AUTOMATED ON-LINE SOLID PHASE EXTRACTION CHROMATOGRAPHY TECHNIQUE FOR THE DIRECT ANALYSIS OF TRICYCLIC ANTIDEPRESSANTS IN BIOLOGICAL MATRICES

INTRODUCTION

NCE’s (new chemical entities) are becoming more potent, in turn the administered dosages are decreasing, which leads to lower limits of detection required [1-10 ng/mL]. Biological samples from in vivo studies need to be analyzed rapidly throughout a NCE lifecycle. Consequently, the analysis of biological samples must be very rapid yet remain selective and sensitive for the compounds of interest. Due to these pressures LC/MS/MS has become the standard analytical technique for the determination of NCE’s in complex matrices. The analysis of NCE’s and their metabolites in biological matrices requires sufficient sample preparation prior to separation, detection and quantification to reach the low detection limits by overcoming ion suppression effects, for example. Sample pretreatment can be time consuming, repetitive and prone to human error due to the large number of samples required for analysis. This means that a generic approach with the direct injection of biological matrices using an on-line system is desirable [11].

The aim of this study was to evaluate an on-line sample extraction system for the direct analysis of tricyclic antidepressants (TCAs) in biological matrices. Selectivity, linearity, precision, recovery and limit of quantification were studied.

EXPERIMENTAL

Methodology

The tandem mass spectrometry system was configured using multiple pumps and switching valves in order to perform on-line sample extraction of biological samples under a single software platform as outlined in Figures 1-6.

Hardware Setup

Figure 1 shows a schematic of the online sample preparation configuration. Figure 2 shows the configuration of the 10-port switching valve. The sample extraction procedure and analysis is outlined below.

In the OPLS position (black line pathway) the diluted plasma is loaded onto the Oasis™ HLB in aqueous mobile phase at a high flow rate (2 mL/min) with the flow directed towards waste. The extraction column is washed in 3% organic at 2 mL/min. Plasma matrix is directed towards waste and the analytes are retained on the sorbent by reverse-phase mechanisms. The valve is switched to the ON position so that the extraction column and analytical column are in-line.

The two Waters 1525 pumps were set up as follows:

Gradient: As shown in Figure 3

B: Acetonitrile 0.05% formic acid
A: Water 0.05% formic acid

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The two Waters 1525 pumps were set up as follows:

Gradient: As shown in Figure 4

B: Acetonitrile 0.05% formic acid
A: Water

The precision of the method was demonstrated by the QC samples having a % RSD of <5%.

Multiple Reaction Monitoring

The sensitivity of the method was determined by comparing the blank plasma to the plasma spiked with the mixture of analytes and the internal standard. No interfering compounds were detected at the same retention times as the studied compounds.

RESULTS

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Sample Preparation

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REFERENCE


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