Original Article

Preliminary Analysis of Methamphetamine Detection in *Lucilia sericata* (Diptera: Calliphoridae) Reared in Methamphetamine-Treated Meat at Various Developmental Stages

Seyedeh Zahra Parkhideh[1], Mehdi Forouzesh[2], Maryam Akhgari[2], Leila Bahmanabadi[2], Farzaneh Jokar[2], Samira Valiyari[2], Kamran Akbarzadeh[1], Sara Rahimi[3], Ali Mohammad Alimohammadi[2], Sayena Rafizadeh[4], *Yavar Rassi[1]

[1]Department of Vector Biology and Control of Diseases, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
[2]Legal Medicine Research Center, Legal Medicine Organization, Tehran, Iran
[3]Medicinal Plants Research Center, Maragheh University of Medical Sciences, Maragheh, Iran
[4]Ministry of Health and Medical Education, Tehran, Iran

***Corresponding author:** Prof Yavar Rassi, E-mail: rassiy@tums.ac.ir

(Received 07 May 2023; accepted 13 June 2023)

Abstract

Background: It is possible to identify drugs and poisons present in cadavers by analyzing blowfly larvae and pupae collected during forensic autopsies. The main purpose of this study was to use *Lucilia sericata's* larvae and pupae to identify drugs and poisons present in human cadavers.

Methods: In an investigation, immature *L. sericata* fed meat treated with methamphetamine (MA) at various concentrations (45, 90, and 180 ng/mg) were analyzed to detect MA. Acetylation derivatization and liquid-liquid extraction (LLE) were used as sample preparation methods prior to gas chromatography-mass spectrometry (GC-MS) analytical instrumentation to find MA.

Results: According to this study, *L. sericata* can be used in toxicological testing to identify MA in a host body. All *L. sericata* larval stages, particularly the third stage larva, pupa and empty pupa tested were positive for MA. Larvae in their first instar produced weak peaks. The *L. sericata* post-feeding instar following the 45 ng/mg treatment showed the highest MA concentration. For the first time, derivatization using the acetylation approach was used to prepare samples, and successfully, excellent results were obtained.

Conclusion: Low quantities of MA can be easily found in immature fly samples using GC-MS. It is important to analyze all samples including human tissues and insect samples, for postmortem drug testing. They can be utilized to find entire MA before they are excreted in excretory samples such as urine. Also, third instar larvae are a great and reliable sample for toxicological study.

Keywords: Entomotoxicology; *Lucilia sericata*; Methamphetamine; Drug testing; GC-MS

Introduction

Autopsies performed on decomposed or even exhumed bodies can provide crucial information about the history of drug use and cause of death. In these situations, forensic toxicology practitioners examine how drugs or poisons affect the rate at which scavengers colonize the scene (1, 2).

Each stage of a human corpse's decomposition is accompanied by a certain insect, from flies to beetles to moths towards the end. However, flies from the family Calliphoridae, particularly those from the subfamilies Chrysomyinae, Calliphorinae, and Lucillinae, are the most significant insects in this process. One of the first insects to visit human cadaver to lay eggs are the specimens of *L. sericata* flies, following egg hatching, the offspring eat dead tissue (3, 4).

After eating the infested tissue, the larvae act as a drug depot. In situations where viable or available host tissue samples cannot be ob-

http://jad.tums.ac.ir
Published Online: Sep 30, 2023

tained, they can be employed in toxicity studies for drug identification. (5).

It is widely known that drug buildup in fly larvae can influence body weight and height fluctuations as well as the typical duration of their life cycle (6, 8). For example, morphine and heroin can delay pupal development while accelerating larval development (9).

In addition to evaluating how medications affect insects, entomotoxicology also employs insects as an alternative type of matrix (10). Insects are a better choice for toxicological investigations on highly decomposed bodies than standard matrices (blood, urine), as there are fewer decomposition-related disruptions. (10).

Drugs including Amitriptyline (11), Phenobarbital (12), Lorazepam (13), Amphetamine (14), Cocaine (15), Codeine (16), Morphine (17), and Methadone (18) have all been detected at various stages of fly development.

Larval specimens are only useful for drug detection in a forensic case if they are taken alive and stored correctly to prevent the production of too many putrefactive fluids during the process of their own decomposition. If these conditions are not met because the sample is tainted with larval rotting fluid, the results of the forensic toxicity study of dead larval tissue will be skewed (19, 20).

We conducted preliminary research to identify the Methamphetamine (MA) in *L. sericata* reared on meat treated with Methamphetamine at three different doses at each developmental stage. This study adopted an unconventional methodology, using meat that had been exposed to methamphetamine as a model rather than an animal.

Materials and Methods

Fly experiments

In the breeding laboratory of Calliphoridae flies at the School of Public Health, Tehran University of Medical Sciences, *L. sericata* fly culture was done in a temperature and humidity-controlled environment (28 °C and 50±10% RH).

Fresh chicken liver was employed to feed *L. sericata* larvae in this investigation. The Tehran Province Forensic Medicine Organization provided 98% Methamphetamine.

The following were the steps to produce minced chicken liver: First, chicken liver was blended in four separate blenders for 20 minutes to ensure that the mince was homogenized evenly. Next, the mince was separated into four equal groups, each of which mostly had 1000 g of chicken liver mince. Each group was then further divided into two subgroups of 500 g. Each subgroup was then again blended for 20 minutes by four blenders, and three different MA dosages (45, 90, and 180 ng/mg), that had been dissolved in 10 cc of saline was then added. Saline solution was the only treatment given to the control mince (2).

