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EUROPEAN UNION REFERENCE LABORATORY FOR MARINE BIOTOXINS

EU-Harmonised Standard Operating Procedure for determination of Lipophilic marine biotoxins in molluscs by LC-MS/MS: technical issues

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ISSUE	SUMMARY	COMMENTS

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Quantification	EU-RL LC-MS/MS method has been validated in an inter-laboratory study using the available tools when that validation was performed. Due to the absence of certified reference material for all lipophilic toxins regulated in the EU legislation, the approach of assuming equi-molar response among toxin with certified reference standard and toxins belonging to the same toxin group was used.	The assumption of an equal response factor for toxin quantification provided satisfactory results in the interlaboratory validation. However, direct quantification using the own compound is advisable when new reference standard materials are available.
Sample injection	EU-RL LC-MS/MS method has been validated by using duplicate injection.	When Single Lab Validation is performed, each laboratory must check within laboratory repeatability if single injection of sample is to be used.
Recovery correction	EU-RL LC-MS/MS method has been validated using non-corrected and corrected results.	When Single Lab Validation is performed, each laboratory must assess matrix effects on their instrument and determine if correction is necessary. Reference material or spiked extract can be used for toxin recovery or matrix correction if necessary. The approach used for each laboratory has to be perfectly proved through in-house validation experiments.
Identification	Identification of each toxin is performed by comparing the retention time of the analytes in the sample with those of the reference standards when available.	It is advisable using relative retention time for identification of those toxins for which there is no reference standard available.

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Retention time drift	Based on the EU-RL LC-MS/MS method, a retention time drift < 3% is allowed	However, if a RT drift of ≥2% is occurring then LC problems could exist.
Confirmation	No criteria for toxin confirmation has been included in the EU-RL LC-MS/MS method	Results obtained in the SLV performed by the EURLMB (Villar-González et al., J. of AOAC Inter., 94(3), 2011) have proved the usefulness of approach described in Commission Decision 2002/657/EC for confirmation purposes. In order to apply this criterion, the second ion/transition should be detected and present with a signal-to-noise ratio of ≥3:1 within all working range.
Hydrolysis	In order to quantify the total content of OA/DTX toxins an alkaline hydrolysis is required before LC-MS/MS analysis.	By weighting the tube or vials before and after heating, it should be checked if there was methanol evaporation during the process. If methanol evaporation is observed, the volume must be completed with methanol to the weight before continuing the process.