

FORENSIC APPLICATIONS OF SANDPAPER SPRAY IONIZATION -
MASS SPECTROMETRY (SPS-MS): DETECTION AND CHEMOMETRIC
PROFILING OF SMALL MOLECULES FOR PROHIBITED PRACTICES

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ABSTRACT

The illicit trade of pharmaceuticals and wood presents threats to public health, ecological stability, and global markets. This thesis examines Sandpaper Spray Ionization Mass Spectrometry (SPS-MS) as a rapid, field-deployable technique for direct analysis of solid samples in two distinct forensic applications. (1) SPS-MS was used to detect and identify active pharmaceutical ingredients (APIs) and excipients in pharmaceuticals, assessing product authenticity. (2) SPS-MS was further developed for direct sampling and analysis, obtaining chemical fingerprints for classification and origin tracing of Canadian and Brazilian wood samples, in the context of forestry crimes. Chemometric analysis using PCA revealed clear species differentiation, supporting its use in species-level classification. SPS-MS thus offers a versatile, cost-effective, and operationally simple screening method to supplement current MS techniques. Its portability, minimal reagent use, and possible integration into current analytical workflows make it well-suited for remote or resource-limited settings for frontline testing of fraudulent drugs and trafficked wood.

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Table of Contents

Abstract	ii
Acknowledgements	iii
Table of Contents	v
List of Abbreviations	vii
List of Tables	ix
List of Figures	x
Chapter 1: Introduction	1
1.1 Principles of Mass Spectrometry.....	2
1.1.1 Ion Source.....	3
1.1.2 Mass Analyzer & Detector.....	4
1.1.3 The Mass Spectrometer System.....	7
1.2 Mass Spectrometry in Forensics.....	8
1.3 Ambient Ionization Mass Spectrometry (AIMS).....	12
1.3.1 Direct Ionization (DI) Techniques.....	12
1.3.2 Sandpaper Spray – Mass Spectrometry (SPS-MS).....	13
1.4 AIMS Applications.....	14
1.4.1 Fraudulent Medicines.....	14
1.4.2 Wood Analysis.....	14
1.5 Research Objectives.....	16
Chapter 2: Forensic application of sandpaper spray ionization mass spectrometry (SPS-MS): Direct analysis of solid pharmaceutical formulations and edible cannabis products	18
2.1 Abstract.....	19
2.2 Introduction.....	19
2.3 Materials and Methods.....	22
2.3.1 Materials.....	22
2.3.1.1 Cannabis sativa and pharmaceutical products.....	22
2.3.1.2 Substrate and solvents.....	23
2.3.2 MS parameters and method.....	24
2.4 Results and Discussion.....	25
2.4.1 <i>Cannabis sativa</i>	25
2.4.2 Viagra® and Cialis®.....	26

2.4.3 Other pharmaceutical compounds	31
2.4.3.1 Metformin.....	32
2.4.3.2 Canagliflozin	32
2.4.3.3 Rosuvastatin	34
2.4.3.4 Ramipril	36
2.5 Conclusion	37
Chapter 3: Forensic application of sandpaper spray ionization mass spectrometry (SPS-MS): Chemotaxonomic Classification of Brazilian and Canadian Woods ...	38
3.1 Abstract.....	39
3.2 Introduction	39
3.3 Materials and Methods.....	42
3.3.1 Materials	42
3.3.1.1 Wood Samples	42
3.3.1.1 Sampling Device and Reagents	42
3.3.2 MS parameters and method	42
3.3.2.1 MS Parameters.....	42
3.3.2.2 Method.....	43
3.3.2.2.1 ESI(+)-MS	43
3.3.2.2.2 SPS(+)-MS.....	43
3.3.2.2.3 Data Processing.....	43
3.3.2.2.4 PLS-DA model (Modelling & Predictive Performance)	44
3.4 Results and Discussion.....	46
3.4.1 Tree Anatomy: Heartwood versus Sapwood	46
3.4.2 SPS Performance	47
3.4.3 Wood Species Discrimination	48
3.4.4 Modelling & Predictive Performance.....	54
3.4 Conclusion	59
Chapter 4: Conclusions and future work	60
Bibliography	62
Appendices	79
Appendix A. Supplementary Materials for Chapter 2.....	79
Appendix B. Supplementary Materials for Chapter 3.....	83

List of Abbreviations

Δ^8 -THC – Δ^8 -tetrahydrocannabinol

Δ^9 -THC – Δ^9 -tetrahydrocannabinol

ACN – Acetonitrile

AGC – Automatic Gain Control

AIMS – Ambient Ionization Mass Spectrometry

API – Active Pharmaceutical Ingredient

CBD – Cannabidiol

CID – Collision-Induced Dissociation

CRM – Charge Residue Model

DAPPI – Desorption Atmospheric Pressure Photoionization

DC – Direct Current

DEA – Drug Enforcement Administration

DEP – Direct Electrospray Probe

DI – Direct Ionization

DPS – Desalting Paper Spray

ED – Erectile Dysfunction

ELISA – Enzyme-Linked Immunosorbent Assay

ESI – Electrospray Ionization

GSR – Gunshot Residue

HPLC – High-Performance Liquid Chromatography

IEM – Ion Evaporation Model

IMS – Ion Mobility Spectrometry

IMS-MS – Ion Mobility Spectrometry–Mass Spectrometry

LC-MS – Liquid Chromatography–Mass Spectrometry

LTQ – Linear Ion Trap Quadrupole

MALDI – Matrix-Assisted Laser Desorption/Ionization

MS – Mass Spectrometry

MS/MS – Tandem Mass Spectrometry

MSⁿ – Multiple Stage Tandem Mass Spectrometry

m/z – mass-to-charge-ratio

NCE – Normalized Collision Energy

PCR – Polymerase Chain Reaction

PCA – Principal Component Analysis

PLS-DA – Partial Least Squares Discriminant Analysis

PS-MS – Paper Spray Mass Spectrometry

RF – Radio Frequency

SIM – Selected Ion Monitoring

SPS-MS – Sandpaper Spray Ionization Mass Spectrometry

SWGDRUG – Scientific Working Group for the Analysis of Seized Drugs

THC- tetrahydrocannabinol

THCA – as Tetrahydrocannabinolic acid

TLC – Thin Layer Chromatography

List of Tables

Table 3-1. Descriptors for heartwood samples used in the chemometric models	45
Table 3-2. Figures of merit of PLS2-DA model for wood classes	55
Table 3-3. Figures of merit of PLS2-DA model for wood classes	57
Table 3-4. Figures of merit for the PLS2-DA model used for region tracing	58

List of Figures

Figure 1-1. Adapted from Banerjee et al. [7]: Basic schematic representation of the process of electrospray ionization (ESI).....	4
Figure 1-2. Adapted from a. Anukunwithaya [11], b. Schwartz et al [12], and c. [13]: Basic schematic representations of a) a quadrupole, b) a linear ion trap, and c) a discrete dynode-type electron multiplier detector	6
Figure 1-3. Adapted from the Thermo Electron Corporation (now ThermoFisher Scientific) LTQ Hardware Manual. [14]: Schematic diagram of the Thermo Finnigan LTQTM Mass Spectrometer	7
Figure 2-1. Photograph of samples analyzed by SPS-MS in this study: (A) Cannabis sativa hard candy, (B) Viagra [®] , (C) Cialis [®] , (D) APO-Metformin [®] , (E) Invokana [®] , (F) Crestor [®] , and (G) Teva-Ramipril [®]	22
Figure 2-2. Chemical structures of the active pharmaceutical ingredients (APIs) found in the formulations evaluated under SPS-MS.	23
Figure 2-3. Mass spectrum for SPS(-)-MS of a Cannabis sativa hard candy with MeOH:H ₂ O (9:1) + 0.1% NH ₄ OH (v/v) as solvent and mass range of <i>m/z</i> 100–1000....	25
Figure 2-4. Mass spectra for (A) SPS(+)-MS and (B) SPS(-)-MS of a Viagra [®] tablet (API sildenafil) using MeOH as solvent for both ionization modes and mass range of <i>m/z</i> 150–650.	28
Figure 2-5. Mass spectra for (A) SPS(+)-MS using MeOH + 0.1% FA as solvent and (B) SPS(-)-MS using MeOH as solvent of a Cialis [®] tablet (API: tadalafil) with a mass range of <i>m/z</i> 100–450.	30
Figure 2-6. Mass spectrum for SPS(+)-MS of a tablet of APO-Metformin [®] (API: metformin), with MeOH + 0.1% FA (v/v) as the solvent and a (magnified) mass range of <i>m/z</i> 50–150.	32
Figure 2-7. Mass spectra for (A) SPS(+)-MS using MeOH + 0.1% FA as solvent and (B) SPS(-)-MS using MeOH as solvent of an Invokana [®] tablet (API: canagliflozin) with a mass range of <i>m/z</i> 100–1000.....	33

Figure 2-8. Mass spectra for (A) SPS(+)-MS using MeOH + 0.1% FA as solvent and (B) SPS(-)-MS using MeOH as solvent of a Crestor® tablet (API: rosuvastatin) with a mass range of <i>m/z</i> 100–1000.	35
Figure 2-9. Mass spectra for (A) SPS(+)-MS using MeOH + 0.1% FA as solvent and (B) SPS (-)-MS using MeOH as solvent of a tablet of Teva-Ramipril® (API: ramipril) and a mass range of <i>m/z</i> 100–1000.	36
Figure 3-1. Chemometric analysis graphing a) a PCA Scores Plot and b) a Q Residuals Plot from different tree anatomies, Heartwood (sample 25), Sapwood (Sample CG3), and Heartwood + Sapwood (Sample CG2) of <i>Carapa guianensis</i>	47
Figure 3-2. a) ESI(+)-MS and b) SPS(+)-MS spectra of a <i>Swietenia macrophylla</i> sample. Mass range: <i>m/z</i> 800 – 1000.	48
Figure 3-3. PCA Scores Plot of different wood samples taken from the heartwood: <i>Carapa guianensis</i> (Sample 25), <i>Swietenia macrophylla</i> (Sample 3269), <i>Prunus serrulata</i> (A1), <i>Populus tremuloides</i> (B1), <i>Fraxinus americana</i> (C1), <i>Juglans nigra</i> (D1), <i>Prunus serotina</i> (E1), <i>Betula papyrifera</i> (F1), <i>Acer saccharum</i> (G1), and <i>Hymenaea courbaril</i> (Sample HC3).	49
Figure 3-4. Dendrogram produced from HCA for various wood samples: <i>Cedrela odorata</i> (Sample 1800) <i>Carapa guianensis</i> (Sample 25), <i>Prunus serrulata</i> (Sample A1), <i>Juglans nigra</i> (Sample D1), <i>Prunus serotina</i> (Sample E1), <i>Acer saccharum</i> (Sample G1), <i>Hymenaea courbaril</i> (HC3), and <i>Swietenia macrophylla</i> (SM2).	50
FIGURE 3-5: ESI(+)-MS/MS spectra of the precursor ions <i>m/z</i> a) 883 (swietenitin I), b) 885 (swietenitin K), c) 911 (2,11-diacetoxyswietenalide D, and d) swietenitin M. Mass range: <i>m/z</i> a,b) 400 – 1000, c,d) 450 – 950.	52
Figure 3-6. ESI(+)-MS/MS spectra of the precursor ion <i>m/z</i> 493 identified as methyl angolensate. Mass range: <i>m/z</i> 200 – 500.	53
Figure 3-7. ESI(+)-MS/MS spectra of the precursor ion <i>m/z</i> a) 360 and 609 identified as a disaccharide and khivorin respectively. Mass range: a) <i>m/z</i> 100 – 400, b) <i>m/z</i> 200 – 700.	53

Figure 3-8. Figure 3-8: Y prediction graph for *Acer saccharum* wood specie PLS2-DA on
a) ESI(+)-MS and b) SPS(+)-MS..... 54

Chapter 1: Introduction

1.1 Principles of Mass Spectrometry

Mass spectrometry (MS) is an indispensable modern analytical technique used across diverse laboratory settings, owing to the breadth of chemical information it provides and the broad spectrum of uses across multiple fields. This analytical capability stems from the ability of the instrument to exploit a fundamental property of ions, subsequently developed to analyze their mass-to-charge ratios (m/z). This now common laboratory instrument is the result of numerous technological advancements throughout the 20th century, with further major developments to this day, across multiple scientific disciplines. The invention of MS is primarily attributed to Sir Joseph John Thomson (J.J. Thomson), who, in 1912, had initially called its first iterations in 1912 a “parabola spectrograph”. He demonstrated that charged particles such as electrons, and later ions with the contributions of Francis Aston, can be made to follow parabolic trajectories when placed in a magnetic field, allowing them to be spatially separated then recorded on photographic plates.[1] Branching out from the field of Spectroscopy (from the ancient Greek *skopeîn*, as the root of the suffix *-scope*, which means “to look at/examine”), [2] the first “true” mass spectrometer (from the Greek *métron*, as the root of the suffix *-meter*, which means “to measure”) [3] was developed by Arthur Jeffrey Dempster in 1918, enabling quantification of charged species by using a Faraday cup electrometer as the detector, moving from the light to the electronic domain. Dempster improved the transmission of ions, focusing the ion beam with a uniform magnetic field through a semicircular ion path. Dempster also developed one of the first ion sources, a device that ionizes and vaporizes the sample, the two properties necessary for analyzing a compound’s m/z via MS, called electron impact (EI), an ion source still commonly used today. [1, 4, 5] Numerous other developments and refinements have been made to the fundamental operation of the MS instrument by various scientists across different fields over the years, with only a few key contributions highlighted here for the purposes of this study.

1.1.1 Ion Source

The ion source is a crucial component of the MS instrument, responsible for initially ionizing, volatilizing, and introducing applicable analytes in a sample, depending on the ion source, into the instrument. For the fundamental property m/z of compounds to be analyzed, analytes must exist or be turned into this volatile ionized form (ie. gas-phase ions). Both these requirements were met for non-gaseous neutral compounds, when John Fenn, crediting prior related experiments conducted by Malcolm Dole, invented electrospray ionization (ESI) in the late 1980s. The underlying principle involves dissolving non-volatile solutes in a volatile solvent to generate highly charged droplets, which, upon progressive solvent evaporation, ultimately produces gas-phase ions. As this does not involve the introduction of high energy into the system, such as with EI, this technique is classified as a “soft” ionization technique, able to ionize large thermolabile compounds without considerable fragmentation and decomposition, which is particularly useful for organic compounds, including nucleic acids and proteins. [1, 6]

In order to first generate the charge, under ESI, an electrical potential is applied to the analyte-containing solution as it passes through a narrow capillary, producing a double layer of separated opposing charges. The interface to the rest of the MS instrument, the ion transfer tube, is set to the opposite electrical charge, effectively functioning as a counter electrode. A Taylor Cone is produced as the charged solution breaks surface tension and is attracted to the oppositely charged ion transfer tube, producing progressively densely charged microdroplets as solvent evaporation occurs. To assist in the nebulization and promote efficient desolvation of droplets, a neutral sheath gas, typically nitrogen, is introduced coaxially around the end of the electrospray capillary. When the charge density of a microdroplet reaches a critical threshold known as the Rayleigh limit, or the point at which the electrostatic repulsive forces between like charges exceeds the forces holding the droplet together, the droplet undergoes coulombic fission, ejecting smaller progeny droplets. This process continues until the “naked charged analyte” is produced). [7] (Figure 1-1) Although extensively studied, the exact mechanism for this last step, wherein free ions emerge from charged droplets, is yet to be fully understood. Various models such as the charge residue model (CRM)

and the Ion evaporation Model (IEM) aim to provide a complete and accurate mechanism, with the operative model for a given ion determined by factors such as ion size, charge density, and droplet volume. [8]

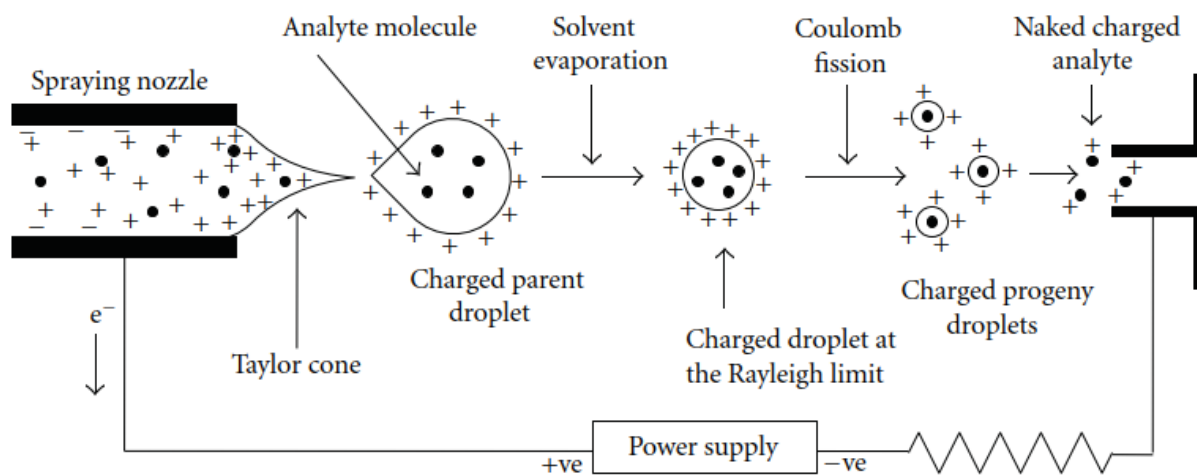


FIGURE 1-1 Basic schematic representation of the process of electrospray ionization (ESI)

1.1.2 Mass Analyzer & Detector

A quadrupole mass filter, commonly and simply referred to as a “quadrupole”, is a type of mass analyzer, or the part of the MS instrument which separates ions according to their m/z by exploiting the stability of ion motion in a quadrupolar electric field. Initially patented in 1953 by Wolfgang Paul, working with Helmut Steinwedel, the device consists of four parallel cylindrical metallic rods arranged in pairs of opposing charges, hence, quadrupole. (Figure 1-2a) A combination of radio frequency (RF) and direct current (DC) are applied to the quadrupole, wherein ions with stable trajectories travel longitudinally across the quadrupole due to their specific m/z , hence a “mass filter”. These ions with stable trajectories at specific RF/DC ratios, also called “resonant ions”, follow sequential stability diagrams based on Mathieu’s equations, wherein the electric potential (V), voltage (U), and angular frequency (ω) dictate the X- and Y- motion of ions, orthogonal to the length of the quadrupole, in an applied electric field. Ions can be isolated by their m/z in a scan mode called SIM (selected ion monitoring). By modulating the amplitude of the RF and DC voltages while maintaining a constant ratio, effectively keeping ω

constant, the quadrupole can also sequentially stabilize ions of varying m/z . This allows ions within a broad range of m/z values to pass through in a scan setting labelled as “full scan”. The device can also function in an RF-only mode, wherein DC voltage is set to zero while varying oscillations of RF. This stabilizes ion trajectories, allowing for the transmission of ions without mass filtering.

The linear ion trap is another type of mass analyzer, similar to a quadrupole, wherein hyperbolic rods are applied with an RF, but also axially with an applied DC potential to electrodes at both ends. (Figure 1-2b) This allows for temporary storage of ions within a defined volume. RF is gradually increased destabilizing ions of specific m/z , ejecting them out of the mass analyzer. Two slits in the rods, one on each side, allow for the ions to escape radially according to their m/z when the RF is modulated. This allows for different types of MS analyses to be performed. [9, 10] Detectors are then positioned on each side where the ions are ejected. Ejected ions are directed towards electron multipliers as the detectors. Upon impact with the first of a series of dynodes, a particularly shaped electrode designed to accelerate and multiply colliding electrons, the ion, now referred to as the incident ion, causes a cascade of electron production or secondary electrons under a maintained potential gradient, producing an amplification effect known as secondary electron emission. This process results in a measurable electrical signal proportional to the incident ion flux. The electrical signal produced can then be inputted and translated by an electronic data system for detection and graphing. (Figure 1-2c) [14]

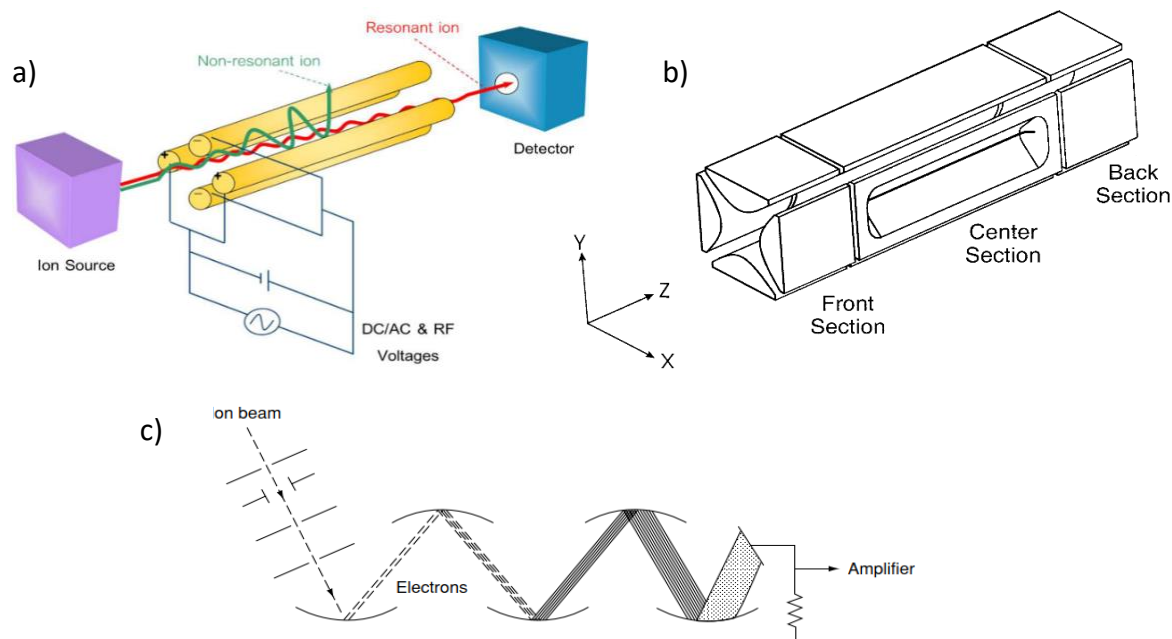


FIGURE 1-2 Basic schematic representations of a) a quadrupole, [11] b) a linear ion trap, [12] and c) a discrete dynode-type electron multiplier detector.[13]

The conceptual foundation of combining and arranging mass analyzers in a sequential configuration, now known as tandem MS (MS/MS), was first proposed by Fred McLafferty in the early 1980s. His interest lied in finding an approach to relate chemical structure to the mass spectrum obtained in MS, which would be particularly useful for detected unknown compounds, or confirming compounds present within a sample. Although molecular weight (MW) offers useful preliminary information, it is frequently inadequate for definitive compound identification, as isomeric and other species can exhibit identical m/z values. Such chemical species are referred to as “isobars”. MS/MS enhances the ability of the MS instrument to identify compounds, greatly reducing the number of possible compounds by their MW alone. The basic operation of MS/MS involves a first stage mass analyzer (q_1) isolating a particular m/z , for an analyte of interest, to pass through called the “precursor ion”. The precursor ion then passes through what is referred to as a reaction cell (q_2), wherein the product ion undergoes collision-induced dissociation (CID). CID is the process of kinetically activating the precursor ion, via further DC modulation, to collide with an inert gas (eg. helium), fragmenting it to smaller fragments called “product ions”. Hence, this type of MS/MS is called a “product ion scan”. The resultant spectrum or “fragmentation profile” from such a scan can now be used for structure elucidation of the molecule. [1, 15]

Various types of MS/MS scans can be performed by varying the operation of the mass analyzers, and the reaction cell, (q1, q2, and q3) for different purposes relating to detecting and quantifying the intact ion, also known as the “molecular ion” (M^+), or the resultant product ions. This can be performed spatially with mass analyzers sequentially attached, or temporally (Tandem in-time) wherein the same mass spectrometer is able to perform each operation (q1, q2, and q3) in the sequence desired. Tandem in-time can trap resultant product ions for additional instrumental operations, instead of immediately ejecting them to be detected. This allows for the additional capability of serially performing multiple CID cycles, called MS^n , wherein n is the number of cycles as resultant product ions, particularly those of complex molecular structures, are further fragmented. However, MS^n is limited by the number of ions remaining after each cycle that can be reliably detected [16]

