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Academic Research Topics in Environmental Measurement and Monitoring

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Detection of Saxitoxins from Source and Drinking water using Solid-Phase Extraction and Hydrophilic Interaction Liquid Chromatography – Mass Spectrometry (HILIC – MS)

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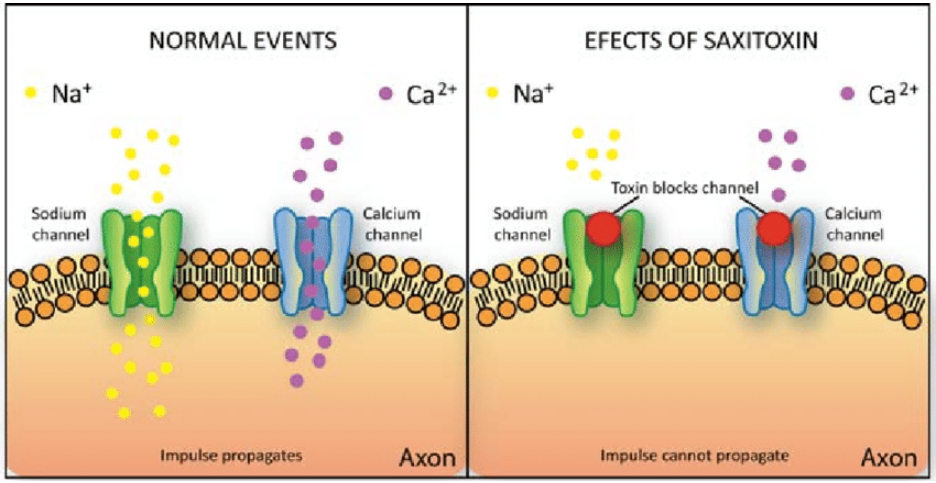
Overall objective

Apply detection method to quantify saxitoxin and its variants in treatment by catalytic ozone membrane filtration

Freshwater cyanobacteria produce saxitoxins that can bind with sodium channels in humans and can lead to paralysis and death in severe cases

Class of toxin	Produced by
Neurotoxin	Dinoflagellates
	Freshwater cyanobacteria



The binding of saxitoxins to sodium and calcium channels leads to paralysis and death by respiratory arrest.[1]

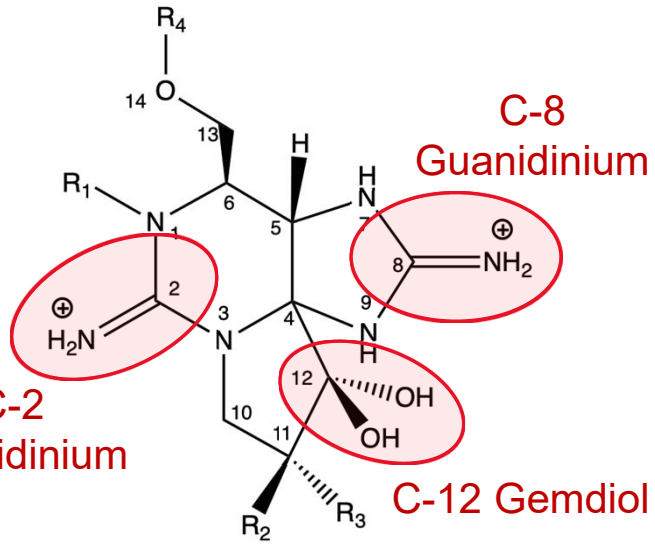
Also binds to potassium channels but blockage is not complete.[1]

Image source: Valério, Elisabete & Chaves, Sandra & Tenreiro, Rogerio. 2010 Toxins. 2. 2359-410.

Cusick, Kathleen D., and Gary S. Sayler. 2013 Marine Drugs 11 (4): 991–1018.

Toxicity of saxitoxins originates from the protonated guanidinium groups and gemdiol leading to an oral LD_{50} of $3 - 10\ \mu g/kg$

Toxicity of Saxitoxins



$LD_{50\ (oral)} = 3 - 10\ \mu g/kg$

Toxin	R_1	R_2	R_3	R_4	Relative Toxicity
STX	- H	- H	- H	$-OC-NH_2$	1
NEO	- OH	- H	- H	$-OC-NH_2$	0.92
GTX1	- OH	- H	$-OSO_3^-$	$-OC-NH_2$	0.99
GTX2	- H	- H	$-OSO_3^-$	$-OC-NH_2$	0.36
GTX3	- H	$-OSO_3^-$	- H	$-OC-NH_2$	0.64
GTX4	- OH	$-OSO_3^-$	- H	$-OC-NH_2$	0.73
GTX5	- H	- H	- H	$-OC-NH-SO_3^-$	0.06
GTX6	- OH	- H	- H	$-OC-NH-SO_3^-$	0.06
C1	- H	- H	$-OSO_3^-$	$-OC-NH-SO_3^-$	0.01
C2	- H	$-OSO_3^-$	- H	$-OC-NH-SO_3^-$	0.01

1. Oshima, Yasukatsu. 1995. *Journal of AOAC International* 78 (2): 528–32.
2. Strichartz, G R. 1984. *Journal of General Physiology* 84 (August 1984): 281–305.

