A COMPARISON OF THE RESULTS OBTAINED FOR THE DIRECT QUANTIFICATION OF GTX6 AND C3,4 TOXINS AND AFTER HYDROLYSIS

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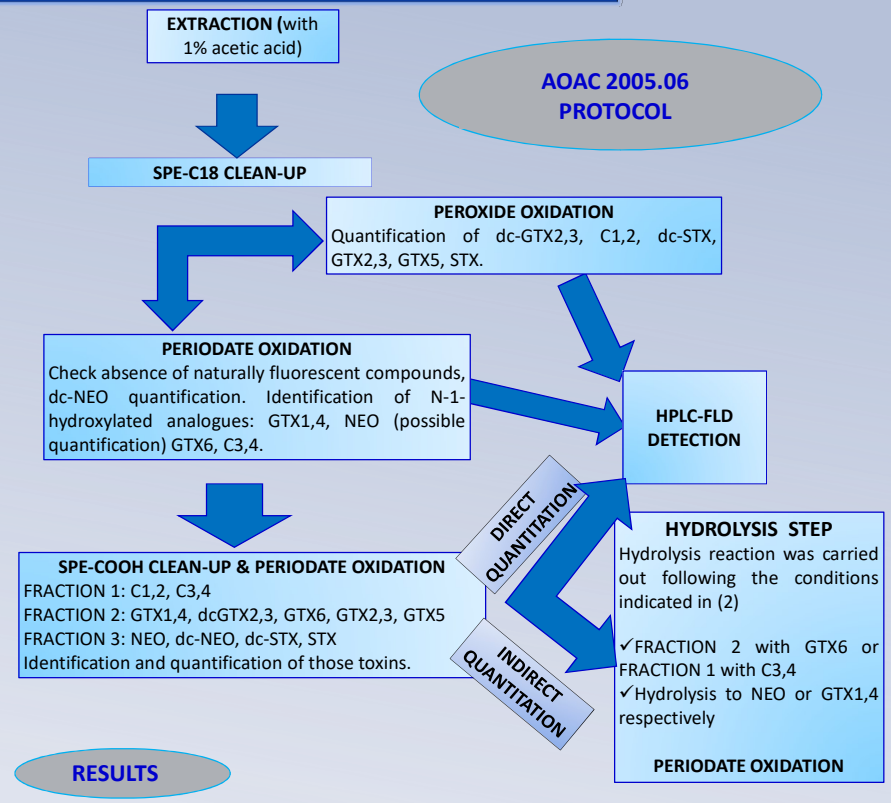
SUMMARY

Gymnodinium catenatum is often primarily responsible of Paralytic Shellfish Poisoning (PSP) episodes worldwide. This dinoflagellate exhibits an extraordinary variation in PST profiles (STX and at least 21 analogues) but consistent regional patterns are evident with regard to the production of C1,2, C3,4, GTX5, GTX6 and NEO analogues. PSP profiles, containing most or even all of the following toxins: dc-GTX2,3, dc-STX, dc-NEO, GTX5, GTX6, C1,2 and C3,4, were found in contaminated samples from toxic events in the Atlantic Coast of Spain (Galicia) and Portugal. Some of these samples were analyzed at the EURLMB by pre-column oxidation HPLC with Fluorescence detection (AOAC 2005.06 OMA).

Although the toxicity of C-toxins, GTX5 and GTX6 seems to be low, their presence in shellfish at high levels, could have a significant contribution to the total sample toxicity, as it was found in previous studies carried out at the EURLMB, in which GTX6 contributed up to 21% of the total sample toxicity. Moreover C3,4 were also detected in several samples, although the lack of standards for these toxins did not allow their quantification. Results obtained in previous studies conducted at the EURLMB, highlighted the importance of quantifying all the PSP toxins involved in the contamination to avoid the underestimation of the PSP toxicity in the samples. As it is well known, the lack of standards for the quantification of GTX6 and C3,4 could be overcome by hydrolyzing these toxins to convert them into NEO and GTX1,4, respectively, with the possibility of indirect quantification.

The results obtained for the quantification of GTX6 and C3,4 after hydrolysis and by direct quantification, using non certified reference materials, are presented and discussed in this work.



RESULTS

Toxin	Sample Code	Direct Quantitation µmol eq STX×diHCl/100g	Indirect Quantitation (Hydrolysis) µmol eq STX×diHCl/100g	Statistical significance*
GTX6	CRL/06/P/08	0,06 (±0,0009)	0,05 (±0,001)	Yes
	CRL/07/HYD/TEST	0,13 (±0,001)	0,10 (±0,003)	Yes
C3,4	CRL/06/P/08	0,027 (±0,001)	0,027 (±0,001)	No
	CRL/07/HYD/TEST	0,046 (±0,004)	0,043 (±0,005)	No

Table 1. Concentrations obtained for GTX6 and C3,4 toxins in shellfish samples by two different quantification methods. Mean of n= 4 (± SD).* t-test: Paired two sample for means

C3,4 AND GTX6 PRELIMINARY VALIDATION DATA

As part of the EURLMB intralaboratory AOAC 2005.06 validation the following parameters were evaluated:

- **Linear range:** evaluated with 8 level calibration curve (each standard prepared in triplicate): **0.01-1 µM** for both toxins.
- **LOD:** S/N=3: **GTX6: 0.014 µM, C3,4: 0.001 µM.**
- **LOQ (theoretical):** S/N=10: **GTX6: 0.004 µM, C3,4: 0.033 µM.**
- **Precision, repeatability conditions: CV_{GTX6} ≤ 2.8 %, $CV_{C3,4}$ ≤ 10.7 %.**

C3.4 STANDARD HYDROLYSIS

0.25 µM C3,4 standard was hydrolyzed to GTX1,4 and quantified with a calibration curve prepared with NRC-CRM-GTX1,4. The C3,4 standard concentration was checked with a calibration curve prepared with NRC-RM-C3,4.

Although C3,4 concentrations obtained by the two methods are the same, we can not guarantee the complete conversion of C3,4 into GTX1,4 since the ratios GTX1,4 second/third peak found in the GTX1,4 standard and in the C3,4 hydrolyzed standard are not the same.

GTX6 HYDROLYSIS EFFICIENCY

The efficiency of GTX6 hydrolysis had already been evaluated in the PSP Hydrolysis study of the EURLMB (2007) where GTX6 toxin was transformed into NEO after hydrolysis with a high yield

CONCLUSIONS

- ❖ Good agreement was obtained for the results obtained by the direct and indirect quantification of C3,4 .
- ❖ Similar results were obtained for GTX6 quantification using both methods.
- ❖ Quantification of C3,4 and GTX6 was achieved after hydrolysis, thus the amount of these toxins in contaminated shellfish samples is possible when standards are not available.
- ❖ C3,4 and GTX6 preliminary validation data were obtained. A complete validation of these toxins would require increased amount of C3,4 and GTX6 standards. This highlights the importance of the availability of these materials.
- ❖ Further studies are needed to evaluate the efficiency of C3,4 hydrolysis.

REFERENCES

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