Development of a High-Throughput Method of Analysis for Selected Neonicotinoids

by

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Abstract

Neonicotinoids are a class of insecticides recently developed as more environmentally friendly alternatives to traditional organophosphate, carbamate, and pyrethroid insecticides. Through continuously expanding global use of neonicotinoids, they have become the most widely use insecticides. In addition, there are increasing concerns regarding their use, particularly regarding their effects on pollinating and aquatic species. Regulatory agencies are beginning the phase-out and restriction of neonicotinoid insecticides in Europe and North America.

This research details the development of a comprehensive method of analysis for neonicotinoid insecticides in aqueous environmental matrices. Analytical methods are developed for routine, high-throughput, quantitative measurements of neonicotinoids; and new sampling, extraction, and pre-concentration techniques are examined.

By combining UHPLC with a simpler method and superficially porous column technology, the elution program for the six neonicotinoids of interest is reduced from a literature average of 9 minutes, to just 2.7 minutes for baseline separation. Additionally, tandem mass spectrometry is used to lower instrumental limits of detection. Separation and extraction procedures can be eliminated entirely through the adaptation of TDU-DART-MS, methods for which were developed and optimized for the neonicotinoids. Finally, the novel MIP-SPE procedure is used with high sensitivity to selectively extract neonicotinoids of interest from environmental matrices. Method

detection limits are lowered to fall between 0.1 and 1 ng L^{-1}, and local waterways are shown to demonstrate a presence of neonicotinoids while using this method.

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List of Abbreviations and Symbols

nAChR	Nicotinic acetylcholine receptor
QuEChERs	Quick, Easy, Cheap, Effective, Rugged, Safe
SPE	Solid phase extraction
PSA	Primary-secondary amine
DLLME	Direct liquid-liquid micro-extraction
LC	Liquid chromatography
GC	Gas chromatography
HPLC	High performance liquid chromatography
UHPLC	Ultra-high performance liquid chromatography
PDA	Photo-diode array
UV	Ultra violet
LOD	Limit of detection
AC	Alternating current
DC	Direct current
APCI	Atmospheric pressure chemical ionization
ESI	Electrospray ionization
DART	Direct analysis in real time
TDU	Thermal desorption unit
API	Atmospheric pressure ionization

DESI	Desorption electrospray ionization
DAPPI	Direct atmospheric photoionization
DCM	Dichloromethane
FTICR	Fourier transform ion cyclotron resonance
QTOF	Quadrupole time of flight
QqQ	Triple quadrupole
SRM	Selected reaction monitoring
MIP	Molecularly imprinted polymer
NIP	Non-imprinted polymer
PPC	Pre-polymerization complex
MAA	Methacrylic acid
ACC	Acrylamide
HEMA	2-hydroxyethyl methacrylate
2-VP	2-vinyl pyridine
AIBN	2,2ázobisisobutyronitrile
DMPA	2,2-dimethoxy-2-phenylacetophenone
PAH	Poly-cyclic aromatic hydrocarbon
NDMA	N-nitrosodimethylamine
RAFT	Reversible addition fragmentation chain transfer
SPME	Solid-phase micro-extraction
IF	Imprinting factor
m/z	Mass-to-charge ratio
CID	Collision-induced dissociation

Chapter 1

Introduction

1.1 Overview

Better methods for the determination of neonicotinoid insecticides in environmental samples is an important area of research amongst environmental and analytical chemists. As the use of neonicotinoids continues to expand on a global scale, so too do the potential effects on pollinators and other non-mammalian species. Since their initial development and sale, regulatory agencies are growing increasingly concerned with their use. In order to better map their distribution, persistence, and effects on environmental systems, better methods of analysis are needed to improve sample throughput, limits of detection, and selectivity.

1.1.1 Introduction to Neonicotinoids

Neonicotinoids are a class of insecticides whose development began in the early 1990s as a more environmentally friendly alternative to widely used organophosphate and methyl carbamate insecticides.[1] Prior to their introduction, the market for insecticides was dominated primarily by organophosphates (43%), pyrethroids (18%), and carba-

mates (16%).[2] Beginning in the 1960s, organophosphate insecticides were drawing increased attention as having detrimental effects on avian species. Shortly following this, concerns arose regarding their continued use and potential effects on mammalian populations, including humans.[3,4] Increasing demand for more targeted insecticides with minimal environmental damage and undesirable side effects grew, leading to the collaborative synthesis and patenting of imidacloprid by Shell and Bayer CropScience in 1991.[1] Following the release of imidacloprid, the class of neonicotinoids has expanded considerably to include acetamiprid, clothianidin, dinotefuran, nitenpyram, thiacloprid, and thiamethoxam.[1,5–7] Together, these seven neonicotinoid insecticides account for more than 25% global insecticides sales by volume,[8] and were reported to be used in more than 120 countries worldwide in 2008.[9] Their usage has expanded as well, to include agricultural, domestic, and veterinary applications.[10]

1.1.2 Pharmacology

Imidacloprid was first synthesized in 1991 by Elbert and Overbech.[5] While aqueous tobacco extract had been used previously, it was found to be largely non-targeted and ineffective as an insecticide. The neonicotinoids are all derived from the lead structure nithiazine (Figure 1.1), which was found to have some low level, but non-targeted insecticidal activity, as well as moderate toxicity towards mammals.[1]

Through the addition of functionalities to a para-chloropyridine moiety, pharmacological active compounds are established, to target those insects which feed on the root systems, stems, and leaves of agricultural crops.[11] Structurally distinct from all other classes of insecticides, neonicotinoids are designed for enhanced binding within the pocket of the nicotinic acetylcholine receptor (nAChR), by inclusion of an electronegative nitro or cyano group on the pharmacologically active chain. The majority of other insecticides inhibit the functionality of acetylchloline esterase. In mammals,

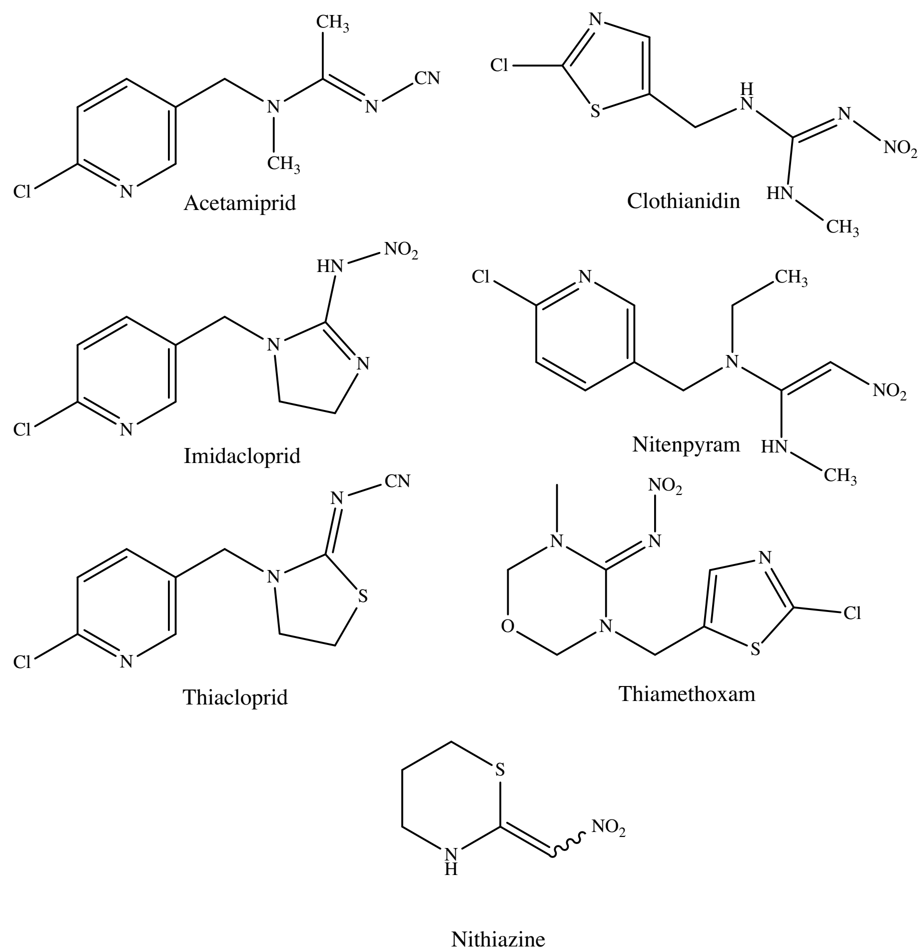


Figure 1.1: Structures of the six neonicotinoid insecticides and nithiazine.

the electronegative moiety is metabolized into a protonated or oxidized version of the neonicotinoid, which does not effectively bind within the nAChR, greatly reducing the toxicity in mammals. In contrast to lower mammalian toxicity, insects have an additional cationic amino acid found within their nAChR. This allows for the electronegative neonicotinoid species to more strongly coordinate within the receptor, making for a potent and highly targeted insecticide.[12] Additionally, vertabrates have a lower number of nACh receptors capable of binding to neonicotinoids than insects, making the insecticides particularly useful at targeting specific species.[10] Upon exposure to neonicotinoids, the nAChR is stimulated, and high enough concentrations in insects can induce paralysis, leading to respiratory arrest and death.[13]

1.1.3 Uses and Applications of Neonicotinoids

The primary use of neonicotinoids is in the treatment of agricultural crops for piercing and sucking insects. These insects include aphids, whiteflies, planthoppers, thrips, micro lepidoptera, and coleopteran pests.[2] One of the reasons the neonicotinoids are exceptionally effective towards these pests is their mode of deployment in agricultural fields. As opposed to spraying crops like in the application of organophosphates, pyrethroids, and carbamates; neonicotinoids are primarily deployed as seed coatings.[14] By treating the seeds of agricultural crops with neonicotinoids prior to planting, the neonicotinoids will become distributed throughout the root system, stem, and leaves of the plants as the plants grow.[15] This follows an increasing trend towards precision agriculture in North America, where farmers are able to ensure pesticide is treating every plant, while also reducing environmental impact through a reduction of spraying applications.[2,16] Neonicotinoids can also be applied by addition to crop irrigation systems, again minimizing environmental impacts through spraying the insecticides. In addition to agricultural treatments, the use of neonicotinoids has expanded to

include a variety of additional applications. These include treatment of household pests, including ants, houseflies, and cockroaches; as well as veterinary applications, such as the treatment of flees.[1,2,8,11,17]

1.1.4 Structure, Synthesis, and Physical Properties

Neonicotinoid insecticides can be divided into three groups based on their similarities in the structure of the pharmacologically active site. These categories are the *N*-nitroguanidines (imidacloprid, nitenpyram, clothianidin, dinotefuran), *N*-cyanoamidines (acetamiprid, thiacloprid), and *N*-nitromethylamines (thiamethoxam) (Figure 1.1). The *N*-nitroguanidines include four of the neonicotinoids and account for the majority of the market for neonicotinoids. All neonicotinoids contain a chloride-substituted 5 or 6 membered nitrogen-containing aromatic ring, onto which a cyclic or acyclic pharmacophore side chain is attached.[1] This unique class of structure amongst insecticides allows them to effectively bind within the nAChR of insects. Imidacloprid, the first synthesized, was found to be incredibly effective towards a variety of pests. It was designed to fit into the nACh receptor in insects, through functionalization of a nitromethylene precursor. All other neonicotinoids are based on this function, with modifications made to the pharmacological active site depending on desired treatment application and pests targeted[1,13]

As the primary application method in agricultural settings is through seed coatings, it was designed to be highly water soluble, and resistant to the influence of rain and sun when found within plants. It is resistant to photo-degradation, yet decomposes by photolysis and hydrolysis when found outside treated species, as a means of minimizing environmental distribution.[13] The molecular weight of the neonicotinoids ranges from 222 to 291 g mol^{-1}, with log P values between -0.66 and 1.26 (Table 1.1).[12,18] The relatively low log P values indicate a strong affinity towards water, as

Table 1.1: Physical data for the neonicotinoid insecticides including molecular weight, logP values, solubilities, and half-lives.

Neonicotinoid	Molecular Weight	Solubility (mg/L)	log(P)	Half-life (Photolysis $_{(aq)}$)(Days)	Half-life (Water-Sediment)(Days)
Acetamiprid	222	2 950	0.80	34	Stable
Clothianidin	249	340	0.70	0.1	56.4
Imidacloprid	255	610	0.57	0.2	Stable
Nitenpyram	270	590 000	-0.66	Stable	Stable
Thiacloprid	252	184	1.26	Stable	28
Thiamethoxam	291	4 100	-0.13	2.7	40

expected. The solubilities of the neonicotinoids in water ranges from 184 to 590 000 mg L^{-1}.[12] They have varying half lives in the envrionment with decomposition primarily through aqueous photolysis. The half lives for the neonicotinoids vary from a minimum of 0.1 days to stable, indicating the compound is resistant to degradation. Although the relatively short half-life by aqueous photolysis is desirable, a challenge is presented in their analysis, caused by the relatively short photolytic half-life in aqueous systems. Care must be taken to avoid exposure to light while the neonicotinoids are stored or sampled in the aqueous phase. In contrast to their aqueous photo-degradation, neonicotinoids show long or immeasurable half-lives when they are found in the sediment, or in plants. The fastest degrading compound, thiacloprid, has a half-life of 28 days, while most other neonicotinoids have half-lives measured in months or years.[18] This data is presented in Table 1.1.

1.1.5 Environmental Persistence

Due to the relatively long half-lives, resistance to degradation, and affinity for water, neonicotinoids have been found to be highly persistent in the environment. Studies of soil in agricultural areas treated with insecticides suggest that only 6% to 20% of the applied treatment is absorbed by the crops.[19] While neonicotinoids are marketed as precision agricultural products, up to 90% of the active ingredient is distributed in the soil, air, and water of the surrounding area. A study which investigated spring snow

melt runoff in the Canadian prairie region found concentrations of thiamethoxam as high as 14 mg L^{-1} in as many as 91% of samples collected.[20]

1.1.6 Concerns Regarding the Continued Use of Neonicotinoids

Neonicotinoids are popular and since their introduction in the early 1990's have grown to be the most widely used insecticides in the world as of 2012.[1] However, continued and widespread use of neonicotinoid insecticides is suggested as a significant contributor to declining populations of pollinating species, particularly honeybees. In addition, they are suspected as having detrimental effects on aquatic and avian species.[19,21] There have been numerous studies published on the effects of neonicotinoids on bees, with results ranging from little demonstrated effect, to results which demonstrate a direct link between neonicotinoid use and colony collapse disorder.[8,21–27] The true effects of neonicotinoids on pollinating species remains an area of active discussion in the scientific community. The general consensus is that the neonicotinoids do have a demonstrated negative impact on pollinating species, however the extent of these effects remains an area of active research. In addition to research, the use of neonicotinoids has become a public phenomenon, as media outlets have begun reporting on the effects of neonicotinoids, and public concern regarding their use is climbing.

1.1.7 Bees and Pollinators

With the demand for fruit, vegetables, and grain increasing with a growing population, the drive to maximize crop production becomes an area of great importance in agriculture. While the use of neonicotinoids remains an important part of minimizing losses due to pests,[2] the role played by pollinators in crop production can often be

overlooked. Bees and other pollinators play a key role in the production of many staple food crops. A study of 107 global crops for direct human use indicated that honeybees and wild pollinators were valuable in ensuring crop production and high yield for 35 crops, and 12 crops were entirely dependent on pollination by honeybees.[28] Additionally, the study found that some flowering crops, such as strawberries, were only fully developed in the presence of both honeybees and wild bees, which provide the necessary pollination for reproduction. Bees are one of the only pollinators to actively gather significant amounts of pollen, moving it between plants.[26] While some studies indicate the contribution of bees towards the crop yield for any particular crop is negligible when compared with other wild insects,[29] other studies indicate that the presence of bees is critical to the successful pollination and production of fruit from crops.[30] There remains a contentious debate in the scientific community regarding the true effects of bees versus other pollinators on wild crops, however, a consensus has been reached that neonicotinoids appear to have a negative impact on bees, and bee populations; and that pollinators are important in the continued production of world crops.[19,23,24,26,28,31]

1.1.8 Effects of Neonicotinoids on Bees

In bees, certain subsets of neonicotinoids such as thiamethoxam and acetamiprid which contain N-cyano moities are classified as nearly non-toxic, while others are classified as extremely harmful.[11] Imidacloprid, the first neonicotinoid, is categorized as highly toxic to bees. Both the neonicotinoid and its metabolites are known to have detrimental effects on the population of bees.[32] Imidacloprid and its metabolites bind irreversibly to the nAChRs, which in bees, are involved in higher-order neuronal processes.[32] These primarily include olfactory learning, and communication; however the presence of neonicotinoids in these receptors has also demonstrated negative ef-

fects on navigation, learning, food collection, longevity, and resistance to disease.[21,32] Through careful examination of molecular recognition sites in bees and other invertebrates, researchers have identified two types of nAChRs, labelled nAChR1 and nAChR2. Certain neonicotinoids target nAChR1, while most target nAChR2.[33] It is the combination of multiple binding sites for neonicotinoids, as well as multiple biological metabolites of the neonicotinoids, that leads to their potency, particularly in bees.

Examining the concentrations of neonicotinoids found in pollen, nectar, leaves, and honey highlights the broader issue of continued use of neonicotinoids. Concentrations of neonicotinoids in crops have generally been found at concentrations that if ingested, would be above the LD_{50} for neonicotinoids for bees, which range from 0.007 to 0.06 μg bee^{-1}.[16,20,24,34,35] The contamination of pollen and honey products with neonicotinoids is also indicative of the exposure of bees to the insecticides.[24] Additionally, the mechanism of seed treatment, widely accepted as environmentally friendly, has recently drawn increasing criticism, as up to 90% of the applied seed treatment is lost to the environment, carried away in water or as dust, to which bees are readily exposed.[19,22]

Honeybees also have demonstrated an affinity for neonicotinoid treated crops. In a study by Kessler et al., bees were shown to prefer sucrose solutions containing the neonicotinoid imidacloprid and thiamethoxam.[36] In the study, researchers realized that bees were unable to detect the presence of neonicotinoids imidacloprid, thiamethoxam, or clothianidin; and no adverse effects were initially observed at field-level concentrations, however the nAChR was observed to be functionally mildly inhibited.[36]

As wild bees and honey bees have shown different metabolic pathways and rates towards neonicotinoid insecticides, this further complicates the studies regarding the

true and complete effects of neonicotinoids on bees.[31,37] Additionally, it remains nearly impossible to replicate true field conditions, and accurate environmental distribution in laboratory tests.

1.2 Existing Methods of Analysis for Neonicotinoids

As neonicotinoids remain a growing regulatory concern, research towards new methods for their analysis becomes increasingly important. Neonicotinoids are present in a wide variety of matrices, including soil, plants, leaves, pollen, honey, produce, waterways, and dust.[1,2,9,11,16,18,20,34,35,38] Analytical methods need to be robust and high-throughput, yet selective for neonicotinoids, as many of these matrices are complex, containing other insecticides, herbicides, and environmental contaminants. To date, there exist a limited number of methods that are moderately effective for extracting and pre-concentrating neonicotinoids from environmental matrices, but do suffer from some significant drawbacks. The most widely used method is the Quick and Easy, Cheap, Effective, Rugged and Safe (QuEChERs) method for pesticide extraction.[39] The other routine method is direct liquid-liquid microextraction.[34] Both methods are often followed by a variety of analytical techniques including liquid or gas chromatography, with photo-diode array or tandem mass spectrometry detection.[34,35]

1.2.1 Solid Phase Extraction

Perhaps the oldest method of analysis for pesticides is solid phase extraction (SPE).[40] Solid phase extraction usually uses cartridges filled with selective sorbent onto which analytes bind under appropriate loading conditions. For example, using a C-18 reverse phase sorbent, analytes such as caffeine will bind under aqueous conditions. These

bound analytes can later be eluted using organic solvents. The advantage of using SPE is its ability to pre-concentrate and clean up analytes from matrices. This allows for lower limits of detection, as large volumes of low concentration sample can be pre-concentrated on the cartridge and later eluted with only a few mLs of solvent. While useful for a variety of analysis, SPE cartridges are not selective enough for the exclusive analysis of neonicotinoids. The most common sorbents for pesticide analysis, C18 and primary-secondary amine (PSA), select for a wide array of pesticides including organophosphates, carbamates, and pyrethroids, as well as neonicotinoids.[40]

1.2.2 Direct Liquid-Liquid Microextraction

DLLME is an analytical technique first developed in 2006 by Rezaee et al.[41] In this technique, small scale liquid-liquid extractions are performed, which depend on analyte affinity for one solvent over another. This technique is primarily suited to the analysis of organic molecules in water. However, since neonicotinoids are highly polar, water-soluble molecules, DLLME may not be the optimal choice for their analysis. While some methods have used DLLME for neonicotinoid analysis, with limited success, most methods make use of the QuEChERs dispersive-SPE procedure, which is far more robust and reliable than DLLME.[42]

1.2.3 QuEChERs Method

The QuEChERs method is a widely adapted technique for the analysis of a variety of pesticide residues in a large number of matrices. It is considered the most widely used method for pesticide residue analysis in industry and environmental monitoring applications, and is validated both in Europe as EN 15662:2008 and the United States as AOAC 2007.[39] QuEChERs is used to extract neonicotinoids, organophosphates, carbamates, and other insecticides, as well as a variety of herbicides from a range

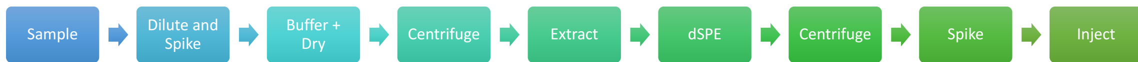


Figure 1.2: Simplified flowchart for the QuEChERs EN 15662 procedure illustrating the extensive sample preparation process.

of matrices including fruits, vegetables, honey, pollen, and soil. Due to its range of applications, it is widely considered the standard method for pesticide residue analysis.[39,43] The method, while useful for analysis in a wide variety of matrices, and for an array of pesticides, has a number of drawbacks. Due to its limited selectivity, instilled due to the capability to assay all pesticides in a sample, LC or GC methods can be more complicated and lengthy, needing to separate the analytes of interest as well as all non-targeted analytes present in the matrix. Additionally, the QuEChERs method can be time consuming and costly. There are a large number of steps which are capable of introducing error in the method, as well as extending the analysis time (Figure 1.2). Pre-fabricated QuEChERs kits are readily available, however, at the time of writing, these can cost up to $7 per sample. While highly useful for broad pesticide residue analysis, more selective sample preparation methods for neonicotinoids could reduce analysis time and cost.

The QuEChERs method has a significant limitation in that it is not optimized for the analysis of neonicotinoids. Its ability to non-selectively target pesticides reduces the number of potential binding sites for neonicotinoids in the dSPE step, as well as its pre-concentration ability for neonicotinoids. This potentially leads to enhanced matrix effects when monitoring for neonicotinoids only.

1.2.4 Liquid Chromatography

Liquid chromatography is a widely used analytical technique for aqueous analysis. It is the most widely used analytical technique for the analysis of neonicotinoids. There

are numerous methods which make use of liquid chromatography coupled to either a photo-diode array or tandem mass spectrometer for quantitative analysis.[34,35,40,42,44–48] The majority of existing methods make use of a C18 reverse phase column and HPLC, which is capable of achieving complete separation of six to eight neonicotinoids in an average of 11 minutes, with method run times varying between 9 and 17 minutes. All of these existing methods make use of a gradient elution program, and many of them use a complex buffering system, which are likely both relics of previous methods. As neonicotinoids are a relatively new class of interest to analytical chemistry, literature methods have been developed rapidly for fast publication. This is most easily achieved by adaptation of existing methods for compounds of similar size, solubility, and polarity.

Existing methods have the potential to be improved through careful method development. The use of isocratic elution programs is preferred, as there is no column equilibration time between runs, which greatly improves sample throughput. Buffering can be eliminated as the neonicotinoids should all be singly protonated in solutions with a pH less than 5. Additionally, the use of ultra high performance liquid chromatography (UHPLC) has significant advantages. UHPLC can use lower flow rates at higher column back-pressures. This is a consequence of using smaller particles to improve separations and reduce peak widths. The smaller particle size provides higher theoretical plates in shorter column lengths compared with HPLC columns by reducing the multi-path and mass transfer factors in broadening. The smaller particles require higher pressures to maintain the linear velocities seen with HPLC.

Separation timescales can potentially be improved further through the use of superficially porous column technology. Superficially porous columns have solid core particles, onto which the active reverse phase stationary substrate is grafted. For example, C18 chains can be grafted onto solid particles. This type of column technology

is widely considered to possess significant advantage over porous silica based particle columns. They typically allow for increased flow rates at reduced pressures, which is highly useful in separating analytes of interest. While superficially porous columns were initially designed to improve HPLC separations, they have since been designed for advanced UHPLC applications. This combination of the two technologies allows for significantly improved separation capabilities at reduced backpressures, leading to narrower peaks.

1.2.5 Photo-diode Array Detectors

Photo-diode-arrays (PDAs) are versatile detectors for a wide variety of molecules. They are routinely coupled to chromatography instrumentation as a first choice for reproducible and robust detection. While they are less sensitive to lower concentrations, and less selective than mass spectrometry methods, they can be a useful alternative provided the method is designed to accommodate their disadvantages.

In a typical PDA detector, light covering the UV and visible spectrum is shone through a flow cell, placed at the outlet of a chromatography column. The light is usually absorbed by compounds passing through the flow cell, which causes changes in the number of photons of various wavelengths detected by the photo-diode array.[49] Unlike UV/Vis spectrophotometers, a photo-diode array is capable of detecting individual wavelengths over a range of concentrations simultaneously, which can be used to improve selectivity and sensitivity. One drawback however, is that the light emitted from the lamp source is only estimated by the detector, as no light is passed to a reference cell. This means that the results are susceptible to baseline drift, and changes in sensitivity as the lamp ages.[49] However, the technique remains highly useful, particularly because the reproducability is often superior to that of mass spectrometry, unless complex and costly standardization is applied.

Table 1.2: Maximum UV absorbance wavelength for neonicotinoids

Neonicotinoid	λ_{max} (nm)
Acetamiprid	245
Clothianidin	266
Imidacloprid	269
Nitenpyram	268
Thiacloprid	242
Thiamethoxam	251

As neonicotinoids are all UV absorptive compounds at different wavelengths (Table 1.2), they are readily detectable with a PDA detector. Due to the limited selectivity of this method, care must be taken to identify individual standards for the analytes of interest, and match their retention times in a multistandard sample. Methods which use PDA for detection need to be pre-concentrated above the LOD, which is higher than that of mass spectrometry methods. Its simplicity and reproducability makes it a useful choice for routine, high-throughput analysis with minimal maintenance or instrument upkeep for a large number of samples, dependant on the efficacy of the extraction, concentration, and clean-up method.

