

ANALYSIS OF PARACETAMOL IN FORENSIC BLOWFLY SAMPLES FROM INTOXICATED-PARACETAMOL CARCASS

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ABSTRACT

Paracetamol is one of the most commonly used analgesic drugs that often reported to be abused and involved in intoxication death cases. Some of these cases involved decomposed biological samples from the dead remains, which cannot be analysed appropriately by methods applied for ante mortem specimens. In this study a method for determining paracetamol in entomological samples obtained from decomposed paracetamol-intoxicated carcass is described. As alternative, entomological samples which is blowflies larvae was used for toxicology analysis since immature blowflies were the main processor for the dead remains. To obtain a paracetamol-exposed carcass, New Zealand white rabbits were ingested with 5600 mg/kg (dose) of paracetamol and were let to decompose. Blowflies larvae consumed the carcasses were collected and paracetamol from the larvae were analysed using High Performance Liquid Chromatography with Diode Array Detector (HPLC-DAD). Paracetamol extraction from blowflies' larvae was done using Solid Phase Extraction (SPE). Separation was conducted under 75:25 ratios of acidified water (pH=3) and acetonitrile, using a C-18 column. Calibration curve was obtained with linear regression (R^2) of 0.9999. Paracetamol peak was eluted out at retention time of 3.7 minutes. The entomological specimen from third instar larvae found out to contain the highest concentration of paracetamol.

Key words: entomotoxicology, HPLC, validation, paracetamol, decomposition

INTRODUCTION

Forensic entomotoxicology is relatively new branch of forensic entomology, which uses insects, mainly fly larvae (maggots), as alternative material for post-mortem toxicological analyses. It deals with the qualitative and quantitative determination of toxins in insects or arthropods feeding on corpse body (Claire and Bryan, 2004). Forensic entomotoxicology maybe useful especially in the cases of human cadavers revealed after long time since death that affected by decomposition (autolytic and putrefaction) processes (Yi *et al.*, 2013). The reliability of entomological evidence in the ways of estimating a postmortem interval (PMI) can depends solely on the toxicology results out of the corpse's tissues (Campobasso *et al.*, 2004). Earlier research stated, the toxins from drugs and illegal narcotics, that accumulates and metabolizes in corpse body

not only can be detected by larvae feeding on the corpse but as well as affects the development rate of the larvae (Goff and Lord, 1994). In forensics investigation, estimation of time of death is estimated from the development of blowflies larvae, as an alternative to physical changes of the dead body. Additional problem arise is the factor of development rate is subsequently affected by the type of tissues area being consumed by the larvae as different toxin substances will metabolizes in different area of body (Pien *et al.*, 2004).

Paracetamol (the active ingredient is Acetaminophen) is one of the most commonly used over-the-counter analgesics and antipyretic drugs available (Moore, 2007). It often found in the forms of tablet and physical state usually white crystalline powder. Paracetamol is classified as a mild analgesic and is commonly used for the relief of headaches and also a major ingredient in numerous cold and flu remedies (Prescott, 2000). Statistically, hospital admissions due to poisoning have steadily increased

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from the 1950s. The easy accessibility and non-prescriptive are the main factors of the misuse of paracetamol. Since the mid-1970s there has been an increase in the number of paracetamol overdoses, it has now become the substance most frequently used in deliberate self poisoning in the United Kingdom where the proportion of overdoses with paracetamol increased from 14.3% in 1976 to 42% in 1990, and in 1993, 47.8% of all overdoses involved paracetamol (McEvoy, 2007). The outcomes of paracetamol poisoning depend on several factors. Sadler *et al.* (1995) found that the quantity of paracetamol ingested and chronic alcohol abuse had been identified as independent risk factors in the development of paracetamol induced hepatotoxicity.

This study comprises the potential in entomology that is to solve crimes by using insects as a toxicological tool applies in the detection of drugs, poisons and toxicant (Bourel *et al.*, 2001) The purposes of this study were to quantitate the concentration of paracetamol that transferred to forensically important insect and also to study the effect of paracetamol to the development of *Chrysomys rufifacies* based on validated and reliable method using HPLC-DAD.

MATERIALS AND METHODS

Standard for paracetamol (Acetaminophen) was obtained from Sigma Aldrich. Methanol used in activation of sorbent in Solid Phase Extraction (SPE) C18 column's sorbent and nitrogen gas was used to make the sample more concentrate in Solid Phase Extraction. Phosphate buffer solution (pH=8.0) used for stabilized the pH in the mixture. Four New Zealand white rabbits weighing from 1.7 kg to 2.0 kg were used as experimental animals.

Sample Preparation

Experimental animals were force feed with over dose paracetamol (5600 mg/kg) orally and were exposed to environment for a week. All entomological samples were prepped by homogenised the sample in phosphate buffer (pH=8.0) and centrifuged at 3000 rpm for 10 minutes. The supernatant was collected and clean-up process of supernatant samples was done using solid phase extraction (SPE). C₁₈ cartridges were preconditioned by using 2 mL methanol as well as 2 mL of phosphate buffer at pH=8.0. The sample was added into the column then washed using 2 mL of distilled water. The elution of the analyte was done using 2 mL of methanol. The eluted sample allowed being concentrated using a gentle stream of nitrogen. Only 20 μ L of the analyte is injected into the liquid chromatograph system.