Saxitoxin is detected in USA but is not regulated by US EPA

EPA National Lakes Assessment 2007[1]:

- STX in 7.7% samples (out of 1161 lakes & reservoirs)
- Mean conc = 0.061 $\mu g/L$
- 82% STX detections occurred in northern half

International drinking water guideline for Saxitoxin = 3 $\mu g/L$[2]

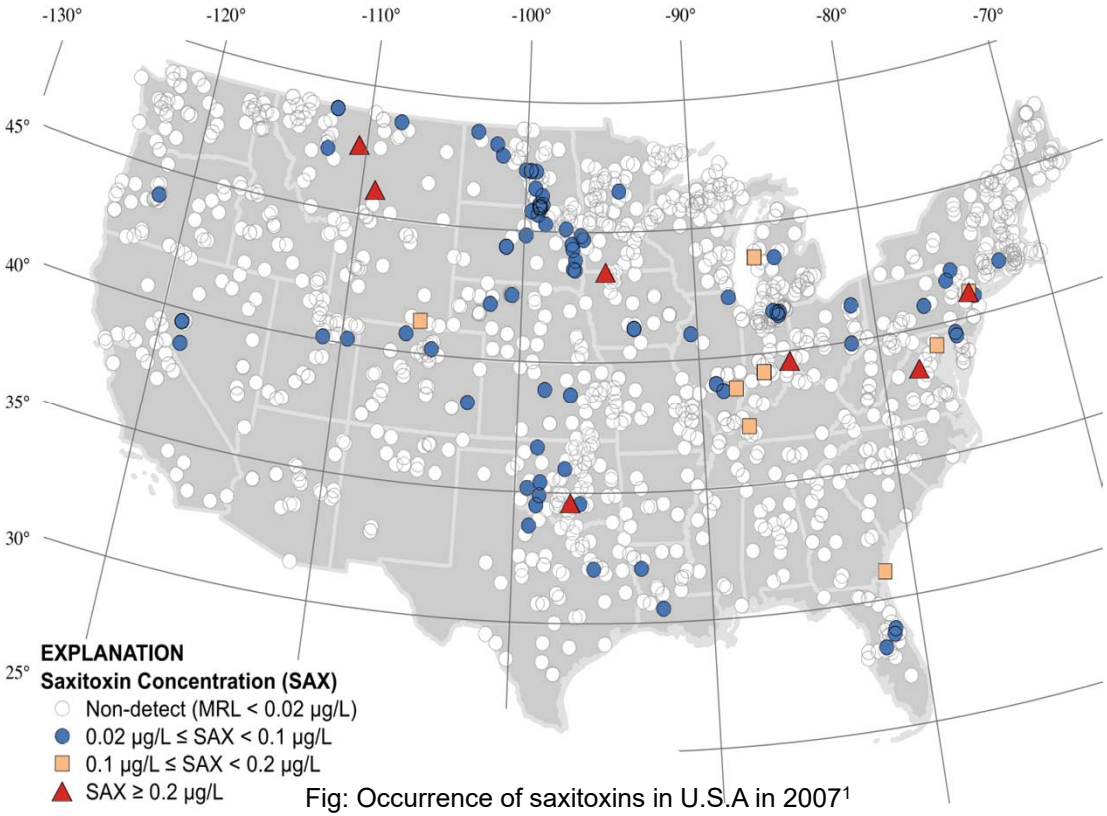


Fig: Occurrence of saxitoxins in U.S.A in 2007[1]

1. Loftin, Keith A, Jennifer L Graham, and Michael T Meyer. 2016 *Harmful Algae* 56: 77–90.
2. AWWA. 2016.

Saxitoxin is detected in source and drinking water in Ohio but is not regulated by US EPA

Ohio Drinking Water Treatment Plants[1] :

- Source waters detections - 0.88 $\mu g/L$
- Treated drinking water detections - 0.064 $\mu g/L$

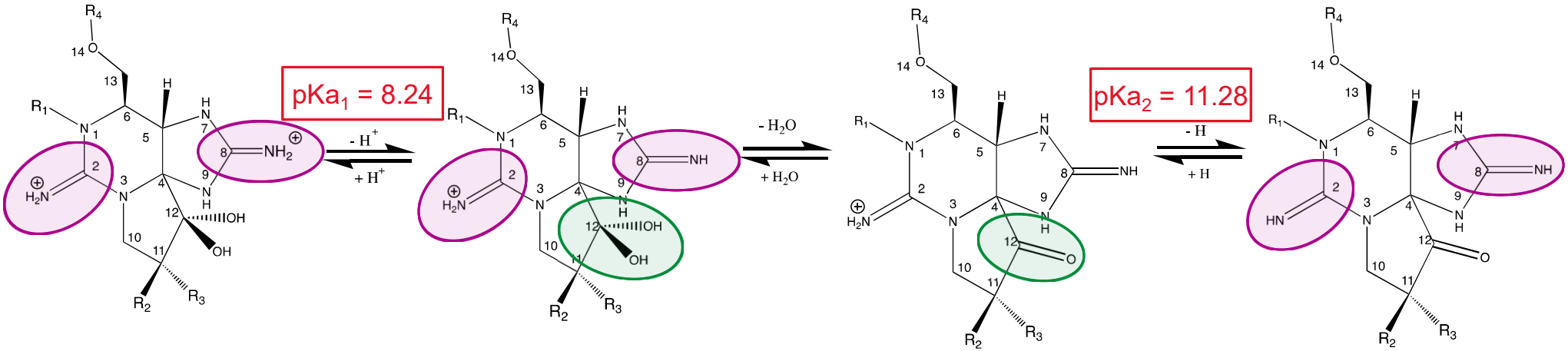
States with drinking water guidelines for saxitoxin:
Ohio = 0.2 $\mu g/L$
Oregon = 1.6 $\mu g/L$

Only Ohio and Oregon have state guidance for saxitoxins in drinking water[2]

Fig: Occurrence of saxitoxins in U.S.A in 2007[1]

1. AWWA. 2016

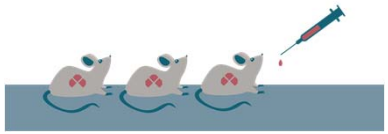
Saxitoxins have a double positive charge at a pH < 8.22 and have no charge at a pH > 11.28



HILIC-MS is suitable for sensitive and accurate analysis of saxitoxin and its variants

Mouse Bioassay (MBA)

