Evaluation of Microcystin Immunoaffinity Columns (IAC)

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Drawbacks of using LC/MS and LC/MS/MS to Quantify Microcystin

- The response of various microcystins is different and requires individual reference compounds for quantification.
- The LC/MS(/MS) standard curve is not linear and its shape is characteristic for each microcystin.
- The level of sensitivity may vary from run to run. Sensitivity can be affected by the matrix and condition of the cone.

Meriluoto, J. A. O.; Codd, G. A., *Toxic: Cyanobacteria Monitoring and Cyanotoxin Analysis*. Abo Akademi University Press.: Abo, Finland, 2005; p. 106.

Summary of Cyanotoxin Recoveries from the Fortified Drinking Water Samples

		Average % Recovery	Standard deviation	Range
Cylindrospermopsin	HPLC/PDA	89	8	68-98
	LC/MS/MS SIM	18	12	5-32
Anatoxin-a	HPLC/PDA	92	5	82-98
	LC/MS/MS SIM	32	14	5-98
Microcystin RR	HPLC/PDA	94	4	86-100
	LC/MS/MS SIM	63	6	54-67
Microcystin LR	HPLC/PDA	94	8	82-101
	LC/MS/MS SIM	157	58	84-207
Microcystin LW	HPLC/PDA	93	7	82-101
	LC/MS/MS SIM	61	29	23-98

Outcomes of EPA Methods Meetings in Cincinnati, OH November 2008

- Sample Collection
  - to limit biological and chemical degradation
- Certified Materials
  - no microcystins
- Sample Preparation
  - SPE and IAC
- Analytical Instrumentation
  - ISO 20179:2005 HPLC/PDA
  - LC/MS/MS

Immunoaffinity Column (IAC) Studies

- Tsutsumi et al. (2000) Immunoaffinity column as clean-up tool for determination of trace amounts of microcystins in tap water. Food and Chemical Toxicology. Food and Chemical Toxicology, 38:7 pg. 593-597.
- Lawrence and Menard. (2001) Determination of microcystins in blue-green algae, fish and water using liquid chromatography with ultraviolet detection after sample clean-up employing immunoaffinity chromatography. Journal of Chromatography A 922 pg. 111-117.
- Kondo et al. (2002) Determination of microcystins in lake water using reusable immunoaffinity column. Toxin, 40:7 pg. 893-899.
- Aranda-Rodriguez et al. (2003) Extraction of 15 microcystins and nodularin using immunoaffinity columns. Toxicon, 42:6 pg 587-599.

Design of the IAC

Publication	Antibody Support Gel	Antibody	MCY	Load MCY
Tsutsumi et al. 2000	Affi-Gel	M8H5 Mab (Kondo et al 1996)	RR, YR , LR	10 ug/mL gel
Kondo et al. 2001	Formyl Cellulofine	M8H5 Mab (Kondo et al 1996)	RR, YR, LR	100 ng
Lawrence and Menard 2002	Sepharose CL-4B	Anti-MCY LR (Health Canada)	RR, YR, LR, LA	800 ng
	Silica-based			420 ng
Aranda-Rodriguez et al. 2003	Sepharose based	Anti-MCY LR (Health Canada)	LA, YR, LF, LW, LR	1200 ng
	Silica-based			420 ng
Abraxis Method 2011	Activated Agarose	mAB ABXMCYMAAB ABXMCTPAB	LR, LA, RR, YR	10-150 ng

Summary of Methods

Publication	Pre IAC	Sample Volume (ml)	Flow (ml/min)	Rinse*	Elute*
Tsutsumi et al. 2000	GF/C	10	gravity	PBS/water	100/0
Kondo et al 2001	GF/C, SPE	5	?	Tris/water	DMF (2.5 ml)
Lawrence and Menard 2002	concentration by RV	5 to 15	1 to 2	25/75	80/20 with 4% acetic acid
Aranda-Rodriguez et al. 2003	1.2 um GF/C	15-20	1	25/75	80/20 with 4% acetic acid
Abraxis Method 2011	GF/C	20	0.5	no rinse	95/5 with acetic acid

*Ratios in Rinse and Elute columns are methanol/water.

