Stereoselective analysis of fluoxetine and norfluoxetine in human milk by direct sample injection using a 2D LC-MS/MS system

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Keywords: *human milk, fluoxetine, enantioselective quantification, restricted-access media, two-dimensional LC, tandem mass spectrometry.*

Abstract

A 2D LC-MS/MS system was used for quantification of fluoxetine and norfluoxetine enantiomers in human milk.

Introduction

Fluoxetine (FLX) belongs to a class of selective serotonin reuptake inhibitors, which is commonly prescribed to the treatment of depression in pregnancy and postpartum depression[1]. FLX is administered as a racemic mixture, in the organism, *R*- and *S*-FLX is N-demethylated to *R*- and *S*-NFLX, which is also pharmacologically active[2].

Some chiral chromatographic separations have been described for quantifications of FLX and NFLX in environmental water and biological fluids. However, sample clean-up procedures were required due to sample matrix complexity. For that, liquid-liquid or off-line solid phase extractions were used furnishing undesired residues.

Regarding sample clean-up of human milk samples, there is only one report on the use of restricted-access media (RAM) columns for protein depletion[3].

This work presents an analytical method for simultaneous quantification of FLX and NFLX enantiomers by direct injection of human milk using a 2D LC-ESI-MS/MS system.

Results e Discussions

For the direct injection of human milk samples a two dimension LC system was required. A RAM-C_{18}-BSA column was used in the first dimension for the milk proteins depletion and in the second dimension was used an antibiotic-based chiral column, Chirobiotic™ V2.

The mobile phase used in the first dimension was 10 mM aqueous ammonium acetate (pH 6,8) and in the second dimension was used a mixture of 10 mM aqueous ammonium acetate (pH 6.8) / ethanol (20/80, v/v) at 25 °C. At this condition the chromatographic run time was of 25 minutes, with the overlap of *S*-FLX and *S*-NFLX. Changes in the organic modifier, in the buffer concentration or even in the modifier did not resolve the observed overlapping. The operation of the MS/MS at SRM conditions, however, did allow the simultaneous quantification of the enantiomers of both analytes.

Figure 1 illustrates the obtained extracted ions chromatograms for a spiked milk sample (FLX, 15 μg.mL^{-1} and NFLX, 20 μg.mL^{-1}).

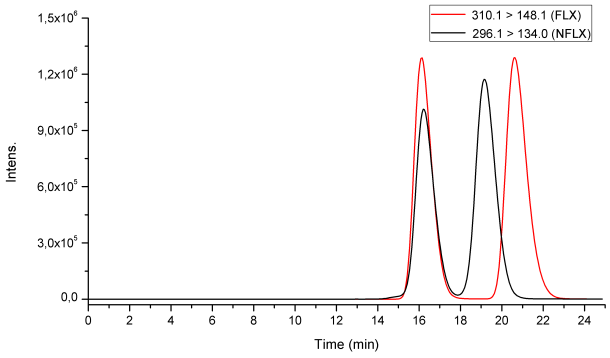

Figure 1. 2D LC-ESI-MS/MS extracted ions chromatograms of a spiked human milk sample

The method validation was carried out in accordance to European Medicines Agency guideline procedure. The calibration curves were linear in the range studied for each enantiomer of FLX (7.50 - 75.0 ng.mL^{-1}) and of NFLX (10.0 - 100 ng.mL^{-1}), with mean correlation coefficients (n = 3) of 0.98 or higher. The extraction efficiency, accuracy, precision and matrix effect was qualitatively measured and the values obtained are in accordance with the established criteria.

Conclusions

The coupling of a RAM-BSA C_{18} column to a Chirobiotic™ V2 column furnished a method with high sensitivity, with no matrix effect and with analysis time of only 25 minutes, with almost no sample preparation and minimal solvent usage (10 mL per run).

Acknowledgment

The authors acknowledge financial support from FAPESP, CAPES and CNPq.

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