

Analysis of Microcystins in Urine with 2D-LC-MS/MS

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INTRODUCTION

Microcystins occur due to the buildup of cyanobacteria in Algae blooms. They are cyclic heptapeptides consisting of a seven-membered peptide ring which is made of five non-protein amino acids (AA) and two protein AA. Human consumption of tainted sea life can lead to exposure. Several analytical techniques have been developed including mouse bioassay, enzyme-linked immunosorbent assay (ELISA) and LC-MS/MS. Little has been done, though, with multi-dimensional chromatography. Thus a method was developed for analysis of biological fluids for microcystin RR, LR, & YR using 2D-LC-MS/MS.

EXPERIMENTAL

ACQUITY UPLC with 2D Technology

Load

Column: Oasis HLB 20 μ m 2.1 x 30 mm
Solvent: MilliQ water (no additives)
Flow: 2 mL/min

Analysis

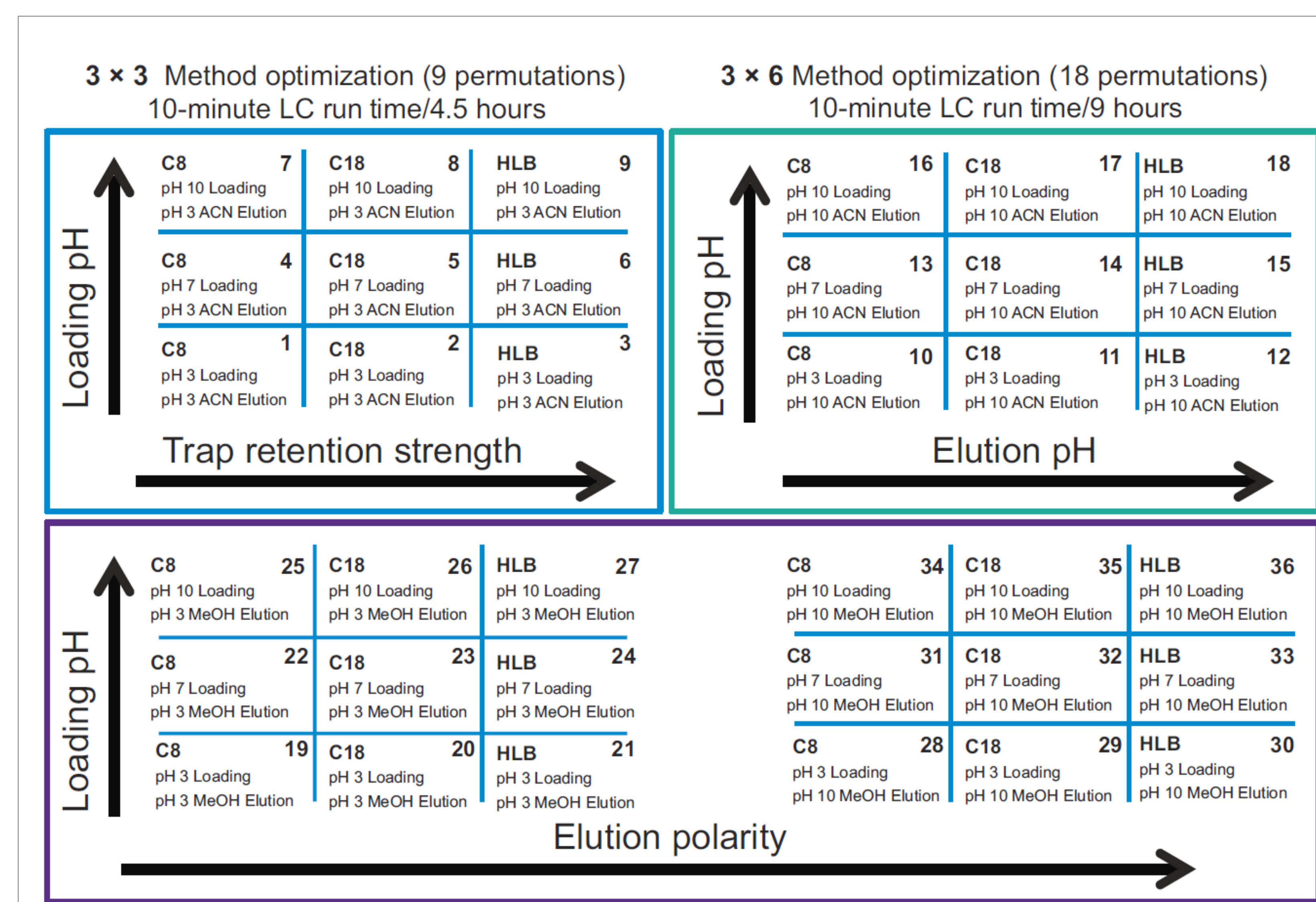
Column: BEH C18, 1.7 μ m, 2.1 x 50 mm
MP: A) H₂O 0.5% FA. B) MeCN 0.5% FA
Inj Vol: 100 μ L Flow: 0.5 mL/min
Gradient: 5 min linear 5%-95% B

Xevo TQ-S Tandem MS, ESI+

Capillary: 3.0 kV Cone: 90 V
Source Temp: 150 C Desolv Temp 550 C
Cone Gas: 50 L/hr.

