A Preliminary Comparative Analysis of Pesticides Residues in Illicit and Regulated Cannabis Inflorescence in Malta

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Abstract

Illicit cannabis consumption presents significant health risks due to the absence of regulatory monitoring in its cultivation, especially with regards to pesticide contamination. In contrast, licensed cannabis produced under regulated frameworks is expected to comply with strict safety standards. This study investigated and compared pesticide residues in illicit cannabis samples seized by law enforcement officers with licensed recreational cannabis inflorescences sourced from Cannabis Associations licensed with the Authority for the Responsible use of Cannabis in Malta. The primary aim was to determine whether illicit cannabis is more likely to contain pesticide contamination. This study thus helped assess public health risks and highlighting the impact of regulatory control.

Twenty-four cannabis inflorescence samples were analysed: twelve illicit samples and twelve licensed samples. The methodology employed a QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) extraction protocol, followed by instrumental analysis using gas chromatography combined with mass spectrometry (GC-MS) and ultra-performance liquid chromatography coupled to tandem mass spectrometry (UPLC-MS/MS).

The findings demonstrated that pesticide residues were detected exclusively in illicit cannabis samples, while no pesticide contamination was found in licensed recreational cannabis provided by Cannabis Associations. Two organophosphate pesticides, dichlorvos and chlorpyrifos, were identified across multiple illicit samples. Statistical analysis using

Fisher's exact test confirmed a statistically significant difference in contamination between the two groups (two-sided analysis, $p = 0.037$).

These results highlight the elevated risk associated with illicit cannabis use and emphasise the protective role of regulatory oversight in licensed production. Inhalation of organophosphate pesticide residues may contribute to acute and chronic toxicological effects, presenting a considerable public health concern for consumers of illicit cannabis. These findings support the implementation of strict monitoring frameworks in order to safeguard cannabis quality and strengthen the importance of consumer access to regulated products.

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List of Abbreviations

ARUC - Authority for the Responsible Use of Cannabis

CBD - Cannabidiol

CNS - Central Nervous System

GC - Gas Chromatography

GCB - Graphitised Carbon Black

HPLC - High-Performance Liquid Chromatography

LC - Liquid Chromatography

M/Z - Mass-to-charge

MeSH - Medical Subject Headings

MRMs - Multiple Reaction Monitoring

MS - Mass Spectrometry

MS/MS - Tandem Mass Spectrometry

QuEChERS - Quick, Easy, Cheap, Effective, Rugged, and Safe

SPE - Solid Phase Extraction

THC - Tetrahydrocannabinol

TIC - Total Ion Chromatogram

UPLC - Ultra-Performance Liquid Chromatography

WHO - World Health Organisation

Chapter 1

Introduction

1.1 Overview

One of the primary concerns regarding the use of illicit cannabis is the potential contamination with pesticides, which may pose serious health risks to consumers (Farrelly, Wardell et al. 2023). The lack of standardised agricultural practices and quality control measures in illicit cannabis cultivation raises questions about the extent of pesticide use and contamination compared to legally regulated cannabis (Farrelly Wardell et al. 2023).

This study had the aim to investigate and compare the presence of pesticides in illicit and licensed cannabis samples in Malta. By conducting a comparative analysis, this research sought to determine whether illicit cannabis poses a higher risk of pesticide contamination than licensed cannabis and how regulatory oversight impacts pesticide residues.

This Chapter begins with a literature review, which describes the search strategy, inclusion and exclusion criteria and identifies gaps in existing knowledge. Following is an overview of cannabis, its use, and current market trends in Europe and Malta. It then examines the legalisation of recreational cannabis in a global and local context, before introducing pesticides, their relevance to cannabis cultivation, and the regulations surrounding their use. The chapter continues with an outline of analytical techniques for pesticide detection, providing the necessary background on sample preparation, separation, and instrumental methods. This is followed by a critically appraises the most relevant studies. Finally, the chapter presents the research question, aim, objectives, and hypothesis of this dissertation.

1.2 Literature Review

This literature review provides a comprehensive overview of current research related to the analysis and screening of pesticides in cannabis, focusing particularly on both illicit and licensed recreational cannabis. As cannabis consumption has gained increased attention due to its legalisation in several countries, the need for rigorous testing methods to detect harmful contaminants, such as pesticides, has become more critical (Abodeely 2025). This review examines relevant studies, methods, and technologies used in the analysis of pesticide residues in cannabis products, comparing findings from various countries with differing regulations. By evaluating recent peer-reviewed articles, this section highlights key trends, methodologies, and gaps in the existing body of knowledge, ultimately contributing to a better understanding of pesticide contamination in cannabis in Malta and its potential health risks.

1.2.1 The Search Strategy

A comprehensive literature search was done between June to September 2024. Databases used, which specialise in science literature, included PubMed, Google Scholar, Science Direct, Scopus and SpringerLink. Cannabis focused databases were also used such as the Cannabis Abstract database and the International Cannabinoids Research Society. For studies conducted on the Maltese islands, the University of Malta library OAR was used.

When searching these databases, keywords like “pesticides”, “cannabis”, “hemp”, “marijuana”, “contaminants”, “residue”, “recreational”, “legal” and other related terms were used to narrow down the research to relevant articles. Additionally Boolean operators (AND,

OR, NOT) and Medical Subject Headings (MeSH) terms helped in refining search queries for more targeted results.

Other terms searched on Google Scholar and other databases included Solid Phase Extraction (SPE), Quick Easy Cheap Effective Rugged and Safe (QuEChERS), Gas Chromatography (GC), Liquid Chromatography (LC) and Mass Spectrometry (MS). These were searched for further understanding of the procedures.

1.2.2 Inclusion and Exclusion Criteria

Articles included were preferably free and peer reviewed as they have a higher standard of quality, validity, and reliability as they undergo a rigorous evaluation process by experts in the field before being published.

In light of the recent legislative shifts regarding the legalisation of recreational cannabis in certain jurisdictions, the sources used in this study were predominantly recent. The selected studies primarily address the screening and analysis of pesticides in both illicit and licensed recreational cannabis. Comparative articles examining licensed versus illicit cannabis were considered particularly valuable, as they provide understanding into international regulatory frameworks and findings, which can be directly applied and implemented to our study. In addition, research concerning medical cannabis was also considered valuable to the present investigation, due to the relevance of the methods used for extraction and analysing cannabis.

Studies examining cannabis products other than the flower, such as vape products, resin, tinctures, edibles, and concentrates, were excluded. Additionally, studies focusing on pesticides in environmental sources like soil and water were also excluded.

Research looking at food products particularly dried leaves like teas were also included for reference to the method adopted for extraction and analysis, keeping in mind its limitations for comparison. Only articles published in English were included in this literature review.

1.2.3 Gap in the Literature

Existing research on cannabis in Malta predominantly focuses on medical cannabis, with studies examining its efficacy, safety, and regulatory frameworks. This study aimed to fill this gap by focusing on recreational cannabis and pesticides. This research will offer new and valuable data on the safety risks posed by pesticide residues in locally available recreational cannabis products. The findings thus are expected to contribute to a better understanding of the risks associated with recreational cannabis use, inform policy decisions related to cannabis safety, and potentially lead to the development of more comprehensive regulatory standards for the production and distribution of recreational cannabis.

Figure 1.10 is a Prisma flow diagram illustrating the search process, specific keywords used and Boolean operators, and the screening and narrowing down to relevant articles for this study, ending up with the final list of studies used for this dissertation.

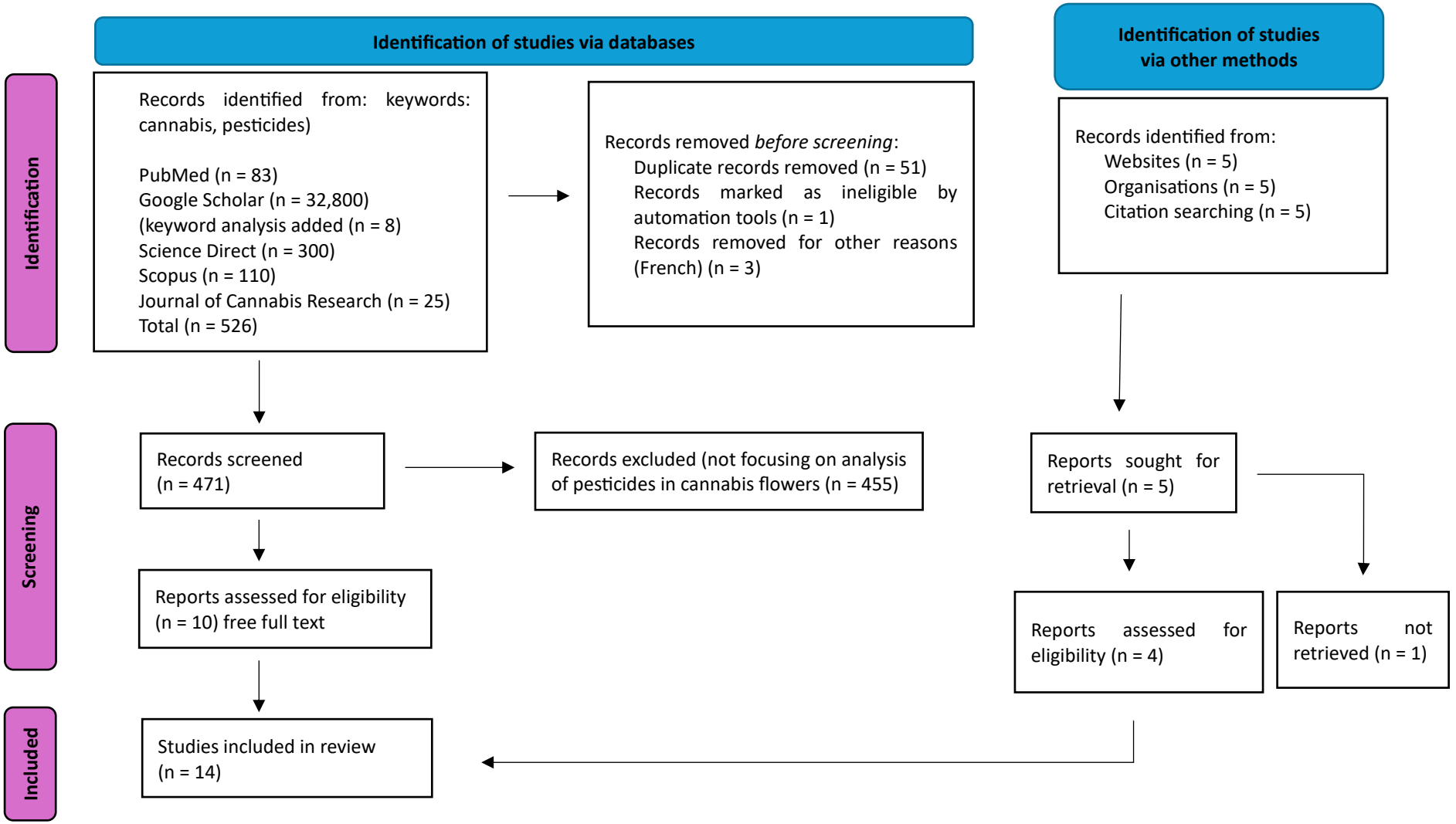

Figure 1.1 PRISMA Flow Diagram (Page Mckenzie et al. 2021)

1.3 Cannabis

Cannabis sativa L., native to Central Asia, is a highly complex plant containing a vast array of chemicals and has been utilised for thousands of years as a source of textile fibre, food, and medicine (Brenneisen 2007). A total of 483 compounds has been identified in cannabis. Among these compounds, over 60 cannabinoids are unique to cannabis, while terpenes, constituting the largest class with approximately 140 compounds, are widely distributed in other plants. (Brenneisen 2007).

The cannabis plant produces phytocannabinoids (phyto-CB), including the bioactive compounds Δ9-tetrahydrocannabinol (THC) and Cannabidiol (CBD) as shown in Figure 1.2 below (Stella 2024). THC is commonly recognised as the primary “psychoactive” compound in cannabis, while CBD is considered the main “non-psychoactive” compound (Stella 2024).

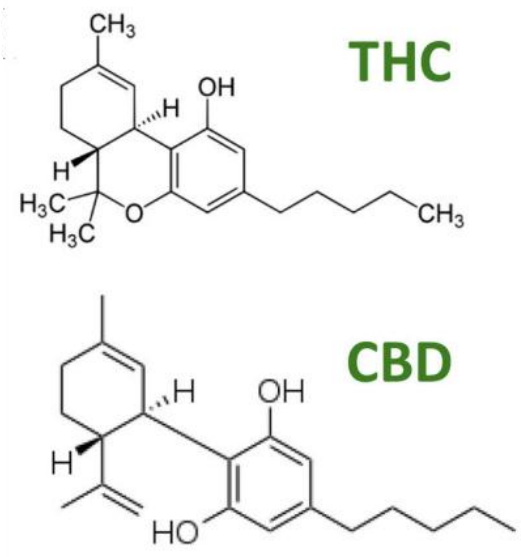

Figure 1.2 Structures of THC and CBD (Stella 2024)

Although THC and CBD are both derived from the same precursor in the Cannabis plant and share a similar chemical backbone, their structural differences significantly alter their biological activity (Stella 2024). The primary difference lies in the presence of a closed cyclic ring in THC versus an open ring structure with a hydroxyl group in CBD (Stella 2024). This seemingly small variation prevents CBD from fitting into the cannabinoid receptor 1 (CB1R) binding site in the same way as THC. As a result, THC acts as a partial agonist at CB1R and produces psychotropic effects, while CBD acts as a negative allosteric modulator at the same receptor, altering receptor activity without directly activating it (Stella 2024). These structural differences are fundamental in determining their distinct pharmacodynamic profiles and therapeutic potentials (Stella 2024).

In the last hundred years, cannabis has gained widespread recognition for its recreational use (Atapattu and Johnson 2019). It is the most extensively used illicit substance worldwide, with increasing legalisation efforts in various countries for medicinal and recreational purposes which is often consumed by smoking (herbal or resin), vaping or ingestion of its extracts (tincture, oils and other edibles) (Stempfer et al. 2021).

1.3.1 Cannabis Use and Market Trends in Europe

Cannabis continues to dominate the illicit drug landscape in the EU, remaining the most widely used controlled substance across all age groups (EMCDDA 2025). According to the European Drug Report 2025, approximately 8.4% of adults aged 15 to 64 years, representing around 24 million individuals, reported using cannabis in the previous year. This prevalence is notably

higher among young adults aged 15 to 34, of whom 15.4% (15.5 million) reported past-year use and even higher among even younger cohorts aged 15 to 24, with reported usage to be 18.6% (8.8 million). The following bar graph (see Figure 1.3) compares this behaviour showing that the younger the population, the higher the percentage of individuals using Cannabis products.

Daily or near-daily cannabis use is defined by the European Drug reports as consumption on 20 or more days within a month. Around 1.5% of adults, or roughly 4.3 million people in Europe, reported using cannabis daily or near-daily. These percentages are illustrated in the pie chart Figure 1.4 below. It is also interesting to note that nearly 75% of these frequent users were men (EMCDDA 2025).

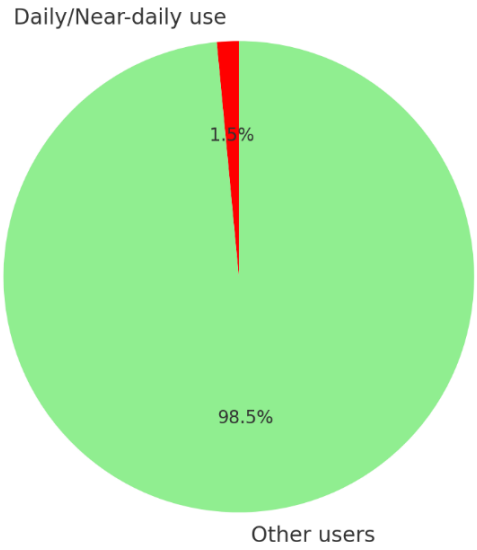

Figure 1.3 Daily/Near-daily cannabis use in the EU (2025) (EMCDDA 2025)

The above figures align with earlier trends reported in the 2024 edition, which also emphasised high cannabis prevalence among youths. Specifically, the 2024 report estimated that 15% of individuals aged 15 to 34 had used cannabis in the past year, with 9.7% reporting use within the last month, and 2% or approximately 2 million adults using cannabis daily or almost daily (EMCDDA 2024).

Herbal cannabis remains the dominant form of cannabis consumed across the continent, with 95% of cannabis users reporting its use in the past year, as compared to 32% for cannabis resin. These percentages are illustrated in the next bar graph Figure 1.5. This preference has coincided with notable shifts in production and trafficking. (EMCDDA 2025). Given its overwhelming predominance in consumption, this study focused on herbal cannabis, since it represents the primary route of exposure to any potential pesticide residues among users.

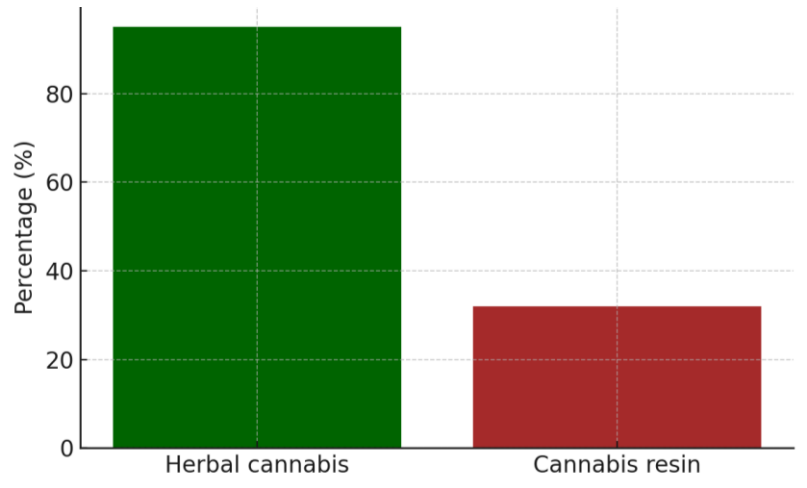

Figure 1.4 Preference for cannabis type in the EU (EMCDDA 2025)

These prevalence figures emphasise why this study is important. Cannabis is not only the most commonly used illicit drug in the EU but also particularly popular among young adults, who are the most vulnerable to potential health risks from contaminated products (EMCDDA 2025). High and sustained levels of use mean that any pesticide residues present in cannabis could affect a very large section of the population, reinforcing the need to investigate the safety of both licensed and illicit supplies.

1.3.2 Cannabis Use and Market Trends in Malta

According to the National Report on the Drug Situation and Responses in Malta 2023, published in 2024, cannabis remains the most widely used illicit drug in Malta, despite significant changes in legislation aimed at reducing criminalisation and promoting harm reduction (Gellel et al. 2024).

The introduction of Act No. LXVI of 2021, which legalised personal possession (up to 7 grams) and home cultivation (up to 4 plants) for individuals aged 18 and over, was a significant shift in Maltese drug policy (Gellel et al. 2024). Additionally, the establishment of Cannabis Harm Reduction Associations (CHRAs) was intended to provide a legal, non-commercial means for distribution. However, the impact of these reforms in 2023 was inconsistent. While they brought certain improvements, several challenges persisted (Gellel et al. 2024).

According to national treatment data, cannabis was the primary substance of concern in 14% of all drug treatment episodes in 2023, ranking behind heroin and cocaine (Muscat et al. 2025). Among individuals entering treatment for cannabis-related issues, daily or near-daily use was reported by 65%. This shows a high prevalence of dependency patterns. The majority of these users were male, predominantly aged between 18 and 30 years (Muscat et al. 2025).

Interestingly, the overall number of cannabis-related treatment cases remained stable compared to 2022, indicating that the decriminalisation policy has not significantly escalated problematic use. However, healthcare professionals have reported a rise in cannabis-related presentations with symptoms such as anxiety, panic attacks, palpitations, and acute psychotic episodes, especially among inexperienced or vulnerable users (Muscat et al. 2025). This trend is likely linked to rising cases involving synthetic cannabinoids, rather than being a direct consequence of recreational cannabis legislation.

In terms of seizures, herbal cannabis also remains dominant, with some samples exceeding 20% THC, raising concerns about high-potency products circulating outside the regulated system (Muscat et al. 2025). Malta's progressive cannabis laws were implemented with the dual goal of reducing the burden on the justice system and minimising harms through education and regulation. However, the slow rollout of Cannabis Harm Reduction Associations, especially during the first years after ARUC was established, has led to a regulatory gap, where demand for legal access exceeds supply, potentially fuelling the parallel illegal market (Muscat et al. 2025).

According to this national report, public opinion remains divided, with harm reduction advocates welcoming the policy shift, while others, particularly within the health and education sectors, express concern about the normalisation of cannabis use, especially among adolescents (Gellel et al. 2024). Although access is legally restricted to adults, youth outreach programmes have reported growing casual use and a decline in the perceived risks of cannabis, prompting renewed investment in prevention campaigns during 2023 (Muscat et al. 2025).

Although Malta has adopted a liberal stance on personal cannabis use since 2021, cannabis trafficking remains a prosecutable offence, with law enforcement maintaining a strong presence against unauthorised supply chains (Muscat et al. 2025). As shown in Figure 1.6 below, cannabis-related trafficking arraignments peaked in 2019 at 78 cases, representing one of the highest points of enforcement action during the six-year period from 2017 to 2022. While a decline was observed in 2020, 2021 and 2022, the number of arraignments in 2021 was 54, surpassing figures for both heroin and cocaine in that year as shown also in years 2017, 2018 and 2019 (Gellel et al. 2024).

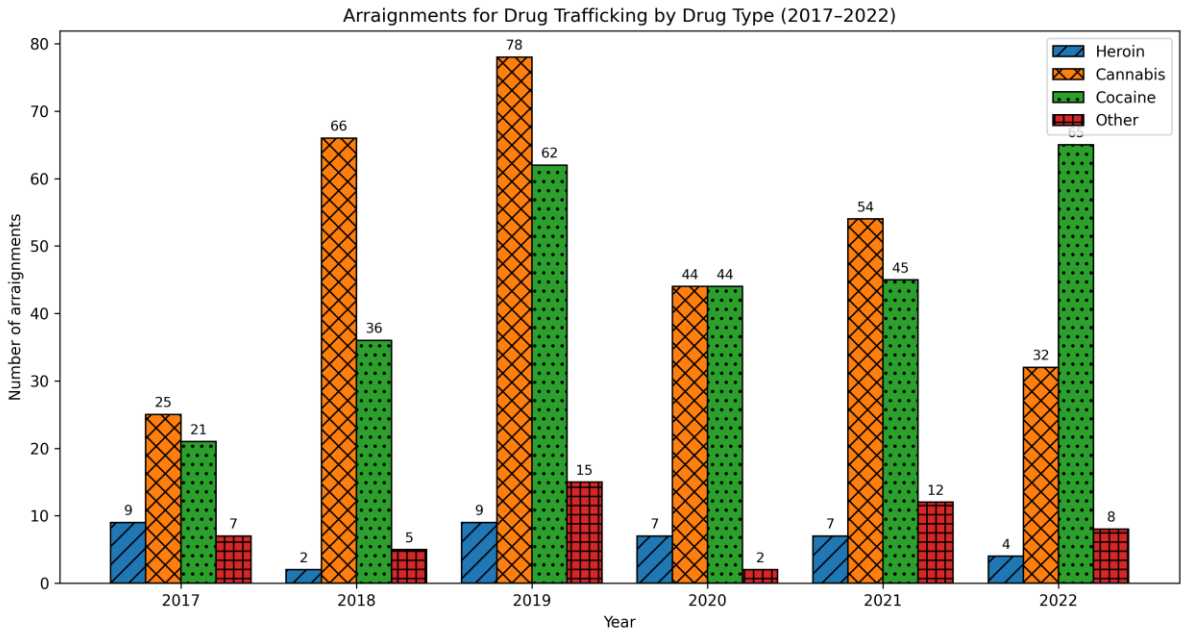

Figure 1.5 Arraignments trafficking by drug type 2017 - 2022 in Malta (Gellel et al. 2024)

Taken together, these statistics highlight why the present study is highly relevant in the Maltese context. Cannabis not only remains the most widely used illicit drug in the country but also accounts for a substantial share of treatment demand, particularly among young men and also court arraignments due to trafficking. The stability in treatment cases despite legislative reform suggests that cannabis will continue to have a strong and enduring presence in Malta (Gellel et al. 2024; Muscat et al. 2025). At the same time, the rise in acute health presentations emphasises the vulnerability of certain user groups. In this setting, ensuring that legally available cannabis is free from harmful pesticide contamination becomes critical. Given its widespread use and combined with patterns of heavy consumption, it can be concluded that even low-level contamination could translate into a meaningful public health risk (EUDA 2025; Gagnon et al. 2023).

1.3.3 Legalisation of Recreational Cannabis in Malta

On 14th December 2021, Malta legalised the cultivation and personal use of recreational cannabis (Government of Malta 2021), following cannabis legalisation for medical and research purposes introduced on the 27th of March 2018 (Government of Malta 2018). Legalisation of Cannabis for personal use in Malta - ACT No. LXVI of 2021 and its regulations aims to standardise and promote consistency, health, and safety within Malta's recreational licensed cannabis industry (Government of Malta 2021). To ensure safe cannabis products to the Maltese population the Authority for the Responsible Use of Cannabis (ARUC) was set up to regulate and give licensing to Harm Reduction Associations which comply with all laws and guidelines deemed necessary (Government of Malta 2023).

Following ARUC's Directive on Technical Standards and Approved Operating Practices, which took effect with Version 1.2 in June 2023, associations must control pests and, where pesticides are used, they have to ensure that these pesticides are suitable for the intended use and safe for use within the food industry (ARUC 2023a). As part of the licence application, applicants must provide a declaration that chemical pesticides will not be used to produce Cannabis, and any nutrients, organic pesticides, and other natural methods they intend to use must be declared (ARUC 2023b).

This study therefore serves to evaluate whether the regulatory framework and ARUC's directives effectively prevent pesticide contamination in licensed Maltese cannabis, by comparing them against illicit samples where such controls are absent.

1.4 Pesticides

According to the Food and Agriculture Organization of the United Nations, the international code of conduct on the distribution and use of pesticides, define pesticides as follows: "Any substance or mixture of substances intended for preventing, destroying or controlling any pest, including vectors of human and animal disease, unwanted species of plants or animals causing harm during, or otherwise interfering with, the production, processing, storage, transport, or marketing of food, agricultural commodities, wood and wood products or animal foodstuffs, or which may be administered to animals for the control of insects, arachnids or other pests in or on their bodies. The term includes substances intended for use as a plant growth regulator, defoliant, desiccant, or agent for thinning fruit or preventing the premature fall of fruit, and substances applied to crops either before or after harvest to protect the commodity from deterioration during storage and transport" (FAO and WHO 2014, p. 3).

The term pesticide encompasses a wide range of compounds including insecticides, mineral oils, fungicides, herbicides, rodenticides, molluscicides, nematicides, plant growth regulators and various other compunds (Aktar et al. 2009). This categorisation is based on the field of usage. However, pesticides may also be categorised by chemical class which includes organochlorines, organophosphates, carbamates, chlorinated hydrocarbons, pyrethroids, and heterocyclic compounds (Tano 2011; Taylor and Birkett 2020).

This is done by categorising pesticides according to the chemical nature of the active ingredient which could be far more useful especially in fields like research due to the

information given on the chemical or physical property of the pesticide. Table 1.3 shows pesticides classification according to their chemical properties. It provides general information and the general chemical structure on each class of pesticides.

Table 1.1 Classification of pesticides based on chemical composition (Tano 2011).

Pesticide	General information	General Chemical Feature
Organochlorines	Chlorinated hydrocarbon insecticides characterised by high environmental persistence and bioaccumulation. Act primarily by disrupting voltage-gated sodium channels in insect nerve membranes, leading to paralysis and death. Examples: DDT, lindane, endosulfan, aldrin, dieldrin, and chlordane.	Clorinated hydrocarbon backbone (multiple C-Cl bonds).
Organophosphates	Organophosphates are phosphorus-containing ester compounds characterised by phosphate or phosphorothioate functional groups. They exert toxicity primarily through irreversible inhibition of acetylcholinesterase, resulting in accumulation of acetylcholine at synapses and neuromuscular junctions, causing continuous nerve stimulation, paralysis, and death. Examples: parathion, malathion, and diazinon.	Phosphorus-containing ester with P=O or P=S bonds and organic (alkoxy/aryloxy) substituents.
Carbamates	Organic pesticide derived from carbamic acid. Act as reversible inhibitors of acetylcholinesterase, producing toxic effects similar to organophosphates but generally of shorter duration due to spontaneous enzyme reactivation. Examples: Carbaryl, carbofuran, aminocarb.	Esters of carbamic acid (–O–C(O)–NH–).
Pyrethroids and pyrethrins	Pyrethrins are naturally occuring insecticides while pyrethroids are synthetic analogues, often containing a cyclopropane carboxylate moiety, developed to improve photostability. Examples include permethrin, cypermethrin, and deltamethrin.	Ester-linked chrysanthemic acid–based structures.

Many pesticides are recognised for their harmful effects on human health and the environment, leading to restrictions or complete bans on their use (WHO 2022). Despite this, over 1000 different pesticides are still widely applied in food production, where their residues are closely monitored (WHO 2022).

Although the acute toxicity of pesticides at high oral doses is well documented, recent systematic reviews and meta-analyses indicate that even modest, chronic exposure can contribute to adverse health outcomes. These include neurobehavioural deficits, endocrine disruption, reproductive problems, and a higher risk of certain cancers, particularly in vulnerable groups (Mnif et al. 2011). Because many pesticides act on the CNS, they present higher risks for individuals with neurological conditions, such as epilepsy, by binding to specific brain receptors, which may pose an additional concern for recreational cannabis users with underlying health issues (Taylor and Birkett 2020).

1.4.1 Pesticides in Cannabis

There is an ongoing debate on the use of pesticides in cannabis cultivation due to the potential adverse effects on human health and the environment (Wylie et al. 2020). In order to gain a better understanding of the pesticides found on illicit versus licensed cannabis for recreational use, it is important to examine the literature available. Although studies on pesticides in recreational cannabis on the Maltese islands may be limited since the legalisation of recreational cannabis is relatively new to the country, one can look at studies conducted internationally where recreational cannabis is also legal. Recreational cannabis has been illegal in most countries around the globe, resulting in recent studies comparing and evaluating pesticide residues on various cannabis samples (Wylie et al. 2020).

The most frequent pests seen on indoor cannabis flowers and leaves are aphids, spider mites, and thrips, while fungal infections can also cause problems, especially in greenhouses and

indoor lighting systems (Salamone and Russo 2016). Consequently, the pesticides mostly used in cannabis production are insecticides, acaricides, and fungicides since most cannabis plants are now cultivated inside to ensure year-round output and stable THC levels. Studies show that indoor plants are at a greater risk of pesticide contamination as opposed to plants grown outdoors (Salamone and Russo 2016).

Since cannabis is mostly smoked, pesticide standards and stated hazardous levels may be inaccurate due to their association with agricultural goods intended for oral ingestion and not for smoking (Voelker and Holmes 2015). Furthermore, it has been reported that many pesticide metabolites are more harmful than their parent chemicals. According to Sullivan study, pesticide metabolites will often be present when cannabis is smoked (Sullivan et al. 2013).

The United Nations Office on Drugs and Crime (UNODC) (2009) claims that the main aim of testing for Cannabis analytically is to confirm its strain and potency, thus these analyses focus on THC and other cannabinoids. (UNODC 2009). Subsequent reviews note that contaminants such as pesticides were often overlooked when compared to potency testing (Taylor and Birkett 2020).

1.5 Pesticide Analysis in Cannabis Samples

Since cannabis' legal status varies globally, it complicates the establishment of consistent quality standards for contaminants, including pesticides. Standardised testing protocols are essential in jurisdictions where cannabis is legal, and their adoption could also support countries moving towards legalisation (Tran et al. 2017).

Developing such protocols remains difficult because of the wide physicochemical diversity of pesticides and the chemical complexity of the cannabis matrix (Taylor and Birkett 2020). Due to the complex cannabis matrix, multi-step sample preparation is usually required to minimise interferences. QuEChERS-based extractions, often combined with dispersive or solid-phase clean-up, are now widely applied for this purpose (Cochran et al. 2017). Following extraction, separation techniques, most commonly GC for volatile, thermally stable pesticides and LC for polar or thermally labile compounds, are employed (Cochran et al. 2017). These are typically coupled to MS, which provides accurate identification of each compound and precise measurement of its levels.

The following subsections examine in more detail the sample preparation strategies, separation techniques, and detection methods currently applied in pesticide analysis of cannabis.

1.5.1 Sample Preparation

Before analysis with either GC or LC methods, the sample must be homogenised and extracted with appropriate solvents and interferences removed. For low-level pesticide residues, an efficient clean-up step is essential (Subritzky et al. 2017).

The EN15662 method is approved for analysing pesticides in plant-based foods. It is not particularly indicated for pesticide analysis in cannabis, although it has been adapted and used for that purpose (Schneider et al. 2013).

The protocol involves sample drying, homogenisation, acetonitrile extraction, and dispersive solid-phase extraction (d-SPE) clean-up using QuEChERS (Lawal et al. 2018). Extraction typically employs acetonitrile and magnesium sulphate ($MgSO_4$) to promote phase separation and improve analyte recovery. The goal is to eliminate sugars, lipids, organic acids, sterols, proteins, pigments, and extra water (Lawal et al. 2018). Although calcium chloride ($CaCl_2$) has been explored as an alternative drying salt in tea matrices, it is not validated for cannabis, and $MgSO_4$ remains the standard due to its inertness and efficiency (Lozano et al. 2012a; 2012b)

Cannabis presents unique challenges for sample preparation. Its resinous glandular trichomes retain hydrophobic pesticides, while cannabinoids and terpenes are themselves hydrophobic and readily co-extracted with organic solvents. In this present study, in order to address this, the present study followed an established Waters method from application note specifically

adapted for pesticide residue analysis in cannabis (James et al. 2019). This method employs QuEChERS extraction with partitioning salts and d-SPE using Graphite Carbon Black (GCB), providing cleaner extracts suitable for GC-MS and UPLC-MS/MS analysis.

1.5.2 Separation Methods

This section outlines the main analytical techniques used for pesticide residue detection in cannabis, focusing on GC, LC, UPLC, MS. It concludes with the justification for selecting GC-MS and UPLC-MS/MS for this present study.

1.5.2.1 Gas Chromatography (GC)

GC is mostly suited to the analysis of volatile and thermally stable pesticide residues. In this technique, analytes are vaporised and separated as they are transported by an inert carrier gas through a heated column, where interactions with the stationary phase determine their retention times (Thet and Woo 2023). Depending on the stationary phase, GC can be performed as gas-solid chromatography (GSC) or gas-liquid chromatography (GLC), though in pesticide analysis the latter is most commonly applied. Figure 1.7 provides a graphic overview of the instrument setup, with each labelled component.

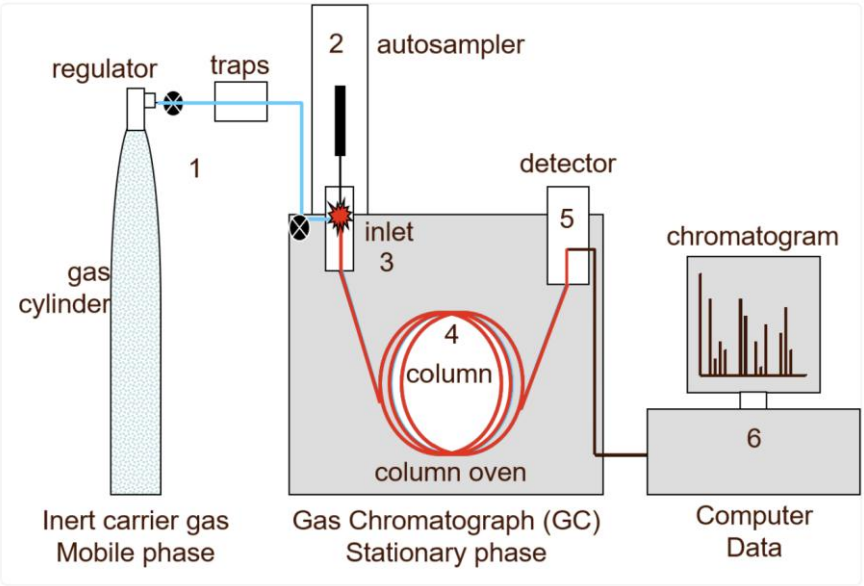

Figure 1.6 A simplified diagram of a GC (Turner 2021).

The sample is introduced into the GC using a syringe or transferred from an autosampler (marked as 2 in Figure 1.7), which may also extract chemical components from solid or liquid samples. The sample is injected through a septum at the GC inlet (3), allowing the sample mixture to be introduced without disrupting the mobile phase. Attached to the inlet is the analytical column (4), a long (10 – 150 m), narrow (0.1 – 0.53 mm internal diameter) fused silica or metal tube that is coated with the stationary phase on the inside walls. The analytical column is placed in a column oven, which is heated during the analysis to help elute the less volatile components. The column's outlet is connected to a detector (5), which detects the chemical components as they elute from the column and generates a signal. The signal generated by the detector is captured by acquisition software on a computer, producing a chromatogram (6) (Turner 2021).

1.5.2.2 Liquid Chromatography (LC)

LC is a crucial separation technique used for separating, and also identifying, and quantifying compounds in a mixture. It is widely employed in pharmaceutical, environmental, and food safety studies due to its high sensitivity and selectivity. LC operates by passing a liquid mobile phase through a stationary phase, allowing different compounds to interact and elute at different times based on their chemical properties. This separation mechanism makes LC particularly useful for complex sample matrices, such as plant extracts, where multiple analytes coexist (Betancourt and Gottlieb 2023).

Figure 1.8 illustrates the fundamental instrumentation of liquid–solid chromatography, with each component labelled by a letter and described in detail in the subsequent paragraph.

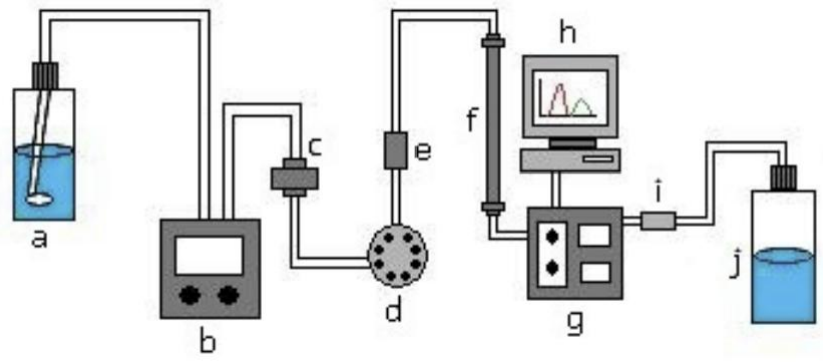

Figure 1.7 A basic LC system (Betancourt and Gottlieb 2023)

The process begins with the solvent inlet (marked as ‘a’ in figure 1.7), which introduces the mobile phase. This phase is pumped (b) through an inline solvent filter (c) before reaching the

injection valve (d), where it combines with the injected sample. The mixture then passes through another filter (e) before entering the column (f), where the sample components are separated. The detector (g) identifies the separated analytes, and the data is recorded by a computer or recorder (h). Finally, the sample moves through a backpressure filter (i) before being directed to waste (j) (Betancourt and Gottlieb 2023).

1.5.2.3 Ultra-Performance Liquid Chromatography (UPLC)

Several types of LC are commonly used in analytical chemistry, including High-Performance Liquid Chromatography (HPLC) and the more advanced Ultra-Performance Liquid Chromatography (UPLC). Both techniques offer high levels of accuracy and precision, making them reliable tools for analytical applications. Although HPLC and UPLC operate on the same fundamental principles and are both used for separating components in complex mixtures, they exhibit distinct characteristics that may differentiate their performance and applications (Van Tran et al. 2021).

Since its development in 1967, HPLC has been the dominant technique in chromatography. However, the advent of UPLC in the mid-2000s marked a significant advancement in the field, contributing to its increasing popularity in recent years (Van Tran et al. 2021).

One of the main distinctions lies in operating pressure. UPLC systems can function at up to 15,000 psi, compared to around 6,000 psi for HPLC, which enables the use of sub-2 µm

stationary-phase particles. This design allows for faster analysis, sharper peak resolution, and higher separation efficiency. As a result, UPLC is particularly suited for challenging trace analyses across complex matrices, including biological fluids, dietary supplements, plant-based products, and pesticide residues in (James et al. 2019).

Cannabis contains numerous cannabinoids, terpenes, and other bioactive compounds that can interfere with accurate pesticide determination. UPLC addresses these challenges by offering ultra-sensitive detection (ng/L to µg/L levels), high selectivity to minimise matrix interferences, shorter run times for high-throughput analysis, and improved reproducibility across sample batches (Van Tran et al. 2021).

UPLC-MS/MS is particularly effective when combined with aqueous or organic extraction protocols, especially following QuEChERS clean-up procedures with sorbents like GCB, which help minimise the co-extraction of cannabinoids while GC-MS can effectively separate volatile pesticides from interfering substances like terpenes, also particularly when paired with clean-up sorbents such as GCB. Finally, the availability of the GC-MS and UPLC-MS/MS systems within the Chemistry Department of the University of Malta, provided a practical yet scientifically justified platform for the analyses. These instruments have been previously qualified and their methods applied to similar validated research contexts, ensuring the robustness and reproducibility of the analysis conducted in this study.

1.6 Critical Appraisal of Existing Literature

Canadian research provides some of the most relevant comparators for Malta, both because of its large-scale cannabis market and the regulatory frameworks that mandate extensive pesticide testing.

In 2023 Gagnon et al. screened 327 pesticides across licensed and illicit cannabis, showing a consistent absence of residues in the regulated cannabis supply chain and widespread contamination in illicit products. The extensiveness of their pesticide panel emphasises both the scale of the contamination problem and the heavy analytical workload needed for regulatory monitoring. The clear strength of this study is its broad-spectrum approach and its direct policy relevance. It demonstrates that strict regulatory measurements can effectively protect consumers from pesticide exposure. However, the study also reflects a Canadian-specific regulatory environment with much greater laboratory capacity than exists in Malta. This limits its immediate transferability. Still, it highlights a regulatory model that Malta currently lacks and demonstrates the benefits of licensed supply chains in reducing risk of contamination (Gagnon et al. 2023).

Craven et al. in 2021 developed and validated a high-throughput Liquid Chromatography couple with Tandem Mass Spectrometry (HPLC-MS/MS) method to screen 82 pesticides, applying it to both a certified and a non-certified facility. This design provided an important comparative element, illustrating how regulatory status directly influences contamination risk. This aligns closely with the present dissertation's comparison between licensed and illicit

Maltese cannabis. Methodologically, the study is a valuable reference point because it demonstrated the feasibility of multi-residue detection in complex cannabis matrices. However, this study suffers from clear limitations. The dataset was restricted to two facilities, limiting generalisability, and sampling conditions varied between sites, raising the possibility of bias. Moreover, the study focused mainly on surface wipe out samples rather than consumer products, which restricts applicability to public health. These weaknesses do not undermine its methodological contribution but do illustrate the need for broader, product-focused studies (Craven et al. 2021).

Atapattu and Johnson (2020) offered another Canadian comprehensive review of 96 pesticide residue analysis in cannabis, confirming Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) for sample preparation and combined with tandem mass spectrometry (MS/MS), as the most reliable and widely used approach. This directly supports the methodology applied in the Maltese context. Their review also highlighted Canada's strict regulatory requirements (screening 96 pesticides at very low detection limits). This underlines how Malta (and E.U.) currently has no comparable regulations. A clear weakness is that it only looks at North America and promotes complex methods that most routine labs cannot afford. Nevertheless, it strengthens the argument that established, internationally recognised methods are essential for credible cannabis testing.

Taken together, Canadian studies provide strong evidence that regulation and the application of validated analytical methods can dramatically reduce consumer exposure to pesticide

residues. Their strengths lie in methodological innovation and direct comparisons between regulated and unregulated supply chains. However, they also reveal limitations in terms of representativeness and geographic transferability. For Malta, where no mandatory pesticide screening currently exists, these studies emphasise both the feasibility of multi-residue testing and the protective role of regulation, while highlighting the need for locally adapted surveillance systems.

Most U.S. studies, such as the Oregon evaluation (Dalmia et al. 2021; Evoy and Kincl 2020; Wylie et al. 2020), used GC-MS/MS and LC-MS/MS with QuEChERS for sample preparation. These studies were rigorous in terms of detection sensitivity but were limited by state-specific pesticide panels according to their regulations (typically 50–100 compounds). This regulatory fragmentation makes results less transferable internationally especially since European jurisdiction lacks a harmonised pesticide list for cannabis.

Evoy and Kincl (2020) analysed pesticide contamination in Oregon cannabis and identified 50 different residues, including highly and extremely hazardous compounds such as carbofuran, aldicarb, dichlorvos and methyl-parathion. A key strength of the study was the comparison between recreational and medicinal products, with the latter showing mean residue levels three to twelve times higher, demonstrating that even within a regulated market pesticide misuse can persist (Evoy and Kincl 2020).

Wylie et al. in 2020 developed a high-resolution Gas Chromatography/Quadrupole Time-of-Flight mass spectrometry (GC/Q-TOF) method capable of screening over 1,000 pesticides and environmental contaminants in cannabis. Their approach demonstrated the extensiveness of possible residues, including banned compounds such as DDT derivatives and dieldrin. It resulted in the contamination of 11 out of 21 illicit samples, in some cases exceeding Canadian maximum residue limits by several thousand-fold. While this extensiveness of screening emphasises the scale of risk from unregulated markets, the study has notable limitations. Many samples had been stored for extended periods, allowing degradation of labile pesticides. Quantification was limited, with several residues falling outside calibration ranges. Furthermore, the work functioned partly as a technology showcase for Agilent, raising issues of accessibility and routine applicability in regulatory laboratories (Wylie et al. 2020).

