Residual pyrethroid content in wet and dry interior decorative coatings- an HPLC study

by
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Declaration

I, the undersigned, hereby declare that the work contained in this thesis is my own work and that I have not previously in its entirety or in part submitted it at any university for a degree.

Joshua Tinashe Johani December 2020

Abstract

Mosquitoes known to be malaria vectors continue to affect many regions in Africa. To mitigate the detrimental health effects arising from the cohabitation of such species with humans, pyrethroid-based interior decorative coatings are being developed. The paints are seen as a viable option to improve the longevity of the insecticides. However, before the insecticidal paints can find widespread application, there is need for the development of analytical methods to quantify the levels of pyrethroid insecticides in the paint. The analytical information is important to determine the quality of the product and the safety profile of the insecticidal paint to humans and the environment.

A normal phase HPLC method was developed for the rapid identification and quantification of bifenthrin, cis- and trans-permethrin based on available analytical standards. A multiple point matrix-matched calibration approach was employed for the determination of insecticide quantities. The limits of quantification for the method are 5.8 ng for bifenthrin, 4.4 ng for cis-permethrin and 5.0 ng for trans-permethrin. The recoveries for the analytes are >99 % and the relative standard deviations are below 2%. Therefore, the validation data for the method are excellent.

In the next step, a method was developed to isolate the insecticides from the matrix of complex paint formulations. A 3 hours Soxhlet extraction protocol with dichloromethane-hexane (95:5 (v/v)) was found to be suitable for the isolation of permethrin and bifenthrin from the paint matrix prior to HPLC analysis. The method can be applied directly to paint films. Wet paints are required to spread over 5 g sand + 0.1 g LiBr and 2.5 g $MgSO_4$ for efficient extraction. The extraction efficiencies are between 86-96 % bifenthrin and 88-99 % permethrin. The relative standard deviations are acceptable and below 20 %.

The new developed method was successfully applied in the analysis of real insecticide paints. Preliminary studies show that decorative coatings may contain >30 % of the initial pyrethroid content after more than 2 years of application. This is remarkable and very promising for the application of pyrethroid paints in contributing towards the fight against malaria.

Opsomming

Malaria, en muskiete wat die parasiet dra, raak steeds baie streke in Afrika. Om die nadelige gevolge van die saamwoon van sulke spesies te versag word daar piretroïed-gebasere verwe ontwikkel. Die verf word gesien as 'n lewensvatbare opsie om die langlewendheid van die insekdoders te verbeter. Voordat die insekdodende verf wydverspreide toepassing kan vind is dit nodig om analitiese metodes te ontwikkel om die vlakke van piretroïed-insekdoders in die verf te kwantifiseer. Die analitiese inligting is belangrik om die kwaliteit van die produk en die veiligheidsprofiel van die insekdodendeverf vir mense en die omgewing te bepaal.

'n Normale-fase hoëdrukvloeistofchromatografie-metode (HPLC) is ontwikkel vir die vinnige identifikasie en kwantifisering van bifentryn, cis- en transpermetrien gebaseer op beskikbare analitiese standaarde. 'n Meervoudige punt-matriks-gekalibreerde benadering is gebruik vir die bepaling van die hoeveelheid insekdoders. Die kwantifiseringsgrense vir die metode is 5.8 ng vir bifentryn, 4.4 ng vir cispermetrien en 5.0 ng vir transpermetrien. Herstel vir die analiete is >99 % en die relatiewe standaardafwykings is onder 2 %. Die bevestigingsdata vir die metode is dus uitstekend.

In die volgende stap is 'n metode ontwikkel om die insekdoders uit die komplekse matriks formulering te isoleer. Daar is gevind dat 'n 3 uur Soxhlet-ekstraksieprotokol met dikloormetaan-heksaan (95:5 (v/v)) geskik is vir die isolasie van permetrien en bifentryn vanaf die verfmatriks voor HPLC-analise. Die metode kan direk op verffilms toegepas word. Nat verf is nodig om oor 5 g sand + 0.1 g LiBr en 2.5 g $MgSO_4$ te versprei te word vir effektiewe ekstraksie. Die ekstraksie-doeltreffendheid is tussen 86 – 96 % bifentryn en 88 – 99 % permetrien. Die relatiewe standaardafwykings is aanvaarbaar en onder 20 %.

Die nuut-ontwikkelde metode is suksesvol toegepas in die ontleding van regte insekdoderverf. Voorlopige studies toon dat dekoratiewe bedekkings na meer as 2 jaar nog >30 % van die aanvanklike piretroïedinhoud kan bevat. Dit is merkwaardig en baie belowend vir die toepassing van piretroïedverf se bydra tot die stryd teen malaria.

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Å	Angstrom
A	Absorbance
α	Selectivity factor
b	Path length across a flow cell
c	Concentration
C_8	Decyl-bonded silica
C_{18}	Octadecyl-bonded silica
$CaCl_2$	Calcium chloride
C_m	Analyte molar concentrations in the mobile phase
C_s	Analyte molar concentrations in the stationary phase
ε	Molar absorptivity coefficient of the analyte at given wavelength
ΔG	Change in Gibbs free energy
ΔH	Change in enthalpic interactions
I	Incident beam of light passing through a sample
I_o	Reference beam of light
K	Retention factor
K_d	Equilibrium distribution coefficient
K_{LAC}	Distribution coefficient
LC_{50}	Median lethal concentrations
LiBr	Lithium Bromide
$MgSO_4$	Magnesium sulphate
N	Theoretical plate number (efficiency)
p	Probability values

r	Correlation coefficient
R	Universal gas constant
r^2	Linear regression coefficient
R_s	Chromatographic resolution
SD, S	Standard deviation
Si	Silica
t_r	Retention time
t_o	Column dead time
V_e	Elution volume
v/v	Composition by volume
V_i	Interstitial volume
V_p	Packed porous stationary phase particles ()
V_S	Volume solids
$w_{1/2}$	Width at half the height of the peak
w_b	Base peak width
wt%	Percentage by weight

List of Abbreviations

^{13}C NMR	Carbon-13 nuclear magnetic resonance
^{1}H NMR	Proton nuclear magnetic resonance spectroscopy
^{31}P NMR	Phosphorus-31 nuclear magnetic resonance
ACE	Acetone
ACT	Artemisinin-based combination therapies
ANOVA	Analysis of variance
APCI	Atmospheric pressure chemical ionisation
ATR	Attenuated total reflectance
CIPAC	Collaborative International Pesticides Analytical Council
DCM	Dichloromethane
DIOX	1,4-Dioxane
DMF	Dimethylformamide
ESI	Electrospray ionization mass spectrometry
Et_2O	Ethoxyethane
EtOAc	Ethyl acetate
FTIR	Fourier transform infrared
GC	Gas chromatography
GLP	Good laboratory practice
GPIRM	Global Plan for Insecticide Resistance Management
H_2O	Deionized water
HCl	Hydrochloric acid
HEX	n-Hexane
HILIC	Hydrophilic interaction Chromatography

HPLC	High perfomance liquid chromatography
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IRS	Indoor residual spraying
ISO	2,2,4-Trimethylpentane
ITN	Insecticide treated nets
LAC	Liquid adsorption chromatography
LC	Liquid chromatography
LLE	Liquid–liquid extraction
LLITN	Long lasting insecticide treated net
LOD	Limit of detection
LOQ	Limit of quantification
MeCN	Acetonitrile
MeOH	Methanol
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
MSPD	Matrix solid phase dispersion
NaCl	Sodium chloride
NEMA	National Environmental Management Agency of Uganda
NMR	Nuclear magnetic resonance
NPLC	Normal phase liquid chromatography
PBO	Piperonyl butoxide
PDA	Photodiode-array UV detector
PLE	Pressurised liquid extraction

PVC	Pigment volume concentration
QuEChERS	Quick, easy, cheap, effective, rugged and safe
RC	Regenerated cellulose
RSD	Relative standard deviation
SFE	supercritical fluid extraction
SLE	Solid-liquid extraction
SPME	Solid phase microextraction
THF	Tetrahydrofuran
TOF-MS	Time-of-flight mass spectrometry
TOL	Toluene
USEPA	United States Environmental Protection Agency
UV	Ultraviolet
WHO	World Health Organisation

Chapter 1

Introduction and Objectives

1.1 Background

Malaria, also known as plasmodium infection, has been identified with untold human morbidities and mortalities for many centuries. Today, nearly half of the world's population is at risk of falling ill with malaria. In 2018, an estimated 228 million malaria infections and 405 thousand deaths due to malaria were recorded worldwide, with Africa bearing 93% of the world's malaria morbidity burden.[1] Pregnant women and children under the age of five years, as well as the poor in Africa have remained the hardest hit by scourge of malaria. Therefore, malaria poses a heavy social and economic burden to Africa that is characterised by low labour productivity due to sickness while government investments are consumed by hefty health bills.[2] In South Africa, malaria is prevalent in the low altitude areas of Limpopo, Mpumalanga and KwaZulu-Natal.[3]

The disease is caused by unicellular parasites of the plasmodium genus (*P. falciparum, P. vivax, P. ovale, P. malariae* and *P. knowlesi*) that have an intricate life cycle which depends upon two hosts – a mosquito vector and a vertebrate host.[4,5] The process of the transmission of malaria is quite complex. The malaria parasites are exclusively transmitted by the female anopheles mosquitoes. The region-wide distribution of the female anopheles mosquitoes *An. gambiae, An. arabiensis* and *An. Funestus* that spread the most virulent *P. falciparum* malaria has been partially attributed to Africa's high malaria burden.[6,7] These mosquitoes need proteins for reproductive purposes hence they feed on human blood every 3-4 days, in between which they rest on surfaces in human dwelling space.[8]

While taking a blood meal, the mosquito can inoculate a human host with the plasmodium parasites or pick up the parasites instead, and remain infective for the rest of its life cycle.[4] The signs and symptoms of malaria are initially non-specific, and vary depending on the type of parasite infecting the host. The general signs and symptoms of uncomplicated malaria include headaches, fever (body temperature >37.5 ˚C), rigors, loss of appetite, diarrhoea, nausea and vomiting etc. Severe malaria is characterised by impaired consciousness, cerebral complications, convulsions, respiratory distress, and jaundice among others.[9]

Malaria is both a preventable and a treatable disease. Remarkable progress has been made in the global fight against malaria to date. The World Health Organisation (WHO) has set out to

attain a world free of malaria through the Global Technical Strategy for Malaria 2016–2030.[10] To reduce morbidity and mortality, the strategy emphasises a package of core interventions which are based on the prevention of new malaria infections and the treatment i.e. early diagnosis and prompt, effective treatment of malaria.[11,12]

Artemisinin-based combination therapies (ACT) are recommended for the treatment of *P. falciparum* malaria. ACTs are mixtures of effective antimalarial drugs with different modes of action which are used together to improve their efficacious therapeutic life span. Chloroquine and primaquine are promoted for the treatment of chloroquine sensitive *P. vivax* malaria to guard against relapse. Sulfadoxine-pyrimethamine is administered as a chemoprevention drug in all malaria risk areas.[11]

The period when mosquitoes rest between blood meals presents an opportune time for vector control methods to intercept the malaria transmission cycle. The capacity of vectors to transmit parasites and their vulnerability to vector control measures vary between mosquito species and is influenced by local environmental factors. The first edition of the guidelines for malaria vector control strongly prioritises as core malaria vector control methods, the distribution of insecticide treated nets (ITNs) and indoor residual spraying (IRS) at high coverage and to a high standard for all populations at risk of malaria.

ITNs are preferred because fewer resources and simpler infrastructure are required for their distribution, and especially that long lasting insecticide treated nets (LLITN) with novel slow release properties from the polymeric fibres retain insecticidal efficacy for between 3–5 years.[13,14] Pyrethroids are the only class insecticides approved by the WHO for ITNs. LLITN programs are now in favour in most African countries because of their affordability and ease of implementation compared to IRS programs.

IRS also is a mainstay of operational vector control interventions to reduce and ultimately eliminate malaria transmission.[15] It involves the application of stable formulations of insecticides to the interior surfaces (walls, ceilings and roofs) of human habitats to kill the mosquitoes. WHO prequalified pesticide formulations for IRS deployment include pyrethroid insecticides which have sodium channel modulatory action against mosquitoes, organophosphate and carbamate insecticides which have acetylcholinesterase inhibitory effects against mosquitoes and the neonicotinoid insecticides which are nicotinic acetylcholine receptor competitors.[16]

IRS residual efficacy is limited to between 3-6 months post application of the insecticide to the substrate, usually cement, sheet metal, mud or wood.[17,18] The development of resistance of the mosquitoes to IRS insecticides has partially been attributed to the rapid decline of the concentration of the insecticides to levels below the median lethal concentrations (LC_{50}) required to kill mosquitoes. The failure of IRS to protect the insecticides is the major cause for this decline.[19] There is, therefore, a need for repeated spray cycles which raises concerns over public health risks, environmental pollution and the increased cost of implementing IRS.

At the same time, the global funding to combat malaria is also shrinking. In 2018, only US$2.7 billion in funding was available against a nominal target of US$5.0 billion.[20] This has necessitated for the development of effective and more environmentally friendly insecticide delivery tools to attain longer lasting protection against malaria transmission. Pyrethroid insecticide-based interior decorative coatings, or paints, have emerged as a viable tool to enhance the durability of the effectiveness of IRS insecticides against malaria disease vectors that rest on ceilings and walls of human dwelling spaces.[17]

Pyrethroid-based interior decorative coatings prolong the insecticidal activity of surfaces against disease vectors such as mosquitoes. The small size of the low molecular weight pyrethroid compounds allows for their leakage out of the coating matrix. This is because coatings are soft materials by virtue of their polymeric composition although they also contain solid fractions.[21] Although the flexibility of the polymers in the paint is very restricted, it is still sufficient for small molecules to be able to move through the coating. It has been learned that over a period of time the quantity of the insecticide would decrease either due to environmental pressure or by transfer to the intended target organisms.[22]

Before insecticide paints can be universally applied, there is need for the development of analytical methods for the determination of insecticide concentrations within the complex paint matrices for quality control or pesticide residue measurement purposes. Liquid chromatography (LC) is a handy separation method for this problem. The aim of the separation is to identify and quantify the analytes in a sample. The samples are often quite complex as they contain a wide range of components with varying solubilities. This presents challenges related to the relative quantities and the physico-chemical properties of different components present in the sample matrix. Consequently a successful analysis requires that the sample preparation and the subsequent separation methods be both highly selective and sensitive. High performance liquid chromatography (HPLC) can satisfy these requirements when combined

with advanced detection systems such as photodiode-array UV detection (PDA) or mass spectrometry (MS).

1.2 Problem Statement

The development, manufacture and distribution of pyrethroid insecticidal interior decorative coatings poses quality, safety and health risks to human beings and the environment. There is no valid HPLC method published in literature for the determination of pyrethroid insecticides in latex interior decorative coatings. Therefore, the lack of analytical methods for the quantification of insecticides in the paint matrix for product quality control, estimation of human exposure risk and modelling of environmental impact contribute effectively to the background of this study.

The expected value of this study is to develop and optimise procedures for the extraction of pyrethroid insecticides from paint matrices, and to develop a valid liquid chromatographic analytical method for the identification and quantification of the insecticides. It is essential to answer questions pertaining to the exact pyrethroid insecticide composition of a paint formulation, the quantity of insecticide remaining after a period of time under storage or service life of the paint, and the processes influencing and the chemical products resulting from the decrease in insecticide quantity. It is envisaged that the preparative quantitative isolation of prethroids prior to analysis with HPLC could present challenges due to the complexity of the paint matrix.

1.3 Objectives

The objectives of this study were to:

1. Develop an HPLC analytical method with the expectations to:
    - Develop an approach for rapid identification of pyrethroid insecticide in a paint formulation based on a standard analytical reference sample
    - Develop and validate a high performance liquid chromatography method for the quantification of pyrethroid insecticides in interior decorative coatings
2. Develop a protocol for the extraction of pyrethroid insecticide from paints. The goals are to:
    - Investigate the relative extractability of pyrethroids from paints using affordable and easily accessible techniques
    - Optimise and evaluate the performance of the method that demonstrates the highest extraction efficiency

3. Apply the valid analytical method for the analysis of pyrethroids in real paint samples with the aim to:
    - Determine the pyrethroid content of aged real wet and dry coatings. The results can be correlated to LC_{50} values to determine the time beyond which the coating is expected to lose its efficacy against mosquitoes
    - Develop a valid LC-MS method for the detection of trace quantities of pyrethroids, and for the identification and characterisation of the products of their transformation

1.4 Layout of thesis

This thesis consists of the following chapters:

Chapter 1

Chapter 1 provides a brief background to the topic and the objectives of the study.

Chapter 2

Chapter 2 presents an overview of the historical and theoretical background to this work. This includes a review of the application of pyrethroid-based latex decorative coatings for the control of mosquito populations in malaria endemic areas. The chapter delves more on the theory of liquid chromatography and its application in the analysis of pyrethroid insecticides, outlining the merits and flaws of each analytical method.

Chapter 3

Chapter 3 addresses the development and optimisation of a liquid chromatography (LC) analytical method for separating and measuring pyrethroid quantities in sheen and matte latex paints by normal phase liquid chromatography (NPLC). The performance of the method is validated with due diligence.

Chapter 4

The extractability studies of the pyrethroids from both wet and dry films of the latex coatings are discussed with the main focus on preparative techniques such as liquid-liquid extraction (LLE) and solid-liquid extraction (SLE) techniques. The pyrethroid quantities are determined by the normal phase liquid chromatography (NPLC) developed in Chapter 3. The most efficient extraction protocol, the Soxhlet method, is selected and its perfomance is evaluated.

Chapter 5

Chapter 5 outlines the preliminary findings of the application of the NPLC method for the quantification of the pyrethroid insecticides in real paint samples. It highlights the need for hyphenation of LC with mass spectrometry (MS) for detection of trace quantities of pyrethroids and their transformation products, and also the need for a time-based study to determine the rate of release or loss of the pyrethroids from the paint matrix.

Chapter 6

In Chapter 6, the results in the Chapters 3 through to 5 are summarised and brought to a conclusion. Also, some future work related to the study is proposed.

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Chapter 2

Historical and Theoretical Background

2.1 Introduction

It was important for this study to review the constituents of the test matrices, the physical and chemical properties of the pyrethroid insecticides to be quantified and the principles behind the analytical methods to be developed and validated for the analysis of the pyrethroids. This information would highlight the important parameters to guide the design of the experimental work in the laboratory.

2.2 Insecticidal Decorative Coatings in Malaria Control

There is not much published literature concerning the application of pyrethroid-based insecticide decorative coatings or paints for the control of mosquito populations in the fight against malaria transmission. A study on the causatives of transformation showed that laboratory ageing conditions (heat, humidity, pH) were not the major impediment to the persistence of IRS insecticides in the environment, but rather water-mediated hydrolysis at high pH as well as high bioactivity in the environment.[1] As a result, the World Health Organisation through the Global Plan for Insecticide Resistance Management (GPIRM) in malaria vectors cited renewed interests in the incorporation of pyrethroid insecticides into interior decorative coatings as controlled release media at the proof of concept level.[2]

Recently, pyrethroid based interior decorative formulations based on modified acrylic emulsions, the Kansai anti-mosquito paints, were developed. These formulations retain the residual effectiveness of a single application against mosquitoes for up to twenty four months.[3] They also have anti-fungus and anti-algae properties to add to their insecticidal activity. The paints can also exhibit spatial repellence, contact irritancy, or insect growth regulation based on the biocidal action of the embedded insecticides. The paints are especially designed to be safe for interior use in residential homes, public and commercial buildings. The arrival of insecticide paint technology coincides with a general shift towards modern building materials which are better suited for the application of paints such as cement, plywood and corrugated metal as a result of increasing urbanisation due to widespread economic development.[4]

The efficacy of these paints was realised through globally recognized Good Laboratory Practice (GLP) testing. The latex paints provide smooth matt or glossy finishes, durability with easy-to-clean surfaces which are available in a range of different colours. These lead-free, low emission products have gained accreditation from the United States Environmental Protection

Agency (USEPA),[3] the Zambia Environmental Management Agency[5] and the National Environmental Management Agency of Uganda (NEMA).[6] This innovation will likely be more welcome because it does not flaw a decorated surface with flecks and stains compared to IRS which many home owners have found highly intrusive.

It has been demonstrated that insecticidal paints meet the minimum quality standards desired for an interior paint, and that the in-service performance of the insecticide paint compares indifferently to a standard paint formulation.[7] The pyrethroids displayed greater potency at an optimum incorporation rate of 0.5 wt% than an organophosphate insecticide and maintained >90% mortality on insect populations daily for 240 days. The mortality rate decreased with increase in the size of insect e.g. the mortality of mosquitoes > house flies > cockroach.