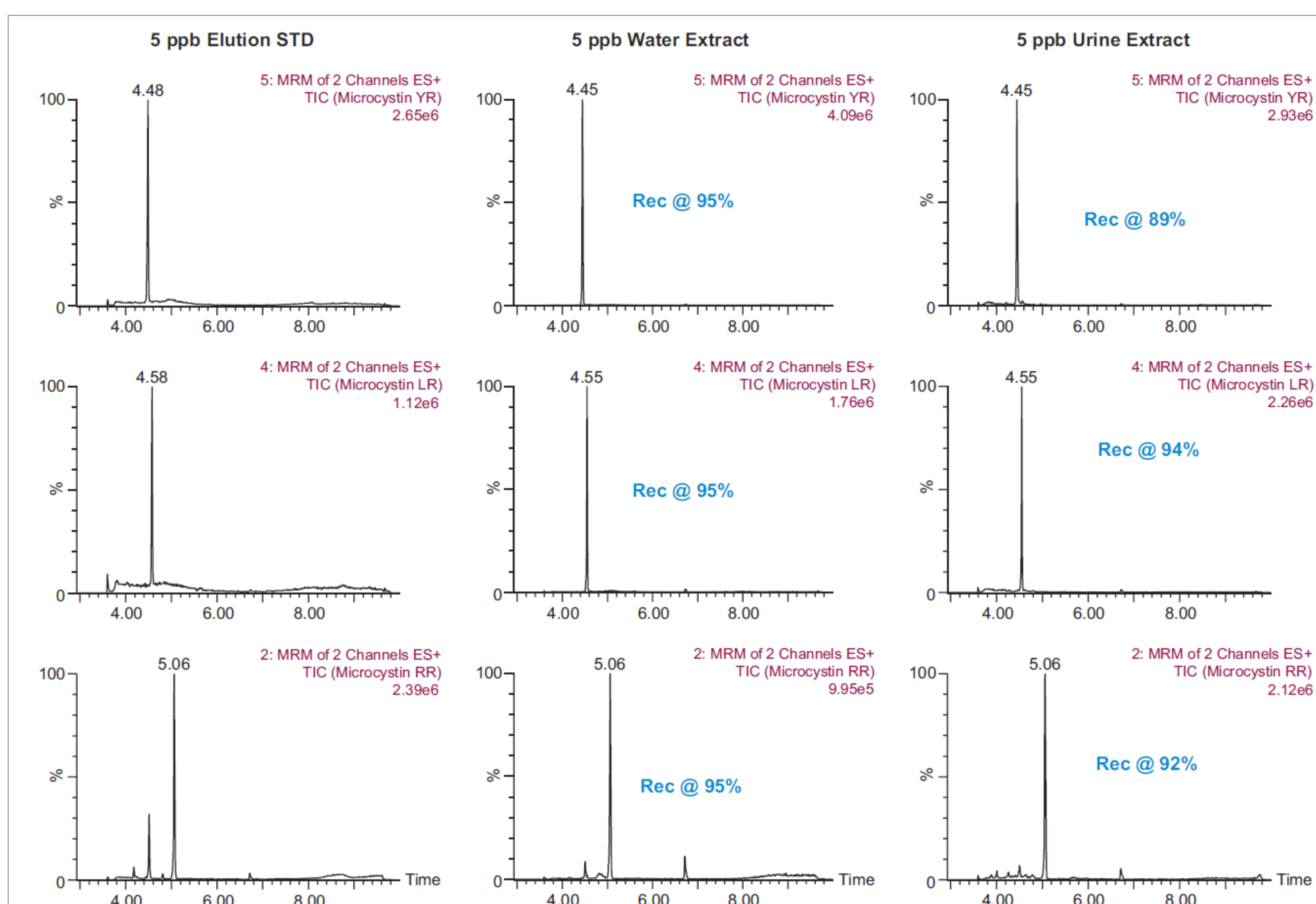
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METHOD