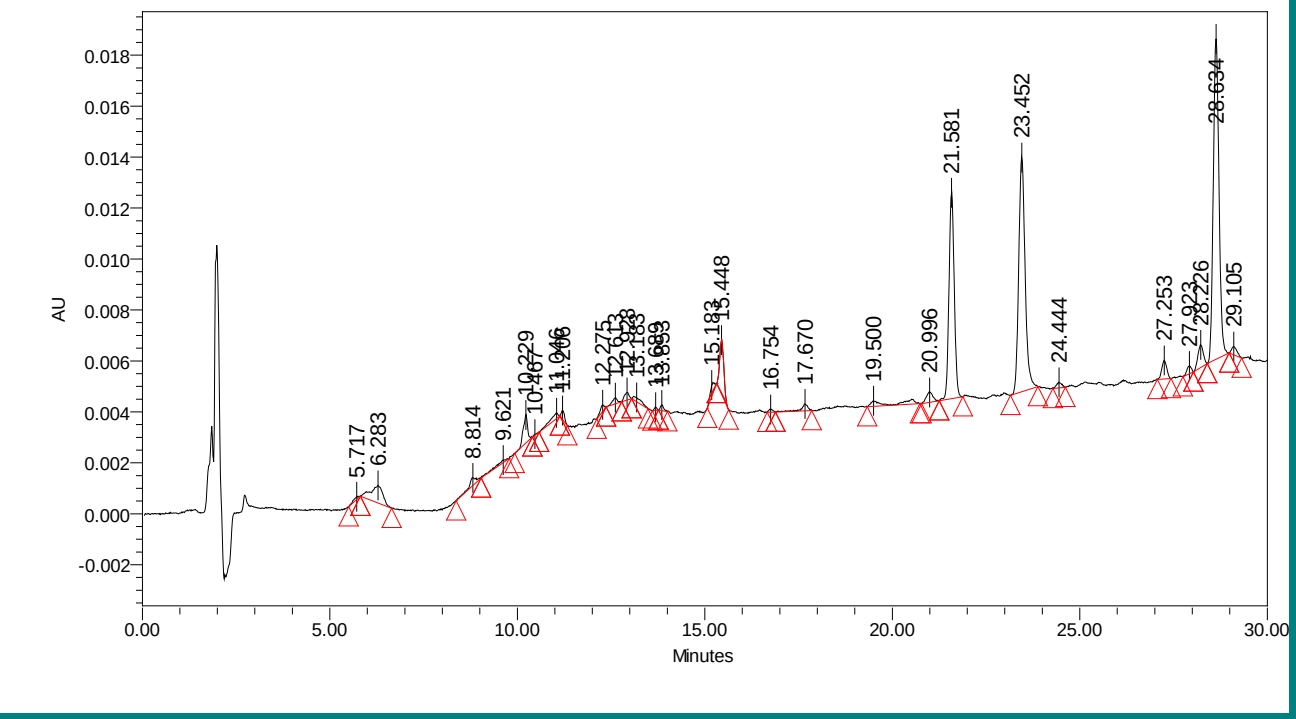
Summary of Percent Recovery

Publication	Antibody Support Gel	% Recovery			
		LR	RR	YR	LA
Tsutsumi et al. 2000	Affi-Gel	92	77	86	
Kondo et al 2001	Formyl Cellulofine	87	88	87	
Lawrence and Menard 2002	Sepharose CL-4B or Silica Based	94	78	86	89
Aranda-Rodriguez et al. 2003	Sepharose based	86	80*	87	89
	Silica-based	88	86*	84	75

