

**DETECTION OF PARAQUAT IN CALLIPHORID MAGGOTS BY
CAPILLARY ELECTROPHORESIS FOR DIAGNOSING FATAL
POISONING**

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UNIVERSITI TEKNOLOGI MALAYSIA

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POISONING**

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A dissertation submitted in partial fulfillment of the requirements for the award of
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This dissertation is dedicated to my beloved family and dearest friends. Thank you for the support, patience and love that all of you have shown.

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ABSTRACT

Entomotoxicology deals with the identification and quantitation of drugs and poisons in maggots for the purpose of diagnosing the possible cause of death, especially in highly decomposed bodies wherein the conventional toxicological specimens are not suitable or available for sampling. In this context, poisons such as paraquat have been reported as one of the agents used for committing suicides as well as accidental deaths and hence the use of Calliphorid maggots for toxicological analysis has been suggested. However, specific analytical method for analyzing paraquat in maggots remains unreported and in view of this problem, this present study that aimed to develop such method acquires forensic significance. The third instar of Calliphorid larvae that fed on paraquat-treated beef substrates in lethal dose for human (sample P1; 40 mg/kg) and three times the lethal dose (sample P2; 120 mg/kg) were collected and extracted using protein precipitation technique. Samples (0.4 g) were mixed with formic acid and ultrapure water, centrifuged and filtered. Paraquat residue was analysed using capillary zone electrophoresis with diode array detector (CZE-DAD). CZE analysis was performed in fused silica capillary under potential +25 kV and detection at 195 nm. The electrolyte was a 50 mM phosphate buffer at pH 2.50. Results of this study indicated that the presence of paraquat was detected in Calliphorid maggot samples that were collected from the paraquat-treated beef substrates in less than 2.5 mins under the optimized conditions and proved the reliability of these samples for qualitative analyses; LOD and LOQ were 0.495 $\mu\text{g/mL}$ and 1.501 $\mu\text{g/mL}$, respectively. Detection of paraquat in maggots may prove useful in diagnosing the cause of death, since paraquat is a poison compared to other drugs of abuse.

ABSTRAK

Entomotoksikologi berurusan dengan pengenalpastian dan penentuan kepekatan dadah dan racun dalam ulat untuk tujuan mencari punca kematian, terutamanya dalam kes mayat yang telah reput. Dalam konteks ini, racun parakuat telah dilaporkan sebagai salah satu ejen yang digunakan untuk membunuh diri serta kematian akibat kemalangan dan dengan itu penggunaan ulat Calliphorid untuk analisis toksikologi telah dicadangkan. Walau bagaimanapun, kaedah analisis yang khusus untuk menganalisis parakuat dalam ulat masih belum dilaporkan dan memandangkan masalah ini, kajian yang dijalankan ini bertujuan untuk membangunkan kaedah tersebut memperoleh kepentingan forensik. Instar peringkat ketiga ulat Calliphorid yang memakan substrat daging lembu yang dirawat dengan parakuat dalam dos maut untuk manusia (sampel P1; 40 mg / kg) dan tiga kali dos maut (sampel P2; 120 mg / kg) dikumpulkan dan diekstrak menggunakan teknik pemendakan protein. Sampel (0.4 g) dicampurkan dengan asid formik dan air tulen, diempar dan ditapis. Sisa parakuat telah dianalisis dengan menggunakan elektroforesis rerambut yang dilengkapi dengan pengesan susunatur diod (CZE-DAD). Analisis CZE dijalankan dengan menggunakan turus rerambut silika terlakur, di bawah keupayaan +25 kV dan pengesanan pada 195 nm . Elektrolit terdiri daripada larutan penimbal fosfat pada kepekatan 50 mM dan pH 2.50. Hasil kajian ini menunjukkan bahawa kehadiran parakuat telah dikesan dalam sampel ulat Calliphorid yang telah dikumpul dari substrat daging lembu dalam masa 2.5 minit dan membuktikan kebolehpercayaan sampel ini untuk analisis kualitatif; LOD dan LOQ ialah 0.495 µg/mL dan 1.501 µg/mL. Pengesanan parakuat dalam ulat berkemungkinan boleh digunakan bagi mendiagnosis sebab kematian, memandangkan parakuat merupakan racun yang boleh menyebabkan kematian berbanding dadah-dadah yang sering disalahguna.