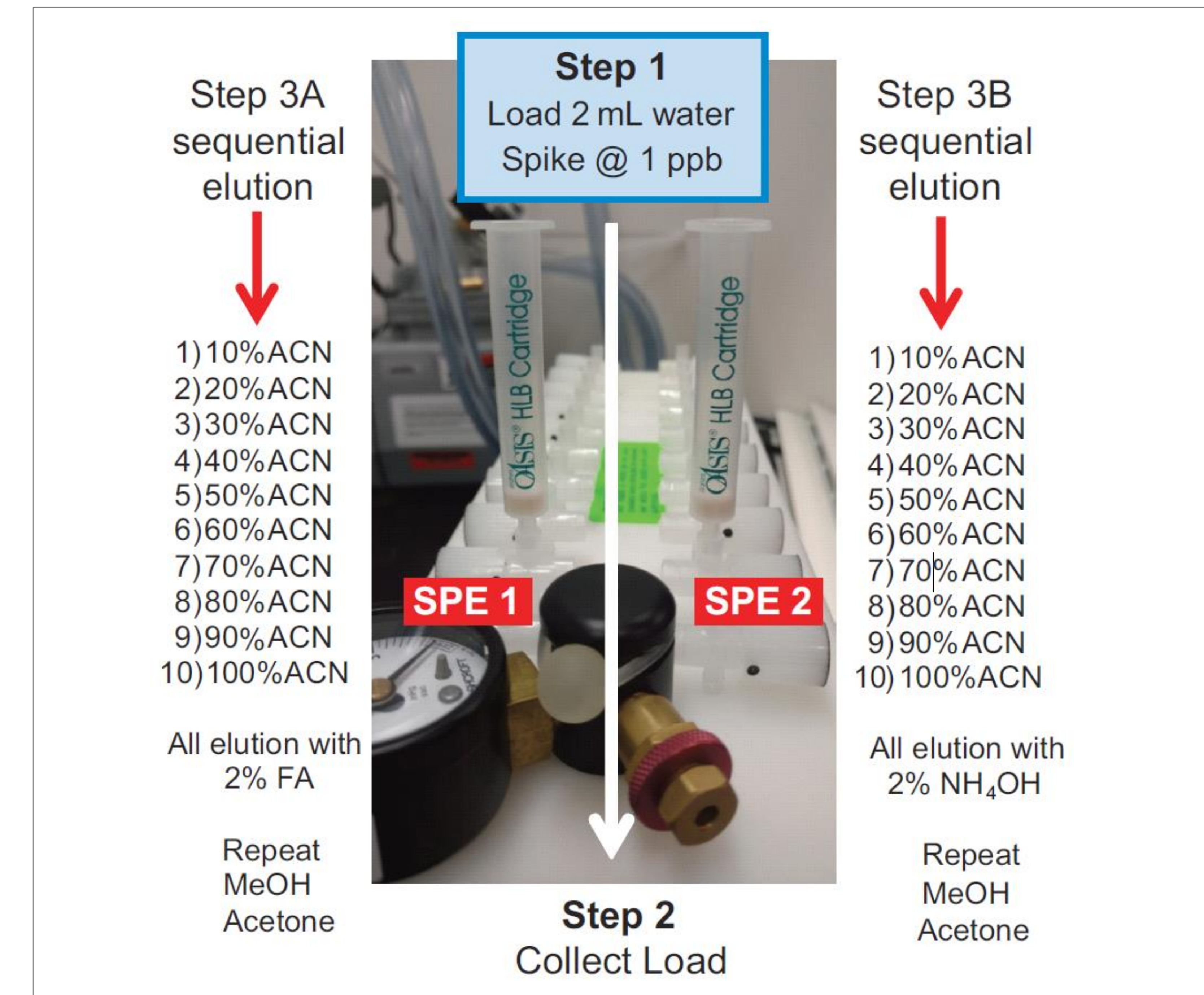
6 X 6 2D-LC Method Optimization Matrix (36 permutations)

RESULTS



2D-LC-MS/MS YR, LR, RR – STD, H₂O Extract, Urine Extract

METHOD



Urine Extraction Protocol using Oasis HLB

Conclusions

- 6 x 6 2D-LC Automated chromatographic method matrix optimization completed overnight.
- Large volume injections of 100% organic extracts eliminate time-consuming evaporation & reconstitution steps, typically needed for 1D-LC.
- Quick captive on-line extraction protocol produced >89% recovery of microcystins YR, LR, and RR from urine.
- 2D-LC-MS/MS is effective for microcystin analysis from urine
- Full Details in [App Note 720006684en](#)