LC-MS/MS Detects Urobilinoids

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OVERVIEW

PURPOSE: To develop a proper methodology to link flies to pathogen transmission via ingestion of infected feces

METHODS: Fly gut extract samples were analyzed via LC-MS/MS

RESULTS: Urobilin and urobilinogen were detected at a retention time of 6.5 min and 8.2 min respectively only in flies that fed on feces

INTRODUCTION

- No clear link stating flies transmit pathogens via ingestion of fecal matter.
- Most analysis are done via molecular biology techniques such as DNA sequencing or bacterial titers
  - Cumbersome
- No compounds found in literature that tie fly consumption of feces to pathogen transmission

METHODS

- Flies were fed based on control group
- Flies were killed, dissected, and DNA extractions were performed
- 50 µL aliquot of organic layer from DNA extraction was evaporated under nitrogen
- Resuspend in 50 µL of 1:1 methanol:water solution
- Vortex for 10 min
- Separate using Agilent 1100 HPLC system
- Identification onThermo Fisher LTQ XL™ Linear Ion Trap Quadrupole

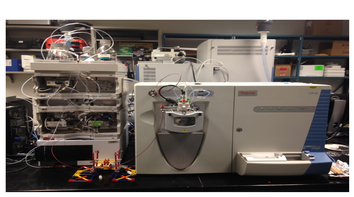
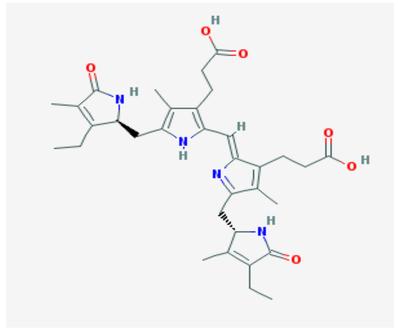


Figure 1: LC-MS instrument set up

RES

Urobilin
Molecular Formula: $C_{33}H_{42}N_4O_6$
Molecular Weight: 590.721 g/mol



Urobilinogen
Molecular Formula: $C_{33}H_{42}N_4O_6$
Molecular Weight: 590.721 g/mol