1.1.3 The Mass Spectrometer System

The MS system used for the following experiments is the Thermo Finnigan LTQ™ Mass Spectrometer. It uses a linear ion trap mass analyzer with a quadrupole mass filter. The basic operation of which have been briefly outlined below. The LTQ is also comprised of various ion optics and an atmospheric pressure ionization (API) source. (Figure 1-3)

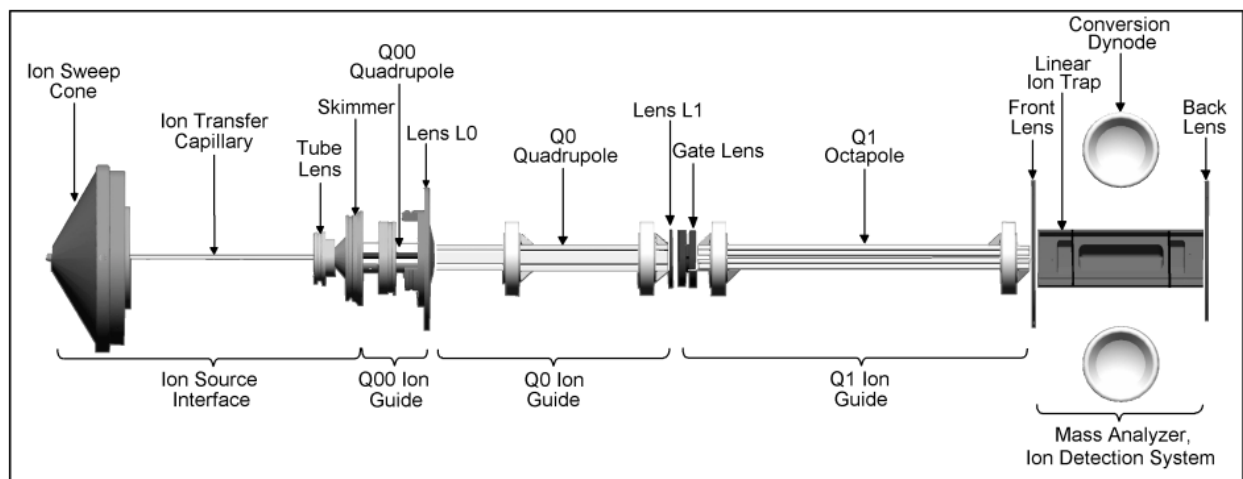


FIGURE 1-3 Schematic diagram of the Thermo Finnigan LTQ™ Mass Spectrometer, adapted from the Thermo Electron Corporation (now ThermoFisher Scientific) LTQ Hardware Manual. [17]

Ion optics, in an MS system, are specially made electrically charged components that act as ion guides, ensuring efficient transmission of ions from one section of the MS system to the next. These can vary in geometry and function from flat and conical metal discs with pinhole openings (eg. gate lenses), to heated metal tubes, to an octapole that ensures minimal ion loss from one mass analyzer to the next by stabilizing ion trajectory, similar to a quadrupole. To achieve such, vacuum is maintained throughout the MS system of progressively decreasing pressures (from ~ 1 Torr to 0.75×10^{-5} Torr in the LTQ). The API interface can be fitted with an ESI ion source or any other atmospheric pressure ion source. As the ions produced by ESI enter the MS system, they pass through the ion transfer capillary (also called the ion transfer tube). The ion transfer tube, on top of being electrically charged, is heated via an adjoined heater block to temperatures of up to 400 °C. This provides additional desolvation, which, in turn, helps achieve greater signal. After which, the skimmer acts as a barrier skimming off neutrals and solvated ions of the expanding plume, as the segment transitions from a low vacuum (~1 Torr) to a high vacuum system (10^{-5} Torr). Injection of the ions, for timing and/or collection, from one section to the next is controlled by gate lenses which block ions from passing through by modulation of DC potential. Ions finally ejected from the mass analyzer hit a pair of conversion dynodes, a type of electron multiplier, for the electrical detection system. The LTQ Mass Spectrometer uses continuous dynode electron multipliers, wherein a single anode is connected to a single horn-shaped dynode for each electron multiplier module. [14, 17]

1.2 Mass Spectrometry in Forensics

Mass spectrometry has a long history of use in forensic science, evolving alongside the field to become one of its most powerful and widely adopted analytical tools. The utility of mass spectrometry (MS) in forensic science was evident even in its early adoption, with one of the first forensic laboratories employing MS in 1973 already demonstrating capabilities that are similar to the many widespread applications today. [18] Its applications have been vast, including but not limited to: drug analysis, gunshot residue analysis, ignitable liquids residue analysis, explosives analysis, document forgery, and doping analysis in sports. [19, 20, 21, 22, 23, 24] MS has demonstrated its utility and

versatility, particularly in the field of forensic science, to the extent that it has been described as “the near universal test for identifying unknown substances.” [25] Although various other techniques and analytical instruments have been used in place of, supplementing, or orthogonal (examining a different property of compounds) to MS techniques, MS has been routinely used to provide chemical information in forensic investigations. The necessary validation of analytical techniques according to established figures of merit (ie. sensitivity, precision, accuracy, limits of detection and quantification, dynamic range, etc.) necessitates that the continual pursuit of novel methodologies, particularly within academic research, be systematically and synergistically integrated into the developmental pipeline within the various sub-disciplines in forensic science. This integration is essential for such innovations to be adopted in practice or even improve and/or replace longstanding protocols and instrumentation. In addition to the validation of the performance of the analytical method, additional considerations are in place for forensic applications, particularly for on-site analyses, such as portability, robustness, level of skill required by the operator, cost of operation, reagent availability, instrumental maintenance, etc. These considerations pose similar challenges for other analytical techniques such as gas or liquid chromatography (GC/LC), immunoassays like enzyme-linked immunosorbent assay (ELISA), polymerase chain reaction (PCR), and ion mobility spectrometry (IMS). These are likewise being actively developed to address these limitations to improve their suitability for forensic applications. In forensic science, analytical techniques are often categorized as either “screening” or “confirmatory”, depending on their intended evidentiary role. Screening methods provide rapid, preliminary assessments, while confirmatory techniques yield definitive identifications that meet admissibility standards. Mass spectrometry (MS) is one of the few analytical techniques capable of fulfilling both roles. Its adaptability, ranging from rapid ambient ionization-based screening to MS-coupled methods such as LC-MS and IMS-MS for confirmatory analysis, has contributed to its classification as a “Category A” method by the Scientific Working Group for The Analysis of Seized Drugs (SWGDRUG) the of the United States Drug Enforcement Administration (DEA), as these MS-coupled methods incorporate orthogonal analytical techniques by design. [26] The importance of such orthogonal

supplementation can be exemplified in a murder case in 1978, wherein MS was used to detect curare, a potent neurotoxin from an alkaloid-containing plant extract, when immunoassay and chromatography had failed. [27] These techniques remain relevant today, particularly in light of legal distinctions between structurally similar cannabinoids (e.g., Δ^9 /delta-9-THC, Δ^8 /delta-8-THC, and THCA), as the ratification of the 2024 Farm Bill amended the 2018 Farm Bill (U.S. Agriculture Improvement Act of 2018) by redefining the threshold of THC-containing products, from allowing no more than 0.3% delta-9-THC by dry weight to no more than 0.3% total THC, hampering industries such as hemp production. This stems from the isomers being detected in the same peak in MS and various chemical interconversions between cannabinoids (ie. thermal degradation of THCA via decarboxylation into delta-9-THC). [28, 29, 30, 31] The versatility of MS in enabling both screening and confirmatory applications establishes it as an invaluable component of contemporary forensic analysis. The modular compatibility of MS instrumentation is a key advantage in forensic science, where samples are often chemically complex, available only in trace quantities, and/or vary in their physical state. MS can be coupled with gas or liquid chromatography (GC-MS, LC-MS) when additional separation is needed, or used without chromatographic separation when rapid screening is prioritized. Instrument parameters, ionization modes, and sampling techniques can all be tailored to the specific requirements of the sample matrix and the type of information required for evidentiary interpretation. The ability of MS to provide both MW and fragmentation profiles allows for the identification of compounds with high specificity and sensitivity, even in the absence of reference standards.

Beyond its broad analytical applicability and classification as a Category A technique by SWGDRUG, the continued legal admissibility and long-standing implementation of MS in forensic laboratories highlight its established role in evidentiary analysis. MS results in legal records are difficult to retrieve, as evidentiary details, including analytical findings, are rarely made publicly available unless reported by courtroom journalists. Different kinds of MS evidence, such as the examples previously discussed, are often found through scientific literature, news media, and case law databases. [32] Institutional bodies such as the Organization of Scientific Area

Committees for Forensic Sciences (OSAC) and the Scientific Working Group for the Analysis of Seized Drugs (SWGTOX) have reinforced MS adoption through standardized and validated protocols in evidentiary frameworks. [33] However, despite its analytical strength, MS-coupled methods such as GC-MS and LC-MS are still constrained by laboratory bottlenecks, including low throughput, complex sample matrices, and limited resources, which slow the integration of novel technologies and techniques.

MS, in certain instrumental configurations, is capable of analyzing samples without the need for extensive sample preparation, which is beneficial in forensic workflows wherein sample quantity is limited or in scenarios with time constraints. Developments in MS, such as ambient ionization methods like desorption electrospray ionization (DESI), desorption atmospheric pressure photoionization (DAPPI), and direct analysis in real time (DART), offer solutions through rapid, minimal-preparation analysis compatible with field deployment. [34] While ambient ionization methods are not yet widely implemented in routine forensic workflows, their demonstrated ability for direct analysis bulk samples, even from surfaces, with minimal preparation and high throughput make them promising options for future integration. Ambient ionization techniques have been successfully applied to a wide array of substances of interest in a forensic setting, including explosives, trace evidence, dyes, and controlled substances, with minimal solvent use and the potential to streamline casework involving high sample volumes or limited material. Although their current use is largely confined to academic settings, they "hold great promise for incorporation into forensic laboratories" [35] as further validation studies, rugged instrument development, and procedural standardization continue to be developed. MS continues to evolve with ongoing advancements to expand applicability to a greater variety of sample types, improve analytical throughput, and increase the level of certainty in chemical identification, all of which are critical in addressing the demands of contemporary forensic science.

1.3 Ambient Ionization Mass Spectrometry (AIMS)

Recent developments have further expanded the range of operational environments in which MS can be utilized. Ambient ionization mass spectrometry (AIMS), as previously mentioned, allows for the direct ionization of samples under ambient conditions with minimal sample preparation, enabling faster analyses and reduced complexity of analytical workflows. [36, 37] Additionally, advances in compact and portable MS systems have introduced the possibility of performing MS analyses outside of traditional laboratory settings. [38] Together, these innovations make MS particularly well-suited for on-site forensic analysis, where the ability to obtain rapid results with minimal sample handling and portable instrumentation is critically important. While these newer instrumental setups are being evaluated for reproducibility, stability, and overall analytical performance under real-case conditions, they represent a technically viable extension and further advancement of conventional MS systems. In the context of forensic science, where analytical confidence, operational efficiency, and adaptability to diverse sample types are critical, MS remains one of the most powerful and broadly applicable techniques currently available.

1.3.1 Direct Ionization (DI) Techniques

AIMS methods have been used in forensic applications from the analysis of gunshot residues (GSR), [39] screening solid pharmaceuticals, [40] determining document authenticity, [41] to chemical imaging of latent fingerprints for illicit drugs. [42] While various ionization methods have enabled these types of analyses, one of the most common and accessible approaches is direct ionization (DI), which can be initially attributed to the invention of the direct electrospray probe ionization (DEP), as a straightforward ionization technique without extensive modification to the MS system. [43] This was achieved by applying a current through a copper coil to ionize solutions, producing the same ESI mechanism of Taylor cone formation and successive microdroplet desolvation. Nowadays, various DI methods, following the same ESI-like ionization mechanism, have been developed utilizing various low-cost and easily-accessible substrates such as thin layer chromatography (TLC) plates, [44] a wooden toothpick, [45], a cotton thread, [46] and even directly from a needle biopsy. [47] One of

the most notable DI techniques is paper spray (PS) ionization, which uses a triangular-cut piece of paper as the substrate. [48] Various paper substrates, geometries, and instrumental parameters have been optimized for stable and strong signal production. [48, 49, 50] PS-MS is versatile in terms of its ability to analyze various sample types by modifying the substrate, such as coating with silica, carbon nanotubes, and even aptamers. PS-MS also allows for supplementary techniques such as LC separation and sampling techniques for complex matrices such as plasma collection paper cards, to be integrated into the analytical workflow. [51] As an illustrative example, recently, PS-MS has been utilized in a pilot test study as a harm reduction tool for on-site checking of illicit drugs in the streets of British Columbia, CA. [52, 53]

1.3.2 Sandpaper Spray – Mass Spectrometry (SPS-MS)

Sandpaper Spray Ionization Mass Spectrometry (SPS-MS) is one of the most recently developed DI techniques, offering an effective and practical approach for the rapid direct analysis of solid polishable materials. [54] Being a variant of PS-MS, SPS-MS shares the same ESI-like mechanism in which ionization occurs at the tip of a triangular substrate oriented toward the MS inlet when solvent and electric current is applied. However, instead of using absorbent paper as the substrate, SPS-MS employs abrasive sandpaper, which provides greater extraction and immediate sampling of materials. SPS-MS has been successfully applied to samples such as coffee beans, contaminated plant leaves, tablets, and lozenges. [54, 55, 56] The method supports fast, inexpensive screening and can be operated with minimal training, requiring no complex extraction steps, large volumes of solvent, or external gas sources as with ESI. Its low cost, portability, and adaptability make SPS-MS particularly advantageous in forensic scenarios where speed, simplicity, and operational flexibility are critical, such as with field inspections, customs enforcement, or settings without access to traditional laboratory infrastructure.

1.4 AIMS Applications

1.4.1 Fraudulent Medicines

The economic impact of substandard and falsified human medicines is estimated at 200 billion USD a year. [57] The issue has become so pervasive that, at one point, 50% of all drugs sold online, even on legitimate-looking dispensaries, may have been fake. [58] This can involve a range of practices from poor quality control, replicating packaging security features, to poor quality contaminated ingredients leading to ineffective treatment at best and adverse health effects at worst. [59] As a recent example, the World Health Organization (WHO) has linked the deaths of 66 children in Gambia to four cough syrup brands from Maiden Pharma, India, due to diethylene glycol and ethylene glycol contamination. [60] The producers involved in this illicit industry manufacture a range of banned or fraudulent medications from cosmetics, cardiovascular drugs, vaccines, and even cancer treatments, just to name a few. [59] Various AIMS methods have been previously used, such as with DESI, [61] DAPPI, [62], and DART, [63] to tackle this very issue. More recently, PS-MS has also been used as an inexpensive method to detect counterfeit drugs, [64] alongside other counterfeit goods such as perfumes, [65] whiskies, [66] cigarettes, [67] and herbal medicines. [68] These applications demonstrate the growing role of AIMS techniques, particularly low-cost DI techniques, in combating pharmaceutical fraud and identifying counterfeit consumer goods through rapid, accessible, and cost-effective analysis.

1.4.2 Wood Analysis

Illegal logging is the third largest transnational crime in the world, following only drug trafficking and counterfeiting, and is a major driver of deforestation, biodiversity loss, and economic instability in developing countries. [69] This highlights the need for rigorous, enforceable methods detecting forestry crimes, verifying wood species and origin tracing. The World Forest ID (WFID) is a global initiative dedicated to combatting illegal logging and enhancing the traceability of timber by creating a scientifically robust, georeferenced database of wood samples. WFID seeks to advance provenance testing, based on the principle that trees from different geographic regions exhibit distinct chemical fingerprints and stable isotope ratios shaped by environmental conditions. By

collecting reference samples from trees across legally harvested forests and protected regions worldwide and analyzing them through various analytical techniques, enforcement agencies and researchers can determine the species and geographic origin of seized wood samples. Organizations participating in the WFID consortium span multiple continents and include governmental, academic, and nonprofit entities such as the United States Forest Service, the Royal Botanic Gardens Kew (UK), the Thünen Institute (Germany), and various national forestry agencies and universities in timber-exporting countries including Ghana, Peru, and Indonesia. The initiative complements existing efforts by the Global Timber Tracking Network (GTTN) and often collaborates with local partners. In contrast, regionally focused programs include the US National Fish and Wildlife Services and their mobile forensic lab, which utilize DART and other analytical instruments for wood analysis. [70] One of the core challenges faced by WFID is the need for field-deployable, rapid verification tools in enforcement scenarios, such as roadside checkpoints, port inspections, and border crossings, where immediate determinations are needed, while conventional laboratory-based methods (eg. polymerase chain reaction (PCR) sequencing) may take several days or weeks. To meet this need for timely field-based identification, DI methods in MS, specifically the recently developed (SPS-MS), supplementing other conventional analytical techniques, offer a promising solution by obtaining chemical fingerprints through rapid, on-site analysis to help prevent illegally sourced timber from entering supply chains. By focusing on scalability, scientific rigor, and applicability in frontline enforcement, WFID represents one of the most comprehensive and actionable frameworks for timber origin verification, supporting both biodiversity conservation and legal trade practices across the globe. [71]

Chemical analysis of wood has found relevance across a diverse range of disciplines, including archaeology, [72] ecological studies, [73] biofuel assessment, [74] and, of particular importance, species identification and/or origin tracing. [75, 76] The latter holds particular significance within the field of forensic science. It plays a critical role in detecting woods involved in forestry crimes, which pose substantial threats to local economies, protected classes of trees, and ecological stability, with corruption

capable of permeating through every stage of the supply chain, from the issuance of illegitimate logging permits to the sale of protected species or classes. [77]

MS techniques, AIMS methods in particular, have also been used to address and aid in combating this serious issue. [78, 79, 80, 81] While the Convention on International Trade in Endangered Species (CITES) has long been keeping track of over 100 species of trees that are or may be endangered, DART seems to have gained enough traction in the scientific and forensic community in recent years to have the spectral database Forensic Spectra of Trees (ForeST[®]) developed around the chemical information it provides. [82] Wood is primarily composed of biopolymers (95% cellulose, hemicellulose, and lignin). Degradation products of holocellulose (cellulose and hemicellulose) and lignin have been used as markers for age and, for the latter, species identification [83] While chemometrics are not established as conclusive evidence under any of the sub-disciplines within forensic science, it has been routinely used for wood discrimination or identification and origin tracing, which can be used as a highly useful supplementary and/or exploratory tool. [79, 82, 84, 85]

1.5 Research Objectives

In the following projects, the main objective was to utilize the novel direct ionization technique Sandpaper Spray Ionization Mass Spectrometry (SPS-MS) in a forensic context. Detection, identity confirmation, and chemical profiling of the compounds acquired via SPS-MS were performed with various MS scanning modes and chemometric analysis.

Outlined below are the specific roles I have played in each of the corresponding projects:

Chapter 2: Forensic application of sandpaper spray ionization mass spectrometry (SPS-MS): Direct analysis of solid pharmaceutical formulations and edible cannabis products (Rodrillo KAM, Costa DM, Viana SFC, Shahbazi H, Augusti R, Ifa DR.)

In this work, I was involved in the sampling procedure, optimization of reagents used and MS parameters, data acquisition, spectral data analysis and interpretation, and structure elucidation with MS/MS fragmentation profiles.

Chapter 3: Forensic application of sandpaper spray ionization mass spectrometry (SPS-MS): Chemotaxonomic Classification of Brazilian and Canadian Woods

(Rodrillo KAM, Diniz MCC, Monteiro TVC, Augusti R, Fasciotti M, Ifa DR.)

In this work, I was involved in acquiring samples, the sampling procedure, optimization of reagents used and MS parameters, data acquisition, spectral data analysis and interpretation, and compound identification with MS/MS fragmentation profiles, and pre-processing, graphing, and interpreting chemometric data. Mariana from Dr. Augusti's group handled modelling of the MS data for predictive classification.

Chapter 2: Forensic application of sandpaper spray ionization mass spectrometry (SPS-MS): Direct analysis of solid pharmaceutical formulations and edible cannabis products
(Rodrillo KAM, Costa DM, Viana SFC, Shahbazi H, Augusti R, Ifa DR.)

Chapter 2 is a version of the published manuscript:

Rodrillo KAM, Costa DM, Viana SFC, Shahbazi H, Augusti R, Ifa DR. Forensic Application of sandpaper spray ionization mass spectrometry (SPS-MS): Direct analysis of solid pharmaceutical formulations and edible cannabis products. *J Forensic Sci.* 2024; 69: 2209–2221. <https://doi.org/10.1111/1556-4029.15592>.

ABSTRACT

In this work, we employed a recently developed ambient ionization mass spectrometry technique, sandpaper spray mass spectrometry (SPS-MS), as a powerful analytical tool for the rapid and minimally destructive analysis of pharmaceutical formulations in solid forms, such as pills and tablets. The technique was employed to analyze a diverse set of pharmaceutical compounds, including antihypertensive agents, lipid-lowering drugs, antidiabetic medications, and therapies for erectile dysfunction (ED). Additionally, we evaluated a cannabis-infused lozenge product containing Δ^9 -tetrahydrocannabinol (Δ^9 -THC) and its isomer cannabidiol (CBD), both of which are key constituents of *Cannabis sativa*. The analysis involved rubbing the untreated surfaces of the samples directly onto triangular segments of sandpaper, followed by SPS-MS acquisition in both positive and negative ionization modes. This approach enabled comprehensive full-scan MS and MS/MS analyses, which allowed for the immediate and accurate identification of active pharmaceutical ingredients (APIs) in each sample. The technique also facilitated the tentative identification of certain excipients, or non-active components included in the formulations. SPS-MS demonstrated its capacity to rapidly screen pharmaceutical products, offering significant advantages in terms of speed and simplicity compared to conventional analytical methods. The ability of SPS-MS to detect APIs and excipients without extensive sample preparation underscores its potential as a robust tool for the detection and analysis of counterfeit and illicit pharmaceutical products. Given the increasing prevalence of counterfeit drugs and the associated risks to public health, the deployment of SPS-MS could represent a significant advancement in pharmaceutical quality control and regulatory enforcement. Our findings underscore the utility of SPS-MS as an efficient and reliable method for ensuring the integrity and safety of pharmaceutical products and other suspected compounds in the global market.

INTRODUCTION

The prevalence of fraudulent activities in pharmaceutical formulations poses a profound and pervasive threat globally, with significant and potentially irreversible consequences for consumers. This form of illicit drug fraud, characterized by its clandestine and deceptive nature, extends far beyond the realm of recreational drugs, infiltrating even

prescription medications. Tampered pharmaceutical products endanger the health and well-being of individuals who rely on these medications for legitimate medical purposes. Beyond the immediate risks, there is warranted concern regarding the long-term health implications associated with ingesting adulterated substances. Such exposure can lead to a range of health complications, from the development of chronic conditions to emerging life-threatening ailments. Therefore, addressing illegal drug fraud is essential, not only in terms of mitigating substance abuse but also in safeguarding public health on a broader scale. Enhancing public awareness, strengthening law enforcement, and implementing comprehensive community outreach initiatives are essential elements in addressing the complex and multifaceted issue of safeguarding individuals from the severe repercussions linked to adulterated pharmaceuticals [86, 87].

Ambient Ionization Mass Spectrometry (AIMS) has gained prominence as a robust analytical technique for the characterization of pharmaceutical formulations. AIMS enables rapid and direct analysis of complex samples with minimal preparation, making it particularly advantageous in the field of pharmaceutical analysis [88, 89]. One of the most significant benefits of AIMS is its capacity to analyze samples in their native state, thereby circumventing the need for extensive sample preparation procedures that are typically required in conventional mass spectrometry and mass-spectrometry-coupled techniques. These conventional methods often involve labour-intensive processes, such as sample extraction, purification, and derivatization. In contrast, AIMS methods allow for the direct analysis of samples while preserving analyte integrity, significantly reducing analysis time. AIMS techniques enable the rapid screening of pharmaceutical formulations, detecting and characterizing active pharmaceutical ingredients (APIs) and various excipients present in the sample. In this context, AIMS has been employed to quickly analyze the composition of pharmaceutical products, enabling the differentiation between authentic and counterfeit drugs. These unique characteristics make AIMS methods well-suited for quickly screening suspected samples, which is crucial in the fight against counterfeit drugs [90]. Additionally, AIMS has demonstrated its utility in drug stability studies, where it is used to monitor the degradation of drugs over time. This application provides valuable insights into the

stability of pharmaceutical formulations, facilitating the optimization of drug storage conditions and ensuring the efficacy and safety of medications throughout their shelf life [91].

