

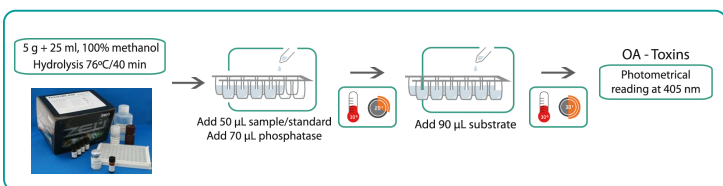
Background

Okadaic acid (OA) and its analogues, DTX1, DTX2, together with their ester forms are known as the OA-toxins group. These toxins cause Diarrhetic Shellfish Poisoning (DSP), which is characterised by symptoms such as diarrhoea, nausea, vomiting and abdominal pain. These symptoms may occur in humans shortly after consumption of contaminated bivalve molluscs such as mussels, clams, scallops or oysters.

Regulation (EC) No 853/2004 states that live bivalve molluscs for human consumption must not contain quantities that exceed 160 µg of OA equivalents per kg for the sum of OA, dinophysistoxins and pectenotoxins. Commission Regulation (EC) No 15/201 indicates that in the case of lipophilic toxins and, among them OA-toxins, liquid chromatography-mass spectrometry technique (LC-MS/MS) is the reference method. Other assays such as the phosphatase inhibition assay can be used for routine testing of official controls or any checks done by food operators providing they fulfil the method performance criteria stipulated by the EU-RL.

OkaTest is a colorimetric phosphatase inhibition assay for OA-toxins determination. The kit has been inter-laboratory (1) and intra-laboratory (2) validated and complies with the EU-RL requirements (3). Therefore, OkaTest can be used in combination with the reference method LC-MS/MS for monitoring of lipophilic toxins.

OkaTest - Colorimetric PP2A method



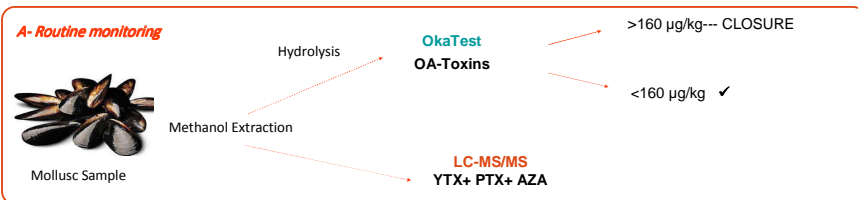
LC-MS/MS - Reference method



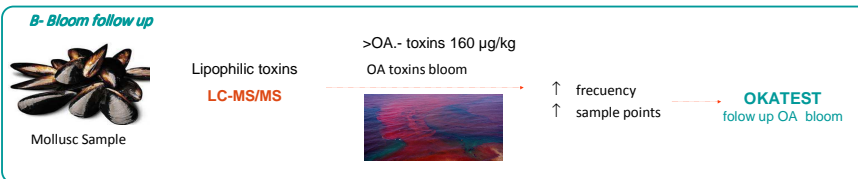
Lipophilic Toxins Monitoring by OkaTest + LC-MS/MS

OkaTest and LC-MS are both reliable analytical tools whose advantages can be combined to bring important benefits to the users. LC-MS is able to identify individual toxins from various groups of lipophilic toxins. However, it requires specialised personnel and large investment. OkaTest detects the potential sample toxicity, quantifying the OA-toxins by PPIA, and does not need either specific equipment or qualified staff. Laboratories with large number of samples or limited access to LC-MS equipment could benefit from using both methodologies in combination. A shorter time-around and low cost per sample could be achieved implementing both methods for routine monitoring of lipophilic toxins.