The degradation of IRS insecticides incorporated into pseudo-paints under accelerated laboratory conditions of elevated temperature, ultraviolet light exposure and high humidity on various substrates related to both rural and urban household surfaces has been reported where the stability of pyrethroids was greater than that of carbamates and organophosphates.[1] Carbamates were particularly the most insecticidally active against mosquito populations when subsumed in acrylic binder during bioassay experiments while pyrethroids were inactivated, suggesting that pyrethroids are somewhat soluble in the latex.[1] The comparison of pyrethroid- and organophosphate-based vinyl paints showed that both formulations retained their efficacies for more than 12 months against *Triatoma infestans*. Although the pyrethroid-based formulation displayed greater potency, the effectiveness of the organophosphate formulation outlasted that of the pyrethroid beyond 12 months.[8]

Organophosphate- and insect growth regulator-based insecticidal paints which demonstrated a residual effect of up to 32 months have also been reported. They are mostly vinyl paints with an aqueous base commonly employed for the control of *Triatoma infestans* - the vector that spreads Chagas diseases.[9,10,11,12,13] They are viewed as alternatives to pyrethroid insecticide paints in areas where resistance to pyrethroids is manifest.

The concept of insecticidal paints is attractive, and will likely gain universal availability relatively quickly once proof of concept information is satisfactory, among which is the development of analytical methods for the quality control determination of insecticide concentrations. The manufacturing of insecticidal paints does not require any special adaptation to current manufacturing facilities, distribution, and sales networks for conventional paints. This critical incentive for commercial development, production, and marketing of

insecticidal paints has already garnered the attention of multinational corporations, such as paint companies Kansai Plascon[3] and Akzo Nobel in partnership with insecticide producer Bayer.[14] The quality of an insecticidal coating can ultimately be judged, in addition to the generic in-service performance, by length of time over which the paint maintains its insecticidal value.

2.3 Pyrethroids

2.3.1 Structure and Chemistry

Synthetic pyrethroids were developed from natural pyrethrins. Pyrethrins are ideal for application in agriculture and public health systems because they are efficient natural pesticides with very good insecticidal properties combined with low mammalian toxicities.[15] However, their instability due to extensive degradation-labile centres within their molecular framework coupled with high costs of production and highly variable supply makes them commercially unviable.[1,16,17] Therefore the synthesis of pyrethroids has been targeted for the reduction of substantial degradation-labile centres that exist in pyrethrins while preserving the overall stereochemical conformation allied with the insecticidal activity of the compounds. The general structure of a pyrethroid insecticide is shown in Figure 2.1.[18] All pyrethroids (except Etofenprox which does not have a cyclopropane moiety) have the characteristic cyclopropane carboxylic ester moiety, dihalogen substituted vinyl moiety at R_1 and a phenoxy-ether moiety at R_2.

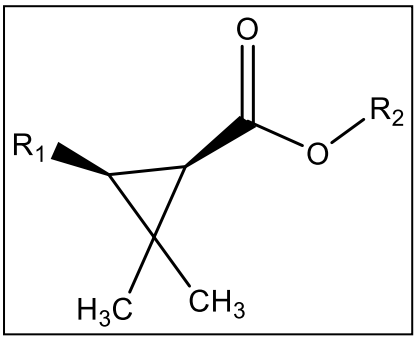

Figure 2.1: General chemical structure of a pyrethroid

Today the pyrethroids are among the largest classes of insecticides used in the world, well favoured for application in agriculture and public health because of their potency and their safety profile related to their ease of metabolisation by enzymes in the body. As a result, most household pest and vector control chemicals today commonly contain pyrethroids as the

principal active ingredient. Pyrethroids are non-systemic insecticides that penetrate rapidly through the cuticle of insects and impair the normal function of insect's nervous system resulting in a characteristic knock down effect, even at small dosages. Insect knock down is seen by excitation and incoordination of movement amounting to paralysis.[15,19] Type I pyrethroids prolong the after-effect of an action potential by delaying the closure of Na^+ channels. Type II pyrethroids disrupt the functioning of Ca^{2+} dependent enzymes to phosphorylate neutral proteins for the impetuous generation of the action potential.[20]

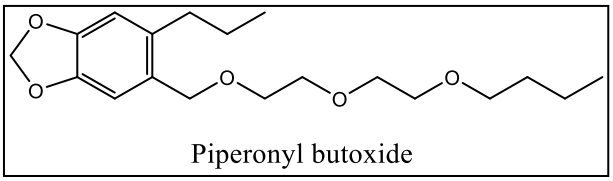

Figure 2.2: The chemical structure of pyrethroid synergist piperonyl butoxide (PBO)

Insects affected by the action of pyrethroids can sometimes recover within a short period of time due to the rapid metabolic breakdown of the pyrethroids. Thus, pyrethroids are commonly used with synergists, i.e. non-toxic or insecticidally benign additives that amplify the activity of an active ingredient. 5-[2-(2-butoxyethoxy)ethoxymethyl]-6-propyl-1,3-benzodioxole (piperonyl butoxide), see Figure 2.2, is the most popular synergist applied with pyrethroids and pyrethrins.[21]

2.3.1.1 Permethrin

3-phenoxybenzyl (1RS,3RS;1RS,3SR)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-carboxylate (permethrin) has four stereoisomers that arise from two cis and trans enantiomer pairs, commonly in the 40:60 or 25:75 ratios, respectively.[22,] Permethrin has non-systemic insecticide properties making it active mainly by contact and stomach action.[23] Cis-permethrin is both insecticidally active and toxic to mammals while the toxicity of trans-permethrin to mammals is below measurable effects because it is intrinsic to the molecule.[24] Although permethrin isomers exhibit equal insecticidal potency, their stability may vary significantly and, therefore, necessitate separate quantification of geometric isomers. Permethrin is less hazardous to mammals because it is rapidly broken down by enzymes in the body to compounds that can be easily excreted from the body.[25] Therefore, permethrin is a potentially safe insecticide for both household and agricultural applications. Figure 2.3 shows the chemical

structure of permethrin and Table A-1 summarises the physical and chemical properties of permethrin.

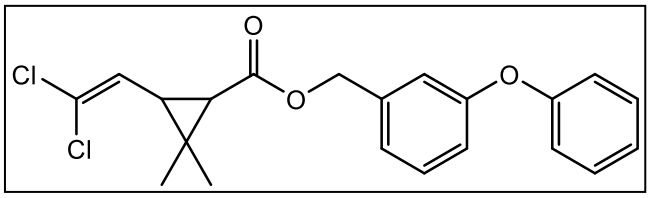

Figure 2.3: Chemical structure of permethrin

2.3.1.2 Bifenthrin

2-Methylbiphenyl-3-yl-methyl (Z)-(1RS,3RS)-3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate (Bifenthrin) exists as a mixture of the E- and the Z-isomers commonly in the ratio of 99.67%: 0.33%, respectively. Bifenthrin is both an insecticide and an acaricide i.e. potent to mites and ticks. It is recommended for public health use against a range of agricultural and veterinary pests because of its low acute toxicity to humans.[26] Although highly lipophilic, bifenthrin particularly does not cause any skin irritation nor does it cause sensitisation to the eyes.[27] As a result of these attributes bifenthrin is preferred for use in mosquito nets, indoor residual spraying, space spraying and insecticidal paints. The degradation pathways for bifenthrin mainly follow the conversion of the cis-isomer to the trans-isomer and cleavage of the ester to yield biphenyl alcohol as the major product of these processes.[27] Figure 2.4 shows the chemical structure of bifenthrin while the physical and chemical properties are summarised in Table A-2.

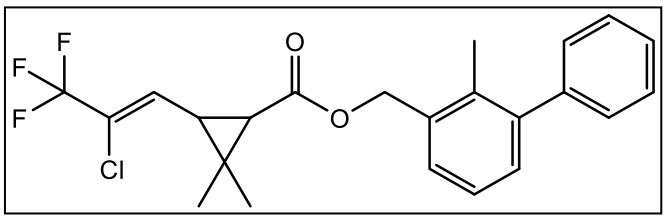

Figure 2.4: Chemical structure of bifenthrin

2.3.2 Transformation of Pyrethroids

2.3.2.1 Photolysis

Decarboxylation is the main route for the photo-decomposition of pyrethroid insecticides because of the susceptibility of the carboxylic ester moiety.[1] The main process of degradation of the pyrethroids is the transformation between cis- and trans- or E/Z isomers when irradiated

with UV light. The minor photo-decarboxylation process depends on the electronegativity of the halogen substituents on the pyrethroid, increasing with increasing electronegativity.[21,22]

2.3.2.2 Thermolysis

The thermal degradation of pyrethroids usually follows transformation between isomer forms, ester cleavage and finally oxidation of the final products.[28] Most pyrethroids disintegrate before reaching the boiling temperature, therefore, secondary oxidation or pyrolysis occurs at temperatures above 300-400 °C and is associated with the production of CO, CO_2 and H_2O.[29,30]

2.3.2.3 Biological transformation

Hydrolysis is the main pathway for biological breakdown of pyrethroid insecticides. Esterase enzymes attack the ester linkage via a reversible acylation of the enzyme. This allows the release of the alcohol moiety of the pyrethroid ester and an acylated enzyme which undergoes base hydrolysis with water to terminate the reaction cycle. Finally the corresponding carboxylic acid moiety of the pyrethroid is released and the enzyme is recovered.[24]

2.4 The Constituents and Formulation of Decorative Coatings

A decorative coating is a stable dispersion of solids. These include pigments, resins, extenders and additives, in a solvent in appropriate proportions that can form a solid film upon drying on the surface of the substrate. Depending on the intended function, the coating can impart decorative, protective or other properties.[31,32] The solid layer (film) left on the substrate consists of the volume solids of the paint i.e. the amount of solids relative to the initial volume of the wet paint. Volume solids, VS, can be calculated using Equation 2.1.

$$VS = \frac{Volume_{pigment} + Volume_{solid\ binder}}{Total\ Volume_{wet\ paint}} \qquad \textbf{Equation 2.1}$$

This parameter is important for determining the distribution per unit area of active ingredients, pigments and extenders in the dry coating film. Another important property is the quantitative measure of the level of pigmentation of a coating known as the pigment volume concentration (PVC). It is calculated using the formula in Equation 2.2.[33]

$$PVC = \frac{Volume_{pigment}}{Volume_{pigment} + Volume_{solid\ binder}} \times 100 \qquad \textbf{Equation 2.2}$$

High PVC (matte) paint contains more binder and pigment than low PVC (sheen) paint, therefore, PVC has a profound effect on the permeability of the final coating film matrix, which can play a significant role towards the release of low molar mass additives like insecticides.[31,34]

The process of manufacturing a paint follows two steps explained in the following sections.

2.4.1 Dispersion or Grind Stage

It involves the high speed dispersion of pigment particles (e.g. iron oxides and TiO_2 to impart opacity and contrast ratio) in the vehicle or solvent i.e. water for latex paints. Dispersing agents e.g. inorganic phosphate salts, organic surfactants e.g. low molar mass polyvinyl acetate or styrene-maleic acid polymers, wetting agents e.g. glycerol or glycols, defoaming agents, biocides and pigment extenders e.g. $CaCO_3$ are also vigorously homogenised into the mixture using a high speed disperser. The mixture is allowed to remain under high shear to allow for stabilisation over a set time.[35,36]

2.4.2 Let Down Stage

This stage involves the low shear mixing of the polymeric binder, usually vinyl- or acrylate-type polymers. Pyrethroids are usually added at this point during the manufacture of insecticide paints.[7] Other additives including colour compatibilisers, colourants or dyes, in-can preservative biocides, coalescing agents (high boiling solvents), defoamers and surfactants to cater for the colourants and lastly the rheology modifiers that stabilise the paint formulation and support the paint system during mixing and spreading are added during this stage.[32,37]

2.5 Principles of Separation in High-Performance Liquid Chromatography (HPLC)

This work focuses on the quantification of pyrethroid insecticides in interior decorative coatings. The coating matrices are a complex concoction of numerous components (Section 2.4) which make it difficult for the direct determination of the pyrethroids. The distribution of the pyrethroids within the paint matrix is not fully understood, but it is expected that they reside both within the inorganic (pigment and extenders) dominated and organic (polymeric binder) dominated micro domains. This complicates the selection of suitable sample preparation methods and demands for a robust, sensitive, selective and accurate analytical technique. Therefore, liquid chromatography (LC) was selected because it is capable of providing good resolution on the diastereomers or enantiomers of the pyrethroids, has no issues with sample volatility and the availability of non-destructive detectors especially for pyrethroids which can be sensitive to heat.[28,29]

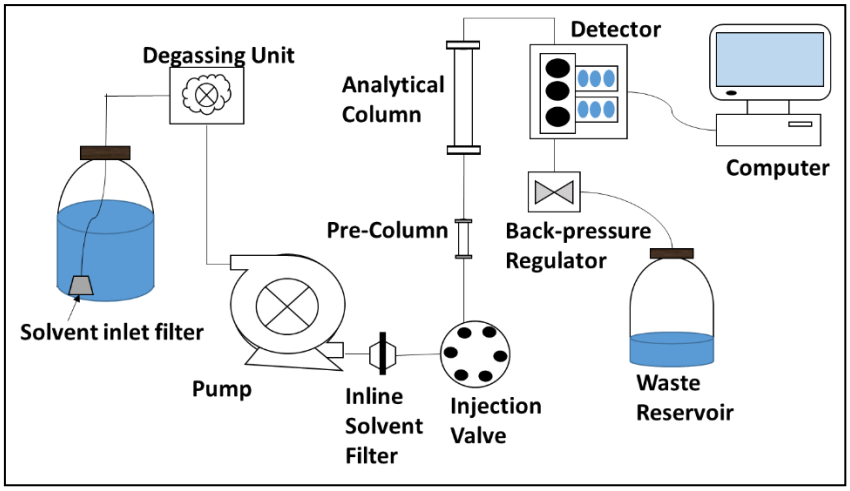

Figure 2.5: Scheme of a modern HPLC system

A modern HPLC system (Figure 2.5) consists of a solvent reservoir, a high-pressure solvent delivery system, an automated sample injector, a separation column, a thermostated column compartment for temperature control, a detector (often a UV or DAD) and a computer to control the system and display the results. Many systems now include an in-built degasser to free the solvent of dissolved gases. HPLC depends upon the mechanisms of interaction between the analyte and the stationary phase. The stronger the analyte-stationary phase interactions, the more the analyte partitions between the stationary and mobile phases. This partitioning depends on thermodynamic effects and is described by the equilibrium distribution coefficient K_d (Equation 2.3). The higher K_d the more an analyte is retained and the later it elutes from the column.

$$K_d = \frac{c_s}{c_m} \qquad \textbf{Equation 2.3}$$

where C_s and C_m represent the analyte molar concentrations in the stationary and mobile phases, respectively.

The total volume of the column is the sum of the volume of the packed porous stationary phase particles (V_p) and the interstitial volume (V_i) between the packed particles. The retention volume of an analyte can be described using Equation 2.4:

$$V_r = V_i + V_p K_d \qquad \textbf{Equation 2.4}$$

The pores of stationary phase particles have limited dimensions into which, depending on the analyte's molecular mass, the molecules can completely or partially penetrate the pores.

Separation by size of the molecules or their chemical nature can occur depending on the strength of the analyte-stationary phase interactions. K_d relates the elution volume to the change in Gibbs free energy (ΔG) through Equation 2.5 where the entropy term (ΔS) denotes the possible conformations an analyte can occupy while the enthalpy term (ΔH) represents the analyte-stationary phase interaction by partitioning or adsorption. Stationary phase pore sizes pose a restriction to the number of possible analyte conformations such that separation occurs through enthalpic interactions, conformational entropy or a combination of both.

$$\Delta G = \Delta H - T\Delta S = -RT \ln K_d \qquad \textbf{Equation 2.5}$$

where *T* is the absolute temperature of the system and *R* is the gas constant.

The modes of separation can be identified into which LC can be categorized depending on the extent to which the enthalpic and entropic effects contribute to the change in Gibbs free energy: steric exclusion as in size exclusion chromatography (SEC) of high molar mass compounds, specific binding interactions as in affinity chromatography, charge-dependant interactions as in ion exchange chromatography (IEC), and adsorption- or partition-based separations as in liquid adsorption chromatography (LAC).[38,39]

Liquid Adsorption Chromatography

The separation in LAC is based on only the analyte-stationary phase adsorptive interactions and, therefore, LAC separates analytes with respect to chemical composition. Under ideal conditions, enthalpic effects govern the separation process while conformational entropy contributions are negligible. Therefore, the distribution coefficient (K_{LAC}) is expressed by the enthalpic term only as shown in Equation 2.6.

$$K_{LAC} = e^{\left(\frac{-\Delta H}{RT}\right)} \qquad \textbf{Equation 2.6}$$

LAC methods in which the mobile phase composition remains constant are called isocratic methods while (solvent) gradient elution methods are where the chemical composition of the mobile phase changes during the separation. Liquid chromatographic methods that employ highly polar stationary phases with nonpolar organic solvents as mobile phases are referred to as normal phase liquid chromatography (NPLC).[40] In NPLC, the least polar component is eluted first while the most polar component elutes last. Methods can operate under isocratic conditions, or gradient elution where the separation begins with the non-polar solvent going over to the more polar one. The polarity of silica-based stationary phases can be adjusted with

chemically attached functional groups in decreasing order of polarity OH<NH_2<CN, with silica being the most polar. [38,39] Hydrophilic Interaction Chromatography (HILIC) is a variation of NPLC which employs hydrophilic neutral stationary phases in combination with aqueous-organic mobile phases.[41] In reversed-phase liquid chromatography (RPLC) the stationary phases are nonpolar while the aqueous-organic mobile phases are relatively polar.[42] The most polar component is eluted first, because it is the least retained on the stationary phase. Gradient elution starts with the polar solvent going to the less polar one. The polarity of the RP stationary phase may be adjusted as well. Silica bonded with large non-polar aliphatic groups is used as stationary phase; the longer the aliphatic groups the greater the lipophilicity or the lower the polarity (C_{18}> C_8>C_5).[38]

The greater the affinity of an analyte to the stationary phase, the more likely it adsorbs onto the stationary phase. Hence the analyte is retained more and elutes later from the column while those with a lower affinity for the stationary phase elute earlier. As a result the sample components will have different retention factors (*k*), usually expressed in terms of retention time (t_r) or elution volume (V_e), by which components can be identified after a separation.[39] The detector's response to the presence of the analyte presented as a plot of detector signal versus elution time or volume is called a chromatogram.

It is important in HPLC to obtain optimum resolution (baseline separation with a resolution factor of 1.5 or greater) between adjacent analyte peaks in the minimum time possible so that the area or height of each peak can be accurately measured.[39,43] Equation 2.7 is the fundamental resolution equation which shows the dependence of optimum chromatographic resolution on efficiency, selectivity (separation factor) and retention (capacity factor).

$$R_s = \frac{1}{4}\sqrt{N} \times \frac{\alpha - 1}{\alpha} \times \frac{k}{1 + k} \qquad \textbf{Equation 2.7}$$

where R_s is chromatographic resolution, N is the theoretical plate number (efficiency), α is the selectivity factor and k is the retention factor.

The retention or capacity factor, *k*, is a quantitative description of an analyte's retention on the chromatographic column. It is calculated using Equation 2.8.

$$k = \frac{t_{r_i} - t_o}{t_o} \qquad \textbf{Equation 2.8}$$

where t_{r_i} is the retention time of component '*i*' and t_o is the column dead time.

The analytes that interact strongly with the stationary phase are highly retained and hence have high *k* values. Factors such as small variations in the mobile phase flow rate and column dimensions have minimal effects on the retention factor, but adjusting the elution strength of the mobile phase is the most effective way to adjust the retention factor of an analyte. At values approximately k>5, increasing retention only provides minimal increases in resolution. Therefore, increasing the selectivity or efficiency of a separation becomes more useful. Selectivity, α, is the ability of the chromatographic system to distinguish between sample components.[44] It is computed as a ratio of the retention factors (k) of the two peaks in question, see Equation 2.9.

$$\alpha = \frac{k_2}{k_1} = \frac{t_{r_2} - t_o}{t_{r_1} - t_o} \qquad \textbf{Equation 2.9}$$

where $k_2 > k_1$ are the retention factors of the two analyte peaks in question.

High α values demonstrate good separation between the apexes of the two peaks but they are not directly indicative of the resolution at the baseline. Selectivity of a separation depends on the chemistry of the analyte, the mobile phase, and especially the stationary phase which must be selected carefully in order to optimise the separation. The theoretical plate number (N) is a measure of the efficiency of the HPLC column. It reflects the dispersion of the analyte band on the column as it migrates through the system and is calculated using Equation 2.10.

$$N = 16\left(\frac{t_r}{w_b}\right)^2 = 5.54\left(\frac{t_r}{w_{1/2}}\right)^2 \qquad \textbf{Equation 2.10}$$

where t_r is the retention time of a sample component, w_b is the base peak width and $w_{1/2}$ is width at half the height of the peak.

$$H = \frac{L}{N} \qquad \textbf{Equation 2.11}$$

where L is the length of the column and N is the theoretical plate number

Each plate is the distance over which an equilibrium for K_d of sample components can be achieved.[44,43] The quality or resolution of a separation improves when there are more theoretical plates available per unit length so that more equilibrations are possible. It is also important that H (Equation 2.11), the height equivalent to a theoretical plate (HETP), be low to accommodate more theoretical plates per unit length of column. The number of theoretical plates is useful in establishing the efficiency of a column. A successful chromatography method requires optimal synergy between the chemistry of the solute, the composition of the mobile

phase, and the stationary phase. Therefore, investigations must be made carefully to arrive at a suitable HPLC column to achieve a good separation and the mobile phase must be optimised to suit the column conditions.