Insect cages were used to maintain *L. sericata* colonies (30x 30x 30 cm). Approximately 28 °C, 50–10% relative humidity, and natural light was present in the room. In the meantime, 150 g of fresh chicken liver is offered as a medium for collecting eggs (2, 21, 22).

To obtain enough eggs, they were collected within 3 hours. Once collected, the eggs were divided into eight groups, each group was then promptly mixed with one of three MA concentrations into chicken liver mince. Two samples from each group were collected at regular intervals (6 h from 12 to 50 h, and 12 h from 50 to 168 h). One sample contained 1 gram of the first, second, and third larvae (L1-L3), post-feeding larvae (PF), pupal (P), adult (A), and even empty pupa (EP), and the other sample contained 10 specimens of each one (2).

In order to determine the MA content of the specimens by GC-MS, a 1 g sample was taken at each development stage and kept at minus 20 °C until the sampling time was over. The procedure was approved using the control EP 100 mg because of the high chitin concentration. Due to this quality, EP was able to offer reliable information for toxicological study. Because EP can persist in a decaying body for a long time (23, 24).

http://jad.tums.ac.ir
Published Online: Sep 30, 2023

Preparation of samples for GC-MS analysis

Dichloromethane (CH2Cl2) was added as the first extractor solvent to a Falcon tube (50 ml) containing larvae (L2, L3, and PF), P, and EP samples. The organic solvent was discarded after washing the L and EP tubes upside-down for 5 minutes. L and EP were individually ground with a glass rod after crystallization in liquid nitrogen, and 100 mg aliquots were then transferred to fresh centrifuge tubes (10 ml).

After that, 2 ml of distilled water was added to the tube, and the tube was kept at 4 °C for 48 h to completely dissolve the matrix.

The addition of 0.5 mg of NaCl solid had a salting-out effect. The method that was optimized for the liquid-liquid extraction was used (LLE). Samples were subjected to a 30-minute ultrasonic treatment prior to LLE extraction (2).

Liquid-Liquid extraction (LLE) procedure

Two ml of phosphate buffer was added to the L and EP tubes to bring the pH level down to 6, after which the tubes were vortexed for 4 minutes, mixed again for 4 minutes, then centrifuged at 4000 rpm for 8 minutes. Fifty µl of Amantadine (internal standard) was mixed with 3 ml of supernatant and shaken for 4 minutes in a wash tube. The pH was then adjusted to 14 by adding 1 ml of 10% NaOH solution.

Samples were mixed and vortexed for 10 minutes. Ethyl acetate: Hexane (1:1.4 ml) was added to the tube after mixing for 4 minutes. The sample was once again mixed and vortexed for 10 minutes. The solution was then mixed and centrifuged at 4000 rpm for 8 minutes. The residual solution was re-extracted with ethyl acetate to increase the extraction recovery of MA. Three mL of the supernatant was transferred to a new tube, and the remaining solution was extracted using ethyl acetate: Hexane (1:1, 2 ml). The mixed solution followed 4 minutes of vortexing, 8 minutes of 4000 rpm centrifugation, and a final 45 °C nitrogen stream drying process. One ml of distilled water was added to each sample after it had dried completely to make it ready for the acetylation process of derivatization (2).

Derivatization with acetic anhydride (acetylation)

Derivatization buffer is prepared from 10 M potassium hydroxide solution and saturated sodium bicarbonate ($NaHCO_3$) solution. To each tube containing prepared sample, 500 microliters of derivatization buffer and 1 ml of distilled water were added. After that, 1500 µl of dichloromethane and 100 µl of acetic anhydride (as derivatizing agent) were added to experiment medium. The samples were then centrifuged at 3000 rpm for 10 minutes after being vortexed for 10 minutes.

Organic phase (bottom layer) was then transferred to another tube and dried for 24 hours. Samples were reconstituted in 50 µl of methanol. One µl of each sample was then injected into the GC-MS.

An Agilent 5975 Mass Series and an Agilent 6850 gas chromatograph were used to conduct the GC-MS analysis. Data gathering and instrument control were performed using Agilent MS Chem Station software (25).

The study was conducted using an Agilent 5-MS capillary column (30 m, 0.25 mm I.D, 0.25 µm film thickness). The GC method's chromatographic conditions were as follows:

The injection volume was 1 ml (split less), the inlet temperature was 25 °C, and the Helium flow rate was constant at 1.5 ml/min (99.999% purity). The oven temperature was set to 90 °C for one minute, then ramped up to 280 °C at 20 °C per minute and held for five minutes. Temperatures of 230 °C for the mass source and 150 °C for the quadrupole were chosen. The ion source was run simultaneously in full scan and selective ion monitoring (SIM) modes. In full scan mode, the scan range was 40–500 m/z, and the ions 58,100 for MA (Figs. 1–3), and 136, 94, 193 for amantadine were chosen for quantitative investigation (25).

Determination of the presence of Methamphetamine in larvae from human

Experiments on human cadaver larvae with suspected methamphetamine use or overdose history were also carried out to confirm the re-

http://jad.tums.ac.ir
Published Online: Sep 30, 2023

sults in authentic samples. Cases with the history of Methamphetamine use were investigated in the autopsy room of the Forensic Medicine Organization as soon as they were discovered to be infected with larvae. Samples of larvae or pupae were collected using the appropriate entomological techniques, and using reliable diagnostic keys, larvae of the third age of *L. sericata* were identified. Additionally, extraction and derivatization were carried out for the detection of Methamphetamine and its metabolite using GC-MS, as the confirmatory method.