- Early detection method for saxitoxins[1]
- Live animals and slow results

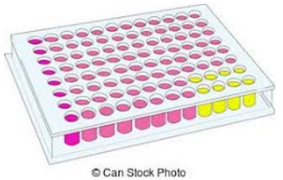


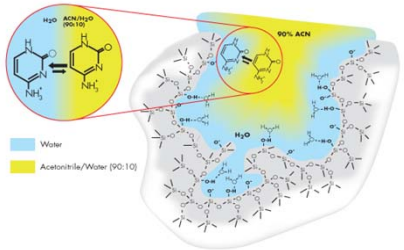
HPLC and LC-MS

- Quick and accurate analysis
- Requires ion-pairing of saxitoxin

ELISA

- Highly sensitive screening tool
- Poor selectivity of variants of saxitoxin[2]





HILIC-MS

- Can quantify variants of saxitoxin
- Has been used to quantify saxitoxins from shellfish and algal extracts[3]

1. Sommer, H, and K F Meyer. 1937. *Arch. Pathol.* 24: 560–98.
2. Humpage, A. R., V. F. Magalhaes, and S. M. Froscio. 2010. *Analytical and Bioanalytical Chemistry* 397 (5): 1655–71.
3. Dell'Aversano, Carmela, Geoffrey K. Eaglesham, and Michael A. Quilliam. 2004. *Journal of Chromatography A* 1028 (1): 155–64.

HILIC Methods have been developed for concentrated solution but not dilute samples like surface water

HILIC-MS used for quantification of saxitoxin from extracts of:

- shellfish[1],
- algal extracts[2],
- urine[3] and
- water (small volume)[4]

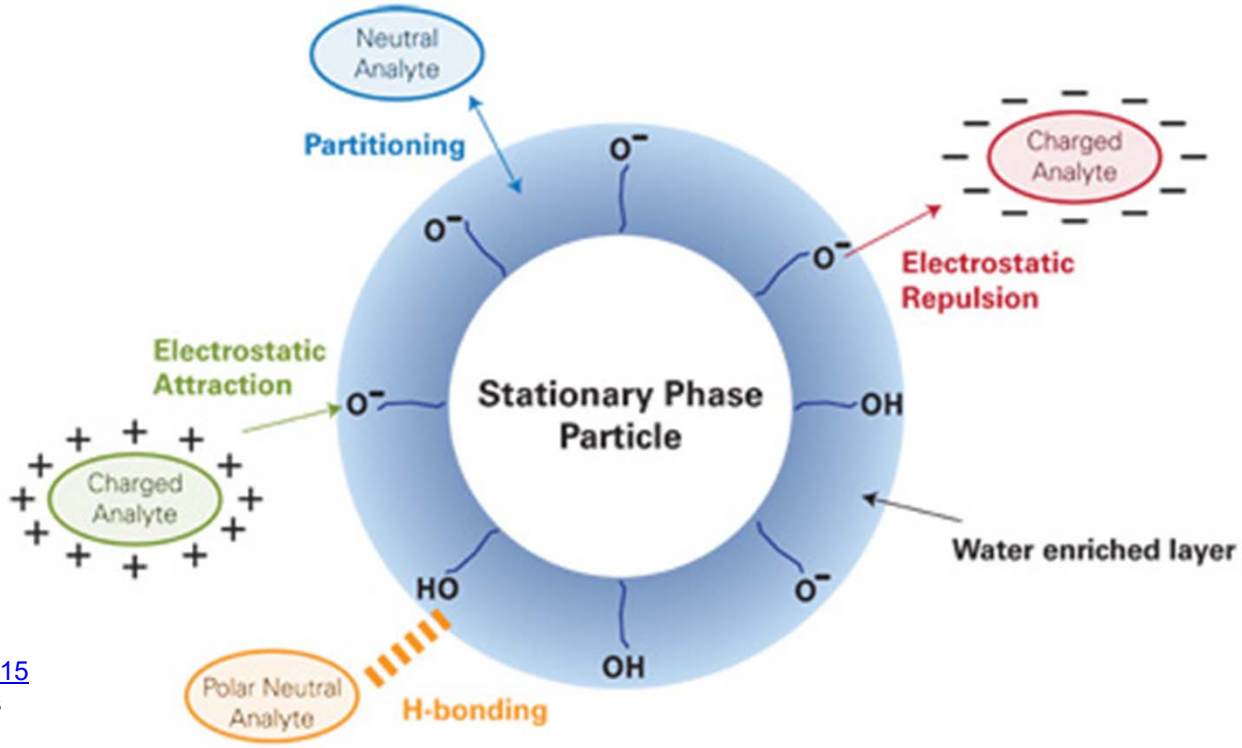
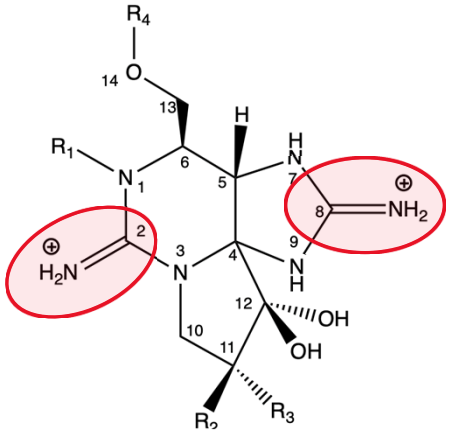


