The development and optimisation of a Large-Volume-Injection method on a GC-MS/MS instrument for selected organophosphate pesticide analysis in selected juice samples.

By

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Thesis submitted in fulfilment of the requirements for the degree

Master of Applied Science in Chemistry

in the Faculty of Applied Sciences

at the Cape Peninsula University of Technology

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Date submitted: November 2024

DECLARATION

I, **KATLEGO EFFECTUATE MAFURA**, declare that the contents of this dissertation/thesis represent my own unaided work, and that the dissertation/thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

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ABSTRACT

During agricultural production, organophosphate pesticides are used to control, destroy and prevent pests. However, excessive application of the chemical pesticides on fruits and vegetables has severe effects on the environment and poses harm to humans due to their high acute toxicity. Food safety is ensured by strict monitoring of residue levels, as organophosphate pesticides and carbamates inhibit the enzyme acetylcholinesterase (AChE) that results in accumulation of acetylcholine (ACh) at cholinergic receptor sites, leading to paralysis. Various standard methods including Liquid chromatography (LC) and Gas chromatography (GC) are used for the detection of multiple classes of pesticides in various sample matrices including juice samples. There are continuous efforts to improve trace analysis and sensitivity of current methods. This study investigated the applicability a large-volume injection method (LVI) for the determination of organophosphate pesticides in juice samples. The study aimed to evaluate the developed and optimised LVI in comparison to a currently used hot splitless injection method (HSI). Investigations were conducted to identify which method was most suitable.

Representative pesticides selected from the organophosphate class was malaoxon (MLXN), malathion (MLTN), chlorpyrifos (CPFS), bromophos-methyl (BRMP), bromophos-ethyl (BRPE), methidathion (MTDN) and profenofos (PRFF). These pesticides present in fruit juice samples were extracted using the QuEChERS extraction protocol and analysis performed using a Gas Chromatography Tandem Mass Spectrometry instrument (GC-MS/MS) instrument. Method development of LVI involved an investigation of various injection techniques, HSI, CSI and LVI at volume of 5, 10 and 25 µL. The injection method selected for further method optimization was the 5 µL LVI method. Parameters investigated for optimal conditions was vent flow, vent pressure, vent time, inlet and oven temperature. Additionally, comparison between the HSI and optimised 5 µL LVI method was conducted by assessing specificity, limit of detection (LOD), limit of quantification (LOQ), precision (repeatability) and trueness (recovery). Proficiency test samples from schemes such as the National Metrology Institute of South Africa (NMISA) and the Food Analysis Performance Assessment Scheme (FAPAS) were analysed for expanded insight.

The LVI method produced greater sensitivity, with larger peak areas and improved peak shapes. Chromatograms generated from the LVI analysis necessitated little to no manual integration during the analysis as better peak shapes and resolution was achieved. The evaluation of LVI and HSI for specificity, LOD, LOQ, repeatability, and trueness in apple and orange juice samples resulted in all pesticides meeting the acceptable criteria of

repeatability below 20% and trueness within the 70%-120% range. Both methods produced satisfactory z-scores from the analysis of proficiency test samples.

This study concluded by confirming the proposed hypothesis, that the LVI method would demonstrate enhanced analytical performance compared to the HSI method for the analysis of juice samples. This implied more accurate determinations of pesticides due to significant improvements in detection limits, sensitivity and recovery.

ACKNOWLEDGEMENTS

The assistance and effort given by the following persons and organisations made this study possible. I will forever show my appreciation. My husband, Dr Kgashane Leroy Malatji, for his continued encouragement to study further. My children, Amohelang and Kgosi, for their understanding and patience. Professor Vernon Somerset of Cape Peninsula University of Technology for the numerous years of mentorship and guidance throughout my career. Marissa Louw, my external supervisor, for being a friend and a great colleague in the field. I am extremely thankful for her significant inputs and willingness to always assist. Dr Meredith Kujawa for her guidance in the final stages of the project. The management of Hearshaw and Kinnes Analytical Laboratory (HKAL), Kevan Hearshaw (Technical Director) and Sue Kinnes (Managing Director), for their excellent guidance and enabling me to make use of the facility, instrumentation, and resources to conduct the study. The research assistance is also acknowledged and appreciated greatly. Opinions expressed in the study and conclusions drawn, are mine, the author, and not necessarily of the organisations.

DEDICATION

For my parents, Mr and Mrs Mafura and little brother, Phenyo

Joel 2:24 - 27

LIST OF CONFERENCE ATTENDANCES

1. Katlego Mafura Malatji, Marissa Louw and Vernon Somerset. (**2021**). Development and optimization of a GC-MS/MS protocol for analysis of selected organophosphorous pesticides in juice samples. Paper presented at the SETAC Europe 31st Annual Meeting. A virtual conference from 3-6 May 2021. [Poster presentation]

2. Katlego Mafura Malatji and Vernon Somerset. (**2023**). The development and optimization of a Large-Volume-Injection method on a GC-MS/MS instrument for selected organophosphate pesticide analysis in selected juice samples. 2023 Chromatography Postgraduate Student Workshop. 21 September 2023, PerkinElmer, Building 21, Thornhill Office Park, 94 Bekker Rd, Midrand, South Africa

3. Katlego Mafura Malatji, Vernon Somerset, Meredith Kujawa. (**2024**). The development of a Large-Volume-Injection method on a GC-MS/MS Instrument for selected organophosphate pesticide analysis in selected juice samples. Oral presentation at the Test and Measurement 2024 International Conference and Workshop. An in-person event from 16 – 18 September 2024, CSIR International Convention Centre, Pretoria, Gauteng, South Africa

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ABBREVIATIONS AND ACRONYMS

AChE	Acetylcholinesterases
AOAC	Association of Official Agricultural Chemist
AP	Apple
BRMP	Bromophos-methyl
BRPE	Bromophos-ethyl
Cal	Calibration
Calc.	Calculated
CEN	European Committee for Standardisation
COA	Certificate of Analysis
Conc.	Concentration
CPFS	Chlorpyrifos
d-SPE	Dispersive solid-phase extraction
EI	Electron Impact
EU	European Union
Exp. Conc.	Expected Concentration
FAPAS	Food Analysis Performance Assessment Scheme
FCHF	Fenchlorphos
GC	Gas Chromatography
HKAL	Hearshaw & Kinnes Analytical Laboratory
HPLC	High Performance Liquid Chromatography
HSI	Hot Splitless Injection
IEC	International Electrotechnical Commission
ISO	International Organization for Standardization
IUPAC	International Union of Pure Applied Chemistry
JU	Juice
L 1 - 10	Level 1 - 10
LLE	Liquid-liquid extraction

LOD	Limit of Detection
LOQ	Limit of Quantification
LVI	Large Volume Injection
MG	Mango
MLTN	Malathion
MLXN	Malaoxon
MM	Matrix-Matched
MMI	Multimode Inlet
MRLs	Maximum Residue Limits
MRM	Multiple Reaction Monitoring
MS	Mass spectrometry
MS	Mass spectrometry
MTDN	Methidathion
MU	Measurement of uncertainty
NMISA	National Metrology Institute of South Africa
NPD	Nitrogen Phosphorous Detector
OPs	Organophosphates
OR	Orange
PAU	Peak Area Unit
ppm	Parts per million (mg/L)
PRFF	Profenofos
psi	Pounds per square inch
psig	Pounds per square in gauge
PT	Proficiency test
PTV	Programmed Temperature Vaporization
QC	Quality control
QuEChERS	Quick, Easy, Cheap, Rugged & Safe
r.p.m	Revolutions per minute
R^2	Coefficient of determination
Resp.	Response
RL	Reporting Limit

RM	Reference materials
RR	Relative Response
RSD	Relative Standard Deviation
RT	Retention time
SANAS	South African National Accreditation System
SOP	Standard Operating Procedure
SP 1 - 6	Spike 1 - 6
SPE	Solid-phase extraction
STD	Standard
STDEV	Standard deviation
SVR	Solvent vent recovery
TIC	Total Ion Chromatogram

CHAPTER ONE
INTRODUCTION

1.1. Introduction

This chapter introduces the study presented in this thesis. The background of the study is given by defining pesticides and their various classes. The importance of monitoring levels of pesticide residue in different sample commodities to ensure food safety is stated. Problem statements, hypotheses, aims, objectives, and delimitations encountered in the study are also presented in the chapter. The chapter concludes by providing the layout of the thesis.

1.2. Background of the study

Pesticides are commonly defined as chemical substances, or a mixture of substances used in the agricultural industry to control or kill pests, weeds, rodents, fungi, and insects that jeopardize food production (Kaur, 2019). The application is not only limited to the agricultural industry, but pesticides are now used as household disinfectants to kill pests such as cockroaches, mosquitoes, rats, and other harmful bugs. Usage of any form of pesticide has adverse effects on the environment and human health. These chemicals can form toxic residues in food, air, soil and water during growth, harvesting and storage resulting in food safety problems (Jin, 2004).

There are numerous ways to classify pesticides depending on their use, toxicity, chemical composition, or mode of entry into the species they intend to destroy (Kaur, 2019). When chemical composition is used to classify pesticides, four (4) main groups are identified. The groups are the pyrethroids or pyrethrins, organochlorines, carbamates, and organophosphates (Kaur, 2019).

The toxicity of pesticides to the health of humans and their negative impact on the environment, has led to strict monitoring of residue levels in fruits and vegetables. These levels need to be accurately determined and below their set maximum residue limits (MRLs) to minimize exposure, especially for consumption (Pano-Farias et al., 2016).

1.2.1. Analysis of pesticide residues

Reliable analytical methods for accurate qualitative and quantitative determinations of pesticide residues have been developed and continue to be optimised due to the adverse effects they possess. Godula et al. (2001:24) indicated in their study that the gas

chromatographic technique is the popular technique to employ for the analysis of pesticide residues. Godula et al. (2001:24) based this assertion on the fact that conventional detectors were coupled to the gas chromatograph to provide sensitivity, selectivity, and separation efficiency during analysis. Considering this is an article written in the year 2001, liquid chromatography has since now emerged on top.

According to Fernandes et al. (2011:49), multi-class and multi residue methods are utilised by pesticide testing laboratories to analyse a variety of pesticides in a singular sample. These laboratories use validated methods to ensure quality results are produced. Accreditation is gained as a sign of quality compliance from accreditation bodies like the South African National Accreditation System (SANAS). The ISO/IEC 17025 is a global standard recognised by SANAS to assess the competence of official testing and calibration laboratories. ISO stands for International Organization for standardization and IEC is an acronym for International Electrotechnical Commission. The standard governs and provides strict guidelines for compliance. The study was conducted in an ISO/IEC 17025:2017 accredited laboratory.

1.2.2. Analytes of interest

The pesticide group of interest was the organophosphates. Feldman, S. (ed. et al. 2001) state that the synthesis of organophosphates was performed by reacting alcohol with phosphoric acid. This group of compounds can be used as chemical fertilizers to increase crop yields in the agricultural sector. Representative analytes chosen for the study were malaoxon (MLXN), malathion (MLTN), chlorpyrifos (CPFS), bromophos-methyl (BRMP), bromophos-ethyl (BRPE), methidathion (MTDN) and profenofos (PRFF). An internal standard method was used with fenchlorphos (FCHF) at the internal standard during analysis. These analytes are evaluated in apple (high-water content sample) and orange juice samples (high acid and high-water content sample) (Pihlström et al., 2021). The different sample matrices will provide an understanding of the behaviour of organophosphates.

1.2.3. Sample injection techniques

An in-depth understanding of two sample injection techniques that can be used during the analysis of organophosphates in apple and orange juice samples was obtained from this study. Highly reliable and accurate methods are required for the analysis of multiresidue pesticides. Therefore, continuous efforts are made to improve trace analysis and sensitivity of current methods. This study embarked on exploring the large-volume-injection (LVI) method as a possibility of achieving lower detection limits and improved

analyte recoveries. Therefore, a new method of LVI was developed, and its method parameters investigated for optimal conditions. The current hot splitless injection (HSI) method being used to analyse pesticides had shortcomings which included thermal degradation of unstable analytes. These analytes were 'breaking down' in the inlet due to high temperature settings required to vaporize the sample. Methods LVI and HSI were compared to determine which one of the sample injection techniques is most suited for the analysis of fruit juice samples.

According to Poole, (2012), sample introduced into the GC system by LVI can be accomplished when a programmable temperature vaporization (PTV) injector is utilized. Godula et al., (2001), states that this injector was introduced by Vogt et.al. in 1979. LVI allows for the injection of large sample volumes, up to several hundreds (µL). The use of LVI mitigates the discrimination of analytes during injection and better recoveries are achieved for thermally degradable analytes as sample is injected into the inlet set at a temperature below the boiling point of the solvent used.

1.3. Problem statement

Health risks and environmental degradation caused by inappropriate usage of pesticides can be minimized by strict monitoring of residue levels. To measure these levels, accurate analytical methods that are highly selective and sensitive must be applied (Grimalt & Dehouck, 2016:23). In this case, the applicability of an LVI method for the determination of organophosphate pesticides in juice samples is assessed against the current HSI method. Investigations were conducted in the study to determine which method is most suitable.

1.4. Hypothesis

The LVI method provides an improved analytical performance, compared to the HSI method for the analysis of organophosphate pesticides in apple and orange juice samples.

1.5. Aims

The aim of the study was to determine if there is a difference in the results obtained when an HSI or an LVI technique is used to inject fruit juice samples into the GC-MS/MS instrument.

Secondly, to determine whether the LVI technique is more sensitive to protect consumers from any possible dangers of organophosphate pesticides in juice samples.

1.6. Objectives

The objectives of the study were to:

i. Develop an LVI method by following an Agilent Technology tutorial approach.
ii. Optimise parameters of the developed LVI method.
iii. Determine the specificity, the limit of detection (LOD), the limit of quantification (LOQ), precision (repeatability) and trueness (recovery) for the different injection techniques.
iv. Analyse a quality control (QC) sample from a proficiency test (PT) scheme using HSI and LVI methods employed in this study to evaluate the z-scores.

1.7. Delimitation

The study was conducted in an ISO/IEC 17025:2017 accredited laboratory for pesticide testing. It is a mandatory requirement to participate in proficiency testing (ISO/IEC 17025 Clause 7.7.2a). The laboratory sets out a plan on which proficiency tests to partake in according to their scope. Upon finalisation of a PT sample, these samples may be treated like QC materials due to the known analyte concentrations. The limitation encountered was the lack of available PT samples that are now treated as QC samples which contained all the analytes investigated in this study. To overcome this, QC samples that contained at least one but not all the analytes were obtained.

1.8. Layout of the thesis

The thesis contains the following chapters:

Chapter 1 introduces the study by providing the background of pesticide residue analysis. The problem statement, hypothesis, aims, objectives, and delimitations of the study are included in the chapter.

Chapter 2 reviews published literature applicable to this study. Organophosphate pesticides are classified, and their toxicity is detailed in this chapter. The extraction method used to extract the analytes of interest from juice samples is defined. Also included in this chapter, is the analysis technique and instrumentation used.

Chapter 3 outlines the experimental design of the study. Herein, the chemicals, reagents and solvents used are listed. Sample protocol such as sample collection and treatment is included in the chapter. Additionally, a detailed description of the instrument and instrument conditions used, together with the analysis software is given.

Chapter 4 of the thesis discussed the overall results obtained during the study. The study is divided into 3 sections, the development of an LVI method, the optimisation of the developed LVI method, and the comparison of the newly designed and optimised method with the current HSI method. The observations made for each section are extensively discussed in this chapter.

Chapter 5 contains the conclusion of the thesis based on the investigations conducted and any prospects of future research work are stated.

Chapter 6 is a list of references employed during the study. The list is structured according to Harvard's style of referencing.

CHAPTER TWO
LITERATURE REVIEW

2.1. Introduction

This chapter embraces published literature related to the presented study. The review sets forth the origin, classification, and toxicity of organophosphates. The chapter then outlines previous work conducted on the analysis of juice samples. Lastly, the objectives of the study point to the crucial area of sample injection techniques. Therefore, a comprehensive review is included. A conclusion is made at the end of the chapter highlighting key aspects.

2.2. Organophosphates

2.2.1. The origin

A book edited by Feldman ed. et al. (2001) states that in the year 1669, phosphorus was the 13th chemical element to be discovered. This naturally occurring element has four oxygen atoms attached to it. It is further indicated that when alcohol reacts with phosphoric acid, one oxygen atom is replaced with a carbon atom, chemical groups attach to other oxygens, and organophosphates are synthesized.

2.2.2. The classification of organophosphates

An extensive article on the classification of pesticides published by Hassan & Nemr (2020), describe how the term 'pesticides' is an umbrella for insecticides, fungicides, rodenticides, household disinfectants, herbicides, and garden chemicals. A distinction is drawn between the classification of natural and chemical pesticides. Furthermore, chemical pesticides are grouped according to their sources. The groups are namely, organochlorine, organophosphate, pyrethroids and carbamates. Hassan & Nemr, (2020:207) also indicated that the organophosphates are compounds formed from esterification from phosphoric acid, this matched up with Feldman ed. et al.'s (2001) theory. This study focused mainly on the organophosphates.

Malaoxon (MLXN), malathion (MLTN), chlorpyrifos (CPFS), bromophos-methyl (BRMP), bromophos-ethyl (BRPE), methidathion (MTDN), and profenofos (PRFF) served as the investigated analytes for the purpose of the study. The chosen compound as an internal standard was fenchlorphos (FCHF). The chemical structures of the analytes are presented in Figures 1 to 8 below.

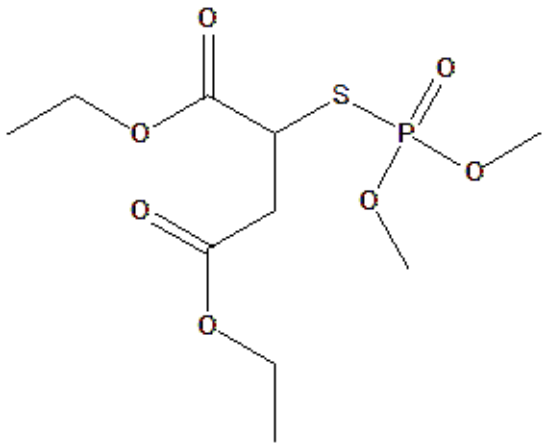

Figure 1: Chemical structure of MLXN.

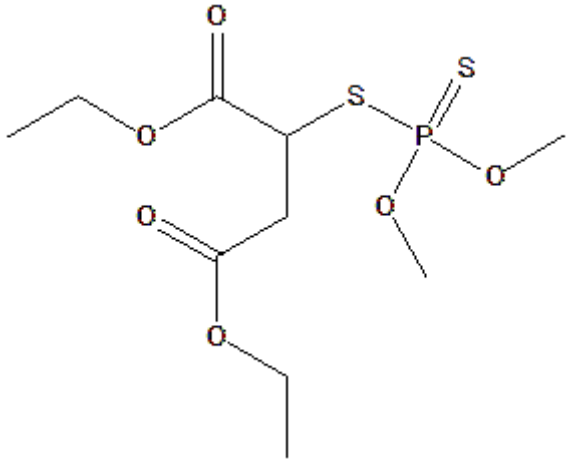

Figure 2: Chemical structure of MLTN.

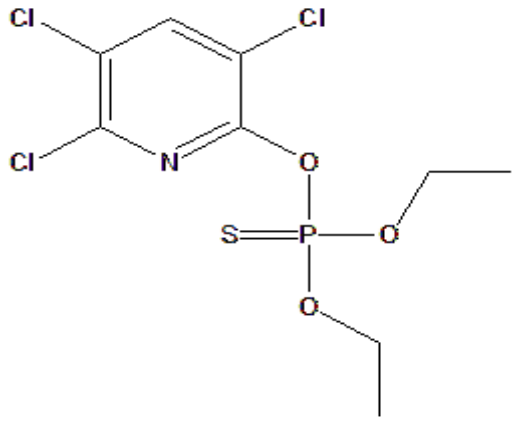

Figure 3: Chemical structure of CPFS.

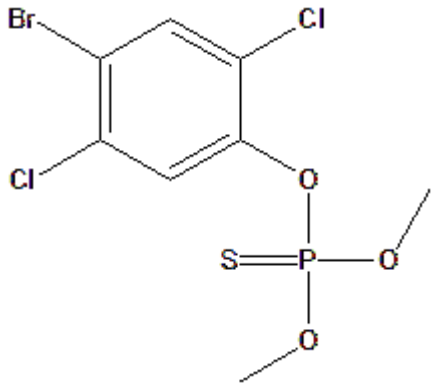

Figure 4: Chemical structure of BRMP.

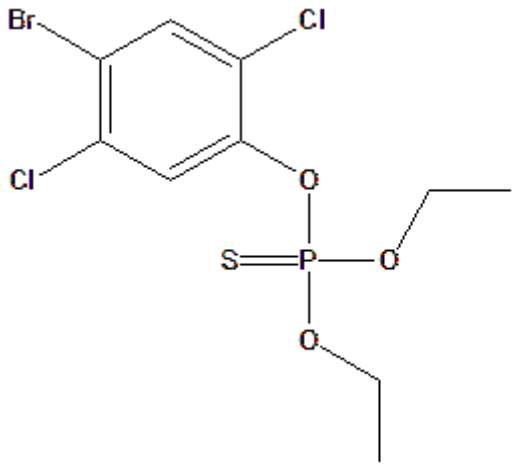

Figure 5: Chemical structure of BRPE.

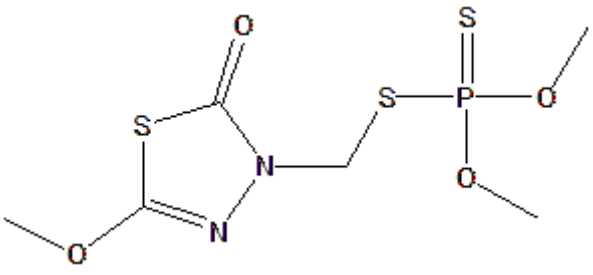

Figure 6: Chemical structure of MTDN.

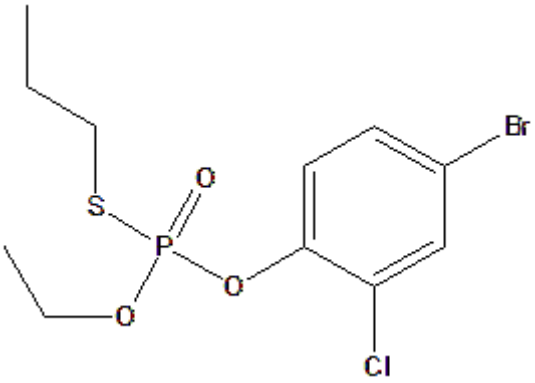

Figure 7: Chemical structure of PRFF.

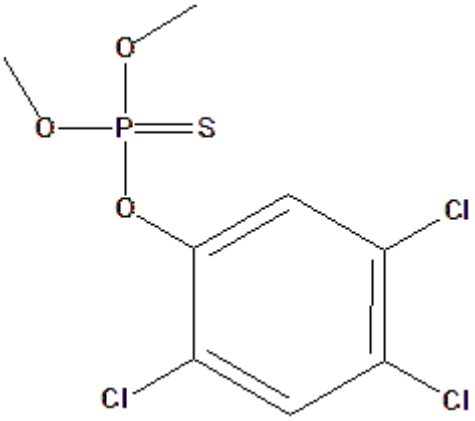

Figure 8: Chemical structure of FCHF (Internal standard)

2.2.3. Toxicity of organophosphates

Dwivedi et al. (2017) state that the rapid growth of the population worldwide has led to an increase in the demand for food. This has caused agricultural productivity to increase to meet the demand. The application of chemical pesticides during agricultural production is for plant protection achieved by destroying, preventing, and controlling pests and increasing production. These pesticides may be applied before or after harvest as growth regulators, defoliant and to ripen the fruit or vegetables, preventing deterioration during storage and transportation (Dwivedi et al., 2017).

Organophosphorus pesticides (OPs) possess hazardous and toxic properties that have been applied in the military as chemical warfare agents. Over the years, these compounds have also been used in the agricultural sector despite their toxicity. It describes how these highly stable esters of phosphoric acid compounds are sprayed

over crops or soil and their residues are found in surface and ground water, drinking water, fruits, and vegetables. These pesticides can last for years, or even decades before breaking down (Dwivedi et al., 2017). These insecticides are used to kill or disrupt the growth and development of insects (Kaur, 2019). OPs have a high acute toxicity to vertebrates and invertebrates by means of obstructing the acetyl cholinesterase (AChE), which participates in nerve-impulse transmission (Zhao, 2009). This causes rapid involuntary twitching of muscles which leads to paralysis and death (Zhao, 2009). Exposure to pesticide residues is through ingestion of contaminated foods that were directly treated or grown on contaminated fields (Dwivedi et al., 2017).

The severe effects of pesticides on the environment and potential harm to humans have led to strict monitoring of environmental pollutants, particularly pesticides due to their high acute toxicity to humans. Organophosphorus pesticides are frequently used for domestic purposes and widely applied in the agricultural industry, equally posing a danger to the ecosystem. These compounds are insecticides used to control insects and arthropod pests on crops of grain, nuts, cotton, and fruits during agricultural production. The residues of OPs have also been found to be responsible for aquatic life toxicity. Accurate detection of the presence of these pesticides in natural waters and foodstuffs is a priority for humans and the environment (Valdés-Ramírez et al., 2008; Kuswandi et al., 2008). The study focused on the presence of OPs in juice samples with their properties and effects evaluated.

2.3. Fruit juice samples

Fruit and vegetable juice drinks are now preferred because of their rich nutrition and positive health effects as compared to carbonated drinks (Meng et al., 2021). According to Velkoska-Markovska et al., (2016) apple juice is the first non-dairy product given to infants and a favourite in older children. Pesticides are applied during the mass production of fruits and vegetables from which comes juice beverages. It has become extremely important to monitor pesticide residue levels because improper use can occur. These include the use of different kinds of pesticides, illegal use of prohibited and restricted pesticides and excessive pesticide levels that can occur which pose a danger to consumers (Meng et al., 2021).

Pesticide residue testing is a necessity for the canning industry, pack-houses, and private farming individuals and for tea and dried fruit producers. These industries all play an integral part in the food supply chain. Therefore, to monitor pesticide residue levels,

highly reliable standard methods should be developed to ensure food safety (Velkoska-Markovska et al., 2016).

The beneficial impact of using pesticides to repel pests that infest various fruits includes an increase in production volumes, shelf-life is extended, production can be conducted in new geographical areas, and improves the overall appearance of the fruit. Despite these positive effects, public health is a great concern. Maximum residue limits (MRLs) in a wide range of commodities have been set for assessing the intake of pesticide residues in foodstuffs. These include foodstuffs of animal origin, fruits, vegetables, and other plant products (Fernandes et al., 2011).

Pesticide testing laboratories monitor residue levels by using standard methods that can detect multiple classes of pesticides and numerous residues in a single sample. The sample can be of any type, which includes fruit juices (Fernandes et al., 2011). A guideline document (SANTE 11312:2021) stipulating the requirements of method validation and analytical quality control for pesticide residue testing laboratories across the EU is published. The validity of the data sent to the EU is assessed for compliance with MRLs. One of the objectives of the document is to support compliance and implementation of ISO/IEC 17025 accreditation (SANTE 11312:2021).

In the presented study, apple and orange juice samples were samples of interest. Detailed in the (SANTE 11312:2021) document are commodity groups and their representative commodities. Apples are classified as high-water content samples and oranges are representative samples of high acid and high-water content commodity (SANTE 11312:2021). The analysis of OPs in fruit juice samples, the analytes present in the sample, need to be extracted effectively.

2.4. Extraction Method

The objective of an extraction method is to obtain appropriate recovery of analytes contained in different sample matrices. Analytical chemists have invested their efforts into developing eco-friendly extraction methods. The aim is to have a cost-effective, quick, simple, and with minimum use of organic solvents and reagents, helping in the reduction of environmental pollution (Santana-Mayor, 2019). Pesticides may be extracted by means of liquid-liquid extraction (LLE), solid-phase extraction (SPE), and dispersive solid-phase extraction (d-SPE) (Dwivedi et al., 2017).

One of the ancient methods still used, with slight modifications, is the Luke method developed in 1981 for the analysis of pesticide residues in fruits and vegetables (Pihlstrom, 2007). The Luke method made use of acetone and methylene chloride mixture for the extraction of pesticides from the food matrix (Pihlstrom, 2007). However, other organic solvents were investigated due to the low recoveries presented by the Luke method. This led to the development of other extraction methods.

In 2003, the QuEChERS extraction method was introduced by Anastassiades, Lehotay, Stajnbeher, and Schenck (Santana-Mayor, 2019). QuEChERS stands for *quick, easy, cheap, effective, rugged, and safe* (Santana-Mayor, 2019). The original QuEChERS technique became popular because it involved only two (2) steps; the partitioning (salting-out process) and clean-up by using sorbents in solid-phase extraction cartridges.

Santana-Mayor et al. (2019) have pointed out factors that influence extraction efficiency such as the solvent and amount thereof used during extraction, the composition of the sample (pH and matrix), comminution, sample size, temperature, extraction time, the agitation mode used, the addition of salts together with the amount and type of sorbent used for clean-up. All these factors were investigated and optimised conditions were applied to maximize the recovery of analytes.

The influence of pH on analyte degradation led to the adoption of two official buffered versions of the method. Lehotay developed the AOAC (*Association of Official Agricultural Chemists*) Official Method 2007.01, whereby an acetate buffer is used (Santana-Mayor, 2019). Whereas Anastassiades developed the CEN (*European Committee for Standardisation*) Standard Method EN15662 and this version makes use of citrate buffer to adjust pH (Santana-Mayor, 2019).

In the presented study, a QuEChERS extraction methodology was used for the extraction of pesticides from juice samples. According to (Dwivedi et al., 2017), the method presents important advantages such as yielding high recovery rates for a wide range of analytes compared to traditional extraction methods. It is further stated that acetonitrile is the chosen solvent due to its property of a large solvent expansion volume and its compatibility with chromatographic application (Dwivedi et al., 2017). An additional advantage of the QuEChERS method is the provision of extracting about 20 samples within 30 – 40 minutes by one analyst. In support of the presented study, fruit juices and vegetables were also analysed for the determination of 229 pesticides using the QuEChERS method by Lehotay for validation experiments in 2005 (Dwivedi et al., 2017).

After extraction of the pesticides, a gas chromatographic technique may be applied for analysis.

2.5. Analysis by Gas Chromatography

Chromatography is defined according to McNair et al., (2009) as a method of separation in which components of a sample partition between two phases, the mobile and stationary phase. A vaporized sample is transported through the stationary phase of a large surface area (column) by the mobile phase. Components of the sample equilibrate with the liquid stationary phase based on solubility at a given temperature. The different components of the sample separate from each other based on their relative vapour pressure and affinities with the stationary phase causing them to elute at different times. McNair et al. (2009) further state that the naming of a chromatographic process is based on the physical state of the mobile phase. Therefore, gas chromatography is based on the mobile phase being gaseous, and liquid chromatography is whereby the mobile phase is liquid. The presented study is based on gas chromatography (GC).

According to Poole (2012), many practitioners have considered Archer Martin as the first person to have presented the idea of gas chromatography during his noble-prize-winning speech for his work in liquid/solid chromatography, making suggestions that a vapour phase can be used as a mobile phase. In 1952, Martin and Millington, used ethyl acetate to desorb fatty acid mixture to an adsorbent placed in a tube. The vapour stream elutes from the tube connected to a titration apparatus. Each eluted acid is neutralised by sequential addition of the base and the steps are presented by a graph obtained by automated titration (Poole, 2012).

Poole (2012), further state that an article published by Leslie Ettre in 2008, suggests the idea of gas chromatography was being considered in 1940 during the Second World War constructed the first prototype of gas chromatography, but published their results in 1951 (Poole, 2012).

The new analytical technique was explored by many petroleum companies in 1953 and a year later, the first crude chromatograph was built. The chromatograph constituted thermal conductivity cells on a Wheatstone bridge connected to a strip chart recorder and a Gaussian peak was observed for every eluting solute (Poole, 2012). The technique has developed over the years and has become a widely used technique. A typical gas chromatographic system is shown in Figure 9. It consists of carrier gas contained in a

cylinder, a flow controller, a sample inlet and sampling device (injection port), a controlled temperature zone (oven), a column, a detector, and a data system (Poole, 2012).

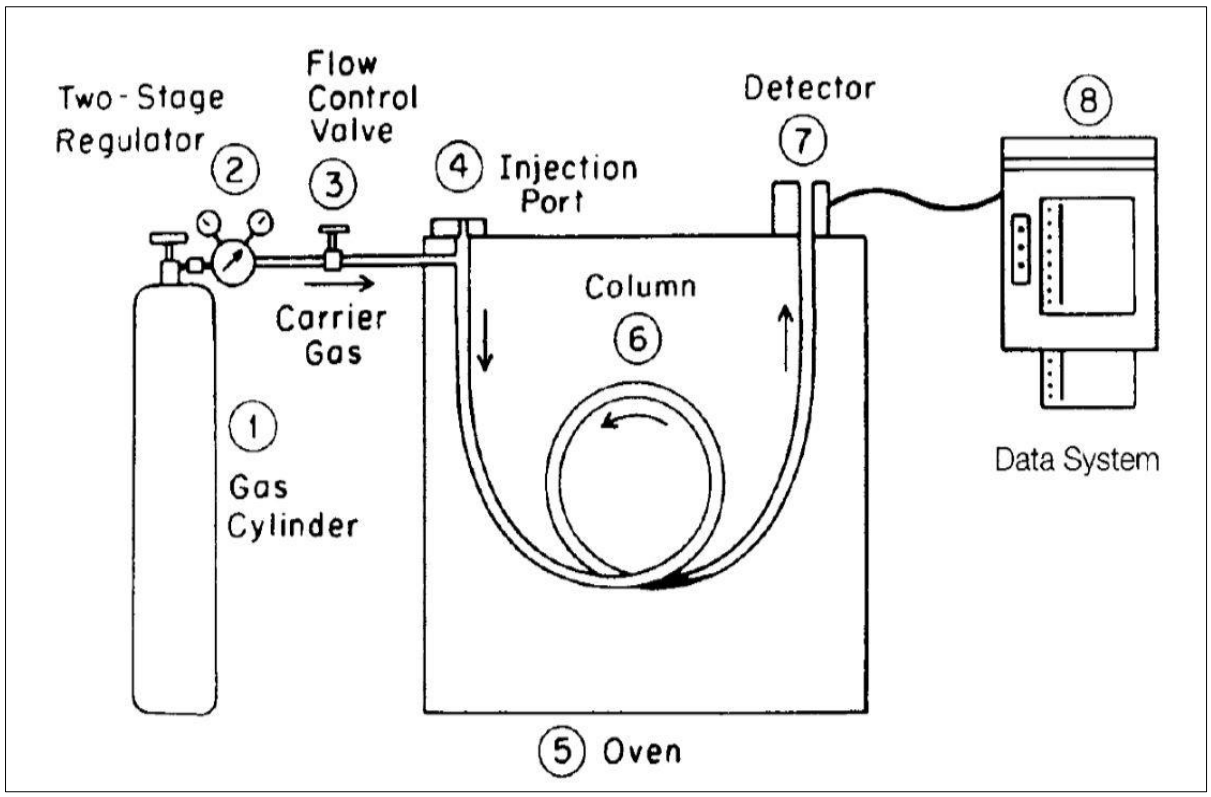

Figure 9: Schematic diagram of gas chromatography (Poole, 2019).

The GC process is initiated when a micro-syringe is used to inject a sample into a hot injection port. The sample gets vaporised and carried by the inert or unreactive gas (helium or nitrogen), which continuously flows from the cylinder through the injection port, the column, and to the detector carrying components of the sample. The flow controller is used to control the rate of the carrier gas for reproducible retention times of the components and decrease detector noise. The mobile phase (carrier gas containing the sample) enters the column which is a glass or metal tubing coated with the stationary phase (Poole, 2012). The stationary phase is a microscopic layer of high-boiling liquid or polymer coated on an inert solid support (Hussain et al., 2014). The sample components partition between the mobile and stationary phases due to their relative vapour pressures and solubility in the liquid phase (Poole, 2012). Intermolecular bonds cause the components to be retained at different times in the column and cause different elution times. The function of the detector is to measure the quantity of the components by generating an electric signal which gets sent to the data system where a peak is observed (Poole, 2012). A chromatogram is the output of the GC, showing peaks of the eluted compounds.

A graphical representation of the chromatographic process is illustrated in Figure 10. The column is represented by the horizontal lines increasing time from top to bottom during the process. A sample consisting of components A and B is injected into the column and carried through by the mobile phase. Each component partitions between the mobile phase and the stationary phase illustrated by the distribution of the peaks above and below the line. Component A can be noted to have a greater distribution in the mobile phase therefore eluting earlier than component B. The components are detected at different times when passing through the detector. The detector gives an output signal of the chromatogram.

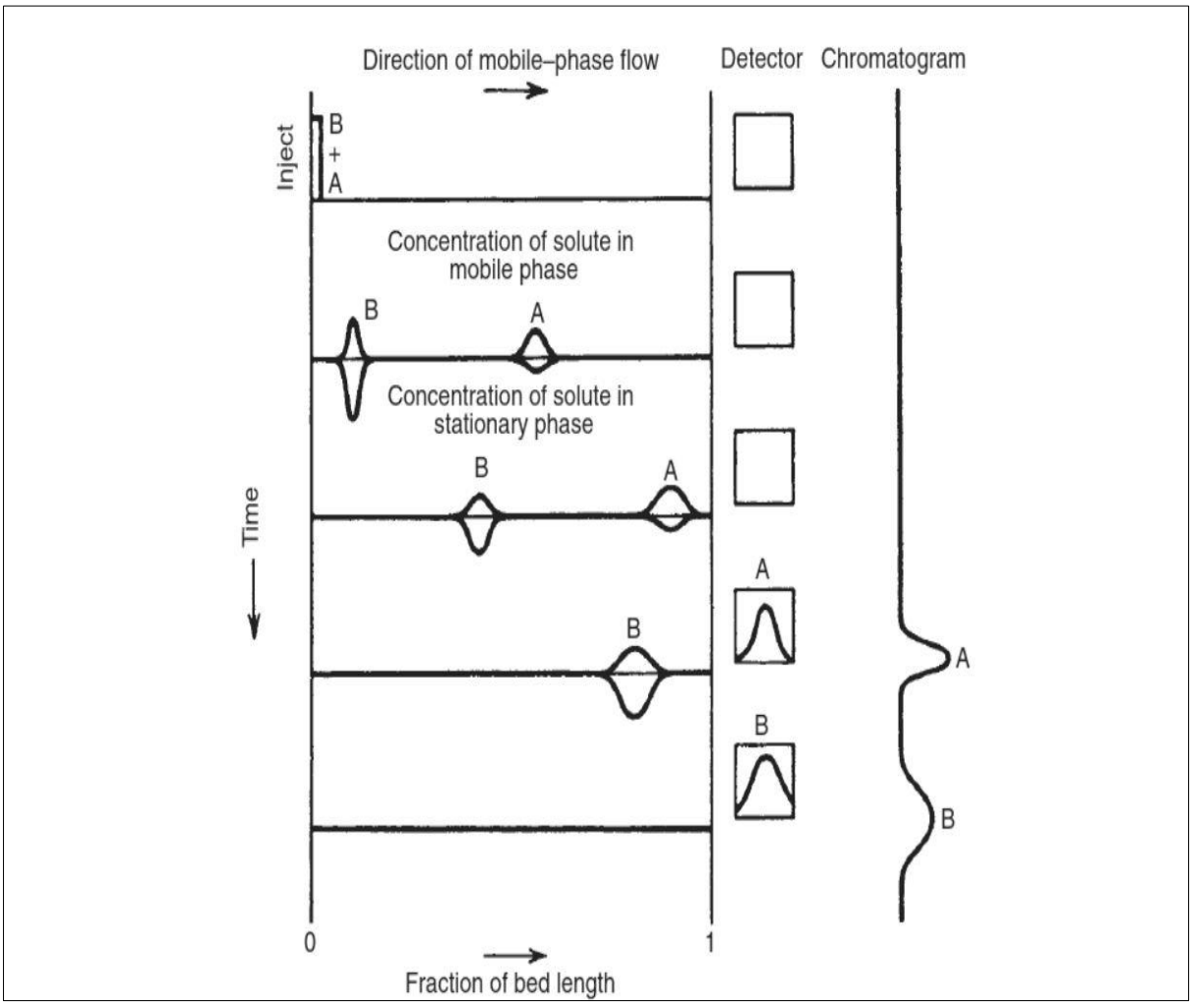

Figure 10: A graphical representation of the chromatographic process (McNair et. al., 2009).

2.5.1. Mass spectrometer (MS/MS) detector

There have been tremendous advancements and improvements since the inception of the GC. The widespread application of the GC in industries such as food, fragrance, petroleum, flavour, chemical, environmental, medical, and biological, has proven to be

an important tool in the isolation and separation of chemical components contained in complex matrices. One such advancement is the use of mass spectrometry as a detector from element selective detectors (Ferrer et. al., 2013).

A triple quadrupole mass spectrometer is coupled to the GC system to function as a detector. The use of MS as a detector has become popular over the years. When molecules leave the column, entering the MS which is equipped with an electron impact (EI) source, they are bombarded with high-energy electrons. This process is called ionization which must be carried out under low pressures. (Taylor, 2015).

The mass spectrometer has three quadrupoles aligned in series. The first and the last quadrupoles are used to filter out selected masses. One quadrupole consists of four (4) rods that filter ions fragmented from the molecular ion according to different mass-to-charge ratios. Using a multiple reaction monitoring (MRM) mode to quantify compounds, specified precursor ions will pass through the first quadrupole while others are restricted. These precursor ions will then collide with a collision gas, producing product ions in the second quadrupole. These product ions are then accelerated to the third quadrupole where only a specified mass-to-charge ratio will be detected. (Van Bramer, 1998). Thereafter, a detailed spectrum is obtained with structural information of a compound.

