Advances in solid-phase microextraction as sample preparation method for food analysis

by

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AUTHOR'S DECLARATION

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners. I understand that my thesis may be made electronically available to the public.

Abstract

Within all steps involved in the analytical process, sample preparation is considered the most time-consuming step. Therefore, substantial efforts have focused on the search for automated sample preparation strategies that minimize sample handling and errors associated with human interference.

Solid phase microextraction (SPME) addresses well the necessity for simple and automated sample preparation, with the integration of sampling, extraction, clean up and instrumental introduction into a single step. In SPME, selective extraction of compounds takes place based on the degree of distribution of the analyte between the SPME coating and the sample matrix. For this reason, the correct choice of SPME coating for a given application has great influence on the acquisition of reliable analytical data. In spite of its great potential, the implementation of SPME in the analysis of complex matrices, such as food, has been hindered by the lack of suitable SPME coatings that possess compatibility with complex matrices while maintaining sufficient sensitivity for trace applications. The main problem resides in the fact that the most matrix compatible coating, PDMS, has limited extraction efficiency towards less hydrophobic analytes, whereas the coating that exhibits best extraction efficiency towards pesticides, in general, is PDMS/DVB. PDMS/DVB as a solid coating suffers from the attachment of matrix components onto the coating surface, known as fouling. Fouling does not only considerably shorten coating reusability, but it also causes

significant changes in extraction efficiency, skewing the reliability of the data obtained. Therefore, in this thesis, a new approach to fabricate a matrix-compatible SPME coating for GC-based analysis of food matrices is presented. The developed matrix-compatible coating was evaluated for its reusability in complex matrices, namely grape pulp and Concord grape juice, as well as for its extraction capabilities towards various analytes bearing different physicochemical properties.

First, a method to impart matrix-compatibility to commercially available solid SPME coatings was developed. The method consists of applying a thin layer of PDMS onto the solid coating, in this case PDMS/DVB. The main premise behind this approach was to create a coating that presents the matrix compatibility of PDMS, while maintaining the sensitivity obtained with PDMS/DVB. The reusability of the obtained PDMS-modified coating was evaluated in grape pulp, and rewarding results were obtained since the coating could be reused for over 100 extractions. Moreover, the PDMS-modified coating presented a similar extraction efficacy to that presented by the original PDMS/DVB coating towards the triazole pesticides, used as model analytes. The developed PDMS-modified coating was then employed to develop a simple and fast DI-SPME-GC-ToFMS method for determination of ten triazole fungicides in grapes and strawberries. The method was successfully validated, and the figures of merit obtained with the SPME method were compared to those obtained with the QuEChERS method. The limits of quantitation reached by SPME were at least one order of magnitude

lower than those achieved by the QuEChERS method, whereas precision and accuracy were comparable for both methods.

Subsequently, given the vast option of commercial PDMS blends available, different types of PDMS were compared for their reusability in complex matrices, and parameters associated with the PDMS-overcoated fiber fabrication were investigated in regards to their effect on fiber longevity. Results showed that the long-term reusability of such coatings is a function of the coating's fabrication process, such as achievement of smooth and uniform PDMS surface, and sealing of both fiber ends by PDMS layer. Regarding PDMS type, best results were obtained with Sylgard ® 184.

Since one of the most important branches of food analysis involves the simultaneous analysis of pesticides with a wide range of polarities and from different classes, the PDMS-modified coating was evaluated for the extraction of analytes of different polarities (log P = 1.43 to 6) from water samples in order to understand the mass transfer of analytes within the PDMS outer layer during the mass uptake process. Results showed that for hydrophobic analytes, the kinetics of extraction of the PDMS-modified coating are quite similar to that of the original PDMS/DVB. However, for more polar analytes, the rate-limiting step is the diffusion through the coating; therefore, the PDMS layer affects the kinetic uptake. The main implication of these results is quite evident if a method aiming at simultaneous determinations of both polar and non-polar analytes is to be developed, such as is the case in multiclass pesticide analysis, since the sensitivity of the method at too short extraction times might not be enough for polar analytes.

Finally, once the PDMS-overcoated fibers were proven to be robust and compatible for use in fruit pulp, the DI-SPME-ToFMS method for multiresidue pesticide determination in grapes was developed and SPME parameters that can affect extraction efficiency were optimized via multivariate methods. Despite a thorough investigation during optimization, the most polar pesticides, acephate and omethoate, could not be detected. Next, a careful evaluation of internal standards was presented and attentively discussed. The results showed that two pairs of internal standards, interchangeable amongst them (i.e. only two internal standards were needed) were sufficient to ensure reliable, precise and accurate analytical data. Interestingly, only two internal standards at the time were needed, and among the choices presented, the use of non-deuterated compounds presents an affordable, cost-effective solution for the method. Next, the method was fully validated for 40 pesticides in compliance to EU/SANCO requirements (R^2 > 0.995, RSD < 20%, and 80% < accuracy < 120%). The validated method exhibited excellent performance for pesticides such as chlorothalonil, dicofol, and folpet, which are considered the weak link in QuEChERS-based multiresidue methods. Pyrethroid pesticides were not validated due to their non-specific adsorption onto the vial walls. For pyrethroids, a solvent pre-extraction step should be incorporated in order to avoid losses due to the interaction of these compounds with glassware.

Overall, despite the challenges and limitations encountered, it is evident that the practical aspects of the PDMS-modified coating demonstrated in this thesis create new opportunities for SPME applied in food analysis.

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Dedication

This thesis is dedicated to the Almighty God for His protection and sustenance during the tough times.

It is also dedicated to two wonderful women, my models, my inspiration, and my motivation to never give up: my mom, Joelita, and my Godmother, Rosali.

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List of abbreviations and acronyms

A Surface area

a Time constant

b Thickness of the coating

BTEX Benzene, Toluene, Ethylbenzene, Xylene

C_0 initial analyte concentration

C18 Octadecyl

C_f^{∞} Equilibrium concentration of analyte on the coating

C_f^{max} Maximum concentration of active sites on the coating

C_{free} free (unbound) concentration of analyte

C_s Analyte concentration in the bulk of the sample matrix

C_{total} total (bound + unbound) concentration of analyte

CW Carbowax

CW-TPR Carbowax-templated resin

DI Direct immersion

DLLME Dispersive liquid-liquid microextraction

D_s Diffusion coefficient of the analyte in the sample matrix

DVB Divinylbenzene

DVB/CAR/PDMS Divinylbenzene/carboxen/polydimethylsiloxane

EI electron impact

EU European Union

f_c fiber constant

GC Gas chromatography

GC-MS gas chromatography mass spectrometry

HM Hollow membrane

HPLC High performance liquid chromatography

HS Headspace

K_{fs} Distribution constant of the analyte between SPME coating and sample

L Length of the coating

LC Liquid chromatography

LC-MS liquid chromatography-mass spectrometry

LLE Liquid-liquid extraction

LLME Liquid-liquid microextraction

LOD Limit of detection

Log P Log of octanol/water partition coefficient

LOQ Limit of quantitation

LPME Liquid-phase microextraction

MAE Microwave-assisted extraction

MEPS Microextraction in a packed syringe

MIP Molecularly imprinted polymer

MPS2 MultiPurpose Sampler

MRL Maximum residue limit

MS Mass spectrometer

MS/MS Tandem mass spectrometry

n amount of analyte extracted by SPME fiber

NA Not applicable

ND Not detected

n_e amount of analyte extracted by SPME fiber at equilibrium

PA Polyacrylate

PAN Polyacrylonitrile

PAN Polyacrylonitrile

PCB Polychlorinated biphenyl

PDMS Polydimethylsiloxane

PDMS/DVB Polydimethylsiloxane/divinylbenzene

PFE Pressured fluid extraction

ppb Parts-per-billion

ppm Parts-per-million

ppt Parts-per-trillion

QC Quality control

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

R Radius of the support wire

R^2 Linear regression coefficient

RAM Restricted access material

Rpm Revolutions per minute

RSD Relative standard deviation

S/N Signal-to-noise ratio

SBSE Stir bar sorptive extraction

SDME Single drop microextraction

SEM Scanning electron microscopy

SFE Supercritical fluid extraction

SFE Superfluid extraction

SPE Solid phase extraction

SPE Solid phase extraction

SPME Solid phase microextraction

t sampling time

TFME Thin film microextraction

TIC total ion chromatogram

TOF time-of-flight mass spectrometer

v/v volume by volume

V_f Volume of SPME coating

VOCs Volatile organic compounds

V_s Volume of sample matrix

w/w weight by weight

XIC Extracted ion chromatogram

δ The thickness of boundary layer

η Viscosity of the solution

1 Introduction

1.1 The importance of food analysis

Food represents an exceedingly diverse and complex composition. Rightfully, food comprises a group of different matrices rather than a specific matrix. The origin of the food commodity, animal or vegetal, gives rise to a universe of components, namely, proteins, carbohydrates, minerals, vitamins, and so on. Such constitutional diversity creates challenges and difficulties that require food analysts to resort to the best science and technology available. Indeed, increasing consumer awareness of food quality and safety issues has led to the relentless pursuance of more competent techniques for the verification of food quality.

Accordingly, research in the field of assessment of food quality has been actively growing in recent years, and several aspects related to food quality and safety have been the subject of studies [1–6]. Food analysis is important for the evaluation of attributes related to quality of fresh and processed products, such as nutritional value, flavor, adulteration, contamination and so on. For example, most food commodities have a defined shelf-life; therefore, knowing the original and modified chromatographic patterns of fresh and processed/stored food commodities is an asset in determining the compositional changes that many food commodities undergo with time as a result, for example, of microbial activity or packaging leaching. As such, analytical methodologies are commonly applied to the detection of foodborne pathogens in food commodities by monitoring volatile

metabolic compounds produced by microorganisms in growth media [1,7–9]. Improper packaging can lead to the deterioration of sensorial properties due to the depletion of aroma constituents [10]. Furthermore, characterization of food packaging materials has become increasingly important due to possible leaching of packaging constituents into food [10,11].

In addition to the aforementioned aspects of food analysis, which are focused on the detection of deteriorative processes and the determination of various food constituents that may affect quality, research in the field of safety has been one of the main goals in food analysis, and as such, analysis of pesticides has been a top-priority. A plethora of substances is used worldwide as pesticides; they belong to many different chemical classes, and the only common characteristic among them is their effectiveness against pests. This means that a wide range of analytes exists, with an extensive array of different physicochemical properties to be considered.