2.6 Detectors Used for Liquid Chromatography Analysis of Pyrethroids

The detection of the pyrethroids following a separation by LC is as important as the LC method chosen for the analysis such as NPLC or RPLC. Therefore, the selection of a suitable detection method could allow for additional insights into the nature of the analyte such as concentration or molecular mass.

The most commonly used detectors in LC are concentration-sensitive detectors. Their functionality depends upon particular spectroscopic properties that are intrinsic to the analytes to facilitate detection, for example the presence of ultraviolet (UV) chromophores. UV, fluorescence (FL) and infrared (IR) detectors find wide application in the analysis of pyrethroids based on this principle. The interaction of a particular wavelength of a beam of light with a given functional group on the analyte is measured as transmittance or absorbance (see Equation 2.12).[45] The Beer-Lambert law (see Equation 2.13) correlates the quantity of analyte to the attenuation of the IR, UV or FL light thus enabling the detectors to measure the quantity of an analyte in LC.[45,46] Although these detectors are quite versatile, their scope of application is limited by the lack of universality because they cannot be applied to analytes which do not possess specific functional groups that are sensitive to the technique for detection to be possible.

$$A = \log \frac{I_o}{I} \qquad \textbf{Equation 2.12}$$

where '*A*' is the absorbance, 'I' is the intensity of the transmitted light and 'I_o' is the reference light intensity

$$A = \varepsilon \times b \times c \qquad \textbf{Equation 2.13}$$

where 'ε' molar extinction coefficient of the sample, '*b'* is the path length across the flow cell in cm and '*c'* is the concentration of the analyte.

The evaporative light scattering detector (ELSD) and differential refractive index detector (dRI) are the popular alternatives for universal detection. This is because the mode of their detection is based on the quantity of the analyte rather than the chemical nature of the analyte.[45] The dRI and the ELSD are two of the most common concentration-dependent universal

detectors available for LC. Their means of detection is independent of the type of analyte hence they are classified as universal detectors.

The dRI detector depends on the principle that a particular analyte will alter the refractive index of the mobile phase relative to its concentration and its own refractive index properties.[45] The dRI detector, however, suffers from a lack of sensitivity. It is desirable, for example in the analysis of residual amounts of pyrethroids in paints, that minute quantities of analyte generate a signal that can be observed. Therefore, the dRI detector is limited in this respect. Furthermore, dRI detectors are not suitable for gradient LC because of sensitivity to mobile phase composition, flow rate and temperature. Gradient LC is actually quite handy in finding optimum mobile phase conditions during method development.

The ELSD provides an alternative to the dRI for the detection of trace amounts of analyte. It is a type of charged aerosol detector. In the detector, the column eluent is nebulised and the mobile phase is evaporated from the analyte droplets. The analyte particles pass through a laser from where the system measures the light scattering of the particles.[47,48] ELSDs can detect non-volatile and semivolatile compounds such as pyrethroids regardless of their spectral or physicochemical properties. The calibration curve obtained from an ELSD response against analyte quantity is usually polynomial. It is from such curve that the limitation in detector sensitivity arises because there is a sharp decrease in signal at lower concentrations of the analyte.[47,49]

The hyphenation of LC to mass spectrometry (MS) is a commonly used approach which combines the resolving power of LC to the sensitivity and selectivity of MS. The limit of detection (as injected mass) in MS (100 ag – 1 ng) is much lower than for UV (1 pg – 1 ng), IR (100 ng – 1 µg), dRI (10 ng – 1 µg) or FL (1 fg -10 pg) detectors, therefore, MS is more sensitive for quantification.[50] In addition, MS is capable of determining the molecular mass of small molecules or their fragments and hence facilitate structure and chemical property elucidation of unknown compounds.

MS involves the formation of ionic chemical species in the ion source, their separation according to mass-to-charge ratio (m/z) and finally detection of the ions in the electron multiplier. Atmospheric pressure ionisation (API) probes such as electrospray ionisation (ESI) and chemical ionisation (APCI) have found the widest use in pyrethroid analyses.

Ionisation in EI occurs on a thermospray interface. The ionisation of analyte species is mediated by bombardment with a beam of high energy electrons. The molecular ions generated by EI

are unstable due to their internal energy hence they fragment into smaller species. Under EI conditions, pyrethroid insecticide molecules tend to produce many unspecific low-mass fragment ions, which usually have similar m/z values. The result is a loss of selectivity especially when the resolution of the chromatographic separation is poor.[51] EI also requires that analytes be sufficiently volatile and stable, a property that not all pyrethroids possess.

Soft ionisation techniques in which the fragmentation of the analyte molecule is minimal so that the molecular ion can be observed are now preferred. ESI is one such technique, where ionisation takes place during final droplet formation following the nebulisation of LC effluent into an electric field to produce a very fine mist of charged droplets. APCI on the other hand employs gas-phase ion-molecule chemistry at atmospheric pressure to generate analyte ions. Molecular ions generated by the soft ionisation techniques are more stable leading up to their detection. Both APCI and ESI interfaces have been evaluated demonstrating that ESI was more suitable for the analysis of pyrethroids.[51,52] In comparison to EI, ESI is better suited for the analyses of non-volatiles and molecules that can be thermally sensitive such as pyrethroid insecticides.

Data acquisition in LC-MS can be executed in the scan mode. In this mode, the mass spectrometer searches for signals over a programmed range of m/z values in a short space of time. Consequently, a loss of sensitivity due to less frequent sampling per m/z value leaves the scan mode most suitable for qualitative analyses. A more sensitive data acquisition mode is the selected ion (SIM) or selected reaction monitoring (SRM). The designation of the terms is to single quadrupole and triple quadrupole mass analysers, respectively. In this mode the mass spectrometer is pogrammed to detect a few select m/z values so that the analyser's sampling frequency increases leading to more sensitivity. It is for this reason that SIM/SRM is better suited for quantitative analyses.[51,53,54]

2.7 Spectroscopic Techniques

2.7.1 Infrared (IR) Spectroscopy

The concept of IR spectroscopy relies on the tendency of molecules to absorb specific frequencies of light when irradiated with light in the IR region of the electromagnetic spectrum. The energy of the light absorbed corresponds to the specific frequency of the bond vibration in a molecule. In addition a bond will only interact with IR radiation if it is polar i.e. exhibits a dipole moment. Absorption of IR radiation leads to stretching, rotation or bending vibrations of the covalent bonds in sample molecules.[46] Bonds with different functional groups where a

dipole moment exists absorb light at different regions of the IR spectrum measured in wavenumbers such that each different molecule will have a distinct absorption pattern according to the number of different functional groups and the different types of bonds present.[46] Therefore, it is common for Fourier transform infrared spectroscopy in attenuated total reflectance mode (FTIR-ATR) to be used as a complimentary analysis technique to liquid chromatography for the identification of unknown compounds.[55]

2.7.2 Nuclear Magnetic Resonance (NMR) Spectroscopy

NMR spectroscopy is a very popular and powerful non-destructive technique for the determination of the structure of compounds. Analytical modes such as proton (^{1}H), carbon (^{13}C) and phosphorus (^{31}P) NMR can reveal the number of atoms and their connectivities, and thus the conformations of the molecules.[46] The information obtained by measuring, analysing and interpreting NMR spectra can be directly correlated to the molecular structures of the components of the sample.[46] ^{1}H-NMR is the most sensitive requiring analyte amounts in the range of 1-10 mg while ^{13}C-NMR is much less sensitive thus requiring sample concentrations of >10 mg.[56] Such quantities may not be available following a laboratory scale sample preparation in the analysis of trace transformation products of pesticides such as pyrethroids in decorative coatings.

2.8 High Performance Liquid Chromatography Analysis of Pyrethroids

Pyrethroids are applied at very low dosages ($\leq$ 0.05g active ingredient/m^2) demonstrating excellent efficacy, low hazard to users and rather low environmental impact. For these reasons the pyrethroids are used widely in food and agriculture, veterinary pest control and disease vector control. The need to identify terminal residues for toxicological purposes has driven the development of residue analysis for pesticides. The parent pyrethroid compounds are the main components of any residue present in a test matrix following normal application, and no metabolites of the pyrethroids have been proven to be significantly toxic. Therefore, there is a need for highly selective and sensitive analytical methods such as chromatography for the measurement of pyrethroid insecticides.

Chen and Wang,[57] LeDoux et al.,[58] Gullick et al.,[52] Tuck et al.[51] and Albaseer et al.[59] wrote comprehensive reviews of research articles and published methods describing methodologies for the analysis of pyrethroid pesticide residues in crops, water, sediment, soil, agrofoods and foods of animal origin. The selection of the extractive solvent and the protocol for the isolation of pyrethroid pesticide residues is governed by the nature of the test matrix. The most common

method for extraction of pyrethroids has been direct solid-liquid extraction (SLE) which involves grinding chopped samples or extracted fats several times at high speed in selected organic solvents. Liquid–liquid extraction (LLE) methods that involve the shaking of liquid samples such as milk and honey repeatedly in selected organic solvents for extracting pesticide residues from the bulk of the sample have also been reported for the extraction of pyrethroids. SLE and LLE have mostly yielded efficient pyrethroid extraction recovery rates between 70 and 120% in samples of animal origin.[58] 120% is an anomalous figure for a recovery because it implies more of the analyte was extracted compared to the original amount in the sample. The traditional Soxhlet extraction method usually performs efficient pyrethroid extractions on a large range of test matrices if solvents are selected well. The well-established Soxhlet extraction method is generally unpopular for long analysis times, being tedious, consumes large volumes of solvents and is energy intense because of the application of thermal energy.

When pyrethroids have been included as additives in polymers, solid-liquid extraction techniques e.g. Soxhlet extraction are often employed.[60] Pyrethroids have been extracted from insecticide treated anti-mosquito nets by heating the polymer fibre in organic solvent while applying ultrasonic energy to aid the extraction.[61,62] Ouattara et al.[63] reviewed the studies that have focused on the extraction and analysis of pyrethroids from different types of insecticide treated nets designed with polymeric fibres, but decorative coating matrices contain different polymers and are far more complex than fibres. Alternative extraction procedures such as QuEChERS (quick, easy, cheap, effective, rugged and safe), supercritical fluid extraction (SFE), pressurised liquid extraction (PLE), automated solvent extraction, and matrix solid phase dispersion (MSPD) have also been employed for pyrethroid extractions in other studies. More novel methods such as solid phase microextraction (SPME) and ionic liquid-linked dual magnetic microextraction (ILDMME) for pyrethroid determination in various matrices continue to be developed.[51,59,64]

GC methodologies have been the most widely used for the analysis of pyrethroid residues because of the possibility of direct analysis without the need for derivatisation and also for better sensitivity compared to LC. LC has been used for the analysis of polar, non-volatile and thermally labile pesticides. For example it was observed in a study that isomers of tralomethrin transformed into deltamethrin under GC analysis conditions.[28]

Various stationary phases have been tested for the LC separation of pesticides, but generally reversed phase C_{18}-bonded silica columns have been preferred because pyrethroids are

nonpolar. In addition, C_8-bonded columns have been employed for separating pyrethroids extracted from beef and poultry meats, paints and food samples (fluvalinate and fenvalerate).[52,65] LC has also been combined with conventional detectors such as fluorescence or UV detectors for identifying and quantifying pesticide residues. Gradient elution liquid chromatography coupled with diode-array detector (LC-DAD) has been used for analysing pyrethroid contamination of rabbit meat and fat (permethrin), and cow milk (deltamethrin, tralomethrin).[57] Seven pyrethroids (acrinathrin, bifenthrin, permethrin, fenvalerate, fenpropathrin, deltamethrin and cyalothrin) extracted from egg and liver, respectively, have been successfully quantified at 0.1 µg/mL level using a DAD after LC separation on a C_{18} column. Therefore, LC-DAD can be employed as a pesticide multiresidue determination technique.[51]

The Collaborative International Pesticides Analytical Council (CIPAC) that promotes the international agreement on methods for the analysis of pesticides and physico-chemical test methods of formulations has approved a NPLC method for the measurement of deltamethrin content. Otherwise, NPLC methods are not widely used for the quantification of pyrethroids. Rather NPLC provides good resolution on the enantiomers and the diastereomers of pyrethroid insecticides and is, therefore, mostly applied where studies are concerned with chiral separations.[66]

The coupling of LC with different kinds of MSDs, single quadrupole, ion trap, tandem-MS (MS/MS), and time-of-flight-MS (TOF-MS) in the determination of pesticide residue levels and the elucidation of their structures in water, sediment and in foods of vegetable and animal origin has overcome the sensitivity issues that LC suffered against GC.[51] ESI-MS and APCI-MS make it possible to identify pesticides of different chemical structures in environmental samples and foods of animal and vegetable origin at trace concentrations. Furthermore, the enhanced selectivity afforded by MS/MS detection provides the feasibility to distinctly identify target analytes that are marginally separated by LC. For example, a RPLC-MS/MS method to determine the amount of pyrethroid insecticide suspected to cause mortality has been reported where limits of detection were in the range 1-1.5 µg/L after a single extraction step.[67] The high-resolution MS technology is considered an excellent tool for identifying and elucidating the structure of metabolites and transformation products of the insecticides in food and environmental samples, but face limitations to accurate quantification due to their narrow dynamic ranges. For this reason these instruments are mainly employed for identification and confirmatory analysis.

A few methods have been published in literature that are related to liquid chromatographic analysis of pyrethroids in latex paints. RPLC methods using C_{18} or C_8 stationary phases with methanol- or acetonitrile-water mobile phases have been reported. Sim and Hsu[68] reported an isocratic C_8-RPLC method with acetonitrile-water (70:30 *v/v*) and UV detection to measure the amount of transferable insecticide from surfaces coated with insecticidal latex paint. Insecticides were extracted sequentially by repeated vortexing of the paint sample in methanol, quantifying the amount of insecticide extracted at each step. Pyrethroid insecticide (deltamethrin) recovery was unsatisfactory, 333% of the nominal values declared by the producers of the paints. This figure is rather peculiar, it is three times more than what was put in. This can mean certain matrix components coeluted with deltamethrin and thus the selectivity of the analytical method was not well optimised or the analyte signal was amplified by matrix effects.

A NPLC method to study the effect of the method of incorporation of pyrethroids into solvent-based alkyd paints using a silica-based column with 2,2,4-trimethyl pentane-1,4-dioxane (95:5 (v/v)) as mobile phase and UV detection has been reported.[65] The extraction of the pyrethroid deltamethrin was based on shaking paint film samples in different solvents and then sonicating the sample. Of the solvent systems investigated, isooctane-dioxane (80:20 (v/v)) had the highest recoveries of 85-90%. The paints into which deltamethrin had been incorporated during the grind stage demonstrated greater retention of deltamethrin compared to those into which it was incorporated during the let-down stage of the manufacturing process.

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Chapter 3

HPLC Method Development and Validation

3.1 Introduction

The materials and methods employed in this project are presented. The research samples are pyrethroid-based insecticide paints manufactured by a commercial supplier in the coatings industry. The exact trade names of the ingredients and the formulation recipes are not presented due to confidentiality considerations. This section hinges on the experimental and validation parameters of the analytical method being developed for the research.

3.2 Experimental

3.2.1 Materials

Bifenthrin (PESTANAL ≥99%, Sigma Aldrich, Buchs, Switzerland), permethrin (PESTANAL ≥99%, 40/60 cis/trans isomers, Sigma Aldrich, Steinheim, Germany, tetrahydrofuran (THF, CHROMASOLV ≥99.9%, Honeywell Riedel-de Haen AG, Hannover, Germany), dichloromethane (DCM, ≥99.8%, Honeywell Riedel-de Haen AG, Hannover, Germany), n-Hexane (HEX, CHROMASOLV ≥99.9%, Honeywell Riedel-de Haen AG, Hannover, Germany), 2-propanol (IPA, HPLC grade, ≥99.9%, Sigma Aldrich, Israel), acetonitrile (MeCN, HPLC grade, ≥99.9%, Sigma Aldrich, Saint-Quentin-Fallavier, France), ethyl acetate (EtOAc, ≥99.8%, Merck, KGaA, Darmstadt, Germany), ethoxyethane (Et_2O, %. Merck, KGaA, Darmstadt, Germany), methanol (MeOH, HPLC grade, ≥99.9%, Sigma Aldrich, Saint-Quentin-Fallavier, France), 1,4-dioxane (DIOX, HPLC grade, ≥99.5%, Sigma Aldrich, Israel), deionized water (H_2O) from a laboratory Millipore purification system, 2,2,4-trimethylpentane (ISO, ≥99%, Honeywell Burdick & Johnson, Muskegon, USA), toluene (TOL, >99.9%, Sigma Aldrich, Steinheim, Germany), acetone (ACE, >99.8%, Sigma Aldrich, Steinheim, Germany) and cellulose thimbles (GE Healthcare, Amersham, UK) were used as received.

3.3 Analytical Instrumentation

3.3.1 High Performance Liquid Chromatography (HPLC)

An Agilent 1200 liquid chromatographic system (Agilent Technologies, Waldbronn, Germany) with ChemStation (Revision B.04.03) software was used. The system comprised the following: autosampler (model G1329A) fitted with a 100µL sample loop, quaternary pump unit with built-in degasser (model G1311C), a thermostated column compartment (model G1316A) operated at 30^{o}C, an automatic fraction collector (AFC, model G1364) and a photodiode array detector (PDA) (model G1315D).

Table 3.1: Methods applied with the HPLC columns

Method I	Isocratic mode: RPLC Column: Waters Symmetry C_{18} (length 250 mm, internal diameter 4.6 mm) Mobile phase: MeCN/H_2O (80:20(v/v)) Pump flow rate: 0.5 mL/min Detector: UV at 215 nm
Method II	Isocratic mode Column: Discovery CN (length 250 mm, internal diameter 4.6 mm) Mobile phase: EtOAc, DCM, MeCN, THF or Hex (100%) Pump flow rate: 0.5 mL/min Detector: UV at 225 nm
Method III	Isocratic mode: NPLC Column: SUPELCO Ascentis Si 100-5 (length 250 mm, internal diameter 4.6 mm) Mobile phase: DIOX, HEX, DCM, MeCN, THF or MeOH (100%) Pump flow rate: 0.5 mL/min (IPA was used at a flowrate of 0.2 $mL.min^{-1}$ with 86 bars back pressure caused by its high viscosity) Detector: UV at 240 nm
Method IV	Isocratic mode: NPLC Column: Macherey Nagel Nucleosil Si 300-5 (length 250 mm, internal diameter 4.6 mm) Mobile phase: DCM (100%) Pump flow rate: 0.5 mL/min Detector: UV at 240 nm
Method V	Isocratic mode: NPLC Column: Macherey Nagel Nucleosil Si 100-5 (length 250 mm, internal diameter 4.6 mm) Mobile phase: DCM-HEX (50:50 (v/v)) Pump flow rate: 0.5 mL/min Detector: UV at 240 nm

20 µL of sample or standard filtered through 0.45 µm regenerated cellulose (RC) membrane filter was injected for analysis. The following analytical columns were used: Waters Symmetry

C_{18} 100–5 column, with the following dimensions: 250 mm × 4.6 mm i.d; Discovery Cyano 100–5 column, with the following dimensions: 250 mm × 4.6 mm i.d; SUPELCO Ascentis Si 100–5 column, with the following dimensions: 250 mm × 4.6 mm i.d; Macherey-Nagel Nucleosil 300–5 column, with the following dimensions: 250 mm × 4.6 mm i.d and Macherey-Nagel Nucleosil Si 100–5 column, with the following dimensions: 250 mm × 4.6 mm i.d. The operating conditions that were applied on the aforementioned columns for the different separation modes used are listed in Table 3.1.

3.3.2 UV-Vis Spectroscopy

The UV spectra of bifenthrin and permethrin prepared in MeCN were measured with an Analytik Jena Specord 210 plus UV-Vis instrument, using a Hellma QS 10.00 mm cuvette.

3.4 Sample Preparation for HPLC Method Development and Validation

Standard stock solutions of bifenthrin and permethrin were prepared by dissolving accurately weighed 10.0 mg of each compound in 10.0 mL of DCM-HEX (50:50 (v/v)) (MeCN for Method I) measured using a micropipette into a tightly capped vial to yield solutions of concentration 1 mg/mL.

Working solutions of both permethrin and bifenthrin were prepared by transferring 1.0 mL of the standard stock solutions and making up to 10 mL with the same solvent in a vial using micropipettes to attain a final concentration of 100 µg/mL.

