Ionic Matrices: Improving the Microcystin Quantitative Analysis by MALDI-TOF-MS.

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Keywords*: MALDI-TOF-MS, ionic matrices, quantification, cyanobateria, microcystin-RR.*

Abstract

In this work, fluidic ionic matrices were used to improve the precision of microcystin-RR (MC-RR) quantitative analysis. Analytical curve was constructed by using the matrix that provided the lowest coefficient of variation (CV) value. Low limits of detection (LOD) and quantitation (LOQ) were obtained.

Introduction

Microcystins are cyclic peptides of great relevance produced by some cyanobacteria genus. These highly toxic metabolites can cause fatal incidents to humans and animals.[1]

Matrix assisted laser desorption/ionization mass spectrometry has been successfully applied to the quantitative analysis of these compounds.[1] However, usual organic crystalline matrices provide a non-homogeneous distribution of the analyte over the sample plate spot, increasing the CV value and, therefore, decreasing the precision of replicate analysis.[2]

Ionic matrices are organic salts, generally prepared by reacting acidic organic matrices with amines. Some of them are fluidic and can be used to reduce the effect of the sample inhomogeneity over the CV.[2]

This work aims the application of fluidic ionic matrices in the quantitative analysis of MC-RR, in order to increase the analysis precision.

Results and Discussion

Five ionic matrices were synthesized through the reaction of the matrix α-cyano-4-hidroxycinamic acid (CHCA) with aniline (ANI), pyridine (Py), isopropylamine (Isoprop), triethylamine (TEA) and diisopropylamine (DIPA). Spots prepared only with the deposition of 1.0 µL of each ionic matrix were evaluated by optical microscopy (**Figure 1**) in order to check their physical state.

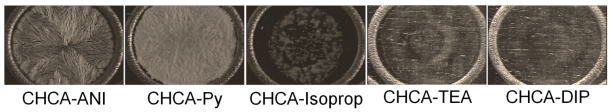

Figure 1. Optical microscopy of the spots prepared with ionic matrices.

Fluidic matrices CHCA-TEA and CHCA-DIPA, were chosen to assess their effect over the CV value. In this stage, a solution containing MC-RR and angiotensin I (AGT), used as internal standard, both at a concentration of 2.5 µmol/L in MeOH:H_2O 40%, was analyzed in quadruplicate. The preparation method was accomplished by adding 1.0 µL of sample over a MALDI plate spot, which was dried under vacuum in a desiccator coupled to a vacuum pump. After addition of 1.0 µL of the matrix solution over the sample, solvent was evaporated under ambient conditions. The peak ratio between MCRR and AGT signals of each replicate was used to calculate the CV values, shown in **Table 1**.

Table 1. CV values for ionic matrices CHCA-DIPA and CHCA-TEA and crystalline CHCA matrix.

Matriz	Coeficiente de variação
CHCA-DIPA	2.04
CHCA-TEA	4.69

Obtained values are much lower compared with those provided by crystalline CHCA matrix in diverse sample preparation protocols, in which the better CV value was 9.5.[2]

The CHCA-DIPA provided the smaller CV value and was used to construct the MCRR analytical curve. AGT concentration was constant (2.5 µmol/L) and MC-RR concentration varied as folows: 0.1; 0.5; 1.0; 2.5; 5.0; 7.0; and 9.3 µmol/L. Coefficients of determination (R^2) and linear correlation (r) were both 0,99. Calculated LOD and LOQ were 0.06 and 0.36 µmol/L respectively.

Conclusions

The ionic matrix CHCA-DIPA demonstrated an impressive effect over the homogeneity of the sample, which could be confirmed by the low CV value obtained, increasing, therefore, the analysis precision. The constructed analytical curve showed low values of LOD and LOQ.

Aknowledgements

FAPESP, CNPq and CAPES.

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