Results

GC-MS analyses demonstrated that the MA artificially added to the food substrate was present throughout *L. sericata*'s various developmental stages. MA was found in *L. sericata* larvae of all ages, particularly in the third stage larva, pupa, and empty pupa.

GC-MS analyses were performed (all ages of larvae, pupae, EP, and adults) following the procedure for liquid-liquid extraction and derivatization by the acetylation method.

As shown in Fig. 4, the chromatograms obtained from the *L. sericata* (L3) larval samples from treatment 2 regimen (90 ng/mg) analysis, retention times for Methamphetamine and Amantadine are 8.1 and 8.5 minutes respectively. The same results were obtained from an empty pupa with treatment 3 regimen (180 ng/mg) (Fig. 5). Methamphetamine peaks in *L. sericata* instar 1 and 2 larvae were not clear and sharp, and low pains could not be detected as in treatment 1 regimen (45 ng/mg), while third instar larvae, pupae and empty pupa were excellent for detection. It can be justified in this way, when the first and second stage larvae are on the corpse, the corpse is fresh and bloated and the tissues and organs have not been seriously damaged yet.

Figure 6 shows the chromatogram obtained from the second stage larvae of treatment regimen number 3 (180 ng/mg). It should be mentioned that at higher doses of Methamphetamine, it can also be detected in second age larva. In the current experiment, when *L. sericata* adults were killed and analyzed one week after eclosion, no Methamphetamine was discovered. Moreover, larvae taken from human cadavers with the history of Methamphetamine use (used for validation purposes) were also positive, and the method was validated.

The chromatogram from larvae samples of *L. sericata* (L3) from the body of a 45-year-old man with a history of Methamphetamine addiction was confirmed by our employed method, as shown in Fig. 7. The chromatogram had a retention time of 5.4 minutes.

A 36-year-old man with a history of Methamphetamine addiction had larvae samples (L3) of *L. sericata* that were similarly positive, demonstrating the effectiveness of the study procedure (Fig. 8).

http://jad.tums.ac.ir
Published Online: Sep 30, 2023

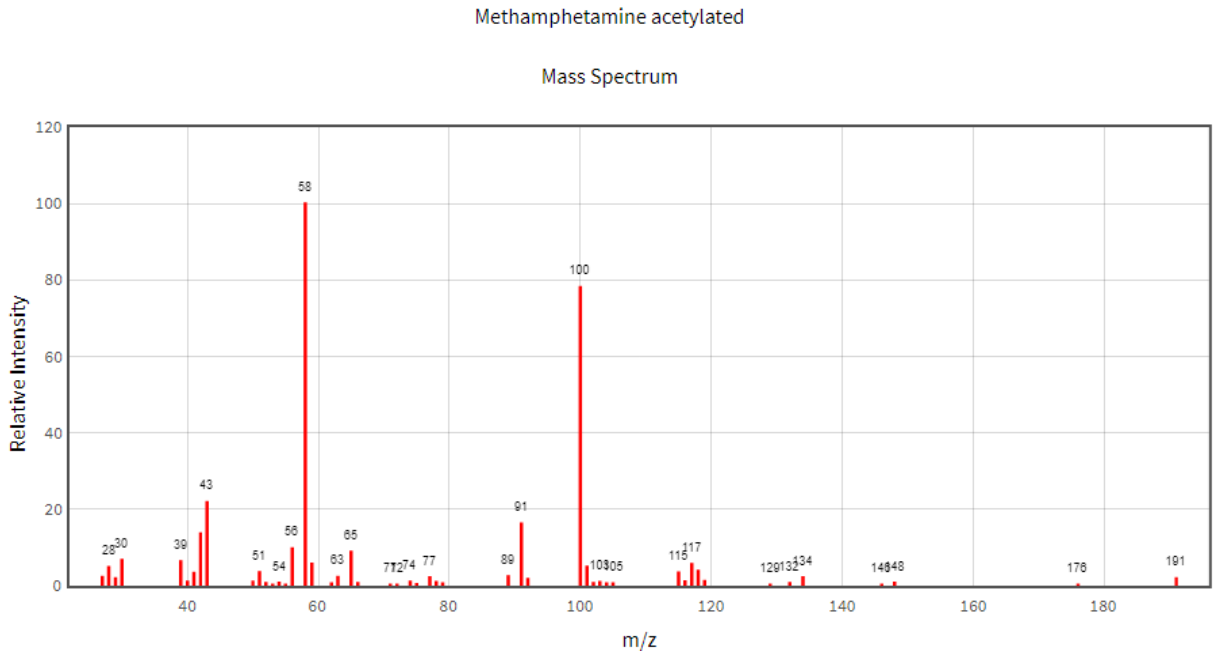

Fig. 1. Electron ionization (EI) mass spectra of the Methamphetamine (MA): MA monitored at m/z -58,100.