2.6. Conclusion

The chapter was structured according to the key elements of the study and the applicable literature was reviewed. It started with the origin of the organophosphate pesticide class and what constitutes their classification. The chemical structures of the representative analytes were presented in the chapter. Additionally, in-depth knowledge of the toxicity of these pesticides was provided. Various published literature on juice samples and ways of efficiently extracting pesticides from these sample commodities was highlighted. The chapter concluded by emphasising the GC-MS/MS technique for analysis of the pesticides. A clear description of the chromatographic process is also included in the chapter.

CHAPTER THREE
EXPERIMENTAL DESIGN

3.1. Introduction

The research was conducted in a laboratory accredited with the IEC/ISO 17025 standard. The laboratory performs pesticide residue testing on a variety of foodstuffs. Documents such as the (SANTE 11312:2021) document stipulate guidelines for analytical quality control and method validation criteria for the analysis of pesticide residues in food. The IEC/ISO 17025 standard combined with (SANTE 11312:2021) guidelines were considered during the research. This chapter outlines the methodology used to meet the 4 objectives stated in section 1.6 of the thesis. All the analytical standards, chemicals, the treatment of the samples, and instrumentation used for analysis are contained in this chapter.

Method development and optimization studies required the preparation of spiked samples from orange and apple juice. The evaluation and comparison of HSI and LVI methods, preparation of spiked samples at different levels (0.01 and 0.1 mg/L), calibration, and matrix-matched standards were required. A flow diagram is provided (Figure 11) to give a summary of the experimental work performed from the preparation of stock solutions to a spiking solution containing all the pesticides. The overall workflow of the thesis is illustrated in Figure 12 below and demonstrates where these solutions and spiked samples played a role.

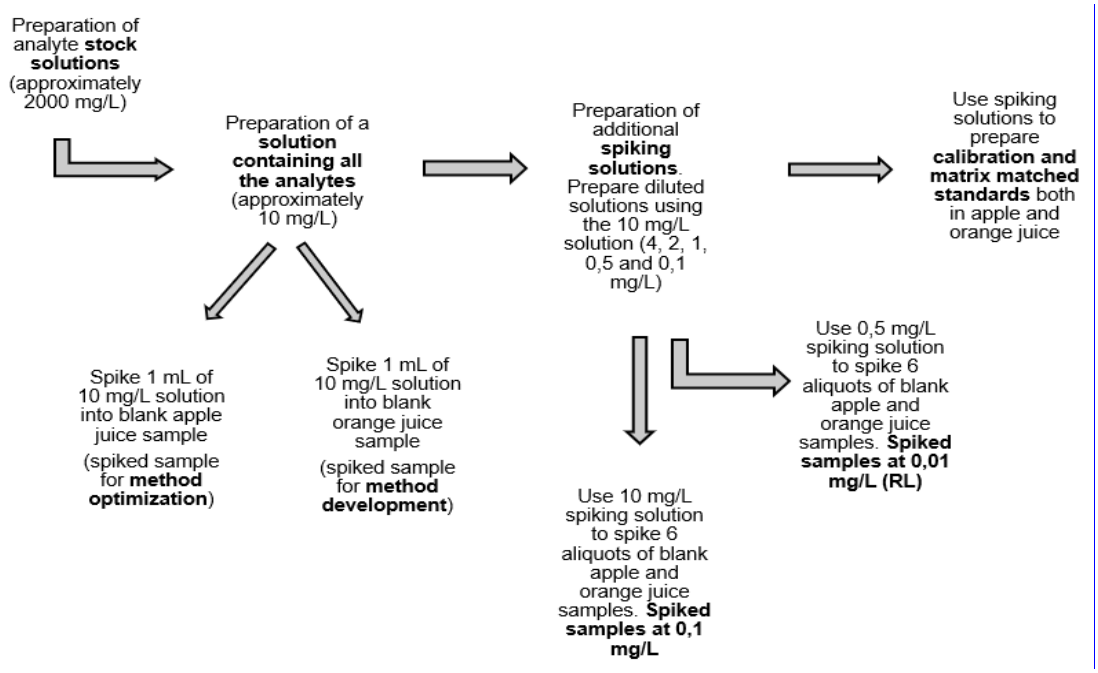

Figure 11: Flow diagram of the experimental work performed for preparation of stock solutions, spiking solutions, calibration/matrix matched standards and spiked samples.

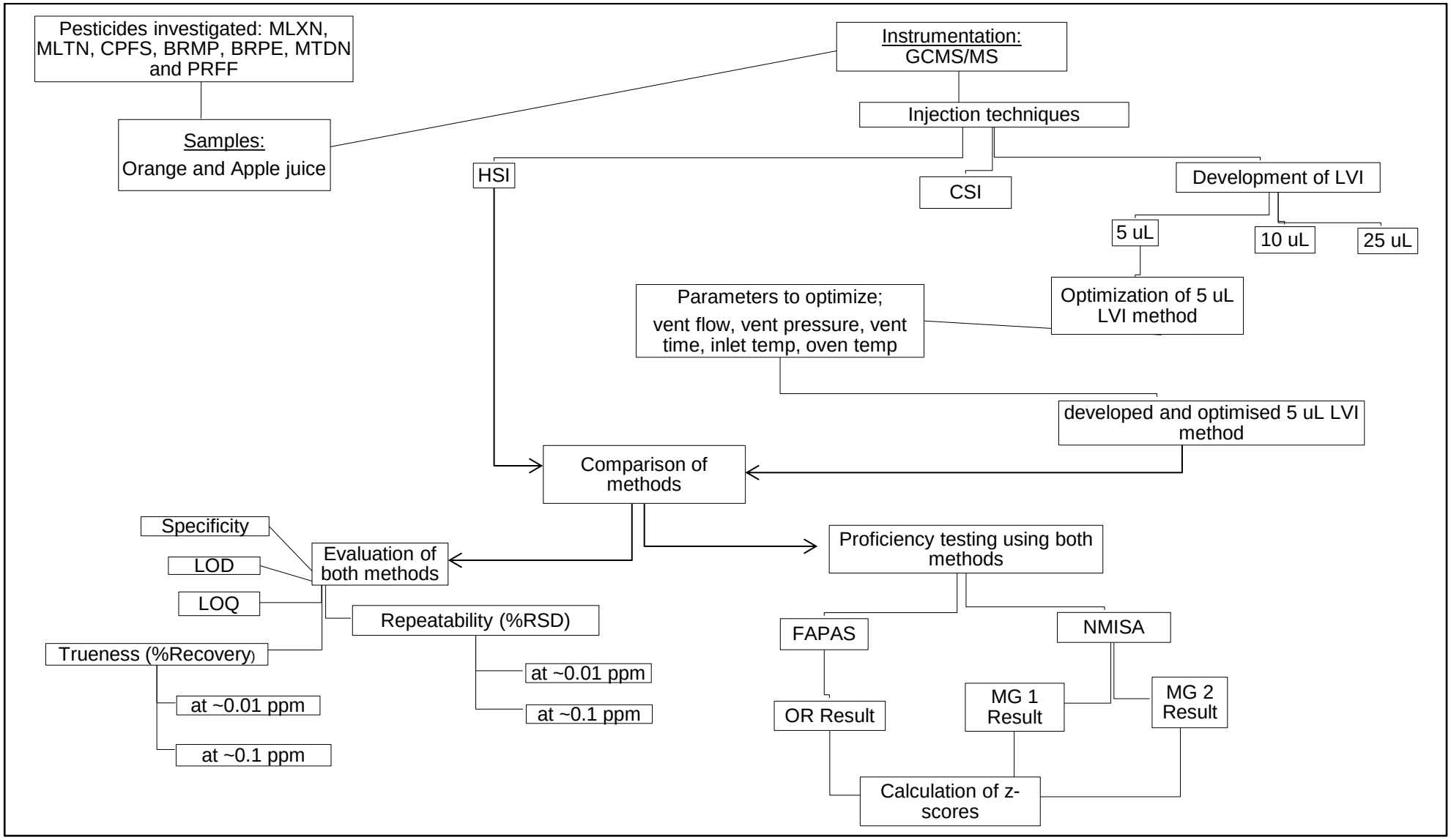

Figure 12: Schematic diagram illustrating the workflow of the thesis for chapter 3 and 4.

3.2. Analytical standards

Certified reference materials (RM) of bromophos- ethyl, chlorpyrifos and fenchlorphos (internal standard) were purchased from Sigma Aldrich (St Louis, MO, USA). Bromophos-methyl, malaoxon, malathion, methidathion and Profenofos were purchased from EhrenstorferTM (Germany). Certificate of analysis (COA) of the pesticides can be found in Appendix A. COA of pesticides are laboratory documents obtained when pesticides are purchased detailing information such as the expiry date of the reference material, required storage conditions, purity, and batch/lot number.

Approximately 2000 mg/L of individual stock solutions were prepared in acetonitrile solution which contains 0.1% formic acid. Pesticide stock solutions served as the source from which diluted solutions (working solutions) can be prepared. Stock solutions and working solutions were prepared using a laboratory-verified pipette. The verification certificate is appended in Appendix B of the thesis. These stock solutions were stored in a freezer set at -18 ºC. Theoretical concentrations of the stock solutions are calculated in Appendix C of the thesis. The purity of the reference material, mass weighed, and volume of the solution prepared are considered when the theoretical concentration of each pesticide was calculated (see Appendix C – Table 31).

A single composite mixture of 10 mg/L was prepared using the stock solutions. The solution served as a working solution with the highest concentration. The 10 mg/L solution was used to spike into blank apple and orange juice samples for purposes of method development and optimisation studies. Additionally, the 10 mg/L solution was used in the preparation of procedural standards and matrix matched standards required for the evaluation of the methods. Other working solutions of approximately 4, 2, 1, 0.5, and 0.1 mg/L were subsequently prepared from the 10 mg/L solution. These working solutions are also used to prepare additional concentration levels of procedural standards and matrix matched standards. Theoretical concentrations of all the pesticides contained in working solutions can be found in Appendix D.

All working solutions were spiked into blank apple and orange juice samples and the QuEChERS extraction method employed to extract the pesticides, preparing the samples for chromatographic analysis.

3.3. Chemicals and reagents

Acetonitrile (ACS/HPLC grade), and toluene were purchased from Honeywell (Muskegon, MI, USA). Formic acid used was purchased from Merck (South Africa).

QuEChERS salts containing magnesium sulphate, sodium chloride, sodium citrate tribasic dihydrate and sodium citrate dibasic sesquihydrate were used during the extraction of samples.

3.4. Sample treatment

3.4.1. Sample collection

The development and optimization of the LVI method required the collection of approximately 2 litres of apple (AP) and orange (OR) juice samples. These were purchased from Organic Zone Fruit & Vegetable, a local consumer store located at 1 Main Road, Lakeside in Cape Town. Upon arrival at the laboratory, the samples were tested as routine samples to ensure the pesticides of interest were not present in the samples.

Samples received by the laboratory from proficiency test schemes (FAPAS orange juice and NMISA mango samples) may be treated as QC materials once finalised. This is because of the known analyte concentrations contained in the samples. Upon arrival at the laboratory, these homogeneous and stable test samples are weighed out in aliquots of 10 g and stored according to specifications detailed by PT schemes. In the study, these samples were used for comparison purposes of the methods.

3.4.2. Sample preparation

All the samples were prepared for analysis on the GCMS/MS using the QuEChERS extraction method. Figure 13 below is a schematic diagram of a modified QuEChERS method employed in the study. Approximately 10 g of juice sample was weighed into a dry and clean 50 mL centrifuge tube and 10 mL of acetonitrile containing FCHF (internal standard). The tube was securely capped and inserted into the mixer to shake for 1 minute. A QuEChERS sachet containing 4 g of magnesium sulfate, 1 g sodium chloride, 1 g sodium citrate tribasic dehydrate and 0.5 g sodium citrate dibasic sesquihydrate was emptied into the tube. The tube was again capped and placed into the mixer to shake for another minute.

The sample was centrifuged at 3000 r.p.m. for 5 minutes to form an organic extract. Approximately 1 mL of the supernatant was transferred into an auto-sampler vial containing 500 µL of toluene. The sample was evaporated on a sample concentrator for 30 minutes. Thereafter, 800 µL of toluene was added. The vial was then capped, and sample analysed on a GC-MS/MS.

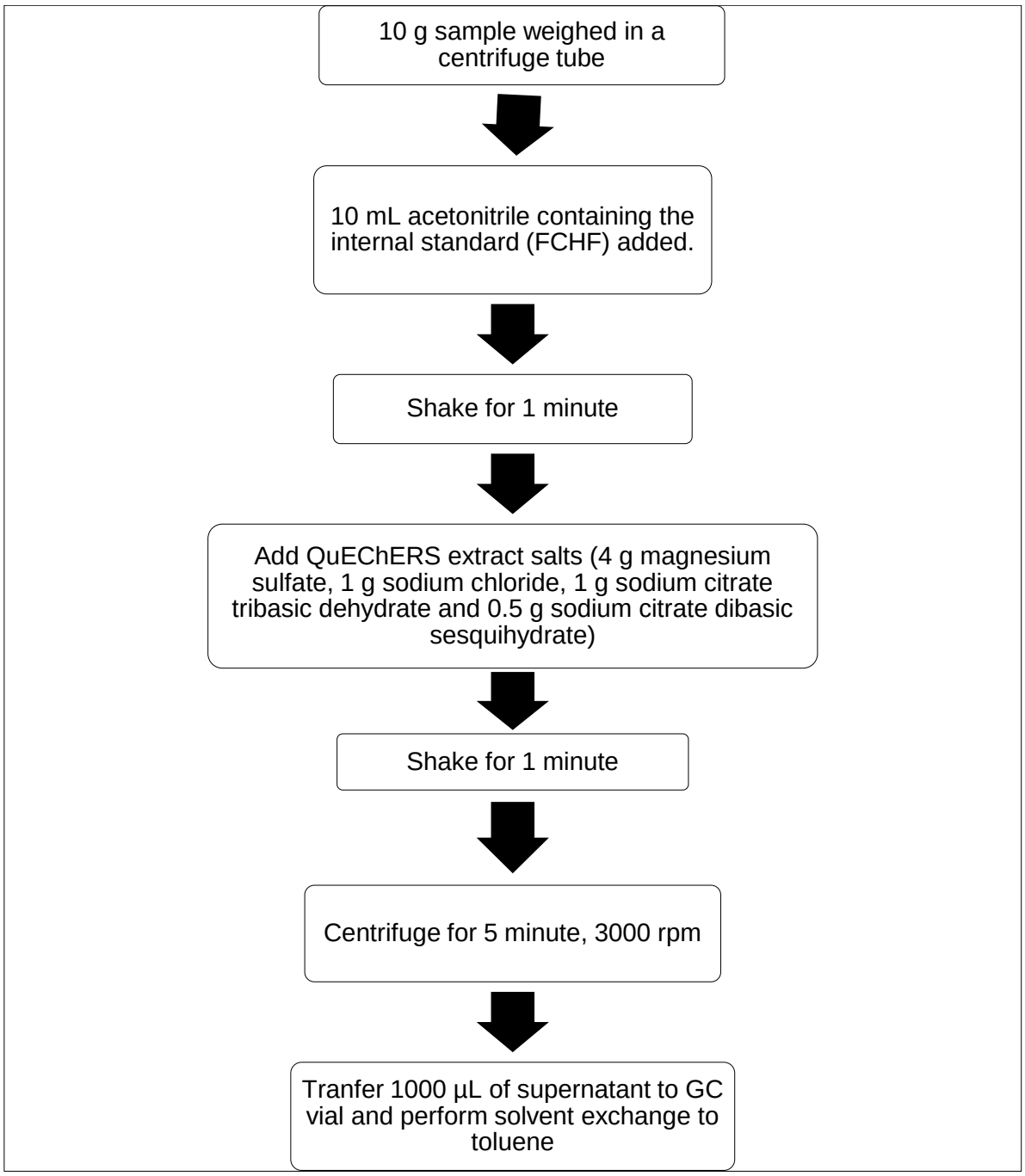

Figure 13: A schematic diagram of a modified QuEChERS extraction method for samples.

3.5. Method development of LVI

One objective of the study is the development of an LVI method. A tutorial published by Agilent Technologies (2009) aimed to help researchers use an LVI technique to improve trace analysis and sensitivity of their current methods. The detailed steps in the tutorial provide an overview of the migration from the HSI method to the CSI and then ultimately the LVI method.

According to the tutorial, the development process may be followed using a checkout sample provided by Agilent or a sample using an existing HSI method. In this study, an artificial sample was made by spiking with a known concentration of organophosphate pesticides. The artificial sample was prepared using a blank orange juice (OR JU) sample. A 10 g sample was weighed and spiked with a mixture of pesticide solution at approximately 1 ppm for purposes of method development studies. The sample was extracted using the modified QuEChERS extraction method illustrated in Figure 13. Triplicate injections of this sample using the different sample injection techniques (HSI, CSI and LVI) were performed.

3.5.1. Sample Injection techniques

A pivotal point in this study is the way a sample is introduced into the gas chromatographic system. There are various techniques that can be used to inject a sample such as the split/splitless injection, cold and hot on-column injection, vaporizing split/splitless injection, programmed split/splitless injection, and LVI (Poole, 2019). The study investigated the HSI, CSI and LVI technique. The focus was mainly on the HSI and LVI methods which consider the aims of the study. Investigation regarding the CSI was merely to illustrate the difference in the injection techniques.

3.5.1.1. Hot-Splitless Injection

According to Poole (2012), splitless injection is the most popular and robust technique to employ when analysing samples. In splitless injection, the sample is injected into a liner which is heated by the surrounding heating block and kept at an isothermal temperature. The sample gets vaporized and enters the column. Unlike the split injection where the split valve is opened to allow excess carrier gas to escape, here the valve is kept closed to allow the whole sample with the carrier gas to enter the column. Poole (2012) describes that after the injection, the split valve is opened to allow sample residue to be flushed out. The single design of a split/splitless injector is shown in Figure 14.

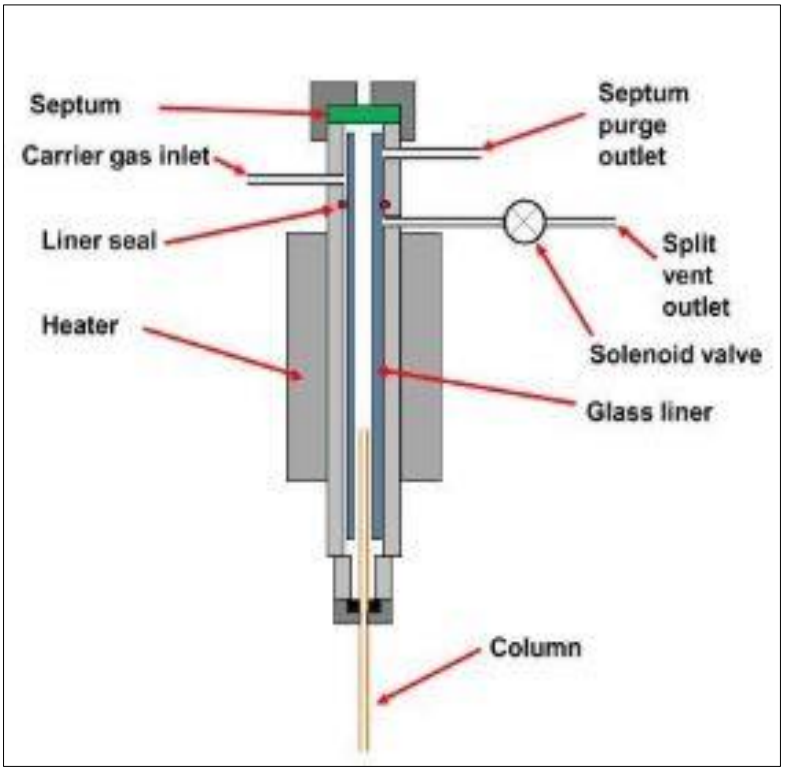

Figure 14: A split/splitless injector diagram of the instrument (Poole, 2012).

Method parameters of the HSI method used are tabulated in Table 1. The method was used to analyse the spiked sample described in section 3.5 of the thesis by performing triplicate injections.

Table 1: Parameters of the HSI method.

Mode	Splitless
Inlet temperature	250 ºC
Final temperature	280 ºC
Injection volume	1 µL

3.5.1.2. Cold splitless injection (CSI)

The CSI method was investigated merely for the purpose of showing the transition from the HSI to the LVI method. In the CSI technique, no vaporization of the sample takes place during sample injection because the temperature is set 5-10 ˚C below the boiling point of the solvent. The boiling point of toluene is 110.6 ˚C, therefore 100 ˚C was a suitable temperature setting. The CSI method was created by lowering the injection port temperature of the HSI. The temperature of the inlet was decreased from 250 ˚C to 100 ˚C and held for 0.1 min with a ramp of 720 ˚C/min to 250 ˚C. Triplicate injections of the spiked sample were then made using the CSI method with method parameters stipulated in Table 2.

Table 2: Parameters of the CSI method.

Mode	Splitless
Inlet temperature	100 ºC
Hold time	0.1 min
Final temperature	280 ºC
Injection volume	1 µL

3.5.1.3. Large volume injection (LVI)

A programmable temperature vaporizing (PTV) injector allows for large-volume injections. The PTV injector has the convenience of performing various injection techniques including split/splitless injections. The LVI technique combines the split and splitless techniques operated under different temperatures for the injection of a single sample (Poole, 2012). The cooling of the liner is mainly due to the cooling fan fitted in the injector as shown in Figure 15. Hoh et. al. (2008) describes the PTV injector as having a temperature control function that enables rapid cooling and heating during injections.

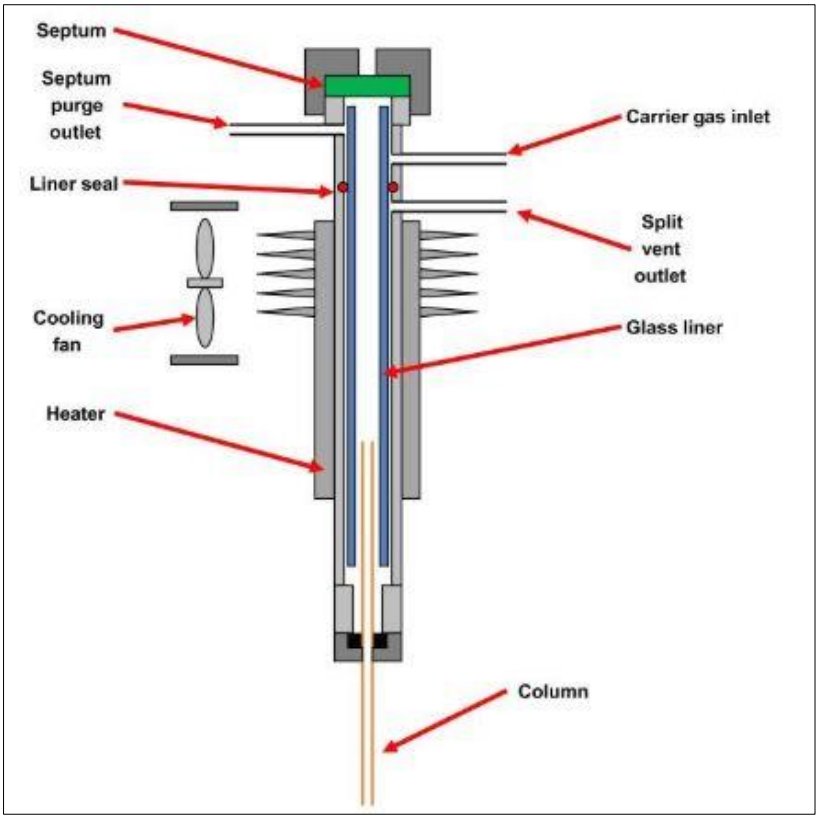

Figure 15: A PTV injector diagram of the instrument (Poole, 2012).

The principle is that a large volume of sample, normally 5 - 100 µL, is injected into a liner which is positioned in low-temperature conditions with a carrier gas flow rate set at 100

$mL.min^{-1}$. The split vent is kept open for some time to allow the solvent to be vaporized and to escape through the split vent. After the solvent vent process, the split vent is closed, and less volatile components are retained in the liner. The injection port temperature is increased to allow the whole sample residue to be vaporized and to enter the column as in the case with the splitless injection (Poole, 2012).

The GC-MS/MS instrument used in this study is equipped with a PTV injector (multimode inlet) which can be operated in solvent vent mode. This enables the possibility of a LVI technique. The tutorial used provides guidance for method development to find standard instrument parameters for large-volume injections. For the instrument to do LVI, the splitless inlet mode parameter used in the CSI method (Table 2) had to be changed to a solvent vent mode. The switch from splitless to solvent vent initiates a software-embedded calculator for the determination of solvent elimination rate. The calculator provides reasonable starting parameters for the LVI method.

The term ‘solvent elimination rate’ is derived from the principle of large-volume injections because solvent is vented out during injection of the sample. Initially, the inlet is set in split mode set at a low pressure, gas flows through the inlet liner and out the vent valve at a specific rate. During sample injection, gas flow is continuous while simultaneously venting out the solvent at a similar rate (Agilent Technologies, 2009). There are factors influencing the solvent elimination rate. Firstly, small changes in inlet temperature cause a significant impact on the rate solvent is eliminated. Solvent elimination rate increases when inlet temperature is increased, and vice versa. The mechanism can be explained by principles of kinetic molecular theory. The kinetic energy of molecules is proportional to temperature. As temperature of a gas increases, particles move more rapidly. A decrease in temperature will cause particles to slow down. Brown et al. (2009:591) states the relation between temperature and rate is that faster rates are observed at higher temperatures due to the increase in the rate constant with increasing temperature. Secondly, changes in vent flow will produce a corresponding change in solvent elimination rate. Thirdly, vent pressure and solvent elimination rate are inversely proportional to each other. Lastly, the boiling point of the solvent used significantly impacts the rate of solvent elimination, as solvents with lower boiling points evaporate more quickly, therefore affecting the elimination rate (Agilent Technologies, 2009).

The wizard needed information such as the solvent to be used, the desired injection volume, and the boiling point of the first eluting analyte as a start (Figure 16). Toluene as the solvent and 5 µL injection volume was appended. The tutorial stipulated a choice could be made between choosing a temperature closest to the boiling point of the first

eluting analyte or keeping the temperature at 150 °C to widen the scope of the analytes to be tested using the method. The first eluting analyte among the pesticides investigated was MLXN that has a boiling point of 114 °C, and the chosen temperature was 150 °C.

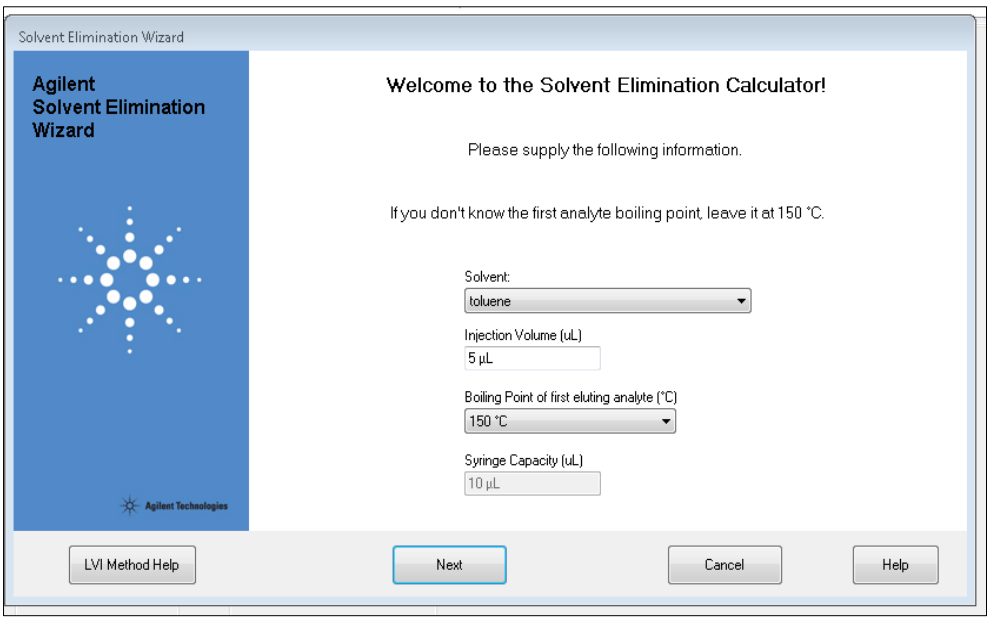

Figure 16: The software makes provision for method development studies with the solvent elimination calculator. The solvent to be used, desired injection volume and boiling point of the first eluting analyte entered.

Next, the wizard provided calculated values for elimination rate (µL/min), suggested injection rate (µL/min), and suggested vent time (min). Figure 17 below presents values obtained for the 5 µL injection volume. Manual changes could be applied to values provided for inlet temperature, vent flow, injection volume, vent pressure and outlet pressure.

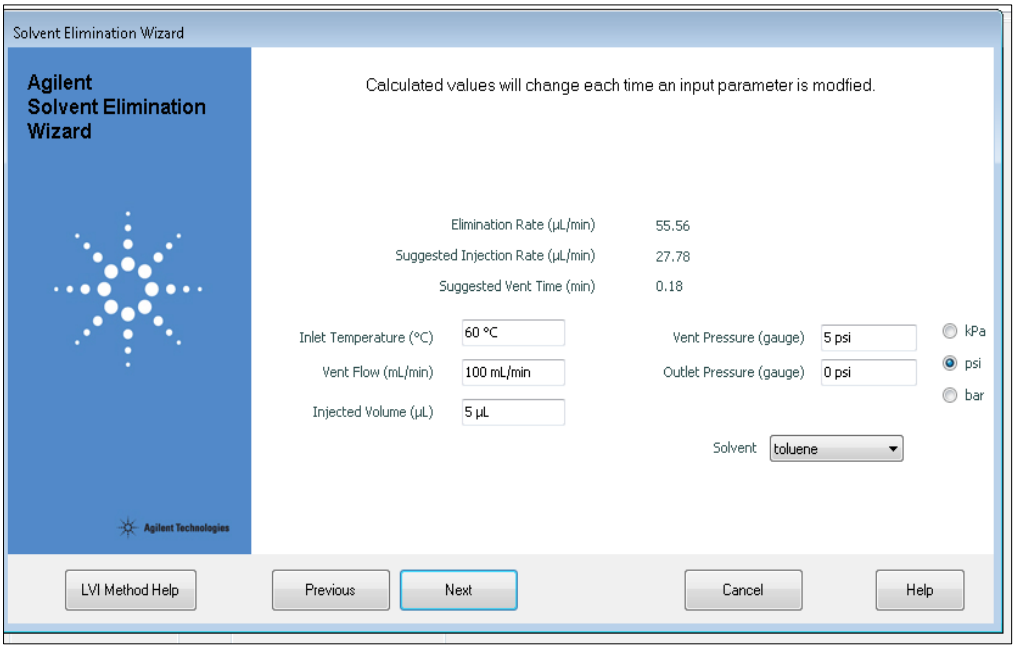

Figure 17: Elimination rate, suggested injection rate, and vent time are calculated by the wizard with the information provided.

The final step of the wizard was to provide a summary of the starting method parameters. Parameters obtained for the 5 µL injection volume are seen in Figure 18 which were loaded onto the method and saved as an LVI method. The oven's initial temperature of 50 °C and oven's initial hold time of 2.68 min were not loaded onto the method. When oven temperature is changed in the different injection methods, the retention times of the analytes would change. This was not desired as it would cause difficulty in comparing the peaks. Therefore, the oven temperature for all the methods was kept at 85 °C.

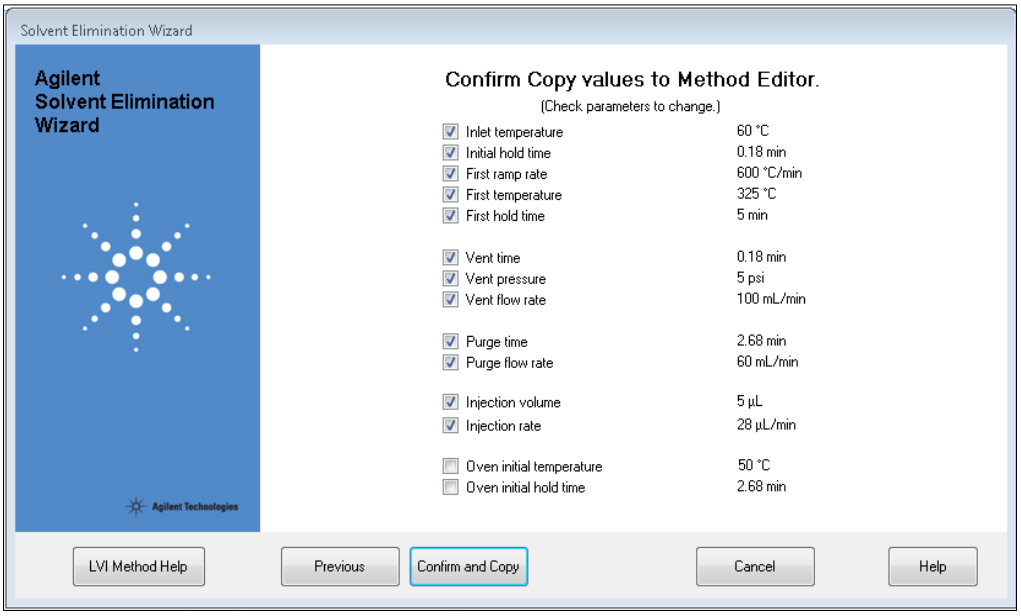

Figure 18: A summary of the parameters provided by the wizard to be copied into the method for application.

The spiked sample previously analysed using the HSI and CSI method was analysed again using the LVI method with parameters stated in Figure 18 except for the oven's initial temperature and hold time.

The developed LVI method was further explored by investigating increases in the desired injection volume of the sample from 5 µL to 10 µL and 25 µL. Changes in the injection volume resulted in changed calculated values for the elimination rate, suggested injection rate, and suggested vent time. Table 3 below shows changes in calculated values obtained for the different injection volumes.

Table 3: Elimination rate, injection rate and vent time values calculated by the wizard for different injection volumes.

Injection volume (µL)	**Elimination rate (µL/min)**	**Suggested injection rate (µL/min)**	**Suggested vent time (µL/min)**
5	55.56	27.78	0.18
10	55.56	27.78	0.36
25	55.56	27.78	0.90

When increasing the injection volume, the 10 µL syringe (Agilent part number 002804) used for previous runs (1 µL HSI, 1 µl CSI, and 5 µL LVI) had to be changed. The maximum injection volume the instrument allows is 50 % of the total volume of the

syringe installed in the instrument (Agilent Technologies, 2009). Therefore, a 25 µL syringe with Agilent part number G4513-80242) was used for the 10 µL injection volume. Furthermore, the 25 µL injection volume required the installation of a 50 µL syringe (Agilent part number 5183-0318).

The spiked sample analysed in previous injection techniques (HSI, CSI, and 5 µL LVI) was analysed again, performing triplicate injections using method parameters obtained for the 10 µL and 25 µL LVI methods. Peak shapes, areas and the overall chromatograms obtained for the spiked OR JU sample analysed using all the different injection techniques are discussed in Chapter 4 of the thesis.

3.6. Optimization of LVI method

After the development of the LVI method followed by the optimisation of method parameters. Firstly, a suitable injection volume was chosen considering all the results obtained during the development process followed in section 3.5 of this study. Parameters that were investigated for optimal conditions were the vent flow, vent pressure, vent time, and inlet and oven temperatures. The usage of different inlet liners was also investigated for the LVI technique. The results of which optimized condition produced an efficient method are discussed in the chapter to follow.

The artificial sample was prepared using a blank apple juice (AP JU) sample. A 10 g sample was weighed and spiked with a mixture of pesticide solution at approximately 1 ppm for purposes of method optimization studies. The sample was extracted using the modified QuEChERS extraction method illustrated in Figure 13.

3.7. Comparison of the HSI and LVI methods

A comparison study was conducted between the HSI method and optimized LVI method using ‘finalised’ proficiency testing (PT) scheme samples (FAPAS orange juice and NMISA mango samples). Finalised PT samples refer to samples completed for analysis within the respective PT scheme the samples were sourced from. In this study, these samples were treated as QC materials because of the know analyte concentrations. These samples were extracted and analysed by both techniques. Additionally, specificity, limit of detection (LOD), limit of quantification (LOQ), repeatability, and recovery were determined for both methods for evaluation. The z-scores obtained of PT samples, total ion chromatograms, peak shapes and areas, resolutions of the peaks and observed retention times will be compared in both methods. The determination of specificity, LOD, LOQ, repeatability, and recovery for both methods required the preparation of calibration standards (procedural and matrix-matched standards), spiked

samples at (~0.01 ppm) reporting limit levels, and (~0.1 ppm) a level higher than the reporting limit.

The rationale for spiking samples at those levels (~0.01 and ~0.1) ppm is based on the reporting limit set by the laboratory for each pesticide studied which is 0.01 ppm. Pesticides have potential health risks and monitoring usage, and exposure is crucial. Regulatory bodies such as the European Union (EU), World Health Organisation (WHO) and Food Agriculture Organisation (FAO) have established restrictive measures in the domain of pesticides and set maximum residue limits (MRLs). Official laboratories and National Reference Laboratories are used to control, enforce and monitor that pesticides residue levels are low and under stipulated MRLs. Analytical methods used for the analysis of pesticides must be reliable and accurate to ensure compliance with the framework of regulatory bodies (Lozano et al., 2016).

In this study, the (SANTE 11312:2021) document was used which guides laboratories within the framework of EU regulations. The reporting limit set by the laboratory is within the set MRL for the pesticide in apple and orange juice samples. Recovery, repeatability and LOQ of an analytical method must be assessed at the reporting limit or MRL and at a level higher (2-10x RL) to assess validity. All samples were prepared and extracted using the QuEChERS method described in section 3.4.2 of the thesis.

3.7.1. Preparation of procedural standards

Ten (10) procedural standards (STD 1 – 10) covering the range of (0.0050 – 1 ppm) were prepared by spiking the working solutions detailed in section 3.2 of the study into aliquots of 10 g samples and the extraction procedure applied.

3.7.2. Preparation of spiked samples at ~0.01 ppm reporting limit (RL)

Six (6) aliquots of 10 g sample of both the apple and orange juice were weighed and spiked with the working solution of 0.5 ppm concentration to obtain measurements at approximately 0.01 ppm reporting limit (RL) (LOQ 1 – 6).

3.7.3. Preparation of spiked samples at ~0.1 ppm

Another six (6) aliquots of 10 g sample of both apple and orange juice were weighed and spiked with the working solution of 10 ppm concentration to obtain measurements of approximately 0.1 ppm (SP 1 – 6).

3.7.4. Preparation of matrix matched (MM) standards

Recovery studies were also conducted and evaluated by preparing ten (10) MM standards (MM STD 1 – 10). These standards were prepared by first extracting blank AP JU and OR JU samples (20 aliquots of each) following the extraction method. The supernatant liquid obtained from the extracts of AP JU samples was combined and the same was done for the OR JU extracts. Standards with similar concentrations to that of the procedural standards (0.0050 – 1 ppm) were prepared in 10 mL volumetric flasks and made up to the mark with the combined supernatant liquid.

3.8. Instrumentation

The instrumentation utilized in the study for the analysis of all samples was a GC-MS/MS instrument. Pico et al., (2020) suggests that volatility and thermal stability of multiclass pesticide residues in any matrix are key characteristics of an analyte which allows for quantitative and qualitative determination by the gas chromatographic technique. Samples and calibration standards were analysed by the GC technique on an Agilent Technologies 7890B GC system fitted with an Agilent Technologies 7693 auto-sampler. An HP-5MS analytical column (30 m x 0.25 mm diameter x 0.25 µm film thickness) was fitted into the GC. A backflush cycle was set up by the addition of a purged ultimate union mid-column controlled by Auxiliary EPC which reduces contamination on the column and the electron impact (EI) source. Additionally, a retention gap of approximately 1 m of deactivated tubing is fitted in front of the analytical column. A retention gap was used to improve peak shapes and to extend the life of the analytical column by preventing its contamination.

The GC system was coupled to Agilent 7000D Triple Quadrupole mass spectrometer (Agilent Technologies, Santa Clara, CA, USA) was coupled to the GC system. The transfer line temperature was 280 °C. Mass spectrometer was fitted with an electron ionization (EI) ion source operated at 300 °C. Electron energy was 70 eV and solvent delay was 1.6 min. Scan mode used was multiple reaction monitoring (MRM) with specified precursor and product ions for each pesticide. A G9250AA GCMSMS database was used to obtain multiple-reaction-monitoring (MRM) transitions, collision energies and dwell time of each pesticide in the study. Solvent solutions of the analytes were scanned on the system to obtain their respective retention times. These transitions and retention times will be typed into the acquisition method. Two (2) transitions at the least for each pesticide (a qualifier and quantifier/s) are used to ensure specificity. Table 4 contains the tabulated MS transition parameters of the pesticides presented in the study. The precursor and product ion, dwell time, and collision energy (CE) are used in the acquisition method (GCMS/MS software database).

Table 4: MS transition parameters of pesticides: precursor and product ion (m/z ratios), dwell time and collision energy used during the acquisition method.