Several ambient ionization techniques have been employed in drug analysis, each offering its unique advantages. Desorption Electrospray Ionization (DESI) and Direct Analysis in Real-Time (DART) are currently among the most widely utilized methods in this area of study [92, 93]. Recently, Sandpaper Spray Ionization Mass Spectrometry (SPS-MS) has emerged as an innovative advancement in AIMS techniques [54]. SPS-MS is a further development of the principles of Paper Spray Ionization Mass Spectrometry (PS-MS), an earlier technique also previously applied in the analysis of illicit substances, amongst various other applications [48, 94, 95]. Both PS-MS and SPS-MS employ an ESI-like mechanism, where ionization occurs at the tip of a triangular substrate directed toward the mass spectrometer. However, SPS-MS distinguishes itself by utilizing textured sandpaper with an expanded surface area, which enhances its efficacy in sampling and analyzing solid samples. This technique facilitates rapid screening of solid samples through a straightforward extraction process, proving particularly effective in the analysis of materials such as coffee beans and leaves infected with various species of fungi [54, 55]. SPS-MS is accessible to non-specialized personnel, offering a user-friendly alternative to techniques such as High-Performance Liquid Chromatography (HPLC) coupled to MS systems, which typically requires trained personnel, larger volumes of solvent, and significantly longer analysis times [96]. SPS-MS boasts several distinctive characteristics, including enhanced surface coverage, the ability to directly analyze solid materials without extensive sample or substrate preparation, versatility in application across diverse substances, cost-effectiveness, and broad applicability.

This work demonstrates the utility of SPS-MS in analyzing pills and tablets of pharmaceutical formulations, including those for blood pressure, cholesterol, diabetes regulation, and erectile dysfunction, as well as phytocannabinoid lozenges, by directly detecting their active ingredients and various excipients. As a potential application of

this technique, the data provided via SPS-MS are also proposed to reveal fraudulent pharmaceutical formulations used to treat erectile dysfunction (i.e., Viagra® and Cialis®).

MATERIALS AND METHODS

Materials

Cannabis sativa and pharmaceutical products

Drugs for the treatment of erectile dysfunction (ED), Viagra® (API: sildenafil) and Cialis® (API: tadalafil) were acquired in drugstores in Brazil. Other medications, such as APO-Metformin® (anti-diabetes; API: metformin), Invokana® (anti-diabetes; API: canagliflozin), Crestor® (cholesterol regulator; API: rosuvastatin), and Teva-Ramipril® (blood pressure regulator; API: ramipril), were acquired at local drugstores in Canada. A hard candy containing Δ^9 -tetrahydrocannabinol (Δ^9 -THC) and its related isomer cannabidiol (CBD) was acquired in a local dispensary in North York, Ontario, Canada. Figure 2-1 shows the medicinal tablets, pill, and lozenge. The chemical structures of all the active ingredients of the samples are shown in Figure 2-2.

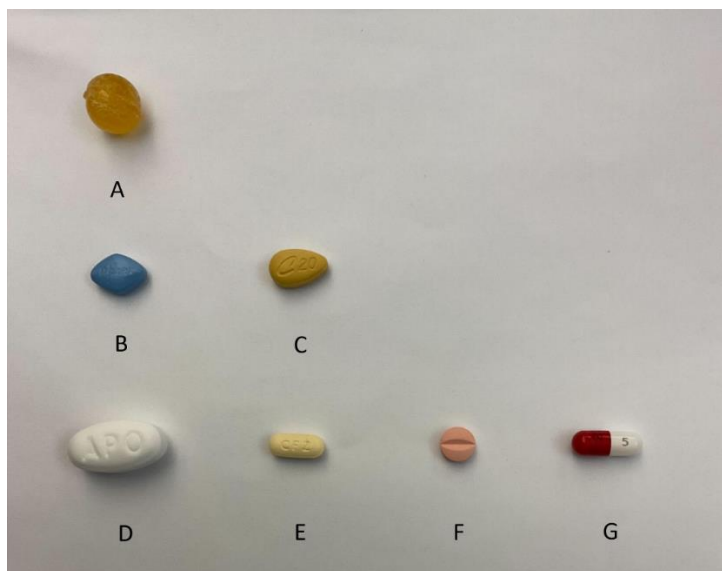


FIGURE 2-1 Samples analyzed by SPS-MS in this study: (A) *Cannabis sativa* hard candy, (B) Viagra®, (C) Cialis®, (D) APO-Metformin®, (E) Invokana®, (F) Crestor®, and (G) Teva-Ramipril®.

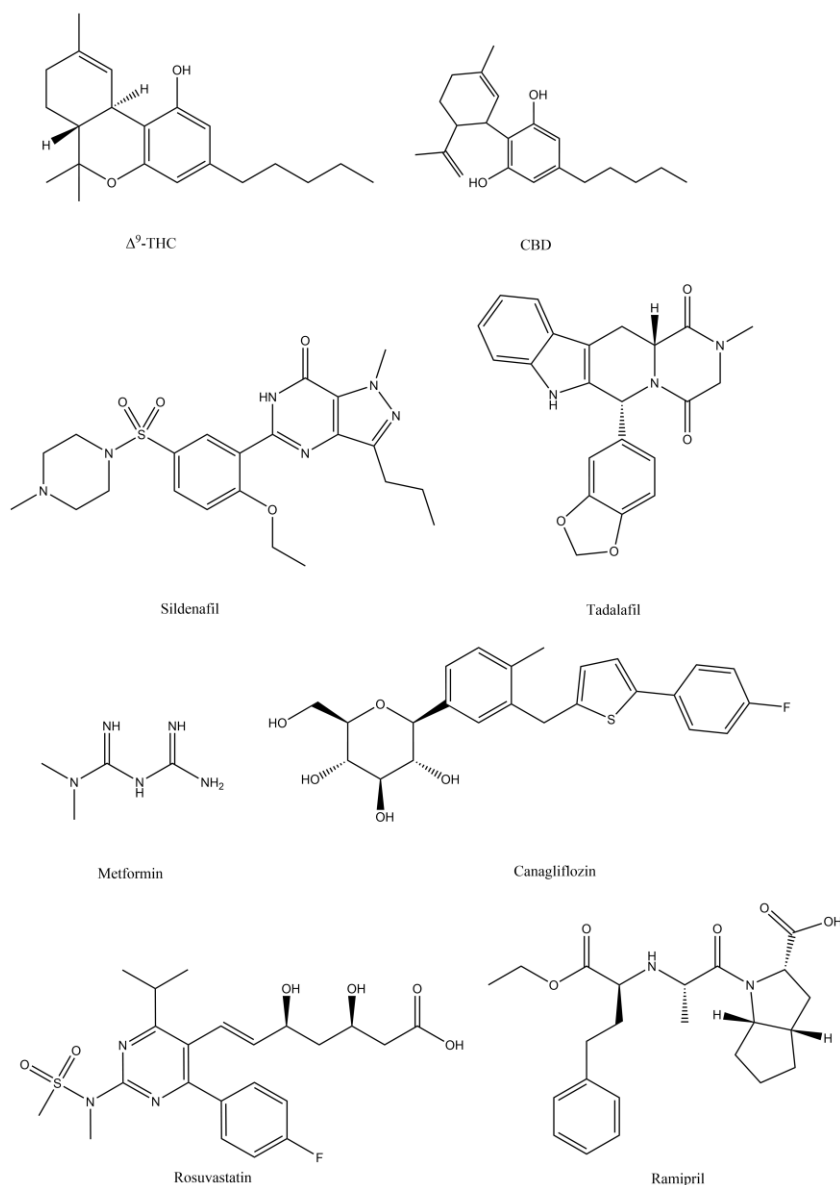


FIGURE 2-2 Chemical structures of the active pharmaceutical ingredients (APIs) found in the formulations evaluated in this study.

Substrate and solvents

Sheets of Duramax[®] sandpaper with a granulometry of G120 were purchased from a local store. HPLC grade methanol (MeOH), acetonitrile (ACN), formic acid (FA), ultrapure water (H₂O), and ammonium hydroxide (NH₄OH) reagents were acquired from Sigma-Aldrich (St. Louis, MO, USA).

MS parameters and method

Data were acquired using a linear ion trap mass spectrometer (LTQ, Thermo Fisher Scientific, San Jose, CA). Mass spectra were recorded under the following parameters: automatic gain control (AGC) enabled and maximum ion trap injection time set to 200 ms with 3 micro scans. The acquisition time for each analysis was 30 s. The instrument was configured with a spray voltage of ± 4 kV, capillary temperature of 300°C, tube lens set to ± 102 V, and capillary voltage of ± 48 V. Full scan mass spectra were acquired in the ionization mode (positive or negative) best suited for the compound, with an initial m/z range of 100–2000, then further adjusted based on the ions detected during the analysis. MS/MS experiments were conducted by mass-selecting a precursor ion of interest, which was then fragmented using collision-induced dissociation (CID), with helium as the collision gas. The dissociation profiles were achieved using normalized collision energies (NCE) between 15 and 40 a.u. (manufacturer's arbitrary unit).

The tests were carried out by vigorously rubbing the samples (tablets, pill, and hard candy) against the sandpaper, [the pill was opened manually and spread across the surface until fully saturated with excess material stored for further analyses] then cutting them into equilateral triangles with dimensions of 1.0 cm for each side. The sandpaper triangle was placed ~ 0.5 cm away from the MS inlet, with the tip pointed directly in line with the mass spectrometer ion transfer tube. An alligator clamp supplying the required voltage was attached to one of the sides of the sandpaper triangle. Solvent was added (25 μ L) to the sandpaper triangle, and high voltage (4 kV) was applied to be analyzed with an acquisition time of 30 s, producing averaged spectra. Blanks were acquired by applying solvent to the substrate, and used for background subtraction to produce the resultant spectra. Solvent selection was optimized to detect the active ingredient and other excipients of the samples evaluated. Fragmentation profiles were compared to annotated mass spectra from the online spectral database DrugBank [97]

RESULTS AND DISCUSSION

Cannabis sativa

SPS-MS has demonstrated its capability to reliably detect and characterize Δ^9 -THC and its isomer cannabidiol (CBD) in a *Cannabis sativa*-derived hard candy (Figure 2-3).

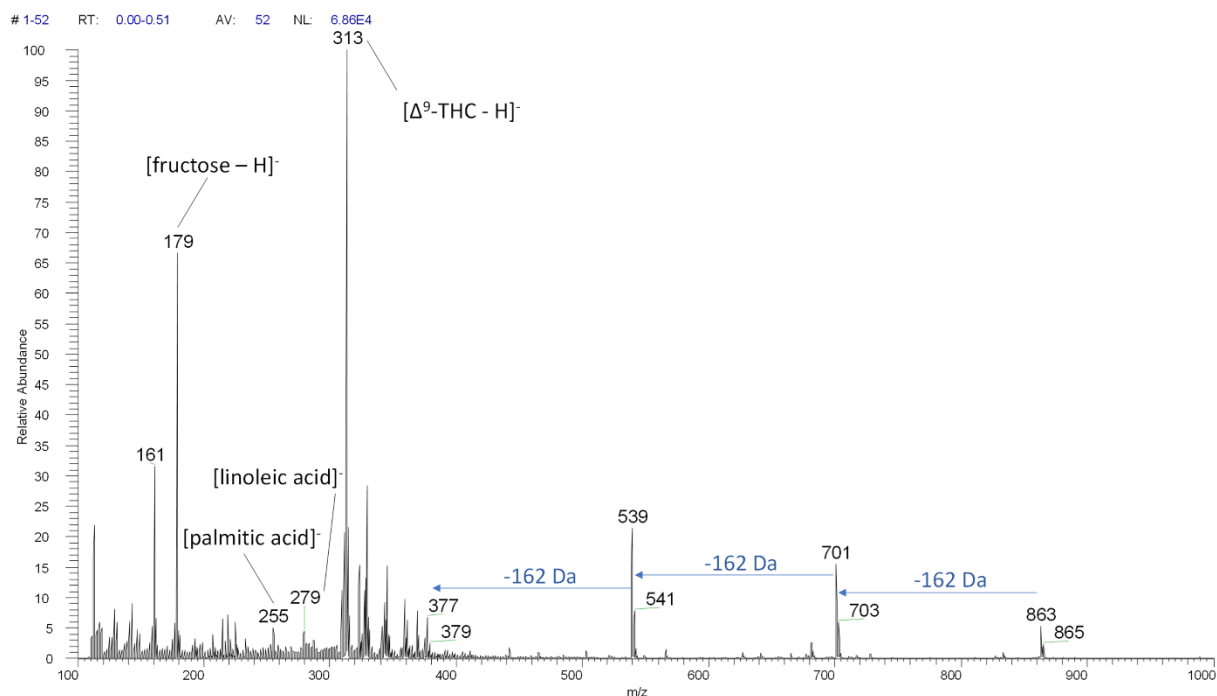


FIGURE 2-3 SPS(-)-MS of a *Cannabis sativa* hard candy with MeOH:H₂O (9:1) + 0.1% NH₄OH (v/v) as solvent and mass range of m/z 100–1000.

The ion of m/z 313 corresponds to the deprotonated molecular ion of these phytocannabinoids (Δ^9 -THC and CBD). Additionally, other significant ions detected via SPS(-)-MS include the monomeric form of oligofructose, an ingredient listed in the formulation (deprotonated fructose at m/z 179), and the chlorinated adducts of oligosaccharide subunits, which exhibit characteristic mass differences of 162 Da between them (m/z 863 and 865, 701 and 703, 539 and 541, and 377 and 379). The dehydrated form of the monosaccharide was also observed at m/z 161.

This result highlights a distinct advantage of SPS-MS over PS-MS, where oligosaccharides are typically not detected due to the retention of sugars by cellulose-based substrates. In contrast, SPS-MS enables the detection of oligosaccharides without requiring an additional desalting step, from a process referred to as DPS

(Desalting Paper Spray) to identify oligosaccharides and related compounds with strong hydrophilic interactions with the paper substrate [98]. Moreover, palmitic acid (m/z 255) and linoleic acid (m/z 279), typical excipients added to the formulation, were also detected.

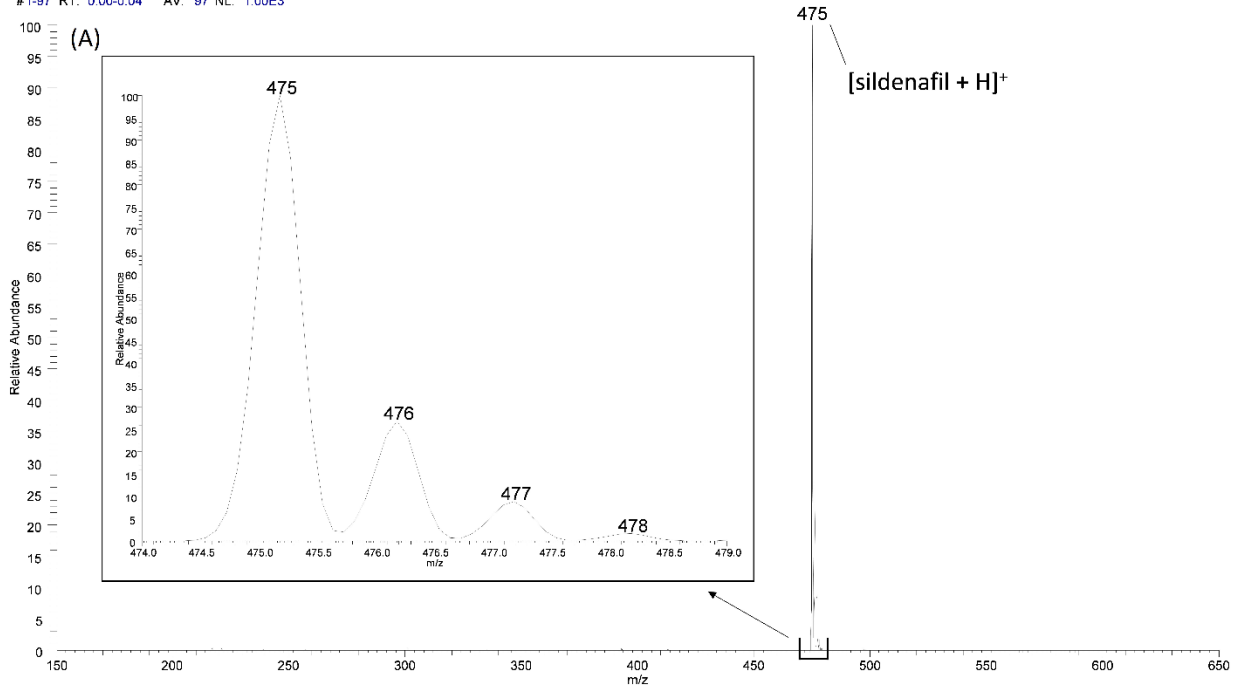
The fragmentation profile of the precursor ion for the deprotonated form of Δ^9 -THC at m/z 313 (Figure A1) is consistent with previously reported data in the literature [99, 42], confirming the presence of Δ^9 -THC in the *C. sativa* hard candy. A challenging analytical problem, however, lies in the differentiation between Δ^9 -THC and CBD even when employing MS/MS, as both compounds are likely present in *C. sativa* edibles and exhibit similar fragmentation patterns. Partial differentiation can be achieved by identifying the ion at m/z 199 in the MS/MS spectrum, which is generated exclusively from the dissociation of Δ^9 -THC. The absence of this diagnostic ion suggests the dissociation of CBD, whereas its presence indicates either the dissociation of Δ^9 -THC or a mixture of the isomers Δ^9 -THC and CBD [42]. Further investigation into the full discrimination between these two compounds could be accomplished through reactive SPS-MS (and MS/MS) analysis, given their differing binding affinities to Ag(I), examining whether different MS/MS profiles are produced from their respective adducts [THC + Ag]⁺ and [CBD + Ag]⁺ via simple pipetting of an AgNO₃ solution [100]. Due to the widened isolation window of 4 Da, special consideration is to be made with highly complex compounds, with further purification or extraction to remove possible isobaric compounds within the isolation window, which may obscure spectral data.

Viagra® and Cialis®

The analysis of a Viagra® tablet yielded a particularly clear SPS(+)-MS spectrum, with the unique presence of a singular intense ion m/z 475, identified as the protonated form of the active pharmaceutical ingredient (API) [sildenafil + H]⁺ (Figure 2-4A). Notably, no other ions from different compounds were detected, with a detection threshold set at >2% relative abundance.

In the corresponding SPS(-)-MS analysis, sildenafil was detected in its deprotonated form of m/z 473 (Figure 2-4B). Additional ions, including m/z 255 and 283, were tentatively identified as the deprotonated forms of palmitic acid and stearic acid, respectively. These ions arise from commonly used excipients in tablet formulation, namely magnesium stearate and magnesium palmitate [101]. A characteristic series of losses, each 14 Da apart (m/z 269, 241, 227, 213, 199, 185, 171, and 157), were identified as saturated long-chain fatty acids. The deprotonated and dehydrated forms of quinic acid, m/z 191 and m/z 173 respectively, were also tentatively identified. Several significant unidentified ions were also observed under SPS(-)-MS, including m/z 299, 301, 317, 349, 363, 367, 381, 405, 442, 489, 505, and 509. Importantly, the fragmentation profile obtained from MS/MS of the precursor ion of m/z 475 (Figure A2) matched previously reported data [102], thus confirming the correct assignment of the proposed structures and the presence of the API sildenafil in the tablet.

1-97 RT: 0.00-0.04 AV: 97 NL: 1.00E3



1-140 RT: 0.00-0.50 AV: 140 NL: 2.41E4

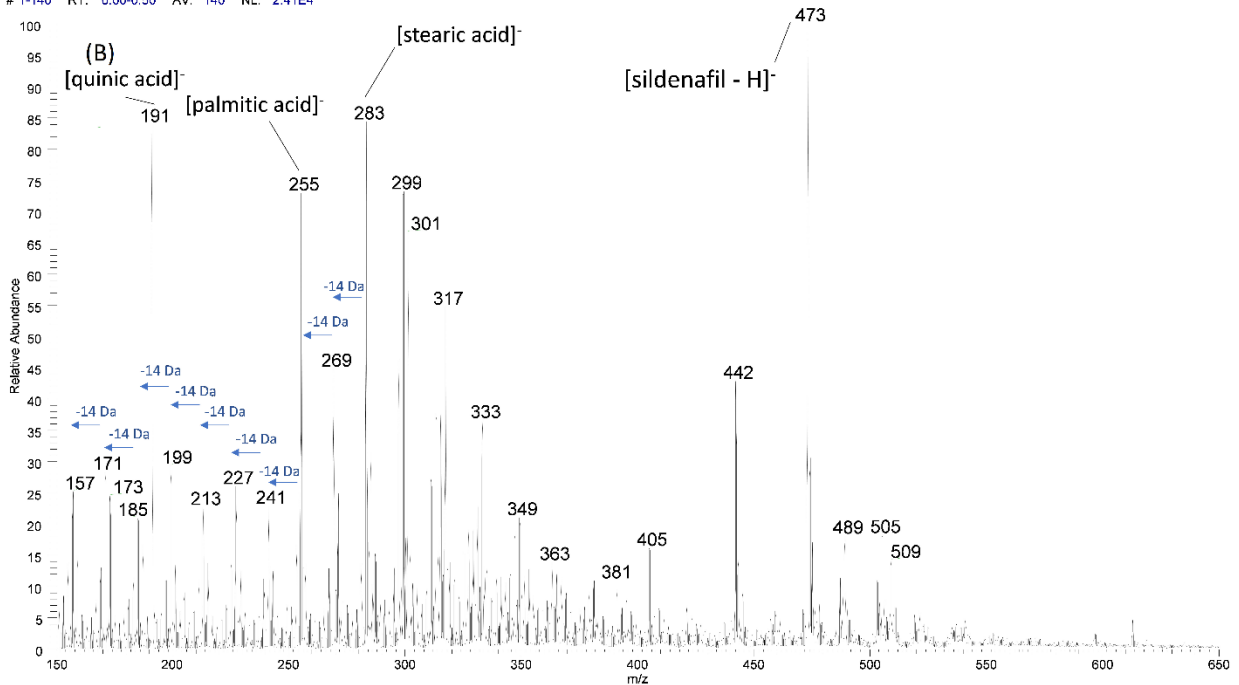


FIGURE 2-4 (A) SPS(+)-MS and (B) SPS(-)-MS of a Viagra[®] tablet (API sildenafil) using MeOH as solvent for both ionization modes and mass range of m/z 150–650.

Typical SPS(+)-MS and SPS(-)-MS spectra obtained from the analysis of a Cialis® tablet are presented in Figures 2-5A and 2-5B, respectively. The API, tadalafil, was clearly detected in both ionization modes, detected in its protonated (m/z 390) and deprotonated (m/z 388) forms. The presence of tadalafil in these tablets was further confirmed through MS/MS analysis, with a fragmentation profile consistent with previously reported data (Figure A3) [103]. Additional ions were detected in SPS(+)-MS (Figure 2-5A: m/z 198, 180, 163, 145, and 127), with the same pattern observed in SPS(-)-MS (Figure 2-5B: m/z 212, 194, 179, 161, and 143). The ion of m/z 198 was identified as the ammonium adduct of glucose [glucose + NH₄]⁺, based on its similarity to the fragmentation profile of the authentic ion [104]. Significant unidentified ions detected under SPS(+)-MS included m/z 117, 149, 181, 355, 371, and 372, while under SPS(-)-MS, significant unidentified ions included m/z 115, 119, 131, 144, 160, and 162.