3.5 Validation

3.5.1 Linearity and Calibration Standards of Bifenthrin and Permethrin

1 µL, 10 µL, 100 µL, 250 µL, 500 µL and 1000 µL aliquots of bifenthrin and permethrin standard working solutions (100 µg/mL) were transferred separately into a series of tightly capped vials and the volume was made up to 1.0 mL with HEX (or MeCN for Method I) using micropipettes. The final concentrations of the standard solutions (the corresponding injected mass for 20 µL injection is in parentheses) were 0.1 µg/mL (2 ng), 1.0 µg/mL (20 ng), 10 µg/mL (200 ng), 25 µg/mL (500 ng), 50 µg/mL (1000 ng) and 100 µg/mL (2000 ng).

3.5.2 Preparation of Matrix-Matched Calibration Standards

For matrix-matched calibration, 1 g of sheen latex paint and 1 g of matte latex paint were spread each over a mixture of 5 g sand + 2.5 g $MgSO_4$ + 0.1 g LiBr followed by Soxhlet extraction using DCM-MeOH (95/5 (v/v)) for 3 hours. The extraction solvent volume was reduced on a rotary evaporator. The residues were re-dissolved in a minimum amount of DCM, precipitated in excess HEX and centrifuged. The volume of the supernatant solution was reduced on a rotary

evaporator, allowed to dry under gentle N_2, dissolved in 10 mL of mobile phase DCM-HEX (50/50 (v/v)) and filtered through 0.45 µm RC membrane filter. 20 µL of the sheen and matte paint matrix solutions were assayed using Method III and Method V to check for interfering matrix components. The sheen and matte paint matrix were also combined 1:1 and used to make up the volume of the calibration standards described in Section 3.5.1 for the construction of the matrix-matched calibration line.

3.5.3 Accuracy

The accuracy of the results was tested by applying the proposed Method V for the determination of 100 ng, 600 ng and 1500 ng of bifenthrin and permethrin injected through 20 µL of 5 µg/mL, 30 µg/mL and 75 µg/mL standard solutions, respectively. Three individual sample preparations were performed for each set of samples, and each analysed in triplicates. In addition, the mean recovery was also evaluated by injecting 100 µg (500 µL of 200 µg/mL) of permethrin and bifenthrin via 25 × 20 µL injections of sample while collecting fractions upon analyte elution. The solvent was allowed to evaporate off under a gentle stream of N_2 gas and the residue reconstituted in 500 µL of hexane and re-injected into the HPLC system. The concentrations were obtained by extrapolation from the corresponding regression equations, and mean % recovery was calculated.

3.5.4 Precision

The precision of Method V was evaluated by calculating the mean and relative standard deviations of the results from the experiments.

3.5.4.1 Repeatability

The variation of the data from the same-day triplicate assays of 100 ng, 600 ng and 1500 ng injected amounts of bifenthrin and permethrin prepared according to the method in Section 3.5.2 was determined under the same experimental conditions. The mean and the standard deviation from the mean were calculated to determine repeatability of the method.

3.5.4.2 Intermediate Precision

The exercise in Section 3.5.4.1 was repeated under the same experimental conditions on three days. The mean and the standard deviation from the mean were calculated to determine the reproducibility of the method.

3.5.5 Selectivity

The selectivity of the adopted Method V was evaluated by comparing the chromatograms of the paint matrix solution alone against the chromatograms of pure permethrin and bifenthrin

prepared in the mobile phase. 20 µL of TOL was assayed in triplicate using method V to determine the column dead time for selectivity calculations.

3.6 Results and Discussion

The use of insecticides in disease vector control requires to develop valid analytical methods for the analysis of both free and microencapsulated insecticide incorporated in the formulations. The Collaborative International Pesticides Analytical Council (CIPAC) leads the international consensus on methods for the analysis of pesticides and the physico-chemical tests for pesticide formulations. The guidelines for analytical method validation from the United States Food and Drug Administration (FDA) and the definitions from the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) are more detailed than those from the United States Pharmacopeia (USP) and Association of Official Analytical Chemists International (AOAC). Hence the most common validation parameters from the ICH and the FDA will be used to validate the analytical method under consideration in this study.[1-3]

3.6.1 Identification of Analytes

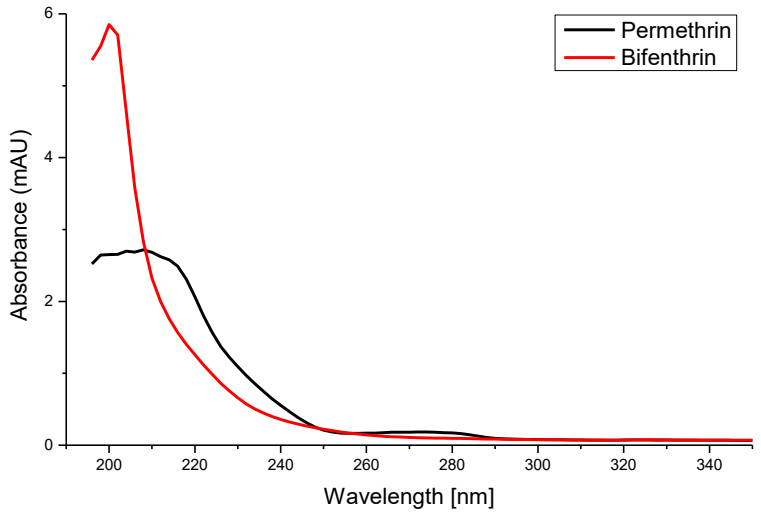

Figure 3.1: The UV spectra of permethrin and bifenthrin in MeCN

The UV absorption spectra of both bifenthrin and permethrin were studied in order to determine the best wavelength at which these pyrethroids could be detected by HPLC coupled to a UV detector. According to Figure 3.1, the wavelengths of maximum absorbance for both bifenthrin and permethrin in MeCN are in the region of 195 nm although they continue to show good absorbance intensities up to wavelengths around 250 nm.

RPLC is the most widely used method in the analysis of pyrethroid insecticides.[4] Separation in RPLC is based on hydrophobicity where the more polar compounds elute first followed by the less polar compounds which are retained more on the stationary phase. In the first part of the research, bifenthrin and permethrin standards were injected individually into the HPLC under the conditions of Method I listed in Table 3.1. Identification of analyte peaks when a mixture of the standards was injected was based on elution volume observed from the assays of the individual standards. Based on chemical structure, bifenthrin is more hydrophobic than permethrin because of an extra methyl group on the non-polar biphenyl moiety and would, therefore, be expected to elute last. This is congruent with the observations in the elugram shown in Figure 3.2.

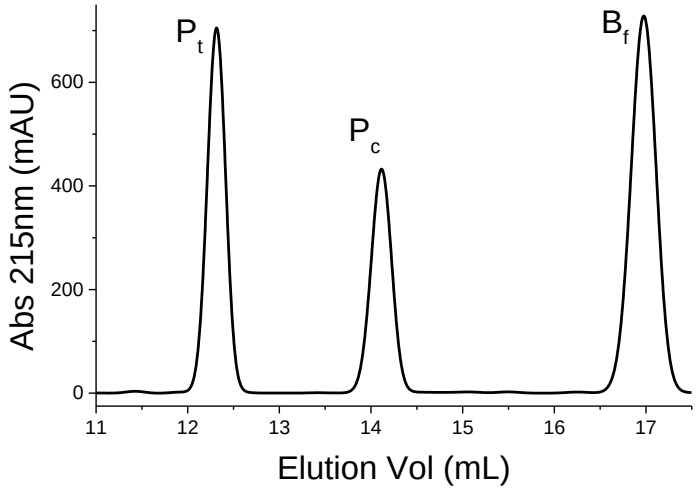

Figure 3.2: RPLC chromatogram of a mixture of 4.0 µg of permethrin and 4.0 µg of bifenthrin injected for HPLC analysis under the experimental conditions of Method I

As shown in Figure 3.2, the compounds are efficiently separated with trans-permethrin (P_t) eluting first at an elution volume of 12.32 mL, cis-permethrin (P_c) at 14.11 mL and bifenthrin (B_f) eluting last at 16.97mL. This confirms that the separation in RPLC is indeed according to hydrophobicity. The assignment of cis- (P_c) and trans-permethrin (P_t) to the chromatographic peaks is based on the ratios of the peak integrals calculated using Equation 3.1. The theoretical 40% cis- and 60% trans-composition of the permethrin standard was confirmed through this method. The recoveries of both permethrin and bifenthrin were >99%. The average composition of the cis- and trans-permethrin isomers as presented in Table 3.2.

$$\% \; cis \; or \; trans = \frac{Peak \; Area_i}{Peak \; Area_{cis} + \; Peak \; Area_{trans}} \times 100$$ **Equation 3.1**

Table 3.2: Identification of the cis/trans isomers

	RPLC		NPLC	
	% Cis	% Trans	% Cis	% Trans
Permethrin	39,46	60,54	38, 65	61, 35

The constituents of latex decorative coatings are mostly hydrophobic and insoluble in water hence the direct injection of analytes from paint matrices would not be possible with RPLC. This is because of the immiscibility of the aqueous mobile phase with the non-polar extraction solvents. NPLC was chosen because it is particularly suitable for water sensitive analytes and chiral compounds such as pyrethroids.[5,6] Separation in NPLC is an adsorptive process. The active sites (polar groups) of the stationary phase are fixed but their spatial arrangement has an effect on the separation which allows for the separation of chemically similar but physically different molecules. Only the average (total) composition of cis- and trans-permethrin enantiomers is considered in this thesis.

Figure 3.3A shows the resultant chromatograms when non-aqueous conditions were explored using Method II to analyse a mixture of bifenthrin and permethrin. As expected for a cyano (CN) stationary phase, the pyrethroids did not elute from the column when HEX (green legend) was used because it is a weak eluent for the system. On the contrary, the semi-polar and polar solvents MeCN (blue legend), EtOAc (black legend), THF (pink legend) and DCM (red legend) were strong solvents that eluted the pyrethroid analytes close to the column dead time. Elution with DCM (red legend) yielded a bimodal peak that indicates some degree of separation of the pyrethroids had occurred, hence, there was need for optimisation.

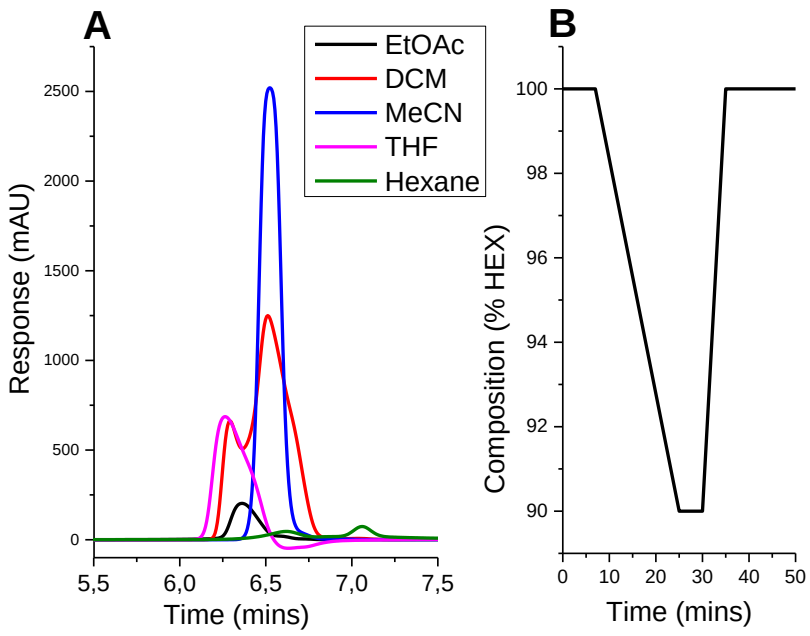

Figure 3.3: (A) NPLC chromatograms of a mixture of 4.0 µg permethrin and 4.0 µg bifenthrin analytical standards as obtained using Method II, (B) Example of solvent gradient showing variation of %HEX in the HEX-DCM gradient.

There are three key parameters that can be manipulated experimentally to optimise a chromatographic separation: the column efficiency (N), the selectivity factor (α), and the retention factor (k). Different combinations of DCM and the non-polar HEX through both isocratic and gradient elution chromatography (Figure 3.3B) were investigated to increase k. Analyte retention was rapidly lost at the addition of small amounts of DCM.

The selectivity (α) of the method was tuned by switching to a more polar silica (Si) stationary phase (SUPELCO Ascentis) under the conditions in Method III. In Figure 3.4 the resolution of the method improved markedly when DCM (blue legend) was applied as the eluent. Bifenthrin (B_f) eluted first after 6.90 min, followed by cis-permethrin (P_c) after 7.57 min and lastly trans-permethrin (P_t) after 8.50 min. As expected, the order of elution in NPLC is directly opposite to that observed in RPLC. This NPLC method also confirmed the theoretical 40% cis- and 60% trans-permethrin composition, see Table 3.2. Elution with the relatively polar solvents caused the analytes to coelute with minimal retention MeOH (6.23 min, purple legend), MeCN (6.22 min, green legend) and THF (6.21 min, orange legend). HEX is non-polar and caused complete retention of the analytes while DIOX (9.82 min, black legend) demonstrated good retention but with no separation of the analytes. The broad peak eluting between 14.5 min and 17 min is a

result of elution with IPA used as eluent at 0.3 mL.min^{-1} due to its viscosity that caused the development of high back pressure,

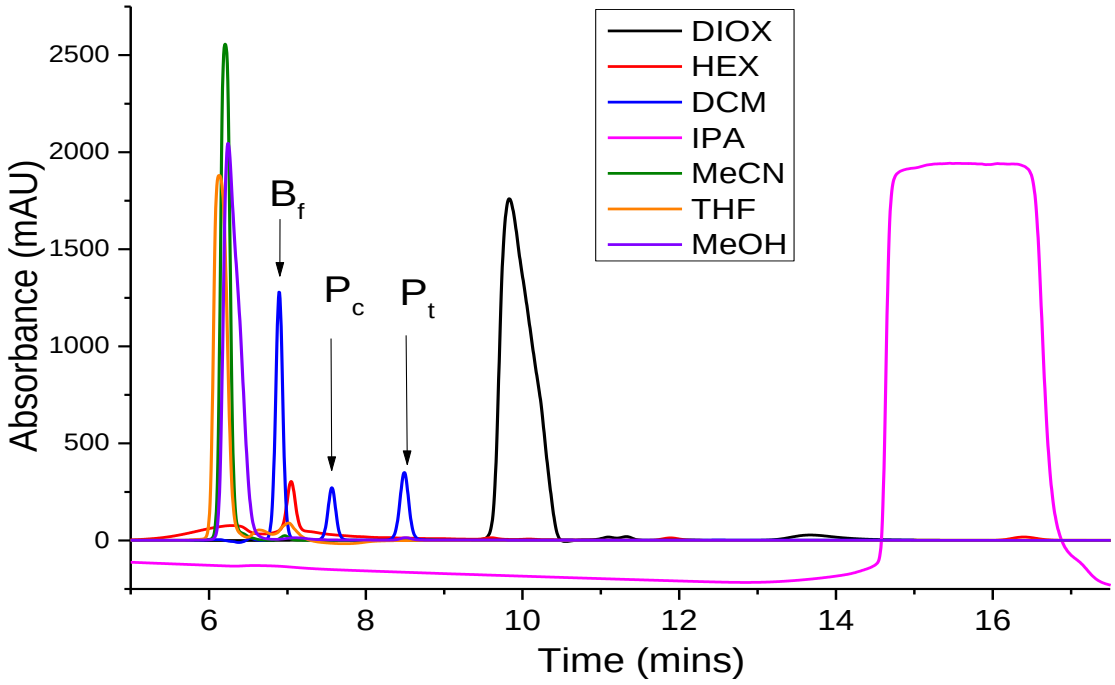

Figure 3.4: NPLC chromatograms showing the elution of a mixture of 4.0 µg permethrin and 4.0 µg bifenthrin analytical standards assayed with different solvents using Method III. Only IPA was used at 0.3 mL.min^{-1} (see text).

Method III (with DCM as mobile phase) was sensitive to the polarity of the injection solvent. In Figure 3.5A the resolution of the analyte peaks was rapidly lost when the samples were prepared in ACE. To counter this effect, sample preparation solvents of low polarity such the mobile phase itself or HEX were selected. Although the resolution was restored successfully, it was difficult to attain reproducible retention times as the peaks for cis-permethrin drifted between 7.13 - 7.35 min and trans-permethrin between 7.78 – 8.16 min as shown by the arrows over the peaks P_c and P_t in Figure 3.5B. This phenomenon is common with bare silica stationary phases because they are highly hygroscopic.[7] Non-polar solvents can contain small quantities of water that can vary significantly. This water can adsorb preferentially to the stationary phase surface ahead of the analytes causing irreproducibility of analyte retention times. Allowing for sufficient column re-equilibration in the mobile phase prior to and between analyses countered these effects.

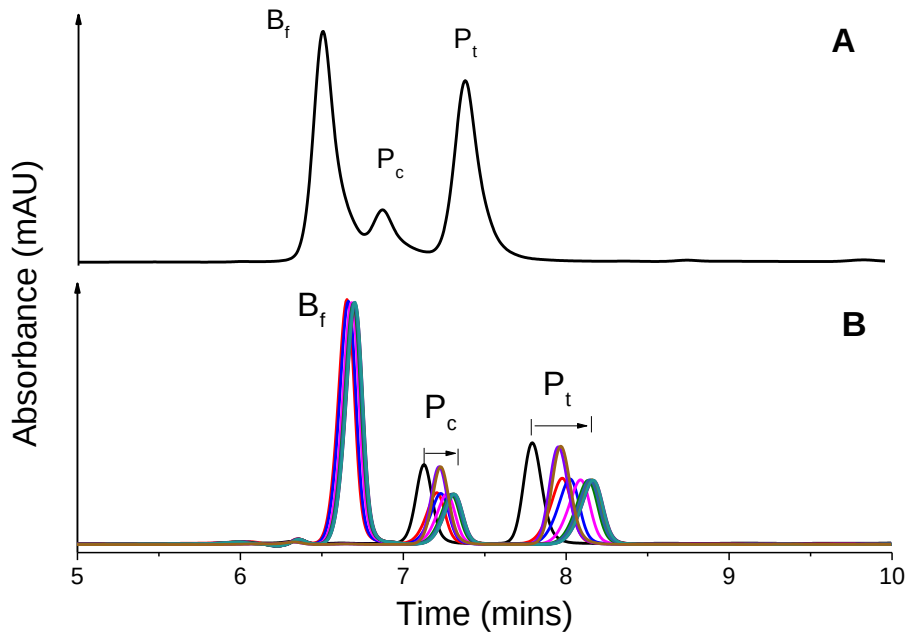

Figure 3.5: NPLC chromatograms of a mixture of 4.0 µg bifenthrin and 4.0 µg permethrin injected for analysis using Method III, A) loss of resolution due to polar injection solvent ACE and B) the different colour codes represent samples analysed sequentially while the arrows above P_c and P_t denote the drifting of retention times from the first analysis through to the last.

The selectivity of Method III was evaluated by the visual inspection of the chromatograms of sheen and matte paint matrix solutions prepared according to section 3.5.2 against the elugram of neat pyrethroid standards prepared according to section 3.5.1. The coelution of matrix components with the analyte standards B_f after 6.80 min, P_c after 7.42 min and P_t after 8.17 min is elaborately shown by the zoomed insert in Figure 3.6A. The coelution problem with Method III was remedied by introducing HEX into the mobile phase to reduce eluent strength (Method V). The retention times of the permethrin isomers increased about two-fold (P_c =12.55 mins and P_t = 17.25 mins). The elugram in Figure 3.6B shows that Method V is selective for the analysis of permethrin and bifenthrin from the test matrix. The mobile phase flow rate was optimum at 0.5 mL/min. Decreasing it to 0.3 mL/min caused excessive peak broadening for P_t at 17.25 mins while increasing it to above 0.7 mL/min caused loss of selectivity between B_f at 9.22 mins and the matrix components.

