K12 An Analysis of Tramadol and Its Metabolites in Rat Skeletal Tissues Following Acute and Repeated Dose Patterns Using High-Performance Liquid Chromatography/Tandem Mass Spectrometry (HPLC/MS/MS)

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Learning Overview: The goal of this presentation is to demonstrate the utility of LC coupled with MS/MS for the semi-quantitative analysis of tramadol and four of its metabolites in animal models that underwent different dosing patterns. Additionally, this presentation seeks to showcase the efficacy of assigning a numerical value representing the frequency of significant differences in order to simplify the results obtained in works where a large number of statistical tests were performed.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by demonstrating the utility of skeletal tissues as a suitable alternative matrix for toxicological analysis. Furthermore, this presentation will demonstrate the importance of a multifaceted approach to toxicological analysis, including appropriate statistical modeling to differentiate between various patterns of drug use.

The use of skeletal elements for the viable analysis of drugs of abuse has seen increased prevalence in the past ten years. Advancements in the analytical methods used, including solid phase extraction and MS, have allowed for increased sensitivity and selectivity. Previous studies have focused on the influence of dose-death interval, microclimate, differential patterns of exposure, and the influence of body position. In this work, the opioid analgesic tramadol was investigated for its pharmacological behavior when administered as part of three dosage patterns to male Sprague Dawley rats. The three exposure patterns consisted of an acute low (n=4, 1 dose, 30mg/kg) group, a repeated high survived (n=5, 3 doses, 30mg/kg) group, and a repeated high overdosed group (n=11, 3 doses, 30mg/kg). Drug-free rats (n=4) served as negative controls.

Following euthanasia by CO_2 asphyxiation, animals were decomposed to skeletons outdoors over the summer of 2019 in Sudbury, ON. Bones were sorted by animal and skeletal element (skull, vertebrae, ribs, pelvis, femur, tibia/fibula), then washed and ground to powder before undergoing dynamic methanolic extraction. Semi-quantitative analysis of tramadol and four of its metabolites—O-desmethyltramadol, N-desmethyltramadol, N,O-didesmethyltramadol, and tramadol N-oxide—was conducted using HPLC/MS/MS in positive ion mode. Analyte levels were expressed as a mass-normalized Response Ratio (RR/m) in order to account for the exact mass of bone used. Method validation for the analysis of tramadol and its metabolites was investigated in accordance with the Scientific Working Group of Toxicologists (SWGTOX) standards of practice, with all criteria except for dilution integrity successfully met at a limit of detection and limit of quantification of 1ng/mL. The effect of exposure pattern on analyte level and analyte level ratio was assessed using the Kruskal-Wallis test for significant differences (P <0.05). A total of 315 pairwise comparisons were performed to assess significant differences, with the ratio of tramadol to N-desmethyltramadol determined to be the metric most commonly able to identify these differences in 91% of tests. Additionally, the effect of skeletal element on analyte level and analyte level ratio was also assessed, with a total of 675 pairwise comparisons. Skeletal element was determined to be a significant factor in all cases.

These data suggest that both skeletal element and dose pattern are important measures to evaluate with respect to the analysis of drugs of abuse in bone tissues. Furthermore, different metrics, including analyte level and analyte level ratios, may be useful for discriminating between these different dosing patterns.

Tramadol, Tandem Mass Spectrometry, Skeletal Tissues

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