1.3 Mass Spectrometry

In MS, ions are generated in a source then separated by mass-to-charge ratio (m/z) in a field. Ions of interest are selected through manipulation of these potentials and are passed to a detector. Detection is performed by one of two methods. Most mass analysis techniques such as time-of-flight, and quadrupoles utilize an electron multiplier, where ions hitting the detector produce a DC current, which is amplified to produce a signal. The other type of detection makes use of a time-domain image current, where ions oscillate in proximity to the detector, but no physical contact is made. The time domain can be converted to a frequency domain, where frequency is

dictated by m/z. This research utilizes multiple types of mass spectrometric detection, as well as numerous ionization and mass selection techniques.

1.3.1 Atmospheric Ionization

Prior to the introduction of atmospheric ionization techniques, mass spectrometry was primarily limited to electron or chemical ionization.[50] Beginning with atmospheric pressure chemical ionization (APCI), the use of atmospheric ionization techniques including electrospray ionization (ESI), have grown substantially. There are a handful of other techniques such as direct atmospheric photoionization (DAPPI), however these other ionization techniques have not seen the same level of adaptation as ESI, and APCI.

Nearly all methods for analysis of neonicotinoids utilize electrospray ionization, as it couples easily with liquid chromatographic techniques and is effective for the ionization of polar molecules.[40,44,46,47] There are only a few methods for neonicotinoid analysis published that make use of ambient ionization such as desorption electrospray ionization (DESI),[51–53] and no published methods exist for the analysis of neonicotinoids using direct analysis in real time (DART) ionization at the time of writing.

Electrospray Ionization

ESI works by the solvent, which contains analytes of interest, passing through a charged needle which forms a Taylor cone at its tip. Ions are generated through two models: the charge residue model, or ion evaporation model.[54] For both models, it is the solvent that is charged by the electric potential applied to the spray needle, the charged solvent then ionizes analytes. In the charge residue model, small charged droplets of analyte undergo radial evaporation in the transfer from Taylor cone to mass spectrometer inlet. By the time the analyte reaches the mass spectrometer

inlet, the solvent has evaporated, leaving a residue of charge on the analyte. The ion evaporation model differs, as the high positive charge in the solvent droplets forces the ejection of charged analytes to reduce the overall charge on the droplet. While it has been theorized that charge-residue occurs primarily with larger molecules, and ion evaporation with smaller molecules, in reality, both charge-residue and ion evaporation likely work in tandem to produce charged analytes for analysis.[54]

Desorption Electrospray Ionization

DESI is a relatively new ionization technique introduced in 2004.[55] It is similar to electrospray ionization, however has a different geometry and sample introduction method. As opposed to ESI, where the sample in contained within the solvent, in DESI, the sample (usually a solid or liquid on a fixed or adjustable stage) is sprayed using DESI-generated charged solvent droplets, and the resulting secondary droplets are redirected into the mass spectrometer. This allows for direct sampling on a variety of matrices, particularly solids, with no complex sample preparation. Previous methods have used DESI to perform direct analysis of neonicotinoids within plant material, including direct analysis of leaves and flowers.[52]

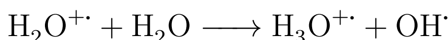
Due to the similarities between ESI and DESI, many of the method parameters from ESI are transferable to DESI, including spray voltage, flow rates, and inlet temperatures. However, there are additional parameters that must be optimized. The majority of these parameters are geometric parameters, including angle of spray, distance from spray tip to surface, and distance and angle from the surface to the mass spectrometer inlet. Also of importance is selecting an appropriate spray solvent so that the sample becomes saturated with solvent, allowing for efficient extraction of analytes from the surrounding matrix. Often times, additives will be used to encourage analyte protonation or extraction efficiency.[55,56]

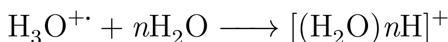
Direct Analysis in Real Time

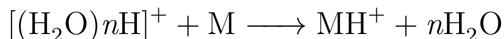
DART is another example of an atmospheric ionization technique; however, it is significantly different from APCI, ESI, or DESI. DART was first published in 2005, one year following DESI.[57] DART utilizes a metastable species to induce ion formation. Carrier gasses such as helium, argon, or neon, are excited using an electrical potential to produce metastable species. The metastable species flows through a gated potential, which removes charged molecules. Both positive and negative ions are produced simultaneously by DART. In positive ionization mode, the metastable gas species reacts with atmospheric water to produce protonated water clusters. These water clusters then transfer charge to analytes of interest. Protonated analytes are then drawn into the mass spectrometer inlet. An external vacuum pump is used to create the additional vacuum required to draw large numbers of gas phase ions into the mass spectrometer.

Ionization mechanism for DART in the positive mode

$He(2\,^3S) + H_2O \longrightarrow H_2O^{+\cdot} + He(1\,^1S) + e^-$


$H_2O^{+\cdot} + H_2O \longrightarrow H_3O^{+\cdot} + OH^{\cdot}$


$H_3O^{+\cdot} + nH_2O \longrightarrow [(H_2O)nH]^+$


$[(H_2O)nH]^+ + M \longrightarrow MH^+ + nH_2O$

DART is arguably more useful for routine analysis of larger samples when compared with DESI. There are far fewer adjustable parameters, which allows for rapid method optimization. Additionally, the parameters are more easily defined and reproducible when running samples. Parameters for DART include metastable gas temperature, ion grid voltage, type of metastable species, and the optional use of dopants. The metastable species selected is dependent on the ionization potential of the analyte of

interest. The choice of metastable species can lead to selectivity amongst analytes, where ionization energies of the metastable species below that of the background will lead to a reduction of background or matrix ions in the mass spectrometer. The use of dopants in the metastable gas stream is also interesting. For example, the addition of DCM vapour in the metastable species produces [M+Cl], and ammonia vapour produces [M+NH_4].[58] It is worth noting that DART is a soft ionization technique, producing only [M+H] or [M-H] mass spectrum peaks with minimal to no fragmentation.[57,58]

1.3.2 Mass Detection

Throughout this project, three types of mass detection were used for the analysis of neonicotinoids. Fourier-transform ion cyclotron resonance (FTICR), quadrupole time of flight (QTOF), and triple quadrupole (QqQ) mass analyzers were used. Both FTICR and QTOF detection offer high resolution mass spactra for accurate detection of compounds of interest in complex matrices. Due to the high-resolution, they can perform accurate mass determination of the neonicotinoids of interest, and can perform non-targeted analysis of environmental samples, which is useful in examining the matrix of samples of interest. By identifying key matrix components, the efficacy of the developed methods in this research can be examined for their ability to selectively extract and detect neonicotinoids over other insecticides.

1.3.3 Tandem Mass Spectrometry

The most used technique for detection of neonicotinoids in this research is tandem MS. Tandem MS is chosen for its selectivity and excellent detection limits. In this work, the MS/MS is accomplished using three quadrupoles in tandem. This configuration is capable of performing a number of mass spectrometric experiments, including product

ion scans, precursor ion scans, neutral loss scans, and selected reaction monitoring (SRM). In SRM, ions of interest are selected in the first quadrupole, fragmented in the second quadrupole, and the fragment ions selected and detected in the third quadrupole. This minimizes matrix effects by selecting only the ions of interest, and viewing only the product of their fragmentation. Using SRM can often lower the limits of detection by full orders of magnitude compared to single quadrupole systems, which is often necessary to achieve envrionmentally-relevant sensitivity.

1.4 Molecularly Imprinted Polymers

Molecularly imprinted polymers (MIPs) are analyte recognition materials. Rigid polymer frameworks are formed by of co-polymerized monomers and cross-linkers. Template molecules are added at the time of polymerization and later removed, leaving a porous polymer structure, capable of selectively binding targeted molecules of interest. MIPs can be fabricated in a variety of formats, including bulk polymer, thin-film, and grafted onto solid supports such as silica. Each format has unique advantages and disadvantages, which will be discussed in this section.

1.4.1 An Overview of Molecularly Imprinted Polymers

Fabrication of molecularly polymers is a relatively simple, yet highly tunable process. MIPs are formed by free-radical polymerization of a pre-polymer complex (PPC) containing template or pseudo-template, monomer, cross-linker, free-radical initiator, and solvent system. The solvent system is often referred to as porogen due to its significant impact on formation of the pore structure.[59–62] Each of these factors can be modified to tailor the function of the resultant MIP.

All MIPs are synthesized by polymerization of a solid structure around a template

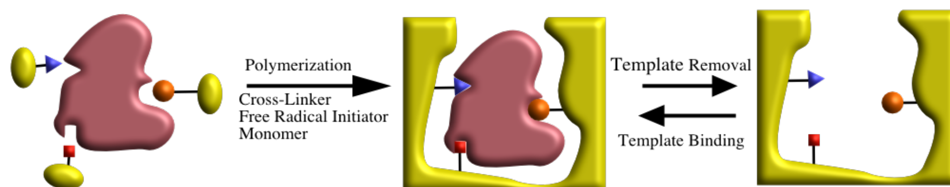


Figure 1.3: Illustration of the MIP templating process, showing an analyte (red) reversibly binding within the porous polymer structure (yellow).

or pseudo-template molecule (Figure 1.3). The template binds to the polymer either through ionic (non-covalent MIPs) or hydrogen bonding or π-π interactions (covalent MIPs). The majority of MIPs for non-metal analysis utilize the non-covalent approach, or a semi-covalent complexation between the template and the functional monomer.[59,62] This type of approach focuses on a strongly bonded polymer backbone, with an easily removable template or analyte binding site. The presence of the removable template ensures the appropriate functional groups in the polymer backbone are oriented such that the MIP has pores of appropriate size and functionality to selectively bind target analytes over the matrix. While the analyte itself may be used as the template molecule, this presents certain issues, such as template bleeding. This occurs when quantitative template removal is not achieved, resulting in artificially high concentrations and false positives. This is often avoided with the use of a pseudo-template molecule, which resembles the target analyte in both size and functional groups. The use of a pseudo-template retains the selective action of the MIP, while eliminating the problems associated with template bleeding, allowing for more accurate results.[59–62]

The monomer provides the majority of functionality to the polymer. Typically, vinyl-terminated monomer units are used in conjunction with an appropriate cross-linker molecule, which provides the rigid structure of the polymer.[60] The monomer

units are selected with the target analyte in mind. Where the binding is expected to occur through hydrogen bonding, monomers with hydrogen-bonding capabilities through carbonyl or hydroxy groups are selected. These typically include methacrylic acid (MAA) or 2-hydroxyethyl methacrylate (HEMA). Where the analyte lacks hydrogen bonding capabilities, π-π interactions can be favoured by selecting monomers with cyclic-aromatic units such as styrene or 2-vinyl pyridine (2-VP).[59,60] Cross-linkers are used to join monomer units together, which allows for control of pore sizes and provides increased rigidity to the polymer, allowing the pore structure to be retained in the absence of the porogen.

Perhaps one of the most important aspects of the PPC is the choice of porogen. The porogen is responsible for the formation of pore structure in the polymer, and is responsible for driving template-monomer interactions during fabrication, as well as is primarily responsible for driving the binding of the analyte into the polymer. The porogen must be carefully selected prior to polymerization. Template-monomer interaction is crucial to the formation of a functional MIP.[60] Solvent systems must be chosen to favour this interaction in the pre-polymer complex, before the polymerization begins. Aprotic or low-polarity solvents favour hydrogen-bonding interactions, while the presence of water in the solvent system favours a more hydrophilic polymer, which is useful for environmental sampling, where the matrix is primarily aqueous.

The functionality of any MIP is dependent on the appropriate selection of a ratio between the template, monomer, cross-linker, and porogen. Once an appropriate formulation is chosen, polymerization is achieved either through thermally-initiated free-radical initiators such as 2,2'-azobisisobutryonitrile (AIBN), or by UV initiated free-radical photoinitiators such as 2,2-dimethoxy-2-phenylacetophenone (DMPA).[59–61,63] Thermal or photo-initiation is selected primarily on type of polymer fabricated and the ease of initiation.

1.4.2 MIP Formats and Fabrication

MIPs can be fabricated using numerous different methods, producing a variety of polymer types useful in different applications. Traditionally, MIPs are fabricated in a simple bulk polymer format.[59,61] This is the most widely used and well understood fabrication procedure for MIPs.[59,61] Using this method, the PPC is placed in a closed vial and using thermal free radical initiation, polymerization is accomplished in an oil bath over 12–24 hours.[60–62] The density, porosity, and pore structure are controllable by the ratio of porogen to all other PPC components. A higher ratio of porogen dilutes the PPC components, resulting in smaller, closely packed MIP particles, while lower ratios of porogen forms more rigid MIPs with complex pore structure.

Although less prevalent in the literature, MIPs can be fabricated in numerous other formats including thin-films, as coatings on stir-bars, and as solid-phase microextraction (SPME) fibres.[63–65] The adaptability of MIPs to differing substrates is highly useful, allowing for sampling of a complex array of analytes and matrices. For example, the thin-film variety can be used for direct sampling in aqueous environments of PAHs, pesticides, pharmaceuticals, and more.[63] MIPs as SPE sorbents are routinely used as superior alternatives to the universal C18-SPE cartridges, demonstrating improved selectivity of analytes in complex matrices, as well as impressive pre-concentration and clean-up capabilities.[59–62]

1.4.3 MIPs as an SPE Sorbent

As previously mentioned, the most common and widely adapted use of MIPs is as a sorbent for solid phase extraction. MIP-SPEs have consistently demonstrated improvements in selectivity, sample cleanup, and pre-concentration ability compared with more extractive sorbents such as reverse phase C18 or polymeric reversed phase

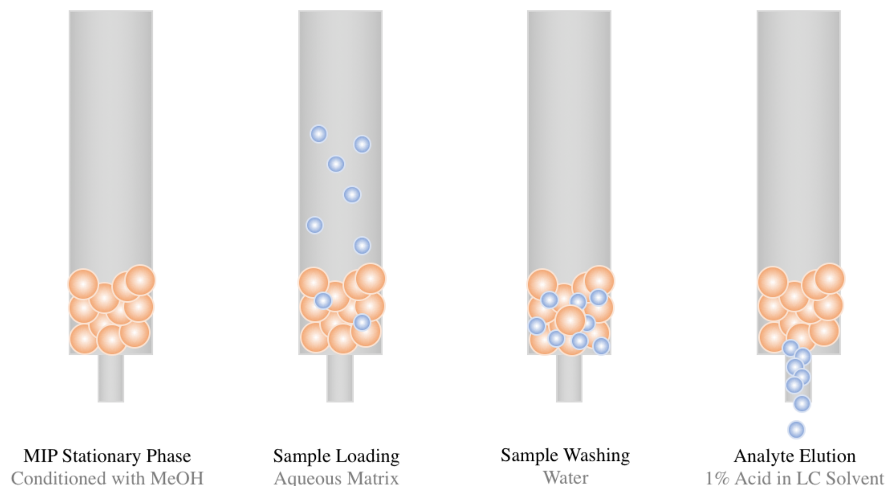


Figure 1.4: Simple sampling procedure for extraction and pre-concentration of analytes from aqueous matrices.

substrates.[60–62] Since the active sites are designed selectively for analytes of interest, the MIP-SPE should retain and pre-concentrate the target analytes, while the matrix components are virtually unretained. Previous work in this area has focused on the analysis of pharmaceuticals and their metabolites in wastewater effluent. It has been determined that MIPs as SPE sorbents were highly effective at selective uptake and analysis of targets such as ibuprofen, metformin, and bisphenol A.[66] MIP-SPEs typically utilize the traditional method of packing the SPE cartridges, and involve multiple steps including cartridge preparation, sample loading, washing, and elution (Figure 1.4)

Preparation of MIPs for use as an SPE sorbent can be referred to as the fabrication of bulk MIP. This process is largely understood, however, can be time-consuming, requiring several steps for preparation.[66] Additionally, many procedures require the grinding and sieving of the bulk phase, which results in reduced yield, higher waste

product, and destroyed pore structure in the MIP. A number of different polymerization strageties have been tried as a means of generating uniform particle size. These include grafting the MIP onto solid supports such as silica or nanoparticles, precipitation polymerization, or reversible addition fragmentation chain transfer (RAFT) polymerization.[59–62] Each of these methods have their advantages and disadvantages, however an in-depth analysis of polymerization methods is not the focus of this research.

1.4.4 Thin-Film MIPs

Perhaps one of the most unique fabrication procedures for MIPs is the thin-film variety. MIPs are fabricated on functionalized glass slides,[63] or as surface coatings on stir bars, nanorods, and SPME fibres.[64,65,67,68] The thin-film format has some advantages over SPE. The primary advantage is the simplified sampling procedure typically found when using thin-film MIPs. For example, on a glass slide, the thin-film MIPs may be deployed directly into environmental samples, eliminating the need for sample collection and transport. This is particularly useful, as large volumes of sample are required for SPE MIPs, however with thin-film MIPs, sampling can be performed in-situ. Another improvement over SPE MIPs is the simplified fabrication process. As shown in Figure 1.5, fabrication of thin-film MIPs can be performed with fewer materials, and on a much shorter timeline than SPE bulk-phase MIPs.[63]

There are however a number of drawbacks when using thin-film MIPs that need to be overcome before they are widely adopted. The thin layer of polymer may show less porosity than their SPE counterparts, as well as fewer binding sites, meaning the thin-film MIP will reach capacity sooner than the MIP-SPE per unit mass. In this regard, the MIP-SPE has the potential to reach lower detection limits, as it is capable of pre-concentrating more analyte from a sample. Additionally, the fabrication

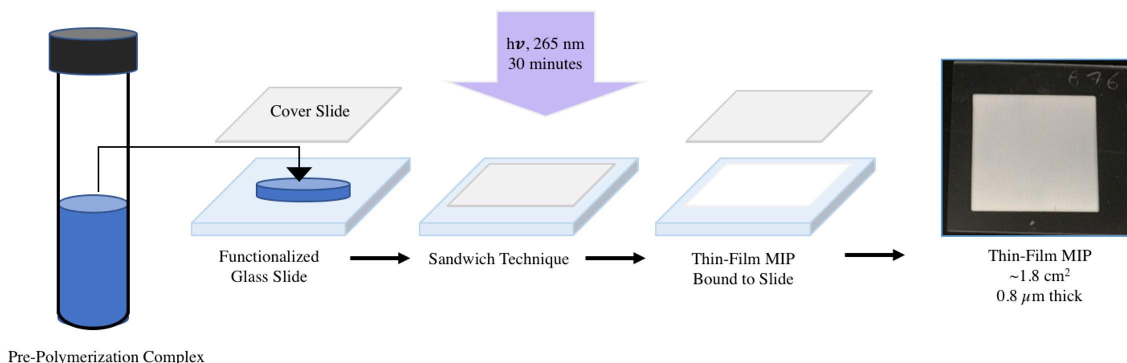


Figure 1.5: Illustrated fabrication procedure for thin-film molecularly imprinted polymers

procedure must be optimized for each system. The synthesis of MIP-SPEs is more widely understood than that of the thin-film MIPs, and typically has more success than the thin-film variety.[59,60,62,64,65]

1.4.5 Analysis and Measurement of MIPs

The success of imprinting and the performance of MIPs can be assessed using a variety of criteria. There are three factors which are widely used as indicators of the performance of MIPs: imprinting factor (IF) (Equation 1.1), mass loading, and pre-concentration factor. The imprinting factor is a measure of the concentration of analyte in the MIP relative to that of a non-imprinted polymer (NIP).[59] NIPs are fabricated using a pre-polymerization complex which is identical to that of the MIP in the absence of a template or pseudo-template molecule. In theory, the NIP should bind no analyte of interest, as the pore structure does not possess the specific

binding pockets for the analytes of interest. However, there will always be some surface adsorption of analytes onto the polymer. The imprinting factor accounts for non-targeted uptake of analytes by the polymer itself, and provides a measure of how effective the MIP is at binding the analytes.

$$IF = \frac{C_{MIP}}{C_{NIP}} \tag{1.1}$$

The mass loading is a measurement of mass of analyte per mass of polymer. This is a useful metric as it provides an indication of the efficiency of the polymer, as well as can provide isotherm data to determine the maximum concentration of analyte that can be loaded into the MIP.[59] The pre-concentration factor is a highly useful metric for measuring the performance of MIPs. It can indicate how well the material concentrates the analyte from a given matrix, and is related to the sorption capacity and the affinity of the analyte for the solid phase.

1.4.6 MIPs for the Analysis of Neonicotinoids

At the time of writing, only one publication was identified as using MIPs for the analysis of neonicotinoids. An SPE based MIP method was developed for the analysis of imidacloprid in environmental samples.[69] Much of the current focus on MIP development is directed towards the analysis of the organophosphorus pesticides.[70,71] This indicates that the development of MIPs for pesticide analysis lags behind regulatory standards, and that there is a significant market for the development of an MIP that could selectively target the full class of neonicotinoids. As the neonicotinoids all contain the *p*-chloropyridine subunit, as well as a pharmacological active chain containing multiple hydrogen bond donors and acceptors, they should show success with MIPs designed to target either π-π or hydrogen bonding interactions. Their high polarity

and solubility mean they are difficult to extract from aqueous samples using other methods, which is where MIPs could have an advantage.

1.4.7 Neonicotinoid MIPs Compared with Other Methods

With the advancement of mass spectrometers, many analytical methods use direct injection coupled with liquid chromatography and tandem mass spectrometry for analysis of environmental contaminants. MIPs need to demonstrate significant advantages over the direct injection method by pre-concentrating the analyte and cleaning the matrix, allowing for far lower limits of detection to be reached.

The most widely used method for analysis of neonicotinoids is the QuEChERs dispersive SPE method.[39,43,47] While this method has been successful for their analysis, there are a number of improvements that could be made by the adaptation of MIPs. QuEChERs is not a selective method; it was designed for non-specific analysis of pesticides in envrionmental samples.[39] This means that all pesticides should be isolated and detectable, including organophosphates, carbamates, and neonicotinoids, as well as any other herbicides or insecticides that might be present. While this could be useful for non-targeted screening, it means the method is not optimized to one specific class of pesticide, and thus will demonstrate decreased efficiency of extraction and pre-concentration across all compounds.[39] The introduction of MIPs could solve this issue by providing a highly selective method of analysis, which eliminates the contamination of non-targeted insecticides and herbicides from the method. This allows for more accurate concentration determination, as the binding sites will not be saturated with undesired compounds, as well as better sample cleanup from complex matrices. With a cleaner sample, analytical methods can be made higher-throughput, as for example, only 7 neonicotinoids would need to be separated during a chromatographic run, compared with up to 60 pesticides in other methods.[43]

1.4.8 Direct Analysis of MIPs

Perhaps one of the primary advantages of using MIPs as opposed to other methods like SPE, QuEChERs, or SPME, is the capability to perform direct analysis on the MIP. While this can only be performed using the thin-film format of MIPs, it is a highly useful technique, particularly for the screening of analytes in environmental samples. Thin-film MIPs can be analyzed directly by DESI and DART. Both ambient ionization methods can be used to perform surface analysis of the thin-films. With DESI, the electrospray saturates the surface of the MIP, which extracts any analytes of interest, ionizes them, and introduces them into the mass spectrometer. With DART, the high temperature metastable gas causes thermal desorption of analytes from the thin-film surface, the analytes are then ionized and introduced into the mass spectrometer. DESI has the additional capability of generating a mass spectrometric map of analyte concentration across the polymer, which is useful in indicating the homogeneity of analyte uptake within the polymer. Each ambient ionization has its advantages. DESI is more suited to larger, polar analytes, while DART is better suited to small non-polar, thermally stable analytes. Additionally, DART has the ability to sample more of the polymer surface (Figure 1.6), which provides a more accurate representation of the concentration of analyte within the entire polymer. However, the high temperature of DART ionization can potentially lead to decomposition of the analytes or the polymer itself.

1.4.9 Extractive Techniques

While ambient ionization techniques are highly useful, they have the significant limitation of providing only semi-quantitative analysis. Due to their inability to fully desorb all analytes of interest, these techniques cannot be used for highly precise or

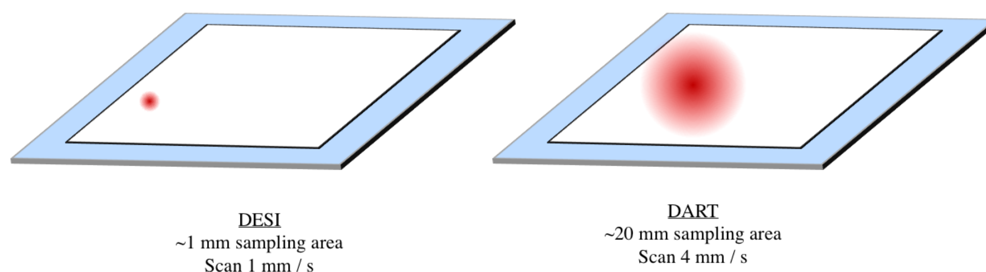


Figure 1.6: Schematic comparision of the sampling capabilities of DESI compared to DART for thin-film MIPs

accurate determination of concentrations of analytes of interest in real samples. To achieve the highest quality quantitative analysis, the analyte must be extracted from the MIP and injected into an LC or GC system. This is true of both bulk and thin-film MIP varieties. Extraction is usually accomplished by exposing the polymer to a suitable solvent system, typically an organic solvent containing some acid, which breaks the interactions between the analyte and active site in the MIP. The solvent can then be evaporated and the analyte reconstituted in an appropriate solvent with addition of an internal standard to improve the analytical data quality in instrumental analysis.

1.5 Objectives

The main goal of this work is to develop of a MIP for the analysis of six neonicotinoid insecticides: acetamiprid, clothianidin, imidacloprid, nitenpyram, thiacloprid, and

thiamethoxam. These six neonicotinoids are representative of the current insecticide market and are facing increased regulatory control and restriction in Europe and North America. It is believed this work is the first of its kind, and will advance the environmental detection of neonicotinoids through the use of high-throughput methods and ultra-trace analysis. The ancillary goals of this work are development of instrumental analytical methods for the detection and quantification of neonicotinoids, development of MIPs as sorbents for solid phase extraction, and investigations into thin-film MIPs coupled to ambient ionization methods, including DESI and DART, for fast screening of neonicotinoids in environmental samples.

A UHPLC method will be developed and coupled to both a photo-diode array detector and tandem mass spectrometry. This method will be used in evaluating each MIP formulation. Through the use of advanced column technologies, such as superficially porous columns, and careful method optimizations, the UHPLC method should provide high-throughput analysis with short run times. The use of a PDA detector offers cheap and readily available detection, while the tandem mass spectrometry method allows for lower limits of detection and more comprehensive, targeted analysis.

As neonicotinoids are facing increased regulatory control throughout Europe and North America, reliable methods of analysis are needed for better understanding of environmental distribution of neonicotinoids, particularly in waterways. At the time of writing, no work is known to incorporate an MIP for use as an SPE sorbent for the analysis of neonicotinoids. As bulk phase MIPs are typically easier to synthesize and validate, this is a logical first step towards fabrication of MIPs for neonicotinoids. The results from the MIP-SPEs developed will be validated against the widely used QuEChERs method. The QuEChERs method will be performed in-house using the same standards as used for the SPE method. Both methods will be applied to real-world samples and results compared.