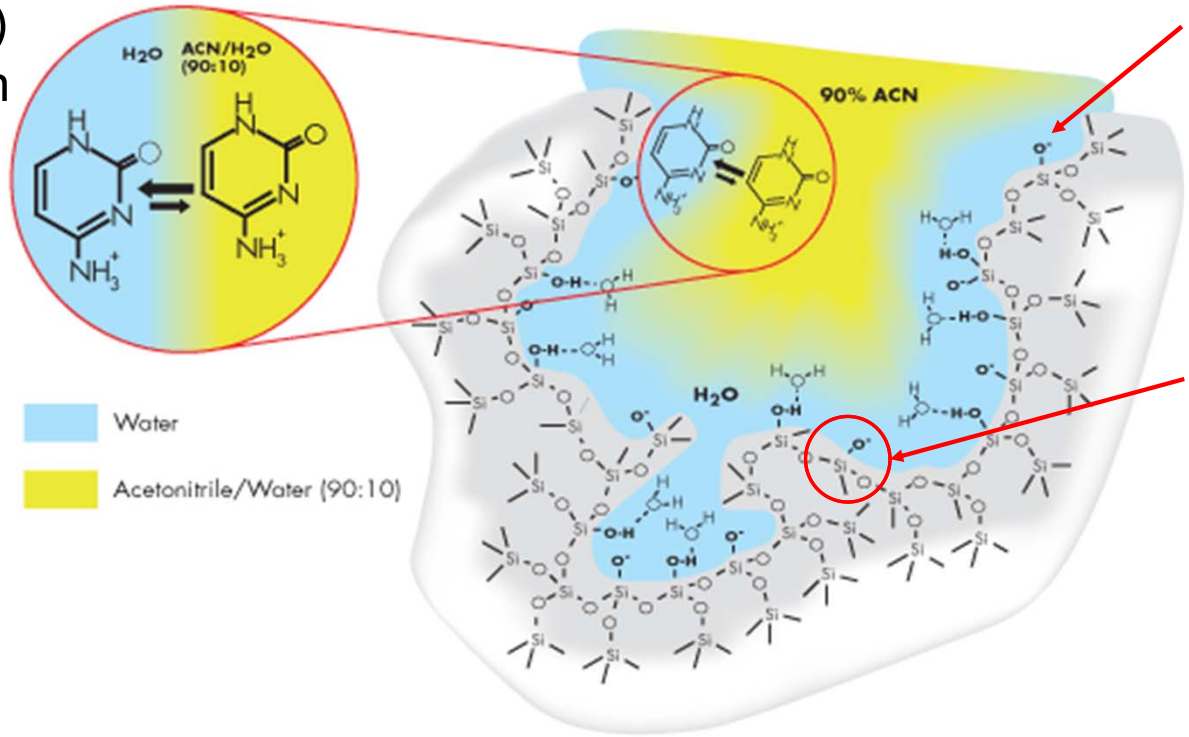
Image source: http://www.ace-hplc.com/products/product.aspx?id=5115

1. M.A. Quilliam, P. Hess, C. Dell'Aversano, Mycotoxins Phycotoxins Perspect. Turn Century. (2001) 383–39
2. C. Dell'Aversano, G.K. Eaglesham, M.A. Quilliam, J. Chromatogr. A. 1028 (2004) 155–164
3. R.C. Johnson et al. J. Anal. Toxicol. 33 (2009) 8–1
4. Jansson, Daniel, and Crister Åstot. 2015. Journal of Chromatography A 1417: 41–48.

Saxitoxins are retained on the HILIC column through partitioning and electrostatic interactions

Step 2: Polar analyte (saxitoxin) partitions between mobile phase and water layer





Step 1: Wetting stationary phase with a polar solvent like water

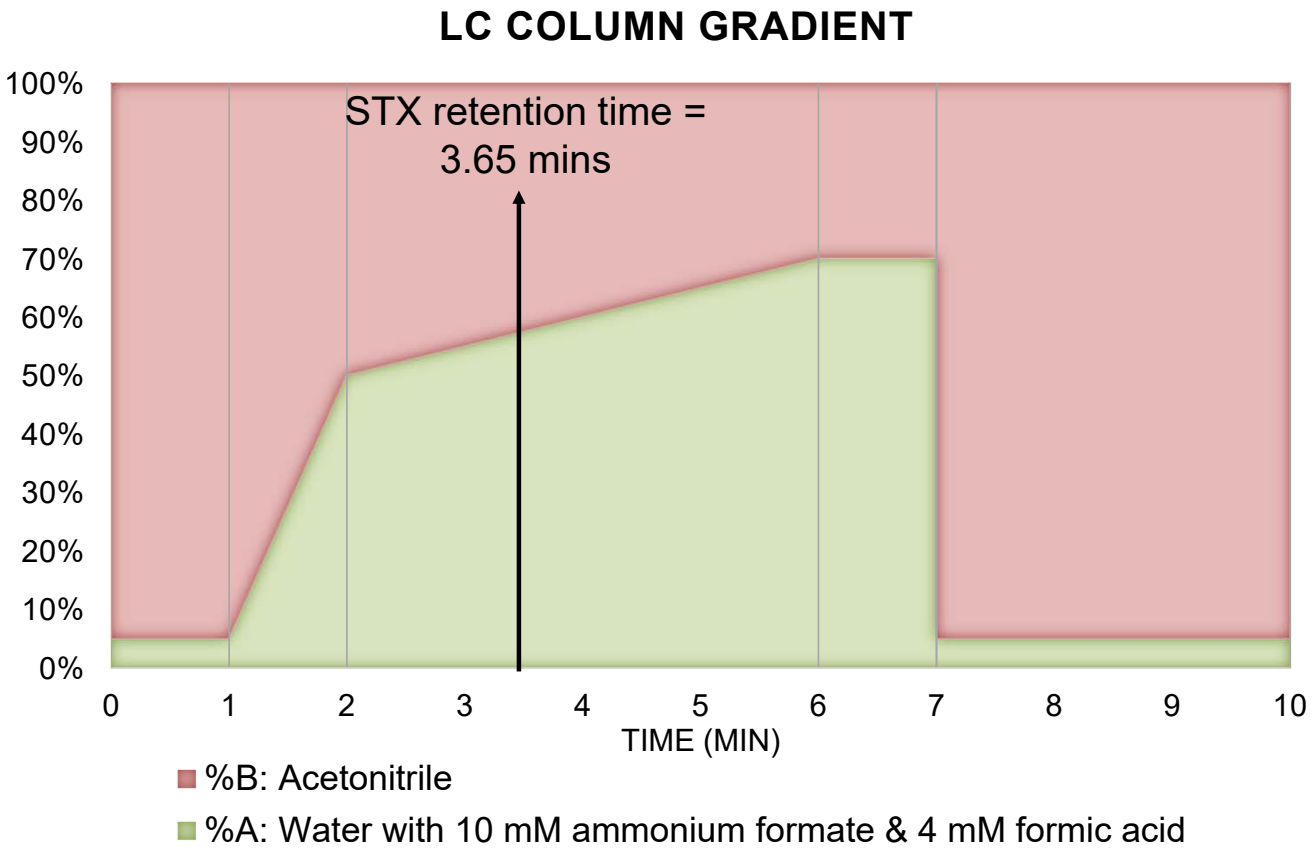
Step 3: Secondary electrostatic interactions