Regardless of the type of information pursuit in the analysis of food, the analytical procedures for complex sample analysis consist of sampling, sample preparation, quantification, statistical evaluation and decision making steps. The sampling step consists of obtaining samples that are properly representative of the system under study. Due to the complexity of the food matrices, and/or the low concentration of the substance being studied, original samples are often not ready for direct introduction into the measuring instruments, often having to be subjected to a sample preparation step. The sample preparation step involves the

isolation of the components of interest from the sample matrix. Once the sample preparation is complete, the analysis can then be carried out by the instrument of choice. Sophisticated instrumentation, such as gas chromatograph-mass spectrometer (GC-MS) and liquid chromatograph-mass spectrometer (LC-MS), are often preferred to perform separation and quantification of analytes from complex mixtures [2,3,5,6,12–19].

Each analytical step is critical for gathering of precise and informative results, and must be followed in order. The slowest step determines the overall speed of the analytical process, and it has been reported that over two-thirds of analysis time is spent on the sampling and sample preparation steps [20–22]. One of the reasons for slow progress in the sample preparation step is that most traditional extraction processes involve multi-step procedures that are not only time-consuming and difficult to be automated, but are also prone to more systematic error due to extensive sample handling. Therefore, regardless of the objective within food analysis, special attention has to be paid to the choice of sample preparation to be used.

1.2 Sample preparation methods for determination of contaminants in food matrices

The analysis of food products is a challenging undertaking due to the variety and complexity of both the matrices and the compounds of interest. Sample preparation and downstream analysis require careful consideration to ensure method robustness as well as accurate and precise quantification.

As mentioned previously, the hyphenation of the sample preparation step to the instrumentation employed for final analysis is not easily achievable, primarily because conventional sample preparation techniques utilizing multi-step procedures and use of organic solvents. For this reason, the development of an automated method that integrates sample preparation with separation methods is greatly hindered. Consequently, over 80% of analysis time is usually spent on conventional sampling and sample preparation procedures.

Focusing onto sample preparation techniques for analysis of contaminants in food, traditional methods for determination of pesticides in food evolved from laborious and environmentally unfriendly methods to simpler methods covering a broader scope of analytes. In order to achieve a practical and reliable method for the analysis of complex food matrices, several solvent-based and sorbent-based extraction methods have been employed [17,23–27].

1.2.1 Solvent-based sample preparation methods for contaminants analysis in food

Solvent-based extraction is a common approach used to extract pesticides from food matrices based on the differences in solubility of pesticides in a selected organic solvent and food matrices. The procedure is generally combined with, but not limited to, homogenization, liquid–liquid partition (using different solvents), clean up, solvent exchange, and instrumentation [23].

Liquid-liquid extraction (LLE) is one of the most well-known solvent-based sample preparation methods; in LLE, the partition of analytes depends on

the relative solubility in each phase. This method allows for pre-concentration of analytes to a desired level by partial evaporation or complete evaporation of the extraction solvent, followed by reconstitution into a smaller volume of solvent. LLE is very simple and does not require complex apparatus; for this reason, it is still frequently used as a sample preparation technique in many methods for food analysis. For example, Banerjee and co-workers reported various works utilizing extraction by ethyl acetate specific for grapes [28–31].

Dichloromethane has also been reported by Arrebola and Vidal [32] as an extraction solvent in a method devised to determine 81 multiclass pesticide residues in vegetables. The method was based on a fast extraction of the pesticides with dichloromethane and a further analysis of the extract by gas chromatography-tandem mass spectrometry (GC-MS-MS). The authors used dichloromethane (50 ml) to extract fruit and vegetable samples (15 g), and the resulting extracts were filtered and evaporated to dryness. The dried residues were reconstituted using cyclohexane and analyzed using GC-MS/MS with a programmable temperature vaporization (PTV) injector. No additional clean up steps were taken, but a carbofrit liner and a guard column were used to prevent potential matrix interferences. However, increasing safety concerns associated with the use of dichloromethane have limited its use as a common extraction solvent for pesticide analysis.

Acetonitrile, as a water-miscible solvent, is suitable for extraction of pesticides from water-rich food samples. In the course of extraction, salt such as

sodium chloride is generally added to help achieve phase separation between water and acetonitrile [23]. In 2003, Anastassiades and co-workers reported an acetonitrile-based extraction procedure for extraction of multiresidue pesticides in a variety of food matrices [33]. This method, named QuEChERS, which stands for quick, easy, cheap, effective, rugged and safe, is the newest-generation method for analysis of pesticide residues in food matrices. QuEChERS is a quick and convenient replacement for the old LLE method, offering great quality results with less labor-intensive sample preparation steps, as well as low consumption of solvents and glassware. The approach was developed to provide a highly flexible sample preparation method that could extract multiple classes of compounds from foods of plant origin, while eliminating or minimizing interferences such as organic acids, pigments and fats. Since its development, several optimization modifications of the QuEChERS method have been reported for different analytes, matrices, instrumentation and analyst preferences [34–42]. Furthermore, two independent and inter-laboratory validated methods have been established: AOAC International (AOAC Official Method 2007.01) and the European Committee for Standardization (CEN Standard Method EN 15662).

In short, QuEChERS is comprised of two steps: extraction followed by dispersive solid-phase extraction (d-SPE). The first step, extraction, relies on the use of acetonitrile and salts that, when mixed with a food sample, cause the target analytes to partition into the organic layer (similar to a liquid-liquid extraction procedure). Once the initial extraction is performed, potential matrix interferences are removed from the organic layer using d-SPE. Dispersive SPE uses SPE

sorbents to specifically remove undesired matrix components. For example, a C18 sorbent can remove hydrophobic interferences such as fats and lipids while a primary/secondary amine (PSA) ion exchange sorbent removes acids, sugars and anthocyanine pigments that might act as instrumental interferences. The d-SPE step can also use graphitized carbon black (GCB), which is effective for the removal of a variety of planar pigments and sterols from the sample. Several different combinations of d-SPE sorbents can be employed; choosing the most suitable d-SPE sorbents is dependent on the characteristics of the commodity type (or food type) being analyzed (i.e. general, fats and waxes, pigmented, highly pigmented, pigmented and fats) [33].

Undoubtedly, QuEChERS is nowadays the sample preparation method of choice in multi-residue analysis of pesticides in fruits and vegetables, and most recently, to food commodities of animal origin. The advantages of the QuEChERS method comprises high recovery, high sample yield, accurate results, low solvent and glassware consumption, lower labor and bench space, less reagent costs and ruggedness. The main drawback of this method is the low pre-concentration capability obtained per initial gram of sample; as such, the final extract must be concentrated to a larger extent in order to provide the high sensitivity needed and to obtain acceptable limits of quantification (LOQ). In addition, QuEChERS, as a multistep method, can be difficult to automate; the combination of sample preparation and instrument introduction steps is not easily accomplished by this method. However, the demand towards improved methodologies never ceases, as regulatory agencies keep lowering the permitted

level of pesticides in given commodities, also known as maximum residue levels (MRLs). Food chemists, regulatory agencies and quality control laboratories are always seeking cheaper and cleaner analytical methodologies capable of detecting contaminants in sub part-per-billion levels [43].

1.2.2 Pressurized Liquid Extraction (PLE)

Pressurized liquid extraction or accelerated solvent extraction (ASE) is a sample preparation technique that combines elevated temperature (80-180 ºC) and pressure (100-140 bar) with conventional solvents to achieve fast and efficient extraction of analytes from solid matrices. The use of higher temperatures implies a reduction in solvent viscosity, thereby increasing the solvent's ability to wet the matrix and to solubilize the target analytes. Temperature also assists in breaking down analyte–matrix bonds and encourages analyte diffusion to the matrix surface. By applying heat and pressure to extraction solvent and samples because the solubility, rate of mass transfer, and extractability of pesticides, as well as sample wetting and penetration, increases with temperature, thus improving extraction speed and efficiency. However, it is paramount to consider the thermal stability of the target analytes due to the relatively high extraction temperatures [44–48].

For example, Celeiro et al. [49] employed pressurized liquid extraction (PLE) followed by gas chromatography-triple quadrupole-mass spectrometry (GC TQ-MS) for the rapid determination of 11 fungicides in white grape bagasse. The

method offered recoveries higher than 80% for the majority of the studied fungicides, and LOD at sub ppb levels for the majority of the target fungicides.

1.2.2.1 Microwave-Assisted (MAE) and Ultrasound-Assisted Extraction (UAE)

To accelerate liquid extraction, microware-assisted extraction (MAE) and ultrasound-assisted extraction (UAE) utilize heat generated by microwave irradiation or ultrasonic waves [50]. Microware irradiation can directly affect the dipole rotation and ionic conduction of molecules that absorb microwaves. Under the influence of microwave irradiation, molecules are aligned toward a specific and aligned direction. Once the aligned molecules return to their normal random movement stage, energy is released in the form of heat, creating a rise in temperature. Because microwave irradiation can be directly absorbed by molecules, the heating efficiency is higher than a conventional convection heating process (e.g., Soxhlet extraction) in which heat is transferred from the container to the extraction solvent. Selection of an appropriate extraction solvent for MAE should factor in whether the solvent can absorb microwaves and how much it can absorb. Polar solvents such as water and methanol have strong microwave absorption, while nonpolar solvent such as hexane does not absorb microwaves at all. Extraction efficiency depends on the operating power of the irradiation source, time of irradiation, extraction solvent, and the water content of the sample [51]. Otake and co-workers successfully developed a method based on MAE for the analysis of organophosphorus and pyrethroid pesticides in green onions by GC/MS [52]. Nonetheless, the limitations of MAE and UAE are that they are only applicable to thermally stable compounds, and are not easily automated nor

suitable for volatile analytes. Moreover, both selectivity and sample enrichment capabilities are limited; as such, further clean up and/or concentrations steps are usually required for determination of trace analytes in food [18,25,46,48].

1.2.3 Sorbent-based sample preparation methods for contaminants analysis in food

The use of different adsorbents for the clean up of complex matrices, such as food, has been increased in recent years by the use of matrix solid phase dispersion (MSPD), solid phase extraction (SPE), or even dispersive SPE (d-SPE), which has been implemented in the QuEChERS method.