Finally, thin-film MIPs will be fabricated using a pre-polymerization complex similar to that of the SPE-MIPs for neonicotinoids. These will be analyzed using both DESI and DART, from which conclusions can be drawn for the use of both ambient ionization techniques for the analysis of MIPs in thin-film formats. The thin-film MIPs for neonicotinoids will be compared to the SPE variant, and will be tested using real environmental samples.

Chapter 2

Materials

2.1 Chemicals, Reagents, and Comsumables

Neonicotinoids were sourced from two suppliers. Acetamiprid, imidacloprid, and thiacloprid were purchased from Sigma-Aldrich (Oakville, ON, Canada), and clothianidin, nitenpyram, and thiamethoxam were purchased from Toronto Research Chemicals (Toronto, ON, Canada). All neonicotinoids were guaranteed at a minimum of 99.5% purity.

Solvents for liquid chromatography (acetonitrile, methanol, and water) were purchased from Fisher Scientific (Hampton, NH, U.S.A.), and were of Optima grade. Solvent additives included formic acid, purchased from Sigma-Aldrich, and acetic acid, purchased from Fisher Scientific. Both were of chromatography grade.

Samples were prepared in either distilled/deionized water (available within the lab), methanol ACS reagent grade (Sigma-Aldrich), or acetonitrile ACS reagent grade (Sigma-Aldrich).

Reagents for polymerization reactions including methacrylic acid (MAA), 2-hydroxy ethyl methacrylate (HEMA), acrylamide (ACC), ethylene glycol

dimethacrylate (EGDMA), 2,2'-azobisisobutryonirile (AIBN), 2-dimethoxy-2-phenylacetophenone (DMPA), and 3-(trimethoxysilyl)-propylmethacrylate were purchased from Sigma Aldrich. ACS reagent grade toluene was purchased from ACP chemicals (Toronto, ON), and 1-octanol was purchased from Fisher Scientific.

For the QuEChERs method, bulk-phase SPE sorbents C18 and primary secondary amine (PSA) were extracted from manufactured SPE cartridges purchased from Canadian Life Science (Peterborough, ON, Canada). Sodium chloride was purchased from ACP chemicals. Buffering salts trisodium citrate dihydrate, disodium hydrogencitrate sesquihydrate, and magnesium sulfate were purchased from Sigma-Alrdich.

2.2 Instrumentation

Chromatographic separations were performed using a Waters H-class UPLC system equipped with a quaternary solvent pump, PDA detector, active column pre-heater, and autosampler (Waters, Mississauga, ON, Canada). The majority of analysis was performed on a Waters Xevo TQS tandem mass spectrometer interfaced with a Waters Z-spray ESI source.

Also available was a Prosolia DESI ion source (Prosolia, Indianapolis, IN, U.S.A.), and through a collaboration with the Ontario Ministry of the Environment (MOE) an Ionsense DART-SVP source (Ionsense, Saugus, MA, U.S.A.), a Varian 901-MS FTICR with linear ion trap (Varian), and Waters G2-XS Q-TOF mass spectrometer.

2.2.1 UHPLC-PDA

For chromatographic separations, which is a large focus of this work, a Waters H-class UHPLC with quaternary solvent manager, auto-sampler, active column pre-heater, photodiode array detector, and sample compartment temperature controller was used.

An elution program was used which consisted of a binary solvent system with an isocratic elution. Solvent A was water (LC grade) containing 0.1% formic acid, and solvent B was acetonitrile (LC grade) containing 0.1% formic acid. The optimized isocratic elution program consisted of 68% A and 32% B. The mobile phase flow rate was set at 0.350 mL min^{-1}, with the column heated at 30 °C to maintain consistency between separations. Samples were warmed to room temperature before being placed in the auto-sampler, maintained at 20 °C. An injection volume of 2.000 μL was used for all samples.

The photo-diode array detector was set to monitor wavelengths between 210 and 400 nm. Neonicotinoid standards were individually analyzed to determine retention times and maximum absorbance wavelength. A multistandard was run following this to confirm baseline separation for all analytes. The total run time varied between 1.8 and 4 minutes, depending on the column stationary phase used.

C18 1.7μm Column Parameters

The C18 reverse phase column was provided by Waters. Column dimensions were 2.1 mm diameter, 50 mm in length. The particle size was 1.7 μm.

Halo 2.7 Column Parameters

The Halo series of columns were supplied by Canadian Life Science. Column dimensions were 2.1 mm in diameter and 100 mm in length. The stationary phase consisted of a 1.7 μm fused core with 0.5 μm porous layer giving a total particle size of 2.7 μm solid particles, onto which C18 was grafted.

Isosceles Column Parameters

The majority of separations and analyses were performed using a Canadian Life Science C18 Isosceles column. This is a superficially porous column similar to the Halo column with 1.7 μm solid core and 0.5 μm coating giving total particle size of 2.7 μm. Column dimensions were 2.1 mm diameter by 100 mm length. To protect the analytical performance a 0.5 μm column filter was fitted between the column pre-heater and analytical column.

2.2.2 Mass Spectrometry

During this research, various mass spectrometric techniques were used for the analysis of the neonicotinoids. For ionization, ESI, DESI, and DART were used, and for detection, FTICR, tandem MS, and Q-TOF were used.

Electrospray Ionization

For UHPLC experiments, the ESI source was operated in positive mode. Capillary voltage was set at 3.00 kV, with a cone voltage of 15 V and source offset of 50 V. Nitrogen nebulizing gas desolvation temperature was set at 300 °C with a flow rate of 800 mL hr^{-1}. For CID for MRM argon collision gas was used at a flow rate of 0.15 mL min^{-1}. The source temperature was set at 150 °C.

Intellistart is a software package provided by Waters which allows for rapid method optimization of mass spectrometric parameters for individual compounds. Standards (1 mg L^{-1}) were directly injected into the ESI source, and Intellistart was used to determine optimal collision energy, cone voltage, and fragmentation pathways (Table 2.1).

Tandem MS Parameters

Mass spectra were collected in MS scanning mode between 50 and 500 m/z units, in addition to individual MRM experiments for the six neonicotinoids, based on the results obtained from the Intellistart method (Table 2.1) Each MRM experiment examined two fragmentation pathways for a single neonicotinoid, and data was collected in agreement with the neonicotinoids elution from the LC method to maximize points per peak. Data was extracted using MassLynx 4.2 by integrating chromatographic peak areas for the MRM experiments.

Table 2.1: Intellistart-determined optimal ESI parameters for analysis of the six selected neonicotinoids by tandem-MS using MRM

Neonicotinoid	MRM_1	MRM_2	Coll. E_1	Coll. E_2	Cone Voltage
Acetamiprid	$223 \rightarrow 126$	$223 \rightarrow 56$	14.0 eV	20.0 eV	2.00 V
Clothianidin	$250 \rightarrow 169$	$250 \rightarrow 132$	16.0 eV	12.0 eV	10.00 V
Imidacloprid	$256 \rightarrow 209$	$256 \rightarrow 175$	16.0 eV	20.0 eV	28.00 V
Nitenpyram	$271 \rightarrow 130$	$271 \rightarrow 126$	12.0 eV	28.0 eV	2.00 V
Thiacloprid	$253 \rightarrow 132$	$253 \rightarrow 90$	20.0 eV	38.0 eV	2.00 V
Thiamethoxam	$292 \rightarrow 211$	$292 \rightarrow 181$	12.0 eV	22.0 eV	8.00 V

Direct Analysis in Real Time

The DART ionization source has relatively few optimizations when compared with other ambient methods such as DESI. The two main variables with this ionization source are the temperature of the metastable gas, which can vary between 200 and 550 °C, and the distance between the metastable gas source and mass spectrometer inlet.

For method optimization for the individual standards, the DART source was placed in-line with the mass spectrometer inlet at a distance of 2.1 cm (Figure 2.1a). Samples were introduced using a melting point capillary dipped in the standard solution. The temperature was varied at 50 °C increments to determine the optimal

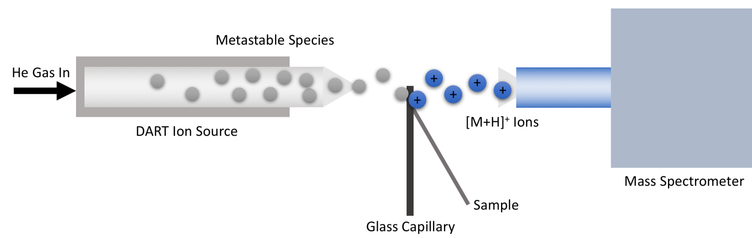
desorption temperature for sample analysis.

For analysis of MIPs, the DART source was placed at a 135° angle to the mass spectrometer inlet (Figure 2.1b). The polymers on glass slides were then introduced into the metastable gas stream using a linear rail. This allowed for sampling across the surface of the polymer.

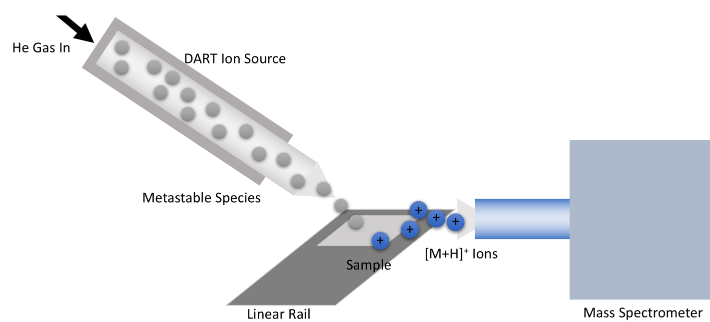
For quantitative analysis, a custom built thermal-desorption unit (TDU) was placed in-line between the DART ionization source and mass spectrometer inlet (Figure 2.1c) The TDU consisted of a Variac controlled heating element placed under an aluminium sampling surface contained within a metal box. The TDU had the capacity to heat between 50 and 220 °C. All samples were desorbed at the maximum temperature of 220 °C. Ceramic tubes were placed at either end, which allowed for contact between the ceramic output of the metastable gas from the DART source, and the ceramic input of the mass spectrometer. Samples were introduced on a folded piece of aluminium foil by spotting known volumes alongside an injection standard and placing the foil inside the TDU. This allowed for quantitative sample desorption and ionization into the mass spectrometer.

FTICR Parameters

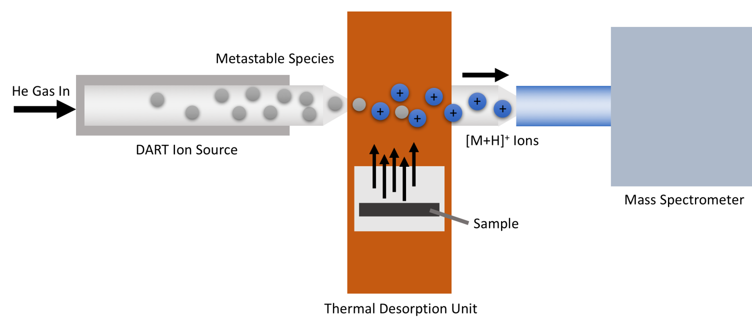
For detection and method optimization of DART ionization for neonicotinoids, a single quadrupole ion trap - FTICR was used at the Ontario Ministry of the Environment. Mass spectra were collected between 150 and 800 m/z. An average of 5 scans per sample were completed with a dataset size of 2048 k, magnetic field strength of 9.4 tesla, ion trap exit-time of 10 seconds, and transient duration of 3 seconds. The cone voltage was set at 45 V.



(a)



(b)



(c)

Figure 2.1: Illustration of DART sampling by (a) in-line sample introduction, (b) thin-film MIP sampling, and (c) sampling using the TDU.

Quadrupole Time-of-Flight Parameters

The Q-TOF mass spectrometer was used with the DART ion source set to acquire spectra between 50 and 1000 m/z units in high-resolution. Spectra were collected continuously as sample was introduced. This produced peaks of high ion intensity corresponding to the presence of sample in the ionization source. These peaks were then integrated using extracted ion data to determine the peak area corresponding to compounds of interest. To ensure accuracy, the exact masses were corrected by calibration against the known mass of a siloxane contaminant.

2.3 Sample Preparation

Stock neonicotinoid solutions were prepared at levels between 80 and 300 mg L^{-1} by dissolution of the solid compounds in acetonitrile. These were stored in amber bottles at 2 °C for up to one month, after which the compounds demonstrated signs of degradation. Working standard solutions and spike solutions were prepared by dilution from these stock solutions as needed. Diluted working standards were stored at room temperature for up to 48 hours.

River water taken from a local river downstream of an agricultural area was used to analyze the efficacy of the QuEChERs method, two different SPE procedures, as well as direct injection methods. Canadian honey (Compliments brand) was purchased and used as a matrix for the QuEChERs method. Both river water and honey were spiked using appropriate working standards to perform spike and recovery and standard addition experiments.

2.4 Instrument Method Validation

All instrumental methods were examined using typical method validation parameters. Linearity of the method was examined over a range of concentrations. The methods were also examined for reproducability, limits of detection, sensitivity, and selectivity.

2.4.1 LC-PDA Validation

For the photo-diode array detector, a calibration curve was constructed using standards at 14 levels (50, 100, 150, 200, 250, 500, 750, 1000, 1500, 2000, 2500, 3000, 4000, and 5000 μg L^{-1}). Linear regression was performed to determine the coefficient of determination (R^2) as a measurement of linearity. Limits of detection and quantification were estimated by injecting decreasing concentrations of standards until no chromatographic peaks were observed. Limits of detection were later quantified by running 9 replicate samples at 20 μg L^{-1}. The limit of detection was then defined as three times the standard deviation of the blank-subtracted peaks from the replicate samples, divided by the slope of the regression line. Limits of quantification were defined using the same equation, replacing 3σ with 10σ.

$$MDL = \frac{3\sigma}{m} \tag{2.1}$$

Reproducability of the method was determined by running replicate samples on the same day, and on multiple days, and calculating variation in instrument response, in terms of peak area, retention time, and maximum wavelength.

2.4.2 LC-MS/MS Validation

Method validation parameters for the LC-MS/MS method are similar to those used to validate the LC-PDA method. The notable exception is the construction of the

calibration curve. For examination of range and linearity, a calibration curve was constructed from neonicotinoid standards at 19 levels including 0, 0.5, 1, 5, 10, 15, 20, 40, 60, 80, 100, 200, 400, 600, 800, 1000, 1500, 2000, and 2500 μg L^{-1}. Limits of detection were determined by running 9 replicates at 5 μg L^{-1}.

2.4.3 TDU-DART-QTOF Validation

The method for TDU-DART-QTOF was validated by constructing a calibration curve at 9 levels (0, 0.5, 1, 5, 10, 50, 125, 250, and 500 μg L^{-1}). This range of concentrations, when injected to the TDU-DART system at 7 μL volume corresponds to between 3.5 and 3500 pg of analyte.

2.5 Overview of MIP Fabrication

MIPs are formed by radical polymerization of a PPC containing cross-linker, monomer, solvent (porogen), template or pseudo-template, and either a thermal or UV induced radical initiator. The formulation can be adjusted to suit various applications, including the thin-film and bulk polymer formats. In general, the template and monomer are dissolved in the solvent, which allows for template-monomer interactions to occur. To this mixture, the cross-linker is added, complexing with the template and monomer. Finally, a free-radical initiator is added and polymerization induced for a specific time, resulting in a solid polymer. The volume of solvent relative to other polymer components as well as the polarity, and hydrogen bonding capacity in the solvent work in tandem to govern the resultant polymer structure.

2.5.1 Pre-polymerization Complex

For detection of neonicotinoid insecticides, the pre-polymerization complex is formed as follows. The monomer can be any molecule that contains at least one hydrogen bonding site, and a vinyl terminated moiety. The most common monomer used in this research is acrylamide, however methacrylic acid has also been used successfully. The cross-linker is an ether-based linker with vinyl-terminated moieties, which allow for incorporation with the monomer. In the case of these MIPs, ethylene glycol dimethacrylate (EGDMA) is used exclusively. The majority of the MIPs were fabricated using 2-chloropyridine-4-carboxylic acid as the template. Other templates tested included nitenpyram, thiamethoxam, and nicotine. The molecular structures for the molecules contained in the PPC are given in Figure 2.2.

2.5.2 Thermal Polymerization

Thermally-initiated polymerization is accomplished through the use of 2,2-azobis-isobutyronitrile (AIBN) as the free-radical initiator. A catalytic amount of AIBN is added to the PPC and the solution capped and placed in an oil bath at 65 °C for 24 h with stirring. After 24 h, the bulk polymer could be scraped from the vial as an opaque white powder.

2.5.3 UV Polymerization

Photo-initiated polymerization is accomplished using 2-dimethoxy-2-phenyl acetophenone (DMPA) as the free-radical initiator. Similarly to the thermally initiated polymerization, a catalytic amount of DMPA is added to the PPC, the solution degassed, then placed under a UV light at wavelength 265 nm for 30 minutes. Provided the PPC containing initiator is free of oxygen, either in a closed vial or under a micro-

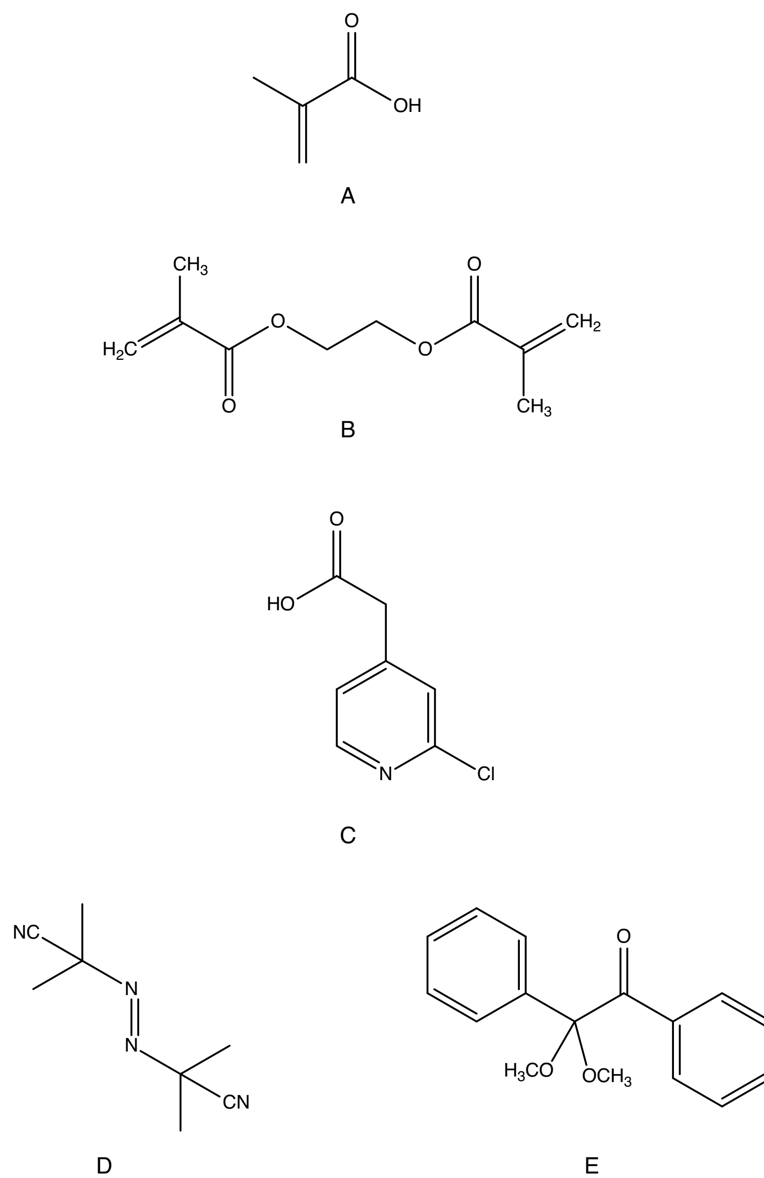


Figure 2.2: Components of the pre-polymerization complex including (a) monomer methacrylic acid, (b) cross-linker ethylene glycol dimethacrylate, (c) template 2-chloropyridine-4-carboxylic acid, (d) thermal initiator AIBN, and (e) photoinitiator DMPA

scope cover slide, polymerization results in an opaque white polymer affixed to the glass microscope slide.

2.6 MIP-SPE Fabrication

MIP for use as an SPE sorbent was fabricated using a modified sol-gel preparation method. A functionalized silica gel is synthesized containing vinyl functional groups onto which the MIP can be grafted. The schematic for this process is shown in Figure 2.3.

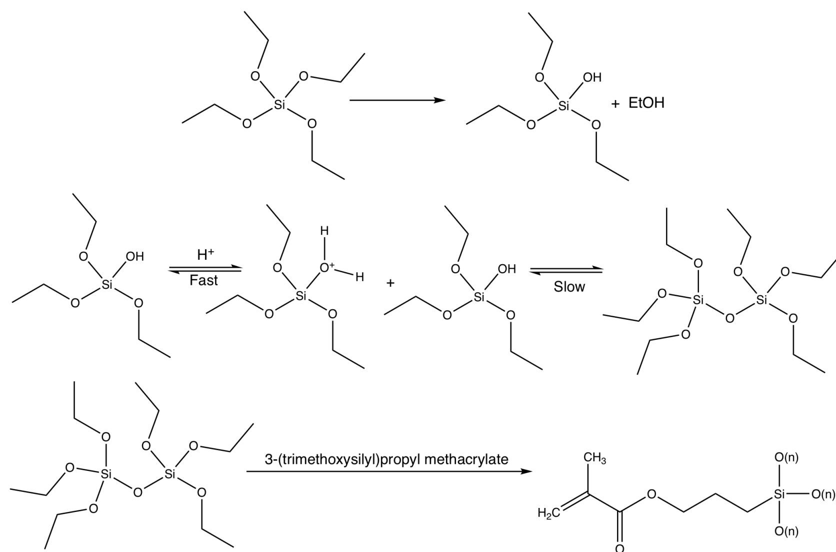


Figure 2.3: Synthesis of MIPs for use as an SPE sorbent

2.6.1 SiO_2 Preparation

The first step in the preparation of MIP-SPEs is the synthesis of the substrate. This provides necessary rigidity to the polymer so that it can be placed under vacuum. 50 mL of 2M HCl is combined with 10 mL of tetraethylorthosilicate (TEOS) slowly

over 10 minutes. This mixture is stirred for 1 hour at 50 °C. Following this, 2.6 mL of derivitizing agent 3-tri(methoxysilyl)propyl methacrylate is added dropwise. The resultant solution is stirred overnight at 80 °C. Following this, the functionalized silica is washed using ethanol, separated by centrifuge (5 min at 5000 rpm), and dried at 40 °C. The product, hereafter referred to as 3TMSPM-SiO_2, is stored either in an oven at 40 °C or in a desiccator.

2.6.2 MIP Grafting

In a glass scintillation vial, pre-polymerization complex consisting of a molar ratio of 60:9:2.5:1 of EGDMA:acrylamide:template:initiator is combined with an appropriate amount of solvent and vortexed for complete dissolution. In a separate vial, previously synthesized 3TMSPM-SiO_2 is weighed in a ratio of 1.6 g per 10 mL porogen. The two vials are combined with stirring and polymerization is carried out overnight in an oil bath at 65 °C. The resultant gel is not dried in an oven, but may be used after drying under atmospheric conditions.

2.7 Thin-Film MIP Fabrication

The fabrication procedure for thin-film MIPs varies significantly from the MIP-SPE format. Glass microscope slides are functionalized using the same derivitizing agent used in the MIP-SPE fabrication. Microscope slides are cut to 2.2 cm square size and placed in a solution of 2% v/v 3-(trimethoxysilyl)propyl methacrylate overnight. A PPC containing template, monomer, cross-linker, and solvent in a molar ratio of 1:4:20:200 along with DMPA photo-initiator at a catalytic concentration is degassed in a glass vial. 8 μL of this PPC is then deposited on a functionalized glass slide and covered with a micro glass cover slide. UV initiation is then carried out by placing

the slide under a UV light source emitting at wavelength 265 nm.[72]

2.8 QuEChERs Procedure

A modified QuEChERs procedure was adopted for analysis of both spiked honey and river water samples during the course of this research. Into a 50 mL centrifuge tube: 2.4 g $MgSO_4$, 0.6 g trisodium citrate dihydrate, 0.6 g NaCl, and 0.3 g disodium hydrogencitrate sesquihydrate were weighed. To this, a 6 mL sample of either honey or water was added, and diluted to 12 mL total volume with acetonitrile.[73] Any analyte spikes for spike and recovery were performed at this point, accounting for a total volume of 12 mL. These centrifuge tubes were shaken for 1 minute, and then centrifuged for 5 minutes at 3500 rpm. Following this, a 1 mL aliquot of the supernatant was added to a 15 mL centrifuge tube containing 30 mg C18, 30 mg PSA, and 90 mg $MgSO_4$. This tube was vortexed and centrifuged for 5 minutes at 5000 rpm. Finally, 600 μL of supernatant, plus an appropriate internal standard spike was diluted to a total volume of 2 mL for injection to the LC system.

2.9 Sampling

Various sampling techniques were utilized for analysis of neonicotinoid insecticides. Samples primarily consisted of spiked distilled water, river water samples, and honey and diluted honey. Samples were analyzed for the presence of neonicotinoids by extraction using commercial C18 cartridges, the modified QuEChERs method, the MIP-SPEs, or by direct LC injection; direct injection was not carried out for honey samples.

2.9.1 C18-SPE

The C18 SPE cartridges (Canadian Life Science) were used for environmental sampling as per the manufacturer's instructions, as follows. The cartridges were conditioned twice with 5 mL of methanol, and washed twice with 5 mL water before the sample was introduced. The sample was then loaded onto the column at a rate of approximately 1.2 mL min^{-1} using a vacuum manifold, with suction continued following loading until the sorbent was dry. Samples were eluted with 3 mL of methanol into a 5-mL volumetric flask, spiked with internal standard, and diluted to volume. An aliquot of this sample was injected onto the LC column.

Either 25 or 100 mL aqueous samples were used for analysis by the C18 SPE method. Calibration curves were constructed by spiking 100 mL of distilled water with an appropriate amount of neonicotinoid multistandard solution to obtain an 8-point calibration curve ranging from 0 to 2000 ng L^{-1}.

2.9.2 QuEChERs

Samples for the QuEChERs method were analyzed using a modified method outlined above. 6-mL samples of spiked distilled water, river water, or honey were used. A calibration curve was constructed using standard addition spikes of neonicotinoid multistandard solution between 0 and 1000 μg L^{-1}.

