Kakvoća mora za kupanje na području Opatije od 2001. do 2016.

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SEA BATHING WATER QUALITY IN OPATIJA AREA 2001 – 2016

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original scientific paper

Summary

Sea bathing water quality is one of the key elements of touristic offer of coastal destinations. During the almost three decades of this programme conduct on the Croatian Adriatic coast, legal requirements have changed, along with statistical elaboration of the data as well as the evaluation criteria. This study elaborates the results of the microbiological analysis according to the actual Directive (Official Gazette No 73/2008). This has enabled the actual picture of seawater quality in Opatija area during the longer time scale. This study presents the quality of seawater at the beaches of the Opatija area (18 locations) during the 16-year period (2001-2016). The highest microbiological pollutions were detected at the locations of the Hotel Kristal and Slatina beach and the lowest at the location of Puntica beach. Moreover, a case of 2013 seawater pollution in the area of Slatina beach was described: a multi-institutional commitment in resolving of this issue, carrying out the additional off-season monitoring 2013/2014 (325 samples), the measures of remediation as well as the results of the implemented measures. The results of the routine monitoring showed that overall quality of the seawater in Opatija area during the observed period was the lowest in the years 2001, 2002 and 2013, when the first notice of pollution of the Slatina beach was registered. After the first Wastewater treatment device in Ičići was put into operation (2012) and the repair of damaged pressurized pipeline was finished (2013), the seawater quality has received excellent grade at all Opatija's locations.

Keywords: bathing water quality, microbiological parameters, water pollution, remedial measures, supplemental program

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METHOD VALIDATION OF MICROCYSTIN-LR IN WATER

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review

Summary

The worldwide occurrence of cyanobacterial blooms due to water eutrophication evokes extreme concerns. These blooms produce cyanotoxins which are hazardous to living organisms. Microcystin-LR (MC-LR) is the most studied and frequently encountered toxin produced by the cyanobacteria in the contaminated water. Microcystin-LR is a hepatotoxic for animals and humans, and the International Agency for Research on Cancer has classified MC-LR as a possible tumor promotion. A liquid chromatography-electrospray ionization tandem mass spectrometry (LC-ESI–MS/MS) method has been developed and validated to identify and quantify trace levels of MC-LR in lake water. The method was validated according to SANTE/11945/2015. The spiked water samples passed through the 45 μm regenerated cellulose filter membranes, and the water sample was concentrated by SPE (solid phase extraction) on Oasis HLB. The eluate was evaporated to near dryness, then resolved in mobile phase and analyzed by LC-MS/MS.The calibration curve of MC-LR was linear within the range of 0.01-0.2 μg/mL. The R^2 was 0.9956. The average recovery (0.1; 0.2 and 0.5 μg/L) was 86.0±7.95%. The LOQ was 0.1 μg/L. The obtained method provides a very high sensitivity, good reproducibility, appropriate linearity and can be applied with a high reliability to the analysis of the MC-LR content in lake water samples.

Keywords: water, MC-LR, validation, LC-MS/MS

Introduction

Nutrient rich, eutrophic, warm and low turbulent conditions in freshwater bodies typically promote the dominance of cyanobacteria within phytoplankton communities. The excessive proliferation of cyanobacteria leads to blooms that disrupt ecosystems, adversely affect the taste and odour of water. Owing to the high surface water temperature and a persistent water column stratification, cyanobacterial water blooms have, in recent years, occurred in eutrophic lakes, rivers and reservoirs worldwide (Cong et al., 2006). There are 234 identified microcystins (MCs) (Meriluoto et al., 2017), but the most commonly analysed are MC-RR, LR, LW and LF. Many of the common cyanobacterial species (called "blue-green algae") produce toxic metabolites, during bloom events (Vuković et al., 2017) and they are