Image source: https://www.waters.com/waters/en_US/Polar-compound-retention-of-broad-analytes/nav.htm?cid=513211&locale=en_US

HILIC – MS Experimental Details

Liquid Chromatography
Column: Acquity UPLC BEH Amide (pore size 130 Å and particle size 1.7 μm)

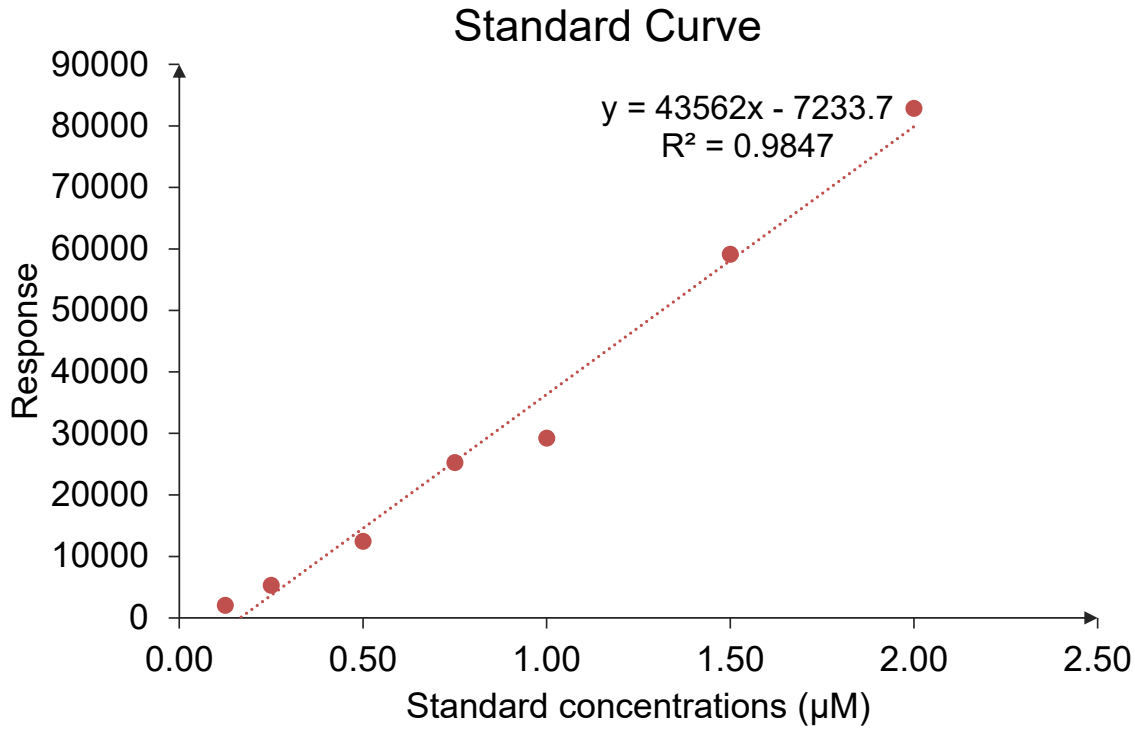
Mass Spectrometry
Instrument: Waters Xevo G2-XS UPLC/MS/MS (Quadrupole/Time-of-Flight)



We achieved a HILIC – MS detection limit of $0.125\ \mu M$ for saxitoxin without SPE