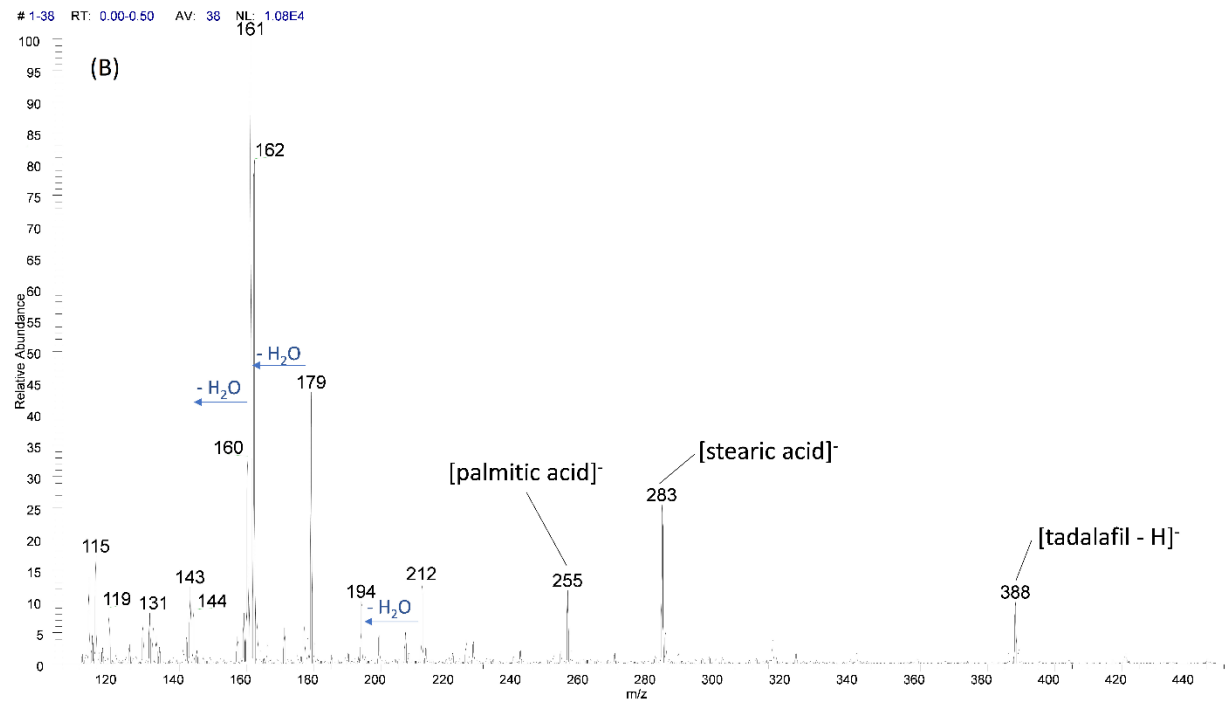
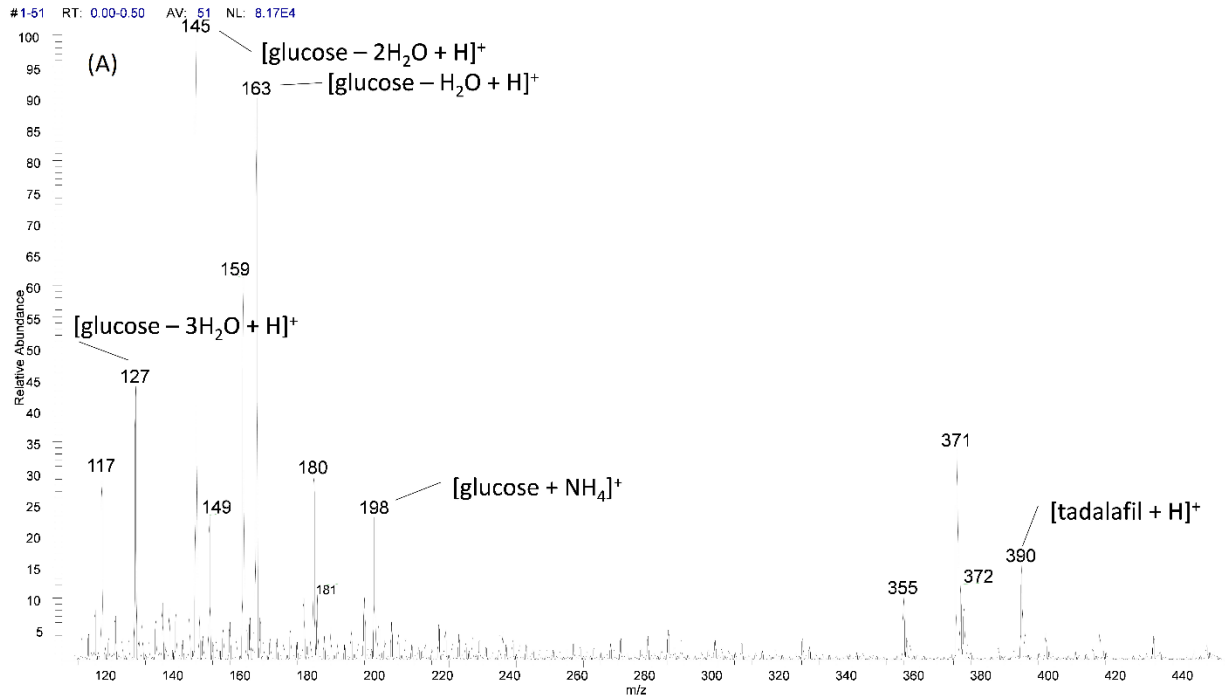


FIGURE 2-5 (A) SPS(+)-MS using MeOH + 0.1% FA as solvent and (B) SPS(-)-MS using MeOH as solvent of a Cialis® tablet (API: tadalafil) with a mass range of m/z 100–450.

These findings are particularly valuable for the detection of fraudulent practices. For instance, homosildenafil, previously reported as an alternative or adulterant to sildenafil, can be detected in its protonated form (m/z 489) or as its sodiated adduct (m/z 497), indicating counterfeit sildenafil. Homosildenafil, which has significantly different pharmacological effects compared to sildenafil, can also be confirmed by its MS/MS fragmentation profile [105]. The results displayed herein demonstrate that SPS(+)-MS can effectively detect such counterfeits.

Another concerning practice involves the fraudulent substitution of tadalafil-containing tablets with sildenafil, driven by the lower cost of sildenafil [106]. This type of fraud has also been detected using more complex methods, such as near-infrared spectroscopy (NIRS) combined with machine learning algorithms (MLAs) to differentiate convoluted spectra between legitimate and counterfeit drug samples [107]. Other spectroscopic techniques, often used in conjunction with chemometric analysis, have also been employed for this purpose [108]. SPS-MS offers considerable advantages over these techniques, primarily due to its ability to rapidly and conclusively detect both sildenafil and tadalafil, which are readily observable in both ionization modes.

Other pharmaceutical compounds

SPS-MS was also employed in the analysis of various additional compounds to demonstrate its broad applicability. The active pharmaceutical ingredients (APIs) of these medications include metformin, a hepatic gluconeogenesis inhibitor; canagliflozin, a sodium-glucose co-transporter 2 inhibitor; rosuvastatin, a 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor; and ramipril, an angiotensin-converting enzyme inhibitor. All these medications are administered orally in tablet or pill form. SPS-MS effectively detected the APIs in their protonated, deprotonated, and adduct forms using both ionization modes. Additionally, certain inactive excipients and loosely-bound dimers of the APIs were also observed. The structures of the detected APIs were confirmed through MS/MS analysis.

Metformin

SPS(+)-MS analysis of a medicinal tablet of APO-Metformin[®] readily detected the API metformin in its protonated form at m/z 130, with an intense signal and an otherwise clear spectrum (Figure 2-6). This compound is most suited to be analyzed under SPS(+)-MS, given its more ionizable structure in this mode. Mass selection and dissociation of the precursor ion produced product ions consistent with the chemical structure of metformin (Figure A4).

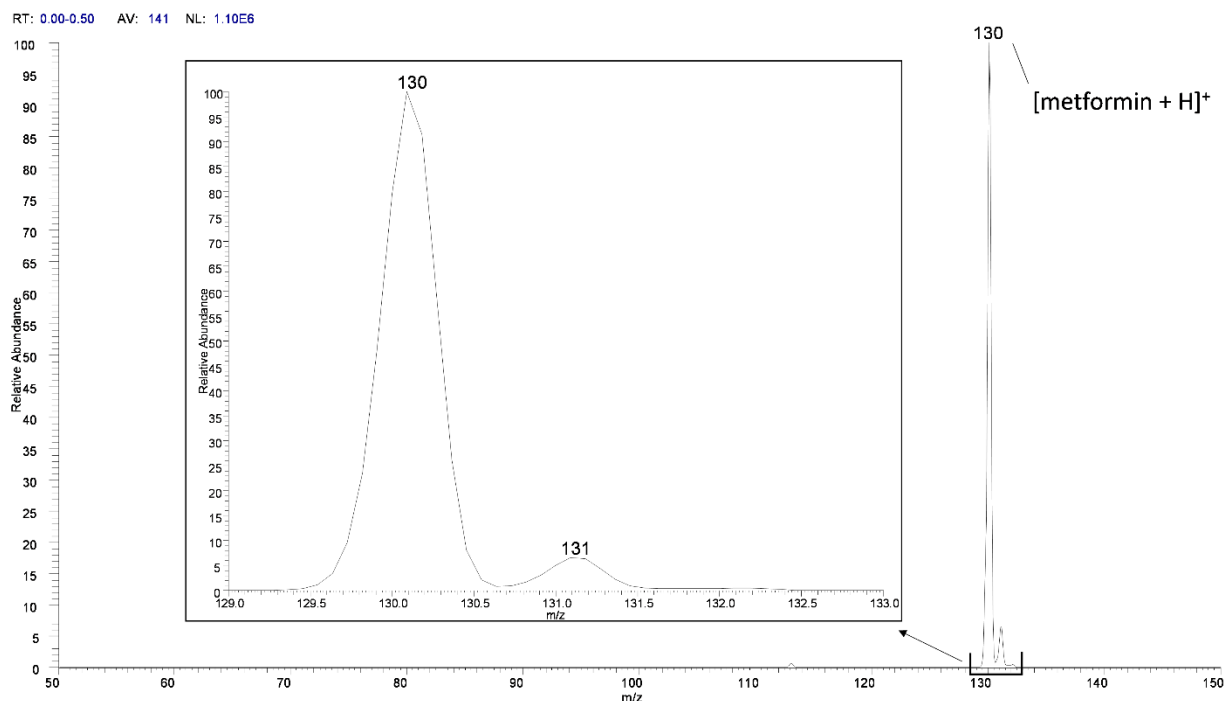


FIGURE 2-6 SPS(+)-MS of a tablet of APO-Metformin[®] (API: metformin), with MeOH + 0.1% FA (v/v) as the solvent and a (magnified) mass range of m/z 50–150.

Canagliflozin

SPS(+)-MS and SPS(-)-MS analyses of a tablet of Invokana[®] successfully detected its API canagliflozin. Under SPS(+)-MS analysis (Figure 2-7A), canagliflozin was detected as its sodium adduct at m/z 467 and as a sodium-bound dimer at m/z 911. Additionally, ions at m/z 365, corresponding to the sodium adduct of lactose, and at m/z 707, corresponding to the sodium adduct of a lactose dimer, were detected, indicating the presence of the matching excipient used in the formulation. Similar observations were made under SPS(-)-MS analysis (Figure 2-7B), where canagliflozin was detected as its

chloride adduct (m/z 467/469) and as a chloride-bound dimer (m/z 923/925). The presence of excipients was also evident in the SPS(-)-MS results. Specifically, palmitic and stearic acids were detected in their deprotonated forms at m/z 255 and 283, respectively. Furthermore, lactose was detected as a chloride adduct at m/z 377/379. Most importantly, the fragmentation profiles of the product ions of the API canagliflozin, at m/z 467 corresponding to $[\text{canagliflozin} + \text{Na}]^+$, and at m/z 479, corresponding to $[\text{canagliflozin} + \text{Cl}]^-$, were entirely consistent with the chemical structure of canagliflozin (Figure A5), as compared to the data reported in the online spectral database DrugBank [97].

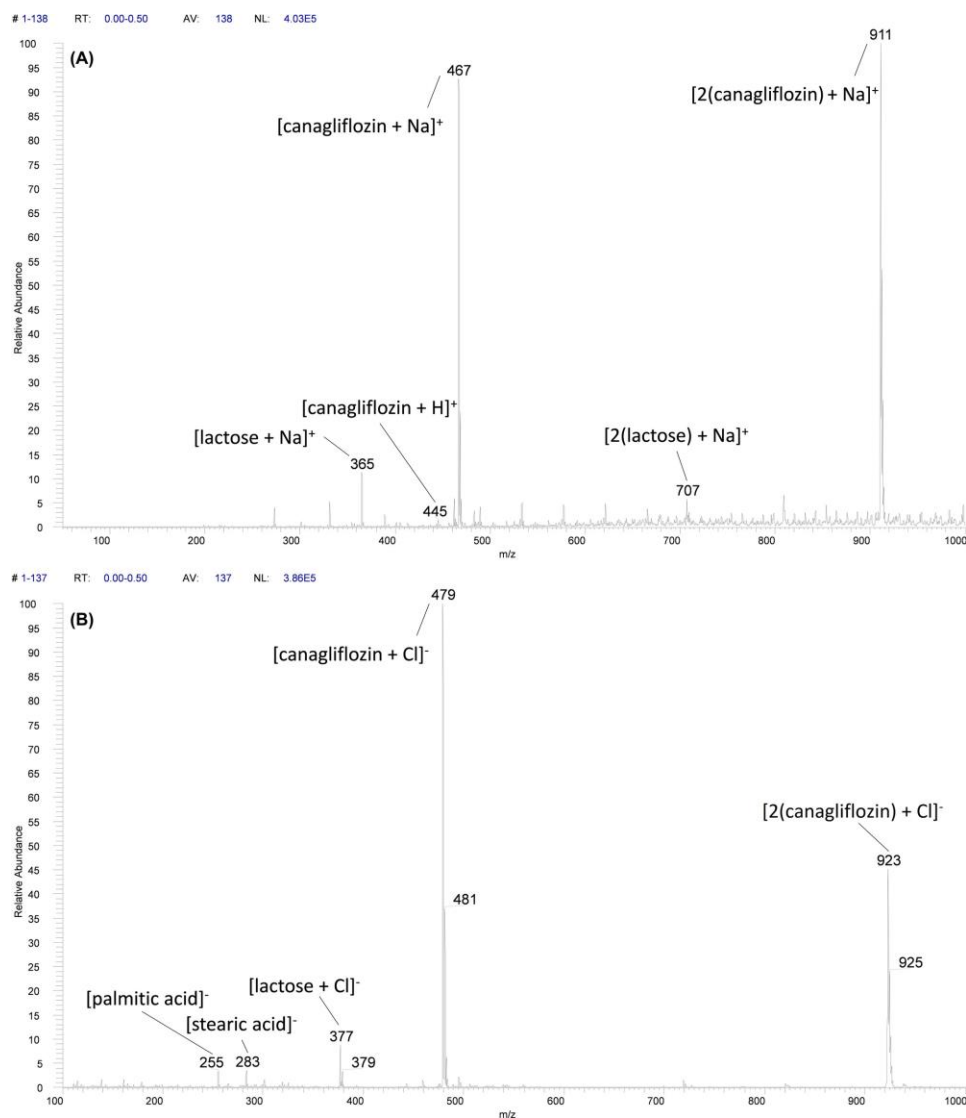


FIGURE 2-7 (A) SPS(+)-MS using MeOH + 0.1% FA as solvent and (B) SPS(-)-MS using MeOH as solvent of an Invokana[®] tablet (API: canagliflozin) with a mass range of m/z 100–1000.

Rosuvastatin

SPS(+)-MS and SPS(-)-MS analyses of a Crestor® tablet readily detected the API rosuvastatin. Under SPS(+)-MS analysis, rosuvastatin was detected in its protonated form (m/z 482) and as a sodium adduct (m/z 504). Additionally, the sodium adduct of a lactose dimer was also detected (m/z 707) (Figure 2-8A). Under SPS(-)-MS analysis, rosuvastatin was detected in its deprotonated form (m/z 480), as its chlorinated adduct at (m/z 516/518), and as its deprotonated dimer (m/z 961/963). Other ions identified include deprotonated palmitic acid (m/z 255), the chlorinated adduct of lactose (m/z 377/379), and the chlorinated adduct of a lactose dimer (m/z 719/721). These ions arise from compounds added as excipients in the formulation. Notably, significant unidentified ions were observed under SPS(+)-MS including m/z 241, 526, 525, 596, 862, and 868. Consistent with the analyses of other APIs, MS/MS spectra were acquired for the precursor ion of m/z 480, and the resulting fragmentation profile matched the expected chemical structure of rosuvastatin (Figure A6).

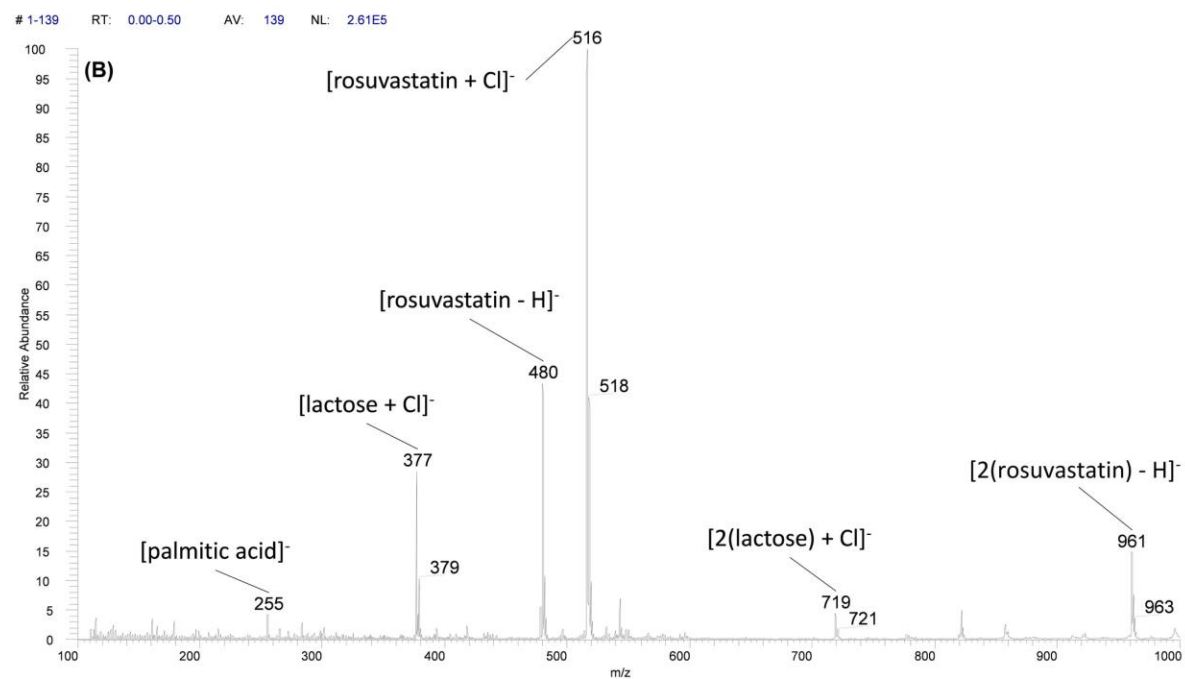
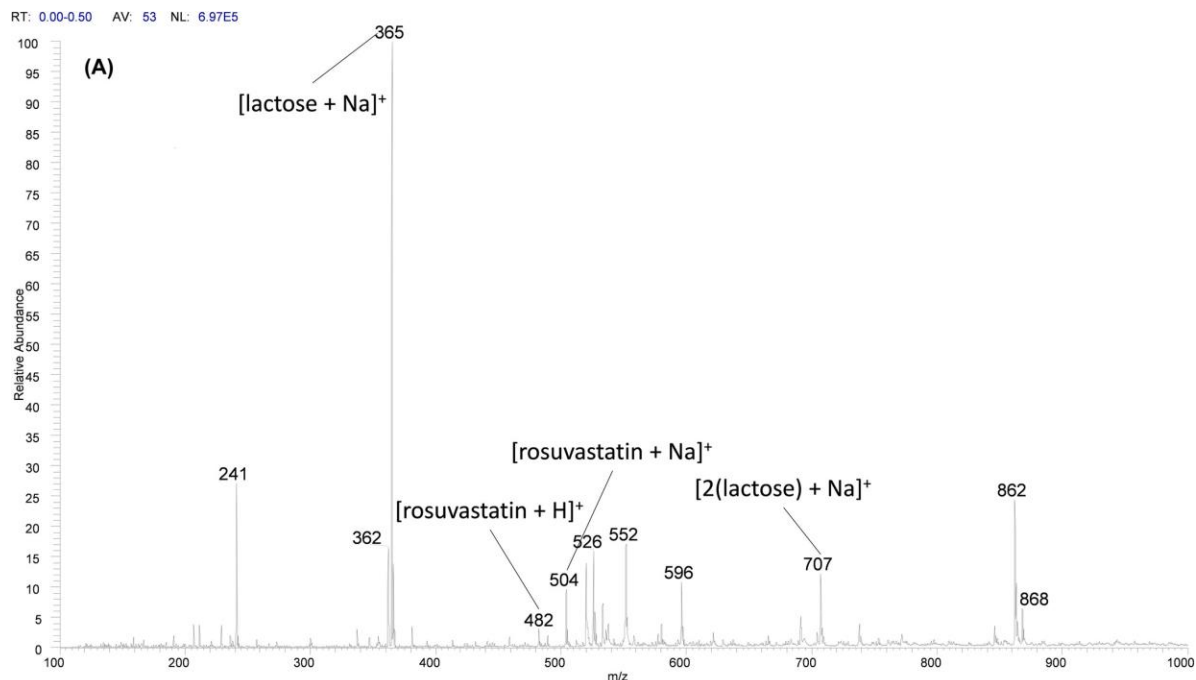


FIGURE 2-8 (A) SPS(+)-MS using MeOH + 0.1% FA as solvent and (B) SPS(-)-MS using MeOH as solvent of a Crestor[®] tablet (API: rosuvastatin) with a mass range of m/z 100–1000.

Ramipril

The API ramipril was readily detected through direct analysis of the medicinal tablet Teva-Ramipril® (Figure 2-9). Under SPS(+)-MS analysis, ramipril was detected in its protonated (m/z 417), sodiated (m/z 439), and sodiated dimer (m/z 855) forms. Under SPS(-)-MS analysis, ramipril was characterized by the presence of the ions m/z 415 ([ramipril - H]⁻), m/z 831 ([2(ramipril) - H]⁻), and m/z 451/453 ([ramipril + Cl]⁻). Significant unidentified ions include m/z 234 and m/z 399. Confirming the presence of the API, the fragmentation profiles of both the protonated (m/z 417) and deprotonated (m/z 415) precursor ions were subjected to mass selection and dissociation. The resulting fragmentation profiles were entirely consistent with the expected structure of ramipril (Figure A7).