Pesticides	Precursor Ion (m/z)	Product Ion (m/z)	Dwell time (min)	Collison energy (eV)
MLXN	267.8	126.9	10	10
	194.8	108.9	10	15
MLTN	126.9	99	10	5
	172.9	99	10	15
	157.8	125	10	5
CPFS	198.9	171	10	15
	196.9	169	10	15
BRMP	330.8	315.8	10	15
	124.9	47	10	10
	332.8	317.8	10	15
	330.8	92.9	10	25
BRPE	358.7	302.8	10	15
	302.8	284.7	10	15
MTDN	144.9	58.1	10	15
	144.9	85	10	5
PRFF	338.8	268.7	10	15
	207.9	63	10	30
FCHF	286.9	272	10	15
	285	269.9	10	15

3.9. Conclusion

The chapter dealt with the experimental design of the study. The methodology was followed to meet the objectives of the study. Details of the analytical standards and chemicals purchased, and suppliers thereof were disclosed. The chapter included the procedure for the preparation of solutions and standards. Additionally, sample collection and treatment were described which entailed providing an overview of the QuEChERS extraction method. The instrumentation used and mass spectrometer parameters for analysis of the samples were described. The thesis is centred around the different sample injection techniques. Therefore, injection methods were described, and method parameters used were provided in the chapter. Furthermore, samples and standards required to evaluate method validation parameters such as specificity, limit of detection (LOD), limit of quantification (LOQ), repeatability, and recovery for both the HSI and LVI methods were stated in section 3.6 of the thesis. Results obtained from the methodology followed are discussed thoroughly in Chapter 4.

CHAPTER FOUR
RESULTS AND DISCUSSIONS

4.1. Introduction

Chapter 4 details the results obtained from investigations conducted during the study. Discussions regarding the results are stipulated. A presented diagram to illustrate the workflow of the thesis (Figure 12 – chapter 3), is illustrated again as a schematic structure of Chapter 4 (Figure 19). The review of the diagram shows that organophosphate pesticides (MLXN, MLTN, CPFS, BRMP, BRPE, MTDN, and PRFF) are investigated in orange and apple juice samples. The samples are analysed using a GC-MS/MS instrument with a focus given on the various sample injection techniques. These techniques include hot splitless (HSI), cold splitless (CSI), and large-volume injection (LVI).

The study embarks on a process to develop an LVI method by first providing a brief overview of HSI and CSI. Three injection volumes (5, 10, and 25 µL) for the LVI method were investigated. The 5 µL injection volume for LVI was selected (based on the results) for further optimization of the method parameters. These parameters include vent flow, vent pressure, vent time, inlet, and oven temperatures. The developed and optimized 5 µL LVI method was then compared to the HSI. The comparison was conducted by evaluating specificity, limit of detection (LOD), limit of quantification (LOQ), repeatability, and trueness at the reporting limit level (0.01 mg/kg) and a level higher (0.1 mg/kg) for both methods. Additionally, the methods were compared using proficiency test samples from NMISA and FAPAS test schemes. Statistical evaluations by means of calculating z-scores from the results obtained were thoroughly discussed.

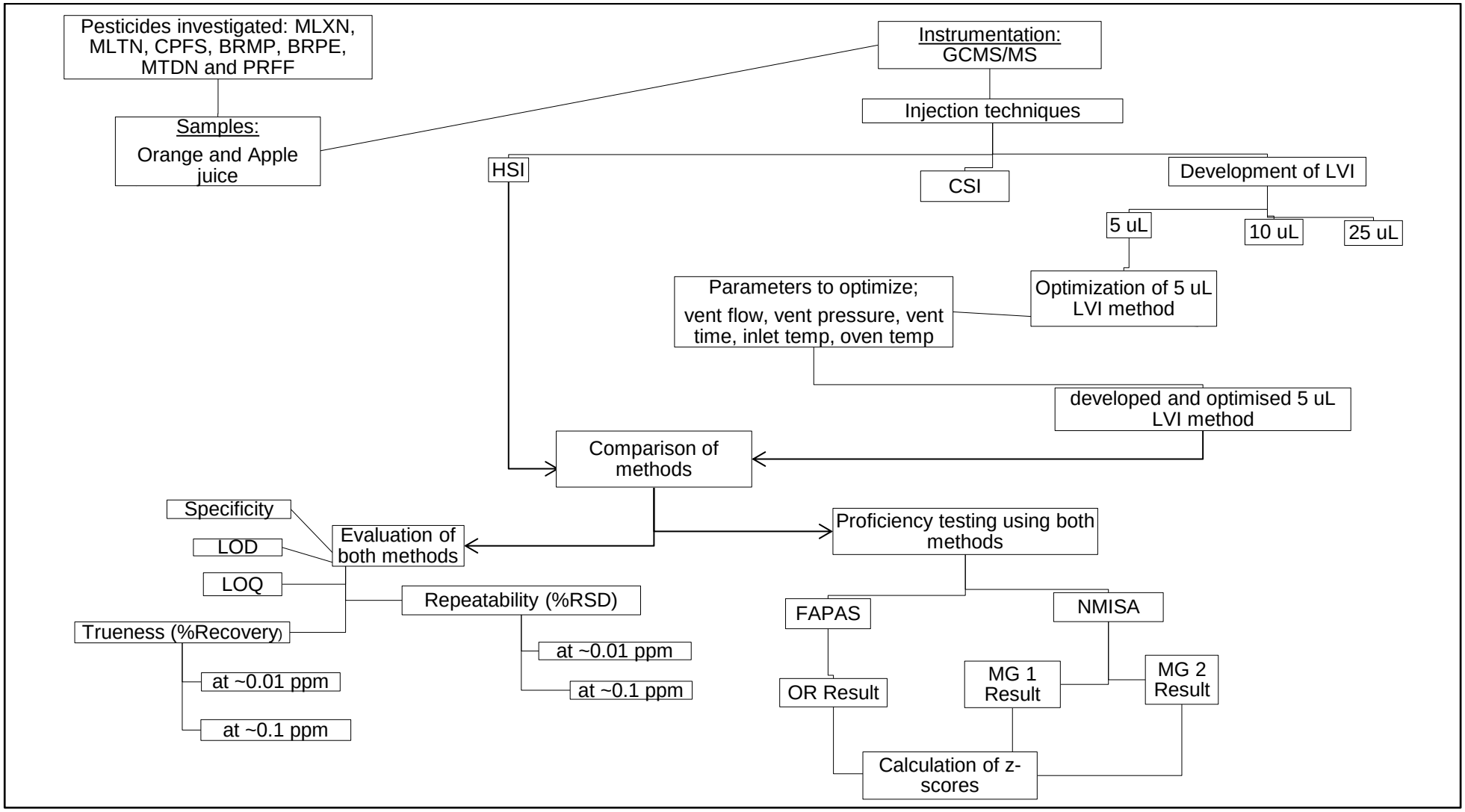

Figure 19: A schematic diagram illustrating the workflow of the study which provides the layout of the results obtained. The figure is a duplicate of Figure 12 in Chapter 3.

4.2. Standard Chromatographic Results

The chromatogram (see Figure 20) obtained from using the HSI method to analyse the spiked orange juice sample described in section 3.5, served as a standard/reference chromatogram. This standard chromatogram was used for the comparison of other injection techniques (1 µL CSI, 5 µL, 10 µL and 25 µL LVI).

According to (Walorczyk, 2012), the HSI is an isothermal splitless technique that is widely applied in trace analysis with injection volume ranging between 1 – 2 µL. In this technique for the study, inlet temperature was kept constant at 250 °C, and 1 µL of the sample was injected (see Table 1 – section 3.5.1.1 for HSI set parameters). Godula et al., (2001) state that high temperatures applied to volatize sample components when a splitless injection is used is constrained by the thermal stability of analytes, resulting in their loss. According to Snow N. H., (2018), inlet temperature is often set to 250 °C in splitless injections as this is a temperature above boiling point of most samples. However, in other injection methods such as LVI, temperature setting is set below or close to the boiling point of the solvent (Godula et al., 2001). The physiochemical properties of the analytes, such as low molecular weight and chemical structure are important because adsorption may occur at active sites in the injection port. According to Godula et al., (2001) this is the extreme for polar analytes as hydroxyl, carbonyl and amino functional groups are contained in the chemical structure. Lara et al., (2017) discusses the challenges of selectively extracting polar analytes from polar solvent, as interferences are co-extracted, affecting the accuracy of measurements.

A study investigating similar injection techniques (HSI, CSI, and LVI) was conducted by Wilson et al., (2009) by using a 40-ppb pesticide standard containing multiclass pesticides and injecting a 2 µL sample volume for HSI. In this study, the focus was on organophosphates and the sample volume used for HSI was 1 µL. Notably, similar observations were encountered in both studies regarding the behaviour of pesticides in the injection methods despite the discrepancies of pesticides studies and sample volume injected. Wilson et al., (2009) observed that with the HSI, certain pesticides were barely visible in the chromatogram compared to chromatograms obtained for 10 µL CSI and 25 µL solvent vent (LVI). Peaks in a chromatogram must appear clearly distinct without being obscured for accurate determinations. The signal-to-noise ratio was improved in other injection techniques compared to HSI, implying improved peaks area measurements for reliable identification which signals quality results.

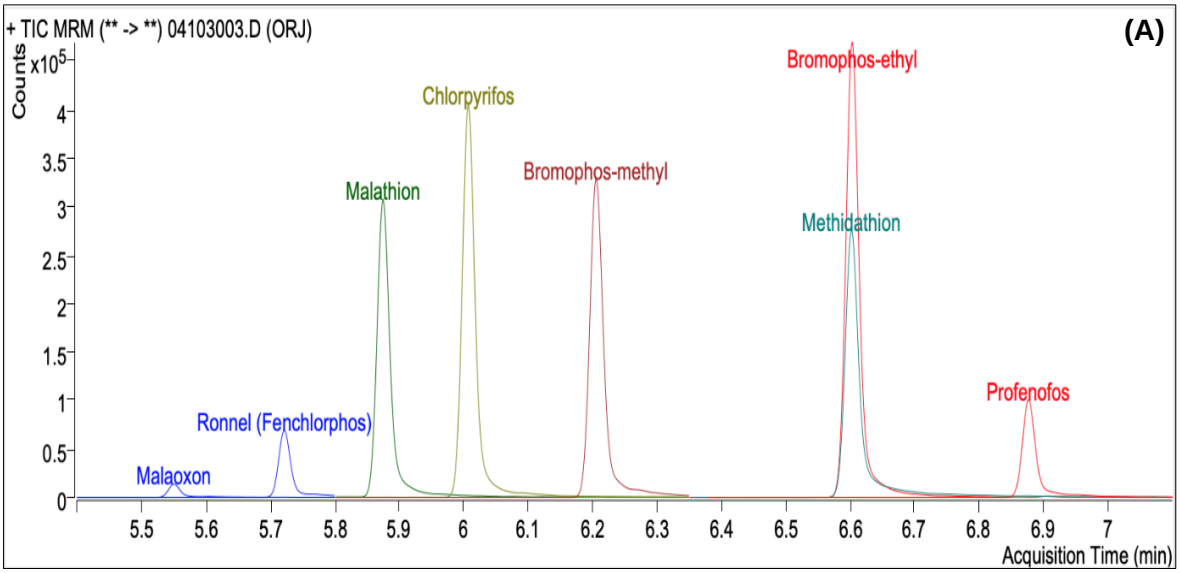

Figure 20: GCMS/MS chromatogram obtained using the HSI method for the injection of 1 µL orange juice sample containing spiked organophosphate pesticides.

Symmetrical peaks were seen eluting at various retention times for all the pesticides. Analyte peaks in the chromatogram showed excellent resolution whereby peaks were effectively separated. MLXN was the first eluting pesticide detected at 5.550 min of the run, exhibiting the smallest peak size of all the other pesticides. BRPE and MTDN were observed co-eluting at 6.6 min. Triplicate injections of the sample were performed and peak size values obtained are tabulated in Table 5 and averaged for all the pesticides.

Table 5: Peak areas of all the pesticides obtained using the 1 µL HSI method.

Peak areas obtained for each pesticide during method development								
		MLXN	MLTN	CPFS	BRMP	MTDN	BRPE	PRFF
RT (min)		5.550	5.875	6.007	6.205	6.602	6.603	6.876
1 µL HSI	Run 1	16386	419979	586401	480994	401779	681011	140732
	Run 2	16132	419766	566785	497189	385597	693078	135525
	Run 3	18329	440456	578666	493827	432385	696704	151750
Average		**16949**	**426734**	**577284**	**490670**	**406587**	**690264**	**142669**

4.2.1. Co-elution of MTDN and BRPE

According to (Hernández et al., 2013), pesticide residue analysis is required to ensure strict control of pesticides in food. This type of analysis is a specialised field in food safety because there is a variety of food matrices comprising various content (fat, acidity, sugar, and water). Additionally, there are representative pesticide families such as herbicides, insecticides, and fungicides which are formulated products from numerous active substances based on distinct polarity and volatility. Hernández et al., (2013) describes

the absence of a reliable identification and accurate quantitative method for the analysis of all pesticides in all the sample matrices. However, a multiresidue method which comprises a few hundred pesticides in the scope of the method is an alternative approach. Jo et al. (2006) refers to multi-residue pesticide analysis as developed methods of monitoring pesticide residues by simultaneously analysing multiple pesticides using a GCMS/MS and LCMS/MS.

The hyphenation of gas chromatography and mass spectrometry (GC-MS/MS) provides great sensitivity and a more accurate method for multi-class pesticide determinations. A mass analyzer such as a triple quadrupole (QqQ) may be used for the capability of having simultaneous selection of multiple transitions for a particular analyte (Kmellár et al., 2011). This technique provides identification and distinct conformation of an analyte (Lee et al., 2015).

Sawikowska et al., (2021) defines co-elution as the inability of two or more compounds to chromatographically separate during analysis and this was seen to be the case for methidathion (MTDN) and bromophos-ethyl (BRPE) (see Figure 20). The two compounds were observed to be co-eluting at approximately 6.6 minutes of the analysis during investigations.

Multiple reaction monitoring (MRM) transitions for each pesticide investigated in this work are tabulated in section 3.8.1 (Table 4) of the thesis. MTDN has quantification (quantifier) MRM transitions of 144.9 – 85 (precursor to product ion) with a collision energy (CE) of 5 eV. Identification MRM transition ions (qualifier) used for MTDN are 144.9 – 58.1 with 15 eV CE. Quantifier transition ions used for bromophos-ethyl (BRPE) was 358.7 – 302.8 with CE of 15 eV. The qualifier transition ions used for the compound were 302.8 – 284.7 with a CE of 15 eV. These set MS parameters for the compounds enable the method to distinguish between MTDN and BRPE. Figure 21 shows the observed peaks for MTDN and BRPE with respective quantifiers and qualifier MRM transition ions used, and qualifier ratios obtained.

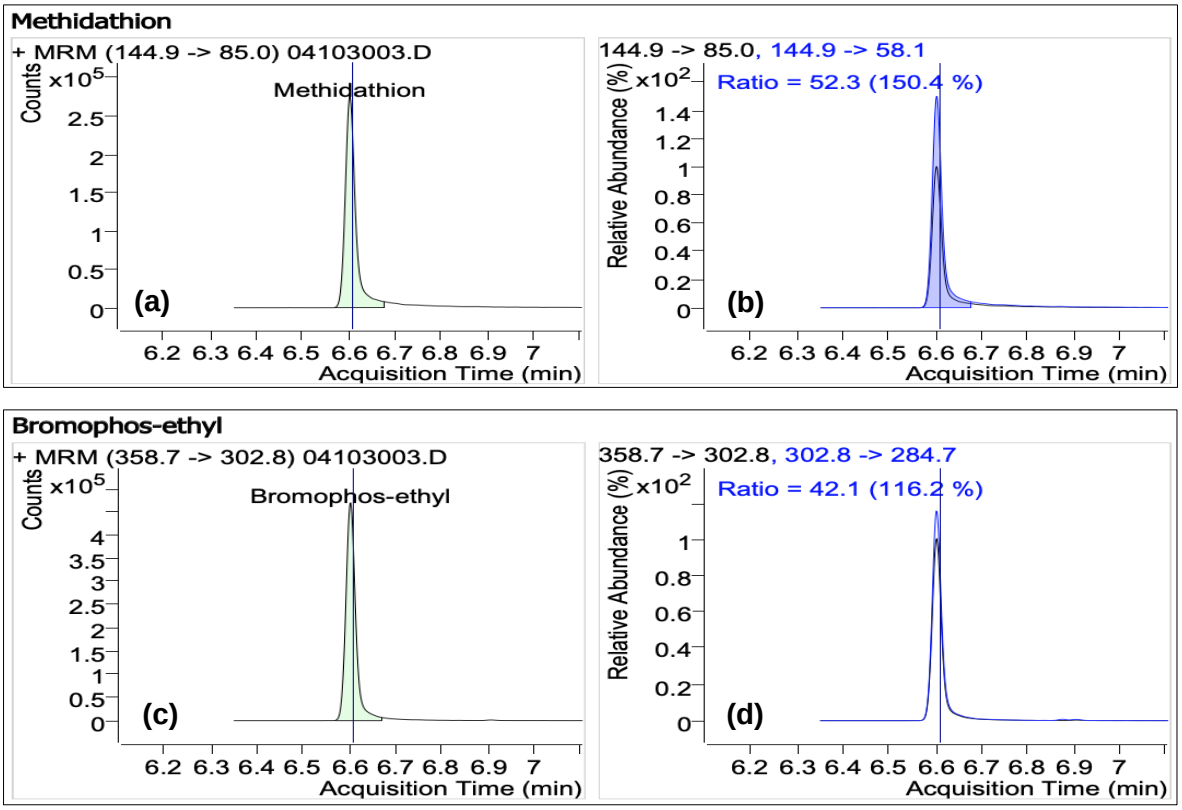

Figure 21: Methidathion (MTDN) and bromophos-ethyl (BRPE) co-elution. **(a)** MTDN quantifier peak observed of 144.9 – 85 transition ions. **(b)** MTDN qualifier peak observed of 144.9 – 58.1 transition ions with a qualifier ratio of 52.3. **(c)** BRPE quantifier peak observed of 358.7 – 302.8 transition ions. **(d)** BRPE qualifier peak of 302.8 – 284.7 transition ions with a 42.1 qualifier ratio.

4.3. Chromatographic results of investigated injection methods

Injection techniques investigated were the 1 µL CSI and LVI at various injection volumes (5, 10 and 25 µL). Figure 22 depicts all the chromatograms obtained from the spiked orange juice sample analysed using the different injection methods including the 1 µL HSI standard chromatogram (A) detailed in section 4.2 of the thesis. There were few variations observed when a comparison of chromatogram (A) with other chromatograms (B) 1 µL CSI, (C) 5 µL LVI, (D) 10 µL LVI and (E) 25 µL LVI was conducted.

Chromatograms depicted in figure 22 is from the third run (Run 3) of each injection and peak areas of all pesticides are listed in Appendix F of the thesis. The most evident observation was that MTDN and BRPE peaks were co-eluting throughout all the various injection techniques. The difference observed was the peak area variations of the two compounds. Analysis by HSI produced a MTDN peak area 62% of the BRPE peak. When CSI was investigated, a slight decrease of MTDN peak area was observed and calculated to be 55% that of the BRPE peak area. When the LVI injection of 5 µL was

introduced, a significant increase was observed, MTDN peak area was 89% of the BRPE peak. Further investigations of LVI at 10 µL and 25 µL resulted in drastic increases of the MTDN peak areas that were 116% and 158% of the BRPE peak area, respectively.

MLXN peak area was the smallest peak area of all the pesticides for all the injection methods. Slight peak distortion of fronting peaks was observed during the application of the 5 µL LVI (chromatogram C – Figure 22). The same distortion of peaks was observed with the 25 µL LVI (chromatogram E – Figure 22) for all pesticides except MLXN which was extremely distorted and resulted in a shift of its retention times. Chromatogram D obtained from the application of the 10 µL LVI method revealed ideal peak shapes with no distortions. The peaks were narrow and symmetrical. An in-depth discussion of the observations encountered during various injections is provided in sections to follow and reasons why the 5 µL LVI method application was selected for further optimization rather than the 10 µL LVI method application. Probable sources of error and variations in the results include thermal degradation, sensitivity of the methods, interferences affecting analytes and large solvent vapours formed from LVI methods.

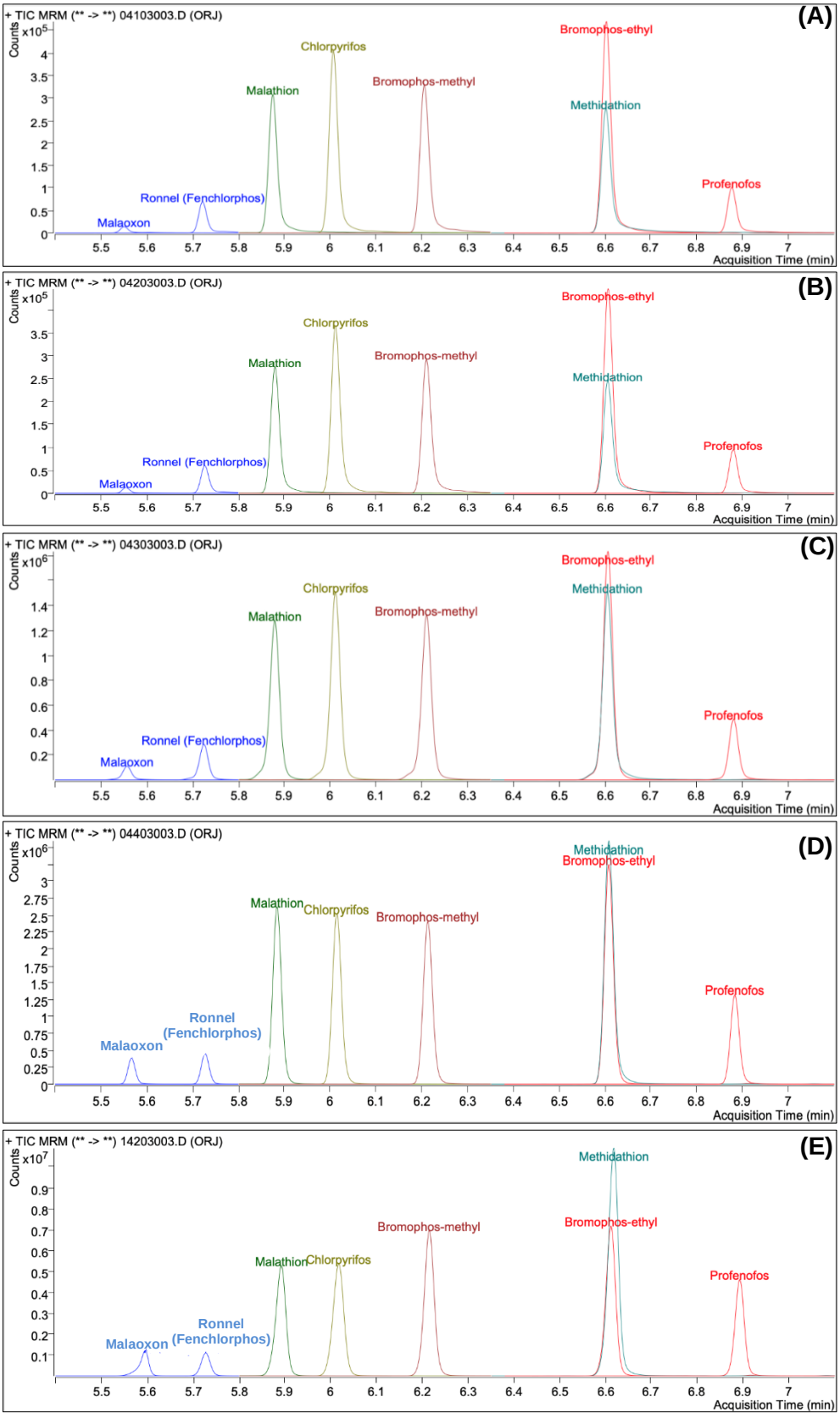

Figure 22: GCMS/MS chromatograms from **(A)** a 1 µL HSI, **(B)** 1 µL CSI, **(C)** 5 µL LVI, **(D)** 10 µL LVI, and **(E)** a 25 µL LVI method runs of the orange juice sample spiked with organophosphate pesticides.

4.3.1. Chromatographic results for the 1 µL CSI

Investigation of the CSI method for purposes of this study, as mentioned, was to demonstrate the migration from the HSI to the LVI method. The HSI method was changed as described in section 3.5.1.2 of the thesis for parameters of the CSI method. The spiked orange juice sample was analysed using the CSI method and chromatogram B (see Figure 22) was obtained. The average peak size values of all the pesticides are seen (Table 6) below for this injection.

Table 6: Peak areas of all the pesticides obtained using the 1 µL CSI method.

Peak areas obtained for each pesticide during method development							
	MLXN	MLTN	CPFS	BRMP	MTDN	BRPE	PRFF
RT (min)	5.554	5.879	6.012	6.209	6.606	6.607	6.879
Average	**15874**	**398365**	**547071**	**442173**	**366535**	**670644**	**137323**

The Agilent tutorial (Agilent Technologies, 2009), merely states that the results of the two techniques (CSI and HSI) were identical for the hydrocarbon sample analysed. In this study, a similar trend was observed. Application of the CSI resulted in extremely slight (~1%) decreases in peak areas of the pesticides. The trend is illustrated in the section to follow where the distribution of peak areas of the pesticides is illustrated between HSI, CSI and 5 µL LVI (Figure 24 – section 4.3.2) injection techniques. Figure 23 is shown to illustrate average peak areas obtained from both techniques (for HSI see Table 5 and for CSI see Table 6). In a study conducted by Wilson et al., (2009), a statement is made that the CSI is an alternative technique for analysis that addresses concerns presented by the HSI. Vaporization of analytes takes place when sample injection is completed due to temperature conditions set lower than the solvent boiling point. This allows analytes to vaporize at the lowest inlet temperature which minimises thermal degradation (Wilson et al., 2009)

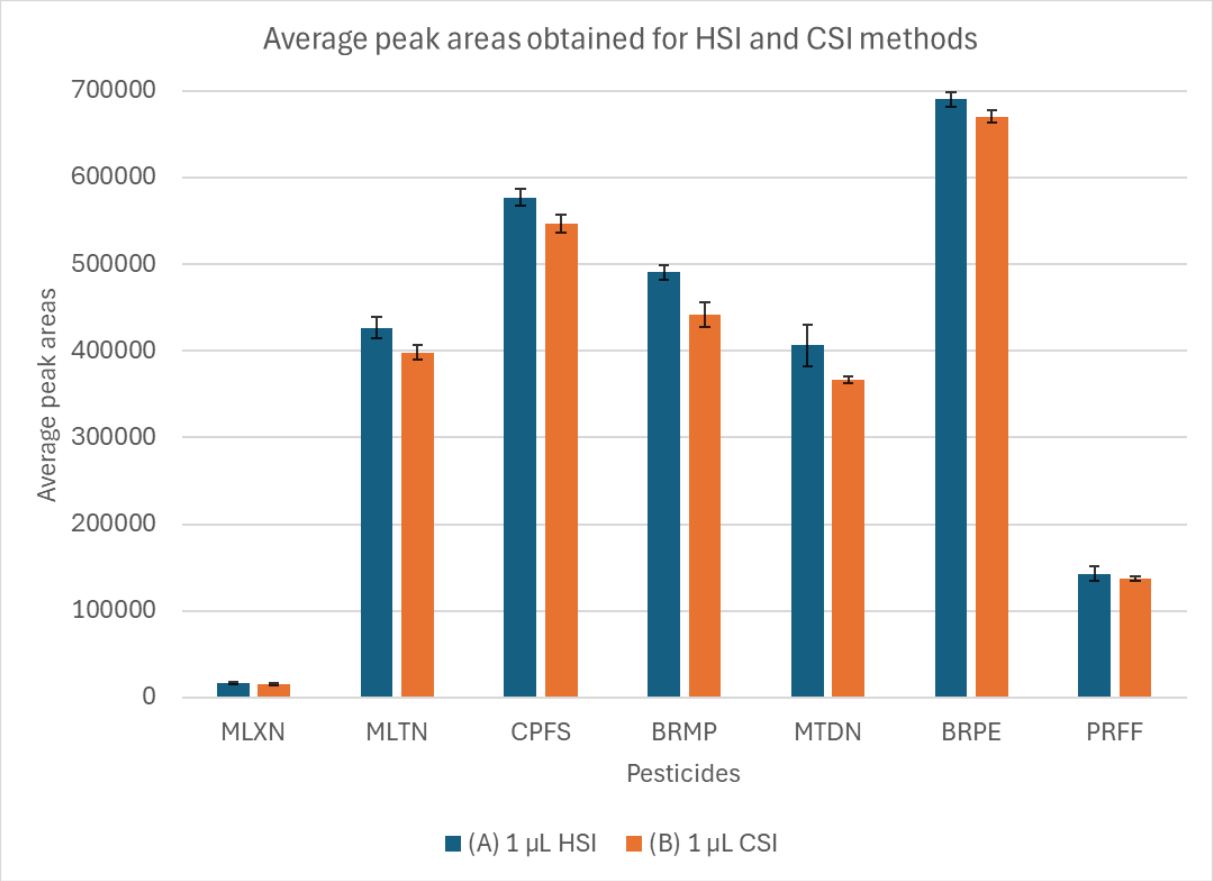

Figure 23: Average peak areas obtained for each pesticide during analysis using (A) HSI method and (B) CSI method.

4.3.2. Chromatographic results of 5 µL LVI

Three (3) sample injection volumes (5, 10, and 25 µL) were investigated in the study for the LVI method using the spiked sample previously analysed for the HSI and CSI methods. The sample was run using the method parameters stated in Figure 18 (section 3.5.1.3) for the 5 µL injection volume. Table 7 contains the average peak area of the pesticides resulting from the 5 µL LVI analysis.

Table 7: Peak areas of all the pesticides obtained from the 5 µL LVI method.

Peak areas obtained for each pesticide during method development							
	MLXN	MLTN	CPFS	BRMP	MTDN	BRPE	PRFF
RT (min)	5.557	5.880	6.012	6.210	6.605	6.607	6.881
Average	**241679**	**2468232**	**2815101**	**2567448**	**3291167**	**3264993**	**1011263**

Chromatogram C (see Figure 22) was obtained which showed an overall improvement in the peak area of all the compounds. MLXN peak observed for the 1 µL HSI greatly increased during analysis. MTDN peak was approximately half the size of the BRPE peak during HSI analysis (Chromatogram A -Figure 22), but significantly improved to

have approximately three-quarters of the BRPE peak (Chromatogram C – Figure 22). This indicated an increase in sensitivity compared to the 1 µL volume of the HSI method.

Vallejo et al., (2010) appended in an article that LVI performed in a PTV injector improves sensitivity by several magnitudes compared to HSI. This is supported by Ferrari et al., (2018), stating LVI analysis provides increased sensitivity. Additionally, the application of LVI produces better recoveries for compounds that easily breakdown. This study supports that great improvements were observed when the LVI technique was used. According to (Zhao et al., 2012), peak shapes of early eluting analytes were improved, and low detection limit requirements were achieved when the LVI method was applied in a similar study investigating different injection modes.

Figure 24 presents the peak area distribution between HSI (Table 5), CSI (Table 6), and 5 µL LVI peak areas (Table 7). The injection of 5 µL LVI resulted in increased peak areas ranging between 70 – 80 %, whereas with HSI and CSI it was less than 20% (Figure 24). A difference of 1 % was seen between the HSI and CSI applications.

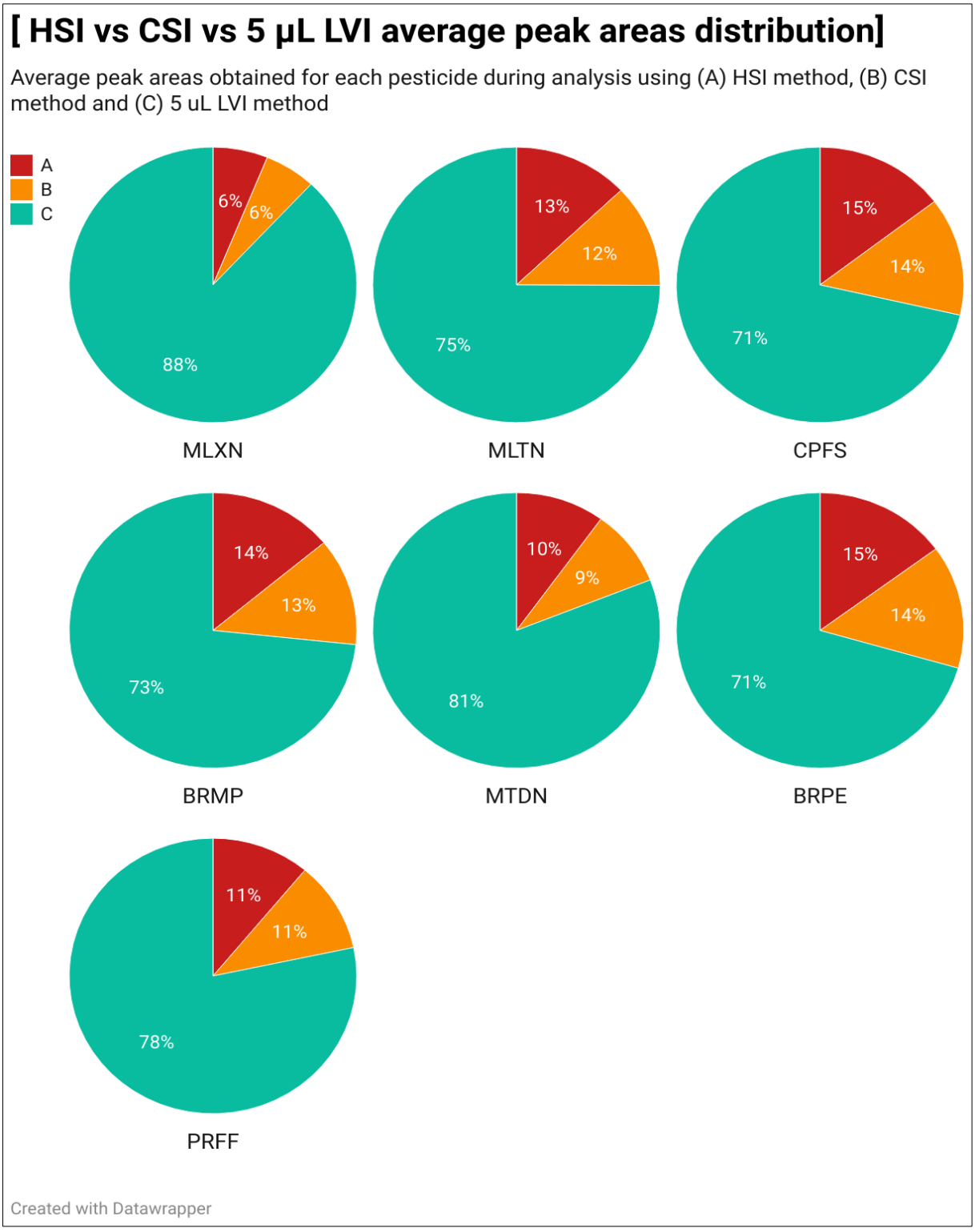

Figure 24: The distribution of average peak areas of pesticides during analysis of (A) HSI method, (B) CSI method and (C) 5 µL LVI method.

4.3.3. Chromatographic results of 10 µL LVI

Another sample injection volume that was investigated was 10 µL. New method parameters were calculated due to the change in volume and uploaded into the method using the solvent elimination wizard. The desired volume changed from 5 µL to 10 µL. The obtained chromatogram for the 10 µL LVI run is depicted as chromatogram D in Figure 22.

The 10 µL injection run showed peaks that were narrow and symmetrically shaped for all the compounds. There was no slight tailing or fronting of the peaks during the 10 µL injection as was observed in previous injections (1 µL HSI, 1 µL CSI, and 5 µL LVI) methods. The chromatogram of the run shows that MTDN was slightly greater in peak size than the BRPE peak (Chromatogram D). No interference was observed for MLXN during the 10 µL LVI run. Table 8 contains the resulting peak areas of the pesticides when the method was applied.

Table 8: Peak areas of all the pesticides for the 10 µL LVI.

Peak areas obtained for each pesticide during method development							
	MLXN	MLTN	CPFS	BRMP	MTDN	BRPE	PRFF
RT (min)	5.567	5.884	6.014	6.213	6.608	6.609	6.883
Average	**433495**	**3288638**	**3308005**	**3209566**	**5092865**	**4275537**	**1749279**

Inspection of Figure 22, of all chromatograms for all injections, conclusions may be drawn that 10 µL LVI (Chromatogram D) is the most satisfactory volume of sample to inject. Peak shapes obtained were all symmetrical even MLXN which was poorly performing in other injections was improved. However, there were factors to consider when choosing the volume of sample to inject. These include high sample throughput to be received for analysis using LVI on a day-to-day basis. Samples received for screening are of various sample matrices and the extraction method (QuEChERS) employed in this study does not include a clean-up step therefore, ‘dirty’ samples would generally be injected. According to (Walorczyk, 2012), loss of inertness and chromatographic efficiency might occur with the injection of ‘dirty’ samples.

The application of 10 µL LVI would require an increased frequency of routine maintenance on the system to ensure quality is maintained. Frequent maintenance becomes costly for a laboratory because consumables such as instrument liners and septa, columns will need to be replaced. This in turn affects the laboratory’s turnaround time which will hinder customer satisfaction. A 5 µL LVI seemed to be a better option considering the above factors because of the increased sensitivity and higher peak

abundance obtained already. A decision was taken to commence with the study using the 5 µL LVI for further investigations of optimizing the parameters of the method.

4.3.4. Chromatographic results of 25 µL LVI

The desired volume to be investigated was the 25 µL LVI. New method parameters that correspond to an injection volume of 25 µL were calculated and uploaded. The orange spiked sample was injected, and chromatogram E obtained for the run is depicted in Figure 22. Peak areas obtained from this injection are tabulated in Table 9.

Extreme peak fronting was observed for all the compounds. There was also a shift in the retention time (RT) for all the compounds. This means that peaks eluted at later times compared to the 1 µL HSI method. The peak for MLXN was greatly increased using an injection volume of 25 µL but the peak showed extreme fronting. MTDN peak size in this run was even greater than the BRPE peak.

Table 9: Peak areas of all the pesticides obtained for the 25 µL LVI.

Peak areas obtained for each pesticide during method development							
	MLXN	MLTN	CPFS	BRMP	MTDN	BRPE	PRFF
RT (min)	5.595	5.893	6.017	6.215	6.618	6.612	6.893
Average	**1413331**	**7197152**	**7709670**	**8823580**	**15581418**	**9450877**	**5782511**

In (Wilson et al., 2009) studies included 25 µL solvent vent (LVI) injection at a starting inlet temperature of 30 °C. Differences were appended between solvent vent run against a 2 µL HSI operated at a constant temperature of 280 °C and 10 µL CSI with a starting inlet temperature of 30 °C. Figure 25 is an extracted image showing chromatograms of the injections for comparison during the study. Similarities of significant improvement in signal-to-noise ratio were also observed in this study. However, in this study, peaks were distorted with the application of the 25 µL LVI method.

The most critical issue in LVI is the huge solvent vapor volume resulting from the expansion of the high amount of the injected solvent (Walorczyk, 2012). The distortion of peaks may be explained by Godula et al., (2001) who suggested that during LVI injections, the solvent must be prevented from reaching the bottom of the inlet. When liquid sample reaches the bottom of the inlet, it may cause column flooding and sample loss may occur through the split exit. This results in peak shape deterioration and contamination of the GC system. In (Godula et al., 2001) studies, an experiment was first conducted to determine the maximum injection volume before commencing further studies on LVI.

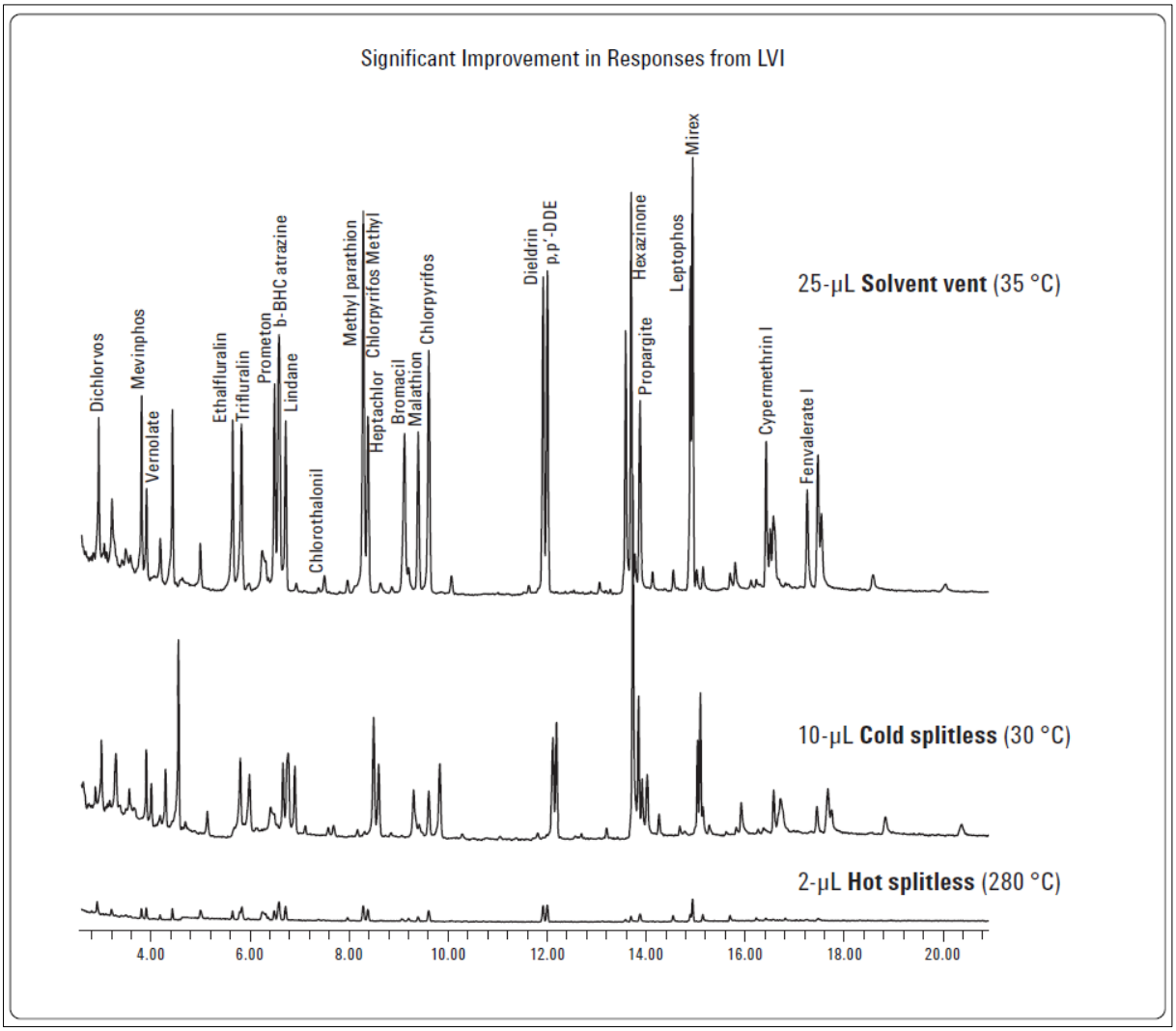

Figure 25: Extracted chromatograms from Wilson et al., (2009) studies in which 25 µL LVI was investigated.

