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Metabolomics Study of Rodenticide Poisoning using Serum Samples with LCMS-QTOF Instrumentation

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Introduction

Rodenticides, commonly known as rat poison are toxic chemicals used to eradicate rodents and are common household poisons.

As per the American Association of Poison Control Centres' rodenticides accounted for 0.3% of 2.3 million human exposures and the most common agent was anticoagulants.

Rodenticide poisoning in India is commonly due to phosphorus and metal phosphides (aluminum and zinc) which are toxic substances resulting primarily in hepatotoxicity. Identifying the toxic substances help the clinicians to initiate appropriate treatment prior to fulminant hepatic failure and salvage the individual.

Most of the packages of rodenticides available in the market do not have appropriate information like composition and its toxicity. Most cases of poisoning are as a result of intentional self harm and often the individuals do not reveal consumption of the toxic substances.

Hence laboratory identification of the chemicals or the metabolites of the toxic substances from biological samples of individuals is important.

In this study we compared the Rat poisoned serum sample with control serum samples using Agilent 6546 LC/Q-TOF for the comprehensive profile of the differentially expressed metabolites.

A total of 5,726 metabolites were detected in all the samples, with 1,728 metabolites upregulated in the poisoned sample.

Experimental

Sample Collection

Serum samples (500 µl volume) was collected from a Individual who had consumed rat poison and 2 healthy individuals. Pooled serum from 3 healthy individuals was used as a control.

Sample Preparation

The metabolites were extracted from serum as per the method described by Broecker et. al. Briefly, the protein from 100 µl serum sample was removed by mixing with 4 volumes (400 µl) of acetonitrile and resultant supernatant was evaporated to dryness under nitrogen. Subsequently, diluent was added to the microcentrifuge tubes.

Experimental

LCMS QTOF Analysis

The Metabolites were detected using Agilent 6546 LC/Q-TOF coupled to Agilent 1290 Infinity II LC using Agilent Zorbax Eclipse Plus 2.1 mm x 100 mm, 300 µm column.

LC Conditions

Mobile Phase A: 10 mM Ammonium Acetate in Water

Mobile Phase B: Methanol

Gradient: 0 Min - 0% B, 20 Min - 100% B

Stop time: 22 Min, Post Time: 3 Min

MS Conditions

The Source parameters and voltages were optimized for efficient detection of the metabolites.

System was tuned using ESI low concentration tune mix for 50 - 250 *m/z* fragile ion tune mode.

Data Processing

The LCMS data was Processed using Agilent MassHunter Profinder software (version B.08.00) in 'Batch Recursive Molecular Feature Extraction' mode. The intensity cutoff was set at 2000 counts. Triplicate injections for each samples were processed and only compounds detected reproducibly in 2 out of 3 injections were carried forward for further processing. The compounds were exported as .cef files.

Statistical Analysis

Agilent MassHunter Mass Profiler Professional (version 15.00) was used for statistical and chemometric analysis of the acquired data. Guided workflow for correlation and fold-change analysis was used to get differentially expressed compounds in the rat poisoned serum sample.

The rat poison was also injected alongside samples to rule out compounds originating from it.

The correlation coefficient of 0.05% and fold change > 3 was used as cut-filters to determine differentially expressed compounds.

Database Search

Agilent Metlin PCDL (B.07) was used to generate custom Human (*Homo Sapiens*) database using Pathways to PCDL (B.08) utility. Wikipathways and Biocyc/Metacyc were used as the pathway source.

A total of 616 pathways in WikiPathways were used to generate a PCDL with 5,779 compounds and 289 pathways from BioCyc were used to generate a PCDL with 1,641 compounds.

