## Original Article

# Validation and Quantification of Methamphetamine in Empty Puparia of *Lucilia sericata* (Diptera: Calliphoridae) Using Liquid-Liquid Extraction and Gas Chromatography-Mass Spectrometry

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## Abstract

**Background:** Entomotoxicology provides an alternative matrix for detecting drugs in decomposed human remains when conventional specimens are unavailable. This study aimed to develop and validate a quantitative method for detecting methamphetamine (MA) in different developmental stages of the blowfly *Lucilia sericata.*

**Methods:** Immature stages of *L. sericata* (larvae, pupae, empty puparia) were reared on chicken liver spiked with MA at concentrations of 45, 90 and 180 ng/mg. Samples underwent liquid–liquid extraction, derivatization via acetylation, and analysis using gas chromatography-mass spectrometry (GC-MS). The method was validated according to international guidelines, evaluating limit of detection (LOD), limit of quantification (LOQ), linearity, precision, accuracy and repeatability.

**Results:** The method showed strong linearity across the studied concentration range ($R^2$= 0.9817–0.9934). The limit of detection and LOQ were 15 ng/mg and 45 ng/mg, respectively, with a relative standard deviation (RSD)≤ 20% at the LOQ level. Intra- and inter-day precision showed RSD values< 10%, and accuracy ranged from 98.55% to 100.73%. Methamphetamine was detected in all immature stages and empty puparia. The highest observed concentrations were detected in third-instar larvae and pupae, whereas no drug residues were detected in adult flies. Methamphetamine retention in empty puparia supports their potential forensic relevance for retrospective drug detection.

**Conclusion:** This validated method demonstrated acceptable sensitivity, precision, accuracy and repeatability for quantitative entomotoxicological analysis. The detection of methamphetamine across insect developmental stages suggests the suitability of this approach as a supplementary tool for forensic investigations involving decomposed remains.

**Keywords:** Entomotoxicology; Liquid–liquid extraction; Method validation; Blow fly; Forensic entomology

## Introduction

Determining the cause of death is a crucial aspect of forensic investigations, particularly in cases where bodies are recovered in an advanced stage of decomposition (1). Under such conditions, conventional biological specimens such as tissues, body fluids and internal organs are often degraded or no longer available, making insects the only reliable alternative matrices for toxicological analysis (2, 3). Entomotoxicology, the study of toxins in necrophagous arthropods, has gained increasing attention after it was observed that drugs accumulated in fly larvae can alter their developmental rates. This finding is of considerable forensic importance since many fly species are used to estimate the post-mortem interval (PMI). Any alteration in



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their developmental timing due to drug exposure may thus influence PMI accuracy. Although the detection of drugs in necrophagous insects is generally accepted, the interpretation of such findings remains controversial. Unreliable results often arise from limited entomological knowledge, such as incomplete understanding of insect metabolism and feeding behavior, as well as from inadequate sensitivity of analytical instruments (4). Cases involving drug abuse, suicide, or delayed recovery of the body further complicate the scenario (1, 5).

In Entomotoxicological research, larvae are most frequently sampled because they are abundant and easily collected at the scene. However, drug concentration in larvae may vary depending on their developmental stage (first, second, or third instar) and feeding condition. After feeding, larvae leave the food source to pupate, forming puparia enclosed within the last larval cuticle (6). Once adult emergence occurs, only empty puparial cases remain near or on the corpse. These puparia are more difficult to locate because of their dark coloration and concealed position (2, 3). Nevertheless, they possess remarkable resistance to chemical degradation, microbial activity, and environmental stressors, and can persist long after adult emergence (7). Although the quantification of drugs in insect tissues has been achieved using sensitive analytical instruments (7–9), the relationship between drug concentrations in empty puparia and corresponding human or animal tissues remains unclear. This gap poses a major challenge in forensic interpretation. Drug pharmacokinetics in insects depend on several factors, including species, developmental stage, mode of action, absorption, redistribution, metabolism and chemical stability (10, 11).

Current research in this emerging field mainly focuses on the use of necrophagous arthropods as alternative toxicological specimens and on evaluating the effects of toxicants on their life cycles (12). Various analytical methods have been applied, including immunoassays, gas chromatography (GC) (13), and liquid chromatography (LC) (14). The coupling of LC with tandem mass spectrometry (MS-MS) enhances both sensitivity and selectivity while reducing preparation and analysis times (9). These advances enable the detection and quantification of drugs in single insects, improving the accuracy and precision of PMI estimation (14). Methamphetamine abuse has emerged as a major public health concern in many countries, particularly among younger populations (15, 16). In forensic toxicology laboratories, analyses of conventional biological matrices such as blood, urine and hair are routinely performed to detect and monitor drug use. However, alternative matrices, including oral fluid, have gained attention for their non-invasive collection and potential for real-time drug monitoring (16).