Chromatographic conditions

Separation achieved by using octadecyl carbon chain (C18)-bonded silica HPLC column. The mobile phases were acidified water (pH=3.0) and acetonitrile with the ratio of 75: 25 respectively. The mobile phase flow rate was set at 1 mL/min. The DAD detector was set at 254 nm. Overall analysis took 5.5 minutes.

Validation Method

Acetaminophen standard was diluted to a number of dilutions to form a calibration curve. A series of six dilutions of 0.1, 1.0, 5.0, 10.0, 20.0, and 50.0 mg/L were run to construct the calibration curve. The analysis was done in triplicates in order to obtain linearity. Limit of detection and limit of quantitation were detected by injecting the lowest concentration of standard into the system until there was no peak seen with S/N ratio 3:1 and 10:1 respectively.

RESULTS AND DISCUSSION

For quantitation purposes of paracetamol, standard of paracetamol, acetaminophen was used to develop a standard calibration curve in Fig. 1. The standard calibration curve that obtained had R² value of 0.9999 and equation $y = 107.15x$. Since the R² value of calibration curve was closed to 1, it is acceptable to be used in quantitation of paracetamol in samples. The calibration curve obtained from the concentration of standard ranging from 0.1 μ g/ml to 50 μ g/ml. From HPLC analysis, all samples except for adult fly were positive for paracetamol at (Fig. 2) retention time ± 3.67 minutes. Based on the calibration curve, standard deviation of the response and the slope, the LOD and LOQ were 0.048 μ g/ml and 0.144 μ g/ml respectively and recovery obtained in this experiment is 63.7% due to the limitation in sensitivity of the instrument. Analysis of paracetamol active ingredient acetaminophen was done using larvae from first, second and third instar and also from pupae and adult of blowflies.

Optimization of elution solvent ratio was done to find out which ratio of solvents was the best for the elution of acetaminophen standard and sample. The 75:25 ratio of water to acetonitrile was selected for elution of acetaminophen from samples. The standard calibration curve has been used for determination and further quantitation of acetaminophen in blowflies' sample. In this study, the presence of acetaminophen had caused the development rate of larvae accelerated compared to control thus may contribute to error in the estimation of PMI. This is caused by the active feeding activity that resulting in accumulation of drugs in *Chrysomya rufifacies* during first, second