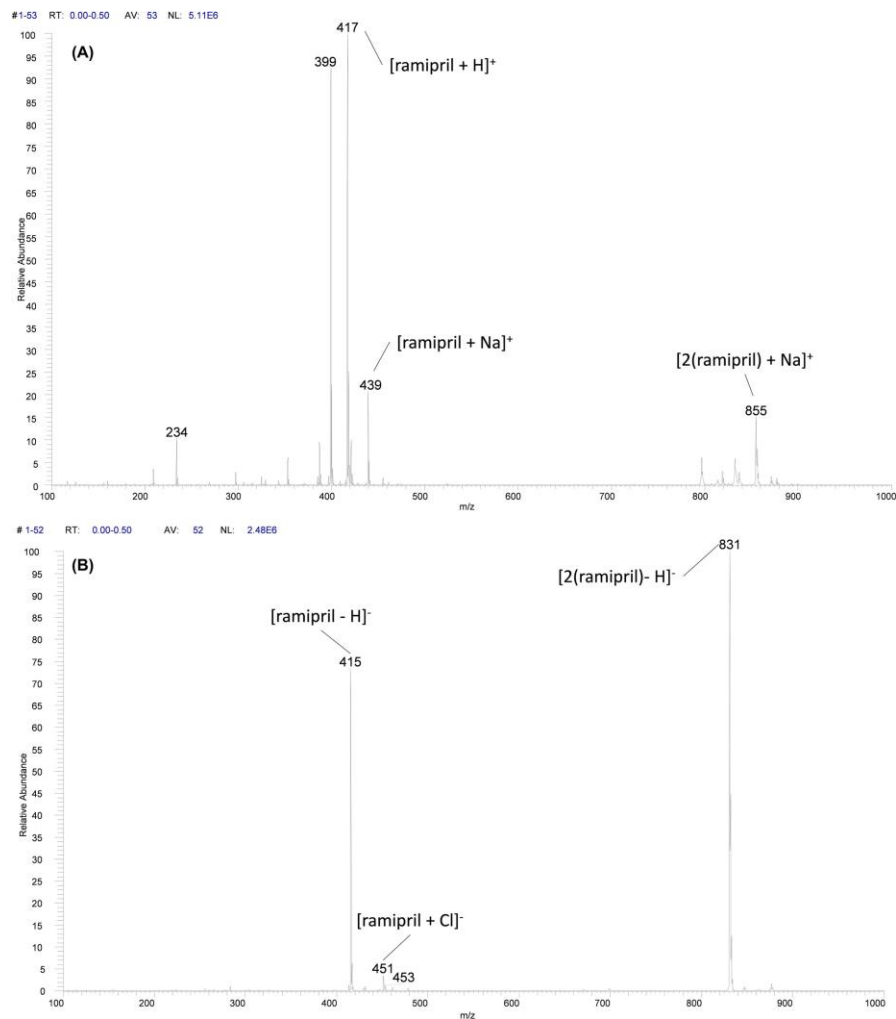


FIGURE 2-9 (A) SPS(+)-MS using MeOH + 0.1% FA as solvent and (B) SPS (-)-MS using MeOH as solvent of a tablet of Teva-Ramipril® (API: ramipril) and a mass range of m/z 100–1000.

Conclusion

The direct analysis of phytocannabinoids and APIs in pharmaceutical formulations using SPS-MS underscores the technique's simplicity and selectivity. SPS-MS demonstrates considerable potential for the rapid screening of suspicious (possible fraudulent or contraband) pharmaceutical products or edibles, making it particularly well-suited for deployment in high-traffic areas such as airport security checkpoints. [109, 110] Moreover, this technique is capable of detecting oligosaccharides and other related compounds retained in the cellulose-based substrate of PS-MS. SPS-MS has proven to be highly effective in the rapid detection of both active ingredients and certain excipients, thus serving as a valuable preliminary screening tool that does not require laboratory-trained personnel. This screening capability can be supplemented with additional analytical techniques where, if needed, samples screened by SPS-MS can be confiscated and subjected to comprehensive qualitative and quantitative analyses. SPS-MS enhances the range of chemical identification methods, particularly for compounds such as phytocannabinoids and various drugs of abuse.

Chapter 3: Forensic application of sandpaper spray ionization mass spectrometry (SPS-MS): Chemotaxonomic Classification of Brazilian and Canadian Woods
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ABSTRACT

The ability to accurately identify wood species and their geographic origins remains a major challenge due to the chemical complexity of such samples, and the limited applicability of most on-site analytical tools. In this work, sandpaper spray ionization mass spectrometry (SPS-MS) is proposed as a field-deployable technique for direct analysis of wood samples for chemical profiling. The method enables direct sampling of polishable wood surfaces with minimal sample preparation and required materials for analysis. Multiple wood specimens from diverse species and regions were collected and analyzed to assess species-level discrimination based on chemical fingerprints. Chemometric classification demonstrated distinct separation between sample classes, highlighting the method's suitability for the creation or supplementation of a reference spectral database. SPS-MS provides a practical approach for the on-site screening of wood materials, with potential application at ports of entry and other checkpoints where timely identification of protected classes is required, aiding regulatory enforcement in combating forestry crimes.

INTRODUCTION

Forestry crime, including the illegal logging and trafficking of protected classes of wood, is an illicit global industry valued at over 150 billion USD annually, with Southeast Asia, Central Africa, and South America comprising up to 90% of illegally obtained timber, continuing to cause widespread harm to biodiversity, environmental sustainability, and economic stability. [69, 111] This illegal trade is often facilitated by high global demand for rare and high-value timbers, limited regulatory enforcement, and complex supply chains that obscure the origin of the materials. A further complication arises from the widespread use of visually similar substitute woods, which are often artificially dyed to mimic protected species such as mahogany, often referred to as 'green gold' due to its excessive logging in the 1990's,[112] thereby undermining enforcement efforts and facilitating illicit trade. Reliable and accurate detection and identification of smuggled woods belonging to protected species or originating from protected regions is critical for combating these forestry crimes. However, reliable identification remains challenging due to the complexity and variability of chemical profiles across different wood species. Current on-site analytical methods in the field of forensic chemistry, suitable for

deployment at ports of entry, are limited, and many existing approaches rely predominantly on exploiting various properties (ie. electrochemical, colorimetric, near infrared, stable isotope ratio analysis (SIRA), etc.) of specific compounds for which they were designed. [113, 114]

Mass spectrometry (MS) is a versatile analytical technique capable of detecting and characterizing a broad range of ionizable compounds by analyzing their mass-to-charge ratios (m/z). Various MS-coupled techniques have been utilized for the purposes of wood analysis, namely, pyrolysis with techniques such as Direct Exposure (DE-MS), Evolved Gas Analysis (EGA-MS), and Pyrolysis-Gas Chromatography (Pyr-GC/MS) to characterize lignin, one of the major components of wood and other lignocellulosic plant material, and its degradation products. [115, 116, 117, 83] These techniques require numerous sample preparation steps (ie. ball-milling and sequential soxhlet extraction), which, in turn, make them difficult to employ for on-site analyses. Ambient ionization techniques with simpler setups have been implemented such as Direct Analysis in Real Time (DART) being used in the development of the wood spectral database “Forensic Spectra of Trees” or ForeST©. [118, 119, 82] However, due to the nature of the harsh ionization technique, similar to pyrolysis, it relies on certain diagnostic ions, primarily from the degradation products of lignin, for genus-level or species-level classification. There are limited wood genera and species with such diagnostic ions, making general classification with this technique difficult, even when supplemented with chemometric analysis. This is reflected in the lower prediction accuracy of DART when compared to a computer vision identification model examining wood microanatomy. [120] Other notable MS techniques used to analyze wood samples include conventional Electrospray Ionization (ESI) [121, 122] and Venturi Easy Ambient Sonic Spray Ionization (V-EASI), [75] which offer more promising prospects for wood typification due to the softer ionization technique, providing less convoluted spectra with more intact molecular ions to detect. V-EASI can also be adapted into what is referred to as “Spartan V-EASI” which switches the source gas to that of a can of compressed air, in attempt to make the technique more portable. [123] To effectively address these issues, rapid on-site analysis with minimal sample preparation combined with the broad

chemical profiling capabilities of MS provides a promising approach, enabling comprehensive chemical fingerprinting of various protected classes of wood.

One of the simplest and most accessible MS techniques developed for ambient ionization has been Paper Spray (PS) – MS, utilizing an electrode attached to a triangularly-cut absorbent material such as cellulose-based paper, which has been extensively characterized for its analytical performance with various substrates and geometries. [48, 49]. PS-MS has been used to analyze various samples from tissue biopsies to explosive material, with a notable pilot study in Vancouver (BC, CA) analyzing over a hundred street drug samples with a large portion found to be laced with fentanyl. [124, 125, 52] Sandpaper spray ionization (SPS) – MS is a variant of PS-MS, able to sample polishable solids for immediate MS analysis. SPS-MS has been previously used to analyze coffee beans, fungi-infected plant leaves, lozenges, pills, and tablets. [54, 55, 56] These techniques offer rapid analysis and accessible materials, without the need for extensive extraction and purification steps or large gas cylinders as the nebulizing gas (ie. nitrogen) for ESI-MS. SPS-MS offers significant advantages in terms of rapid analysis, operational simplicity, accessibility of sampling materials, and field portability. These attributes make it well-suited for on-site screening scenarios, such as with food authentication [126], but also forensic analysis at ports of entry and customs facilities, as well as deployment in remote or resource-limited settings where conventional laboratory infrastructure is unavailable.

In this study, Sandpaper Spray Ionization Mass Spectrometry (SPS-MS) is employed for direct sampling, ionization, and chemical analysis of wood specimens representing multiple species and diverse geographical origins. The primary objective is to evaluate the method's capability for accurate species-level classification based on chemical fingerprinting. Furthermore, the study explores the feasibility of generating a comprehensive spectral dataset, either for the creation of a new reference database or integration into existing spectral libraries. Such a resource could significantly enhance traceability and authentication protocols in the context of combating forestry crimes, including the illegal logging and trade of protected or regionally restricted wood species.

MATERIALS AND METHODS

Materials

Wood Samples

Plant fibres from different tree anatomy (heartwood, sapwood, or both) of various Brazilian woods (*Carapa guianensis*, *Cedrela odorata*, *Hymenaea courbaril*, and *Swietenia macrophylla*) were generously provided by various institutions, namely: Laboratório de Produtos Florestais (LPF), Distrito Federal, Brazil, Instituto Nacional de Pesquisas da Amazônia (INPA) Manaus, Amazonas, Brazil, Museu Paraense Emílio Goeldi (MPEG), Belém, Pará, Brazil, and Universidade Federal Rural do Rio de Janeiro (UFRRJ), Seropédica, Rio de Janeiro, Brazil). *Prunus serrulata*, and *Acer saccharum* were acquired from York University, North York, Ontario, Canada. *Populus tremuloides*, *Fraxinus americana*, *Juglans nigra*, *Prunus serotina*, and *Betula papyrifera* were acquired within 150 km of Toronto, Ontario, Canada from Grain & Bark, a local wood artisanal shop.

Sampling Device and Reagents

HPLC grade methanol (MeOH), ultrapure water (H₂O), isopropanol (IPA), and ≥ 99.0% NaCl were acquired from Sigma-Aldrich (St. Louis, MO, USA). Dremel[®] 7350 rotary tool (Robert Bosch GmbH, Gerlingen, Germany) and included circular sandpaper bits with a granulometry grade of G150 were purchased from a local hardware store.

MS Parameters and Method

MS Parameters

Mass spectra were acquired using a linear ion trap mass spectrometer (LTQ, Thermo Fisher Scientific, San Jose, CA, USA) under the following parameters: automatic gain control (AGC): On, maximum ion trap injection time set to 100 ms with 3 micro scans, and acquisition time of 30 s for each analysis. Applied voltage was set to +3.0 kV, capillary temperature to 200°C, capillary voltage to +44 V, and tube lens to +120V. Full scan mass spectra were acquired in the positive ionization mode with an initial *m/z* range of 50–2000, further adjusted based on the ions detected during the analysis. MS/MS experiments were conducted by mass-selecting a precursor ion of interest, which was then fragmented using collision-induced dissociation (CID), with helium as

the collision gas. The dissociation profiles were achieved using normalized collision energies (NCE) between 19 and 26 a.u. (manufacturer's arbitrary unit).

Method

ESI(+)-MS

ESI(+)-MS was carried out from a sample solution of the wood produced with a previously performed sample preparation protocol by Fasciotti et al. (2015). [122] Briefly, ~4.50 mg of the sample was vortexed with 400 μ L of solvent (3:1 MeOH:H₂O), centrifuged at 10,000 rpm for 20 mins., diluted 100:1, then added with 6 μ L of 0.05 M aqueous NaCl. MS analyses were performed with 3 analytical and 3 technical replicates (n = 9) and blank subtracted with clean solvent solution containing the NaCl spike. ESI line and MS inlet were flushed with IPA and 1:1 MeOH:H₂O after every analysis until sample signal disappeared.

SPS(+)-MS

Sampling for SPS(+)-MS was carried out using a portable handheld electric rotary tool (Dremel[®] 7350), attached with 150-grit sandpaper bits, applied on the region of interest against the cross-section of a wood sample. The sandpaper was removed from the tool and cut into equilateral triangles with dimensions of 1.0 cm for each side and placed ~0.5 cm away from the MS inlet, with the tip pointed directly in line with the MS ion transfer tube. Equivalent mass of the donated wood fibres was simply placed on the sandpaper triangles proceeded by the same procedures. An alligator clamp supplying the required voltage was attached to one of the sides of the sandpaper triangle. Solvent was added (25 μ L) to the sandpaper triangle producing averaged spectra. Blanks were acquired by applying solvent to the substrate alone and used for background subtraction to produce the resultant spectra. The same rinsing procedure from the ESI(+)-MS analyses was performed with the source reattached after every SPS(+)-MS analysis.

Data Processing

Spectral data from both ESI(+)-MS and SPS(+)-MS were extracted from the Qual Browser of the Xcalibur software (version 4.1.50) using the following parameters optimized for chemical profiling: mass range: m/z 200 – 1000; Noise threshold: 3;

Window size: 1s. The resultant spectral data, relative abundances and m/z , were extracted, converted to .csv format, and uploaded to the web-based platform MetaboAnalyst 6.0 for pre-processing, namely: binning, m/z set to 0.5, and feature (peak) alignment with retention time tolerance of 30s, as per the total acquisition time. A standard deviation filter of 25% and an abundance filter of 15% were also applied. The pre-processed data was then transposed and imported, with additional pre-processing was performed with a cube root transformation and mean centering chemometric analysis. Chemometric software used were Solo and Mathworks MATLAB 2010b (Natick, EUA) and PLS toolbox 5.2.2 (Eigenvectors Research Inc., Manson, USA).

PLS-DA model (Modelling & Predictive Performance)

For the construction of the partial least squares discriminant analysis (PLS2-DA) model, 9 samples per class ($n = 9$) were used. The data were pre-processed as described in the section “Data Processing”. Subsequently, the mass spectra were organized into a matrix X (135×544), containing all m/z values, sample names, and corresponding class labels. Table 1 presents the wood samples and the number of replicates per wood type.

To split the dataset into training and test sets, the Kennard-Stone algorithm was applied within each class (based on species and location), [127, 128] allocating two-thirds of the samples to the training set and one-third to the test set. Random subset cross-validation was performed using the maximum number of iterations, and the number of latent variables was selected based on the lowest cross-validation classification error (CVCE). The following figures of merit were made: sensitivity (true positive rate), specificity (true negative rate), and efficiency.

Table 3-1: Description of heartwood samples used in the chemometric models

Species	Comon Name	Code	Colection Location	Samples
<i>Acer saccharum</i>	sugar maple	G1	Toronto, Ontario, Canada	9
<i>Betula papyrifera</i>	white birch	F1	Toronto, Ontario, Canada	9
<i>Carapa guianensis</i>	crabwood	S25	Flona Tapajós - BR 163 - Satarém/PA	9
<i>Carapa guianensis</i>	crabwood	CG1	Presidente Figueiredo - Amazonas	9
<i>Carapa guianensis</i>	crabwood	CG4	Manaus - Amazonas	9
<i>Fraxinus americana</i>	white ash	C1	Toronto, Ontario, Canada	9
<i>Hymenaea courbaril</i>	courbaril	HC1 HC3	Itacoatiara - Amazonas	18
<i>Juglans nigra</i>	black walnut	D1	Toronto, Ontario, Canada	9
<i>Populus tremuloides</i>	aspen	B1	Toronto, Ontario, Canada	9
<i>Prunus serotina</i>	black cherry	E1	Toronto, Ontario, Canada	9
<i>Prunus serrulata</i>	cherry blossom	A1	Toronto, Ontario, Canada	9
<i>Swietenia macrophylla</i>	mahogany	SM2 SM4	Presidente Figueiredo - Amazonas	9
<i>Swietenia macrophylla</i>	mahogany	SM5	Amazonas	18
Total				135

RESULTS AND DISCUSSION

Tree Anatomy: Heartwood versus Sapwood

Spectra data were acquired via ESI(+)-MS from three anatomical sections of *Carapa guianensis* (Crabwood): heartwood, sapwood, and a composite sample containing both tissue types. These were subjected to chemometric evaluation, namely, examining the types and number of chemical features detected by MS in order to assess viability for species discrimination and/or classification. A PCA Scores Plot was produced, initially employed as an unsupervised approach to observe clustering within the dataset, from the data acquired sampling from heartwood, sapwood, and a combination of the two, with a total variance of 84.35% from the first 3 principal components to build the 2D graph. (Figure 3-1a) This shows distinct separation of the tree anatomies with clear non-overlapping 95% confidence intervals (ellipses), indicating distinct chemical profiles produced when sampling at different anatomies. A corresponding Hotelling T² plot was also produced, indicating sampling from the heartwood to be statistically distinct, with orthogonal variance already present in PC1 from the different tree anatomy combinations. PC1 and PC 2 have a cumulative variance of 80.87%, comprising a significant portion of the total variance. Q Residuals lied at 15.65%, indicating no significant oversampling. Although the initial rationale for sampling both heartwood and sapwood, on the basis that combining chemical information from both to enhance class separation, may seem reasonable, the results suggest otherwise. The more abundant ions originating from the sapwood region can mask lower abundance ions that may primarily lie in the heartwood. Given that chemical profiling is based on relative abundances rather than absolute amounts, this can hinder chemometric discrimination and further classification of samples. These findings are congruent with the common practice of analyzing heartwood for chemical analysis, whether it be for profiling or detecting and analyzing species-specific compounds, in various ionization techniques including ESI and DART. [129, 130, 131]

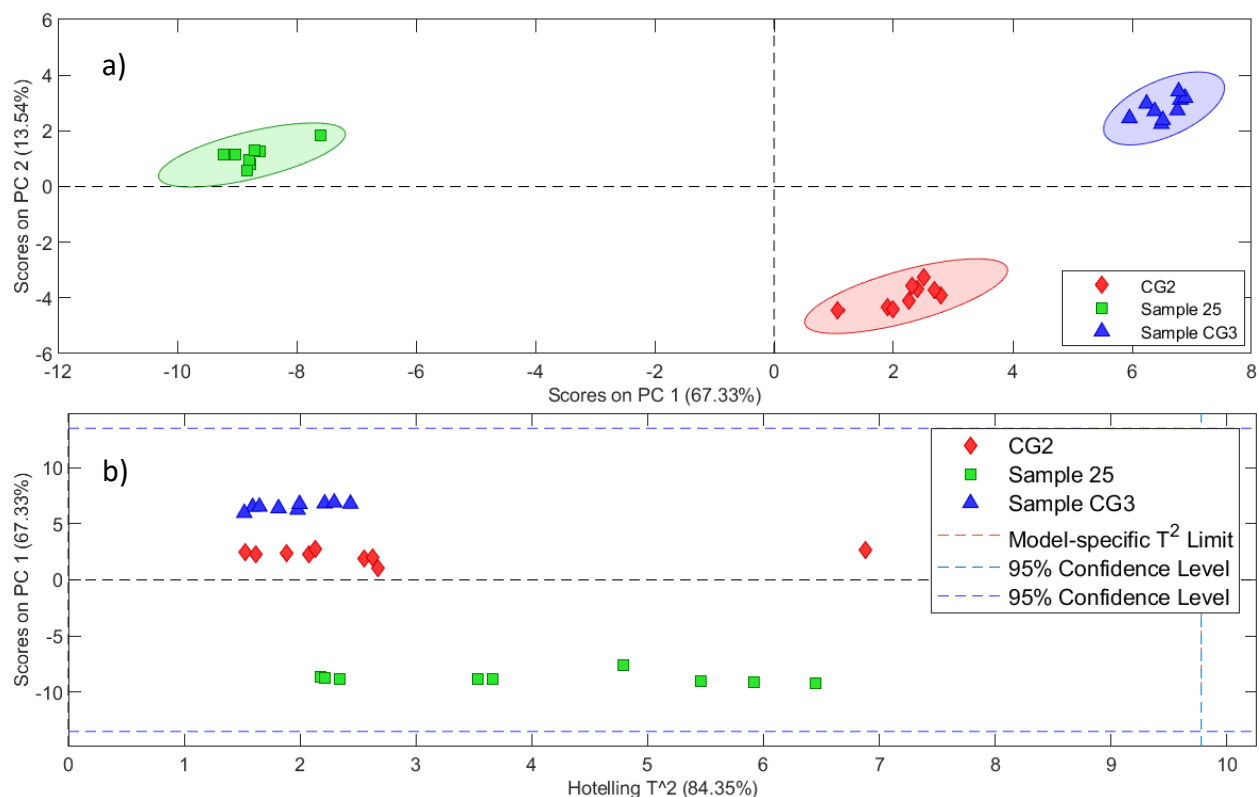


FIGURE 3-1: Chemometric analysis graphing a) a PCA Scores Plot and b) a Q Residuals Plot from different tree anatomies, Heartwood (sample 25), Sapwood (Sample CG3), and Heartwood + Sapwood (Sample CG2) of *Carapa guianensis*

SPS Performance

While PS – MS has been extensively characterized and assessed comparatively with ESI – MS and nanoESI – MS, [51, 132] the relatively new SPS – MS technique has had only a few early-stage comparisons with other direct ionization techniques. Figure 3 – 7 compares ESI and SPS – MS spectra of a sample of mahogany, labelling detected ions with a 1% threshold. Similar to initial findings, [54] SPS – MS employs enhanced extraction, which can be observed with the greater signal produced. This better deconvolutes the spectrum of highly complex matrices without dilution of the sample, which is particularly effective at detecting high abundance compounds, seen here with m/z 911, without prior enrichment or purification.

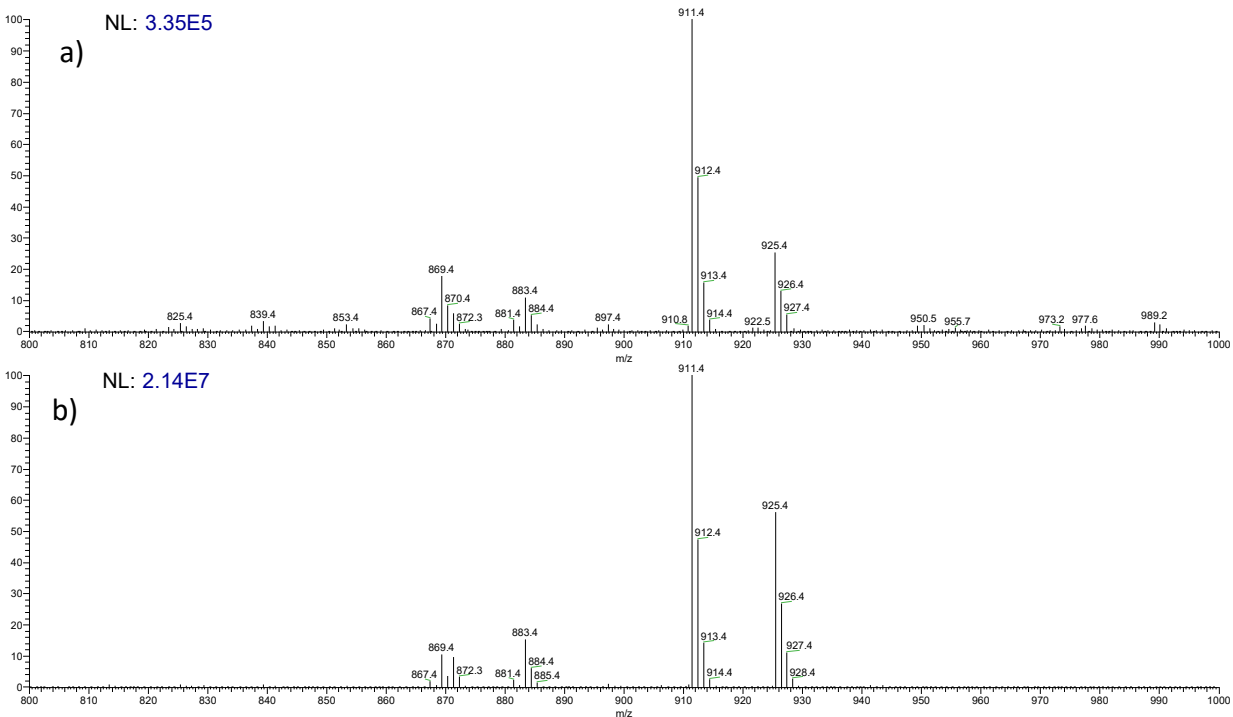


FIGURE 3-2: a) ESI(+)-MS and b) SPS(+)-MS spectra of a *Swietenia macrophylla* sample. Mass range: m/z 800 - 1000

For chemometric applications, both high and low abundance compounds, may significantly affect species classification. While SPS-MS enhances signal intensity and reduces matrix effects, it may also mask low-abundance compounds that serve as key discriminants. Initial unsupervised chemometric tools such PCA with clear groupings may serve as an initial validation method.