## Materials and Methods

### Fly experiments

*Lucilia sericata* colonies were maintained in the Calliphoridae breeding laboratory at the School of Public Health, Tehran University of Medical Sciences, under controlled temperature (28 °C) and relative humidity (50±10% RH). Fresh chicken liver served as the larval feeding substrate, and methamphetamine (98%) was obtained from the Tehran Province Forensic Medicine Organization. The rearing and extraction procedures were adapted from a previous qualitative study on *L. sericata*; however, the present study extends this approach through the validation of a quantitative analytical method for m;ethamphetamine determination in empty puparia.

To prepare the feeding substrate, chicken liver was homogenized in four separate blenders for 20 minutes to achieve uniform consistency. The resulting mince was divided into four portions (~1000 g each), and each portion was further subdivided into two 500 g subgroups, which were re-homogenized for 20 minutes. Methamphetamine, previously dissolved in 10 mL of saline, was added to achieve


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concentrations of 45, 90 and 180 ng/ mg. The control group received saline only (17).

Colonies were maintained in 30× 30× 30 cm insect cages under controlled environmental conditions. For oviposition, 150 g of chicken liver was provided as an oviposition substrate. Eggs were collected within 3 hours and assigned to four experimental conditions (three methamphetamine concentrations and a control), each performed in duplicate. Sampling was performed at regular intervals from each experimental group: every 6 hours from 12 to 50 hours, and every 12 hours from 50 to 168 hours. At each sampling time point, three independent biological replicates (~1 g each) were collected from each developmental stage (L1–L3 larvae, post-feeding larvae, pupae, adults and empty puparia) for GC-MS analysis. Each processed extract was injected into the GC–MS system in triplicate to assess analytical repeatability (17).

Samples for GC-MS analysis were stored at –20 °C until processing. Empty puparia were used as the primary matrix due to their high chitin content, persistence after adult emergence, and suitability for forensic toxicological studies (2, 18).

**Sample preparation for gas chromatography-mass spectrometry (GC-MS)**

Larval, pupal, and empty puparial samples were initially washed with dichloromethane ($CH_2Cl_2$) by gentle inversion for 5 minutes, after which the solvent was discarded. Samples were then rapidly frozen in liquid nitrogen and individually ground using a glass rod. Homogenized material (100 mg) was transferred to fresh 10 mL centrifuge tubes, and 2 mL of distilled water was added.

Samples were maintained at 4 °C for 48 hours before extraction to facilitate tissue softening and matrix disruption, following laboratory practice previously applied for biological tissue processing. Sodium chloride (0.5 g) was then added to induce a salting-out effect, followed by ultrasonic treatment for 30 minutes to enhance analyte release (17).

**Liquid–liquid extraction (LLE)**

Samples were adjusted to pH 6 using 2 mL of phosphate buffer and vortexed for 4 minutes, then gently mixed for another 4 minutes. After centrifugation (4000 rpm, 8 min), 50 µL of amantadine (internal standard, selected according to a previously validated laboratory protocol (17)) was added to 3 mL of the supernatant and vortexed for 4 minutes. The pH was raised to 14 using 1 mL of 10% NaOH solution, followed by thorough vortexing for 10 minutes. Ethyl acetate and hexane (1:1, v/v; 1.4 mL and 1 mL, respectively) were added, vortexed for 10 minutes, and centrifuged at 4000 rpm for 8 minutes. The aqueous phase was re-extracted with 2 mL ethyl acetate: hexane (1:1) to improve recovery. Combined extracts were dried under nitrogen at 45 °C and reconstituted in 1 mL of distilled water for derivatization (17).

**Derivatization with acetic anhydride**

A derivatization buffer was prepared by mixing 10 M KOH with saturated $NaHCO_3$ solution. For each sample, 500 µL derivatization buffer, 100 µL acetic anhydride and 1500 µL dichloromethane were added. Samples were vortexed for 10 minutes, centrifuged (3000 rpm, 10 min) and the lower organic layer was collected and dried at room temperature for 24 hours. Residues were reconstituted in 50 µL methanol, and 1 µL was injected into the GC-MS system (Agilent 5975 MS coupled to Agilent 6850 GC) using an HP-5MS column (30 m× 0.25 mm× 0.25 µm). Injection was splitless (1 µL), inlet temperature 250 °C, helium carrier gas (1.5 mL/min), oven program: 90 °C (1 min) → 280 °C at 20 °C/min (hold 5 min). Both the prepared samples and a methamphetamine standard reference compound were analyzed under identical GC–MS conditions. The MS was operated in both full scan (m/z 40–500) and selected ion monitoring (SIM) modes. For identification and confirmation, characteristic methamphetamine ions (m/z 58 and 100) were monitored (Fig. 1) (16).