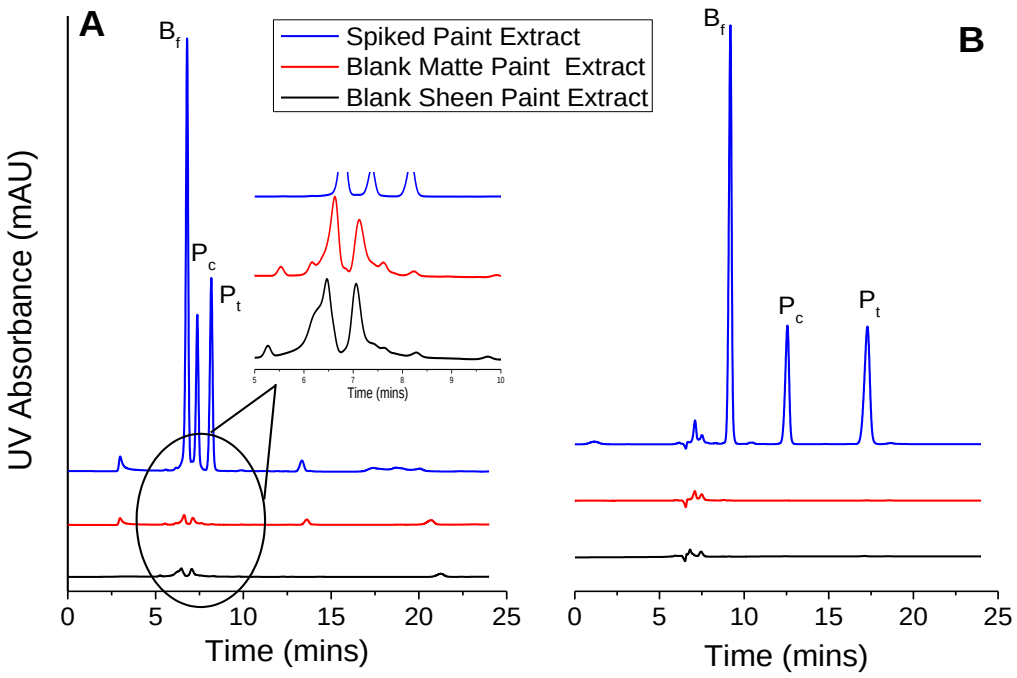

Figure 3.6: NPLC chromatograms of A) Method III and B) Method V individual assays of the sheen and matte paint matrix solutions and 1 µg injected masses of permethrin and bifenthrin.

Using Method IV, the effect of the porosity of the stationary phase (at a constant particle diameter of 5 µm) on the performance of the method was investigated. A loss of resolution was observed by the coelution of permethrin and bifenthrin where a 300Å pore diameter stationary phase (Macherey-Nagel Nucleosil 300-5) was employed. This happened because the accessible surface area for adsorptive interactions with the analytes on the stationary phase decreased together with the (loadability) capacity of the column when the pore size was increased.[8] Therefore a Si column (250 × 4.6 mm) with 5 µm particle size and 100Å pore diameter was selected as the optimum stationary phase conditions for the method. Finally the Macherey-Nagel Nucleosil Si column in Method V was selected for the superior reproducibility of the retention times of P_c and P_t when column re-equilibration was allowed prior to and between successive analyses.

The system suitability parameters for Method V were calculated and presented in Table 3.3. The resolution between peaks B_f and P_c is 4.20, and between peaks P_c and P_t is 4.98. Toluene (TOL) was analysed under the same conditions because of its UV absorptivity to determine the time it takes an unretained analyte to elute from the column, from which the resolution of 3.94 for peak B_f was determined. The peaks P_t and P_c present excellent symmetry denoted by tailing

factors close to unity (1.03 and 0.94, respectively). Peak P_t has a tailing factor value of 0.69 that depicts a leading peak. This is a result of dispersion mechanisms that cause the spreading of the analyte into a band the yields shorter, broader and sometimes unsymmetrical peaks. Dispersion effects influence peak height but have minimal effects on peak area. Therefore, only the integral values of analyte peak areas can be used for quantification with the proposed Method V.

Table 3.3: System suitability parameters for Method V

Parameter	Bifenthrin (B_f)	Cis-Permethrin (P_c)	Trans-Permethrin (P_t)
Tailing factor	1.03	0.94	0,69
Resolution factor	3.94	4.20	4.98
Theoretical plates, N	2653	3751	4624
HETP (cm)	0.0094	0.0067	0.0054

3.6.2 Quantification of Bifenthrin and Permethrin

The quantification of bifenthrin and permethrin was carried out using multipoint external calibration of Method V with the DAD set at 240 nm. The calibration method was chosen because of the simplicity of the sample preparation and the robustness of the analytical instruments. The quantities of the assayed analytes were determined by extrapolation from the standard calibration graph.

3.6.2.1 Selectivity

The term selectivity describes an analytical method that, despite being responsive to a number of components, has the ability to distinguish the analyte's signal from all other responses. In this study, the selectivity of Method V was investigated by comparing chromatograms of pure sheen and matte paint matrix solutions with pure permethrin and bifenthrin standard solutions. The chromatograms presented in Figure 3.6(B) show that there are no signals interfering with those of bifenthrin (B_f, 9.22 min), cis-permethrin (P_c, 12.55 min) or trans-permethrin (P_t, 17.25 min). Therefore, the method is selective for the accurate quantification of these analytes.

3.6.2.2 Linearity, Range and Calibration

The decorative coatings supplied for this study were formulated with 0.5 wt% and 1.0 wt% pyrethroid which amounts to 5 mg and 10 mg of active ingredient per 1 g of coating sample, respectively. There is need for detector calibration in order to quantify the pyrethroids by

HPLC. The calibration is derived from the linear range of the detector's response to analyte quantity.[9-11] Linearity is normally expected to be evaluated at concentrations ranging from 80 to 120% of the target concentration range.[12,2] Instead, for the purpose of enabling for the determination of trace amounts of insecticides in paints, the lowest possible range of linearity was investigated beginning at 1 ng/mL all the way up to 1 mg/mL for a 20 µL sample injection. Both bifenthrin and permethrin were difficult to detect at injected quantities below 1 ng. This is in accordance with literature limits of detection of the UV-Vis detector.[13,14] In the nanogram range the assay provided an acceptable degree of linearity, precision and accuracy for analyte samples.

It is recommended that a calibration should consist of a minimum of five non-zero standard samples, and it is important that calibration standards be prepared in the same matrix as the samples of the intended study to cater for matrix effects.[13,15] Matrix-matched calibration standard solutions of cis/trans-permethrin and bifenthrin were prepared in triplicates. The analytes were injected at six levels: 2 ng, 20 ng, 200 ng, 500 ng, 1000 ng and 2000 ng. The analyses were performed in triplicate to detect and minimise errors arising from serial dilutions. The mean peak area at each analyte quantity level was calculated, and the results are shown in Table 3.4.

Table 3.4: Results of the HPLC method regarding linearity testing in the assay of bifenthrin and permethrin

	Bifenthrin		Cis-permethrin		Trans-permethrin	
Injected amount (ng)	Mean Peak Area (n = 3)	Mean Peak Area RSD (%)	Mean Peak Area (n = 3)	Mean Peak Area RSD (%)	Mean Peak Area (n = 3)	Mean Peak Area RSD (%)
2	2,19	2,25	1,03	2,12	1,30	2.20
20	4,26	1,20	1,73	2,23	2,46	2,30
200	12,8	1,25	4,81	1,63	7,35	1,50
500	38,96	1,90	14,3	1,87	22,3	1,69
1000	77,9	1,55	29,3	0,81	46,2	1,60
2000	144,8	0,51	54,5	0,50	86,4	0.45

The variation of the data was described by the relative standard deviations from the mean which were generally 2% or less and therefore acceptable.[16] This range of linearity would suffice because the residual concentrations of both cis- and trans-permethrin and bifenthrin in paint sample extracts at the time of injection were well within this linearity range.

Standard calibration graphs were plotted in Figure 3.7 using the information in Table 3.4. Linear regression analysis was performed to validate the acceptability of the linearity of the data.[17]

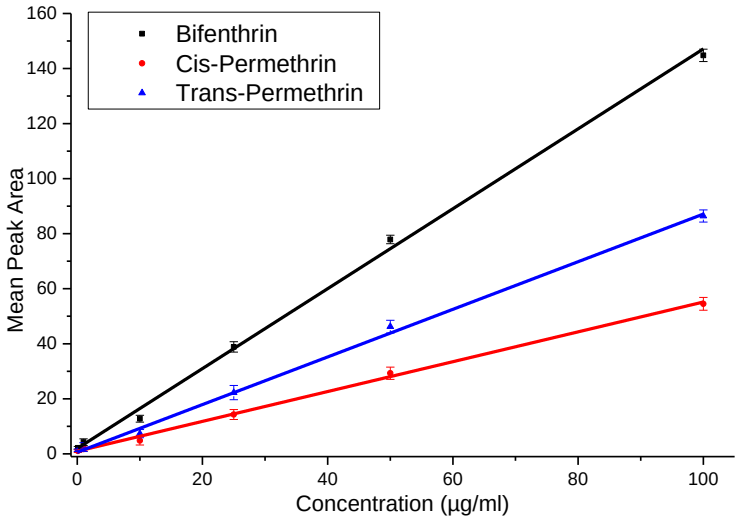

Figure 3.7: Linear calibration plots for 20 µL injections of matrix-matched standard concentrations of solutions of bifenthrin, cis- and trans-permethrin in the linear range of detector response determined using Method V.

The linear regression equation for the calibration line of bifenthrin is y = 1.45x + 1.95, while those for cis- and trans-permethrin are y = 0.54x + 0.96 and y = 0.87x + 0.60, respectively, where 'y' is the detector response as a function of the analyte concentration 'x'. The y-intercepts for the regression equation are close to zero and, therefore, the calibration lines are acceptable. The linear regression coefficient (r^2) values for the calibration plots of bifenthrin, cis- and trans-permethrin are 0.9958, 0.9974 and 0.9969, respectively. It is widely agreed that the linear correlation coefficient for a linear calibration should exceed 0.99.[18,19] The values demonstrate strong linear proportionality between peak area and concentration of the analytes.[20] The standard error values of 0,042, 0,012 and 0,022 were observed in the slopes of the bifenthrin, cis- and trans-permethrin standard calibration lines, respectively. These numbers are small signifying that the mean along the calibration lines is closely related to the true mean of the samples.

Statistical treatment of the data was performed to validate the linearity for each analyte separately. The results are shown in Table 3.8. The F-tests prove that the slope of the calibration line (bifethrin, cis- or trans-permethrin) is significantly different from zero at the 0.05 confidence level. The variation in the amount of analyte (concentration, x-axis) which can account for >99% of the variation observed in the response (absorbance, y-axis). This information coupled with the regression equations, the 'r' and 'r^2' values that are approaching unity serve to prove the validity of the linear fit, and the strength of the interdependence between detector response and analyte concentrations. The analytical method is, therefore, accurate and proportional for the quantification of both permethrin and bifenthrin within the valid linear ranges. This was confirmed through Student's t-tests of proportionality that showed that for all the analytes the R values were not significantly different from the expected 100% at the 0.05 level of confidence.[16,19,21]

In each case (bifenthrin, cis-permethrin, trans-permethrin) there is a strong correlation between the x- and y-axis variables to fit a linear model proved by the F-test probability. Finally, the analytical method is proportional for the quantification of bifenthrin, cis- and trans-permethrin demonstrated by the Student's t-test probability values greater than 0.05.

3.6.2.3 Sensitivity

The minimum detectable concentrations of bifenthrin, cis- and trans-permethrin, and the slopes of their calibration graphs, were determined to evaluate the sensitivity of Method V. The lowest amount of analyte that can be detected above baseline noise, usually three times the magnitude of baseline noise i.e. S/N = 3, is the LOD while the LOQ is lowest amount of analyte that can be reproducibly quantitated above the baseline noise that gives S/N = 10 as summarised in Equation 3.2 and 3.3.[22]

$$LoD = 3.3\,X\frac{\delta}{S} \qquad \textbf{Equation 3.2}$$

$$LoQ = 10\,X\frac{\delta}{S} \qquad \textbf{Equation 3.3}$$

where σ is the residual standard error of the slope of the calibration line and S is the slope of the calibration line.

The LOD for a 20 µL injection of bifenthrin analytical standard was 2.0 ng, and the LOQ was 5.8 ng. The LODs for cis- and trans-permethrin standards were 1.4 ng and 1.6 ng, respectively, while the LOQs were 4.4 ng and 5.0 ng in the same order.

Table 3.5: Sensitivity Parameters

Parameter	Bifenthrin	Cis-Permethrin	Trans-Permethrin
LOD (ng)	2.0	1.4	1.6
LOQ (ng)	5.8	4.4	5.0
Sensitivity (ng^{-1})	1.45	0.54	0.87

3.6.2.4 Accuracy and Recovery

Accuracy is a measure of the deviation from the true value due to systematic error. It is often estimated as the deviation of the mean from the true value.[16] Firstly, the accuracy of Method V was assessed by analysing samples of neat bifenthrin and permethrin standards prepared in the laboratory at three injection mass levels of 100 ng, 300 ng and 750 ng. The quantity of analyte was determined by extrapolating of the peak areas from the standard calibration line. The measured values for the laboratory prepared samples are presented in Table 3.6. The results were evaluated as percentage recovery using Equation 3.4.

$$\%\ Recovery\ = \frac{concentration\ of\ laboratory\ prepared\ sample}{expected\ concentration} \qquad \textbf{Equation 3.4}$$

Table 3.6: Bifenthrin and permethrin recovery for the laboratory prepared samples

Injected mass (ng)	Bifenthrin		Cis-Permethrin		Trans-Permethrin	
	Sample mass (ng)	Recovery (%)	Sample mass (ng)	Recovery (%)	Sample mass (ng)	Recovery (%)
100	98.9	98.9	96.6	96.6	101	101
	104	104	102	102	96.6	96.6
	97.6	97.6	98.4	98.4	102	102
300	295	98,4	305	101	301	100
	304	101	298	99.3	308	103
	307	102	296	98.5	296	98.8
750	761	101	748	99.7	761	101
	754	100	751	100	746	99.5
	743	99,1	757	100	738	98.4

The second approach to assessing the accuracy of the method was based on the recovery of known amounts of analyte injected into the HPLC system by collecting fractions during the analysis and reinjecting into the same system. In the present study, a method was designed where 100 µg of bifenthrin and 100 µg of permethrin standards was injected into the HPLC via a 25 x 20µL injection sequence of 200 µg/mL standard solution with fraction collection. The fractions were combined and the eluent was reduced to dryness by evaporation under a gentle stream of N_2 gas. The residue was reconstituted in 500µL of hexane and analysed by HPLC. The percent recoveries were calculated using Equation 3.4. The recoveries for both bifenthrin and permethrin are presented in Table 3.7.

Table 3.7: Bifenthrin and permethrin recovery values from injected amount

Sample	Peak Area of Standard	Average Peak Area of Fraction (±SD)	%Recovery	%RSD
Bifenthrin	398	405 ± 3.83	102	3,75
Cis-Permethrin	103	102 ± 0.73	98,6	0,74
Trans-Permethrin	149	153 ±1.93	103	1,88

Table 3.8: Statistical parameters for the linearity and accuracy studies of bifenthrin and permethrin samples prepared in 1:1 DCM-HEX

	Bifenthrin	Cis-Permethrin	Trans-Permethrin
Number of data points	6	6	6
Range (ng)	0.1 – 100	0.1– 100	0.1– 100
Correlation Coefficient (r)	0.998	0.999	0.999
Determination Coefficient (r^2)	0.996	0.997	0.997
Slope (m)	1.45	0.54	0.87
Standard deviation of slope	0.042	0.012	0.022
Intercept (y)	1.95	0.96	0.60
Standard deviation of intercept	2.09	0.46	0.87
F-test of regression (p)	1194,5 ($4,2\times10^{-6}$)	1915,6 ($1,\times10^{-6}$)	1618,4 ($2,3\times10^{-6}$)
t-test of proportionality (p)	1.87 (0.09)	0.96 (0.02)	1.10 (0.05)

(p) is the probability value of the statistic

The results for the statistical treatment of the accuracy data for permethrin and bifenthrin are presented in Table 3.8. One-way analysis of variance (ANOVA) was performed on the sets of

data and it was shown that the concentration level of the pesticides, whether high or low, had no significant influence on the percentage recovery from one concentration level to another since the probability values (p) of the F-test were all less than 0.05 (the critical value of the test at a confidence level of 0.05). Based on this information one average recovery (R) was calculated from the three concentration levels.[17,22,23]

3.6.2.5 Precision

Precision is a measure of repeatability (intra-assay variance) and reproducibility (interday assay variance) of an analytical method due to random error. Since data from the measurements were limited, the arithmetic mean was computed to estimate the mean, accompanied by the unbiased standard deviation (s). The coefficient of variation (CV) or relative standard deviation (RSD) is the preferred parameter for expressing precision in analytical sciences.[24]

Table 3.9: statistical parameters for accuracy and precision

	Bifenthrin	Cis-Permethrin	Trans-Permethrin
Precision ($\bar{x} \pm \sigma$)	100,66 ± 1,88	99,66 ± 1,70	100,40 ± 1,97
Levene's Test, F (p)	2.56 (0.16)	1.83 (0.24)	2.99 (0.13)
Average Recovery (R)	100,66	99,66	100,40
Standard deviation of 'R'	1,88	1,70	1,97
RSD (%)	1,86	1,71	1,96
No. analyses for precise result	3.46	3.09	4.06

Accuracy			
F-test for recovery (p)	9512 (3×10^{-11})	23156 (2×10^{-12})	6733 (8×10^{-11})
Student t-test (p)	1.06 (0.32)	0.60 (0.56)	0.61 (0.55)
95% Confidence Interval	99.25-102.08	98.38-100.94	98.92-102.54

Precision can be calculated from the same data of laboratory prepared samples as accuracy (see Table 3.9).[2] This is according to one of the ICH recommendations for repeatability of the method to be measured by the analysis of three determinations at three different concentration levels especially where the concentration of the analytes would be expected to vary. Through Levene's tests for homogeneity of variance, it was determined that for a 0.05 level of

significance the variances of the responses obtained for each of 5, 30 and 75 µg/mL concentration levels of bifenthrin and permethrin were homogeneous. All the data per analyte were then grouped together to calculate the overall coefficients of variation and the respective confidence intervals to express the precision of the analytical method. This information is tabulated in Table 3.9. It is commendable that the precision for quality control should be better than 2 %. The results for the assays of permethrin and bifenthrin varied within 2 %RSD overall hence the method demonstrates remarkable precision. The number of determinations required to obtain a precise result were calculated for a 95% confidence level with 2.5% margin of error.[3,25]

The intermediate precision shows the variations affected in day-to-day analysis, by different analysts, different instruments etc. Reproducibility, as above, represents the precision obtained between different laboratories and is not usually required if intermediate precision is determined.[3,9] Table 3.10 contains the information pertaining to the daily repeatability of retention times and reproducibility of peak areas for the same sample analysed on three consecutive days.

Table 3.10: Peak area integrals and retention times from the reproducibility study

	Bifenthrin		Cis-permethrin		Trans-permethrin	
Analysis Day	Retention Time (min)	Peak Area	Retention Time (min)	Peak Area	Retention Time (min)	Peak Area
1 (n=3)	8,99	141	12,13	33,4	16,52	48,9
2 (n=3)	9.00	142	12,14	33,4	16,52	49.0
3 (n=3)	9.00	142	12,14	33,5	16,53	48,9
Mean	9.00	141	12,14	33,5	16,52	48,9
RSD (%)	0,01	0,02	0,05	0,02	0,01	0,04

Both the retention times and peak areas of bifenthrin, cis- and trans-permethrin were quite reproducible throughout the assays. The RSD values were well below 2% for all the analytes signifying minimal variability and commendable robustness of the analytical method.

3.7 Conclusions and Recommendations

Method V has been proposed for the quantification of pyrethroid insecticides in latex paint matrices. It was shown that the paint matrix solution did not produce interferences in the quantification of bifenthrin and permethrin. Therefore, this work effectively shows that the

proposed NPLC analytical Method V for the determination of bifenthrin and permethrin in the presence of the latex paint matrix is selective, proportional, accurate, precise and linear in the concentration range from 0.1 µg/mL to 100 µg/mL for a 20 µL injection (2-1000 ng) of matrix-matched standard bifenthrin and permethrin solutions.

However, this NPLC method cannot be applied directly for the analysis pyrethroid insecticides that are incorporated into latex paints developed for malaria vector control. One condition that must be fulfilled is the development of an efficient insecticide extraction protocol from the paint matrix prior to HPLC analysis. The pyrethroid must be isolated from other paint matrix components to minimise the levels of background noise arising from the test matrix. Such a procedure needs to be validated to show that the extraction solvents selected do not produce interferences in the quantification of neat or microencapsulated bifenthrin and permethrin embedded in latex matrices.

3.8 References

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Chapter 4

Development and Validation of Analyte Extraction Protocol

4.1 Introduction

The importance of analytical sample preparation cannot be overstated as it serves to bring about a processed sample that is relatively devoid of interferences and compatible with the HPLC column leading to better analytical results. The suitability of a sample for introduction into the analytical column is particularly important as damage to the column could nullify the whole analytical process. In this regard, the analysis of pyrethroid insecticides in complex polymeric and inorganic decorative coating matrices requires sample pre-treatment that eliminates the less soluble components that can easily clog analytical columns such as macromolecular latexes and the inorganic pigments. Simple and selective sample preparation techniques are always preferable even for complex matrices but often are superintended by both the nature and size of the samples as well as the selectivity of separation in the analytical method and the detection system employed. Although a number of techniques can be applied in a single sample treatment, it is ideal that the chosen methodology is simple, fast, cheap, available and easy to operate. The aim of the project was to quantitatively isolate bifenthrin and permethrin from interior paint sample matrices, so that a minimal amount of potentially interfering species was carried through to the analytical separation phase.