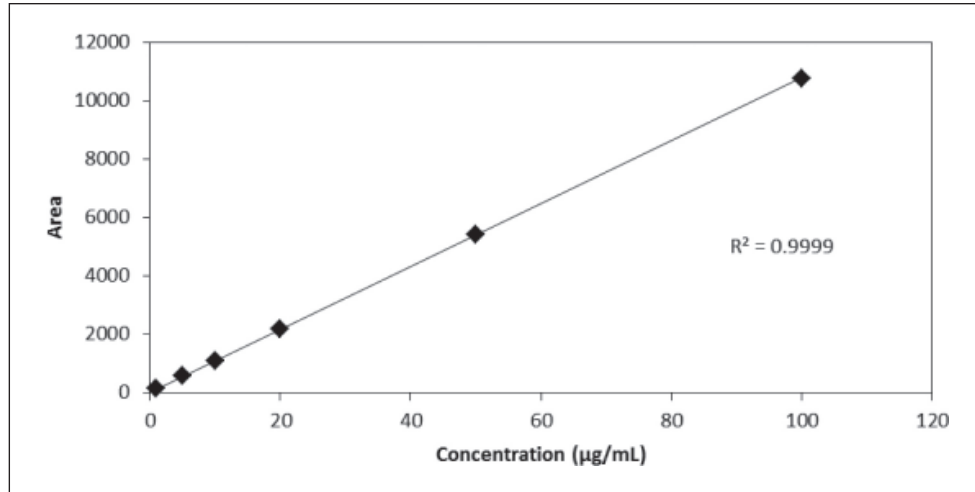


Fig. 1. Standard calibration curve for Acetaminophen standard

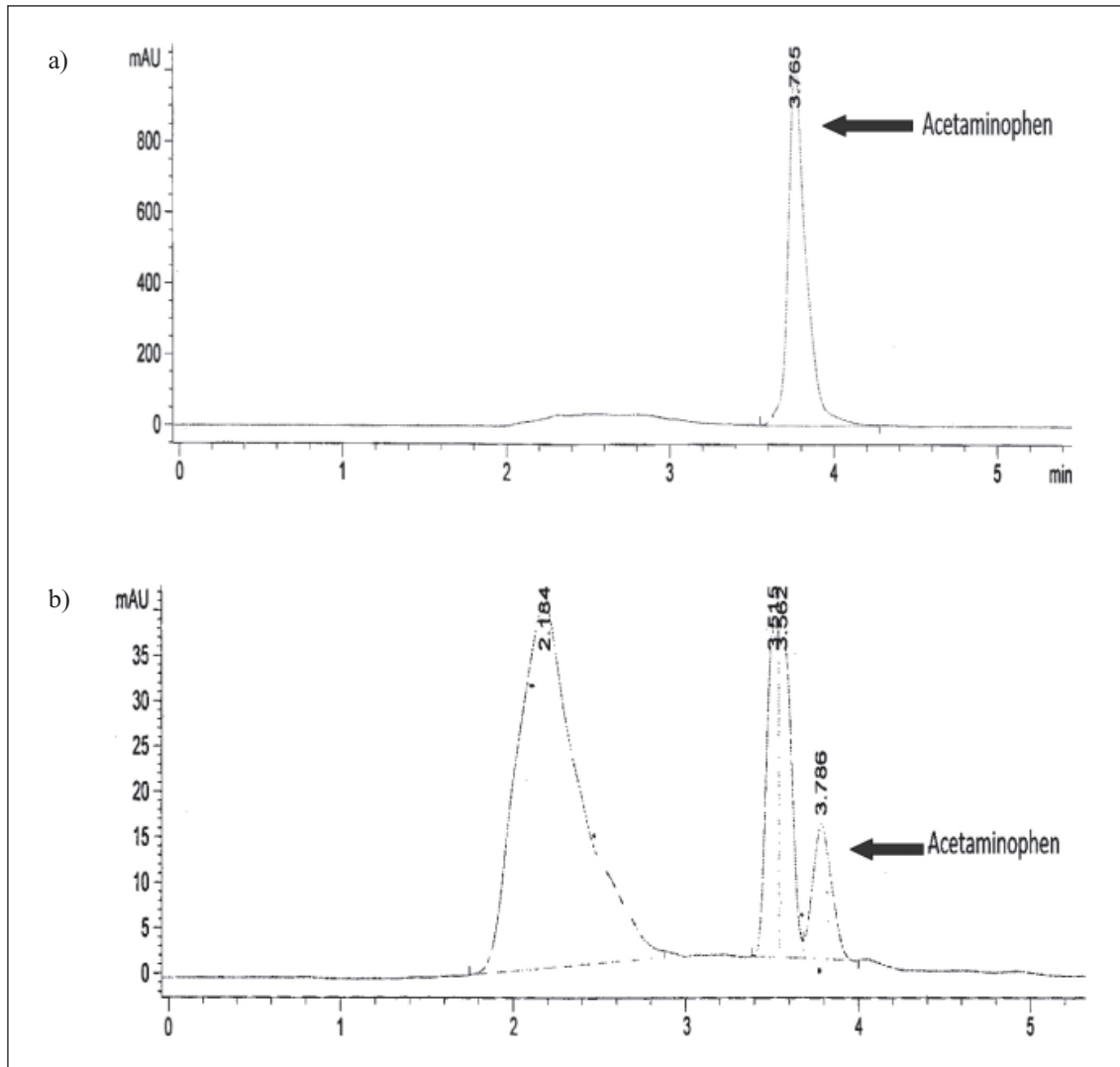


Fig. 2. Chromatogram peak of Acetaminophen a) at 80 µg/mL and b) chromatogram peak from sample on first instar larvae. Acetaminophen was eluted out at retention time 3.7 min

Table 1. Concentration of Acetaminophen (ppm) from entomological samples from Paracetamol – ingested carcass

Stages of development	Concentration of Acetaminophen (ppm)
First instar	0.13
Second instar	0.37
Third instar	3.05
Pupae	0.43
Adult	ND

ND – not detected

and third instar. As stated by Rashid *et al.* (2013), accumulation of ketum extract that present in the *Chrysoma rufifacies* in larvae stages accelerating their development rate hence affect the estimation of post-mortem interval. Concentration of acetaminophen detected was the highest in third instar larvae which is the largest in larvae stage, compared to others (Table 1). The accumulation of acetaminophen in the system may contribute to high concentration of acetaminophen detected throughout the larvae stages. In post feeding stages (pre pupa, pupa and adult) the concentrations is either lower and not detected may due to the pharmacokinetics of the drugs which is the study of action of drugs in the body, involving rate of excretion and duration of effects over a period of time (Carvalho *et al.*, 2001). Due to lower concentration of Paracetamol in pupae compared to third instar, there may be elimination where the drug had been metabolised and eliminated as stated in study by Rumiza *et al.* (2008). The drug in the pupae system did not retained until adult stage reached. The metabolism of paracetamol in the blowfly system had lowering the concentration of paracetamol as all paracetamol had been converted into metabolites thus, lower concentration detected worse, no detection of paracetamol as seen in adult sample.

CONCLUSION

In conclusion, we had proved that entomological samples are evidence that is important in estimating post-mortem interval (PMI) for paracetamol intoxicated carcasses. Our method provides a simple, reliable and fast instrumentation technique for toxicological analysis. A well-documented analysis on the relation of each toxin with forensically-important arthropods will be beneficial towards estimating an accurate PMI of intoxicated cases.

Data obtained from this study hope to be useful for possible application in interpretation and estimation of PMI for human cadavers intoxicated by paracetamol.

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