Wood Species Discrimination

Various wood samples, consisting of primarily angiosperms (hardwood), *Carapa guianensis* (crabwood), *Swietenia macrophylla* (mahogany), *Prunus serrulata* (cherry blossom), *Populus tremuloides* (aspen), *Fraxinus americana* (white ash), *Juglans nigra* (black walnut), *Prunus serotina* (black cherry), *Betula papyrifera* (white birch), *Acer saccharum* (sugar maple), and *Hymenaea courbaril* (courbaril) were sampled from their heartwood and analyzed under ESI(+)-MS. This has been subjected to preliminary chemometric analysis, assessing viability for further modelling. (Figure 3-3) The resultant PCA Scores plot was produced with a total variance of 76.50% from the first 4 principal components. While variation was expected to be much greater than that of the different tree anatomy combinations due to the greater number of chemical features,

there was still no significant oversampling signified by the low Q Residuals value of 23.50%. Overlap between 95% CI of the groupings is reduced compared to a PCA scores plot including a sample of *Cedrela odorata* (cedar). The PCA scores plot produced including the sample of cedar also produced a much lower total variance of 62.57 with the first 2 principal components. This indicates further convolution of the data when hardwood and softwood samples aren't differentiated, and that improved class discrimination and predictive modelling can be achieved by treating hardwood and softwood as distinct groups, since their differences introduce increased confounding variation.

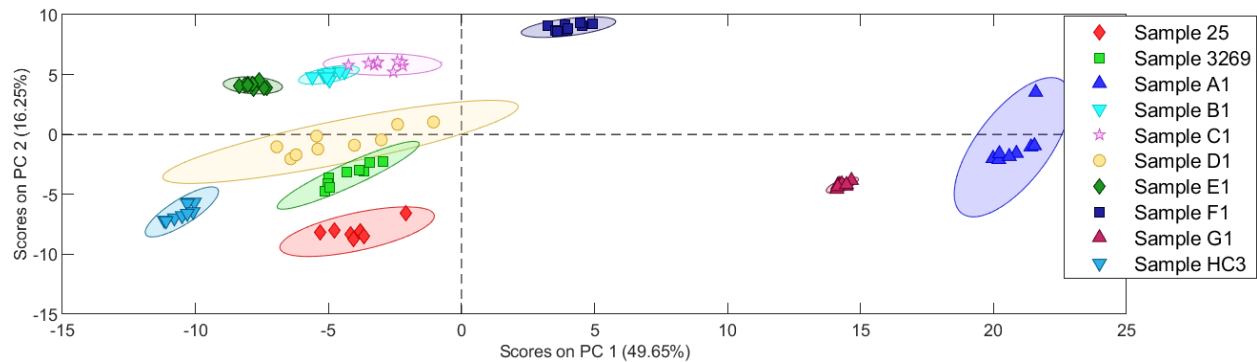


FIGURE 3-3: PCA Scores Plot of different wood samples taken from the heartwood: *Carapa guianensis* (Sample 25), *Swietenia macrophylla* (Sample 3269), *Prunus serrulata* (A1), *Populus tremuloides* (B1), *Fraxinus americana* (C1), *Juglans nigra* (D1), *Prunus serotina* (E1), *Betula papyrifera* (F1), *Acer saccharum* (G1), and *Hymenaea courbaril* (Sample HC3).

Hierarchical Cluster Analysis (Ward's Method) was also performed with the first 4 principal components, using the same processing procedures, to produce a dendrogram displaying clustering within wood species. The results show complete clustering by species, further validating the viability of chemometric modelling. (Figure 3-4)

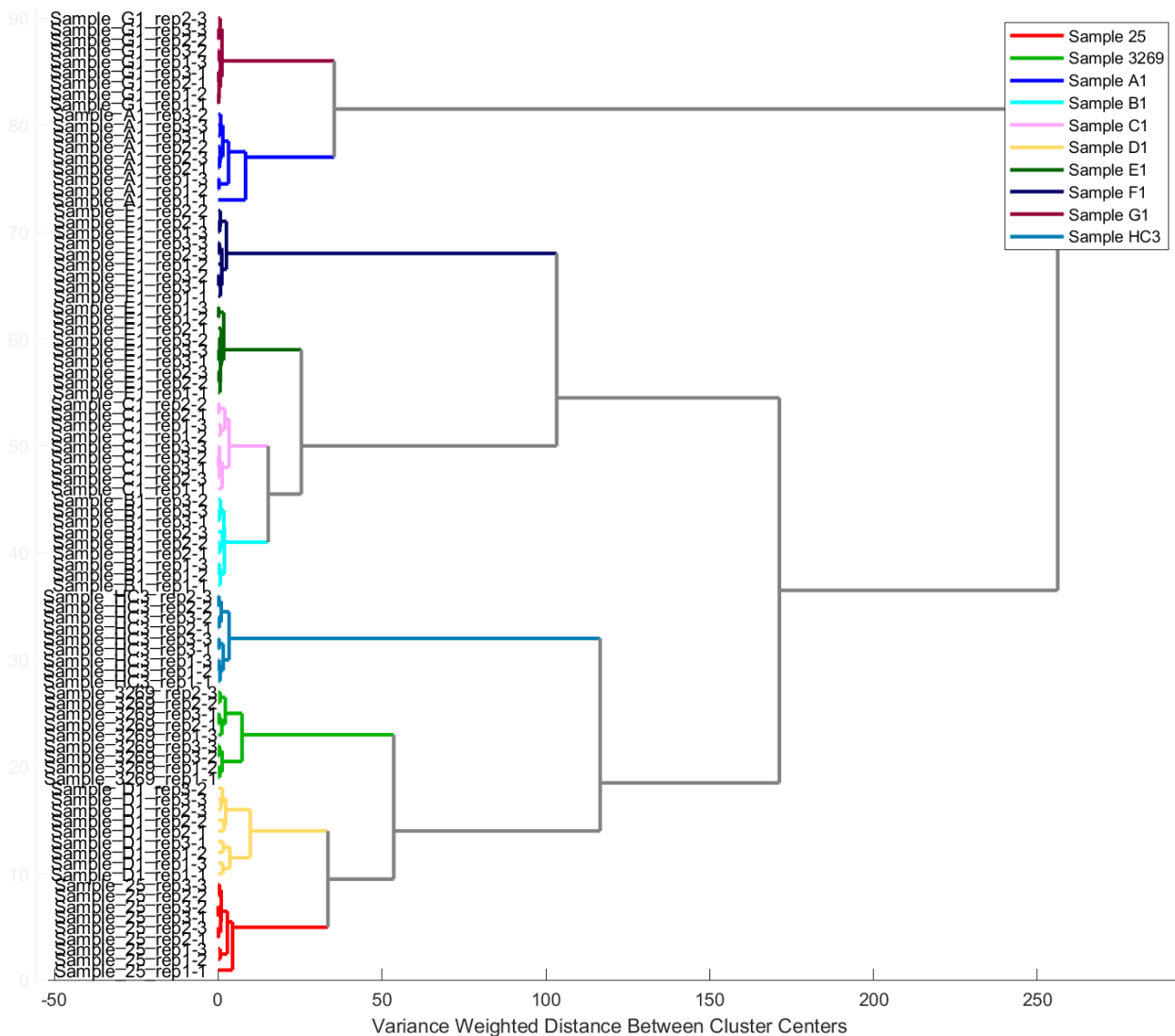


FIGURE 3-4: Dendrogram produced from HCA for various wood samples: *Cedrela odorata* (Sample 1800) *Carapa guianensis* (Sample 25), *Prunus serrulata* (Sample A1), *Juglans nigra* (Sample D1), *Prunus serotina* (Sample E1), *Acer saccharum* (Sample G1), *Hymenaea courbaril* (HC3), and *Swietenia macrophylla* (SM2).

Several detected ions in varying abundances across different wood samples, or “marker ions”, have been observed. Various other unidentified ions could also be observed. (Figure B2-9) MS/MS spectra for a few of these ions have been produced to confirm their identities.

Consistent with related literature, [122, 78] various limonoids have been detected, in the m/z 800 – 1000 range, and confirmed in their sodiated forms ($[M+Na]^+$), via their distinct neutral losses of acetic acid (60 Da). These were found in *Juglans nigra* or black walnut and *Swietenia macrophylla* or mahogany via MS/MS of the precursor ions m/z 883 (NCE 26), 885 (NCE 20), 911 (NCE 25), and 927 (NCE 22), identified as sweitenitin I, swietenitin K, 2,11-diacetoxyswietenalide D, and swietenitin M respectively. (Figure 3-5) The limonoid methyl angolensate was also observed in the same sodiated state via the ion precursor m/z 493 (NCE 19), noticeably in a sample of *Prunus serrulata*, or cherry blossom, as well as cedar, with the same distinct neutral loss. (Figure 3-6)

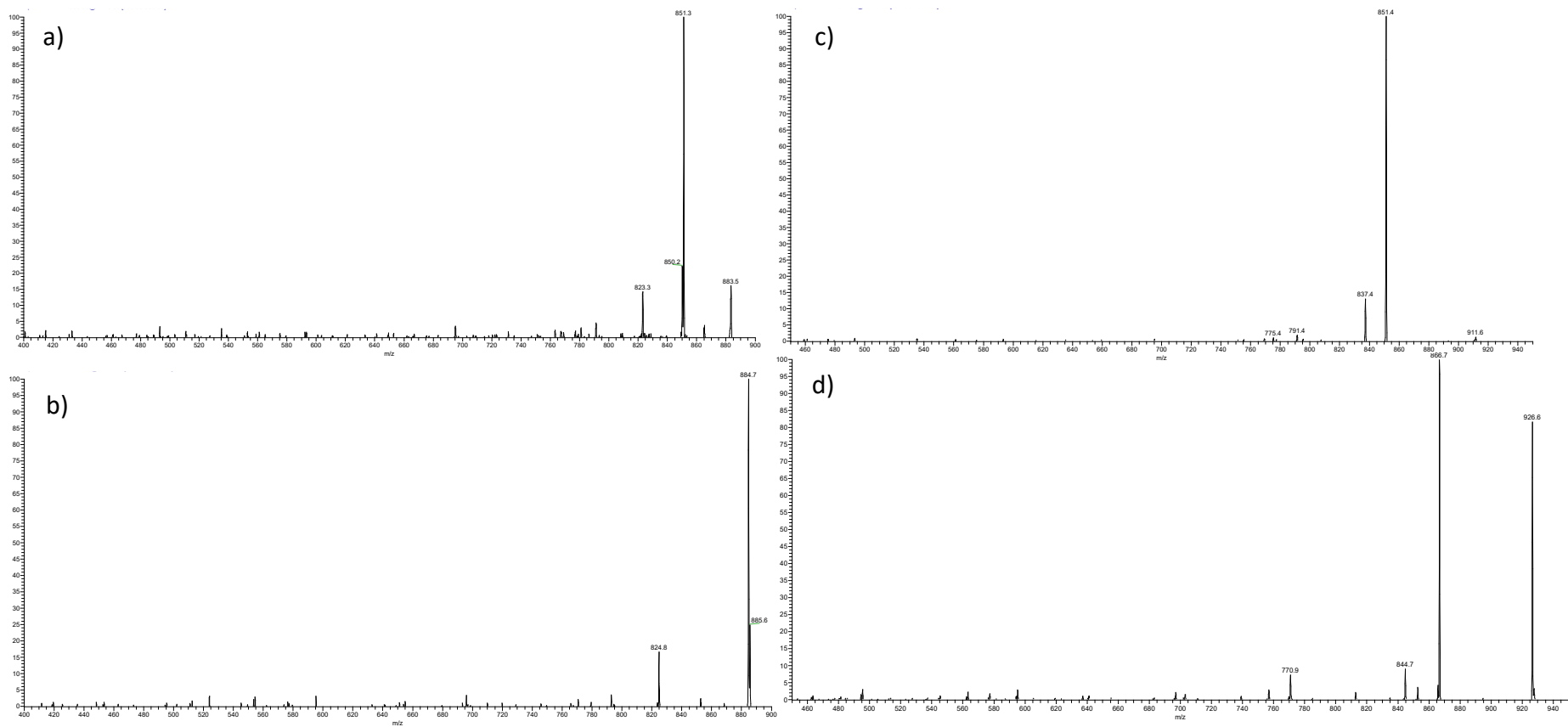


FIGURE 3-5: ESI(+)-MS/MS spectra of the precursor ions m/z a) 883 (swietenitin I), b) 885 (swietenitin K), c) 911 (2,11-diacetoxyswietenalide D, and d) 927 (swietenitin M). Mass range: m/z a,b) 400 – 1000, c,d) 450 – 950.

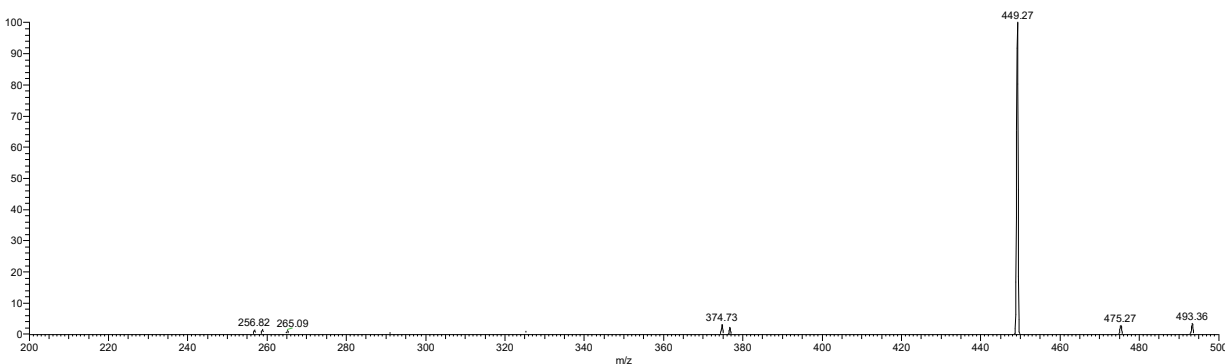


FIGURE 3-6: ESI(+)- MS/MS spectra of the precursor ion m/z 493 identified as methyl angolensate. Mass range: m/z 200 – 500.

Other wood species exhibit distinct marker ions as well. *Acer saccharum*, or sugar maple, sampling from the heartwood, unsurprisingly contains sugar, identified via the ion precursor m/z 360 (NCE 20) in the form of the ammonium adduct of a dehydrated disaccharide $[2M-H_2O+NH_4]^+$. (Figure 3-7a) Neutral loss of 18 Da can be observed, such as with glucose, mannose, and galactose. [133] The limonoid khivorin could also be observed with the precursor ion m/z 609 (NCE 19), also in the sodiated form $([M+Na]^+)$. (Figure 3-7b)

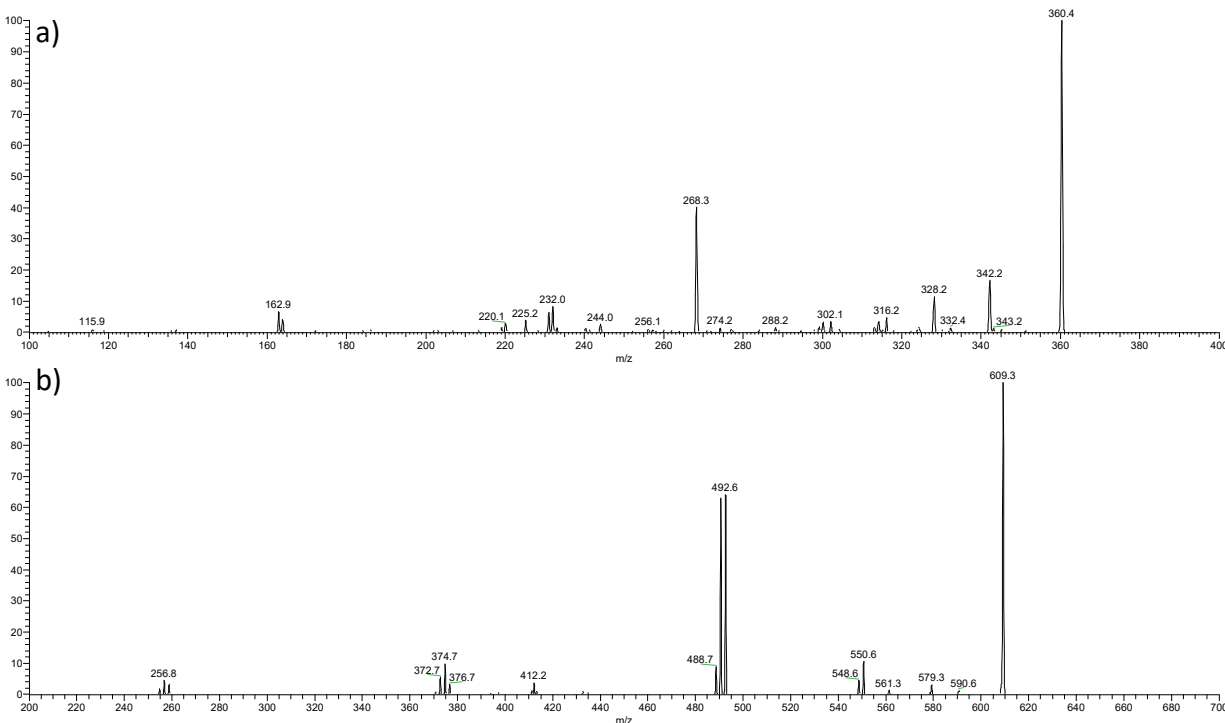


FIGURE 3-7: ESI(+)- MS/MS spectra of the precursor ion m/z a) 360 and 609 identified as an ammonium adduct of a dehydrated disaccharide and khivorin, respectively. Mass range: a) m/z 100 – 400, b) m/z 200 – 700.

Modelling & Predictive Performance

PLS2-DA models were constructed using ESI(+)-MS and SPS(+)-MS data for the prediction of wood species and the location origin of the samples. The PLS2-DA models based on ESI-MS and SPS-MS for species classification (Fig. 7a and Fig. 7b, respectively) demonstrated effective performance in discriminating among wood classes.

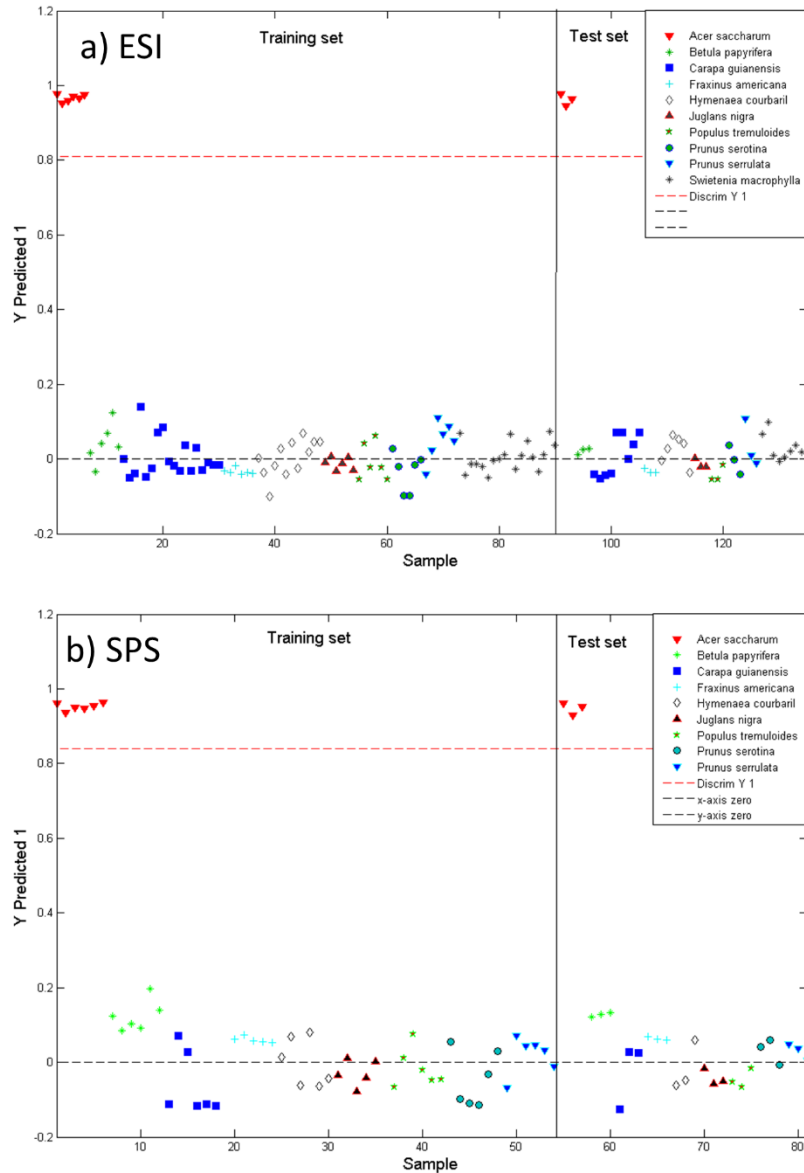


Figure 3-8: Y prediction for *Acer saccharum* wood specie PLS2-DA on a) ESI(+)-MS and b) SPS(+)-MS.

The predictive models built from both ESI-MS and SPS-MS data exhibited comparable efficiency, thereby highlighting the effectiveness of the proposed SPS-MS methodology (Table 2).

Table 3-2: Figures of merit of PLS2-DA model for wood classes

		Training set			Test set		
Ionization technique	Class	Sensitivity	Specificity	Efficiency	Sensitivity	Specificity	Efficiency
SPS	<i>Acer saccharum</i>	100%	100%	100%	100%	100%	100%
	<i>Betula papyrifera</i>	83%	85%	84%	100%	88%	94%
	<i>Carapa guianensis</i>	100%	89%	94%	100%	88%	94%
	<i>Fraxinus americana</i>	100%	92%	96%	100%	100%	100%
	<i>Hymenaea courbaril</i>	100%	98%	99%	100%	100%	100%
	<i>Juglans nigra</i>	100%	100%	100%	100%	100%	100%
	<i>Populus tremuloides</i>	100%	94%	97%	100%	88%	94%
	<i>Prunus serotina</i>	100%	91%	96%	67%	88%	76%
	<i>Prunus serrulata</i>	100%	98%	99%	100%	100%	100%

	<i>Acer</i>						
	<i>saccharum</i>	100%	100%	100%	100%	100%	100%
	<i>Betula</i>						
	<i>papyrifera</i>	83%	91%	87%	100%	76%	87%
	<i>Carapa</i>						
	<i>guianensis</i>	94%	97%	96%	100%	100%	100%
	<i>Fraxinus</i>						
	<i>americana</i>	100%	99%	99%	100%	100%	100%
	<i>Hymenaea</i>						
	<i>courbaril</i>	100%	94%	97%	100%	100%	100%
ESI	<i>Juglans</i>						
	<i>nigra</i>	100%	100%	100%	100%	100%	100%
	<i>Populus</i>						
	<i>tremuloides</i>	100%	99%	99%	100%	98%	99%
	<i>Prunus</i>						
	<i>serotina</i>	100%	92%	96%	67%	76%	71%
	<i>Prunus</i>						
	<i>serrulata</i>	100%	99%	99%	100%	100%	100%
	<i>Swietenia</i>						
	<i>macrophylla</i>	94%	94%	94%	89%	86%	87%

To explore chemical significance of the separation between wood types, VIP scores were analyzed for each class in the PLS2-DA model based on SPS-MS data. The results shown in Table 3 indicate the specific m/z values that contributed significantly to class discrimination.