1.2.3.1 Solid phase extraction (SPE)

Although still highly used today, liquid–liquid extraction (LLE) was once the most popular technique for sample clean up and concentration. However, the use of high amounts of environmentally harmful solvents, the slowness and laboriousness of its performance, and the necessity of improving recoveries have displaced it in favor of SPE. Furthermore, SPE offers important advantages over LLE, such as faster and easier manipulation, lower consumption of organic solvents, higher concentration factors, and the possibility of automation [18,24,43,48,53].

In a typical SPE method, the first step to be undertaken is the selection of the best SPE sorbent/desorption conditions and solution. In most cases, food samples are diluted, centrifuged, and/or filtered prior to analysis, with an aim to eliminate clogging of SPE cartridges [27,43,54–56]. In dispersive SPE (d-SPE), the

extraction is not carried out in a column or in a disk, but in the bulk solution [57]. As one can deduce, its performance is easier, faster, and less tedious. Nevertheless, what is normally intended is to use the sample extraction solvent as the dissolving solvent in the d-SPE step, as it occurs in the QuEChERS method [18,37].

As there are a variety of SPE adsorbents available, the choice of adsorbent is dependent on the food matrix, the analyte of interest and the interferences. There is a wealth of scientific literature on the applications of SPE for preparation of different food samples (e.g. vegetables, fruits, juices, grains, milk, etc.) [55,58–67]. Additionally, when the objective is the analysis of a broad range of analytes, compounds with different physicochemical properties might have different breakthrough volumes, which limits the sample volume according to the analyte with the smallest breakthrough [27,55]. In the determination of multiple analytes, the sensitivity of the SPE method for analytes with higher breakthrough volumes might be sacrificed, owning to the abovementioned limiting breakthrough volume.

1.2.3.2 Matrix solid phase dispersion (MSPD)

MSPD is as ample preparation method for solid, semisolid, or liquid-viscous samples that are mechanically blended together with a solid support material in a mortar, with the aim of obtaining the total disruption and dispersion of the sample on the solid support. The technique uses bonded-phase solid supports as abrasives to produce disruption of sample architecture, and a bound solvent to aid complete sample disruption during the sample blending process

[24,43]. The sample disperses over the surface of the bonded-phase support material to provide a new mixed phase for isolating analytes from various sample matrices. Afterwards, the sample, together with the support material, is carefully packed in an empty column, where elution takes place through the employment of an adequate solvent. In some cases, a clean up procedure is necessary depending on the analytes and the instrumentation that will be used later for their determination. The final extracts obtained are ready for direct injection into the chromatographic instrument in the vast majority of applications [24].

Chu and co-workers developed a method based on MSPD with diatomaceous earth to extract 266 pesticides from apple juice samples by GC-MS [68]. In another work, Valsamaki et al. took advantage of the MSPD with Florisil for removal of high molecular weight fats when developing a method for the determination of 20 organochlorine pesticides and eight PCB congeners in chicken eggs [69].

The advantages offered by MSPD are short extraction times; small amounts of sorbents and solvents needed; low costs; and, the possibility to perform extraction and clean up. However, due to a possible irreversible adsorption of analytes into the sorbent, inappropriately low recoveries may be obtained.

Despite the type of sample preparation used in food analysis, nowadays, the trend has shift into the miniaturization of chemical procedures in order to lower the consumption of solvents and reagents that could damage the

environment. In fact, sometimes, only small amounts of sample are available to be analyzed. Furthermore, the development of automated methodologies to analyze pesticides in food matrices can present the important advantage of high sample throughput with the entire analysis being completely automated, thus diminishing errors associated to human mistakes and allowing for high throughput of samples analyzed.

1.2.4 Solid phase microextraction (SPME)

1.2.4.1 Brief introduction to SPME

Nowadays, extensive efforts towards modernization of analytical instrumentation have greatly simplified, and at times even eliminated, the need for complex and laborious sample preparation methods prior to analysis. However, in most cases, sample preparation still represents the bottleneck in the pursuit of optimum analytical methodologies. Particularly, the determination of trace levels analytes in complex matrices often requires the employment of extensive sample preparation protocols prior to analysis. Steps often involved in sample preparation include extraction of analytes from the matrix, clean-up, and pre-concentration, which is conducted in order to achieve enough sensitivity for a particular analytical method. As such, simpler sample preparation regimens are sought not only to decrease the time required to process samples, but also to diminish errors associated with each step of the procedure, as statistically, the amount of uncertainty in a method is directly related to the number of steps it contains. Automation of the complete workflow, including sample preparation, also allows

for improved reproducibility, while decreasing the labor required to prepare samples.

Thus, one of the main goals of sample pre-treatment is to reduce the time and labor involved in multistep sample preparation techniques. Introduced in the early 1990s, solid-phase microextraction (SPME) addresses several challenges in traditional sample preparation, as it successfully integrates a number of analytical steps such as sampling, extraction, pre-concentration, and, in case of gas chromatography (GC) applications, sample introduction for instrumental analysis [70]. Additionally, SPME is a simple, sensitive, time-efficient, cost-effective, reliable, easy-to-automate, and portable sample preparation technique that minimizes solvent consumption.

In contrast to conventional exhaustive extraction methods such as SPE, SPME technology is a non-exhaustive technique based on the partition equilibrium of the analytes between the sample matrix and the extraction phase. In SPME, the extraction phase can be exposed directly to the sample media (DI) or to the headspace (HS) above the sample. When the SPME extraction phase is placed directly in contact with the sample, the amount of analyte extracted at equilibrium (n_e) can be described as [71]:

$$n_e = \frac{K_{fs} \times V_s \times V_f}{K_{fs} \times V_f + V_s} \times C_s \qquad \textit{Equation 1-1}$$

In Equation 1-1, n_e is proportional to the distribution coefficient of the analyte between the coating and sample matrix (K_{fs}), volume of the extraction

phase (V_f), volume of the sample (V_s) and analyte concentration in the sample matrix (C_s). Equation 1.1 indicates that the amount of analyte extracted onto the coating (n_e) is linearly proportional to the analyte concentration in the sample (C_s), which is the analytical basis for quantitative analysis using SPME.

It is very important to point out that Equation 1.1 assumes that sample matrix is homogenous. For multi-phase systems and heterogeneous samples, especially in the case of complex matrices like food, this equation should be modified to Equation 1-2.

$$n_e = \frac{K_{fs} V_f C_0 V_s}{K_{fs} V_f + \sum_{i=1}^{i=m} K_{is} V_i + V_s} \qquad \textit{Equation 1-2}$$

where $K_{is} = C^{\infty}_i / C^{\infty}_s$ is the distribution constant of the analyte between the i^{th} phase and the matrix being analyzed, and the other terms are the same as previously defined. For example, i^{th} phase can be binding matrix components with affinity for the analyte, and the contribution of this phase should be taken into account. Equation 1-2 has two very important consequences: it shows that the amount of analyte extract is proportional to the unbound analyte concentration and that the exact composition of sample matrix can impact the amount of analyte extract by SPME when dealing with complex heterogeneous samples. From quantitative perspective, Equation 1-2 shows that strict control is necessary to ensure calibration samples exactly match the composition of real samples, and that small variations in matrix composition can have adverse effects on method performance.

Furthermore, if the sample volume is very large, that is, $V_f K_{fs} << V_s$, Equation 1-1 can be simplified to:

$$n_s = K_{fs} \times V_f \times C_s \qquad \textit{Equation 1-3}$$

Equation 1-3, in practice, denotes that there is no need to collect a defined sample prior to analysis, because the fiber can be exposed directly to the sample matrix, for example, in applications involving large sample volume.

From theoretical perspective, time required to reach equilibrium is required to reach equilibrium is infinitely long; as such, equilibrium time is assumed to be achieved when 95% of the equilibrium amount of an analyte is extracted from the sample [71,72]. As depicted in *Figure 1-1*, pre-equilibrium extraction can also be performed to shorten the time required for analysis. Although extraction equilibrium is not reached, there is still a linear relationship between the amount of analyte extracted onto the fiber and the concentration of analyte in the sample matrix. [71–76].

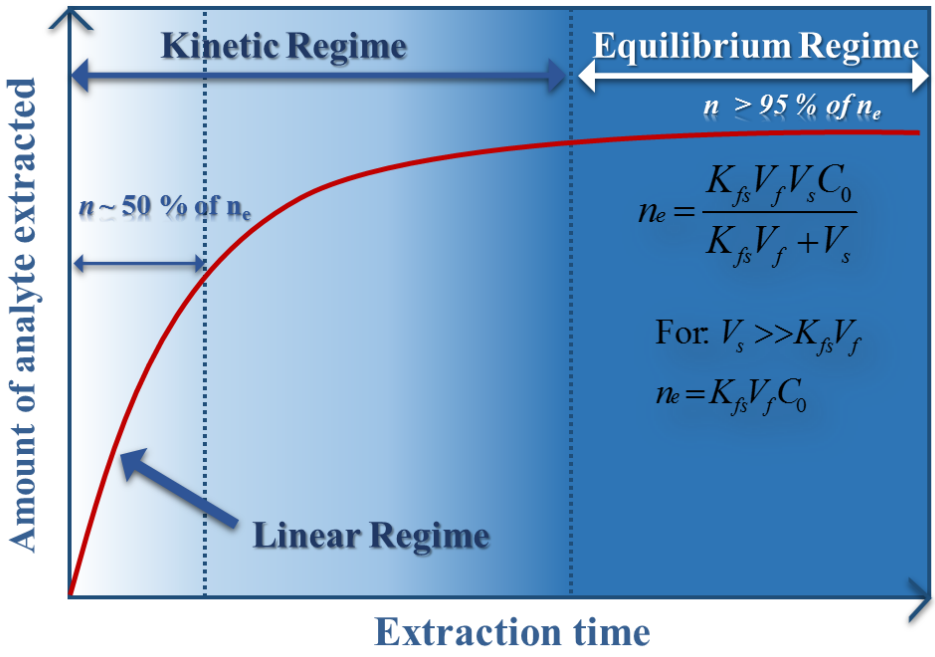


Figure 1-1 Graphical representation of SPME extraction time profile

The extraction efficiency in SPME techniques can therefore be enhanced by increasing the magnitude of K_{fs} (chemistry of the extraction phase), and/or by increasing the volume or active surface area of the extraction phase (geometry of the extraction phase).