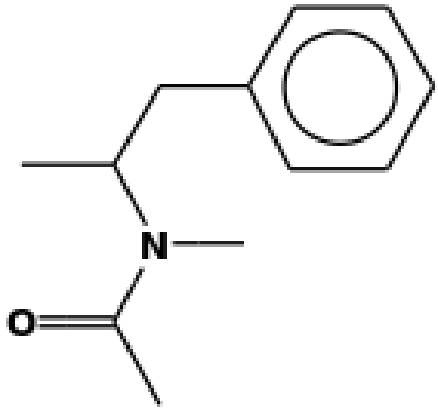

Fig. 2. The structure of acetylated Methamphetamine

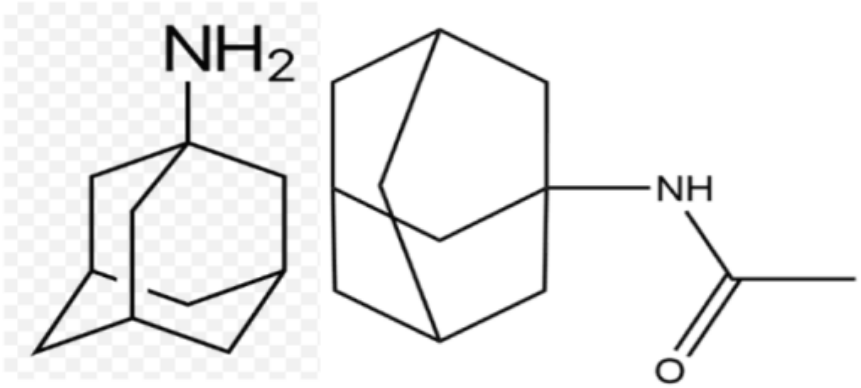

Fig. 3. The structure of Amantadine (left) and acetylated Amantadine (right)

http://jad.tums.ac.ir
Published Online: Sep 30, 2023

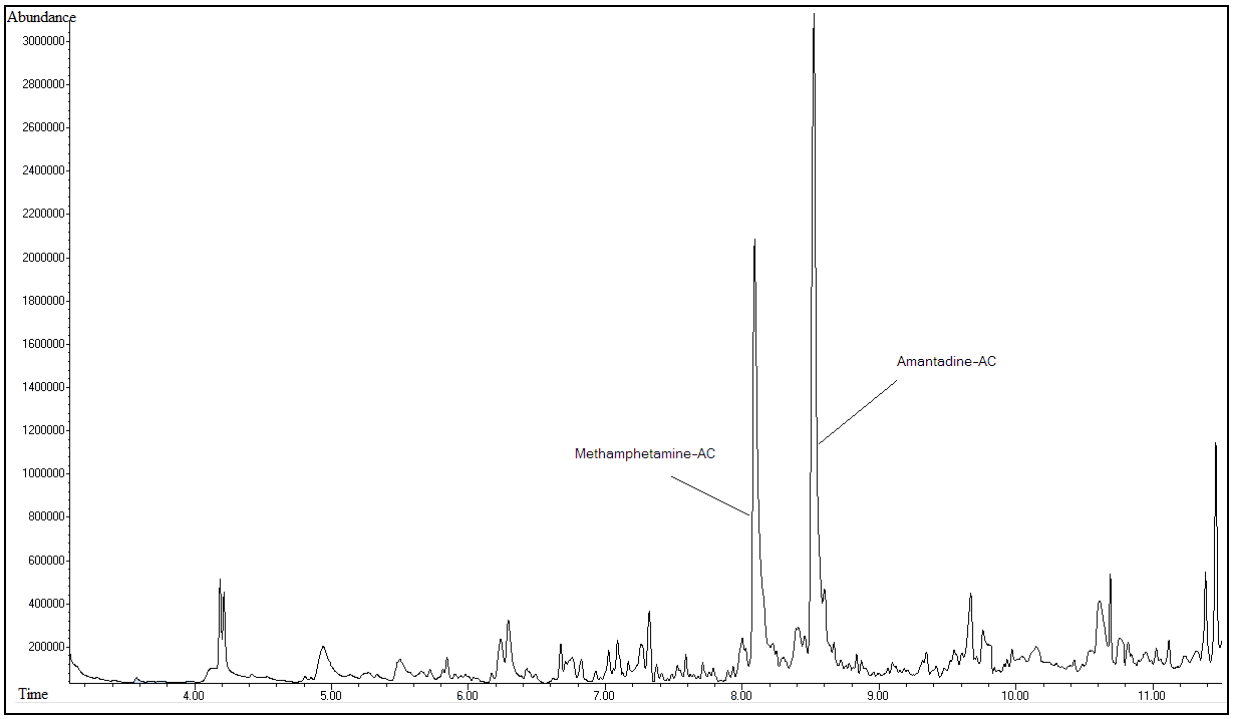

Fig. 4. Methamphetamine and Amantadin chromatograms generated by GS-MS. Retention time (minutes) of each peak is marked below the chromatogram. Chromatograms derived from *L. sericata* (L3) larval samples that were fed on chicken liver mince treated with 90 ng/mg MA