4.4. Total Ion Chromatograms (TIC)

Total ion chromatograms (TIC) of all the investigated injection techniques using different volumes injected are depicted in Figures 26 and 27 below. The shifts in the retention times are clearly seen with an extreme shift happening with the MLXN peak. TICs are not able to show overlapping peaks that co-elute. In Figure 26, TICs for all the injection techniques are generated using Excel for purposes of enlarging MLXN peaks obtained. Analysis software generated TICs are also depicted in Figure 27. The largest peak abundance was observed in the 25 µL LVI chromatogram and this is due to it being the largest volume injected. The 1 µL HSI (black) and the 1 µL CSI (red) chromatograms overlaid perfectly. There was an insignificant change in peak areas between both runs for all pesticides.

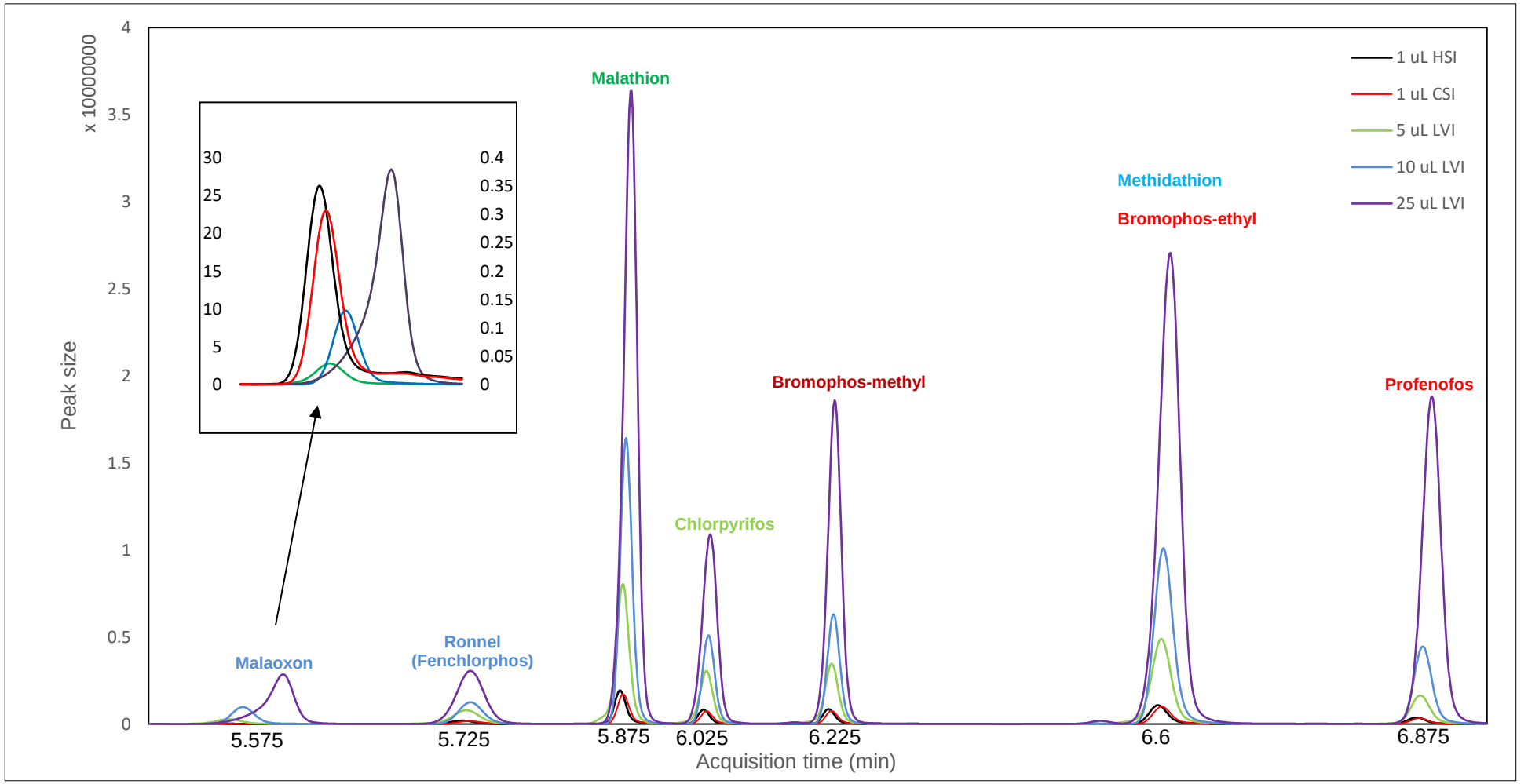

Figure 26: Excell generated graph – TIC with enlarged MLXN peaks produced using chromatographic data of the analysis of spiked orange juice sample illustrating difference of investigated injection techniques studied.

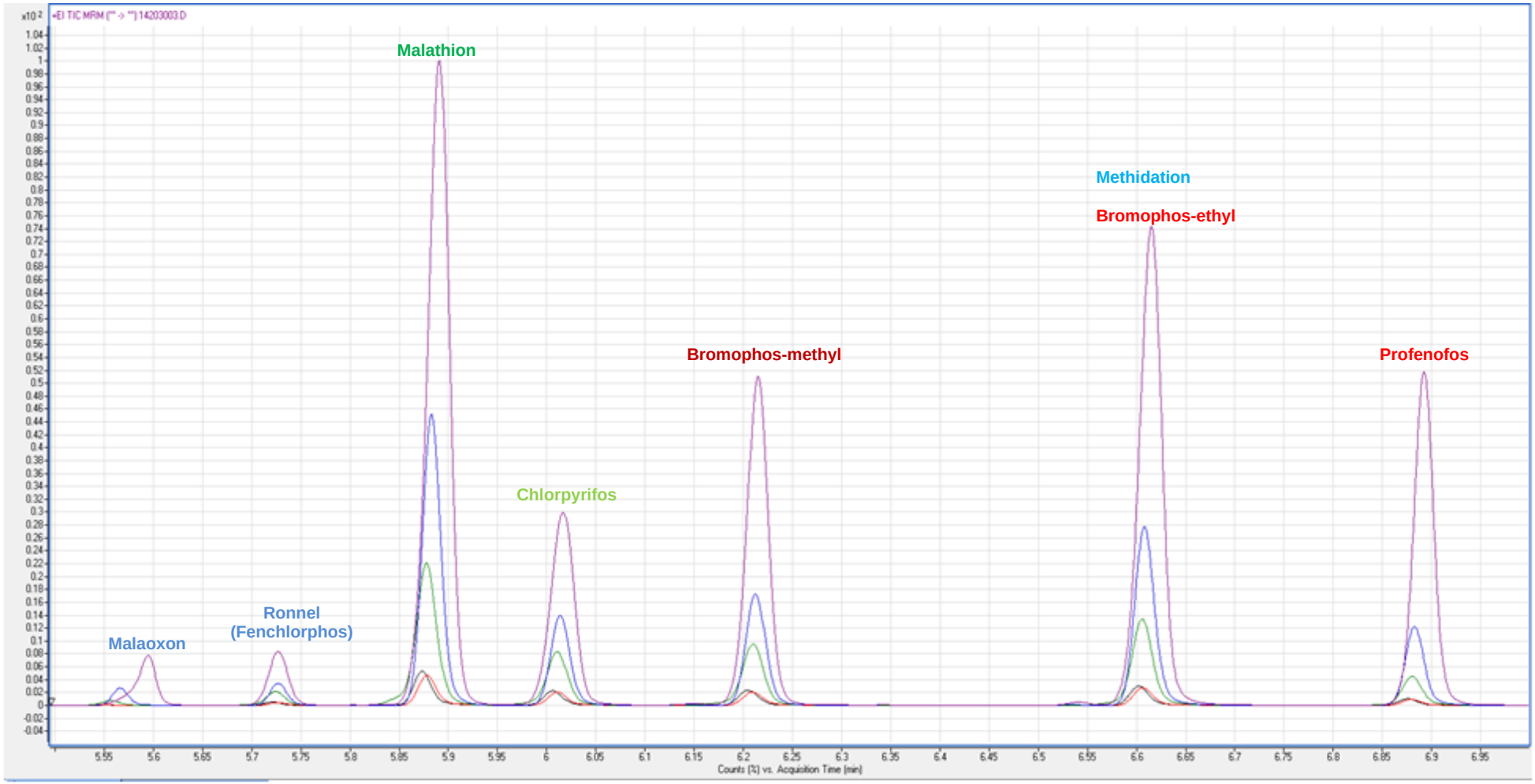

Figure 27: Analysis software generated TICs for the orange spiked sample analysed using different injection techniques.

Chromatographic data (peak area values obtained from all the injections) of the depicted TICs illustrated in Figures 26 and 27 is appended in Appendix F of the thesis for method development studies. The averaged peak area values of the pesticides are graphically presented in Figure 28 below. There was no significant difference in the HSI and CSI peaks of pesticides obtained. The 25 µL LVI method application result in greater peak responses for all the pesticides due to it being the largest volume injected.

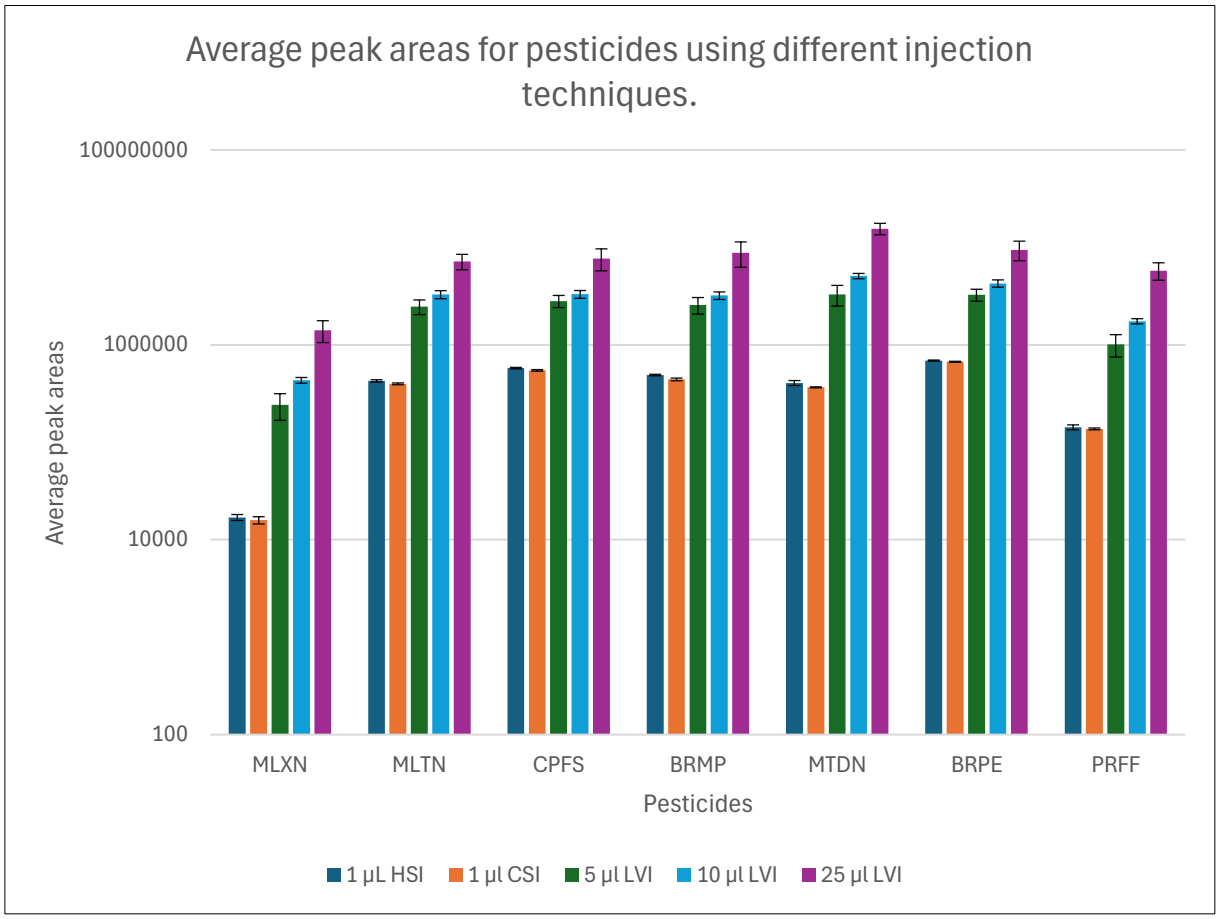

Figure 28: Graphical representation of average peak area values obtained for each pesticide using different injection techniques.

4.5. Chromatographic observations of Malaoxon (MLXN).

Theoretically, an analyte that is present in different samples should have the same retention time and peak shape when analysis is conducted. The difference should only be the intensity of the peak or amount of analyte present in the sample (He et al., 2018). However, the shift of retention times analyte peaks may be observed in the different samples. He et al. (2018) states that changes in instrument temperature and pressure, unavoidable deviations during experiments, and the analytical column aging as factors that contribute to shifts in retention times.

In this study, column aging was controlled by performing instrument maintenance prior to the experiment as prescribed by the laboratory standard operating procedures (SOP) for equipment. Routine maintenance included changing the analytical column or retention gap/guard column or even trimming the column at the first sign of deterioration. The instrument is positioned in an environment that is temperature controlled, and the instrument is autotuned weekly. Additionally, instrument performance is evaluated by analysing quality control samples with every batch ran on the instrument.

Malaoxon (MLXN) pesticide was the most affected peak with observed shifts in retention times and peak distortions. The shift in retention times for the peak during method development studies is shown in Figure 29. When the HSI method was conducted the peak was observed at a retention time of 5.550 min (Table 5). Gradual increases were observed in other injection techniques with a noticeable shift to 5.595 min for the 25 µL LVI method (Table 9).

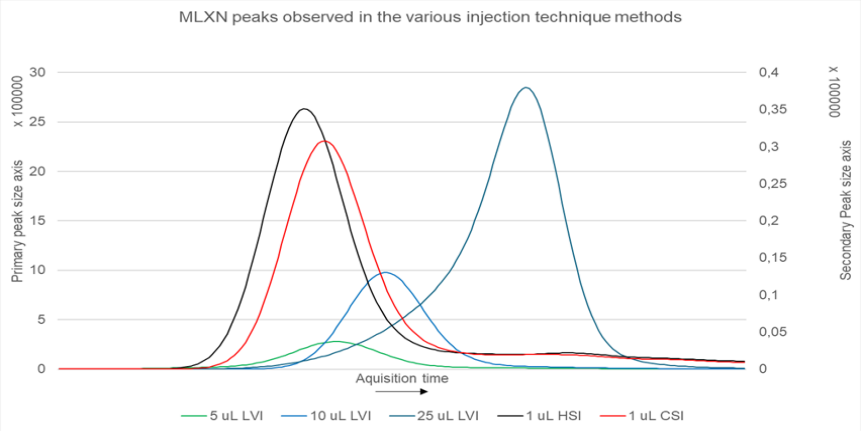

Figure 29: MLXN peaks obtained from HSI, CSI and LVI injections illustrating RT shifts and peak distortion.

Several aspects were investigated for probable reasons for this observation. The first aspect was possibilities of interferences that might have occurred. In Figure 30, MLXN peaks are shown separately for all injections investigated. Interference was observed for (a) 1 µL HSI method and (b) 1 µL CSI method. The peak produced for (c) 5 µL LVI method was slightly fronting. A shift in retention time was noted when (d) 10 µL LVI method was used. The peak obtained when (e) 25 µL LVI was applied resulted in the peak being extremely distorted.

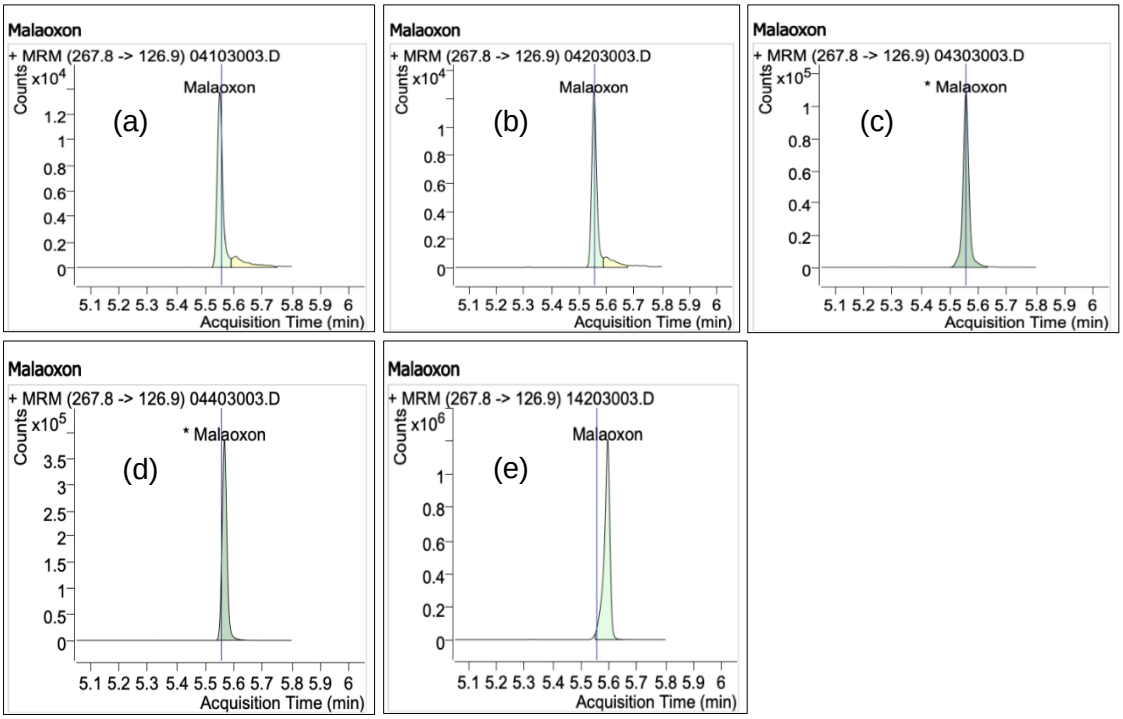

Figure 30: Malaoxon (MLXN) peaks obtained during method development studies. **(a)** MLXN observed during 1 µL HSI method. (b) MLXN peak observed for the 1 µL CSI method. (c) MLXN peak observed for the 5 µL LVI method. (d) MLXN peak observed for the 10 µL LVI method. (d) MLXN peak observed for the 25 µL LVI method.

The synthesis of malaoxon was explored. According to Inge and Whatling (2010:1527), bioactivation of malathion produces malaoxon because of oxidative desulfuration during insect metabolism. This would qualify malaoxon as a metabolite of malathion. Therefore, this study investigated a 'parent' pesticide with its metabolite. There are published analytical residue methods for analysis of these pesticides together such as one referenced EN-CAS method No ENC4/93 by the U.S. Environmental Protection Agency. Therefore, the presence of malathion in the sample could not have affected the analysis of malaoxon in the presented study. Additionally, these pesticides elute at extremely different retention times.

Physiochemical characteristics of malaoxon like molecular weight, boiling point and chemical structure was another aspect explored. The molecular weight of the malaoxon is 314.30 g/mol which is not the smallest as methidathion molecular weight is 302.00 g/mol. Malaoxon eluted first in the analysis and has a boiling point of 114 °C. According to Moldoveanu et al. (2013), shifts in retention times is not only influenced by the chemical structure of the analyte. The nature of the mobile and stationary phase, dimensions of the column and the flow rate of the mobile phase are other factors that impact on the retention time. Parameters of the methods used in this study were carefully examined following statements by Moldoveanu et al. (2013). A summary of the

parameters is listed in Table 10 below. Focus was given to the injection rates of the LVI methods which was a measure of how fast the injection was for that method. An injection rate of 28 µl/min for 5 µL, 340 µL/min for 10 µL and 68 µL/min for the 25 µL sample volume injected. The largest sample volume of 25 µL was injected at a slower rate compared to the 10 µL sample volume. Conclusions are drawn that the slow injection rate is a probable reason for peak distortion and shift in retention time. Solvent could have not completely vented out. Godula et al. (2001) warns against flooding the column with excessive solvent vapours and prompts carefully optimising injection parameters. Additionally, Godula et al. (2001) states peak band broadening is formed when residual solvent volume (solvent left after the vent process) is too high.

Table 10: Summary of method parameters of HSI, CSI and LVI (5, 10 and 25 µL) during method development studies.

Parameters	**HSI**	**CSI**	**5 µL LVI**	**10 µL LVI**	**25 µL LVI**
Injection volume (µL)	1	1	5	10	25
Mode	Splitless	Splitless	Solvent vent	Solvent vent	Solvent vent
Inlet temperature (°C)	250	100	60	85	85
Initial hold time (min)	-	0.1	0.18	0.15	0.36
First ramp rate (°C/min)	-	-	600	600	600
Final temperature (°C)	280	280	325	325	325
Ramp1 hold time (min)	-	-	5	5	5
Vent time (min)	-	-	0.18	0.15	0.36
Vent pressure (psi)	-	-	5	5	5
Vent flow rate (mL/min)	-	-	100	100	100
Purge time (min)	-	-	2.68	2.65	2.86
Purge flow rate (mL/min)	-	-	60	60	60
Injection rate (µL/min)	-	-	28	340	68

Peak distortion is observed when analytes have undesirable interaction (occurrence of adsorption) with active sites in the inlet and column. In previous section (4.2), states probably reasons for the occurrence of adsorption in the liner. These active sites may be filled with matrix components to minimize interaction and improve peak quality (Anastassiades et al., 2003). According to Pai, (2003), there is a common occurrence of poor chromatographic peak shapes in practice rather than the usual assumption that peaks are symmetrical in literature. Gaussian peak shapes are desired for the improvement of instrument sensitivity which lowers detection limits (Farooq et al., 2017). Pai (2003) states that routine analysts ignore peak distortions as instruments malfunctions or artifacts which does not affect quantification. This is further supported by Keunchkarian et al., (2006), that non-gaussian peaks that are not excessively distorted

are accepted by analysts. In contrast, Anastassiades et. al., (2003) state that quantification by means of identifying and integrating analytes with poor peak shapes is difficult and prone to interferences.

4.6. Solvent vent recovery

A Multimode Inlet (MMI) will perform large-volume injections (LVI) when the mode of sample injection is set to the solvent vent. (Wilson et.al., 2009). The recovery of analytes during different injections is evaluated by calculating the percentage solvent vent recovery (SVR). Comparison is made of the 5 µL, 10 µL and 25 µL LVI technique against the 1 µL HSI. Equation 1 below is used to calculate the percentages by dividing the average solvent vent peak area by the value obtained by multiplying the injection volume of the solvent vent method with the average peak size of the 1 µL HSI method.

Equation 1: Calculation equation of percentage solvent vent recovery.

$$SVR = \frac{average\ solvent\ vent\ peak\ area}{injection\ volume\ of\ the\ solvent\ vent\ method\ \times\ average\ peak\ area\ of\ 1uL\ HSI} \times 100$$

Theoretically, a peak area obtained during the 1 µL HSI method must be enlarged proportionally to the volume injected for the LVI method. In the tutorial, (Agilent Technologies, 2009), peak areas obtained for the 1 µL HSI for the sample used which contained a mixture of hydrocarbons, increased approximately in 5-folds for the 5 µL LVI and in 10-folds for the 10 µL LVI.

Experimentally in this study, analyte peak areas observed for the LVI method were several degrees higher than expected from the peak areas obtained for the HSI method. Table 10 is tabulating peak areas obtained for each analyte during the injection of 1 µL HSI method and different LVI method injections. The average peak areas are calculated from the triplicate run. The expected theoretical peak area value is calculated by multiplying the average 1 µL HSI peak area with the appropriate injection volume. A column of the FCHF internal standard (ISTD) peak areas are included, and relative response (RR) is calculated for each run. RR is calculated by dividing the analyte peak area by the ISTD peak area (Equation 2).

Equation 2: Calculation formula for relative response (RR).

$$RR = \frac{analyte\ peak\ area}{ISTD\ peak\ area}$$

Consistent peak areas were observed for the 3 runs during the application of the 1 µL HSI method for all the analytes (Table 10). However, during the application of the LVI method at different injection volumes, variations in peak areas obtained were noted between the 3 runs at different volumes. The ISTD peak area plays a crucial role as RR may be calculated for each run and inconsistency evaluated. RR obtained during method development studies was not significantly inconsistent as the ISTD peak area decreased/increased in the same magnitude as the analyte peak area. In quantitative determinations that apply the ISTD method, the calibration curve used for quantification is plotted with the y-axis as RR against concentration as the x-axis.

Additionally, peak areas obtained for the LVI methods were several degrees higher than what was expected considering the theoretical peak area calculated for some analytes (MLXN, MLTN, BRMP, MTDN and PRFF).

Solvent vent recovery (SVR) was more than 100% for MLXN, MTDN, and PRFF when comparing the HSI method to all volumes of the LVI method. This was a result of the average peak areas calculated which were higher than the expected theoretical value calculated from the average peak area observed during the HSI run. The calculated SVR for MLTN and BRMP was higher than 100% only during the application of the 5 µL LVI method but lower than 100% for other LVI volumes. Less than 100% solvent vent recovery was observed for CPFS and BRPE when comparing HSI with all LVI method injections. There was a decreasing trend in the percentage of SVR as the injection volumes of LVI increased.

Table 11: Peak areas observed for the HSI method and different volumes of the LVI method. Average peak areas are calculated from each method and the percentage SVR for analytes is calculated at the different LVI volumes.

		FCHF (ISTD)	**MLXN**		**MLTN**		**CPFS**		**BRMP**	
	Name	Area	Area	RR	Area	RR	Area	RR	Area	RR
HSI 1 µl	Run 1	91063	16386	0,1799	419979	4,6120	586401	6,4395	480994	5,2820
	Run 2	87516	16132	0,1843	419766	4,7964	566785	6,4763	497189	5,6811
	Run 3	91683	18329	0,1999	440456	4,8041	578666	6,3116	493827	5,3863
	Average		**16949**		**426734**		**577284**		**490670**	
	Theoretical:									
	x5		**84745**		**2133668**		**2886420**		**2453350**	
	x10		**169490**		**4267337**		**5772840**		**4906700**	
	x25		**423725**		**10668342**		**14432100**		**12266750**	
LVI 5 µl	Run 1	607439	307563	0,5063	2832659	4,6633	3178387	5,2324	3070301	5,0545
	Run 2	520488	255521	0,4909	2568789	4,9354	2881208	5,5356	2550649	4,9005
	Run 3	417860	161954	0,3876	2003247	4,7941	2385709	5,7094	2081395	4,9811
	Average		**241679**		**2468232**		**2815101**		**2567448**	
	%SVR		**285**		**116**		**98**		**105**	
LVI 10 µl	Run 1	473747	403754	0,8523	2990540	6,3125	2997329	6,3269	2939716	6,2052
	Run 2	534577	434376	0,8126	3262838	6,1036	3316396	6,2038	3179101	5,9469
	Run 3	572233	462355	0,8080	3612535	6,3131	3610289	6,3091	3509881	6,1337
	Average		**433495**		**3288638**		**3308005**		**3209566**	
	%SVR		**256**		**77**		**57**		**65**	
LVI 25 µl	Run 1	907135	1028289	1,1336	5792763	6,3858	5472573	6,0328	5922298	6,5286
	Run 2	1484959	1479475	0,9963	7447528	5,0153	8528178	5,7430	9723016	6,5477
	Run 3	1571112	1732228	1,1025	8351166	5,3154	9128258	5,8101	10825426	6,8903
	Average		**1413331**		**7197152**		**7709670**		**8823580**	
	%SVR		**334**		**67**		**53**		**72**	

Table 11 continued: Peak areas observed for the HSI method and different volumes of the LVI method. Average peak areas are calculated from each method and the percentage SVR for analytes is calculated at the different LVI volumes.

		FCHF (ISTD)	**MTDN**		**BRPE**		**PRFF**	
	Name	Area	Area	RR	Area	RR	Area	RR
HSI 1 µl	Run 1	91063	401779	4,4121	681011	7,4785	140732	1,5454
	Run 2	87516	385597	4,4060	693078	7,9194	135525	1,5486
	Run 3	91683	432385	4,7161	696704	7,5991	151750	1,6552
	Average		**406587**		**690264**		**142669**	
	Theoretical:							
	x5		**2032935**		**3451322**		**713345**	
	x10		**4065870**		**6902643**		**1426690**	
	x25		**10164675**		**17256608**		**3566725**	
LVI 5 µl	Run 1	607439	4056905	6,6787	3715972	6,1174	1277219	2,1026
	Run 2	520488	3331816	6,4013	3282465	6,3065	1003472	1,9279
	Run 3	417860	2484780	5,9464	2796543	6,6925	753098	1,8023
	Average		**3291167**		**3264993**		**1011263**	
	%SVR		**162**		**95**		**142**	
LVI 10 µl	Run 1	473747	4846736	10,2310	3941945	8,3208	1664254	3,5130
	Run 2	534577	4980966	9,3176	4213080	7,8811	1715069	3,2083
	Run 3	572233	5450892	9,5257	4671586	8,1638	1868515	3,2653
	Average		**5092865**		**4275537**		**1749279**	
	%SVR		**125**		**62**		**123**	
LVI 25 µl	Run 1	907135	13456312	14,8340	7032754	7,7527	4474829	4,9329
	Run 2	1484959	15663834	10,5480	10182394	6,8570	6126633	4,1258
	Run 3	1571112	17624108	11,2180	11137482	7,0889	6746071	4,2938
	Average		**15581418**		**9450877**		**5782511**	
	%SVR		**153**		**55**		**162**	

When evaluating MLXN, 285% SVR was calculated for the 5 µL LVI method. The average peak area calculated from the triplicate injection of the HSI method was 16494 (Table 10). Theoretically, a 5 µL volume of LVI should produce a peak area of 84745 (5 × 16494), for a 10 µL volume of LVI a peak area of 164940 (10 × 16494) and peak area of 423725 (25 × 16494) for the 25 µL LVI run. However, experimentally, the peak areas observed were more than 2 times larger. This resulted in higher than 100% SVR being calculated.

A probable explanation for the higher than 100% in SVR was the possibility that peak area observed for HSI was not a true reflection of the analyte present in the sample. This meant that analyte loss might have occurred during the HSI method application. According to (Wilson et. al., 2009), HSI method application becomes a challenge for analytes that are thermally unstable, and loss of analyte might occur due to the high constant temperature applied in HSI. Thermal degradation of the analyte is observed, hence the small peak area.

A minor experiment was conducted to supplement this reasoning. A comparison between an HSI method of 1 µL and 3 µL of the analytes was conducted. The aim of the additional experiment was to evaluate if the 1 µL HSI run would produce 3 times the peak size during the injection of 3 µL of the sample. Additionally, the purpose of the experiment was also to check the consistency of instrument injections. Figure 31 below shows the tabulated peak area values observed during the experiment.

Peak areas observed for the 3 µL HSI method were more than 3 times the peak areas observed for the 1 µL HSI method. It is evident that the probable reason is that with the 1 µL HSI method, loss of analyte occurs resulting in an even smaller peak area of the analyte being observed.

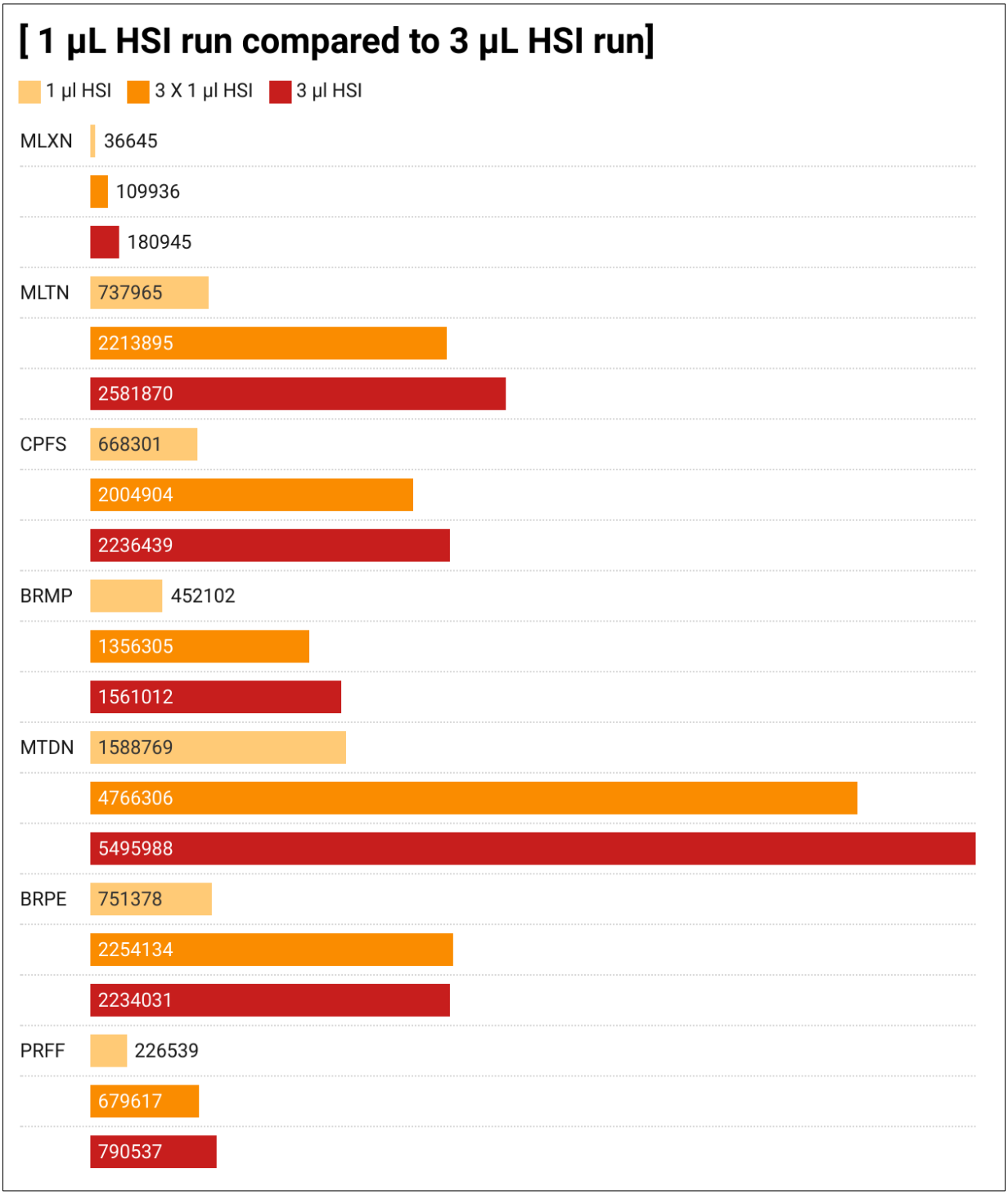

Figure 31: Additional experiment to evaluate differences in the injections for 1 µL and 3 µL HSI methods.

4.7. Optimization of parameters

Method development studies had been concluded at this stage. An appropriate injection volume from those investigated (5, 10, and 25 µL) was chosen to commence method optimization studies. Although, 10 µL LVI was a satisfactory sample injection volume observing results obtained during method development, factors mentioned in section 4.3.3 had to be considered. These factors included sample throughput and instrument maintenance that would be required (lifetime of column, consumables, etc). The chosen volume for method optimization studies was 5 µL LVI.

Four (4) method parameters of 5 µL LVI were investigated for optimal conditions. Parameters include vent flow, vent pressure, vent time, inlet, and oven temperatures. Additionally, different instrument liners were evaluated for efficiency. When investigating a certain parameter, other parameters were kept constant. Table 11 below provides a summary of the parameter settings (extracted from Figure 18) given by the instrument which was investigated. Peak areas, peak shapes and resolution of the peaks are presented and extensively discussed.

Table 12: Summary of starting parameter settings given by the instrument for the 5 µL LVI method which was investigated for optimal conditions.

Vent flow rate	100 mL/min
Vent pressure	5 psi
Vent time	0.18 min
Inlet temperature	60 ºC
Oven initial temperature	50 ºC

It is important to note that because the study was conducted in a laboratory that has a high sample throughput, the instrument used for the study was concurrently used for routine samples. Regular maintenance such as a change in the retention gap and analytical column was performed in the interim. Changes in the retention gap and analytical column meant retention times of the pesticides would change. This was observed when method optimization studies commenced. A blank apple juice sample was spiked and prepared as described in section 3.6 of the thesis. The sample was analysed using the 5 µL LVI method parameters given by the instrument as starting parameters (Figure 18 – Chapter 3). The chromatogram obtained from the analysis (Figure 32) was treated as a standard chromatogram and a comparison of each method parameter was investigated against it.

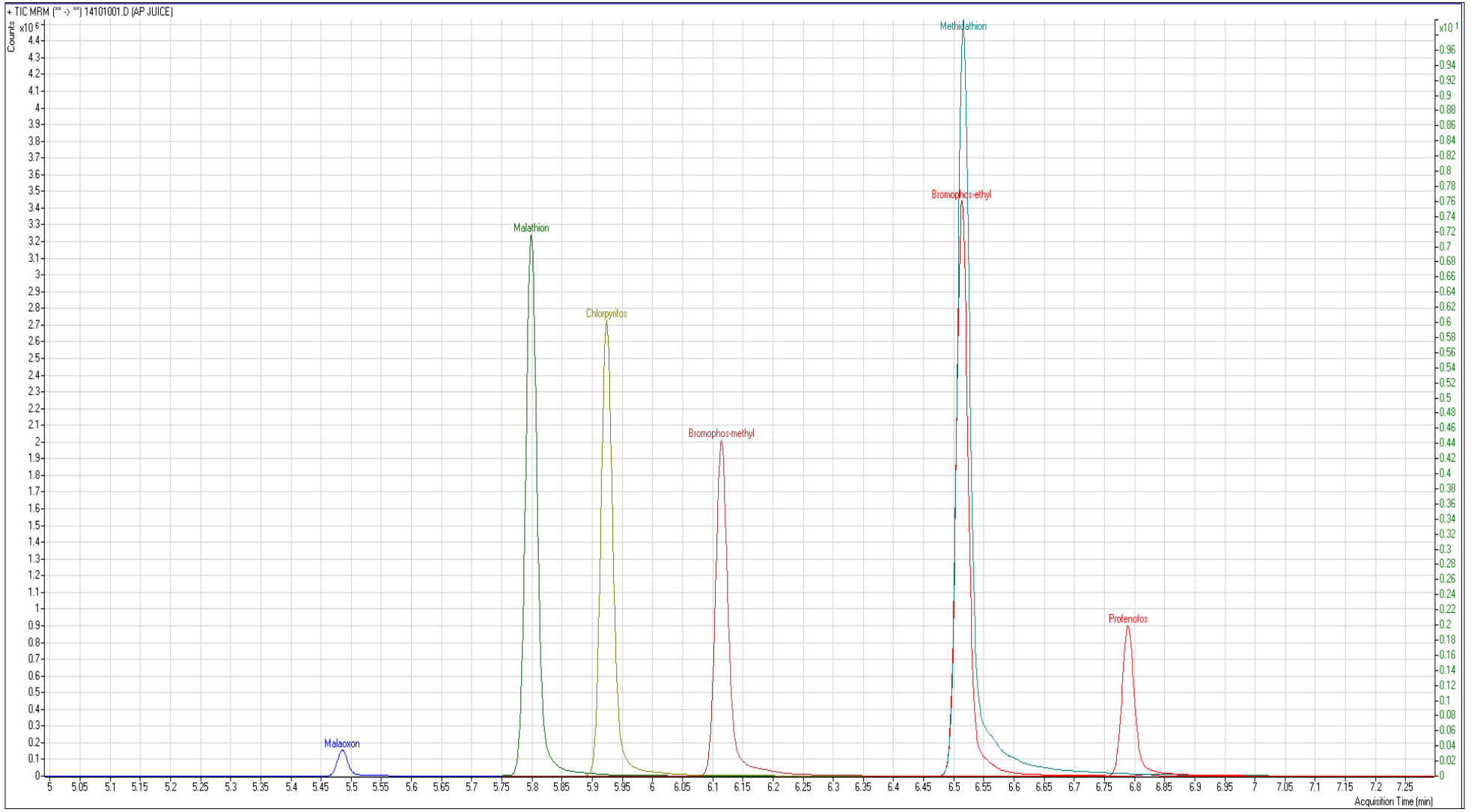

Figure 32: A GCMS/MS standard chromatogram obtained using the 5 µL LVI method for the analysis of spiked apple juice sample.