Detection Limit = $0.125\ \mu M$

Within 7 replicates:
Minimum Reporting Limit = $0.25\ \mu M$

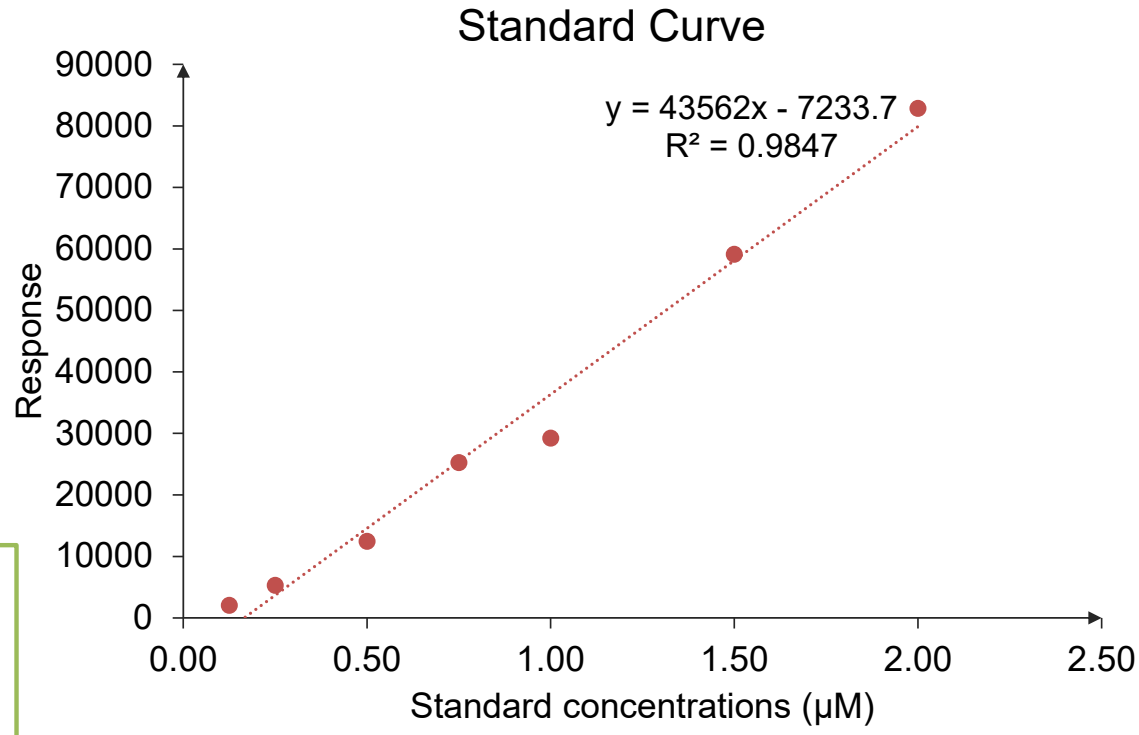


Our detection limit is expected to achieve $0.1\ nM$ saxitoxin detections with SPE, which is well below the surface water detection needs

Detection Limit = $0.125\ \mu M$

Within 7 replicates:
Minimum Reporting Limit = $0.25\ \mu M$

With 50% SPE recovery and concentration by 5000, surface water STX conc = $0.1\ nM\ (0.03\ \mu g/L)$



Solid Phase Extraction (SPE) will be employed to concentrate saxitoxins from $500\ mL$ of water

Weak cation exchange

Graphite carbon

Strong cation exchange

Hydrophilic-Lipophilic Balance (HLB)[3]

Weak cation exchange (WCX)

Extraction of saxitoxin from human urine $(0.5 - 1\ mL)$[1] and plasma $(3\ mL)$[2]

Not tested for extraction from large volume of water

1. R.C. Johnson, et al, 2009. *J. Anal. Toxicol*. 33 8–14
2. Peake, Roy W.A., et al. 2016. *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences* 1036–1037: 42–49.

Graphitized carbon extraction

Extraction of highly basic polar compounds: anatoxin and cylindrospermopsin[1]

Clean up of saxitoxin containing extracts from mussels[2]

Extraction of saxitoxin from $1\ mL$ water samples[3]

1. Zervou, Sevasti Kiriaki, et al. 2017. *Journal of Hazardous Materials* 323: 56–66.
2. Rey, Veronica, et al. 2018. Food Chemistry 269 (June): 166–72.
3. Jansson, Daniel, and Crister Åstot. 2015. *Journal of Chromatography A* 1417: 41–48.

Strong cation exchange (SCX)

Extraction of gonayutoxins[1] and C-toxins[2] from small volumes of urine and water

1. Eangoor, Padmanabhan, Amruta Indapurkar, and Jennifer S Knaack. 2015. 2–5.
2. Jansson, Daniel, and Crister Åstot. 2015. *Journal of Chromatography A* 1417: 41–48.

Hydrophilic Lipophilic Balance (HLB)

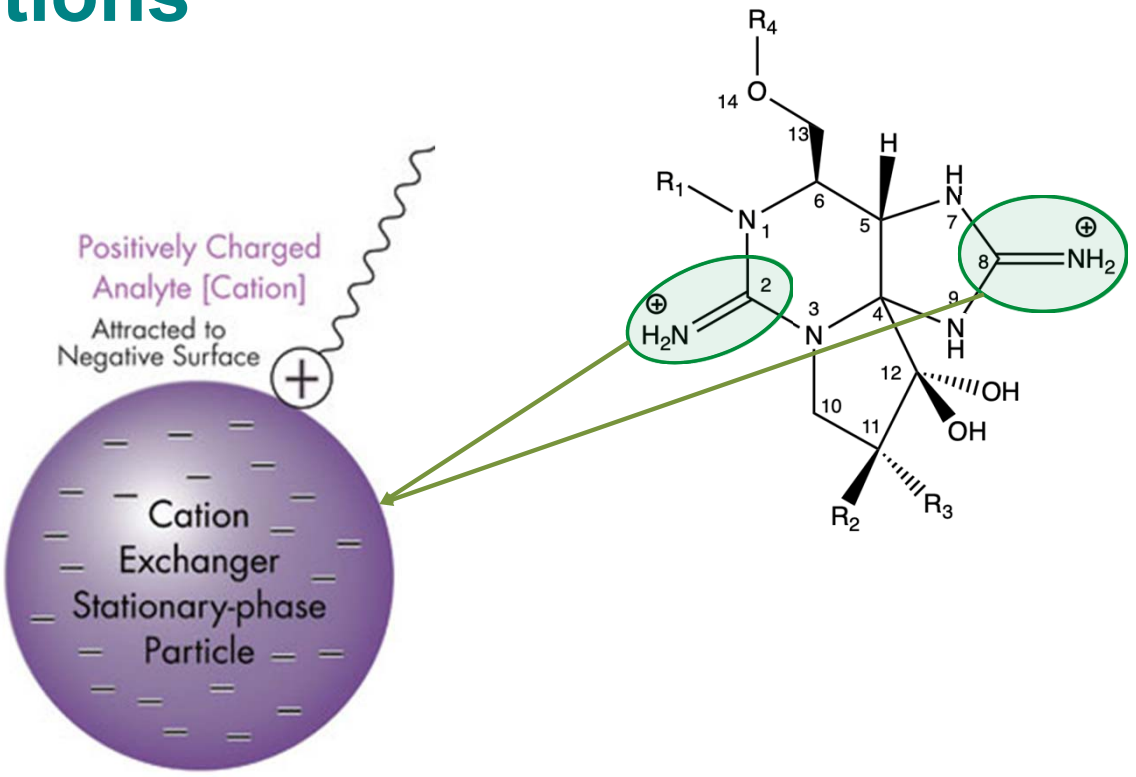
Commonly used for extraction of Microcystins from surface water[1]

Used for clean-up of extracts containing saxitoxins[2]

1. J.A. Shoemaker, D.R Tettnhorst, and A. de la Cruz. 2015. *United States Environmental Protection Agency*.
2. Quilliam, Michael A., Phillip Hess, and Carmela Dell'Aversano. 2001 *Mycotoxins and Phycotoxins in Perspective at the Turn of the Century*, 383–91.

