



K16 Amitriptyline and Morphine Determination in Larvae of *Lucilia Sericata* and Decomposed Liver Using LC-MS/MS

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After attending this presentation, attendees will understand the reliability of insect larvae as samples for toxicological investigations and the methods that were developed in analysis of drugs in larvae and liver samples.

This presentation will impact the forensic science community by providing the real toxicological evidence from corpse and larvae.

Analytical entomotoxicology is a basic new of forensic toxicology, where a few studies exist in literature. The goal of this study is to evaluate the use of insects as alternative specimens for toxicological evidence. For this purpose, larvae of *Lucilia sericata* were reared on samples of minced chicken liver treated with different concentrations of amitriptyline and morphine; regarding therapeutic, toxic, and potentially lethal doses. A method was developed for amitriptyline determination in larvae and liver and morphine detection in larvae. Amitriptyline and morphine was detected in all tested larvae samples, confirming the reliability of these specimens for qualitative toxicology analysis. Quantitative concentrations of amitriptyline measured in larvae were correlated with levels in liver tissue. The recoveries for morphine was not repeatable and the method could only be used to detect this drug qualitatively. These observations bring new elements regarding the potential use of drug analysis in larvae for estimation of drug levels in human tissues.

Introduction: Insect colonization patterns are the most common factors utilized for postmortem interval (PMI) estimation, especially when the discovery of the corpse is delayed and the soft tissues are decomposed. Diptera larvae feed on decomposed tissues containing chemical substances because of antemortem drug exposure. Because of this, the use of necrophagous insect specimens can be valuable as evidence for qualitative drug detection and sometimes quantitative drug determination, when the liver is almost completely decayed.

Materials and Methods: Breeding - Approximately 400 eggs of *Lucilia sericata* which is a common necrophage species of Diptera in Europe, were deposited on different concentrations of amitriptyline with homogenized tissues of chicken liver (250.0 g). Each chicken liver homogenate was spiked with different amitriptyline concentrations. C₁, C₂, C₃, C₄ were 500.00, 3000.00, 7000.00, 10000.00 ng/g, respectively.

The non-spiked blank liver was regarded as C₀. **Sampling** - At the end of the 117(±0.5) hours period (beginning from the egg phase to third feeding larvae phase), the larvae were collected from each liver, along with the corresponding liver sample for analysis. **Extraction procedure**- Approximately 0.500 g larvae and liver were homogenized in a 0.9% NaCl solution and an original LLE extraction procedure is developed. Organic layer of each sample was evaporated to dryness under N₂, reconstituted in methanol and analyzed by ESI LC-MS/MS. **Results and Discussion:** Amitriptyline and morphine were analyzed in this study. MRM method was developed with determined R_t and selected m/z values. The ions 286.1→201.1 for morphine and 278.0→91.0 for amitriptyline were monitored. The method was validated in terms of specificity, linearity, accuracy (recovery ≥82%), and precision and LOD and LOQ values were determined using Eurachem method. Validation results established that routine quantitative amitriptyline and qualitative morphine determination can be achieved in liver and larvae matrices. Also at this study; a formula was suggested for a back-calculation from the results that were obtained from the decomposed liver matrix and larvae collected at the end of the 117(±0.5) hours period, to the 0th hour liver concentration.

Entomotoxicology, Tricyclic Antidepressants, Opiates