A- Routine monitoring: In labs with limited access to LC-MS/MS equipment (used for other analysis rather than marine toxins), analysis of lipophilic toxins using OkaTest would reduce the total analysis time and costs. LC-MS/MS requires 2 injections per sample. One of them to determine yexotoxins, azaspiracides, free OA and pectenotoxins and a second injection to identify the OA derivatives. The second injection could be diverted to OkaTest, as this kit is able to determine total OA-toxins and can analyse 43 samples in few hours.



B- Bloom follow up: OkaTest can be used in combination with LC-MS/MS once an OA-toxins bloom has been identified by LC-MS/MS. The number of samples and sampling points would increase in a toxic bloom. OkaTest can be used to quantify positive samples following up the bloom. The kit is able to test more samples than LC-MS per day, so a faster turnaround time will be achieved. LC-MS/MS could be used to confirm that the levels of toxins are below the legal limit.



Besides, in areas with historical okadaic acid blooms and at times when samples are likely to be positive, samples can be tested by OkaTest in first instance to reduce the waiting time. Negative samples to OkaTest would need LC-MS/MS testing for other lipophilic toxins.

	OkaTest	LC-MS/MS
Samples/Run*	86 samples/day	40 samples/day
No. Test/sample	2 replicates	4 replicates
Sample Extraction	1 extraction + 40 min hydrolysis	2 extractions + 40 min hydrolysis
Assay time*	2 min/sample	30 min/sample
Equipment costs	< 5.000€	> 200.000€
Estimated cost of analysis	< 30€/sample	> 100€/sample

*samples preparation not included

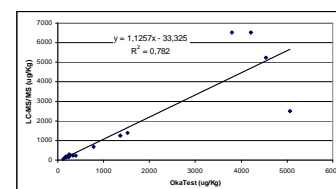
Benefits of OkaTest + LC-MS/MS

- Faster turnaround time: able to make faster decisions
- Lower cost: less investment, depreciation and maintenance costs
- LC-MS available for other analyses

Sample ID	LC-MS/MS	OkaTest
A1	280,2	247
A2	< 40	<LoQ
A3	35,5<40	<LoQ
A4	31,4<40	<LoQ
A5	<40	<LoQ
A6	18,6<40	<LoQ
A7	12,3<40	<LoQ
A8	691,8	777
A9	158,9	169

Sample ID	LC-MS/MS	OkaTest
B1	1256,7	1364
B2	175,6	180
B3	235,4	328
B4	1395,6	1525
B5	5234	4541
B6	2517	5057
B7	6506	3802
B8	6516	4215
B9	238,1	392

Sample ID	LC-MS/MS	OkaTest
C1	24,1<40	<LoQ
C2	32,8<40	<LoQ
C3	182,4	220
C4	197,9	232
C5	179,4	241
C6	152,5	166
C7	69,5	117
C8	246,2	265
C9	<40	<LoQ



Results from twenty seven samples analysed by LC-MS and OkaTest were compared. Samples were fully extracted and analysed according to the reference LC-MS/MS. In case of OkaTest, the methanolic extract was obtained following the LC-MS method and then hydrolysed according to the OkaTest assay procedure. Most results obtained by both methods showed good correlation ($R^2 = 0.991$ up to 1400 µg/Kg). Samples with concentrations higher than 1400 µg/kg showed bigger differences ($R^2 = 0.782$). Nevertheless, a comparison of results from both methods showed no statistical significant differences ($P > 0.05$) and discrepancies at high levels do not pose any health hazard.

References & Acknowledgments

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- Smiernik H., Domínguez E., Rodríguez-Velasco M.L. Clarke D., Katrin K., Katikou P., Cabado A.G., Otero A., Vieties J.M. Razquin P., and Mata L. *Quantitative Determination of the Okadaic Acid Toxins Group by a Colorimetric Phosphatase Inhibition Assay: Interlaboratory Study.* *Journal AOAC*, 2013. 96,1,77-85
- EU-RL website: http://www.aesan.msp.es/en/CRLMB/web/otros_procedimientos/other_crlmb_standard_operating_procedures.shtml

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