Optimized SPE for the detection of the cyanotoxin Cylindrospermopsin from cultured cyanobacteria.

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Introduction

The problem of cyanobacterial toxins in Brazilian water reservoirs is widely known and a current public health issue. [1] The accident occurred in 1996 at the Institute of Kidney Diseases in Caruaru (PE), transformed the history and the clinical practice of hemodialysis. In this case, the water used for hemodialysis was contaminated with cyanotoxins, causing the death of at least 65 patients. [2] This incident made it clear that studies of harmful cyanobacteria and their secondary metabolites is a relevant topic worldwide.

Cylindrospermopsin (CYN) is a hepatotoxin produced by some species of freshwater cyanobacteria (*Cylindrospermopsis spp., Anabaena spp., Aphanizomenon ovalisporum, Umezakia natans, among others),* some of them present in almost all latitudes of the world. [1]

For this experiment 4,5 L of a toxic *Cylindrospermopsin sp*. culture were centrifuged. Since CYN and its less toxic analog 7-deoxy-cylindrospermopsin (7-deoxy-CYN) are highly polar and secreted by the cells, only the medium (supernatant) was used on the experiments. Four types of solid phase extraction (SPE) cartridges were tested using samples of 10 mL of centrifuged culture medium. We recovered the fraction after the sample passage through the cartridge as well as the eluate. As a mean of comparison of the total amount of the analytes in the sample, 10 mL of culture medium were lyophilized. All the collected fractions and also the lyophilized medium were evaporated with N_2 and resuspended in Milli-Q H_2O. These extracts were analyzed by HPLC-DAD (Shimadzu LC20AD) using a C18 (5µm, 250x3mm) column.

Results and Discussion

The SPE's absorption capacity for our analytes is compared on figure 1.

After application of the sample, elution of both CYN and 7-deoxy-CYN was carried out using 1,5 mL of pure methanol (MeOH). Diferential elution of CYN and 7-deoxy-CYN were optimal at 10% MeOH-H_2O and 50% MeOH-H_2O respectively.

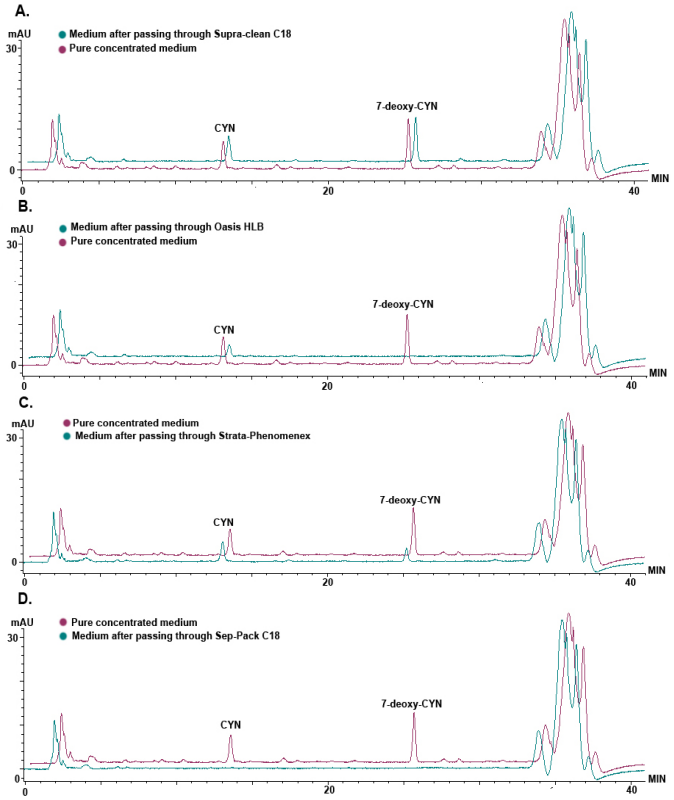

Figure 1. Overlay of the chromatograms of 10 mL of pure concentrated culture medium and the recovered culture medium after the application of 10mL on: A) Supra-clean® C18 Perkin Elmer, B) Oasis® HLB Waters, C) Strata® C18 Phenomenex and D) Sep-Pack® C18 Oasis. (n=3).

Conclusions

These results show that a specific brand of C18 SPE cartridges (Sep-Pack®) yielded the best absorption for CYN and 7-deoxy-CYN from cyanobacterial cell culture. Interestingly enough, the capacity of the SPE column to retain the analytes varies among different brands of C18 columns.

Aknowledgements

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