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unicellular organisms often growing in colonies or filaments (Vuković et al., 2014). Approximately 50-70% of cyanobacterial blooms have been proved to be acutely toxic since some of the cyanobacteria species had the ability to produce toxins. MCs are common toxins produced mainly by cyanobacteria belonging to the genera *Microcystis, Anabaena, Planktothrix,* and *Nostoc*. To date, nearly 80 variants of MCs have been reported in cyanobacteria in natural or laboratory cultivated waters (Li et al., 2011).
Many of the common cyanobacterial species produce toxic metabolites which can be lethal to wildlife, domestic livestock and humans (Mekerbi et al., 2009). Moreno et al. (2005) pointed out that the toxins produced by cyanobacterial bloom can be transferred from cyanobacterial cells to different fish tissues. MCs can induce hepatic diseases and liver cancer. Human deaths from drinking water polluted by MCs were reported by various countries since 1878 (Xu et al., 2008).
Microcystin-LR is one of the most prevalent and potent microcystins (Li et al., 2011), it is designated as possibly carcinogenic to humans by the International Agency for Research on Cancer (IARC). The variable residues of MC-LR are L-arganine and L-leucine (Fig. 1). The World Health Organization guideline recommended limitation of MC-LR to 1 µg/L in drinking water.

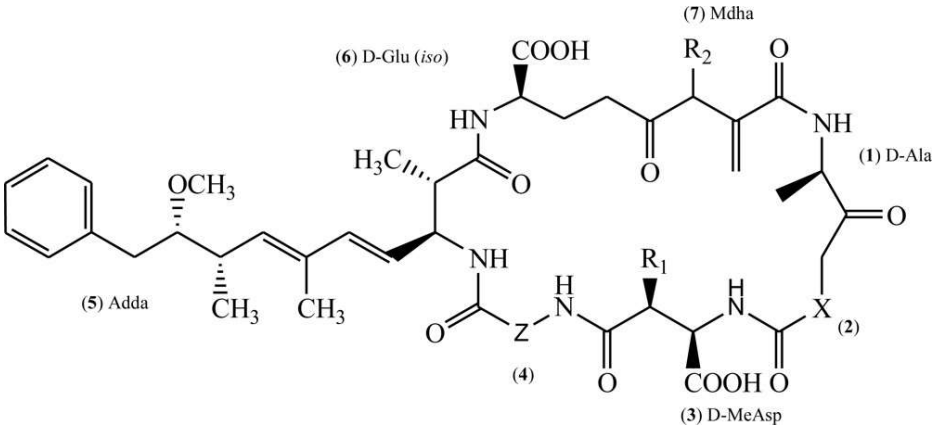

Fig.1. General structure of microcystin: MC-LR X represents l-Leucine; Z l-Arginine; R_1 and R_2 CH_3 (Campos and Vasconcelos, 2010)

Waterblooms of cyanobacteria and their cyanotoxins are also common in the majority of surface waters in Serbia (Natić et al., 2012), so the need for a rapid, sensitive, and reliable analytical method for determination of trace amounts of microcystin-LR in water was the main objective of this paper. In that manner, the validation of liquid chromatography electrospray ionization tandem triple quadrupole mass spectrometry (LC-ESI-MS/MS) method with the multiple reaction monitoring mode (MRM) for the determination of MC-LR in lake water was done.

Materials and methods

Analytical standards The MC-LR and nodularin (internal standard) were purchased from Sigma (St. Louis, MO, USA) in the concentration of 10 μg/mL in methanol.Stock standard solution of MC-LR was prepared in methanol, and stored at -20 ºC. At the same conditions, the nodularin in the concentration of 0.05 μg/mL, was stored. Methanol (for HPLC, Ultra Gradient Grade), was purchased from J.T.Backer (United Stated). Formic acid (98/100%, laboratory reagent grade) was from Fischer Scientific (Loughborough, UK). Pure water was obtained from Purelab® ELGA water purification system (Vivendi Water Systems Ltd UK). Glass microfiber filters (GF/A) were from Whatman, Cat. No.6880-2504 (Maidstone, UK). Econofilters regenerated cellulose (0.45 μm) were from Agilent, Germany and OASIS HLB from Waters.