Weak cation exchange has been most frequently used in the past for extraction for saxitoxins from concentrated solutions[1–5]

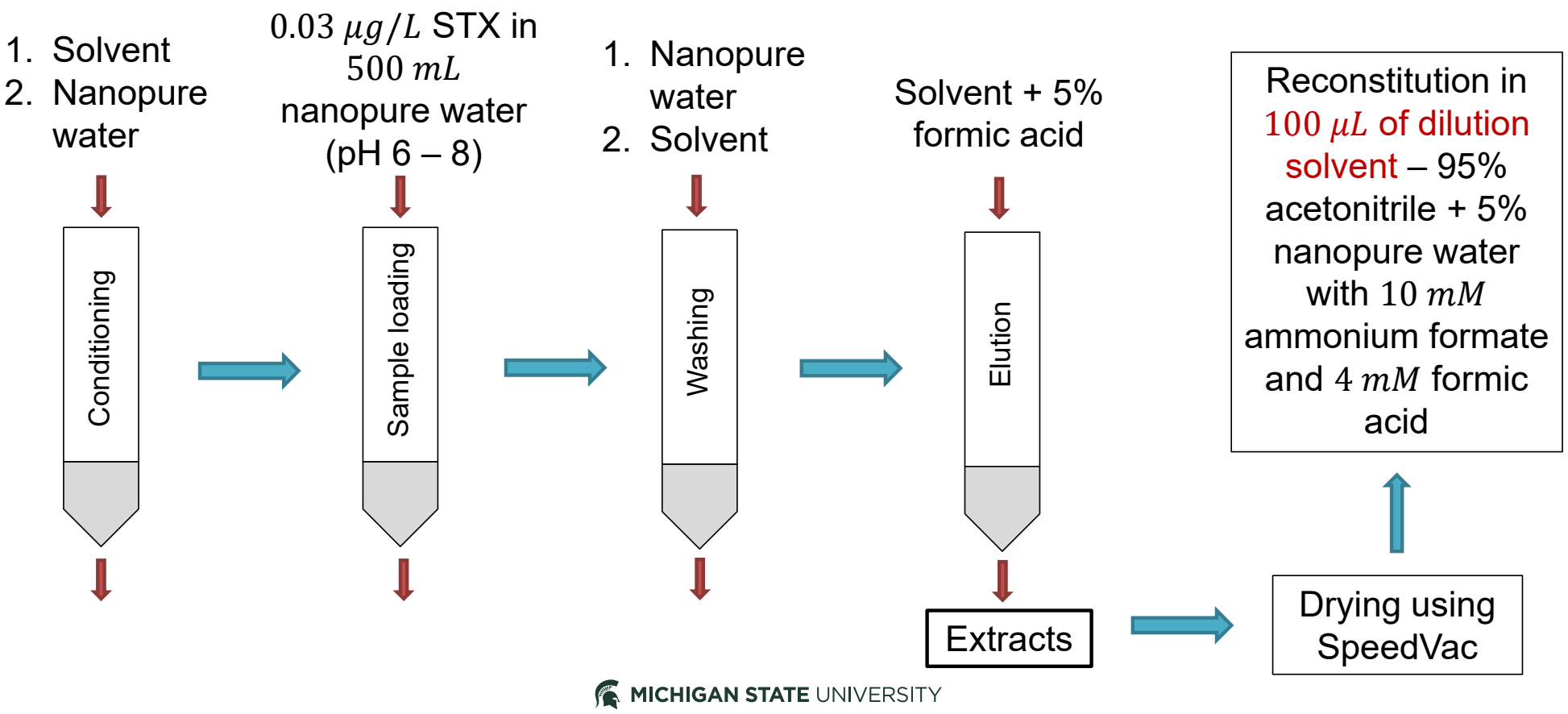
Positively charged guanidinium groups of saxitoxin interact with negatively charged sorbent of the resin



1. R.C. Johnson, et al, 2009. *J. Anal. Toxicol*. 33 8–14
2. Peake, Roy W.A., et al. 2016. *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences* 1036–1037: 42–49.
3. Stafford, Robert G., and Harry B. Hines. 1994. *Journal of Chromatography B: Biomedical Sciences and Applications* 657 (1): 119–24.
4. Eangoor, Padmanabhan, et al. 2015. 2–5.
5. Bragg, William A, et al. 2015. *Toxicon* 99: 118–24.

GS6

WCX SPE procedure modified from Johnson et al 2009 that extracted STX from a concentrated solution of 1 mL



Slide 20

GS6 Can talk about this first and then relate it to the other cartridges in the next slide
Gawankar, Shardula, 7/13/2020

Step	WCX	SCX	HLB	Graphite carbon
Procedure derived from	Jonson et al 2009	Recommended procedure by Biotage	EPA Method 544	Zervou et al 2017
Condition	$15\ mL$ MeOH	$15\ mL$ MeOH	$15\ mL$ MeOH	$6\ mL$ DCM $6\ mL$ MeOH
Equilibration	$15\ mL$ water (pH = 6.5)	$15\ mL$ water (pH = 7)	$15\ mL$ water	$6\ mL$ water + $2\ M$ NaOH (pH > 11.22)
Load $500\ mL$ water + $0.1\ nM$ Saxitoxin @ < $5\ mL/min$ (pH 6.5 – 7)				pH > 11.22
Wash	$6\ mL$ water $6\ mL$ MeOH	$6\ mL$ water $6\ mL$ MeOH	$6\ mL$ water	$6\ mL$ MeOH
Elute	$10\ mL$ 95% MeOH + 5% FA	$10\ mL$ 5% NH_4OH in 50:50 DCM:MeOH	$10\ mL$ 95% MeOH + 5% FA	$10\ mL$ 40:60 DCM:MeOH + 0.5% FA
Dry all extracts completely with SpeedVac				
Reconstitute dried extracts with $100\ \mu L$ of 95% acetonitrile + 5% water with $10\ mM$ ammonium formate and $4\ mM$ FA				