TABLE OF CONTENTS

CHAPTER	TITLE	PAGE
	SUPERVISOR’S DECLARATION	ii
	AUTHOR’S DECLARATION	iii
	DEDICATION	iv
	ACKNOWLEDGEMENT	v
	ABSTRACT	vi
	ABSTRAK	vii
	TABLE OF CONTENTS	viii
	LIST OF TABLES	xi
	LIST OF FIGURES	xii
	LIST OF ABBREVIATIONS	xiii
	LIST OF APPENDICES	xv
1	INTRODUCTION	1
	1.1 Background of Study	1
	1.2 Statement of Problem	3
	1.3 Objectives	4
	1.4 Scope of Study	4
	1.5 Significance of Study	5
2	LITERATURE REVIEW	6
	2.1 Forensic Entomology	6
	2.2 Insect and Death	7
	2.3 Types of Flies	9
	2.4 Pesticides of Abuse	13

2.5	Paraquat Fatal Poisoning and Toxicity	15
2.6	Sample preparation	17
2.7	Analysis of Paraquat in Biological Fluids	20
2.7.1	Capillary Electrophoresis	21
2.7.2	Liquid Chromatography	22
2.7.3	Gas Chromatography-Mass Spectrometry	22
2.8	Analysis of Drugs and Poisons in Entomological Specimens	23
2.9	Capillary Electrophoresis	28
2.9.1	Component of a Capillary Electrophoresis Instrument	28
2.9.2	Principle of a Capillary Electrophoresis	30
2.9.3	Chemical Composition of Buffer	32
3	EXPERIMENTAL	34
3.1	Introduction	34
3.2	Apparatus	34
3.3	Chemicals and Materials	35
3.4	Instrumentation	35
3.5	Sampling on Real Entomological Specimens	36
3.6	Sample Preparation	39
3.7	Analysis Procedure Employing Capillary Electrophoresis	39
3.7.1	Preparation of Standard Solutions	39
3.7.2	Buffer Solution	40
3.8	Method Validation	40
3.8.1	Blank and quality control samples	40
3.8.2	Detection of Paraquat from Larvae	41
3.8.3	Linearity and Sensitivity	41
3.8.4	Repeatability and Reproducibility	42
3.8.5	Recovery	42

4	RESULTS AND DISCUSSION	43
4.1	Introduction	43
4.2	Examination of Entomological Specimens	44
4.3	Capillary Zone Electrophoresis Analysis of Paraquat	45
4.4	CE Optimization	46
4.4.1	Selection of Wavelength	46
4.4.2	Effect of pH of BGE	46
4.4.3	Effect of Potential	47
4.4.4	Effect of Concentration of BGE	48
4.4.5	Effect of Temperature	49
4.5	Validation of Method	50
4.5.1	Linearity and Sensitivity	50
4.5.2	Repeatability and Reproducibility	51
4.5.3	Recovery	52
4.6	Extraction Method	54
4.7	Detection of Paraquat in Samples of Calliphorid Maggot	54
5	CONCLUSIONS AND RECOMMENDATIONS	59
5.1	Conclusions	59
5.2	Limitation of Study	60
5.3	Recommendations for Future Work	60
	REFERENCES	61
	APPENDICES	68

LIST OF TABLES

TABLE NO.	TITLE	PAGE
2.1	General properties of pesticides of abuse	14
2.2	Phases of paraquat toxicity and associated clinical effects	17
2.3	Summary of the analysis of quaternary ammonium herbicides in biological matrices	20
2.4	Summary of the previous entomotoxicological studies	27
4.1	Effects of applied potential on migration time, peak area and peak height	48
4.2	Effects of BGE concentration on migration time, peak area and peak height	49
4.3	Effects of temperature on migration time, peak area and peak height	50
4.4	Repeatability and reproducibility assay (n = 6) in paraquat standard solutions for concentrations of 5 µg/ml and 80 µg/ml	52