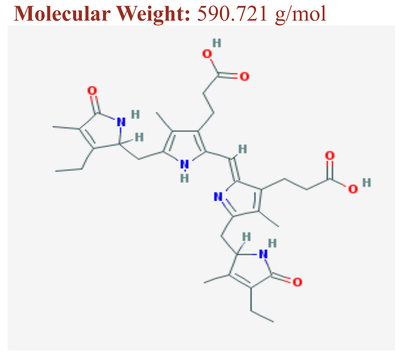


Figure 2: Structures for urobilin and urobilinogen

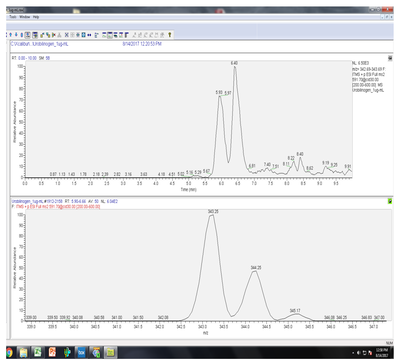


Figure 3A: Extracted ion chromatogram and mass spectra for urobilin

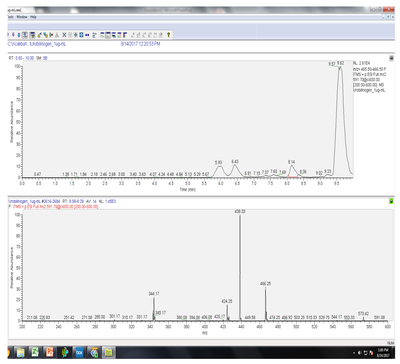


Figure 3B: Extracted ion chromatogram and mass spectra for urobilinogen

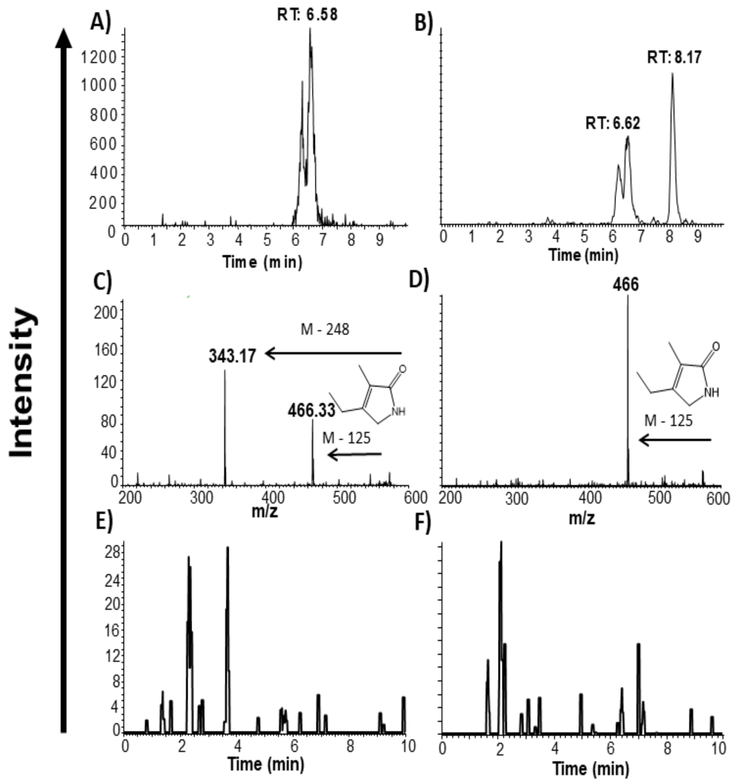


Figure 4: Extracted ion MS/MS chromatograms (XIC) and MS/MS spectra for fly samples (absolute intensities).

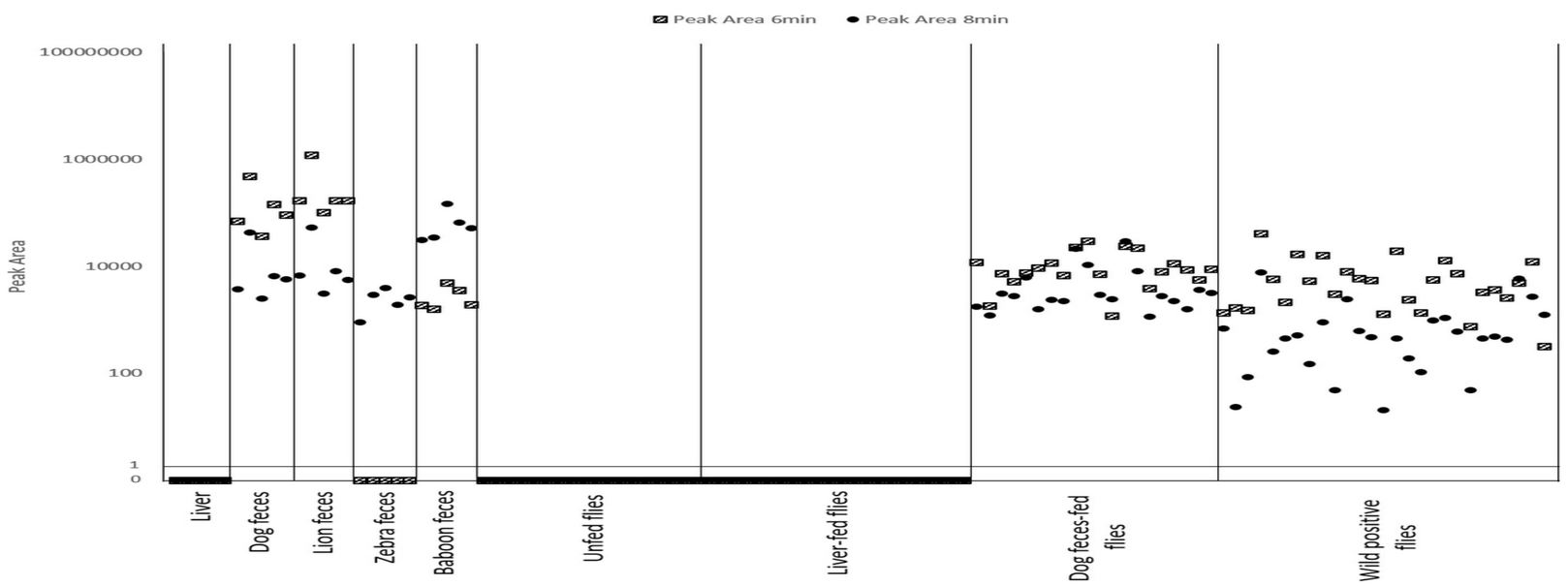


Figure 5: Scatterplot comparison of LC MS/MS 6.3 – 6.6 and 8.2 min peak area data for all tissue and fecal controls, as well as all experimental and wild flies

