A rapid UPLC-MS/MS method for serotonin, tryptophan and its kynurenine metabolites in mice plasma and spinal cord.

Gabriela A. Buqui[1] (PG), Guilherme R. Souza[2] (PQ), Thiago Mattar Cunha[2] (PQ), Norberto P. Lopes[1*] (PQ)

***Correspondence author: nplopes@fcfrp.usp.br**

[1]*Núcleo Pesquisas em Produtos Naturais e Sintéticos, Departamento de Física e Química, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Zipcode 14040-903, Ribeirão Preto, SP, Brazil.*

[2]*Departamento de Farmacologia, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Zipcode 14040-903, Ribeirão Preto, SP, Brazil.*

Palavras Chave: *kynurenine metabolites, UPLC-MS/MS, quantification, tryptophan*

.

Abstract

We have developed a rapid, sensitive and specific UPLC-MS/MS for the quantification of serotonin and tryptophan metabolites of the kynurenine pathway in mice plasma and spinal cord.

Introduction

The clinical relevance of serotonin, tryptophan and the kynurenine metabolites have been stimulate the development of several chromatographic methodologies hyphenated in mass spectrometry analyzers. Among them we have gas chromatography–mass spectrometry[1] and high voltage electrophoreses[2], but liquid chromatography coupled to mass spectrometry have been the most common technique to investigate kynurenines metabolism in biological fluid[3].

UPLC-MS/MS protocols have also been emerging as an important tool for rapid quantification of low amount samples in addition to other vantages. The relevance of protocols using mice models and the clinical importance to monitor serotonin, tryptophan and the predominant kynurenines metabolites in a large series of samples stimulated the development of a single UPLC-MS/MS protocol in order to quantify it in plasma and spinal cord samples.

Results and Discussion

A UPLC-MS/MS method was developed and validated following the FDA´s guideline for Bioanalytical Method Validaton. Caffeine was used as internal standard (IS). Chromatographic analysis was performed on an Acquity UPLC system (Waters®) and the separation was performed at 40°C using a kinetex F5 column (3 mm x 50mm, 2.6 µm) with a linear gradient elution, using water (containing 0.5% formic acid) and acetonitrile (containing 1% formic acid) as the mobile phase. Mass spectrometry detection was performed using a TQ detector (Waters®.) equipped with an electrospray ionization (ESI) source. All the parameters for ionization of the analytes were optimized. The MS/MS detection was carried out by MRM mode, monitoring the fragmentation of *m/z* 168→150 for QA, 177→160 for 5-HT, 209→192 for KYN, 154→136 for 3-OHAA, 205→188 for TRP, 190→144 for KYNA and 195→138 for IS.

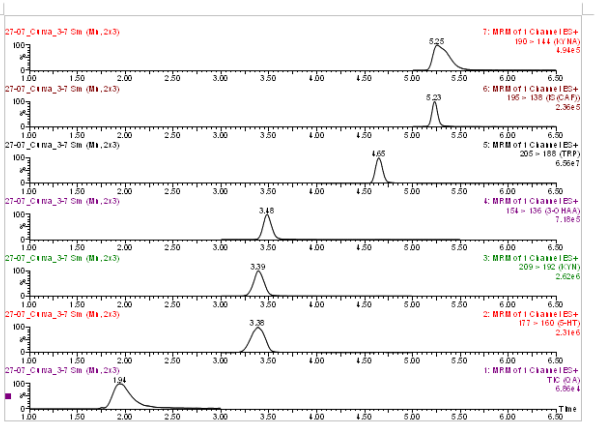

Figure 1. Chromatogram with the MRM transitions for 5-HT, TRP and kynurenine metabolites (KYN, KYNA, 3-OHAA and QA).

The method developed showed good linearity in a wide range concentration for all the analytes (about 2.8 to 5000 ng mL^{-1}) with r^2 > 0,99 also did not showed matrix effect. The plasma samples were diluted added IS and then filtered using a 3kDa 0.5mL Millipore Amicon Ultra filter with good extraction recovery, as well as the spinal cord samples. Precision and accuracy were measured for each analyte in three concentrations and the coefficient of variation was less than 10%.

Conclusions

The UPLC–MS/MS was successfully applied to the investigation of 5-HT and TRP metabolites of the kynurenine pathway in plasma and spinal cord mice samples.

Ackowledgments

To FAPESP, CNPQ and CAPES foundation.

[1]Giorgini, F. et al. J. Biol Chem. **2013**, 288, 36554.
[2] Furlanetto, S. et al. Pharm. Biomed. Anal.**1998**, 17, 1015.
[3] Möller, M. et al. J. Chrom. B. **2012**, 898, 121.