

UPLC-TOF for fast screening and quantitation of microcystins in water and blue-green algae products.

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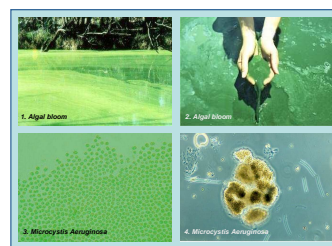
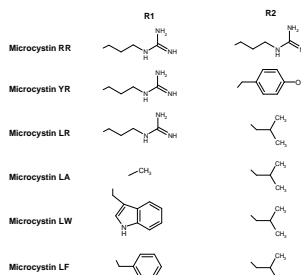
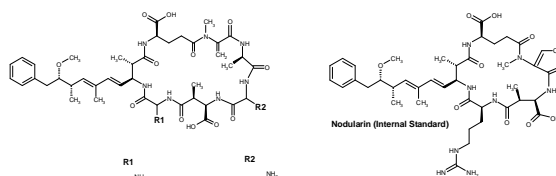
Swiss Centre for Applied Toxicology
www.scaht.org

Official Food Control Authority of Geneva
www.geneve.ch/consommation



Introduction

Cyanobacteria, commonly called "blue-green algae", are unicellular organisms often growing in colonies or filaments. Cyanobacteria may accumulate in surface water supplies as "blooms" and may concentrate on the surface as blue-green "scums" (picture 1 and 2). Algal bloom concentrations may reach millions of cells per milliliter. Colors observed are green, yellowish-brown, or red. Some species of cyanobacteria produce toxins, which are classified according to their mode of action into hepatotoxins, neurotoxins, skin irritants, and other toxins. One of the groups of toxins produced and released by cyanobacteria is called microcystin named from the species *Microcystis aeruginosa* (picture 3 and 4). The microcystins are a group of cyclic heptapeptide hepatotoxins and at least 50 congeners have been identified. Microcystins are of relevance to water supplies and to blue-green algae products sold as food supplement often in tablets, capsules or powder form. Considering a TDI of 0.04 µg/kg of body weight per day, the WHO has proposed a guideline value for total microcystin-LR (free plus cell-bound) of 1 µg/L in drinking water. For algae products, the Swiss federal office of public health proposed a maximum daily intake of 2 µg of microcystins for adults.



Analytical method

Sample preparation

Water samples : Water sample preparation need to include sonification to break up cells and release toxins. Sample are then centrifuge 5 min at 2000 g. Add 50 µl of Nodularin at 100 ng/ml (I.S.) to 5 ml of sample. Samples are filtered on LCR (PTFE) 0.45µm (Millex®) before direct injection.

Blue-Green algae : 3 g of algae powder is extract with 30 ml of MeOH/H₂O 75/25. Sample is placed in an ultrasonic bath for 15 min and then mechanically shaken another 15 min. Sample is centrifuged 10 min at 2000 g. 100 µl of supernatant and 50 µl of Nodularin at 100 ng/ml (I.S.) are placed in a 5 ml volumetric flask and completed with water. Sample is directly injected without any further purification.

UPLC

Column: Acquity UPLC BEH C18 1.7 µm, 100 x 2.1 mm
 Temperature : 30°C
 Run Time : 4 minutes
 Injection : 50 µl full loop
 Mobile phase A : H₂O + 0.1 % formic acid
 B : MeCN + 0.1 % formic acid

Gradient :

| Time [min] | Flow [ml/min] | A [%] | B [%] |
|------------|---------------|-------|-------|
| 0 | 0.35 | 95 | 5 |
| 3 | 0.5 | 30 | 70 |
| 3.1 | 0.7 | 0 | 100 |
| 3.9 | 0.7 | 0 | 100 |
| 4 | 0.35 | 95 | 5 |

TOF (LCT Premier XE)

Source : ES⁺, V mode, DRE extended
 Acquisition : Mass 400-1100 m/z, time 1.5 - 3.5 min
 Voltage : Cone 40 V, Aperture 5 V
 Capillary : 3000 V
 Desolvation : 380 °C
 Source temp. : 120 °C
 Cone gas flow : 20 L/Hr
 Desolvation gas : 750 L/Hr
 Reference scan : Leucine enkephalin 0.5 ng/µl, freq 25
 Detection : Mass window 0.02 Da, quantification ions as indicated in the table