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**Calibration curve**

Calibration standards were prepared in empty puparia spiked with methamphetamine at 0, 45, 67.5, 90, 135, 180 and 360 ng/mg. Intermediate calibration levels (67.5 and 135 ng/mg) were included between the nominal exposure concentrations (45, 90 and 180 ng/mg) to improve curve fitting across the analytical range.

**Method validation**

The LLE method was validated according to FDA bioanalytical guidelines and recent literature (18, 22, 23), evaluating LOD, LOQ, linearity, precision, accuracy and repeatability. The limit of detection (LOD) was defined as a signal-to-noise ratio (S/N) of 3, whereas LOQ was defined as the lowest spiked concentration meeting an RSD< 10–25% at S/N= 10. All analytical concentrations, including LOD and LOQ, were expressed on a matrix basis (ng/mg of sample). Linearity was assessed across the calibration range in the larval feeding substrate, whereas calibration standards were prepared in methamphetamine-spiked empty puparia to reflect the analytical matrix used for quantitative detection. Regression equations, $R^2$ and CV% were calculated. Inter- and intra-day precision and repeatability were determined using QC samples at multiple concentration levels over three consecutive days. Recovery experiments were performed using quality-control samples at multiple concentration levels to evaluate extraction efficiency and analytical performance.

## Results

Analysis of empty puparial cases of *L. sericata* using liquid–liquid extraction followed by acetylation derivatization and GC-MS confirmed the presence of methamphetamine. Representative chromatograms from treatment group 2 (90 ng/mg) showed retention times of 8.1 minutes for methamphetamine and 8.5 minutes for the internal standard, amantadine (Fig. 2).

**Linearity of mthamphetamine quantification in empty puparia**

Linearity of methamphetamine quantification in empty puparia was evaluated using triplicate analyses across the calibration range, and three independent calibration curves were constructed for Days 1–3. The regression equations and correlation coefficients demonstrated excellent linearity between methamphetamine concentration and GC-MS response. The coefficient of determination ($R^2$) values were 0.9891, 0.9934 and 0.9817 for Days 1, 2 and 3, respectively (Figs. 3–5). These results indicate consistent analytical performance across independent calibration sets.

**Limit of detection (LOD) and limit of quantification (LOQ)**

The limit of detection (LOD) and limit of quantification (LOQ) for methamphetamine in empty puparial cases were determined to be 15 ng/mg and 45 ng/mg, respectively. At the LOQ level, the relative standard deviation (RSD) was 8.75%, which was within the predefined acceptance criteria (RSD≤ 20%), indicating adequate sensitivity and reliable quantification performance of the method for this biological matrix.

**Accuracy and precision in empty puparia**

Accuracy and precision were evaluated using QC samples in intra-day and inter-day analyses. Results are summarized in Table 1.

**Repeatability and method reliability**

Repeatability was assessed using QC samples analyzed in triplicate over three consecutive days (n= 27). Relative standard deviation values were consistently low, indicating acceptable precision. Accuracy ranged from 98.55% to 100.73%, supporting the suitability of the method for quantitative analysis of methamphetamine in empty puparial cases of *L. sericata*.


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**Table 1.** Intra-day and inter-day accuracy and precision of methamphetamine quantification in the empty puparia of *Lucilia sericata* over three consecutive days

| MA Conc. (ng/mg, Nominal) | Day 1 | | | Day 2 | | | Day 3 | | |
|---|---|---|---|---|---|---|---|---|---|
| | SD | RSD (%) | Accuracy (%) | SD | RSD (%) | Accuracy (%) | SD | RSD (%) | Accuracy (%) |
| **45** | 1.3638 | 3.0 | 100.44 | 0.6236 | 1.37 | 100.73 | 0.894 | 2.0 | 98.97 |
| **90** | 0.8165 | 1.04 | 99.11 | 0.4082 | 0.51 | 100.38 | 0.4836 | 0.62 | 98.55 |
| **180** | 0.5715 | 0.51 | 99.11 | 1.2356 | 1.09 | 100.62 | 0.8165 | 0.72 | 99.55 |