* variant of RR

Evaluation of Abraxis Microcystin LR IAC

Water Fortified with Microcystin LR	
HPLC/PDA	LC/MS/MS
TOC (0 mg/L)	
Monoclonal - 81-101%	Monoclonal - 85-104%
Polyclonal - 101-102%	Polyclonal - 98-95%
TOC (0 mg/L)	
Monoclonal - 100-128%	Monoclonal - 80-85%
Polyclonal - 100-116%	Polyclonal - 25-100%



DI TOC 0

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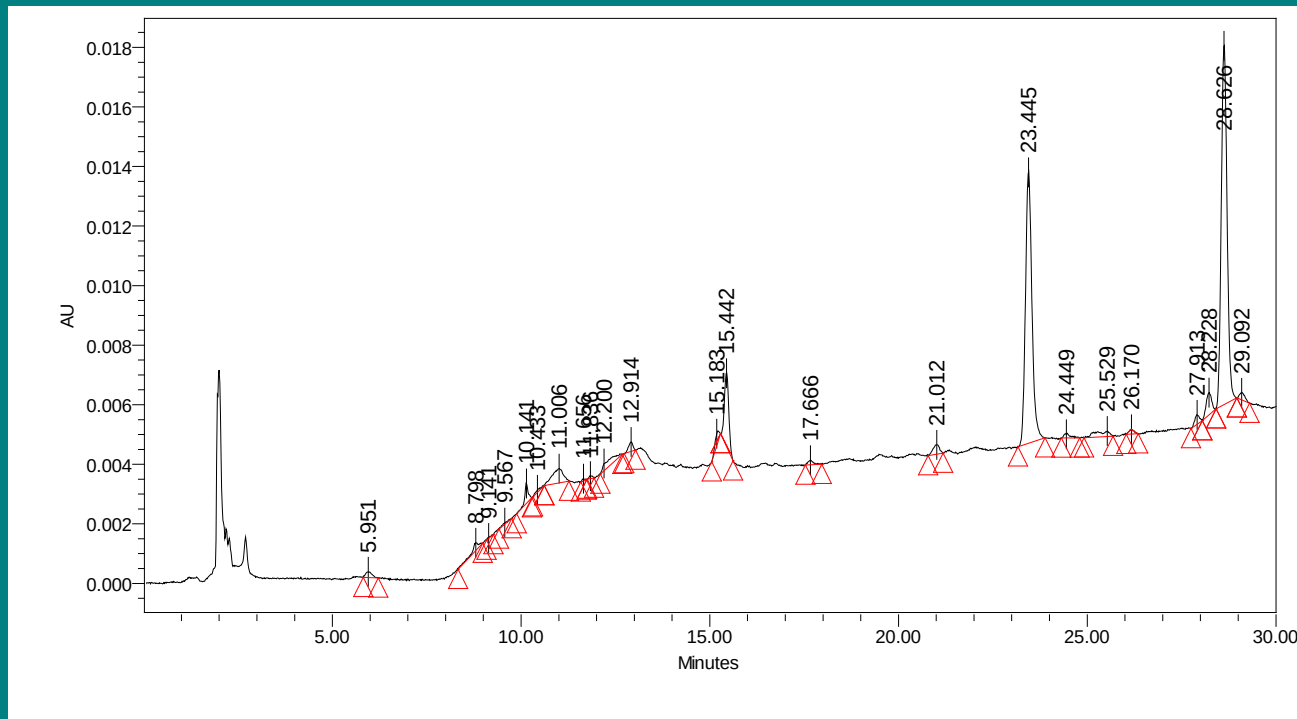
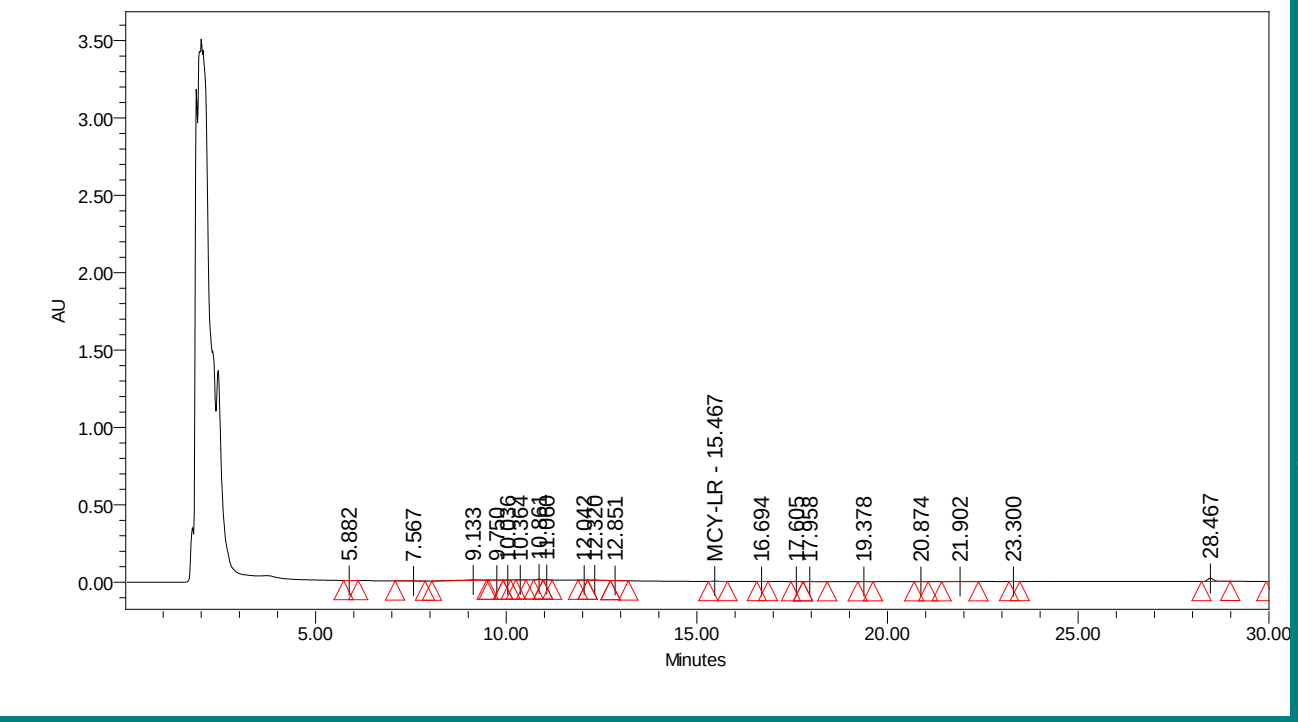


