# MQOs[i] for Determination of Cyanotoxins[ii] in Water and Tissue Samples by Liquid Chromatography and Mass Spectroscopy

[ii] *MQOs developed specifically for determination of microcystins, anatoxin-a, and nodularin (toxins produced by cyanobacteria)*

**Table 1. Lab Quality Control for Microcystins, Anatoxin-a, and Nodularin in Water and Tissue Samples by Liquid Chromatography and Mass Spectroscopy**

| Lab Quality Control | Frequency of Analysis | Measurement Quality Objective | *DQ Indicator or Reasoning* |
|---|---|---|---|
| Tuning [1] (not reported to SWAMP database) | Per analytical method | Per procedures specified by instrument manufacturer (5) | Instrument setup |
| Calibration (not reported to SWAMP database) | Initial method setup and when calibration verification fails | • $r^2 > 0.990$ (for single standard) *OR* • $r^2 > 0.900$ (for 2 or more standards) • RSD ≤20% (9) • Minimum of 5 points per curve • At least 1 calibration point at or below the RL(5)) | Bias, Instrument QC |
| Calibration Verification (not reported to SWAMP database) | After every 20 environmental samples [2] or every 12 hours, whichever is more frequent | • Expected response or expected concentration ±20% | Bias, Continued Instrument QC |
| Laboratory Blank | Per 20 environmental samples or per analytical batch, whichever is more frequent | <MDL | Representativeness of analytical system |
| Filter Blank [3] (only required if filter is used for process) | Per 20 environmental samples or per analytical batch, whichever is more frequent | <Reporting Limit | Check of processing materials |
| Reference Material [4] (preferred) *OR* Laboratory Control Sample | Per 20 environmental samples or per analytical batch, whichever is more frequent | • For certified reference material recovery of 70-130% • For non-certified reference materials recovery of 50-150% | Lab accuracy |
| Reference Material Duplicate *OR* Laboratory Control Sample Duplicate | Per 20 environmental samples or per analytical batch, whichever is more frequent | RPD ≤25% of true value | Lab precision |
| Matrix Spike | Per 20 environmental samples or per analytical batch, whichever is more frequent | Recovery 50-150% of true value | Matrix accuracy |

[i] These MQOs have been developed for current SWAMP methodology. This does not limit the use of the MQOs for other laboratory methods. Please feel free to contact the OIMA Helpdesk (OIMA-Helpdesk@waterboards.ca.gov) to request assistance to adapt the MQOs for an additional laboratory method.

| Lab Quality Control | Frequency of Analysis | Measurement Quality Objective | *DQ Indicator or Reasoning* |
|---|---|---|---|
| Environmental Sample Duplicate *OR* Matrix Spike Duplicate [5] | Per 20 environmental samples or per analytical batch, whichever is more frequent | RPD ≤25% (water)<br>RPD ≤30% (tissue) | Matrix precision |
| Surrogate [6] | Per analytical method | Based on historical laboratory control limits (recovery of 50-150% or better) | Sample processing QC and process bias |
| Internal Standard [6] | Per analytical method | Per analytical method | Instrument QC (after extraction) |

[1] Applicable for mass spectroscopy instrument.

[2] The term "environmental samples" refers to the unknown samples; thus, quality control samples should not be included when calculating every 20 environmental samples.

[3] Anytime a filter is used in the processing of the sample, one filter should be analyzed as a filter blank sample.

[4] If reference material (RM) sample and RM sample duplicate is processed, then a laboratory control sample (LCS) and LCS duplicate is not required. Recommend preparing the RM or LCS from a second source or different manufacturing lot than was used to prepare the calibration standard. If a second source is unavailable, the RM or LCS should be prepared independently from the calibration standard (i.e. not a serial dilution from the stock solution used for calibration).

[5] Performance of the duplicate on the matrix spike is preferred when environmental samples have low or non-detections. If an environmental sample duplicate is used, result of original must be > RL.

[6] As appropriate compounds become available. Inclusion of surrogate and internal standard is not standard practice in currently available analytical methods. To ensure data of the highest quality, the use of internal standards and surrogates is preferred by SWAMP. These MQOs will be reviewed annually and may be revised pending further research and applicable method developments.