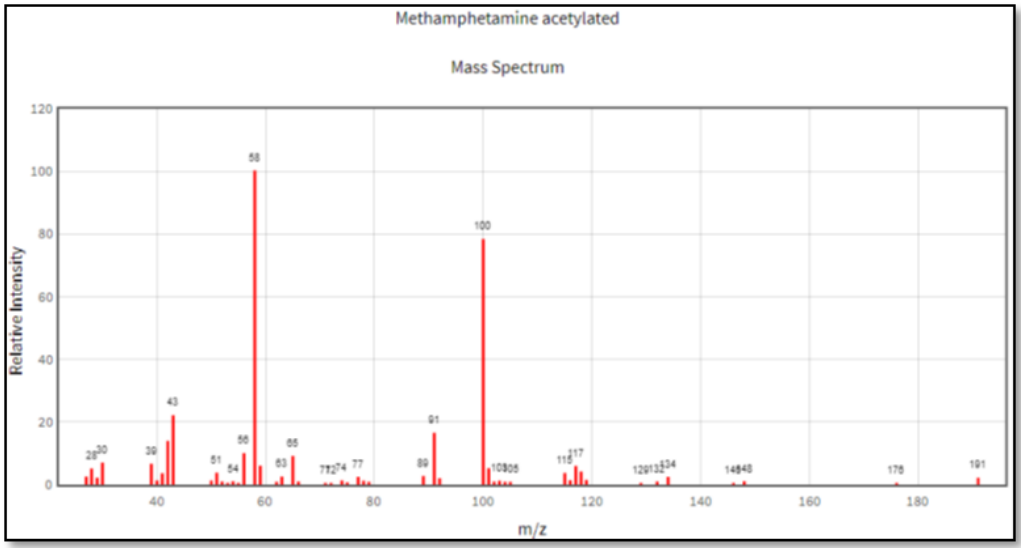


**Fig. 1.** Reference EI mass spectrum of derivatized methamphetamine standard obtained by GC–MS analysis. The characteristic fragment ions at m/z 58 and 100 were selected for methamphetamine identification and selected ion monitoring (SIM) in the analyzed samples

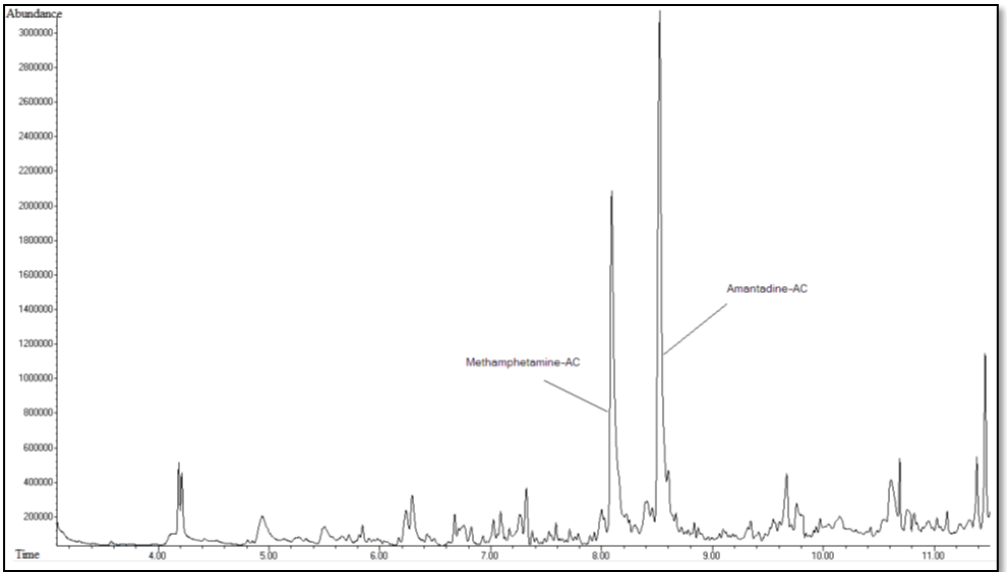


**Fig. 2.** Methamphetamine and amantadin chromatograms generated by GS-MS. Retention time (minutes) of each peak is marked below the chromatogram. Chromatograms derived from *Lucilia sericata* empty pupa samples that were fed on chicken liver mince treated with 90 ng/mg MA


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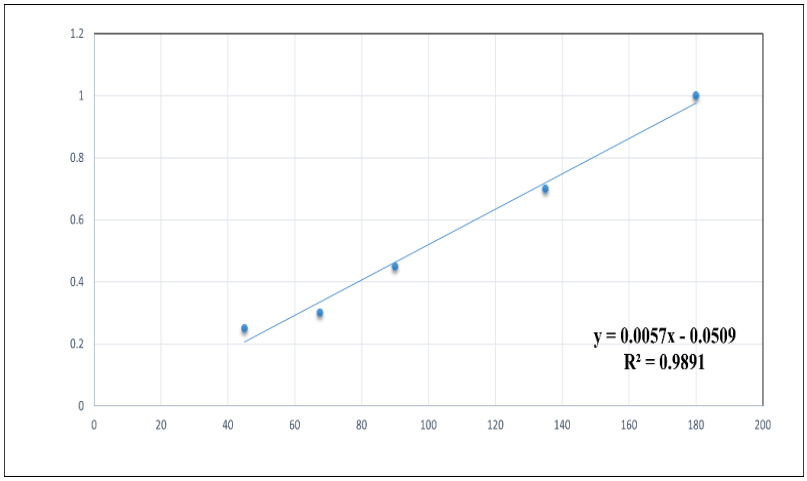


