K51 Detection and Quantification of Antidepressants in Aqueous Matrices

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The goal of this presentation is to delineate the process of developing a detection method capable of simultaneously identifying and quantifying antidepressant compounds in aqueous matrices using a Liquid Chromatograph/Tandem Mass Spectrometer (LC/MS/MS), the determination of quantitative differences between detection methods, the effect of stationary phase composition on the separation of analytes, and applications to wastewater samples pursuant to a relevant EPA method.

This presentation will impact the forensic science community by showing how the methodologies and results presented will provide widely applicable LC/MS/MS method for the detection of small molecules in a variety of aqueous matrices, including wastewater, blood, and urine. The development process can be adapted to produce a quantitative screening method for any compounds of interest suitable for LC/MS/MS identification.

Antidepressants are psychiatric medications that are taken with the intent to alleviate mood disorders. These drugs and their metabolites enter the environment as a byproduct of use, and may pose a danger to the health of humans and the environment. In the U.S., there are few regulations concerning the discharge, fate, and transport of such pharmaceuticals, many of which remain biochemically active after passing through current treatment processes. An important first step toward developing proper regulation is the creation of a selective and sensitive detection method in relevant matrices.

This presentation will discuss the methodology developed for the detection and identification of a variety of commonly used antidepressant drugs and their metabolites. High Pressure Liquid Chromatography (HPLC) on fused core silica phases is used for the separation of analytes, and a triple-quadrupole mass spectrometer is employed using both Scheduled Multiple Reaction Monitoring (s-MRM) and Information Dependent Acquisition (IDA) detection methods with Electrospray Ionization (ESI). Sample preparation is performed in accord with EPA method 1694, using solid-phase extraction with an Oasis® HLB cartridge suitable for retention of acidic, basic, and neutral compounds.

A library containing reference spectra was created using direct syringe pump infusion of standards of each antidepressant in methanol. Generalized parameters capable of ionizing and fragmenting each compound were optimized and compiled. The composite method was used to create and assess the efficacy of identifying and quantifying components of a mixture using both SMRM and IDA.

Preliminary testing was performed on a C18 column, the currently most-used column for analysis of antidepressants. The variance of s-MRM, which scans for analytes at specified expected retention times, was determined to be within acceptable limits to provide reproducible results. Information Dependent Acquisition, a method that scans using s-MRM and produces additional library matching spectra for analytes with intensities over a certain threshold, supposedly at a cost of quantitative reproducibility. The difference in quantification between the two methods was determined using labeled analogues of each compound as internal standards.

The optimized detection method was then applied to chromatographic separations using different stationary phase compositions including C8, C18, phenyl-hexyl, and amide. The goal was to determine how chemical interactions between analytes and the column influence separation and analysis. Parameters such as efficiency, reproducibility, and selectivity were considered in method optimization. Retention times, elution order, and peak shapes were compared when possible.

Antidepressant, LC/MS, Wastewater

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