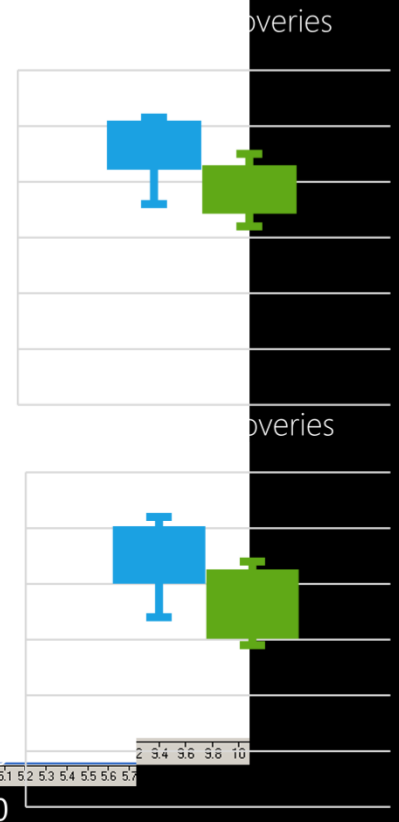
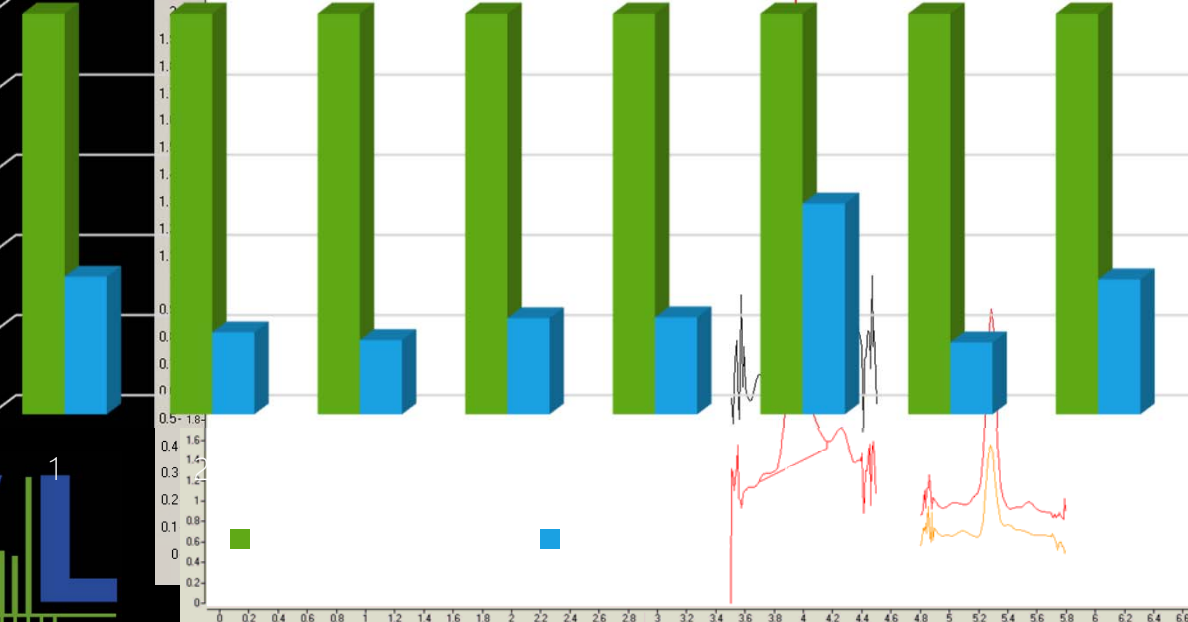
Domoic

Large c

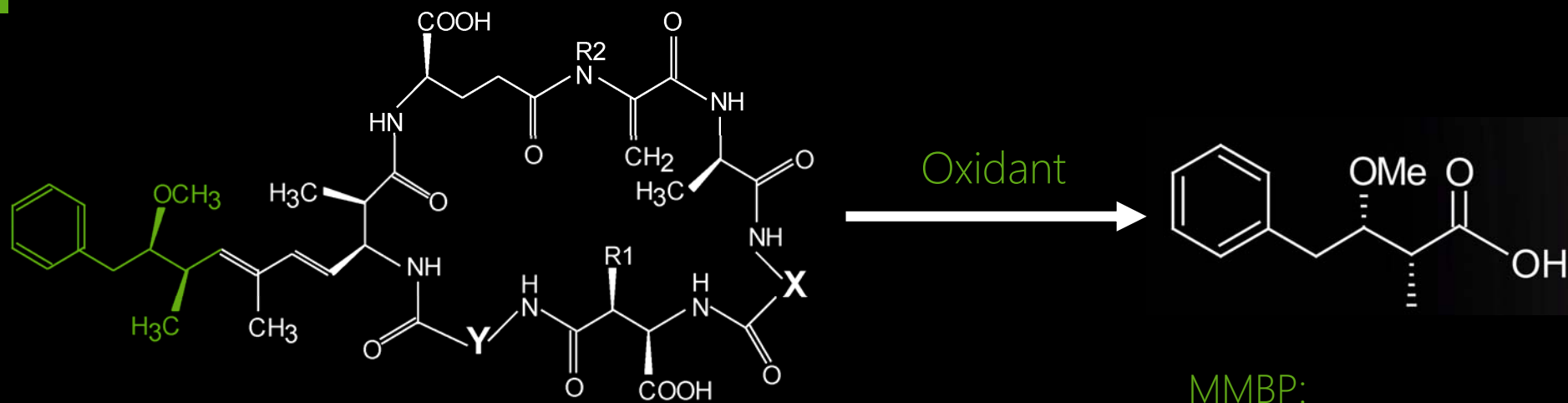
Tar

Relative MC concentrations

100
80
60
40
20
0



Ongoing efforts for reconciliation



Screen -> Oxidize -> C
 concentrate -> Analyze
 Not suitable for finished
 Low recoveries (<30%)
 Standard addition





Questions?

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