Dalmia et al. 2021 presented study describing a method to test all 66 pesticides mandated by California. This study used a single LC–MS/MS system with dual ionisation. The method was efficient, avoided complex preparation steps, and reduced matrix interferences, suggesting practical advantages. However, this study is compromised by clear conflicts of interest, as it was produced by the instrument manufacturer. The method was relying on Atmospheric Pressure Chemical Ionisation (APCI) makes the method harder to reproduce in ordinary labs. Thus, while promising, it remains more of a technological showcase than an established alternative to existing methods (Dalmia et al. 2021).

Sullivan et al. (2013) provide crucial evidence that pesticide residues present on cannabis are not eliminated during combustion but can transfer in significant quantities into the mainstream smoke inhaled by consumers. Using spiked plant material and GC-MS, the study demonstrated recovery rates of up to 69% for pesticides such as diazinon, permethrin, and bifenthrin when smoked through a glass pipe, with lower but still notable recoveries when water pipes were used, especially without filters. These findings underline the substantial risk of inhalation exposure, especially given that cannabis smoking typically lacks the filtration steps present in tobacco consumption. Although the study is limited due to its use of artificially contaminated samples and a narrow pesticide scope, it remains highly relevant to the present research by showing that residues detected in illicit Maltese cannabis are likely to reach the end-user. This reinforces the argument that contamination in unregulated cannabis markets represents not only a regulatory gap but also a direct public health concern (Sullivan et al. 2013).

European investigations, such as the Belgian plantation study Cuypers et al. (2017), confirm widespread pesticide use in illicit cultivation. This study investigated pesticide use in 43 illicit indoor cannabis plantations in Belgium, detecting residues in 64% of plant samples and 65% of carbon filter cloths. A clear strength of the study lies in its forensic design. The sampling was conducted in real confiscated plantations, and both plant and environmental matrices were analysed using validated EN15662 QuEChERS with LC–MS/MS, demonstrating widespread and repeated pesticide use throughout cultivation cycles. The analysis also highlighted occupational risks, showing that residues persisted in the air via carbon filters, exposing police and dismantling staff to inhalation hazards (Cuypers et al. 2017).

However, the study has notable limitations. It provided only qualitative data without quantification, making it impossible to judge exposure levels or compare against toxicological thresholds. The sampling was opportunistic, depending on police raids, which limits representativeness and so the findings cannot be generalised to the wider cannabis market. Furthermore, the inclusion of labelled pesticide bottles at crime scenes without always matching them to detected residues risks over-interpreting their actual use (Cuypers et al. 2017).

Italian (Amendola et al. 2021) and Austrian (Stempfer et al. 2021) studies also used LC–MS/MS with QuEChERS, reflecting a methodological agreement, but again lacked standardisation in pesticide panels or reporting criteria. This inconsistency illustrates a broader European weakness showing that while methods are robust, regulatory frameworks remain irregular.

Amendola et al. (2021) analysed 31 hemp inflorescence samples marketed as “light cannabis” in Italy, detecting pesticides in 87% of cases and metals such as lead, arsenic and cadmium at levels often exceeding US Pharmacopeia limits for inhaled products. A strength of this study is its comprehensive multi-contaminant design: cannabinoids, pesticides and metals were all measured using validated QuEChERS with LC–MS/MS and GC–MS/MS, (alongside ICP-MS for metals). This extensiveness provided a toxicological risk assessment that moved beyond simple residue detection, linking findings directly to consumer health. Another strength was the geographical spread of samples across six Italian regions, which allowed regional variability to be explored (Amendola et al. 2021).

However, the study also has weaknesses. It examined only legally marketed hemp with THC <0.6%, which means the most hazardous illicit cannabis products were excluded. Furthermore, the use of surrogate borrowed residue limits (such as chamomile inflorescences or tobacco general residue limits) highlights the absence of cannabis-specific European benchmarks, which restricts regulatory interpretation (Amendola et al. 2021).

The Austrian investigation by Stempfer et al. (2021) analysed 93 seized cannabis samples using a validated non-targeted LC-MS/MS workflow designed to screen for pesticides, mycotoxins, and synthetic cannabinoids. Their QuEChERS-based method was benchmarked against 182 pesticide. When applied to casework samples, 44% tested positive, with residues. A strength of this study lies in its non-targeted screening capacity, enabling possible detection of a wide panel of contaminants beyond predefined pesticide lists. However, the method showed reduced sensitivity and the reliance on only confiscated material limits the samples' representativeness across Austria. Nevertheless, the work demonstrates that pesticide contamination in illicit cannabis is both frequent and diverse, reinforcing the public health significance of regulatory gaps for cannabis products in the European Union (Stempfer et al. 2021).

Overall, the literature consistently shows higher contamination in illicit cannabis compared to licensed products, across North America and Europe. The methodological agreement around QuEChERS + MS/MS strengthens confidence in these findings. However, the differences in pesticide panels, validation depth, and regulatory requirements leaves a clear gap.

1.7 Research Question

As already described, the use of pesticides in cannabis cultivation raises significant safety concerns, particularly regarding consumer exposure to harmful chemical residues. Licensed recreational cannabis is subject to regulatory oversight, with strict limits on pesticide use, whereas illicit cannabis is grown without such controls, potentially leading to higher contamination levels. Understanding the differences in pesticide residues between illicit and licensed recreational cannabis is crucial for evaluating consumer risks and regulatory effectiveness.

This study will investigate the extent of pesticide contamination in cannabis samples from both illicit and licenced sources in Malta. Specifically, it seeks to answer the following questions:

- How do pesticide residues differ between licensed recreational cannabis and illicit cannabis samples?
- Do licensed recreational cannabis samples comply with established safety regulations for pesticide residues?
- Are illicit cannabis samples more contaminated with pesticides than licensed ones?

1.8 Aim of the Study

This study aims to identify the presence of pesticides in illicit cannabis inflorescence, if any, and compare these findings with licensed recreational samples sourced from various Harm Reduction Cannabis Associations in Malta.

1.9 Objectives of the Study

The objectives for this study include

- Determine pesticide prevalence by analysing and comparing the prevalence of pesticides in cannabis samples sourced from licensed and illicit sources.
- Assess regulatory compliance by evaluating whether licensed cannabis samples comply with established pesticide regulations and standards, contrasting this with pesticide levels in illicit cannabis samples.
- Establish awareness for potential health risks associated with pesticide contamination in recreational cannabis products
- Identify and compare (if any) the most commonly detected pesticides in both licenced and illicit cannabis samples to determine patterns of contamination.
- Investigate the differences in cultivation practices (if any) between licensed and illicit recreational cannabis producers and their influence on pesticide contamination levels.
- Evaluate the effectiveness of current regulatory oversight in minimising pesticide contamination in the licensed recreation cannabis market versus the lack of oversight in the illicit market.

1.10 Hypothesis

Licenced cannabis has lower pesticide contamination due to regulatory controls, while illicit cannabis has higher and more varied pesticide residues.

Chapter 2

Methodology

2.1 Overview

This Chapter provides an overview of the method adopted in this study for the analysis and detection of pesticide residues in cannabis. It covers a comprehensive overview of the experimental framework, detailing the materials, equipment, and step-by-step procedures used for sample collection, preparation, and analysis. By providing this level of methodological transparency, the study may support future replication and contributions to the broader or deeper scientific understanding of pesticide contamination in cannabis products in Malta.

As discussed in Chapter 1, in Malta, there is currently no legal requirement for mandatory testing of pesticide residues in recreational cannabis flowers prior to their distribution to consumers. In late 2024, the ARUC formalised an agreement with the Department of Chemistry at the University of Malta (see Appendix 1) to enable this study on licensed recreational cannabis, with the aim of generating evidence to support regulatory development and strengthen product safety standards in Malta. This study received ethical approval from the University of Malta Research Ethics Committee, ensuring that the investigation of pesticide residues in cannabis was conducted in accordance with institutional and public health standards (See Appendix 2). In addition to the collaboration with ARUC, the study required authorisation from the Malta Law Courts to analyse illicit cannabis samples. For material obtained through police seizures, formal requests were submitted to the Inquiring Magistrate to release a portion of the confiscated samples for research. This procedure follows the established legal framework for the use of seized substances in scientific investigations. (See Appendix 3).

2.2 Research Setting and Design

Although Malta has recently legalised recreational cannabis leading to the establishment of Harm Reduction Cannabis Associations to cultivate and distribute cannabis for recreational use, the illicit cannabis market remains a challenge in terms of regulatory compliance and public health (Gellel et al. 2024).

This comparative study used a cross-sectional design to assess pesticide residues in illicit cannabis inflorescences and licensed samples from Cannabis Associations in Malta. The cross-sectional approach provided a relatively cost-effective means to analyse samples from multiple sources at a single time point. This analysis took place in three laboratories located at the Department of Chemistry, University of Malta between February 2025 and April 2025. One of the laboratories was used for sample preparation, while the other two laboratories were used for analysis (UPLC/MS/MS Laboratory and GC/MS Laboratory).

2.3 Sample Collection

In order to ensure that the study reflected, as realistically as possible the sources of cannabis florets available to the Maltese population, samples were obtained from both licensed associations and illicit sources. At the time of sampling, 12 licensed associations were registered with the ARUC. In consultation with ARUC, it was agreed that one representative sample would be provided from each association, resulting in a total of 12 licensed samples. To maintain balance and comparability, an equal number of illicit samples (n = 12) were

subsequently requested and obtained through court approval from law enforcement seizures submitted to the forensic laboratory.

Confidentiality of the sample sources was ensured through the anonymisation of the source of the samples through an intermediary. A letter of acceptance from the intermediary of this study can be found in Appendix 4. Thus, no Cannabis Associations or court case can be associated with any samples used in this study. This analysis was carried out purely for research purposes.

2.4 Apparatus and Reagents

The following apparatus/laboratory ware was used during the sample preparation process:

(i) two precision balances (AND FZ-1200i Precision Balance and Sartorius LA230S Analytical Balance),

(ii) disposable weighing boats,

(iii) 15 mL polypropylene centrifuge tubes,

(iv) 2-20 μL and 100-1000 μL micropipettes,

(v) laboratory blender,

(vi) high-speed vortex mixer (Stuart Scientific Autovortex SA6), and

(vii) centrifuge (Jouan C3i).

Sample extraction was carried out using a QuEChERS kit obtained from Waters Corporation®. The kit consisted of:

(i) DisQuE 150 mg $MgSO_4$, 50 mg PSA, and 50 mg C18, 2 mL tubes, 100/pk (SKU number 186004830), and

(ii) Graphitised Carbon Black, 25 g Bottle (SKU number 186004835).

For pesticide analysis, two analytical platforms were employed. A Hewlett Packard (HP) 6890 Series gas chromatograph coupled with HP 5973 mass selective detector (GC-MS) was used initially for method assessment. The GC was equipped with a Restek Rxi-5Sil MS column and an autosampler. For enhanced sensitivity and selectivity, UPLC-MS/MS was conducted using the Waters® ACQUITY UPLC® System with a TQ-D Detector, fitted with an Acquity UPLC® BEH C18 column (1.7 µm).

Ultra-pure water with a resistivity of 18 MΩ·cm was generated using the Elga Purelab Classic purification system to ensure minimal contamination during sample preparation and analysis. Reagents used specifically for UPLC-MS/MS analysis were of LC/MS grade to ensure reliable detection by high-sensitivity mass spectrometry. Pesticide Mixture used for spiking cannabis samples for GC-MS preliminary assessment was the LGC Pesticide-Mix 20 (10 µg/mL in cyclohexane, 10 mL, Product Code DRE-L18000020CY; Laboratory of the Government Chemist, UK).

2.5 Sample Preparation

QuEChERS extraction was applied to homogenised cannabis inflorescence to isolate pesticide residues. The protocol used in this local study was adapted from a Waters® application note to suit the analytical measures requirements (James et al. 2019). The Waters® application note can be found in Appendix 5.

Between 5 g and 20 g of cannabis inflorescence were homogenised using a laboratory blender. The photos below were taken during the first sample preparation session done for this study. Figure 2.1 shows cannabis flowers as acquired from either illicit sources or licenced cannabis associations (not homogenised). Figures 2.2 and 2.3 show the homogenisation process using a laboratory blender provided. The final photo Figure 2.4 shows homogenised product ready for the QuEChERS sample preparation.

Figure 2.1 Cannabis flower – non homogenised

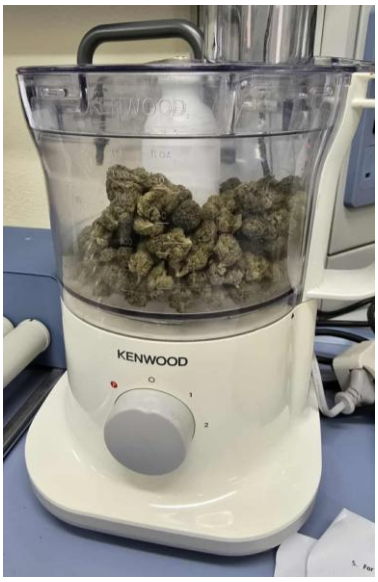

Figure 2.2 Cannabis flowers in the laboratory blender, non-homogenised

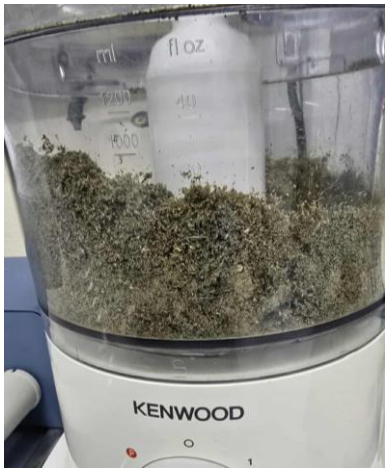

Figure 2.3 Cannabis flowers in the laboratory blender, homogenised

Figure 2.4 Cannabis flowers - homogenised

From the homogenate, 0.05 g of sample was transferred into a 10 mL centrifuge tube using a glass weighing boat and a spatula as shown in Figure 2.5.

Figure 2.5 0.05 g of homogenised cannabis to be transferred to a centrifuge tube

5 mL of acetonitrile (LC-MS grade) was added to each tube using a micropipette. The mixture was then vortexed for 5 minutes using the Stuart Scientific Autovortex SA6 and centrifuged at 5000 rpm for 5 minutes using the Jouan C3i centrifuge. Figure 2.5 shows a subset of samples (0.05 g of homogenised cannabis) after being added 5 mLs of acetonitrile, vortexed and centrifuged.

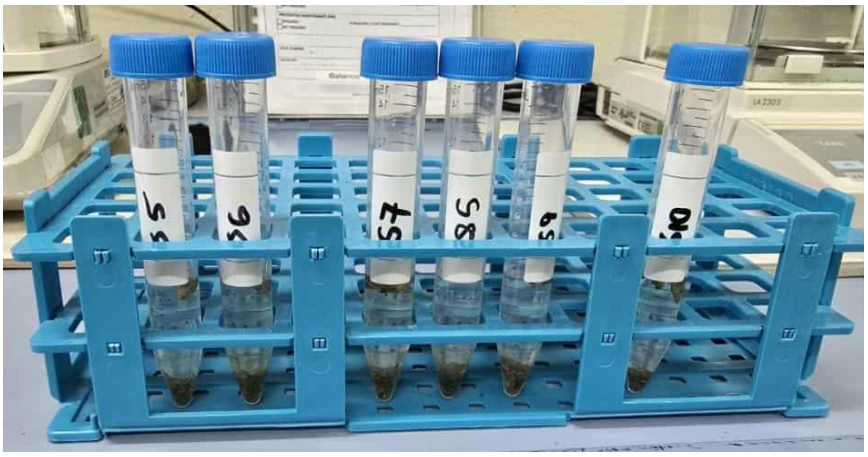

Figure 2.6 Cannabis (0.05 g) in acetonitrile after being vortexed and centrifuged

Following centrifugation, 1 mL of the resulting supernatant was transferred into a 2 mL DisQuE tube containing 150 mg MgSO4, 50 mg PSA, and 50 mg C18. To this, 7.5 mg of graphitised carbon black (GCB) was added to enhance clean-up by removing pigments and interfering matrix components. The 2 ml tube was then sealed, vortexed thoroughly for another 5 mins, and centrifuged again at 5000 rpm for 5 minutes under the same conditions. The final extract (1 µL) was transferred into either GC-MS or UPLC-MS/MS vials for instrumental analysis. The next flow diagram Figure 2.7 illustrates the steps carried out for sample preparation.

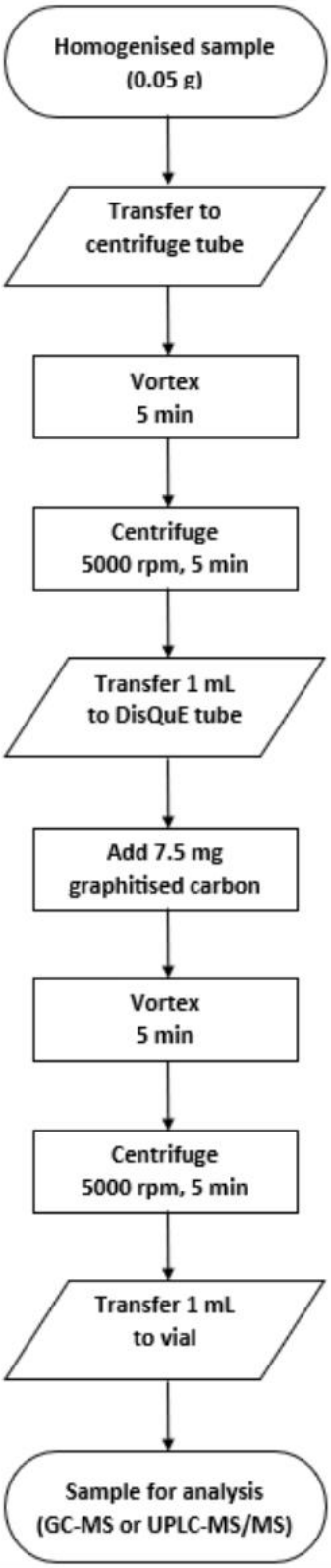

Figure 2.7 Sample preparation flow diagram

2.6 Analytical Procedure

This study used a methodology that had already been established by Waters® (James et al. 2019). See Appendix 5. Their protocol combines QuEChERS extraction with dispersive solid-phase clean-up and analysis by UPLC-MS/MS and GC-MS/MS. In their validation work, Waters reported limits of quantification as low as 20 ppb, recovery rates between 81.7% and 117.6%, and relative standard deviations under 20%. These results show that the method meets accepted international standards for pesticide analysis and can reliably detect the compounds on Health Canada's cannabis pesticide list (James et al. 2019).

For the present dissertation, the Waters validated protocol was followed with changes to fit the instruments and materials available in the local laboratory. While the Waters protocol included GC-MS/MS analysis, the local laboratory was equipped with a GC-MS system. This meant that detection was limited to single quadrupole capability. The GC oven program and column conditions were adapted from the Waters application note. But since only a single-quadrupole GC-MS was available, MRM acquisition was not possible. The spectra that were acquired in EI mode, were compared against the instrument's internal spectral library for compound identification (James et al. 2019).

The essential steps of extraction, clean-up, and detection remained the same. Because this project was intended as a preliminary comparison of illicit and licensed cannabis samples, no new validation experiments were carried out. The method was applied solely for screening

purposes, as the study focused on comparing illicit and licensed cannabis samples rather than generating new validation data.

2.6.1 Preliminary Evaluation of GC-MS for Pesticide Detection

In order to determine the suitability of GC-MS for pesticide residue analysis in cannabis (since Waters (James et al. 2019) protocol used GC-MS/MS), an initial screening was performed using two illicitly seized cannabis flowers. No pesticide residues were detected in either sample. This outcome may be explained either by the true absence of residues or by the limited sensitivity and selectivity of the single-quadrupole GC-MS system employed. The absence of GC-MS/MS capability, which is typically needed for trace-level pesticide detection in complex plant matrices, points to the latter explanation.

In order to investigate this limitation, a spiking experiment was conducted using a reference pesticide mixture (LGC Pesticide-Mix 20, 10 µg/mL in cyclohexane; Product Code DRE-L18000020CY), referred to as working solution 1 (WS1). The mixture contained 17 organochlorine pesticides, including DDT isomers, aldrin, dieldrin, endrin, heptachlor, hexachlorobenzene, and various HCH and endosulfan isomers. A secondary working solution (WS2, 1 µg/mL) was prepared by diluting 2 mL of WS1 to 20 mL with acetone, as confirmed by the dilution equation ($C_1V_1 = C_2V_2$).

Cannabis homogenate was spiked at four concentration levels (C1-C4). The three lowest levels employed WS2, while the highest level used WS1 to minimise spiking volume. Details are provided in Table 2.1. Spiked samples were vortexed for five minutes and equilibrated for 30 minutes prior to QuEChERS extraction. Subsequent GC-MS analysis again failed to detect any of the target pesticides, even at the highest spiked level. This outcome suggested that the system lacked sufficient sensitivity and/or compound compatibility for reliable pesticide detection in cannabis.

Table 2.1 Spiking experiment to test method performance

Concentration Level Solution	**Solution Used**	**Concentration (µg/mL)**	**Volume [µL]**	**Mass spiked (µg per 1g of cannabis)**
C1 (LOW)	WS2	1	20	0.02
C2	WS2	1	200	0.20
C3	WS2	1	400	0.40
C4 (HIGH)	WS1	10	500	5.00

As a final check, a direct injection of WS1 was performed. Under these conditions, only DDE, DDT, and dieldrin were detected, while the remaining compounds were not observed. This selective response indicated that volatility and thermal stability were a limiting factor under the GC parameters applied.

Collectively, these findings demonstrated that the available GC-MS platform was inadequate for the scope of this study. The results provided clear justification for transitioning to UPLC-MS/MS, which offers improved sensitivity, selectivity, and compatibility with a broader range of pesticide chemistries.

2.6.2 UPLC-MS/MS Analysis

Given the limitations demonstrated with GC-MS, all subsequent analyses of illicit and association-derived cannabis samples were conducted using UPLC-MS/MS under the conditions outlined in Section 2.8.2. Sample preparation again employed QuEChERS extraction to maintain method consistency. The UPLC-MS/MS method was operated in MRM mode, monitoring transitions for 84 pesticide residues spanning major chemical classes such as organophosphates, pyrethroids, and carbamates. For each analyte, quantifier and qualifier transitions with compound-specific retention times were monitored (see Appendix 5 for full transition list in table labelled appendix A). This approach provided superior sensitivity and selectivity for trace-level residues in complex herbal matrices and allowed detection of thermally labile compounds not suited to GC-MS analysis, therefore offering a more appropriate analytical platform for the objectives of this study.

2.7 Instrumentation Conditions

In order to ensure accurate detection of pesticide residues in cannabis inflorescence samples, both GC-MS and UPLC-MS/MS systems were optimised to achieve high sensitivity, specificity, and reproducibility. GC-MS was initially applied as a preliminary assessment tool during method development, however, due to its limited sensitivity for certain analytes, UPLC-MS/MS was subsequently adopted as the primary analytical platform. The following subsections describe the chromatographic and mass spectrometric conditions used for each system in detail.

2.7.1 GC-MS Conditions

GC was performed on an Agilent 6890 Series GC system coupled to an Agilent 5973 mass selective detector (MSD). Separation was achieved using a Restek Rxi-5Sil MS column (30 m × 0.25 mm i.d., 0.25 µm film thickness; Serial No. 1654949), with helium as the carrier gas at a constant flow of 1.0 mL/min. Samples (1 µL) were introduced in pulsed splitless mode at 280 °C, with an equilibration time of 1 min.

The GC oven temperature programme is shown in Table 2.2 and Figure 2.7. The gradient was optimised to ensure efficient volatilisation and separation of analytes within the cannabis matrix, with a final hold at 320 °C to allow elution of late retained compounds. The total run time was 18.35 min.

Table 2.2 GC oven conditions

Rate [°C / min]	Final Temp [°C]	Hold time [min]
-	60	0.45
18.70	320	3.65

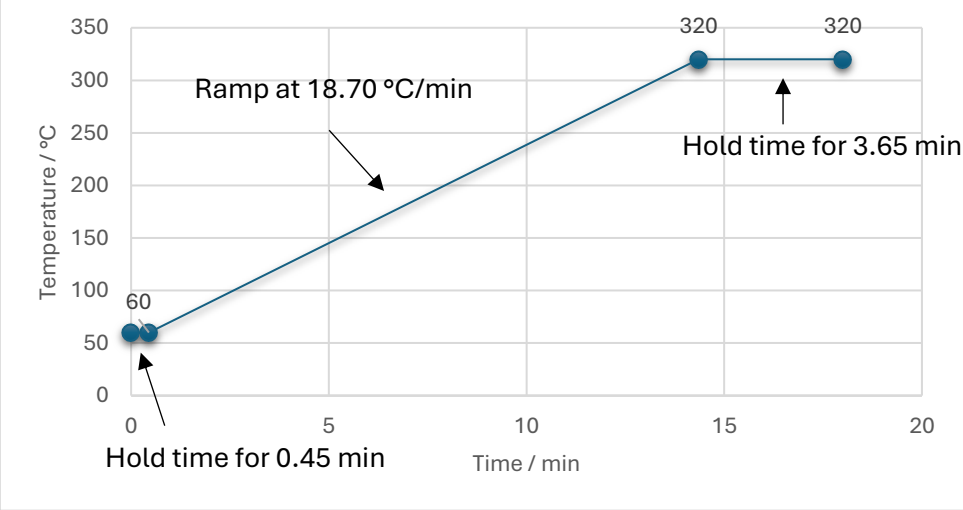

Figure 2.8 Gas chromatographic oven temperature program applied for pesticide analysis

Mass spectrometric detection was carried out in electron ionisation (EI) mode. The interface (transfer line) was held at 280 °C, with an ion source temperature of 230 °C and a solvent delay of 3 min. Data was acquired in EI mode. An autosampler was used for all injections, with three needle rinses before and after each run to minimise carryover.

The GC oven temperature program shown in Figure 2.8 was adapted from a validated Waters® application note for pesticide residue analysis in cannabis, with modifications to suit the available single-quadrupole GC-MS system and column configuration (James et al., 2019). A relatively steep ramp rate of 18.7 °C/min up to a final temperature of 320 °C was selected to ensure elution of higher-boiling, non-polar pesticide residues within a reasonable analysis time, while maintaining chromatographic resolution.

The target compounds assessed by GC-MS during preliminary evaluation consisted primarily of organochlorine pesticides and selected organophosphates, which are known to exhibit sufficient thermal stability for GC analysis under electron ionisation conditions. Organochlorine pesticides such as DDT, DDE, and dieldrin have boiling points above 250 °C and are routinely analysed using final oven temperatures of 300–320 °C without evidence of thermal degradation (Wylie et al. 2020; Cuypers et al. 2017). Chlorpyrifos and dichlorvos, although more volatile, have also been widely reported as GC-amenable compounds when analysed using short high-temperature ramps (Evoy and Kincl 2020).

In the present study, no evidence of thermal breakdown, peak splitting, or abnormal tailing was observed for compounds detected during direct standard injections, indicating that the selected oven program did not induce detectable thermal degradation. Furthermore, the

Restek Rxi-5Sil MS column used is rated for continuous operation up to 330 °C, supporting the suitability of the final oven temperature selected.

2.7.2 UPLC-MS/MS Conditions

UPLC was performed on an ACQUITY UPLC BEH C18 column (100 mm × 2.1 mm, 1.7 µm particle size) operated in gradient mode. The mobile phases consisted of LC-MS grade methanol (shown in table 2.3 as %A) and 2% formic acid in reverse osmosis (RO) water (shown in table 2.3 as %B). The flow rate was maintained at 0.50 mL/min, with the column temperature set to 50 °C. Samples were held at 15 °C in the autosampler, and the injection volume was 2 µL. The gradient programme is shown in Table 2.3

Table 2.3 UPLC gradient program

Time (min)	%A (MeOH)	%B (2% Formic acid)
0.00	2%	98%
8.00	95%	5%
9.00	95%	5%
12.00	2%	98%

Mass spectrometric detection was carried out using a TQ-D triple quadrupole mass spectrometer equipped with an electrospray ionisation source operating in positive ion mode (ESI+). The following parameters were applied: capillary voltage, 1.2 kV; cone voltage, 30 V; source temperature, 150 °C; desolvation temperature, 500 °C; desolvation gas flow, 1000 L/h; cone gas flow, 50 L/h.

Multiple reaction monitoring (MRM) transitions for each target pesticide were adapted from a validated cannabis multi-residue method (James et al. 2019) and applied to the pesticides

targeted in this study. Precursor and product ion pairs, collision energies, and retention times are provided in Appendix 5.

2.8 Quality Control Measures

Quality control measures were added to confirm the reliability of the analyses. Blank samples were injected between runs to monitor cross-contamination and carryover. This step confirmed that the system remained clean throughout the sequence and that any detected signals originated from the cannabis samples rather than residual compounds from previous injections or laboratory materials.

As part of the preliminary GC-MS evaluation, cannabis homogenates were spiked at four concentration levels (in the range of 0.02-5.00 µg/g) with a reference pesticide mixture. These spiked samples were intended as positive controls to demonstrate detection capability. However, no pesticides were detected following QuEChERS extraction and GC-MS analysis, even at the highest concentration level. A subsequent direct injection of the pesticide mixture resulted in detection of only DDE, DDT, and dieldrin, while the remaining compounds did not produce distinct peaks (see Figure 3.7 in Chapter 3). The chromatogram instead showed a rising baseline after 13 minutes. This pattern indicated that several pesticides were either insufficiently volatile to elute as sharp peaks or underwent thermal degradation under the GC conditions applied.

These findings confirmed that the available GC-MS system lacked suitability for multi-residue pesticide analysis in cannabis. While spiking was not repeated for the UPLC-MS/MS analyses, the inclusion of blanks in every batch ensured ongoing monitoring of background noise and instrument performance during the main study.

2.9 Data Collection and Handling

A quantitative approach was adopted to determine the presence or absence of pesticide residues in cannabis inflorescences. Analytical data were generated using both GC-MS and UPLC-MS/MS platforms. In the preliminary GC-MS analyses, compounds were screened by spectral matching against the instrument's internal library. For UPLC-MS/MS, detection relied on MRM transitions, which provided higher selectivity and sensitivity for targeted pesticides. Data acquisition and processing were carried out using MassLynx v4.1, and results were evaluated based on peaks, retention times, and signal-to-noise ratio criteria.

2.10 Disposal of Chemicals

Cannabis samples were consumed during the extraction and analytical procedures, with bulk reference material retained by the laboratory. All solvents and reagents were collected in designated organic waste containers and disposed of according to the Department of Chemistry's hazardous chemical waste management protocol (ZRH-007-02) (Katarzyna et al. 2023).

3 Chapter 3

Results

3.1 Overview

This Chapter presents the findings from the analysis of pesticide residues in cannabis samples as outlined in Chapter 2. In total, 24 cannabis inflorescence samples were examined, comprising 12 illicit samples obtained through law enforcement seizures and 12 licensed samples provided from Cannabis Associations through ARUC. Two complementary analytical techniques were employed to screen for a broad range of pesticides, GC-MS and UPLC-MS/MS.

The GC-MS analysis primarily identified naturally occurring cannabinoids and terpenes characteristic of the cannabis plant which included Δ^9-tetrahydrocannabinol (THC), cannabidiol (CBD), cannabinol (CBN), and related metabolites. Pesticide residues were not revealed in the unspiked sample tested by this method.

In contrast, the UPLC-MS/MS method provided the sensitivity and specificity necessary to detect tract organophosphate pesticide residues (specifically dichlorvos and chlorpyrifos) in a subset of illicit samples, while all licensed samples tested negative for pesticides.

The combined results indicate that while GC-MS was suitable for confirming the expected chemical profile of cannabis, it lacked the sensitivity to detect trace-level pesticide residues in the complex plant matrix. UPLC-MS/MS therefore served as the more effective technique for residue pesticide analysis in this study, uncovering contaminants that the GC-MS had missed.

3.2 GC-MS Analysis

Two illicit samples were analysed in order to see if any pesticide peaks were evident. The GC-MS results were organised into three parts. Section 3.2.1 shows results obtained from unspiked illicit cannabis samples to observe any inherent pesticide signals. Section 3.2.2 shows results of illicit cannabis samples spiked with a known pesticide mixture to test recovery while section 3.2.3 are the results of the pesticide standard solution run as a positive control.

3.2.1 Unspiked Illicit Samples

Two illicit cannabis samples were analysed by GC-MS without any pesticide spiking. In both cases, no pesticide related peaks were observed in the Total Ion Chromatograms (TIC) (see Figure 3.1 and 3.2). Instead, the chromatograms showed only typical plant compounds. The major peaks identified with spectral library matching, were mainly cannabinoids including THC (with its Δ^{10}-THC isomer), THCV, CBC, CBG, and CBN and terpenes such as caryophyllene and related sesquiterpenes like valencene. These identifications confirm the expected cannabis matrix composition which was not the focus of this study.

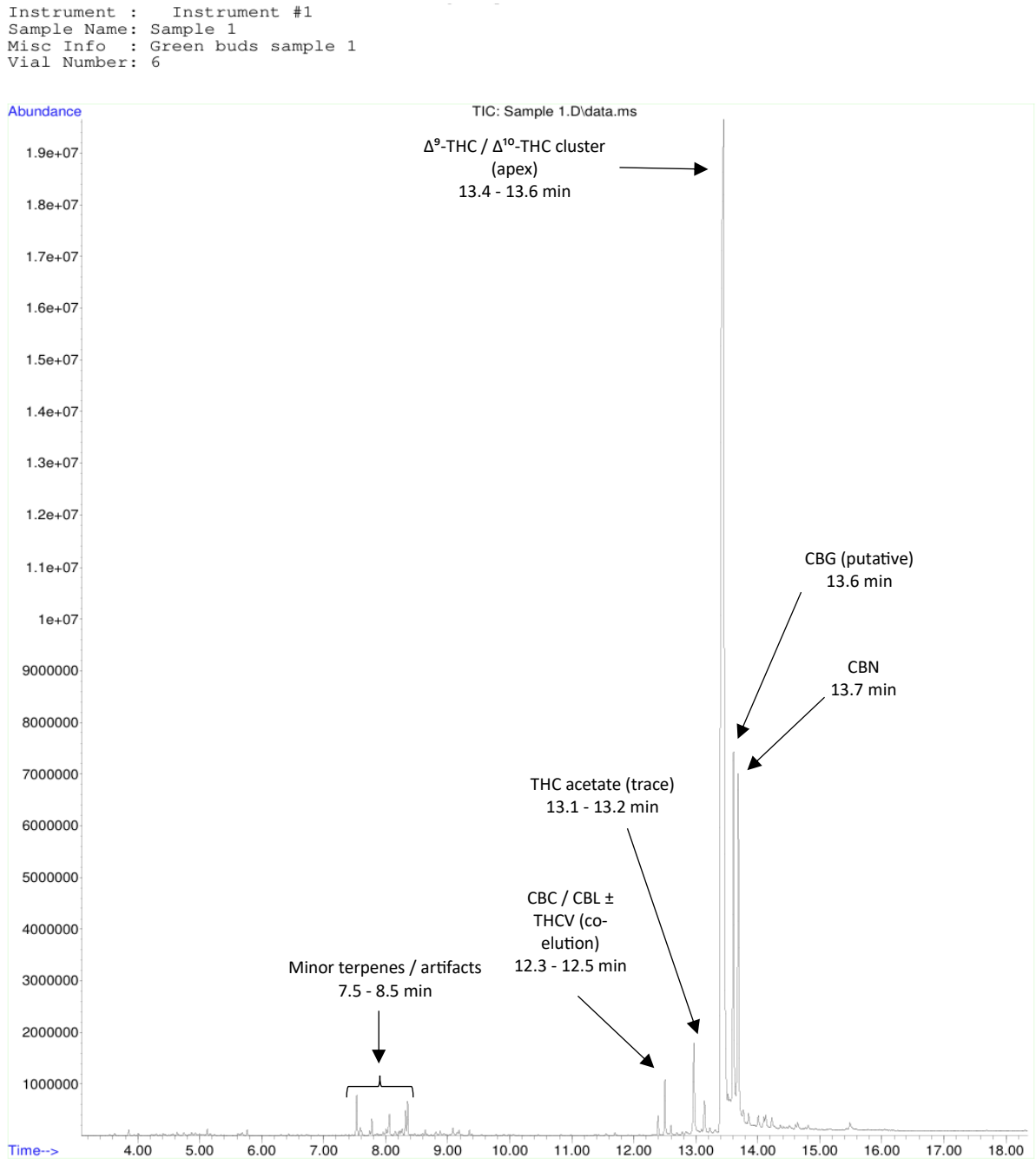

Figure 3.1 TIC of illicit cannabis Sample 1 (Unspiked, GC-MS)

TIC of illicit cannabis sample (Sample 1). Major peaks correspond to cannabinoids including CBC/CBL (12.3 min), THCV (12.5 min), THC acetate (13.1 min), Δ^9/Δ^{10}-THC cluster (13.4 - 13.6 min), cannabigerol (13.6 min), and cannabinol (13.7 min). Earlier minor peaks (7.5 - 8.5 min) represent terpenes or thermal rearrangement products.

```
Instrument :   Instrument #1
Sample Name: Sample 2
Misc Info  : Green buds sample 2
Vial Number: 7
```

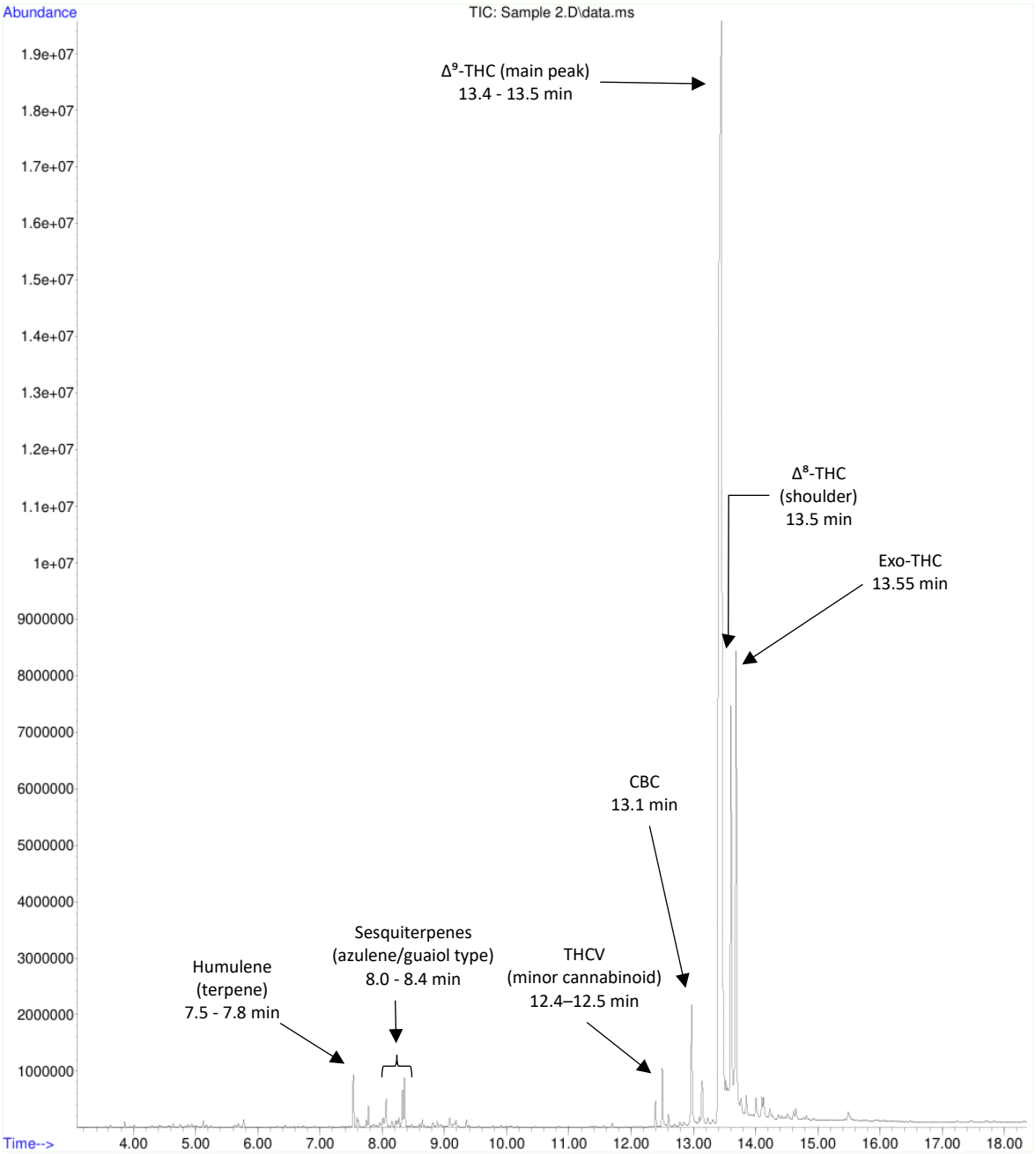

Figure 3.2 TIC of Cannabis Sample 2 (Unspiked, GC-MS)

TIC of illicit cannabis Sample 2 showing major cannabinoid peaks: THCV (12.5 min), Δ^9-THC cluster (13.4–13.6 min), CBG (13.6 min), and CBN (13.7 min). Minor terpenes and library artefacts omitted for clarity.

Table 3.1 summarises the primary compounds detected in the two unspiked illicit samples (Illicit sample 1 and 2). As shown, each of these samples yielded almost exclusively cannabis-derived compounds, and no pesticides could be detected in either sample under the GC-MS screening conditions. This table presents the main compounds identified in the two illicit cannabis samples by GC-MS. In both samples, the chromatograms were dominated by Δ^9-THC/Δ^{10}-THC isomers (retention time ~ 13.45 min), with very high library match scores of 99%, confirming Δ^9-THC as the principal psychoactive constituent. Alongside this dominant signal, several secondary cannabinoids were also detected. These included cannabichromene CBC, cannabigerol (CBG), cannabinol (CBN), and tetrahydrocannabivarin (THCV), each with match values above 93%, indicating a complex cannabinoid profile. Trace levels of Δ^9-THC acetate were observed in both samples, which may represent a derivative, thermal artefact, or by-product of processing.

Differences between the two samples were also evident. Sample 1 contained primarily cannabinoids, with THCV detected only as a minor peak. Sample 2, however, showed both cannabinoids and a detectable terpene fraction. Caryophyllene and valencene (sesquiterpenes) were identified with match scores above 90%, which can suggest a more complete preservation of the plant's terpene profile. These differences only show that illicit cannabis samples can vary in their chemical composition.

This lack of detection pesticides is likely attributable to matrix effects, as the high abundance of cannabinoids and terpenes can suppress trace-level pesticide signals and reduce the

sensitivity of single-quadrupole instruments. Full details of the GC-MS library search outputs of the two unspiked samples, named green bud 1 and 2 are presented in Appendix 6. These runs therefore served as a baseline, where pesticide residues could not be detected in either sample.

Table 3.1 GC-MS library identifications from two unspiked illicit cannabis samples

Sample	Retention time (min)	Compound Identified	Class	Library Match (%)	Notes
Illicit Sample 1	12.50	Tetrahydrocannabivarin (THCV)	Cannabinoid	95	Minor peak
	12.97	Cannabichromene (CBC) / Cannabicyclol	Cannabinoid	93	Co-elution
	13.14	Δ^9-THC acetate	Cannabinoid	99	Trace derivative
	13.45	Δ^9-THC / Δ^{10}-THC isomers	Cannabinoid	99	Dominant peak
	13.60	Cannabigerol (CBG)	Cannabinoid	99	Present
	13.68	Cannabinol (CBN)	Cannabinoid	95	Present
Illicit Sample 2	7.53	Caryophyllene	Terpene	97	Sesquiterpene
	8.32	Valencene / related sesquiterpenes	Terpene	90	Minor
	12.40	Cannabicitran	Cannabinoid	98	Detected
	12.97	Cannabichromene (CBC) / Cannabicyclol	Cannabinoid	94	Present
	13.14	Δ^9-THC acetate	Cannabinoid	99	Trace derivative
	13.45	Δ^9-THC / Δ^{10}-THC isomers	Cannabinoid	99	Dominant peak
	13.60	Cannabigerol (CBG)	Cannabinoid	99	Present
	13.68	Cannabinol (CBN)	Cannabinoid	96	Present

3.2.2 Spiked Cannabis Samples

Illicit Sample 1 was re-analysed after spiking it with the pesticide standard mixture at four different concentrations. Spiking levels corresponded to approximately 0.02 µg/g for C1, 0.20 µg/g for C2, 0.40 µg/g for C3, and 5.00 µg/g for C4 of cannabis flower (see Table 2.1 in Method Chapter 2 for calculation). Because no method validation was carried out, the exact detection limits of the GC-MS for these pesticides in the cannabis matrix remain unknown. However, spiking at up to approximately 5 µg/g (C4) represents a concentration well above the maximum residue limits typically set for food crops (usually 0.01–0.1 µg/g). Despite these additions, no pesticide-related peaks were detectable under the GC-MS conditions. The fact that these spiked levels were still not visible supports the interpretation that matrix suppression and non-targeted scan severely limited detection capability with GC-MS method.