**Fig. 3.** Calibration curve of methamphetamine in the empty puparia of *Lucilia sericata* on Day 1 showing linear regression between concentration (ng/mg) and GC–MS response, with correlation coefficient ($R^2$= 0.9891)

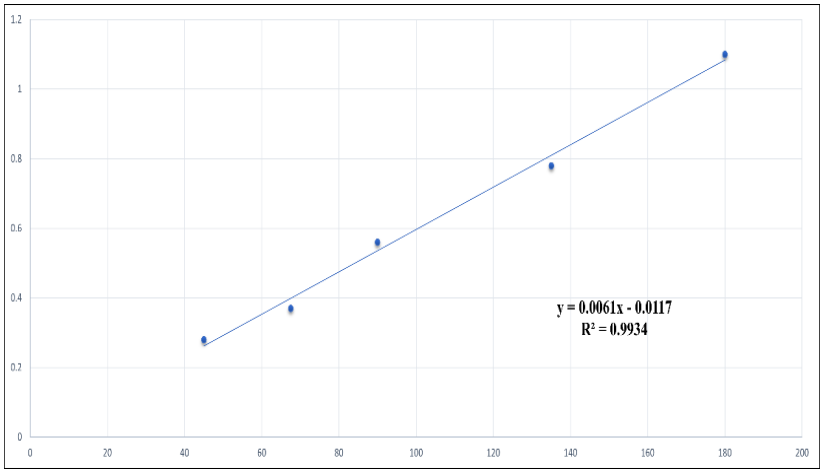


**Fig. 4.** Calibration curve of methamphetamine in the empty puparia of *Lucilia sericata* on Day 2 showing linear regression between concentration (ng/mg) and GC–MS response, with correlation coefficient ($R^2$= 0.9934)

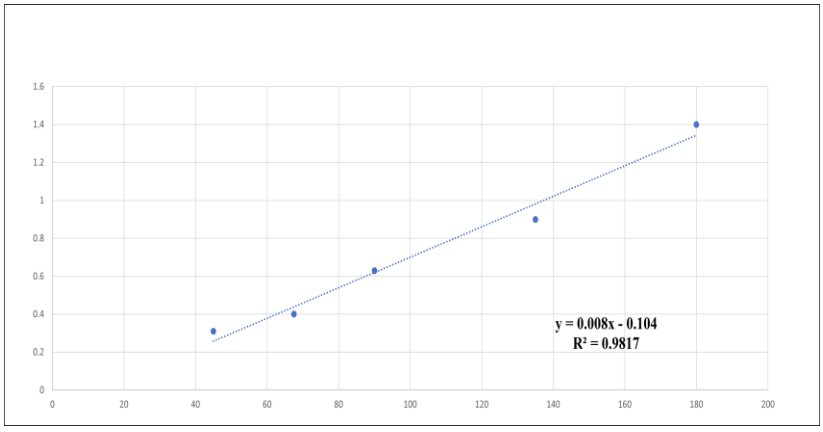


**Fig. 5.** Calibration curve of methamphetamine in the empty puparia of *Lucilia sericata* on Day 3 showing linear regression between concentration (ng/mg) and GC–MS response, with correlation coefficient ($R^2$= 0.9817)


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## Discussion

Entomotoxicology evaluates the effects of drugs on insects and also employs insects as an alternative biological matrix for toxicological analysis (8, 19). Compared to traditional matrices such as blood or urine, the toxicological analysis of highly decomposed bodies is more practical using insects, as interference from decomposition is considerably reduced (20, 21).

In entomotoxicology studies, the use of drug-treated meat presents an operational advantage over methamphetamine-treated animal models. This approach enables precise control of drug concentration in the meat before larval feeding. In contrast, in live animals such as drug-injected rats or rabbits, the amount of drug accessible to larvae is highly unpredictable, varying with factors including the animal's metabolic processes, other physiological variables, and environmental conditions (20).

Using drug-prepared meat ensures a fixed initial concentration, thereby allowing accurate quantitative comparison of pre- and post-feeding results across an adequate number of replicates. Although live animals may provide more realistic models that closely simulate forensic conditions, previous studies have shown that establishing a clear correlation between drug concentrations in human or animal tissues and the levels detected in feeding larvae remains challenging due to the complexity and variability of multiple influencing factors (2, 20).

Gosselin et al. (2) reported that larvae exhibited fewer matrix effects compared to putrefied human tissues when analyzing the opioid methadone. Similarly, other studies have noted that the analysis of insect samples may provide more sensitive results with reduced interference from decomposition by-products. Collectively, these and other investigations highlight the potential of larval analysis as a complementary tool for detecting a range of chemicals ingested by the deceased, particularly in situations where human remains are scarce, severely decomposed, or both (22, 23).

