K16 Detection of Amitriptyline and Nortriptyline in Decomposed Skeletal Tissues by Microwave-Assisted Extraction and Ultra High-Performance Liquid Chromatography

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After attending this presentation, attendees will understand how to develop a microwave-assisted extraction methodology using bone tissue and be provided with an example of its practical application using vertebral bone.

This presentation will impact the forensic science community by adding to the body of data illustrating the utility of skeletal tissues as a matrix for toxicological analysis and by demonstrating an efficient method for preparation of skeletal tissue samples.

The use of Microwave Assisted Extraction (MAE) followed by Microplate Solid Phase Extraction (MPSPE) and Ultra High Performance Liquid Chromatography (UHPLC) to detect Amitriptyline (AMI) and Nortriptyline (NORT) from postmortem skeletal tissues is described. Rats (n=4) received 60mg/kg amitriptyline and were euthanized by CO^2 asphyxiation approximately 20min post-dose. The remains decomposed to skeleton outdoors and vertebral bone was collected. Bones were cleaned with phosphate buffer (PBS, 0.1M, pH 6), methanol, and acetone, then dried under ambient conditions and pulverized to a powder. Bone samples (n=3, 0.5g), as well as one drug-free sample, underwent MAE using methanol in a closed vessel system for a total of 60 minutes. The extraction solvent was replaced with fresh methanol after 10, 20, 30, and 60 minutes of irradiation. The methanolic extracts were evaporated and reconstituted in 1mL PBS. Internal standard (Desipramine, DMI, 500ng) and 100µL glacial acetic acid were added to each extract. Acetonitrile:methanol (1:1, 3mL) was added to each extract, followed by storage at -20oC overnight to precipitate proteins and lipids. Following centrifugation, the supernatants were evaporated to 1mL, diluted to 3mL using PBS and acidified with 100µL glacial acetic acid.

Diluted supernatants underwent further clean-up by MPSPE, using CleanScreen® XCEL™ 1 48 well plates. Wells were conditioned with methanol (3mL), distilled water (3mL) and PBS (3mL). Following loading of samples, columns were washed with PBS (3mL) and 0.1M acetic acid (3mL). Columns were dried (~5 in Hg, 5min) and washed with methanol (3mL). Columns were then dried again under vacuum (~10 in Hg, 10min). Basic compounds were eluted with 3% NH_4OH in 20:80 isopropanol: dichloromethane (3mL). Extracts were evaporated to dryness and reconstituted in 0.1% formic acid in 10:90 acetonitrile:water (500µL). Samples were analyzed using an Acquity UHPLC with a Photo-Diode Array (PDA) detector. The column used was a Selectra DA (100mm x 2.1mm, 3.0µm particle size). Samples were run using a binary gradient elution (A: 0.1% (v/v) formic acid, 10% (v/v) acetonitrile, and 90% (v/v) water; B: 0.1% (v/v) formic acid, 10% (v/v) water, and 90% (v/v) acetonitrile). The mobile phase gradient began with 90:10 A:B, held for 3min, followed by a linear increase to 40:60 A:B over 8min, followed by reversion back to 90:10 over 1min for a total run time of 11min at a constant flow rate of 0.300mLl/min. The autosampler was maintained at 25oC, with the column temperature set to 50oC. The wavelengths chosen for analysis were 245nm for AMI and NORT as well as 290nm for DMI. Analyte stability to MAE in methanol was assessed and both analytes were stable for at least 60mins irradiation time. Recovery was at least 95% of maximal value within the first 10min of MAE for all samples assayed. The MPSPE/UHPLC method was linear between 25-10,000ng/mL, with precision and accuracy <20% in triplicate analyses, with a limit of detection of 25ng/mL for both AMI and NORT. The vertebral bone analyzed using this method detected AMI (2.8µg/g -15µg/g) and NORT (1.8µg/g-4.3µg/g) in all samples assayed.

Forensic Toxicology, Bone, Microwave-Assisted Extraction