Library matches results again in cannabis-derived compounds rather than pesticides. The Total ion chromatograms (Figures 3.3 – 3.6) for all four spiked runs continued to show dominant peaks for cannabinoids such as THC, CBN, CBC, THCV, and CBG, together with terpenes such as caryophyllene. This is summarised in Table 3.2. while full library search details of the results can be seen in Appendix 7.

Table 3.2 GC-MS outcome for Sample 1 spiked at four concentrations (C1–C4)

Spiked run	Concentration level (µg/g)	Dominant compounds detected	Pesticide residues detected
C1	0.02	Cannabinoids (THC, CBC, CBN, CBG), terpenes (caryophyllene)	None
C2	0.20	Cannabinoids (THC, CBC, CBN, CBG), terpenes (caryophyllene)	None
C3	0.40	Cannabinoids (THC, CBC, CBN, CBG), terpenes (caryophyllene)	None
C4	5.00	Cannabinoids (THC, CBC, CBN, CBG), terpenes (caryophyllene)	None

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Sample Name: C1
Misc Info  : spiked C1
Vial Number: 7
```

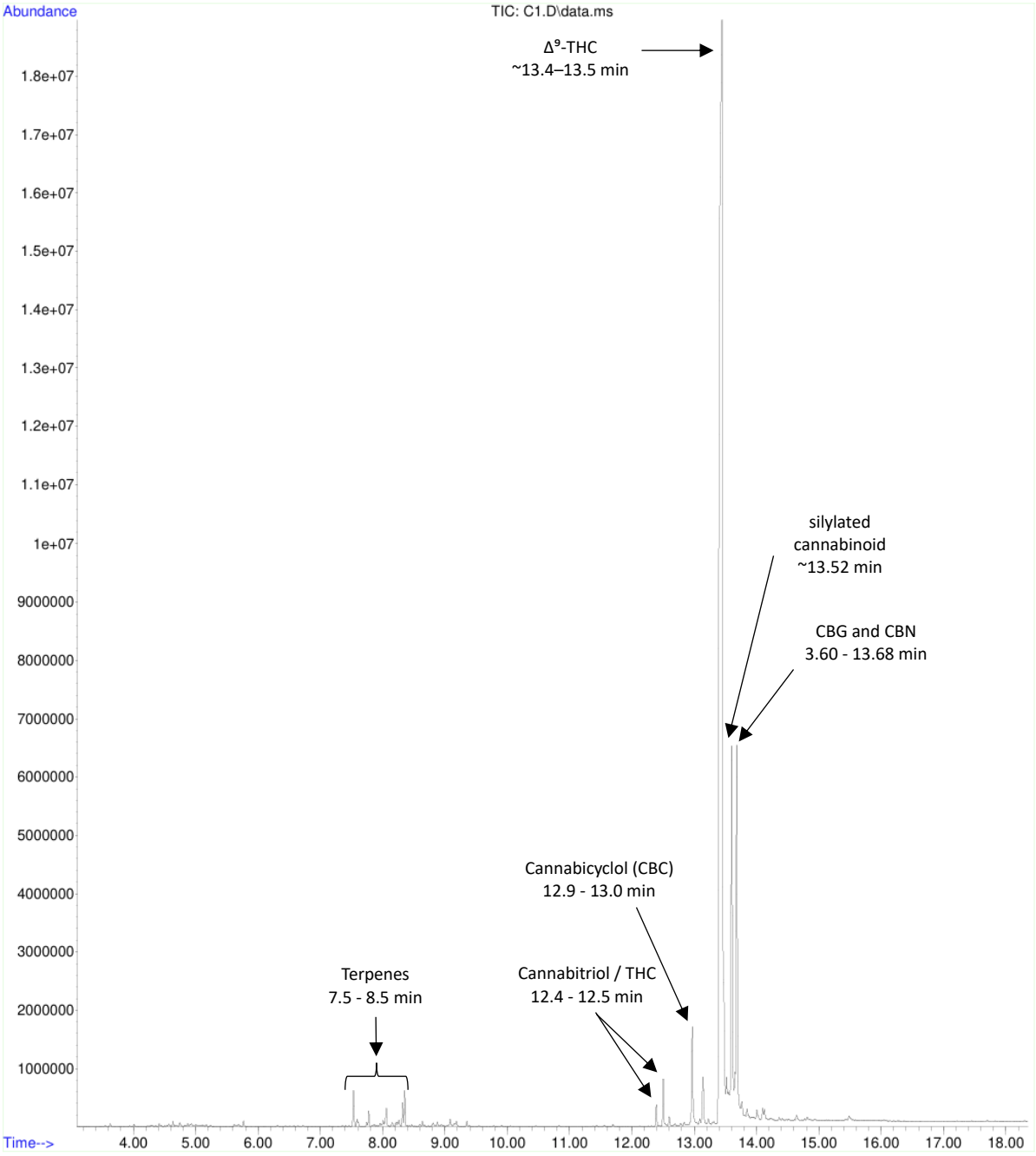


Figure 3.3 GC-MS TIC of illicit Sample 1 spiked with pesticide standard (C1)

Two small peaks followed the major Δ⁹-THC signal (~13.5–13.7 min). The first (~13.52 min) was a trimethylsilyl-derivatised artefact, while the later peaks (~13.6–13.7 min) were putatively identified as minor cannabinoids (CBG, CBN).

Instrument : Instrument #1
Sample Name: C2
Misc Info : spiked C2
Vial Number: 8

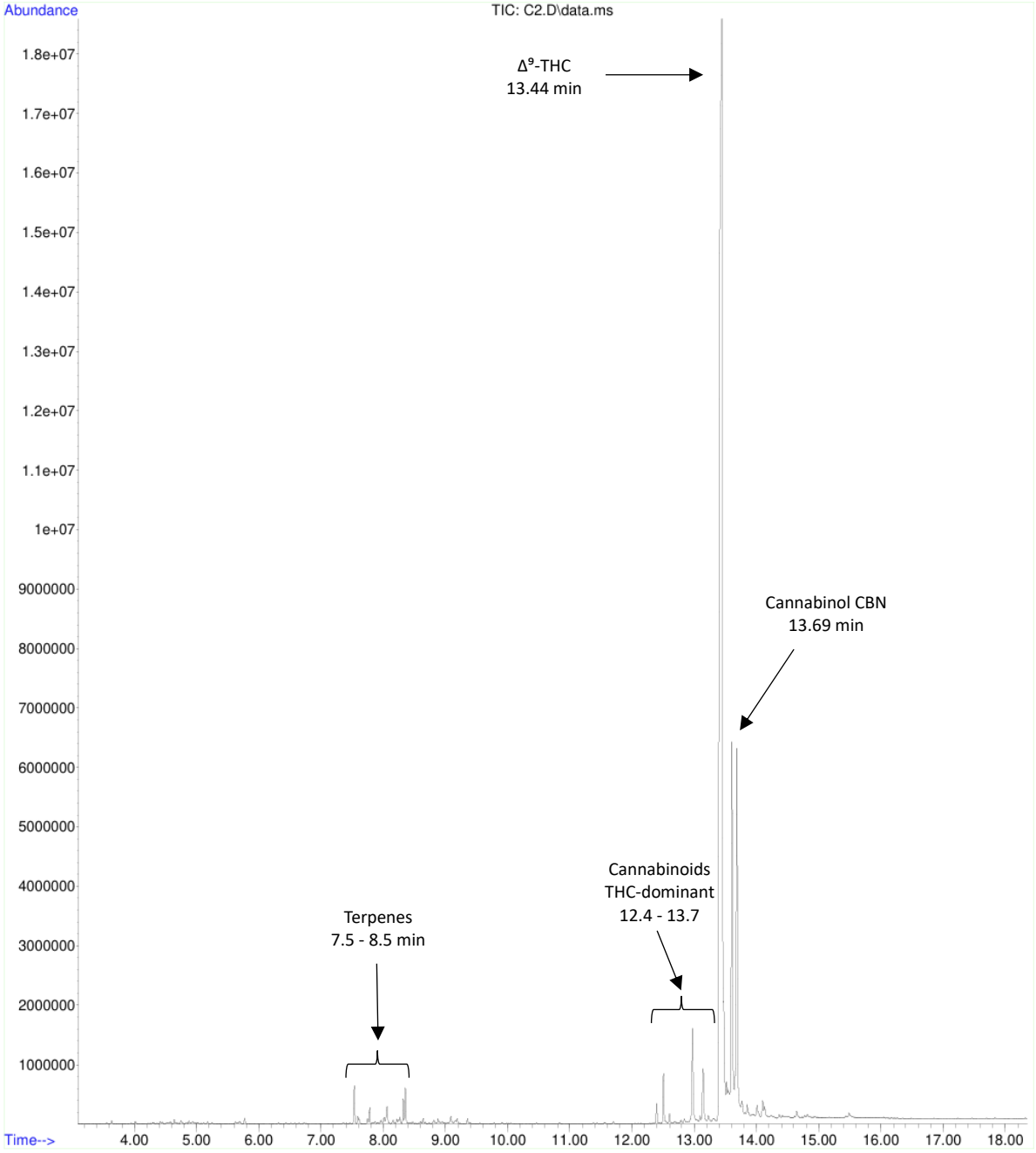


Figure 3.4 GC-MS TIC of illicit Sample 1 spiked with pesticide standard (C2)

```
Instrument :   Instrument #1
Sample Name: C3
Misc Info  : spiked C3
Vial Number: 9
```

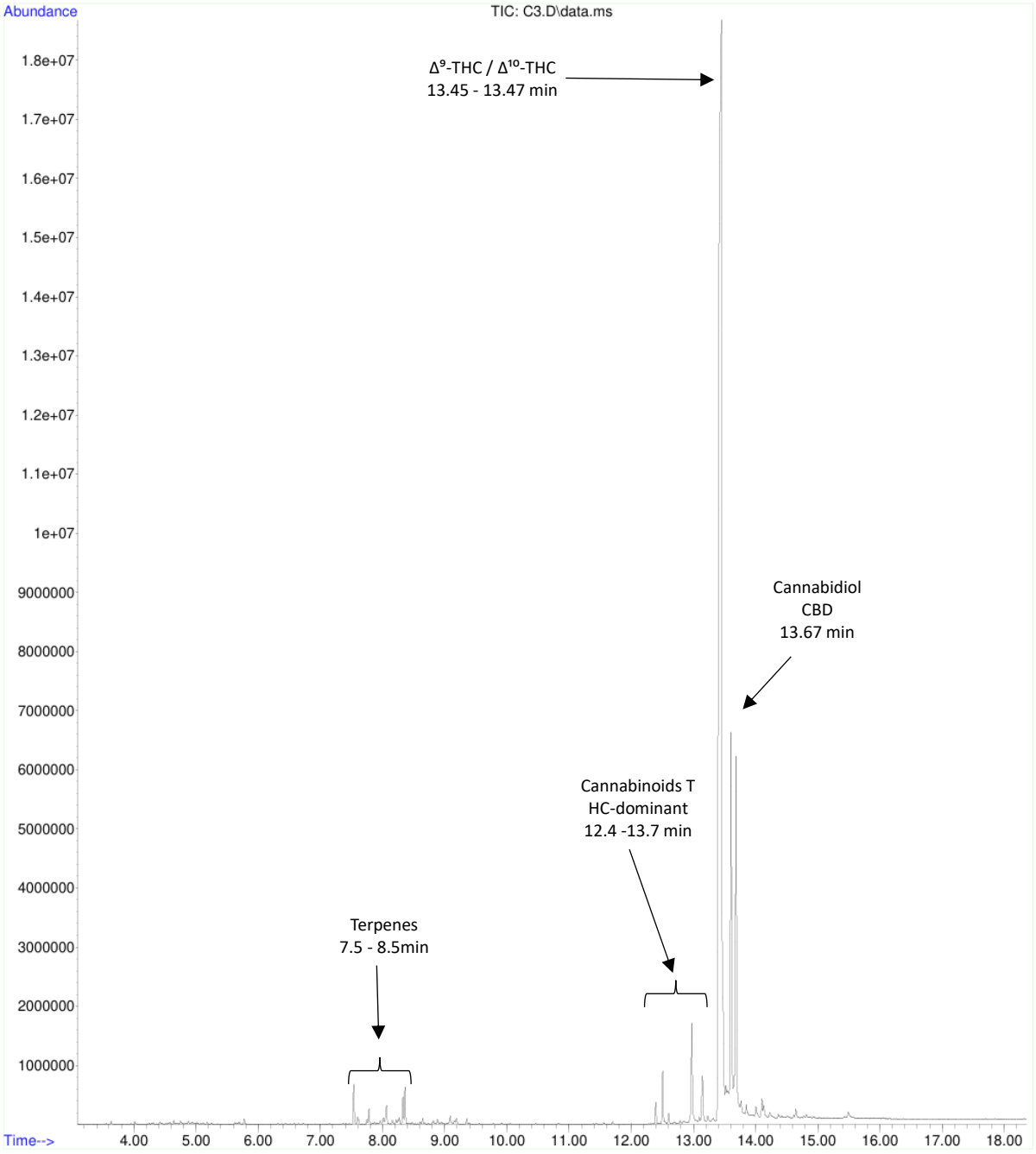


Figure 3.5 GC-MS TIC of illicit Sample 1 spiked with pesticide standard (C3)

Instrument : Instrument #1
Sample Name: C4
Misc Info : spiked C4
Vial Number: 10

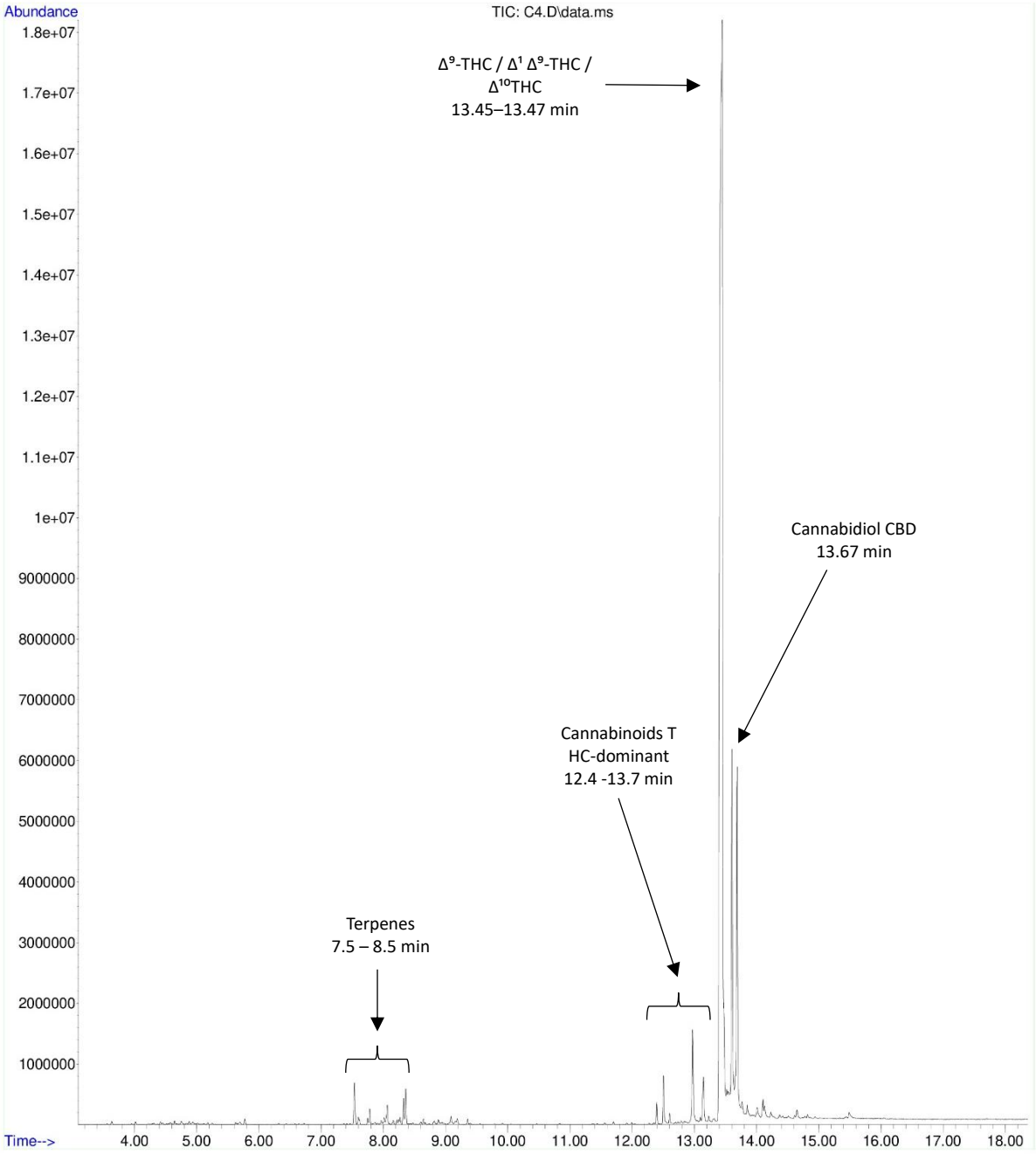


Figure 3.6 GC-MS TIC of illicit Sample 1 spiked with pesticide standard (C4)

3.2.3 Pesticide Standards (WS1)

In order to confirm instrument performance, the pesticide working solution (WS1) was injected directly into the GC-MS as a positive control. The chromatogram (Figure 3.7) showed distinct peaks corresponding to three organochlorine pesticides: DDE, DDT, and dieldrin, with retention times between ~8.5 and 11.5 minutes (see Table 3.3).

The appearance of these clear peaks demonstrates that the GC-MS was able to detect pesticide residues in a clean solution, confirming that the instrument itself was functioning correctly. However, only 3 out of the 17 pesticides in the WS1 mixture were detected. The remaining compounds were not observed. This could be most likely because they were either insufficiently volatile for GC analysis or underwent thermal degradation.

Table 3.3 Pesticides detected in GC-MS positive control (WS1)

Retention Time (min)	Compound Identified	Class	Notes
~8.5 - 9.0	DDE (Dichlorodiphenyldichloroethylene)	Organochlorine pesticide	Clear peak
~9.5 - 10.0	DDT (Dichlorodiphenyltrichloroethane)	Organochlorine pesticide	Clear peak
~11.0 - 11.5	Dieldrin	Organochlorine pesticide	Clear peak

This result highlights a key limitation of single-quadrupole GC-MS. While it can identify certain organochlorine pesticides, it lacked the scope and sensitivity to reliably detect a broader panel of pesticides. Therefore, the inability to observe pesticide residues in cannabis matrix samples cannot be attributed to instrument failure, but instead reflects the combined effects of matrix suppression and the restricted detection capability of GC-MS method compared with tandem MS/MS methods.