4.2 Experimental

4.2.1 Materials and Samples

Bifenthrin (PESTANAL ≥99%, Sigma Aldrich, Buchs, Switzerland), permethrin (PESTANAL ≥99%, 40/60 cis/trans isomers, Sigma Aldrich, Steinheim, Germany), tetrahydrofuran (THF, CHROMASOLV ≥99.9%, Honeywell Riedel-de Haen AG, Hannover, Germany), dichloromethane (DCM, CHROMASOLV ≥99.8%, Honeywell Riedel-de Haen AG, Hannover, Germany), n-Hexane (HEX, CHROMASOLV ≥99.9%, Honeywell Riedel-de Haen AG, Hannover, Germany), 2-Propanol (IPA, HPLC grade, ≥99.9%, Sigma Aldrich, Israel), ethyl acetate (EtOAc, ≥99.8%, Merck, KGaA, Darmstadt, Germany), ethoxyethane (Et_2O, ≥99.8%. Merck, KGaA, Darmstadt, Germany), methanol (MeOH, HPLC grade, ≥99.9%, Sigma Aldrich, Saint-Quentin-Fallavier, France), deionized water (H_2O) from a laboratory Millipore purification system, N,N-dimethylformamide (DMF, HPLC grade, ≥99.5%, Fischer Scientific, 1,4-dioxane (DIOX, HPLC grade, ≥99.5%, Sigma Aldrich, Israel), hydrochloric acid (HCl, 37%, Fisher Scientific, Loughborough, UK), 2,2,4-trimethylpentane (ISO, ≥99%,

Honeywell Burdick & Johnson, Muskegon, USA), toluene (TOL, >99.9%, Sigma Aldrich, Steinheim, Germany), acetone (ACE, >99.8%, Sigma Aldrich, Steinheim, Germany), sand (White Quartz, 50–70 mesh, Sigma Aldrich, Steinheim, Germany), lithium bromide (LiBr, ≥99%, Sigma Aldrich, St. Louis, USA), anhydrous magnesium sulphate ($MgSO_4$, >99%, Merck, KGaA, Darmstadt, Germany), anhydrous calcium chloride ($CaCl_2$, 98%, Merck, KGaA, Darmstadt, Germany), sodium chloride (NaCl, >99% Merck, KGaA, Darmstadt, Germany) and cellulose extraction thimbles (GE Healthcare, Amersham, UK) were used as received.

The pyrethroid content of the interior decorative coating formulations collected for the study are summarized in Table 4.1.

Table 4.1: Pyrethroid content of the paint samples

Paint Type	Sheen										Matte		
Sample	P1	P2	P3	P4	P5	P6	P7	P8	P9	P12	P10	P11	P13
% Bifenthrin	0.5	0.5	0.5	0.5	-	-	-	-	-		1.0	-	
% Permethrin	-	-	-	-	1.0	1.0	1.0	0.5	0.5*		-	1.0	

Values with * represent microencapsulated insecticides

The details of the specific decorative coating formulation recipes are beyond the scope of this study, and have been excluded to protect the supplier's trade confidentiality.

4.2.2 Sample Preparation for Extractability Tests

4.2.2.1 Solid-Liquid Extraction

1 g of paint sample was mixed with 30 mL extraction solvent DMF, DCM, HEX, EtOAc, TOL, or ACE in a stoppered conical flask. The mixture was allowed to stand for 6 hours with frequent shaking. The sample was filtered into a round bottomed flask containing $MgSO_4$, and the residue washed three times. The volume of solvent was reduced with a rotary evaporator, allowed to dry under N_2 gas and then re-dissolved in a set volume of the mobile phase DCM-HEX (50/50 (v/v)) for analysis by HPLC.

4.2.2.2 Ultrasonic Extraction

1 g of paint sample prepared and treated with a solvent as in Section 4.2.2.1 was sonicated. The extraction solvent was decanted into a round bottomed flask containing $MgSO_4$ and replaced with fresh solvent after every 0.5 hour, up to 2 hours. The volume of solvent was reduced with

a rotary evaporator, allowed to dry under N_2 gas and then re-dissolved in a set volume of the mobile phase for analysis by HPLC.

4.2.2.3 Liquid-Liquid Extraction

1g of wet paint sample was dispersed in 20 mL of water in a separating funnel. Also separately, 1 g of wet paint sample was dispersed in 20 mL of 1% (w/v) aqueous solution of LiBr, $MgSO_4$, NaCl, HCl or $CaCl_2$.

LLE was performed three times with 30 mL of HEX, EtOAc, DCM or Et_2O. The organic phase was collected into a beaker containing $MgSO_4$ to remove moisture. Then the volume of the organic phase collected was transferred into a round bottomed flask, reduced with a rotary evaporator and allowed to dry under N_2 gas flow. The residue was re-dissolved in the mobile phase to the target quantities for analysis by HPLC.

4.2.2.4 Soxhlet Extraction

1 g of wet paint sample was weighed into a thimble for extraction. Also, a separate 1 g of wet paint sample was carefully spread over 5g of sand, 2.5g of $MgSO_4$ and 0.1g of LiBr and transferred into a thimble for extraction. In a variation of the experiment 2.5 g $CaCl_2$ was used in place of the 2.5g $MgSO_4$.

A 100 µm dry paint film was prepared by spreading a 200 µm film of wet paint on a glass panel using a bar-coater machine. The same was allowed to dry for 72 hours in the laboratory. A 10 cm^2 section of the film was scrapped off from the glass panel using a scalpel and transferred into a thimble for extraction.

The Soxhlet extraction was performed for 3 and 6 hours with 100 mL of HEX, HEX-ACE (99/1), (95/5), (90/10) or (80/20), EtOAc, DCM, DCM-MeOH (98.8/1.2) or (95:5). All solvent compositions were prepared v/v. The extract volume was reduced with a rotary evaporator and allowed to dry under a gentle N_2 gas stream. EtOAc and HEX extract residue was re-dissolved in the mobile phase to attain 50 and 100 µg/mL target concentrations for 0.5 wt.% and 1.0 wt.% pyrethroid formulations, respectively. DCM extract residue was re-dissolved in DCM and precipitated in cold HEX. The residue was separated on a centrifuge operating at 5000 rpm for 5 min and the supernatant decanted into a round bottom flask. The process was repeated three times. The final volume of supernatant was reduced with a rotary evaporator, allowed to dry under N_2 gas flow and finally the residue was re-dissolved in the mobile phase for HPLC analysis.

4.2.3 Validation Strategy

To validate the extraction procedures, 1 g of wet paint sample was carefully spiked with either permethrin at 1.0 wt.% (10 mg) or bifenthrin at 0.5 wt.% (5 mg) by dissolving the standards in a minimal amount of acetonitrile and mixing thoroughly into the paint. The analyte was extracted by the method that demonstrates the best extraction efficiency prior to analysis by HPLC. The method was examined regarding matrix components that would interfere with NPLC method V. The validation experiments to study the precision of the method were carried out in duplicates daily for three days. The accuracy of the method is determined from the same experiments and expressed in terms of recovery (%). The extraction efficiency (accuracy) was computed as a ratio of analyte concentration against the nominal concentration (% recovery).

4.3 Analysis of Pesticide Formulated in Interior Decorative Coatings

4.3.1 HPLC analysis

The experimental procedure for Method V was described in Table 3.1 which was presented in in Section 3.3.1 of **Error! Reference source not found.**. All samples were filtered through a 0.45 µm RC membrane filter prior to analysis.

4.3.2 FTIR Analysis

Attenuated total reflectance(ATR)–FTIR measurements were performed on a Thermo Scientific Nicolet iS10 spectrometer (Thermo Scientific, Massachusetts, USA). The spectra were recorded from 4000 to 650 cm^{-1} with automatic background subtraction, collecting 64 scans at a resolution of 4 cm^{-1}. The software used for data collection and processing was Thermo Scientific OMNIC (version 8.1). The measurements were a qualitative test to detect the presence of pyrethroids in the test matrix.

4.4 Results and Discussion

4.4.1 Extractability Tests

It is usually necessary to extract compounds of interest from polymeric matrices before analysis in order to accurately determine their quantities. The conventional extraction techniques for compounds in polymeric samples are liquid-liquid extraction (LLE), usually through dissolution and re-precipitation of the polymer, and solid-liquid extraction (SLE) methods. Some new techniques including supercritical fluid extraction (SFE), microwave-assisted extraction (MAE) and accelerated solvent extraction (ASE) are now available at a cost.[1]

The development of an extraction protocol for the pyrethroids was carried out using the paint sample formulations listed in Table 4.1 as they were supplied in modest quantities. This was

done in order to save on expensive analytical standards that were available in small quantities. The advantage of using real samples lies in their representation of the true test matrix. A failure to account for the matrix effects can result in a negative bias in the analytical results.[2,3]

LLE and SLE procedures were investigated in order to select one that combines high extraction efficiency with minimal matrix dependency. Once extracted, the samples were analysed by NPLC Method V according to Table 3.1. The extracted quantity was determined by extrapolation of the experimental peak area from the matrix-matched calibration line in Figure 3.7. The extraction efficiency is determined by comparing the recovered amount against the nominal quantity and presented as percentage recovery. The method that would demonstrate the highest extraction of the pyrethroids relative to the nominal amount in the paint formulation would be selected for validation.

The paints are formulated with technical grade permethrin (93% pure, with 25:75 cis/trans enantiomer composition) and bifenthrin (98% pure). The purity was taken into account to make up for the amount of active ingredient's exact quantity nominated for each paint formulation. The extraction efficiency for permethrin is reported as the sum-average value of the cis- and trans- isomers together because there is no significant difference between the experimental and the theoretical average isomer composition as shown in Table 4.2.

Table 4.2: Ratio of cis/trans isomers extracted from paint formulated with technical permethrin (25/75) and also from paints spiked with analytical grade permethrin (40/60)

	Technical permethrin		Analytical Grade Permethrin	
	Cis (%)	Trans (%)	Cis (%)	Trans (%)
	26,2	73,8	40,2	59,8
	26,7	73,3	39,5	60,5
	24,7	75,3	39,6	60,4
	24,8	75,3	38,9	61,1
	26,7	73,3	38,8	61,2
	23,8	76,2	39,9	60,1
Average ± SD	25,5±1,10	74,5±1,10	39,5±0,506	60,5±0,506

SD is the standard deviation

4.4.1.1 Liquid-Liquid Extractions

LLE is a well-established purification method for pyrethroid separations.[4,5] It is based on the partitioning of the analyte between an aqueous phase and an immiscible organic phase.[6] This is a particularly useful technique for the extraction of pyrethroids from aqueous media since these insecticides are extremely hydrophobic.

Latexes employed in decorative coatings are generally insoluble in organic solvents, but can be easily dispersed in their emulsions by dilution with water. Therefore, paint samples were diluted with water to increase the surface area for extraction. However, such an experimental setup for the extraction of bifenthrin and permethrin with HEX, DCM, EtOAc or Et_2O was unsuccessful due to a difficulty in visually identifying the line of phase separation between the immiscible aqueous and organic phases. This is attributed to the presence of polymeric surfactants that can interact with both the aqueous and organic phase. In addition, some components are simply insoluble in either aqueous or organic phase and are, therefore, distributed in the phase separation boundary by virtue of their densities. These insoluble solids and the polymer self-assemblies can entrap the analytes. This effect is further compounded by the high affinity of pyrethroids towards solid substrates and, therefore, lead to low extraction efficiency.

The distribution of pyrethroids during LLE can be influenced by the polarity of the organic solvent, the pH or ionic strength of the aqueous solution. Careful tuning of these parameters can increase the distribution ratio of the pyrethroids in the organic phase. The results presented in Figure 4.1 show the extraction efficiencies for permethrin and bifenthrin from paint samples that were dispersed in 1% aqueous solutions of LiBr, NaCl, $CaCl_2$, $MgSO_4$ and Na_2SO_4 when HEX was used as the extraction solvent. A volume ratio of 1:5 between the aqueous phase and the organic phase was used to monitor the experimental conditions. The visibility of the phase separation between the aqueous and immiscible organic phases improved slightly with the use of the salts.

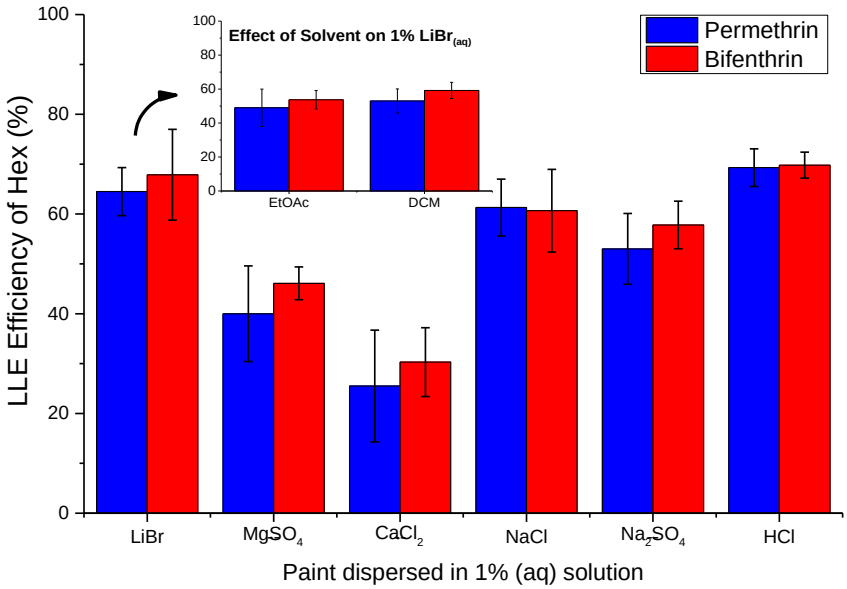

Figure 4.1: Pyrethroid extraction efficiency of HEX on paints dispersed in 1% aqueous solution of different salts. The small insert in the figure compares the efficiencies of DCM and EtOAc to HEX on paints dispersed in 1% LiBr solution.

Higher extraction efficiencies are obtained where the salts with monovalent cations are applied in the order LiBr > NaCl > Na_2SO_4 for both permethrin and bifenthrin compared to salts with divalent cations such as $MgSO_4$ and $CaCl_2$. It can also be seen that the extraction efficiencies are higher with the cations of smaller ionic radii. 67.9 ± 9.11% of bifenthrin and 64.5 ± 4.82% of permethrin were recovered from the paint sample in 1% LiBr solution while 25.5 ± 11.2% of permethrin and 30.3 ± 6.9% of bifenthrin were recovered from paints in 1% $CaCl_2$ solution. The ineffectiveness of salts with divalent cations is attributed to their poor solubility in water and hence lower ionic strengths of their aqueous solutions compared to their monovalent counterparts. The high variability in the mean extraction efficiency shown by the error bars in the graph is directly indicative of the magnitude of error associated with the method.

The highest extraction efficiencies of 69.8 ± 2.60% for bifenthrin and 69.3 ± 3.77% for permethrin are observed in Figure 4.1 when the paint samples are diluted with 1% HCl solution prior to extraction with HEX. A gas evolved upon dilution with the acid followed by a spontaneous disintegration of the consistency of the diluted sample. The evolution of gas is attributed to the dissolution of $CaCO_3$ from pigment microcapsules while the increase in ionic strength of the solution disrupted the interactions of the polymeric surfactants resulting in the deterioration of the consistency of the sample. However, this approach is unattractive due to its low pH potential to hydrolyse the pyrethroid esters with which the analysis is concerned.

The effect of the choice of an extraction solvent on paints dispersed in 1% LiBr was evaluated with DCM and EtOAc. The results are shown in the small insert in Figure 4.1. Both solvents were less efficient compared to HEX. Recoveries of 53.0 ± 7.10% for bifenthrin and 59.2 ± 4.77% for permethrin were attained with DCM, while 53.7 ± 5.45% for bifenthrin and 49.0 ± 11.0% for permethrin were achieved with EtOAc.

The LLE approach was prone to error as the organic phase needed to be filtered to remove some paint matrix residues that contained moisture. The presence of moisture causes the pyrethroids to precipitate and adsorb to solid surfaces. Although a filter packed with $MgSO_4$ was used to clarify and dry the organic fractions upon collection to eliminate both residual solids and moisture, the pyrethroids were less likely to cross the layer of hydration around solid particles into the organic phase due to intense hydrophobic interactions. The result was a negative impact on the efficiency of the extraction. Semi-automated and automated LLE methods have been developed to minimise these sources of errors and limitations in order to improve the recovery of the analytes, but it is not known if such methods can cope with high residue systems such as latex paints.

4.4.1.2 Solid-Liquid Extractions

The process of extraction of organic compounds from solids is based on desorption of solutes from the sample matrix followed by dissolution into the solvent. Here, the analyte is extracted from the solid medium by a liquid, which is separated by physical means, such as filtration. There are many methods for carrying out SLE including Soxhlet, sonication and shake-flask extractions. The transport of the analyte from the core of the matrix to the solvent depends on the diffusion coefficient of the analyte, particle size and structure of the matrix, temperature, agitation speed, solvent viscosity and the ability of the solvent to penetrate the matrix.[6] Critically, there is not a single solvent that is universally suitable for all analytes and all matrices. Therefore the importance of the selection of a suitable extraction solvent cannot be overstated.

A simple shake-flask extraction was carried with an array of extraction solvents including DMF, DCM, HEX, EtOAc, TOL and ACE. A paint sample containing permethrin or bifenthrin was allowed to stand in a volume of solvent with occasional shaking for up to 6 hours. The recoveries per solvent applied are presented in Figure 4.2. The recovery of the analytes was generally low. For example, where ACE was used, the extraction efficiencies were 23.1 ± 3.7% for permethrin and 25.4 ± 4.2% for bifenthrin. The performances of DCM and EtOAc were

very similar and followed closely after ACE with extraction efficiencies of 19.8 ± 5.3% and 19.4 ± 4.3% for permethrin and 22.1 ± 3.5% and 22.6 ± 3.1% for bifenthrin, respectively. Extraction efficiencies decreased for hexane while DMF provided the lowest extraction efficiencies of 11.1 ± 4.1% for bifenthrin and 9.3 ± 3.8% for permethrin. Therefore, the extraction efficiencies were poor for all solvents. The paint samples typically formed into a globule that massively reduced the surface area for extraction, hence the low efficiencies. When mechanical mixing was applied through stirring, the paint samples only dispersed into ACE as smaller globules and flakes but retained the single globular form in the rest of the solvents.

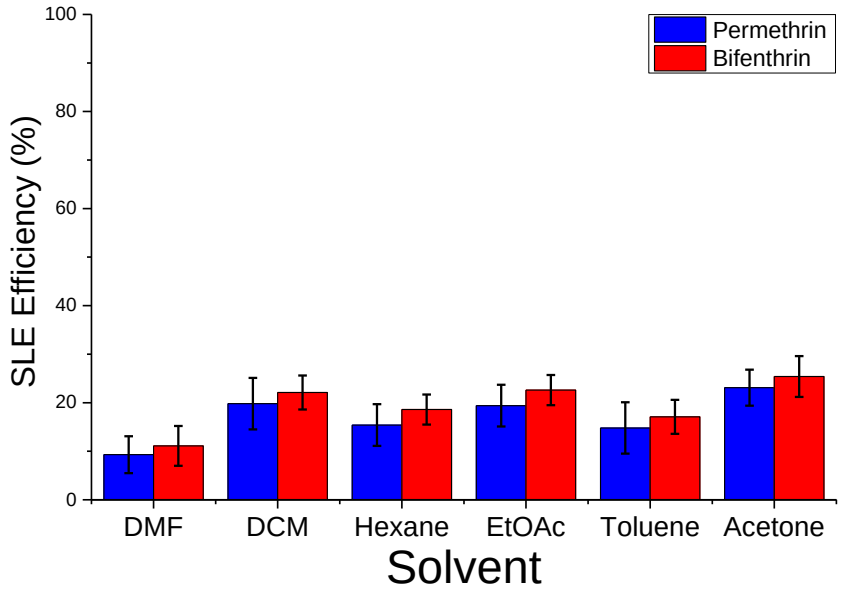

Figure 4.2: Solvent efficiency in shake-flask SLE of permethrin and bifenthrin from paint samples.

Ultrasonic energy was then employed to improve the extraction efficiencies of the shake-flask approach. Its principle of operation relies on agitating the solution using ultrasonic vibration to produce cavitations in the liquid ensuring intimate contact between sample and solvent. Only the rate of transfer of analytes across the polymeric substrate and solvent boundary would be expected to increase, but not the diffusion coefficient of the analytes. The results shown in Figure 4.3 are not reflective of this expectation. After the sonication of paint samples in EtOAc, DCM, TOL and DMF, each experiment produced an emulsion that was stable and inseparable when allowed to sit for a whole day. Also, the emulsion could not be separated by filtration.