The detectability of chemicals within larvae is influenced by multiple factors. Foremost, the larval feeding site determines the specific compounds to which the larvae are exposed and subsequently ingest. In addition, the developmental stage of the larvae influences the extent of chemical accumulation, as metabolic activity and tissue composition change throughout larval growth. Together, these variables shape both the types and concentrations of chemicals that can be reliably detected in larval specimens (24, 25). Furthermore, it is essential to consider the physicochemical characteristics and pharmacokinetic properties of the drug in both humans and larvae.

In this study, larval samples were extracted using a liquid–liquid extraction method, and derivatization was applied to these samples via an acetylation-based approach. This method appears practical and may represent an improvement over the previous technique. A rapid, cost-effective and efficient protocol was developed for the extraction and preparation of samples for the analysis of amantadine-related compounds, particularly methamphetamine, using derivatization with acetic anhydride. Moreover, quantitative analysis based on this derivatization method was performed in larval matrices.

To date, four studies in entomotoxicology have examined the effects of methamphetamine (MA) on necrophagous flies; however, no previous research has specifically investigated the presence or effects of MA in *L. sericata* (17, 26–28). The effects of methamphetamine (MA) on the development rate of *Phormia ruficornis* larvae were investigated by Lee Goff et al. (26) employing radioimmunoassay (RIA). Because Mullany et al. (28) did not include quantitative data for *Calliphora stygia* reared on MA-spiked sheep liver, the study by Magni et al. (27) may be useful for comparison of MA concentrations in blowflies. Larvae of *Calliphora vomitoria* fed homogenized liver containing the drug showed


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MA levels of 5 and 10 ng/mg, according to Magni et al. (27).

The procedure was validated in accordance with international recommendations (29), and in the current work, MA was identified using GC-MS following liquid–liquid extraction (LLE). In line with Wang et al. (17), MA was detected in all juvenile stages and empty puparia of *L. sericata*, but not in adults. Upon emergence, adult flies rapidly eliminate drug residues acquired during immature stages (30). All treatments showed peak methamphetamine concentrations in third-instar (L3) larvae, likely due to physiological characteristics of this stage, such as the crop, a laterally diverging and enlarged diverticulum of the esophagus (31). A substantial portion of the drug was primarily localized within the puparial cuticle, which may explain the detection of methamphetamine in EP, with concentrations comparable to the pupal stage (P) (32).

The present validation was performed exclusively on EP, based on both analytical and forensic considerations. The chitinous puparial structure is chemically stable and resistant to enzymatic or microbial degradation, providing a reproducible, interference-free matrix for GC-MS analysis. In forensic scenarios, EPs are often the only entomological evidence recovered from decomposed remains, as adult flies have dispersed and soft tissues have disintegrated. Previous studies demonstrated that a significant portion of methamphetamine and other xenobiotics remains trapped within the puparial cuticle even after adult emergence (32, 33). Our previous qualitative study reported the detection of methamphetamine residues in puparial exuviae of *L. sericata*. Building upon that work, the present research quantitatively validated the analytical method using EP, demonstrating methodological robustness and highlighting the forensic relevance of puparial remains for retrospective drug detection (33).

The development of specific and validated analytical methods for detecting drugs and toxins in necrophagous samples underscores the importance of selecting analytical techniques appropriate for the physicochemical properties of the substances and the required levels of sensitivity and selectivity. Moreover, the choice of sample preparation and extraction methods critically influences detection reliability. To date, entomological samples have been either processed using protocols adapted from conventional toxicological matrices (for example, visceral tissues and fluids) or subjected to extraction methods similar to those applied to hard tissues, such as nails (34, 35).

Reliability, and overall robustness. Since necrophagous insects differ substantially in composition and morphology from typical toxicological samples, applying methods developed for human or animal tissues may lead to less accurate interpretations. Given the importance of entomological evidence in criminal investigations (36), the use of inappropriate analytical methods may raise concerns regarding the validity of findings, potentially affecting the admissibility of evidence in court (37). The validated method presented in this study provides supportive entomotoxicological evidence, contributing to the assessment of methamphetamine exposure.

## Conclusions

Insects can provide valuable insights into a person’s drug use before death, as drugs ingested by the deceased may be detected in arthropods feeding on the body. The validated method developed in this study demonstrates potential applicability in entomotoxicology, providing supportive evidence for the assessment of methamphetamine exposure. It showed acceptable sensitivity, precision, accuracy and repeatability according to international validation standards. The detection of methamphetamine across different insect developmental stages suggests the suitability of the method for forensic applications. Overall, this approach may serve as a supplementary tool in entomo-


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toxicology, improving the detection of drugs in decomposed remains and supporting forensic investigations.

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## Ethical consideration

This study was approved by the Research Ethics Committee of Tehran University of Medical Sciences (TUMS) under the code IR.TUMS.SPH.REC.1399.027IR.