4.7.1. Vent flow rate

A 100 mL/min was the standard condition for vent flow given by the software (Table 11). To investigate how vent flow affects the analytes, 50, 150, 200, and 300 mL/min conditions were investigated. Table 12 below indicates the peak areas of each analyte obtained for the vent flows. A graphical representation of the changes in peak areas for each pesticide during vent flow changes is depicted in Figure 33.

Table 13: Resulting peak areas for various vent flow conditions.

	Vent Flow (mL/min)								
	100	**50**		**150**		**200**		**300**	
MLXN	198241	110131	44%↓	120567	39%↓	94624	52%↓	49806	75%↓
MLTN	4475632	3011704	33%↓	3647363	19%↓	3589149	20%↓	3325981	26%↓
CPFS	3911233	2689018	31%↓	4112150	5%↑	4097608	5%↑	4027544	3%↑
BRMP	3037100	2010378	34%↓	3272197	8%↑	3271280	8%↑	3146661	4%↑
BRPE	5003548	3571210	29%↓	6334470	27%↑	6386941	27%↑	6354291	27%↑
MTDN	7296988	4517192	38%↓	3987966	45%↓	3661247	50%↓	2624700	64%↓
PRFF	1364451	794643	42%↓	1100985	19%↓	1054100	23%↓	917686	33%↓

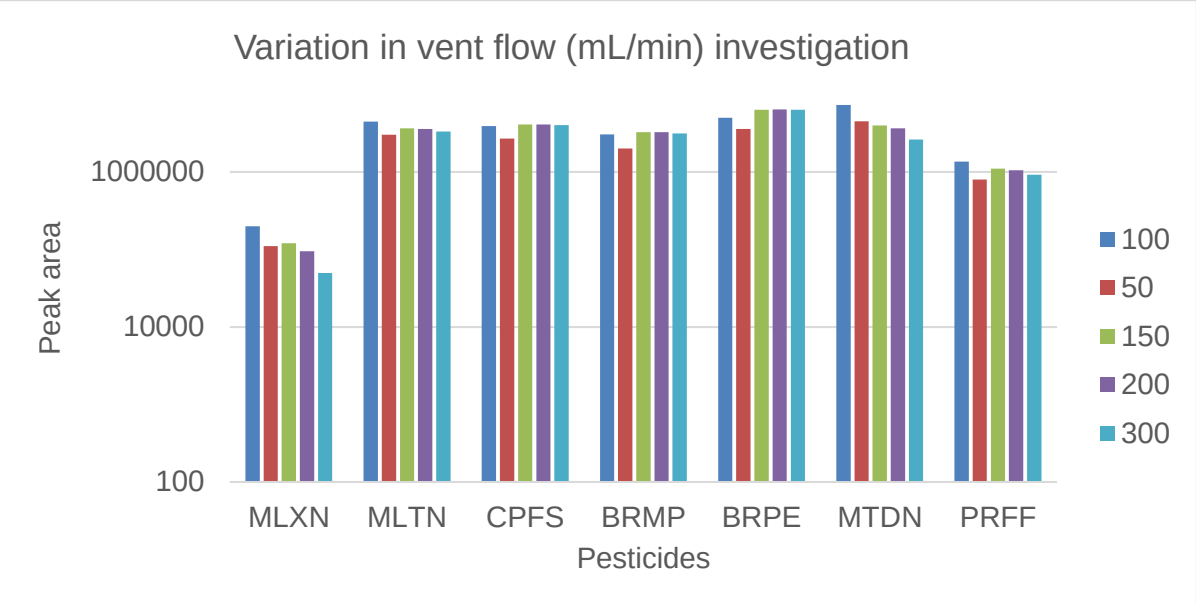

Figure 33: Graphical representation of the peak areas obtained at different vent flows.

The 100 mL/min vent flow peaks showed greater peak areas for the early eluting compounds and the late eluting pesticides. CPFS, BRMP, and BRPE showed peak areas had slightly increased with the change in vent flow. Slight peak tailing of MTDN and BRPE was observed for the 100 mL/min vent flow run. This remained the case for all the changes in vent flow conditions. The 150, 200, and 300 mL/min vent flows caused the MTDN peak to reduce in size compared to the 100 mL/min vent flow. In both the 50

mL/min and 100 mL/min, MTDN was greater than the BRPE peak. The 100 mL/min vent flow was believed to be the optimal condition and was not changed.

4.7.2. Vent pressure

The starting condition for vent pressure given by the solvent elimination calculator integrated into the software is 5 psi (Table 11). The chromatogram obtained using a vent pressure of 5 psi is seen in Figure 32. Vent pressure of 1 and 2,5 psi was investigated. Similarly, these vent pressure conditions were investigated in the studies presented by (Zhao et al., 2012). In their studies, higher peak responses were observed for 2,5 psi and 5 psi compared to the 1 psi vent pressure peaks. The opposite was observed in this study. The peaks obtained for 1 psi and 2,5 psi vent pressure were relatively similar in peak size for all the pesticides. The early and late eluting pesticides decreased in peak size compared to the peaks obtained using a vent pressure of 5 psi. Whereas the middle eluting pesticides increased slightly in peak size compared to the 5-psi vent pressure run. Table 13 below shows the peak areas obtained for the pesticides at different pressures. Figure 32 is a graphical illustration of the peak areas obtained at different vent pressures.

In the studies of Zhao et al., (2012), a vent pressure of 2,5 psi was chosen as the optimal condition. The reason for this as stated, is that 2,5 psi was the better option due to less solvent being loaded into the column before the transfer of analytes when low vent pressure is applied. It must be noted that in their studies (Zhao et al., 2012), all the investigated vent pressures showed peak splitting, and this can be attributed to the use of acetonitrile as the solvent. In this presented study, no peak splitting was observed for all the pesticides (Figure 34). Different from their studies, the solvent used was toluene. MLXN showed a low peak response as lower vent pressures were applied (Figure 35). It is for this reason that vent pressure was kept at 5 psi as the optimal condition.

Table 14: Resulting peak areas for various vent pressure conditions.

	Vent pressure (psi)				
	5	1		2,5	
MLXN	198241	97519	51%↓	96105	52%↓
MLTN	4475632	3846033	14%↓	3867784	14%↓
CPFS	3911233	4318191	10%↑	4307961	10%↑
BRMP	3037100	3429061	13%↑	3377339	11%↑
BRPE	5003548	6725784	34%↑	7051269	41%↑
MTDN	7296988	3568386	51%↓	3568372	51%↓
PRFF	1364451	1132174	17%↓	1111546	19%↓

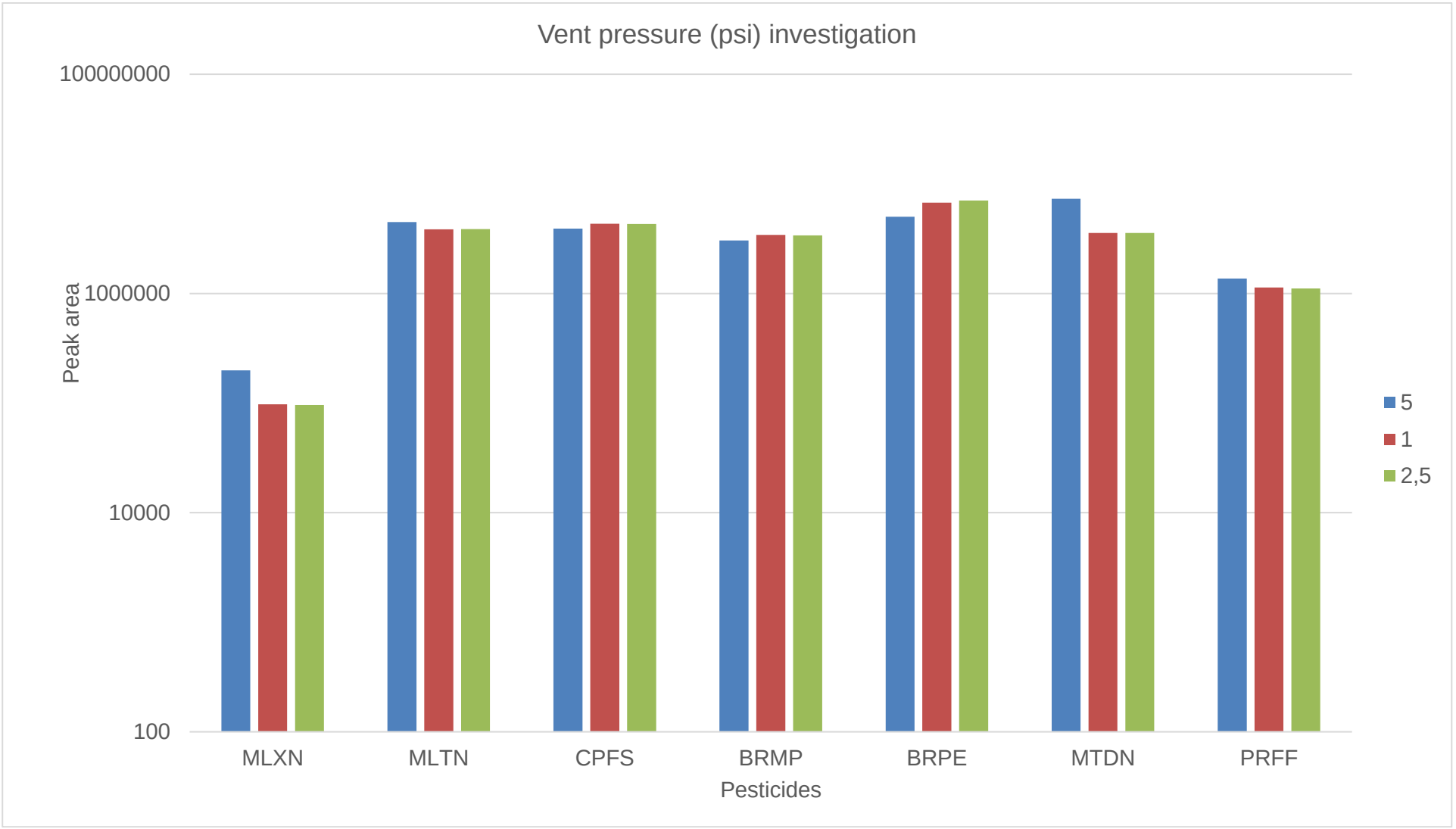

Figure 34: Graphical representation of pesticide peak area variations at different vent pressure.

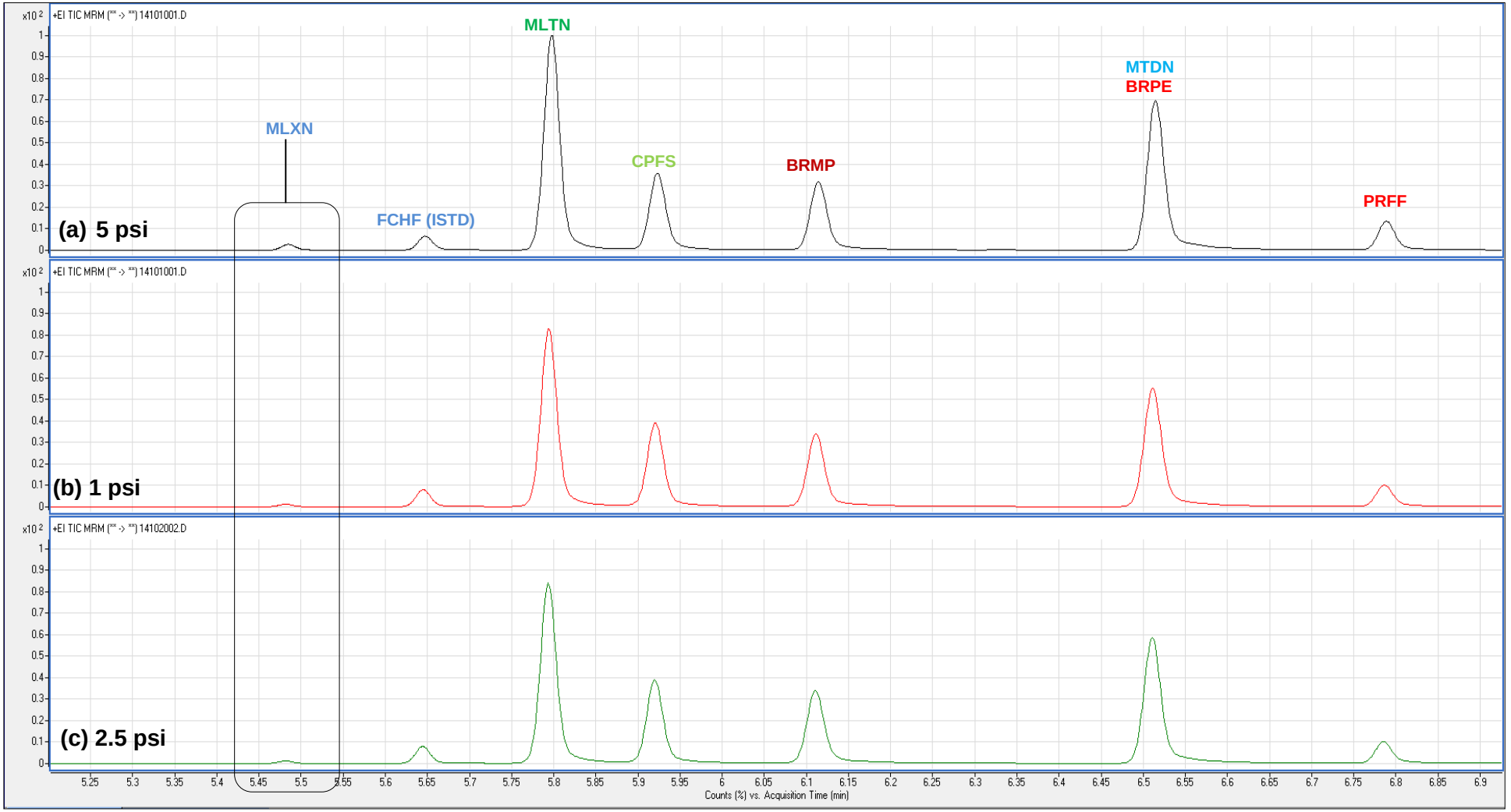

Figure 35: TIC obtained using vent pressure of **(a)** 5 psi, **(b)** 1 psi and **(c)** 2,5 psi.

4.7.3. Vent time

When vent time was investigated in the (Zhao et al., 2012) studies, parameter settings that were already optimized (vent flow, inlet temperature, and vent pressure) were applied to the method. The new calculated values of elimination rate, injection rate, and vent time were obtained from the software. During the investigation of vent time in this presented study, parameters that had already been investigated (vent flow and vent pressure) were not changed from the initial parameters calculated by the software (Table 11). This is because no changes needed to be applied. The values calculated by the software proved to be the optimal conditions for vent flow and vent pressure.

The chromatogram (Figure 32) was used as a reference point and the vent time was 0.18 min. Additional vent times of 0,06 min, 0.09 min, 0,21 min and 0.5 min were investigated. Peak areas obtained for each pesticide for the different vent times can be seen in Table 14 and a vent time of 0,5 min resulted in an increase of peak size for all the pesticides. Whereas, 0.09 min and 0,21 min vent times, only middle eluting compounds slightly increased in peak size compared to the 0,18 min vent time run. When 0,06 min vent time was applied, all the pesticides reduced in peak size except BRPE which increased by 22% from the 0,18 min vent time run (Table 14).

It was noted that holding the vent time for 0,5 min was better as peak areas increased for all analytes. This can be a result of most solvents being vented out and the analytes being focused on the liner before entering the column. This description agrees with what is published by (Zhao et al., 2012) who suggests that extending vent time improves the peak shape and reduces the amount of solvent reaching the column. A graphical representation of peak areas obtained from all pesticides is noted in Figure 36. The optimal condition for vent time was 0.5 min.

Table 15: Resulting peak areas for various vent time conditions.

	Vent time (min)								
	0,18	**0,06**		**0,09**		**0,21**		**0,5**	
MLXN	198241	147099	26%↓	52552	73%↓	135675	32%↓	289915	46%↑
MLTN	4475632	3674761	18%↓	3505562	22%↓	4267474	5%↓	5666169	27%↑
CPFS	3911233	3891610	1%↓	4313663	10%↑	4427487	13%↑	5128123	31%↑
BRMP	3037100	2998995	1%↓	3220390	6%↑	3744572	23%↑	4597778	51%↑
BRPE	5003548	6106649	22%↑	6655945	33%↑	6888116	38%↑	7911575	58%↑
MTDN	7296988	4261868	42%↓	2204606	70%↓	4206983	42%↓	7596383	4%↑
PRFF	1364451	1229939	10%↓	839689	38%↓	1228951	10%↓	1864697	37%↑

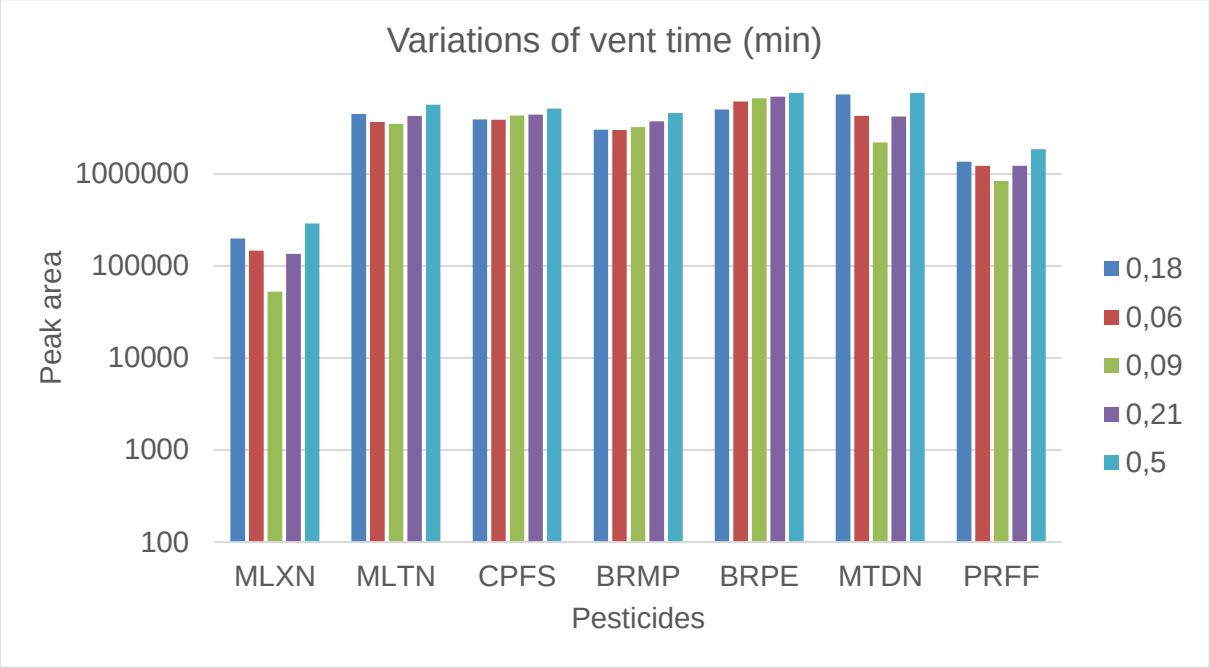

Figure 36: Graphical representation of the obtained peak area of the pesticides at different vent times.

4.7.4. Inlet temperature

An inlet temperature of 60 ˚C was the starting temperature chosen by the software (Table 11) during method development. An investigation into the changes in inlet temperature commenced with gradual increases from 60 ˚C to 70 ˚C, 85 ˚C and 100 ˚C. According to (Zhao et al., 2012), satisfactory recoveries of analytes during LVI analysis are achieved by an inlet temperature setting that does not exceed 5 ˚C below the boiling point of the solvent used. This implied that, toluene being the solvent used in these studies, inlet temperature must not exceed 105,6 ˚C because the boiling point of toluene is 110,6 ˚C. Additionally, the inlet temperature setting should not be set too close to the boiling point of the solvent or higher (Zhao and Meng, no date).

The resulting peak areas can be seen in Table 15 below. Decreases and increases of peak areas are indicated with respect to the inlet temperature of 60 ˚C run peak area obtained for all pesticides. Increasing inlet temperature from 60˚C showed a decrease in peak areas for the early and late eluting pesticides. Slight increases were observed for the middle eluting pesticides. MLXN peak showed a greater reduction of peak size of 81% during the 70 ˚C inlet temperature run, 71% for the 85 ˚C, and 83% for the 100 ˚C run. According to (Zhao et al., 2012) analyte losses are due to a faster solvent evaporation rate which is due to higher inlet temperature settings. This indicated that the inlet temperature must be kept at 60 ˚C.

Table 16: Resulting peak areas for various inlet temperature conditions.

	Inlet temperature (°C)							
	60	**70**		**85**		**100**	
MLXN	198241	38275	81%↓	57027	71%↓	33117	83%↓
MLTN	4475632	3385528	24%↓	3219694	28%↓	3192944	29%↓
CPFS	3911233	4069370	4%↑	4073287	4%↑	4135827	6%↑
BRMP	3037100	3038610	0,05%↑	3087109	2%↑	3100641	2%↑
BRPE	5003548	6385856	28%↑	6307386	26%↑	6525221	30%↑
MTDN	7296988	2222858	70%↓	2399266	67%↓	1889770	74%↓
PRFF	1364451	852004	38%↓	912734	33%↓	830228	39%↓

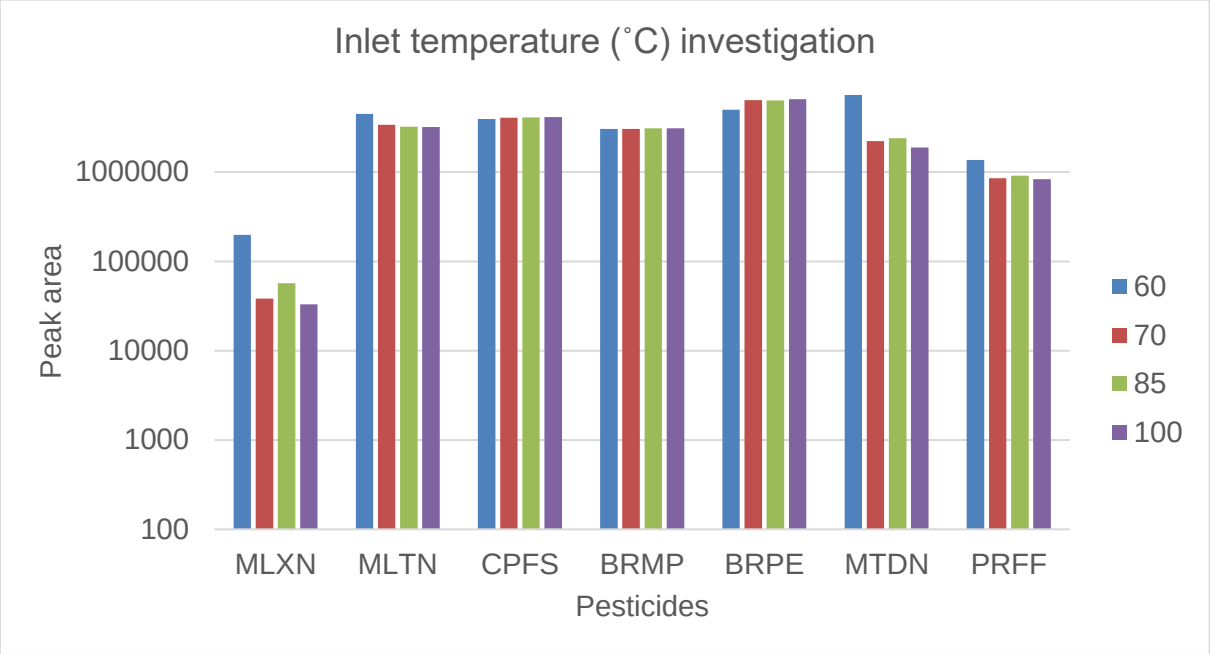

Figure 37: Graphical representation of changes in inlet temperature for all the pesticides.

4.7.5. Oven temperature

McNair et al., (2009) supports the use of temperature programming in GC analysis as it can improve analyte separation significantly. Partition coefficients of various analytes is decreased with temperature increases, causing analytes to move through the column quickly. A challenge encountered during the investigation of oven temperature is that the instrument refused to start when there was a big difference between the oven and inlet temperatures. When the oven is too hot, lowering the inlet temperature is nearly impossible due to the oven and injection port positioned close to each other. Cryogenic conditions must be applied to cool the injection port and compressed air was the coolant used in the study.

Figure 18 shows the solvent elimination wizard suggested an initial oven temperature of 50 ˚C. All the suggested conditions are optional, initial oven temperature and initial oven hold time of 2,68 min were not loaded into the method. When oven temperature is changed, there will be a

shift in the RT of the pesticides. The higher the oven temperature, the earlier the pesticides elute. A lower oven temperature meant the pesticides would elute later. Therefore, the run method changes had to be applied to see all the pesticides within the run time.

Starting oven temperature was set at 85 ˚C instead of 50 ˚C to avoid longer run times. Oven temperatures of 60, 70, and 100 ˚C were investigated, and the effects of the pesticides were studied. The inlet temperature was kept at 60 ˚C while investigating the various oven temperatures. Figures 38, 39, and 40 illustrate the TIC obtained for the different oven temperatures compared to the 85 ˚C run (Figure 32). Improvements in the sensitivity of the method was observed at lower temperature settings. Figure 38 (b), 60 °C and figure 39 (c), 70 °C illustrates the efficiency of the method to detect at lower levels which could not be detected when oven temperature was set at 85 °C. Peak areas were observed to also improve with the lowering of oven temperature conditions from 85 °C (see Table 16). A higher temperature of 100 ˚C caused some peak areas to decrease such as MLXN, MLTN, BRPE, and PRFF. A 23% decrease in peak area was observed for MLXN, 3% for MLTN, 8% for BRPE and 1% for PRFF compared to the peak area obtained from an oven temperature of 85 °C. The decrease indicated loss of analytes during sample analysis. The significance of this observation is the inaccurate measurements that would be produced using a 100 °C oven temperature in this regard.

When the oven temperature was set to 70 ˚C, peak areas for all pesticides increased more than the 60 ˚C oven temperature run (Table 16). Enhanced peak sizes obtained when 70 °C was used indicated sensitivity of the method had improved, chances of detecting analytes at lower levels are increased at this temperature setting. An oven temperature of 70 ˚C was set as the optimal condition. Figure 41 illustrates graphically, peak size increases observed for 60°C and 70 °C temperature setting and the decrease observed for most pesticides when the 100 °C was applied.

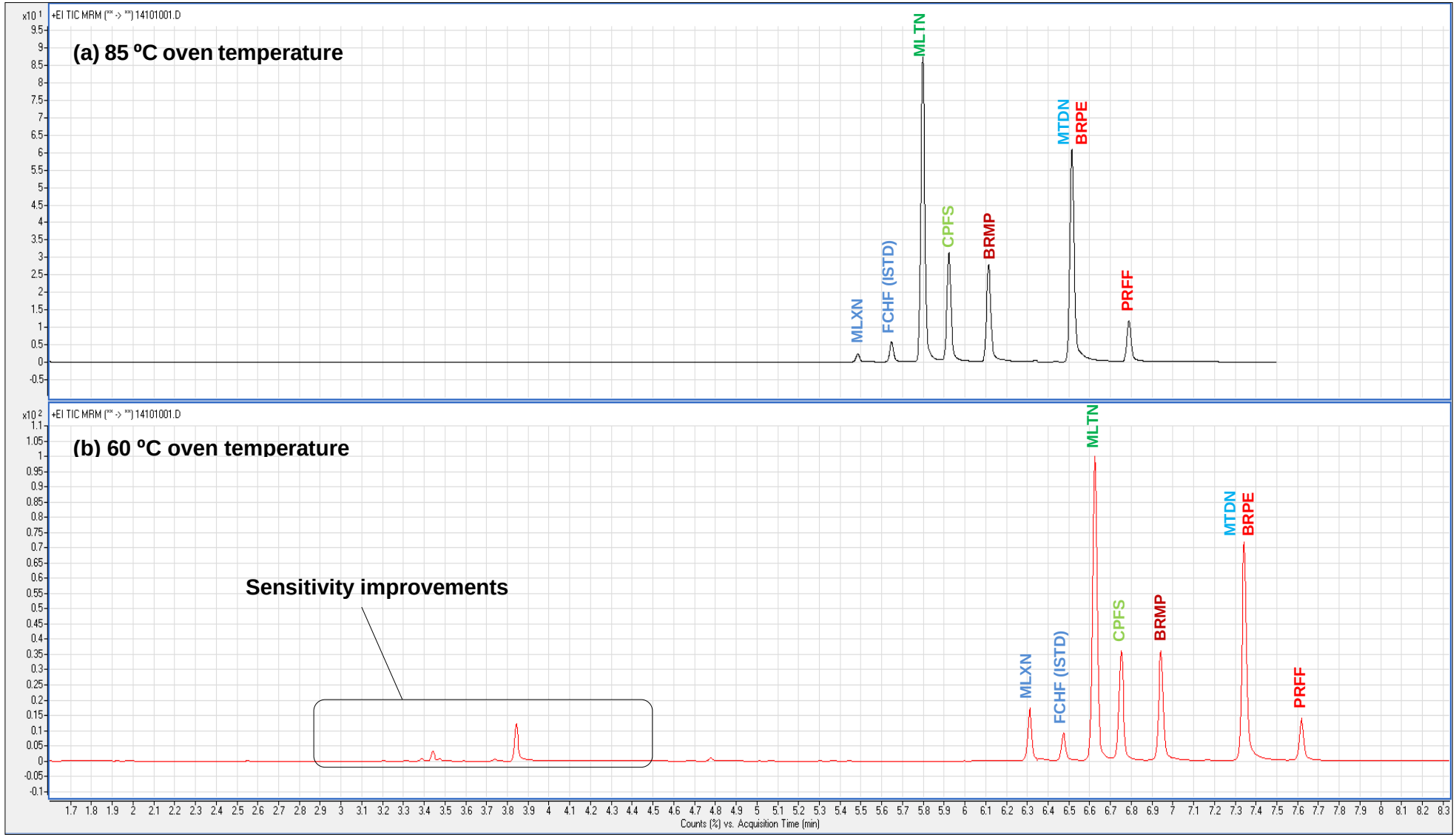

Figure 38: TIC showing effects of changes in oven temperature setting of **(a)** 85 ˚C and **(b)** 60 ˚C.

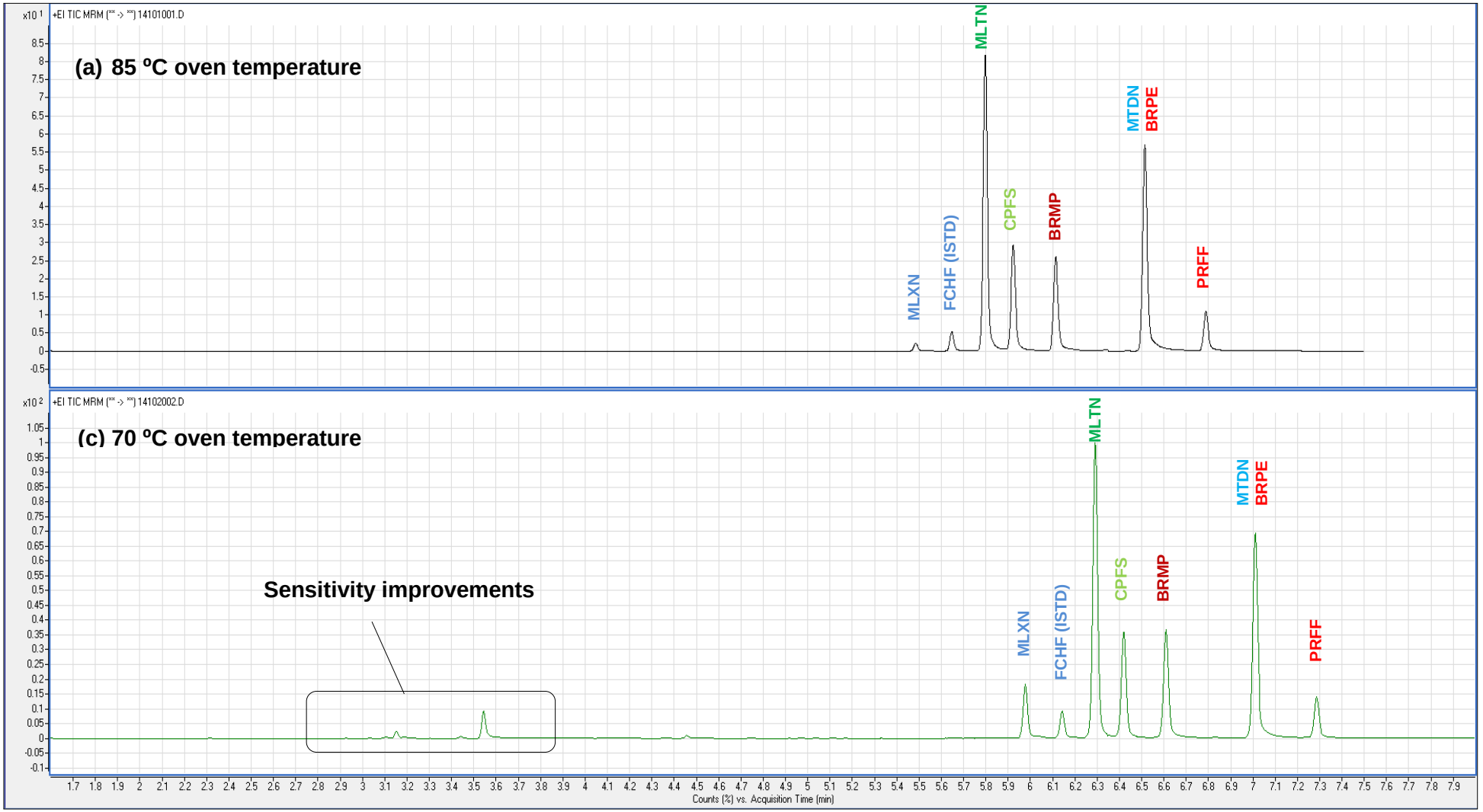

Figure 39: TIC showing effects of changes in oven temperature setting of **(a)** 85 ˚C and **(c)** 70 ˚C

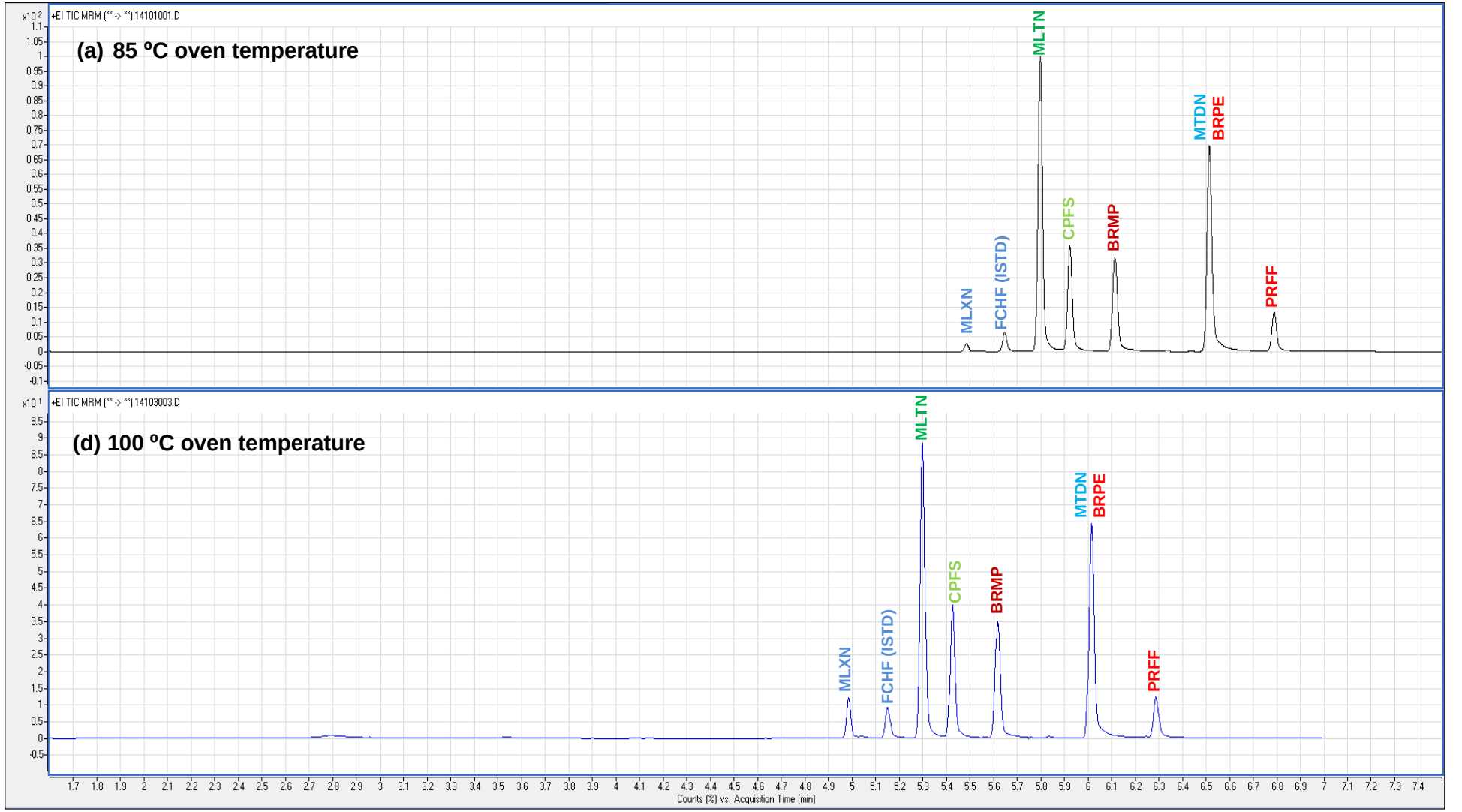

Figure 40: TIC showing effects of changes in oven temperature setting of **(a)** 85 ˚C and **(d)** 100 ˚C.

Table 17: Resulting peak areas for various oven temperature conditions.

	Oven temperature (°C)							
	85	60		70		100		
MLXN	198241	259969	31%↑	297101	50%↑	152167,4	23%↓	
MLTN	4475632	5704646	27%↑	6204247	39%↑	4331466	3%↓	
CPFS	3911233	5116336	31%↑	5295523	35%↑	4599987	18%↑	
BRMP	3037100	4586519	51%↑	4859940	60%↑	3736871	23%↑	
BRPE	5003548	7960826	59%↑	8079036	61%↑	4602238	8%↓	
MTDN	7296988	7383704	1%↑	8251975	13%↑	7309775	0,2%↑	
PRFF	1364451	1757796	29%↑	1917788	41%↑	1353937	1%↓	

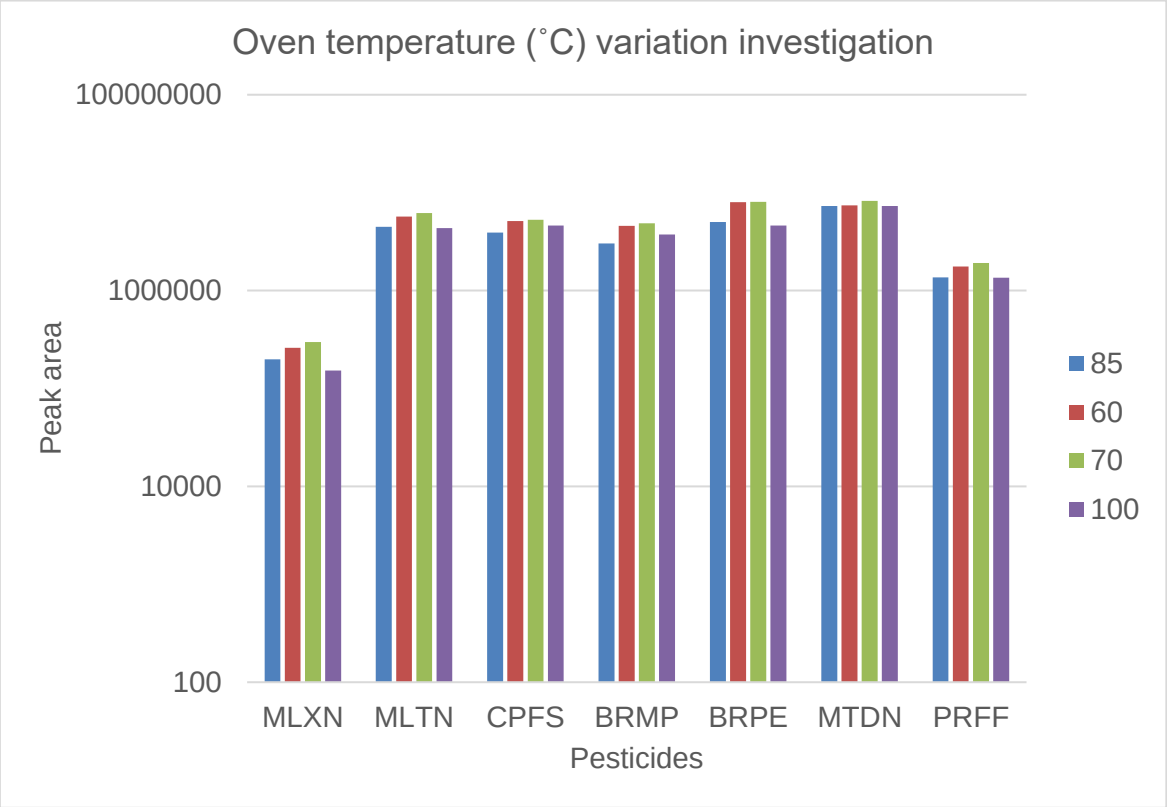

Figure 41: Graphical representation of peak areas obtained at different oven temperatures.

