Analysis of Tramadol and its Metabolites in decomposed Rat Skeletal Tissue following Repeated and Acute Exposure to Tramadol over Various Dose Death Intervals via UPLC-MS/MS

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Introduction

Throughout the known list of opioids there exists a relatively new synthetic opioid called Tramadol. In death investigations drugs of abuse can be identified using various analytical chemistry techniques from collected biological samples including blood, serum, urine, and organ tissue samples. It is not uncommon to see a death investigation occur with nothing but skeletal tissue as biological samples. However, skeletal tissues are not currently analyzed in toxicological analyses [1]. Complications in extractions occur that other biological samples do not have. When extracting drugs from bone tissues it is impossible to remove 100% of the analyte which leads to semi-quantitative data [2].

Tramadol hydrochloride is a synthetic opioid used for pain relief, often after surgery. The opioid was first approved for clinical use in 1995 as it functioned like and structurally resembled opiates (morphine, oxycodone) but was considered safer. After having seen the addictive effects of tramadol the FDA decided to list tramadol as a controlled substance ultimately tightening up restrictions for acquisition methods [3]. Currently tramadol is classified as a Schedule IV drug which includes drugs that are considered to have a low potential for abuse and a low risk of dependence [4].

The objective of this study was to examine how dose-death interval (DDI) effects the concentration analyzed within skeletal tissue. To obtain the data required for analysis a methanolic solvent extraction followed by filtration pass-through extraction followed by UPLC-MS/MS analysis was used.

Methods

Male Sprague Dawley rats (n = 52) were divided into 3 groups (AL, n = 16; AH, n = 16; RH, n = 16). Each group was further subdivided into 4 dose-death interval times (1 hour, 2 hours, 4 hours, or 24 hours). The animals in the AL group received one does of TRAM (30mg/kg) through intraperitoneal injection and were subsequently euthanized by CO2 asphyxiation either 1 hour, 2 hours, 4 hours, or 24 hours after dosing based on DDI assigned. The animals in the AH group received one does of TRAM (60mg/kg) through intraperitoneal injection and were subsequently euthanized by CO2 asphyxiation either 1 hour, 2 hours, 4 hours, or 24 hours after dosing based on DDI assigned. The animals in the RH group received three doses of TRAM (30mg/mL) through intraperitoneal injections having 45 minutes pass between each administered dose. However, of the 16 animals used in the RH group 11 of them died within one hour of the last dose (RH OD). The remaining 5 animals were euthanized based on their labelled DDI. Four animals were used as drug-free negative controls. After each animal was euthanized, all animals were placed outdoors in Sudbury, Ontario in July of 2019, contained within a wire mesh, left to decompose to a skeleton with full exposure to surrounding weather conditions (sunlight, precipitation, humidity etc.) for approximately 3 weeks. Once decomposition was complete the bones were further subdivided by skeletal element.

Extracting the analyte from ground skeletal tissue was done by dynamic methanolic extraction using a Tissuelyzer® apparatus (Qiagen, Germantown, MD, USA). Bone samples (0.05g, n = 1) from a given animal, skeletal element and DDI was combined with 975 µL of methanol and 25 µL of the internal standard (ISTD) mixture solution (1µg/mL ISTD) in 2 mL screw-ap vials. Followed by filtration pass through extraction (FTPE) performed using Waters Ostro® Protein Precipitation & Phospholipid Removal Plate a with a 50:50 methanol:acetonitrile mixture to achieve five-fold dilution.

The analysis of the extracts of 2 µL was done using an Acquity® H-Class UPLC coupled to a TQS Micro triple quadrupole mass spectrometer (Waters Corp, Milford, MA). The chosen column was an Acquity® HSS C18 column (150mm x 2.1mm, 1.7µm particle size, Waters Corp, Milford, MA). A flow rate of 0.4 ml/min was used for the gradient elution using two mobile phases. Mobile phase A (MPA) was comprised of LCMS grade deionized water acidified with 0.1% formic acid. Mobile phase B (MPB) was comprised of LCMS grade acetonitrile acidified with 0.1% formic acid. Initially, mobile phase MPA ran through at 90% for 1 min, followed by an 80% linear decrease over 2 min, then decreasing to 75% MPA for 1 min, followed by a decrease to 50% over 2 min, then an increase to 90% MPA over the duration of 1 min, and then maintaining this condition for 1 min. The total runtime for 1 sample was 10 min (Table 2.1).