K_{fs} values play a critical role in the recovery and distribution of analytes when changing the geometry of the extraction phase is not convenient. The above Equation 1.1, for calculation of analyte amount extracted at equilibrium conditions, takes into account the actual extraction phase amount that participates in the extraction process. For liquid coatings, it is appropriate to take into account the entire volume (V_f) of the extraction phase that is involved in the extraction. However, in the case of solid porous coatings, the interaction between analyte and

extraction phase is limited to superficial active sites that constitute the active surface area of the porous polymer. For this reason, in order to accurately calculate the amount extracted at equilibrium by solid coatings, it is necessary to substitute the value related to the extraction phase volume (V_f) in the aforementioned equation for the total surface area (S_a). This implies that the amount of analytes extracted by solid porous coatings is limited by the number of active sites available for extraction.

Although the occurrence of saturation is rarely encountered in most of the applications of SPME, the reader should be aware of this possibility in order to better understand possible bias in results obtained, and correct his *modus operandi* accordingly. A direct consequence of coating saturation is inter-analyte competitive adsorption [77].

In summary, a mathematical description of the phenomenon can be given by the following equation for a two-analyte system at equilibrium conditions [77]:

$$C_{fA}^{\infty} = \frac{C_{fmax} K_A C_{sA}^{\infty}}{1 + K_A C_{sA}^{\infty} + K_B C_{sB}^{\infty}} \qquad \text{Equation 1-4}$$

where K_A and K_B are the adsorption equilibrium constants for the analytes A and B, and C_{fmax} represents the maximum concentration of active sites on the coating. C_{fA}^{∞} and C_{fB}^{∞}, C_{fA}^{∞} and C_{sB}^{∞} represent the equilibrium concentrations on the fiber and in the sample of the analyte A and B, respectively. Considering B as

the analyte that induces coating saturation, it possible to notice that when K_B and C_{sB}^{∞} are large enough to render the term $K_A C_{sA}^{\infty}$ negligible, the amount of analyte A extracted at equilibrium will decrease when the concentration of saturating compound B in the matrix is higher. Saturation of the coating, especially in complex mixtures, may affect HS-SPME mode more than DI. This is mainly related to the mass transfer resistance of the analytes at the interface of the HS/matrix and to their Henry's law constants. Compounds characterized by higher affinities for the coating and high Henry's law constants readily enrich the headspace above the matrix and are extracted by the coating, occupying consistently its active sites. On the contrary, semi volatile and hydrophilic analytes bearing higher solubilities in aqueous media display an increased mass resistance at the interface between different phases, thus enriching the headspace at slower rate. DI extraction provides more balanced coverage in terms of analytes extracted compared to HS sampling, even at pre-equilibrium conditions, because the kinetic of extraction is faster for hydrophilic compounds, while the extraction of hydrophobic compounds will be more affected by their diffusion in the boundary layer surrounding the extraction phase.

SPME is available in different configurations, as depicted in *Figure 1-2*. In its most known configuration, the SPME device consists of an extraction phase coated onto a fused silica rod. Fiber SPME allows for complete automation of the entire analytical workflow when coupled to GC applications due to the

commercialization of autosamplers dedicated to SPME, and its similarity to a common injection syringe used in GC applications [71,78].

Automated coupling of SPME to liquid chromatography (LC) was first achieved through the development of in-tube SPME. In contrast to fiber SPME, in-tube SPME consists of an extraction phase coated onto the inner walls of fused-silica tubing [79].

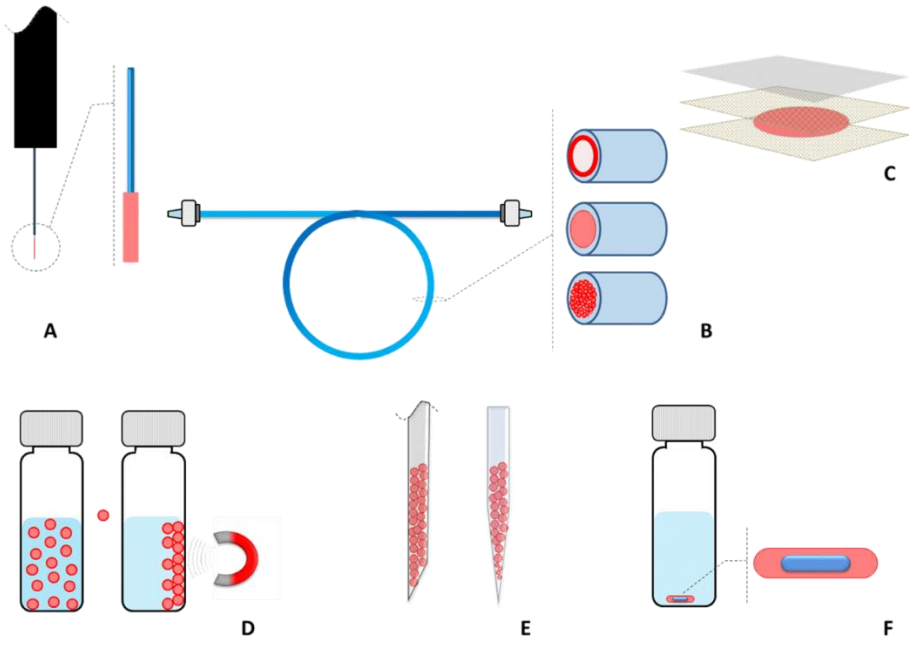


Figure 1-2 Various SPME configurations: (A) fiber; (B) in-tube; (C) thin-film; (D) magnetic; (E) in-tip; and (F) stir-bar.

The simplified features of SPME contributed to its expanded applications, which in turn motivated the ever-growing set of novel advances in SPME sorbents, configurations, and applications to a broader scope of sample matrices and analytes.

However, in spite of the numerous advantages presented by SPME and the ever-increasing number of reports regarding applications of SPME, analyses of complex matrices, such as food, impose substantial challenges, especially when dealing with trace-level analysis. For instance, the direct immersion of the extraction phase in a complex sample matrix can cause damage to the extraction phase. Fouling of the extraction phase by irreversible adsorption of macromolecules from the sample matrix could not only lead to a substantial decrease in the coating lifetime, but also possibly change the extraction properties of the coating, while carrying of matrix to the instrument may cause matrix effects. As such, the search for robust SPME coatings to improve the performance of SPME in complex matrices is an active research topic. Additionally, since SPME is an equilibrium technique that extracts free concentrations of analytes, when studying traces of analytes in food matrices, extensive binding of analytes to food matrix constituents might occur, leading to diminished free concentrations of analytes, which in turn reduces method sensitivity. In such cases, SPME advances have focused on the development of highly specific extraction phases capable of improved extraction efficiency.

1.2.4.2 SPME applied to determination of contaminants in food: and overview

Ideally, a method for pesticide analysis in food commodities should be rapid and easy to perform, requiring a minimum amount of chemicals (especially solvents), while providing a certain degree of selectivity and covering a wide scope of analyte–matrix combinations [12]. The development of automated methodologies to analyze pesticides in food matrices can present the important

advantage of high sample throughput with the entire analysis being completely automated, thus diminishing errors associated to human mistakes. Moreover, with the development of sample preparation in the direction of miniaturization, easy operation and automation, there has been a growing interest in the employment of SPME in the analysis of pesticides and other contaminants in food.

SPME has been able to fulfill most of the aforementioned requirements by integrating sampling, extraction, concentration and sample introduction into a single solvent-free step. The major advantages of SPME are its easy miniaturization, automation of devices, and its convenience in coupling with chromatographic instruments. However, the facilitation of high-quality analytical methods in combination with SPME requires optimization of the parameters that affect the extraction efficiency; namely, extraction phase chemistry, extraction mode, agitation method, sample modification (pH, ionic strength, organic solvent content), sample temperature, extraction time and desorption conditions [78]. Within the above-mentioned parameters, the appropriate selection of fiber coating is one of the most critical steps of SPME method development. The suitability of the fiber coating for a specific analyte of interest is determined by the polarity of the coating and its selectivity towards the analytes of interest in contrast to other matrix components. Since SPME is based on the equilibrium distribution of analytes between sample and extraction phase, the properties of the sorbent play a key role in its performance.

However, despite the advantages offered by SPME, some critical limitations hindered its application to food analysis, such as (i) a limited number of commercially available sorbent coatings; (ii) relatively low operating temperatures due to the poor thermal stability of the physically held sorbent coating; (iii) instability and swelling of the coating in organic solvents and, (iv) the short lifetime of the physically held sorbent coating. In the past years, substantial research efforts have been made in the pursuit of the optimum SPME coating/fiber/device. In this sense, the development of new extraction phases has greatly benefited from sol–gel technology [58,80–82]. Sol–gel technology is undoubtedly one of the most ingenious ways of creating tailor-made extraction phases. Indeed, since its introduction to SPME by Malik and co-workers, sol–gel technology has greatly broadened the scope of application of SPME in the analysis of contaminants in food, owing to the enhanced thermal and mechanical stability achieved by sol–gel coatings. Coatings produced via sol–gel technology display improved thermal stability, which, in cases of GC applications, allows the implementation of higher temperatures for desorption. Additionally, the enhanced mechanical stability conferred by covalently binding the sorbent to the substrate enhances the robustness of sol–gel coating towards organic solvents and acidic/alkali solutions, improving their feasibility in LC-based SPME methods [58,81,83,84]. This is especially important given the diverse nature of constituents in food matrices, where less volatile compounds could be successfully desorbed from the fiber coating, which would result in a reduction of carryover issues. One of the most cited sol–gel based coatings for SPME application in food analysis is

based on vinyl crown ether structures. Crown ether is a heterocyclic chemical compound with a cavity structure, medium polarity, and a strong electronegativity effect. The degree of polarity of crown ether increases with the number of rings. These vinyl crown ether coatings exhibit a strong electronegative effect via heteroatoms on the ring, which makes the fiber coatings selective towards polar compounds. In addition, these coatings possess a porous 3-dimensional network that provides a higher surface area for extraction, affording a faster mass transfer rate. However, the porous 3D structure might pose a challenge in terms of fouling of the coating surface, a common issue encountered during direct immersion in food samples. In fact, most of the studies presented in the following paragraph that applied such porous sol–gel coatings in food analysis were performed in HS mode [81,85,86].