cont. Evaluation of Abraxis Microcystin LR IAC

Finished Low TOC Waters Fortified with Microcystin LR	
HPLC/PDA	LC/MS/MS
TOC 1.23 mg/L	
Monoclonal - 92-135%	Monoclonal - 27-277%
Polyclonal - 89-107%	Polyclonal - 94-122%
TOC 3.96 mg/L	
Monoclonal - 113- 146%	Monoclonal - 237-200%
Polyclonal - 76-95%	Polyclonal-375-412%



Florida TOC 3.96

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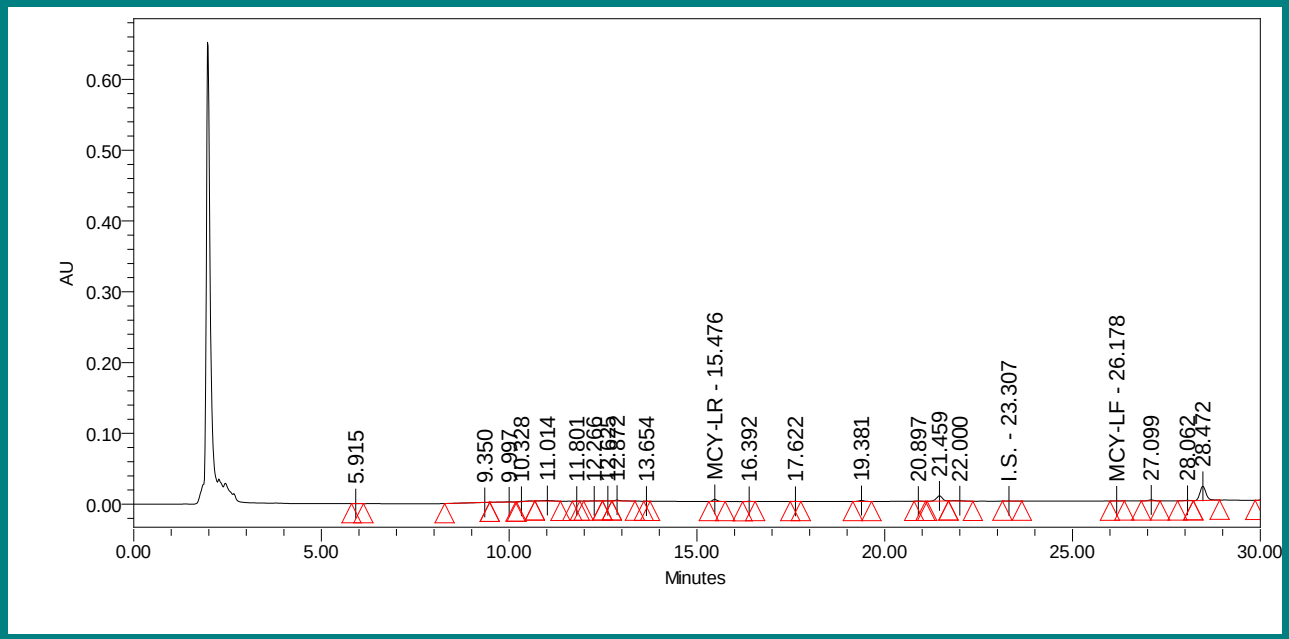