| Substances | Retention time | Formula | MW | Quantification ions |
|------------------|----------------|--|--------|---|
| Microcystin RR | 2.13 | C ₂₄ H ₃₇ N ₇ O ₁₂ | 1038.2 | 519.7907 + 520.2924 + 520.7941 + 1038.5738 |
| Nodularin | 2.28 | C ₂₄ H ₃₅ N ₇ O ₁₂ | 825.0 | 825.4511 + 826.4544 + 827.4500 |
| Microcystin YR | 2.34 | C ₂₂ H ₃₁ N ₇ O ₁₂ | 1045.2 | 1045.5359 + 1046.5392 + 1047.5426 + 523.2718 + 523.7735 |
| Microcystin LR | 2.39 | C ₂₄ H ₃₇ N ₇ O ₁₂ | 995.2 | 995.5566 + 996.5599 + 997.5633 + 498.2822 + 498.7839 |
| Microcystin LA | 2.85 | C ₂₄ H ₃₇ N ₇ O ₁₂ | 910.1 | 910.4926 + 911.4960 + 912.4993 + 927.5040 + 928.5070 |
| # Microcystin LW | - | C ₂₄ H ₃₇ N ₇ O ₁₂ | 1025.2 | 1025.5348 + 1026.5382 + 1027.5415 + 513.271 + 513.7730 |
| # Microcystin LF | - | C ₂₄ H ₃₇ N ₇ O ₁₂ | 986.2 | 986.5239 + 987.5273 + 988.5306 + 493.7659 + 494.2675 |

Microcystin LW and LF are not commercially available. Quantifications ions were determined theoretically on molecular ions and ¹³C isotopes for single and double charged species.

Validation results for Blue-green algae samples (3 days, 3 levels and 4 replicates)

Microcystin RR

| Level | Accuracy | Fidelity | LOD : 0.1 µg/g |
|----------|----------|----------|----------------------------|
| 0.5 µg/g | 70 % | 20 % | CC _a : 1.2 µg/g |
| 1 µg/g | 78 % | 15 % | CC _B : 1.4 µg/g |
| 2 µg/g | 79 % | 10 % | |

Microcystin YR

| Level | Accuracy | Fidelity | LOD : 0.1 µg/g |
|----------|----------|----------|----------------------------|
| 0.5 µg/g | 86 % | 22 % | CC _a : 1.2 µg/g |
| 1 µg/g | 101 % | 16 % | CC _B : 1.6 µg/g |
| 2 µg/g | 102 % | 16 % | |

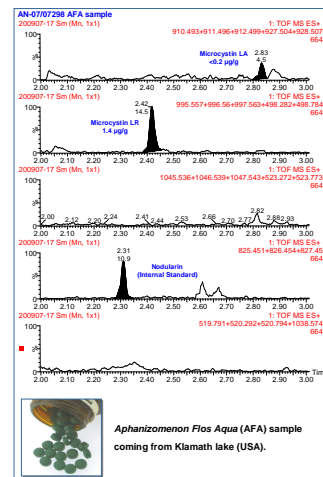
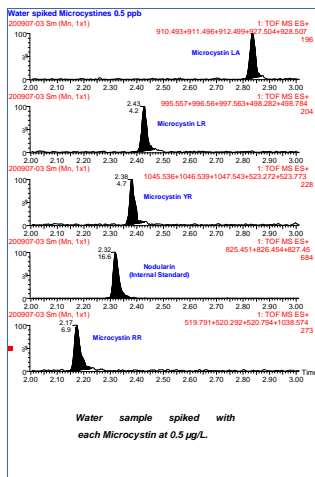
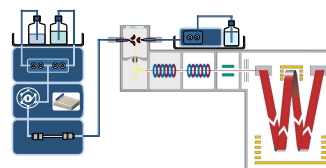
Microcystin LR

| Level | Accuracy | Fidelity | LOD : 0.1 µg/g |
|----------|----------|----------|----------------------------|
| 0.5 µg/g | 88 % | 12 % | CC _a : 1.2 µg/g |
| 1 µg/g | 102 % | 16 % | CC _B : 1.4 µg/g |
| 2 µg/g | 105 % | 11 % | |

Microcystin LA

| Level | Accuracy | Fidelity | LOD : 0.2 µg/g |
|----------|----------|----------|----------------------------|
| 0.5 µg/g | 89 % | 14 % | CC _a : 1.4 µg/g |
| 1 µg/g | 98 % | 14 % | CC _B : 2.1 µg/g |
| 2 µg/g | 98 % | 9 % | |

LOD : Limit of detection
 CC_a : The Decision Limit is the limit above which it can be decided that the identified analyte concentration is truly above the MRL.
 CC_B : The Detection Capability is the concentration of analyte, at which the method is able to detect MRL concentrations.



Food supplements analyses

A wide variety of food supplement containing microalgae can be found on the market. Among them, Spirulina (*athrospira platensis*) is probably the most common variety. Most of samples comes from culture and therefore, the risk of contamination of production with other toxic species such as *Microcystis Aeruginosa* is reduced. Among 10 samples of Spirulina found on the Swiss market, none has been found to contain microcystins above limits of detection. However, other algae products that are harvested in lake may be contaminated other algae species and with toxins. 9 samples of *Aphanizomenon Flos Aquae* (AFA) coming from Klamath lake (USA) were analysed and 6 of them showed the presence of microcystins LR and LA at harmful level. We found up to 4.0 µg/g of microcystins with a recommended intake of 2 g of algae per day. With such products, the TDI value (0.04 µg/kg of body weight) of WHO is largely exceeded.