LCMS Data

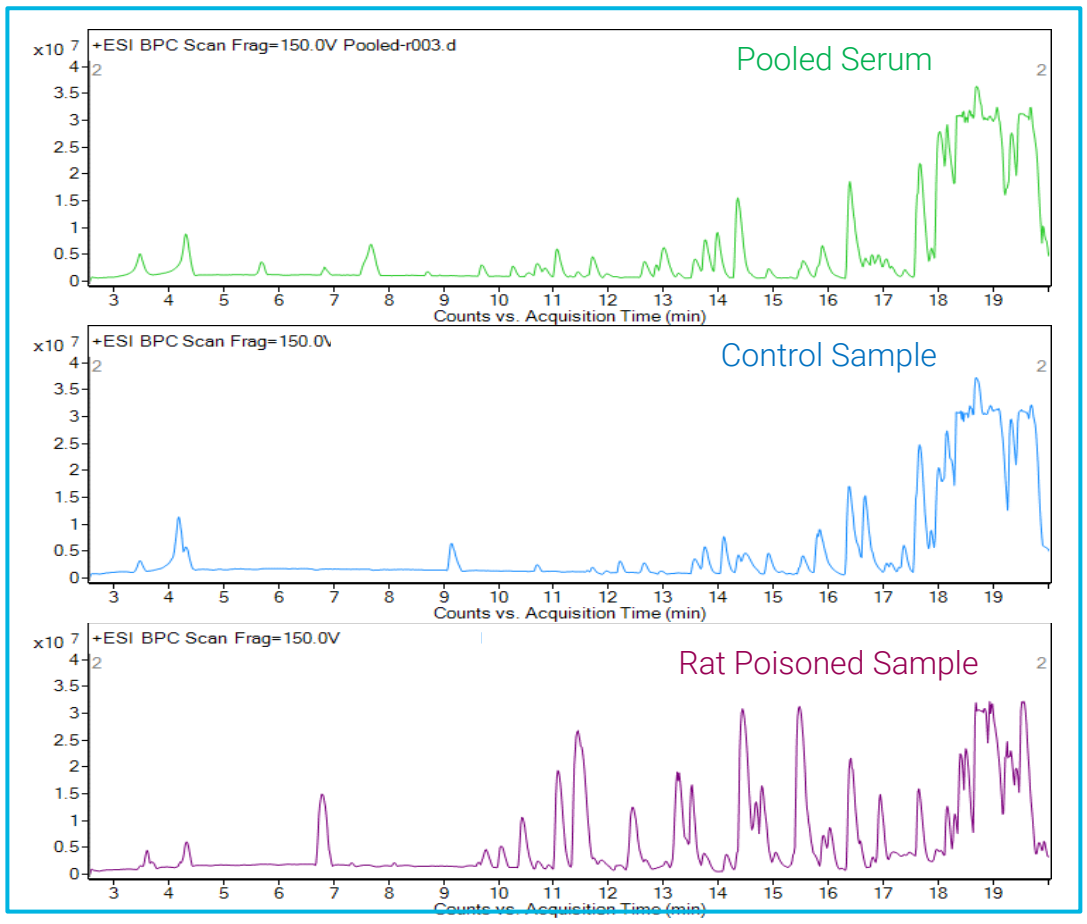
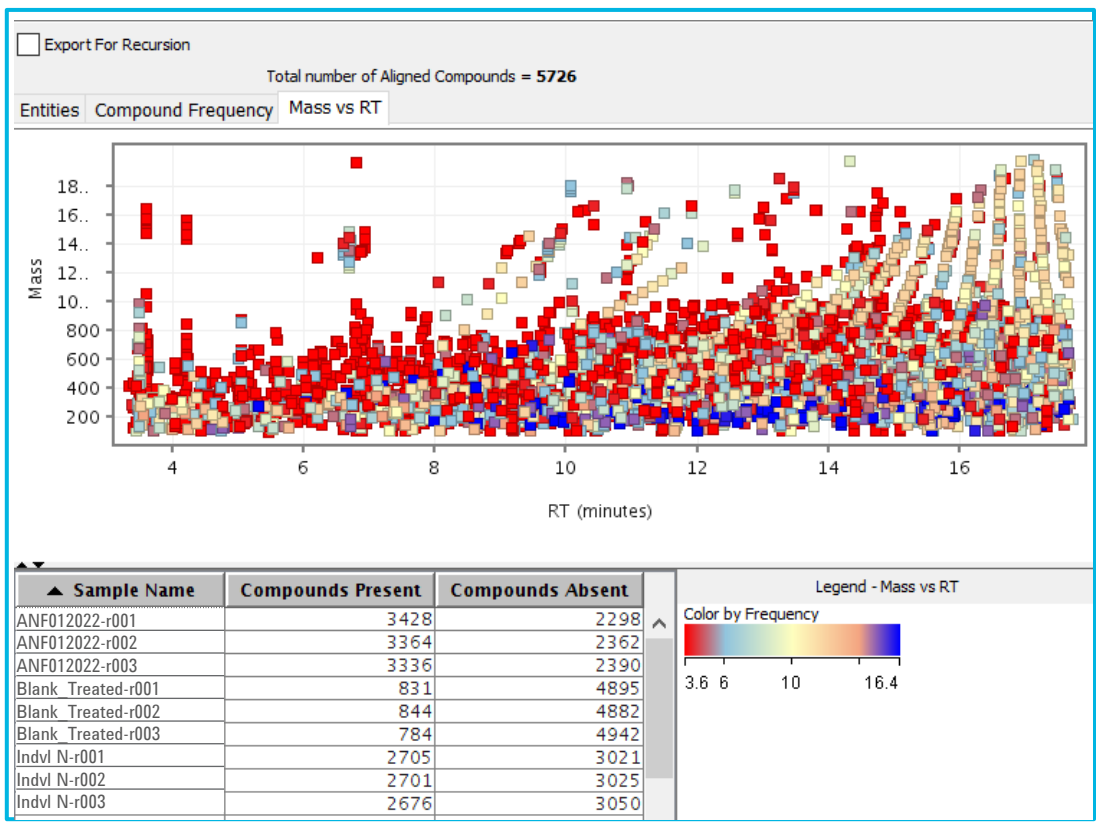