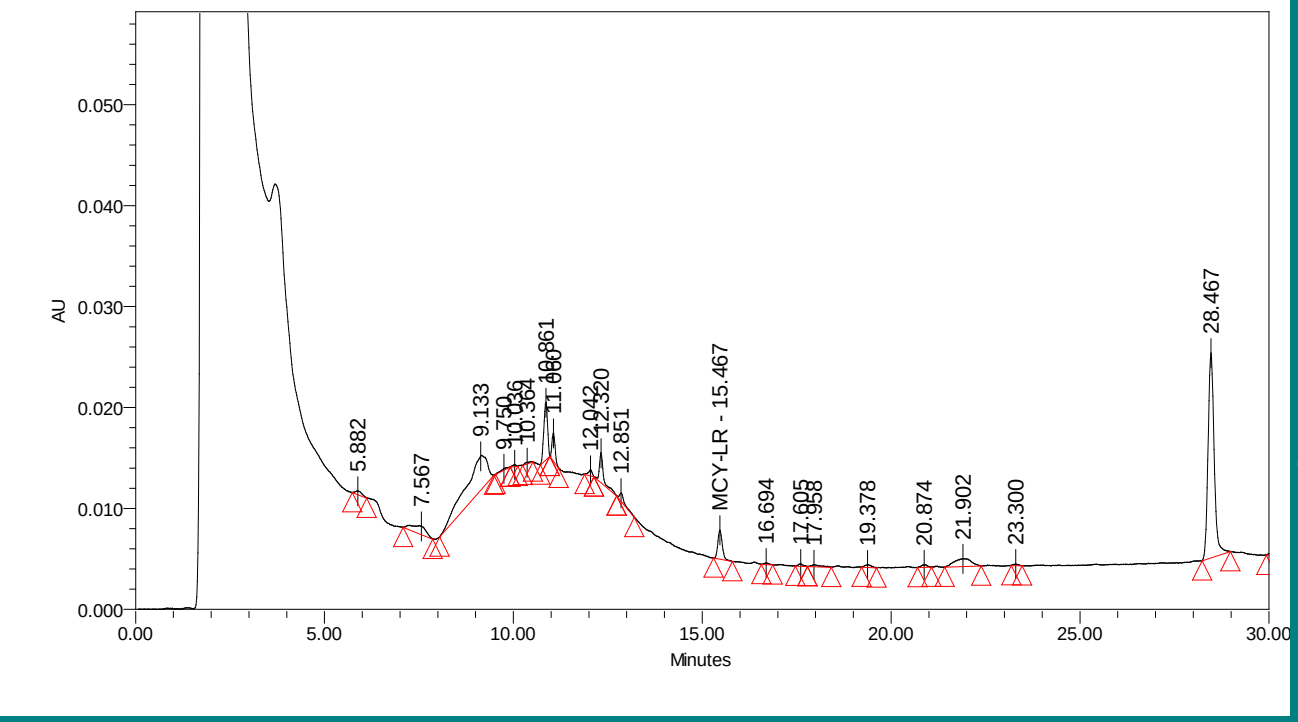

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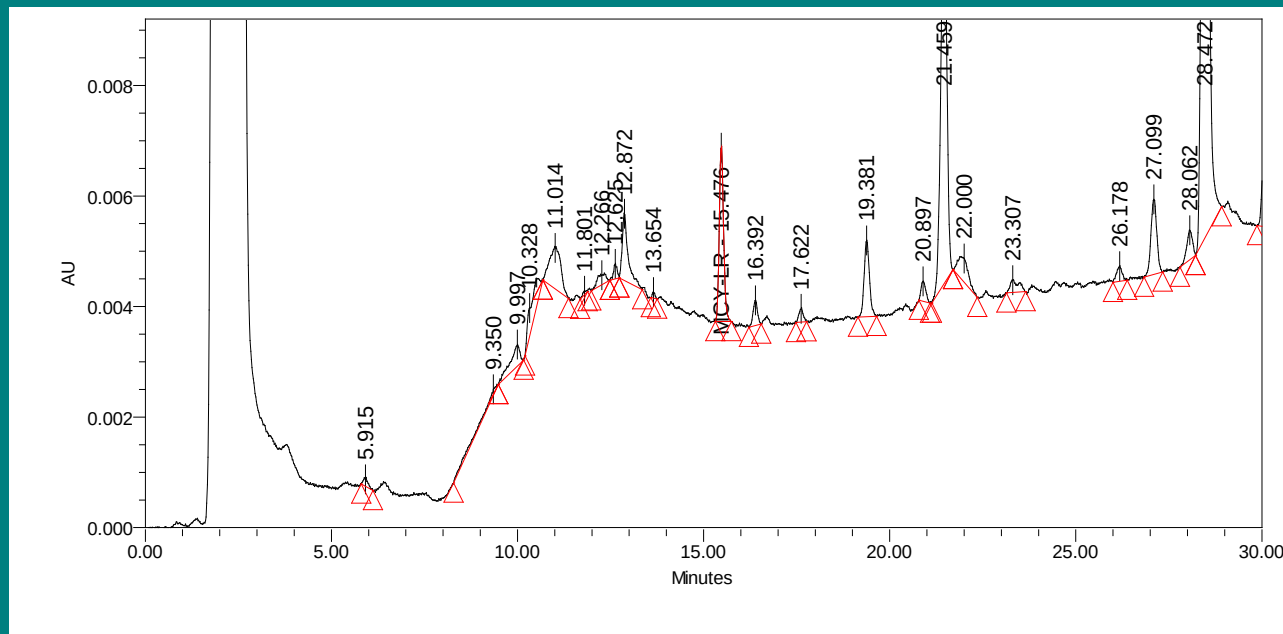
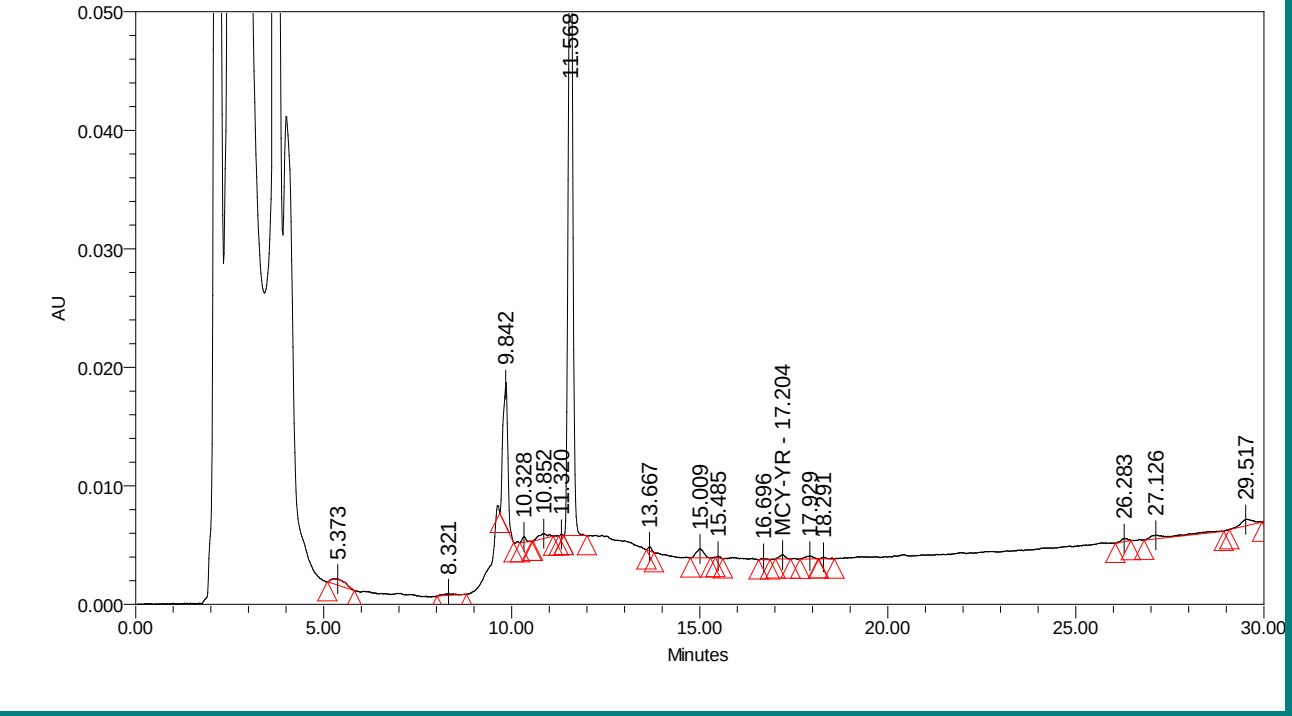


cont. Evaluation of Abraxis Microcystin LR IAC

Finished High TOC Waters fortified with Microcystin LR	
HPLC/PDA	LC/MS/MS
TOC (not reported)	
Monoclonal - 41-84%	Monoclonal - 7-10%
Polyclonal - 20-65%	Polyclonal - 7-26%
TOC 90 mg/L	
Monoclonal-34-85%	Monoclonal - 8-10%
Polyclonal - 42-81%	Polyclonal - 17-33%