Cai and co-workers used a sol–gel process via radical cross-linked vinyl crown ether to prepare three kinds of coatings. The benzo-15-crown-5 coating was applied for HS-SPME coupled to GC-FPD analysis of organophosphorous pesticides (OPPs) in apple, tomato, and apple juice, reaching limits of detection between 3 to 90 pg/g, and showing a higher extraction efficiency and sensitivity for organophosphorus pesticides compared with commercial fibers (85μm PA and 65μm PDMS-DVB) [86]. In a similar fashion, different types of sol–gel hybrid coatings based on crown ether structures have been synthesized and applied to the determination of OPPs in strawberry, green apples, grapes, honey, juice, orange and pakchoi [85,87]. Recently, an SPME fiber coated with polypyrrole/sol–gel (Ppy/sol–gel) composite was prepared through electrochemical deposition. The

thin coating presented a porous surface and was stable for over 150 extraction/desorption cycles. The polypyrrole/sol–gel coating was applied for the determination of three organophosphorus pesticides in lettuce and cucumber samples via DI-SPME, followed by gas chromatography and nitrogen phosphorus detection (GC-NPD) [88]. Under the optimized conditions, the Ppy/sol–gel coating showed better extraction efficiency than polypyrrole and commercial PDMS and PDMS/DVB fibers. The affinity of the Ppy/sol–gel coating for the analytes is attributed to the phenyl and hydrophilic groups in the coating that enhanced the π–π interaction, hydrogen bonding, and dipole–dipole interactions with the selected pesticides [88].

Additionally, problems associated with the poor mechanical stability of fibers coated onto fused-silica capillaries have been addressed by sol–gel technology through the utilization of unbreakable cores such as platinum, gold and stainless steel [84,89]. Supelco® also commercializes fibers coated onto a metal core, though such coatings are physically deposited onto the substrate rather than covalently bound.

Similarly to sol–gel technique, the employment of ionic liquids (ILs) as extraction phases in SPME has been growing considerably in the past decade [90]. The main characteristic of ILs that makes them a very attractive option in developing selective extraction phases is that they can be structurally tuned for a given application. They are also compatible with GC given their thermal stability, present negligible vapor pressure, high viscosity, and the tailoring of the structure

towards hydrophobicity and/or hydrophilicity by functionalizing cations and anions. By optimizing all of these properties, one can design the desired extraction phase to be employed in trace levels determinations of different classes of analytes/contaminants in a variety of environmental and food samples. Recently, Zhang reported a novel IL SPME coating tailor-made for the selective and sensitive extraction of pyrethroids. The IL, namely, 1-vinyl-3-hexadecylimidazolium hexafluorophosphate ($ViHDIm^+$ $PF6^-$), was applied to the determination of seven pyrethroids in vegetables by direct immersion into hexane extracts, followed by GC-ECD [91]. This work brings up important outcomes, considering that: (i) pyrethroids constitute a major proportion of the insecticide market worldwide; thus, the residue analysis of pyrethroids is of importance in terms of agricultural and environmental control; (ii) given the physical-chemical properties of pyrethroids, successful methods of sample preparation employed for these analytes are usually exhaustive, solvent-based methods, since these analytes possess a high ability to undergo unspecific adsorption onto labware surfaces; (iii) PDMS would be the best coating towards extraction of pyrethroids; however, a pre-solvent extraction step is needed due to the aforementioned fact, and PDMS suffers from chemical incompatibility with non-polar organic solvents.

Another breakthrough in SPME for applications in food matrices involves the development of molecularly imprinted polymer (MIP) coatings to enhance method sensitivity and selectivity. In SPME, the volume of adsorbents is usually much smaller than that of SPE; thus, the amount extracted by SPME can be very small. Additionally, in food matrices, analytes are usually highly bound to matrix

components such as proteins and high molecular weight carbohydrates. The utilization of task-specific molecularly imprinted polymer (MIP) as SPME coatings has demonstrated outstanding capabilities to overcome the limitations associated with low sorbent volumes, rendering enhanced selectivity and improving method sensitivity [58,89,92–99]. A DI-SPME method coupled to GC-MS employing a monolithic MIP coating for selective extraction of triazines herbicides in onions and rice demonstrated high extraction efficiency, yielding LODs between 20 - 88 ng/mL^{-1} due to the direct immersion method and the high quantities of recoveries. The reliability of the prepared fiber in the extraction of atrazine and other analogues in real samples has been investigated and proved by implementation of SPME in spiked samples such as tap water, onion and rice. It is worth noting that after extraction, the authors rinsed the fibers prior to desorption in order to eliminate possible organic matter from the matrix adsorbed to the coating surface. Next, a rinsing step was added, comprised of water followed by methanol. Although the authors do not mention possible losses due to rinsing with methanol, it can be understood that due to the specific affinity of the coating to the analyte of interest, such losses would not be significant [100,101]. In another work, a high throughput method to fabricate more than 20 unbreakable molecularly imprinted polymer (MIP) coated solid-phase microextraction fibers per batch was introduced by Hu and co-workers: it used Sudan I as template and stainless steel fibers as a substrate. The obtained MIP-coated stainless steel fibers were characterized by high extraction capacities, and specific selectivities to Sudan I–IV dyes (carcinogen compounds). MIP-based SPME with high-

performance liquid chromatography was applied to the fast and selective determination of trace Sudan I–IV dyes in hot chili powder and poultry feed samples, achieving limits of detection of Sudan I–IV dyes within 2.5–4.6 ng/g [94,97].

The combination of these technologies, namely sol–gel and MIP coatings, led to the manufacturing of a water-compatible molecularly imprinted polymer (MIP) coating via sol–gel using diazinon as a template and polyethyleneglycol as a functional monomer. The MIP-coated fiber demonstrated much better selectivity to diazinon and its structural analogs, and was applied to the analysis of cucumber, green pepper, Chinese cabbage, eggplant, and lettuce samples. Thanks to its specific adsorption as well as rough and porous surface, the coating revealed a rather larger extraction capability than the non-imprinted polymer and commercial fibers. In addition, the fiber exhibited superior thermal and chemical stability [102].

Fatty matrices are especially cumbersome when developing an SPME method. The binding of the analytes to the matrix results in very low free concentrations of the analytes. Analytes bearing mid to high hydrophobicity (log P > 3) also display a low Henry's Law constant in fatty matrices, which negatively affects the implementation of HS mode. Direct immersion SPME is even more complicated, given the possibility of fouling onto the extraction phase surface. In such cases, pre-treatment of samples prior to SPME extraction is unavoidable.

Gonzalez-Rodriguez and co-workers developed a method combining SPME and low pressure-gas chromatography (LP-GC) to analyze residues of 40 different pesticides in milk. The authors were able to achieve successful results without employing solvent extraction as a sample pre-treatment. Instead, milk samples were diluted in water, and triethylamine was added to promote protein denaturation (in order to increase free concentration of analytes in aqueous phase). SPME was performed in direct immersion mode employing a commercial PDMS/DVB coating [103].

The burdensome extraction of polycyclic aromatic hydrocarbons (PAHs) from vegetable oils by SPME methods was accomplished by Purcaro and co-workers in two distinct studies, coupling SPME to either fast GC or GCxGC. Oils samples were either extracted in acetonitrile and co-extracted in hexane, or directly diluted in hexane, and SPME was latter performed via direct immersion from a hexane extract. In both studies, a commercial SPME fiber Carbopack Z/PDMS was employed. Unlike the carboxen particles constituting the commercial coatings DVB/Car/PDMS and PDMS/Car, CarbopackZ is a porous graphitized carbon black with a pore size of approximately 100 Å. This solid coating possesses a low degree of microporosity and consequently, this phase does not suffer from the same limitations as carboxen coatings towards the extraction of high molecular weight analytes. The primary mode of extraction for this fiber is the π–π interaction between the carbon surface and the analytes. Planar compounds, such as PAHs, can have a greater interaction with the carbon surface and are retained, while other analytes are not retained as efficiently. This

becomes especially critical in a nonpolar solvent matrix. Under these conditions, the effect of PDMS is greatly minimized, although sorption into the PDMS phase would play a much greater role in a water-based matrix. The proposed methods depicted rapid and sensitive solutions to reduce the interference of triglycerides, saving the column life and avoiding frequent cleaning of the mass spectrometer ion source. In addition, solvent consumption and sample manipulation were minimized as compared to alternative methods [104,105].

Yang et al. utilized carbon nanotube-reinforced hollow fiber SPME (CNTs-HF-SPME) combined with HPLC to extract and determine diethylstilbestrol in milk products. Wall pores of the hollow fiber were filled with multi-walled carbon nanotubes (MWCNTs) using sol–gel technology. The use of hollow fibers can limit large molecules, such as proteins, from entering small pores. The MWCNTs in the wall pores of the hollow fiber can absorb target molecules, thus effectively and selectively extracting DES from milk products. Moreover, in that particular study, CNTs were used only once to prevent possible carryover issues. This study showcased CNTs-HF-SPME combined with HPLC as a simple, rapid, and cost-effective technique to monitor diethylstilbestrol residues in milk products, and as such, the approach should be further pursued in order to expand the scope of analytes being determined in milk samples. Even though the method has shown excellent sensitivity, its main disadvantage lies in the lack of automation and the need for multiple sample handling, which could add substantial error to the method [106].

A dispersive solid-phase microextraction (dispersive-SPME) method was proposed in which 30 mg of different silica-based and polymeric sorbents were evaluated for their capacity to simultaneously pre-concentrate tetracyclines (TCs) from water and milk samples. In the proposed dispersive solid-phase microextraction method, after extraction with acetonitrile and salt-promoted partitioning, TCs were adsorbed onto a small amount of dispersive silica-based primary and secondary amine sorbents, desorbed with a small volume of desorption solution, and determined by high-performance liquid chromatography with diode-array detection. In brief, the method comprised of a miniaturized version of QuEChERS, in which the dispersive step has the goal of pre-concentrating the analytes rather than cleaning-up the extract from matrix compounds, as is the case in the QuEChERS method [107]. While an enrichment factor was observed with the dispersive-SPME method, the LODs reported (7.9 to 35.3 ng/g) do not present a significant improvement over the levels reported by conventional QuEChERS methods. Since the adsorbents studied do not display specificity towards tetracyclines, one could expect that the co-extraction of matrix endogenous components would still occur to some extent, and as such, matrix effects would be observed.