2.9.3 MIP-SPE

Approximately 0.5 g of the MIP bulk phase was packed into a standard SPE cartridge and compressed using a glass frit. The SPE cartridges were then conditioned by washing under vacuum twice with 5 mL of 1:1 acetonitrile:water containing 2% acetic acid, and twice with 5 mL distilled water to remove any traces of acid. The cartridges

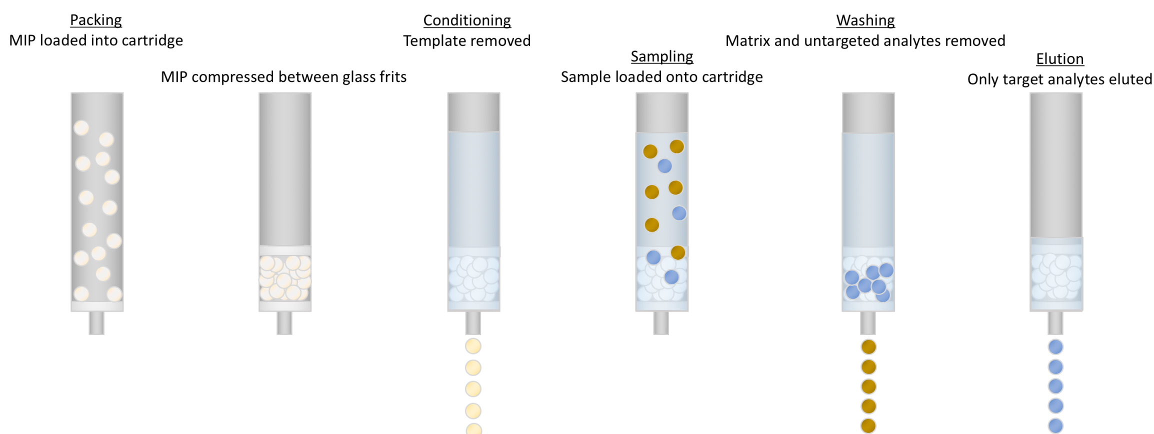


Figure 2.4: Illustrated sampling procedure for analysis of neonicotinoids by SPE-MIP

were not allowed to dry during the conditioning procedure.

Once conditioned, aqueous sample was loaded at a flow rate of approximately 2 mL min^{-1}. The analytes retained on the cartridge were eluted using 3 mL of HPLC grade 1:1 acetonitrile:water containing 1% HPLC grade acetic acid into a 5 mL volumetric flask, spiked with an appropriate amount of internal standard, and made to volume using 1:1 acetonitrile:water. An aliquot of this solution was then subjected to analysis using the method developed earlier in this project. The use of MIP-SPEs for sampling is illustrated in Figure 2.4

The performance of the MIP-SPEs was validated by constructing both a low concentration range, and high concentration range calibration curve. The high concentration curve was constructed at 8 levels between 0.1 ng L^{-1} and 100 ug L^{-1}. The low concentration curve was constructed at 8 levels between 0 and 2000 ng L^{-1}. All samples were spiked distilled water with 100 mL volume loaded for each. Masses of polymer were obtained to determine mass loading of the polymer. Limits of detection

were determined by running 5 replicates loaded at 0.5 ng L^{-1}, taking the standard deviation multiplied by three, and dividing this value by the slope of the regression equation. Polymer breakthrough was analyzed by running a 1 mg L^{-1} solution at 100 mL volume through cartridges loaded with a decreasing polymer mass, to determine at which mass loading volume, no more analyte could be retained. Also examined were analyte retention, analyte extraction, and percent recovery.

2.9.4 Thin-Film MIP

Fabricated thin-film MIPs were placed in a petri dish and rinsed with 50 mL of 9:1 methanol:acetic acid for 1 hour, followed by 50 mL of 100% methanol for one hour. Following this conditioning phase, the MIPs were exposed to aqueous samples for a 2-hour upload period. The aqueous samples were primarily neonicotinoid spiked distilled water, at a volume of 50 mL, into which 3 thin-film MIP slides were placed.

After completion of upload, the thin-film MIPs were analyzed directly by DART ionization, or extracted for analysis by LC. For direct analysis, the thin-films were placed directly in front of the ionization source, according to method procedures described previously. For extraction, individual slides were placed in a beaker with 5 mL of 1% acetic acid in methanol for 1 hour with stirring. The extract solution containing neonicotinoids was dried by rotary evaporation, and reconstituted in 1:1 acetonitrile:water for injection into the LC system. Analysis was performed using the LC method outlined previously with either PDA or MS/MS detection.

2.10 Environmental Monitoring and Applications

To verify the applicability and use of the MIP-SPE phase, an environmental study was conducted, using the MIP-SPE cartridges to examine concentrations of neoni-

cotinoids in local waterways. In collaboration with the City of St. John's, a water table distribution map was obtained, and the Waterford river, and its in-flows were selected as the optimal monitoring site. As shown in Figure 2.5, 11 sampling sites were selected for this study. The sampling sites were divided as follows: samples 7 through 11 were designated as upstream, and samples 1 through 6 were designated as downstream. For the upstream samples, samples 7 and 9 were taken from an urban, heavily populated area, and are expected to contain minimal to no neonicotinoids. Sample 8 was taken from a river which collects the runoff from agricultural fields. Samples 7-9 are combined downstream at sample site 5. Sample 10 and 11 were taken from the same river, which feeds sample 6 downstream. Sample 10 was taken downstream from sample 11, which was taken from a heavily agricultural area, however the waterway is not designated as agricultural runoff. For downstream samples, sample 5, fed from samples 7-9, and samples 6, fed from samples 10-11 were collected. Samples 5 and 6 are combined into one river, from which samples 1-4 was collected. Sample 1 was collected at the outflow of this river, sample 2 in the geographic centre of this river, and samples 3 and 4 collected at the same site, near the location where all upstream samples are combined.

All samples were collected in 500 mL amber sampling bottles. All bottles were purchased new, certified clean, and rinsed 3 times at the sampling site prior to collection. Samples were all collected on the same day, during a period of snow-melt runoff, where water levels were determined to be elevated. Samples were stored at 2 °C for 3 days prior to analysis.

For analysis, SPE cartridges were packed with approximately 1 g of the 3-TMSPM-SiO_2-MIP bulk phase. 100 mL of sample was passed through the cartridge following pre-conditioning, and eluted with 3 mL of 1% acetic acid in 1:1 acetonitrile:water. Concentration was determined using existing calibration curves constructed using

spiked water samples extracted with the MIP SPE cartridges.

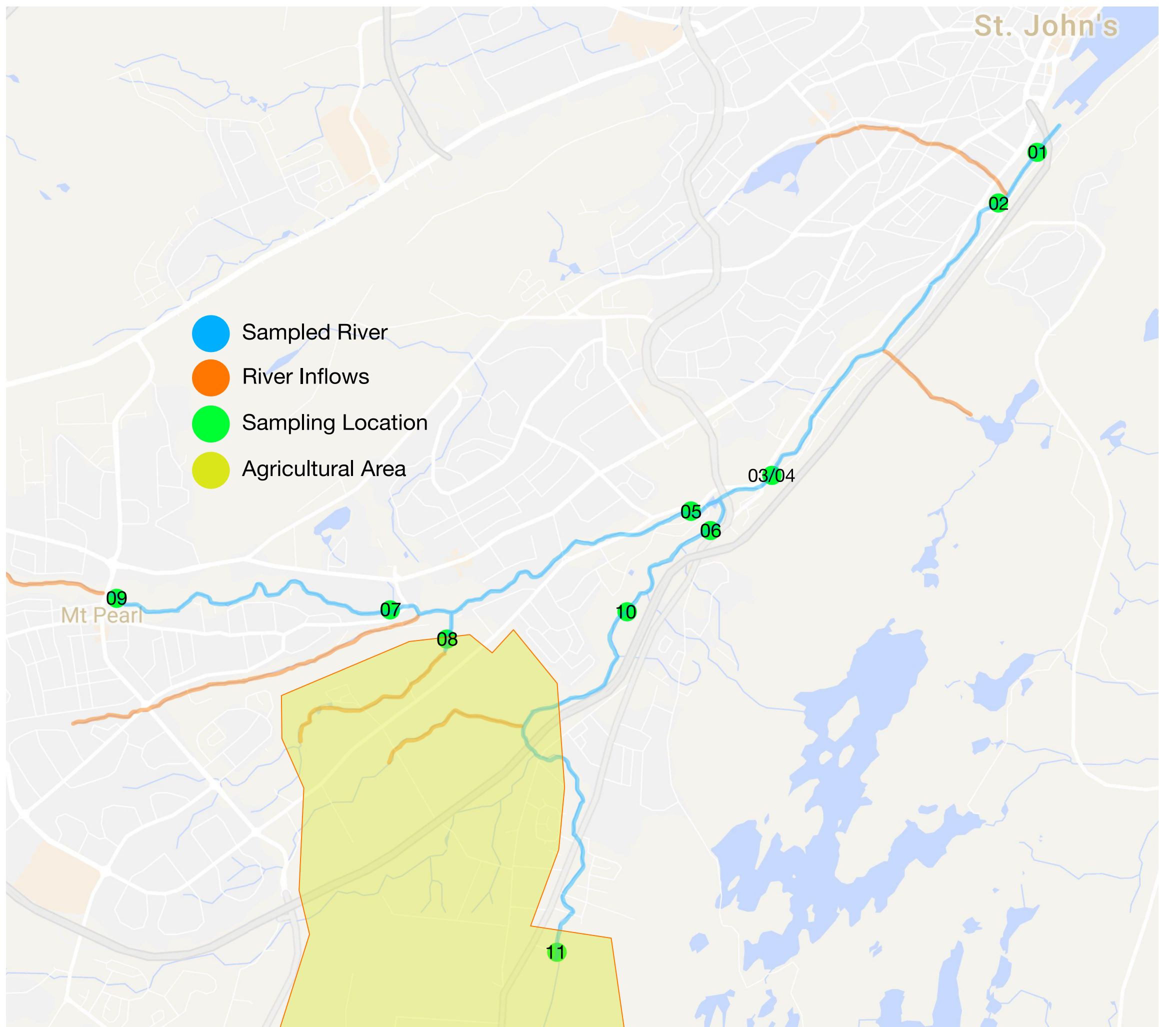


Figure 2.5: Map of sampling locations for neonicotinoids along the Waterford river and selected inflow rivers

Chapter 3

Results and Discussion

This thesis focuses on three specific aspects, which when combined, allow for complete examination of environmental samples for the analysis of neonicotinoids. The development of appropriate instrumental techniques and method was the first objective, and is critically important for both sample analysis, and validation of extraction and pre-concentration methods. Two different methods were developed for the analysis of neonicotinoids: an LC method using either PDA or MS/MS detection, and a DART method, using a high-resolution Q-TOF MS for detection. Both methods provide different advantages, which will be discussed further in this chapter.

Following the development, validation, and optimization of appropriate analytical methods, work began on the fabrication of a molecularly imprinted polymer which could be used to extract, pre-concentrate, and clean-up the matrix in environmental samples suspected to contain neonicotinoids. The MIP phase was used primarily as a packing for SPE cartridges, and results from the MIP-SPE were validated against commercial C18 cartridges, as well as the industry-standard QuEChERs method for pesticide analysis.

Finally, the MIP-SPE phase was tested using real environmental samples. River

water samples were spiked with neonicotinoid solution to obtain percent recovery values, which were compared to various other methods including C18 SPE, QuEChERs, and direct aqueous injection. Samples were also collected along a local river, both upstream and downstream of agricultural areas, including river inflows and outflows, and analyzed for the presence of neonicotinoids using the validated MIP-SPE cartridges.

3.1 Liquid Chromatography

Previous methods for neonicotinoid separation by LC are either complex, or inefficient. Most methods take an average of 9 minutes to complete one separation, and utilize complex buffering systems or gradients, which are often relics of past methods adapted to the separation of neonicotinoids. Here, a novel and simplified method is proposed using UHPLC with a superficially porous column, allowing for far faster separations.

3.1.1 Method Development

Method development was completed using individual standards of each neonicotinoid plus internal standard nicotine, and a multistandard solution, all at 2 mg L^{-1} concentration. Starting with a high organic content in the mobile phase (strong solvent), individual standards were injected to determine their retention times and elution order. Following this, the percentage of aqueous phase in the solvent system was increased until sufficient separation was achieved for all neonicotinoids plus internal standard. Following this, adjustments were made to flow rate and column temperature to shorten the method run time, while maintaining baseline separation. The final parameters for the isosceles column included a flow rate of 0.350 mL min^{-1}, with column temperature of 30 °C. A solvent composition of 68% aqueous and 32% organic were used, where the aqueous phase was water with 1% formic acid, and the

Table 3.1: Column comparison results including experimentally determined plate counts and capacity factors

Column		Nitenpyram	Thiamethoxam	Clothianidin	Imidacloprid	Acetamiprid	Thiacloprid
Waters C18	Plate Count	3281.7	340.2	53.4	900.0	1650.6	1259.7
	k'	0.0025	0.2738	0.9288	1.5688	1.9963	2.6713
Isosceles 2.7	Plate Count	243.0	865.1	560.1	2020.7	892.3	6434.8
	k'	0.06562	0.3672	0.6641	0.8438	0.9953	1.8828
Halo 2.7	Plate Count	2081.8	1909.0	2898.9	1722.6	2978.7	1496.8
	k'	0.0683	0.3523	0.6238	0.7952	0.9492	1.7635

organic phase was acetonitrile also with 1% formic acid. As each neonicotinoid has a dissociable pK_a value greater than 5, the introduction of formic acid in the mobile phase ensures complete protonation of the non-ionized neutral form.

3.1.2 Column Analysis

Three columns were available for this analysis. Each column was a C18 reverse phase column, however they differed in the stationary phase fabrication and endcapping. Theoretical plate count was calculated using equation 3.1, where t_r is the retention time and W is the peak width at baseline, for each analyte peak, and averaged to determine an approximate plate count for the column. The capacity factor of each compound was also calculated using equation 3.2, where t_r is the retention time of the analyte and t_0 is the retention time of the solvent peak. Full results are shown in Table 3.1.

$$N = 16(\frac{t_r}{W})^2 \tag{3.1}$$

$$k' = \frac{(t_r - t_0)}{(t_0)} \tag{3.2}$$

For method development, a Waters C18 column was used. This column had dimensions 2.1 × 50 mm, with a particle size of 1.7 μm. The particles were solid and

fully-porous, as opposed to the superficially porous particles found in the two other columns. Baseline separation of the six neonicotinoids was achieved in 3 minutes with Gaussian peak shape (Figure 3.1a). Calculation of the average theoretical plate count gave a result of 1248, which is lower than the other two columns, both having slightly higher numbers of theoretical plates. The backpressure averaged 4200 psi during the separation, which is again higher than the superficially porous columns, but understandable given the smaller particle size. However, despite some drawbacks compared to superficially porous columns, baseline separation of the six neonicotinoids in under 3 minutes demonstrates significant advantages over all literature methods at the time of writing, which average 9 minutes in length. The isocratic elution program is also a significant advantage, as no time is required for system equilibration between runs, allowing for direct injection following elution of the last compound.

The Halo column provided by Canadian Life Science demonstrates significant advantages over the Waters C18 column. The Halo column has dimensions of 2.1 × 100 mm, with a 2.7 μm particle size. The use of a superficially porous column allows for faster separations. More uniform and narrow pore distribution in the column, as illustrated by the manufacturers allows for shorter diffusion paths, and a narrower range of paths (related to the multipath term in the van Deemter equation) and therefore narrower peak widths compared to fully porous systems. The lower multi-path broadening and better mass transfer leads to superior resolving power and greater number of theoretical plates when applied to the van Deemter equation (Equation 3.3).[74] This is demonstrated experimentally by running the same separation and comparing the results to the Waters column. There is an increased plate count from 1248 to 2181 plates simply by changing the stationary phase to a superficially porous one. Another advantage is reduced backpressure. The backpressure at 0.300 mL min^{-1}, the same flow rate as the Waters column, decreased from an average of 4200 to 2500 psi. When

the flow rate was increased to 0.400 mL min^{-1}, the backpressure averaged at 3500 psi during the run. The superficially porous columns allows both for faster separations at increased flow rates, and longer column and instrument lifetimes by running methods at lower backpressure. The lower pressures required to achieve a suitable separation also means a HPLC can be used rather than the less common UHPLC instrumentation.

$$H = A + B/u + (C_s + C_m) \tag{3.3}$$

Canadian Life Science provided a prototype column, the Isosceles C18 reversed phase. Column dimensions were 2.1 × 100 mm, with a particle size of 2.7 μm. The stationary phase in this column is also a superficially porous solid-core type, however the difference between this column and the Halo column is in the end-capping of the C18 phase. The Isosceles column is fabricated with a proprietary end-capping designed to prolong column life and improve reproducibility for separations. Experimental calculations of theoretical plate count indicated a lower plate count than the Halo column, but still significantly higher than the Waters column. The capacity factor for nitenpyram, the first eluting analyte, in the Isosceles column is similar to that of the Halo column, and both are improved when compared with the Waters column. The Isosceles column was selected for all further analysis and validation studies, while it did have slightly lower separation efficiency than the Halo column, it promised improved reproducibility in terms of retention time and peak shape, as well as column life. A comparison of the results from all three columns is shown in Figure 3.1 a,b, and c.

In Figure 3.1 it is clear there is an advantage to using the superficially porous columns over the Waters column. Both the Halo and Isosceles columns afford narrower peak widths, and faster baseline separation. As these results were obtained using

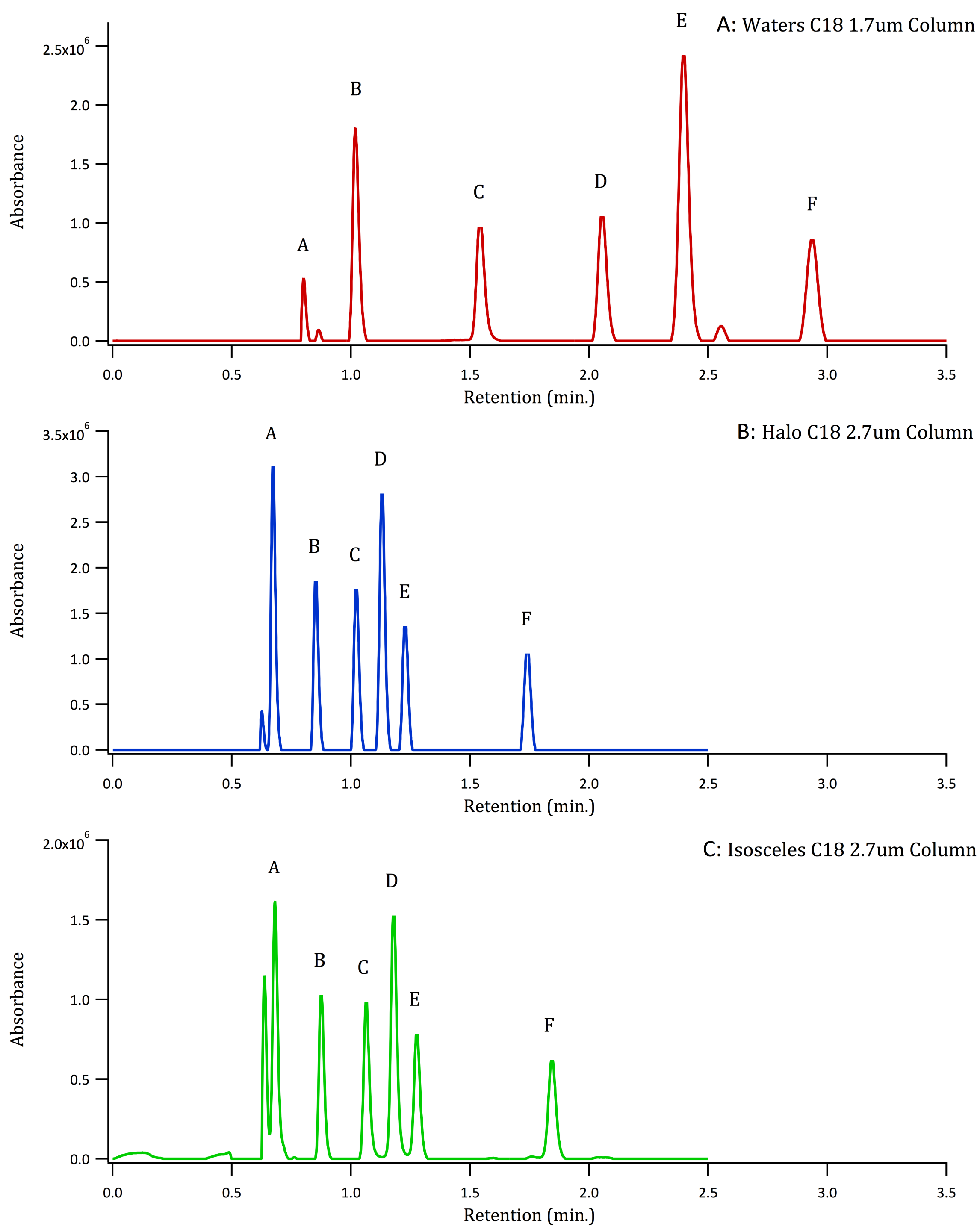


Figure 3.1: Comparison of LC columns used in this research. Chromatographic peaks: A: nitenpyram, B: thiamethoxam, C: clothianidin, D: imidacloprid, E: acetamiprid, F: thiacloprid

Table 3.2: Reproducability of the optimized LC method, examining changes in retention time and peak area

Neonicotinoid	Retention Time (n=7) (95% CI)	%RSD in Retention Time	Absorbance Peak Area (n = 7) 2 mg L^{-1}	%RSD in Peak Area
Nitenpyram	0.676 ± 0.007	0.81%	79605	0.45%
Thiamethoxam	0.866 ± 0.017	1.55%	50960	0.56%
Clothianidin	1.048 ± 0.032	2.47%	51646	1.03%
Imidacloprid	1.158 ± 0.032	2.24%	82459	0.58%
Acetamiprid	1.256 ± 0.035	2.22%	43693	0.25%
Thiacloprid	1.802 ± 0.075	3.34%	44320	0.25%

a PDA detector, it is expected to see a fronting solvent peak. Due to the high water solubility of nitenpyram, the first eluting peak, the solvent and analyte peaks often overlap, with nitenpyram showing no retention as shown in 3.1a. However, the superficially porous columns are able to offer additional separation, which causes the appearance of a peak before the nitenpyram (A) peak.

3.1.3 Optimized LC Parameters

The Isosceles C18 column required some further optimizations to take full advantage of the separation capability and column efficiency. The flow rate was increased from 0.300 mL min^{-1} to 0.350 mL min^{-1} to both sharpen peaks and decrease run time. The mobile phase ratio was adjusted from 70% aqueous to 68% aqueous. Although this appears to be a small change, due to the precise nature of UHPLC this results in a significant change in retention time and capacity factor. The 2% reduction in aqueous content results in improved separation. These two adjustments allowed for adequate separation in under 2 minutes, and baseline separation in 2.7 minutes, the latter of which was used for routine analysis of all samples. A comparison of retention times and peak areas using the same standard and method over multiple runs during the same, and on different days was performed to establish the reproducibility of the method. Results from this experiment are shown in Table 3.2.

Table 3.3: Method validation parameters for the optimized LC-PDA method

Neonicotinoid	Regression Equation	R^2	LOD (μg L^{-1})	LOQ
Nitenpyram	y = 1090x + 16442	0.9997	1.907	6.358
Thiamethoxam	y = 1120x + 26654	0.9996	4.800	15.999
Clothianidin	y = 680x + 8768	0.9995	3.576	11.920
Imidacloprid	y = 695x + 9041	0.9998	3.173	10.578
Acetamiprid	y = 306x + 1937	0.9997	9.368	31.225
Thiacloprid	y = 426x - 2352	0.9997	6.008	20.027

3.2 Photo-Diode Array Detection

The maximum wavelength for all neonicotinoids ranged between 215 and 280 nm. Even in complex environmental matrices, or honey extracts each neonicotinoid had a unique maximum absorbance wavelength and there were no spectra interferences from the matrix, allowing for baseline separation and good detection of each neonicotinoid in all samples. The advantage of using a PDA method for detection is the high level of reproducibility and low cost of use. PDA is a significantly cheaper and easier instrument to run compared to mass spectrometric methods, requiring only replacement and calibration of a new lamp once or twice a year, and minimal parameter optimization such as slit width, maximum wavelength, and reference wavelength. The selectivity for neonicotinoids, ease of use, and low cost make PDA detection highly useful for the routine analysis. Limits of detection, while higher than mass spectrometry, remain environmentally relevant, ranging between 1.91 and 9.37 μg L^{-1} (Table 3.3). Limits of detection were calculated using three times the standard deviation of 9 replicate samples at 20 μg L^{-1} over the slope of the linear regression line. The linear regression line indicated high linearity for all neonicotinoids, with regression coefficient R^2 greater than 0.9990 for all analytes, as shown in Figure 3.2 and Table 3.3.

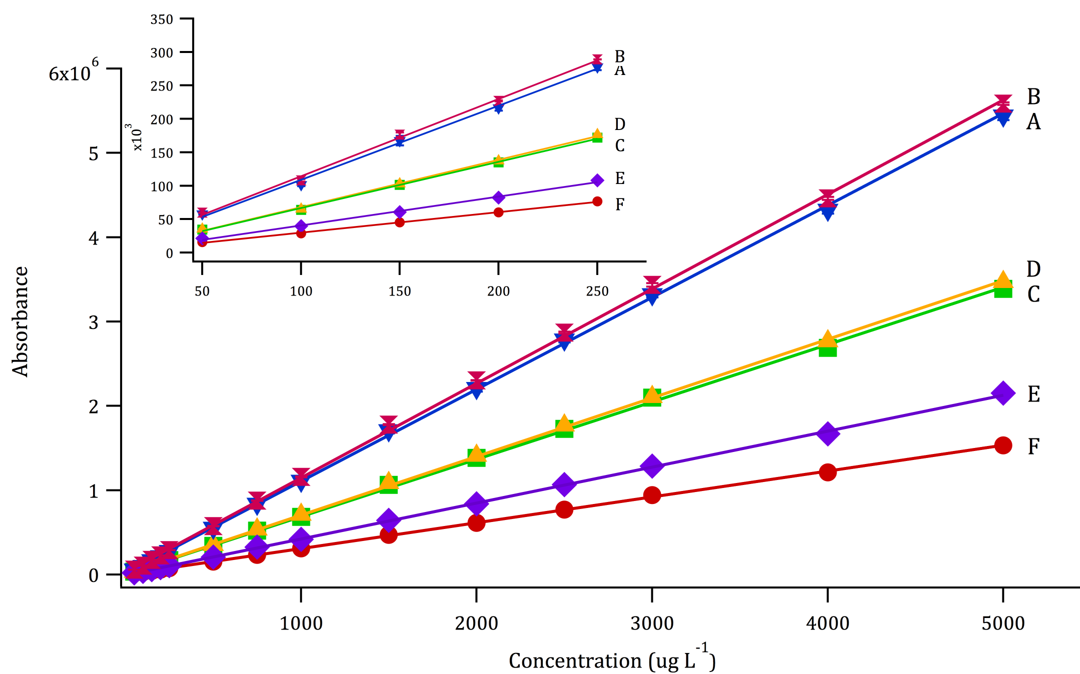


Figure 3.2: LC-PDA calibration curve from 50 - 5000 μg L^{-1} for neonicotinoid standards. n=3. A: nitenpyram, B: thiamethoxam, C: clothianidin, D: imidacloprid, E: acetamiprid, F: thiacloprid

3.3 Tandem MS Method Validation

Intellistart was used to determine appropriate fragmentation patterns, cone voltage, and collision energy for the six neonicotinoids of interest. Intellistart examines samples for the presence of analytes based on an input of molecular formula. By rapidly adjusting mass spectrometric parameters including cone voltage, collision energy, source temperature, desolvation temperature, atmospheric pressure ionization (API) gas pressures, collision gas pressures and more; then using an algorithm to calculate optimal parameters for each analyte of interest, this program allows for rapid method development, reducing what might be days of careful tuning of the mass spectrometer to minutes.