## Conflict of interest statement

The authors declare there is no conflict of interest.

## References

1. Boulkenafet F, Dob Y, Karroui R, Al-Khalifa M, Boumrah Y, Toumi M, Mashaly A (2020) Detection of benzodiazepines in decomposing rabbit tissues and certain necrophagic dipteran species of forensic importance. Saudi J Biol Sci. 27 (7): 1691–1698.
2. Gosselin M, Di Fazio V, Wille SM, Fernandez MdMR, Samyn N, Bourel B, Rasmont P (2011) Methadone determination in puparia and its effect on the development of *Lucilia sericata* (Diptera, Calliphoridae). Forensic Sci Int. 209(1–3): 154–159.
3. Badenhorst R, Villet M (2018) The uses of *Chrysomya megacephala* (Fabricius, 1794) (Diptera: Calliphoridae) in forensic entomology. Forensic Sci Res. 3(1): 2–15.
4. da Silva EI, Wilhelmi B, Villet M (2017) Forensic entomotoxicology revisited-towards professional standardisation of study designs. Int J Legal Med. 131(5): 1399–412.
5. Archer MS, Elgar MA, Briggs CA, Ranson D (2006) Fly pupae and puparia as potential contaminants of forensic entomology samples from sites of body discovery. Int J Legal Med. 120(6): 364–368.
6. Charabidze D, Bourel B, Morvan G, Hedouin V, Gosset D (2008) ForenSeek, un programme de simulation et d'expertise dédié à l'entomologie médico-légale. J Med Leg Droit. 51(3): 131–140.
7. Reiter C, Wollenek G (1985) Status of disintegration and forensic value of empty fly pupae. J Archiv fur Kriminologie. 175 (1–2): 47–56.
8. Introna J, Dico CH, Caplan YH, Smialek J (1990) Opiate analysis in cadaveric blowfly larvae as an indicator of narcotic intoxication. J Forensic Sci. 35(1): 118–122.
9. Wood M, Laloup M, Pien K, Samyn N, Morris M, Maes R, De Bruijn EA, Maes V, De Boeck G (2003) Development of a rapid and sensitive method for the quantitation of benzodiazepines in *Calliphora vicina* larvae and puparia by LC-MS-MS. J Anal Toxicol. 27(7): 505–512.
10. Rashid RA, Osman K, Ismail MI, Zuha RM, Hassan RA (2008) Determination of malathion levels and the effect of malathion on the growth of *Chrysomya megacephala* (Fibricius) in malathion-exposed rat carcass. Trop Biomed. 25(3): 184–190.
11. Charabidze D, Gosselin M, Hedouin V (2017) Use of necrophagous insects as evidence of cadaver relocation: myth or reality? Peer J. 5: e3506.
12. Boulkenafet F (2016) Characterization of necrophagous insects, their usefulness in