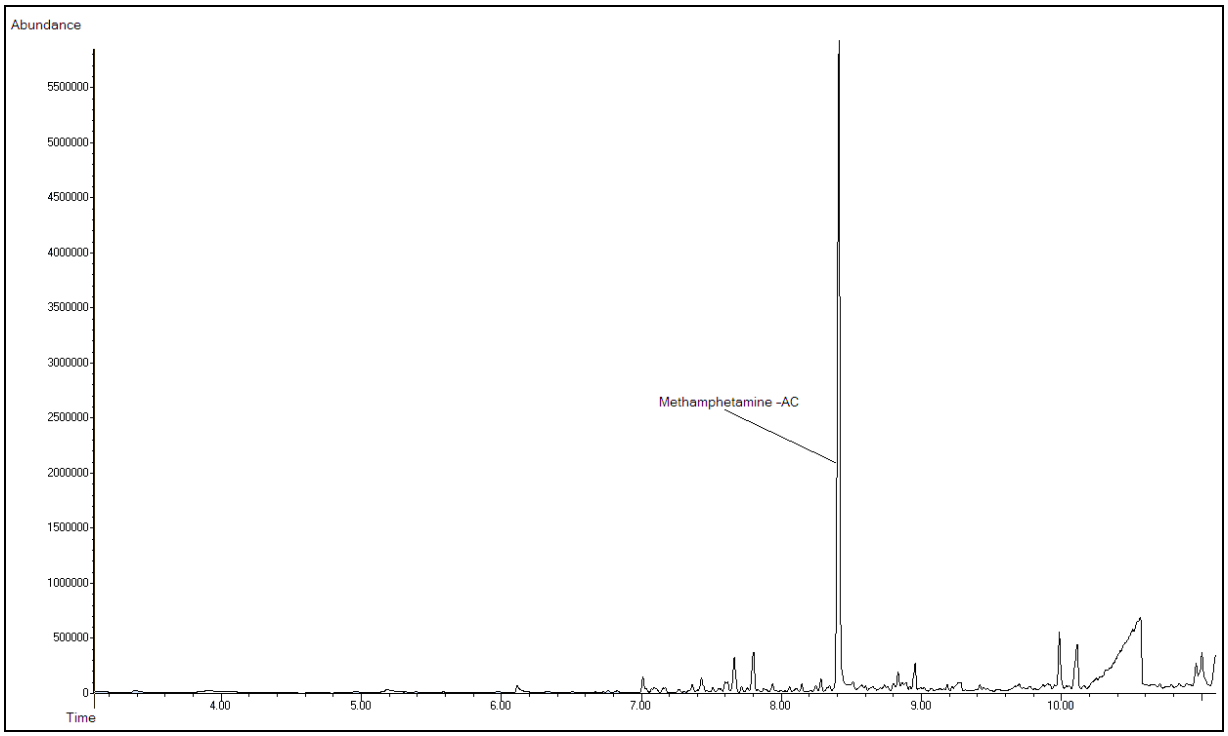

Fig. 5. Methamphetamine chromatogram generated by GS-MS. Retention time (minutes) of each peak is marked below the chromatogram. Chromatogram derived from *L. sericata* (EP) empty pupa samples that were fed on chicken liver mince treated with 180 ng/mg MA

http://jad.tums.ac.ir
Published Online: Sep 30, 2023

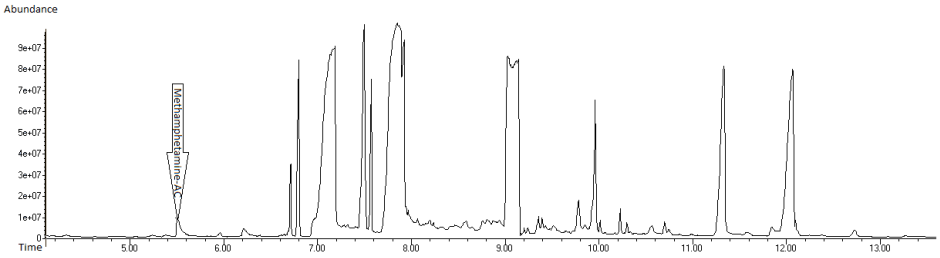

Fig. 6. Methamphetamine chromatogram generated by GS-MS. Retention time (minutes) of each peak is marked below the chromatogram. Chromatogram derived from *L. sericata* larval samples (L2) that were fed on chicken liver mince treated with 180 ng/mg MA

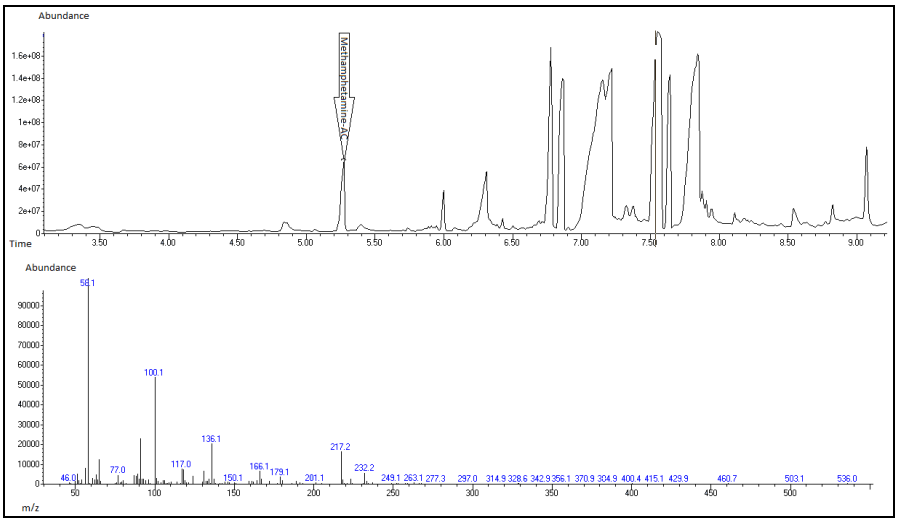

Fig. 7. The Methamphetamine chromatogram derived from larvae samples of *L. sericata* (L3) collected from the body of a 45-year-old man with a history of Methamphetamine addiction. The retention time (minutes) of MA peak is marked below the chromatogram (upper) and electron ionization (EI) mass spectra of the MA monitored at m/z -58,100 (below)