Continuing with the analysis of troublesome animal food products, an on-line fiber-in-tube solid-phase microextraction (SPME) method was developed by Hu et al. to analyze four fluoroquinolones in pork liver and chicken samples. The technology consisted of longitudinally packing molecularly imprinted fibers (MIP-fibers) into a PEEK tube as the online extraction unit. Reduced back

pressure and rapid kinetics were obtained, as well as improved extraction capacity thanks to an increase in coating volume. In addition, the extraction of analytes was specifically tailored by using molecularly imprinted coatings (multiple ofloxacin imprinted fibers), which greatly reduced the interference of the sample matrix. Sensitive results were achieved with limits of detection as low as 0.016–0.11 μg/L. Indeed, MIP ensured specific recognition towards the template and its structural analogues. Conversely, the specificity of the MIP coating makes it unsuitable for simultaneous extractions of multiclass compounds. Since the contaminants residues are generally required to be analyzed simultaneously, to address this limitation and expand the applicability of the method, the PEEK tube was filled with two different fibers imprinted by ofloxacin and sulfamethazine, respectively, in order to obtain simultaneous extraction of these two categories of antibiotic drugs. Preliminary results showed the hybrid packing strategy could simultaneously enrich the target analytes from complicated samples. The possibility of applying the method to pork liver samples spiked with fluoroquinolones and sulfonamides was also studied [96,98].

Currently, the major limitations of SPME methods for multiclass determinations are the polarity of the extraction phases, and their cut-off in terms of extraction capabilities towards more polar analytes. Melo and co-workers encountered such problems when developing a method to analyze 10 pesticides in lettuce by SPME-HPLC-DAD. The method was not satisfactory for most polar analytes (Log K_{ow}< 2), even though authors had employed a carbowax/templated resin (CW/TPR). CW/TRP is a polar coating that had its commercialization

discontinued due to the low chemical stability caused by swelling when in contact with aqueous samples [108].

Nanotechnology was used to develop an effective and sensitive method to determinate five carbamate pesticides in apples using carbon nanotubes-reinforced hollow fiber solid-phase microextraction (CNTs-HF-SPME) combined with high performance liquid chromatography-photodiode array detection (HPLC-DAD). The CNTs were dispersed in water by adding a surfactant, and then held in the pores of the HF supported by capillary forces and sonification. The SPME device, which was wetted with 1-octanol, was placed in stirred apple samples to extract target analytes. After extraction, analytes were desorbed and analyzed using HPLC-DAD. Under the optimized extraction conditions, the enrichment factors were achieved in a range of 49- to 308-fold, obtaining good inter-fiber repeatability and batch-to-batch reproducibility, as well as good linearity ranges and recoveries. The limits of detection ranged from 0.09 to 6.00 ng/g. Therefore, the results demonstrated that this novel method was an efficient pretreatment and enrichment procedure for the determination of trace carbamate pesticides in apples [109].

For new developments in SPME, key issues involve the reduction of analysis time and the enhancement of sample throughput. The early automation of SPME in GC applications can be credited to the simplicity of the SPME fiber design. Over the years, autosamplers have evolved to perform a complete SPME-GC workflow, including fiber exchange, without manual interference.

Increases in sample throughput have been successfully achieved in SPME coupled to LC applications on the 96-well plate format, as exemplified in the previous sections. Hagehri and co-workers employed the concept of 96-well plate format and successfully automated a fiber-SPME method coupled to GC for the determination of pesticide residues in cucumber. In that work, the authors used a PDMS coating for extraction, and liquid desorption in acetonitrile, followed by solvent evaporation and re-concentration of extract on n-octane. The developed method exhibited clear advantages such as high-throughput, good sensitivity, and convenient suitability for the analysis of studied pesticides in cucumber samples. Conversely, the method was developed and validated for a few semi-volatile pesticides. Regarding its application to more volatile pesticides, some limitations in the implementation of an open-well method, which includes solvent exchange, can be foreseen [110].

In spite of the advantages achieved by such technologies, their main drawback is the lack of standardized procedures in this field, which causes problems with inter laboratory reproducibility, and as such, are not feasible for routine applications. For this reason, despite the drawbacks presented by the commercially available coatings, those are still the first choice for routine and inter laboratory validations.

The hyphenation of HS-SPME with a comprehensive two-dimensional gas chromatography instrument coupled with high-speed time-of-flight mass spectrometry (HS-SPME–GC×GC/TOF MS) proved to be a quick and sensitive

alternative to detect 36 pesticides that may contaminate tea samples (green, black and fruit tea). Comparisons between the SPME protocol, employing a commercial DVB/Car/PDMS coating, and a procedure involving ethyl acetate extraction and high-performance gel permeation chromatography (HPGPC) yielded comparable results. Nonetheless, the HS-SPME procedure resulted in cleaner extracts compared to the conventional HPGPC extraction strategy, as well as less interfering matrix components. A decrease of interfering matrix components, in turn, resulted in a cleaner background, providing means for better identifications of compounds due to chemical noise, and the elimination of non-volatiles deposits built in the injector and front part of the separation capillary [111]. A similar HS-SPME approach was also employed by Tranchida et al. to evaluate a fast GC system hyphenated to a fast-speed quadruple MS analyzer capable of providing full scan and MS/MS information. Information regarding the quality attributes associated with the volatile content of tea samples was obtained in untargeted full scan acquisition mode, whereas the MRM mode provided sensitive and accurate determination of pesticide contamination in such samples [112].

Although most food applications use headspace-SPME mode, since it protects the fiber coating from damage, HS-SPME is not suitable for all cases; major limitations lie in the low rates of extraction for poorly volatile or polar analytes, as well as a larger possibility of fiber saturation in the case of solid coatings. In such cases, direct immersion SPME, where the extraction phase is placed directly in contact with the sample, should be used in order to guarantee higher sensitivity as well as better representativeness of the analytes extracted

from the matrix. However, in DI-SPME, direct coating exposure in a complex matrix may lead to extraction-phase fouling and subsequent loss in extraction sensitivity, reproducibility, accuracy and extract integrity.

For this reason, despite the vast literature available regarding the application of direct immersion SPME methods to analyze pesticides in complex matrices such as fruits and vegetables, in most of the cases, the sample has to be subjected to extensive pretreatment or clean-up prior to SPME extraction such as centrifugation, dilution or pre-extraction in organic solvent [113–118]. This limitation has so far hindered the scope of application of SPME in food analysis for less volatile analytes.

Table 1-1 Selected applications of SPME for analysis of contaminants in food.

Matrix	Analytes	Mode/ Configuration	Coating	Separation/ Detection	Quantitation	Remarks	Ref
Apple Tomato	Pesticides (OPPs)	HS-SPME Fiber (~80 μm)	Vinyl crown ether Sol–gel (polar)	GC-FPD	Matrix-matched	Fiber exhibited extraction capabilities superior to PDMS/DVB 65 μm and PA 80 μm fibers.	86
Honey Orange Pakchoi	Pesticides (OPPs)	DI-SPME Fiber (~40μm)	Crown ether Sol–gel (hybrid)	GC-FPD	Standard Addition	Fiber exhibited extended lifetime (over 200 extraction cycles).	87
Green apple Strawberry Grapes (aqueous extracts)	Pesticides (OPPs)	HS-SPME Fiber (~17 μm)	Crown ether Sol–gel (hybrid)	GC-FPD	External Calibration (water)	Fiber exhibited extraction capabilities similar or superior to PDMS 100 μm fiber.	85
Lettuce Cucumber	Pesticides (OPPs)	DI-SPME Fiber (~18 μm)	Polypyrrole/ sol–gel	GC-NPD	Standard Addition	Coating was electrochemically deposited on stainless steel wire	119
Vegetables (hexane	Pesticides (Pyrethroids)	DI-SPME Fiber	Ionic Liquid ViHDIm^{+}PF6^{-}	GC-ECD	External Calibration	PIL coating displayed superior chemical stability towards non-polar organic solvent	91

Matrix	Analytes	Mode/ Configuration	Coating	Separation/ Detection	Quantitation	Remarks	Ref
extracts)		(15-20 μm)			(hexane)	compared to PDMS coating.	
Onion Rice (aqueous extracts)	Pesticides (Triazines)	DI-SPME Fiber (15-20 μm)	MIP (atrazine, ametryn templates)	GC-MS GC-FID	External Calibration (water)	Specificity of coating enabled effective rinsing of fiber prior to desorption. Unbreakable fibers: MIP-ametryn coated onto anodized silylated aluminum wire.	100, 120, 121
***Tomato Sauce *Hot chilli powder Poultry feed**	Sudan dyes (I-IV)	DI-SPME Fiber (~19.8 μm) *Fiber (~0.55 μm)	MIP (Sudan-I template)	HPLC-UV *LC-MS/MS	External Calibration (acetone/water)	(i) Unbreakable fiber: MIP coated onto stainless steel wires; (ii) Batch production of coatings; (iii) Solvent and pH resistant; *(iv) Ultra-thin coatings prepared via chain transfer.	94,97
Cucumber Green pepper Chinese cabbage Eggplant Lettuce	Pesticides (OPPs)	HS-SPME Fiber (~50 μm)	Sol–gel MIP (PEG diazinon-template)	GC-NPD	Matrix-matched	Water compatible polar coating. LOQs at ppt levels.	102
Milk	Multiclass 40 pesticides	DI-SPME Fiber	Commercial PDMS/DVB	LP-GC-MS/MS	Matrix-matched + IS	Protein denaturation on diluted milk samples Single IS (pentachlorobenzene)	103

Matrix	Analytes	Mode/ Configuration	Coating	Separation/ Detection	Quantitation	Remarks	Ref
			(65 μm)				
Vegetable Oils (hexane extracts)	PAHs	DI-SPME	Commercial Carbopack Z/PDMS	GCxGC-ToFMS GC-MS	Matrix-matched (olive oil) Multiple ISs	Fiber was rinsed with hexane prior to desorption. Coating provided good π-π interaction with planar compounds.	104, 105
Milk products	Diethylstilbestrol	Hollow fiber HF-SPME	MWCNTs Sol–gel	HPLC		Lack of automation.	106
Milk	Tetracyclines	Dispersive SPME	Silica-based and Polymeric sorbents (30 mg)	HPLC-DAD	Matrix-matched	Employed commercial SPE sorbents. Procedure is similar to QuEChERS (acetonitrile extraction)	107
Pork liver Chicken (acetornitrile extract)	Fluoroquinolones Sulfonamides	In tube-SPME (Packed fibers)	MIP	HPLC-UV	External Calibration (acetonitrile)	PEEK tube packed with MIP fibers of different functionalities. On line coupled to HPLC.	96
Lettuce	Multiclass 10 Pesticides	DI-SPME	Commercial CW/TRP	HPLC-DAD	Matrix-matched IS	Coating has been discontinued commercially due to low chemical and mechanical stability.	108
Tea	Multiclass	HS-SPME	Commercial	GCxGC-	Matrix-matched	SPME protocol was compared to solvent	111