```
Instrument :   Instrument #1
Sample Name: Pesticide WS1
Misc Info  : MS Scan 50-500
Vial Number: 6
```

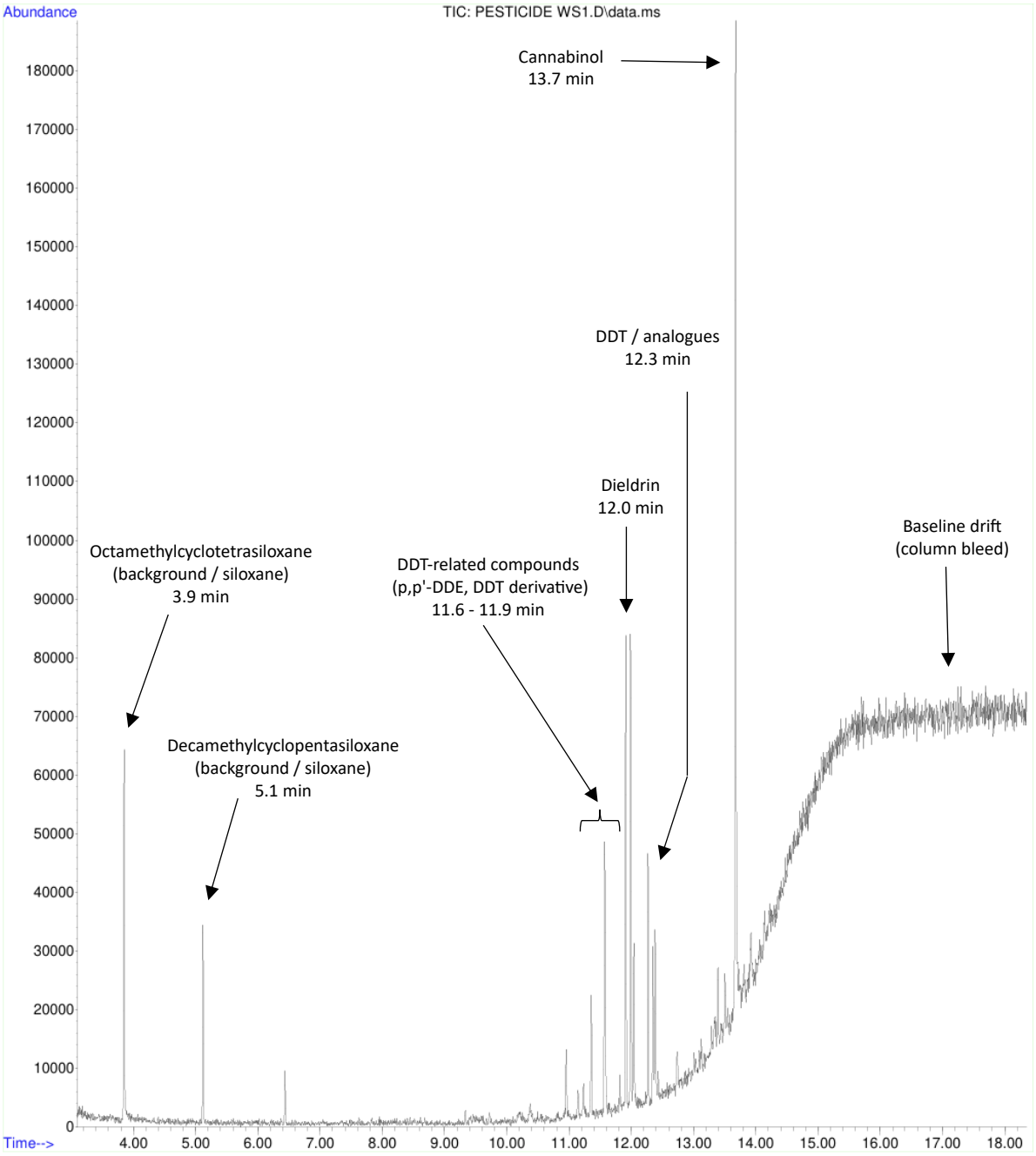


Figure 3.7 TIC of the pesticide mixture (WS1) analysed by GC-MS

Alternative oven temperature programs could have employed slower ramp rates or multi-step gradients to further optimise separation of early-eluting volatile compounds. For example, ramp rates in the range of 5–10 °C/min are commonly used in food pesticide analysis to

maximise peak resolution. However, such approaches substantially increase total run time and may not significantly improve performance for complex cannabis matrices, where co-elution from terpenes and waxes remains a limiting factor.

In addition, extended exposure of late-eluting compounds to high oven temperatures under slower ramp conditions may increase column bleed and reduce signal-to-noise ratios, particularly when using single-quadrupole GC-MS systems. Given that the present study was designed as a preliminary screening comparison rather than a full quantitative validation, the selected ramp rate represented a practical compromise between analytical efficiency, compound elution, and instrument limitations.

Ultimately, the limitations observed during GC-MS evaluation supported the transition to UPLC-MS/MS for definitive pesticide detection, where thermal constraints are avoided entirely.

3.2 UPLC-MS/MS Analysis

3.2.1 The UPLC-MS/MS method was applied to all 24 cannabis samples to provide a more sensitive and selective assessment of pesticide residues. This technique enabled detection of trace-level organophosphate compounds that were not seen by the GC-MS. Full detailed MRM chromatograms showing that no pesticides were detected are available in Appendix 9. Samples 1 - 12 showed the illicit samples results while the rest, samples 13 - 24, represent the licensed samples from regulated associations through ARUC.Licensed Samples

All 12 licensed cannabis samples tested negative for pesticide residues. No peaks corresponding to the target compounds were observed, confirming that these samples were free from detectable contamination under the conditions applied.

The MRM chromatograms displayed only baseline fluctuations and non-specific matrix signals, with no peaks corresponding to the retention times or transitions of the targeted pesticide panel. Within the limits of detection of this UPLC-MS/MS method, no quantifiable signals were observed, indicating that the samples did not contain any of the 84 screened pesticides at detectable levels.

3.2.2 Illicit Samples

Out of the 12 illicit cannabis samples analysed by UPLC-MS/MS, 5 produced clear signals for organophosphate pesticides. Identification of dichlorvos and chlorpyrifos was confirmed by matching both retention times (5.89 and 6.47 min, respectively) and their characteristic MRM transitions. This dual requirement improves specificity by ensuring that signals correspond to the target pesticides rather than unrelated matrix compounds. This also minimise the chance for false positives from matrix interferences.

Samples 1 and 2, which had previously been assessed by GC-MS without detectable residues, yielded clear pesticide peaks under UPLC-MS/MS conditions. Representative simplified chromatograms for dichlorvos and chlorpyrifos are shown in Figures 3.8 - 3.13, illustrating the peaks observed in the organophosphate-contaminated samples. Both quantifier and qualifier transitions yielded co-eluting peaks at the expected retention times, confirming compound identity.

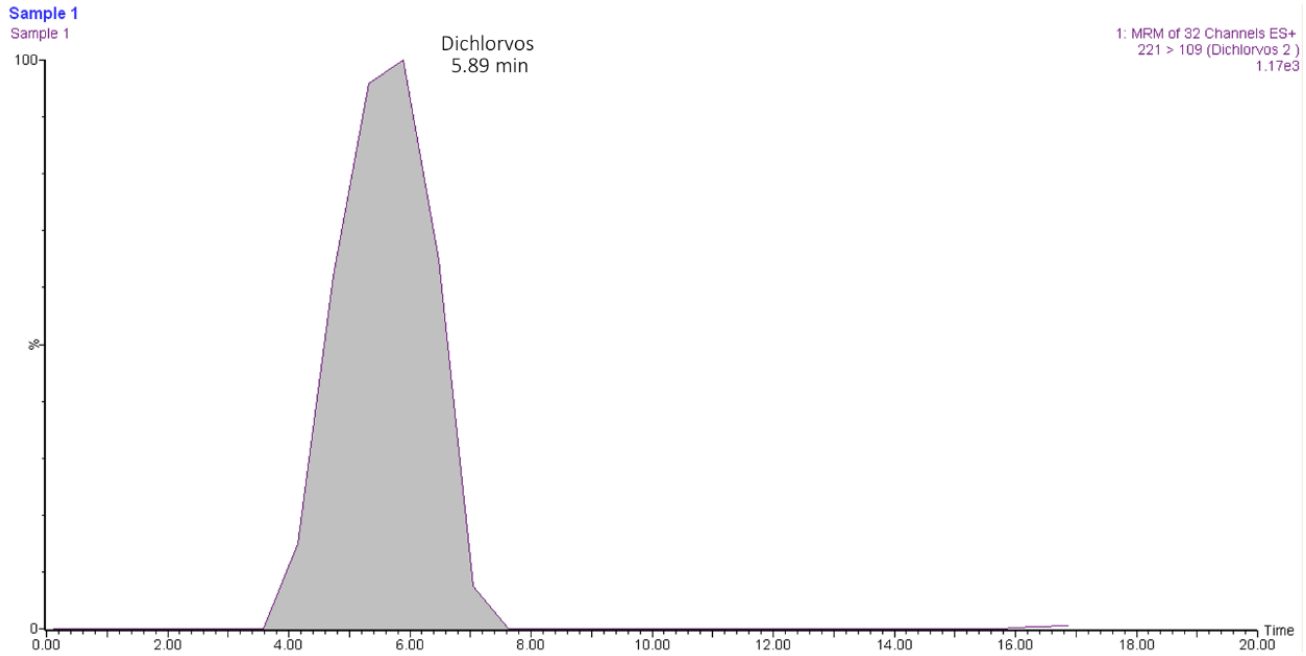


Figure 3.8 MRM chromatogram of dichlorvos in illicit cannabis Sample 1, transition 221 - 109, retention time 5.89 min

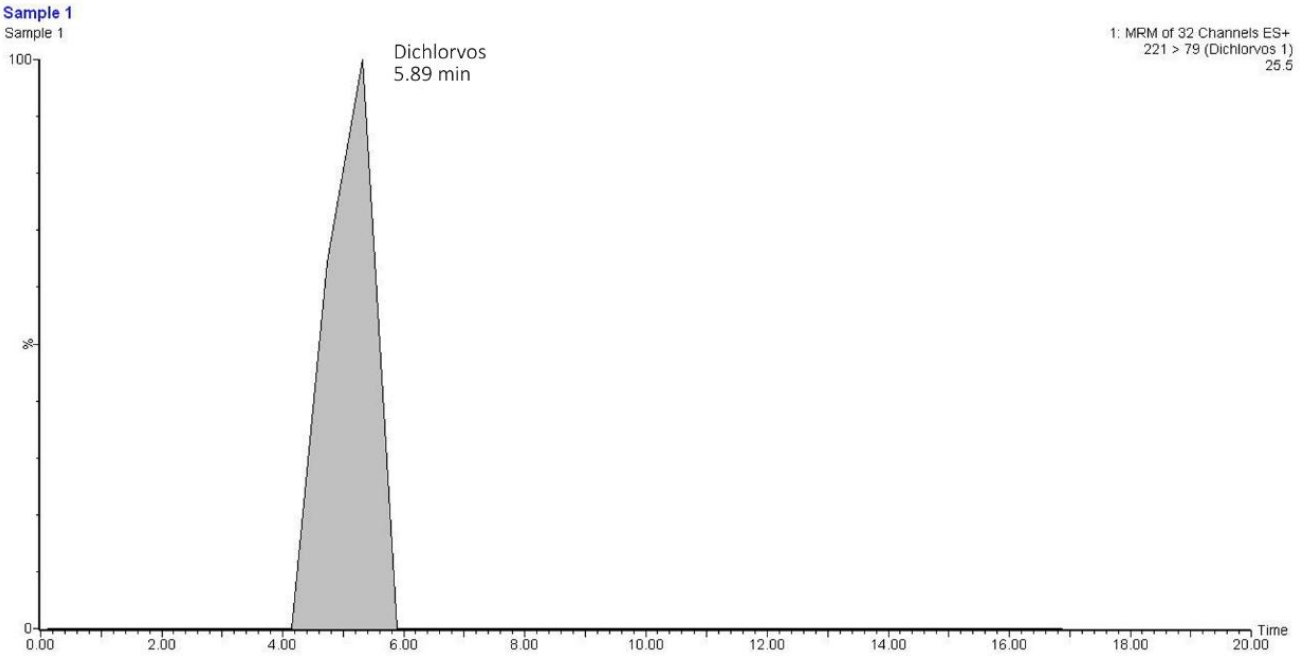


Figure 3.9 MRM chromatogram of dichlorvos in illicit cannabis Sample 1, transition 221 - 79, retention time 5.89 min

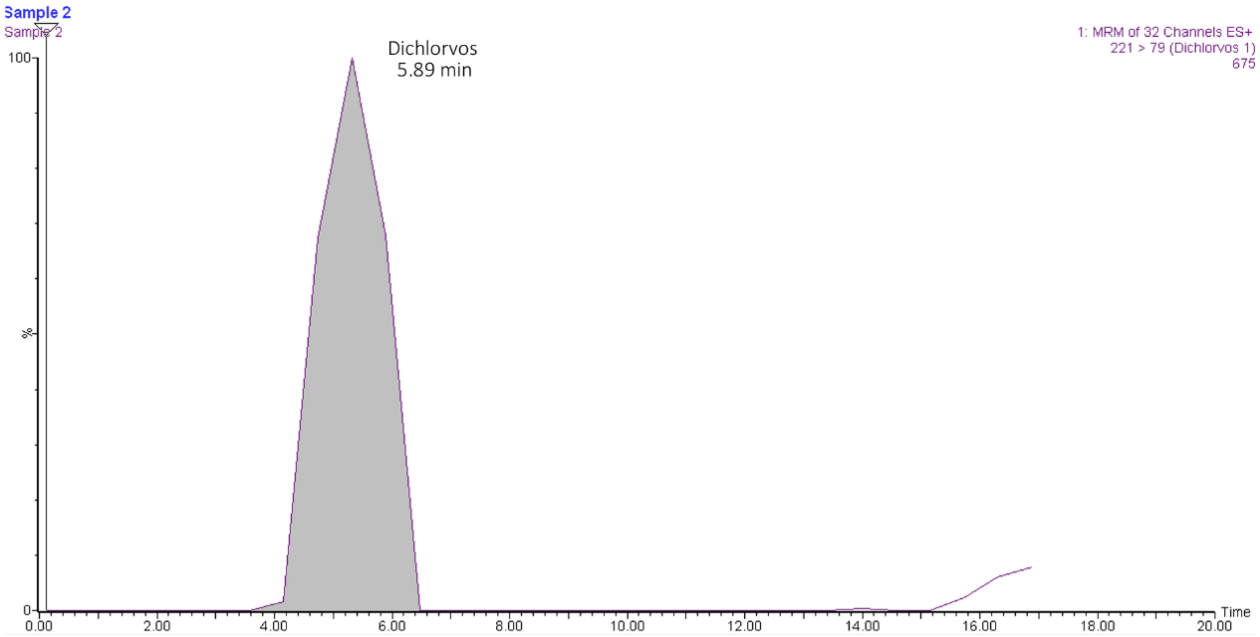


Figure 3.10 MRM chromatogram of dichlorvos in illicit cannabis Sample 2, transition 221 - 79, retention time 5.89 min

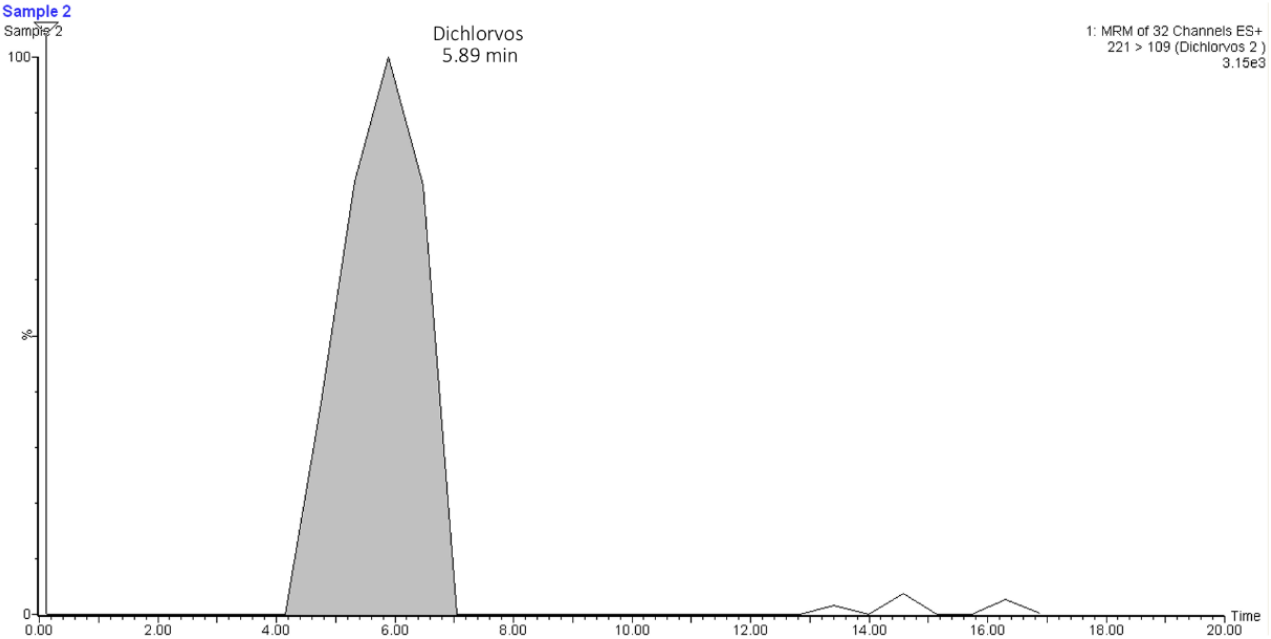


Figure 3.11 MRM chromatogram of dichlorvos in illicit cannabis Sample 2, transition 221 - 109, retention time 5.89 min

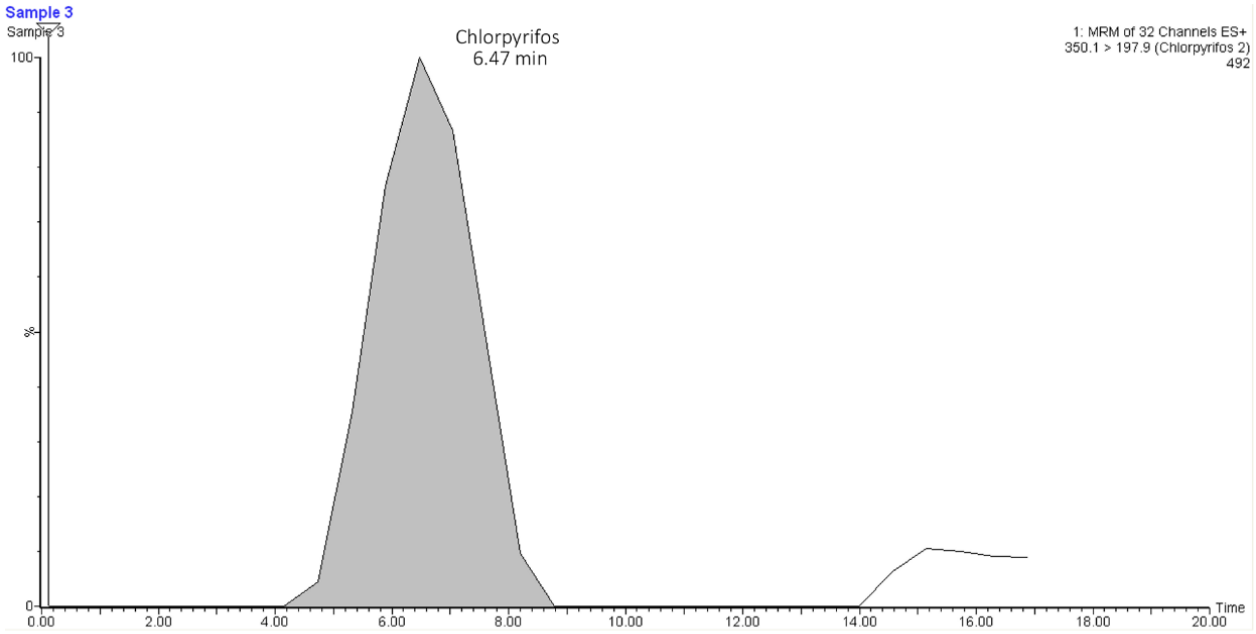


Figure 3.12 MRM chromatogram of chlorpyrifos in illicit cannabis Sample 3, transition 350.1 - 97, retention time 6.47 min

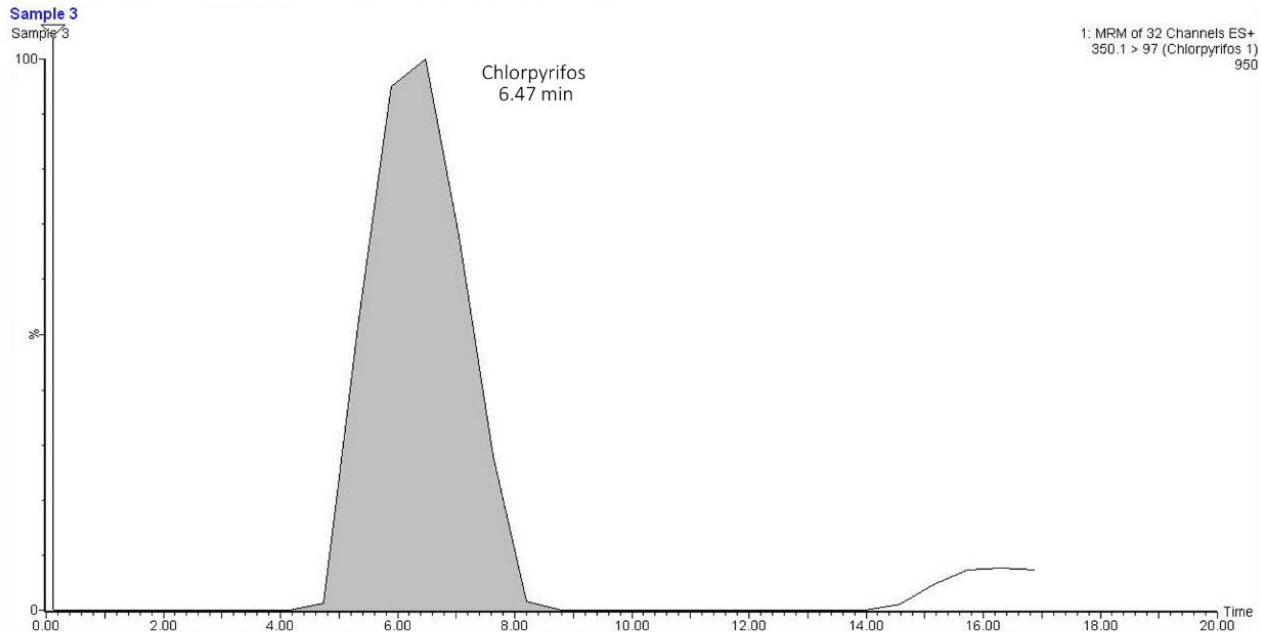


Figure 3.13 MRM chromatogram of chlorpyrifos in illicit cannabis Sample 3, transition 350.1 - 197.9, retention time 6.47 min

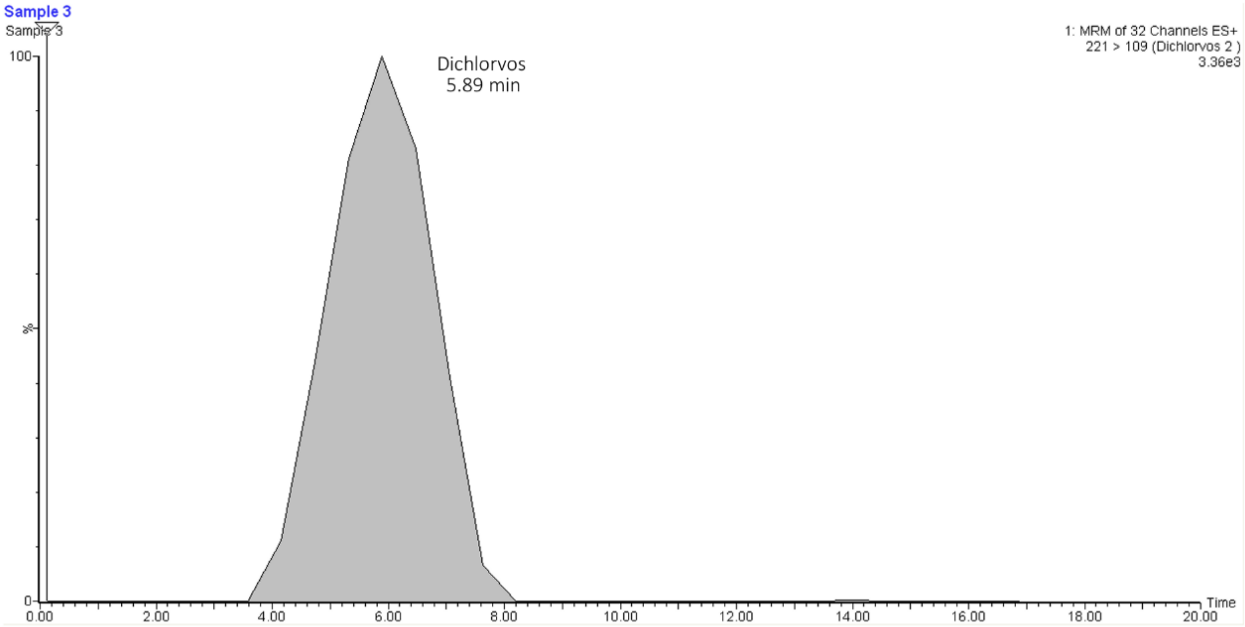


Figure 3.14 MRM chromatogram of dichlorvos in illicit cannabis Sample 3, transition 221 - 79, retention time 5.89 min

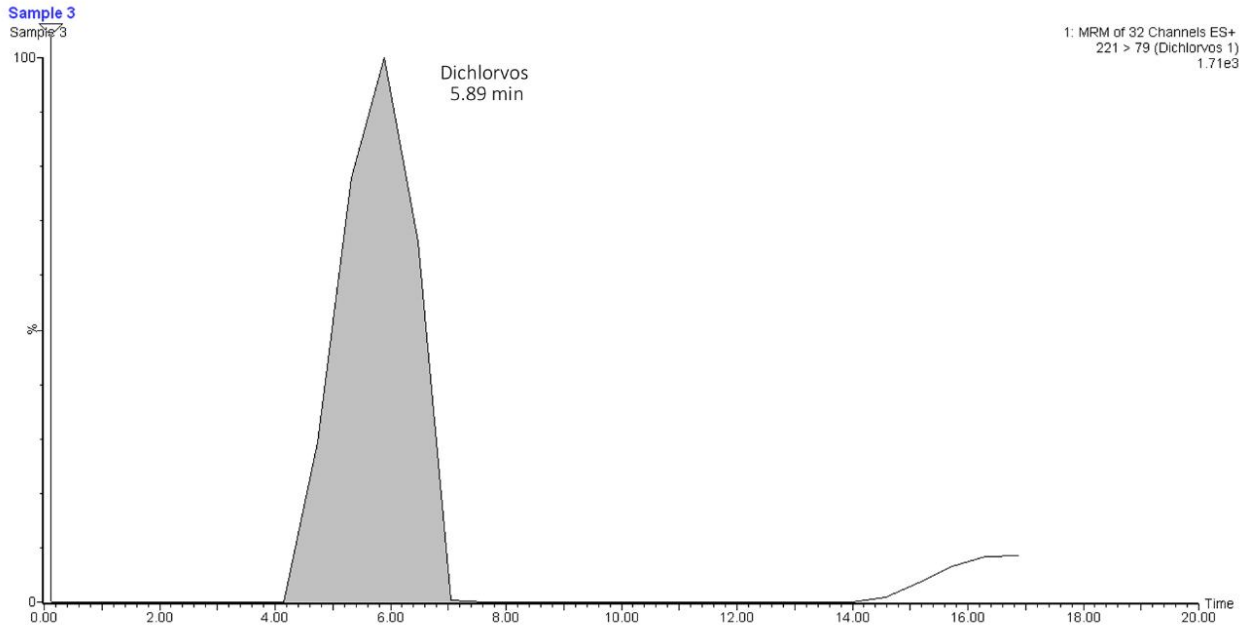


Figure 3.15 MRM chromatogram of dichlorvos in illicit cannabis Sample 3, transition 221 - 109, retention time 5.89 min

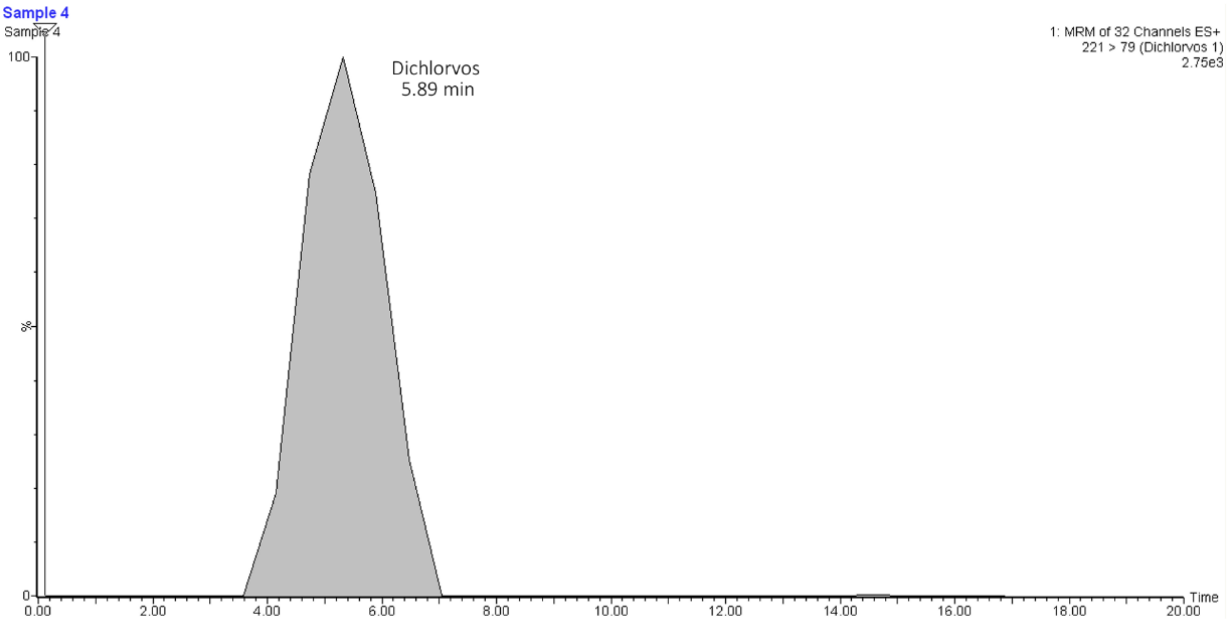


Figure 3.16 MRM chromatogram of dichlorvos in illicit cannabis Sample 4, transition 221 - 79, retention time 5.89 min

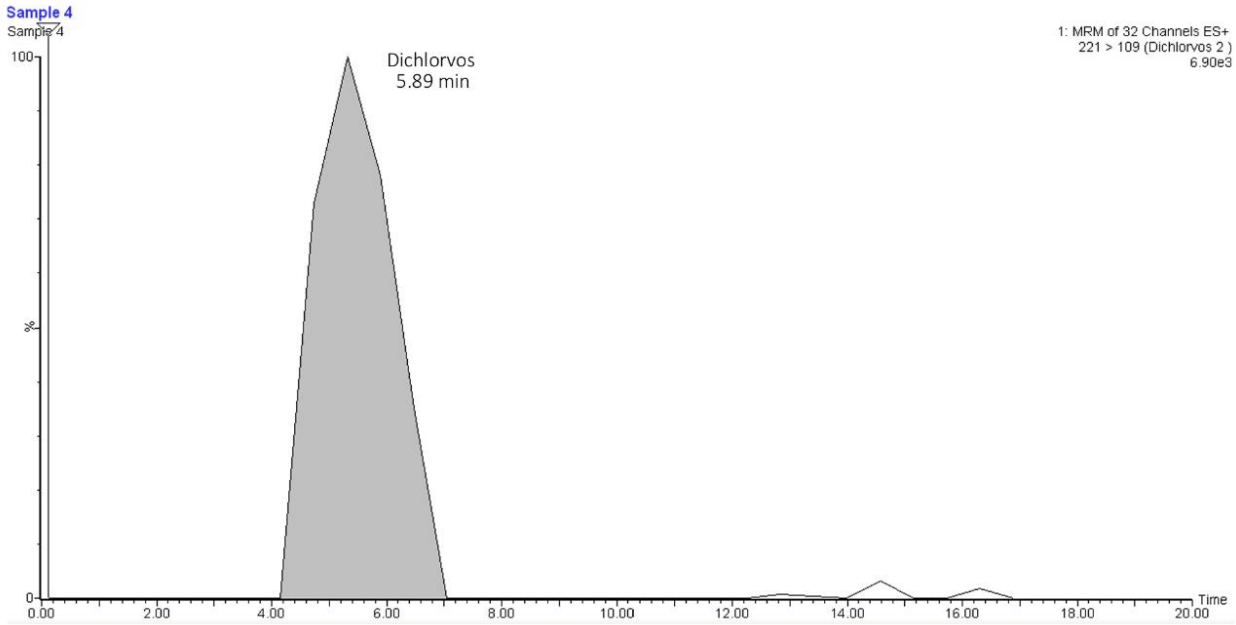


Figure 3.17 MRM chromatogram of dichlorvos in illicit cannabis Sample 4, transition 221 - 109, retention time 5.89 min

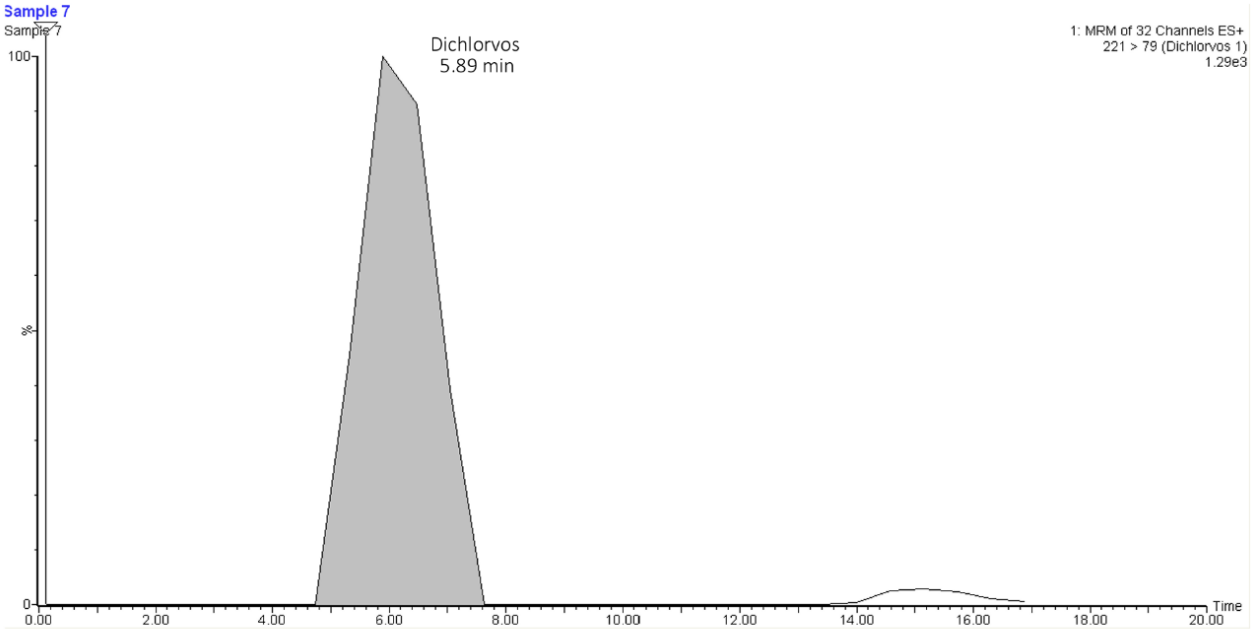


Figure 3.18 MRM chromatogram of dichlorvos in illicit cannabis Sample 7, transition 221 - 79, retention time 5.89 min

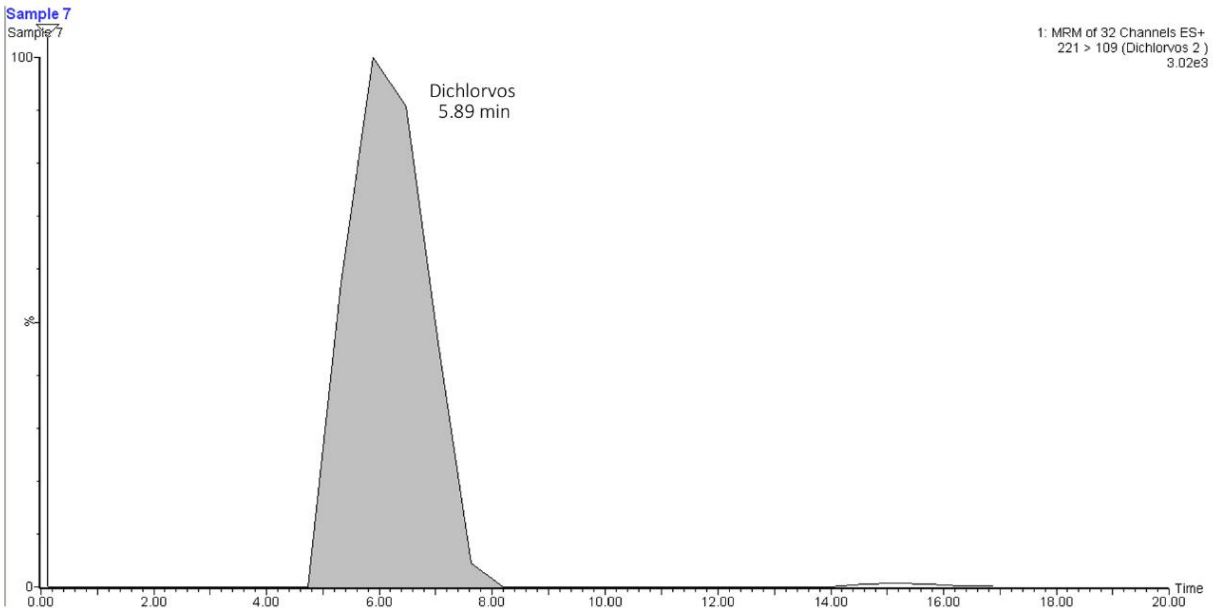


Figure 3.19 MRM chromatogram of dichlorvos in illicit cannabis Sample 7, transition 221 - 79, retention time 5.89 min

As shown also in Table 3.4, out of the 12 illicit cannabis samples analysed, five (41.7%) were found to be contaminated with pesticide residues. Dichlorvos was the most frequently detected compound, identified in five samples (Samples 1, 2, 3, 4, and 7). Chlorpyrifos was detected in a single sample (Sample 3), which also contained dichlorvos, indicating an overlap of pesticide residues in that case. The remaining 7 illicit samples, together with all twelve licensed samples, showed no evidence of pesticide contamination.

Table 3.4 Pesticide detection in illicit cannabis flowers

<table>
<tr><th>Sample ID</th><th>Source</th><th>Pesticide Detected</th><th>Retention Time (mins)</th><th>Compound Class</th></tr>
<tr><td>Sample 1</td><td rowspan="12">Illicit</td><td>Dichlorvos</td><td>5.89</td><td rowspan="4">Organophosphate</td></tr>
<tr><td>Sample 2</td><td>Dichlorvos</td><td>5.89</td></tr>
<tr><td>Sample 3</td><td>Chlorpyrifos + Dichlorvos</td><td>5.89, 6.47</td></tr>
<tr><td>Sample 4</td><td>Dichlorvos</td><td>5.89</td></tr>
<tr><td>Sample 5</td><td colspan="3" rowspan="2">None detected</td></tr>
<tr><td>Sample 6</td></tr>
<tr><td>Sample 7</td><td>Dichlorvos</td><td>5.89</td><td>Organophosphate</td></tr>
<tr><td>Sample 8</td><td colspan="3" rowspan="5">None detected</td></tr>
<tr><td>Sample 9</td></tr>
<tr><td>Sample 10</td></tr>
<tr><td>Sample 11</td></tr>
<tr><td>Sample 12</td></tr>
</table>

The bar graph below Figure 3.15 illustrates the number of contaminated samples (both illicit and licenced) with pesticides.

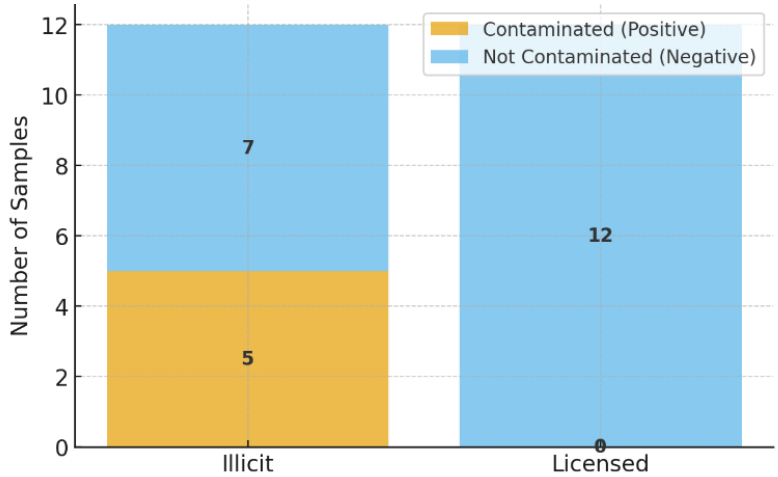


Figure 3.20 Pesticide contamination in illicit vs licensed cannabis samples

The next pie chart Figure 3.16 illustrates the percentage of contaminated samples comparing illicit and licenced samples.

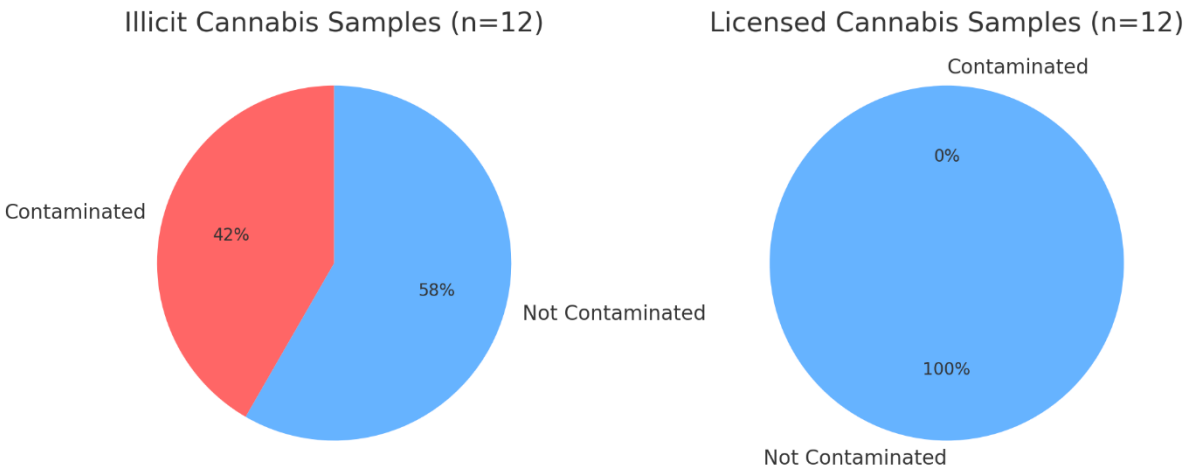


Figure 3.21 Contaminated vs not contaminated samples in percentage

The analysis identified pesticide contamination exclusively in illicit cannabis samples, with licensed samples remaining free of residues. Statistical testing confirmed this difference to be significant. These findings form the basis for the discussion in the next chapter, where their implications for public health and regulatory practice are considered.

3.3 Statistical Analysis

In order to evaluate whether pesticide contamination differed between illicit and licensed cannabis samples, a contingency table was prepared (see Table 3.6). Fisher's exact test was selected instead of the Chi-square test because of the small sample size and the presence of a zero value in the licensed group.

Table 3.5 shows a general contingency table with the letters representing the numbers are explained further below.

Table 3.5 General contingency table

Group	Contaminated	Not Contaminated	Total
Illicit	a	b	a + b
Licensed	c	d	c + d
Total	a + c	b + d	n

Where:
a = number of contaminated illicit samples
b = number of uncontaminated illicit samples
c = number of contaminated licensed samples
d = number of uncontaminated licensed samples
n = a + b + c + d = total number of samples

The next contingency table, Table 3.6, shows the true values of the explained general table above Table 3.5.

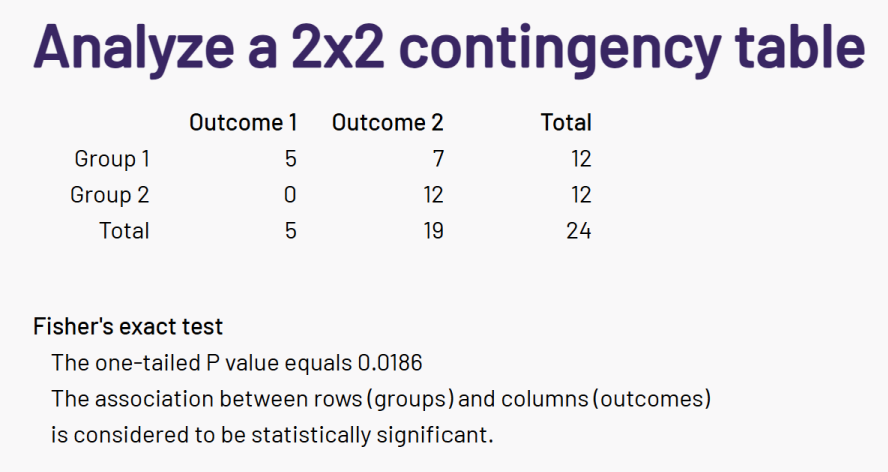
Table 3.6 Contingency table showing contaminated vs non contaminated samples

Group	Contaminated	Not Contaminated	Total
Illicit	5	7	12
Licensed	0	12	12
Total	5	19	24

Since this study compared proportions of contaminated vs non-contaminated samples between two groups, the Chi-square test (χ^2) would work for categorical data (presence vs absence), but with the small sample sizes (12 vs 12) and especially a zero cell (licensed = 0 positives), χ^2 assumptions may not hold. A better choice for this study is the Fisher's exact test which is designed for small sample sizes and will give an exact p-value for the difference in contamination between illicit vs licensed cannabis (Kim 2017). Fisher's exact test was performed using GraphPad QuickCalcs (GraphPad Software, San Diego, USA).

The Null hypothesis (H_0) states that there is no significant difference in the proportion of pesticide-contaminated samples between illicit and licensed cannabis groups. The Alternative hypothesis (H_1) states that there is a significant difference in the proportion of pesticide-contaminated samples between illicit and licensed cannabis groups. If p value is less than 0.05 the null hypothesis can be rejected and the alternative hypothesis showing statistically significant difference can be adopted.

Figures 3.17 and 3.18 shows 2 screenshots taken from the online free tool used to get a p value from Fisher's exact test. Figure 3.16 shows p-values of one-sided results while figure 3.17 gives two-sided value which is the recommended by the tool and has stronger results.



Analyze a 2x2 contingency table

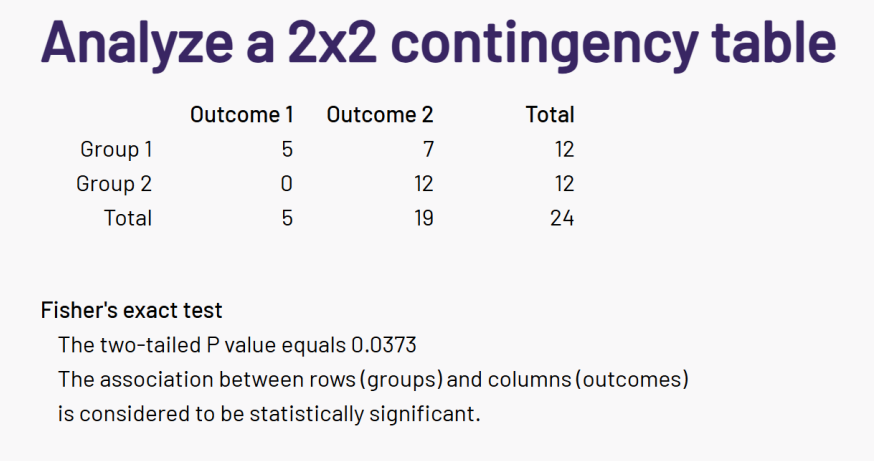
	Outcome 1	Outcome 2	Total
Group 1	5	7	12
Group 2	0	12	12
Total	5	19	24

Fisher's exact test

The one-tailed P value equals 0.0186

The association between rows (groups) and columns (outcomes) is considered to be statistically significant.

Figure 3.22 Results of one side Fisher's exact test from contingency table



Analyze a 2x2 contingency table

	Outcome 1	Outcome 2	Total
Group 1	5	7	12
Group 2	0	12	12
Total	5	19	24

Fisher's exact test

The two-tailed P value equals 0.0373

The association between rows (groups) and columns (outcomes) is considered to be statistically significant.

Figure 3.23 Results of two-sided Fishers exact test from contingency table

Fisher's exact test demonstrated a statistically significant difference in the presence of pesticide residues between illicit and licensed cannabis samples ($p < 0.05$), with contamination observed exclusively in illicit sources. The test was significant on both the two-sided analysis ($p = 0.037$) and the one-sided analysis ($p = 0.019$), confirming that illicit cannabis was more likely to contain pesticide residues.

Even with the limited sample size, it could be determined that illicit cannabis was more contaminated than licensed cannabis. The 95% confidence interval (calculated to be 13.8%–69.6%) shows that the true difference is unlikely to be zero, confirming that the effect is real rather than due to chance.

Chapter 4

Discussion and Conclusion

4.1 Overview

As discussed in previous Chapters, this present study set out to compare pesticide contamination in illicit cannabis inflorescences seized by Maltese law enforcement with licensed samples obtained from Cannabis Associations regulated by the ARUC. Twenty-four samples were analysed in total, which were evenly split between illicit and licensed sources (n = 12).

Using a QuEChERS extraction protocol followed by GC-MS and UPLC-MS/MS analysis, residues of dichlorvos and chlorpyrifos were identified in a subset of illicit samples, while none were detected in the licensed material. Statistical analysis confirmed a significant difference between groups (Fisher's exact, two-sided p = 0.037). These findings represent the first Maltese dataset directly comparing pesticide residues across illicit and licensed recreational cannabis. The discussion below provides an interpretation for these findings, compares them with international literature, and considers the broader public health and regulatory implications.

4.2 Interpretation of Findings

The key outcome of this study is that 41.7% (5/12) of illicit cannabis samples were found contaminated, whereas 0% (0/12) of licensed samples contained detectable pesticide residue. Among positives, dichlorvos was detected in all five contaminated samples, and chlorpyrifos in one.

This directly addresses the central hypothesis that stated that illicit (unregulated) cannabis would carry a higher burden of pesticide residues, especially banned or hazardous compounds, when compared to regulated cannabis. Our results confirm this expectation. Organophosphate pesticides residues were detected exclusively in illicit samples, while none were detected in the licensed samples. This exclusive presence of organophosphates in illicit cannabis strongly supports the hypothesis that regulatory oversight leads to safer, cleaner products. In practical terms, it suggests that Malta's regulatory frameworks and the adherence of Cannabis Associations to this regulation, have effectively prevented the use of certain high-risk pesticides, whereas illicit cultivators, operating outside these controls, resorted to these pesticides.

Organophosphates are commonly employed to control insect pests due to their broad-spectrum efficacy and low cost. This pattern of using opportunistic pesticide is common in unregulated cultivation (Taylor and Birkett 2020). It is important to emphasise that this outcome of this study shows that the type of pesticide found (organophosphates) is significant given its toxicity. This highlights how regulation filters out the worst offenders.

Dichlorvos and Chlorpyrifos are widely restricted in the European Union due to of their neurotoxic potential, but they still remained accessible in informal agricultural markets (European Commission 2012; European Commission 2020).

Chlorpyrifos and chlorpyrifos-methyl were withdrawn from approval under Regulation (EC) No 1107/2009 following the European Commission's decision not to renew their authorisation, with effect from 31 January 2020 (European Commission, 2020). This decision was informed by the European Food Safety Authority's conclusion that a safe exposure threshold could not be established.

EFSA identified consistent evidence of neurodevelopmental toxicity, particularly affecting children, alongside concerns regarding the genotoxic potential of chlorpyrifos. Additional uncertainties related to possible endocrine-disrupting effects further contributed to the overall risk assessment. In the absence of a demonstrable safe level of exposure, and in line with the hazard-based approach adopted within EU pesticide regulation, chlorpyrifos was therefore considered unsuitable for continued approval (European Commission, 2020).

Dichlorvos was subject to earlier regulatory restriction under the EU Biocidal Products Directive (98/8/EC), where it was ultimately not included in the list of approved active substances. As a consequence, the placing of dichlorvos-containing biocidal products on the EU market was prohibited from 1 November 2012 (European Commission, 2012). This decision was based on evidence that dichlorvos posed an unacceptable risk to both human health and the environment.

Regulatory assessments identified acute cholinesterase inhibition in applicators, alongside the compound's high volatility, which significantly increased the potential for inhalation exposure. Additional concerns were raised regarding its ecotoxicity towards non-target organisms. As an organophosphate insecticide, dichlorvos exerts its toxic effects through inhibition of acetylcholinesterase, disrupting normal nerve transmission. Although this mechanism is effective for pest control, the narrow margin between pesticidal efficacy and adverse effects in humans rendered its continued use incompatible with EU safety standards (European Commission, 2012).

These findinds are notable because they include both older organophosphate compounds associated with high acute toxicity (such as dichlorvos) and more recently restricted compounds (such as chlorpyrifos), reflecting different regulatory histories but similarly unacceptable toxicological profiles. This targeted discussion of dichlorvos and chlorpyrifos directly supports the study's objective of assessing whether regulatory oversight reduces exposure to high-risk pesticide residues in recreational cannabis.

The persistence of these chemicals in illicit cannabis samples in Malta therefore suggests that unregulated cultivators may be sourcing not only pesticides in general, but specifically substances that are banned within the European Union, which are outdated or illegally traded, either through informal agricultural supply chains or cross-border leakage. This not only contravenes EU pesticide policy but also re-introduces compounds that regulators have already deemed too hazardous for any approved agricultural use.

It may also be possible that some contaminated illicit cannabis in Malta is imported from North Africa or other nearby regions where regulatory controls are weaker and banned pesticides remain in circulation. Investigations show that pesticides banned in the EU are still manufactured and sold in parts of Africa, often due to lax enforcement, weak regulatory capacity, or informal markets. If this is the case, it complicates efforts to mitigate contamination because domestic regulation alone cannot address imported illegal or unsafe supply chains.

Moreover, the presence of these banned pesticides in cannabis, which is a product consumed predominantly through smoking or vaping, increases the risk of toxicity when compared to risks associated with ingested food products, because inhalation provides a direct and efficient exposure route for neurotoxic organophosphates (Sullivan et al. 2013).

The complete absence of residues in licensed samples demonstrates that ARUC's regulatory framework is effective in preventing pesticide contamination. If such compounds had been used, our analytical methods would have detected them given their sensitivity. Under ARUC Directive 1.2 (2023), associations must declare the absence of chemical pesticides and rely instead on integrated pest management, organic nutrients, and biological controls (ARUC 2023a; ARUC 2023b). The clean laboratory findings suggested that these measures are functioning as intended.

The statistically significant difference between groups, though based on a modest sample size, supports the hypothesis that illicit cannabis poses a greater toxicological risk to consumers. Importantly, the contamination rate observed here (41.7%) is within the range reported internationally for illicit markets, which often varies between 20% and 60% depending on jurisdiction. This suggests that Malta's illicit supply chain is not unique but reflects wider patterns of unregulated cultivation through the European Union.

4.3 Comparison with International Studies

Our findings align with, and reinforce, trends observed in other international studies, highlighting that the contrast between regulated and illicit cannabis is not unique to Malta.

Gagnon et al. (2023) reported a very similar pattern in Canada after recreational legalisation. The Canadian study, screened 327 pesticides in cannabis, found that illicit samples contained multiple residues at far higher frequencies than licensed products. Only 6% of licensed cannabis samples contained any pesticide residues and then only at trace levels, whereas a staggering 92% of illicit samples were pesticide-positive. Moreover, the Canadian illicit cannabis contained 23 different pesticide active ingredients (averaging approximately 3 - 4 different pesticides per sample). These pesticides also included organophosphates like chlorpyrifos, compared to just two low-level pesticide detections in the legal products. This mirrors our result that shows how organophosphates, and other hazardous pesticides proliferate in illicit supply chains but are effectively absent under licensed cultivation.

Craven et al. (2021) provide further evidence. They performed environmental wipe sampling at two cultivation sites and found 41 different pesticides present at the unlicensed (illicit) grow, compared to only six (and at very low levels) at a licensed facility. This dramatic 41 vs. 6 difference in pesticide use between an illicit operation and a regulated one, echoes the core of our findings. Illicit cultivation tends to be chemically intensive and uncontrolled, whereas licensed cultivation, under compliance requirements, is far more judicious in pesticide application. The consistency of this theme across regions strengthens the interpretation that regulation universally curtails pesticide contamination. It is notable too that even when regulated growers do use pest control, they appear to favour compounds that either degrade before harvest or remain at negligible residual levels. By contrast, illicit growers may apply pesticides without adherence to pre-harvest intervals or approved lists, leading to persistent residues (Craven et al. 2021).

In Oregon US, post-legalisation surveillance of dispensary products revealed residues of myclobutanil, bifenthrin, and abamectin in illicit or unlicensed cannabis, while licensed products consistently complied with state standards (Evoy and Kincl 2020). Similarly, California data show that illegal market products frequently contain organophosphates and carbamates, whereas regulated dispensary samples rarely breach limits (Dalmia et al. 2021). In Malta, dichlorvos and chlorpyrifos were detected, similar to findings in the US with regards to organophosphates. However, the US illicit market resulted in a wider range of pesticides detected.

In Europe, similar concerns have been documented. Cuypers et al. (2017) in Belguim, analysed illicit indoor cannabis plantations in Belgium and found pesticide residues in approximately 64% of sampled plants. In total, they identified 19 different pesticides spanning various chemical classes, including insecticides and fungicides commonly encountered in illicit grows such as imidacloprid, propamocarb, propiconazole, tebuconazole, and others. This extent of pesticide types in Belgian illicit cannabis aligns with what we observed in others studies and in Malta's illicit samples which shows a broad, and often unregulated, use of agro-chemicals. Although Cuypers et al. did not explicitly highlight organophosphates in their summary, the prevalence of insecticidal and fungicidal residues reinforces the same core issue: illicit cultivation environments are characterised by extensive chemical. The overlap in commonly detected substances like imidacloprid, a neonicotinoid insecticide, appeared in the Belgian study and was also among those found in other jurisdictions' illicit cannabis. This suggests that certain pesticides have become go-to solutions for illicit growers across different countries. These are often pesticides that regulated industries would avoid. Cuypers et al. 's findings, point out that even without legal access to cannabis, illicit production which is often in clandestine indoor setups, introduces significant pesticide-related health risks to consumers and even to those handling the plants like law enforcement or cleanup crews.

Even in quasi-legal contexts, a lack of strict regulation can yield high pesticide contamination rates. Amendola et al. (2021) examined so-called "light cannabis" inflorescences which are low-THC hemp flowers legally sold in Italy as herbal products. They discovered that 87% of samples contained pesticide residues. They quantified a range of fungicides and insecticides at levels from 0.01 up to 185 µg/g, with spinosad (an insecticide derived from bacterial toxins)

and cyprodinil (a fungicide) being the most frequently detected compounds. Importantly, these Italian hemp products were outside food and tobacco regulations at the time, effectively forming a lightly regulated or grey market because they lacked formal pesticide standards. The high positivity rate reported by Amendola et al. highlights that without explicit pesticide regulations and oversight, even supposedly legal cannabis/hemp markets can become chemically contaminated (Amendola et al. 2021).

In our study, by contrast, the Cannabis Association samples showed no organophosphate residues or any other pesticides residue, hinting that Malta's requirements, or at least the culture of compliance among licenced growers, may be stricter than the situation Italy had for hemp. Nonetheless, the Italian data can serve as a warning for Malta and Europe: they highlight how crucial it is to establish and enforce pesticide limits in all cannabis markets, whether medical, recreational, or hemp, to avoid exposing consumers to potentially harmful substances (Amendola et al. 2021).

International evidence from North America and Europe shows the same pattern: illicit or weakly regulated cannabis is heavily contaminated, while regulated products are far cleaner. Collectively, the Maltese results are not an outlier but instead reinforce a consistent international trend. The slight difference lies in the narrower range of pesticides observed in Malta, which may reflect the small scale of cultivation compared to larger producer nations.

4.4 Toxicological and Public Health Implications

The detection of dichlorvos and chlorpyrifos has serious toxicological implications. Both belong to the organophosphate class, which act as irreversible acetylcholinesterase inhibitors. This causes accumulation of acetylcholine at synapses which leads to cholinergic overstimulation (Jameson et al. 2022). Acute exposure can trigger nausea, respiratory distress, seizures, and in severe cases death. Chronic low-dose exposure is associated with neurodevelopmental deficits, endocrine disruption, and increased risk of certain cancers (Mnif et al. 2011).

Inhalation is a particularly concerning route of exposure. Unlike dietary ingestion, where residues may degrade or be metabolised, combustion or vaporisation of cannabis flowers delivers pesticide residues directly to the pulmonary system, bypassing first-pass metabolism. Studies have shown that up to 70% of pesticide residues present on cannabis plant material can transfer into smoke or vapour (Sullivan et al. 2013). Thus, the health risk to consumers of contaminated cannabis is magnified compared to foodborne exposure.

The implications are especially acute for vulnerable populations, including adolescents, pregnant women, and individuals with pre-existing neurological conditions such as epilepsy (Taylor and Birkett 2020). For regular users, chronic low-dose exposure may accumulate over time, which then increases health risks. Importantly, cannabis users often perceive the plant as “natural” and therefore safer, which can obscure awareness of pesticide hazards (Wylie et al. 2020).

The absence of residues in licensed samples underscores the importance of providing safe alternatives. If consumers migrate from the illicit to the regulated market, population-level exposure to neurotoxic pesticides could be substantially reduced. This is a central harm reduction argument for regulation.

4.5 Regulatory Implications

From a regulatory standpoint, the results enforced ARUC's framework and emphasised its necessity. All licensed cannabis samples analysed with our method showed no detectable pesticide residues, showing that regulatory declarations and inspection processes are not merely bureaucratic but have real toxicological benefits. This positions Malta as an early EU case study demonstrating that non-profit cannabis associations can provide clean, safe products to consumers.

However, the persistence of contaminated illicit cannabis highlights ongoing challenges. Despite the 2021 reform, illicit supply chains remain active, driven partly by the slow rollout of licensed associations and unmet demand (Muscat et al., 2025). Consumers unable to access licensed cannabis may still turn to the illicit market, thereby exposing themselves to harmful residues.

Internationally, the Maltese findings provide support for harmonised EU pesticide standards in cannabis. Currently, only Canada and some US states maintain comprehensive multi-residue

testing regimes (Gagnon et al. 2023; Dalmia et al. 2021). The EU has not yet standardised cannabis pesticide limits, leaving member states to devise their own rules. This study demonstrates that without regulation, harmful organophosphates enter the supply, while effective oversight keeps them out. The regulatory implication is clear, cannabis markets require the same pesticide surveillance as food products.

Furthermore, these findings can inform future ARUC policy. As the Maltese cannabis club model matures, regulators may consider mandatory third-party laboratory testing, routine audits, and publication of pesticide monitoring results to enhance consumer trust. This could parallel the transparency standards already seen in Canada and several US states.

4.6 Limitations of the Study

While this dissertation provides the first systematic comparison of pesticide residues in illicit and licensed recreational cannabis in Malta, several limitations must be acknowledged.

The dataset consisted of only 24 samples in total, evenly split between illicit and licensed sources. Although Fisher's exact test indicated statistical significance, the small sample size limits generalisability. Larger sample sets across multiple seasons and associations would provide greater confidence in prevalence estimates and strengthen inferential statistics.

The analytical method focused on detecting pesticides through retention time matching, rather than screening a broad multi-residue panel. By contrast, international studies such as Gagnon et al. (2023) employ screening of hundreds of compounds simultaneously. It is therefore possible that other pesticide residues were present but undetected.

This study reported binary detection (present/absent) without concentration values. Without quantitative levels (e.g., µg/kg), toxicological risk characterisation remains limited. Even trace detections could be clinically relevant if above inhalation reference doses, but this cannot be established here.

Licensed samples were obtained directly via ARUC, ensuring traceability and regulatory compliance. Illicit samples were obtained from seizures by law enforcement. While these are representative of what reaches consumers, seizure bias cannot be excluded. Samples may reflect cultivation practices of certain suppliers rather than the broader illicit market.

Cannabis is a highly resinous, cannabinoid-rich matrix, which presents challenges for extraction and clean-up. Despite following validated QuEChERS adaptations, matrix effects such as ion suppression may have influenced detection sensitivity (Lozano et al. 2012b).

Another limitation of the present study is the lack of tandem MS couples with GC, which restricts both sensitivity and compound coverage, particularly for non-volatile or thermally

labile pesticides. This constraint may have led to underestimation of certain residues. Future work employing GC-MS/MS or non-targeted LC-MS/MS is recommended to validate and expand upon the present findings.

This dissertation represents a preliminary comparative investigation; therefore, full validation procedures (e.g., determination of LOQ, recovery studies) were outside the scope of this study. Instead, reference to previously validated protocols (Appendix 5) ensured methodological reliability, while the focus remained on identifying relative differences in pesticide contamination between licensed and illicit samples.

This study was limited by the absence of replicate analyses and spiked recoveries during the UPLC-MS/MS phase. While blanks confirmed the absence of contamination, additional quality control measures would have strengthened precision and recovery estimates. Future work should incorporate these steps to strengthen the assessment of method performance.

This study did not include replicate analyses or continuing calibration verification, which would have strengthened the assessment of instrument precision and stability. Future work should incorporate these measures to provide a more complete evaluation of method performance.

Acknowledging these limitations is essential for contextualising the results. They do not undermine the finding that illicit cannabis carried detectable residues while licensed cannabis did not, but they temper the strength of broader generalisations.

4.7 Recommendations

Based on the findings and limitations of this study, several recommendations can be given for practice, regulation, and future research.

Mandatory multi-residue testing for cannabis. Licensed Cannabis Associations should undergo routine testing using expanded LC-MS/MS and GC-MS/MS panels, covering at least 200–300 pesticide compounds. This would align Malta with Canadian standards and set a precedent within the EU (Health Canada 2023). Transparency measures can include routine publication of testing results. This could strengthen consumer trust and encourage migration away from the illicit market.

Another recommendation is for stronger enforcement against illicit cultivation. Since 41.7% of illicit samples contained banned pesticides, seizures could be accompanied by chemical analyses including pesticides. Public reporting of the findings can help to highlight the risks to consumers.

Adoption of integrated pest management (IPM). While ARUC guidelines already mandate pesticide-free cultivation, associations should be supported with IPM training, including use of beneficial insects, controlled humidity, and organic nutrients. Third-party audits provide independent verification of cultivation practices. This can further demonstrate compliance and pre-empt regulatory breaches.

Consumer education campaigns which messaging should stress that illicit cannabis not only carries legal risk but also poses higher toxicological risks due to pesticides. This reframes regulation as a health protection measure rather than merely a legal restriction.

Future Maltese studies should analyse larger, longitudinal sample sets and incorporate quantitative pesticide residue analysis. Cross-jurisdictional comparisons by collaborative research with other EU states could support development of harmonised standards.

Toxicological modelling by conducting simulation studies that estimate inhaled doses under realistic smoking or vaping conditions would allow a deeper risk assessment which can be tailored to Maltese consumers.

4.8 Conclusion

This dissertation added new evidence to Maltese cannabis research. It suggests that Cannabis Associations provide cleaner cannabis than illicit suppliers achieving one of the central aims of legalisation which is harm reduction. At the same time, the persistence of contaminated illicit cannabis revealed the unfinished nature of reform. Until regulated products are widely available and affordable, consumers will continue to rely on illicit sources, sustaining their exposure to harmful residues. Policymakers must therefore expand legal access while maintaining pressure on unregulated markets.

The study also highlighted the analytical and regulatory challenges of pesticide testing in cannabis, from matrix complexity to inconsistent international standards. Despite these hurdles, even a small-scale investigation can yield insights when carefully designed and interpreted.

By comparing 12 licensed samples with 12 seized illicit samples, this work found organophosphate contamination (dichlorvos and chlorpyrifos) in five illicit samples (41.7%), but none in licensed products. Statistical testing confirmed this difference was significant. These results mirror international findings that illicit cannabis is more prone to pesticide contamination, with serious public health consequences given the neurotoxicity of organophosphates and the risks of inhalation exposure.

While the sample size was modest and the pesticide scope limited, the findings validate ARUC's regulatory approach and underline the consumer protection benefits of oversight. Future research should broaden pesticide screening and enlarge the dataset, while regulators should embed routine monitoring across all associations.

The key message is simple: regulation protects, the illicit market endangers. Malta's early cannabis association model offers a chance to build pesticide safety into its regulatory foundations and set an example for other European countries.

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L-Università ta' Malta

FRAMEWORK COLLABORATION AGREEMENT
BETWEEN
THE UNIVERSITY OF MALTA
AND
THE AUTHORITY FOR THE RESPONSIBLE USE OF CANNABIS
(UM REF: 2024_295_UM_ARUC)

This Framework Collaboration Agreement (hereinafter the **"Framework Agreement"**) is being made by and between:

(1) On the first part, the University of Malta of Msida, MSD2080, Malta, (hereinafter the **"University"**) as established under the Education Act (Cap. 327, laws of Malta), represented hereon by its Rector, Prof. Alfred J. Vella (I.D. 919150M); and

(2) On the second part, the Authority for the Responsible Use of Cannabis, of Level 4, Onda Business Centre, ARUC, Aldo Moro Rd, Marsa MRS 9065 (hereinafter **"ARUC"**), represented hereon by its Executive Chairperson, Dr Joey Reno Vella (I.D. 143095M).

Hereinafter also referred to each as a **"Party"** and collectively the **"Parties"**.

PREAMBLE

(a) Whereas the remit of the University's is to provide students with an outstanding education and experiences and participate in research;

(b) Whereas ARUC mission is to establish an effective and efficient system that ensures the responsible use of cannabis;

(c) Whereas the Parties wish to build and foster a collaborative relationship, in order to draw on each other's mission and strengths; and

(d) Whereas the Parties are desirous to regulate their collaboration under the terms of this Framework Agreement.

THE PARTIES NOW THEREFORE HEREBY AGREE AS FOLLOWS:

1. Interpretation

1.1 For the purposes of this Framework Agreement, unless the context otherwise requires, the following terms shall have the following meanings:

(a) **"Background IP"** means Intellectual Property owned or controlled by either of the Parties at the Effective Date or which shall at any time thereafter become so owned or controlled otherwise than as a result of this Framework Agreement;

(b) **"Collaborative Initiatives"** shall have the meaning attributed to it in clause 3.

(c) **"Effective Date"** means the latest date of the signature of either of the Parties of this Agreement, as indicated on the last page of this document;

(d) **"Force Majeure"** means any acts of nature including fire, flood, earthquake, storm, hurricane, volcanic eruption, pandemic or other natural disaster, war, invasion, act of foreign enemies, hostilities (whether war is declared or not), civil war, rebellion, revolution, insurrection, military

or usurped power or confiscation, terrorist activities, nationalisation, government sanction, blockage, embargo, labour dispute, strike, lockout or interruption or failure of electricity;

(e) **"Foreground IP"** means Intellectual Property resulting directly from and authored, conceived, developed, reduced to practice or otherwise created during the performance of this Framework Agreement and therefore by reason of a Collaborative Initiative;

(f) **"Intellectual Property"** or **"IP"** means inventions, discoveries, developments, methods, processes, compositions, works, concepts and ideas (whether or not patentable or copyrightable or constituting trade secrets) conceived, made, created, developed or reduced to practice and which may result in Intellectual Property Rights;

(g) **"Intellectual Property Rights"** or **"IPR"** means patents, rights to inventions, copyright and related rights, trade-marks, trade names and domain names, rights in get-up, goodwill and the right to sue for passing off or unfair competition, rights in designs, rights in computer software, database rights, rights to preserve the confidentiality of information (including know-how and trade secrets) and any other intellectual property rights, in each case whether registered or unregistered and including all applications (or rights to apply) for and be granted, renewals or extensions of, and rights to claim priority from, such rights and all similar or equivalent rights or forms of protection which may now or in the future subsist in any part of the world; and

(h) **"Term"** has the meaning assigned to it in clause 7.1.

1.2 In this Framework Agreement, a reference to a "clause" shall mean a reference to a provision of this Framework Agreement.

1.3 The headings in this Framework Agreement are inserted for convenience only and shall not affect its construction.

1.4 In this Framework Agreement, a reference to a particular law is a reference to it as it is in force at the time of its application, therefore taking account of any amendment, extension, or re-enactment and includes any subordinate legislation for the time being in force made under it.

1.5 In this Framework Agreement, unless the context otherwise requires, words in the singular include the plural, and words in the plural, include the singular.

1.6 In this Framework Agreement, any phrase containing the term "include", "including", "in particular", or any similar expression will be construed as illustrative and will not limit the meaning or sense of the words preceding that term.

1.7 Any reference to this Framework Agreement herein shall be construed as a reference to this Framework Agreement inclusive of its annexes, which shall form an integral part hereof.

2. Purpose

The purpose of this Framework Agreement is to establish an understanding in terms of which the Parties shall undertake efforts to collaborate and work closely through the development and implementation of collaborative initiatives as outlined in clause 4.

3. Statement of Principles

The Parties shall undertake collaborative initiatives in the context of this Framework Agreement (hereinafter **"Collaborative Initiatives"**) in order to facilitate collaboration on research and analysis of cannabis.

4. Collaboration

4.1 The Parties shall endeavour to undertake Collaborative Initiatives in the following areas:

(a) Conduct analytical tests as required;

(b) Research collaboration in the areas of mutual interest;

(c) Exchange of academic materials which are made available by both Parties in the areas of mutual interest;

(d) Cooperative symposia, seminars, workshops and conferences in the areas of mutual interest;

(e) Undertaking other Collaborative Initiatives as may be jointly agreed in writing from time to time.

4.2 All Collaborative Initiatives, together with the specifics thereof, need to make reference to this Framework Agreement and be the subject of a separate and more specific agreement (hereinafter **"Specific Agreement"**) that shall be annexed to this Framework Agreement and formally endorsed in writing by and between the Parties.

4.3 For the avoidance of doubt, no financial obligations are being undertaken hereunder, and any such obligations will be agreed with respect to each Collaborative Initiative under the respective Specific Agreement.

5. Contact Persons, Coordination and Notices

5.1 The following shall be the contact persons (hereinafter the **"Contact Persons"**) for the purpose of ensuring that any Collaborative Initiatives are effectively coordinated and implemented by the Parties:

(a) **For the University**:
Contact: Prof. Emmanuel Sinagra or their delegate
Email: emmanuel.sinagra@um.edu.mt

(b) **For the ARUC:**
Contact: Dr Joey Reno Vella or their delegate or successor in title as
Email: joey-reno.vella@aruc.mt

or such other person as duly notified by one Party to the other from time to time, in accordance with the terms hereof.

5.2 A notice required to be given under this Framework Agreement (or a Specific Agreement made hereunder) or information or other documentation required to be sent under this Framework Agreement (or a Specific Agreement made hereunder) shall be validly given if sent by e-mail to the addresses stipulated in clause 5.1 or to any other email address as duly notified by one Party to the other from time to time.

5.3 For the purposes of this Framework Agreement, and any Specific Agreement made hereunder, any notice duly sent by email shall be deemed to be delivered and received up on transmission, provided that if it is sent after 17:00 hours on a working day (referring to the time in the place of receipt), or if it is sent during the weekend or on any public or national holiday in the place of receipt, it shall be deemed to have been received at 09:00 hours of the immediately-following working day in the place of receipt.

5.4 This clause 5 does not apply to the service of any proceedings or other documents in any legal action.

5.5 The Parties may also, if they agree, establish a standing committee to oversee this Framework Agreement.

6. Reporting Obligations

The University will prepare an annual report containing a description of the activities undertaken by the Parties further to this Framework Agreement, from the Effective Date up to termination of the same. The

annual report shall fall due on expiry of the thirteenth (13th) month from the Effective Date and annually thereafter.

7. Term and Termination, Review

7.1 This Framework Agreement shall come into force on the Effective Date and shall be valid for three (3) years therefrom (the **"Term"**). This notwithstanding, any one of the Parties can terminate this Framework Agreement by giving to the other Party three (3) months' notice in writing; provided that the following shall apply:

(a) Notwithstanding any termination of this Framework Agreement for any reason whatsoever, where the effective term of a Specific Agreement made hereunder subsists beyond the date of such termination, the provisions hereof shall continue to apply for the duration of the term of the relevant Specific Agreement, and this for the purposes of the fulfilment of the Parties' obligations in respect of the Specific Agreement; and

(b) Where a provision of this Framework Agreement (or a Specific Agreement made hereunder) specifically stipulates any obligation of a Party that is to subsist beyond the termination of this Framework Agreement, the relevant provision shall continue to have full force and effect for the period referred to therein.

7.2 Either Party may, at any given time, request that this Framework Agreement be reviewed, provided that any changes to this Framework Agreement shall not be effective or valid unless agreed to in writing by both Parties.

7.3 Termination of or changes to this Framework Agreement in the manner prescribed by this clause 7 shall not in any way affect or compromise any Collaborative Initiatives already underway between the Parties in furtherance of this Framework Agreement. The Parties shall therefore ensure to honour their obligations under any Collaborative Initiative, notwithstanding termination of this Framework Agreement for whatsoever reason.

8. Intellectual Property

8.1 Title in Foreground IP developed by a Party in execution of this Framework Agreement shall be vested in that Party, who shall grant the other Party a royalty-free, non-exclusive, irrevocable, world-wide license for the use of such Intellectual Property exclusively for non-commercial purposes.

8.2 Where Foreground IP is developed jointly by the Parties in execution of this Framework Agreement and title is therefore vested in them jointly, they shall grant each other a royalty-free, non-exclusive, irrevocable, world-wide license for the use of such Intellectual Property.

8.3 Each Party accepts and agrees that the Background IP of a Party shall be and remain at all times the sole and exclusive property of that Party. Access to and use of a Party's Background IP by the other Party shall be limited to what is necessary for that other Party to perform its obligations under this Framework Agreement and/or any Specific Agreement. Access by a Party to the other Party's Background IP for the use of Foreground IP owned by a Party shall be granted on a case-by-case basis and subject to fair and reasonable conditions.

8.4 Neither Party (hereinafter for the purposes of this clause, the **"First Party"**) is permitted to use the Intellectual Property of the other Party (hereinafter for the purposes of this clause, the **"Other Party"**) or to publish, alter or modify in any way the Intellectual Property of the Other Party except with the express written consent of that Other Party. To such effect, the First Party shall be required to notify the Other Party of its request in connection with the above, and the Other Party shall in turn notify the First Party of its decision in writing. For the avoidance of doubt, if no communication is received from the Other Party in reply to the said request, this shall not grant any rights whatsoever to the First Party in connection therewith, and only a written confirmation in writing by the Other Party shall entitle the First Party to affect such use, publication, alteration of modification.

8.5 This clause 8 shall indefinitely survive the expiration or termination of this Framework Agreement and is without prejudice to any specific conditions related to Intellectual Property that may be agreed upon by the Parties in a Specific Agreement.

8.6 Any use of the name of a Party, including but not limited to its trademark and logo under this Framework Agreement and/or any subsequent Specific Agreements will require the prior written permission of that Party. All joint promotional materials shall bear the official corporate logos of the Parties and shall be subject to editorial checks by the Parties, according to the Parties' internal procedures.

9. Confidentiality

9.1 Each Party shall keep confidential and shall not disclose or attempt to disclose to any person/entity any information of the other Party which has come to its knowledge during the operation of this Framework Agreement (**"Confidential Information"**), unless with the consent of the other Party and only to the extent it is required for the purposes of the fulfilment of its obligations hereunder or under a Specific Agreement; provided that nothing in this provision shall prevent a Party from disclosing Confidential Information to those persons/entities that have a duty of confidentiality towards that Party and which need to know the same for the purposes of the fulfilment of the terms of this Framework Agreement or of any Specific Agreement. All Confidential Information disclosed by one Party to the Party hereunder shall at all times remain the Property of the disclosing Party.

9.2 Each Party shall use the Confidential Information of the other Party only to the extent necessary for that Party to perform its obligations under this Framework Agreement and/or any Specific Agreement.

9.3 The restrictions contained in the foregoing sub-clauses shall not apply to Confidential Information which is:

(a) required by law to be disclosed;

(b) in the public domain without breach of the terms of this Framework Agreement;

(c) is already known by receiving Party at the time of disclosure under no obligation of confidentiality, as evidenced by written records;

(d) is subsequently developed by or on behalf of the receiving Party independently of the Confidential Information as evidenced by the receiving Party's written records; or

(e) becomes known from a third party who is under no obligation of confidentiality towards the Party to whom the Confidential Information belongs or relates, as evidenced by written records.

9.4 The duties of confidentiality and non-disclosure under this clause 9 shall survive the termination of this Framework Agreement for a period of five (5) years and are without prejudice to any specific conditions related to confidentiality and non-disclosure that may be agreed upon by the Parties in any Specific Agreement.

10. Liability and Warranties

10.1 Each Party (herein referred to as the **"indemnitor"**) shall irrevocably indemnify the other Party (herein referred to as the **"indemnitee"**) from and against any and all liability, loss, damage, harm, costs or expenses which the indemnitee may suffer, incur or sustain as a result of any breach of this Framework Agreement by the indemnitor or as a result of any unlawful act, wilful misconduct or negligence of the indemnitor or of any of its employees except to the extent that any such liability, loss, damage, harm, costs or expense arises from the indemnitee's breach of this Framework Agreement, unlawful act, wilful misconduct, or negligence.

10.2 Notwithstanding the provisions of clause 10.1, the following shall apply:

(a) Each Party shall not, under any circumstances, be liable towards the other Party for consequential, incidental, special or indirect damages arising out of or related exclusively to the purpose of this Framework Agreement. In particular, the University nor does it accept any liability for any use or reliance that the ARUC may make or place on the University's work, information or results;

(b) The University's aggregate liability hereunder in respect of each Specific Agreement shall be limited to the amounts received by it from the ARUC under the respective Specific Agreement; and

(c) The ARUC hereby fully acknowledges that the University cannot and does not provide any warranties on any work carried out hereunder or under any Specific Agreement made pursuant hereto.

10.3 Notwithstanding the preceding provisions of this clause 10, neither Party shall be liable for failure to perform any of its obligations under this Framework Agreement in the event that such failure arises by reason of Force Majeure. Provided that the Party affected by Force Majeure shall immediately notify the other Party of the Force Majeure and, if possible, take all reasonable steps to remedy or reduce the effects thereof on the implementation of this Framework Agreement.

11. Processing of Personal Data

The Parties agree that any personal data made known to them by virtue of this Framework Agreement shall be processed in accordance with the provisions of the General Data Protection Regulation (EU) 2016/679 (GDPR).

12. General

12.1 The Parties acknowledge that this Framework Agreement complements other negotiations, commitments and agreements that the Parties have already entered into.

12.2 Each Party acknowledges that it has not relied on any statement, promise or representation made or given by on or on behalf of the other Party in relation to the subject-matter of this Framework Agreement prior to the date hereof and which is not set out in this Framework Agreement.

12.3 This Framework Agreement (inclusive of its annexes) constitutes the entire agreement between the Parties with respect to the subject matter hereof and may only be modified by a written document signed by the Parties.

12.4 Neither Party shall assign, transfer, or subcontract any rights and obligations arising under this Framework Agreement and/or any Specific Agreement without the prior written consent of the other Party.

12.5 No modification, amendment or waiver of this Framework Agreement or provision hereof shall be binding upon any Party unless made in writing or confirmed in writing by their duly authorised representatives.

12.6 Should any part, term or provision of this Framework Agreement or any document required herein to be executed be declared invalid, void or unenforceable, all remaining parts, terms and provisions hereof shall remain in full force and effect and shall in no way be invalidated, impaired or affected thereby.

12.7 Each Party is an independent contractor under this Framework Agreement. The Parties agree that this Framework Agreement (or any Specific Agreement made hereunder) shall not create any partnership, agency or any other relationship under which either Party may be deemed responsible for the acts or omissions of the other Party and this Framework Agreement should not be construed so as to render the parties liable as partners or as creating a partnership or agency or any other similar relationship.

12.8 No failure or delay on the part of either Party hereto to exercise any right or remedy under this Framework Agreement shall be construed or operated as a waiver thereof nor shall any single or partial exercise of any right or remedy as the case may be. The rights and remedies provided in this Framework Agreement are cumulative and are not exclusive of any rights or remedies provided by law.

12.9 This Framework Agreement may be executed in any number of counterparts, each of which, when executed and delivered, shall be an original, and all the counterparts together shall constitute one and the same instrument.

12.10 Notwithstanding any other provision hereof, the signature of the Parties via an electronic signature (e.g., DocuSign) shall have the same force and effect as an original handwritten signature.

12.11 No person other than a Party to this Framework Agreement may enforce any of its terms.

12.12 This Framework Agreement and any dispute or claim arising out of or in connection with it or its subject-matter or formation (including non-contractual disputes or claims) shall be governed by and construed in accordance with the laws of Malta and any dispute arising from this Framework Agreement shall be referred to final and binding arbitration in terms of Part IV of the Arbitration Act (Chapter 387, Laws of Malta) under the applicable Arbitration Rules of the Malta Arbitration Centre.

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Signed –

For the ARUC

Dr Joey Reno Vella
Executive Chairperson
Date: 16/10/2024

For the University of Malta

L-Università ta' Malta
OFFICE OF THE RECTOR

Prof. Alfred J. Vella
Rector
Date: 20/9/2024

Prof. Emmanuel Sinagra
Head of Department of Chemistry

SPECIFIC AGREEMENT
(ANNEX 1 to Framework Agreement Ref 2024_295_UM_ARUC)
BETWEEN
THE UNIVERSITY OF MALTA
AND
THE AUTHORITY FOR THE RESPONSIBLE USE OF CANNABIS
(UM REF: 2024_295_UM_ARUC_ANX1)

This Specific Agreement (**"SA"**) is made by and between:

(1) On the first part, the University of Malta of Msida, MSD2080, Malta, (hereinafter the **"University"**) as established under the Education Act (Cap. 327, laws of Malta), represented hereon by its Rector, Prof. Alfred J. Vella (I.D. 919150M); and

(2) On the second part, the Authority for the Responsible Use of Cannabis, of Level 4, Onda Buisness Centre, ARUC, Aldo Moro Rd, Marsa MRS 9065 (hereinafter **"ARUC"**), represented hereon by its Chairperson, Dr Joey Reno Vella (I.D. 143095M).

Hereinafter also referred to each as a **"Party"** and collectively the **"Parties"**

PREAMBLE

(A) Whereas the Parties concluded a framework collaboration agreement (hereinafter the **"Framework Agreement"**) on the Effective Date, to which this SA is to be attached as **Annex 1** and this in pursuance of clause 4.2 of the Framework Agreement;

(B) Whereas the ARUC is desirous for the University to provide comparative analysis of pesticides in illicit and licenced cannabis inflorescence in Malta; and

(C) The Parties are therefore desirous to enter into this SA.

THE PARTIES THEREFORE HEREBY AGREE AND COVENANT THE FOLLOWING -

1. Interpretation

1.1 For the purposes of this SA, unless the context otherwise requires, the following terms shall have the following meanings:

(a) **"Effective Date"** shall have the meaning attributed to it in the Framework Agreement;

(b) **"Raw Data"** means primary data as collected directly from a source during the course of the Research Project and in such a format where such data has not been subjected to any analysis; and

(c) **"Study"** shall have the meaning attributed to it in section 2.1;

(d) **"Study Commencement Date"** means 1st October 2024; and

(e) **"Termination"** means termination of this SA for whatsoever reason.

1.2 Capitalised terms used in this SA that are not defined herein shall have the meaning attributed to them in the Framework Agreement.

1.3 The headings in this SA are inserted for convenience only and shall not affect its construction.

1.4 In this SA, a reference to a particular law is a reference to it as it is in force at the time of its application, therefore taking account of any amendment, extension, or re-enactment and includes any subordinate legislation for the time being in force made under it.

1.5 In this SA, unless the context otherwise requires, a reference to one gender shall include a reference to the other gender.

1.6 In this SA, unless the context otherwise requires, words in the singular include the plural and words in the plural include the singular.

1.7 In this SA, any phrase containing the term "include", "including", "in particular", or any similar expression will be construed as illustrative and will not limit the meaning or sense of the words preceding that term.

1.8 In this SA, any reference to a "clause" shall mean a reference to a provision in the Framework Agreement and a reference to a "section" shall mean a reference to a provision in this SA.

2. Specific Collaborative Initiatives

2.1 Further to clause 4 of the Framework Agreement, the Parties hereby wish to reinforce the purpose and principles of the Framework Agreement. By means of this SA, the Parties therefore wish to undertake a research study (the **"Study"**), by **October 2025**, consisting of comparative analysis for the presence of pesticides in illicit cannabis inflorescence and licenced cannabis inflorescence in Malta.

2.2 ARUC shall provide the anonymised licenced samples of the cannabis for the Study.

2.3 UM shall obtain the illicit samples of the cannabis for the Study by Inquiring Magistrate (subject to the approval, in every case, of the said Magistrate) and shall also obtain all necessary ethical approvals.

2.4 By the end of October 2025, the University shall provide ARUC the analysis and the report of the Study, together with recommendations based on the findings of the research undertaken by way of the Study (the **"Report"**). For the avoidance of doubt, the Report shall not include Raw Data or any personal data of individuals, which the University is obliged to process in accordance with the provisions of the Regulation (EU) 2016/679 (General Data Protection Regulation – the "**GDPR**").

2.5 The Parties agree that their respective contribution in the execution of this Agreement shall occur on *pro bono basis*; provided that each Party will bear its own costs in relation to the Study. For the avoidance of doubt, no funds or financial compensation of whatsoever nature shall be due by either Party to the other Party hereunder, unless specifically agreed to in writing by the Parties.

3. Intellectual Property

3.1 Further to clause 8 of the Framework Agreement, the Parties acknowledge that all Foreground IP arising from the Study shall be the exclusive property of the University, inclusive of all Raw Data.

3.2 Notwithstanding clause 8.1 of the Framework Agreement, the licence for use granted by the University to the ARUC of all Foreground IP shall be limited to the findings in the Report and shall specifically exclude Raw Data. Provided that while Raw Data shall at all times remain the property of the University, the University may grant the ARUC access to Raw Data for specific purposes, as may be required by the ARUC from time to time, and upon granting such access, the University shall stipulate any conditions in respect of the dissemination and publication of the same. The foregoing shall be subject to any data anonymization procedures as may be required at law for the protection of personal data. For the avoidance of doubt, the University shall use all Foreground IP as it sees fit, provided that the right for publication of the Foreground IP by ARUC shall occur solely following written consent by the University, unless otherwise agreed in writing between the Parties, which consent by the University shall not be unreasonably withheld.

3.3 The foregoing provisions of this section 3 shall not operate in such a way so as to impinge on work undertaken by University students further to the collaboration hereunder, which shall be governed by the University's Intellectual Property Policy as in force from time to time. For the

avoidance of doubt, University students shall in every case be entitled to publish findings hereunder for the purposes of the submission of their dissertation or thesis. This notwithstanding, the said students will be required to acknowledge the role of the ARUC in the collaboration hereunder.

4. Visibility

4.1 It is agreed that during all dissemination activities in connection with the above-mentioned Collaborative Initiatives, the following shall apply:

(a) The Parties shall promote each other's involvement;

(b) The ARUC and University logos shall feature in all printed and visual media; and

(c) Information has to be provided to the effect that the Study has been undertaken in collaboration between the ARUC and the University.

5. Term, Default Notice, and Termination

5.1 Subject to the terms of the Framework Agreement, this SA shall be deemed to have come into force on the Study Commencement Date and shall thereafter continue in full force until 31st October 2025 or until the complete discharge of all obligations undertaken by the Parties hereunder, whichever is the latest (the **"SA Term"**). This SA shall not be subject to automatic renewal.

5.2 Notwithstanding section 5.1, should a Party be in breach of its obligations under this SA (the **"Defaulting Party"**), the other Party (the **"Non-Defaulting Party"**) has the right to issue default notices in writing to the Defaulting Party, which shall be served in the manner stipulated in clause 5 of the Framework Agreement. A default notice serves to outline any shortfalls by the Defaulting Party, as perceived by Non-Defaulting Party, in respect of the terms of this SA, which shortfalls are to be rectified by the Defaulting Party from the date of receipt by it of the said default notice. In the event of failure by the Defaulting Party to rectify the default within the stipulated time-frame, the following shall apply:

(a) the Non-Defaulting Party may, at its absolute discretion, and *bona fide*, grant the Defaulting Party an additional one (1) month period to rectify the default by issuing a second default notice; or

(b) the Non-Defaulting Party may terminate this SA forthwith by written notification to the Defaulting Party.

5.3 In the event of termination of this SA pursuant to section 5.2 above, the following shall apply:

(a) Where the University is the Defaulting Party, the University shall be required, within one (1) month from the effective date of termination, to reimburse the ARUC, *pro rata* to the remaining term hereof, all pre-financing made to the University by the ARUC hereunder which has not be already expensed or committed for the purposes of the Study;

(b) Where the ARUC is the Defaulting Party, the ARUC shall be required, within one (1) month from the effective date of Termination, to reimburse the University for any expenses properly incurred or committed by it pursuant to section 2.3 and the University's obligations hereunder.

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Signed –

For the ARUC

Dr Joey Reno Vella
Executive Chairperson
Date: 16/10/2024

For the University of Malta

L-Università ta' Malta
OFFICE OF THE RECTOR

Prof. Alfred J. Vella
Rector
Date: 20/09/2024

Prof. Emmanuel Sinagra
Head of Department of Chemistry

Appendix 2

REDP Application ID

MED-2024-00486

Current Status

Approved

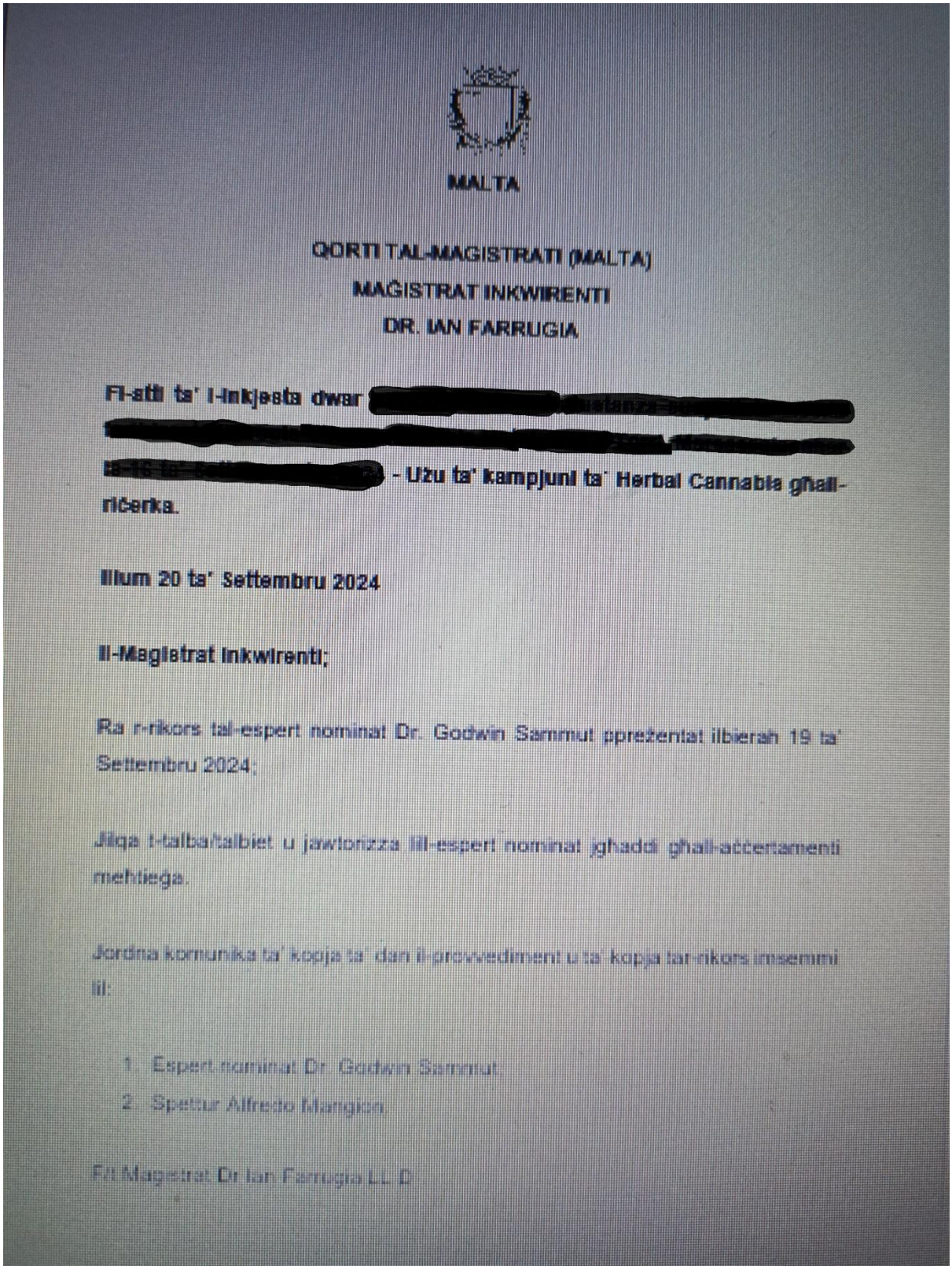
Audit Trail

04/12/2024 18:29
Vanessa Vella
Submitted by researcher for review

04/12/2024 18:30
Janet Mifsud
Form set to Endorsed by supervisor
endorsed

30/01/2025 09:38
FACULTY RESEARCH AND ETHICS COMMITTEE
Approved by F/REC

Appendix 3



MALTA

QORTI TAL-MAGISTRATI (MALTA)
MAĠISTRAT INKWIRENTI
DR. IAN FARRUGIA

Fl-atti ta' l-inkjesta dwar [redacted] - Użu ta' kampjuni ta' Herbal Cannabis għall-riċerka.

Illum 20 ta' Settembru 2024

Il-Magistrat Inkwirenti;

Ra r-rikors tal-espert nominat Dr. Godwin Sammut ppreżentat ilbieraħ 19 ta' Settembru 2024;

Jiliqa t-talba/talbiet u jawtorizza lill-espert nominat jgħaddi għall-aċċertamenti meħtieġa.

Jordna komunika ta' kopja ta' dan il-provvediment u ta' kopja tar-rikors insemmi lil:

1. Espert nominat Dr. Godwin Sammut
2. Spettur Alfredo Mangion

Fit Magistrat Dr Ian Farrugia LL.D.

MALTA

QORTI TAL-MAGISTRATI (MALTA)
MAĠISTRAT INKWIRENTI
DR. IAN FARRUGIA

Fl-atti ta' l-inkjesta dwar [redacted] - Użu ta' kampjuni ta' Herbal Cannabis għall-riċerka.

Illum 20 ta' Settembru 2024

Il-Magistrat Inkwirenti;

Ra r-rikors tal-espert nominat Dr. Godwin Sammut ippreżentat illbieraħ 19 ta' Settembru 2024;

Jilqa t-talba/talbiet u jawtorizza lill-espert nominat jgħaddi għall-aċċertamenti mehtieġa.

Jordna komunika ta' kopja ta' dan il-provvediment u ta' kopja tar-rikors insemmi lil:

1. Espert nominat Dr. Godwin Sammut;
2. Spettur Alfredo Mangion.

Fil-Magistrat Dr Ian Farrugia LL.D.

Appendix 4

L-Università ta' Malta

Forensic Analysis Laboratory
DEPARTMENT OF CHEMISTRY

18th October 2024

Dear Dr. Sammut,

With reference to your letter regarding the approval obtained from the Inquiring Magistrate for the use of cannabis samples for research purposes, as a member of the Forensic Analysis Laboratory, I would like to confirm my acceptance to act as the Intermediary for the research project to be carried out by Ms. Vanessa Vella.

In accordance with your request, I will ensure the anonymisation of the cannabis samples and maintain the appropriate storage conditions of the samples as per our standard laboratory procedures. This includes securing the samples in a monitored, alarmed and restricted-access storage room.

Thank you and kind regards,

SSchembri

Ms. Sarah Schembri B.Sc. (Hons), M.Sc.
Scientific Officer
Faculty of Science, Department of Chemistry, Porta Cabin Room 25, Carpark 3 +356 2340 6162

1

[APPLICATION NOTE]

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

A Multi-Residue Method for the Analysis of Pesticides in Cannabis Using UPLC-MS/MS and APGC-MS/MS to Meet Canadian Regulatory Requirements

David James,[1] Nichole Taylor,[1] and Christopher Stadey[2]
[1] Bloom Labs at Perennia Laboratories, Nova Scotia, Canada
[2] Waters Corporation, Mississauga, Canada

APPLICATION BENEFITS

- Sensitive and reproducible workflow for screening cannabis for the Canadian list of pesticides
- Minimal sample preparation followed by rapid UPLC and GC separations
- UPLC-MS/MS and APGC-MS/MS analysis of the same sample extracts on one tandem quadrupole mass spectrometer
- Analysis of large suites of pesticides in a single injection per chromatographic inlet
- Analysis of cannabis at legislatively relevant levels

WATERS SOLUTIONS

ACQUITY™ UPLC™ H-Class PLUS System

ACQUITY UPLC BEH Column

QuEChERS Dispersive Solid Phase Extraction (d-SPE)

Xevo™ TQ-S micro Tandem Quadrupole Mass Spectrometer

MassLynx™ Software v4.2

Atmospheric Pressure Gas Chromatography (APGC™)

KEYWORDS

Canada, cannabis, pesticides, ACQUITY UPLC, Xevo TQ-S micro, APGC, MassLynx 4.2 Software, hemp, marijuana

INTRODUCTION

Health Canada requires mandatory testing for the presence of pesticide residues in cannabis before the product can be sold to consumers.[1,2] The regulations are present to ensure the highest safety and quality standards possible when it comes to the supply of cannabis for medical or recreational use. To adhere to testing requirements, licensed cannabis producers must demonstrate that no unauthorized pesticides have been used on their products and that there is no contamination of the products within the limits set out by Health Canada. Currently, the target list consists of 96 pesticides, with limits of quantitation as low as 20 ppb in dried cannabis. Tandem mass spectrometry is a sensitive and selective technique. When coupled with both gas (GC) and liquid chromatography (LC), it provides a comprehensive analysis for a wide range of pesticide residues with sufficient sensitivity to meet the Health Canada regulations. The advantage of ultraperformance liquid chromatography (UPLC) coupled with tandem quadrupole mass spectrometry (MS/MS) for multi-residue pesticide analysis is widely reported.[3] More recently, the use of GC-MS/MS operated at atmospheric pressure (APGC) has been shown to offer significant improvements in performance over EI for challenging pesticides, in terms of selectivity, specificity and speed of analysis.[4,5] Regulations for cannabis testing will most likely evolve and possibly become even more rigorous. The use of both LC-MS/MS and GC-MS/MS ensures system flexibility that can be adapted in the event that more pesticides are regulated.

In this Application Note, we present the use of a simple sample extraction and d-SPE (dispersive solid phase extraction) cleanup where the resulting extract is analyzed by UPLC-MS/MS or APGC-MS/MS. A single workflow for the multi-residue analysis of pesticides in cannabis is demonstrated. Utilizing the universal source of the Waters™ Xevo TQ-S micro allows for LC and GC analyses to be completed on the same tandem quadrupole MS instrument. The performance of the method will be highlighted in terms of sensitivity, recovery, and linearity for both LC and GC analysis.

EXPERIMENTAL CONDITIONS

Materials and reagents

1. Pesticide standards

Pesticide analytical standards were purchased from LGC Standards. Mix 1 consisted of 35 pesticides at 50 ppm in acetonitrile, Mix 2 consisted of 45 pesticides at 100 ppm in acetonitrile, and Mix 3 consisted of 14 pesticides at 100 ppm in toluene. Dimethomorph was also purchased from LGC Standards at 10 ppm in acetonitrile. Benzovindiflupyr was purchased separately from Chem Service at 100 ppm in methylene chloride solution. All 96 pesticides were combined in a 1 ppm stock solution of each.

2. Reagents

LC-MS-grade methanol, LC-MS-grade acetonitrile, and RO (reverse osmosis) water were all purchased from Fisher Scientific and were used as received. Formic acid was purchased from Waters (p/n: 186006691) and was used as received.

3. Miscellaneous

Helium and argon gases were obtained from Air Liquide. A Thermo Fisher Scientific vortex (0-3200 rpm), a Fisher Scientific accuSpin 400 centrifuge, a Fisher Scientific 60L gravity oven, and a Mettler Toledo AE50 analytical balance (0.1 mg) were all used in the sample preparation procedure.

Sample preparation

Preparation

The representative samples were dipped in liquid nitrogen and frozen before grinding. After freezing, but before grinding, all stems and seeds were removed from the sample. The ground sample was equilibrated to room temperature. Several 0.5-g portions of ground cannabis were weighed. The initial mass was recorded. To ensure that all the liquid nitrogen had evaporated, and an accurate sample mass was obtained, the sample sat on the scale until there was <1 mg change in mass over a 10-minute period.

Pesticide extraction

The 0.5-g samples of ground cannabis were placed in a 10-mL centrifuge tube and 5 mL of LC-MS/MS-grade acetonitrile was added. The sample was then vortexed for five minutes followed by centrifugation at 5000 rpm for five minutes. One milliliter of the supernatant was removed and used in the clean-up step.

Clean-up

One milliliter of the supernatant from the pesticide extraction was placed in a d-SPE cartridge (150 mg $MgSO_4$, 50 mg PSA, 50 mg C_{18}, and 7.5 mg graphitized carbon black). The cartridge was shaken for one minute and centrifuged for five minutes at 5000 rpm. The resulting cannabis extracts were directly pipetted into clean 2-mL vials in preparation for analysis by LC-MS/MS and APGC-MS/MS.

Calibration preparation

Calibration standards were made using a stock solution of 96 pesticides (1 ppm stock). Matrix-matched calibrations were used to ensure that the signals obtained in the analysis were representative of what the signal would be in cannabis samples. Standards ranging from 1–6400 ppb were made to accommodate the different ionization efficiencies of all analytes. Pesticides with low detection limits used the lower concentration standards and the pesticides with higher detection limits used higher concentration standards for their calibration curves.

Instrumentation and software

A Waters ACQUITY UPLC H-Class PLUS System coupled with a Waters Xevo TQ-S micro Tandem Quadrupole Mass Spectrometer (MS/MS) with electrospray as the ionization mode was used to carry out the analysis of 84 of the pesticides by LC-MS/MS (see Appendix A). An Agilent 7890B gas chromatograph (GC) coupled with a Waters Xevo TQ-S micro Tandem Quadrupole Mass Spectrometer was used to carry out the analysis of the remaining 12 pesticides with APGC as the ionization mode. A nitrogen generator (Peak Scientific) was used as the source of the N_2 gas. MassLynx MS Software v4.2 was used for data acquisition and processing for both LC-MS/MS and GC-MS/MS methods.

Method conditions

UPLC method

Separation mode:	Gradient
Column:	ACQUITY UPLC BEH C_{18}, 1.7 µm, 2.1 × 100 mm
Solvent A:	Methanol
Solvent B:	Water
Solvent C:	2% formic acid in RO water
Flow rate:	0.500 mL/min
Column temp.:	60 °C
Sample temp.:	10 °C
Injection volume:	2 µL

Gradient conditions

Time (min)	%A	%B	%C
0.00	2%	93%	5
8.00	95%	0%	5
9.00	95%	0%	5
9.10	2%	93%	5
12.00	2%	93%	5

Xevo TQ-S micro conditions

Ionization mode:	ESI+
Capillary voltage:	1.2 kV
Cone voltage:	30 V
Collision energy:	Various eV (see Appendix)
Desolvation temp.:	600 °C
Source temp.:	150 °C
Desolvation gas flow:	1000 L/hr
Cone gas:	50 L/hr

All MS/MS parameters including precursor ion (*m/z*), product ion (*m/z*), cone voltage (V), and collision energy (CE) for the 84 pesticides analyzed by LC-MS/MS can be found in Appendix A.

GC method

GC system:	Agilent 7890B
Column:	Agilent DB-5 MS (30 m × 0.250 mm × 0.25 µm)
Carrier gas:	Helium
Flow rate:	2 mL/min
Injection type:	Pulsed splitless
Injector temp.:	280 °C
Equilibration time:	1.5 min
Injection volume:	2 µL
Makeup gas:	Nitrogen at 350 mL/min

GC oven program

Rate (°C/min)	Temp. (°C)	Hold (min)
–	60	0.45
18.70	320	3.65

Total run time = 18.0 min

GC-MS/MS parameters

MS system:	Xevo TQ-S micro
Ionization mode:	APGC+
Corona:	2.0 µA
Transfer line temp.:	320 °C
Source temp.:	150 °C
Solvent delay:	3.5 min
Acquisition mode:	MRM

All MS/MS parameters including precursor ion (*m/z*), product ion (*m/z*), cone voltage (V), and collision energy (CE) for the 12 pesticides analyzed by GC-MS/MS can be found in Appendix B.

Method development and optimization

LC-MS/MS and GC-MS/MS data analysis

The UPLC and GC parameters were optimized to ensure adequate separation of pesticide peaks with reduced background noise and optimum peak shapes. Upon completion of the sample run, a "multiplier" must be input into the UPLC and GC to account for the dilutions and sample mass weighed. The following formula is used to calculate the multiplier:

$$Multiplier\% = \frac{Vextraction}{Mass} \times 100$$

where Vextraction is the total volume of the extract used (5 mL) and Mass is the mass of the dried cannabis weighed for the extraction (0.5 g). This will convert all results in ppb in cannabis (µg of pesticide/g of cannabis).

Validation of method (sample spiking and recovery)

To validate the method, sample spikes were performed on ground cannabis prior to the extraction and clean-up. The pesticide mixes were spiked into 0.5 g of fresh ground "pesticide-free" cannabis samples. Extraction and clean-up were performed resulting in 2000, 1000, 500, 250, 100, 50, 25, 10, 5, and 2 ppb spiked samples. After applying the multiplier (described above), the concentration of the pesticides mentioned above are 10x higher in the cannabis sample.

The spiking procedure was performed at nine different spike concentrations for each pesticide to obtain the limit of quantification (LOQ) for each individual pesticide. Once the LOQ was established, three spikes of each analyte at their respective LOQ were performed to obtain average spike recoveries and relative standard deviations (RSD) for each pesticide individually.

As shown in Table 1, spike recoveries for all pesticides at their LOQs averaged between 81.7% and 117.6%. The acceptable % recovery limits for method validation are between 70% and 120%. Low relative standard deviations (RSD) were also reported for all 96 spike recoveries (all <20%). The acceptable RSD for method validation is <20%.

It should be noted that the recovery for daminozide is determined separately since it is strongly retained by the PSA sorbent. For spike recoveries and to test for the presence of daminozide in cannabis samples, a separate LC-MS/MS run is performed following sample extraction but before clean-up.

Limits of quantification (LOQs)

The LOQs were calculated for all 96 pesticides. To determine the LOQs, pesticide-free cannabis samples were spiked with various concentrations of standards ranging from 1–2000 ppb. Sample spike recoveries between 80% and 120% were deemed acceptable. Once the lowest acceptable spike recoveries (lowest concentrated spike) were determined for each pesticide, three separate runs were performed and only after all three runs fell within the acceptable limits was the LOQ established. As shown in Table 2, all LOQ values are within Health Canada's limits.

Table 1. Spike recoveries for the 96 pesticides in dried cannabis sample.

#	Pesticide (conc. in ppb)	Average spike recovery (%)	RSD (%)	Method
1	Abamectin (20)	97.4	10.7	LC
2	Acephate (20)	91.5	2.5	LC
3	Acequinocyl (100)	98.5	3	LC
4	Acetamiprid (50)	81.7	0.5	LC
5	Aldicarb (50)	105.7	2.9	LC
6	Allethrin (100)	84.6	2.5	LC
7	Azadirechtin (50)	99.5	5.3	LC
8	Azoxystrobin (20)	91.3	14.9	LC
9	Benzovindiflupyr (20)	110.8	8.8	LC
10	Bifenzate (20)	87.3	3	LC
11	Bifenthrin (250)	93.5	14.5	GC
12	Boscalid (20)	93.8	16.1	LC
13	Buprofenzin (20)	82.8	3.3	LC
14	Carbaryl (20)	93.6	1.9	LC
15	Carbofuran (20)	86.5	5.1	LC
16	Chlorantraniliprole (20)	90.7	16.7	LC
17	Chlorphenapyr (20)	97.3	14.3	LC
18	Chlorpyrifos (20)	117.6	3.2	LC
19	Clofentezine (20)	111.8	1.3	LC
20	Clothiandin (20)	87.7	5.6	LC
21	Coumaphos (20)	90.8	4.2	LC
22	Cyantranilipole (20)	84.4	2.9	LC
23	Cyfluthrin (250)	109.9	13.4	GC
24	Cypermethrin (100)	98.4	16.1	LC
25	Cyprodinil (20)	82	2.8	LC
26	Daminozide (100)	82	3.2	LC
27	Deltamethrin (100)	111.9	7.2	GC
28	Diazinon (20)	88.4	2.6	LC
29	Dichlorvos (20)	87.3	4.5	LC
30	Dimethoate (20)	82.2	0.4	LC
31	Dimethomorph (20)	98.3	4.9	LC
32	Dinotefuran (20)	85.8	5	LC
33	Dodemorph (20)	87.4	8.4	LC
34	Endosulfan-alpha (500)	107.6	12.4	GC
35	Endosulfan-beta (500)	99.1	11.8	GC
36	Endosulfan-sulfate (500)	89.3	0.9	LC
37	Ethoprophos (20)	83.6	2.2	LC
38	Etofenprox (20)	90	6.7	LC
39	Etoxazole (20)	81.7	0.4	LC
40	Etridiazole (500)	85.2	1.5	LC
41	Fenoxycarb (20)	91.9	12	LC
42	Fenpyroximate (20)	86.6	4.4	LC
43	Fensulfothion (20)	89.1	1.8	LC
44	Fenthion (50)	102.2	5.5	LC
45	Fenvalerate (1000)	87.5	9.7	GC
46	Fipronil (50)	98.9	19.9	LC
47	Flonicamid (20)	88.9	1.9	LC
48	Fludioxinil (20)	96.5	16.8	GC
49	Fluopyram (20)	85.9	2.5	LC
50	Hexythiazox (250)	103.6	10	LC
51	Imazalil (50)	83.3	1.4	LC
52	Imidacloprid (20)	86.4	0.5	LC
53	Iprodione (20)	115.1	6.5	LC
54	Kinoprene (5000)	96	4.8	GC
55	Kresoxim-methyl (20)	117.1	1.9	LC
56	Malathion (20)	83.8	1.7	LC
57	Metalaxyl (20)	91.1	2.8	LC
58	Methiocarb (20)	106.9	8.2	LC
59	Methomyl (20)	82.2	2.5	LC
60	Methoprene (100)	100.5	3.3	LC
61	Methyl parathion (100)	101.2	11.3	LC
62	Mevinphos I (20)	82	0.5	LC
63	MGK-264 (500)	100.2	7.6	GC
64	Myclobutanil (20)	98.5	3.1	LC
65	Naled (20)	91.1	9.3	LC
66	Novaluron (50)	107.4	0.8	LC
67	Oxamyl (50)	84.7	3.9	LC
68	Paclobutrazol (20)	85.4	6.3	LC
69	Permethrin (100)	89.1	6.4	GC
70	Phenothrin (20)	92.4	17.1	LC
71	Phosmet (50)	106.3	12.1	LC
72	Piperonyl butoxide (50)	92.7	2.6	LC
73	Pirimicarb (20)	84.5	2.1	LC
74	Prallethrin (50)	109.5	5.4	LC
75	Propiconazole (100)	102.9	5.1	LC
76	Propoxur (20)	109.3	1.4	LC
77	Pyraclostrobin (20)	83.9	3.1	LC
78	Pyrethrin II (20)	109.1	4.6	LC
79	Pyridaben (20)	114.2	1.1	LC
80	Quintozene (250)	98.5	11.7	GC
81	Resmethrin (100)	100.8	6	GC
82	Spinetoram (50)	102.5	7.2	LC
83	Spinosad A (100)	95.4	7.7	LC
84	Spirodiclofen (20)	102	15.6	LC
85	Spiromesifen (100)	82.5	2.9	LC
86	Spirotetramat (20)	88.6	3.7	LC
87	Spiroxamine II (20)	90.1	6.7	LC
88	Tebuconazole (20)	87.6	1.1	LC
89	Tebufenozide (20)	104.5	9	LC
90	Teflubenzuron (50)	94.4	5.2	LC
91	Tetrachlorvinphos (20)	92.9	9.8	LC
92	Tetramethrin (20)	82	5	LC
93	Thiacloprid (20)	88.1	0.4	LC
94	Thiamethoxam (20)	84.6	0.7	LC
95	Thiophanate-methyl (50)	104.3	11.4	LC
96	Trifloxystrobin (20)	107.2	3.1	LC

Table 2. Experimental limits of detection for all 96 pesticides using the LC-MS/MS and GC-MS/MS methods.

#	Analyte	LOQ in cannabis (ppb)	LOQ Health Canada (ppb)	Method
1	Abamectin	20	N/A	LC-MS/MS
2	Acephate	20	20	LC-MS/MS
3	Acetamiprid	50	100	LC-MS/MS
4	Acequinocyl	100	N/A	LC-MS/MS
5	Aldicarb	50	1000	LC-MS/MS
6	Allethrin	100	200	LC-MS/MS
7	Azadirachtin	50	1000	LC-MS/MS
8	Azoxystrobin	20	20	LC-MS/MS
9	Benzovindiflupyr	20	20	LC-MS/MS
10	Bifenazate	20	20	LC-MS/MS
11	Bifenthrin	250	N/A	GC-MS/MS
12	Boscalid	20	20	LC-MS/MS
13	Buprofezin	20	20	LC-MS/MS
14	Carbaryl	20	50	LC-MS/MS
15	Carbofuran	20	20	LC-MS/MS
16	Chlorantraniliprole	20	N/A	LC-MS/MS
17	Chlorphenapyr	20	N/A	LC-MS/MS
18	Chlorpyrifos	20	N/A	LC-MS/MS
19	Clofentezine	20	20	LC-MS/MS
20	Clothianidin	20	50	LC-MS/MS
21	Coumaphos	20	20	LC-MS/MS
22	Cyantranilipole	20	N/A	LC-MS/MS
23	Cyfluthrin	250	N/A	GC-MS/MS
24	Cypermethrin	100	N/A	LC-MS/MS
25	Cyprodinil	20	N/A	LC-MS/MS
26	Daminozide	100	N/A	LC-MS/MS
27	Deltamethrin	100	N/A	GC-MS/MS
28	Diazinon	100	N/A	LC-MS/MS
29	Dichlorvos	20	100	LC-MS/MS
30	Dimethoate	20	20	LC-MS/MS
31	Dimethomorph	20	N/A	LC-MS/MS
32	Dinotefuran	20	100	LC-MS/MS
33	Dodemorph	20	N/A	LC-MS/MS
34	Endosulfan-alpha	500	N/A	GC-MS/MS
35	Endosulfan-beta	500	N/A	GC-MS/MS
36	Endosulfan sulfate	500	N/A	LC-MS/MS
37	Ethoprophos	20	20	LC-MS/MS
38	Etofenprox	20	N/A	LC-MS/MS
39	Etoxazole	20	20	LC-MS/MS
40	Etridiazol	20	N/A	LC-MS/MS
41	Fenoxycarb	20	20	LC-MS/MS
42	Fenpyroximate	20	20	LC-MS/MS
43	Fensulfothion	20	20	LC-MS/MS
44	Fenthion	50	N/A	LC-MS/MS
45	Fenvalerate	1000	N/A	GC-MS/MS
46	Fipronil	50	60	LC-MS/MS
47	Flonicamid	20	50	LC-MS/MS
48	Fludioxonil	20	20	GC-MS/MS

#	Analyte	LOQ in cannabis (ppb)	LOQ Health Canada (ppb)	Method
49	Fluopyram	20	20	LC-MS/MS
50	Hexythiazox	250	N/A	LC-MS/MS
51	Imazalil	50	N/A	LC-MS/MS
52	Imidacloprid	20	20	LC-MS/MS
53	Iprodione	20	1000	LC-MS/MS
54	Kinoprene	5000	N/A	GC-MS/MS
55	Kresoxim-methyl	20	N/A	LC-MS/MS
56	Malathion	20	20	LC-MS/MS
57	Metalaxyl	20	20	LC-MS/MS
58	Methiocarb	20	20	LC-MS/MS
59	Methomyl	20	50	LC-MS/MS
60	Methoprene I	100	N/A	LC-MS/MS
61	Methyl parathion	100	N/A	LC-MS/MS
62	Mevinphos I	20	50	LC-MS/MS
63	MGK-264	5000	N/A	GC-MS/MS
64	Myclobutanil	20	20	LC-MS/MS
65	Naled	20	N/A	LC-MS/MS
66	Novaluron	50	50	LC-MS/MS
67	Oxamyl	50	3000	LC-MS/MS
68	Paclobutrazol	20	20	LC-MS/MS
69	Permethrin	1000	N/A	GC-MS/MS
70	Phenothrin	20	50	LC-MS/MS
71	Phosmet	50	N/A	LC-MS/MS
72	Piperonyl butoxide	50	N/A	LC-MS/MS
73	Pirimicarb	20	20	LC-MS/MS
74	Prallethrin	50	N/A	LC-MS/MS
75	Propiconazole	100	N/A	LC-MS/MS
76	Propoxur	20	20	LC-MS/MS
77	Pyraclostrobin	20	20	LC-MS/MS
78	Pyrethrins II	20	50	LC-MS/MS
79	Pyridaben	20	50	LC-MS/MS
80	Quintozene	250	N/A	GC-MS/MS
81	Resmethrin	100	100	GC-MS/MS
82	Spinetoram	50	N/A	LC-MS/MS
83	Spinosad A	100	N/A	LC-MS/MS
84	Spirodiclofen	20	N/A	LC-MS/MS
85	Spiromesifen	100	3000	LC-MS/MS
86	Spirotetramat	20	20	LC-MS/MS
87	Spiroxamine (II)	20	N/A	LC-MS/MS
88	Tebuconazole	20	N/A	LC-MS/MS
89	Tebufenozide	20	20	LC-MS/MS
90	Teflubenzuron	50	50	LC-MS/MS
91	Tetrachlorvinphos	20	20	LC-MS/MS
92	Tetramethrin	20	100	LC-MS/MS
93	Thiacloprid	20	20	LC-MS/MS
94	Thiamethoxam	20	20	LC-MS/MS
95	Thiophanate-methyl	50	50	LC-MS/MS
96	Trifloxystrobin	20	20	LC-MS/MS

RESULTS AND DISCUSSION

PESTICIDES ANALYSIS BY UPLC-MS/MS

Using the LC-MS/MS method, 84 pesticides were analyzed. The compounds analyzed by LC-MS/MS and the parameters used are listed in Table 2 and Appendix A. Representative MRM chromatograms for the pesticides acetamiprid (50 ppb), cyprodinil (25 ppb), fenoxycarb (25 ppb), and tetrachlorvinphos (25 ppb) in a pesticide-free extracted cannabis matrix are shown in Figure 1.

Matrix-matched calibration curves were generated using pesticide-free extracted cannabis. An example of the calibration curves for the pesticides acetamiprid, cyprodinil, fenoxycarb, and tetrachlorvinphos are shown in Figure 2. Linear calibration curves (R^2>0.990) for all pesticides were obtained over the range tested as shown in the figure.

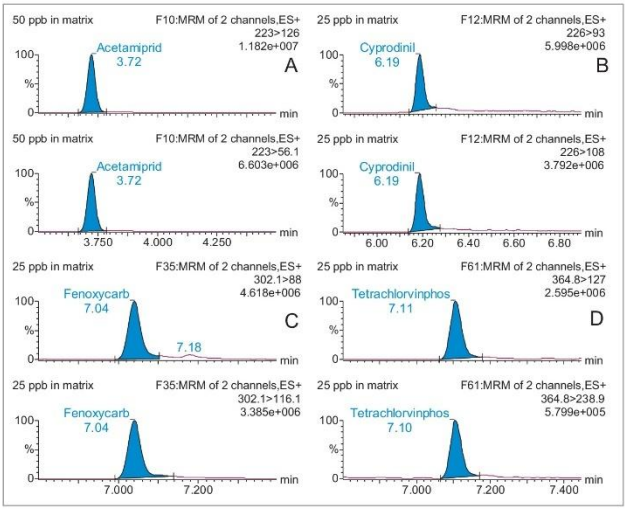


Figure 1. Representative MRM chromatograms showing the primary quantification and the secondary qualifier transition for acetamiprid (A, 50 ppb), cyprodinil (B, 25 ppb), fenoxycarb (C, 25 ppb), and tetrachlorvinphos (D, 25 ppb) in pesticide-free cannabis extracted using the sample preparation protocol reported.

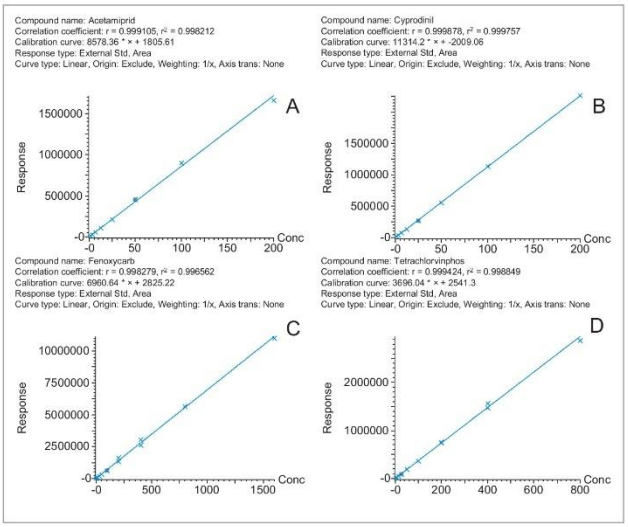


Figure 2. Representative examples of calibration curves for acetamiprid (A, 0.78–200 ppb), cyprodinil (B, 0.78–200 ppb), fenoxycarb (C, 0.78–1500 ppb), and tetrachlorvinphos (D, 0.78–800 ppb), demonstrating linearity over the ranges tested for these compounds.

PESTICIDES ANALYSIS BY GC-MS/MS

Analysis of pesticide residues in cannabis also required the use of GC-MS/MS to meet the Canadian pesticide regulations. A complete list of compounds analyzed by GC-MS/MS and the parameters used is provided in Table 2 and Appendix B. Example chromatograms for endosulfan alpha and fenvalerate are shown in Figure 3.

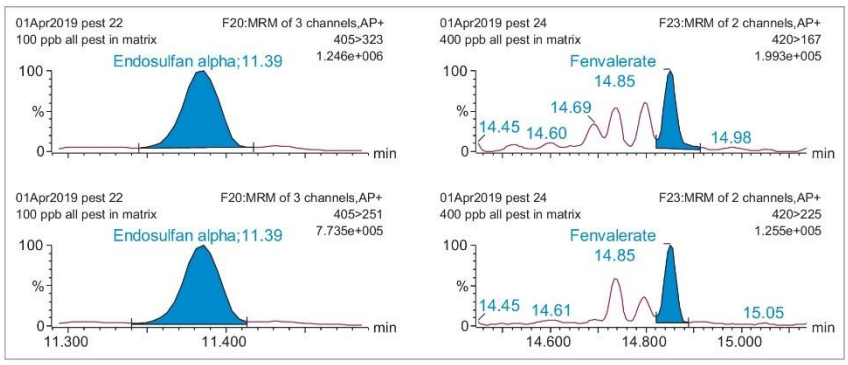


Figure 3. Representative MRM chromatograms showing the primary quantification and the secondary qualifier transition for endosulfan alpha (100 ppb) and fenvalerate at a level and 400 ppb (ng/g) in pesticide-free cannabis extracted using the sample preparation protocol reported.

An example of the calibration curves for the pesticides endosulfan alpha and fenvalerate are shown in Figure 4. Linear calibration curves (R^2>0.990) for both pesticides were obtained over the range tested, as shown in the figure.

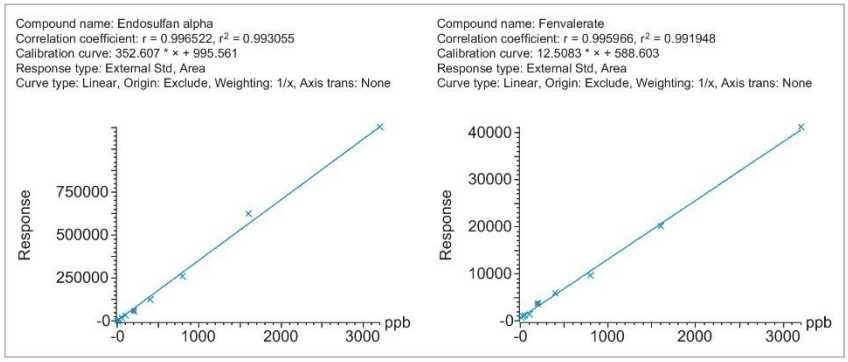


Figure 4. Representative examples of calibration curves for endosulfan alpha and fenvalerate demonstrating linearity over the ranges tested for these compounds.

CONCLUSIONS

The simple sample extraction and d-SPE clean-up method followed by UPLC-MS/MS and GC-MS/MS analysis provides a rapid, sensitive, and robust workflow for the determination of the Canadian pesticide list in challenging cannabis matrices. Complex multi-residue pesticide analysis in a cannabis matrix was demonstrated using both UPLC and APGC analysis on the same tandem quadrupole instrument (Xevo TQ-S micro) with detection at the maximum action levels for each of the 96 pesticides in the Canadian pesticide list. Having the flexibility of universal source architecture to provide access to both UPLC-MS/MS and GC-MS/MS on the same instrument, allows for an increase of laboratory efficiency, while maintaining required sensitivity and repeatability. This method meets the action levels for the Canadian pesticide list and mycotoxins in cannabis matrices.

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Appendix A.

MS/MS parameters for pesticides using UPLC.

	Analyte	Retention time (min)	MW (g/mol)	Precursor (*m/z*)	Product (*m/z*)	CV	CE	Health Canada detection limit (ppb)
1	Abamectin	8.65	873.09	895.46	182.9	76	48	N/A
				895.46	327.02	76	52	
				895.46	751.22	76	44	
2	Acephate	1.88	183.2	183.9	94.6	20	25	20
				183.9	142.8	20	10	
3	Acetamiprid	3.7	222.67	223	56.1	30	15	100
				223	126	30	20	
4	Acequinocyl	9.4	384.51	343.2	115	35	40	N/A
				343.2	189.1	35	20	
5	Aldicarb	4.37	190.261	213.1	89.1	35	20	1000
				213.1	116.1	35	11	
6	Allethrin	7.92	302.4079	303.03	90.95	20	44	200
				303.03	134.94	20	10	
7	Azadiractin	5.6	720.721	703.2	567	10	10	1000
				703.2	585	10	10	
				703.2	685	10	10	
8	Azoxystrobin	6.29	403.394	404.1	328.9	15	30	20
				404.1	372	15	16	
9	Benzovindiflupyr	7.25	398.235	398	322	20	18	20
				398	342	20	10	
10	Bifenazate	6.72	300.3523	301.1	170	25	20	20
				301.1	198	25	10	
11	Boscalid	6.46	343.2067	342.9	139.9	25	20	20
				342.9	307	25	45	
12	Buprofezin	7.77	305.44	306.1	115.9	20	16	20
				306.1	201	20	12	
13	Carbaryl	5.23	201.22	202.1	127	30	22	50
				202.1	145	30	28	
14	Carbofuran	5.08	221.256	222.11	123	5	20	20
				222.11	165.1	5	10	
15	Chlorantraniliprole	6.08	483.15	481.6	283.9	15	23	N/A
				481.6	450.9	15	25	
16	Chlorfenapyr	7.5	407.6	409	59	58	16	N/A
				409	379	58	10	
17	Chlorpyrifos	8.04	350.59	350.1	97	25	33	N/A
				350.1	197.9	25	19	
18	Clofentezine	7.37	303.146	303	102	20	35	20
				303	138	20	15	
19	Clothianidin	3.3	249.678	250	132	25	15	50
				250	169	25	10	
20	Coumaphos	7.2	362.77	363	289	32	24	20
				363	307	32	16	
21	Cyantranilipole	5.49	473.715	475	286	20	13	N/A
				475	444	20	17	
22	Cypermethrin	7	416.3	415.8	375.12	6	4	N/A
				415.8	225.12	6	20	
23	Cyprodinil	6.22	225.29	226	93	5	35	N/A
				226	108	5	25	
24	Daminozide	0.9	160.171	161	61	24	12	N/A
				161	143	24	12	
25	Diazinon	7.27	304.25	305.1	96.9	20	35	N/A
				305.1	169	20	22	
26	Dichlorvos	4.92	220.98	221	79	23	34	100
				221	109	23	22	
27	Dimethoate	3.58	229.26	230	124.8	20	22	20
				230	198.8	20	10	
28	Dimethomorph	6.41	387.9	388.1	165	15	30	N/A
				388.1	300.9	15	20	
29	Dinotefuran	2.22	202.214	203	113	15	10	100
				203	129	15	10	
30	Dodemorph	5.6	281.48	282.1	98	40	28	N/A
				282.1	116	40	21	
31	Endofulfan Sulfate	6.59	422.903	423.04	124.97	14	34	N/A
				423.04	204.12	14	24	
32	Ethoprophos	6.87	242.332	242.97	97	18	31	20
				242.97	130.95	18	20	
33	Etofenprox	8.83	376.496	394.3	106.9	20	43	N/A
				394.3	177	20	15	

	Analyte	Retention time (min)	MW (g/mol)	Precursor (m/z)	Product (m/z)	CV	CE	Health Canada detection limit (ppb)
34	Etoxazole	8.2	359.417	360.2	57.2	60	25	20
				360.2	141.1	60	25	
35	Etridiazol	4.21	247.518	247.02	148.99	10	12	N/A
				247.02	205.97	10	12	
36	Fenoxycarb	7.03	301.34	302.1	88	10	20	20
				302.1	116.1	10	11	
37	Fenpyroximate	8.31	421.497	422.2	138.1	5	30	20
				422.2	366.1	5	20	
38	Fensulfothion	5.83	308.347	309	157.1	36	25	20
				309	173.1	36	22	
39	Fenthion	7.12	278.33	279	104.9	25	25	N/A
				279	168.9	25	18	
40	Fipronil	7.03	437.15	453.9	250	42	25	60
				453.9	330	42	13	
				453.9	368.1	5	25	
41	Flonicamid	2.74	229.1586	230.1	148.08	35	25	50
				230.1	203.7	35	15	
42	Fluopyram	6.78	396.717	397	173.2	30	41	20
				397	208.1	30	35	
43	Hexythiazox	8.11	352.877	353	168.1	10	25	N/A
				353	228.1	10	15	
44	Imazalil	5.25	297.18	297	69	25	20	N/A
				297	159	25	20	
45	Imidacloprid	3.36	255.661	256.1	174.9	25	20	20
				256.1	209	25	12	
46	Iprodione	6.99	330.165	330	245	35	15	1000
				330	288.1	35	15	
47	Kresoxim-methyl	7.13	313.353	314.2	115.9	30	12	N/A
				314.2	131	30	25	
48	Malathion	6.48	330.358	331	98.9	30	25	20
				331	126.9	30	12	
49	Metalaxyl	5.88	279.33	280.1	192.1	10	20	20
				280.1	220.1	10	15	
50	Methiocarb	6.29	225.306	226	121	25	20	20
				226	169	25	10	
51	Methomyl	2.74	162.2101	162.9	88	15	10	50
				162.9	105.9	15	10	
52	Methoprene	6.05	310.48	312.41	72.08	82	38	N/A
				312.41	81.06	82	38	
53	Methyl parathion	6.11	263.204	264	125.1	38	18	N/A
				264	232.1	38	14	
54	Mevinphos	3.75	224.1483	225.1	127.1	15	15	50
				225.1	193.1	15	10	
55	Myclobutanil	6.62	288.779	289.1	70.2	25	15	20
				289.1	125.1	25	30	
56	Naled	5.94	380.778	382.8	109	30	27	N/A
				382.8	127	30	17	
57	Novaluron	7.77	492.706	493.02	141	5	30	50
				493.02	158.03	5	15	
58	Oxamyl	2.66	219.259	237	72	15	10	3000
				237	90	15	10	
59	Paclobutrazol	6.49	293.79	294.1	70.2	10	20	20
				294.1	125.1	10	35	
60	Phenothrin	6.48	350.451	352.89	195.02	32	14	50
				352.89	227.14	32	16	
61	Phosmet	6	317.314	318	77	28	46	N/A
				318	160	28	22	
62	Piperonyl butoxide	7.98	338.438	356.3	119	20	35	N/A
				356.3	176.9	20	10	
63	Pirimicarb	4	238.29	239.1	72	25	20	20
				239.1	182.1	25	15	
64	Prallethrin	7.62	300.4	301.2	133	5	12	N/A
				301.2	169	5	9	
65	Propiconazole	7.37	342.22	342.1	69.1	35	30	N/A
				342.1	158.9	35	20	
66	Propoxur	5.02	209.2417	210.1	92.9	15	25	20
				210.1	110.9	15	12	
67	Pyraclostrobin	7.34	387.82	388.1	163	25	25	20
				388.1	193.9	25	12	
68	Pyrethrin	7.64	371.461	373.2	133	37	19	50
				373.2	161	37	8	

	Analyte	Retention time (min)	MW (g/mol)	Precursor (m/z)	Product (m/z)	CV	CE	Health Canada detection limit (ppb)
69	Pyridaben	8.55	364.93	365.1	147.1	5	24	50
				365.1	309.1	5	12	
70	Spinetoram	7.49	748.011	748.53	98.07	60	35	N/A
				748.53	142.16	60	30	
71	Spinosad	7.05	731.968	732.6	98.1	35	35	N/A
				732.6	142	35	30	
72	Spirodiclofen	8.37	411.319	411.14	71.16	35	15	N/A
				411.14	313.1	35	10	
73	Spiromesifen	8.24	370.4819	371.1	273.1	35	5	3000
				388.2	273.1	35	25	
74	Spirotetramat	6.81	373.449	374	302	20	30	20
				374	330	20	15	
75	Spiroxamine	6.06	297.476	298	100	40	32	N/A
				298	144	40	20	
76	Tebuconazole	7.18	307.82	308.2	70.1	30	24	N/A
				308.2	124.9	30	40	
77	Tebufenozide	7.05	352.478	353.22	105.13	10	20	20
				353.22	133.14	10	10	
78	Teflubenzuron	7.92	381.108	381	141	25	30	50
				381	158	25	15	
79	Tetrachlorvinphos	7.1	365.952	364.8	127	32	16	20
				364.8	238.9	32	20	
80	Tetramethrin	6.49	331.406	330.91	98.95	34	18	100
				330.91	126.99	34	10	
81	Thiacloprid	4.02	252.72	253	90	35	40	20
				253	125.8	35	20	
82	Thiamethoxam	2.86	291.71	292	132	25	20	20
				292	211.2	25	10	
83	Thiophanate methyl	4.92	342.39	343	93	25	35	50
				343	151	25	20	
84	Trifloxystrobin	7.59	408.37	409.2	145	25	40	20
				409.2	185.9	25	14	

Appendix 6

Operator : Godwin Sammut / Vanessa Vella
Sample : Sample 1
Misc : Green buds sample 1
ALS Vial : 6 Sample Multiplier: 1

Search Libraries: C:\Database\SWGDRUG 3.13.L Minimum Quality: 20
C:\Database\NIST129K.L Minimum Quality: 0

Unknown Spectrum: Apex
Integration Events: ChemStation Integrator - autoint1.e

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
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			Bicyclo[5.2.0]nonane, 2-methylene-4,8,8-trimethyl-4-vinyl-	36364	000000-00-0	97
			Caryophyllene	120854	000087-44-5	95
			Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene- $$ Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-, (Z)- $$ cis-4,11,11-Trimethyl-8-methylenebicyclo(7.2.0)undeca-4-ene	36479	013877-93-5	94
2	7.778	0.41	C:\Database\SWGDRUG 3.13.L			
			.alpha.-Humulene	2841	006753-98-6	97
			N-phenylpropanamide	927	000620-71-3	4
			Betahistine	446	005638-76-6	2
3	8.059	0.52	C:\Database\NIST129K.L			
			Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1S-(1.alpha.,7.alpha.,8a.beta.)]-	120817	003691-11-0	99
			Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1S-(1.alpha.,7.alpha.,8a.beta.)]-	120818	003691-11-0	99
			Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1S-(1.alpha.,7.alpha.,8a.beta.)]- $$ Guaia-1(10),11-diene $$ .alpha.-Bulnesene $$ .delta.-Guaiene	36442	003691-11-0	98
4	8.321	0.50	C:\Database\NIST129K.L			
			Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethylidene)-, (4aR-trans)-	120815	000515-17-3	90
			Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethylidene)-, (4aR-trans)-	120784	006813-21-4	90
			.beta.-Panasinsene	36365	000000-00-0	90
5	8.356	0.75	C:\Database\NIST129K.L			
			Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethylidene)-, (4aR-trans)-	120784	006813-21-4	98
			Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-, (1.alpha.,4a.beta.,8a.alpha.)- $$ .gamma.-Cadinene	36474	039029-41-9	78
			Isoledene	36341	000000-00-0	70
6	12.393	0.41	C:\Database\SWGDRUG 3.13.L			
			Cannabicitran	2580	031508-71-1	52
			Cannabichromene	1111	020675-51-8	45
			exo-THC	3545	027179-28-8	10
7	12.503	1.12	C:\Database\SWGDRUG 3.13.L			
			Tetrahydrocannabivarin	2128	031262-37-0	99
			Delta8-Tetrahydrocannabivarin	3219	031262-38-1	60

Operator : Godwin Sammut / Vanessa Vella
Sample : Sample 1
Misc : Green buds sample 1
ALS Vial : 6 Sample Multiplier: 1

Search Libraries: C:\Database\SWGDRUG 3.13.L Minimum Quality: 20
C:\Database\NIST129K.L Minimum Quality: 0

Unknown Spectrum: Apex
Integration Events: ChemStation Integrator - autoint1.e

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
			5-Androstenedione	844	000571-36-8	25
8	12.972	2.61	C:\Database\SWGDRUG 3.13.L			
			Cannabicyclol	1064	021366-63-2	93
			Cannabichromene	1111	020675-51-8	93
			Cannabidiol	1112	000521-37-9	43
9	13.144	0.99	C:\Database\SWGDRUG 3.13.L			
			delta-9-THC Acetate	3572	023132-17-4	98
10	13.450	70.81	C:\Database\SWGDRUG 3.13.L			
			(6aR,9R)-delta10-THC	3176	095543-62-7	99
			delta-9-Tetrahydrocannabinol	1229	001972-08-3	99
			(6aR,9S)-delta10-THC	3173	095588-87-7	96
11	13.523	0.87	C:\Database\SWGDRUG 3.13.L			
			(6aR,9R)-delta10-THC	3176	095543-62-7	92
			(.+/-.)-cis-Delta-9-THC	3568	006087-73-6	92
			(6aR,9S)-delta10-THC	3173	095588-87-7	66
12	13.607	10.96	C:\Database\SWGDRUG 3.13.L			
			Cannabigerol	1056	002808-33-5	99
			9(R)-Hexahydrocannabinol	3549	036403-90-4	9
			Carbamazepine	1114	000298-46-4	1
13	13.687	9.19	C:\Database\SWGDRUG 3.13.L			
			Cannabinol	1113	000521-35-7	95

Operator : Godwin Sammut / Vanessa Vella
Sample : Sample 2
Misc : Green buds sample 2
ALS Vial : 7 Sample Multiplier: 1

Search Libraries: C:\Database\SWGDRUG 3.13.L Minimum Quality: 20
C:\Database\NIST129K.L Minimum Quality: 0

Unknown Spectrum: Apex
Integration Events: ChemStation Integrator - autoint1.e

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	7.536	1.01	C:\Database\SWGDRUG 3.13.L			
			Stearidonic Acid methyl ester	2434	073097-00-4	23
			4-Acetoxyindole	3057	005585-96-6	9
			1-Phenyl-1-Cyclohexanol	1066	001589-60-2	9
2	7.784	0.38	C:\Database\SWGDRUG 3.13.L			
			.alpha.-Humulene	2841	006753-98-6	97
			Betahistine	446	005638-76-6	2
			N-phenylpropanamide	927	000620-71-3	1
3	8.065	0.66	C:\Database\NIST129K.L			
			Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1S-(1.alpha.,7.alpha.,8a.beta.)]- $$ Guaia-1(10),11-diene $$ .alpha.-Bulnesene $$ .delta.-Guaiene	36442	003691-11-0	99
			Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1S-(1.alpha.,7.alpha.,8a.beta.)]-	120818	003691-11-0	99
			Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1S-(1.alpha.,7.alpha.,8a.beta.)]-	120817	003691-11-0	99
4	8.327	0.64	C:\Database\NIST129K.L			
			Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethylidene)-, (4aR-trans)-	120815	000515-17-3	90
			(+)-Cyclosativene	36338	000000-00-0	90
			Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-, [1R-(1.alpha.,7.beta.,8a.alpha.)]-	120791	004630-07-3	89
5	8.361	0.98	C:\Database\NIST129K.L			
			Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethylidene)-, (4aR-trans)-	120784	006813-21-4	98
			Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethylidene)-, (4aR-trans)- $$ Eudesma-3,7(11)-diene $$ Selina-3,7(11)-diene	36403	006813-21-4	97
			Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-, (1.alpha.,4a.beta.,8a.alpha.)- $$ .gamma.-Cadinene	36474	039029-41-9	78
6	12.396	0.47	C:\Database\SWGDRUG 3.13.L			
			Cannabicitran	2580	031508-71-1	98
			Cannabicyclol	1064	021366-63-2	68
			Cannabichromene	1111	020675-51-8	59
7	12.505	1.16	C:\Database\SWGDRUG 3.13.L			
			Tetrahydrocannabivarin	2128	031262-37-0	91
			Delta8-Tetrahydrocannabivarin	3219	031262-38-1	50
			5-Androstenedione	844	000571-36-8	18

Operator : Godwin Sammut / Vanessa Vella
Sample : Sample 2
Misc : Green buds sample 2
ALS Vial : 7 Sample Multiplier: 1

Search Libraries: C:\Database\SWGDRUG 3.13.L Minimum Quality: 20
C:\Database\NIST129K.L Minimum Quality: 0

Unknown Spectrum: Apex
Integration Events: ChemStation Integrator - autoint1.e

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
8	12.975	2.74	C:\Database\SWGDRUG 3.13.L			
			Cannabichromene	1111	020675-51-8	93
			Cannabicyclol	1064	021366-63-2	93
			Cannabidiol	1112	000521-37-9	64
9	13.144	1.31	C:\Database\SWGDRUG 3.13.L			
			delta-9-THC Acetate	3572	023132-17-4	98
10	13.452	65.89	C:\Database\SWGDRUG 3.13.L			
			(6aR,9R)-delta10-THC	3176	095543-62-7	99
			delta-9-Tetrahydrocannabinol	1229	001972-08-3	99
			(6aR,9S)-delta10-THC	3173	095588-87-7	96
11	13.526	1.04	C:\Database\SWGDRUG 3.13.L			
			(.+/-.)-cis-Delta-9-THC	3568	006087-73-6	93
			(6aR,9R)-delta10-THC	3176	095543-62-7	92
			THCA-A	1701	023978-85-0	35
12	13.551	0.96	C:\Database\SWGDRUG 3.13.L			
			(6aR,9R)-delta10-THC	3176	095543-62-7	92
			exo-THC	3545	027179-28-8	70
			(.+/-.)-cis-Delta-9-THC	3568	006087-73-6	64
13	13.608	9.75	C:\Database\SWGDRUG 3.13.L			
			Cannabigerol	1056	002808-33-5	99
			9(R)-Hexahydrocannabinol	3549	036403-90-4	18
			Carbamazepine	1114	000298-46-4	1
14	13.690	11.80	C:\Database\SWGDRUG 3.13.L			
			Cannabinol	1113	000521-35-7	93
15	13.855	0.55	C:\Database\NIST129K.L			
			Methyltribenzocentrotriquinanol	74698	000000-00-0	58
			2-Propanone, 1-phenyl-1-(5-phenyl-3H-1,2-dithiol-3-ylidene)-	74529	038489-98-4	50
			Mepazine	126202	000060-89-9	46
16	14.012	0.48	C:\Database\NIST129K.L			
			D-Homo-24-nor-17-oxachola-20,22-diene-3,16-dione, 7-(acetyloxy)-1,2:14,15:21,23-triepoxy-4,4,8-trimethyl-, (5.alpha.,7.alpha.,13.alpha.,14.beta.,15.beta.,17a.alpha.)-	102239	055658-66-7	28
			Phenol, 2,4-bis(1-methyl-1-phenylethyl)-	126833	002772-45-4	28
			Androstane-3,17-dione, bis(O-methyloxime), (5.beta.)-	83468	056210-80-1	28
17	14.105	0.18	C:\Database\SWGDRUG 3.13.L			
			HU-331	46	137252-25-6	42
			Flutoprazepam	2861	025967-29-7	11
			.delta.9-THCH	3461	036482-24-3	9

Appendix 7

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  Operator  : Godwin Sammut / Vanessa Vella
  Sample    : C1
  Misc      : spiked C1
  ALS Vial  : 7   Sample Multiplier: 1

  Search Libraries:   C:\Database\SWGDRUG 3.13.L        Minimum Quality:  20
                      C:\Database\NIST129K.L            Minimum Quality:   0

  Unknown Spectrum:   Apex
  Integration Events: ChemStation Integrator - autoint1.e
```