The paint sample sonicated in ACE is separable after a short time of standing, and can be further filtered to remove the solid residues. The extraction efficiency improved marginally from the shake-flask approach, and remained low i.e. 34.3 ± 5.4% for bifenthrin and 30.0 ±

7.0% for permethrin. The paint sample sonicated in HEX or Et_2O remained in a very neat globular state that presents a limited surface area for extraction in the solvent. Hence the low recoveries of 30.7 ± 4.5% for bifenthrin and 26.0 ± 6.35% for permethrin are achieved with HEX and 19.6 ± 5.3% for bifenthrin and 13.0 ± 6.4% for permethrin with Et_2O. Although sonication is known to be fast, its extraction efficiencies are generally lower than other techniques. Also, it has been reported that for a group of organophosphorus insecticides closely related to the pyrethroids, ultrasonic irradiation induced decomposition of the compounds.[7] Therefore, sonication might be unsuitable for the extraction of pyrethroids.

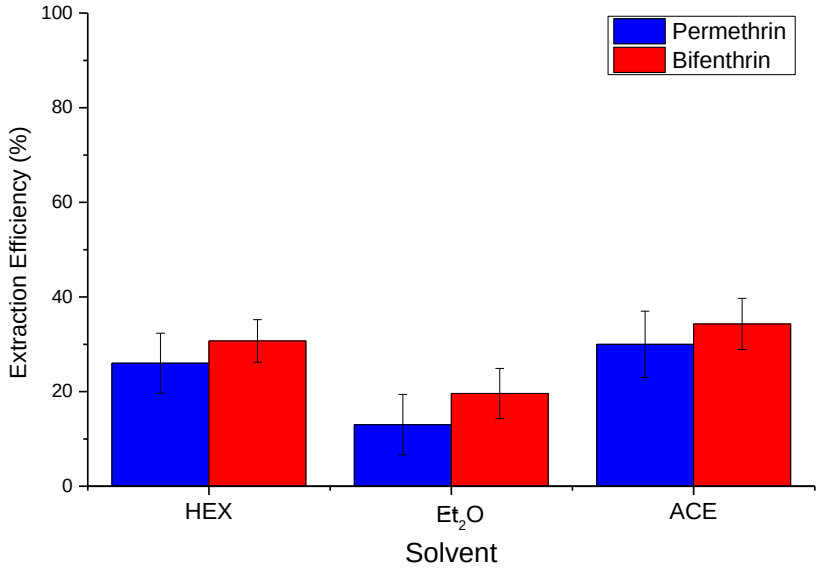

Figure 4.3: Extraction efficiencies when paint samples were sonicated in extraction solvent for 2 hours.

A method involving the combination of shake-flask extraction and sonication for the extraction of the pyrethroid insecticide deltamethrin using ISO-DIOX (80/20 (v/v)) from solvent-based paints has been reported.[8] The same method was applied on emulsion paints for the extraction of permethrin and bifenthrin. As it can be seen in Figure 4.4, the extraction efficiencies are extremely low for both permethrin and bifenthrin at 5 ± 2.1% and 5 ± 1.9%, respectively, when the method was operated directly on wet paint samples. The polymer had fused the residue obtained after the extraction into a globule, meaning there was minimum surface area for extraction hence the low recoveries. In addition, it shows that the solvents lack penetrating power into the paint matrix.

The results for overnight Soxhlet extraction shown in Figure 4.5A are similar to the observations made in the sonication experiments in Figure 4.3. DCM, EtOAc, HEX and ACE were investigated as extraction solvents. The extraction efficiencies for the four solvents ranged between 13% – 26% for permethrin and 27% – 32% for bifenthrin. The paint residue collected in the Soxhlet thimble after the extraction showed that the polymer had fused the whole sample together leaving minimal surface area for interaction with the solvent.

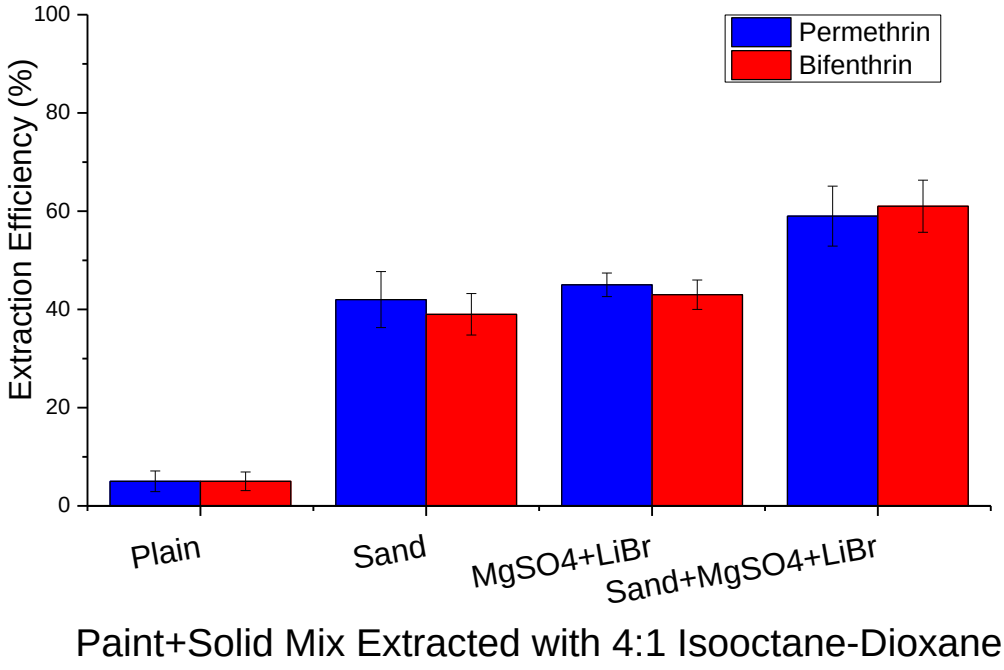

Figure 4.4: Extraction efficiencies from combined shake-flask and ultrasonic extraction of permethrin and bifenthrin with ISO-DIOX (80/20 (v/v)).

It is common practice that polymeric samples are air-dried and ground to a fine powder before extraction since most analyte extraction procedures generally perform better on dry samples with small particle size distributions. However, this approach is objectionable in this study in order to minimise human exposure to the insecticides in the sample. Instead, the samples were dried by mixing with anhydrous $MgSO_4$ (and $CaCl_2$ for performance comparisons). LiBr was included in the mixture to disrupt hydrophilic interactions in the sample. Figure 4.4 shows that the extraction efficiency increased up to 44.9 ± 2.4% for permethrin and 43.2 ± 3.00% for bifenthrin. There was still a tendency of the polymer in the sample to fuse the matrix together. The samples were then dispersed in sand before extraction to assist with preventing the polymer fusing together. The results were closely comparable to those where the drying salts were

employed. The two approaches were then combined and resulted in improved analyte recoveries of up to 59.0 ± 6.10% for permethrin and 60.9 ± 5.30% bifenthrin.

Pyrethroids have been extracted with mixtures of water-miscible solvents, usually ACE, with non-miscible ones, usually HEX or DCM, from biological tissues using Soxhlet extraction, LLE and ultrasonic extraction.[4,5] The polar solvents penetrate the layer of moisture on the surface of the solid particles to extract the hydrophilic organic components while the non-polar solvents extract the hydrophobic organic components. Figure 4.5(B) displays the results of Soxhlet extraction performed with HEX with varying amounts of ACE on paint samples treated with LiBr and$MgSO_4$. Bifenthrin and permethrin recoveries of 72.8 ± 4.66% and 75.0 ± 2.90%, 73.0 ± 1.90% and 74.1 ± 3.11%, and 81.2 ± 3.7% and 79.0 ± 4.20% were obtained with HEX, 1:1 HEX-ACE and 4:1 HEX-ACE, respectively.

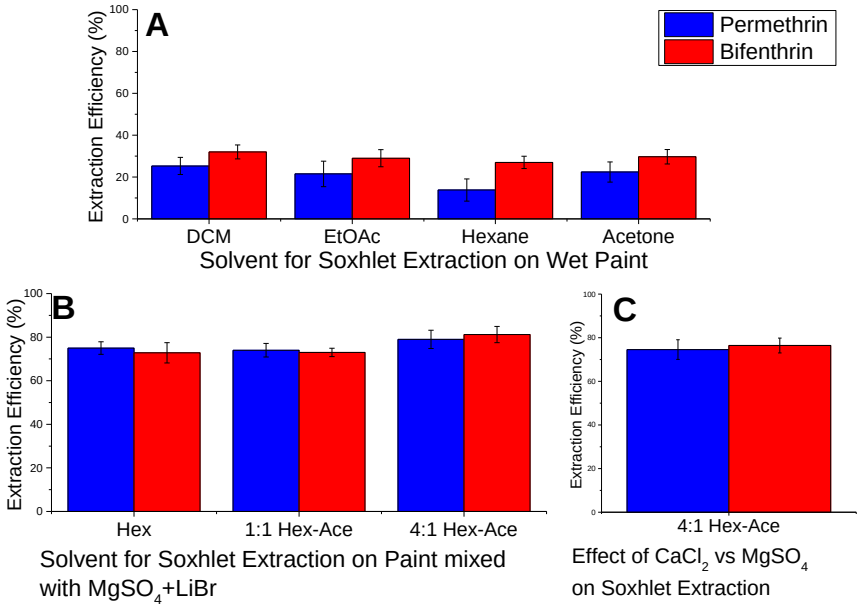

Figure 4.5: Extraction efficiencies for 6-hour Soxhlet extractions of permethrin and bifenthrin, A) using different solvents directly on paint samples; (B) using varying compositions of HEX-ACE on paint samples treated with $MgSO_4$, LiBr and sand; and (C) using HEX-ACE (4:1 v/v)) with $CaCl_2$ instead of $MgSO_4$ to evaluate the effect of the drying salt.

The effect of the amount of ACE in the extraction increased for 0 – 20% (v/v), above which the extraction efficiency of the system decreased. The effect of the drying salt on extraction efficiency of HEX-ACE (80/20 (v/v)) was investigated using $CaCl_2$ in place of $MgSO_4$ while everything else was held constant. Figure 4.5(C) shows that there was a marginal decrease in the recoveries of both bifenthrin and permethrin to 76.5 ± 3.40% and 74.6 ± 4.50%, respectively. Therefore, $MgSO_4$ was considered the more efficient selection.

Challenges were still faced with the tendency of the polymer to fuse the sample matrix together during extraction. Therefore, sand was now included in the sample pre-treatment step in addition to $MgSO_4$ and LiBr. A marked increase in the extraction efficiency with HEX-ACE (80/20 (v/v)) for both bifenthrin (89.5 ± 5.31%) and permethrin (87.4 ± 4.66%) was observed which could not be improved by allowing longer extraction times. This information is shown in Figure 4.6, where the effect of solvent selection on the extraction was also investigated by comparing HEX-ACE with EtOAc and DCM-MeOH (95:5 (v/v)). Sample extracts from HEX-ACE contained very little solids and were, therefore, suitable for re-dissolution in the mobile phase, filtration and direct analysis by HPLC.

Lower extraction efficiencies for bifenthrin (69.0 ± 7.20%) and permethrin (73.0 ± 3.70%) were observed where EtOAc was the extraction solvent. The extract residues obtained contained small amounts of components that were insoluble in the mobile phase DCM-HEX (50/50 (v/v)) that made it very difficult to filter the sample through the 0.45µm RC membrane filter. Extraction values greater than 100% (bifenthrin (133 ± 5.0 %) and permethrin (120 ± 6.7%)) were observed where 19:1 DCM-MeOH was employed as the extraction solvent. Once concentrated, the residue was made up of substantial amounts of gel-like material. The very high extractability of analytes was due to the coelution of the analytes with the matrix components as was shown in Figure 3.6A when method III was operated. This prompted the modification of the separation conditions to Method V to achieve selectivity of the analytical method for both permethrin and bifenthrin.

Similar to EtOAc extract residues, the residues from DCM-MeOH also formed a milky suspension when dissolved in the mobile phase DCM-HEX (50/50 (v/v)). This then required the inclusion of a sample clean-up step. The polymeric residues were dissolved in DCM and re-precipitated in excess amounts of cold HEX. The samples were centrifuged and the supernatant solution was decanted into a round-bottomed flask for concentration by rotary evaporation. The analytes were then clearly soluble in the mobile phase, easy to filter and analyse by HPLC.

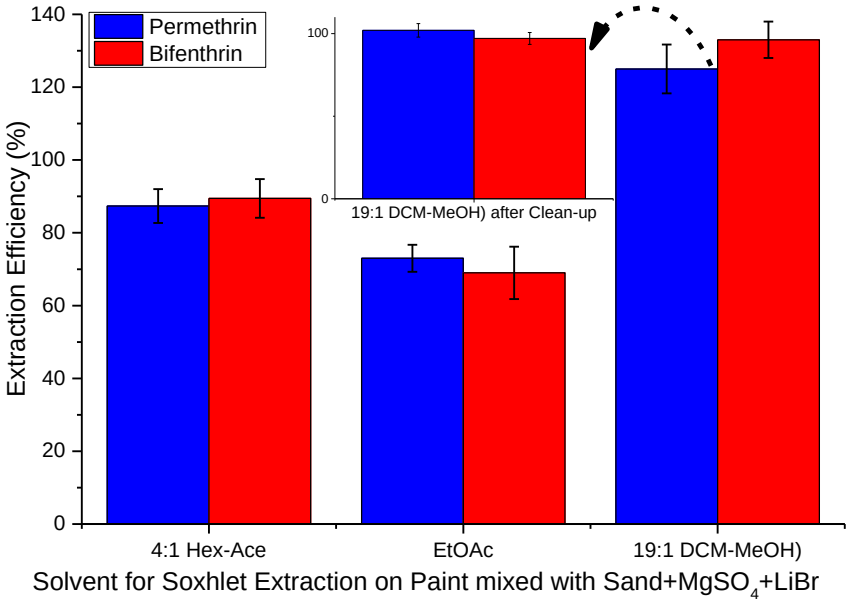

Figure 4.6: The effect of solvent selection on Soxhlet extraction efficiencies of permethrin and bifenthrin from paint samples treated with sand, LiBr and $MgSO_4$.

The IR spectra of blank paint samples (without any permethrin or bifenthrin) isolated using the same method were obtained for comparison against the IR spectra of bifenthrin and permethrin analytical standards, see Figure 4.7.

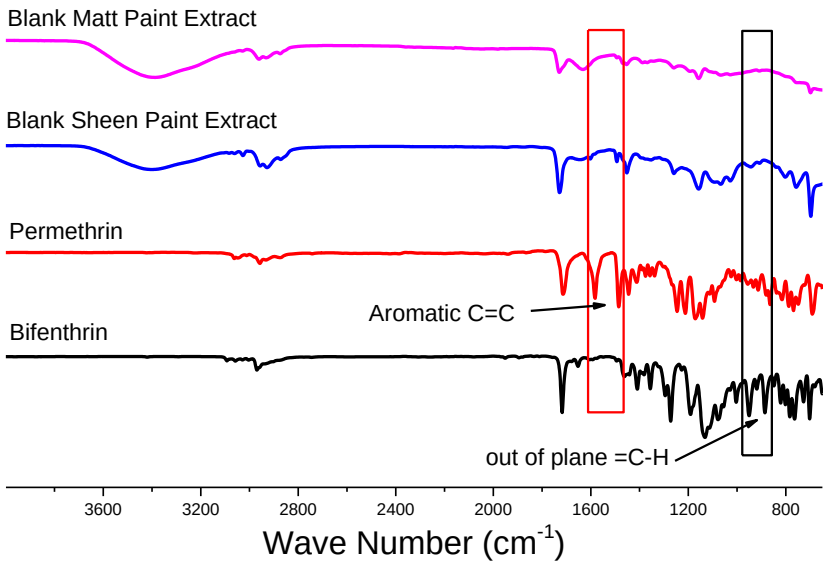

Figure 4.7: FTIR of bifenthrin and permethrin analytical standards, and blank extract residues of sheen and matt wet paints prepared by Soxhlet extraction with DCM-MeOH (95:5 (v/v)).

FTIR was used as a qualitative determination to see if the analytes could be identified in extract residues. The IR spectra in Figure 4.7 show that Permethrin (red legend) could be uniquely identified by the C=C aromatic ring stretch absorption peaks at 1582 and 1485 cm^{-1} while the presence of bifenthrin (black legend) in the extract matrix could be identified by the =C-H out of plane bending peaks at 950 and 877 cm^{-1}. Therefore, FTIR could be used prior to HPLC analysis to determine the success of the analyte extraction step from wet paints.

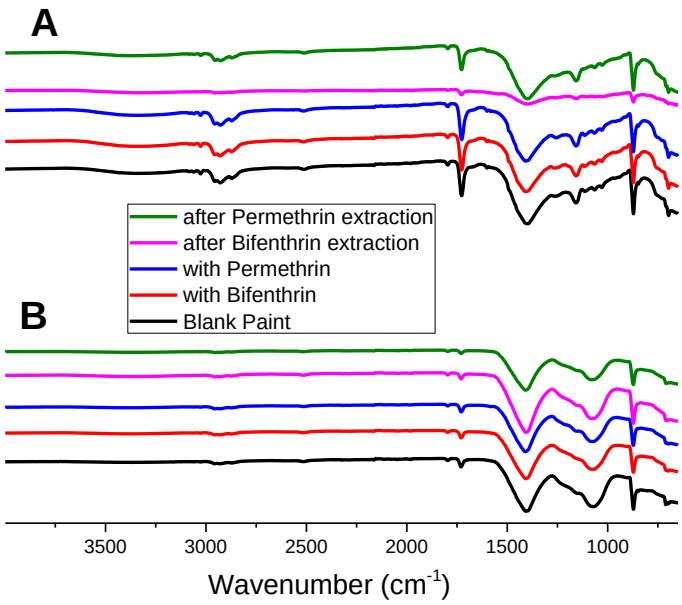

Figure 4.8: FTIR spectra of dry films of (a) sheen paint and (b) matte paint.

The application of the method was extended to study the extractability of the pyrethroid insecticides from dry paint films. It was observed that there was no significant difference in the extractability of pyrethroid insecticides from dried films compared to wet paint samples. FTIR was not suitable for tracking the presence of pyrethroids in the dry paint film. This is evident in the similarity of the spectra of the blank paints, the paints with permethrin or bifenthrin and the residue of the paints post extraction with the Soxhlet method (see Figure 4.8). The detection limit of FTIR is 100 ng – 1 µg, therefore, the distribution of the pyrethroids in the dry paint film which results in a low concentration per unit area cannot be detected.

Above all, after the sample clean-up step the extraction efficiencies were pleasing and highly acceptable by ICH recommendations for both bifenthrin (97.1 ± 3.6%) and permethrin (102.0 ± 4.1%). These high extraction efficiencies could be achieved with 3 hours Soxhlet extraction, beyond which there is no significant improvement in analyte recoveries. Hence this optimised

Soxhlet extraction method for pyrethroids in latex paints was selected for performance evaluation in the following sections.

4.4.2 Evaluation of Method Performance

4.4.2.1 Assessment of Matrix Effects

The quantification of pesticide residues in decorative coating matrices may be adversely affected by chromatographic matrix effects. This phenomenon can be induced by factors such as the nature of pesticide and the matrix, and the ratio of the pesticide to the matrix. In liquid chromatography with UV detection, this phenomenon occurs through coelution of the analyte with certain matrix components.[6,9-11] The major consequence of matrix effects is either signal enhancement or suppression with respect to the same amount of analyte in solvent or standard solution. The test matrix did not impose any negative effects on the chromatographic method. This was further demonstrated by the selectivity parameters of the analytical method discussed in section 3.6.2.1.

The matrix effect was evaluated by comparison of the elugrams of pyrethroids prepared in pure solvent, matrix matched standard pyrethroid solutions against blank paint extract solutions. The chromatograms shown in Figure 3.6B demonstrate clearly that the matrix had no negative impact on the chromatographic separation of the pyrethroids.

4.4.2.2 Linearity of the Method

The selected and optimised Soxhlet extraction method in discussion was employed for the construction of a matrix-matched calibration used in this study. This was intended to minimise quantitative errors arising from matrix effects. The linearity was discussed in section 3.6.2.2 in **Error! Reference source not found.** because the Soxhlet extraction method was used for the preparation of the matrix solution used for the construction of the matrix-matched calibration graphs.

4.4.2.3 Limits of Detection and Quantification

The LOD and LOQ considered for the method are in line with the dicussion of the NPLC method development for which the matrix effects of the paints have been accounted for in section 3.6.2.3. Paints spiked with insecticides at 0.5 mg/g, 3 mg/g and 7.5 mg/g were analysed for their pyrethoid content. A linear fit was performed connecting the three assay results and statistical treatment of data followed. Table 4.3 shows the LOD and LOQ values as a mass fraction (in ng/g) obtained from the paint extracts analysed by the NPLC method.