MeOH – Methanol
DCM – Dichloromethane
NaOH – Sodium hydroxide
FA – Formic acid

Saxitoxins will be extracted by SPE using 4 different cartridges and reconstituted into solvent to achieve a concentration factor of 5000

Starting conc. of saxitoxin = $\mathbf{0.1\ nM\ (0.03\ \mu g/L)}$;
Expected conc. in $100\ \mu L$ = $\mathbf{0.5\ \mu M\ (150\ \mu g/L)}$ with 100% recovery

Prepare 7 replicates of $500\ mL$ nanopure water samples

Spike $0.1\ nM$ $(0.03\ \mu g/L)$ Saxitoxin into these samples

Perform SPE using:

1. WCX
2. Graphite carbon
3. SCX
4. HLB

Dry extracts

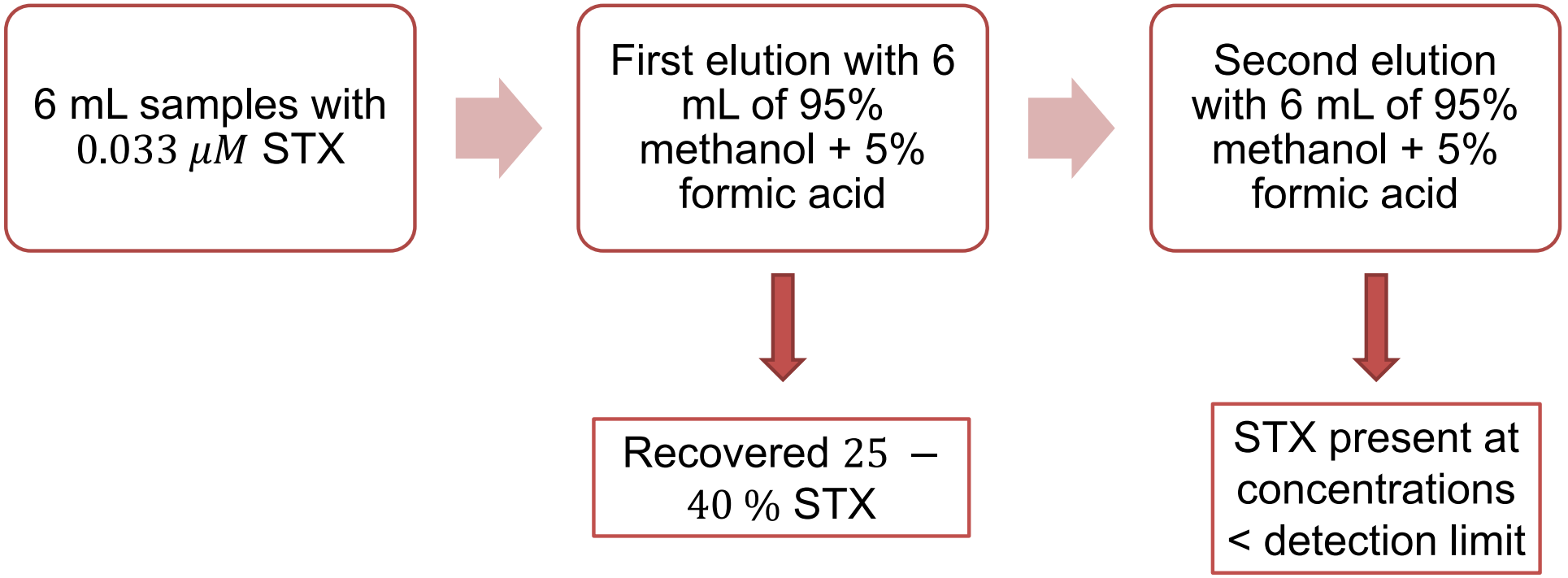
Reconstitute with $100\ \mu L$ dilution solvent ⇒ $5000\times$ concentration

Select cartridge with highest recovery

↓

filtered surface water samples

Less than 40% saxitoxin was recovered from WCX, all within the first $6\ mL$ of elution



Low recoveries obtained from WCX and HLB cartridges

Cartridge	Recovery
WCX Strata™-X-CW 33 µm Polymeric Weak Cation Exchange	Using 5% formic acid in methanol elution solvent = $\leq 40\%$ Using 5% ammonium hydroxide in methanol = no recovery
WCX Enviro-Clean – Carboxylic Acid – PTFE Frits 500mg 6mL	Using 5% formic acid in methanol elution solvent = 10%
HLB Oasis HLB 6cc 500 mg sorbent 30 μm	< Detection limit toxin in load effluent 10% concentration present in wash effluent < 10% toxin in elution

Future steps

- Perform SPE of saxitoxin using SCX and graphite carbon cartridges

- Optimization:
  - Matrix addition to find the loss of saxitoxin at various steps (i.e. drying, adsorption to sample preparation surfaces, inadequate reconstitution)
  - Use stronger solvents like dichloromethane

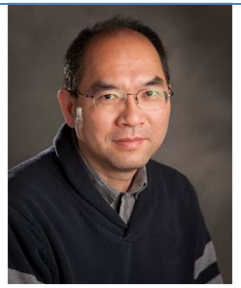
- Apply developed method to quantify saxitoxins in treatment by catalytic ozone membrane filtration

Acknowledgements

Committee


Rebecca H. Lahr


Thomas C. Voice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Xiaoyan Li


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Thank you!

Questions?