Adding MS detection based on the Intellistart optimization to the UHPLC method development with the PDA, which is unchanged, allows for highly efficient monitoring of neonicotinoids. The PDA results are used to confirm the chromatographic peaks in the MS results. By monitoring the MRM transitions corresponding to the neonicotinoids of interest only when they are eluting from the column, as opposed to collecting data on 6 MRM channels simultaneously, the sensitivity of the instrument is greatly increased. This allows for more points per peak to be collected, an optimization demonstrated in Figure 3.3.

As shown in Figure 3.4, some neonicotinoids have isobaric fragments when analyzed by MRM. This presents challenges when ambient ionization techniques such as DESI or DART are used, as it becomes difficult to distinguish which fragments correspond to which precursor ions. This issue is overcome with the use of separatory methods such as chromatography, or high-resolution mass spectrometry, which will be discussed later.

The tandem mass spectrometry method lowered limits of detection as compared to the PDA detection method. Limits of detection were determined using three times

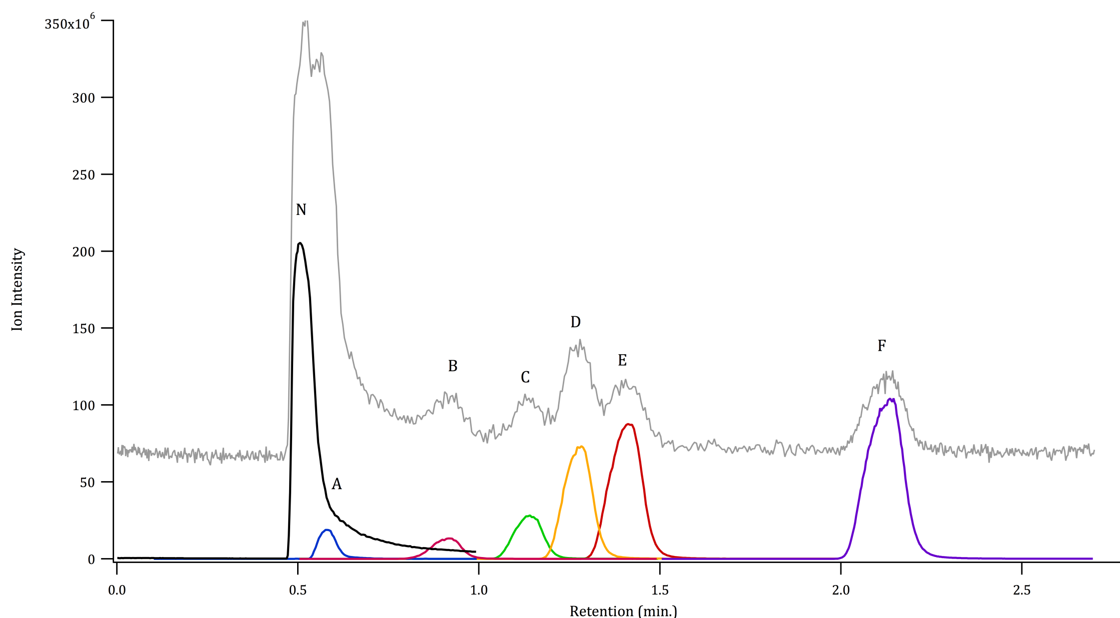


Figure 3.3: LC-MS/MS MRM chromatograms highlighting the selectivity of the MRM method compared to a total ion chromatogram. A: nitenpyram, B: thiamethoxam, C: clothianidin, D: imidacloprid, E: acetamiprid, F: thiacloprid, N: nicotine internal standard

the standard deviation of 9 replicate samples at 2 μg L^{-1}. Limits of detection ranged from 0.19 to 0.37 μg L^{-1}. The regression coefficient R^2 remained above 0.990 for all analytes, although the variance in the signals were slightly higher than with the PDA yielding slightly poorer fit to the linear model. As expected, the relative standard deviation in replicate standards was also slightly increased in comparison to the PDA method, which is expected due to the higher signal variability in mass spectrometry compared to absorbance measurements. Full results for method validation of the MS/MS detection method are given in Table 3.4. The MS/MS method is highly sensitive, by examining two product ions of a single precursor, and accounting for elution results from the separation and PDA method, there is a high probability that the only compounds being detected are the analytes of interest. This is particularly useful for examining complex matrices, or performing direct aqueous injection of environmental samples.

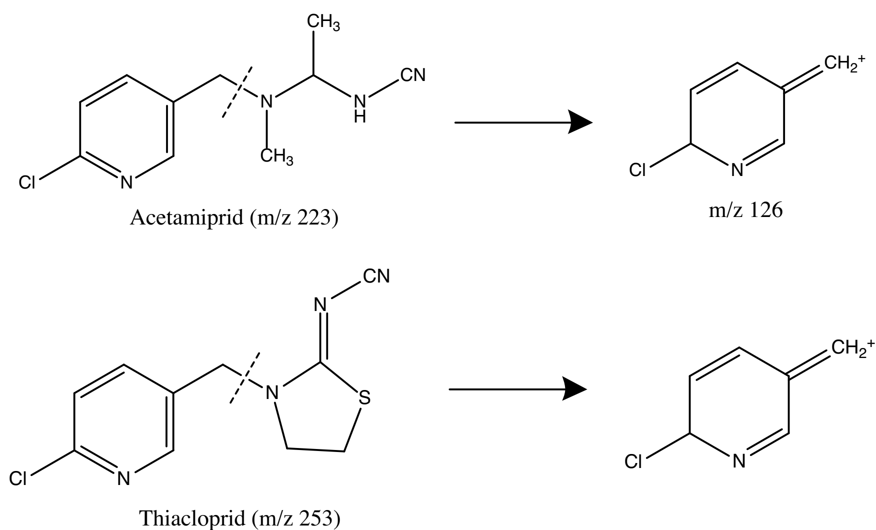


Figure 3.4: Illustration of potential isobaric fragments at m/z 126 for thiacloprid and acetamiprid

Table 3.4: Method validation parameters for the optimized LC-MS/MS method

Neonicotinoid	Regression Equation	R^2	LOD (μg L^{-1})	%RSD in Regression
Nitenpyram	y = 2586x + 20248	0.9997	0.203	1.31%
Thiamethoxam	y = 3077x + 14927	0.9998	0.366	2.21%
Clothianidin	y = 1348x + 9828	0.9997	0.195	2.06%
Imidacloprid	y = 4704x + 59998	0.9979	0.211	1.72%
Acetamiprid	y = 7963x + 172799	0.9980	0.284	0.95%
Thiacloprid	y = 7648x + 129362	0.9989	0.359	1.83%

3.4 Direct Analysis in Real Time

Through a collaboration with the Ontario Ministry of the Environment, a DART method was able to be developed for the analysis of neonicotinoids. DART is a type of ambient ionization which provides a direct sampling to analysis workflow, removing time-consuming sample processing, extraction, and concentration steps. The DART ion source was operated in positive mode, which as discussed previously generates protonated water clusters that subsequently transfer charge to analytes of interest, producing only $[M+H]^+$ analyte peaks in the MS. A number of experiments were performed to optimize the relatively simple source, including investigating the effects of metastable species temperature, solvent doping, and different metastable species. Optimization for neonicotinoids was performed using an FTICR, before switching to a Q-TOF for quantitative analysis.

3.4.1 DART-FTICR

A series of experiments were performed to optimize a DART method for neonicotinoid analysis. The majority of optimizations were performed using high-concentration standards on a quadrupole ion trap FTICR instrument, due to availability. The FTICR was configured to collect spectra from pulses of trapped ions every 5 seconds, the ions were trapped for 5 seconds in the quadrupole ion trap, then released into the FTICR where they were quickly excited and an image current domain collected on the frequency by which they precessed to the centre of the instrument. This occurred over 800 ms. Due to the age of the instrument, limits of detection were estimated at 5 mg L^{-1}. While not capable of performing low concentration environmental analysis, it did provide an opportunity to examine the capabilities of the DART ionization source, and its capability for ionization of neonicotinoids.

3.4.2 Temperature Optimization

One of the only user-variable parameters related to DART ionization is the temperature of the metastable species. An increased temperature corresponds to an increased production of metastable species, and the generation of more ions from analytes. However, if the temperature is too high, it can cause thermal degradation of the analytes, or the sampling substrate. To optimize this critical parameter with DART, high-concentration standards of each of the six neonicotinoids were introduced on a glass melting point capillary, and their ion counts recorded using FTICR. As shown in Figure 3.5, there is a region of high ion counts between 300 and 350 °C for all analytes. Below these temperatures, not enough of the analyte is ionized, due to the lack of energetically favourable metastable species. Above these temperatures, it is possible that thermal degradation of the neonicotinoids occurred, as the peak corresponding to the ion count of $[M+H]^+$ was reduced, while some potential fragmentation peaks increased in ion count.

3.4.3 Solvent Doping

Another interesting experiment was investigation of the capabilities of solvent doping with the DART source.[75] By exposing a small quantity of solvent, either DCM or ammonia, to the stream of metastable species, the solvent vapours should form adducts with analytes of interest. To test this, 3 – 5 mL of DCM was placed in a small vial directly below the stream of metastable species so that solvent vapours might be ionized. Using a standard of thiacloprid, introduced on a glass melting point capillary, the spectrum was collected using FTICR, and examined for the presence of chlorine adducts. It was determined, as shown in Figure 3.6, that the presence of DCM vapour in the metastable stream caused a reduction in ion count for $[M+H]^+$

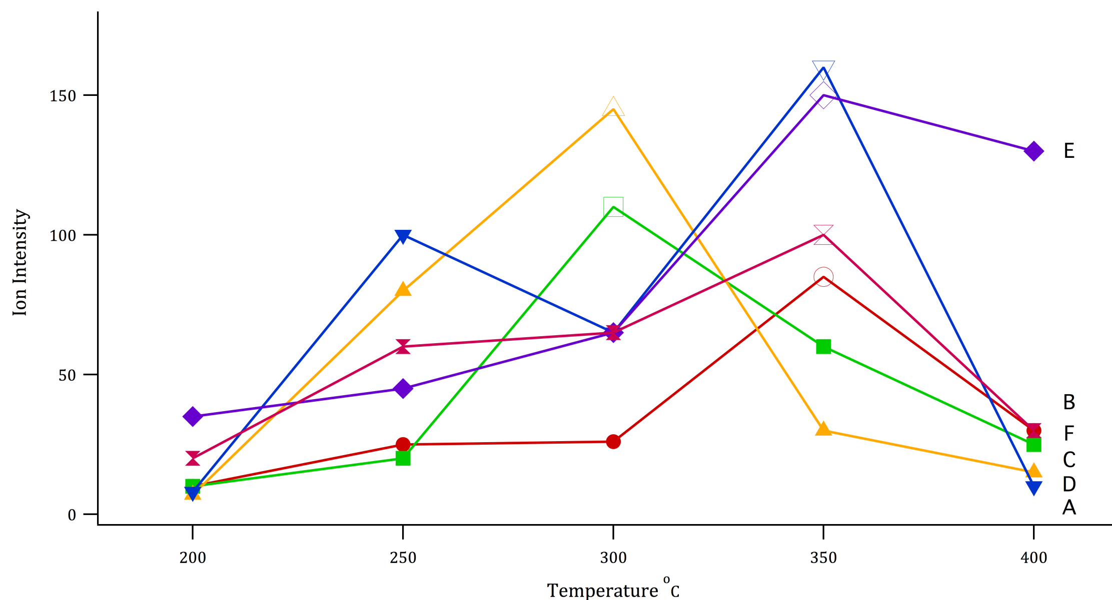


Figure 3.5: DART-FTICR temperature optimization to maximize ion count for each of the six neonicotinoids. A: nitenpyram, B: thiamethoxam, C: clothianidin, D: imidacloprid, E: acetamiprid, F: thiacloprid

from 80 for m/z 253 to just 2.5 for m/z 253, and the appearance of a new $[M+Cl]^+$ peak. While solvent doping was not used for further analysis of neonicotinoids, it does provide useful insight into methods of improving selectivity and sensitivity of DART.

3.4.4 Metastable Species Variations

One of the interesting aspects of the underlying mechanism behind DART ionization, is in the generation of metastable species, and ions. As was outlined in the introductory chapter, in positive mode, DART generates protonated water clusters using metastable species which interact with atmospheric water to generate H^+. The ability to generate these protonated water clusters, and in general, the ability to ionize analytes of interest, is largely dependent on the ionization energy of the metastable species, and the ionization potential of the analytes. Helium, the most commonly used metastable species, has an ionization energy of 26.4 eV,[76] and is therefore capa-

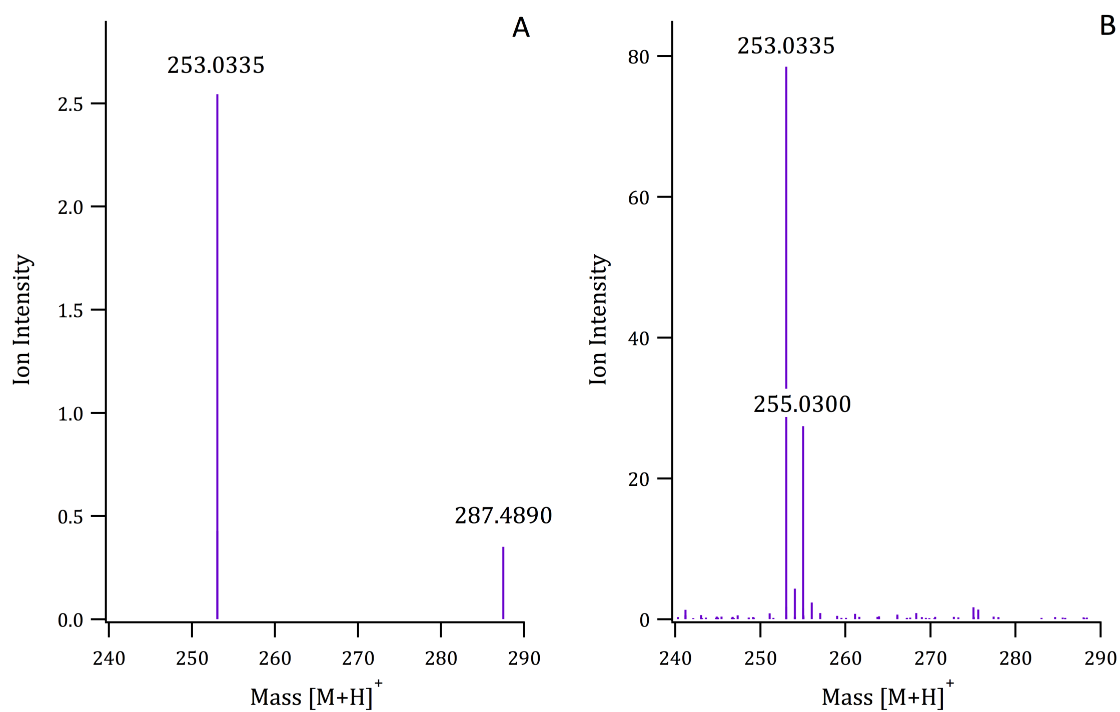


Figure 3.6: The mass spectrum demonstrating the effects of solvent doping on the ionization mechanism of DART. A: DCM solvent vapours introduced into the metastable species, B: routine DART ionization

ble of ionizing most analytes. Argon however, has an ionization energy of only 11.55 eV,[77] which is less than the ionization potential of water, at 12.60 eV. This means that argon is unable to generate the required protons or ions. When the metastable species was switched from helium to argon, the selectivity in ionization was observed experimentally, with no ions produced while using argon as the metastable species. An interesting result from this experiment, was the ability to utilize the metastable source gas to selectively ionize analytes dependant on their ionization potential. For example, when nitrogen was used as the metastable species, with an ionization energy of 14.50 eV,[78] it was able to ionize 3 of the 6 neonicotinoids. Likely those neonicotinoids with ionization potentials less than that of nitrogen. In addition, the ion counts were significantly higher than those from the helium metastable species, due to a reduction of background interferences entering the FTICR. This suggests that selective ionization could be performed by matching an appropriate metastable source gas with the ionization potential of analytes. While the results using the other metastable species were interesting, helium was used for further analysis of neonicotinoids.

3.4.5 Sampling with DART and TDU-DART

Sampling with DART is relatively straightforward, however does present with some issues for routine analysis. Unlike DESI, which has many geometric parameters which can be optimized, DART can sample at an angle of 180° to the mass spectrometer inlet, or 135° to the mass spectrometer inlet, as shown in Figures 2.1a and 2.1b. The 180° angle is useful for method development using droplets of standard on melting point capillaries, while the 135° angle is useful for analysis of solid substrates, such as thin-film MIPs. Both these sampling methods have issues with reproducibility, due to the method of sample introduction. It becomes challenging to introduce the sample in a reproducible way, or at a reproducible volume, leading to large %RSD values. While

this is adequate for screening methods and method optimization, the error in sample introduction is unacceptable for quantitative analysis of analytes. To overcome this, the TDU-DART concept is employed. By placing the sample inside a TDU, heated at 220 °C, then ionized by the DART, quantitative ionization and transfer to the mass spectrometer can be achieved.

Analytes of interest are spotted in known volume on an aluminium substrate and placed into the heated, custom-built TDU. The temperature of the TDU is variable, however anything below the maximum temperature of 220 °C did not show improvement in ionization efficiency, and the maximum temperature was selected to ensure the maximum thermal desorption of analytes from the substrate was achieved. It is possible that better results could be achieved at increased temperatures, but due to limitations of the TDU, it was determined that the maximum achievable temperature should provide consistent desorption of analytes, reducing sampling error. By being able to dispense a known sample volume, and ensure it is consistently thermally desorbed in an enclosed system, the sample introduction and ionization mechanism is made more reproducible. For these quantitative experiments, a Waters Q-TOF mass spectrometer was used, which still provided highly accurate m/z measurements with lower limits of detection than the FTICR.

Accurate Mass Measurements

The high mass resolution of the Q-TOF gave accurate mass determinations for the neonicotinoids, which agreed well with the theoretical masses (Table 3.5). All spectra for the neonicotinoids, shown in Figure 3.7, were fairly clean, with limited background noise. All neonicotinoids peaks appeared with at least 5× the ion intensity of the surrounding background spectra, and the characteristic chlorine isotopic 3:1 ratio was observable for all neonicotinoids.

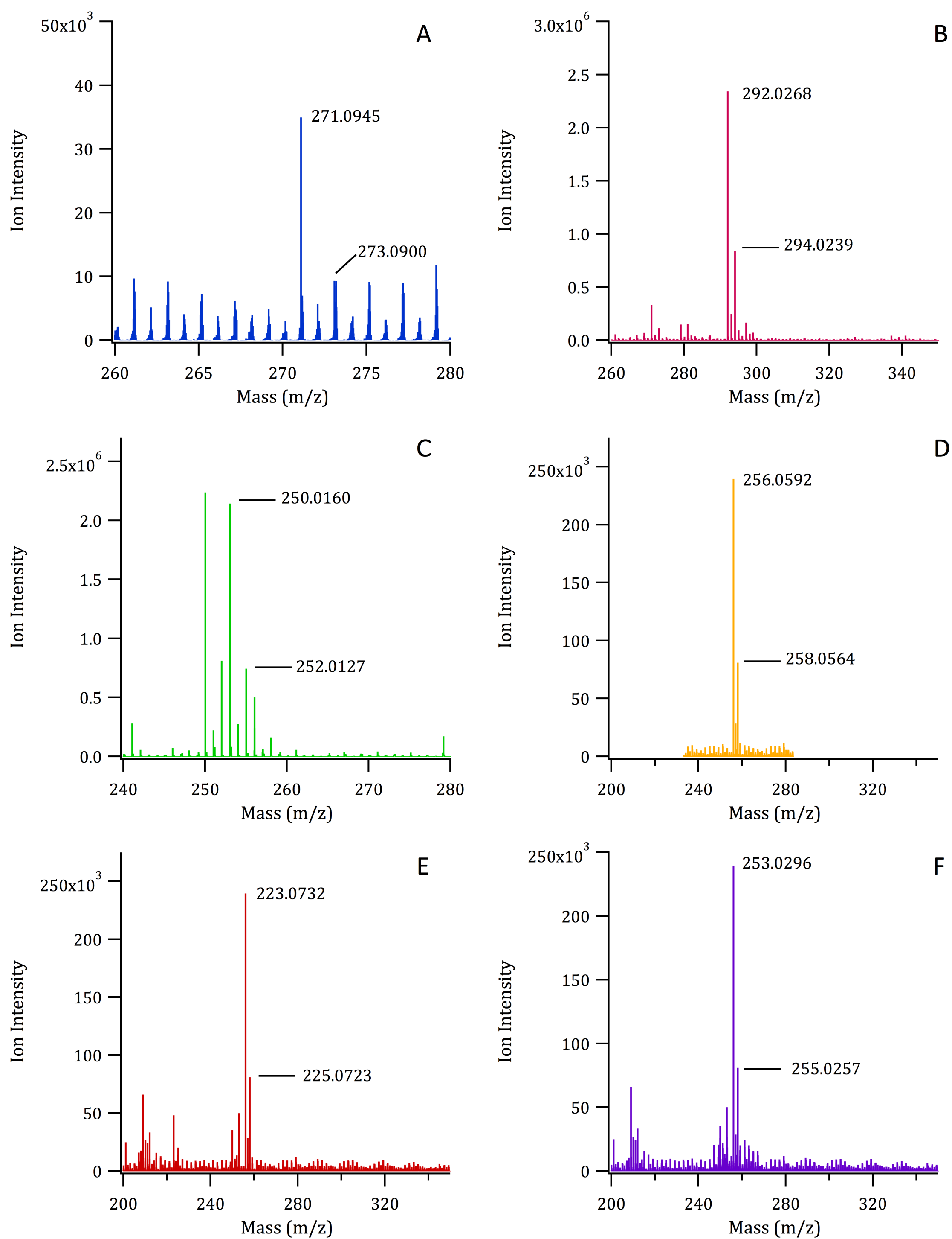


Figure 3.7: Mass spectrum measured by TDU-DART-QTOF-MS of 6 neonicotinoids at 0.5 mg L^{-1}.A: nitenpyram, B: thiamethoxam, C: clothianidin, D: imidacloprid, E: acetamiprid, F: thiacloprid

Table 3.5: Comparison of theoretical calculated masses and actual mass measurements by DART-Q-TOF for the six neonicotinoids of interest

Neonicotinoid	Formula	Theoretical $[M+H]^+$	Measured $[M+H]^+$
Nitenpyram	$C_{11}H_{11}ClN_4H^+$	271.0962 / 273.0935	271.0945 / 273.0900
Thiamethoxam	$C_8H_{10}ClN_5O_3SH^+$	292.0271 / 294.0242	292.0268 / 294.0239
Clothianidin	$C_6N_5H_8SO_2ClH^+$	250.0165 / 252.0136	250.0160 / 252.0127
Imidacloprid	$C_9H_{10}ClN_5O_2H^+$	256.0601 / 258.0574	256.0592 / 258.0564
Acetamiprid	$C_{10}H_{11}ClN_4H^+$	223.0750 / 225.0723	223.0732 / 225.0896
Thiacloprid	$C_{10}H_9ClN_4SH^+$	253.0315 / 255.0723	253.0296 / 255.0257

Calibration Curve

As a direct result of the quantitative nature of the TDU-DART method, it was possible to construct a calibration curve using neonicotinoid standards ranging from 0.1 – 500 μg L^{-1}. A known volume of standard was dispensed on the aluminum substrate, dried, and an injection standard of *N*-nitrosodimentylamine (NDMA) was added in known volume and concentration, with the solvent allowed to evaporate before placing the substrate inside the TDU. By continuously collecting spectra, and introducing the sample-containing substrate at regular intervals, a chronogram was generated, where the peaks for the extracted ions of interest correspond to increased ion intensity as a result of introduction of sample into the TDU (Figure 3.8). Using this method, data for a 9-point calibration curve was collected in just 15 minutes. The peak area from the extracted ion chronograms was integrated and normalized against the injection standard and used for the calibration curve (Figure 3.9).

The calibration curve for the TDU-DART method, shown in Figure 3.9, indicates good linearity and reproducibility for neonicotinoids, particularly for an ambient atmospheric ionization technique. The regression coefficients were greater than 0.990 for all analytes, which corresponds well with those from the LC-MS/MS method. Limits of detection were higher than both the LC-PDA and LC-MS/MS methods, however this is to be expected, as the method requires much more optimization. For example,

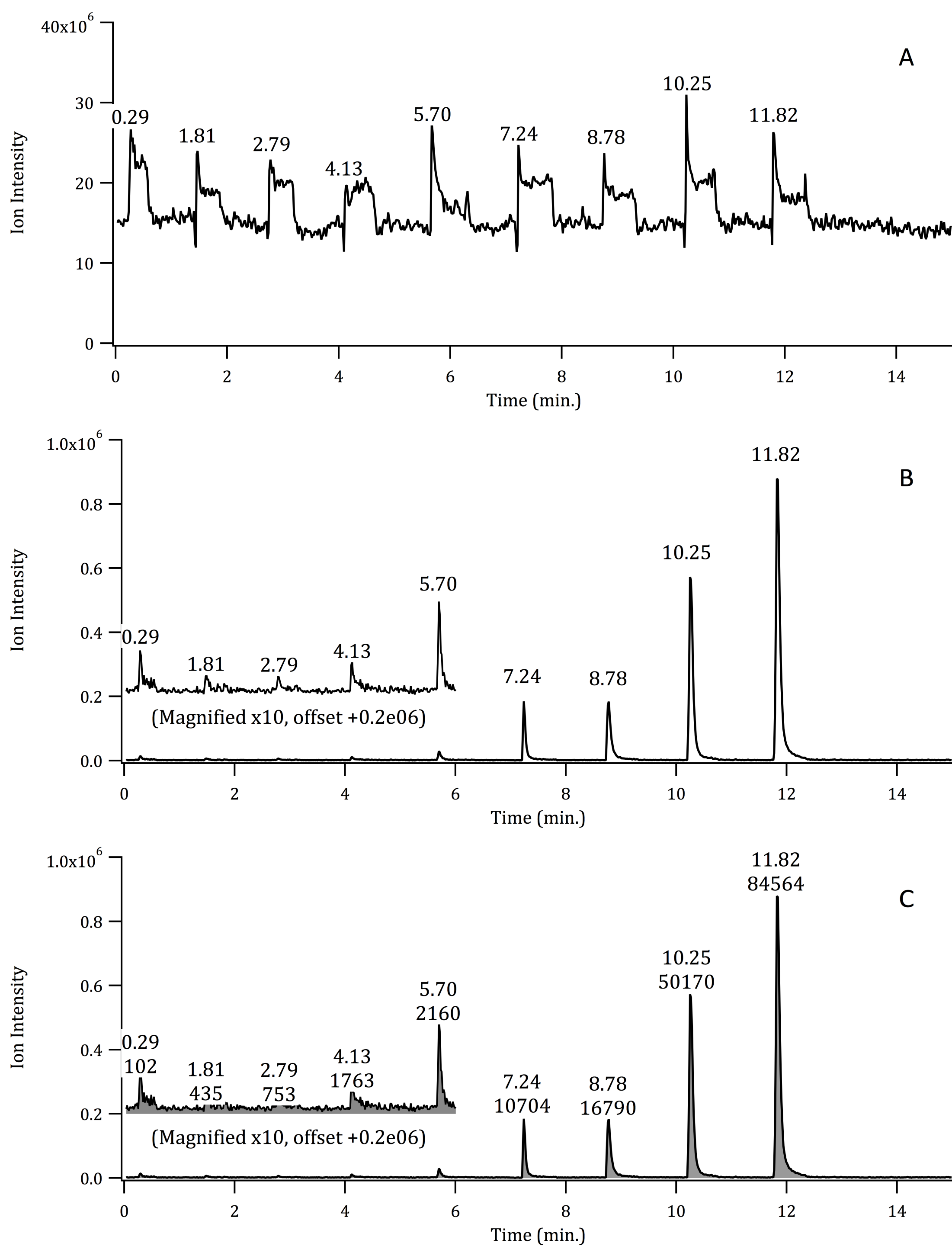


Figure 3.8: Chronogram of a 0.5 mg L^{-1} neonicotinoid multistandard collected by TDU-DART-QTOF-MS (A) showing the extracted ion chronogram for imidacloprid (B), and subsequent peak integration (C).