4.7.6. Inlet liners

Numerous publications including Walorczyk, (2012) and Godula et al., (2001) place emphasis on the effects of inlet liners used during the analysis of samples. Walorczyk, (2012) states liners are manufactured in different shapes and sizes, therefore the volume of the sample injected depends on the liner volume. When considering both methods, (HSI and LVI), the sample is vaporized in the liner and a vapour 'cloud' is formed. In HSI, the vapours stay in the liner until transfer to the column is completed while the split valve is kept closed. In LVI, an even larger vapour cloud is formed because of the larger volume

of sample injected. Godula et al., (2001) explains this in terms of the importance of the internal diameter of the liner. There is better heat transfer and analytes are transferred further to the column when liners with smaller internal diameters are used. Additionally, liners with larger internal diameters are beneficial for injecting larger sample volumes.

According to Walorczyk, (2012), another important aspect to consider is the packing material of a liner. In the presented study, liners packed with wool were utilized. Walorczyk, (2012) regards these liners as frequently used even though for pesticide analysis, thermal degradation, and adsorption often occur. According to Walorczyk, (2012), peak tailing of analytes is a result of pesticides interacting with active sites of the packing material of the liner. Zhao et al., (2012) supports the use of wool-packed liners because those liners help retain analytes while the solvent is being vented out during LVI.

Figure 42 below shows the 2 liners investigated in the study for any significant changes. The packing material was wool for both liners. However, suppliers were different. Slight shifts in retention times for the analytes were observed with the change in liners. There was no major change in the peak shapes and peak areas. Therefore, the decision on which liner would be suitable was purely based on the price of the consumable.

Figure 42: Different inlet liners used during the study.

In a study conducted by Godula et al., (2001), wherein a PTV injector was optimized for application in the analysis of pesticide residues, an empty liner and a liner packed with silanized glass wool were investigated. Peak losses were observed for certain analytes due to thermal degradation and certain analytes adsorbed on active sites present in the glass wool surface. It was only the inert pesticides that had improved peak shapes.

4.8. Evaluation of HSI and LVI methods

In this section, method validation parameters such as specificity, limit of detection (LOD), limit of quantification (LOQ), repeatability, and trueness of the developed LVI and HSI method was determined and discussed. Each method parameter for all the pesticides of interest in the study was evaluated in apple juice sample and orange juice sample to determine if the methods are fit for the purpose of the analysis of organophosphates. The results are summarized in Table 17 for the apple juice and Table 18 for the orange juice sample for all the method validation parameters evaluated.

4.8.1. Specificity

The term ‘specificity’ is a preferred term in sectors such as the pharmaceutical industry over ‘selectivity’. However, both terms relate to the method’s ability to identify and in some cases quantify analytes of interest in the presence of similar behaving substances without any interference (Magnusson et al., (eds.) Eurachem Guide:2014). United Nations Office on Drugs and Crime Laboratory and Scientific Section., (2009) states that specificity is a method parameter that is concentration-based and is to be determined at a low-end range of the calibration curve. Additionally, impurities and cross-reacting substances present in the sample matrix should be known and effects determined.

Specificity is determined by assessing if the response in the reagent blank and sample blank is less than 30% of the response at the reporting limit of the analyte (SANTE 11312:2021). The reporting limit of all the compounds is 0.01 ppm. The second standard of the calibration standards (STD 2) was spiked at approximately 0.01 ppm. Therefore, 30% of the response of STD 2 was calculated. The response obtained for the reagent blank and sample blank was inspected if it was higher or lower that 30% of the response at the reporting limit level. Appendix I contains the calculations done to determine specificity in the juice samples using both methods for each analyte. All the pesticides met the criteria of < 30 % of RL deeming specificity accepted for determination of these pesticides in both acquisition methods (Table 35 – 38 Appendix I)

4.8.2. Limit of detection (LOD)

LOD is the lowest concentration at which the method can determine an analyte with a specified level of confidence (Magnusson et al., (eds.) Eurachem Guide:2014). Additionally, it can be defined as the lowest concentration of analyte not confused with background noise with certainty (United Nations Office on Drugs and Crime. Laboratory and Scientific Section., 2009). According to (Magnusson et al., (eds.) Eurachem Guide:2014) parameter is determined using Equation 3 below.

Equation 3: Equation for the calculation of LOD.

$$LOD = 3 \times S'_0$$

S'_O is standard deviation used to calculate LOD and LOQ. It is calculated using Equation 4 below.

Equation 4: Equation for the calculation of standard deviation.

$$S'_0 = \frac{S_0}{\sqrt{n}}$$

Whereby, S_0 is the estimated standard deviation of m single results at or near zero concentration, m being the number of observations. A significant number of observations would typically be between 6 – 15 replicates to obtain adequate estimation of standard deviation. Most methods use $n = 1$ which is the number of replicate analyses carried out on each reporting result where each replicate is obtained following the entire procedure (Magnusson et al., (eds.) Eurachem Guide:2014).

Comprehensive calculations of LOD for apple juice sample using the HSI are found in Appendix J, and Appendix L for the LVI method. Orange juice sample calculations for LOD using HSI method is seen in Appendix N, and Appendix P for LOD calculations using the LVI method. The results are summarized in Table 17 for apple juice and Table 18 for orange juice below.

4.8.3. Limit of quantification (LOQ)

LOQ refers to the lowest concentration of analyte which meets the criteria of method performance acceptable for precision and trueness (SANTE 11312:2021). LOQ is calculated using Equation 5.

Equation 5: Equation used for the calculation of LOQ.

$$LOQ = 5 \times S'_0$$

LOQ was determined for all the pesticides using different methods. The apple juice sample results are noted in Appendix J for the HSI method, and Appendix L for the LVI method. Appendix N and P contain orange juice sample results for HSI and LVI methods, respectively.

4.8.4. Repeatability (%RSD)

The HSI and LVI methods were also assessed for precision and presented as repeatability. The parameter is expressed as relative standard deviation (RSD) and tests the closeness of a series of replicate results under the same conditions of the method. These conditions include the same analyst, measurements are taken on the same day using the same instrument in the same laboratory (United Nations Office on Drugs and Crime. Laboratory and Scientific Section., 2009). According to (SANTE 11312:2012), this parameter should be tested at the targeted RL (approximately 0.01 ppm) and a higher level within the working range (approximately 0.1 ppm). RSD is calculated using Equation 6.

Equation 6: Equation used to calculate RSD.

$$RSD = \frac{Standard\ deviation\ (S_0)}{Average}$$

According to (SANTE 11312:2021), the acceptable method performance criteria for repeatability is that RSD must be ≤ 20.

Appendix J and L contain repeatability calculations for the apple juice samples using HSI and LVI methods. Repeatability results are the orange juice sample are noted in Appendix N and P for both methods.

4.8.5. Trueness (%Recovery)

The trueness of the methods was also evaluated to determine if the acceptable analyte recovery criteria of 70 – 120% were met. Analyte recovery is the extent of the difference between detected responses of analyte when it is added into a sample and extracted and in a pure authentic standard (United Nations Office on Drugs and Crime. Laboratory and Scientific Section., 2009). Matrix matched (MM) calibration standards are prepared

and used to determine recovery at targeted RL (approximately 0.01 ppm) and at a higher level in the calibration range (approximately 0.1 ppm). The parameter was calculated for each pesticide using Equation 7.

Equation 7: Equation used for the calculation of recovery.

$$Recovery = \frac{observed\ concentration}{true\ concentration}$$

The data pertaining to calculations of recovery is contained in Appendix K and M for HSI and LVI methods for the analysis of apple juice samples. Recovery calculations for orange juice samples are appended in Appendix O and Q of the thesis.

LOD and LOQ showed higher concentration levels in the LVI method compared to the HSI method for the pesticides except for MLXN in orange juice sample. LOQ for MLXN in the HSI method was 0.0068 (Table 17) ppm which boarded very close to the reporting limit concentration of 0.01 ppm.

All the pesticides in both methods during the analysis of the AP JU sample were less than 20%, deeming %RSD as acceptable at both levels of 0.01 and 0.1 ppm. Even though the recoveries of all the pesticides in both methods were within an acceptable range of 70 – 120 %, it was higher in the LVI method compared to the HSI method.

The OR JU sample LOQ of MLXN in the LVI method was 0.0084 ppm (Table 18), contradictory to what was observed in the AP JU sample. Repeatability of the methods was also satisfactory for the analysis of the OR JU sample as it was below the 20% mark for all the pesticides. MLXN, MLTN and MTDN were not effectively extracted from the sample when the LVI method was used for analysis compared to the HSI method because a decrease in % recovery was observed even if the recovery was within range.

Table 18: Summary of apple juice sample with tabulated results of specificity, LOD, LOQ, Repeatability and Trueness.

Apple juice sample results								
Pesticides	Injection technique	Specificity	LOD (mg/L)	LOQ (mg/L)	Repeatability [% RSD]		Trueness [% Recovery]	
					0.01 mg/L	0.1 mg/L	0.01 mg/L	0.1 mg/L
MLXN	1 µL HSI	Acceptable	0.0041	0.0068	13.8	7.2	98.87	107.92
	5 µL LVI	Acceptable	0.0012	0.0020	3.5	7.1	112.88	115.09
MLTN	1 µL HSI	Acceptable	0.0015	0.0026	3.5	3.0	90.68	94.18
	5 µL LVI	Acceptable	0.0021	0.0036	4.4	1.9	97.68	96.89
CPFS	1 µL HSI	Acceptable	0.0015	0.0025	4.8	1.8	83.23	88.81
	5 µL LVI	Acceptable	0.0026	0.0043	8.0	2.3	98.49	94.24
BRMP	1 µL HSI	Acceptable	0.0008	0.0013	2.7	1.3	86.96	91.02
	5 µL LVI	Acceptable	0.0033	0.0054	10.7	1.3	102.41	98.44
BRPE	1 µL HSI	Acceptable	0.0007	0.0012	1.8	1.5	85.72	89.36
	5 µL LVI	Acceptable	0.0032	0.0053	7.1	1.1	95.12	92.12
MTDN	1 µL HSI	Acceptable	0.0011	0.0018	3.6	2.7	99.72	98.27
	5 µL LVI	Acceptable	0.0013	0.0022	3.8	3.3	110.02	107.72
PRFF	1 µL HSI	Acceptable	0.0018	0.0030	5.5	4.4	96.26	98.15
	5 µL LVI	Acceptable	0.0009	0.0016	2.7	0.8	98.89	101.56

Table 19: Summary of orange juice sample with tabulated results of specificity, LOD, LOQ, Repeatability and Trueness.

Orange juice sample results								
Pesticide	Injection technique	Specificity	LOD (mg/L)	LOQ (mg/L)	Repeatability [% RSD]		Trueness [% Recovery]	
					0.01 mg/L	0.1 mg/L	0.01 mg/L	0.1 mg/L
MLXN	1 µL HSI	Acceptable	0.0020	0.0034	6.3	4.7	92.47	94.49
	5 µL LVI	Acceptable	0.0050	0.0084	16.2	12.6	85.41	88.31
MLTN	1 µL HSI	Acceptable	0.0023	0.0038	5.2	3.0	94.12	96.92
	5 µL LVI	Acceptable	0.0035	0. 0058	7.2	6.5	89.31	91.84
CPFS	1 µL HSI	Acceptable	0.0010	0.0016	3.1	3.2	85.28	91.50
	5 µL LVI	Acceptable	0.0014	0.0024	4.2	2.5	92.12	96.40
BRMP	1 µL HSI	Acceptable	0.0009	0.0016	3.1	2.9	91.25	92.49
	5 µL LVI	Acceptable	0.0012	0.0020	3.9	2.9	99.35	101.06
BRPE	1 µL HSI	Acceptable	0.0016	0.0027	3.8	3.6	96.56	99.73
	5 µL LVI	Acceptable	0.0019	0.0031	4.0	2.6	97.72	98.35
MTDN	1 µL HSI	Acceptable	0.0009	0.0015	3.1	4.0	96.90	101.77
	5 µL LVI	Acceptable	0.0011	0.0019	3.4	2.3	95.33	98.29
PRFF	1 µL HSI	Acceptable	0.0033	0.0055	9.5	4.8	95.83	102.91
	5 µL LVI	Acceptable	0.0006	0.0011	1.8	2.9	98.96	102.62

4.9. Proficiency testing by HSI and LVI methods

Accredited IEC/ISO 17025 laboratories are required to participate in proficiency testing. This is evident in clause 7.7.2a of the standard. According to, (Magnusson et al., (eds.) Eurachem Guide:2014), proficiency testing participation forms part of a Quality Assurance (QA) system. Wherein, the validity of results of a laboratory is monitored by comparison with the results of other laboratories. An independent PT scheme coordinates the overall process. Participants are furnished with information on available PT items that are applicable to their scope. A homogeneous and stable sample is distributed to all the participating laboratories. The sample should be treated as a routine sample and reported on within a specific time frame.

The scheme evaluates the submitted results of all the laboratories. Herein, the assigned value is calculated by statistical analysis of the reported results, considering standard deviation. A laboratory is furnished with a detailed report of the overall performance. Z-scores are used to evaluate performance on each reported result. The laboratory's standard operating procedure (SOP) on proficiency testing details how the z-score is calculated and interpreted. It is a requisite to investigate non-conforming work.

Equation 8 below indicates how a z-score is calculated using the assigned value and standard deviation to assess a laboratory's performance.

Equation 8: Statistical z-score calculation formula.

$$z = \frac{(x - x_a)}{\sigma_p}$$

Whereby;

x = laboratory's reported result,

x_a = assigned value

σ_p = standard deviation for proficiency

In this study, the z-score is interpreted as follows; $|z| \leq 2$ is satisfactory, $2 < |z| < 3$ is questionable and may be investigated. A $|z| \geq 3$ is deemed unsatisfactory and must be investigated.

Finalised PT samples that contained any of the OP analytes investigated in this study were sought. Finalised PT samples were samples whose analysis on them have been completed and are treated as quality control samples due to the laboratory being in

possession of the report. An orange juice sample that had PRFF and a Mango sample that had CPFS was obtained from 2 different PT schemes, FAPAS and NMISA. The samples were analysed using the HSI method and the developed and optimised LVI method.

LVI method was a more sensitive method compared to the HSI method for the analysis of organophosphates in juice samples. A suitable way to demonstrate this was by analysing QC samples from PT schemes. The results from both methods produced satisfactory z-scores for the analysis. However, the chromatograms produced from both methods showed the LVI method as the most suitable method to be used. Figures 45 and 46 are TIC of highest calibration standards for the OR JU during the analysis of the FAPAS OR JU sample using HSI and LVI. These chromatograms illustrated the peaks shapes obtained and resolution. The HSI method produced peak shapes that were tailing and had an overall poor peak resolution. Manual integration had to be administered during analysis which presented room for inaccurate results (Figure 43). However, the LVI method produced symmetrical peak shapes of the analytes. There was also stability in the baseline and good peak resolution. Additionally, peak distortion had decreased exceptionally (Figure 44).

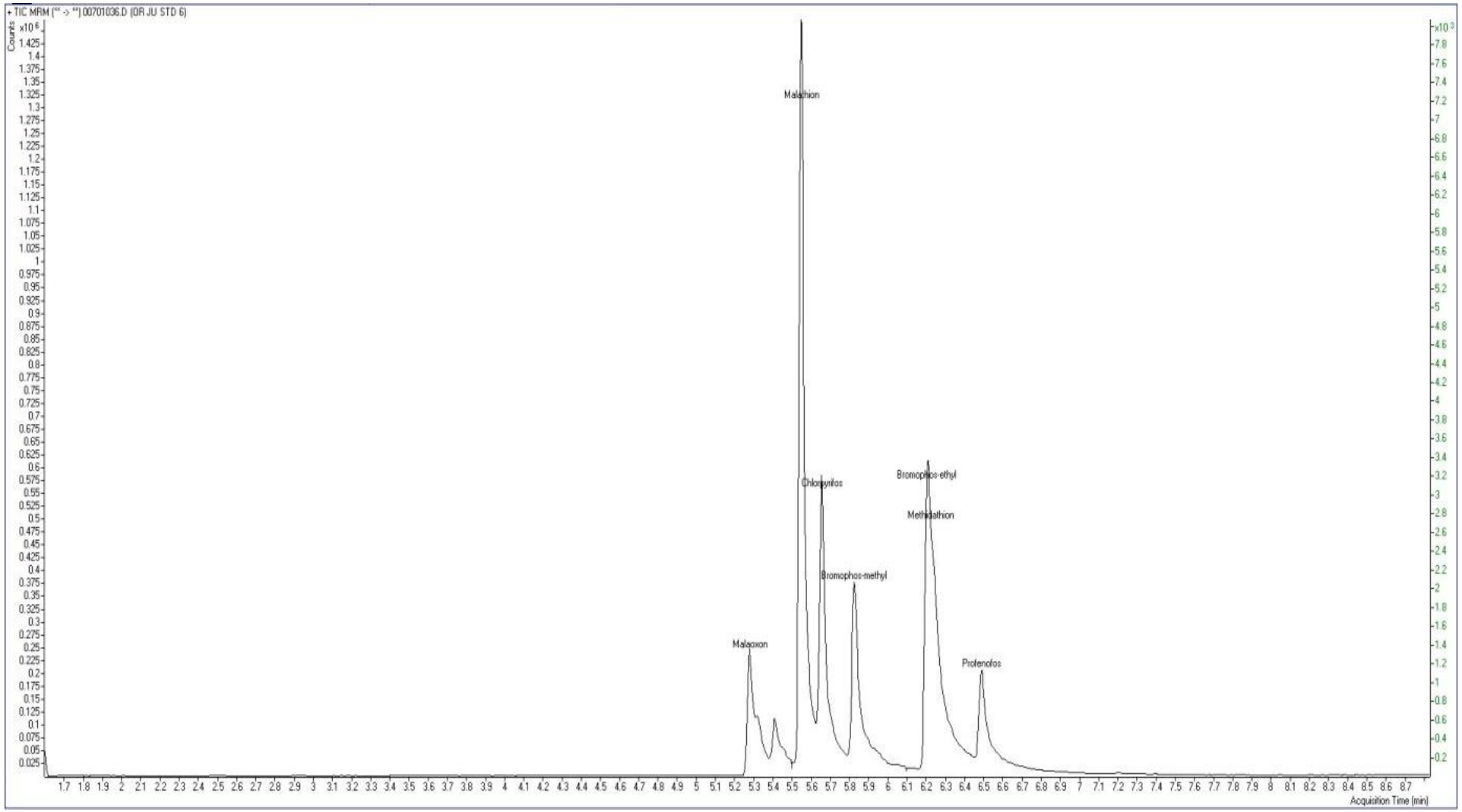

Figure 43:TIC of orange juice spike 6 chromatogram during analysis of FAPAS sample using HSI method.

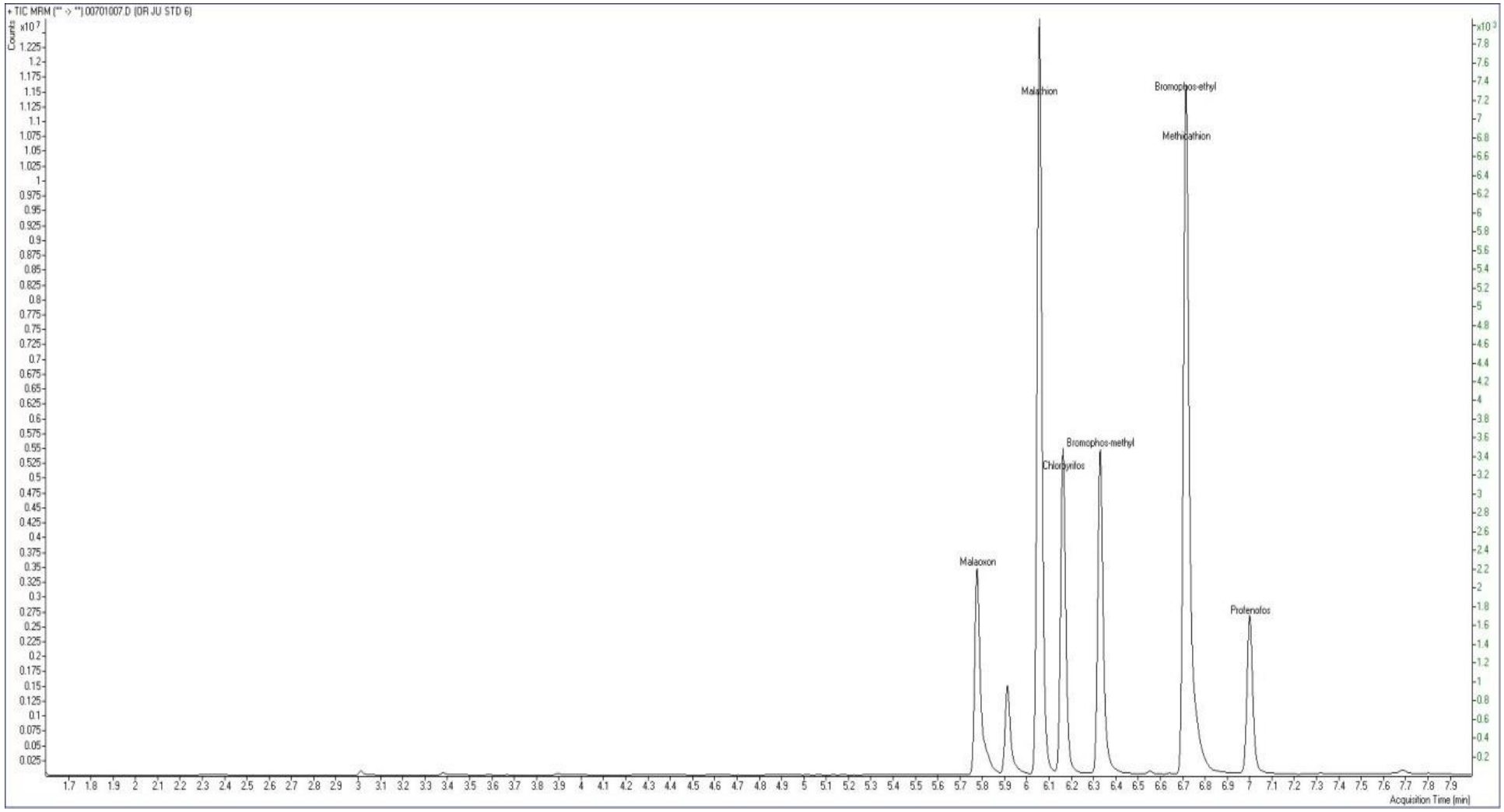

Figure 44:TIC of orange juice spike 6 chromatogram of FAPAS sample using LVI method.

4.9.1. FAPAS orange juice sample

PRFF had been previously detected on an orange juice FAPAS sample. Considering the objective of determining which injection technique was best suited for the analysis of OPs, the sample was analysed using both injection techniques. Appendix R is appended which contains the calibration data and calibration curves obtained from analysis by both HSI and LVI methods. PRFF was determined to be 0.0512 mg/kg using the HSI method (see Table 19) and using the LVI method, 0.0422 mg/kg was found as seen in Table 20.

Satisfactory z-scores were obtained for both injection techniques. A z-score of 0.6 for the HSI and 0.3 for the LVI was calculated. The score of the LVI method illustrated that the obtained concentration of 0.0422 mg/kg PRFF was closer to the assigned value of 0.045 mg/kg than 0.0512 mg/kg obtained from the HSI method.

4.9.1.1. Analysis by HSI method

Table 20: HSI results obtained for the FAPAS orange juice sample.

RESULTS				
Name	PRFF Results			FCHF (ISTD) Results
	RT	Resp.	Calc. Conc.	Resp.
OR FAPAS Run 1	6.49	10029	0.0531	120186
OR FAPAS Run 2	6.49	9469	0.0492	122383
OR FAPAS Run 3	6.49	9517	0.0514	117771
Average:			**0.0512**	

Z-score calculation

Conversion of obtained result, x: 0.0512 mg/kg = 51.2 µg/kg

Assigned value on FAPAS report, x_a: 45 µg/kg

Standard deviation for proficiency, σ_p: 9.90

$$|z| = \frac{(x - x_a)}{\sigma_p} = \frac{(51.2 - 45)}{9.90} = 0.6$$

The result is satisfactory.

4.9.1.2. Analysis by LVI method

Table 21:LVI results obtained for FAPAS orange juice sample.

RESULTS				
Name	PRFF Results			FCHF (ISTD) Results
	RT	Resp.	Calc. Conc.	Resp.
OR FAPAS Run 1	7.01	97951	0.0423	1139728
OR FAPAS Run 2	7.01	100050	0.0422	1167921
OR FAPAS Run 3	7.01	98071	0.0422	1143238
Average			**0.0422**	

Z-score calculation

Conversion of obtained result, x: 0.0422 mg/kg = 42.2 µg/kg

Assigned value on FAPAS report, x_a: 45 µg/kg

Standard deviation for proficiency, σ_p: 9.90

$$|z| = \frac{(x - x_a)}{\sigma_p} = \frac{(42.2 - 45)}{9.90} = 0.3$$

The result is satisfactory.

4.9.2. NMISA Mango (MG) samples

The NMISA MG was also an interesting sample to test using the different injection techniques. This was because the sample was not a juice sample, but CPFS had been previously detected on the sample. Blank apple pulp was used to prepare calibration standards and the data obtained and plotted calibration curves using the two methods are noted in Appendix S of the thesis. The sample was received from NMISA PT scheme in duplicate and for the purpose of this study depicted as MG A and MG B. The results obtained from the HSI method are tabulated in Table 21 and Table 22 for the LVI method.

Like what was observed with the FAPAS sample in the previous analysis, the z-score obtained for the LVI method during the analysis of the MG samples was also lower than that obtained for the HSI method. An absolute z-score value of 1.2 was observed for the HSI method but 0.3 for the LVI method attributed by 0.0435 mg/kg for sample MG A. CPFS determined whereas the assigned value is 0.041 mg/kg. Even though the z-scores were acceptable for both methods, the LVI method giving lower scores proved to be a method better suited.

4.9.2.1. Analysis by HSI method:

Table 22:HSI results obtained for the NMISA mango (MG) samples.

RESULTS					
Name	Type	CPFS Results			FCHF (ISTD) Results
		RT	Resp.	Calc. Conc.	Resp.
NMISA MG A Run 1	Sample	5.66	26684	0.0302	129121
NMISA MG A Run 2	Sample	5.66	23648	0.0296	116477
NMISA MG A Run 3	Sample	5.66	22706	0.0295	112128
Average				**0.0297**	
NMISA MG B Run 1	Sample	5.66	25486	0.0333	112600
NMISA MG B Run 2	Sample	5.66	23046	0.0299	112299
NMISA MG B Run 3	Sample	5.66	24903	0.0376	98116
Average				**0.0336**	

Z-score calculation of MG A

Obtained result, x: 0.0297 mg/kg

Assigned value on NMISA report, x_a: 0.041 mg/kg

Standard deviation for proficiency, σ_p: 0.0091

$$|z| = \frac{(x - x_a)}{\sigma_p} = \frac{(0.0297 - 0.041)}{0.0091} = -1.2$$

The result is satisfactory.

Z-score calculation of MG B

Obtained result, x: 0.0336 mg/kg

Assigned value on NMISA report, x_a: 0.041 mg/kg

Standard deviation for proficiency, σ_p: 0.0091

$$|z| = \frac{(x - x_a)}{\sigma_p} = \frac{(0.0336 - 0.041)}{0.0091} = -0.8$$

The result is satisfactory.

4.9.2.2. Analysis on the LVI method

Table 23: LVI results obtained for the analysis of NMISA mango (MG) samples.

RESULTS					
Name	Type	CPFS Results			FCHF (ISTD)
		RT	Resp.	Calc. Conc.	Resp.
NMISA MG A Run 1	Sample	6.16	295173	0.0427	1339397
NMISA MG A Run 2	Sample	6.16	281153	0.0436	1247677
NMISA MG A Run 3	Sample	6.16	273863	0.0442	1199128
Average				**0.0435**	
NMISA MG B Run 1	Sample	6.16	283385	0.0442	1240752
NMISA MG B Run 2	Sample	6.16	282696	0.0439	1247320
NMISA MG B Run 3	Sample	6.16	306012	0.0429	1379466
Average				**0.0437**	

Z-score calculation of MG A

Obtained result, x: 0.0435 mg/kg

Assigned value on NMISA report, x_a: 0.041 mg/kg

Standard deviation for proficiency, σ_p: 0.0091

$$|z| = \frac{(x - x_a)}{\sigma_p} = \frac{(0.0435 - 0.041)}{0.0091} = 0.3$$

The result is satisfactory.

Z-score calculation of MG B

Obtained result, x: 0.0437 mg/kg

Assigned value on NMISA report, x_a: 0.041 mg/kg

Standard deviation for proficiency, σ_p: 0.0091

$$|z| = \frac{(x - x_a)}{\sigma_p} = \frac{(0.0437 - 0.041)}{0.0091} = 0.3$$

The result is satisfactory.

4.10. Conclusion

This chapter discusses the results obtained throughout the study. To reach the objectives of the study, conclusions must be reached based on the results obtained. The study was initiated by the development of an LVI method. A detailed description of how the development was conducted was described in this chapter. Chromatograms obtained throughout the various injection techniques are included with resulting peak areas. Parameters of the developed LVI method were optimised and graphical representations for the different peak areas obtained as the changes in conditions were applied are provided to give a clear picture.

The chapter also included the evaluation of both the developed-optimized method of LVI and the HSI method. The evaluation was conducted by a comparison of specificity, LOD, LOQ, repeatability at different levels and recovery at different levels obtained for each method. The chapter concluded with an evaluation using PT samples and comparing the results obtained using the different injection techniques.

CHAPTER FIVE
CONCLUSIONS AND RECOMMENDATIONS

5.1. Introduction

In this chapter, conclusions are drawn based on the problem statement, hypothesis, aims and objectives of the presented study. A summary of the findings is explained, and implications described. Recommendations are provided and justified based on factors that emerged during the execution of the study.

5.2. Summary and Key Findings

Emphasis was made throughout the study on the importance of reliable analytical methods for accurate and precise determinations of pesticide residue levels in foods of different commodities including juice samples. The study assessed the applicability of a newly developed LVI method used to analyse MLXN, MLTN, CPFS, BRMP, MTDN, BRPE and PRFF in apple and orange juice samples and comparing against the HSI method currently being used. Investigations were conducted to determine which method was more suitable between the two methods.

The hypothesis presented was that the LVI method would provide an improved analytical performance compared to the HSI method for the analysis of juice samples. The results obtained proved the hypothesis to be true, LVI method produced better sensitivity with the increased peak areas and shapes. Chromatograms obtained from analysis by LVI had better peak shapes and resolution and required minimum to no manual integration during analysis.

The aims of the study were fulfilled by pursuing stipulated objectives. The first objective included the development of the LVI method by following steps provided in a published tutorial (Agilent Technologies, 2009). Firstly, an in depth understanding of different injection techniques was pursued during method development studies. Method development studies entailed the investigation of HSI, CSI, 5. 10 and 25 µL LVI methods. Investigating the first injection technique, HSI, it was discovered that BRPE and MTDN co-elute. The definition of co-elution was explained as the inability of two or more compounds to chromatographically separate. The description of the critical role of the mass spectrometer (MS) as a detector to distinguish between the compounds was provided. Transition ions of each pesticide were obtained from the database and specified in the acquisition method during a multiresidue analysis. Therefore, MTDN and

BRPE have different transition ions, making it possible to distinguish between the two. It was noted that co-elution of the compounds was encountered throughout the study.

CSI method was investigated for purposes of illustrating the transition from HSI to LVI and providing an explanation of how each injection method functions. When CSI was investigated, the peak areas obtained differed slightly (approximately 1%) to peak areas obtained for the HSI method.

The LVI methods (5, 10 and 25 µL injection volumes) produced improved peak areas in general. Specific to MTDN which was half the peak size of BRPE for application of HSI and CSI methods. When LVI was applied, MTDN was seen to greatly improve as injection volume was increased to be even greater than the BRPE peak. MLXN was the smallest peak size observed for all the injection technique. However, MLXN peak size improvements was observed for LVI application compared to the HSI method application. The retention time of MLXN peak was consistent at 5.55 minute during the application of HSI and CSI methods. When LVI method was applied, drastic shifts in retention time was observed. Slight peak distortions were observed for the 5 µL and 25 µL LVI applications. The 10 µL presented satisfactory peak shapes that were symmetrical.

Analysis using different LVI volumes were compared against HSI analysis by means of determining percentage solvent vent recoveries. Compounds such as MLXN and PRFF produced peak areas that were several degrees larger than expected for LVI. For example, the peak areas obtained for the 5 µL LVI method must be 5 times larger than the peak areas obtained for the HSI method. This resulted in high solvent vent recoveries of more than 100%.

The second objective of the study was the optimization of a developed LVI method. The 5 µL LVI was chosen for further optimization for practical reasons stated such as routine sample throughput received daily for analysis, instead of the 10 µL LVI which produced more satisfactory results. Optimization of method parameters entailed investigating vent flow rate, vent pressure, vent time, inlet and oven temperature parameters for efficiency. The effects of the use of different inlet liners were also investigated in the study.

The third objective was to evaluate both the LVI and HSI methods by determining specificity, LOD, LOQ, repeatability and trueness of the methods for each pesticide and conducting a comparison. Specificity was acceptable for all pesticides regardless of which method was applied in apple and orange juice samples. Repeatability was determined at levels of 0.01 and 0.1 mg/L. The criteria to determine acceptable

repeatability was ≤ 20 % and was below 20% for all the pesticides in both apple and orange juice samples for all methods. Trueness of a method was determined for the methods (LVI and HSI). The criteria which was applied was a range of 70% - 120% and calculated values for all the pesticides fell within this range for apple and orange juice samples.

The last objective of the study was to analyse proficiency test samples using the developed and optimized LVI method and HSI method and the results compared. Statistical z-scores were calculated from the results obtained and interpreted. A z-score below or equals to 2 is deemed satisfactory. Questionable results are z-scores of higher than 2 but less than 3 and these results may or may not be investigated. Z-scores which are higher than 3 are unsatisfactory and must be investigated. The z-scores calculated from analysis by both methods were acceptable as they were below 2 in all the test samples. It was noted that z-scores obtained for LVI were lower than those obtained using HSI.

5.3. Conclusion and Recommendations

The aim of the study was to determine, firstly, if there is a difference in the results obtained when a HSI or a LVI technique is used to analyse juice samples using the GC-MS/MS instrument. It was discovered that there is no significant difference in the results obtained and both methods may be applied for analysis. The results obtained from evaluating both methods met specified criteria and satisfactory z-scores were calculated.

The second aim of the study was the determination of whether the LVI method was more sensitive compared to HSI method for organophosphate pesticide analysis in juice samples. It was discovered that LVI is indeed sensitive as it provided increased peak areas, better chromatogram of improved peak shapes. There was also minimal manual integration required with LVI as compared to HSI.

The findings presented the conclusion that satisfies the hypothesis of the study. The LVI method provided an improved analytical performance for organophosphate analysis in juice samples.

Recommendations made after the conclusion of the study is to broaden the scope of the analytes. Multi-residue analysis is now the preferred methods for pesticide testing. This entails multiple classes of pesticides analysed in a singular sample at once. It is therefore recommended the scope of the study be broadened to investigate other class pesticides such as pyrethroids, carbamates, organochlorines etc.

Recommendations include the improvement of poor performing pesticides during the study that degraded due to high thermal conditions of the inlet, such as MLXN. These pesticides would be further investigated to improve their analysis and method parameters optimized.

Additionally, the available proficiency test samples analysed in the study included CPFS and PRFF. There were limitations in obtaining test samples that contained all the pesticides of interest. Therefore, recommendations are made to analyse more test samples which cover all the analytes.

CHAPTER SIX
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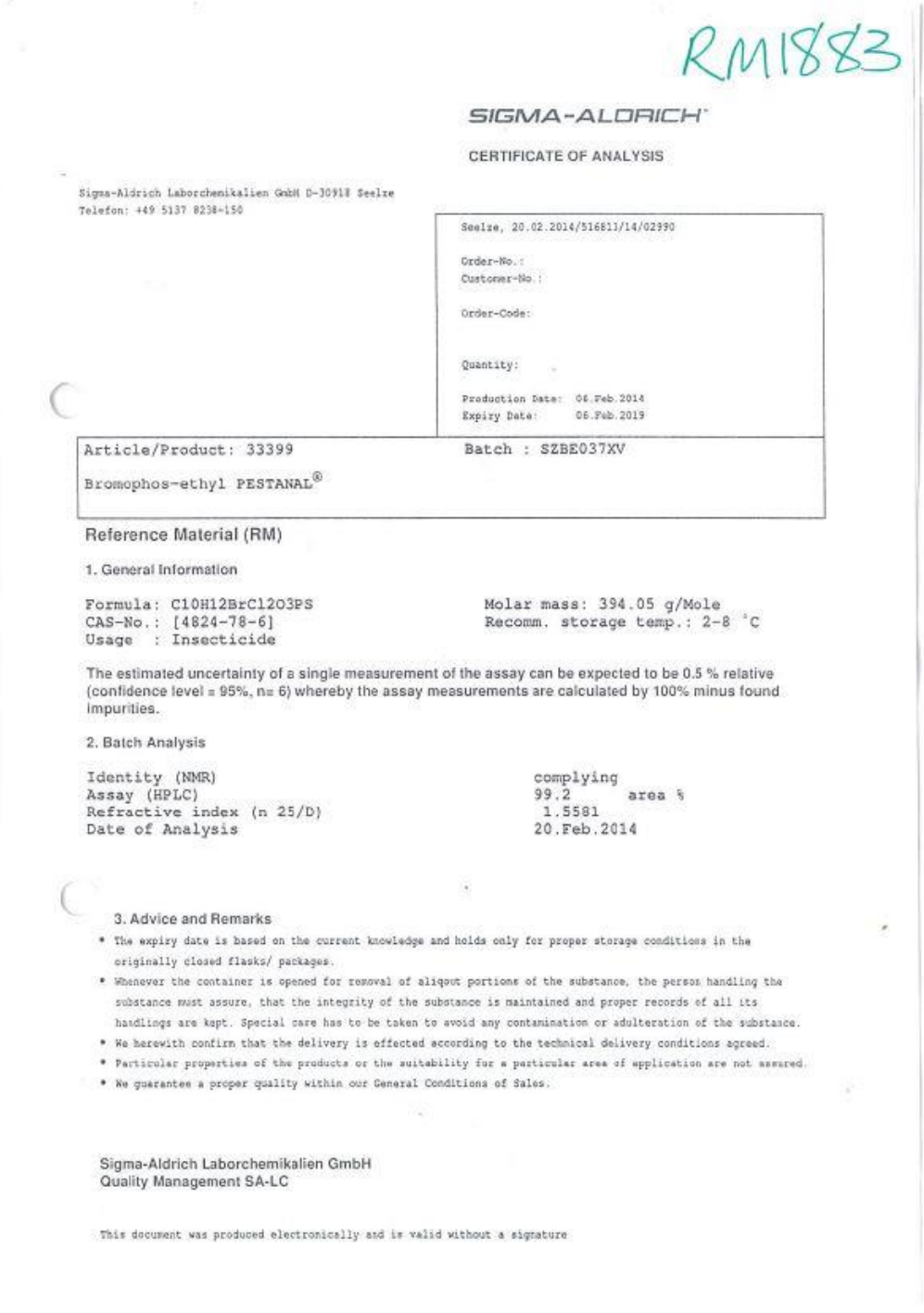
APPENDICIES

Appendix A: Certificate of analysis of compounds



RM1883

SIGMA-ALDRICH

CERTIFICATE OF ANALYSIS

Sigma-Aldrich Laborchemikalien GmbH D-30918 Seelze
Telefon: +49 5137 8238-150

Seelze, 20.02.2014/516811/14/02990

Order-No.:
Customer-No.:

Order-Code:

Quantity:

Production Date: 06.Feb.2014
Expiry Date: 06.Feb.2019

Article/Product: 33399 Batch : SZBE037XV

Bromophos-ethyl PESTANAL®

Reference Material (RM)

1. General Information

Formula: C10H12BrCl2O3PS
CAS-No.: [4824-78-6]
Usage : Insecticide

Molar mass: 394.05 g/Mole
Recomm. storage temp.: 2-8 °C

The estimated uncertainty of a single measurement of the assay can be expected to be 0.5 % relative (confidence level = 95%, n= 6) whereby the assay measurements are calculated by 100% minus found impurities.

2. Batch Analysis

Identity (NMR)	complying
Assay (HPLC)	99.2 area %
Refractive index (n 25/D)	1.5581
Date of Analysis	20.Feb.2014

3. Advice and Remarks

- The expiry date is based on the current knowledge and holds only for proper storage conditions in the originally closed flasks/ packages.
- Whenever the container is opened for removal of aliqout portions of the substance, the person handling the substance must assure, that the integrity of the substance is maintained and proper records of all its handlings are kept. Special care has to be taken to avoid any contamination or adulteration of the substance.
- We herewith confirm that the delivery is effected according to the technical delivery conditions agreed.
- Particular properties of the products or the suitability for a particular area of application are not assured.
- We guarantee a proper quality within our General Conditions of Sales.

Sigma-Aldrich Laborchemikalien GmbH
Quality Management SA-LC

This document was produced electronically and is valid without a signature

Figure 45: Certificate of analysis (COA) of BRPE.

RM3192

Certificate of Analysis

ISO 17034 Reference Material

Product Identification

Article Code: DRE-C10745000
Article Name: Bromophos-methyl
Formula: C8H8BrCl2O3PS
Mol. Weight: 366.00
CAS No.: 2104-96-3

Lot Number: G974380
Expiry Date: 09.10.2024
Storage Temperature: -18°C ± 4°C

Storage and handling: The RM should be stored in the original sealed bottle at the temperature given above. After use the bottle should be tightly closed and protected from moisture.