Fig 1: Base Peak Chromatogram of the poisoned serum sample along with control and pooled serum. Other control and blank chromatograms are not included.

A large number of peaks were observed in the rat poisoned samples as compared to the control and pooled samples. We have not shown the BPCs of other control, blank and Ratol poison here. These peaks were reproducibly seen in all three replicates of the poisoned serum sample.



Sample Name	Compounds Present	Compounds Absent
ANF012022-r001	3428	2298
ANF012022-r002	3364	2362
ANF012022-r003	3336	2390
Blank_Treated-r001	831	4895
Blank_Treated-r002	844	4882
Blank_Treated-r003	784	4942
Indvl N-r001	2705	3021
Indvl N-r002	2701	3025
Indvl N-r003	2676	3050

Fig 2: Summary plot of all 5,726 aligned compounds observed across all samples. A large number of compounds were found with frequency of 3 (triplicate injections) as denoted here.

PCA Plot of the Samples

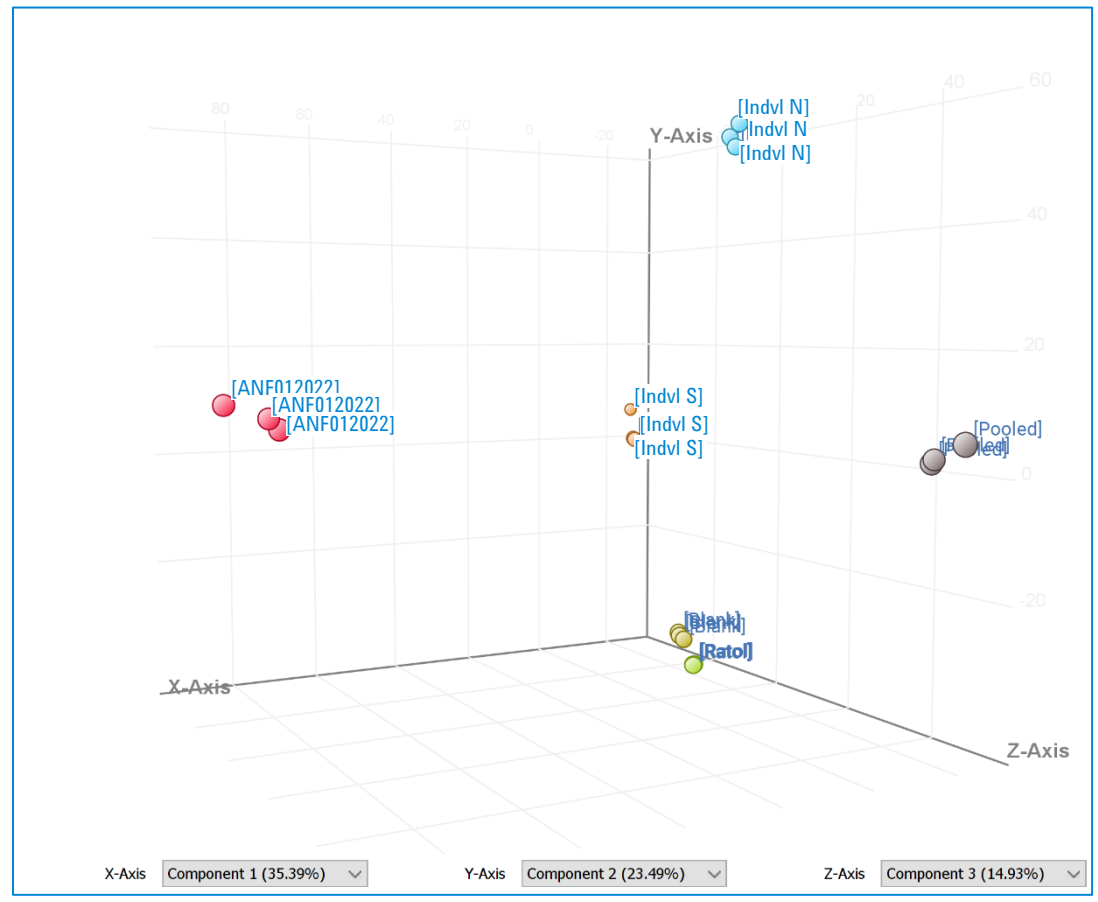


Fig 3: PCA plot on all 5,726 compounds for all replicates.