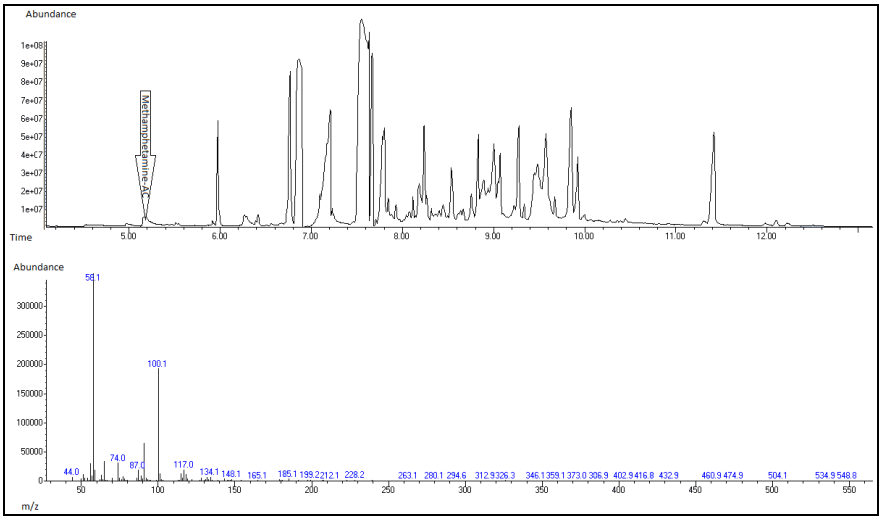

Fig. 8. The Methamphetamine chromatogram derived from larvae samples of *L. sericata* (L3) collected from the body of a 36-year-old man with a history of Methamphetamine addiction. The retention time (minutes) of MA peak is marked below the chromatogram (upper) and electron ionization (EI) mass spectra of the MA monitored at m/z -58,100 (below)

Discussion

The current study demonstrated that MA could be found in the larvae and empty pupae of *L. sericata* fed with spiked livers containing MA using GC-MS analysis.

However, according to our research, *L. sericata* was sensitive to the GC-MS method when it consumed liver that contained 45 ng/mg, 90 ng/mg, and 180 ng/mg MA. The biological phenomena, such as MA exposure and biological absorption, MA toxicity-dose correlations, and blowfly physiology, were unpredictable and variable by nature because of the exposure phase. This could explain why the liver MA concentrations of 45 ng/mg, 90 ng/mg, and 180 ng/mg only showed minor variations.

Groth et al. demonstrated in a study conducted in 2022 that entomological samples have a very high capability in field research. In addition to various benzodiazepines and synthetic cannabinoids, almost every chemical found in human samples were also found in larvae. Unexpectedly, several drugs, such as the brand-new psychoactive compounds EAM-2201 and U-47700, were only discovered in larvae and human hair. Only the liver, lungs, and stomach contents contained the benzodiazepine etizolam, probably due to its distinctive tissue distribution in people and/or larvae (26).

In another investigation by Gosselin et al. (27), larvae produced fewer matrix effects than the putrefied human specimens during the examination for the opioid Methadone. Similar to this, other entomotoxicologists stated that the examination of insects provided more sensitive results with fewer interferences from by-products of decomposition (10).

The human putrefaction metabolites that often interfere with analytical techniques may be metabolized by larvae (28). Most published investigations have found that all drugs discovered in human tissues could also be found in insect specimens (29–31).

In actuality, the toxicological examination of necrophagous insects occasionally revealed chemicals that were not found in the readily accessible human remains (32).

These and other studies (33, 34) point to the significant potential for the study of larvae as a supplemental technique to identify at least most of the chemicals consumed by the deceased, when human remains are uncommon, highly decomposed, or both.

The ability to detect chemicals in larvae may be influenced by a variety of variables. The chemicals that the larvae ingest and the degree of their bioaccumulation in larvae are determined by their feeding region and developmental stage (5, 31, 35). Additionally, it is important to take into account the drug's physicochemical characteristics and pharmacokinetic behavior in both humans and larvae.

The metabolism and accumulation of drugs in necrophagous larvae are now unknown (16). Like people, the tissues of larvae should bioaccumulate drugs if their rate of absorption exceeds their rate of excretion.

However, it also exhibits a weak link with drug absorption rates and raises the possibility that environmental factors may have influenced the final drug concentration discovered in insect tissue. This shows that exposure concentration had a substantial impact on toxicological consequences. The relationship between ambient temperature and drug metabolism, excretion, and stability has been studied in previous works (36–38). Numerous drug studies have shown that during the post-feeding stage, when the larvae were excreting drugs from their organs, some of the drugs also appear to be absorbed into the cuticle of the pupae (39, 40). Due to the fact that pupal cases can sometimes recover years, and even hundreds of years, after death, such specimens may be of major importance in toxicological analysis (41).

According to our study, negative results in *L. sericata* adults were predicted because it is known that when the flies become adults, they quickly eliminate the drugs (38, 42). Metals have

http://jad.tums.ac.ir
Published Online: Sep 30, 2023

been released into meconium in the pupal hindgut by Calliphoridae fed on mercury-containing fish tissue, according to Nuorteva and Nuorteva (1982) (43). They also discovered that the adult fly only had 50% of the mercury seen in the developing larvae two days after hatching (43). However, no Methamphetamine was found when *L. sericata* adults were killed and examined one-week following eclosion in the current investigation.