http://jad.tums.ac.ir
Published Online: March 31, 2026

forensic medicine and judicial investigations. [PhD dissertation]. Entomology, Université des Frères Mentouri Constantine, Constantine, Algeria.
13. Kintz P, Tracqui A, Mangin P (1994) Analysis of opiates in fly larvae sampled on a putrefied cadaver. J Forensic Sci Soc. 34 (2): 95–97.
14. Lee Goff M, Miller ML, Paulson JD, Lord WD, Richards E, Omori A (1997) Effects of 3, 4-methylenedioxymethamphetamine in decomposing tissues on the development of *Parasarcophaga ruficornis* (Diptera: Sarcophagidae) and detection of the drug in postmortem blood, liver tissue, larvae, and puparia. J Forensic Sci. 42(2): 276–280.
15. Wu LT, Woody GE, Yang C, Li JH, Blazer DG (2011) Recent national trends in *Salvia divinorum* use and substance-use disorders among recent and former *Salvia divinorum* users compared with nonusers. Subst Abuse Rehabil. 4(2): 53–68.
16. Bahmanabadi L, Akhgari M, Jokar F, Sadeghi H (2017) Quantitative determination of methamphetamine in oral fluid by liquid–liquid extraction and gas chromatography/mass spectrometry. Hum Exp Toxicol. 36(2): 195–202.
17. Wang S, Zhang C, Chen W, Ren L, Ling J, Shang Y, Guo Y (2020) Effects of methamphetamine on the development and its determination in *Aldrichina grahami* (Diptera: Calliphoridae). J Med Entomol. 57 (3): 691–696.
18. Sukontason KL, Ngern-Klun R, Sripakdee D, Sukontason K (2007) Identifying fly puparia by clearing technique: application to forensic entomology. J Parasitol Res. 101(5): 1407–1416.
19. Goff ML, Lord WD (1994) Entomotoxicology: a new area for forensic investigation. Am J Forensic Med Pathol. 15(1): 51–57.
20. Ishak N, Ahmad AH, Mohamad Noor SA, Ahmad A (2019) Detection of heroin metabolites at different developmental stages of *Lucilia cuprina* (Diptera: Calliphoridae) reared in heroin-treated meat: a preliminary analysis. Egypt J Forensic Sci. 9: 65.
21. Kharbouche H, Augsburger M, Cherix D, Sporkert F, Giroud C, Wyss C, Champod Ch, Mangin P (2008) Codeine accumulation and elimination in larvae, pupae, and imago of the blowfly *Lucilia sericata* and effects on its development. Int J Legal Med. 122(3): 205–211.
22. Kintz P, Tracqui A, Ludes B, Waller J, Boukhabza A, Mangin P, Lugnier AA, Chaumont, AJ (1990) Fly larvae and their relevance in forensic toxicology. Am J Forensic Med Pathol. 11(1): 63–65.
23. de Aguiar França J, Brandao M, Sodré FF, Caldas ED (2015) Simultaneous determination of prescription drugs, cocaine, aldicarb and metabolites in larvae from decomposed corpses by LC–MS–MS after solid–liquid extraction with low temperature partitioning. Forensic Toxicol. 33(1): 93–103.
24. Sadler D, Fuke C, Court F, Pounder D (1995) Drug accumulation and elimination in *Calliphora vicina* larvae. Forensic Sci Int. 71(3): 191–197.
25. Nolte KB, Pinder RD, Lord WD (1992) Insect larvae used to detect cocaine poisoning in a decomposed body. J Forensic Sci. 37(4): 1179–1185.
26. Lee Goff M, Brown WA, Omori A (1992) Preliminary observations of the effect of methamphetamine in decomposing tissues on the development rate of *Parasarcophaga ruficornis* (Diptera: Sarcophagidae) and implications of this effect on the estimations of postmortem intervals. J Forensic Sci. 37(3): 867–872.
27. Magni PA, Pacini T, Pazzi M, Vincenti M, Dadour IR (2014) Development of a GC–MS method for methamphetamine detection in *Calliphora vomitoria* L.(Diptera:


http://jad.tums.ac.ir
Published Online: March 31, 2026

Calliphoridae). Forensic Sci Int. 241: 96–101.
28. Mullany C, Keller PA, Nugraha AS, Wallman JF (2014) Effects of methamphetamine and its primary human metabolite, p-hydroxymethamphetamine, on the development of the Australian blowfly *Calliphora stygia*. Forensic Sci Int. 241: 102–111.
29. Raposo F (2016) Evaluation of analytical calibration based on least-squares linear regression for instrumental techniques: A tutorial review. Trends Anal Chem. 77: 167–185.
30. Norris K (1965) The bionomics of blow flies. Annu Rev Entomol. 10(1): 47–68.
31. Boonsriwong W, Sukontason K, Olson JK, Vogtsberger RC, Chaithong U, Kuntalue B, Ngern-Klun R, Upakut S, Sukontason K (2007) Fine structure of the alimentary canal of the larval blow fly *Chrysomya megacephala* (Diptera: Calliphoridae). J Parasitol Res. 100(3): 561–574.
32. Bourel B, Tournel G, Hedouin V, Deveaux M, Goff ML, Gosset D (2001) Morphine extraction in necrophagous insects remains for determining ante-mortem opiate intoxication. Forensic Sci Int. 120(1–2): 127–131.
33. Parkhideh SZ, Forouzesh M, Akhgari M, Bahmanabadi L, Jokar F, Valiyari S, Akbarzadeh K, Rahimi S, Alimohammadi AM, Rafizadeh S, Rassi Y (2023) Preliminary analysis of methamphetamine detection in *Lucilia sericata* (Diptera: Calliphoridae) reared in methamphetamine-treated meat at various developmental stages. J Arthropod-Borne Dis. 17(3): 229–240.
34. Introna F, Campobasso CP, Goff ML (2001) Entomotoxicology. Forensic Sci Int. 12 (2): 42–47.
35. Campobasso CP, Gherardi M, Caligara M, Sironi L, Introna F (2004) Drug analysis in blowfly larvae and in human tissues: a comparative study. Int J Legal Med. 118 (4): 210–214.
36. Mahat N, Zulkifli N, Abdul-Wahab R, Nordin H (2019) Evidential values of insects in crime investigations. In Mahat NA and Huri MAM (Eds.), *Scientific approaches to crime investigations*. Penerbit UTM Press, Malaysia. pp. 37–89.
37. Sari SA, Muda NW, Mohamed Huri MA, Abdul Keyon AS, Azman AR, Mahat NA (2023) Analysis of poisons and drugs in entomological specimens for forensic applications: a review. Arab J Basic ApplSci. 30(1): 401–428.


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