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	7.532	0.86	C:\Database\NIST129K.L			
			Bicyclo[5.2.0]nonane, 2-methylene-4,8,8-trimethyl-4-vinyl-	36364	000000-00-0	95
			Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene- $$ Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-, (Z)- $$ cis-4,11,11-Trimethyl-8-methylenebicyclo(7.2.0)undeca-4-ene	36479	013877-93-5	90
			Caryophyllene	120854	000087-44-5	90
2	8.323	0.49	C:\Database\NIST129K.L			
			Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-, [1R-(1.alpha.,7.beta.,8a.alpha.)]- $$ 4.beta.H,5.alpha.-Eremophila-1(10),11-diene $$ (+)-Valencene $$ Valencen $$ Valencene $$ 4.alpha.,10.alpha.-Dimethyl-6.beta.-isopropyl-.delta	36423	004630-07-3	90
			Isoledene	36341	000000-00-0	76
			Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethylidene)-, (4aR-trans)-	120784	006813-21-4	76
3	8.357	0.74	C:\Database\NIST129K.L			
			Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethylidene)-, (4aR-trans)-	120784	006813-21-4	98
			Epizonarene	36340	000000-00-0	83
			Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-, (1.alpha.,4a.beta.,8a.alpha.)- $$ .gamma.-Cadinene	36474	039029-41-9	78
4	12.393	0.43	C:\Database\SWGDRUG 3.13.L			
			Cannabicitran	2580	031508-71-1	98
			Cannabidiolic Acid	1640	001244-58-2	78
			Cannabidiol	1112	000521-37-9	72
5	12.503	1.01	C:\Database\SWGDRUG 3.13.L			
			Tetrahydrocannabivarin	2128	031262-37-0	95
			Delta8-Tetrahydrocannabivarin	3219	031262-38-1	60
			Cannabidivarin	2416	024274-48-4	42
6	12.971	2.53	C:\Database\SWGDRUG 3.13.L			
			Cannabicyclol	1064	021366-63-2	93
			Cannabichromene	1111	020675-51-8	93
			Cannabicitran	2580	031508-71-1	64
7	13.144	1.43	C:\Database\SWGDRUG 3.13.L			
			delta-9-THC Acetate	3572	023132-17-4	99
			Amlodipine	487	088150-42-9	2
8	13.445	71.73	C:\Database\SWGDRUG 3.13.L			
			(6aR,9R)-delta10-THC	3176	095543-62-7	99
			delta-9-Tetrahydrocannabinol	1229	001972-08-3	99
			(6aR,9S)-delta10-THC	3173	095588-87-7	96