Table 3-3: Significant ions from each wood species from VIP scores of the PS2-DA model

Species	<i>m/z</i>
<i>Acer saccharum</i>	439;500
<i>Betula papyrifera</i>	217;301;353;381;441
<i>Carapa guianensis</i>	301;353;381;413;441;500
<i>Fraxinus americana</i>	301;353;381;413;441;500
<i>Hymenaea courbaril</i>	251;374;301;355;413;441;500;529;630;660
<i>Juglans nigra</i>	413;439;500
<i>Populus tremuloides</i>	251;355;439;500;630;660
<i>Prunus serotina</i>	353;381;413;441;500
<i>Prunus serrulata</i>	441;457;630;660

In contrast, the PLS2-DA models for predicting the geographical origin of the wood samples showed limited discriminative performance (Table 4), both for ESI-MS and SPS-MS data. This may be attributed to the presence of samples from different species collected at the same location, as well as samples from the same species collected at different locations. As a potential alternative, one-class classification models focusing on species-location pairs could be explored.

Table 3-4: Figures of merit for the PLS2-DA model used for region tracing

Ionization technique	Class	Training set			Test set		
		Sensitivity	Specificity	Efficiency	Sensitivity	Specificity	Efficiency
SPS (5LV's)	Toronto	98%	92%	95%	100%	100%	100%
	Flona Tapajos	83%	98%	90%	100%	100%	100%
	Itacotiara	100%	98%	99%	100%	100%	100%
ESI (5LV's)	Toronto	93%	92%	92%	100%	40%	63%
	Presidente Figueiredo	100%	96%	98%	0%	83%	0%
	Flona Tapajos	100%	87%	93%	83%	82%	83%
	Manaus	89%	93%	91%	0%	100%	0%
	Itacotiara	100%	97%	99%	100%	95%	97%

While the model gives promising predictive performance for chemometric modelling of wood samples, it was expectedly less robust compared to simple class discrimination (ie. species-level grouping). Various improvements can be made to aid overall performance, which could include additional refinements to the analytical workflow, particularly at the sample preparation stage. Employing discrete solvent systems tailored to selectively extract and enrich specific chemical classes, could enhance the detection of marker ions and other low abundance but diagnostically relevant compounds, in order to improve predictive performance. [134] These improvements would support the continued development of SPS-MS as a viable tool for on-site screening and rapid analysis of potentially fraudulent wood samples in various settings.

CONCLUSION

The results herein demonstrate the viability of using SPS(+)-MS, combined with chemometric analysis, for discrimination and predictive classification for species and origin. It has also been demonstrated that the sampling of heartwood is more suitable for chemical profiling. Heartwood consistently exhibited more distinct and chemically informative chemical profiles than sapwood. Preliminary modelling based on ESI(+)-MS successfully identified certain marker ions, including known limonoids and saccharide derivatives, further substantiating species-level differences. While the SPS-MS model demonstrated strong predictive performance, its comparatively lower robustness in blind testing underscores the importance of expanding reference datasets and optimizing sample preparation, such as selective solvent extraction, to improve the detection of diagnostically relevant features and enhance overall classification reliability. Given the complexity of wood species traceability, SPS-MS may prove to be more useful as a complementary technique, available to be used at every stage of trade involved in forestry crimes, rather than an outright alternative to other MS techniques (eg. pyrolysis, DART, SIRA, and others) with their own pros and cons and different chemical information provided, aiding in more accurate chemometric classification.

Chapter 4: Conclusions and future works

The works presented demonstrate the utility of a recent ambient ionization mass spectrometry (AIMS) method, sandpaper spray ionization – mass spectrometry (SPS), in a forensic setting, due to its versatility, low-cost, and without requiring extensive sample preparation steps. Its ease-of-use also allows for non-laboratory personnel with minimal to no training to perform initial screening of suspected materials with a simple outlined protocol. This makes SPS-MS suitable for rapid on-site analyses, and, due to being an MS-coupled ionization technique, a wide array of sample types from purified samples such as pharmaceuticals to highly complex biological samples can be analyzed.

As outlined in Chapter 2, SPS-MS has demonstrated itself as a capable MS technique in detecting and identifying pharmaceutical formulations. In the form of a tablet, pill, and hard candy, the samples were highly suited for sampling via abrasion and, readily ionized in order to detect their active pharmaceutical ingredients (APIs). Various excipients within the formulation of the samples were also detected, which can be used to detect fraudulent products with an inferior formulation with possible adverse health effects. Further investigations into reaction monitoring on the substrate can be made, particularly for differentiating detected isomers that may be pharmacologically distinct and relevant to legal determinations, in the case of differentiating Δ^8 -THC and Δ^9 -THC.

In Chapter 3, SPS-MS was used to develop a method which is able to sample and analyze various wood samples for possible species-level classification and origin tracing. The technique was contrasted with spectral data acquired from a more established ionization technique, ESI, displaying the viability of integrating with existent MS spectral databases related to identifying protected classes of wood/trees, not needing to create its own database with all the challenges therein. The technique was further developed to utilize a handheld rotary tool which not only ensures sufficient sampling and minimizes handling for portable use, but also makes the abrasion of hard surfaces, such as hardwood, quicker and easier for the operator. Further development of the method can be made to increase the number of spectral features produced,

which, in turn, would provide more reliable classification. Concurrently, further investigations can be made to the metabolomics of trees after being cut down, in various conditions and stages of development. This would further ensure reliable classification, not only from the increased spectral features, but also in examining validity of use, understanding age and shelf-life of acquired samples, whether in their raw unprocessed state or with the collection of wood fibres and fine particles.

In addition to these, I have been involved in other projects utilizing an electrospray ionization / matrix-assisted laser desorption/ionization (ESI/MALDI) dual ion source (Spectrograph LLC, Kennewick, WA, USA) coupled to a quadrupole-orbitrap mass spectrometer (Q Exactive™ Orbitrap Mass Spectrometer from Thermo Fisher Scientific, USA), becoming accustomed to its basic operations and related maintenance procedures. A recently developed quantification method using MALDI-MS by Parasecolo et al. [135] was used for a high-throughput targeted metabolomic analysis of phytoalexins, or plant-synthesized small molecules in a stressed state due to external stimuli and/or physiological conditions. [136] My contributions to the project(s) include the sample preparation steps for MALDI-MS analysis, standard checks, and the acquisition and extraction of the MS data. Two additional publications are planned from the MS data acquired, to be finished by the end of the year.

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Appendices

Appendix A. Supplementary materials for Chapter 2

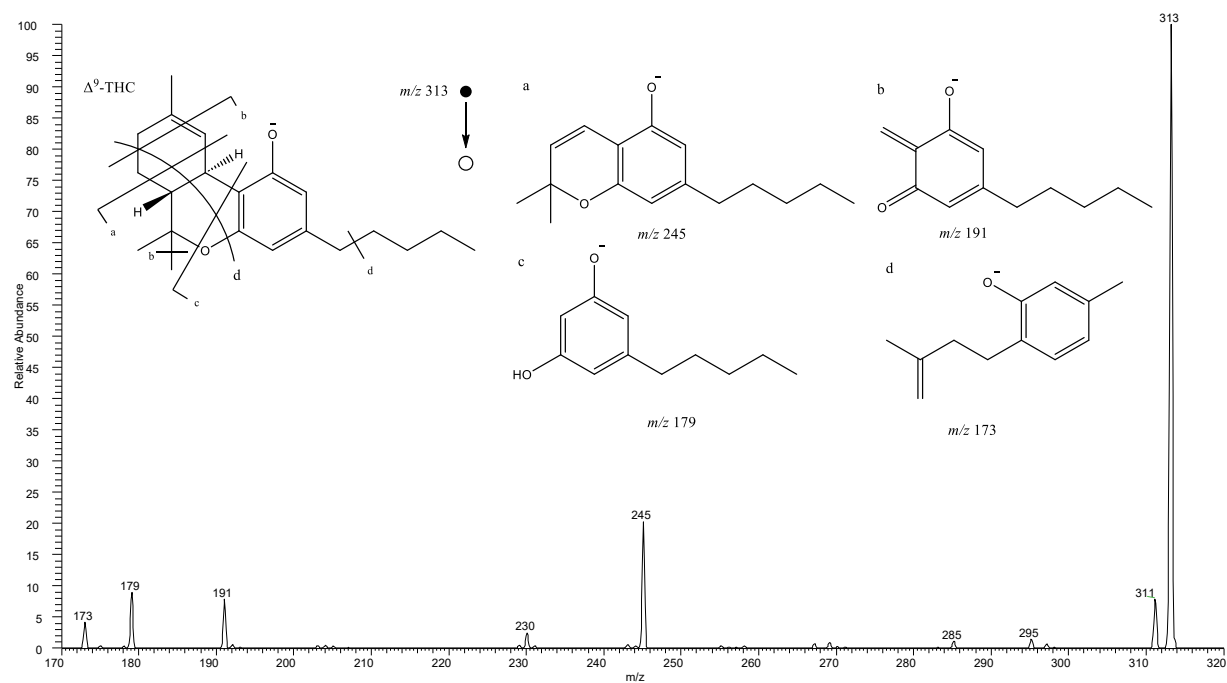


FIGURE A1 MS/MS (CID 20) and proposed fragment ions of the precursor ion of m/z 313 (deprotonated Δ^9 -THC). The SPS(-)-MS comes from a *Cannabis Sativa* lozenge.

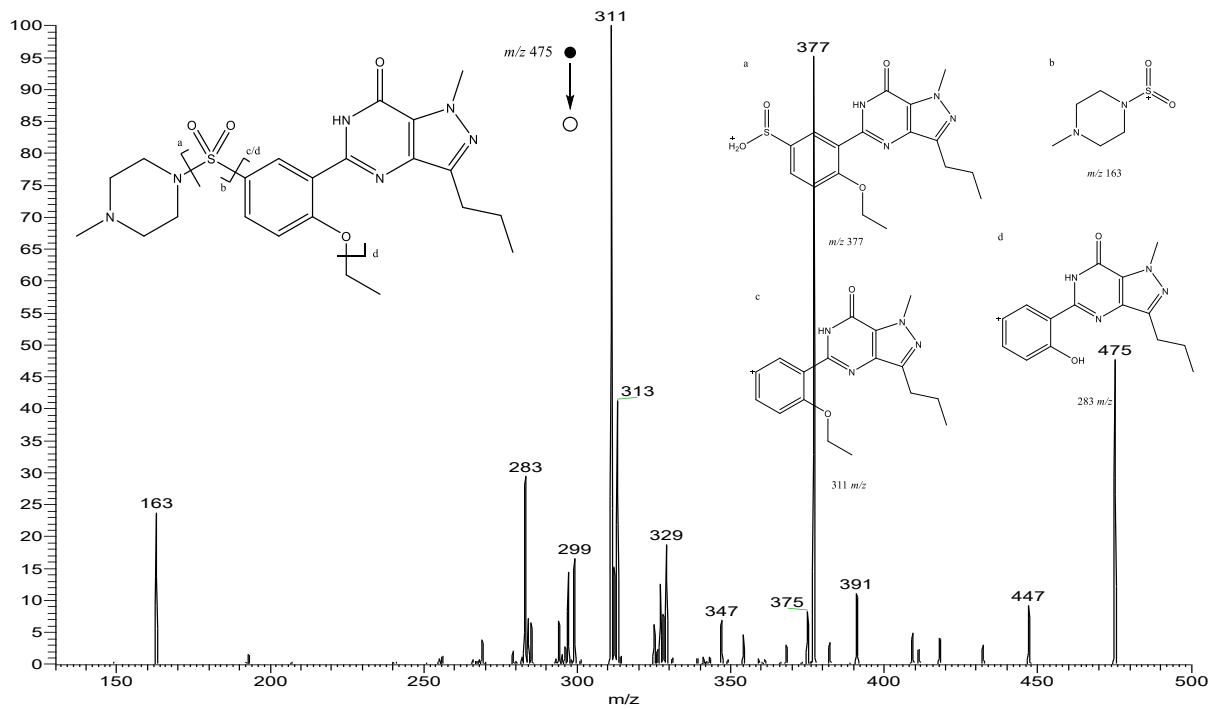


FIGURE A2 MS/MS (CID 20) and proposed fragment ions of the precursor ion of m/z 475 (protonated sildenafil). The SPS(+)-MS comes from a Viagra® tablet.

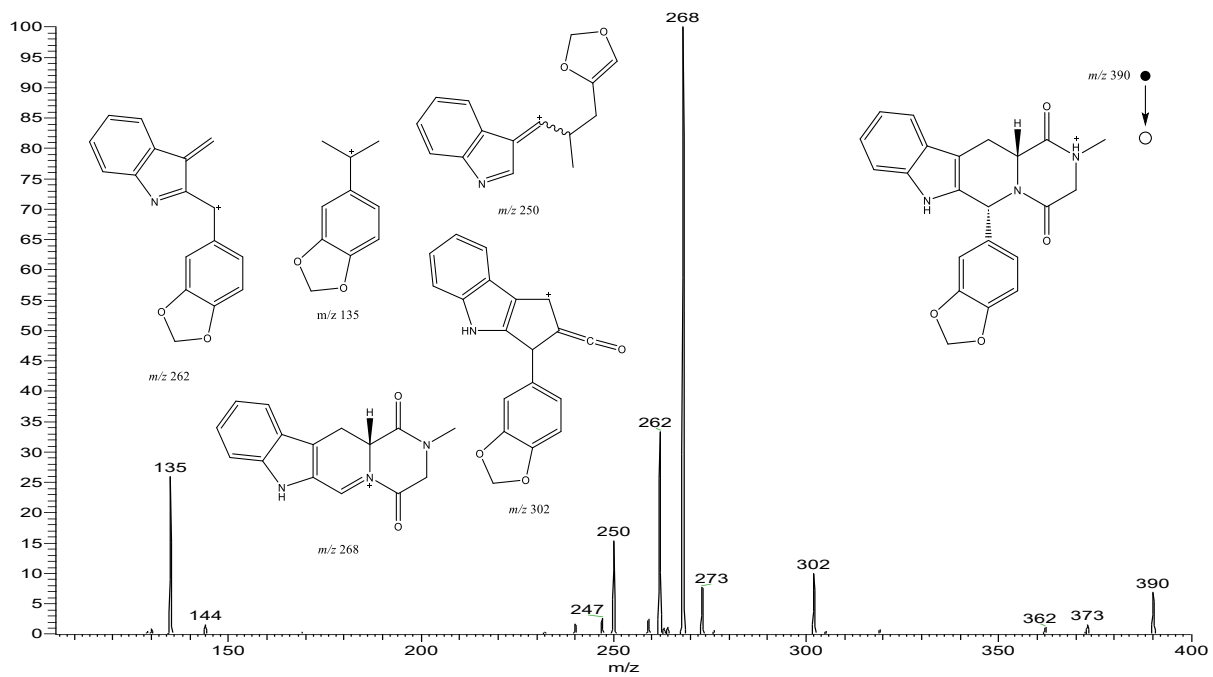


FIGURE A3 MS/MS (CID 19) and proposed fragment ions of the precursor ion of m/z 390 (protonated tadalafil). The SPS(+)-MS comes from a Cialis® tablet.

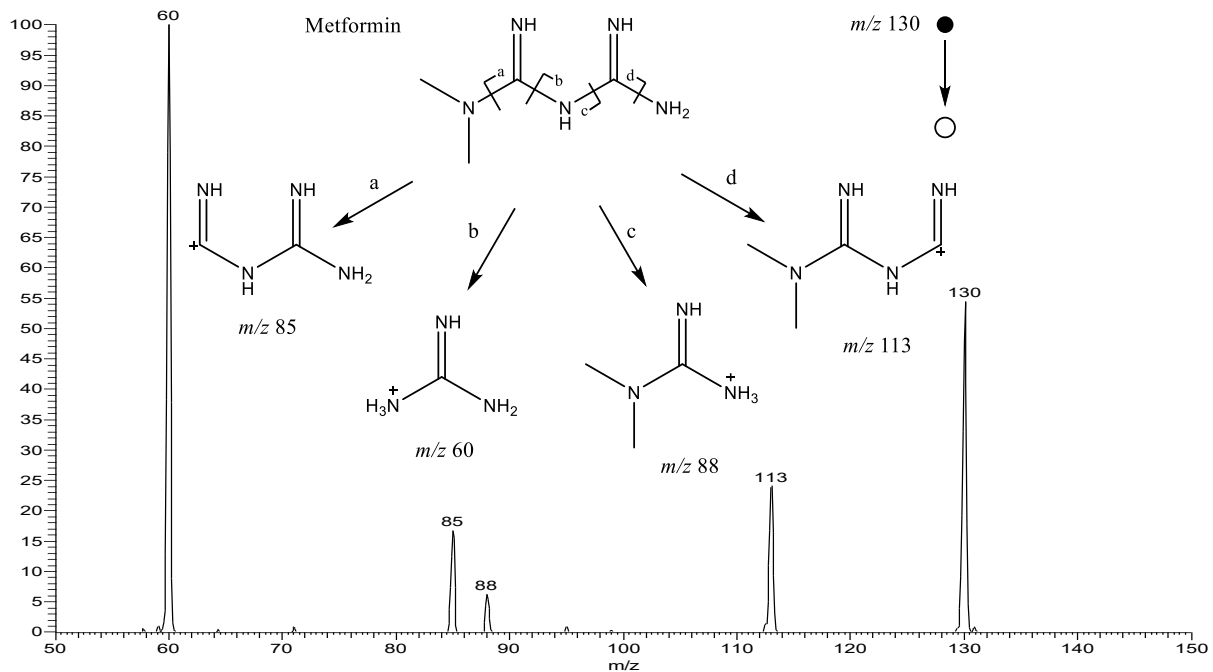


FIGURE A4 MS/MS (CID 39) and proposed fragment ions of the precursor ion of m/z 130 (protonated metformin). The SPS(+)-MS comes from a APO-Metformin[®] tablet.

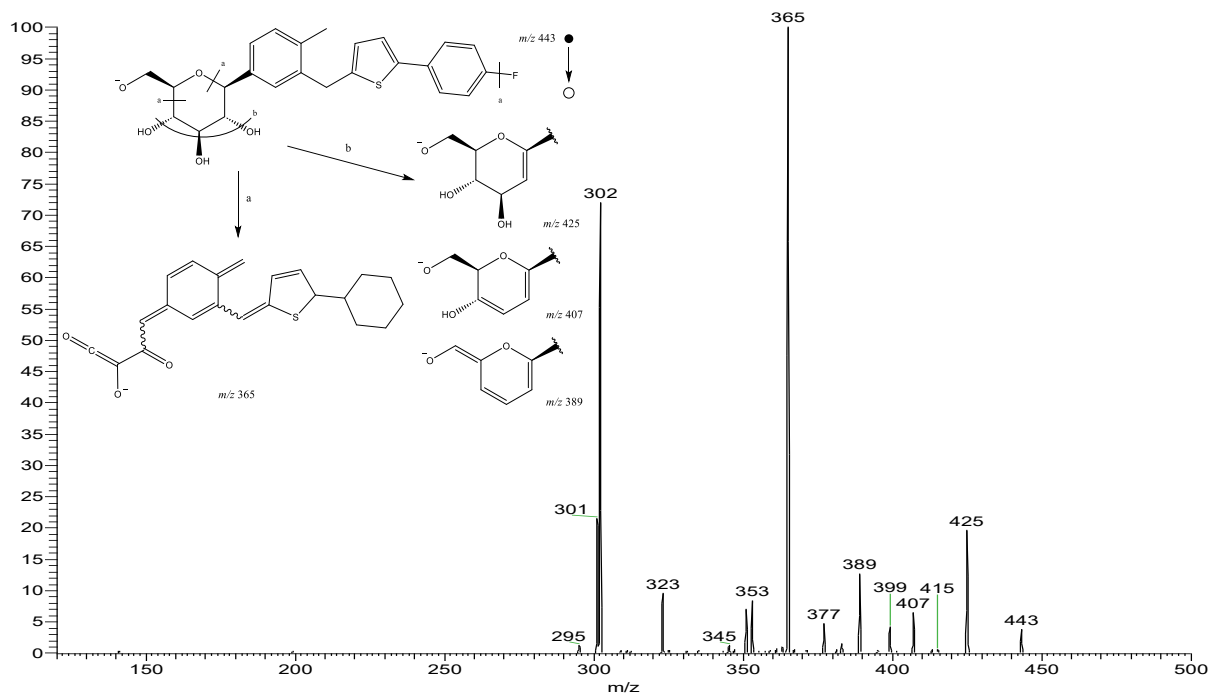


FIGURE A5 MS/MS (CID 21) and proposed fragment ions of the precursor ion of m/z 443 (deprotonated canagliflozin). The SPS(-)-MS comes from an Invokana[®] tablet.

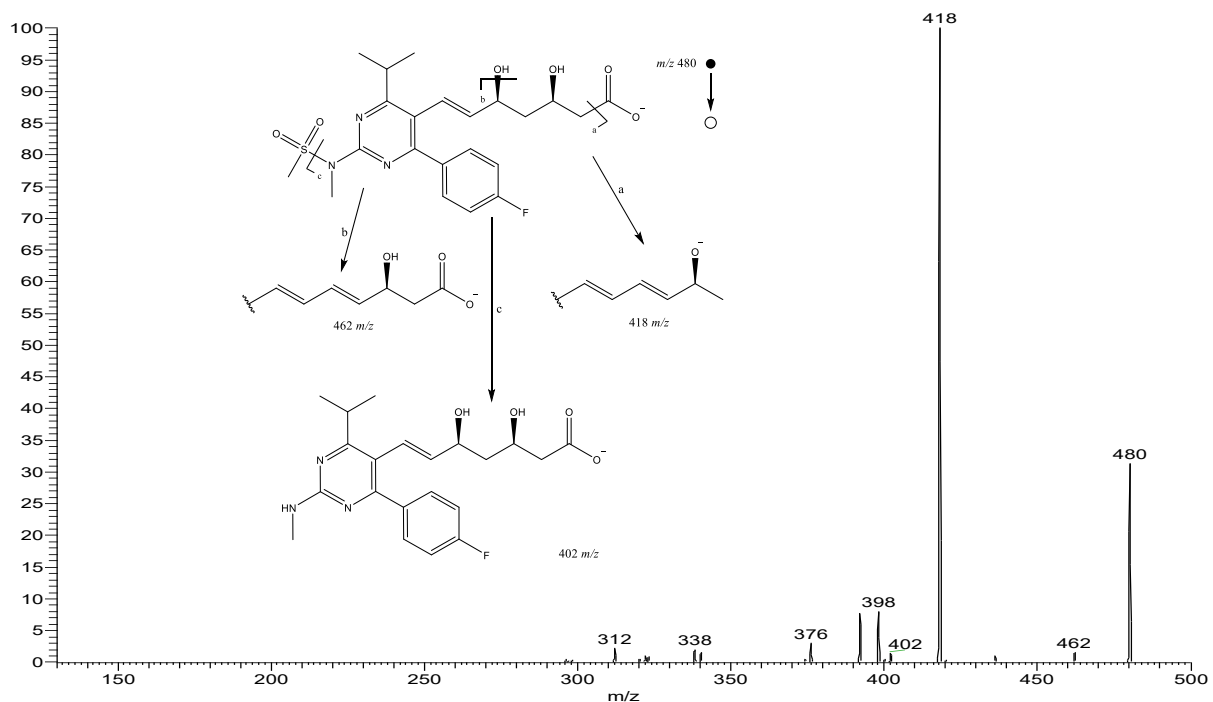


FIGURE A6 MS/MS (CID 21) and proposed fragment ions of the precursor ion of m/z 480 (protonated rosuvastatin). The SPS(-)-MS comes from a Crestor® tablet.

