Determination of anatoxin-a in environmental water samples by solid-phase microextraction and gas chromatography - mass spectrometry

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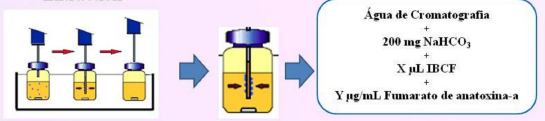
Instruction

The present work was similarly tested by Rodríguez et al, 2006 and summarizes the development of an automated method combining Solid phase microextraction (SPME) with Gas Chromatography coupled with Mass Spectrometry (GC-MS) for the fast and sensitive determination of Anatoxin-a in water samples [1]. This method is based on the direct derivatization of the analyte by adding isobutylchoroformate (IBCF) to the sample extract in alkaline conditions. The derivatized anatoxin-a was extracted by SPME procedure, submersing a PDMS fiber in an amber vial for 20 min under magnetic stirring. GC-MS is used to identify and quantify the analyte in the SIM mode (quantification ions underlined): 191,164 and 265. Parameters affected to the extraction such as: salt concentration, time of extraction, time of reaction and stirring speed, were initially evaluated. The calibration curve showed linearity in the range of 30 – 150 ng/mL and the LOD was 10 ng/ml. The results obtained in this study let us to conclude that this method is an efficient and fast alternative for the reliable control of anatoxin-a present in contaminated waters.

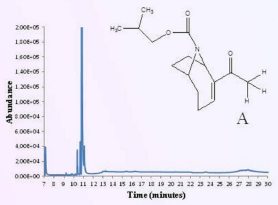
Experimental

Fig.1- SPME- GC/MS System



GC/MS Sistem	HP 6890 Series GC-5973 ms
Capillary column	30m× 0.25mm×0.25 µm d.i. HP-5MS
Oven temperature program	80°C → 275°C (15°C/min)
Injection	5min, splitless; 250 ºC
Ionization	Electronic impact (70eV)
Injector Temperature	250ºC
SIM selected ion mode	m/z 265,209, 164, 150, 136,122

Fig.2- SPME- GC/MS Conditions



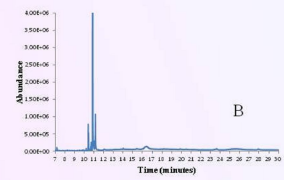
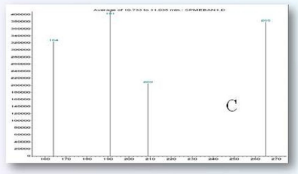


Fig.3- SIM (A) and SCAN (B) analysis of anatoxin-a derivatized with IBCF (100 µg/mL anatoxin-a fumarate).



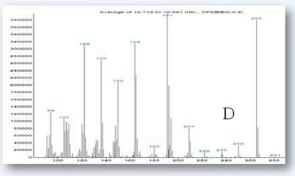
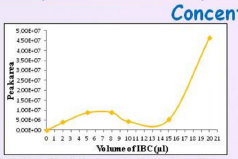


Fig.4- Mass spectrum of anatoxin-a derivatized with IBCF: C) SIM mode, D) SCAN

Optimization of parameters affecting the SPME

Concentration of IBCF



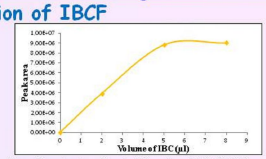


Fig.6 - Effect of IBCF concentration in the efficiency of extraction of anatoxin-a (1µg/mL) using a PDMS 100 µm fiber.

➢The results of the capability of hexylchloroformate to derivatize anatoxin-a in seven different concentrations (1053, 2633, 4212, 5265, 7898, 10530 µg/mL) were checked;

➢ In the figure 6b can see that the best efficiency of derivatization was obtained with the concentration of 2633 µg/mL;

➢ For concentrations higher than 2633 µg/mL the response is unstable, associated with increased interaction of IBCF with the fiber PDMS;

➢ It was also observed that the larger the quantity of derivatization reagent used the shorter was the fiber lifetime. This probably happened because the fiber coating could be attacked by chlorinated molecules of the IBCF.

Salt concentration

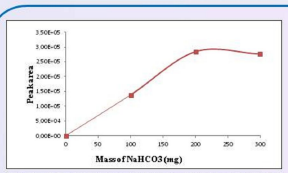


Fig.5 - Effect of amount of sodium bicarbonate in the efficiency of extraction of anatoxin-a (1µg/mL) derivatized with IBCF using a PDMS 100 µm fiber.