**Table 2. Lab Quality Control Corrective Actions for Microcystins, Anatoxin-a, and Nodularin in Water and Tissue Samples by Liquid Chromatography and Mass Spectroscopy**

| Lab Quality Control | Recommended Corrective Action [1] |
|---|---|
| Calibration | If calibration does not meet acceptance criteria, then recalibrate instrument prior to analyzing samples. |
| Calibration Verification | If verification fails, either recalibrate the instrument or verify with fresh calibration standard as a second attempt. If second attempt verification fails, recalibrate instrument. If further verification fails, maintenance instrument prior to analysis. (9) |
| Laboratory Blank | If value is ≥RL then all samples with detections are considered invalid. Investigate the source contamination. Prepare fresh samples and re-analyze analytical batch. |
| Filter Blank | If value is ≥RL then all samples with detections are considered invalid. Investigate the source contamination. Prepare fresh samples and re-analyze analytical batch. |
| Reference Material *OR* Laboratory Control Sample | Reanalyze the reference material or LCS to confirm the result. If reanalysis fails, prepare fresh samples and reanalyze the analytical batch.(6) |
| Reference Material Duplicate *OR* Laboratory Control Sample Duplicate | Reanalyze the sample to confirm result. Review the recovery obtained for the sample. Review the results of other quality control samples to determine if other analytical problems are the potential source. (6) |
| Matrix Spike | The concentration of the spike solution should be near the midrange of the calibration curve. Reanalyze the sample to confirm the result. Compare result to the recovery of the duplicate sample if possible. Review the results of other quality control samples to determine if other analytical problems are the potential source. (6) |

| Lab Quality Control | Recommended Corrective Action |
|---|---|
| Environmental Sample Duplicate<br>*OR*<br>Matrix Spike Duplicate | The concentration of the spike solution should be near the midrange of the calibration curve. Reanalyze the sample to confirm the result. Compare result to the recovery of the matrix spike sample. Review the results of other quality control samples to determine if other analytical problems are the potential source. (6) |
| Surrogate | Complete actions per analytical method. If applicable, include surrogate in all samples and all QC samples. If acceptance criteria are not met, reprocess samples. Consider behavior of surrogate compound during trouble shooting. If reprocessing of samples is not possible, stop analysis and flag batch. (6) |
| Internal Standard | Complete actions per analytical method. If acceptance criteria are not met, reprocess samples if possible. (6) |

[1] Documentation should be included when a MQO is not met and appropriate corrective actions are taken. Please include this documentation in the "LabBatchComments" field of the SWAMP data template or in a Corrective Action Report. The documentation should provide justification for excluding the record(s) from the lab batch **or** why the record(s) should be considered in the lab batch after corrective actions.

**Table 3. Field Quality Control for Microcystins, Anatoxin-a, and Nodularin in Water and Tissue Samples by Liquid Chromatography and Mass Spectroscopy**

| Field Quality Control | Frequency of Analysis | Measurement Quality Objective | *DQ Indicator or Reasoning* |
|---|---|---|---|
| Equipment Blank [1] | 5% of analytical batch or 5% of project samples, whichever is fewer | <Reporting Limit | Field Process Bias |
| Field Duplicate | 5% of analytical batch or 5% of project samples, whichever is fewer | RPD ≤25% | Sample Collection Precision |
| Filter Blank (only required if filtration done in field) | 5% of analytical batch or 5% of project samples, whichever is fewer | <Reporting Limit | Sample Process Bias |

[1] Equipment blank refers to preparing a sample bottle blank or sampling equipment blank. Sample bottle blank is only required if the sample bottle is re-cleaned from prior sampling. Sampling equipment blank is only required if a device (e.g. cup, pump) is used to transfer the environmental sample from water body to the sample container.

**Table 4. Field Quality Control Corrective Actions for Microcystins, Anatoxin-a, and Nodularin in Water and Tissue Samples by Liquid Chromatography and Mass Spectroscopy**

| Field Quality Control | Recommended Corrective Action |
|---|---|
| Equipment Blank | Investigate the source of contamination. The laboratory should report evidence of field contamination as soon as possible so corrective actions can be implemented. Samples collected in the presence of field contamination should be flagged. (6) |
| Field Duplicate | Visually inspect the sample to determine if a high RPD between results could be attributed to sample heterogeneity. For duplicate results due to matrix heterogeneity, or where ambient concentrations are below the reporting limit, qualify the results and document the heterogeneity. All failures should be communicated to the project coordinator for further actions. (6) |
| Filter Blank | Investigate the source of contamination. The laboratory should report evidence of field contamination as soon as possible so corrective actions can be implemented. Samples collected in the presence of field contamination should be flagged. (6) |

[1] Documentation should be included when a MQO is not met and appropriate corrective actions are taken in the field at the time of collection. Please include this documentation in the collection comments found on the field entry form or in a Corrective Action Report. The documentation should provide justification for excluding the record(s) from the data set **or** why the record(s) should be considered in the data set after corrective actions.

