K3 Analysis of Promethazine, Chlorpromazine, and Selected Metabolites in Decomposed Skeletal Tissues by Microwave-Assisted Extraction/Microplate Solid Phase Extraction/Ultra High-Performance Liquid Chromatography (MAE/MPSPE/UHPLC)

Courtney A. Campbell, BS, Laurentian University, 935 Ramsey Lake Road, Sudbury, ON P3E 2C6, CANADA; James Watterson, PhD, Laurentian University, 935 Ramsey Lake Road, Sudbury, ON P3E 2C6, CANADA; and Caroline C. Betit, MSc, 935 Ramsey Lake Road, Sudbury, ON P3E 2C6, CANADA*

After attending this presentation, attendees will understand how to develop a microwave-assisted extraction methodology using bone tissue. An example of this methodology's practical application using vertebral bone will be presented.

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 purpose of this study was to develop and validate a Microwave Assisted Extraction (MAE) followed by a Microplate Solid Phase Extraction (MPSPE) method for the detection and semi-quantitation of Promethazine (PMZ), Chlorpromazine (CPZ), and selected metabolites (promethazine sulphoxide, desmethylpromethazine, chlorpromazine sulphoxide, and desmethylchlorpromazine) in the skeletal remains of rats using Ultra High Performance Liquid Chromatography (UHPLC).

Male Wistar rats (n=10) received either a dose of PMZ (50mg/kg, i.p.) or CPZ (50mg/kg, i.p.). Five rats acted as drug-free controls. The rats were euthanized by CO_2 asphyxiation 20min after exposure and placed outdoors to decompose to skeleton. Vertebral bone was recovered and washed with 3mL Phosphate Buffer (PBS: 0.1M; pH6), 3mL methanol, and 3mL acetone and air-dried. Bones were pulverized and samples underwent MAE in 5mL of methanol at 80oC in a MARS™ 6 microwave oven for a total of 60min, with extraction solvent recovered and replaced with fresh solvent at 15min and 30min. All solvent extracts were recovered, evaporated to dryness, and reconstituted in 1mL of Phosphate Buffer Saline (PBS). Promazine was added as an internal standard (500ng) and extracts then underwent protein precipitation by adding 1mL of PBS along with 3mL of acetonitrile-methanol (1:1) followed by storage at -20oC for 24h. The samples were centrifuged and the supernatant was collected and evaporated to 1mL. PBS (2mL) was utilized to dilute the samples which were then acidified with 100µl of acetic acid. All samples were subjected to SPE using XCEL I (130mg) 48 microwell plates. Wells were conditioned by sequential addition of 3mL methanol, water, and PBS. Samples were loaded by gravity. Wells were washed with PBS (3mL) and 0.1M acetic acid (3mL) and dried for 5min under vacuum (10 in Hg). Wells were washed with methanol (3mL) and dried for 10min (10 in Hg). Analytes were eluted using 3% NH_4OH in 20:80 isopropanol:dichloromethane (3mL). Extracts were evaporated to dryness and reconstituted in 500µL of mobile phase A (0.1 % formic acid in 90:10 water:acetonitrile). Samples were centrifuged for 10min and then 15µl of sample was injected into the UPHLC with Photodiode Array Detection (PDA). The column used was a Raptor™ biphenyl column (150mm x 2.1mm, 1.7µm) with a column temperature set to 50oC. The mobile phase gradient began with 95:5 A:B (B: 0.1%formic acid in 90:10 acetonitrile:water) held for 1min, then increased to 70:30 A:B over 4min, held for 1min, then increased to 20:80 A:B over 3min, and reversion back to 95:5 A:B which was held for 1min, for a total run time of 10min at a constant flow rate of 0.400mL/min. Quantitative measurements were made using the response ratio measured at 240nm for the sulphoxide metabolites and 250nm for the remaining analytes.

Method validation involved preparation of standard analyte samples in drug-free Bone Tissue Extract (BTE). The response ratio was linear from 10ng/mL to 5,000ng/mL (R^2=0.990-0.999). The precision was <25% (n=3 on each of three different days). The limit of detection was approximately 10ng/mL for each analyte, and the limit of quantification for the method was approximately 25ng/mL for each analyte. The majority of analytes were recovered after 30min extraction interval. Analytes were stable under the microwave extraction for at least 60min.

Promethazine, Chlorpromazine, Bone