As previously mentioned, immature fly samples had low levels of MA that GC-MS was able to easily identify. Insect samples as well as samples taken from dead bodies can be used for postmortem drug testing. The utilization of decaying tissues and fluids makes toxicological studies extremely difficult. There are typically considerable compromises in chromatographic performance and separation, extraction recovery, and ionization efficiency. Additionally, post-mortem tissues have a significant loss in drug stability, which makes it challenging to discover new drugs (44, 45).

In this study, larvae were extracted using the liquid-liquid approach, and for the first time, derivatization was carried out using the acetylation method in larval samples

Conclusions

GC-MS made it simple to detect small amounts of MA in samples of immature flies. Testing for mortality should make use of all toxicological investigation tools, including human tissue and insect samples. Third instar larvae were an excellent and trustworthy sample for toxicological studies since they could recognize every MA before they were excreted. There is, however, little information in the literature about the discovery of drug metabolites in fly larvae, and less understanding of the mechanisms by which larvae metabolize drugs. The pharmacokinetics of drugs in various species of larvae may therefore be the main topics of future investigation.

If results from standard methods are unsatisfactory, this would open up the possibility to use insect samples as an additional matrix for toxicological research. As a result of our research, we advise the preservation of larvae in all postmortem situations where they may be located on or inside the corpse.

Acknowledgment

This article is part of Seyedeh Zahra Parkhideh's Ph.D. thesis, funded and supported by the Tehran University of Medical Sciences (TUMS) [grant no. 46885]. We would like to extend our sincere gratitude to the entire toxicology department staff for providing the facilities, ongoing support, advice, and helpful recommendations that made doing this study possible. For all the facilities used in this investigation, we are incredibly grateful to the Tehran Province Forensic Medicine Organization.

Ethical consideration

Research ethics approval ID: IR.TUMS.SPH.REC.1399.027IR.

Conflict of interest statement

The authors declare that there is no conflict of interest.

References

1. Basilicata P, Pieri M, Simonelli A, Faillace D, Niola M, Graziano V (2019) Application of a chemiluminescence immunoassay system and GC/MS for toxicological investigations on skeletonized human remains. Forensic Sci Int. 300: 120–124.
2. 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* (Dip-

http://jad.tums.ac.ir
Published Online: Sep 30, 2023

tera: Calliphoridae). J Med Entomol. 57(3): 691–696.
3. Wells JD, Introna F, Di Vella G, Campobasso CP, Hayes J, Sperling FA (2001a) Human and insect mitochondrial DNA analysis from maggots. J Forensic Sci. 46: 685–687.
4. Wells JD, Pape T, Sperling FA (2001) DNA-based identification and molecular systematics of forensically important Sarcophagidae (Diptera). J Forensic Sci. 46 (5): 1098–1102.
5. Sadler DW, Fuke C, Court F, Pounder DJ (1995) Drug accumulation and elimination in *Calliphora vicina* larvae. Forensic Sci Int. 71(3): 191–197.
6. Goff ML, Omori AI, Goodbrod JR (1989) Effect of cocaine in tissues on the development rate of *Boettcherisca peregrina* (Diptera: Sarcophagidae). J Med Entomol. 26(2): 91–93.
7. Bourel B, Fleurisse L, Hedouin V, Cailliez JC, Creusy C, Gosset D, Goff ML (2001) Immunohistochemical contribution to the study of morphine metabolism in Calliphoridae larvae and implications in forensic entomotoxicology. J Forensic Sci. 46 (3): 596–599.
8. Oliveira HG, Gomes G, Morlin JJJ, Von Zuben CJ, Linhares AX (2009) The effect of Buscopan on the development of the blow fly *Chrysomya megacephala* (F.) (Diptera: Calliphoridae). J Forensic Sci. 54(1): 202–206.
9. Carvalho LM, Linhares AX, Trigo JR (2001) Determination of drug levels and the effect of diazepam on the growth of necrophagous flies of forensic importance in southeastern Brazil. Forensic Sci Int. 120 (1–2): 140–144.
10. 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.
11. Wilson Z, Hubbard S, Pounder DJ (1993) Drug analysis in fly larvae. Am J Forensic Med Pathol. 14(2): 118–120.
12. Beyer JC, Enos WF, Stajic M (1980) Drug identification through analysis of maggots. J Forensic Sci. 25(2): 411–412.
13. Wood M, Laloup M, Pien K, Samyn N, Morris M, Maes RAA, 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.
14. Sadler DW, Richardson J, Haigh S, Bruce G, Pounder DJ (1997) Amitriptyline accumulation and elimination in *Calliphora* vicina larvae. Am J Forensic Med Pathol. 18(4): 397–403.
15. 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.
16. Kharbouche H, Augsburger M, Cherix D, Sporkert F, Giroud C, Wyss C, Champod C, Mangi 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.
17. Hedouin V, Bourel B, Martin-Bouyer L, Becart A, Tournel G, Deveaux M, Gosset D (1999) Determination of drug levels in larvae of *Lucilia sericata* (Diptera: Calliphoridae) reared on rabbit carcasses containing morphine. J Forensic Sci. 44(2): 351–353.
18. Gosselin M, Ramirez Fernandez MDM, Wille SMR, Samyn N, De Boeck G, Bourel B (2010) Quantification of methadone and its metabolite 2-ethylidene-1,5- dimethyl-3,3-diphenylpyrrolidine in third instar larvae of *Lucilia sericata* (Diptera: Calliphoridae) using liquid chromatography-tandem mass spectrometry. J Anal Toxicol. 34(7): 374–380.