Minnesota TOC 90

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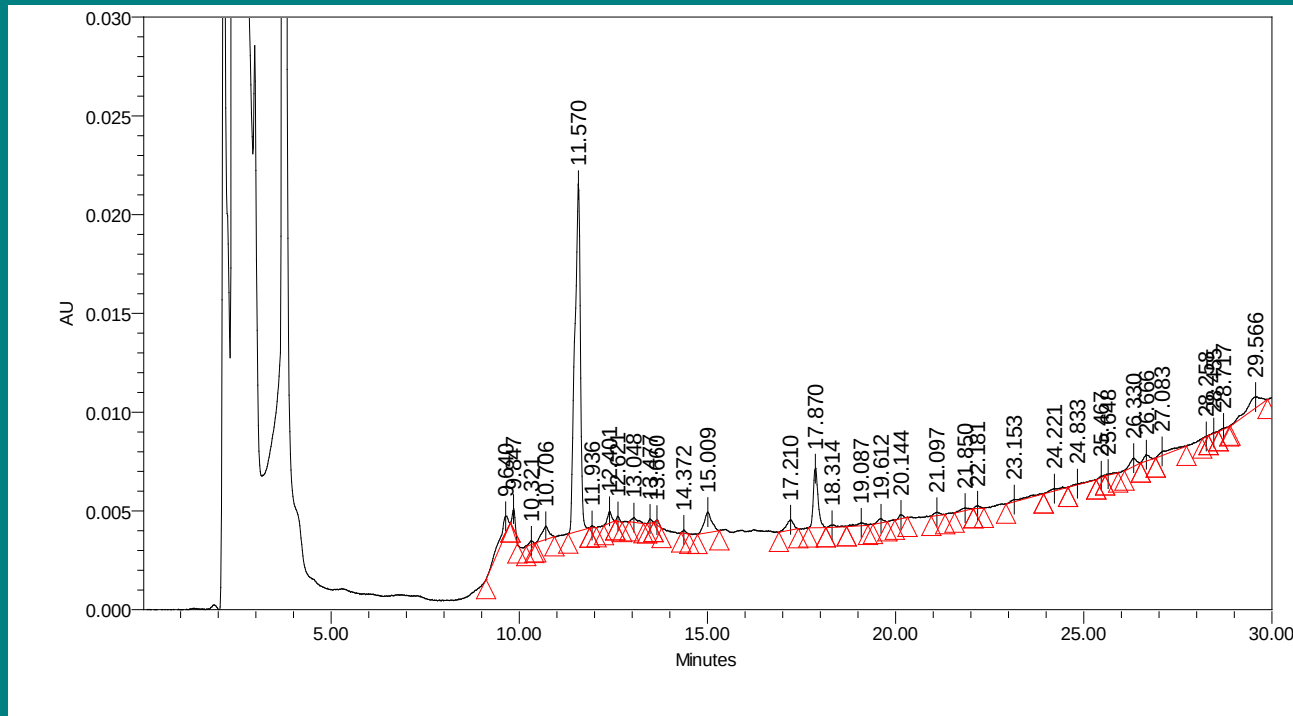

Outcomes

- The HPLC/PDA suggests microcystin IAC were very effective with DI water.
- The HPLC/PDA suggests microcystin IAC were effective for TOC <4.0 mg/L.
- The HPLC/PDA suggests microcystin IAC were not effective for TOC with 90 mg/L.
- The LC/MS/MS analyses on the samples were performed 9 months after samples were processed. Since they were stored in acidic method the LC/MS/MS results are not reliable. (Acidic methanol is believed to methylate and denature microcystin.)
- This study was inconclusive to whether the microcystin IAC has removed the TOC enough to reduce matrix effects for LC/MS/MS.

Recommendations

Repeat the IAC study with the following changes.

- LC/MS/MS analyses must be performed immediately.
- Perform a DI positive control as well as fortified samples.
- Finished water must include a reducing agent to remove excess oxidant.
- Water samples must include natural and synthetic waters with TOC concentrations above 5 mg/L.
- Review concentrating method.
- Rinse the column before elution.
- A discussion regarding the load and load capacity of the column.

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