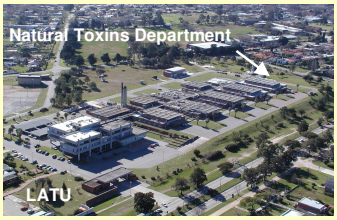

TECHNOLOGICAL LABORATORY OF URUGUAY

DETERMINATION OF MICROCYSTIN-LR IN DRINKING WATER AND SURFACE FRESHWATERS

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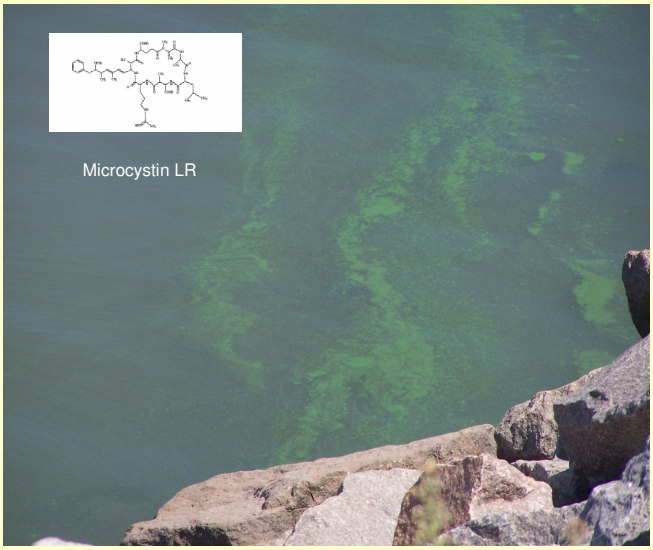
ABSTRACT

Blue-green algae, correctly known as cyanobacteria, are well known for their ability to produce potent toxins called cyanotoxins. One group of them are classified as hepatotoxins incluiding the microcystins. Uruguay had not available a reference analitycal method to determine microcystins.
The objective of this work was to validate a method for determining total microcystin LR and quantifying it in samples of drinking water and surface freshwaters.
86 samples (river and drinking water) were collected between October 2005- 2006.
Analysis were performed following a method based on Lawton et al., 1994.
The performance parameters evaluated were those suggested in the AOAC Peer-Verified Methods Program. Recovery percentage was determined at 3 levels 0,5, 1, 2 µg/L using internal material. Mean value was 76, 73 and 69 % respectively. Detection limit and quantification limit was 0,03 and 0,04 µg/L respectively. Calibration curve covered the range from 0,03 to 1 µg/L, with a linear correlation coefficient of 0.999827.
Precision study showed acceptable results according to Horwitz equation.
Positive samples ranged from 0,130 to 2900 µg/L.
Results obtained confirmed the cause of death in some animals, showing the importance for the country to have implemented a reference method. It was accreditated following ISO17025 by United Kingdom Accreditation Service (UKAS).

Keywords: cyanobacteria, cyanotoxins, hepatotoxins, microcystin LR, detection limit , quantification limit, calibration , precision, Horwitz equation.



INTRODUCTION

The increasing prevalence of algal blooms in freshwaters arising through increasing eutrophication is placing greater pressures on the uses of water for both drinking and recreational purposes. Blue-green algae, more correctly known as cyanobacteria, are well known for their ability to produce potent toxins which have been responsible for numerous animal deaths (Schwimmer and Schwimmer, 1968; Carmichael *et al*., 1985; Beasley *et al*., 1989; Kuiper-Goodman *et al*., 1999). Cyanobacteria produce several toxins, including the neurotoxins, (anatoxins and saxitoxins), hepatotoxins, cylindrospermopsin and lipopolysaccharide (LPS) endotoxins (Carmichael and Falconer, 1993; Carmichael, 1997).
The hepatotoxins are cyclic peptides with the most frequently encountered compounds being the microcystins, cyclic heptapeptides produced most commonly by *Microcystis aeruginosa* (Sivonen and Jones,1999).
Currently there are more than 60 variants of microcystin which have been characterised (Rinehart *et al*., 1994:Sivonen and Jones, 1999). Of these 60 compounds, microcystin-LR would appear to be the microcystin most commonly found in cyanobacteria. It is also common for more than one microcystin to be found in a particular strain of cyanobacterium (Namikoshi *et al*., 1992; Lawton *et al*., 1995). The microcystin variants may also differ in toxicity (Carmichael, 1992).
The literature indicates that hepatotoxic blooms of *M. aeruginosa* containing microcystins occur commonly worldwide (Sivonen and Jones, 1999).
In Uruguay some cases of animal death were reported and the toxicity of the water detected by biological analysis.
Up to 2005 Uruguay had not available a reference analitycal method to determine microcystins.

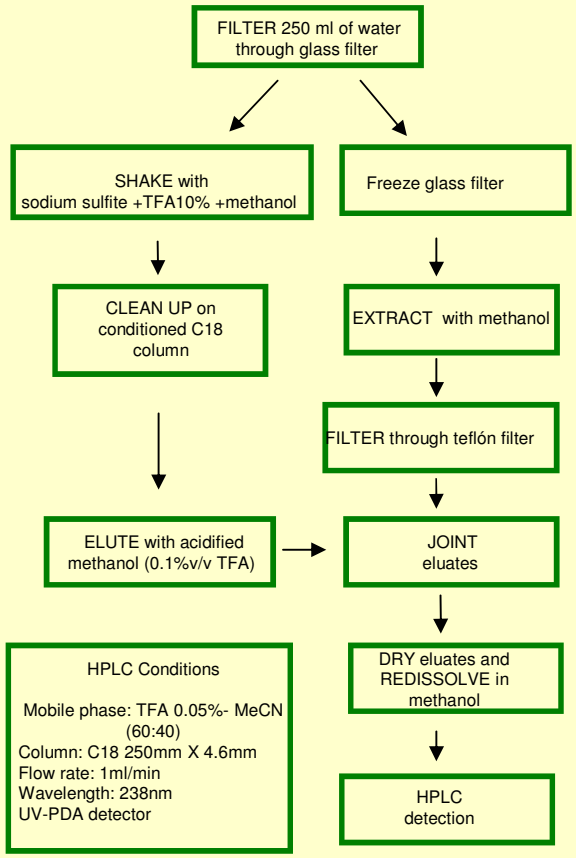
OBJECTIVE

The objective of this work was to validate and accredite a method for determining total microcystin LR and quantifying it in samples of drinking water and surface freshwaters.

RESULTS

Calibration Curve	Area=1,76e+003ng +(2,35e+002)	
Linearity	R=0,999827	
Range	0,03 µ/L to 1 µg/L	
Detection limit	0,03 µg/L	
Quantitation limit	0,04µg/L	
Recovery percentage	Mid-level (0,5µg/L) Specification level (1µg/L) High level (2µg/L)	76% 73 % 69 %
Repeatability	Mid-level (0,5µg/L) Specification level (1µg/L)	RSD= 21% RSD=23%
Reproducibility	Mid-level (0,5µg/L)	RSD=8.5%

EXPERIMENTAL



CONCLUSIONS

Following the AOAC Peer-Verified Methods Program, the results obtained for performance parameters were satisfactory .
The need of reference materials is detected as a priority.
Samples analysed confirmed the cause of death in some animals, showing the importance for the country to have implemented a reference method.
This method was accreditated following ISO17025 by United Kingdom Accreditation Service (UKAS).