19. Levine B, Golle M, Smialek JE (2000) An unusual drug death involving maggots. Am J Forensic Med Pathol. 21(1): 59–61.
20. Kintz P, Godelar B, Tracqui A, Mangin P, Lugnier AA, Chaumont AJ (1990) Fly larvae: a new toxicological method of investigation in forensic medicine. J Forensic Sci. 35(1): 204–207.
21. Zhu GH, Ye GY, Hu C (2004) Development profile of hemolymph soluble proteins during the larval and pupal stages in *Aldrichina grahami*. J Zhejiang Univ. (Agric. Life Sci.). 30: 69–72.
22. Bugelli V, Campobasso CP, Verhoff MA, Amendt J (2017) Effects of different storage and measuring methods on larval length values for the blow flies (Diptera: Calliphoridae) *Lucilia sericata* and *Calliphora vicina*. Sci Justice. 57: 159–164.
23. Sukontason KL, Radchadawan NK, Duanghatai S, Kom S (2007) Identifying fly puparia by clearing technique: application to forensic entomology. Parasitol Res. 101: 1407–1416.
24. Gosselin M, Fazio VD, Wille SMR, 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: 154–159.
25. 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.
26. Groth O, Franz S, Fels H, Krueger J, Roider G, Dame T, Musshof F, Graw M (2022) Unexpected results found in larvae samples from two postmortem forensic cases. Forensic Toxicol. 40: 144–155.
27. Gosselin M, Fernandez MdMR, Wille SM, Samyn N, De Boeck G, Bourel B (2010) Quantification of methadone and its metabolite 2-ethylidene-1, 5-dimethyl-3, 3-diphenylpyrrolidine in third instar larvae of *Lucilia sericata* (Diptera: Calliphoridae) using liquid chromatography-tandem mass spectrometry. J Anal Toxicol. 34(7): 374–380.
28. Gagliano-Candela R, Aventaggiato L (2001) The detection of toxic substances in entomological specimens. Int J Legal Med. 114: 197–203.
29. Kintz P, Tracqui A, Mangin P (1990) Toxicology and fly larvae on a putrefed cadaver. J Forensic Sci Soc. 30: 243–246.
30. 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: 210–214.
31. Nolte KB, Pinder RDLW (1992) Insect larvae used to detect cocaine poisoning in a decomposed body. J Forensic Sci. 37: 1179–1185.
32. Levine B, Golle M, Smialek JE (2000) An unusual drug death involving maggots. Am J Forensic Med Pathol. 21: 59–61.
33. Kintz P, Tracqui A, Ludes B, Waller J, Boukhabza A, Mangin P, Lugnier A, Chaumont A (1990) Fly larvae and their relevance in forensic toxicology. Am J Forensic Med Pathol. 11: 63– 65.
34. De Aguiar FJ, Brandão M, Fabriz Sodré F, Dutry Caldas E (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: 93–103.
35. 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: 1–9.
36. Tracqui A, Keyser-Tracqui C, Kintz P, Ludes B (2004) Entomotoxicology for the forensic toxicologist: much ado about nothing?

Int. J Legal Med. 118: 194–196.
37. Parry S, Linton SM, Francis PS, O'Donnell MJ, Toop T (2011) Accumulation and excretion of morphine by *Calliphora stygia*, an Australian blow fly species of forensic importance. J Insect Physiol. 57: 62–73.
38. 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: Calliphoridae). Forensic Sci Int. 241: 96–101.
39. Sadler DW, Robertson L, Brown G, Fuke C, Pounder DJ (1997) Barbiturates and analgesics in *Calliphora vicina* larvae. J Forensic Sci. 42(6): 1214–1215.
40. Hedouin V, Bourel B, Bécart A, Tournel G, Deveaux M, Goff ML, Gosset D (2001) Determination of drug levels in larvae of *Protophormia terraenovae* and *Calliphora vicina* (Diptera: Calliphoridae) reared on rabbit carcasses containing morphine. J Forensic Sci. 46: 12–14.
41. Miller M, Lord W, Goff M, Donnelly D, McDonough E, Alexis J (1994) Isolation of amitriptyline and nortriptyline from fly puparia (Phoridae) and beetle exuviae (Dermestidae) associated with mummified human remains. J Forensic Sci. 39: 1305–1313.
42. Goff ML, Lord WD (2010) Entomotoxicology-Insects as toxicological indicators and the impact of drugs and toxins on insect development, in: JH Byrd, JL Castner (Eds.), Forensic Entomology-The Utility of Arthropods in Legal Investigations, 2ed CRC Press, pp. 427–436.
43. Nortueva P, Nortueva SL (1982) The fate of mercury in Sarcosaprophagous flies and in insects eating them. Ambio. 11: 34–37.
44. Skopp G, Pötsch L, Klingmann A, Mattern R (2001) Stability of morphine, morphine-3-glucuronide, and morphine-6-glucuronide in fresh blood and plasma and postmortem blood samples. J Anal Toxicol. 25(1): 2–7.
45. Schloegl H, Dresen S, Spaczynski K, Stoertzel M, Wurst FM, Weinmann W (2006) Stability of ethyl glucuronide in urine, postmortem tissue and blood samples. Int J Legal Med. 120: 83–88.

http://jad.tums.ac.ir
Published Online: Sep 30, 2023