LIST OF FIGURES

FIGURE NO.	TITLE	PAGE
2.1	Calliphorid larval structures	10
2.2	Morphology of <i>Ch. megacephala</i>	11
2.3	Morphology of <i>Ch. rufifacies</i>	11
2.4	Development of Calliphorids larvae	12
2.5	Statistics on paraquat poisoning in Hospital Taiping from the year 2008-2011	16
2.6	The schematic of the CE system	28
2.7	Schematic diagram showing the origin of EOF	31
3.1	Placement of substrates	38
4.1	<i>Chrysomya megacephala</i> 's third instar larvae	44
4.2	<i>Chrysomya rufifacies</i> 's third instar larvae	44
4.3	Three spiracular slits in third instar larvae of <i>Chrysomya megacephala</i>	45
4.4	Three spiracular slits in third instar larvae of <i>Chrysomya rufifacies</i>	45
4.5	Calibration curve for paraquat by CZE	51
4.6	Electropherogram of paraquat standard at concentration of 5 µg/mL	56
4.7	Electropherogram of blank BGE	56
4.8	Electropherogram of blank extract	57
4.9	Electropherogram of paraquat in P1 sample	57
4.10	Electropherogram of paraquat in P2 sample	58

LIST OF ABBREVIATIONS

AAS	-	Atomic absorption spectroscopy
BGE	-	Background electrolyte
CE	-	Capillary electrophoresis
CNS	-	Central nervous system
CZE	-	Capillary zone electrophoresis
DAD	-	Diode array detector
DNA	-	Deoxyribonucleic acid
ECD	-	Electron capture detector
EOF	-	Electro-osmotic flow
GC	-	Gas chromatography
HCl	-	Hydrochloric acid
HPLC		High performance liquid chromatography
LC	-	Liquid chromatography
LLE	-	Liquid-liquid extraction
LOD	-	Limit of detection
LOQ	-	Limit of quantification
MPH	-	Methylphenidate
MS	-	Mass spectrometry
NaOH	-	Sodium hydroxide
RIA	-	Radioimmunoassay

RSD	-	Relative standard deviation
SD	-	Standard deviation
SPE	-	Solid phase extraction
TCA	-	Trichloroacetic acid
UV	-	Ultraviolet

LIST OF APPENDICES

APPENDIX	TITLE	PAGE
A	Paraquat	67
B	Abstract for NCFMS 2014	68

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Forensic entomology deals primarily with the utility of insects and other arthropods which infest human remains in criminal investigations, particularly those involving unlawful or unexplained death. Insects lay eggs on or in human remains, as well as utilize the corpse for food or habitat. Insect development and successional patterns can be an indication of the post-mortem interval when time of death is unknown. Insects also provide an alternate specimen for toxicological analysis when traditional specimens are no longer useful. Entomological evidence has also been implicated in cases of abuse or neglect.

Being a subset of forensic entomology, entomotoxicology emphasizes on the developmental patterns of necrophagous insects infesting on decomposing carcasses/ corpses as well as the value of entomological evidence in suggesting the possible cause of death (Gennard, 2007). While the detection of drugs of abuse and therapeutic drugs in entomological specimens would only be useful in suggesting circumstances surrounding death, detection of poisons such as malathion and paraquat may possibly

be indicative in deciding the actual cause of death (Mahat *et al.*, 2012; Mahat and Jayaprakash, 2013).

Death investigation involving a badly decomposed body is a challenge because of decomposition process that begins immediately after death. Decomposition process consists of several stages: fresh stage, bloated stage, decay stage, postdecay stage and skeletal stage (Goff, 2011). The availability of tissues and body fluids for toxicological analysis is influenced by the state of decomposition. As an alternative, fly larvae found on decomposing dead bodies can be used for toxicological analysis in cases where the conventional sample such as tissues and body fluids are not suitable or available (Tracqui *et al.*, 2004; Introna *et al.*, 2001). Flies are attracted to dead bodies due to the odour produced during decomposition process, infested on them and deposited eggs (Goff, 2011). While feeding on dead bodies as a source of food, the larvae will accumulate drugs and poisons that are present in the cadaveric tissue (Sadler *et al.*, 1995).