Table 4.3: LOD and LOQ in ng/g of paint pyrethroids in paint samples

Insecticide	Bifenthrin	Cis-Permethrin	Trans-Permethrin
LOD (ng/g)	3.8	3.0	3.4
LOQ (ng/g)	7.8	5.8	5.6
Sensitivity (ng^{-1})	1.15	0.32	0.58

These obtained values are higher than those for the NPLC matric matched calibration. This is due to the margin of error incurred during the extraction and sampling process prior to HPLC analysis, meaning there is greater variation in the analytical result obtained post analyte extraction. Therefore, the LOD and LOQ demonstrate that the method provides adequate sensitivity for residual pyrethroid content determination within the target paint matrix.

4.4.2.4 Recovery

Extraction efficiency is an important factor when considering the performance of an analytical method. A recovery study to evaluate the bias of the optimised Soxhlet extraction protocol in the extraction of paint samples spiked with permethrin at 10 mg/g and bifenthrin at 5 mg/g in duplicates was performed. Triplicate measurements are recommended for validation across numerous guiding references but duplicate measurements were performed to balance between extraction time and analysis time in a day. The recovered amounts were determined using the external calibration for the NPLC Method V described in section 3.6.2.2. The recovery was reported as a percentage of the spike quantity together with the corresponding variance of the data.

Figure 4.9 shows the recoveries achieved for permethrin and bifenthrin with the Soxhlet extraction method. The analytical recovery range for bifenthrin was 86 – 96% and permethrin 88-99%. The standard deviation, shown as error line on the bar chart, describing the variation of the recovery results was 5.6 – 11.1 for bifenthrin and 3.5 – 10.7 for permethrin

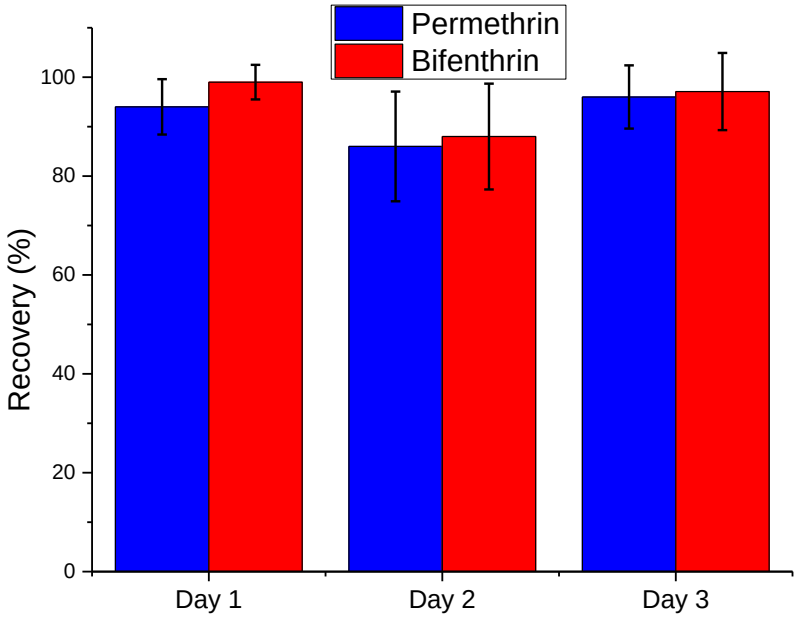

Figure 4.9: Recovery (%) of the pesticides from paints (n = 2 × 3 days) with error bars representing the standard deviation for permethrin at 10 mg/g and bifenthrin at 5 mg/g.

4.4.2.5 Precision

The repeatability (duplicate analyte extractions carried out on the same day) and intermediate precision (duplicate sample extractions performed on three different days) was determined separately for a standard concentration of 5 mg/g for bifenthrin and 10 mg/g for permethrin by calculating the relative standard deviation (RSD, %). A graphical presentation of the results is shown in Figure 4.9.

The repeatability for the duplicate analyses performed on the same day with the optimised Soxhlet method is expressed as relative standard deviation. The repeatability was generally acceptable below 20 % for both permethrin (RSD between 3.9 and 10.8%) and bifenthrin (RSD between 6.5 and 11.8%).[12,13] These figures appropriate the adequacy of the repeatability and ruggedness of this Soxhlet extraction method.

4.5 Conclusion

Factors influencing the extraction efficiency of LLE and SLE were optimized by experimental design. The comparison of the extraction methods by means of extractability tests carried out on real samples showed that there was no significant difference between the extraction of permethrin and bifenthrin from sheen or matte type coatings. The best result for the extractability of pyrethroids from the paint matrix was obtained with the Soxhlet extraction on

1.0 g of paint sample supported on 5.0 g sand, 2.5 g $MgSO_4$ and 0.1 g LiBr extracted with DCM-MeOH (95:5 (v/v)) for a minimum of 3 hours.

The recoveries were excellent and the sensitivity of the method was determined by calculation of the LOD and LOQ based on the matrix-matched calibration line. Soxhlet extraction is known to be more sensitive and efficient than others due to repeated cycles of extraction with the same solvent and with minimal supervision. The proposed method provides a simple, selective, robust and easily accessible procedure for the quantitative extraction of bifenthrin and permethrin in decorative latex paint samples.

4.6 References

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Chapter 5

Analysis of Real Insecticide Paint Samples- Preliminary Results

5.1 Introduction

The analytical method for residual pyrethroid content determination was applied to different commercial paints of the sheen and matte type. The information acquired from the extraction tests on paint formulations can be important for the development of manufacturing and regulatory policies.[1,2] Since the decorative coatings under study are intended to contain pyrethroid insecticides under interior household environments, extractability studies were performed under the same conditions for in-can stability and residual content in the dry state. An extension to study the different interactions between the coating matrix and the pyrethroids was made for prolonged release durations under the same conditions.

5.2 Materials

Dichloromethane (DCM, CHROMASOLV ≥99.8%, Honeywell Riedel-de Haen AG, Hannover, Germany), n-Hexane (HEX, CHROMASOLV ≥99.9%, Honeywell Riedel-de Haen AG, Hannover, Germany), methanol (MeOH, HPLC grade, ≥99.9%, Sigma Aldrich, Saint-Quentin-Fallavier, France), sand (White Quartz, 50–70 mesh, Sigma Aldrich, Steinheim, Germany), lithium bromide (LiBr, ≥99%, Sigma Aldrich, St. Louis, USA), anhydrous magnesium sulphate ($MgSO_4$, >99%, Merck, KGaA, Darmstadt, Germany), and cellulose extraction thimbles (GE Healthcare, Amersham, UK) were used as received.

5.3 Sample Preparation

5.3.1 Wet Paint Samples

The samples are listed in Table 4.1. 1 g of wet paint sample carefully spread over 5 g of sand, 2.5 g of $MgSO_4$ and 0.1 g of LiBr. The mixture was transferred to a thimble for Soxhlet extraction.

5.3.2 Fresh and Naturally Aged Dry Paint Films

A fresh 100 µm dry paint film was prepared by applying 200 µm wet paint film (see Table 4.1) using a bar-coater machine on a glass panel. The sample was allowed to dry in open air for 72 hours in the laboratory. A 10 cm^2 section (5 cm × 2 cm) was scrapped off using a scalpel and transferred into a thimble for extraction.

Naturally aged 100 µm paint films were prepared on wooden boards, and kept for 2-3 years in the laboratory. A 10 cm^2 section (5 cm × 2 cm) was chipped off using a scalpel and transferred into a thimble for extraction.

5.3.3 Soxhlet Extraction

The Soxhlet extractions of the samples in Sections 5.3.1 and 5.3.2 were performed with 100 mL DCM-MeOH (95:5 (v/v)), for 3 hours. The extracts were evaporated to a small volume using a rotary evaporator, and precipitated in excess HEX in a centrifuge tube. The sample was centrifuged at 5000 rpm for 5 min. The supernatant solution was transferred into a round bottomed flask and evaporated to a small volume which was allowed to dry off under a gentle stream of N_2 gas. The residue was dissolved in the mobile phase DCM-HEX (50/50 (v/v)), and diluted suitably for analysis by HPLC.

5.4 Analytical Methods

The HPLC method V described in Table 3.1 was employed for the quantification of bifenthrin and permethrin from real paint samples. The calibration plots in Figure 3.7 were used for the determination of the quantities of the analytes. All samples were filtered through a 0.45 µm RC membrane filter prior to analysis.

5.5 Results and Discussion

The wet paint samples available for analysis (listed in Table 4.1) had been stored in the laboratory for over 1 year. Therefore, determination of their pyrethroid content would give valuable insight to the in-can stability of the insecticidal paint formulations. The paints were analysed using the developed Soxhlet extraction and NPLC with UV detection. The quantity of insecticide in the paint was determined based on the calibration in section 3.6.2.2. The results of the pyrethroid content determination are displayed in Figure 5.2 as percentage of the initial quantity in each formulation. Paint film drawdowns of the same wet paints were prepared on glass panels and stored in the laboratory. They were only analysed 4 months later due to the Covid 19 pandemic lockdown.

The bifenthrin content of the wet paints was between 76-88 % (Figure 5.1A) of the initial, while that in the corresponding paint film was 68-90 % (Figure 5.1B) The permethrin content of the wet paints was 72-82 % (Figure 5.1C) and in the corresponding film 61-75 % (Figure 5.1D). The dercrease in pyrethroid content from the initial quantities in the wet paints is very small signifying that pyrethroids were very stable over the duration (18 months) of the storage of the wet paint formulations. In general, the paint films contain less insecticide than the parent

wet paints. This is because the film panels were stored in the open environment in the laboratory during the lockdown period hence environmental forces e.g. light, humidity or microbes may have had influence.[3]

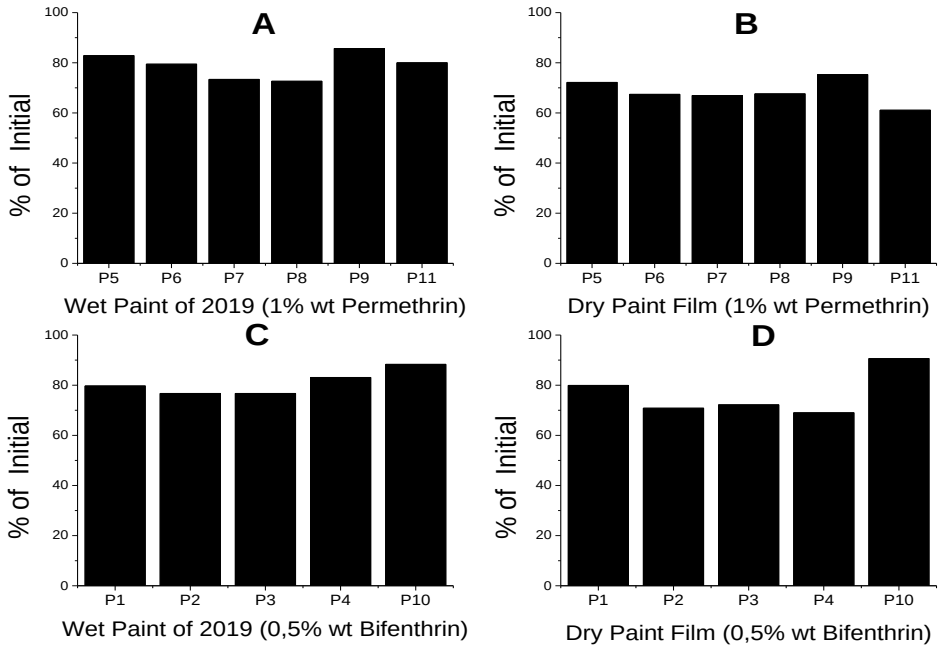

Figure 5.1: Pyrethroid content presented as percentage of the initial in (A) wet paint with 1% wt permethrin, (B) dry paint films from paint in (A), (C) wet paint with 0.5% wt bifenthrin and (D) dry paint films from paint in (C), manufactured in the year 2019.

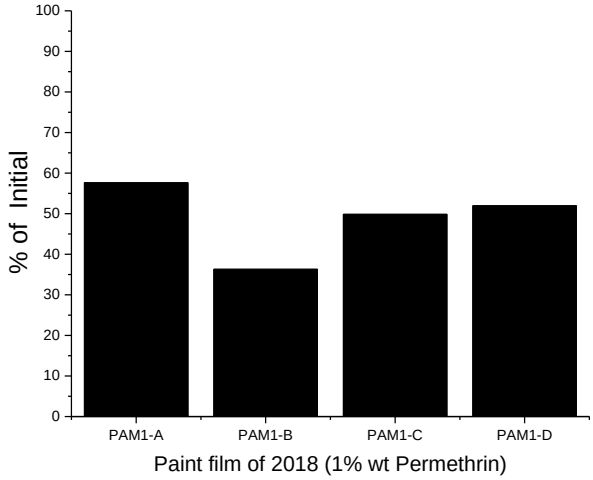

Figure 5.2: Permethrin content of dry paint films prepared in the year 2017 presented as a percentage of the initial composition.

Also the paints retain more bifenthrin than permethrin. There are a number of possible reasons for this observation. In the wet paint, bifenthrin is more hydrophobic than permethrin (section 3.6.1). Therefore, bifenthrin could be hid farther inside the core of the micro-domains of the paint matrix due to stronger hydrophobic interactions in the presence of water (the solvent). In the dry paint bifenthrin could also be more soluble than permethrin in the latex polymers making up the binder of the paint, hence the greater retention.[3]

Paint films that were prepared in 2018 with 1 wt.% permethrin (Figure 5.2) and in 2017 with 0.5 wt.% bifenthin were also analyed (Figure 5.3). Permethrin content in the paint films of 2018 was between 36 – 57 %. Bifenthrin content in the paint drawdowns of 2017 ranged between 27 – 36 %.

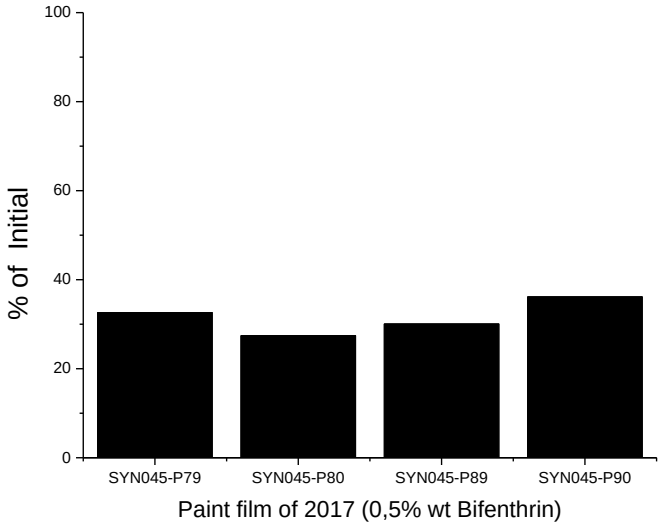

Figure 5.3: Bifenthrin content of dry paint films prepared in the year 2017 presented as a percentage of the initial composition.

Meanwhile the samples were grouped by year of manufacture, and the mean pyrethroid content in dry paint films (regardless of whether permethrin or bifenthrin because samples are not available for each year) was calculated for each group as percent of the initial quantity. The change in mean percent of composition was plotted against time as shown in Figure 5.4. The standard deviation about the mean pyrethroid content per sample group was calculated to describe the varance of the data as shown by the error bars.

The pyrethroid content of the paints clearly decreases progressively with increasing age of the paint.[3-5] Although the mechanisms by which the pyrethroid content of the paints decreases are

not fully understood, the preliminary studies have demontrated that the latex paints can prolong the lifespan of the pyrethroids.

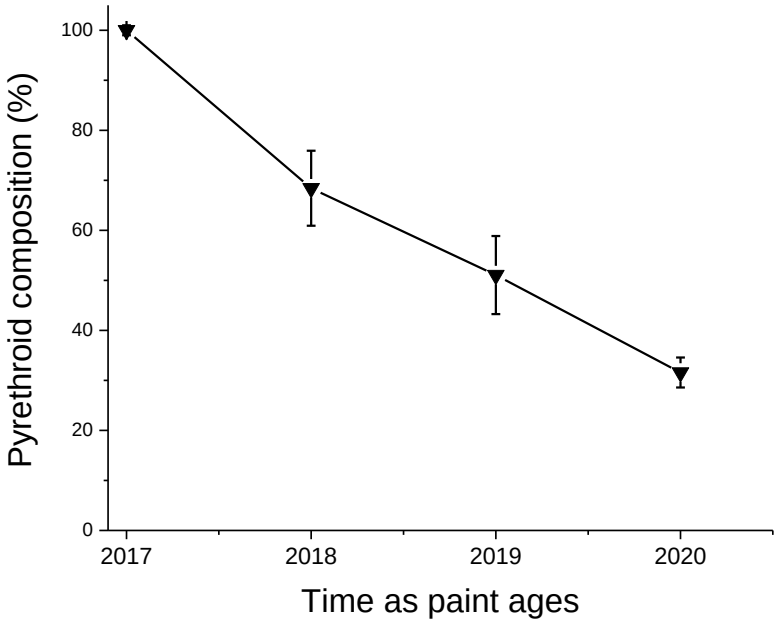

Figure 5.4: Average pyrethroid content of paint from the year 2017 to 2020 (regardless of whether permethrin or bifenthrin)

5.6 Conclusion

The optimised Soxhlet extraction method and the developed NPLC method have been successfully applied for the analysis of real pyrethroid-based latex paints. The method is proportional and precise for the analysis of the pyrethroids at their target concentrations in the paint formulations. The pyrethroids remained stable to a great extent during the 18 months shelf life of the wet paint formulations. Preliminary results have demonstrated that latex paints can retain pyrethroids for periods in excess of 2 years.[6]

5.7 References

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Chapter 6

Conclusions and Recommendations

6.1 Summary and Conclusions

The aim of the study was to develop a HPLC method for the quantitation of pyrethroid residues in interior decorative coatings developed as an intervention against the transmission of malaria. The decorative coatings were manufactured with neat or microencapsulated pyrethroid insecticides.[1,2] The complexity of the paint formulation would demand that a sample purification be included to make the sample suitable for HPLC analysis.

The first objective was achieved by the development of a NPLC method with UV-detection. The method was successfully validated for its selectivity, sensitivity, linearity range, accuracy and precision. A multiple point external calibration plot was constructed in the case of each analyte: bifenthrin, cis- and trans-permethrin. Some results for permethrin were presented as the average of the isomers together because the calibration curves for the isomers could be combined for total permethrin determination. However, it was important for the method to be able to quantify the analytes individually since it is known that their susceptibility to degradation may differ significantly.[3]

Secondly, a 3 hours Soxhlet extraction method with DCM-MeOH (95:5 (v/v)) was developed to determine permethrin and bifenthrin in interior decorative latex paints. The approach can be employed directly on dried paint films. However some sample treatment is required for wet paints prior to the Soxhlet extraction. Sand was used as a solid support to increase the surface of the wet paint, LiBr was employed to keep the ionisable species in a neutral state while $MgSO_4$ was employed as dessicant to dry the sample. The performance of the method was evaluated to prove the sensitivity, repeatability and extraction efficiency.

Finally the method was applied in the analysis of the real pyrethroid-based paints. The developed method is precise, proportional and rugged. The preliminary findings show signs of the usefulness of pyrethroid paints in curbing malaria transmission. With the developed method, it is possible to assure the quality of a manufactured pyrethroid-based emulsion paint product.

6.2 Future work

Due to time contraints, the development of a more sensitive LCMS method for the detection of trace pyrethroid residues or their degradation by-products was not possible. It is of interest to investigate the causes of the losses in pyrethroid content of the paint, to nominate the possible degradation pathways and to identify and characterise the degradation products. There is need to perform accelerated ageing studies under artificial laboratory conditions and studying naturally incurrred paint samples may not be appropiate. The importance of such an exercise lies in mapping a time-based study of pyrethroid content loss from paints to the period of time it would take for the pyrethroid composition to diminish below significant values as the median lethal concentrationn (LC_{50}) against mosquitoes.

6.3 References

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Appendix A

Table A-1: Physical and Chemical Properties of Permethrin

Property	Quantity
Molecular Weight ($g.mol^{-1}$)	391.3
Relative density at 20°C ($g.cm^{-3}$)	1.19 – 1.27
Boiling Point and thermal stability	vaporises intact at temperature range >220 °C
Octanol-water partition coefficient (log K_{ow})	6.10
Vapour Pressure at 20°C (mmHg)	1.5×10^{-8}
Solubility in Water at 25°C ($mg.L^{-1}$)	5.50×10^{-3}
Toxicity, LD_{50} (ng/mg mosquito)	0.90 – 2.34

Table A-2: Physical and Chemical Properties of Bifenthrin

Bifenthrin Property	Quantity
Molecular Weight ($g.mol^{-1}$)	422.9
Relative density at 20°C ($g.cm^{-3}$)	1.42
Boiling Point and thermal stability	Decomposition starts at 168.3 °C before boiling
Octanol-water partition coefficient (log K_{ow})	6.40
Vapour Pressure at 20°C (mmHg)	1.8×10^{-7}
Solubility in Water at 25°C ($mg.L^{-1}$)	1.4×10^{-5}
Toxicity, LD_{50} (ng/mg mosquito)	0.13 – 0.19