from Feces in Fly Guts

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ULTS

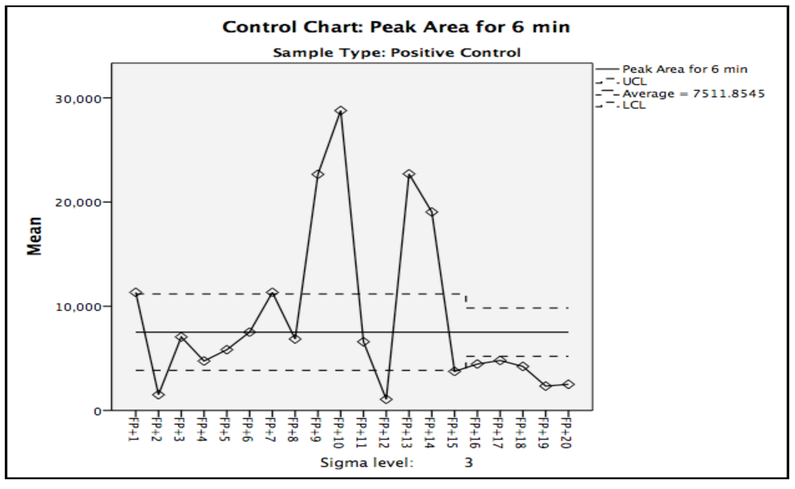


Figure 6A: Xbar control chart for the urobilin positive control samples (RT ~6.5 min) showing overall process variation

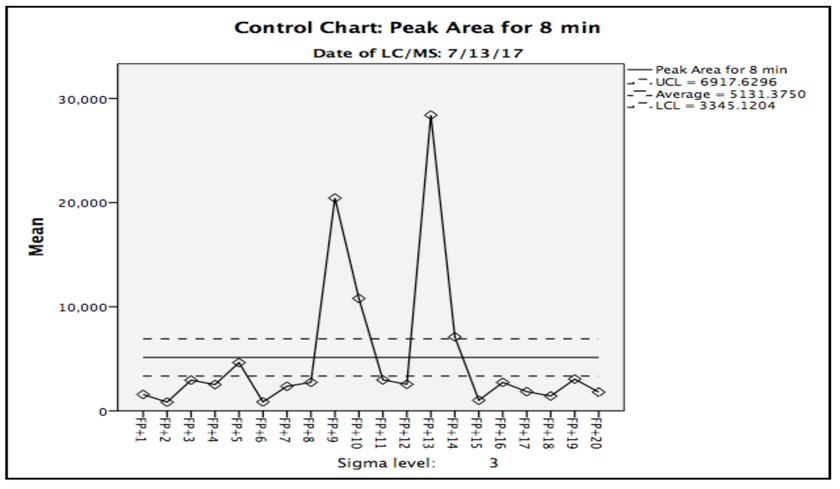


Figure 6C: Xbar control chart for the urobilinogen positive control samples (RT ~8.2 min) showing overall process variation

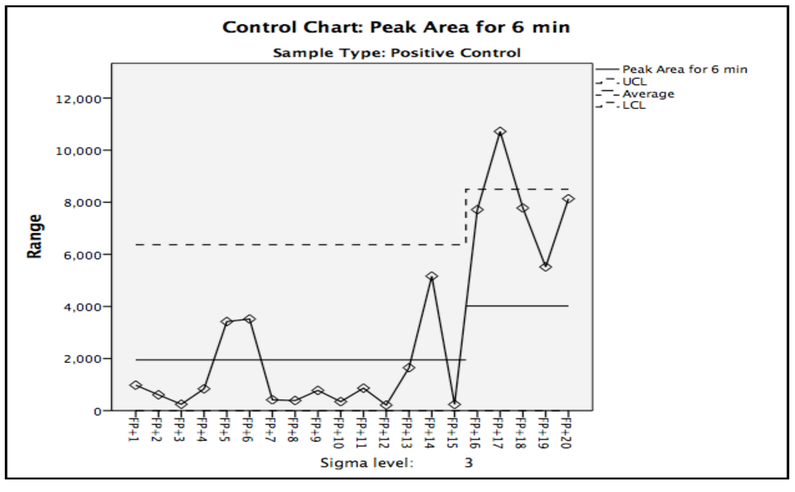


Figure 6B: Range control chart for the urobilin positive control samples (RT ~6.5 min) showing overall process variation

