K30 Mass Spectral Library for Phosphodiesterase Type 5 Inhibitors by Ultra High-Performance Liquid Chromatography/Quadrupole-Time-of-Flight/Mass Spectrometry (UHPLC/Q-TOF/MS)

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After attending this presentation, attendees will understand the process of creating and verifying a mass spectral library for Phosphodiesterase Type 5 (PDE5) enzyme inhibitors. In addition, attendees will better understand the analysis of these compounds in dietary supplements.

This presentation will impact the forensic science community by demonstrating the utility of mass spectral library searching for the detection of PDE5 inhibitors in dietary supplements.

According to recent statistics, PDE5 inhibitors were the most common adulterant detected in the Food and Drug Administration (FDA) -recalled dietary supplements from January 2004 through December 2012. PDE5 inhibitors such as sildenafil (Viagra®), vardenafil (Levitra®), and tadalafil (Cialis®) are commonly prescribed for treatment of Erectile Dysfunction (ED); however, when included in dietary supplements as an off-label ingredient, PDE5 inhibitors will cause serious drug-drug interactions for men taking certain heart medications. In competitive sports, athletes may use PDE5 inhibitors for their potential performance-enhancing effects. PDE5 inhibitors are vasodilators and thus may allow for excess blood flow and oxygenation. These compounds are not currently banned by the World Anti-Doping Agency, though there is some debate as to whether they would provide an unfair advantage to athletes who compete at higher altitudes or in long-distance sporting events such as cycling.

The objective of this study was to utilize UHPLC/qTOF/MS to develop a screening method for PDE5 inhibitors based on mass accuracy and mass spectrum library searching.

To build the library, mass spectra were obtained by injecting 1.0µg/mL neat reference standards in Information Dependent Acquisition (IDA) mode. The mass spectral data was analyzed utilizing PeakView® and MasterView® software. The mass spectral library was created using LibraryView® software. Extraction efficiencies of fortified PDE5 inhibitors were compared between Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) and methanolic extractions of two dietary supplements, a powdered hydration supplement and an oral form of hyaluronic acid. The UHPLC/qTOF/MS utilized reversed phase separation operated in positive electrospray ionization mode.

Nine analytes were included in the PDE5 inhibitor library and all successfully triggered a library match at100ng/mL or greater for fortified extracted samples. The results were scored based on mass error, retention time error, isotope ratio error, and library match; an evaluation of the combined scores showed a threshold of at least 80 to be considered a non-negative sample. Regardless of extraction procedure or dietary supplement matrix investigated, all analytes produced mass spectral library matches and showed a combined score ranging from 84.8 to 98.2. The instrumental limit of detection was analyzed by injecting neat reference standards of each analyte at concentrations ranging from 5ng/mL to 500ng/mL. The retention time and mass accuracy show that all of the analytes are detectable as low as 5ng/mL, but Information Dependent Acquisition (IDA) product ion scans were not triggered for all of the analytes until the concentration reached 100ng/mL.

Fortified matrix samples and four FDA recalled over-the-counter male enhancement supplements were analyzed to verify the utility of the library. The dietary supplement matrices were fortified from 25 to 200ng/mL with a PDE5 inhibitor standard mix. For samples fortified at 200ng/mL, library matches were successfully produced for all nine analytes in the PDE5 inhibitor mix. The extraction of the FDA-recalled supplements found the active pharmaceutical ingredients sildenafil (Viagra®) and tadalafil (Cialis®) in all four supplements.

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A mass spectral library was successfully developed for PDE5 inhibitors and verified by both methanol and QuEChERS extractions as low as 100ng/mL for the supplements tested. The method was challenged by extracting two different fortified dietary supplements and four FDA-recalled, over-the-counter male enhancement supplements; it was effective at identifying PDE5 inhibitors in these matrices.

PDE5 Inhibitors, LC/qTOF/MS/MS, Dietary Supplements

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