Instrumentation and chromatographic conditions for LC-MS/MS LC was performed with an Agilent 1200 HPLC system equipped with a G1379B degasser, a G1312B binary pump, a G1367D autosampler and a G1316B column oven (Agilent Technologies, USA). Chromatography separation was achieved by Zorbax Ecllipse XDB C18 column (50 x 4.6 mm, 1.8 μm) (Agilent, USA) maintained at 30 °C. The analytical separation was performed using methanol as mobile phase A, and water as mobile phase B, both containing 0.1% formic acid with gradient mode (0 min: 60% B, 10 min: 5% B, 15min: 5% B, stop time: 17 min, post time: 5 min. The flow rate was maintained at 0.5 mL/min. The mass analysis was carried out with an Agilent 6410B Triple Quadrupole mass spectrometer equipped with multi-mode ion source (MMI, Agilent Technologies, Palo Alto, CA, USA). The data acquisition and quantification was conducted using MassHunter Workstation software B.04.01 (Agilent Technologies 2010). The following ionization conditions were used: electrospray ionisation (+ESI) positive ion mode, drying gas (nitrogen) temperature 325 °C, vaporizer 220 °C, drying gas flow rate 5 L/min, nebulizer pressure 40 psi and capillary voltage 2500 V. The dwell time was 100 ms.

Validation parameters

The method was validated according to SANTE/11945/2015.
The limit of detection (LOD) was determined as the lowest concentration giving a response of three times the average baseline. The ratio signal/noise in the obtained chromatograms for the LOD was calculated by MassHunter Qualitative Software.
The linearity was checked at the concentrations of 0.01, 0.025, 0.05, 0.1 and 0.2 μg/mL.
The recovery was checked, by enriching of a blank sample (lake water with no MC-LR detection) with the working standard of MC-LR, at the concentration levels of 0.1, 0.2 and 0.5 μg/mL (in five replicates).

The precision of the method in terms of repeatability (% RSD_r) (intra-day precision) and reproducibility (% RSD_R) (inter-day precision) was investigated by the spiked samples analysed in five replicates on the same and different days.

MC-LR extraction

The spiked water samples were filtered through the 45 μm regenerated cellulose filter membranes, the water sample was concentrated by SPE (solid phase extraction) on Oasis HLB. The first step was the column conditioning with 5 mL of MeOH, then loading 100 mL of sample, drying the cartridges during 5 min with vacuum, MC-LR elution with 1 mL of MeOH (twice), evaporation and reconstitution in 0.5 mL of mobile phase. After that, the extract is ready for the LC-MS/MS analyses.

Results and discussion

For the quantification the ion with the best signal sensitivity (Q) was preferred and for the confirmation the second transition (q) and the ratio of abundances between both ion transitions (Q/q) was used. The cone voltages were selected according to the sensitivity of the precursor ions and the collision energies were chosen to give the maximum intensity of the fragment ions obtained. The product-ion spectra obtained on triple quadrupole instrument generally provide fragments which are of diagnostic value for structural elucidation and confirmation.
The MRM transitions (*m/z*) for MC-LR and nodularin were given in the Table 1.

Table 1. Acquisition data

Compound Name	Precursor Ion		Product Ion	Dwell	Frag (V)	CE (V)	Polarity
MC-LR	996.2	→	213.2	100	135	70	Positive
MC-LR	996.2	→	135.2	100	170	90	Positive
Nodularin	825.5	→	135	100	100	70	Positive
Nodularin	825.5	→	103.1	100	80	100	Positive

Method validation data

The calibration curve, in the range of 0.01 to 0.2 μg/mL for MC-LR, was linear in the studied working range with the correlation coefficients higher than 0.99 ($y=6.828833E-004x^2+0.417885x-0.060655$, $R^2=0.9956$) (Fig. 2).