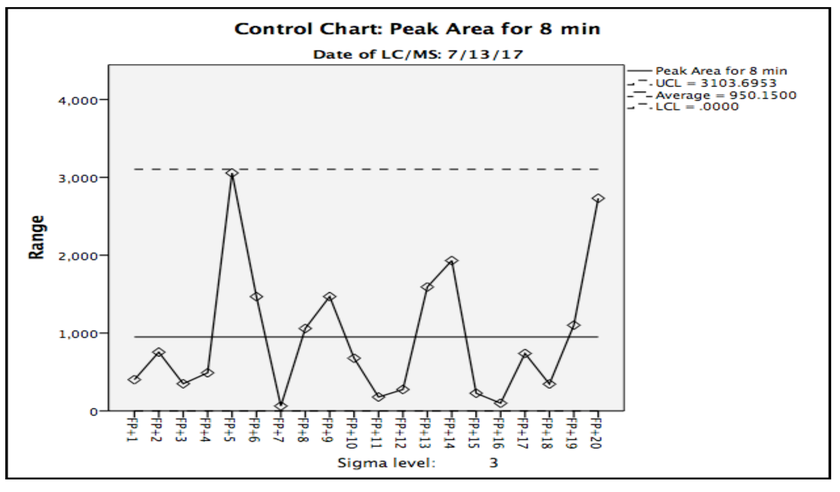
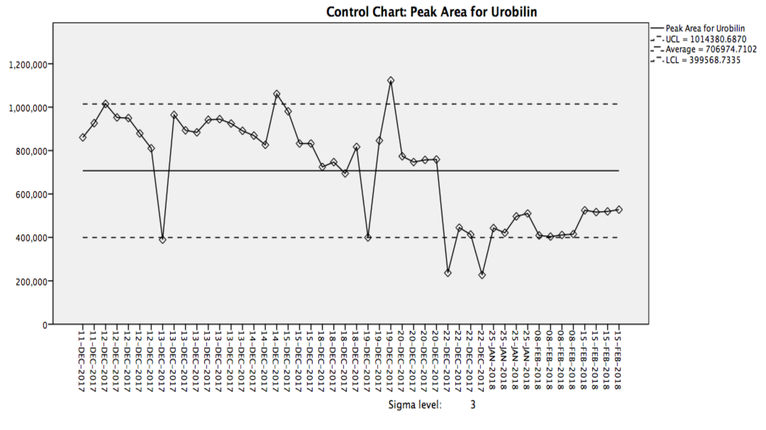


Figure 6D: Range control chart for the urobilinogen positive control samples (RT ~8.2 min) showing overall process variation



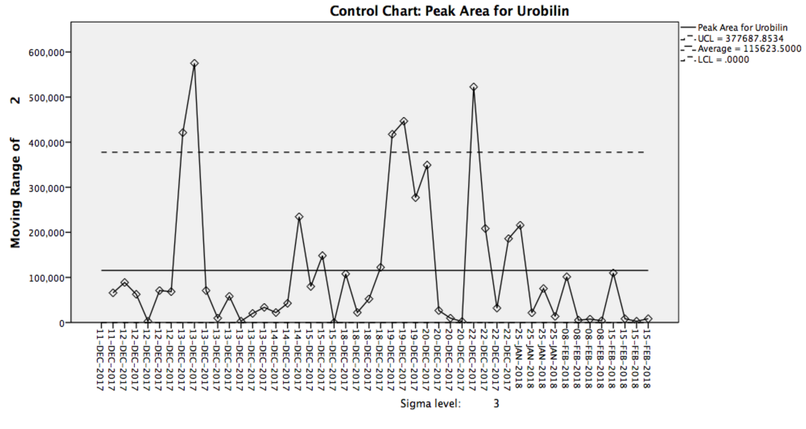


Figure 7: Individual moving range control charts of the peak areas for the urobilinoid standard showing overall instrument variation

CONCLUSIONS

Conclusions:

- LC MS/MS is a good qualitative test to detect fecal urobilinoids
- No false positives or negatives have been observed
- According to the Kruskal-Wallis test, there was a significant effects of treatments at 6 min (chi-squared = 72.8, df = 3, $P < 0.0001$) and 8 min (chi-squared = 78.3, df = 3, $P < 0.0001$)

Potential Problems:

- Many factors can impact the urobilinoid signal intensity
  - Feces consumed by the fly
  - Exposure of samples to light and air
  - Gender of the fly
- Overall instrument variation
- Difficult to get ”pure” urobilin standards
- Difficult to fully resolve urobilinoid isomers
  - Second peak at ~8.2 min helped with identification of these
- High variation among positive “control” samples
  - Standard was used to help with this

Future Directions:

- Apply method using multiple species of filth flies and other coprophagous insects
- Combine the method with microbial culturing and sequencing methods
- Combine the method with vertebrae sequencing methods to identify the source of pathogens

REFERENCES

[1] CG Owings, C Skaggs, W Sheriff, N Manicke, CJ Picard. Chemical assay for the detection of vertebrate fecal metabolites in adult blow flies (Diptera: Calliphoridae). Environmental Entomology

ACKNOWLEDGEMENTS

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