Operator : Godwin Sammut / Vanessa Vella
Sample : C1
Misc : spiked C1
ALS Vial : 7 Sample Multiplier: 1

Search Libraries: C:\Database\SWGDRUG 3.13.L Minimum Quality: 20
C:\Database\NIST129K.L Minimum Quality: 0

Unknown Spectrum: Apex
Integration Events: ChemStation Integrator - autoint1.e

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
9	13.522	1.95	C:\Database\NIST129K.L			
			Cannabinol, O-trimethylsilyl-	90243	000000-00-0	83
			7H-Dinaphtho[2,3-b:2',3'-H]carbazole	87679	000242-45-5	22
			Estra-1,3,5,7,9-pentaen-17-one, 3-[(trimethylsilyl)oxy]-, O-methyloxime	87662	069833-43-8	22
10	13.603	9.45	C:\Database\SWGDRUG 3.13.L			
			Cannabigerol	1056	002808-33-5	99
			9(R)-Hexahydrocannabinol	3549	036403-90-4	7
			Carbamazepine	1114	000298-46-4	1
11	13.685	9.37	C:\Database\SWGDRUG 3.13.L			
			Cannabinol	1113	000521-35-7	95

Operator : Godwin Sammut / Vanessa Vella
Sample : C2
Misc : spiked C2
ALS Vial : 8 Sample Multiplier: 1

Search Libraries: C:\Database\SWGDRUG 3.13.L Minimum Quality: 20
C:\Database\NIST129K.L Minimum Quality: 0

Unknown Spectrum: Apex
Integration Events: ChemStation Integrator - autoint1.e

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	7.536	0.87	C:\Database\NIST129K.L			
			Caryophyllene	120854	000087-44-5	95
			Caryophyllene	120853	000087-44-5	95
			Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene- $$ Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-, (Z)- $$ cis-4,11,11-Trimethyl-8-methylenebicyclo(7.2.0)undeca-4-ene	36479	013877-93-5	89
2	8.327	0.51	C:\Database\NIST129K.L			
			Epizonarene	36340	000000-00-0	89
			Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)-	120872	000483-76-1	86
			Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethylidene)-, (4aR-trans)-	120815	000515-17-3	83
3	8.361	0.77	C:\Database\NIST129K.L			
			Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethylidene)-, (4aR-trans)-	120784	006813-21-4	98
			Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethylidene)-, (4aR-trans)- $$ Eudesma-3,7(11)-diene $$ Selina-3,7(11)-diene	36403	006813-21-4	83
			Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-, (1.alpha.,4a.beta.,8a.alpha.)- $$ .gamma.-Cadinene	36474	039029-41-9	78
4	12.396	0.44	C:\Database\SWGDRUG 3.13.L			
			Cannabicitran	2580	031508-71-1	98
			Cannabicyclol	1064	021366-63-2	74
			Cannabidiol	1112	000521-37-9	64
5	12.506	1.07	C:\Database\SWGDRUG 3.13.L			
			Tetrahydrocannabivarin	2128	031262-37-0	91
			Delta8-Tetrahydrocannabivarin	3219	031262-38-1	60
			5-Androstenedione	844	000571-36-8	12
6	12.974	2.60	C:\Database\SWGDRUG 3.13.L			
			Cannabichromene	1111	020675-51-8	94
			Cannabicyclol	1064	021366-63-2	93
			Cannabidiol	1112	000521-37-9	72
7	13.146	1.64	C:\Database\SWGDRUG 3.13.L			
			delta-9-THC Acetate	3572	023132-17-4	99
			Amlodipine	487	088150-42-9	2
8	13.445	71.27	C:\Database\SWGDRUG 3.13.L			
			(6aR,9R)-delta10-THC	3176	095543-62-7	99
			delta-9-Tetrahydrocannabinol	1229	001972-08-3	99
			(6aR,9S)-delta10-THC	3173	095588-87-7	96
9	13.524	0.93	C:\Database\NIST129K.L			

Operator : Godwin Sammut / Vanessa Vella
Sample : C2
Misc : spiked C2
ALS Vial : 8 Sample Multiplier: 1

Search Libraries: C:\Database\SWGDRUG 3.13.L Minimum Quality: 20
C:\Database\NIST129K.L Minimum Quality: 0

Unknown Spectrum: Apex
Integration Events: ChemStation Integrator - autoint1.e

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
			Cannabinol, O-trimethylsilyl-	90243	000000-00-0	35
			Pentachlorophenyl methyl sulfoxide	74307	070215-07-5	22
			2-[1-Methylimidazol-2-yl]methoxy-6-methoxy-8-nitroquinoline	75517	000000-00-0	15
10	13.547	0.83	C:\Database\SWGDRUG 3.13.L			
			(6aR,9R)-delta10-THC	3176	095543-62-7	91
			exo-THC	3545	027179-28-8	46
			Cannabicitran	2580	031508-71-1	38
11	13.605	9.58	C:\Database\SWGDRUG 3.13.L			
			Cannabigerol	1056	002808-33-5	99
			9(R)-Hexahydrocannabinol	3549	036403-90-4	9
			Carbamazepine	1114	000298-46-4	1
12	13.687	9.49	C:\Database\SWGDRUG 3.13.L			
			Cannabinol	1113	000521-35-7	96

Operator : Godwin Sammut / Vanessa Vella
Sample : C3
Misc : spiked C3
ALS Vial : 9 Sample Multiplier: 1

Search Libraries: C:\Database\SWGDRUG 3.13.L Minimum Quality: 20
C:\Database\NIST129K.L Minimum Quality: 0

Unknown Spectrum: Apex
Integration Events: ChemStation Integrator - autoint1.e

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	7.537	0.90	C:\Database\NIST129K.L			
			Caryophyllene	120854	000087-44-5	98
			Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene- $$ Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-, (Z)- $$ cis-4,11,11-Trimethyl-8-methylenebicyclo(7.2.0)undeca-4-ene	36479	013877-93-5	93
			Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-	120869	013877-93-5	89
2	8.328	0.52	C:\Database\NIST129K.L			
			Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethylidene)-, (4aR-trans)-	120815	000515-17-3	87
			(+)-Cyclosativene	36338	000000-00-0	83
			Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)- $$ Cadina-1(10),4-diene $$ .delta.-Cadinene, (+)- $$ (+)-.delta.-Cadinene $$ .delta.-Cadinene	36480	000483-76-1	76
3	8.362	0.78	C:\Database\NIST129K.L			
			Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethylidene)-, (4aR-trans)- $$ Eudesma-3,7(11)-diene $$ Selina-3,7(11)-diene	36403	006813-21-4	99
			Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethylidene)-, (4aR-trans)-	120784	006813-21-4	98
			Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-, (1.alpha.,4a.beta.,8a.alpha.)- $$ .gamma.-Cadinene	36474	039029-41-9	91
4	12.396	0.44	C:\Database\SWGDRUG 3.13.L			
			Cannabicitran	2580	031508-71-1	98
			Cannabicyclol	1064	021366-63-2	87
			Cannabidiol	1112	000521-37-9	64
5	12.506	1.10	C:\Database\SWGDRUG 3.13.L			
			Tetrahydrocannabivarin	2128	031262-37-0	99
			5-Androstenedione	844	000571-36-8	35
			Delta8-Tetrahydrocannabivarin	3219	031262-38-1	25
6	12.975	2.69	C:\Database\SWGDRUG 3.13.L			
			Cannabichromene	1111	020675-51-8	93
			Cannabicyclol	1064	021366-63-2	93
			Cannabicitran	2580	031508-71-1	64
7	13.146	1.42	C:\Database\SWGDRUG 3.13.L			
			delta-9-THC Acetate	3572	023132-17-4	99
			Amlodipine	487	088150-42-9	2
8	13.447	71.21	C:\Database\SWGDRUG 3.13.L			
			(6aR,9R)-delta10-THC	3176	095543-62-7	99

Operator : Godwin Sammut / Vanessa Vella
Sample : C3
Misc : spiked C3
ALS Vial : 9 Sample Multiplier: 1

Search Libraries: C:\Database\SWGDRUG 3.13.L Minimum Quality: 20
C:\Database\NIST129K.L Minimum Quality: 0

Unknown Spectrum: Apex
Integration Events: ChemStation Integrator - autoint1.e

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
			delta-9-Tetrahydrocannabinol	1229	001972-08-3	99
			(6aR,9S)-delta10-THC	3173	095588-87-7	96
9	13.525	1.68	C:\Database\SWGDRUG 3.13.L			
			(6aR,9R)-delta10-THC	3176	095543-62-7	90
			delta-9-Tetrahydrocannabinol	1229	001972-08-3	56
			delta-9-THC Acetate	3572	023132-17-4	7
10	13.606	9.72	C:\Database\SWGDRUG 3.13.L			
			Cannabigerol	1056	002808-33-5	99
			9(R)-Hexahydrocannabinol	3549	036403-90-4	18
			Carbamazepine	1114	000298-46-4	2
11	13.688	9.28	C:\Database\NIST129K.L			
			Cannabinol $$ 6H-Dibenzo[b,d]pyran -1-ol, 6,6,9-trimethyl-3-pentyl- $ $ 3-Amyl-1-hydroxy-6,6,9-trimethyl -6H-dibenzo(b,d)pyran $$ 6,6,9-Tri methyl-3-pentyl-6H-dibenzo(b,d)pyr an-1-ol $$ CBN $$ 6,6,9-Trimethyl- 3-pentyl-6H-dibenzo[b,d]pyran-1-ol . Product derived	74677	000521-35-7	95
			Cannabinol	126217	000521-35-7	93
			4,5'-Bipyrimidine, 2,2'-dimethoxy- 4',6-bis(methylthio)-	74361	059549-55-2	72
12	14.104	0.27	C:\Database\NIST129K.L			
			Hexadecanoic acid, tert-butyldimet hylsilyl ester	88261	000000-00-0	46
			2-p-Tolyl-6-(4-trifluoromethyl-phe nyl)-pyridine	75364	000000-00-0	46
			Estra-1,3,5(10)-trien-17-one, 3-(a cetyloxy)-4-nitro-, 17-(O-methylox ime)	90833	077883-07-9	37