**Table 5. Sample Handling for Microcystins, Anatoxin-a, and Nodularin in Water and Tissue Samples**

| Matrix | Container [3,6] | Microcystin & Nodularin Temperature & Holding Time | Anatoxin-a Temperature & Holding Time [4] |
|---|---|---|---|
| Water *(for total toxin [1])* | Amber glass (recommended) or dark colored HDPE [3] | Cool to <6 °C (in dark) for up to 5 days, then freeze at <-20°C. [(2)] Long term storage of up to 6 months at -80°C. [(10,11)] | Cool to <6 °C (in dark) for up to 3 days, then freeze at <-20°C. [(2)] Long term storage of up to 6 months at -80°C. [(10,11)] |
| Water *(for dissolved phase or filtrate)* | Amber glass (recommended) or dark colored HDPE [3] | Cool to <6 °C (in dark) for up to 48 hours, then freeze at <-20°C. Long term storage of up to 6 months at -80°C. [(10,11)] | Cool to <6 °C (in dark) for up to 48 hours, then freeze at <-20°C. Long term storage of up to 6 months at -80°C. [(10,11)] |
| Water *(for particulate phase [2] or periphyton)* | Amber glass (recommended) or dark colored HDPE [3,5] | Cool to <6 °C (in dark) for up to 24 hours, then freeze at <-20°C. Long term storage of up to 6 months at -80°C. [(7)] | Cool to <6 °C (in dark) for up to 24 hours, then freeze at <-20°C. Long term storage of up to 6 months at -80°C. [(7)] |
| Tissue [7] *(for dissected tissue)* | Amber glass (recommended) or dark colored HDPE | Freeze short term at <-20°C. Long term storage of up to 6 months at -80°C. [(1,5)] | Freeze short term at <-20°C. Long term storage of up to 6 months at -80°C. [(1,5)] |

[1] Analysis of intracellular and extracellular cyanotoxins.
[2] Analysis of intracellular cyanotoxins.
[3] Glass containers recommended to prevent adsorption of toxin to plastic material. [(3, 8)]
[4] Limit holding time for anatoxin-a analysis to reduce toxin degradation.
[5] Filtering conducted in the field may utilize petri dishes as an alternative container to store filters.
[6] If amber or dark colored containers are not available, foil may be used to cover containers. Ensure foil completely covers container.
[7] Table 5 has been developed for analysis of muscle and organ tissue from fish and shellfish. This does not limit the use of the guidelines for other tissue types. Please feel free to contact the OIMA Helpdesk (OIMA-Helpdesk@waterboards.ca.gov) to request assistance to adapt the guidelines for an alternative tissue.

**References:**


(1) Al-Sammak, M. A, Hoagland K.D., Cassada, D., and Snow, D.D., 2014, Co-occurrence of the Cyanotoxins BMAA, DABA and Anatoxin-a in Nebraska Reservoirs, Fish, and Aquatic Plants, Toxins, Volume 6, 488-508.
(2) Graham, J.L., Loftin, K.A., Ziegler, A.C., and Meyer, M.T., 2008, Guidelines for design and sampling for cyanobacterial toxin and taste-and-odor studies in lakes and reservoirs: U.S. Geological Survey Scientific Investigations Report 2008–5038, 39 p. [Also at http://pubs.acs.org/doi/abs/10.1021/es1008938]
(3) Hyenstrand, P., Metcalf J.S., Beattie K.A., Codd G.A., 2001, Effects of adsorption to plastics and solvent conditions in the analysis of the cyanobacterial toxin microcystin-LR by high performance liquid chromatography, Water Research, Volume 35, Issue 14, 3508-3511.
(4) Mekebri, A., Blondina, G.J., Crane, D.B., 2009, Method validation of microcystins in water and tissue by enhanced liquid chromatography tandem mass spectrometry, Journal of Chromatography, Volume 1216, Issue 15, 3147-3155.
(5) Smith G.A., Pepich B.V., Munch, D.J. Method 536: Determination of Triazine Pesticides and their Degradates In Drinking Water by Liquid Chromatography Electrospray Ionization Tandem Mass Spectrometry (LC/ESI-MS/MS), US EPA Ohio: Office Of Ground Water and Drinking Water, Version 1, 2007.
(6) SWAMP. Water - Quality Control and Sample Handling Tables. Web. 10 December 2014.
(7) Szlag, D.C., Sinclair, J.L., et al., 2015, Cyanobacteria and Cyanotoxins Occurrence and Removal from Five High-Risk Conventional Treatment Drinking Water Plants, Toxins, Volume 7, 2198-2220.
(8) US EPA. "Detection." Nutrient Policy and Data. 1 October 2014. Web. 7 January 2015.
(9) US EPA. Forum on Environmental Measurements. Calibration Curves – Program Use/Needs. 2010.
(10) US EPA, Ohio. Total (Extracellular and Intracellular) Microcystins - ADDA by ELISA Analytical Methodology. Version 2, January 2015.
(11) US EPA, Region 9 Laboratory. SOP 1305 Microcystin by ELISA Analysis. Revision 4, November 2012.