Can the casings of maggots or fly larvae reveal if a drug was present in the tissues the larvae fed on? A 'proof-of-concept' study.

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Introduction

Can drugs consumed by an individual prior to death be detected in fly detritus? This was the key question in my project.
A 'proof-of-concept' study was carried out using blowflies who fed on caffeine infused meat to see if caffeine was detectable in the fly detritus after a number of life cycles.

Background Information
In the later stages of tissue decay, maggots and their detritus are often found on or near the decaying tissue. If a dead body was stored for a period of time in one location and subsequently moved, perhaps only fly casings or detritus may be found at the original location.
Various factors affect fly larvae and development such as temperature and the presence of drugs and these had to be considered in the project design. I commenced this project earlier than my fellow students to ensure I had sufficient time for a number of blowfly lifecycles to occur and observe their development.

Blowfly raising station design

Fig 1 - Blowfly egg collection

Fig 2 - Meat on a tray in a fly raising station

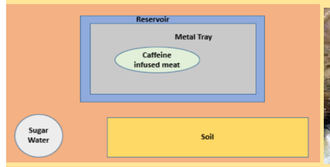

Fig 3 - Graphical representation of fly raising station

Fig 4 - Maggots which emerged over time

Fig 5 - Fly emerging from pupal casing of a maggot which had fed on caffeine infused meat

Fig 6 - Fly emerging from pupal casing of a maggot which fed the negative control meat

I designed a system to raise blowflies by initially catching two adult blowflies and getting them to lay eggs on a meat source. These eggs were deposited on caffeine infused meat and on a negative control meat sample. Both fly raising stations consisted of a metal tray, which I placed on a reservoir of paper to absorb any residual meat juices. I placed soil in the station to facilitate larvae pupation and also sugar water to give adult flies that emerged energy to continue their life cycles. Both stations were confined to separate areas and multiple blowfly life cycles were achieved. The temperature at both stations was kept above 15 degrees Celsius to ensure any developmental observations I made were due to the effect of caffeine and not due to cold temperatures which are known to affect fly development.

Extraction strategy

Mechanical breakdown of pupal casings / detritus
Addition of dichloromethane as solvent
Vortex, centrifuge and collection of supernatant
Evaporation of supernatant to near dryness
Addition of a suitable solvent or mobile phase prior to analytical analysis.

Thin Layer Chromatography Analysis

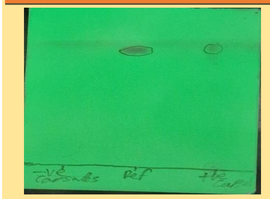

Fig 7 - TLC extracts from pupal casings (infused and negative control)

Fig 8 - TLC showing caffeine presence in adult fly, fly faeces and pupal casings

TLC system Methanol:Ammonia (100:1.5).
Caffeine was detected in fly pupal casings, faeces and adult flies and not in the negative control specimens (Visualization using UV 254 nm).

High Pressure Liquid Chromatography

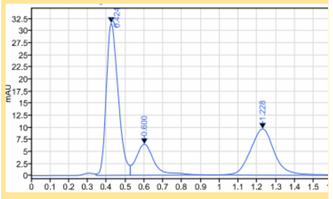

Fig 9 - Pupal casings extract from caffeine infused meat. Caffeine RT 1.2mins.

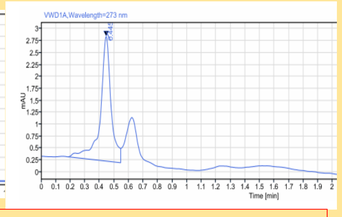

Fig 10 -Pupal casings extract of negative control.
No Caffeine detected

The HPLC mobile phase was Water : Methanol (60:40) with a UV detector set at 273nm. Caffeine was observed in the pupal casing extracts of the caffeine infused meat with a retention time of 1.2 minutes. No caffeine was detected in the negative control pupal casings extract. The peak RT 0.4 mins was identified as water while the peak at RT 0.6mins was identified as a possible protein within the pupal casings.

References used in my undergraduate project are available upon request.
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Gas chromatography Mass Spectroscopy

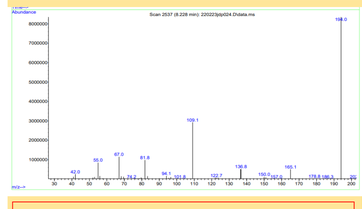

Fig 11 - Caffeine standard GC-MS spectra
RT 8.23 min
194m/z base peak

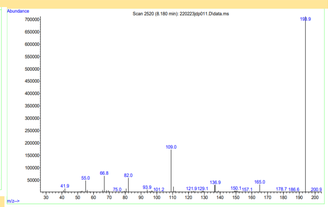

Fig 12 - GC-MS spectra of caffeine +ve pupal casings using SIM mode to search for m/z 194, initially.

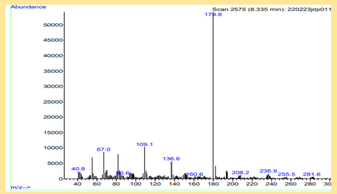

Fig 13 - GC-MS of theobromine in pupal casings NIST library match

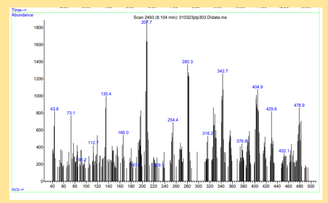

Fig 14 - GC-MS SIM mode 194m/z negative control pupal casings

Caffeine was present in the pupal casings of the specimens which fed on caffeine infused meat. Theobromine a metabolite of caffeine was also present in pupal casing extracts. The negative control specimens contained no caffeine or theobromine proving that cross contamination did not occur between specimens.

Observations on fly development stages

Life stage	Caffeine infused	Negative control
Egg	24hrs.	24hrs.
1st instar	108hrs. (4.5 days)	48hrs. (2 days)
2nd instar	144hrs. (6 days)	48hrs. (2 days)
3rd instar	192hrs. (8 days)	96hrs. (4 days)
Pupa	420hrs. (17.5days)	336hrs. (14days)
Total time	888hrs.(37 days)	552hrs. (23 days)

The total life cycle from egg to adult fly took approximately two weeks longer for the maggots which fed on caffeine infused meat compared to the negative controls. Additionally, fly pupal casings of the caffeine infused specimens were lighter in colour than the negative controls.

Discussion/Conclusion

Caffeine was detected by HPLC in pupal casings, faeces and adult flies. GC-MS detected caffeine in all positive specimens. Importantly theobromine a caffeine metabolite was detected in pupal casings and fly faeces. Caffeine was observed to slow down larval development. The core question which is of forensic interest can drugs be detected based on the tissues fly larvae fed on has been answered.