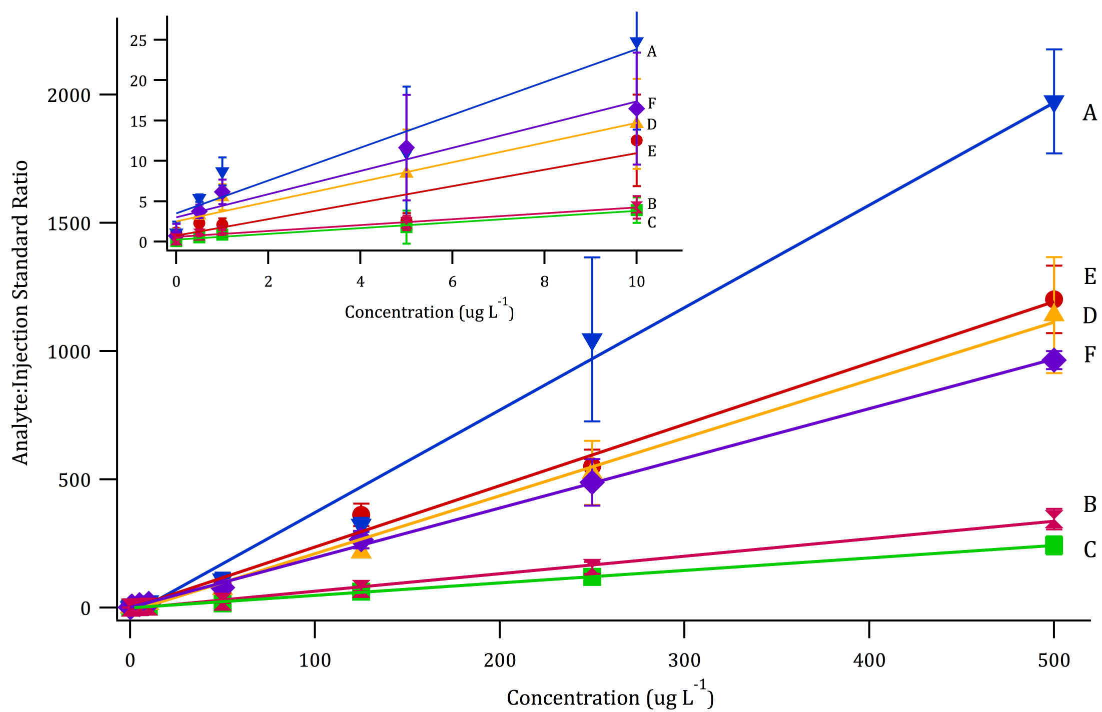


Figure 3.9: Calibration curve of neonicotinoid standards collected by TDU-DART-QTOF-MS, injection standard NDMA at 0.5 mg L^{-1}. A: nitenpyram, B: thiamethoxam, C: clothianidin, D: imidacloprid, E: acetamiprid, F: thiacloprid

Table 3.6: Method validation parameters for the TDU-DART-QTOF-MS method for neonicotinoid analysis

Neonicotinoid	Regression Equation	%RSD in Regression	R^2	LOD (pg analyte)
Nitenpyram	y = 3.996x - 28.9	36.0%	0.9906	0.438
Thiamethoxam	y = 0.682x - 4.11	5.97%	0.9964	0.434
Clothianidin	y = 0.487x - 1.08	27.4%	0.9989	0.480
Imidacloprid	y = 2.257x - 15.4	22.4%	0.9952	0.693
Acetamiprid	y = 2.392x - 2.90	7/43%	0.9947	0.409
Thiacloprid	y = 1.937x + 1.25	21.8%	0.9989	0.206

spectra and data were collected using a total ion chromatogram mode; limits of detection could be improved through the use of tandem mass spectrometry and MRM. Unfortunately, there was not enough time available to develop an MS/MS method on the Q-TOF. Limits of detection were found to be between 29.4 and 98.9 ug L^{-1}. However, when these are expressed in terms of mass loading, the LOD ranges from 0.206 to 0.693 pg per sample (Table 3.6). While higher than methods that use separations and tandem mass spectrometry, this demonstrates a good result for a simple high-throughput method that can be applied to a variety of substrates and sampling matrices.

3.5 QuEChERs

For a number of years, the QuEChERs method has been the industry standard for pesticide residue analysis in environmental samples. It is applicable to produce, vegetation, water, honey, and various other consumer products which may have been contaminated with pesticides. It has been validated in the European Union as method EN 15662 and in the United States as method AOAC 2007. In this research, a modified QuEChERs method is used for the examination of river water and honey samples. The difference between the modified QuEChERs method, and method EN 15662, which forms the basis for the modified method, is the sample dilution step. In the

Table 3.7: QuEChERs results for neonicotinoid extraction from spiked honey samples

Neonicotinoid	Regression Equation	%RSD in Regression	R^2	LOD (μg L^{-1})	%Recovery
Nitenpyram	y = 5.528E-05x + 1.7E-03	6.44%	0.9942	0.711	58.6%
Thiamethoxam	y = 1.552E-04x + 5.6E-03	4.84%	0.9888	5.07	13.4%
Clothianidin	y = 1.589E-04x + 4.8E-03	5.36%	0.9913	0.714	34.6%
Imidacloprid	y = 2.672E-04x - 8.8E-03	10.5%	0.9818	0.775	71.4%
Acetamiprid	y = 5.421E-04x + 1.6E02	6.73%	0.9910	0.206	6.73%
Thiacloprid	y = 7.465xE-02x + 2.11E-02	7.28%	0.9912	0.449	35.6%

EN 15662 method, samples are first added to the centrifuge tube, and diluted with 10 mL of water. In the modified method, developed for the analysis of honey and water, samples are added to the centrifuge tube. Honey samples were further diluted with 6 mL of water before continueing with the EN 15662 procedure of adding 6 mL of acetonitrile, buffer salts, and $MgSO_4$ for drying.

3.5.1 Honey Samples

Honey samples (6.0 g) were spiked with various concentrations of neonicotinoid multi-standard and extracted using the QuEChERs method. A matrix-matched calibration curve was constructed and the method examined for linearity, reproducibility, limits of detection, and percent recovery of analytes. The previously validated LC-MS/MS method was used for the analysis of extracts.

The calibration curve for the QuEChERs honey extracts is shown in Figure 3.10. It indicates good linearity and moderate reproducibility for neonicotinoids analysis over a range of concentrations from 0 – 1000 μg L^{-1}. Only 5 points were used due to the time-consuming and complex nature of performing the extraction, which requires numerous weighings, centrifuging, vortexing, and transferring steps. Each sample requires approximately 30 minutes of labour-intensive preparation.

Limits of detection for the QuECheRs method were good (Table 3.7), indicating the method is applicable to spiked samples, and standard addition could be used for analysis of pesticides in honey, however, the method does not demonstrate signifi-

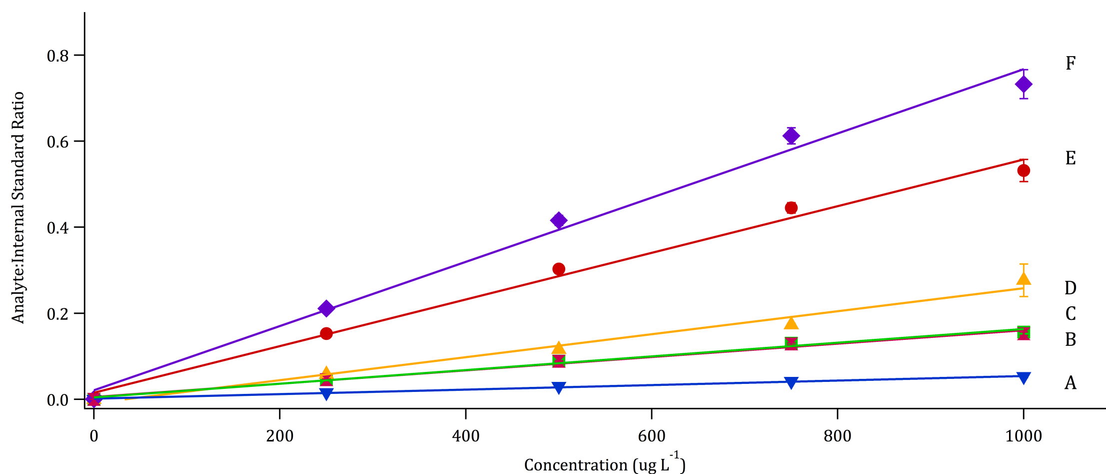


Figure 3.10: Calibration curve for the QuEChERs method. Data points were extracted using the method, spiking relevant concentrations in honey. A: nitenpyram, B: thiamethoxam, C: clothianidin, D: imidacloprid, E: acetamiprid, F: thiacloprid

cant pre-concentration of analytes. In addition, limits of detection are higher than those achieved using direct injection methods, which are goverend by the instrument LOD. With percent recovery values varying between 13 and 71%, the method appears to be non-ideal for the analysis of neonicotinoids. It is possible both that there is competitive absorption and high levels of matrix components in the complex honey matrix causing ion suppression, or that not all neonicotinoids are being extracted by the method. The QuEChERs method makes use of dispersive-SPE, which involves placing the bulk SPE phase in a centrifuge tube and extracting the neonicotinoids into acetonitrile. The analysis of river water extracts by QuEChERs will be further investigated later.

Results from the QuEChERs method highlight a need for faster, more reliable methods of analysis for neonicotinoids. The limited selectivity, combined with the limited pre-concentration capability of the method does not allow for analysis of dilute solutions, such as environmental river water or agricultural runoff. Method detection limits for environmental samples must be lower than those achievable by

the QuEChERs method, to allow for regulatory analysis of neonicotinoids.

3.6 Solid Phase Extraction

As a means of validation, as well as improving those results from the QuEChERs method, solid phase extraction was run using a commercial C18 reverse phase cartridge. C18 was selected due to availability and universal extraction capability of small, organic molecules. All SPE results were analyzed using the previously developed LC-MS/MS method for neonicotinoids.

3.6.1 Initial Testing

An important factor in developing an SPE method, is to examine the extraction capability of the cartridges. Ideally, the cartridge should retain 100% of the analyte as the liquid-sample matrix passes through, and should be capable of pre-concentrating the analyte by reducing a large sample volume to a few mLs of solvent. Also of importance is the ability to selectively extract only those analytes of interest, which simplifies further separation methods such as LC or GC, and simplifies the mass spectrometric analysis.

To test the retentive capabilities of the C18 SPE cartridges, three concentrations of neonicotinoid standard in 100 mL of water were loaded onto the cartridges. The extracts were collected and analyzed by LC-MS/MS. The effluent from the first loading of the cartridge, hereafter referred to as flow-through was collected, and passed through another C18 cartridge to assess breakthrough. Comparisons between the extract concentration and flow-through concentrations allowed for an estimate of the percent of analyte which passed through the cartridge. Results from these experiments are shown in Table 3.8. For concentrations of 1, 5, and 10 μg L^{-1} loaded onto the C18

Table 3.8: Initial test of the C18 SPE method demonstrating its capabilities in analyte retention, % recovery, pre-concentration capability, and reprodicability.

Neonicotinoid	Pre-concentration Factor	% Recovery	% RSD
Nitenpyram	17.69	99.1	19.9
Thiamethoxam	14.16	99.6	74.2
Clothianidin	16.29	99.6	43.4
Imidacloprid	14.36	99.7	58.2
Acetamiprid	19.65	99.8	18.9
Thiacloprid	16.92	99.8	45.5

SPE cartridges, greater than 99.6% of analytes were retained for all neonicotinoids.

The pre-concentration factor (PCF) for the cartridge was determined by comparing the concentration of extract calculated using a standard calibration curve linear regression with the concentration loaded onto the cartridge. An average PCF was taken from the pre-concentration factor determined for each concentration level. The PCF for the C18 cartridges was determined to range from 14.2 to 19.7 for the neonicotinoids, as shown in Table 3.8. As 100 mL of sample was loaded onto the cartridge and eluted in 5 mLs, the expected PCF is 20. When the results from PCF are compared against % recovery values it becomes clear that the C18-SPE cartridges are not ideal for neonicotinoid analysis. Due to the limited sensitivity and selectivity of the cartridge, it is possible that some of the binding sites are occupied by other analytes present in the matrix, or that not all neonicotinoids are being efficiently extracted by the cartridge. It is hypothesized that a more selective SPE phase could improve efficiency of the extraction, as well as increase the pre-concentration factor.

3.6.2 C18-SPE Validation

The C18-SPE method was assessed for the typical method-validation parameters of linearity, range, reproducibility, and limits of detection. An 8-point calibration curve was constructed at levels 0, 1, 5, 10, 100, 500, 1000, and 2000 ng L^{-1}. Using SPE

Table 3.9: Regression parameters and method detection limits for the C18 SPE method

Neonicotinoid	Regression Equation	R^2	LOD (ng L^{-1})
Nitenpyram	y = 2.106E-06x + 8.1E-05	0.99676	1.229
Thiamethoxam	y = 4.498E-06x + 8.7E-06	0.99997	2.591
Clothianidin	y = 9.257E-06x + 7.4E-06	0.99997	3.802
Imidacloprid	y = 2.359E-05x - 7.6E-05	0.99975	2.776
Acetamiprid	y = 3.637E-05x + 5.5E-05	0.99997	0.998
Thiacloprid	y = 4.768E-05x + 2.2E-04	0.99997	5.788

and its pre-concentration capabilities necessitates an order of magnitude reduction in the concentration range, relative to the instrumental method, where the concentration of standards is in the μg L^{-1} range. This range of concentrations represents an environmentally applicable range for the analysis of river waters, and agricultural run-off. For all analysis, an internal standard of nicotine at 500 μg L^{-1} is added prior to sample injection. As shown in Table 3.9, the regression coefficients for all neonicotinoid curves are very good. The nitenpyram curve has a regression coefficient of 0.996, while the other 5 neonicotinoid curves have regression coefficient values of 0.999 or better. Method detection limits for the C18-SPE method ranged between 1.0 and 5.8 ng L^{-1}, which demonstrates excellent applicability to environmental analysis of dilute river samples. However, it is possible that these results could be further improved with a more selective phase for detection of neonicotinoids specifically, as the universal nature of a C18-SPE cartridge allows for many matrix components to be pre-concentrated from the samples, which could potentially cause ion suppression of the neonicotinoids signal.

3.7 Molecularly Imprinted Polymers

In an effort to improve selectivity, extractive capabilities, and pre-concentration of analytes from environmental matrices, MIPs were fabricated with affinity for neoni-

cotinoid insecticides. There are two mechanisms of fabrication, bulk fabrication for use as an SPE sorbent, and thin-film fabrication for direct sampling to analysis workflow. While some work has been completed on thin-film fabrication for use with DART ambient ionization, the focus of this research is on MIPs for use as an SPE sorbent.

3.7.1 Bulk MIP Fabrication

By using a pseudo-template similar in structure and functionality to neonicotinoids during the development of the cross-linked polymer network, it is theorized that pore structures form with suitable structure, size, and functionality for binding neonicotinoids. Bulk molecularly imprinted polymers are fabricated using a thermal free-radical initiator. The first step in their fabrication is selecting a monomer which interacts strongly enough with the template, along with a solvent system that will not disrupt its complexation. The monomer, acrylamide, was selected as it is expected to provide relatively strong interactions with the pharmacophore functionality of the neonicotinoids through hydrogen-bonding. Acryalmide can also interact with the chloro-substituted aromatic ring present on all neonicotinoids. The combination of the template and monomer in an aprotic solvent creates favourable conditions for these hydrogen-bonding interactions to occur before the addition of other pre-polymerization components. Once the mixture has been sufficiently stirred, cross-linker EGDMA is added. Finally, the initiator is added directly before placing the sealed pre-polymerization complex in an oil bath to thermally activate the AIBN and induce polymerization.

Polymerization occurs by vinyl-terminated carbon-carbon bond formation between the functional monomer, acrylamide, and cross-linker. The presence of the carbonyl groups on the acrylamide allow for free-radical initiation, and free-radical chain polymerization provides propagation. The cross-linker interacts with the monomer allow-

ing for pores to be developed with sufficient rigidity, maintaining structure when the template is removed. Figure 3.11 depicts a proposed structure for the formation of polymer surrounding a template molecule, imidacloprid. In this figure, multiple free-radical pathways from differently initiated molecules combining to form a complete bulk polymer are illustrated. Each potential radical pathway is depicted using unique colours, joined by carbon-carbon bonds (orange in colour). This figure presents a theorized formation of a pore with an MIP surrounding a template molecule. The template molecule can then be removed from the polymer with a solvent system that can interrupt the monomer-template interactions. In this case, acetonitrile with 0.5 % acetic acid disrupts the hydrogen bonding interactions to remove the template.

The initial attempts to fabricate the bulk-phase MIP, presented an issue regarding the structural rigidity of the polymer. It was determined that under the vacuum of the SPE manifold, the polymer, with delicate pore structure, collapsed, which blocked solvent flow through the MIP. The collapse of the pore structure resulted in non-ideal analyte retention and low solvent flow rate. These challenges were overcome by grafting the MIP onto a solid support structure.

3.7.2 Grafted MIPs

To overcome the challenges of the soft polymer matrix, MIPs were fabricated on a silica sub-structure using a modified sol-gel procedure, outlined in Figure 3.12. This procedure is similar to, but modified from that by Kia et al.[79] In this procedure, silica gel was synthesized by acid hydrolyzed polymerization of tetraethylorthosilicate, which resulted in a white gelatinous silica network. Typically, sol-gel methods dry this gel before use, or aerogel can be created by super-critically drying the silica gel. Instead, this method functionalizes the silica gel by attaching vinyl substituents, used for grafting the polymer to the silica substrate.

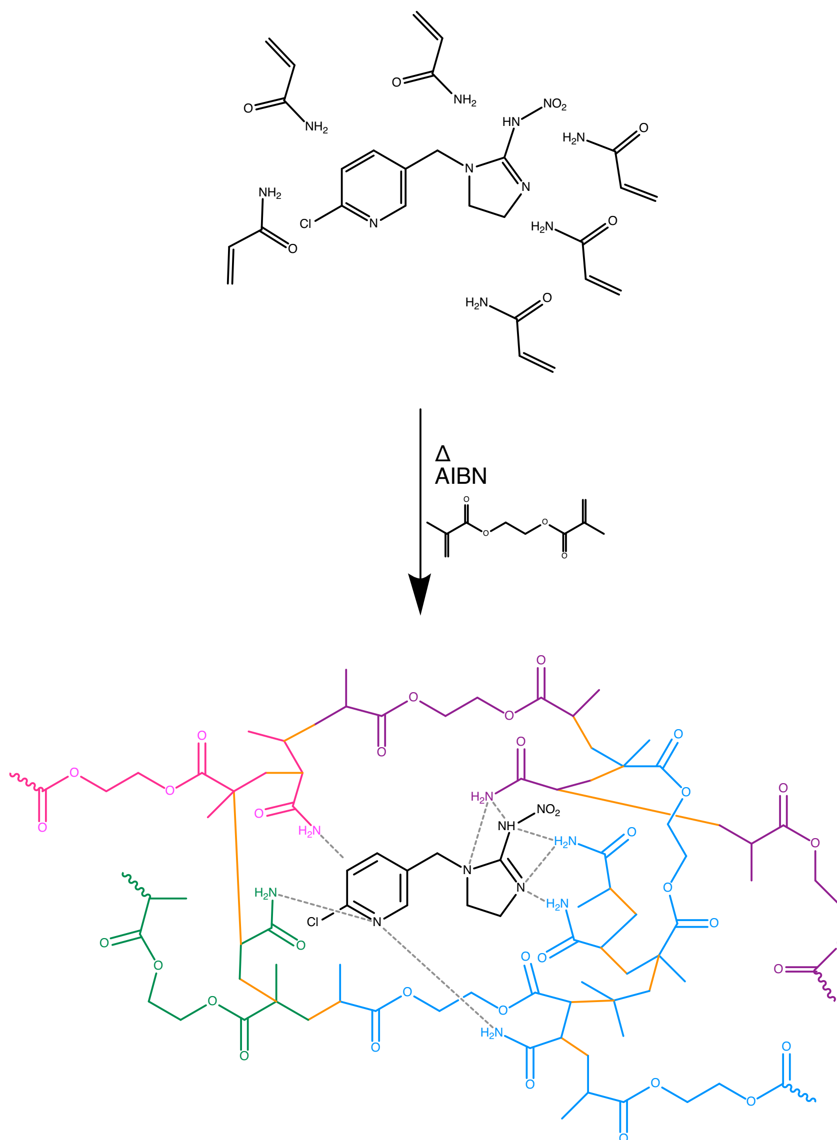


Figure 3.11: Proposed self-assembly of a structure for the formation of a molecularly imprinted polymer, highlighting potential hydrogen-bonding sites between the template (imidacloprid) and monomer/cross-linker co-polymer.

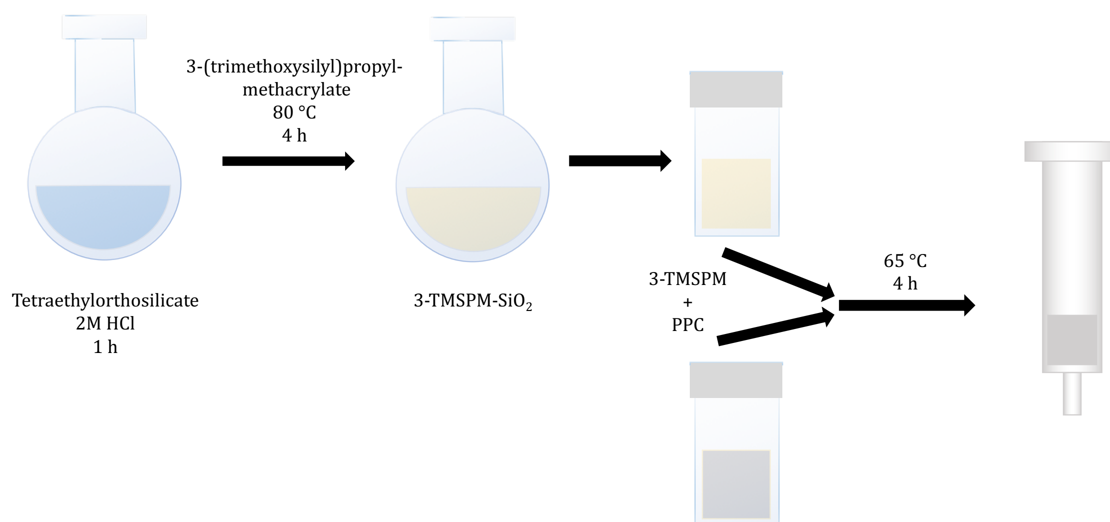


Figure 3.12: Illustrated simplified MIP grafting procedure to incorporate the modified silica and pre-polymerization complex (PPC).

The reaction to produce the vinyl-modified silica network has a measured 96% yield with minimal unreacted starting material. The yield was determined using mass of resultant gel compared with total mass of starting materials. The modified silica, hereafter referred to as 3-TMSPM-SiO_2 indicitative of the functionalization of the SiO_2 network with 3-(trimethoxysilyl)propyl methacrylate, was then dried and ground to appropriate particle size before use as a solid support structure for the MIP. The pre-polymerization complex is created identically to the fabrication of bulk MIPs. This allows for most bulk MIP polymerization methods to be adopted to this modified sol-gel procedure, which is potentially useful for improving performance of many other MIP formulations. The primary difference between the bulk polymerization method and grafting method is the inclusion of the particulate solid support 3-TMSPM-SiO_2 in the pre-polymerization complex. Due to the free-radical polymer formation mechanism of vinyl-terminated chain-growth polymerization as illustrated

previously in Figure 3.13, the mechanism readily incorporates the vinyl-modified silica network as part of the polymer.

Through experimentation, the incorporation of the polymer synthesis with the silica gel substrate lead to far superior MIPs in terms of performance for pressurized flow through column applications. The flow rate when the grafted MIP was used as packing for SPE was improved compared with the MIP only bulk polymer, and was self-regulating respect to flow, achieving an appropriate flow rate for SPE at 0.6 atm of pressure without manipulation of the manifold valves. This bounded flow rate (for low pressure systems like SPE) is likely due to the small and reproducible particle size and pore structure. This alone greatly simplifies the SPE process by making it easier to establish an appropriate flow rate for sample loading and analyte elution. The silica-grafted MIP, referred to as 3-TMSPM-SiO_2-MIP, was packed into 6 mL SPE cartridges between 2 glass frits. As the MIP is fabricated as a gel and contains residual porogen from the radical synthesis, the polymers were weighed after drying under vacuum following removal and elution of the analytes to determine mass loading. While this residual porogen does not impact analysis, and is eluted during cartridge conditioning, it is simpler to pack the MIP as a gel rather than drying the phase before use.