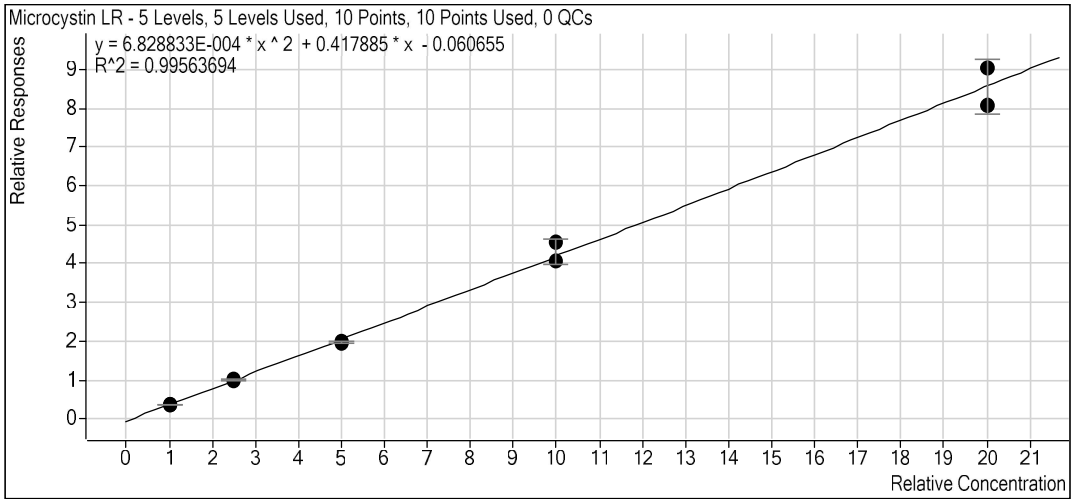

Fig. 2. MC-LR calibration curve

The average recoveries of MC-LR spiked on three levels (0.1, 0.2 and 0.5 µg/L) were 86.0% with obtained RSD value of 7.95% (Table 2).

Table 2. Recovery

Spiking levels (µg/L)	Recovery (%)	RSD (%)
0.02	88.4	14.44
0.04	78.8	2.41
0.1	90.9	6.99

The precision of the method in terms of repeatability (RSD_r) and reproducibility (RSD_R) was evaluated calculating the relative standard deviation (%RSD) of spiked samples at three levels in five replicates on the same and different days. The good accuracy and precision results were obtained in intra-day and inter-day analysis. The LOD was calculated by MassHunter Software, for those concentrations that provide a signal to noise ratio of 3:1. The obtained LOD value for MC-LR was 0.03 µg/L. The limits of quantifications (LOQs) were set and experimentally confirmed at level of 0.1 µg/L. This limit is well below the established World Health Organization guideline recommended for the limitation concerning MC-LR of 1µg/L in drinking water.