Purity: 98.54% (g/g)

Expanded Uncertainty U= 0.39% (g/g)

The uncertainty of this standard is calculated in accordance with the ISO 17034 and EURACHEM/CITAC Guide - Quantifying Uncertainty in Analytical Measurement, Second Edition. The expanded uncertainty is U(exp) = u(RM) x k, where k is the coverage factor at the 95% confidence level (k=2). Uncertainty u(RM) is based on the combination of the uncertainties associated with each individual operation involved in the analysis of the product: $u(RM) = \sqrt{u(char)^2 + u(bb)^2 + u(lts)^2 + u(sts)^2}$; u(char) is the uncertainty of characterisation; u(bb) uncertainty of homogeneity test; u(lts) uncertainty of stability test long-term; u(sts) uncertainty of stability test short-term. u(lts) and u(sts) are not included in the calculation as the stability statement is based on real evidence opposed to simulation.
Minimum sample: 1 mg is recommended as the minimal sample amount. If less material is used, it is recommended to increase the certified uncertainty by a factor of two for half sample and a factor of four for a quarter of sample.
Intended use: Use this RM as calibrant for chromatography or any other analytical technique.

Analytical Data

Traceability of chromatography: To the International System of Units (SI).

Instrument:	GC/FID	Injector:	320°C
Detection:	FID	Initial Temp:	120°C for 4 min
Column:	Optima-5MS, 0.25 µm, 0.25 mm	End Temp:	320°C for 3 min
Inj.-Vol.:	1 µl	Gradient:	15°C/min
Flow:	1.0 ml/min		
Ret.Time:	12.89 min		

Comment

Traceability: The balances used are calibrated with weights traceable to the national standards (DKD).
Calibrated class A glassware is used for volumetric measurements.
Water Content: 0.33% (g/g) by Karl-Fischer-Titration (U(exp) = 0.25% (g/g)).
Purity was determined by chromatographic assay, corrected by water content and/or residue solvents.
Identity: EA, NMR, RT, IR, MS
Attachment: Exemplary chromatogram of given method

Certificate Revision 1 - 09.10.2018 - M. Beck

Certified on: 09.10.2018
Certified by: M. Beck
RM Release

Authorized copy from the original
02. NOV. 2018
Sign.:

The LGC Labor GmbH, accreditated by DAkkS as indicated by the accreditation number D-RM-19883-01 & D-PL-19883-01, has shown competence based on ISO 17034:2017 with relevant parts of DIN EN ISO/IEC 17025:2018 for production of certified reference materials in form of organic pure substances and in form of single and multi-component solutions of organic pure substances.

LGC Labor GmbH - Bgm.-Schlosser-Straße 6A - 86199 Augsburg - Germany
Phone +49 821 906080 - Fax +49 821 9060888 - augsburg.inquiry@lgcgroup.com
The warranty for this product is limited to the purchasing price of this product.

Figure 46: COA of BRMP.

RM3025

SIGMA-ALDRICH

3050 Spruce Street, Saint Louis, MO 63103 USA
Email USA: techserv@sial.com Outside USA: eurtechserv@sial.com

Certificate of Analysis

Product Name:	Chlorpyrifos PESTANAL™, analytical standard
Product Number:	45395
Batch Number:	BCCC1983
Brand:	Sigma-Aldrich
CAS Number:	2921-88-2
Formula:	$C_9H_{11}Cl_3NO_3PS$
Formula Weight:	350.59
Storage Temperature:	2-8 C
Expiration Date:	AUG 2024
Quality Release Date:	11 OCT 2019

TEST	SPECIFICATION	RESULT
APPEARANCE (COLOR)	WHITE TO LIGHT YELLOW AND FAINT BEIGE TO LIGHT BEIGE	LIGHT BEIGE
APPEARANCE (FORM)	POWDER OR CRYSTALS OR SOLID OR CHUNKS	SOLID
PURITY (HPLC AREA %)	= 98.0 %	99.0 %
MELTING POINT	40 - 46 C	41 C
WATER	≤ 1.0 %	0.1 %
PROTON NMR SPECTRUM	CONFORMS TO STRUCTURE	CONFORMS

Dr. Reinhold Schwenninger
Quality Assurance
Buchs, Switzerland

Figure 47: COA of CPFS.

RM3199

LGC | DR EHRENSTORFER™

REFERENCE MATERIAL CERTIFICATE ISO 17034

Reference Material

This certificate is designed in accordance with ISO 17034 and ISO Guide 31. This reference material (RM) was designed, produced and verified in accordance with ISO/IEC 17025, ISO 17034 and a registered quality management system ISO 9001.

Product Name
Malaoxon

Product Code
DRE-C14700000

CAS No.
1634-78-2

Mol. Weight
314.29

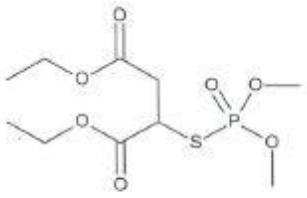
Mol. Formula
$C_{10}H_{19}O_7PS$

Lot Number
G1014619

Format
Neat

Expiry Date
25 Sep 2023

Storage Temp
-18°C ± 4°C



CERTIFIED	CERTIFIED
Purity 97.20% (g/g)	Expanded Uncertainty (U) 2.00% (g/g)

Uncertainty
The certified value(s) and uncertainty(ies) are determined in accordance with ISO 17034 with an 95% confidence level (k=2). Uncertainty is based on the Total Combined Uncertainty, including uncertainties of characterisation, homogeneity and stability testing. Stability values are based on real evidence opposed to simulation.

The producer certifies that this reference material meets the specification stated in this certificate until the expiry date, provided it is stored unopened at the recommended temperature herein. Product warranties for this reference material are set out in the terms and conditions of purchase.

CERTIFIED BY	CERTIFIED ON		
N. Müller	25 Sep 2019		RM Release

Certificate Verified
26 Sep 2019

Version 1

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Figure 48: COA of MLXN.

RM3419

LGC | DR EHRENSTORFER

REFERENCE MATERIAL CERTIFICATE ISO 17034

Reference Material

This certificate is designed in accordance with ISO 17034 and ISO Guide 31. This reference material (RM) was designed, produced and verified in accordance with ISO/IEC 17025, ISO 17034 and a registered quality management system ISO 9001.

Product Name
Malathion

Product Code
DRE-C14710000

CAS No.
121-75-5

Mol. Weight
330.36

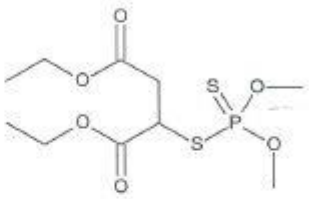
Mol. Formula
$C_{10}H_{19}O_6PS_2$

Lot Number
G994660

Format
Neat

Expiry Date
16 Jan 2025

Storage Temp
4°C ± 4°C



CERTIFIED	CERTIFIED
Purity 98.33% (g/g)	Expanded Uncertainty (U) 0.48% (g/g)

Uncertainty
The certified value(s) and uncertainty(ies) are determined in accordance with ISO 17034 with an 95% confidence level (k=2). Uncertainty is based on the Total Combined Uncertainty, including uncertainties of characterisation, homogeneity and stability testing. Stability values are based on real evidence opposed to simulation.

The producer certifies that this reference material meets the specification stated in this certificate until the expiry date, provided it is stored unopened at the recommended temperature herein. Product warranties for this reference material are set out in the terms and conditions of purchase.

CERTIFIED BY	CERTIFIED ON		
N. Müller	16 Jan 2019	HLD.	RM Release

Certificate Verified
Gabriele Mastrad
21 Jan 2019

Version 1

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Figure 49: COA of MLTN (mercaptothion).

RM3090

LGC | DR EHRENSTORFER

REFERENCE MATERIAL CERTIFICATE ISO 17034

Reference Material

This certificate is designed in accordance with ISO 17034 and ISO Guide 31. This reference material (RM) was designed, produced and verified in accordance with ISO/IEC 17025, ISO 17034 and a registered quality management system ISO 9001.

Product Name
Methidathion

Product Code
DRE-C15020000

CAS No.
950-37-8

Mol. Weight
302.33

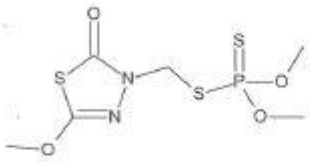
Mol. Formula
$C_6H_{11}N_2O_4PS_3$

Lot Number
G1038949

Format
Neat

Expiry Date
18 Oct 2023

Storage Temp
-18°C ± 4°C



CERTIFIED	CERTIFIED
Purity 98.32% (g/g)	Expanded Uncertainty (U) 1.02% (g/g)

Uncertainty
The certified value(s) and uncertainty(ies) are determined in accordance with ISO 17034 with an 95% confidence level (k=2). Uncertainty is based on the Total Combined Uncertainty, including uncertainties of characterisation, homogeneity and stability testing. Stability values are based on real evidence opposed to simulation.

The producer certifies that this reference material meets the specification stated in this certificate until the expiry date, provided it is stored unopened at the recommended temperature herein. Product warranties for this reference material are set out in the terms and conditions of purchase.

CERTIFIED BY	CERTIFIED ON		
N. Müller	18 Oct 2019		RM Release

Certificate Verified
22 Oct 2019

Version 1

Page 1 of 2

Figure 50: COA of MTDN.

RM3099

LGC | DR EHRENSTORFER

REFERENCE MATERIAL CERTIFICATE ISO 17034

Reference Material

This certificate is designed in accordance with ISO 17034 and ISO Guide 31. This reference material (RM) was designed, produced and verified in accordance with ISO/IEC 17025, ISO 17034 and a registered quality management system ISO 9001.

Product Name
Profenofos

Product Code
DRE-C16330000

CAS No.
41198-08-7

Mol. Weight
374.00

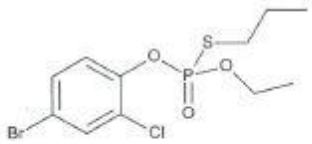
Mol. Formula
$C_{11}H_{15}BrClO_3PS$

Lot Number
G1058110

Format
Neat

Expiry Date
06 Mar 2024

Storage Temp
-18°C ± 4°C



CERTIFIED	CERTIFIED
Purity 95.80% (g/g)	Expanded Uncertainty (U) 1.97% (g/g)

Uncertainty
The certified value(s) and uncertainty(ies) are determined in accordance with ISO 17034 with an 95% confidence level (k=2). Uncertainty is based on the Total Combined Uncertainty, including uncertainties of characterisation, homogeneity and stability testing. Stability values are based on real evidence opposed to simulation.

The producer certifies that this reference material meets the specification stated in this certificate until the expiry date, provided it is stored unopened at the recommended temperature herein. Product warranties for this reference material are set out in the terms and conditions of purchase.

CERTIFIED BY	CERTIFIED ON		
N. Müller	06 Mar 2020		RM Release

Certificate Verified
09 Mar 2020

Figure 51: COA of PRFF.

SIGMA-ALDRICH

3050 Spruce Street, Saint Louis, MO 63103 USA
Email USA: techserv@sial.com Outside USA: eurtechserv@sial.com

Certificate of Analysis

RM 325

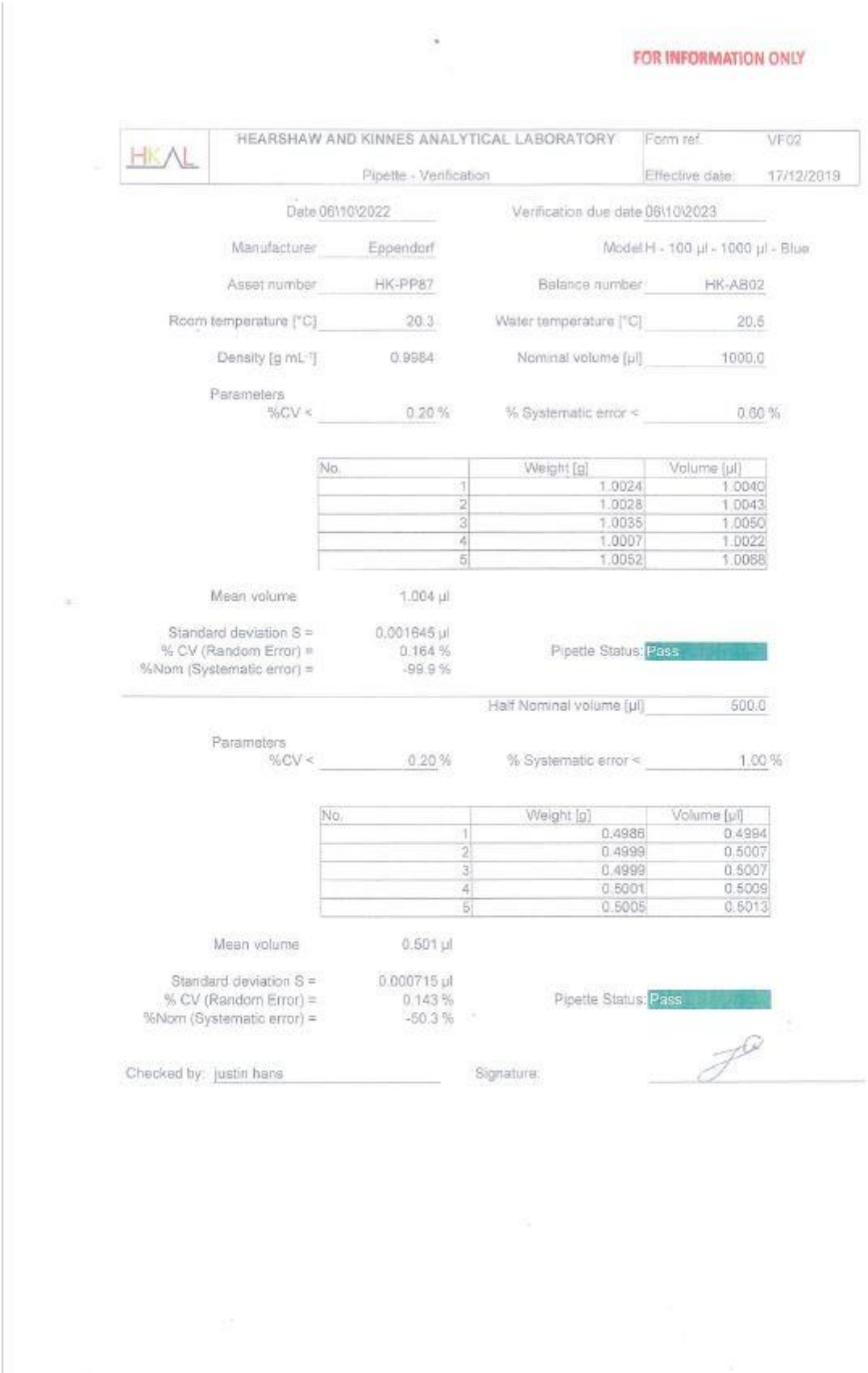
Product Name:	Fenchlorphos PESTANAL™, analytical standard
Product Number:	45485
Batch Number:	BCBZ2890
Brand:	Sigma-Aldrich
CAS Number:	299-84-3
Formula:	$C_8H_5Cl_3O_3PS$
Formula Weight:	321.55
Storage Temperature:	2-8 C
Expiration Date:	OCT 2024
Quality Release Date:	07 NOV 2019

TEST	SPECIFICATION	RESULT
PEARANCE (COLOR)	WHITE TO LIGHT YELLOW AND FAINT BEIGE TO LIGHT BEIGE	WHITE
APPEARANCE (FORM)	POWDER OR CRYSTALS	POWDER
PURITY (HPLC AREA %)	≥ 98.0 %	99.7 %
MELTING POINT	39 - 44 C	42 C
WATER	≤ 1.0 %	0.2 %
PROTON NMR SPECTRUM	CONFORMS TO STRUCTURE	CONFORMS

Dr. Reinhold Schwenninger
Quality Assurance
Buchs, Switzerland

Figure 52: COA of FCHF (Internal standard).

Appendix B: Verification of pipette



FOR INFORMATION ONLY

HKAL | HEARSHAW AND KINNES ANALYTICAL LABORATORY | Form ref: VF02
Pipette - Verification | Effective date: 17/12/2019

Date 06\10\2022 Verification due date 06\10\2023

Manufacturer Eppendorf Model H - 100 µl - 1000 µl - Blue

Asset number HK-PP87 Balance number HK-AB02

Room temperature [°C] 20.3 Water temperature [°C] 20.5

Density [g mL⁻¹] 0.9984 Nominal volume [µl] 1000.0

Parameters
%CV < 0.20 % % Systematic error < 0.60 %

No.	Weight [g]	Volume [µl]
1	1.0024	1.0040
2	1.0028	1.0043
3	1.0035	1.0050
4	1.0007	1.0022
5	1.0052	1.0068

Mean volume 1.004 µl

Standard deviation S = 0.001645 µl
% CV (Random Error) = 0.164 %
%Nom (Systematic error) = -99.9 %
Pipette Status: Pass

Half Nominal volume [µl] 500.0

Parameters
%CV < 0.20 % % Systematic error < 1.00 %

No.	Weight [g]	Volume [µl]
1	0.4986	0.4994
2	0.4999	0.5007
3	0.4999	0.5007
4	0.5001	0.5009
5	0.5005	0.5013

Mean volume 0.501 µl

Standard deviation S = 0.000715 µl
% CV (Random Error) = 0.143 %
%Nom (Systematic error) = -50.3 %
Pipette Status: Pass

Checked by: justin hans Signature:

Figure 53: Verification of pipette used to prepare cocktail solutions.

Appendix C: Theoretical concentrations of stock solutions

Equation 9: Calculation of concentration of stock solutions.

$$Concentration\ (mg\backslash L) = \frac{mg\ \times\ purity\ \%}{L}$$

Table 24: Percentage purity, final volume of solution and mass weighed for the preparation of stock solutions.

Compounds	Percentage purity (%)	Final volume (ml)	Mass weighed (g)
MLXN	97,2	10	0,02221
MLTN	98,33	10	0,03165
CPFS	99	10	0,02204
BRMP	98,54	10	0,02014
BRPE	99,2	10	0,02949
MTDN	98,32	10	0,02134
PRFF	95,8	10	0,02503
FCHF	99,7	200	0,09331

MLXN:

$$Concentration\ (mg\backslash L) = \frac{22.21\ mg\ \times\ 97.2\ \%}{0.01\ L} = 2158.812\ mg/L$$

MLTN:

$$Concentration\ (mg\backslash L) = \frac{31.65\ mg\ \times\ 98.33\ \%}{0.01\ L} = 3112.1445\ mg/L$$

CPFS:

$$Concentration\ (mg\backslash L) = \frac{22.04\ mg\ \times\ 99\ \%}{0.01\ L} = 2181.96\ mg/L$$

BRMP:

$$Concentration\ (mg\backslash L) = \frac{20.14\ mg\ \times\ 98.54\ \%}{0.01\ L} = 1984.5956\ mg/L$$

BRPE:

$$Concentration\ (mg\backslash L) = \frac{29.49\ mg\ \times\ 99.2\ \%}{0.01\ L} = 2925.408\ mg/L$$

MTDN:

$$Concentration\ (mg\backslash L) = \frac{21.34\ mg\ \times\ 98.32\ \%}{0.01\ L} = 2098.1488\ mg/L$$

PRFF:

$$Concentration\ (mg\backslash L) = \frac{25.03\ mg\ \times\ 95.8\ \%}{0.01\ L} = 2397.874\ mg/L$$

FCHF:

$$Concentration\ (mg\backslash L) = \frac{93.31\ mg\ \times\ 99.7\ \%}{0.2\ L} = 465.1503\ mg/L$$

Appendix D: Theoretical concentrations of working solutions

Equation 10: Concentration calculation formula for dilution of solutions.

$C_1 \times V_1 = C_2 \times V_2$

STD 6: $\pm$ 10 mg/L

Volume (V_2): 200 mL
$\pm 2000\ mg/L \times V_1 = \pm 10\ mg/L \times 100\ mL$
$V_1 = 1\ mL$

STD 5: $\pm$ 4 mg/L

Volume (V_2): 100 mL
$\pm 10\ mg/L \times V_1 = \pm 4\ mg/L \times 100\ mL$
$V_1 = 40\ mL$

STD 4:$\pm$ 2 mg/L

Volume (V_2): 100 mL
$\pm 10\ mg/L \times V_1 = \pm 2\ mg/L \times 100\ mL$
$V_1 = 20\ mL$

STD 3:$\pm$ 1 mg/L

Volume (V_2): 100 mL
$\pm 10\ mg/L \times V_1 = \pm 1\ mg/L \times 100\ mL$
$V_1 = 10\ mL$

STD 2:$\pm$ 0.5 mg/L

Volume (V_2): 100 mL
$\pm 10\ mg/L \times V_1 = \pm 0.5\ mg/L \times 100\ mL$
$V_1 = 5\ mL$

STD 1:$\pm$ 0.1 mg/L

Volume (V_2): 100 mL
$\pm 10\ mg/L \times V_1 = \pm 0.1\ mg/L \times 100\ mL$
$V_1 = 1\ mL$

Table 25: Theoretical concentrations of compounds in stock solutions and working solutions.

Compounds	Stock solution concentration (ppm)	Concentrations of working solutions (ppm)					
		STD 6	STD 5	STD 4	STD 3	STD 2	STD 1
		±10	±4	±2	±1	±0,5	±0,1
MLXN	2158,8120	10,7941	4,3176	2,1588	1,0794	0,5397	0,1079
MLTN	3112,1445	15,5607	6,2243	3,1121	1,5561	0,7780	0,1556
CPFS	2181,9600	10,9098	4,3639	2,1820	1,0910	0,5455	0,1091
BRPM	1984,5956	9,9230	3,9692	1,9846	0,9923	0,4961	0,0992
BRPE	2925,4080	14,6270	5,8508	2,9254	1,4627	0,7314	0,1463
MTDN	2098,1488	10,4907	4,1963	2,0981	1,0491	0,5245	0,1049
PRFF	2397,8740	11,9894	4,7957	2,3979	1,1989	0,5995	0,1199

Appendix E: Expected concentrations of spiked standards.

Table 26: Expected concentrations of pesticides in STD 1 - 10 during method evaluation for apple and orange juice samples.

				Expected concentrations in spiked standards						
STDs	**Working solution added (ppm)**	**Volume of spike added (µl)**	**Final volume (10 mL)**	**MLXN**	**MLTN**	**CPFS**	**BRPM**	**BRPE**	**MTDN**	**PRFF**
1	**0,1**	**500**	**10**	0,0054	0,0078	0,0055	0,0050	0,0073	0,0052	0,0060
2	**0,1**	**1000**	**10**	0,0108	0,0156	0,0109	0,0099	0,0146	0,0105	0,0120
3	**0,5**	**400**	**10**	0,0216	0,0311	0,0218	0,0198	0,0293	0,0210	0,0240
4	**0,5**	**1000**	**10**	0,0540	0,0778	0,0545	0,0496	0,0731	0,0525	0,0599
5	**1**	**750**	**10**	0,0810	0,1167	0,0818	0,0744	0,1097	0,0787	0,0899
6	**1**	**1000**	**10**	0,1079	0,1556	0,1091	0,0992	0,1463	0,1049	0,1199
7	**4**	**625**	**10**	0,2699	0,3890	0,2727	0,2481	0,3657	0,2623	0,2997
8	**10**	**500**	**10**	0,5397	0,7780	0,5455	0,4961	0,7314	0,5245	0,5995
9	**10**	**750**	**10**	0,8096	1,1671	0,8182	0,7442	1,0970	0,7868	0,8992
10	**10**	**1000**	**10**	1,0794	1,5561	1,0910	0,9923	1,4627	1,0491	1,1989

Appendix F: Peak areas of pesticides obtained during method development studies.

Table 27: Peak area values of pesticides obtained during method development studies for all injection techniques.

Peak areas obtained for each pesticide during method development								
		MLXN	MLTN	CPFS	BRMP	MTDN	BRPE	PRFF
1 µL HSI	Run 1	16386	419979	586401	480994	401779	681011	140732
	Run 2	16132	419766	566785	497189	385597	693078	135525
	Run 3	18329	440456	578666	493827	432385	696704	151750
Average		**16949**	**426734**	**577284**	**490670**	**406587**	**690264**	**142669**
1 µL CSI	Run 1	17139	406556	551768	454210	371537	668038	140600
	Run 2	14429	389738	554298	445567	363660	679041	134930
	Run 3	16054	398800	535148	426743	364408	664853	136438
Average		**15874**	**398365**	**547071**	**442173**	**366535**	**670644**	**137323**
5 µL LVI	Run 1	307563	2832659	3178387	3070301	4056905	3715972	1277219
	Run 2	255521	2568789	2881208	2550649	3331816	3282465	1003472
	Run 3	161954	2003247	2385709	2081395	2484780	2796543	753098
Average		**241679**	**2468232**	**2815101**	**2567448**	**3291167**	**3264993**	**1011263**
10 µL LVI	Run 1	403754	2990540	2997329	2939716	4846736	3941945	1664254
	Run 2	434376	3262838	3316396	3179101	4980966	4213080	1715069
	Run 3	462355	3612535	3610289	3509881	5450892	4671586	1868515
Average		**433495**	**3288638**	**3308005**	**3209566**	**5092865**	**4275537**	**1749279**
25 µL LVI	Run 1	1028289	5792763	5472573	5922298	13456312	7032754	4474829
	Run 2	1479475	7447528	8528178	9723016	15663834	10182394	6126633
	Run 3	1732228	8351166	9128258	10825426	17624108	11137482	6746071
Average		**1413331**	**7197152**	**7709670**	**8823580**	**15581418**	**9450877**	**5782511**

Appendix G: Apple juice calibration data

1. HSI method - Procedural standards

Table 28: Procedural standard data for apple juice samples using HSI method.

MLXN	R^2	0.99572057
	weight	1/x^2
	origin	Ignore
	type	Quadratic
	Equation	y = 6.049305E-004 * x ^ 2 + 0.040257 * x - 3.091570E-004
	RT	5.427
MLTN	R^2	0.99963614
	weight	None
	origin	Force
	type	Quadratic
	Equation	y = 0.001399 * x ^ 2 + 0.273964 * x
	RT	5.742
CPFS	R^2	0.99901273
	weight	1/x^2
	origin	Ignore
	type	Quadratic
	Equation	y = 0.004333 * x ^ 2 + 0.408175 * x - 0.001016
	RT	5.864
BRMP	R^2	0.99909605
	weight	1/x^2
	origin	Force
	type	Quadratic
	Equation	y = 0.006565 * x ^ 2 + 0.482223 * x
	RT	6.052
BRPE	R^2	0.99972033
	weight	None
	origin	Force
	type	Quadratic
	Equation	y = 0.001392 * x ^ 2 + 0.474856 * x
	RT	6.45
MTDN	R^2	0.9995484
	weight	None
	origin	Force
	type	Quadratic
	Equation	y = 0.005529 * x ^ 2 + 0.833416 * x
	RT	6.535
PRFF	R^2	0.99994832
	weight	None
	origin	Force
	type	Quadratic
	Equation	y = 0.001764 * x ^ 2 + 0.178764 * x
	RT	6.757

2. HSI method - Matrix matched (MM) standards

Table 29: MM standard data for apple juice samples using HSI method.

MLXN	R^2	0.99578001
	weight	1/x^2
	origin	Force
	type	Quadratic
	Equation	y = 8.383681E-004 * x ^ 2 + 0.034301 * x
	RT	5.438
MLTN	R^2	0.99991727
	weight	None
	origin	Force
	type	Quadratic
	Equation	y = 0.002556 * x ^ 2 + 0.284503 * x
	RT	5.74
CPFS	R^2	0.99951774
	weight	1/x^2
	origin	Force
	type	Quadratic
	Equation	y = 0.003053 * x ^ 2 + 0.464875 * x
	RT	5.864
BRMP	R^2	0.99790291
	weight	1/x^2
	origin	Force
	type	Quadratic
	Equation	y = 0.004295 * x ^ 2 + 0.537419 * x
	RT	6.05
BRPE	R^2	0.99993236
	weight	None
	origin	Force
	type	Quadratic
	Equation	y = 0.004390 * x ^ 2 + 0.517158 * x
	RT	6.448
MTDN	R^2	0.9998126
	weight	None
	origin	Force
	type	Quadratic
	Equation	y = 0.014387 * x ^ 2 + 0.784171 * x
	RT	6.451
PRFF	R^2	0.99989267
	weight	None
	origin	Force
	type	Quadratic
	Equation	y = 0.002852 * x ^ 2 + 0.174142 * x
	RT	6.725

3. LVI method - Procedural standards

Table 30: Procedural standard data for apple juice samples using the LVI method.

MLXN	R^2	0.99989458
	weight	None
	origin	Ignore
	type	Quadratic
	Equation	y = 2.878564E-004 * x ^ 2 + 0.034821 * x - 1.726364E-005
	RT	5.942
MLTN	R^2	0.99885556
	weight	None
	origin	Force
	type	Linear
	Equation	y = 0.245755 * x
	RT	6.238
CPFS	R^2	0.99981722
	weight	None
	origin	Ignore
	type	Quadratic
	Equation	y = -5.565312E-004 * x ^ 2 + 0.411940 * x + 0.003092
	RT	6.363
BRMP	R^2	0.99937097
	weight	1/x^2
	origin	Force
	type	Quadratic
	Equation	y = -0.001409 * x ^ 2 + 0.523701 * x
	RT	6.551
BRPE	R^2	0.99991268
	weight	None
	origin	Force
	type	Quadratic
	Equation	y = -7.726853E-004 * x ^ 2 + 0.464985 * x
	RT	6.951
MTDN	R^2	0.99345181
	weight	1/x^2
	origin	Force
	type	Quadratic
	Equation	y = -0.001997 * x ^ 2 + 0.798321 * x
	RT	6.954
PRFF	R^2	0.99984871
	weight	None
	origin	Force
	type	Quadratic
	Equation	y = 4.566591E-005 * x ^ 2 + 0.196254 * x
	RT	7.228

4. LVI method - Matrix matched (MM) standards

Table 31: MM standard data for apple juice samples using the LVI method.

MLXN	R^2	0.99927509
	weight	None
	origin	Force
	type	Quadratic
	Equation	y = 9.377804E-004 * x ^ 2 + 0.032409 * x
	RT	5.942
MLTN	R^2	0.99955787
	weight	None
	origin	Force
	type	Quadratic
	Equation	y = 0.001554 * x ^ 2 + 0.260747 * x
	RT	6.245
CPFS	R^2	0.99959252
	weight	None
	origin	Force
	type	Quadratic
	Equation	y = 0.002013 * x ^ 2 + 0.443921 * x
	RT	6.367
BRMP	R^2	0.99951331
	weight	None
	origin	Force
	type	Quadratic
	Equation	y = 0.003925 * x ^ 2 + 0.517330 * x
	RT	6.555
BRPE	R^2	0.99972456
	weight	None
	origin	Force
	type	Quadratic
	Equation	y = 2.477754E-004 * x ^ 2 + 0.502798 * x
	RT	6.951
MTDN	R^2	0.99932946
	weight	None
	origin	Force
	type	Quadratic
	Equation	y = 0.007728 * x ^ 2 + 0.775225 * x
	RT	6.954
PRFF	R^2	0.99926505
	weight	None
	origin	Force
	type	Quadratic
	Equation	y = 0.002062 * x ^ 2 + 0.188421 * x
	RT	7.232

Appendix H: Orange juice calibration data
5. HSI method - Procedural standards

Table 32: Procedural standard data for orange juice samples using HSI method.

MLXN	R^2	0.99989021
	weight	None
	origin	Force
	type	Quadratic
	Equation	y = 8.471156E-004 * x ^ 2 + 0.038774 * x
	RT	5.441
MLTN	R^2	0.99989536
	weight	None
	origin	Force
	type	Quadratic
	Equation	y = 0.002173 * x ^ 2 + 0.289898 * x
	RT	5.75
CPFS	R^2	0.99890927
	weight	1/x^2
	origin	Force
	type	Quadratic
	Equation	y = 0.004270 * x ^ 2 + 0.415576 * x
	RT	5.838
BRMP	R^2	0.99969265
	weight	1/x^2
	origin	Force
	type	Quadratic
	Equation	y = 0.004868 * x ^ 2 + 0.461858 * x
	RT	6.033
BRPE	R^2	0.99985145
	weight	None
	origin	Force
	type	Quadratic
	Equation	y = 0.003018 * x ^ 2 + 0.453956 * x
	RT	6.451
MTDN	R^2	0.99987308
	weight	None
	origin	Force
	type	Quadratic
	Equation	y = 0.004886 * x ^ 2 + 0.858265 * x
	RT	6.443
PRFF	R^2	0.99936949
	weight	None
	origin	Ignore
	type	Quadratic
	Equation	y = 0.002316 * x ^ 2 + 0.162230 * x + 6.647261E-004
	RT	6.686

6. HSI method - Matrix matched (MM) standards

Table 33: MM standard data for orange juice samples using HSI method.

MLXN	R^2	0.99957148
	weight	None
	origin	Force
	type	Quadratic
	Equation	y = 0.001074 * x ^ 2 + 0.041774 * x
	RT	5.437
MLTN	R^2	0.9995584
	weight	None
	origin	Force
	type	Quadratic
	Equation	y = 0.004339 * x ^ 2 + 0.288492 * x
	RT	5.738
CPFS	R^2	0.99917067
	weight	1/x^2
	origin	Force
	type	Quadratic
	Equation	y = 0.004636 * x ^ 2 + 0.463844 * x
	RT	5.859
BRMP	R^2	0.99922649
	weight	1/x^2
	origin	Force
	type	Quadratic
	Equation	y = 0.004100 * x ^ 2 + 0.515326 * x
	RT	6.045
BRPE	R^2	0.99944954
	weight	None
	origin	Force
	type	Quadratic
	Equation	y = 0.007348 * x ^ 2 + 0.447883 * x
	RT	6.442
MTDN	R^2	0.99848654
	weight	None
	origin	Force
	type	Quadratic
	Equation	y = 0.020050 * x ^ 2 + 0.797371 * x
	RT	6.448
PRFF	R^2	0.99953456
	weight	None
	origin	Force
	type	Quadratic
	Equation	y = 0.003464 * x ^ 2 + 0.168287 * x
	RT	6.726

7. LVI method - Procedural standards

Table 34: Procedural standards data for orange juice samples using LVI method.

MLXN	R^2	0.99995959
	weight	None
	origin	Force
	type	Quadratic
	Equation	y = 1.419266E-005 * x ^ 2 + 0.048616 * x
	RT	6.064
MLTN	R^2	0.99994473
	weight	None
	origin	Force
	type	Quadratic
	Equation	y = -5.117616E-004 * x ^ 2 + 0.277888 * x
	RT	6.234
CPFS	R^2	0.99984636
	weight	None
	origin	Force
	type	Quadratic
	Equation	y = -2.326174E-004 * x ^ 2 + 0.411935 * x
	RT	6.367
BRMP	R^2	0.99987521
	weight	None
	origin	Force
	type	Quadratic
	Equation	y = 9.136553E-004 * x ^ 2 + 0.505883 * x
	RT	6.555
BRPE	R^2	0.99981458
	weight	None
	origin	Force
	type	Linear
	Equation	y = 0.451637 * x
	RT	6.944
MTDN	R^2	0.99960607
	weight	None
	origin	Force
	type	Linear
	Equation	y = 0.919832 * x
	RT	6.947
PRFF	R^2	0.9998871
	weight	None
	origin	Force
	type	Quadratic
	Equation	y = -4.833198E-005 * x ^ 2 + 0.212394 * x
	RT	7.228

8. LVI method - Matrix matched (MM) standards

Table 35: MM standard data for orange juice sample using LVI method.

MLXN	R^2	0.99986589
	weight	None
	origin	Force
	type	Quadratic
	Equation	y = 3.346102E-004 * x ^ 2 + 0.054177 * x
	RT	5.931
MLTN	R^2	0.99984944
	weight	None
	origin	Force
	type	Quadratic
	Equation	y = 3.360562E-004 * x ^ 2 + 0.320160 * x
	RT	6.234
CPFS	R^2	0.99983848
	weight	None
	origin	Force
	type	Quadratic
	Equation	y = 5.321793E-004 * x ^ 2 + 0.457769 * x
	RT	6.353
BRMP	R^2	0.9996424
	weight	None
	origin	Force
	type	Quadratic
	Equation	y = 0.003557 * x ^ 2 + 0.515870 * x
	RT	6.541
BRPE	R^2	0.99965452
	weight	None
	origin	Force
	type	Quadratic
	Equation	y = -1.771879E-004 * x ^ 2 + 0.489828 * x
	RT	6.94
MTDN	R^2	0.99934528
	weight	None
	origin	Force
	type	Linear
	Equation	y = 1.034420 * x
	RT	6.943
PRFF	R^2	0.99952133
	weight	None
	origin	Force
	type	Quadratic
	Equation	y = 0.001482 * x ^ 2 + 0.213137 * x
	RT	7.221

Appendix I: Specificity Calculations

Table 36: Specificity calculated for apple juice sample using the HSI method.

	MLXN	MLTN	CPFS	BRMP	BRPE	MTDN	PRFF
Name	Resp.	Resp.	Resp.	Resp.	Resp.	Resp.	Resp.
Reagent Blank	9	681	1725	2113	2185	1298	112
AP JU blank	10	227	645	685	901	874	30
Response at Reporting limit (0.01 ppm) (STD 2)	748	7241	7395	7308	11299	15552	3412
30 % of Response at Reporting limit	225	2172	2218	2192	3390	4666	1024

Table 37: Specificity calculated for apple juice sample using the LVI method.

	MLXN	MLTN	CPFS	BRMP	BRPE	MTDN	PRFF
Name	Resp.	Resp.	Resp.	Resp.	Resp.	Resp.	Resp.
Reagent blank	123	865	1232	1513	1555	1794	747
AP JU blank	22	72	244	494	56	915	35
Response at Reporting limit (0.01 ppm) (STD 2)	5431	47893	53270	59154	82960	108872	27112
30% of Response at reporting limit	1629	14368	15981	17746	24888	32662	8134

Table 38: Specificity calculated for orange juice sample using the HSI method.

	MLXN	MLTN	CPFS	BRMP	BRPE	MTDN	PRFF
Name	Resp.	Resp.	Resp.	Resp.	Resp.	Resp.	Resp.
Reagent blank	11	2	117	0	12	16	13
OR JU blank	3	2	118	4	9	62	26
Response at the reporting limit (0.01 ppm) (STD 2)	518	6179	6184	6249	8484	11736	2380
30% of Response at reporting limit	155	1854	1855	1875	2545	3521	714

Table 39: Specificity calculated for orange juice sample using LVI method.

	MLXN	MLTN	CPFS	BRMP	BRPE	MTDN	PRFF
Name	Resp.	Resp.	Resp.	Resp.	Resp.	Resp.	Resp.
Reagent blank	5	10	268	138	5	29	34
OR JU blank	4	16	86	250	29	1052	12
Response at reporting limit (0.01 ppm) (STD 2)	7209	58381	57871	61126	84425	132322	30379
30% of response at reporting limit	2163	17514	17361	18338	25328	39697	9114

Appendix J: HSI calculations of LOD, LOQ and Repeatability - AP JU sample

Table 40: HSI - Data results used to calculate LOD, LOQ, %RSD at 0.01 ppm and at 0.1 ppm for the apple juice sample.

Name	MLXN		MLTN		CPFS		BRMP		BRPE		MTDN		PRFF	
	Resp.	Calc. Conc.	Resp.	Calc. Conc.	Resp.	Calc. Conc.	Resp.	Calc. Conc.	Resp.	Calc. Conc.	Resp.	Calc. Conc.	Resp.	Calc. Conc.
AP LOQ 1	593	0.0118	5436	0.0149	5366	0.0100	6168	0.0096	8761	0.0138	11038	0.0099	2716	0.0110
AP LOQ 2	515	0.0100	5652	0.0150	5689	0.0103	6504	0.0098	8909	0.0136	10943	0.0095	2741	0.0107
AP LOQ 3	443	0.0088	5608	0.0149	5847	0.0106	6579	0.0100	8745	0.0134	10931	0.0096	2931	0.0116
AP LOQ 4	411	0.0080	5283	0.0137	6262	0.0110	6277	0.0092	9273	0.0139	11470	0.0098	2576	0.0099
AP LOQ 5	518	0.0100	5692	0.0149	6308	0.0113	6611	0.0099	9306	0.0141	11859	0.0102	2949	0.0115
AP LOQ 6	601	0.0109	6079	0.0150	5995	0.0101	6815	0.0096	9504	0.0135	12816	0.0104	2987	0.0109
Average		**0.0099**		**0.0147**		**0.0106**		**0.0097**		**0.0137**		**0.0099**		**0.0109**
STDEV		**0.0014**		**0.0005**		**0.0005**		**0.0003**		**0.0002**		**0.0004**		**0.0006**
So		**0.0014**		**0.0005**		**0.0005**		**0.0003**		**0.0002**		**0.0004**		**0.0006**
LOD		**0.0041**		**0.0015**		**0.0015**		**0.0008**		**0.0007**		**0.0011**		**0.0018**
LOQ		**0.0068**		**0.0026**		**0.0025**		**0.0013**		**0.0012**		**0.0018**		**0.0030**
%RSD at 0.01 ppm		**13.8**		**3.5**		**4.8**		**2.7**		**1.8**		**3.6**		**5.5**
AP SP 1	6135	0.1056	61174	0.1548	65393	0.1088	71572	0.1023	98184	0.1439	115825	0.0965	30405	0.1140
AP SP 2	5791	0.1055	56218	0.1506	61210	0.1078	65254	0.0988	92378	0.1433	111323	0.0982	28175	0.1118
AP SP 3	6704	0.1065	63966	0.1495	70170	0.1078	76197	0.1006	104447	0.1413	130631	0.1004	32361	0.1120
AP SP 4	6105	0.1068	57842	0.1489	61909	0.1047	68318	0.0993	95006	0.1415	111266	0.0943	29305	0.1117
AP SP 5	6265	0.0959	71685	0.1610	74564	0.1101	79470	0.1008	112783	0.1467	136844	0.1012	36102	0.1201
AP SP 6	4789	0.0891	57088	0.1556	61383	0.1100	64867	0.0999	92170	0.1454	111697	0.1002	25871	0.1045
Average		**0.1016**		**0.1534**		**0.1082**		**0.1003**		**0.1437**		**0.0985**		**0.1124**
STDEV		**0.0074**		**0.0046**		**0.0020**		**0.0013**		**0.0021**		**0.0027**		**0.0050**
%RSD at 0.1 ppm		**7.2**		**3.0**		**1.8**		**1.3**		**1.5**		**2.7**		**4.4**

Appendix K: HSI calculations of Recovery - AP JU sample

Table 41: HSI - Data results used to calculate % Recovery at 0.01 ppm and at 0.1 ppm for the AP JU sample..

