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

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### 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 spectrometry¹ and high voltage electrophoreses², but liquid chromatography coupled to mass spectrometry have been the most common technique to investigate kynurenines metabolism in biological fluid³. 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 Validation. Caffeine was used as internal standard (IS). Chromatographic analysis was performed on an Acquity UPLC system (Waters⁴) 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 (Waters⁵) 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.

### 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.

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