3.7.3 3-TMSPM-SiO_2-MIP Validation

The silica-grafted MIPs were validated against a commercial C18 SPE cartridge for their performance, examining pre-concentration factor, selectivity, analyte retention, percent recovery, range, linearity, and limits of detection. As the selectivity, and therefore the MIP performance is dependant largely on the template molecule, two templates were used: 2-chloropyridine-4-acetic acid (2CP4A), and nitenpyram. 2CP4A was selected as it is a potential useful pseudo-template for all neonicotinoids. It has

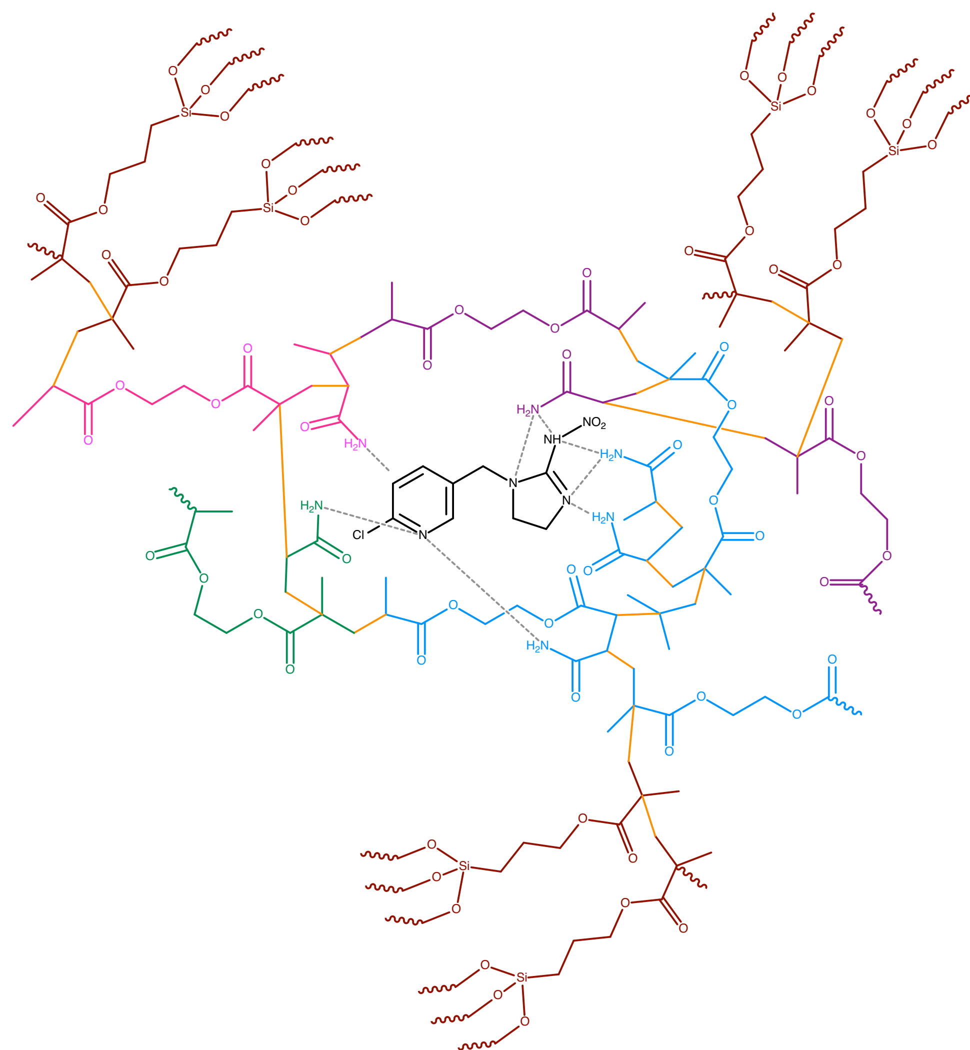


Figure 3.13: Proposed structure of an imidacloprid-containing MIP pore, indicating how the vinyl-modified silica (3-TMSPM-SiO_2) can incorporate into the structure of the MIP. Different colours indicate differently initiated free-radical polymerizations.

the bulky 2-chloropyridine structure, as well as a hydrogen-bond donor/acceptor in a para position to the chlorine, similar to the pharmacophore of all neonicotinoids. Nitenpyram was chosen to examine the potential highly selective capability of the MIP to selectively uptake individual neonicotinoids.

Pseudo-templates are preferred in MIP fabrication due to the effects of template bleeding. It is unlikely that all template will be removed during the acid washing step; this residual template could be eluted with the sample. If the template is a neonicotinoid or other environmental contaminant, it could appear at elevated concentrations, which would invalidate the results for that particular analyte. While templates that match the analyte should provide better results and higher selectivity, they have some significant disadvantages. All method validation studies were performed using 2CP4A as the pseudo-template, and nitenpyram was used selectively to demonstrate the potential selective power of the MIP-SPE method.

To determine the retentive capabilities of the MIP phase, 100 mL of a multistandard solution at 10 μg L^{-1} were loaded onto the MIP-SPE cartridge. The eluate was collected and passed through a second MIP-SPE cartridge, similar to the experiment performed for the C18 cartridge. Like the C18 cartridge, the retention was excellent, with greater than 99.2% retention up to 100 μg L^{-1}. There was an issue with nitenpyram however, where only 81.5% of the analyte was retained. It is possible that the significantly different structure and increased water solubility of nitenpyram from the other 5 neonicotinoids causes reduced extraction performance of the MIP when 2CP4A is used as a pseudo-template. When nitenpyram is used as a template molecule, the retention of nitenpyram during a routine extraction of sample increases to 99.6%, however it is also possible that this increased result is due to template bleeding. It should be noted that 2CP4A is present in all sample elutions from the 2CP4A-MIP-SPE cartridge, evidence of the template not being fully removed from

Table 3.10: Results from the silica grafted 2CP4A-MIP-SPE cartridges showing the performance capabilities in terms of analyte retention, reproducability, and pre-concentration factor

Neonicotinoid	% Analyte Retention	Pre-concentration Factor	%RSD (n=9)
Nitenpyram	81.5%	6.56	7.34%
Thiamethoxam	99.2%	15.2	7.86%
Clothianidin	100.0%	15.9	5.03%
Imidacloprid	100.0%	13.9	6.09%
Acetamiprid	100.0%	18.5	2.42%
Thiacloprid	100.0%	15.8	5.70%

the cartridge during pre-conditioning steps. Results from the initial tests are shown in Table 3.10.

A test of the MIP-SPEs for pre-concentration capability places them nearly on-par with the commercial C18 variant, with PCFs ranging from 13.8 to 18.5, with approximately 5% variation relative to the C18 cartridges. PCFs were again calculated by comparison of eluted analyte to a standard calibration curve to determine the concentration of analyte eluted. The exception again is nitenpyram, with a PCF of only 6.6, indicating lower overall performance for this analyte, while the other analytes are performing as expected with an expected PCF of 20. Changing the template from 2CP4A to nitenpyram dramatically improved the performance of the MIP-SPEs, outperforming the C18 cartridges in terms of pre-concentration factor for all 6 neonicotinoids. PCFs ranged between 39.3 and 89.9 for the six neonicotinoids using nitenpyram as a template molecule (Table 3.11). It is possible that due to increased mass of polymer in the cartridge or the complex pore structure which allows for more neonicotinoids to be adsorbed by the SPE phase relative to the C18, as well as the increased sensitivity from the nitenpyram template leads to an increase of the PCF above the expected 20. This suggests that not all analyte is retained on the C18 and 2CP4A-MIP SPE as previously theorized. This also further highlights the importance of an appropriate template. The disadvantage here however, is the concentration of

Table 3.11: Examination of three templates to improve the pre-concentration factor and analyte retention, and by extension, performance of the SPE-MIPs

Template	2CP4A		Nitenpyram		Thiamethoxam	
	PCF	%Ret.	PCF	%Ret.	PCF	%Ret
Nitenpyram	6.56	26.8%	89.9	24.3%	2.78	61.4%
Thiamethoxam	15.2	92.3%	51.8	95.6%	820.5	53.0%
Clothianidin	15.9	100%	41.7	100%	22.6	100%
Imidacloprid	13.9	100%	37.6	100%	23.7	100 %
Acetamiprid	18.5	100%	39.3	100%	28.9	100%
Thiacloprid	15.8	100%	41.6	100%	20/4	100%

Table 3.12: Method validation study for the SPE MIP procedure, including method detection limits and regression parameters

Neonicotinoid	Regression Equation	R^2	%RSD (n=3)	LOD (ng L^{-1})	LOQ (ng L^{-1})
Nitenpyram	y = 3.770E-07x + 7.7E-05	0.9623	25.6%	1.961	6.535
Thiamethoxam	y = 2.862E-06x + 1.8E-04	0.9636	4.24%	0.987	3.261
Clothianidin	y = 1.047E-05x - 1.4E-04	0.9954	2.22%	0.081	0.271
Imidacloprid	y = 2.626E-05x - 5.0E-04	0.9882	2.21%	0.102	0.339
Acetamiprid	y = 4.048E-05x - 4.1E-04	0.9962	3.49%	0.276	0.919
Thiacloprid	y = 4.974E-05x - 1.3E-03	0.9934	1.51%	0.170	0.568

nitenpyram in the flow-through and extract was significantly higher than the other 5 neonicotinoids. With the flow-through concentration being 600× that of the other neonicotinoids. While performance is improved with an analyte matched template, it is necessary to use a template that does not have an environmental presence to avoid false positive results.

A calibration curve was constructed for the 2CP4A MIP-SPE cartridges, matching the 8 levels that were used for the C18 validation study. Concentrations of 0, 1, 5, 10, 100, 500, 1000, and 2000 ng L^{-1} were loaded onto the MIP-SPE cartridges at a volume of 100 mL. The extracts were analyzed using the previously developed LC-MS/MS method for the presence of the six neonicotinoids of interest. Results from this calibration curve are shown in Table 3.12.

The 2CP4A-SPE-MIPs demonstrate similar results to the commercial C18-SPE cartridges. The method detection limits range between 0.08 and 1.96 ng L^{-1}, which is

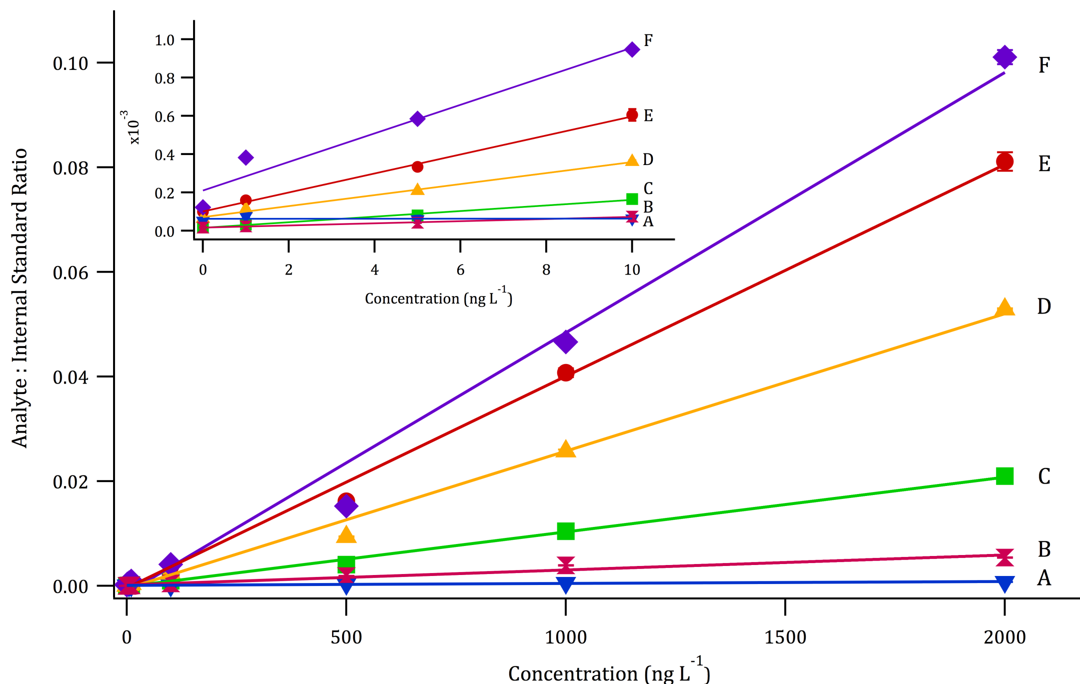


Figure 3.14: Calibration curve for MIPs used as solid phase extraction sorbents for the selective detection of neonicotinoids. A: nitenpyram, B: thiamethoxam, C: clothianidin, D: imidacloprid, E: acetamiprid, F: thiacloprid

well below the detection limits required for regulatory environmental analysis, and significantly lower than those achieved using the C18-SPE method. The lower method detection limits are likely a result of increased selectivity for analytes of interest, excluding more matrix components, which can improve signal-to-noise thereby enhancing instrument response. While the 2CP4A-MIP-SPE results do not fit a linear regression as well as the commercial C18 cartridges, the results are still very good, with regression coefficients greater than 0.96 for all analytes. The calibration curves for the 2CP4A-SPE-MIPs are shown in Figure 3.14. As with the pre-concentration factor and analyte retention studies, the method is underperforming for nitenpyram, having the lowest slope and response of the neonicotinoids.

Comparing the results of the 2CP4A templated SPE-MIPs to those templated using nitenpyram, there is clearly a need to find a better template molecule, which

will provide the high selectivity for neonicotinoids as demonstrated by the nitenpyram MIP-SPEs, but is not a concern for environmental analysis.

3.8 Thin-Film MIPs with DART

The other fabrication method for molecularly imprinted polymers of interest in the work presented here is the thin-film format. The advantage of fabricating MIP films on a solid substrate such as a glass slide, is the ability to take the polymer directly to the sampling site and extract the target analytes directly from water without need for additional equipment such as vacuum manifolds and pumps. This eliminates the need for transporting the sample to a lab for analysis. Another important feature is the ability to directly sample from the thin-film polymer using ambient ionization techniques such as DESI or DART.

Previous work on thin-film MIPs for neonicotinoids indicated a need for improvement of their performance, as they were only able to uptake thiacloprid, while other neonicotinoids demonstrated no significant mass loading on the polymer. For this reason, the project initially focused on MIP-SPEs, which may then be transferred to a thin-film format. Nevertheless, it is important to mention thin-film MIPs, as they were used to demonstrate the applicability of the DART ambient ionization method for neonicotinoids.

As mentioned in the introduction, DART can be used for sampling in three configurations: 180° to the mass spectrometer inlet, 135° to the mass spectrometer inlet, or using the thermal desorption unit, previously described. Although the thin-film MIPs on microscope slides were too large to fit in the TDU, they were sampled using the 135° configuration. By placing the MIPs on a linear rail, and passing them in front of the metastable gas source, mass spectra could be collected. The mass spec-

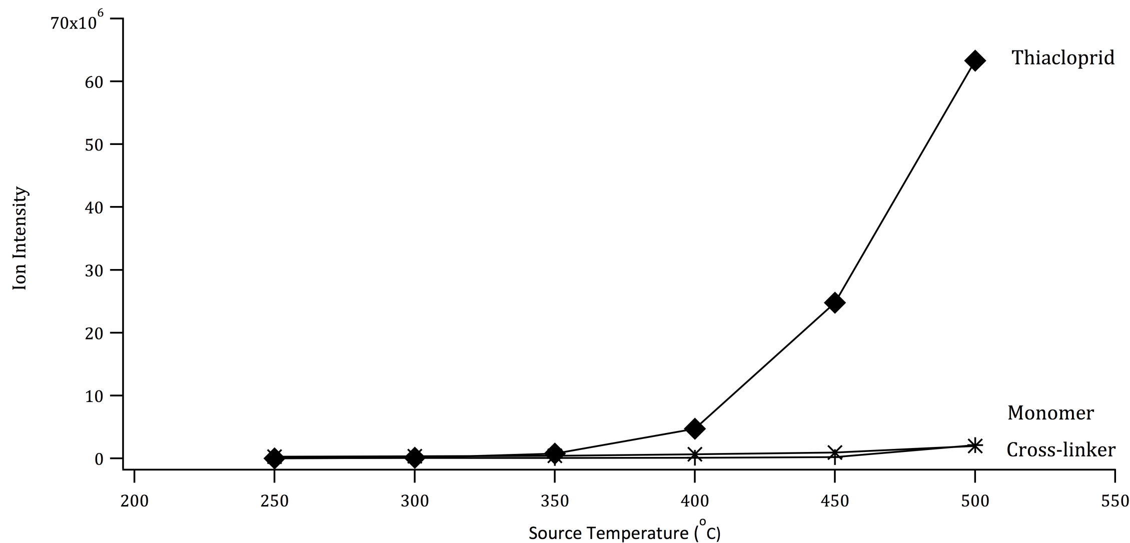


Figure 3.15: Thermal degradation of MIP components as the temperature of the metastable source increases.

tra collected using the Q-TOF MS indicated only the presence of thiacloprid from thin-film MIPs that were exposed to 1 mg L^{-1} of multistandard neonicotinoid solution for 2 hours. This suggests only thiacloprid is binding to the polymer, which is consistent with previous lab studies. This does effectively demonstrate the capability of the DART method to thermally desorb and ionize analytes from the polymer, and also provided a unique opportunity to study the potential thermal degradation of the polymer. As shown in Figure 3.15, as the temperature of the metastable species was increased, the ion count of individual polymer components: monomer methacrylic acid, and cross-linker ethylene glycol dimethacrylate; remained consistent, suggesting no thermal degradation of the polymer under temperatures of up to 550 °C. This is a promising finding for future studies, since the stability of the polymer at high temperatures is confirmed, suggesting the polymer matrix will not lead to interferences upon direct interrogation with the DART source

3.9 Environmental Analysis using SPE-MIPs, C18 SPE, QuEChERs, and Direct Injection

To demonstrate the applicability of the MIPs as well as compare their performance to existing analytical methods, river samples were analyzed using each of the methods validated in this work thus far. 2CP4A-MIP-SPEs, C18-SPE cartridges, the QuEChERs procedure for pesticide extraction, and direct injection of filtered river sample into the LC-MS/MS instrument. The percent recovery of a spiked sample, and the pre-concentration factors for each of the methods were examined.

3.9.1 Direct Injection

The primary regulatory method of analysis for neonicotinoids used by the Ontario MOECC is direct injection of filtered environmental samples. This is widely viewed as the fastest method for regulatory analysis of neonicotinoids, due to the absence of sample preparation, extraction, or pre-concentration steps. However, there are many perceived disadvantages of this method. By neglecting to perform extraction steps, the matrix interferences are plentiful, and could potentially interfere with the complete ionization of the analytes of interest. Additionally, this method requires the use of a highly sensitive triple quadrupole instrument to achieve the necessary limits of detection, which is an instrument not readily available in many laboratories.

For the direct injection UHPLC-MS/MS analysis 3 mL of river water spiked at 10 μg L^{-1} was filtered, spiked with internal standard nicotine, and diluted to 5 mL total volume. 2 μL of this was injected into the LC-MS/MS system, and analyzed using the previously developed method. While all neonicotinoids were detected, the dilution which occurs during the addition of internal standard makes this method less than ideal, with limits of detection matching the instrument limits of detection

Table 3.13: Results of direct aqueous injection of filtered and spiked river water samples into the LC-MS/MS system

Neonicotinoid	Calibration Range	Spiked Concentration (μg L^{-1})	%Recovery	%RSD (n=3)
Nitenpyram	0 - 40 μg L^{-1}	10 μg L^{-1}	83.3%	6.29%
Thiamethoxam	0 - 40 μg L^{-1}	10 μg L^{-1}	24.8%	7.15%
Clothianidin	0 - 40 μg L^{-1}	10 μg L^{-1}	123.6%	8.44%
Imidacloprid	0 - 40 μg L^{-1}	10 μg L^{-1}	97.3%	6.28%
Acetamiprid	0 - 40 μg L^{-1}	10 μg L^{-1}	57.1%	6.85%
Thiacloprid	0 - 40 μg L^{-1}	10 μg L^{-1}	83.3%	11.4%

which range between 0.21 and 0.37 μg L^{-1}, previously determined. Due to the lack of analyte preconcentration, slight dilution, and potential for high matrix effects, the MDLs for direct injection are expected to be higher than instrumental LODs, but were not calculated as direct injection is not the focus of this research. Results for concentrations determined using the direct injection method are given in Table 3.13.

Concentrations determined by direct aqueous injection ranged from 2.4 to 12.4 μg L^{-1}, giving percent recoveries between 24 and 123%. While this range is large, it does demonstrate variation among analytes, and given the large error associated with direct injection, is as expected. This does highlight a need for a more selective and less error prone method of analysis. This method, while easy, is limited by the instrument limits of detection, and can therefore only be used for environmental analysis where the concentration is greater than 1.5 μg L^{-1}, or by a standard addition method, which creates additional sample preparation.

3.9.2 QuEChERs of River Water

The QuEChERs method outlined previously for honey analysis was adapted for the analysis of river water. Spiked river water was extracted using the modified procedure, and results analyzed using the previously developed LC-MS/MS method. The method was largely ineffective at extracting neonicotinoids from river water, possibly due to the higher than normal aqueous content of the sample. As QuEChERs is typically

Table 3.14: Results from QuEChERs-extracted spiked river water samples

Neonicotinoid	Calibration Range	Spiked Concentration (μg L^{-1})	%Recovery	%RSD (n=3)
Nitenpyram	0 - 1000 μg L^{-1}	10 μg L^{-1}	54.7%	2.55%
Thiamethoxam	0 - 1000 μg L^{-1}	10 μg L^{-1}	188.4%	21.7%
Clothianidin	0 - 1000 μg L^{-1}	10 μg L^{-1}	87.6%	3.14%
Imidacloprid	0 - 1000 μg L^{-1}	10 μg L^{-1}	122.0%	1.41%
Acetamiprid	0 - 1000 μg L^{-1}	10 μg L^{-1}	72.8%	0.90%
Thiacloprid	0 - 1000 μg L^{-1}	10 μg L^{-1}	51.7%	3.22%

used for homogenized solid samples, pollen, or honey analysis, the introduction of 6 mL of water potentially overpowered the effects of drying agent $MgSO_4$, resulting in inefficient extraction of neonicotinoids from the sample. However, thiamethoxam did show 90% recovery, which was good compared to other analytes, which showed less than 30% recovery. Where the recoveries are greater than 100% it is possible that neonicotinoids are being detected in the samples of river water. This is the case for imidacloprid and thiamethoxam, which are often used together for crop treatments. Full results from the QuEChERs method for river water samples are shown in Table 3.14.

It is possible that due to the applicability of the QuEChERs method to a number of samples and analytes, that there is competition for binding sites on the extractive phase between neonicotinoids and the vast array of other matrix compounds. It is also possible that since neonicotinoids were developed after the introduction of the QuEChERs method, the method is not well suited towards their analysis. Perhaps more modifications of the method are needed to improve selectivity and efficiency for neonicotinoids.

3.9.3 C18 River Analysis

Using the commercial C18-SPE cartridges, spiked river water samples were tested for percent recovery, selectivity, and pre-concentration factor. River samples spiked with

Table 3.15: Results from spiked river water samples extracted by the C18 SPE method, previously developed and validated

Neonicotinoid	Calibration Range	Spiked Concentration (μg L^{-1})	%Recovery	%RSD (n=3)	PCF
Nitenpyram	0.1 - 10 μg L^{-1}	10 μg L^{-1}	< MDL	0.19%	-
Thiamethoxam	0.1 - 10 μg L^{-1}	10 μg L^{-1}	13.0%	3.13%	7.23
Clothianidin	0.1 - 10 μg L^{-1}	10 μg L^{-1}	31.1%	3.88%	6.64
Imidacloprid	0.1 - 10 μg L^{-1}	10 μg L^{-1}	21.3%	4.21%	6.36
Acetamiprid	0.1 - 10 μg L^{-1}	10 μg L^{-1}	31.7%	0.93%	6.67
Thiacloprid	0.1 - 10 μg L^{-1}	10 μg L^{-1}	42.8%	4.15%	3.87

10 μg L^{-1} of neonicotinoid multistandard were loaded onto the C18-SPE cartridges at a volume of 100 mL. The concentration determined from the extracts was calculated using the previously developed C18 calibration curves, as well as the calibration curves of standards injected directly to determine a percent recovery of the analytes and pre-concentration factors. Results demonstrated the C18-SPE method to be superior to the QuEChERs method, which is to some extent unexpected, as QuEChERs is developed exclusively for the analysis of pesticide residues, however SPE is typically intended to be used for preconcentration from water. The C18-SPE method was able to pre-concentrate the spiked river samples by 4 to 7 times, giving concentrations between 38 and 72 μg L^{-1}, when results were extrapolated from a standard-based calibration. These PCFs are superior to the QuEChERs method for both river and honey analysis, which did not demonstrate any significant pre-concentration ability, however the PCFs for the SPE method are lower than expected (20), suggesting less than 100 % extraction efficiency. Full results are given in Table 3.15.

Percent recoveries were generally lower than those determined using the direct injection method. However, there was significantly less variation amongst the percent recoveries of the six neonicotinoids, indicating an improvement in selectivity for the neonicotinoids, or a reduction of matrix interferences by using a sample cleanup step. Recoveries ranged from 0 to 43%. The only analyte to have poor recovery was nitenpyram, which had concentrations below the MDL. This is interesting, as this

neonicotinoid also demonstrated problems with binding to the cartridge when using the MIP-SPE method. This low performance is again potentially due to its higher water solubility relative to the other neonicotinoids. The significant advantage of using this method, or the SPE-MIP method over direct aqueous injection, is the ability to clean up and preconcentrate the analyte from the complex river matrix. This lowers method detection limits to the ng L^{-1} range, which is far more applicable for environmental analysis.

3.9.4 MIP River Analysis

The goal of the MIP-SPE phase is to extract analytes from spiked river water with better percent recovery, selectivity, and efficiency than the commercial C18 cartridge. To test this, the same river spiked with 10 μg L^{-1} neonicotinoid multistandard was loaded onto the 2CP4A-MIP-SPE cartridges at a volume of 100 mL. Concentrations obtained from the extract were calculated using an external standard-based calibration curve, and a calibration curve constructed using the 2CP4A-MIP-SPEs, to provide pre-concentration factors and mass loading, as well as percent recovery, respectively.

The 2CP4A-MIP-SPEs demonstrated increased selectivity for neonicotinoids compared with the C18 cartridge. When concentration of neonicotinoids was calculated using the standard-based calibration curve, the concentration of neonicotinoids detected ranged from 75 to 87 μg L^{-1}, indicating a pre-concentration of 7 to 9 times, greater than the 4 to 7 times pre-concentration obtained while using the C18-SPE cartridges. However, in following the trend of the earlier results for the MIP-SPEs, nitenpyram is underperforming, with a concentration of 35 μg L^{-1}, indicating a pre-concentration factor of only 3.5 times. Results for the river water analysis by MIP-SPEs are shown in Table 3.16.