Matrix	Analytes	Mode/ Configuration	Coating	Separation/ Detection	Quantitation	Remarks	Ref
	36 Pesticides		DVB/Car/PDMS	ToFMS		extraction followed by gel permeation chromatography	
Apples	Pesticides (Carbamates)	HF-SPME	CNTs	HPLC-DAD	Matrix-matched IS	CNTs were wet in 1-octanol before immersion in apple samples.	109
Cucumber	Multiclass 7 Pesticides	DI-SPME Fibers	PDMS	GC-MS	Matrix-matched IS	96-well plate format (open); Solvent desorption for GC.	110

1.2.4.3 SPME Quantification in Food Analysis – Special Considerations

Another topic broadly discussed is the concern frequently expressed regarding SPME's ability to provide reliable quantitation in complex samples due to its non-exhaustive nature. Owing to the equilibrium nature of the microextraction technique, the amount extracted by SPME is proportional to the free concentration of the analytes in the samples. This principle often causes misconceptions regarding the quantification capabilities of SPME, especially when dealing with complex matrices, where the absolute recovery of analytes may lie within a small percentage of the total amount. In such cases, the achievement of accurate quantification methods demands the choice of a proper calibration technique.

For complex matrices, aqueous external calibration is rarely applicable. In cases where the matrix presents a high water content and moderate to low complexity, external calibration might be employed. In some cases, the initial complex food matrix is extensively diluted (most often with water) and thus, external calibration in aqueous models may be suitable due to the decreased concentration of matrix components. However, each matrix/analytes combination must be studied in order to ensure that matrix effects are absent. Matrix effects must be accounted for, in particular, if the sample matrix contains organic solvents, such as in alcoholic beverages, and quantitation generally requires matrix-matched standards or the method of standard additions [71,78,122,123].

The most commonly employed calibration approach in food analysis where complex matrices are present is matrix-matching to the unknown sample [107]. The use of an isotopically labeled internal standard should also be considered. For instance, the quantitative determination of triazoles in fruits by means of DI-SPME-GC was achieved by means of a matrix-matched calibration curve to account for possible matrix effects; in addition, an isotopically labeled internal standard was also used. The internal standard offers enhanced accuracy and precision to the developed method, as it corrects for instrumental response drifts and for variabilities that often occur when dealing with biological matrices. However, such advantages can only be achieved if the internal standard closely resembles the analytes of interest in terms of its affinity for the extraction phase and any competing phase in the matrix, mimicking the behavior of the analytes during the extraction process. Despite this, it is common to find examples in the literature of SPME applied to the determination of multiclass pesticides in food utilizing one or two internal standards, mostly directly translated from exhaustive sample preparation methods. However, in such exhaustive extraction methodologies - mainly involving solvent extraction-, the totality of analytes is extracted, and internal standards are mainly applied to correct for instrumental drifts. It can be concluded that in such cases, the SPME procedures would fall short in correcting inter-matrix variations that could lead to different coating-analyte-matrix interactions.

Standard addition is the method of choice when endogenous matrix compounds are the target analytes, as it is not possible to acquire matrix blank

samples. Such methods also work well for matrices presenting high inter-sample differences (such as pH, salt content, sugar content, water content, etc.) which could lead to substantial matrix effects (statistically different slopes of calibration curves) [124]. Conversely, careful attention should be paid when using standard addition and when solid or semi-solid matrices are under investigation, since the mass-transfer mechanism can be different for the standards added and the native analytes [125,126]. As well described by Mirnaghi et al., when investigating the phenolic content in berries by SPME, the authors stated that differences in binding and adsorption sites for the endogenous matrix compounds and added standard led to an underestimation of the analytes within the complex matrices under study (study validated against solvent extraction) [127].

In addition to the traditional calibration methods employed in food analysis, SPME offers additional calibration approaches. Equilibrium extraction is the simplest approach for SPME calibration. By directly applying Equation 1.1, one can easily calculate sample concentration. In SPME, achieving the distribution equilibrium is important because in this situation, the variation of mass transfer does not affect the final mass extracted. However, equilibration times can be quite long, and when adsorbent extraction phases (such as PDMS/DVB, DVB/Car/PDMS, PDMS/Car) are employed for determinations in multi-component, complex samples, a careful examination for inter-analyte displacement occurrence must be performed (Figure 1-3) [128,129].

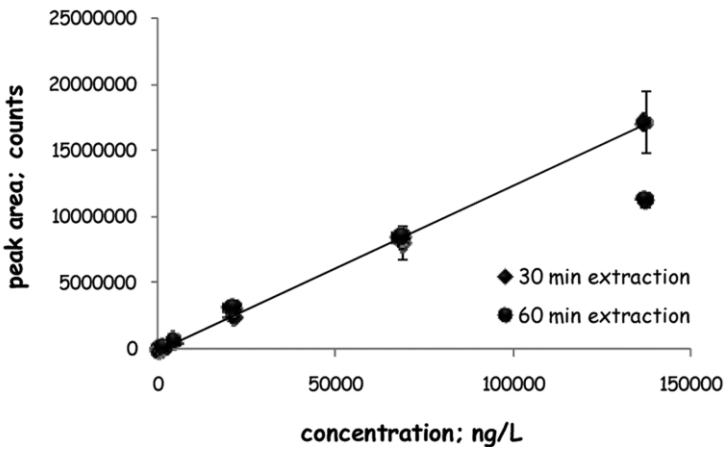


Figure 1-3 - HS-SPME calibration curves for 2-pentanol at 60 (displacement occurrence) and 30 min (no displacement occurrence). Reprinted with permission from [129]*, © American Chemical Society 2013.*

When analysis of complex matrices is performed by SPME solid coatings, the competition between matrix components, analytes and internal standards may result in narrower linear dynamic ranges for polar compounds that are potentially easily displaced by other adsorbates having a higher concentration and affinity for the extraction phase, or when coating saturation occurs. However, in this case, good linearity can still be achieved by limiting the factors that promote coating saturation (extraction time, extraction mode, sample dilution, sample and or HS volume adjustment etc.). Alternatively, for hydrophobic compounds, linear dynamic ranges may be limited by their solubility in water-based samples, by the level of binding to matrix components, or by competitive adsorption to the vial walls. Moreover, sensitivity permitting, pre-equilibrium extraction times can be employed; procedures involving pre-doping of fibers with standards, or kinetic

calibration regimes (n < 95% of n_e in *Figure 1-1*) have been reported to yield satisfactory quantitation.

It is important to note that the phenomena of analyte migration/desorption into the pores of solid and semi-solid matrices can invalidate the application of the kinetic calibration method via loading of standard on the fiber for some food applications (*Figure 1-4*). The rate of unbinding of native matrix compounds may not resemble the rate of desorption of the loaded analytes from coating to matrix, leading to erroneous assumptions of isotropy between the loaded standard and endogenous analytes.

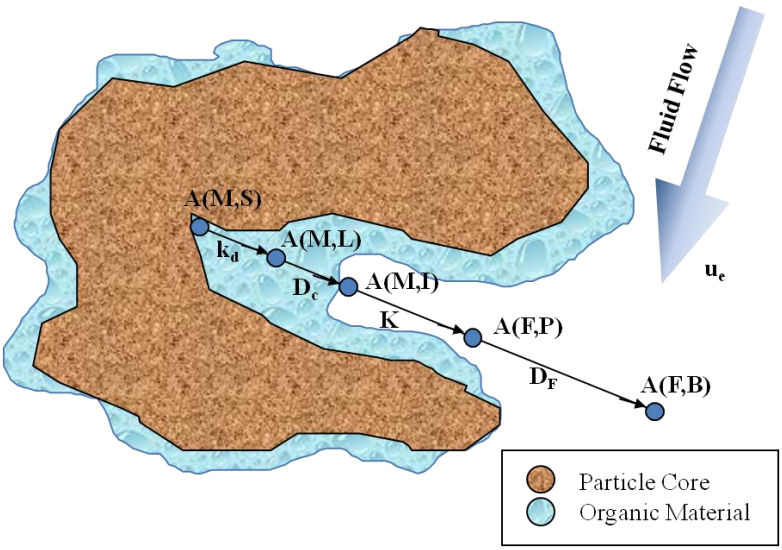


Figure 1-4 Schematic representation of the individual steps in the extraction process of solid particulate. A(M,S): analyte molecules adsorbed on the solid surface of the matrix; A(M,L): analyte in the organic material attached to the matrix particle; A(M.I): analyte at the matrix-fluid interface; A(F,P): analyte dissolved in the fluid located inside particle pore; and A(F,B): analyte in the bulk of the flowing fluid. k_d is the dissociation rate constant of the analyte-matrix complex; D_c is diffusion coefficient of the analyte in the swollen organic component of the matrix, K is the analyte's matrix fluid distribution constant, and D_F is the diffusion coefficient in the fluid.

Lastly, SPME is mainly an equilibrium extraction method, and in most cases, only a fraction of the analytes is extracted. However, if the distribution constant (K_{fs}) is very large, then $V_s << K_{fs}V_f$, and the totality of a given analyte can be extracted; in other words, exhaustive extraction may occur. The calibration

for exhaustive extraction is not often used in SPME because it is typically only suitable for small sample volumes and very large distribution coefficients. By utilizing special devices such as cold-fiber, achieving SPME exhaustive extraction in food matrices is possible [130,131]. The application of thin film geometry has also demonstrated exhaustive extraction capabilities due to the large surface are/volume ratio of the sorbent [127]. A special application of exhaustive extraction is multiple SPME, in which the sample is repeatedly extracted, and the total amount of the analyte can be extrapolated from only a few extractions, even if the analyte in the sample matrix is not extracted exhaustively [125,132]. Multiple HS-SPME was employed to quantify VOCs released by the mushroom *Agaricus bisporus* [132]. The main advantage of such method is that matrix effects can be avoided by calculating the total amount of analytes in the sample. However, the extracted amount cannot be negligible, or the areas of successive extractions would be the same and the approach invalid.