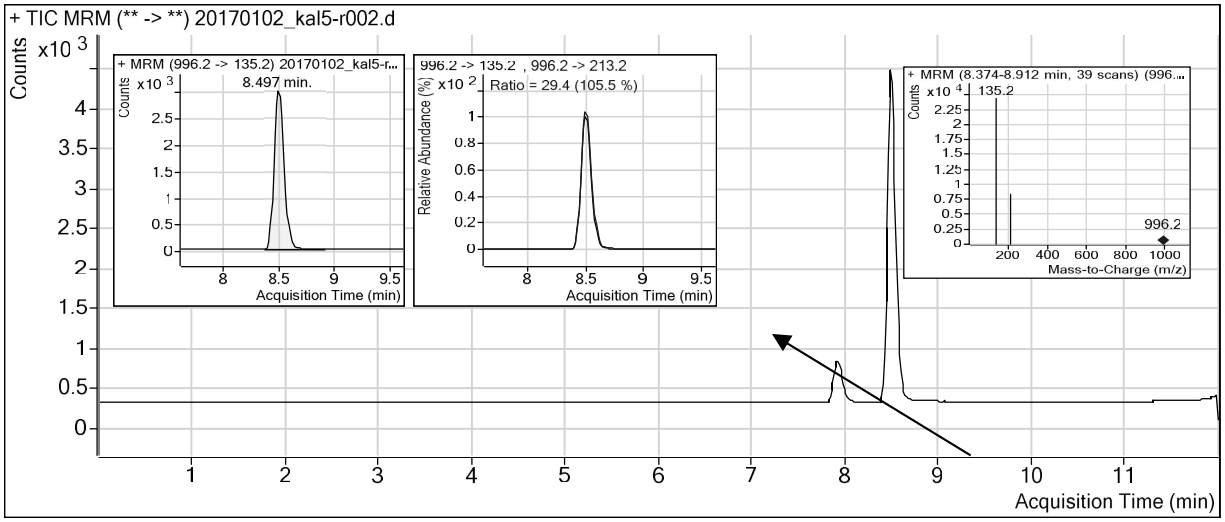

Fig. 3. TIC chromatogram of MC-LR with MRM chromatograms at the concentration level of 0.02 µg/mL

The method occurance was checked out through PT-LGC AQUACHECK PT Scheme, Round 519 with z score of 0.31.

Conclusions

The validation of the LC-MS/MS method using OASIS HLB for the extraction of MC-LR has been successfully performed in accordance with SANTE 11945/2015. The method provides a very high sensitivity, good reproducibility, appropriate linearity and can be applied with a high reliability to the analysis of the MC-LR content in lake water samples.
The basic validated parameters were obtained: good linearity with R^2>0.99 within the calibration ranged from 0.01-0.2 µg/mL; the average recovery (0.1, 0.2 and 0.5 µg/kg) was 86.0±7.95%. The good accuracy and precision results were also obtained in intra-day and inter-day analysis. The LOQ was 0.1 µg/L.
The method occurance was checked out through PT-LGC AQUACHECK PT Scheme, Round 519 with z score of 0.31.

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TECHNOLOGIES FOR REMOVAL OF UV FILTERS

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review

Summary

UV filters are compounds used in cosmetics and in materials such as plastics, adhesives, rubber for prevention of damage due to sunlight irradiation etc. These compounds are considered to be emerging contaminants, due to their presence in the environment and the fact that the risks associated with their presence are still insufficiently and inadequately explored. As a result of widespread use of UV filters, the majority of these compounds ends up in wastewater, where they are usually not removed or degraded in wastewater treatment plants (WWTPs). The consequence could be contamination of natural water resources, i.e. rivers, lakes and oceans. The removal of UV filters in WWTPs greatly depends on the implemented technology. In this paper, the removal technologies for two UV filters, benzophenone-3 ((2-Hydroxy-4-methoxyphenyl)-phenylmethanone) and octocrylene (2-ethylhexyl 2-cyano-3,3-diphenylprop-2-enoate) were studied. These two compounds were selected for research because benzophenone-3 is considered to be more hydrophilic and octocrylene more lipophilic. The best technology for removal of both of these compounds is reported to be membrane filtration, especially reverse osmosis.

Keywords: UV filters, octocrylene, benzophenone-3, wastewater

Introduction

UV filters are ultraviolet-absorbing or reflecting compounds, which are used in personalcare products and some materials for protection from sunlight irradiation. The increased use of these compounds is the result of the growing concern about negative consequences of exposure to sunlight. This intensification of use has awakened the attention on the fate and behaviour of such substances in the environment, especially in aquatic ecosystems, due to the worldwide contamination from widespread use in human activities. Also, some of the UV filter compounds can accumulate in biota and act as endocrine disruptors (Hernández-Leal et al., 2011; Li et al., 2015).

Due to the fact that very little data on the fate of these contaminants is available, it is of great interest to further research the possibility of decreasing the amounts or removing UV filters from the environment.

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