Percent recoveries for the 2CP4A-MIP-SPE extracted river water samples were

Table 3.16: Results from spiked river water samples extracted by the 2CP4A-MIP-SPE method, previously developed and validated

Neonicotinoid	Calibration Range	Spiked Concentration (μg L^{-1})	%Recovery	%RSD (n=3)	PCF
Nitenpyram	0.01 - 100 μg L^{-1}	10 μg L^{-1}	41.4%	9.62%	1.77
Thiamethoxam	0.01 - 100 μg L^{-1}	10 μg L^{-1}	32.5%	4.20%	8.67
Clothianidin	0.01 - 100 μg L^{-1}	10 μg L^{-1}	33.6%	7.21%	8.10
Imidacloprid	0.01 - 100 μg L^{-1}	10 μg L^{-1}	19.2%	14.1%	8.07
Acetamiprid	0.01 - 100 μg L^{-1}	10 μg L^{-1}	44.3%	6.03%	8.11
Thiacloprid	0.01 - 100 μg L^{-1}	10 μg L^{-1}	30.2%	9.74%	7.51

slightly higher than those extracted using the C18 SPE method. For both methods, a method blank was subtracted prior to calculating % recovery. Concentrations determined from an external calibration of MIP-SPE extracted neonicotinoid standards in water ranged from 1.9 to 4.4 μg L^{-1}. Giving 19 to 44% recoveries of neonicotinoids. While not as high as expected for a selective SPE phase, the recoveries were generally on par, or higher than those of the C18-SPE cartridges, indicating improved selectivity for neonicotinoids. It is hypothesized that by using a more appropriate template molecule in the MIP, these percent recoveries could be significantly improved. Interestingly, the nitenpyram recovery at 19%, while lower than all other analytes, is performing better than its C18-SPE counterpart. Pre-concentration factors were increased for all analytes excepting nitenpyram when compared to the C18-SPE cartridges.

3.10 Environmental Sampling

To demonstrate the performance of the MIP-SPEs, an environmental monitoring campaign was undertaken along local waterways. The goal of this study was to determine if there was any correlation between agricultural activities, and presence of neonicotinoids in the surrounding rivers. As expected, the concentrations of neonicotinoids in river water were in the low ng L^{-1} range, direct aqueous injection did not allow

for detection of any neonicotinoids, however, the MIP-SPE phase, with its method detection limits ranging from 0.08 to 1.96 ng L^{-1}, could potentially be highly useful in examining low-level concentrations.

Eleven sampling sites were chosen along a local river basin for this experiment, shown in Figure 3.16. Control sites 07, 09, and 11 were located upstream of all agricultural activity, and were not expected to indicate the presence of any neonicotinoids. Site 08 was taken from a waterway which is sourced directly from agricultural run-off, and connects downstream of sites 07 and 09. Site 08 was expected to have elevated concentrations of neonicotinoids. Sites 10 and 06 were taken downstream of site 11, after the river passes through a heavy agricultural area, and also were expected to have elevated concentrations of neonicotinoids. Site 06 is downstream of site 10, and is fed by many isolated fresh water sources, and was expected to have reduced concentrations of neonicotinoids compared to site 10 due to dilution. Site 05 was taken downstream of 07, 08, and 09. It was expected to have a reduced concentration of neonicotinoids as compared to site 08, due to dilution of the river from isolated fresh water sources, and expected to be free of neonicotinoids. Finally, sites 04, 03, 02, and 01 are taken at different locations along the same river, which is fed from all previous sampling locations. Sampling sites 04 and 03 are at the top of the river, and sample 02 and 01 are downstream. 04 and 03 are taken from the same location, to examine potential errors in the method.

Results shown in Table 3.17 are largely as expected. Nitenpyram presented with elevated concentrations in all sampling sites, with concentrations calculated between 317 and 1473 ng L^{-1}. As the river samples were analyzed using the nitenpyram-MIP-SPE, due to its enhanced sensitivity and selectivity for neonicotinoids relative to the 2CP4A-MIP-SPE, it is expected that template bleeding may occur causing false positive results. It was decided that nitenpyram would be discounted from the results.

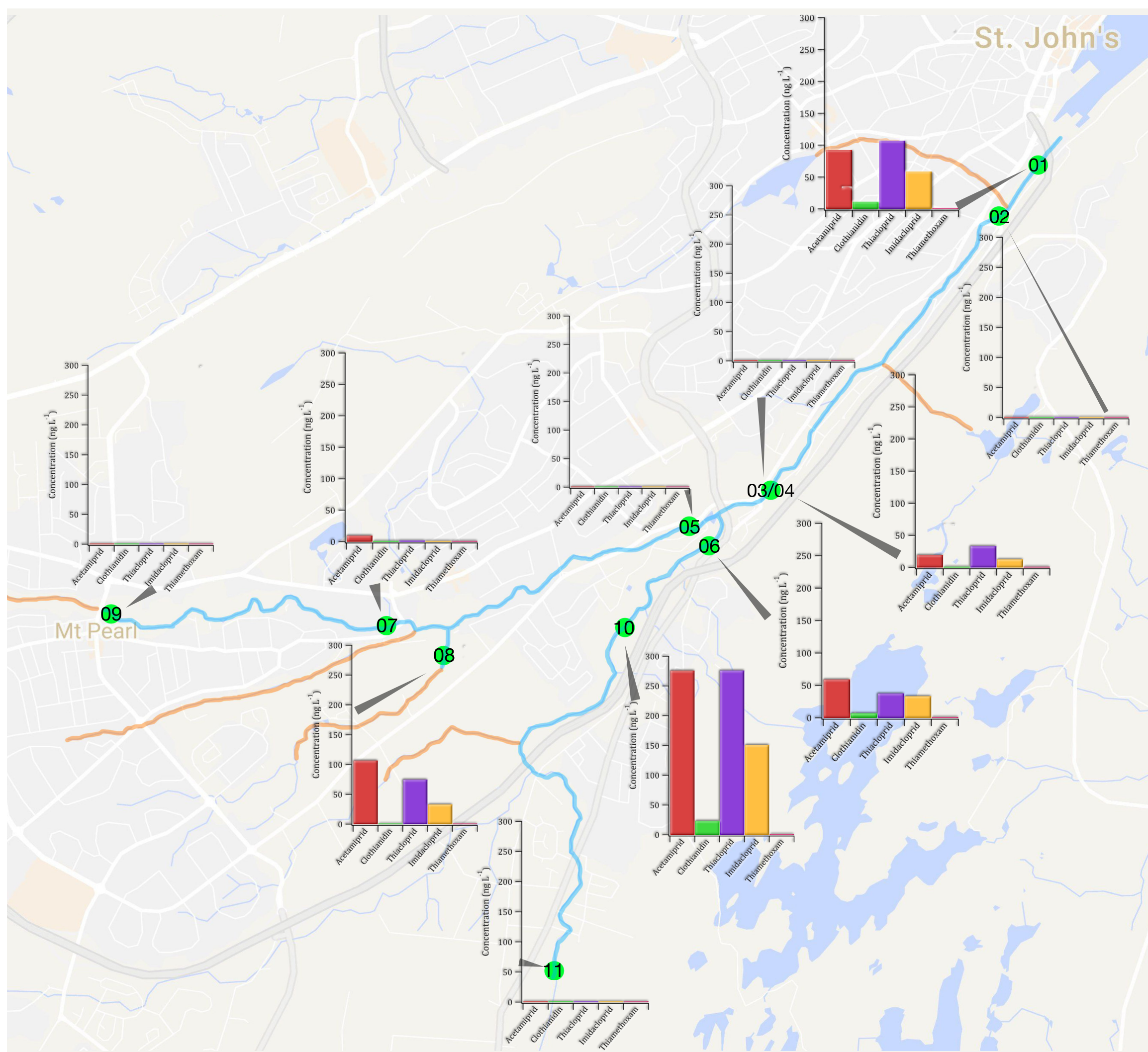


Figure 3.16: Illustrated diagram of sampling locations along a local waterway with concentrations of neonicotinoids as determined by applying unspiked river samples to the SPE-MIP method.

Table 3.17: Concentrations of neonicotinoids determined along a local waterway using the validated SPE-MIP method. All concentrations in ng L^{-1}.

Site	Acetamiprid	Clothianadin	Thiacloprid	Imidacloprid	Nitenpyram	Thiamethoxam
River 01	92.6	11.4	107.9	58.6	465.2	ND
River 02	$<$ LOD	$<$ LOD	$<$ LOD	$<$ LOD	1473	ND
River 03	$<$ LOD	$<$ LOD	$<$ LOD	$<$ LOD	687.1	ND
River 04	19.6	$<$ LOD	33.8	12.8	606.1	ND
River 05	$<$ LOD	$<$ LOD	$<$ LOD	$<$ LOD	415.9	ND
River 06	59.4	7.31	37.8	33.6	498.9	ND
River 07	9.66	$<$ LOD	1.97	$<$ LOD	316.9	ND
River 08	106.9	$<$ LOD	74.9	33.3	958.2	ND
River 09	$<$ LOD	$<$ LOD	$<$ LOD	$<$ LOD	1280	ND
River 10	276.1	23.5	276.2	151.2	351.9	ND
River 11	$<$ LOD	$<$ LOD	$<$ LOD	$<$ LOD	578.7	ND

Thiamethoxam was not detected in any samples. As expected, the control sites 11 and 09 had no detectable concentration of neonicotinoids. At control site 07, neonicotinoids were detected at 9.66 ng L^{-1} of acetamiprid and 1.97 ng L^{-1} of thiacloprid. As acetamiprid and thiacloprid are often used in tandem for crop treatment, it is likely that this site could contain some neonicotinoids. Sites 08 and 10 indicated elevated levels of all neonicotinoids, as expected due to their source of agricultural industrial areas. Similarly, sites 06 and 05 had reduced concentrations of neonicotinoids due to dilution of the river by isolated fresh water sources, as was expected. Site 05 had no detectable concentration of neonicotinoids. Site 04 had detectable, but low levels of neonicotinoids, however site 03 did not. As these were at the same location, the results indicate it is possible that at lower concentrations there is more error in the method. Site 02 showed no measurable presence of neonicotinoids. Site 01, which was downstream of 02 had elevated concentrations. It could be possible that the sampling location for site 02 was from stagnant water, rather than the higher-current area, causing no detectable concentrations of neonicotinoids. Or that site 01, located next to an outflow pipe of unknown origin, had an increased concentration of neonicotinoids arising from a source feeding into the drainage outfall.

The results from this study indicate that it is possible to use the MIP-SPE phase to

correlate agricultural run-off with neonicotinoid concentrations along a local waterway, however, further validation of the material is required before this can be used for regulatory routine analysis. Additionally, thiamethoxam was not detected in any samples, which was not expected. As thiamethoxam and clothianidin are usually used in tandem for crop treatment, the presence of clothianidin should also indicate the presence of thiamethoxam. It is possible that thiamethoxam has degraded before sampling.

Previous literature surveys of neonicotinoids in waterways have detection limits of neonicotinoids in water between 2 and 7 ng L^{-1} while loading 100 mL of sample.[80] This is comparable to the results from the MIP-SPE phase, which achieved MDLs below 1 ng L^{-1} with the same sample loading volume. There are currently no regulatory maximum residue limits for neonicotinoids in Canadian or U.S. waterways, but it is expected that the low MDLs achieved in this research will help contribute to better environmental analysis.

Despite certain drawbacks and unexpected results, this experiment is a nice conclusion to the project. It demonstrates the achievements made in developing the MIP-SPE phase thus far, and highlights the direction the project needs to take in the future. It has demonstrated, as proof-of-concept, the capability of the MIP phase to detect low levels of neonicotinoids from complex environmental matrices, and correlates this data to expected concentrations based on geographical information. It has also demonstrated that the MIP is useful for improving regulatory-driven analysis of neonicotinoids, which is currently performed by direct injection, and limited by the instrument limits of detection. This methodology demonstrates the ability to decrease the method detection limits from μg L^{-1} to low ng L^{-1} range, with potentially lower LODs with further optimization of the MIP formula.

Chapter 4

Conclusions and Future Work

4.1 Conclusions

Through careful examination of the drawbacks of existing sampling and instrumental methods, this project has demonstrated the ability of a MIP-SPE phase coupled with a simplified LC-MS/MS method to achieve lower detection limits, enhanced sensitivity, faster analyte throughput, and ease of use for the analysis of neonicotinoids.

The LC method has been improved compared to literature methods by reducing method run times, and eliminating complex gradient elutions and buffering systems. This was achieved by switching from HPLC to advanced UHPLC instrumentation, and by using superficially porous column technologies. Analysis was performed using MS/MS with MRM to lower detection limits compared to PDA and single quadrupole MS detectors. In addition, a direct sampling method, TDU-DART-MS, was demonstrated as proof of principle for faster analytical throughput.

Literature sampling techniques of direct injection, C18-SPE, and QuEChERs were examined for their efficacy in extracting neonicotinoids from river water samples. The drawbacks of these methods were identified and a novel MIP for neonicotinoids

was fabricated to combat these issues. The MIP, fabricated using a modified sol-gel procedure demonstrated lower MDLs than existing methods, achieved through enhanced selectivity and sensitivity for neonicotinoids.

Using the MIP-SPE, in combination with the optimized LC-MS/MS method, an environmental study of a local river basin was performed which demonstrated the presence of neonicotinoids in the environment. This study also demonstrated the capability of the MIP-SPE phase to outperform literature sampling techniques, making them potentially useful for routine, regulatory analysis.

4.2 Future Work

Using a more appropriate pseudo-template could potentially enhance the performance of the MIP-SPEs, and this is something that will need to be investigated further.

Immediate future work for this project involves the completion of additional sampling along the river basin to further demonstrate the performance of the MIPs. An expansion on the template studies will be necessary to enhance the capability of the MIPs, allowing for the detection of more neonicotinoids while minimizing template bleeding. The DART method can also be expanded to perform analysis on the silica-grafted MIP phase directly.

Longer term goals include fabrication of this formulation on a solid substrate, such as a glass microscope slide, for direct environmental sampling, as well as testing for shelf-life, reconstitution capability, reusability and potential competitive interactions with other environmental analytes such as the organophosphates or carbamates, to determine the selectivity for neonicotinoids relative to these insecticides.

Bibliography

[1] Jeschke, P.; Nauen, R.; Schindler, M.; Elbert, A. *Journal of Agricultural and Food Chemistry* **2010**, 1–7.

[2] Elbert, A.; Haas, M.; Springer, B.; Thielert, W.; Nauen, R. *Pest Management Science* **2008**, *64*, 1099–105.

[3] Peoples, S. A.; Maddy, K. T. *The Western Journal of Medicine* **1978**, *129*, 273–7.

[4] D, A. *British Medical Journal* **1996**, *313*, 690–1.

[5] Elbert, A.; Overbeck, H. Imidacloprid, a novel systemic insecticide for crop protection. Proc British Crop Port Conf - Pests and Diseases. 1990; pp 21–28.

[6] Isao M, Koichi I, Takanori T, Isao A, Takafumi F, Hitoshi I, O. T. *Journal of Pesticide Science* **1993**, *48*, 41–48.

[7] Maienfisch, P.; Huerlimann, H.; Rindlisbacher, A.; Gsell, L.; Dettwiler, H.; Haettenschwiler, J.; Sieger, E.; Walti, M. *Pest Management Science* **2001**, *57*, 165–176.

[8] Bass, C.; Denholm, I.; Williamson, M. S.; Nauen, R. *Pesticide Biochemistry and Physiology* **2015**, *121*, 78–87.

[9] Nauen, R.; Jeschke, P.; Copping, L. *Pest Management Science* **2008**, *64*, 1081–1081.

[10] Simon-Delso, N. *et al. Environmental Science and Pollution Research International* **2015**, *22*, 5–34.

[11] Jeschke, P.; Nauen, R. *Pest Management Science* **2008**, *64*, 1084–1098.

[12] Tomizawa, M.; Casida, J. E. *Annual Review of Pharmacology and Toxicology* **2005**, *45*, 247–268.

[13] Elbert, A.; Becker, B.; Hartwig, J.; Erdelen, C. *Pflanzenschutz-Nachrichten Bayer* **1991**, *44*, 113–136.

[14] Douglas, M. R.; Tooker, J. F. *Environmental Science and Technology* **2015**, *49*, 5088–97.

[15] Alford, A.; Krupke, C. H. *PLoS ONE* **2017**, *12*, 1–19.

[16] Hladik, M. L.; Kolpin, D. W.; Kuivila, K. M. *Environmental Pollution* **2014**, *193*, 189–196.

[17] Caloni, F.; Cortinovis, C.; Rivolta, M.; Davanzo, F. *Science of The Total Environment* **2016**, *539*, 331–336.

[18] Bonmatin, J. M.; Giorio, C.; Girolami, V.; Goulson, D.; Kreutzweiser, D. P.; Krupke, C.; Liess, M.; Long, E.; Marzaro, M.; Mitchell, E. A.; Noome, D. A.; Simon-Delso, N.; Tapparo, A. *Environmental Science and Pollution Research* **2015**, *22*, 35–67.

[19] Goulson, D. *Journal of Applied Ecology* **2013**, *50*, 977–987.

[20] Main, A. R.; Headley, J. V.; Peru, K. M.; Michel, N. L.; Cessna, A. J.; Morrissey, C. A. *PloS one* **2014**, *9*, e92821.

[21] Van Der Sluijs, J. P. *et al. Environmental Science and Pollution Research* **2015**, *22*, 148–154.

[22] Rundlöf, M.; Andersson, G. K. S.; Bommarco, R.; Fries, I.; Hederström, V.; Herbertsson, L.; Jonsson, O.; Klatt, B. K.; Pedersen, T. R.; Yourstone, J.; Smith, H. G.

[23] Sánchez-Bayo, F.; Goulson, D.; Pennacchio, F.; Nazzi, F.; Goka, K.; Desneux, N. *Environment International* **2016**, *89-90*, 7–11.

[24] Sanchez-Bayo, F.; Goka, K. *PLoS ONE* **2014**, *9*, 1–16.

[25] Blacquière, T.; Smagghe, G.; van Gestel, C. A. M.; Mommaerts, V. *Ecotoxicology* **2012**, *21*, 973–992.

[26] Spivak, M.; Mader, E.; Vaughan, M.; Euliss, N. H. *Environmental Science & Technology* **2011**, *45*, 34–8.

[27] Whitehorn, P. R.; O'Connor, S.; Wackers, F. L.; Goulson, D. *Science (New York, N.Y.)* **2012**, *336*, 351–2.

[28] Klein, A.-M.; Vaissiere, B. E.; Cane, J. H.; Steffan-Dewenter, I.; Cunningham, S. A.; Kremen, C.; Tscharntke, T. *Proceedings of the Royal Society B: Biological Sciences* **2007**, *274*, 303–313.

[29] Garibaldi, L. A. *et al.*

[30] Rucker, R. R.; Thurman, W. N.; Burgett, M. *American Journal of Agricultural Economics* **2012**, *94*, 956–77.

[31] The European Commission, *EFSA Journal* **2013**, *11*, 1–68.

[32] Simon-Delso, N. *et al. Environmental Science and Pollution Research International* **2015**, *22*, 5–34.

[33] Bodereau-Dubois, B.; List, O.; Calas-List, D.; Marques, O.; Communal, P.-Y.; Thany, S. H.; Lapied, B. *Journal of Pharmacology and Experimental Therapeutics* **2012**, *341*, 326–339.

[34] López-Fernández, O.; Rial-Otero, R.; Simal-Gándara, J. *Analytical and Bioanalytical Chemistry* **2015**, *407*, 7101–7110.

[35] Rossi, S.; Sabatini, a. G.; Cenciarini, R.; Ghini, S.; Girotti, S. *Chromatographia* **2005**, *61*, 189–195.

[36] Kessler, S. C.; Tiedeken, E. J.; Simcock, K. L.; Derveau, S.; Mitchell, J.; Softley, S.; Stout, J. C.; Wright, G. A. *Nature* **2015**, *521*, 74–76.

[37] Wu-smart, J.; Spivak, M. *Scientific Reports* **2016**, *6:32108*, 1–11.

[38] Douglas, M. R.; Tooker, J. F. *Environmental Science and Technology* **2015**,

[39] Bruzzoniti, M. C.; Checchini, L.; De Carlo, R. M.; Orlandini, S.; Rivoira, L.; Del Bubba, M. *Analytical and Bioanalytical Chemistry* **2014**, *406*, 4089–4116.

[40] Viglino, L.; Aboulfadl, K.; Mahvelat, A. D.; Prévost, M.; Sauvé, S. *Journal of Environmental Monitoring* **2008**, *10*, 482–9.

[41] Rezaee, M.; Assadi, Y.; Milani Hosseini, M.-R.; Aghaee, E.; Ahmadi, F.; Berijani, S. *Journal of Chromatography A* **2006**, *1116*, 1–9.

[42] Jovanov, P.; Guzsvány, V.; Lazić, S.; Franko, M.; Sakač, M.; Šarić, L.; Kos, J. *Journal of Food Composition and Analysis* **2015**, *40*, 106–113.

[43] Calatayud-Vernich, P.; Calatayud, F.; Simó, E.; Picó, Y. *Methods X* **2016**, 452–58.

[44] Xie, W.; Han, C.; Qian, Y.; Ding, H.; Chen, X.; Xi, J. *Journal of Chromatography. A* **2011**, *1218*, 4426–33.

[45] California Department of Food and Agriculture, **2008**, 1–20.

[46] Hao, C.; Morse, D.; Zhao, X.; Sui, L. *Rapid Communications in Mass Spectrometry* **2015**, *29*, 2225–32.

[47] Dankyi, E.; Carboo, D.; Gordon, C.; Fomsgaard, I. S. *Journal of Food Composition and Analysis* **2015**, *44*, 149–157.

[48] Bonmatin, J. M.; Moineau, I.; Charvet, R.; Fleche, C.; Colin, M. E.; Bengsch, E. R. *Analytical Chemistry* **2003**, *75*, 2027–2033.

[49] Jones, D. G. *Instrumentation* **1985**, *57*, 1057–70.

[50] Fenn, J. B.; Mann, M.; Meng, C.; Wong, S.; Whitehouse, C. M. *Science* **1989**, *246*, 64–71.

[51] Vaikkinen, A.; Schmidt, H. S.; Kiiski, I.; Rämö, S.; Hakala, K.; Haapala, M.; Kostiainen, R.; Kauppila, T. J. *Rapid Communications in Mass Spectrometry* **2015**, *29*, 424–430.

[52] Gerbig, S.; Brunn, H. E.; Spengler, B.; Schulz, S. *Analytical Bioanalytical Chemistry* **2015**, *407*, 7379–7389.

[53] Mattarozzi, M.; Bianchi, F.; Milioli, M.; Cavazza, A.; Careri, M. *Talanta* **2016**, *147*, 416–421.

[54] Konermann, L.; Ahadi, E.; Rodriguez, A. D.; Vahidi, S. *Analytical Chemistry* **2013**, *85*, 2–9.

[55] Takats, Z. *Science* **2004**, *306*, 471–473.

[56] Takáts, Z.; Wiseman, J. M.; Cooks, R. G. *Journal of Mass Spectrometry* **2005**, *40*, 1261–1275.

[57] Cody, R. B.; Laramee, J. A.; Nilles, J. M.; Durst, H. D. *Analytical Chemistry* **2005**, *77*, 2297–2302.

[58] Cody, R. B.; Laramee, J. A.; Nilles, J. M.; Durst, H. D. *JEOL News* **2005**, *40*, 8–12.

[59] Alexander, C.; Andersson, H. S.; Andersson, L. I.; Ansell, R. J.; Kirsch, N.; Nicholls, I. A.; O'Mahony, J.; Whitcombe, M. J. *Journal of Molecular Recognition* **2006**, *19*, 106–180.

[60] Martín-Esteban, A. *Trends in Analytical Chemistry* **2013**, *45*, 169–181.

[61] Beltran, a.; Borrull, F.; Marcé, R. M.; Cormack, P. a. G. *Trends in Analytical Chemistry* **2010**, *29*, 1363–1375.

[62] Turiel, E.; Martín-Esteban, A. *Analytica Chimica Acta* **2010**, *668*, 87–99.

[63] Egli, S. N.; Butler, E. D.; Bottaro, C. S. *Analytical Methods* **2015**, *7*, 2028–2035.

[64] Lei, Y.; Xu, G.; Wei, F.; Yang, J.; Hu, Q. *Journal of Pharmaceutical and Biomedical Analysis* **2014**, *94*, 118–124.

[65] Hu, X.; Fan, Y.; Zhang, Y.; Dai, G.; Cai, Q.; Cao, Y.; Guo, C. *Analytica Chimica Acta* **2012**, *731*, 40–48.

[66] He, C.; Long, Y.; Pan, J.; Li, K.; Liu, F. *Journal of Biochemical and Biophysical Methods* **2007**, *70*, 133–150.

[67] Wei, X.; Zhou, Z.; Hao, T.; Li, H.; Zhu, Y.; Gao, L.; Yan, Y. *RSC Advances* **2015**, *5*, 44088–44095.

[68] Chen, P.-Y.; Vittal, R.; Nien, P.-C.; Liou, G.-S.; Ho, K.-C. *Talanta* **2010**, *80*, 1145–1151.

[69] Tang, J.; Zhang, M.; Cheng, G.; Lu, Y. *Journal of Liquid Chromatography & Related Technologies* **2008**, *32*, 59–71.

[70] Boulanouar, S.; Mezzache, S.; Combès, A.; Pichon, V. *Talanta* **2018**, *176*, 465–478.

[71] Sanagi, M. M.; Salleh, S.; Ibrahim, W. A. W.; Naim, A. A.; Hermawan, D.; Miskam, M.; Hussain, I.; Aboul-Enein, H. Y. *Journal of Food Composition and Analysis* **2013**, *32*, 155–161.

[72] Egli, S. N.; Butler, E. D.; Bottaro, C. S. *Analytical Methods* **2015**, *7*, 2028–2035.

[73] Rahman, M. M.; Aty, A. M. A. E.; Kim, S.; Shin, S. C.; Shin, H.; Shim, J. *Journal of Separation Science* **2015**, *40*, 203–212.

[74] DeStefano, J. J.; Schuster, S. A.; Lawhorn, J. M.; Kirkland, J. J. *Journal of Chromatography A* **2012**, *1258*, 76 – 83.

[75] Culzoni, M. J.; Dwivedi, P.; Green, M. D.; Newton, P. N.; Fernandez, F. M. *Med. Chem. Commun.* **2014**, *5*, 9–19.

[76] Kandula, D. Z.; Gohle, C.; Pinkert, T. J.; Ubachs, W.; Eikema, K. S. E. *Phys. Rev. Lett.* **2010**, *105*, 063001.

[77] Velchev, I.; Hogervorst, W.; Ubachs, W. *Journal of Physics B: Atomic, Molecular and Optical Physics* **1999**, *32*, L511.

[78] Eriksson, K. B. S.; Pettersson, J. E. *Physica Scripta* **1971**, *3*, 211.

[79] Kia, S.; Fazilati, M.; Salavati, H.; Bohlooli, S. *RSC Adv.* **2016**, *6*, 31906–31914.

[80] Hao, C.; Morse, D.; Zhao, X.; Sui, L. *Rapid Communications in Mass Spectrometry* *29*, 2225–2232.