Acknowledgements

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Results

Table 1: Coefficients of variation from day 3 of the demonstration of proficiency shown in percentages.

	CV %				
[Analyte]	TRAM	TRAM-NO	NODMT	ODMT	NDMT
1	73	3	2	3	4
2	8	5	4	4	5
5	8	6	7	6	6
10	1	2	3	2	2
25	4	4	4	5	5
50	2	2	2	2	1
100	2	3	3	3	3
250	5	4	4	5	4
500	2	2	2	3	3

Figure 1: Bias percentages shown to be within the ± 20% window run on day 2 of the demonstration of proficiency.

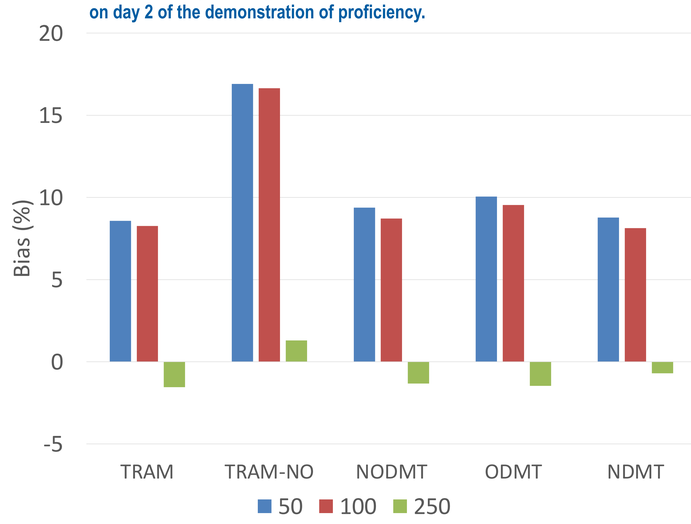
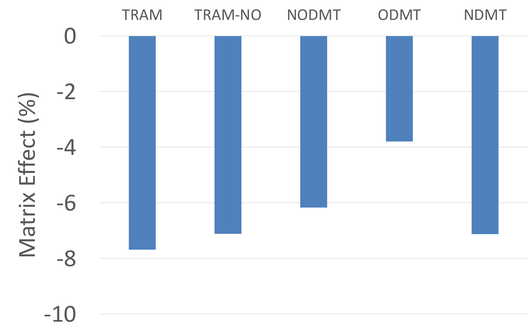


Table 2: ANOVA outputs indicating P values for Comparison of DDI of TRAM using response ratios.

ANOVA of DDI for vertebrae with Acute Low Exposure						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.000644	3	0.000215	0.063001	0.978198	3.708265
Within Groups	0.034061	10	0.003406			
Total	0.034705	13				

Figure 3.9: Bar graph showing the percentage of response deviation caused by matrix effect evaluated for day 3 of the demonstration of proficiency.



Discussion/Conclusion

- Standard curves showed a strong linear trend, with coefficients of correlation (R2) above 0.99 for all analytes.
- Calculations using the linear regression model showed bias values within the acceptable range of ± 20%.
- For all analyte concentration levels above 1 ng/mL and below 500 ng/mL precision fell within the ± 20%
- Matrix effects calculated fell within the ± 20% window in all cases (negative values indicating ion suppression)
- An analysis of variance (ANOVA) was conducted to compare the analyte levels across dose-death intervals (DDI).
    - Separated into 4 groups based on skeletal element (vertebrae, femur, skull, and tibia / fibula)
    - ANOVA conducted showed no statistical difference between each DDI (P > 0.05).
    - 3 of the ANOVA conducted for the response ratios and 4 of the ANOVA conducted for the mass-normalized response ratios showed statistically significant difference across DDI. No observable trends were present.
- The null hypothesis cannot be rejected due to lack of observable trends.

References

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