Operator : Godwin Sammut / Vanessa Vella
Sample : C4
Misc : spiked C4
ALS Vial : 10 Sample Multiplier: 1

Search Libraries: C:\Database\SWGDRUG 3.13.L Minimum Quality: 20
C:\Database\NIST129K.L Minimum Quality: 0

Unknown Spectrum: Apex
Integration Events: ChemStation Integrator - autoint1.e

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	7.537	0.88	C:\Database\NIST129K.L			
			Caryophyllene	120854	000087-44-5	99
			Caryophyllene	120853	000087-44-5	98
			Bicyclo[7.2.0]undec-4-ene, 4,11,11 -trimethyl-8-methylene-	120869	013877-93-5	86
2	8.328	0.51	C:\Database\NIST129K.L			
			(+)-Cyclosativene	36338	000000-00-0	90
			Naphthalene, 1,2,3,4,4a,5,6,8a-oct ahydro-4a,8-dimethyl-2-(1-methylet hylidene)-, (4aR-trans)-	120784	006813-21-4	90
			Epizonarene	36340	000000-00-0	89
3	8.362	0.77	C:\Database\SWGDRUG 3.13.L			
			5,6-Methylenedioxyindole	3060	000267-48-1	22
			Phenobarbital	1792	000050-06-6	2
			Pentobarbital-d5	2631	052944-66-8	2
4	12.397	0.46	C:\Database\SWGDRUG 3.13.L			
			Cannabicitran	2580	031508-71-1	98
			Cannabicyclol	1064	021366-63-2	90
			Cannabichromene	1111	020675-51-8	59
5	12.506	1.07	C:\Database\SWGDRUG 3.13.L			
			Tetrahydrocannabivarin	2128	031262-37-0	94
			Delta8-Tetrahydrocannabivarin	3219	031262-38-1	64
			Famprofazone	855	022881-35-2	14
6	12.976	2.60	C:\Database\SWGDRUG 3.13.L			
			Cannabicyclol	1064	021366-63-2	93
			Cannabichromene	1111	020675-51-8	93
			Cannabidiol	1112	000521-37-9	78
7	13.147	1.36	C:\Database\SWGDRUG 3.13.L			
			delta-9-THC Acetate	3572	023132-17-4	99
8	13.447	69.98	C:\Database\SWGDRUG 3.13.L			
			(6aR,9R)-delta10-THC	3176	095543-62-7	99
			delta-9-Tetrahydrocannabinol	1229	001972-08-3	99
			(6aR,9S)-delta10-THC	3173	095588-87-7	96
9	13.525	1.95	C:\Database\SWGDRUG 3.13.L			
			(6aR,9R)-delta10-THC	3176	095543-62-7	91
			delta-9-Tetrahydrocannabinol	1229	001972-08-3	90
			delta-9-THC Acetate	3572	023132-17-4	10
10	13.607	9.92	C:\Database\SWGDRUG 3.13.L			
			Cannabigerol	1056	002808-33-5	99
			9(R)-Hexahydrocannabinol	3549	036403-90-4	9
			Carbamazepine	1114	000298-46-4	1
11	13.688	10.22	C:\Database\SWGDRUG 3.13.L			
			Cannabinol	1113	000521-35-7	97
12	14.106	0.27	C:\Database\SWGDRUG 3.13.L			
			Triazolam	649	028911-01-5	49
			HU-331	46	137252-25-6	38
			.delta.9-THCH	3461	036482-24-3	12

Operator : Godwin Sammut / Vanessa Vella
Sample : C4
Misc : spiked C4
ALS Vial : 10 Sample Multiplier: 1

Search Libraries: C:\Database\SWGDRUG 3.13.L Minimum Quality: 20
C:\Database\NIST129K.L Minimum Quality: 0

Unknown Spectrum: Apex
Integration Events: ChemStation Integrator - autoint1.e

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual

Appendix 8

```
  Operator  : Godwin Sammut / Vanessa Vella
  Sample    : Pesticide WS1
  Misc      : MS Scan 50-500
  ALS Vial  : 6   Sample Multiplier: 1

  Search Libraries:   C:\Database\SWGDRUG 3.13.L        Minimum Quality:  20
                      C:\Database\NIST129K.L            Minimum Quality:   0

  Unknown Spectrum:   Apex
  Integration Events: ChemStation Integrator - autoint1.e
```

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	3.851	8.44	C:\Database\NIST129K.L			
			Cyclotetrasiloxane, octamethyl-	125682	000556-67-2	90
			5H-Naphtho[2,3-c]carbazole, 5-meth yl-	65619	100025-44-3	56
			7H-Dibenzo[b,g]carbazole, 7-methyl	65620	003557-49-1	45
2	5.119	4.75	C:\Database\NIST129K.L			
			Cyclopentasiloxane, decamethyl- $$ Decamethylcyclopentasiloxane $$ D imethylsiloxane pentamer $$ Dekame thylcyklopentasiloxan	88049	000541-02-6	83
			Cyclopentasiloxane, decamethyl-	127685	000541-02-6	80
			Silane, [[4-[1,2-bis[(trimethylsil yl)oxy]ethyl]-1,2-phenylene]bis(ox y)]bis[trimethyl- $$ Silane, [[4-[1,2-bis[(trimethylsilyl)oxy]ethyl] -1,2-phenylene]bis(oxy)]bis@trimet hyl-	99473	056114-62-6	50
3	11.575	8.62	C:\Database\NIST129K.L			
			DDMU $$ Benzene, 1,1'-(chloroethen ylidene)bis[4-chloro- $$ Ethylene, 2-chloro-1,1-bis(p-chlorophenyl)- $$ p,p'-DDD olefin $$ p,p'-DDM $$ p,p'-DDMU $$ p,p'-DME $$ p,p'-TDE olefin $$ p,p'-TDEE $$ TDEE $$ 1- Chloro-2,2-Bis(p-chlorophenyl)ethy lene $$ 1,1-Bis(p	65776	001022-22-6	96
			DDMU	125145	001022-22-6	95
			Benzene, 1-chloro-2-[2-chloro-1-(4 -chlorophenyl)ethenyl]-	125149	014835-94-0	95
4	11.913	11.90	C:\Database\NIST129K.L			
			p,p'-DDE	126434	000072-55-9	99
			p,p'-DDE	126431	000072-55-9	99
			p,p'-DDE	126432	000072-55-9	99
5	11.993	12.39	C:\Database\SWGDRUG 3.13.L			
			DDT	395	000050-29-3	40
			5-Fluoro MDMB-PICA-d5	3214	000000-00-0	9
			Ipriflavone	3121	035212-22-7	9
6	12.042	4.49	C:\Database\NIST129K.L			
			Dieldrin	127836	000060-57-1	70
			Dieldrin $$ 2,7:3,6-Dimethanonapht h[2,3-b]oxirene, 3,4,5,6,9,9-hexac hloro-1a,2,2a,3,6,6a,7,7a-octahydr o-, (1a.alpha.,2.beta.,2a.alpha.,3 .beta.,6.beta.,6a.alpha.,7.beta.,7 a.alpha.)- $$ 1,4:5,8-Dimethanonap hthalene, 1,2,3,4,10,10-hexachloro -6,7-epoxy-1,4,4a	89496	000060-57-1	46
			Dieldrin	127837	000060-57-1	43
7	12.271	7.26	C:\Database\NIST129K.L			
			2-Methyl-6-phenylimidazo[2,1-b]-1, 3,4-selenadiazole	59283	000000-00-0	18
			Bicyclo[2.2.1]hepta-2,5-diene, 1,2	125680	003389-71-7	12

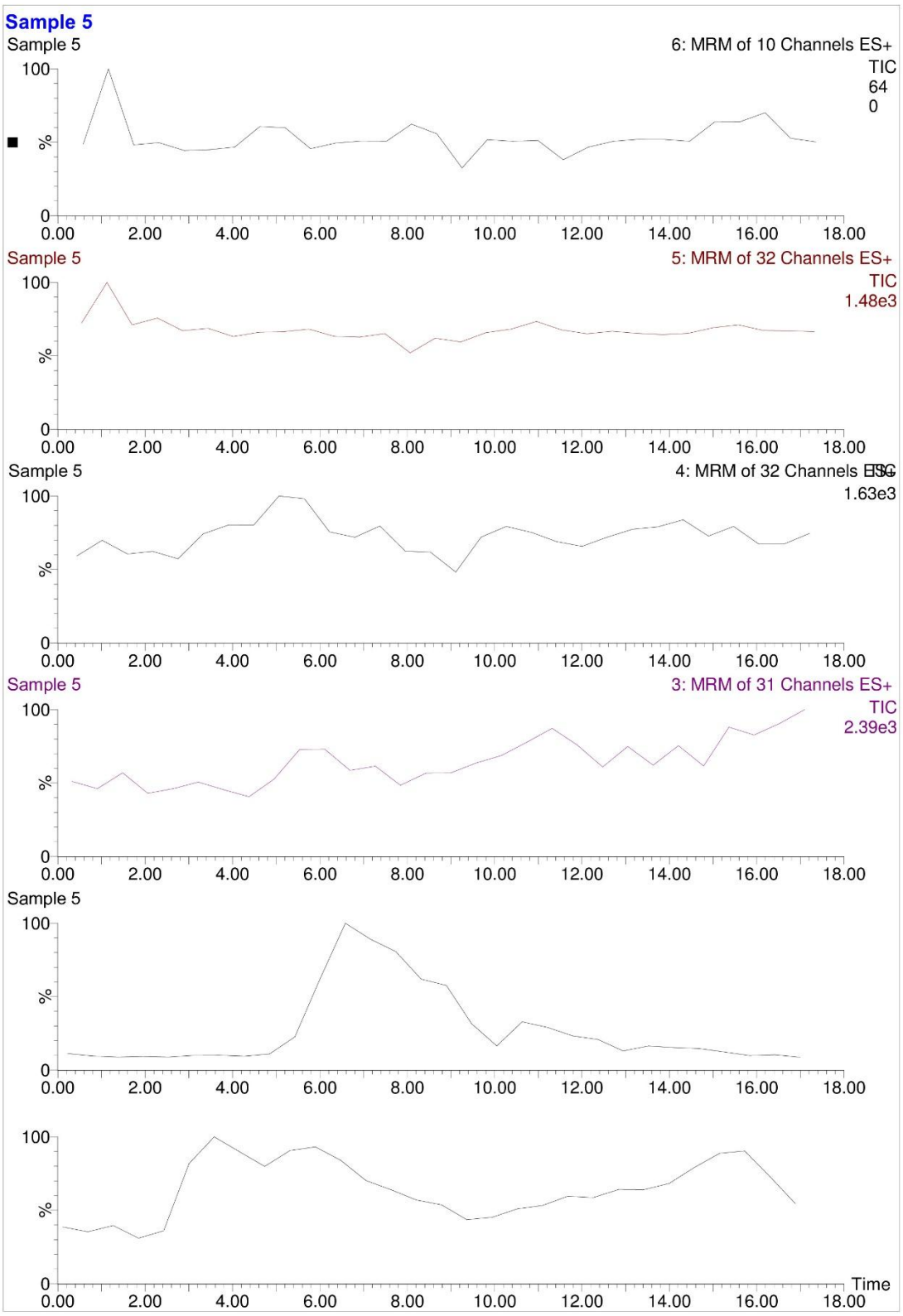
Operator : Godwin Sammut / Vanessa Vella
Sample : Pesticide WS1
Misc : MS Scan 50-500
ALS Vial : 6 Sample Multiplier: 1

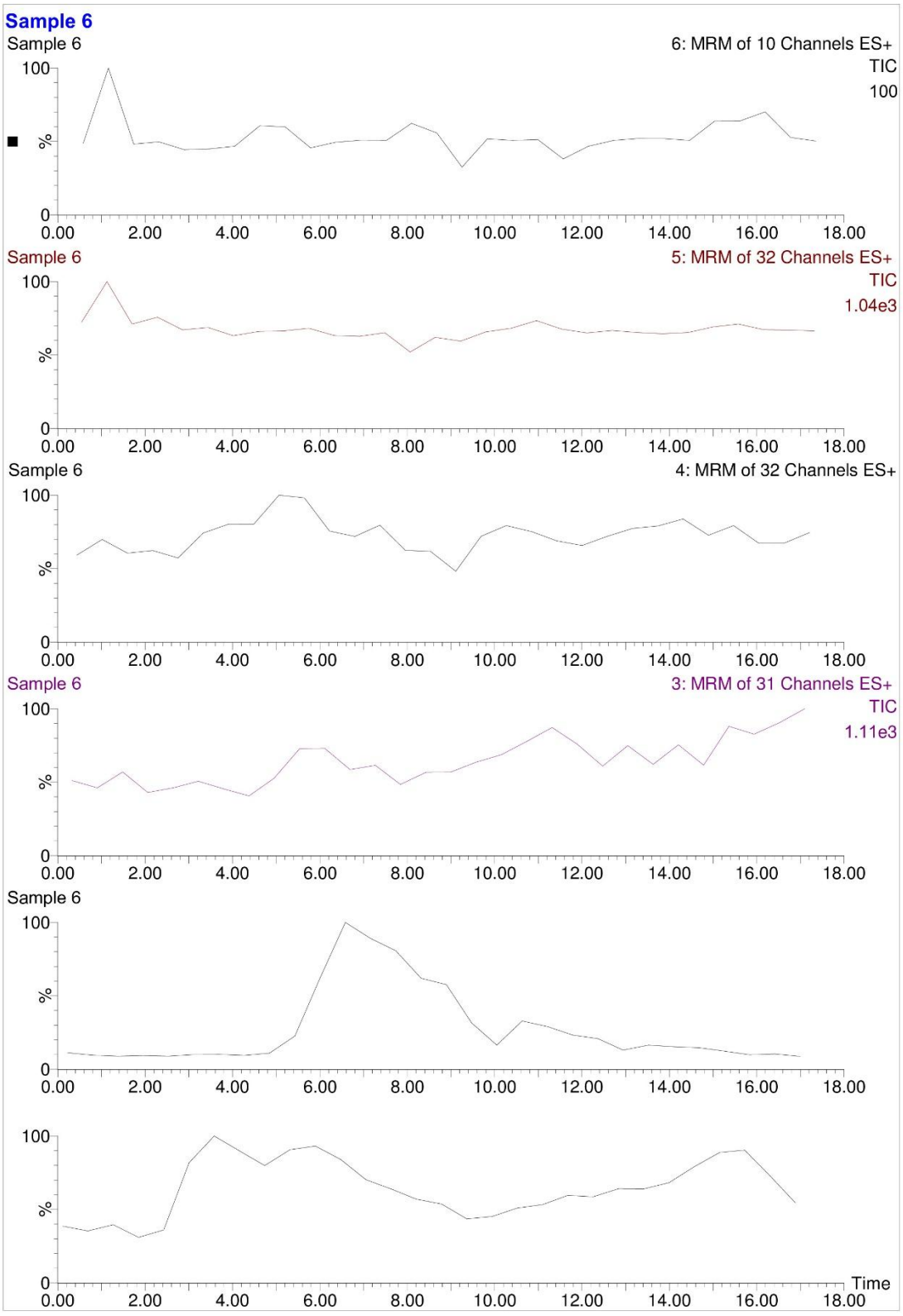
Search Libraries: C:\Database\SWGDRUG 3.13.L Minimum Quality: 20
C:\Database\NIST129K.L Minimum Quality: 0

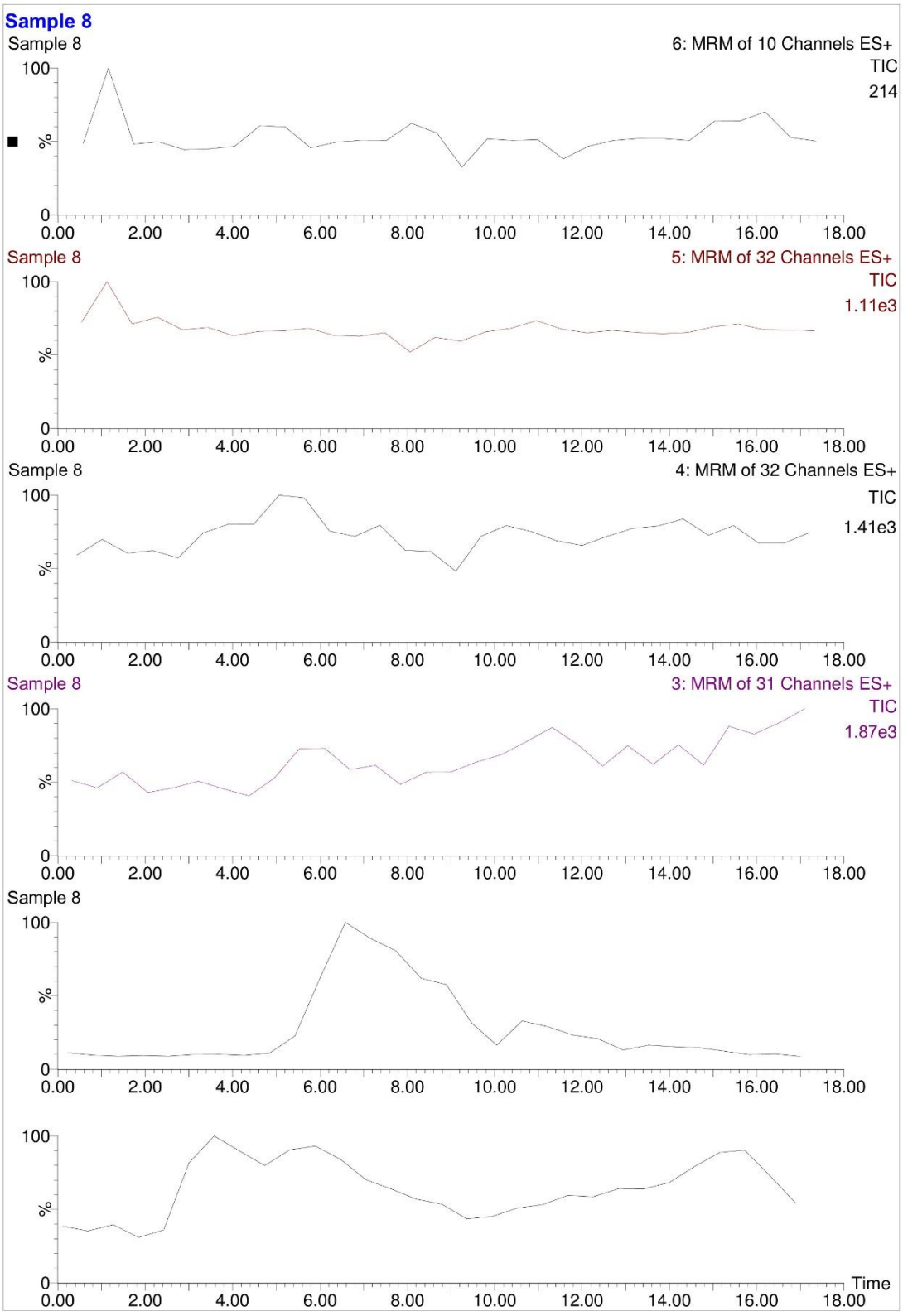
Unknown Spectrum: Apex
Integration Events: ChemStation Integrator - autoint1.e

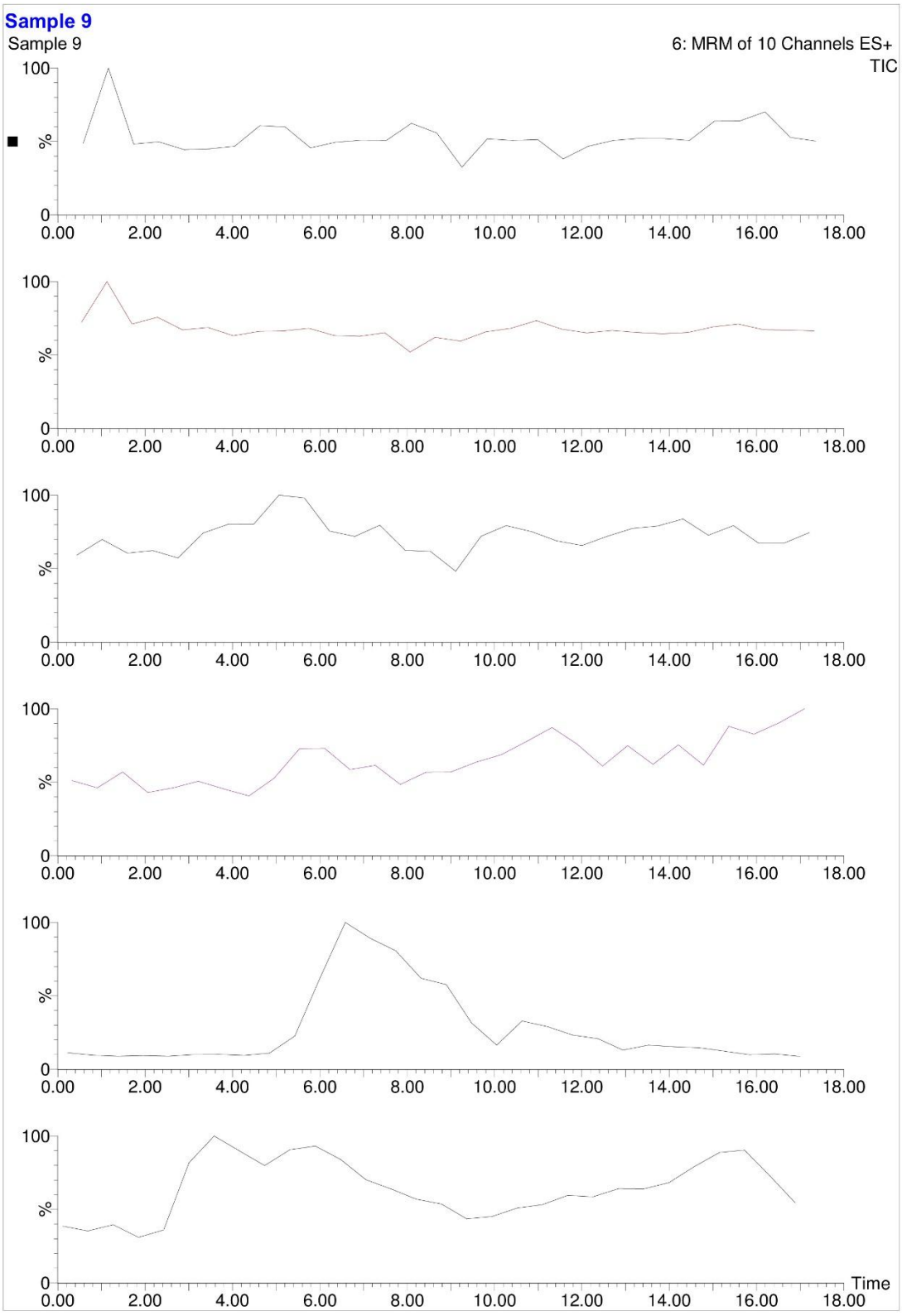
Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
			,3,4,7,7-hexachloro-			
			Flufenamic acid	125100	000530-78-9	12
8	12.350	3.93	C:\Database\SWGDRUG 3.13.L			
			DDT	395	000050-29-3	38
			Mebroqualone	1718	004260-20-2	2
			5-Fluoro MDMB-PICA-d5	3214	000000-00-0	2
9	12.387	4.44	C:\Database\NIST129K.L			
			Chlorophenothane $$ p,p'-DDT $$ Benzene, 1,1'-(2,2,2-trichloroethylidene)bis[4-chloro- $$ .alpha.,.alpha.-Bis(p-chlorophenyl)-.beta.,.beta.,.beta.-trichlorethane $$ p,p'-Dichlorodiphenyltrichloroethane $$ Aavero-extra $$ Agritan $$ Arkotine $$ Azotox $$ A	84517	000050-29-3	99
			o,p'-DDT	127291	000789-02-6	95
			Chlorophenothane	127293	000050-29-3	94
10	13.677	33.78	C:\Database\SWGDRUG 3.13.L			
			Cannabinol	1113	000521-35-7	95

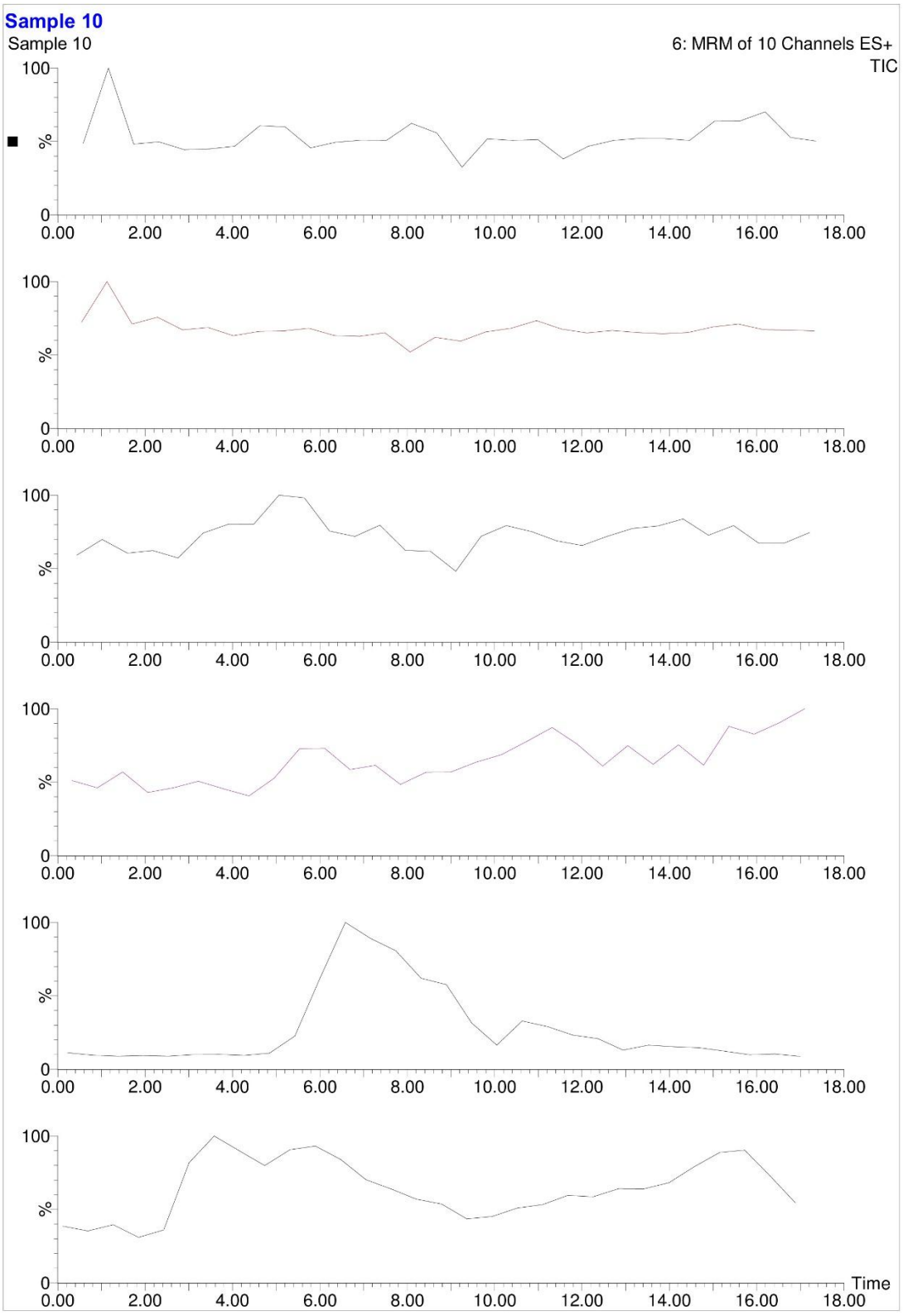
Appendix 9

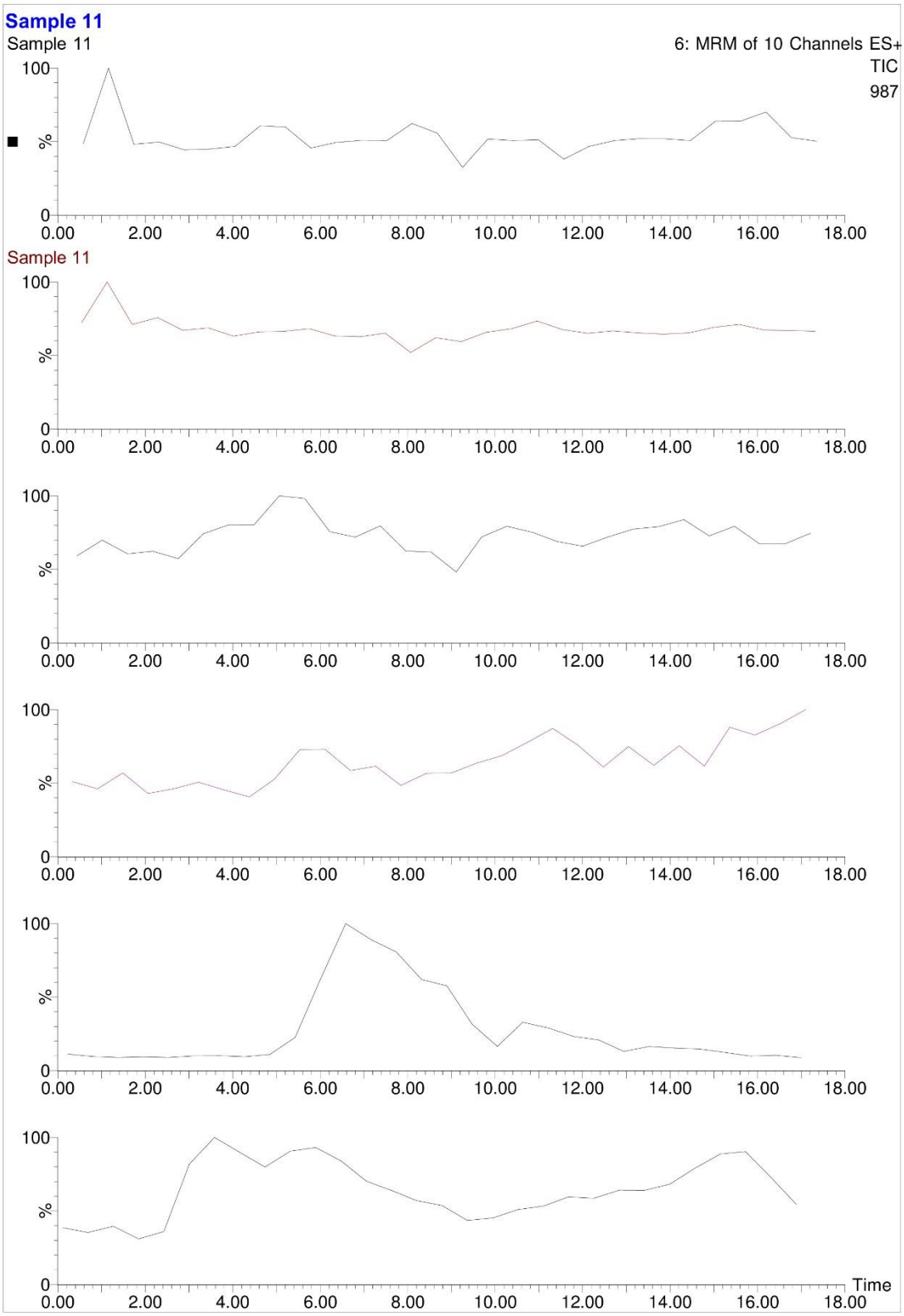

Sample 5
Sample 5
6: MRM of 10 Channels ES+
TIC
64
0
100
%
0
0.00
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
Sample 5
5: MRM of 32 Channels ES+
TIC
1.48e3
Sample 5
4: MRM of 32 Channels ES+
TIC
1.63e3
Sample 5
3: MRM of 31 Channels ES+
TIC
2.39e3
Sample 5
Time

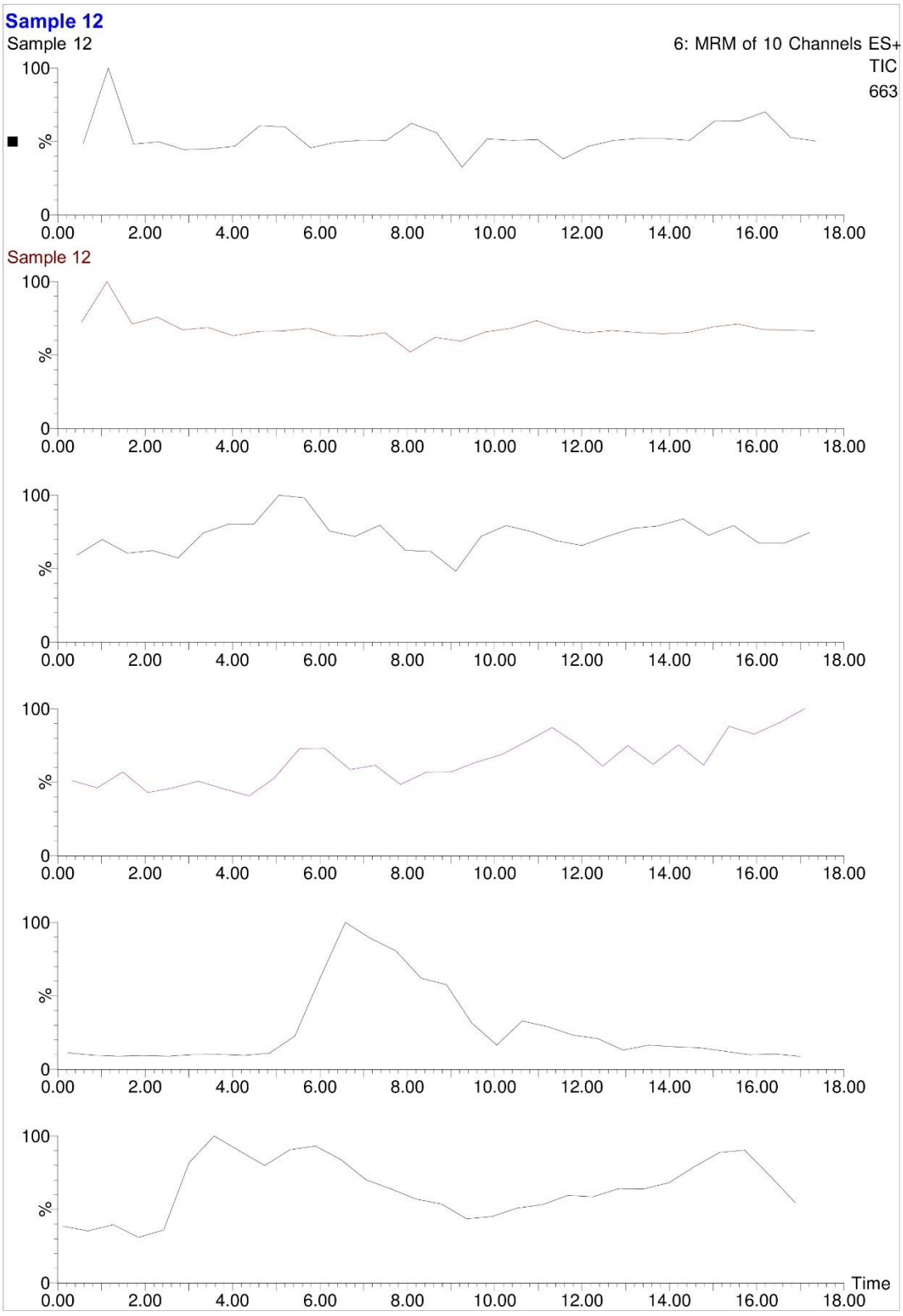

Sample 6
Sample 6
6: MRM of 10 Channels ES+
TIC
100
100
%
0
0.00 2.00 4.00 6.00 8.00 10.00 12.00 14.00 16.00 18.00
Sample 6
5: MRM of 32 Channels ES+
TIC
1.04e3
100
%
0
0.00 2.00 4.00 6.00 8.00 10.00 12.00 14.00 16.00 18.00
Sample 6
4: MRM of 32 Channels ES+
100
%
0
0.00 2.00 4.00 6.00 8.00 10.00 12.00 14.00 16.00 18.00
Sample 6
3: MRM of 31 Channels ES+
TIC
1.11e3
100
%
0
0.00 2.00 4.00 6.00 8.00 10.00 12.00 14.00 16.00 18.00
Sample 6
100
%
0
0.00 2.00 4.00 6.00 8.00 10.00 12.00 14.00 16.00 18.00
100
%
0
Time
0.00 2.00 4.00 6.00 8.00 10.00 12.00 14.00 16.00 18.00

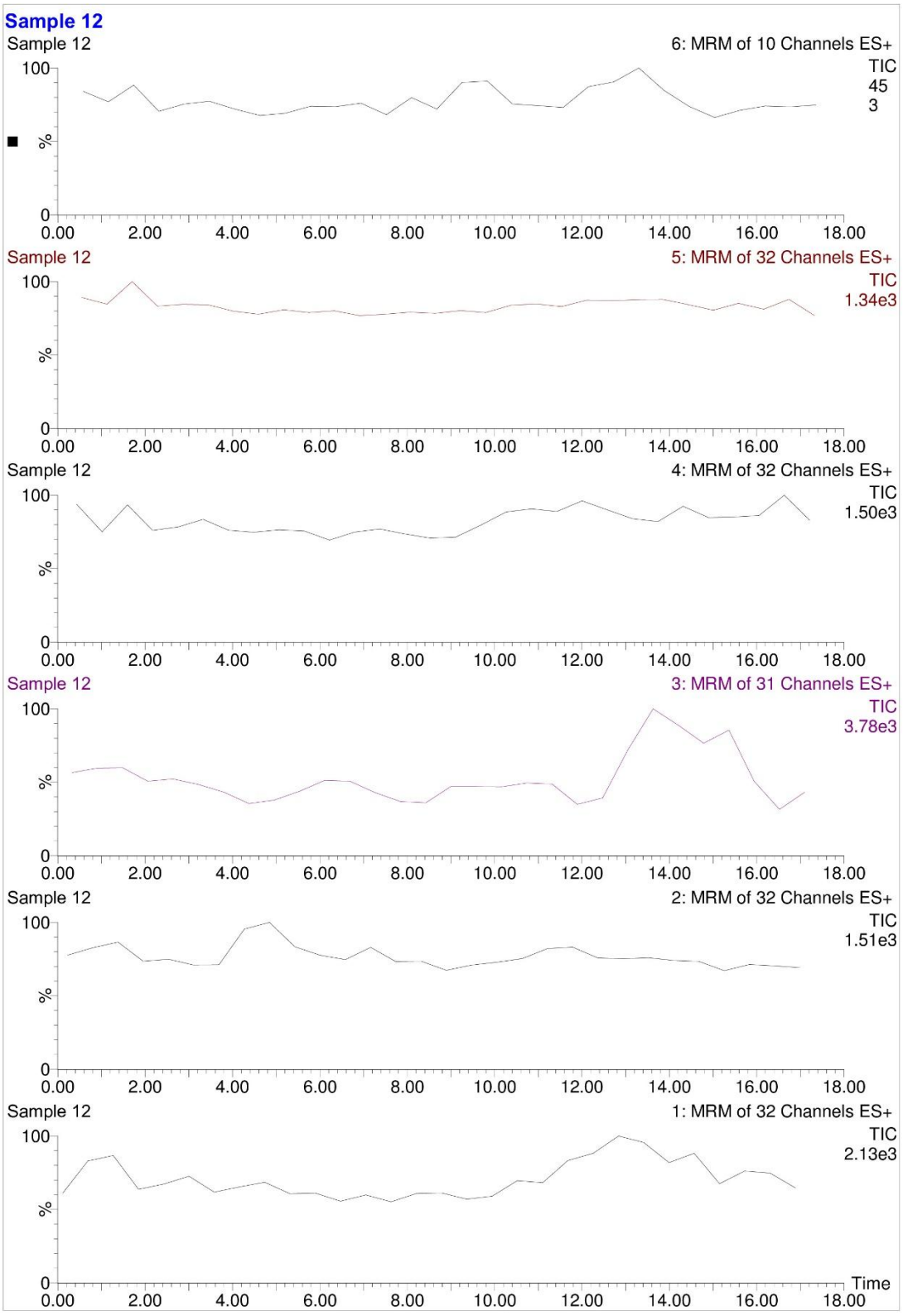

Sample 8
Sample 8
6: MRM of 10 Channels ES+
TIC
214
100
%
0
0.00 2.00 4.00 6.00 8.00 10.00 12.00 14.00 16.00 18.00
Sample 8
5: MRM of 32 Channels ES+
TIC
1.11e3
100
%
0
0.00 2.00 4.00 6.00 8.00 10.00 12.00 14.00 16.00 18.00
Sample 8
4: MRM of 32 Channels ES+
TIC
1.41e3
100
%
0
0.00 2.00 4.00 6.00 8.00 10.00 12.00 14.00 16.00 18.00
Sample 8
3: MRM of 31 Channels ES+
TIC
1.87e3
100
%
0
0.00 2.00 4.00 6.00 8.00 10.00 12.00 14.00 16.00 18.00
Sample 8
100
%
0
0.00 2.00 4.00 6.00 8.00 10.00 12.00 14.00 16.00 18.00
100
%
0
Time
0.00 2.00 4.00 6.00 8.00 10.00 12.00 14.00 16.00 18.00

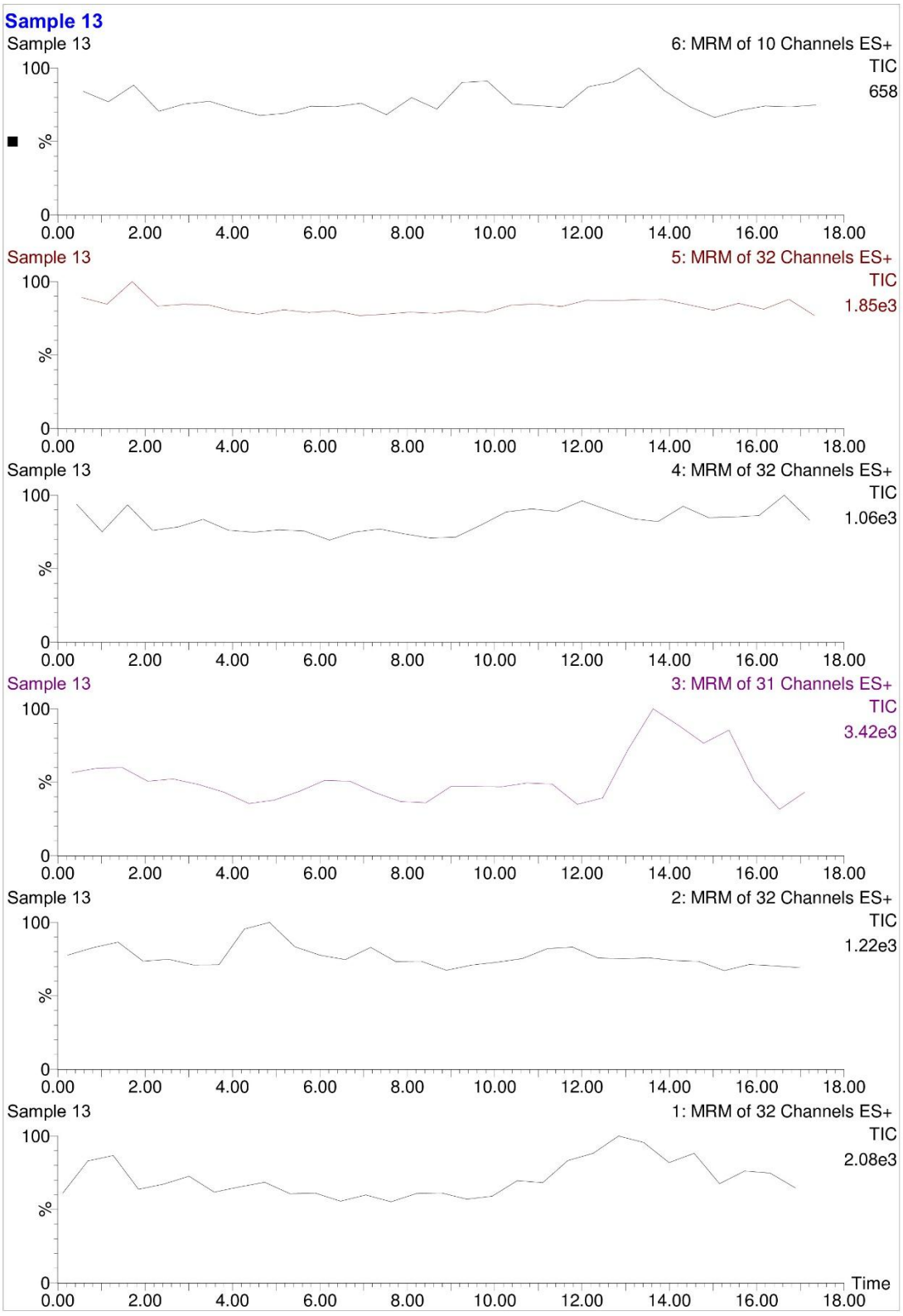

Sample 9
Sample 9
6: MRM of 10 Channels ES+
TIC
100
%
0
0.00
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
Time