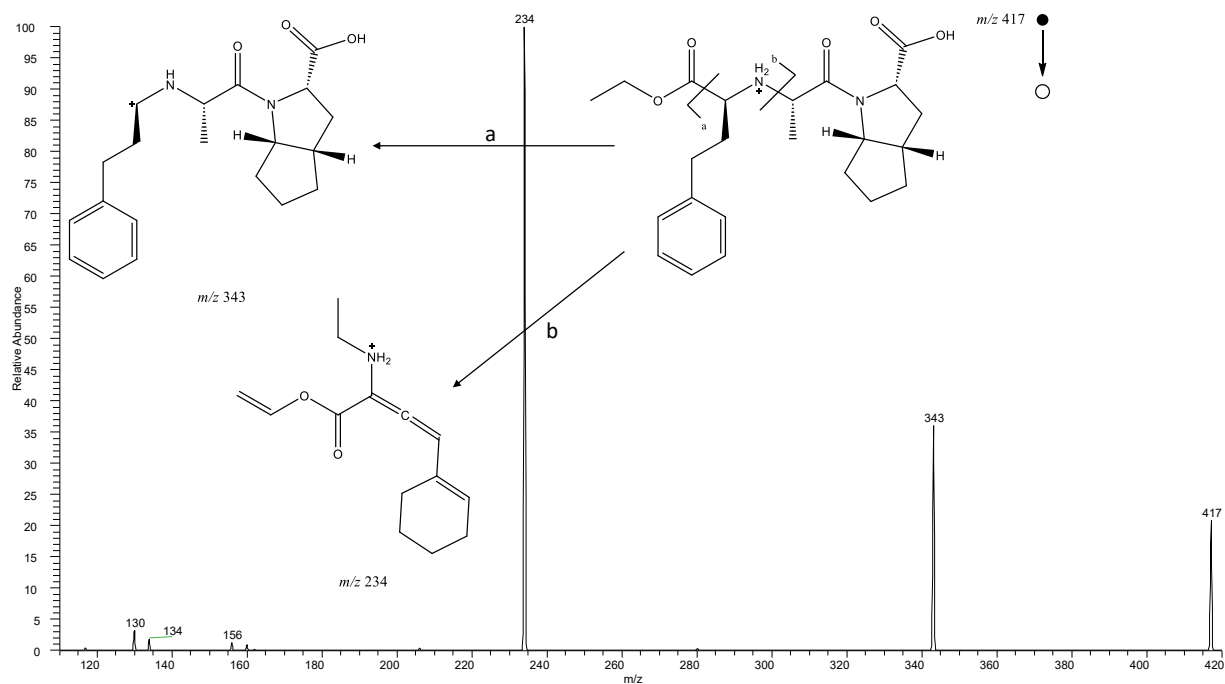


FIGURE A7 MS/MS (CID 16) and proposed fragment ions of the precursor ion of m/z 417 (protonated ramipril). The SPS(+)-MS comes from a Teva-Ramipril® pill.

Appendix B. Supplementary materials for Chapter 3

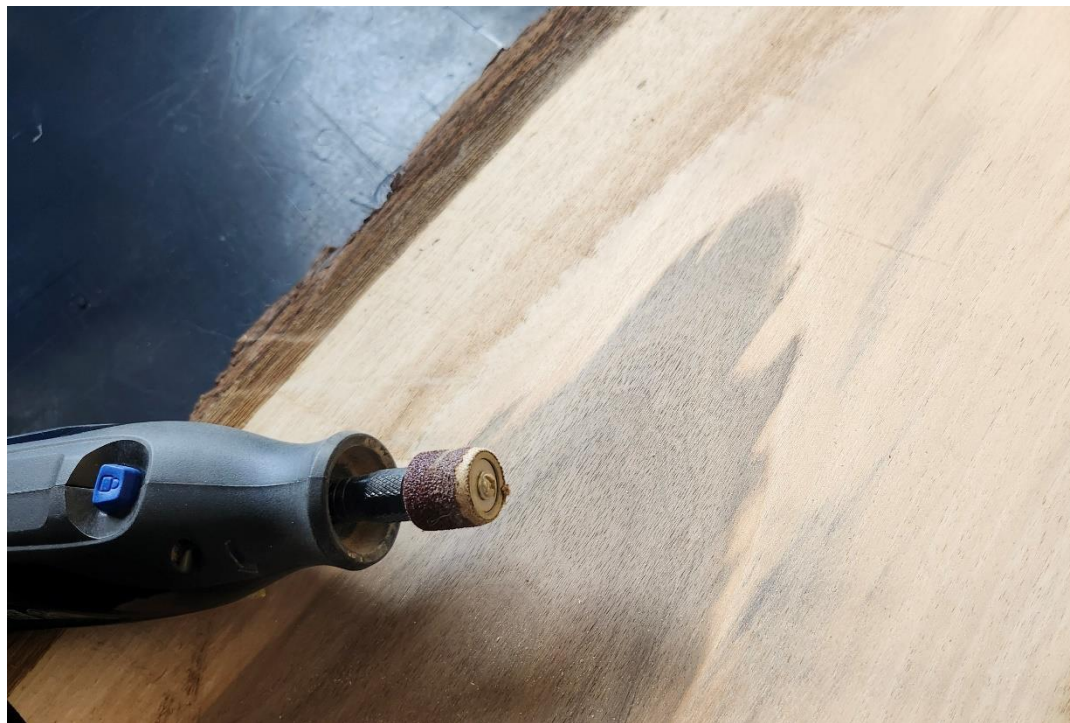


FIGURE B1 Photograph of rotary tool on wood sample used in SPS – MS.

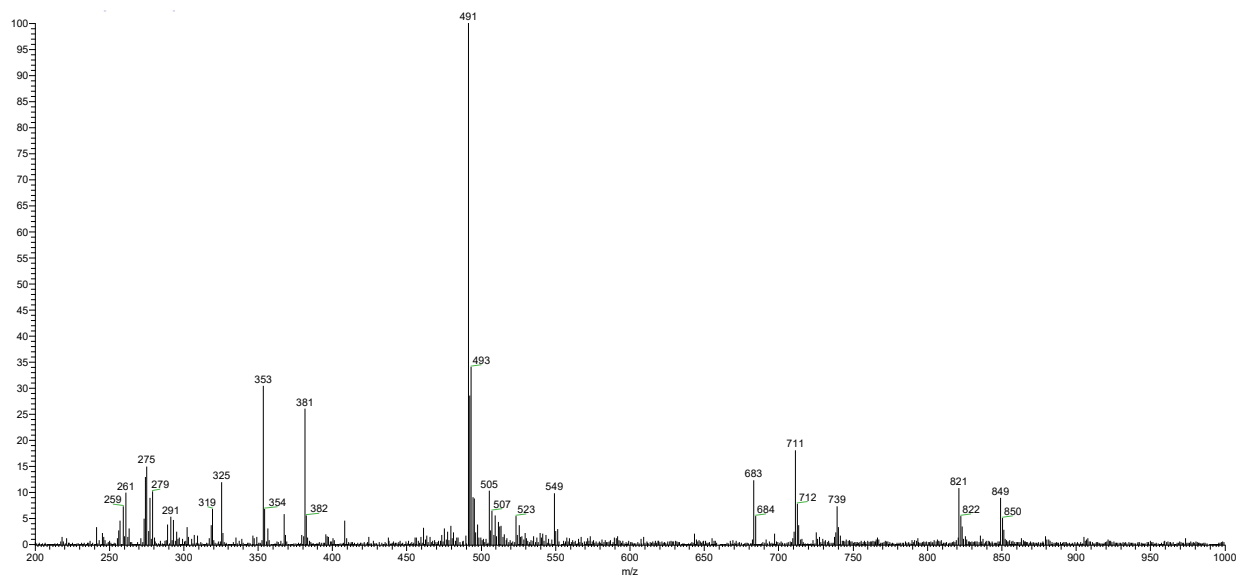


FIGURE B2 ESI(+)-MS spectrum of *Cedrela odorata* sampled from the heartwood.
Mass range: m/z 200 – 1000.

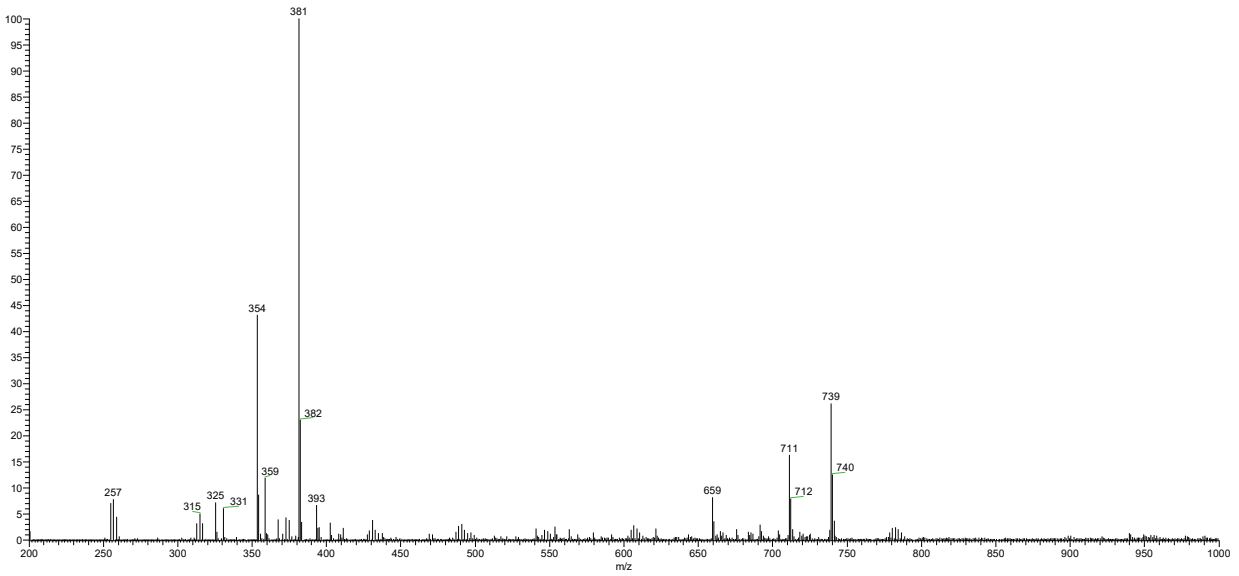


FIGURE B3 ESI(+)-MS spectrum of *Carapa guianensis* sampled from the heartwood.
Mass range: m/z 200 – 1000.

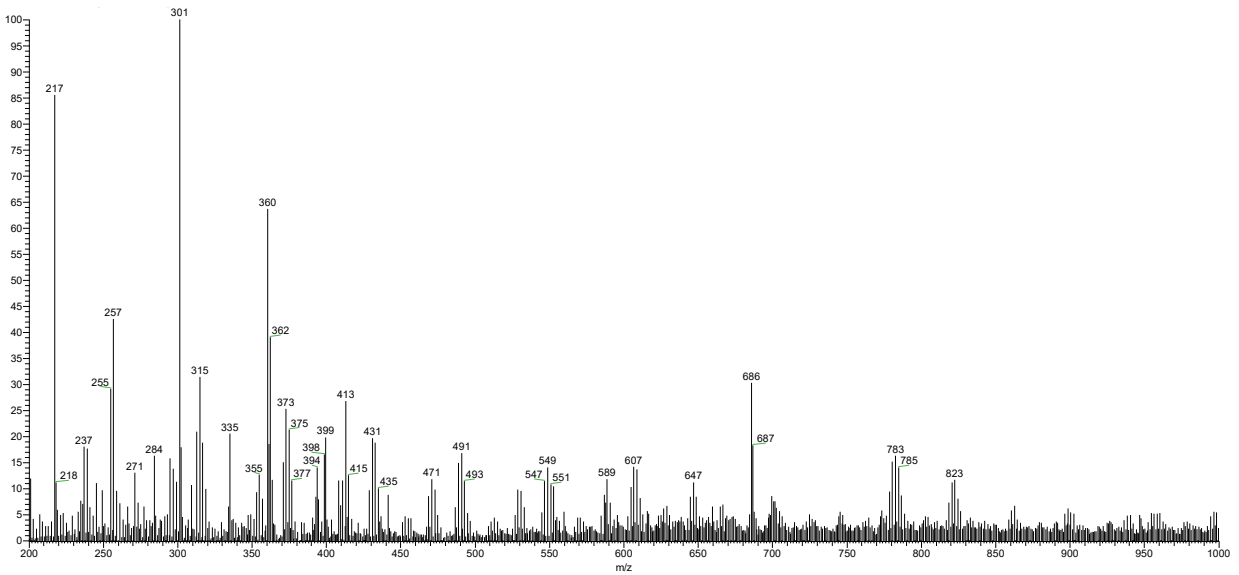


FIGURE B4 ESI(+)-MS spectrum of *Prunus serrulata* sampled from the heartwood.
Mass range: m/z 200 – 1000.

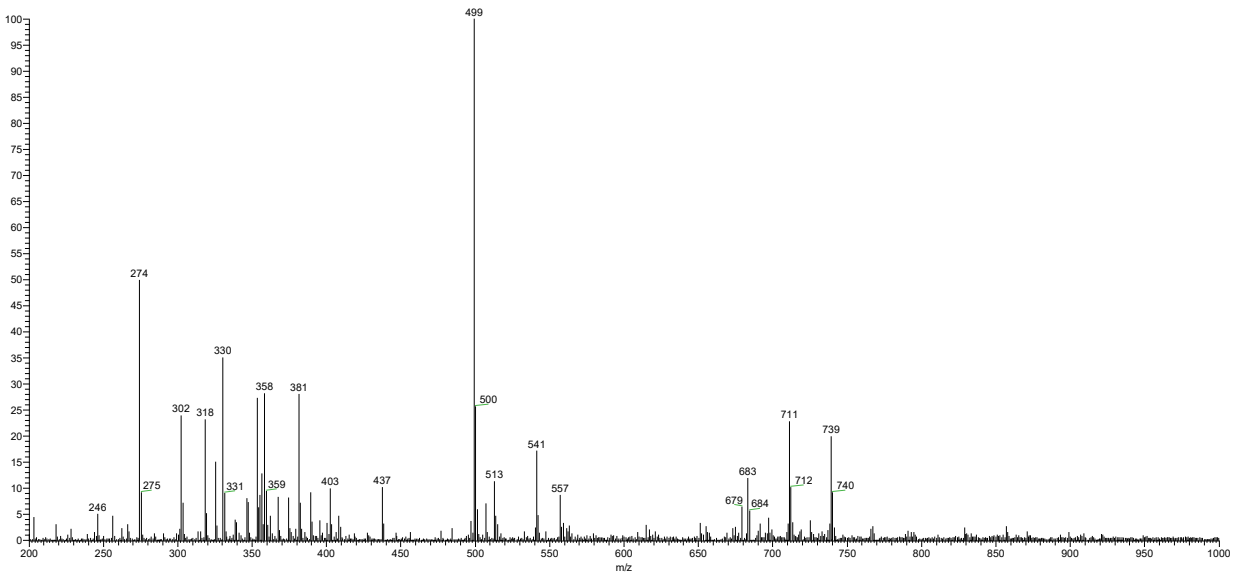


FIGURE B5 ESI(+)-MS spectrum of *Juglans nigra* sampled from the heartwood. Mass range: m/z 200 – 1000.

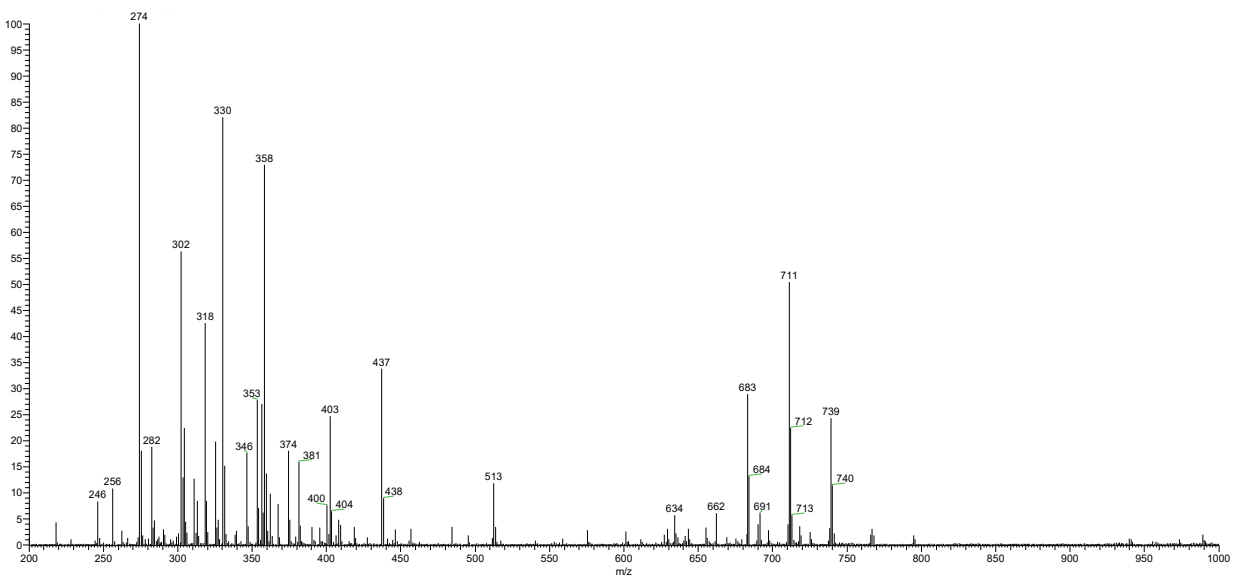


FIGURE B6 ESI(+)-MS spectrum of *Prunus serotina* sampled from the heartwood. Mass range: m/z 200 – 1000.

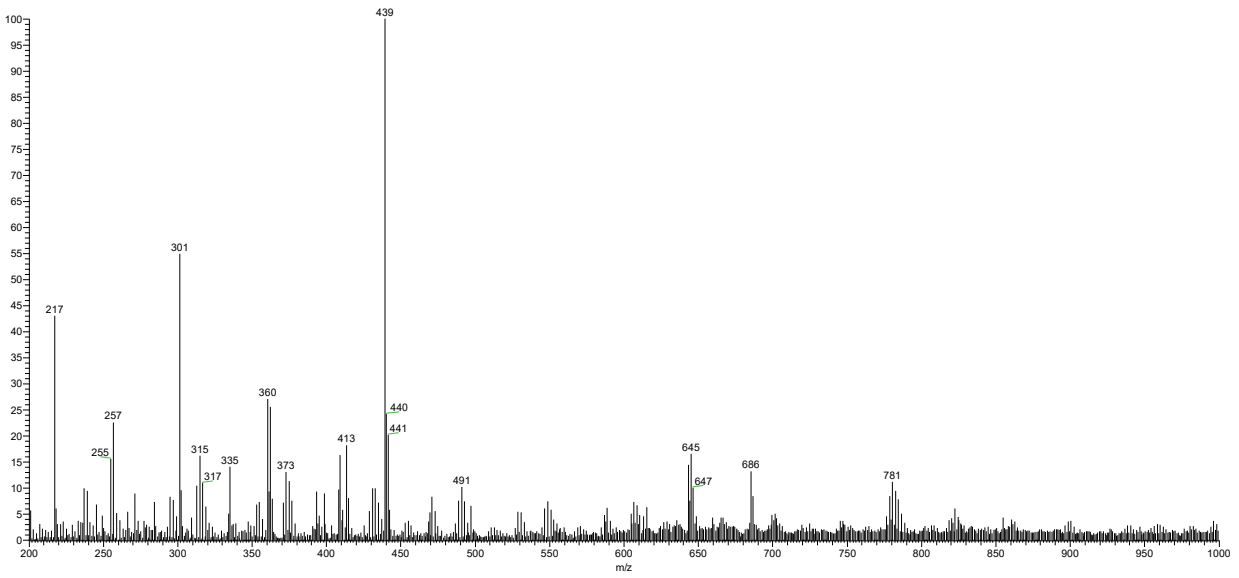


FIGURE B7 ESI(+)-MS spectrum of *Acer saccharum* sampled from the heartwood.
Mass range: m/z 200 – 1000.

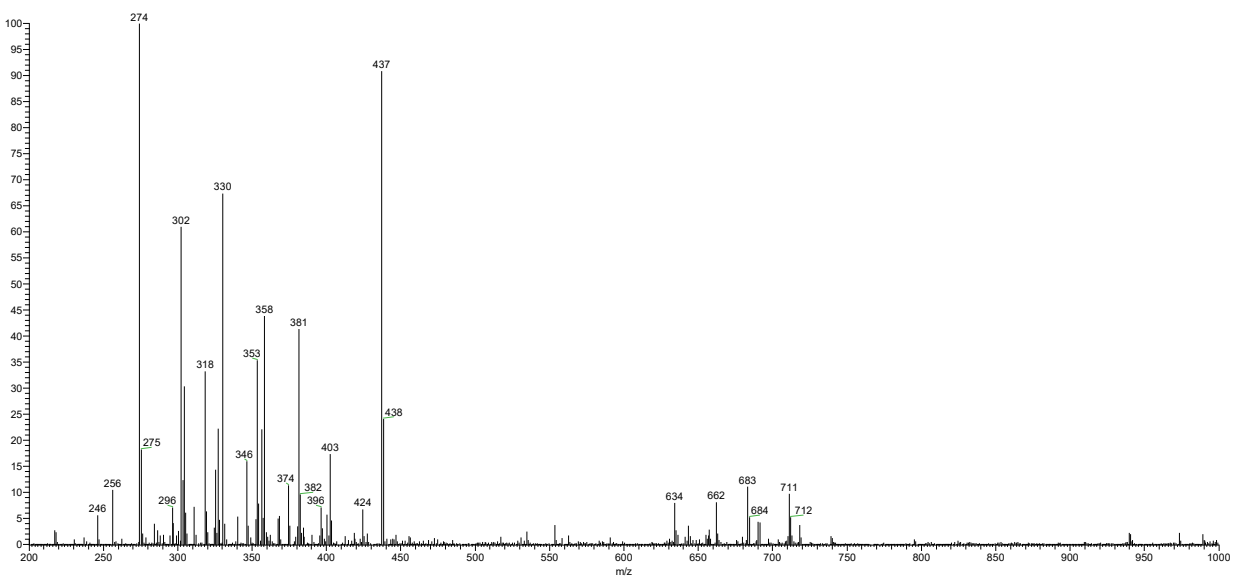


FIGURE B8 ESI(+)-MS spectrum of *Hymenaea courbaril* sampled from the heartwood.
Mass range: m/z 200 – 1000.

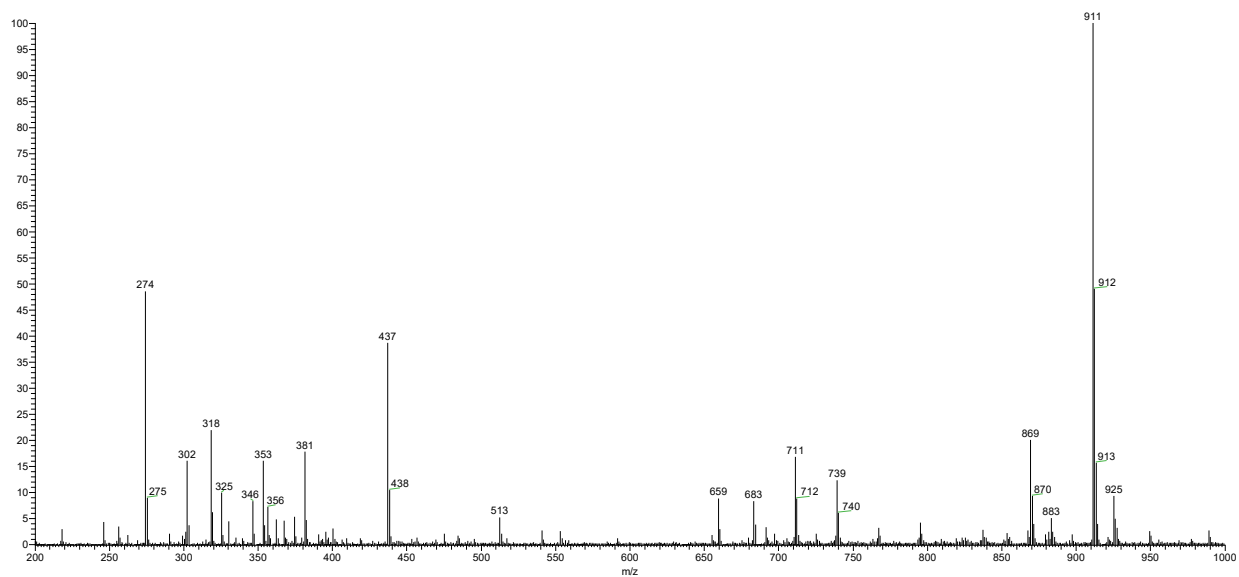


FIGURE B9 ESI(+)-MS spectrum of *Swietenia macrophylla* sampled from the heartwood. Mass range: m/z 200 – 1000.