The PCA Plot reveals that all control samples (Pooled, Indvl_N and Indvl_S) are clustering together in the same plane. The rat poisoned serum sample (ANF012022) is found to be significantly different from the other groups.

The blank and rat poison (Ratol) were found to cluster together and away from all the serum samples. Good reproducibility is observed as all Triplicate injections were found to overlap.

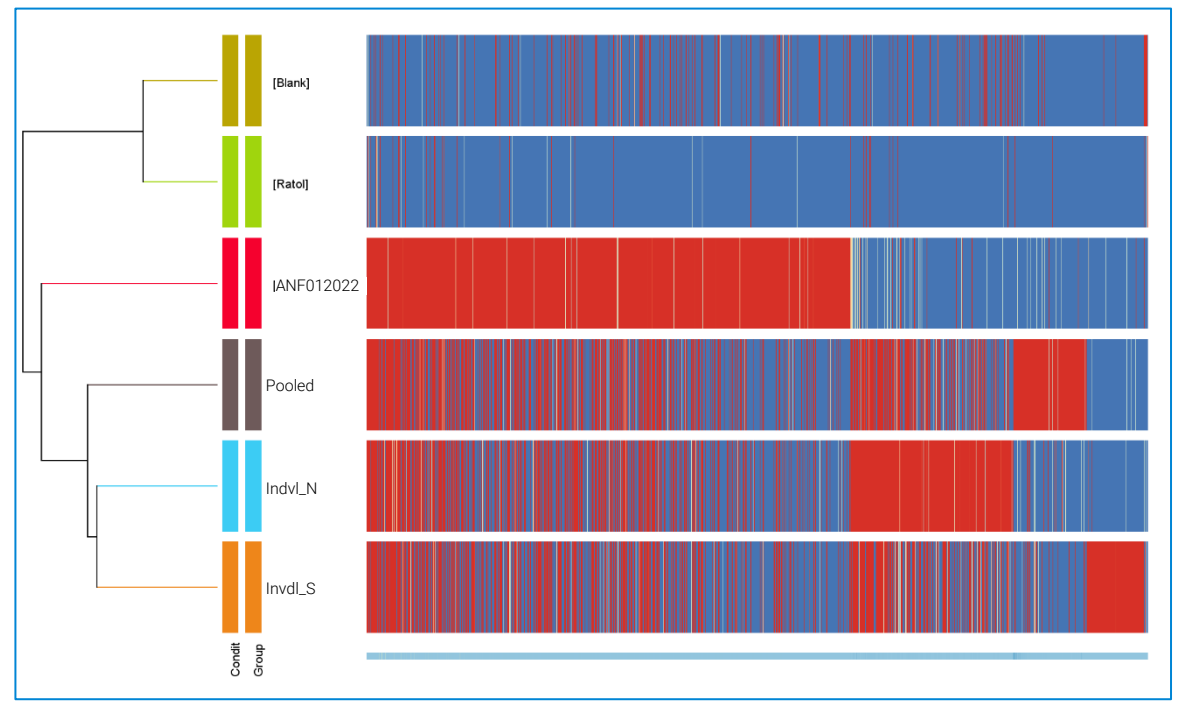


Fig 4: Clustering plot of all samples on all entities.

The Hierarchical Clustering plot based on all entities (5,726) or on all differentially expressed entities with fold change > 5 (4,335) both show that the rat poisoned serum sample is markedly different from the other serum samples.

Results and Discussion

Database Search and Pathway Analysis

Annotations were performed using a mass error window of 5 ppm against the custom Databases using the ID Browser utility in Mass Profiler Professional.

The annotated metabolites were mapped to pathways in MPP using Single Experiment Analysis.

Number of Pathways 102

Pathways

Pathway	Matched Entities(Ne...	Pathway Entities of ...	Organism
Hs_One-carbon_metabolism_and_related_pathways_...	11	41	Homo sapiens
Hs_Lidocaine_metabolism_WP2646_106722	4	8	Homo sapiens
Hs_Eicosanoid_metabolism_via_lipooxygenases_(LOX)...	4	40	Homo sapiens
Hs_Cysteine_and_methionine_catabolism_WP4504_1...	4	39	Homo sapiens
Hs_16p11.2_proximal_deletion_syndrome_WP4949_...	4	42	Homo sapiens
Hs_Glutathione_metabolism_WP100_107114	4	19	Homo sapiens
Hs_22q11.2_copy_number_variation_syndrome_WP4...	4	30	Homo sapiens
Hs_Melatonin_metabolism_and_effects_WP3298_120...	3	18	Homo sapiens
Hs_Heroin_metabolism_WP2645_95179	3	3	Homo sapiens

Fig 5: Pathway Entity inspector showing details of the matched pathways.