Name	MLXN		MLTN		CPFS		BRMP		BRPE		MTDN		PRFF	
	Resp.	Calc. Conc.	Resp.	Calc. Conc.	Resp.	Calc. Conc.	Resp.	Calc. Conc.	Resp.	Calc. Conc.	Resp.	Calc. Conc.	Resp.	Calc. Conc.
AP LOQ 1	593	0.0129	5436	0.0143	5366	0.0087	6168	0.0086	8761	0.0127	11038	0.0105	2716	0.0117
AP LOQ 2	515	0.0109	5652	0.0144	5689	0.0089	6504	0.0088	8909	0.0125	10943	0.0101	2741	0.0114
AP LOQ 3	443	0.0094	5608	0.0144	5847	0.0092	6579	0.0089	8745	0.0123	10931	0.0102	2931	0.0123
AP LOQ 4	411	0.0085	5283	0.0132	6262	0.0096	6277	0.0083	9273	0.0127	11470	0.0104	2576	0.0105
AP LOQ 5	518	0.0108	5692	0.0144	6308	0.0098	6611	0.0088	9306	0.0129	11859	0.0109	2949	0.0122
AP LOQ 6	601	0.0118	6079	0.0144	5995	0.0087	6815	0.0086	9504	0.0124	12816	0.0110	2987	0.0116
Average		**0.0107**		**0.0142**		**0.0091**		**0.0087**		**0.0126**		**0.0105**		**0.0116**
True value		**0.0108**		**0.0156**		**0.0110**		**0.0100**		**0.0147**		**0.0105**		**0.0120**
% Recovery at 0.01 ppm		**98.87**		**90.68**		**83.23**		**86.96**		**85.72**		**99.72**		**96.26**
AP SP 1	6135	0.1214	61174	0.1483	65393	0.0977	71572	0.0924	98184	0.1312	115825	0.1013	30405	0.1197
AP SP 2	5791	0.1213	56218	0.1443	61210	0.0968	65254	0.0892	92378	0.1307	111323	0.1031	28175	0.1174
AP SP 3	6704	0.1224	63966	0.1432	70170	0.0968	76197	0.0908	104447	0.1289	130631	0.1054	32361	0.1176
AP SP 4	6105	0.1228	57842	0.1426	61909	0.0940	68318	0.0897	95006	0.1291	111266	0.0990	29305	0.1173
AP SP 5	6265	0.1103	71685	0.1541	74564	0.0988	79470	0.0911	112783	0.1337	136844	0.1062	36102	0.1260
AP SP 6	4789	0.1025	57088	0.1490	61383	0.0987	64867	0.0902	92170	0.1326	111697	0.1052	25871	0.1098
Average		**0.1168**		**0.1469**		**0.0971**		**0.0905**		**0.1310**		**0.1034**		**0.1180**
True value		**0.1082**		**0.1560**		**0.1094**		**0.0995**		**0.1466**		**0.1052**		**0.1202**
% Recovery at 0.1 ppm		**107.92**		**94.18**		**88.81**		**91.02**		**89.36**		**98.27**		**98.15**

Appendix L: LVI calculations of LOD, LOQ and Repeatability - AP JU sample.

Table 42: LVI - Data results used to calculate LOD, LOQ and %RSD at 0.01 ppm and 0.1 ppm for the AP JU sample.

Name	MLXN		MLTN		CPFS		BRMP		BRPE		MTDN		PRFF	
	Resp.	Calc. Conc.	Resp.	Calc. Conc.	Resp.	Calc. Conc.	Resp.	Calc. Conc.	Resp.	Calc. Conc.	Resp.	Calc. Conc.	Resp.	Calc. Conc.
AP LOQ 1	3921	0.0112	43180	0.0174	55630	0.0126	65100	0.0123	80811	0.0172	96091	0.0119	23181	0.0117
AP LOQ 2	3828	0.0111	38233	0.0157	47249	0.0108	50873	0.0098	69538	0.0151	86300	0.0109	22172	0.0114
AP LOQ 3	4267	0.0115	41536	0.0158	50167	0.0106	54718	0.0098	73949	0.0149	95329	0.0112	23107	0.0110
AP LOQ 4	4271	0.0119	40185	0.0157	48446	0.0106	52985	0.0097	70960	0.0147	92383	0.0111	23000	0.0113
AP LOQ 5	3852	0.0109	39242	0.0157	45605	0.0102	50985	0.0096	68304	0.0145	86975	0.0107	22319	0.0112
AP LOQ 6	4717	0.0119	46720	0.0167	53961	0.0107	56730	0.0095	76051	0.0144	104539	0.0115	26457	0.0118
Average		**0.0114**		**0.0162**		**0.0109**		**0.0101**		**0.0151**		**0.0112**		**0.0114**
STDEV		**0.0004**		**0.0007**		**0.0009**		**0.0011**		**0.0011**		**0.0004**		**0.0003**
S'o		**0.0004**		**0.0007**		**0.0009**		**0.0011**		**0.0011**		**0.0004**		**0.0003**
LOD		**0.0012**		**0.0021**		**0.0026**		**0.0033**		**0.0032**		**0.0013**		**0.0009**
LOQ		**0.0020**		**0.0036**		**0.0043**		**0.0054**		**0.0053**		**0.0022**		**0.0016**
%RSD at 0.01 ppm		**3.5**		**4.4**		**8.0**		**10.7**		**7.1**		**3.8**		**2.7**
AP SP 1	45518	0.1208	424402	0.1611	479743	0.1080	541506	0.0967	721745	0.1451	954197	0.1118	250833	0.1192
AP SP 2	45184	0.1251	410413	0.1626	470456	0.1106	519193	0.0968	692880	0.1454	924652	0.1131	238155	0.1181
AP SP 3	51300	0.1299	456679	0.1655	525188	0.1130	583646	0.0995	775639	0.1489	1031009	0.1153	263508	0.1195
AP SP 4	38077	0.1087	383754	0.1566	449601	0.1088	506254	0.0972	670704	0.1450	841827	0.1060	229408	0.1172
AP SP 5	39043	0.1092	404628	0.1618	467099	0.1108	515857	0.0971	692799	0.1468	879559	0.1086	237081	0.1187
AP SP 6	41257	0.1201	392354	0.1634	465031	0.1150	506075	0.0992	669103	0.1476	892908	0.1148	229604	0.1197
Average		**0.1190**		**0.1618**		**0.1110**		**0.0977**		**0.1465**		**0.1116**		**0.1187**
STDEV		**0.0085**		**0.0030**		**0.0026**		**0.0013**		**0.0016**		**0.0037**		**0.0010**
%RSD at 0.1 ppm		**7.1**		**1.9**		**2.3**		**1.3**		**1.1**		**3.3**		**0.8**

Appendix M: LVI calculations of Recovery - AP JU sample.

Table 43: LVI - Data results used to calculate % Recovery at 0.01 ppm and 0.1 ppm for the AP JU sample.

Name	MLXN		MLTN		CPFS		BRMP		BRPE		MTDN		PRFF	
	Resp.	Calc. Conc.	Resp.	Calc. Conc.	Resp.	Calc. Conc.	Resp.	Calc. Conc.	Resp.	Calc. Conc.	Resp.	Calc. Conc.	Resp.	Calc. Conc.
AP LOQ 1	3921	0.0120	43180	0.0164	55630	0.0124	65100	0.0125	80811	0.0159	96091	0.0123	23181	0.0122
AP LOQ 2	3828	0.0119	38233	0.0148	47249	0.0107	50873	0.0099	69538	0.0139	86300	0.0112	22172	0.0119
AP LOQ 3	4267	0.0123	41536	0.0149	50167	0.0106	54718	0.0099	73949	0.0138	95329	0.0115	23107	0.0115
AP LOQ 4	4271	0.0126	40185	0.0148	48446	0.0105	52985	0.0099	70960	0.0136	92383	0.0115	23000	0.0117
AP LOQ 5	3852	0.0117	39242	0.0148	45605	0.0101	50985	0.0097	68304	0.0134	86975	0.0110	22319	0.0117
AP LOQ 6	4717	0.0127	46720	0.0157	53961	0.0107	56730	0.0096	76051	0.0133	104539	0.0118	26457	0.0123
Average		**0.0122**		**0.0152**		**0.0108**		**0.0102**		**0.0140**		**0.0116**		**0.0119**
True value		**0.0108**		**0.0156**		**0.0110**		**0.0100**		**0.0147**		**0.0105**		**0.0120**
% Recovery at 0.01 ppm		**112.88**		**97.68**		**98.49**		**102.41**		**95.12**		**110.02**		**98.89**
AP SP 1	45518	0.1264	424402	0.1505	479743	0.1003	541506	0.0969	721745	0.1338	954197	0.1135	250833	0.1225
AP SP 2	45184	0.1308	410413	0.1519	470456	0.1027	519193	0.0970	692880	0.1341	924652	0.1148	238155	0.1215
AP SP 3	51300	0.1356	456679	0.1546	525188	0.1049	583646	0.0997	775639	0.1373	1031009	0.1171	263508	0.1229
AP SP 4	38077	0.1140	383754	0.1463	449601	0.1011	506254	0.0974	670704	0.1337	841827	0.1077	229408	0.1205
AP SP 5	39043	0.1146	404628	0.1511	467099	0.1029	515857	0.0973	692799	0.1353	879559	0.1103	237081	0.1220
AP SP 6	41257	0.1257	392354	0.1526	465031	0.1067	506075	0.0994	669103	0.1361	892908	0.1165	229604	0.1231
Average		**0.1245**		**0.1512**		**0.1031**		**0.0979**		**0.1350**		**0.1133**		**0.1221**
True value		**0.1082**		**0.1560**		**0.1094**		**0.0995**		**0.1466**		**0.1052**		**0.1202**
% Recovery at 0.01 ppm		**115.09**		**96.89**		**94.24**		**98.44**		**92.12**		**107.72**		**101.56**

Appendix N: HSI calculations of LOD, LOQ and Repeatability - OR JU sample.

Table 44: HSI - Data results used to calculate LOD, LOQ and %RSD at 0.01 ppm and at 0.1 ppm for the OR JU sample.

Name	MLXN		MLTN		CPFS		BRMP		BRPE		MTDN		PRFF	
	Resp.	Calc. Conc.	Resp.	Calc. Conc.	Resp.	Calc. Conc.	Resp.	Calc. Conc.	Resp.	Calc. Conc.	Resp.	Calc. Conc.	Resp.	Calc. Conc.
OR LOQ 1	594	0.0103	6608	0.0154	6188	0.0101	7152	0.0105	9893	0.0147	11358	0.0089	3060	0.0123
OR LOQ 2	663	0.0115	6311	0.0147	6298	0.0102	6651	0.0097	9113	0.0135	12124	0.0095	2986	0.0120
OR LOQ 3	680	0.0110	6196	0.0134	6881	0.0104	7418	0.0101	10002	0.0139	12731	0.0093	2651	0.0099
OR LOQ 4	645	0.0103	6906	0.0147	7017	0.0104	7821	0.0105	9806	0.0134	13439	0.0097	3016	0.0111
OR LOQ 5	743	0.0115	7438	0.0154	7453	0.0108	7999	0.0104	10704	0.0141	13882	0.0097	3594	0.0129
OR LOQ 6	579	0.0099	6154	0.0141	6823	0.0109	6895	0.0099	9907	0.0145	12410	0.0096	2770	0.0109
Average		**0.0108**		**0.0146**		**0.0105**		**0.0102**		**0.0140**		**0.0095**		**0.0115**
STDEV		**0.0007**		**0.0008**		**0.0003**		**0.0003**		**0.0005**		**0.0003**		**0.0011**
S'o		**0.0007**		**0.0008**		**0.0003**		**0.0003**		**0.0005**		**0.0003**		**0.0011**
LOD		**0.0020**		**0.0023**		**0.0010**		**0.0009**		**0.0016**		**0.0009**		**0.0033**
LOQ		**0.0034**		**0.0038**		**0.0016**		**0.0016**		**0.0027**		**0.0015**		**0.0055**
%RSD at 0.01 ppm		**6.3**		**5.2**		**3.1**		**3.1**		**3.8**		**3.1**		**9.5**
OR SP 1	6369	0.1069	65969	0.1498	69238	0.1097	70126	0.1000	98749	0.1435	126851	0.0979	30489	0.1226
OR SP 2	6915	0.1113	72553	0.1581	75424	0.1147	76671	0.1049	108675	0.1515	140421	0.1040	34842	0.1343
OR SP 3	5932	0.1059	59870	0.1446	62460	0.1053	64832	0.0984	89038	0.1376	118812	0.0975	28937	0.1237
OR SP 4	5787	0.1124	58978	0.1552	61999	0.1138	63775	0.1054	89082	0.1500	116214	0.1039	27606	0.1286
OR SP 5	5677	0.1064	59895	0.1520	63590	0.1125	63375	0.1010	89673	0.1456	114653	0.0988	27971	0.1256
OR SP 6	6305	0.1194	59579	0.1531	63276	0.1134	64598	0.1042	91083	0.1497	123011	0.1073	30396	0.1380
Average		**0.1104**		**0.1521**		**0.1116**		**0.1023**		**0.1463**		**0.1016**		**0.1288**
STDEV		**0.0052**		**0.0046**		**0.0035**		**0.0029**		**0.0052**		**0.0041**		**0.0062**
%RSD at 0.1 ppm		**4.7**		**3.0**		**3.2**		**2.9**		**3.6**		**4.0**		**4.8**

Appendix O: HSI calculations of Recovery - OR JU sample.

Table 45: HSI - Data results used to calculate % Recovery at 0.01 ppm and at 0.1 ppm for the OR JU sample.

Name	MLXN		MLTN		CPFS		BRMP		BRPE		MTDN		PRFF	
	Resp.	Calc. Conc.	Resp.	Calc. Conc.	Resp.	Calc. Conc.	Resp.	Calc. Conc.	Resp.	Calc. Conc.	Resp.	Calc. Conc.	Resp.	Calc. Conc.
OR LOQ 1	594	0.0096	6608	0.0155	6188	0.0090	7152	0.0094	9893	0.0149	11358	0.0096	3060	0.0123
OR LOQ 2	663	0.0107	6311	0.0147	6298	0.0092	6651	0.0087	9113	0.0137	12124	0.0102	2986	0.0120
OR LOQ 3	680	0.0102	6196	0.0135	6881	0.0093	7418	0.0091	10002	0.0140	12731	0.0100	2651	0.0099
OR LOQ 4	645	0.0095	6906	0.0148	7017	0.0094	7821	0.0094	9806	0.0135	13439	0.0104	3016	0.0111
OR LOQ 5	743	0.0107	7438	0.0155	7453	0.0096	7999	0.0093	10704	0.0143	13882	0.0104	3594	0.0128
OR LOQ 6	579	0.0092	6154	0.0142	6823	0.0098	6895	0.0089	9907	0.0147	12410	0.0103	2770	0.0109
Average		**0.0100**		**0.0147**		**0.0094**		**0.0091**		**0.0142**		**0.0102**		**0.0115**
True value		**0.0108**		**0.0156**		**0.0110**		**0.0100**		**0.0147**		**0.0105**		**0.0120**
% Recovery at 0.01 ppm		**92.47**		**94.12**		**85.28**		**91.25**		**96.56**		**96.90**		**95.83**
OR SP 1	6369	0.0990	65969	0.1489	69238	0.0984	70126	0.0900	98749	0.1434	126851	0.1032	30489	0.1178
OR SP 2	6915	0.1031	72553	0.1570	75424	0.1029	76671	0.0944	108675	0.1513	140421	0.1095	34842	0.1289
OR SP 3	5932	0.0981	59870	0.1438	62460	0.0944	64832	0.0884	89038	0.1376	118812	0.1028	28937	0.1188
OR SP 4	5787	0.1041	58978	0.1542	61999	0.1021	63775	0.0948	89082	0.1498	116214	0.1095	27606	0.1235
OR SP 5	5677	0.0986	59895	0.1510	63590	0.1010	63375	0.0908	89673	0.1455	114653	0.1042	27971	0.1207
OR SP 6	6305	0.1106	59579	0.1521	63276	0.1017	64598	0.0937	91083	0.1496	123011	0.1130	30396	0.1325
Average		**0.1022**		**0.1512**		**0.1001**		**0.0920**		**0.1462**		**0.1071**		**0.1237**
True value		**0.1082**		**0.1560**		**0.1094**		**0.0995**		**0.1466**		**0.1052**		**0.1202**
% Recovery at 0.1 ppm		**94.49**		**96.92**		**91.50**		**92.49**		**99.73**		**101.77**		**102.91**

Appendix P: LVI calculations of LOD, LOQ and Repeatability - OR Juice

Table 46: LVI - Data results used for the calculate LOD, LOQ and % RSD at 0.01 ppm and at 0.1 ppm for the OR JU sample.

Name	MLXN		MLTN		CPFS		BRMP		BRPE		MTDN		PRF	
	Resp.	Calc. Conc.	Resp.	Calc. Conc.	Resp.	Calc. Conc.	Resp.	Calc. Conc.	Resp.	Calc. Conc.	Resp.	Calc. Conc.	Resp.	Calc. Conc.
OR LOQ 1	6574	0.0106	59979	0.0169	61269	0.0117	70001	0.0108	95863	0.0166	138483	0.0118	32248	0.0119
OR LOQ 2	6587	0.0112	54520	0.0162	53920	0.0108	60373	0.0099	82904	0.0152	122788	0.0110	30382	0.0118
OR LOQ 3	6987	0.0108	57380	0.0155	58170	0.0106	65822	0.0097	90274	0.0150	132259	0.0108	32849	0.0116
OR LOQ 4	7718	0.0118	64015	0.0172	62530	0.0113	67909	0.0100	92108	0.0152	138752	0.0112	34357	0.0120
OR LOQ 5	4073	0.0070	46295	0.0140	57456	0.0117	60620	0.0101	83640	0.0156	121797	0.0111	30748	0.0122
OR LOQ 6	6188	0.0103	56946	0.0166	58544	0.0115	64471	0.0103	89049	0.0160	131712	0.0116	31629	0.0120
Average		**0.0103**		**0.0161**		**0.0113**		**0.0101**		**0.0156**		**0.0113**		**0.0119**
STDEV		**0.0017**		**0.0012**		**0.0005**		**0.0004**		**0.0006**		**0.0004**		**0.0002**
S'o		**0.0017**		**0.0012**		**0.0005**		**0.0004**		**0.0006**		**0.0004**		**0.0002**
LOD		**0.0050**		**0.0035**		**0.0014**		**0.0012**		**0.0019**		**0.0011**		**0.0006**
LOQ		**0.0084**		**0.0058**		**0.0024**		**0.0020**		**0.0031**		**0.0019**		**0.0011**
%RSD at 0.01 ppm		**16.2**		**7.2**		**4.2**		**3.9**		**4.0**		**3.4**		**1.8**
OR SP 1	71468	0.1168	605843	0.1738	590836	0.1140	637352	0.0999	875021	0.1540	1316319	0.1137	324732	0.1215
OR SP 2	64857	0.1091	574427	0.1696	600017	0.1192	657462	0.1061	891257	0.1614	1336607	0.1189	331483	0.1277
OR SP 3	61243	0.1113	513267	0.1637	528949	0.1136	567137	0.0989	769692	0.1506	1168001	0.1122	286796	0.1194
OR SP 4	57519	0.1123	501971	0.1720	522473	0.1205	556857	0.1043	748847	0.1574	1149004	0.1186	283288	0.1267
OR SP 5	37732	0.0801	389149	0.1449	471204	0.1181	510485	0.1039	696777	0.1592	1049673	0.1178	258528	0.1257
OR SP 6	54710	0.1130	471119	0.1708	487841	0.1190	530719	0.1052	718707	0.1599	1067009	0.1165	271340	0.1284
Average		**0.1071**		**0.1658**		**0.1174**		**0.1031**		**0.1571**		**0.1163**		**0.1249**
STDEV		**0.0135**		**0.0108**		**0.0029**		**0.0029**		**0.0041**		**0.0027**		**0.0036**
%RSD at 0.1 ppm		**12.6**		**6.5**		**2.5**		**2.9**		**2.6**		**2.3**		**2.9**

Appendix Q: LVI calculations of Recovery - OR Juice

Table 47: LVI - Data results used to calculate % Recovery at 0.01 ppm and 0.1 ppm for the OR JU sample.

Name	MLXN		MLTN		CPFS		BRMP		BRPE		MTDN		PRFF	
	Resp.	Calc. Conc.	Resp.	Calc. Conc.	Resp.	Calc. Conc.	Resp.	Calc. Conc.	Resp.	Calc. Conc.	Resp.	Calc. Conc.	Resp.	Calc. Conc.
OR LOQ 1	6574	0.0095	59979	0.0147	61269	0.0105	70001	0.0106	95863	0.0153	138483	0.0105	32248	0.0118
OR LOQ 2	6587	0.0100	54520	0.0141	53920	0.0097	60373	0.0097	82904	0.0140	122788	0.0098	30382	0.0118
OR LOQ 3	6987	0.0096	57380	0.0134	58170	0.0095	65822	0.0095	90274	0.0138	132259	0.0096	32849	0.0115
OR LOQ 4	7718	0.0106	64015	0.0149	62530	0.0102	67909	0.0098	92108	0.0140	138752	0.0100	34357	0.0120
OR LOQ 5	4073	0.0063	46295	0.0122	57456	0.0106	60620	0.0099	83640	0.0144	121797	0.0099	30748	0.0121
OR LOQ 6	6188	0.0092	56946	0.0144	58544	0.0103	64471	0.0101	89049	0.0147	131712	0.0103	31629	0.0120
Average		**0.0092**		**0.0139**		**0.0101**		**0.0099**		**0.0144**		**0.0100**		**0.0119**
True value		**0.0108**		**0.0156**		**0.0110**		**0.0100**		**0.0147**		**0.0105**		**0.0120**
% Recovery at 0.01 ppm		**85.41**		**89.31**		**92.12**		**99.35**		**97.72**		**95.33**		**98.96**
OR SP 1	71468	0.1042	605843	0.1501	590836	0.1024	637352	0.0975	875021	0.1420	1316319	0.1011	324732	0.1201
OR SP 2	64857	0.0973	574427	0.1465	600017	0.1071	657462	0.1035	891257	0.1489	1336607	0.1057	331483	0.1261
OR SP 3	61243	0.0993	513267	0.1415	528949	0.1020	567137	0.0965	769692	0.1389	1168001	0.0998	286796	0.1180
OR SP 4	57519	0.1002	501971	0.1486	522473	0.1082	556857	0.1018	748847	0.1452	1149004	0.1055	283288	0.1251
OR SP 5	37732	0.0716	389149	0.1253	471204	0.1061	510485	0.1014	696777	0.1469	1049673	0.1047	258528	0.1241
OR SP 6	54710	0.1008	471119	0.1476	487841	0.1069	530719	0.1026	718707	0.1475	1067009	0.1036	271340	0.1268
Average		**0.0956**		**0.1433**		**0.1055**		**0.1006**		**0.1449**		**0.1034**		**0.1234**
True value		**0.1082**		**0.1560**		**0.1094**		**0.0995**		**0.1466**		**0.1052**		**0.1202**
% Recovery at 0.1 ppm		**88.31**		**91.84**		**96.40**		**101.06**		**98.85**		**98.29**		**102.62**

Appendix R: Calibration data for FAPAS Proficiency testing

Table 48: Orange juice calibration standards data and blank sample for the analysis of PRFF using HSI method.

Name	Type	Level	PRFF Method	PRFF Results			FCHF (ISTD) Results
			Exp. Conc.	RT	Resp.	Calc. Conc.	Resp.
OR JU blank	Sample			6.52	70	0.0003	145241
OR JU STD 1	Cal	L1	0.0120	6.49	1758	0.0109	102383
OR JU STD 2	Cal	L2	0.0599	6.49	12151	0.0654	118174
OR JU STD 3	Cal	L3	0.1199	6.49	19040	0.1201	100813
OR JU STD 4	Cal	L4	0.2398	6.49	34558	0.2352	93444
OR JU STD 5	Cal	L5	0.4796	6.49	73911	0.4923	95556
OR JU STD 6	Cal	L6	1.1989	6.49	172231	1.1861	92551

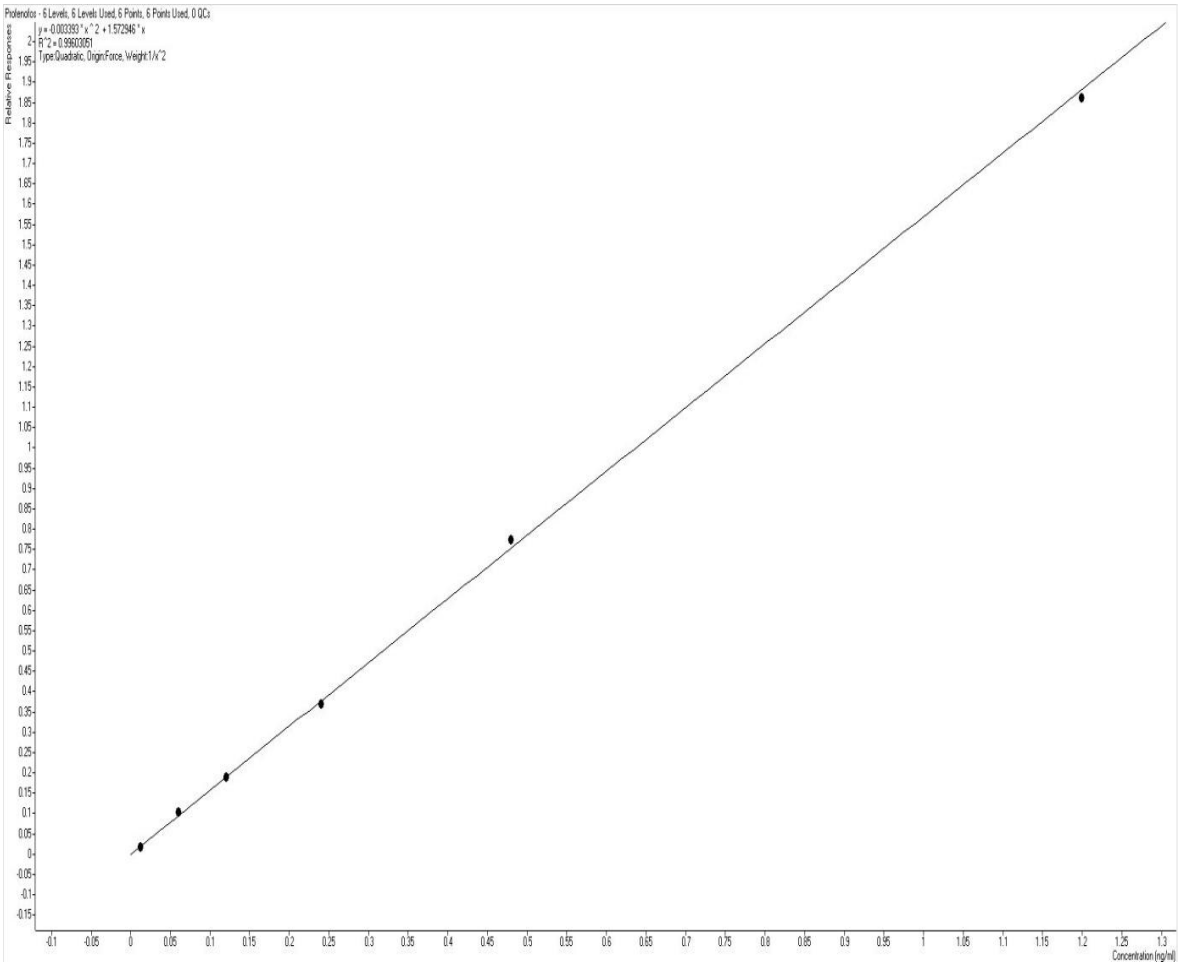


Figure 54: Calibration curve obtained for the analysis of PRFF in the FAPAS orange juice sample using HSI method.

Table 49: Orange juice calibration standards data and blank sample for the analysis of PRFF using LVI method.

Name	Type	Level	PRFF Method	PRFF Results			FCHF (ISTD) Results
			Exp. Conc.	RT	Resp.	Calc. Conc.	Resp.
OR JU BLANK	Sample			7.00	717	0.0003	1057769
OR JU STD 1	Cal	L1	0.0120	7.00	22593	0.0125	889211
OR JU STD 2	Cal	L2	0.0599	7.00	120775	0.0603	985699
OR JU STD 3	Cal	L3	0.1199	7.00	188821	0.1288	722340
OR JU STD 4	Cal	L4	0.2398	7.00	276305	0.2262	602759
OR JU STD 5	Cal	L5	0.4796	7.00	981026	0.4852	1001754
OR JU STD 6	Cal	L6	1.1989	7.00	2107837	1.1984	880975

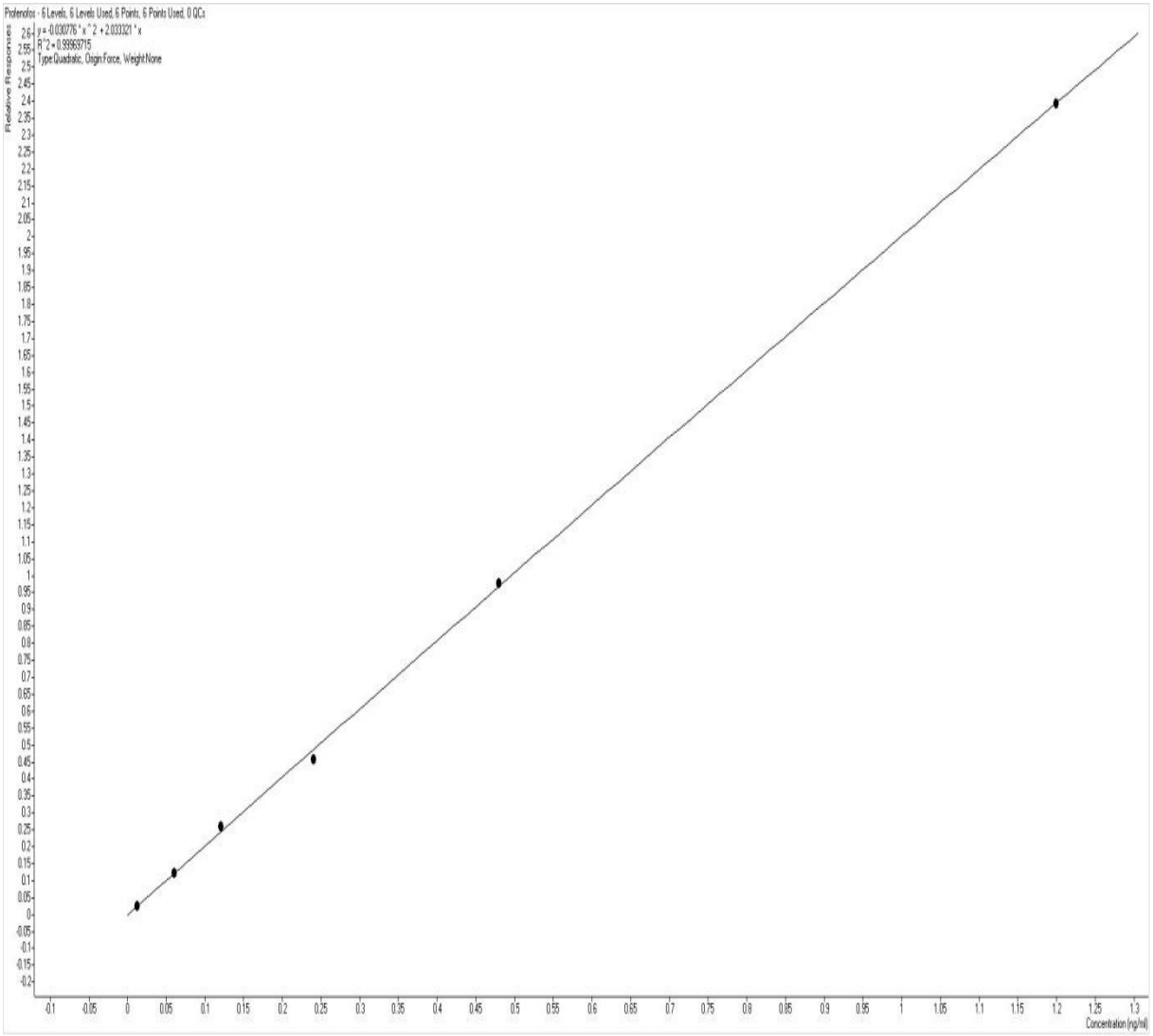


Figure 55: Calibration curve obtained for the analysis of PRFF in the FAPAS orange juice sample using LVI method.

Appendix S: Calibration data for NMISA Proficiency testing

Table 50: HSI calibration curve data for the analysis of NMISA MG sample.

Name	Type	Level	CPFS Method	CPFS Results			FCHF (ISTD) Results
			Exp. Conc.	RT	Resp.	Calc. Conc.	Resp.
AP BLANK	Sample			5.66	1642	0.0000	140002
AP STD 1	Cal	L1	0.0109	5.66	8394	0.0109	98608
AP STD 2	Cal	L2	0.0546	5.66	35621	0.0563	96013
AP STD 3	Cal	L3	0.1091	5.66	85208	0.1050	126215
AP STD 4	Cal	L4	0.2182	5.66	222383	0.2183	161888
AP STD 5	Cal	L5	0.4364	5.66	365534	0.4405	135098
AP STD 6	Cal	L6	1.0910	5.66	1069615	1.0891	169439

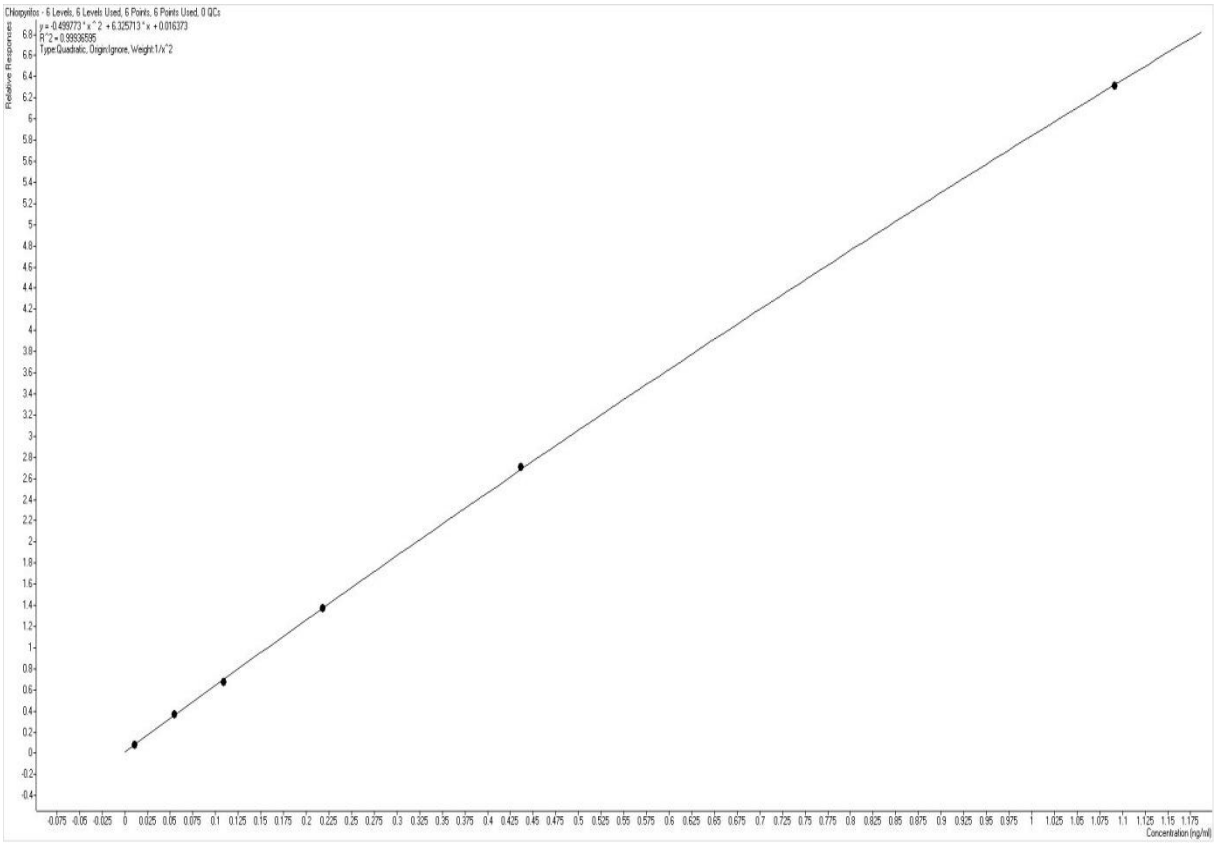


Figure 56: HSI calibration curve obtained for the analysis of CPFS in the NMISA MG sample.

Table 51: LVI calibration data for the analysis of MNISA MG sample.

Name	Type	Level	CPFS Method	CPFS Results			FCHF (ISTD)
			Exp. Conc.	RT	Resp.	Calc. Conc.	Resp.
AP BLANK	Sample			6.16	7077	0.0008	1616513
AP STD 1	Cal	L1	0.0109	6.16	77544	0.0121	1240814
AP STD 2	Cal	L2	0.0546	6.16	388760	0.0553	1361846
AP STD 3	Cal	L3	0.1091	6.16	793269	0.1084	1420479
AP STD 4	Cal	L4	0.2182	6.16	1634597	0.2187	1455838
AP STD 5	Cal	L5	0.4364	6.16	3166956	0.4361	1426191
AP STD 6	Cal	L6	1.0910	6.16	6926977	1.0910	1277979

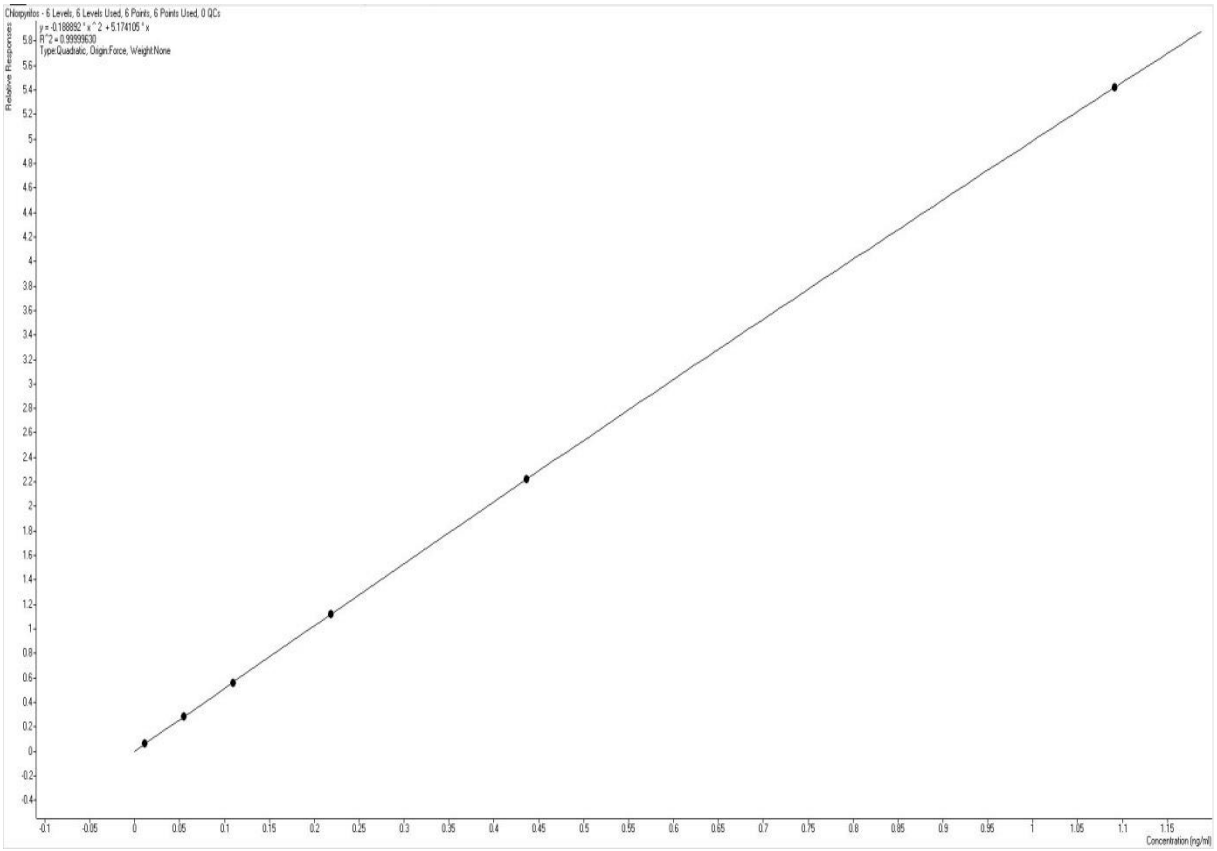


Figure 57: LVI calibration curve of CPFS for the analysis of NMISA MG sample.