➢The 200 mg of $NaHCO_3$ was the amount they that provide a better extraction efficiency. The extraction profile obtained in this experiment was the expected, since it was observed the saturation of the solution when is tested 300 mg of salt, which would hinder the contact of anatoxin-a with the PDMS fiber.

Time of reaction

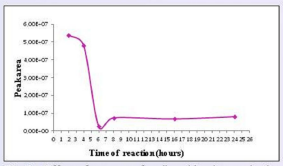


Fig.7 - Effect of amount of sodium bicarbonate in the efficiency of extraction of anatoxin-a (1µg/mL) derivatized with IBCF using a PDMS 100 µm fiber.

➢The equilibrium of the fiber is only reached after 8hours reaction;

➢Although the peak obtained after 2h reaction be of high intensity, its purity is low;

➢The analyte is not extracted only in derivatized form, and also the dispersion of the peaks is much higher peaks in the chromatogram with only 2 hours of reaction.

Time of extraction

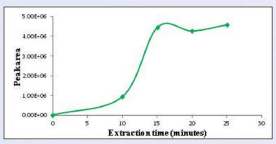


Fig.8 - Effect of the extraction time in adsoption of anatoxin-a (1µg/mL) derivatized with IBCF using a PDMS 100 µm fiber.

➢ Twenty minutes of extraction time was chosen as appropriate time of extraction as it was equivalent to run a GC chromatogram and samples could be analyzed continuously.

➢Although the LOD/LOQ could be lightly improved by increasing the time of extraction, our objective was to develop a simple and relatively fast method.

Validation of the method SPME-GC/MS

Linearity

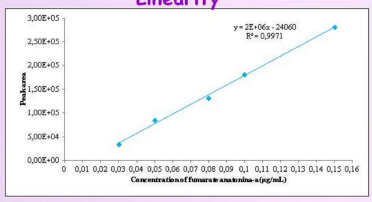


Fig.9- Calibration curve for the determination of anatoxin-a in water for SPME-GC/MS. Concentration range from 30 to 150 ng / mL.

➢ The calibration curve was linear over the specified range (30–150 ng/mL). The linear regression and correlation coefficient were:

$Y = 2E+06x - 24060$, $r^2 = 0.997$

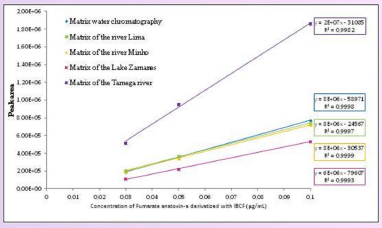
Accuracy of the method

Concentration(µg/mL)	Standard deviation	% RDS	σt (n=4, 95%)
0.03	5920.7	18.4	32167.5 ± 18827.8
0.05	7586.5	10.2	74332,0 ±24125.1
0.1	16956.9	13.4	126933,0 ±53922.9

→ Repeatability

These values were considered satisfactory as the complete method variability was determined including derivatization efficiency, SPME extraction, and chromatographic analysis. To evaluate the reproducibility the RSD (%) were calculated for each level: low (15.6%), medium (6.9%), and high (12.9%).

Evaluation of matrix effect



➢ There is no significant matrix effect, except when is considerate the sample with contaminated water (Microcystins) from Tamega River;

➢ The slope obtained for the different lines do not vary considerably, so we present a very sensitive method for detection of anatoxin-a derivatized with a IBCF SPME-GC/MS.

Conclusions

1. A simple and practical method was proposed for the determination of anatoxin-a in water samples by SPME-GC/MS;
2. The validation procedure confirmed that this method was reliable for the analysis of this important toxin and appropriate for the quality control of water;
3. Up to today there is no guideline value for the concentration of anatoxin-a that may be present in water, however 1ug/ml provide an margin of safety [2].

References

[1] Rodriguez, V., et al., Determination of anatoxin-*a in environmental water* samples by solid-phase microextraction and gas chromatography-mass spectrometry, J. Sep. Sci. 2006, 29, 2085 – 2090.

[2] Fawell,J. K., et al., The toxicity of cyanobacterial toxins in the mouse: II Anatoxin-a, Human & Experimental Toxicology, 1999, 18, 168-173.