A total of 102 pathways were matched with all the entities exhibiting fold change > 5 . The most significant pathway was observed to be Central Carbon Metabolism. Lidocaine metabolism pathway was found to be highly implicated with 4 out of the 8 known metabolites matched from this pathway.

More than 30 pathways were found to be up-regulated in the rat poisoned sample as compared to the pooled serum sample. Melatonin metabolism was observed to be up-regulated with multiple matching metabolites.

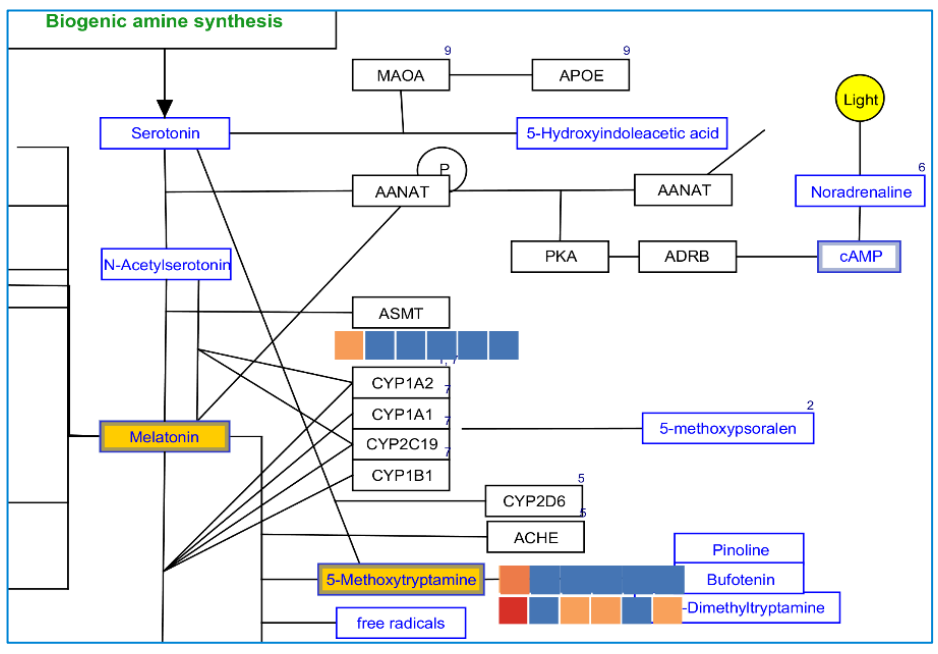


Fig 6: Screenshot of the Melatonin metabolism pathway with matching entities.

BioCyc Pathways also showed similar results with 153 pathways being matched to the entities exhibiting fold change > 5. Out of these 46 pathways were the ones where 2 or more matching compounds were found. Morphine Biosynthesis and Melatonin degradation were the pathways with most hits observed.

Although many compounds were found in rat poisoned serum, few were matched to the pathway database. This could be due to the heavy doses of the therapeutic compounds given during treatment.

Many of the abundant peaks in rat poisoned samples were found to match with pharmaceuticals and personal care compounds (data not shown).

Conclusions

The methodology highlighted in this study clearly establishes itself as a powerful tool to reveal novel insights into mechanism of action of rodenticides in humans.

- Melatonin degradation and Tryptophan metabolism were found to be greatly impacted.
- Including more Biological replicates will be essential to making robust conclusions.
- Orthogonal LC separation using HILIC chromatography will reveal more entities from the already implicated pathways or newer ones.

References

1) Sebastian Broecker et. al. Toxicological Screening with the Agilent 6500 Series Accurate-Mass Q-TOF LC/MS and the Personal Compound Database and Library using the Broecker, Herre and Pragst Accurate Mass Spectral Library. (Agilent Appication note 5990-6419EN).

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4) Gopalakrishnan S, Kandasamy S, Iyyadurai R. Rodenticide Poisoning: Critical Appraisal of Patients at a Tertiary Care Center. Indian J Crit Care Med. 2020 May;24(5):295-298. doi: 10.5005/jp-journals-10071-23426. PMID: 32728318; PMCID: PMC7358862.

https://explore.agilent.com/asms

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