Poisoning by pesticides and other agricultural chemicals is a major problem especially in developing countries such as Malaysia (Eddleston, 2000). Pesticides are used in the agriculture sector to kill, repel or control certain forms of plant or animal life that are considered to be pests. Paraquat is a herbicide widely used for broad leaf weed control. It is a quick-acting, nonselective compound that destroys green plant tissue on contact and by translocation within the plant. In addition to the common use of herbicide such as paraquat; it is also ingested for committing suicide or in accidental manner (Morris and Maniam, 2001; Jeyaratnam, 1990).

1.2 Statement of Problem

Paraquat remains as one of the most common poisons used by suicides in many countries including Malaysia (Eddleston, 2000). Studies have indicated that suicides are increasingly committed in remote areas (Page *et al.*, 2007) with least ease for access (Hayati *et al.*, 2008) leading to more frequent findings of highly decomposed bodies. In such highly decomposing condition, entomological specimens have been suggested as alternative samples for drawing toxicological inferences (Gennard, 2007; Mahat *et al.*, 2012).

Entomological specimens can be used for diagnosing the presence of drugs and poisons in badly decomposed bodies recovered at the scene of crime. Previous studies focused on drugs of abuse or therapeutic drugs that seldom caused death, however the detections were useful in indicating the circumstances surrounding death such as addiction but not the actual cause of death. Detection of poisons such as paraquat and malathion in larvae, in contrast to the detection of drugs of abuse, would enable in diagnosing the cause of death. In this study, detection of paraquat in larvae would enable diagnosis of cause of death, since paraquat is a known poison that is used to commit suicide and not drug of abuse.

The circumstances necessitating the use of larvae for paraquat detection would include situations where body fluids are not available, organs are not identifiable for sampling due to the advanced stages of decomposition (Campobasso *et al.*, 2004; Gennard, 2007) or absence of body in instances of secondary disposal. Despite the vast availability of analytical methods for analyzing paraquat in human tissues/organs reported in literature, specific validated methods for analyzing such compound in entomological specimens remain unreported. Due to differences in its compositions and structures, utilization of invalidated general methods for analyzing paraquat in entomological specimens may carry certain negative legal implications viz. inadmissibility of the evidence in court due to the lack of empirical evidence and dispute in the accuracy of the general method for detecting paraquat in entomological

specimens. Hence, this specific study focusing on developing and validating method for detecting paraquat in entomological specimens for diagnosing fatal poisoning acquires forensic significance.

1.3 Objectives of Study

This study was designed with the following objectives:

- i. To study the potential use of insect larvae as samples for forensic investigation.
- ii. To establish extraction method for detection of paraquat in larvae sample.
- iii. To develop a method for the qualitative determination of paraquat in larvae sample using capillary electrophoresis (CE).

1.4 Scope of Study

Despite its common use as herbicide in agricultural countries such as Malaysia, paraquat has been attributable to suicidal and accidental death cases. This present study investigated the presence of paraquat residue in fly larvae from simulated human body decomposition using in vitro minced-beef substrates. Following the estimated lethal dose of paraquat reported for humans, two of the substrates designated as P1 and P2 were treated with the estimated lethal dose (40 mg/kg) and three times the estimated lethal dose (120 mg/kg), respectively, while one substrate was used as control (C). The larvae samples that infested and fed on

beef substrates were collected and extracted. Identification of the presence of paraquat residue was carried out using capillary electrophoresis (CE) with diode array detector (DAD).

1.5 Significance of Study

The specific analytical method for detecting paraquat in Calliphorid maggot developed in this study may prove of forensic significance in providing the empirical evidence that may lead to the admissibility of such evidence in the court of law.

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