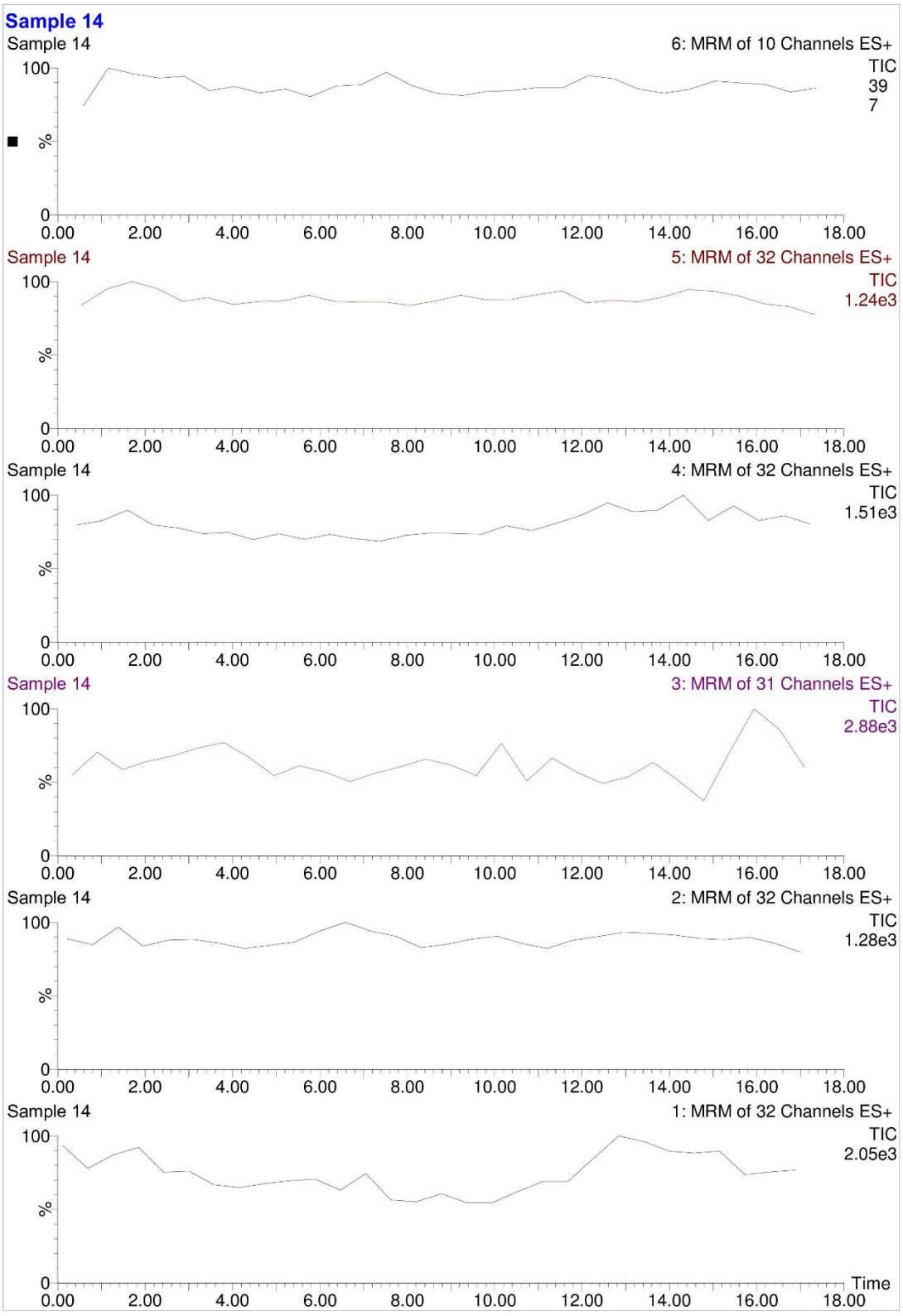

Sample 10
Sample 10
6: MRM of 10 Channels ES+
TIC
100
%
0
0.00
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
Time

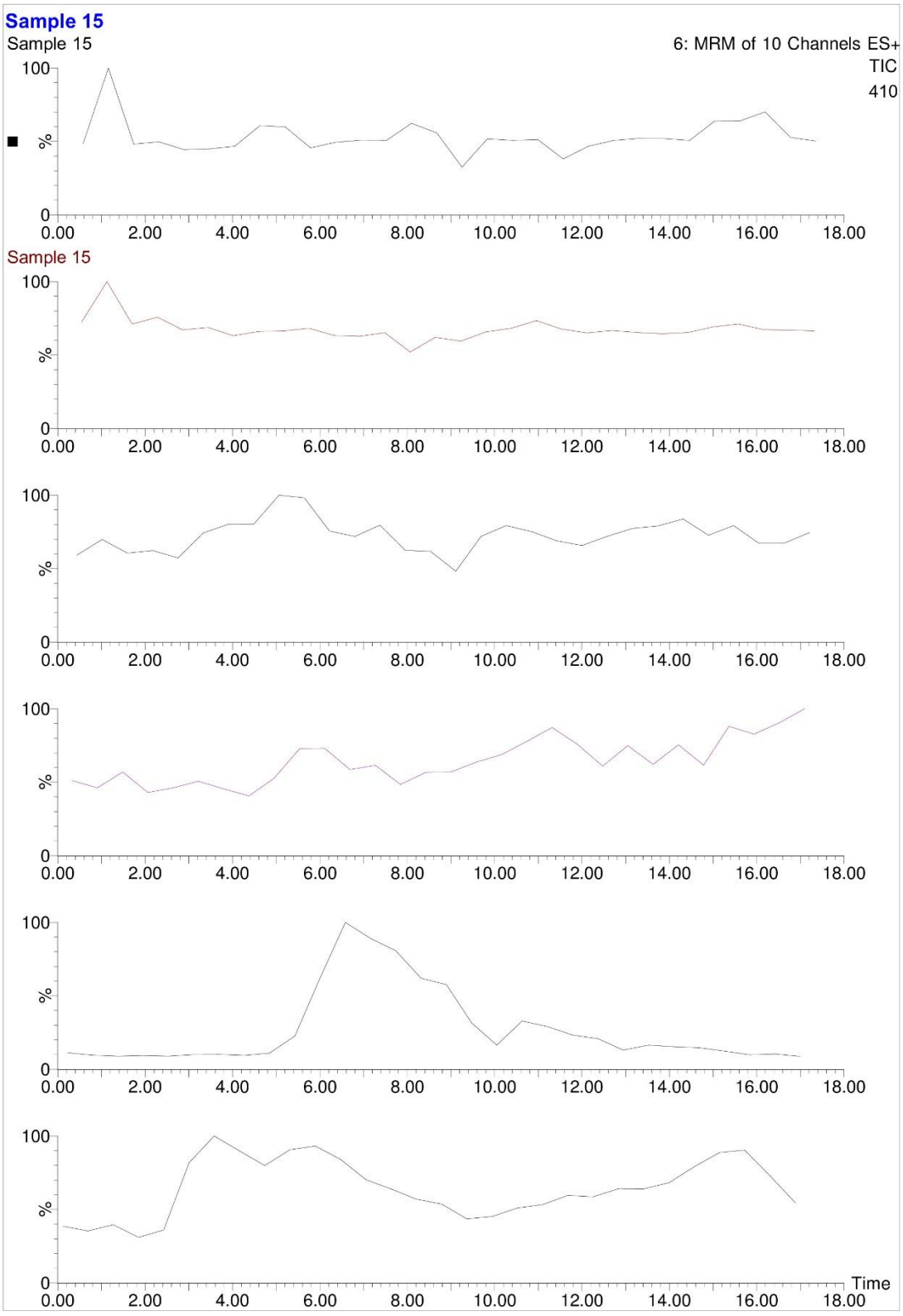

Sample 11
Sample 11
6: MRM of 10 Channels ES+
TIC
987
100
%
0
0.00
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
Sample 11
Time

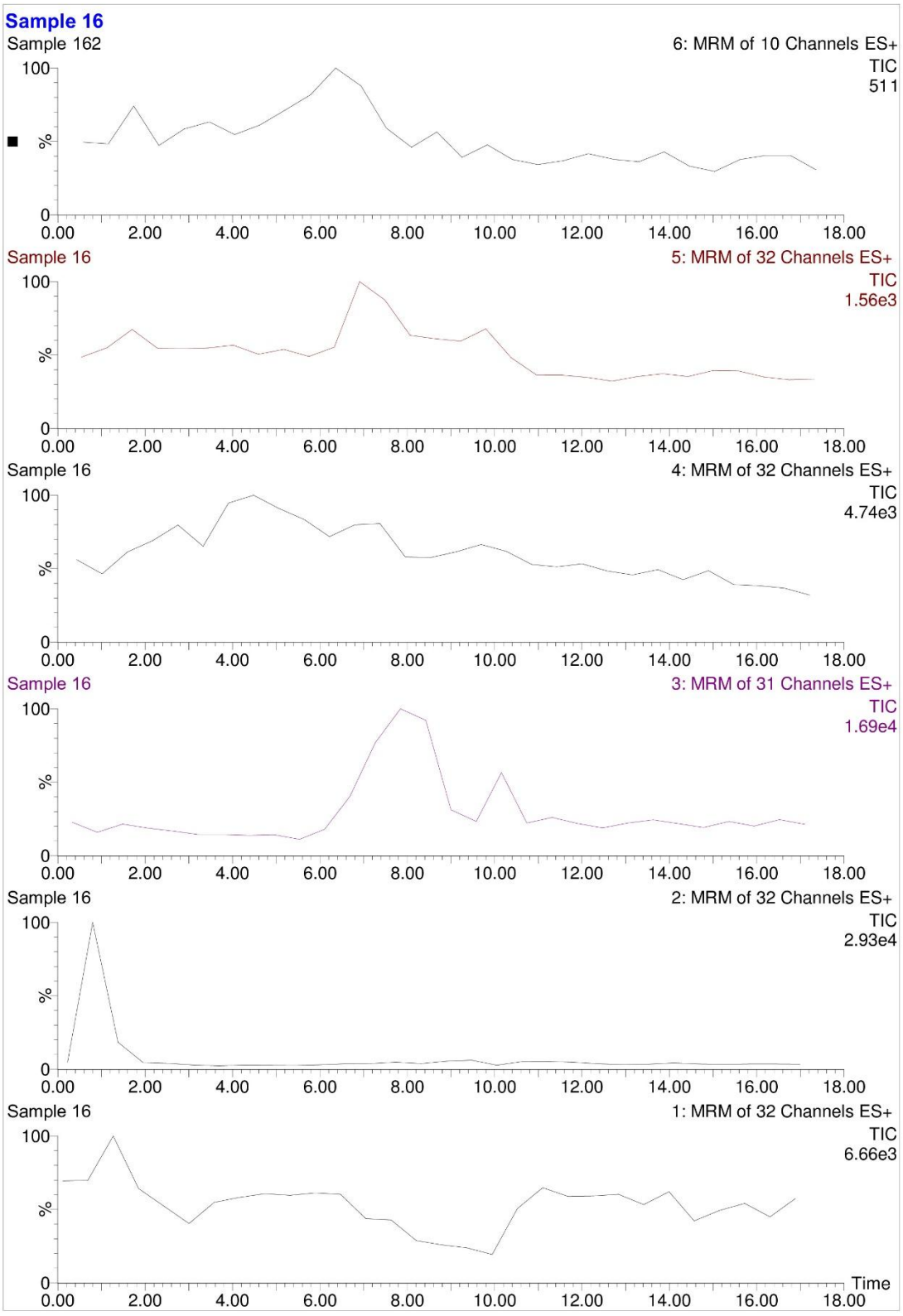

Sample 12
Sample 12
6: MRM of 10 Channels ES+
TIC
663
100
%
0
0.00
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
Sample 12
Time

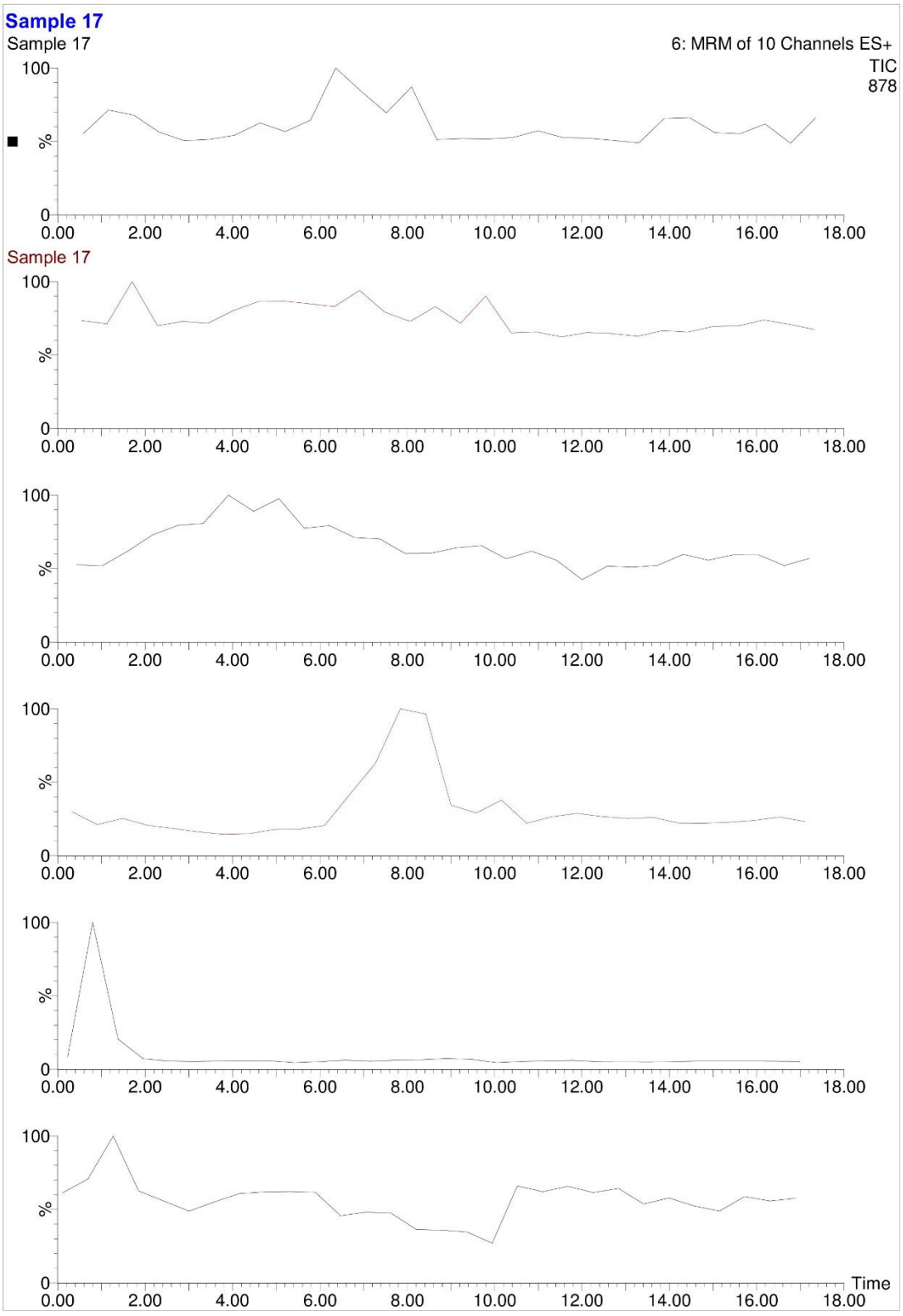

Sample 12
Sample 12
6: MRM of 10 Channels ES+
TIC
45
3
100
%
0
0.00
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
Sample 12
5: MRM of 32 Channels ES+
TIC
1.34e3
Sample 12
4: MRM of 32 Channels ES+
TIC
1.50e3
Sample 12
3: MRM of 31 Channels ES+
TIC
3.78e3
Sample 12
2: MRM of 32 Channels ES+
TIC
1.51e3
Sample 12
1: MRM of 32 Channels ES+
TIC
2.13e3
Time

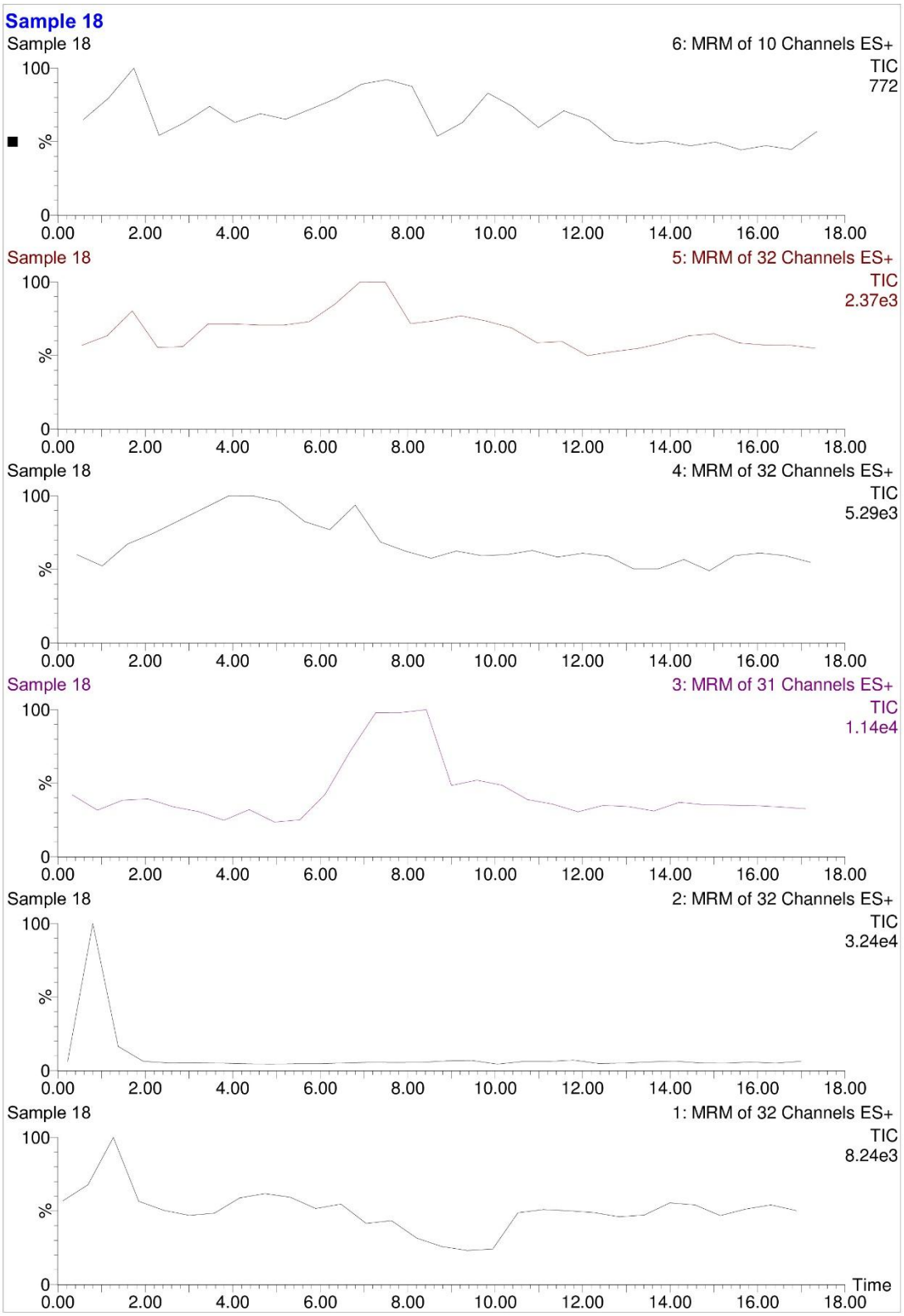

Sample 13
Sample 13
6: MRM of 10 Channels ES+
TIC
658
100
%
0
0.00
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
Sample 13
5: MRM of 32 Channels ES+
TIC
1.85e3
Sample 13
4: MRM of 32 Channels ES+
TIC
1.06e3
Sample 13
3: MRM of 31 Channels ES+
TIC
3.42e3
Sample 13
2: MRM of 32 Channels ES+
TIC
1.22e3
Sample 13
1: MRM of 32 Channels ES+
TIC
2.08e3
Time

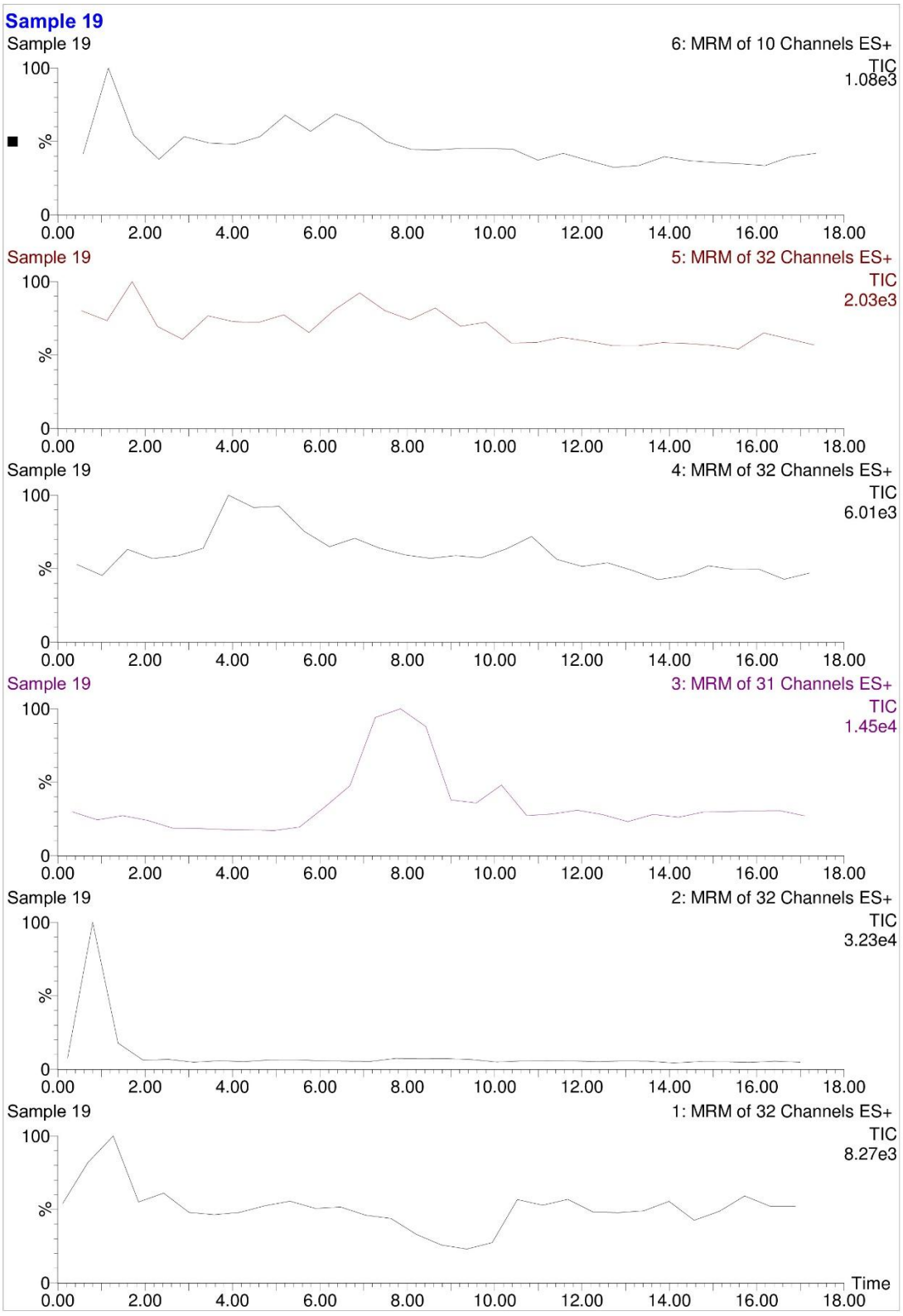

Sample 14
Sample 14
6: MRM of 10 Channels ES+
TIC
39
7
100
%
0
0.00
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
Sample 14
5: MRM of 32 Channels ES+
TIC
1.24e3
Sample 14
4: MRM of 32 Channels ES+
TIC
1.51e3
Sample 14
3: MRM of 31 Channels ES+
TIC
2.88e3
Sample 14
2: MRM of 32 Channels ES+
TIC
1.28e3
Sample 14
1: MRM of 32 Channels ES+
TIC
2.05e3
Time

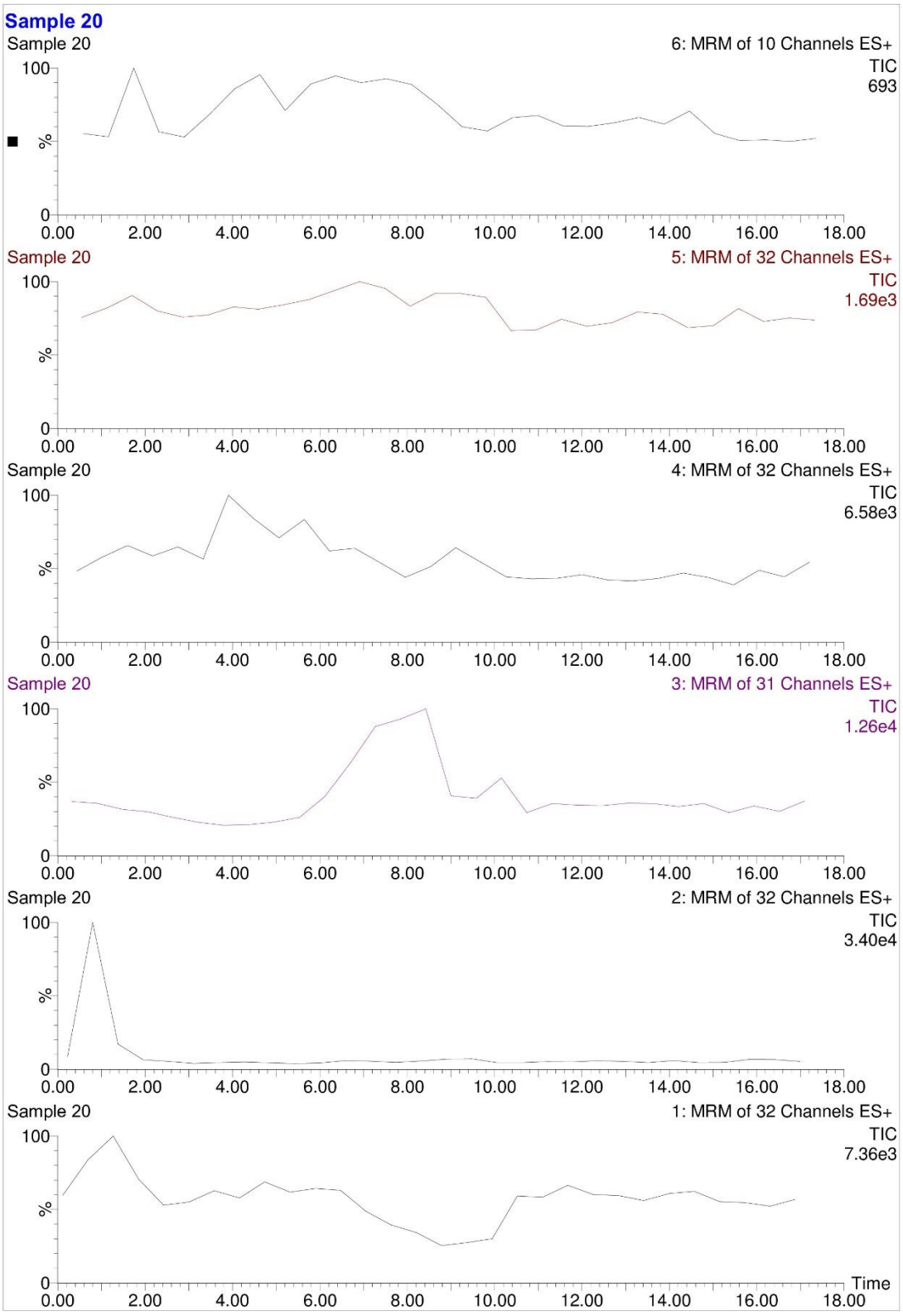

Sample 15
Sample 15
6: MRM of 10 Channels ES+
TIC
410
100
%
0
0.00
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
Sample 15
Time

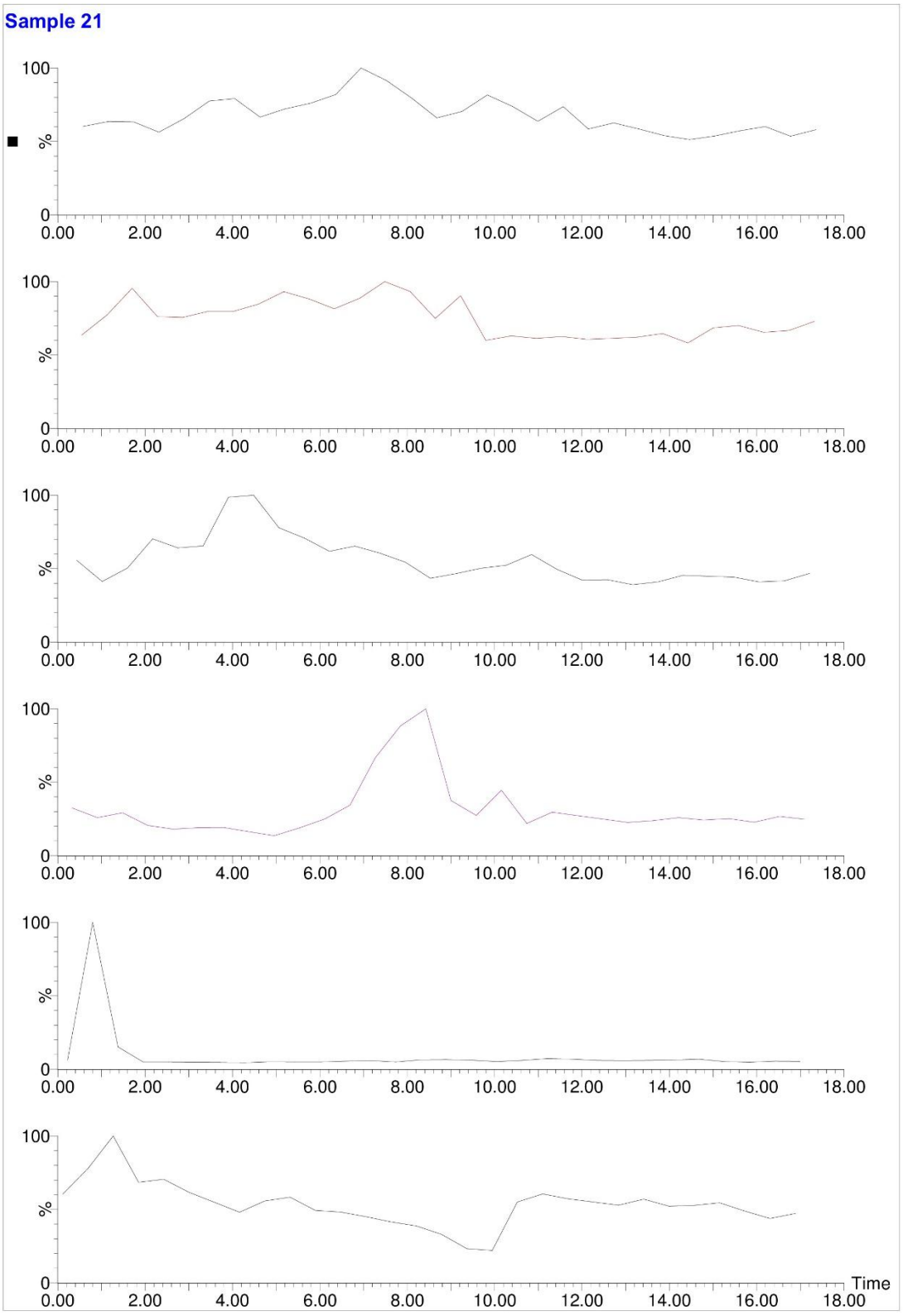

Sample 16
Sample 162
6: MRM of 10 Channels ES+
TIC
511
Sample 16
5: MRM of 32 Channels ES+
TIC
1.56e3
Sample 16
4: MRM of 32 Channels ES+
TIC
4.74e3
Sample 16
3: MRM of 31 Channels ES+
TIC
1.69e4
Sample 16
2: MRM of 32 Channels ES+
TIC
2.93e4
Sample 16
1: MRM of 32 Channels ES+
TIC
6.66e3
100
%
0
0.00
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
Time

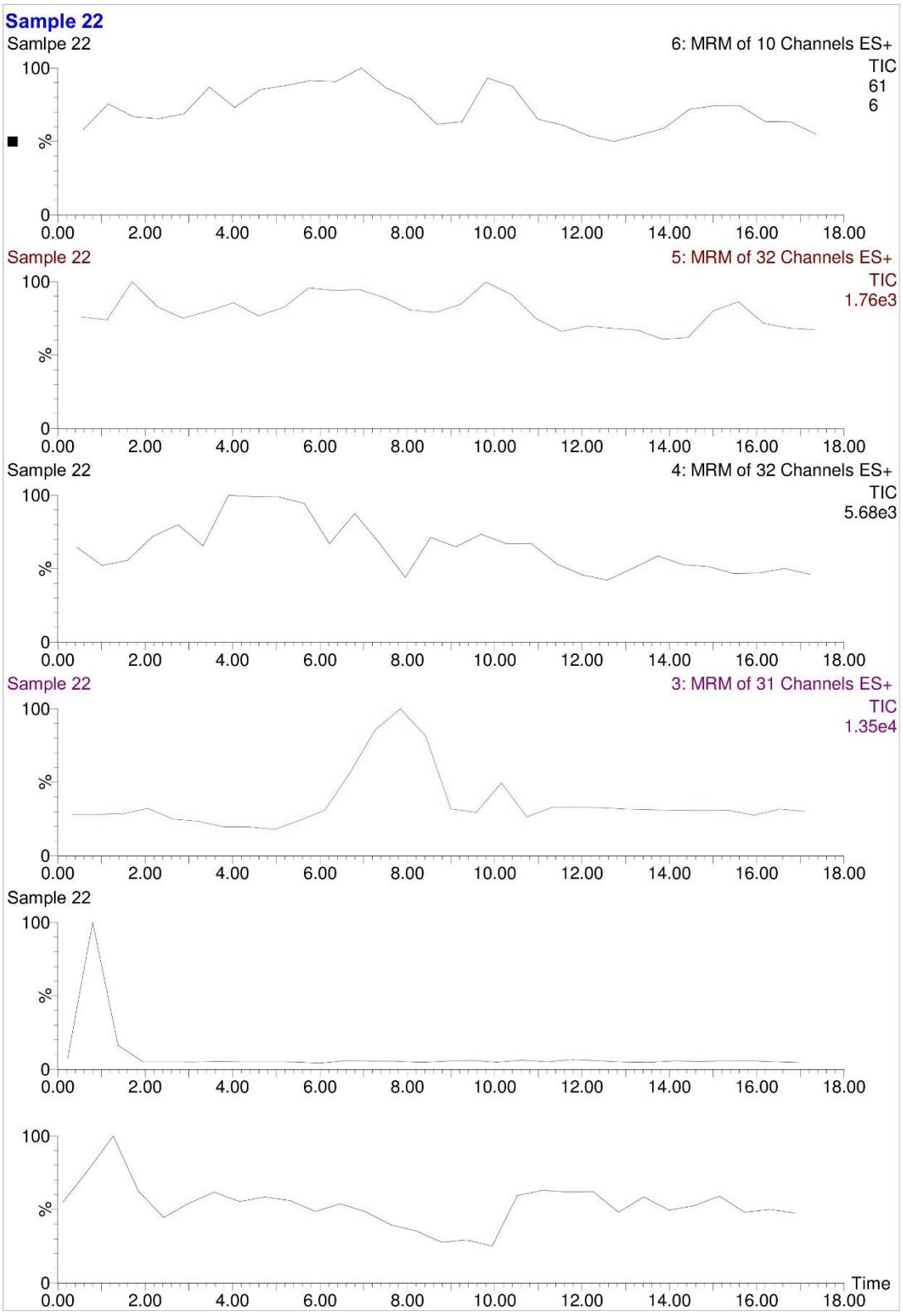

Sample 17
Sample 17
6: MRM of 10 Channels ES+
TIC
878
100
%
0
0.00
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
Sample 17
Time

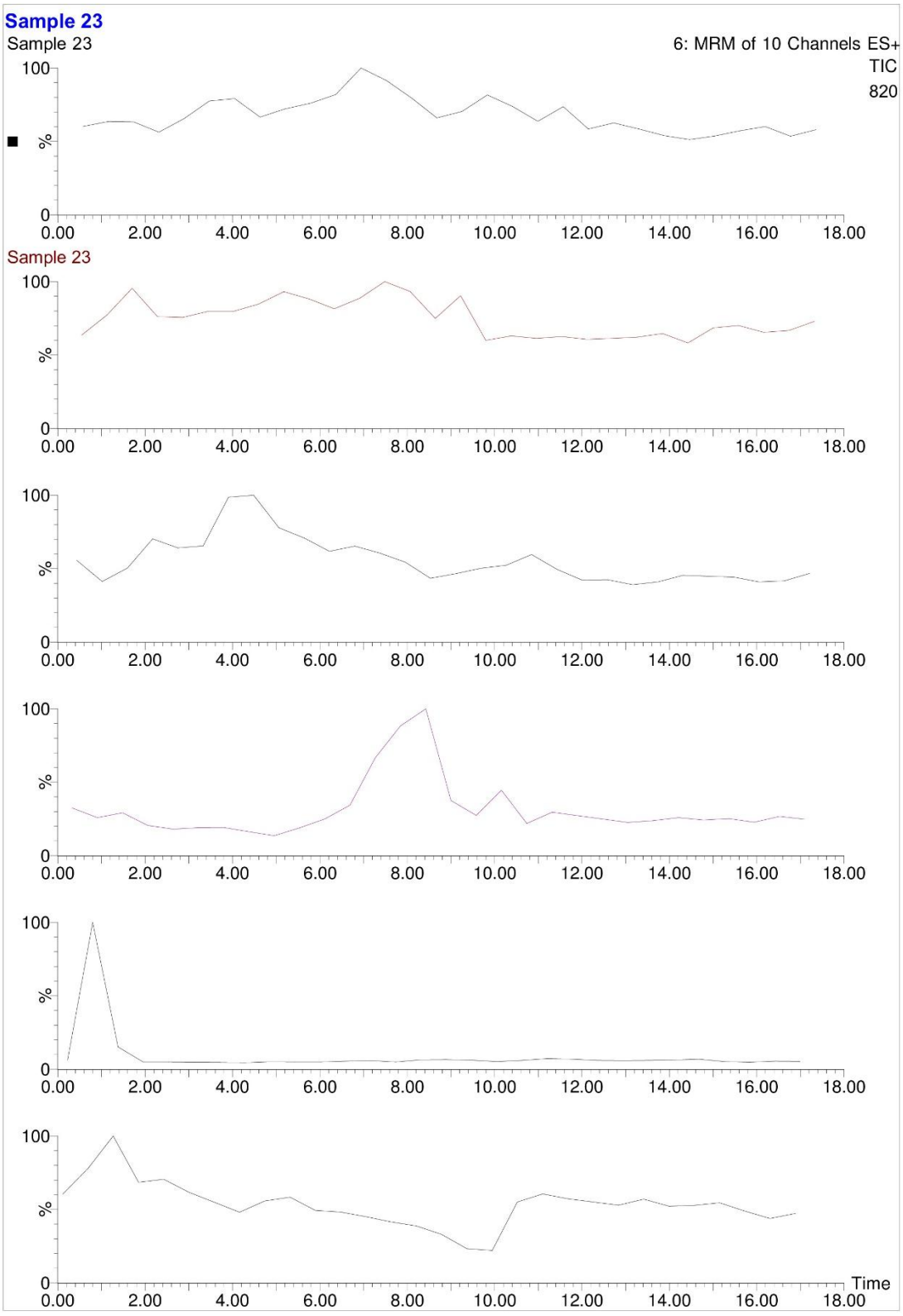

Sample 18
Sample 18
6: MRM of 10 Channels ES+
TIC
772
100
%
0
0.00
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
Sample 18
5: MRM of 32 Channels ES+
TIC
2.37e3
Sample 18
4: MRM of 32 Channels ES+
TIC
5.29e3
Sample 18
3: MRM of 31 Channels ES+
TIC
1.14e4
Sample 18
2: MRM of 32 Channels ES+
TIC
3.24e4
Sample 18
1: MRM of 32 Channels ES+
TIC
8.24e3
Time

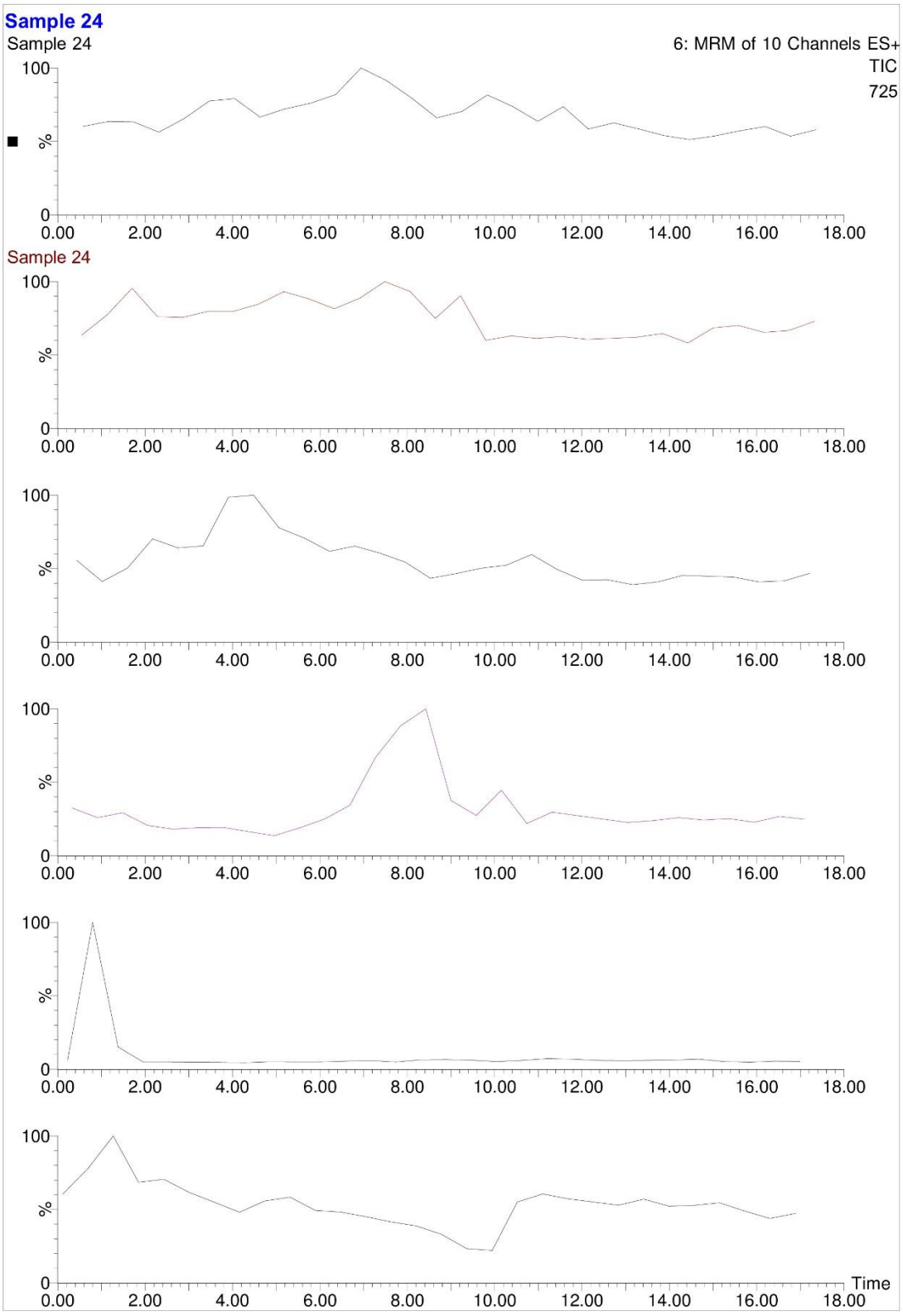

Sample 19
Sample 19
6: MRM of 10 Channels ES+
TIC
1.08e3
100
%
0
0.00
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
Sample 19
5: MRM of 32 Channels ES+
TIC
2.03e3
Sample 19
4: MRM of 32 Channels ES+
TIC
6.01e3
Sample 19
3: MRM of 31 Channels ES+
TIC
1.45e4
Sample 19
2: MRM of 32 Channels ES+
TIC
3.23e4
Sample 19
1: MRM of 32 Channels ES+
TIC
8.27e3
Time


Sample 20
Sample 20
6: MRM of 10 Channels ES+
TIC
693
100
%
0
0.00
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
Sample 20
5: MRM of 32 Channels ES+
TIC
1.69e3
Sample 20
4: MRM of 32 Channels ES+
TIC
6.58e3
Sample 20
3: MRM of 31 Channels ES+
TIC
1.26e4
Sample 20
2: MRM of 32 Channels ES+
TIC
3.40e4
Sample 20
1: MRM of 32 Channels ES+
TIC
7.36e3
Time


Sample 21
100
%
0
0.00
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
Time


Sample 22
Samlpe 22
6: MRM of 10 Channels ES+
TIC
61
6
100
%
0
0.00
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
Sample 22
5: MRM of 32 Channels ES+
TIC
1.76e3
Sample 22
4: MRM of 32 Channels ES+
TIC
5.68e3
Sample 22
3: MRM of 31 Channels ES+
TIC
1.35e4
Sample 22
Time


Sample 23
Sample 23
6: MRM of 10 Channels ES+
TIC
820
100
%
0
0.00
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
Sample 23
Time


Sample 24
Sample 24
6: MRM of 10 Channels ES+
TIC
725
Sample 24
100
%
0
0.00
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
Time