1.3 Research objectives

As emphasized in the literature revision described above, there is currently a lot of effort directed towards the development of competent protocols for food analysis, and in particular, for determinations of contaminants in food. This has led to the development of various analytical methods and techniques with a focus on the simplification of sample preparation, minimization of sample handling, and complete automation. To address the limitations of the current GC-based commercial SPME coatings (e.g. fouling issues) for DI applications, the main objective of this thesis was to develop a matrix-compatible and long-lasting coating that can be used for simultaneous determinations of multiple contaminants in food samples with minimal sample handling. Thus, the breakdown of the thesis is as follows.

Chapter 2 focuses on the improvement of SPME applied to the analysis of semivolatile analytes in complex food matrices through direct immersion sampling. It presents the development of a new approach for improving the coating structure imparting matrix-compatibility to the SPME coating. In the present work, the modified coating was evaluated using selected triazole pesticides as model analytes, given their low volatility, while whole grape pulp was selected as a complex matrix model. Since most DI-SPME methods for pesticide determinations in food require extensive sample pre-treatment prior to SPME, a critical part of this study was to carry out no sample treatment other than blending the grapes prior to analysis. Chapter 3 describes the application of the

developed SPME matrix-compatible coating for the determination of pesticides in fruits. A simple, fast and automated DI-SPME-GC-ToFMS method was developed for the determination of ten triazole pesticides in grapes and strawberries. The method was successfully validated, and the figures of merit obtained were compared to those obtained using the QuEChERS method. In order to address the requirement for simultaneous analysis of compounds from different classes and polarities, Chapter 4 discusses the implications associated with the PDMS overcoating on the kinetics and thermodynamics of extraction. Chapter 5 presents the evaluation of different PDMS types and overcoating strategies to prolong coating lifetime. In Chapter 6, the optimized coating strategy with the best matrix-compatibility characteristic was then used for the development of a multiresidue method for determination of 40 pesticides in grapes. Finally, Chapter 7 summarizes the main research findings of the current work and proposes future directions and challenges for this type of application.

2 Towards More Robust SPME Coatings for Direct Immersion Analysis in Food

2.1 Preamble and introduction

2.1.1 Preamble

Most of the data presented in this chapter has been already published as a paper: Souza Silva, E A. & Pawliszyn, J., Optimization of fiber coating structure enables direct immersion solid phase microextraction and high-throughput determination of complex samples. *Anal. Chem.*, 84, 6933-6938 (2012). The figures and tables are reprinted from this publication with the permission from the American Chemical Society (Copyright 2012 American Chemical Society).

2.1.2 Introduction

As discussed in Chapter 1, there is a growing demand in the area of food analysis to increase the performance, reliability and speed of analytical process, while reducing the cost of the methods, and providing better sensitivity and selectivity as well.

To meet these demands, unceasing efforts have been direct towards the development of new techniques and strategies for sample preparation. The development of automated methodology to analyze pesticides in food matrices can present an important advantage for high sample throughput, with the entire analysis being completely automated.

In this context, SPME coupled to GC analysis addresses the need to facilitate rapid sample preparation and integrate sampling, extraction, concentration and sample introduction to an analytical instrument into one solvent-free step. However, the facilitation of high-quality analytical methods in combination with SPME requires optimization of the parameters that affect extraction efficiency [78,123,133,134].

Amongst all factors, the appropriate selection of fiber coating is one of the most critical steps on SPME method development. The suitability of the fiber coating for a specific analyte of interest is determined by the polarity of the coating and its selectivity towards the analytes in contrast to other matrix components. Given the great diversity of analyte-matrix combinations, significant developments are still being made in some critical areas of SPME coatings development [58,84,135,136].

Despite the challenges, in the last several years, extensive applications of SPME in the extraction of pesticide residues in vegetal foodstuffs have been reported [18,88,111,137–140]. However, most food applications use headspace-SPME mode, the preferred method for extraction of complex matrices, in which the extraction phase is placed in the headspace above the sample rather than immersed into the sample. However, HS-SPME is not suitable for all cases; the major limitation of this approach is that the rates of extraction are low for poorly volatile or polar analytes. In such cases, direct immersion SPME should be used.

In spite of the vast literature available regarding the application of DI-SPME methods to analyze pesticides in complex matrices such as fruits and vegetables, in most of the cases, samples are subjected to additional pre-treatment or clean-up steps prior to SPME extraction. These may include centrifugation, dilution or pre-extraction in organic solvents [113–116,141–144]. Due to the complex nature of food matrices, DI-SPME can be difficult; in such cases, sample pre-treatment is necessary to protect the coating and avoid fouling of the extraction phase by irreversible adsorption of macromolecules from complex matrices at the interface. These could not only led to a substantial decrease in the fiber lifetime, making it unusable for more than a few samples, but also change the coating extraction properties [145].

As such, the search for new coatings to improve the performance of SPME for pesticide residues in complex matrices is an active research topic [58,84,118,146]. Recently, the preparation of tailor made fibers has been the focus of scientific interest. For instance, sol-gel technology, as well as molecular imprinted polymer technologies, have been used to prepare different SPME coatings applied to the extraction of pesticides in food matrices [86,88,98,106,110,147].

In spite of the drawbacks presented by the commercially available coatings, those are still first choice for routine and inter laboratory validations. A review published by Beltran et al. shows that many of the analyses of pesticides in food matrices employ DI-SPME using a PDMS coating [148]. The main reason supporting this fact is that PDMS as a liquid coating suffers less from the

irreversible fouling effect caused by the matrix components compared to solid coatings, making the PDMS coating the most robust option for the direct analysis of complex matrices. As such, PDMS has often been selected as the coating of choice regardless of its sensitivity towards the analytes of interest. Jahnke and Mayer corroborate this hypothesis in a recent publication investigating the effects of non-volatile matrix macromolecules fouling the PDMS, concluding that the sorptive properties of PDMS were not modified, and consequently, that PDMS is suitable for sampling of highly complex matrices [149]. Moreover, the authors mentioned that fouling of the PDMS coating might still occur in highly complex matrices, but a physical cleaning of the polymer is sufficient to circumvent this problem.

All the aforementioned limitations, together with preliminary experimental findings, have led to the exploration of the possibility of modifying existing commercial SPME fiber coatings to achieve matrix-compatibility while retaining their original coating sensitivity towards the analytes of interest. In the present study, grapes were chosen as a complex matrix model, and triazoles pesticides, which are vastly applied in vineyards, as model analytes due to their low volatility. The modified SPME fiber was tested for extraction efficiency and robustness when directly subjected to grape matrix.

2.2 Materials and Methods

2.2.1 Chemicals and Materials

Triazole pesticide standards (triadimefon, penconazole, triadimenol, hexaconazole and diniconazole) were Pestanal grade purchased from Sigma-Aldrich (Oakville, ON, Canada). Individual solutions (c.a. 20 mg/mL) of each pesticide were prepared in methanol. A mixture standard stock solution containing 2000 mg/L of each pesticide was prepared in methanol. Different working standard solutions (0.1 - 200 ng/µL of each pesticide) were prepared by dilution in the same solvent. Benzene, toluene, ethylbenzene, and o-xylene were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sylgard 184 (PDMS prepolymer and curing agent) was purchased from Dow Corning (Midland, MI, USA). Commercial SPME fibers (PDMS 100 µm, PDMS/DVB 65 µm, DVB/Car/PDMS 50/30 µm, Car/PDMS 75 µm and PA 85 µm) were purchased from Supelco (Bellefonte, PA, USA).

2.2.2 Preparation of PDMS-modified coating

Sylgard 184 PDMS prepolymer and curing agent were mixed at a 10:1 ratio, according to the manufacturer's manual, into a polypropylene centrifuge tube and subjected to centrifugation for 3 min at 4000 rpm for degassing. The coating procedure consisted of immersing the commercial PDMS/DVB fiber into the PDMS solution, and subsequently pulling out at a slow rate of approximately 0.5 mm s^{-1}. Passing it through a micropipet tip of about 350 µm diameter aperture ensured that a thinner layer was formed, with excess polymer being removed. After the coating process, the coated fiber was placed in a vacuum oven at 50 °C

under N_2 flow for 12 h. The coating/curing process was repeated twice to ensure complete and uniform coverage. Prior to use, the fiber was conditioned in a GC injection port (PTV) under helium flow from 100 °C (hold for 5 min) to 250 °C (hold for 30 min) at 5 °C/min. The fiber was then conditioned again at 250 °C for 10 min. The 10-min conditioning cycle was repeated a few more times until a stable GC baseline was obtained. After curing and conditioning, the modified coatings were inspected using an optical stereomicroscope to ensure that a thin layer of smooth surface was achieved.

2.2.3 Instrumentation

In order to verify the topography of the coating surface as well as the thickness of the PDMS outer layer, scanning electron microscopy (SEM) images were acquired using an LEO 1530 field emission (Carl Zeiss NTS GmbH, Germany).

Analysis of triazole pesticides was performed on a Saturn 3800 GC/2000 ITMS system fitted with a HP-5MS column (30 m, 0.25 mm i.d., 0.25 μm film thickness) (Hewlett-Packard, Avondale, PA). Helium as the carrier gas was set to 1.5 mL/min. The 1079 injector was set to a temperature of 260 °C (unless otherwise specified). The column temperature program was initially set at 70 °C for 2 min, ramped at 40°C/min to 235°C for 1 min, ramped at 3 ºC/min to 250 ºC, and then ramped at 40 ºC/min to 280 ºC and held for 12.12 min, giving a total run time of 24 min.

For water sample analysis, the ion trap was operated in full scan mode (MS), whereas for grape pulp matrix, the analyzer was operated in tandem mode (MS/MS). The MS operational conditions were: electron ionization (EI) was always 70eV; temperatures of 180, 50 and 260 °C for the trap, manifold and transfer line respectively; initially a mass range of 55-325 m/z was scanned to confirm the retention times of the analytes. The multipler voltage ($1x10^5$ gain) was 1600V with a multipler offset of +200V. Automatic gain control (AGC) was turned on with an AGC target value of 20000 counts for EI-MS and 2000 counts for EI-MS/MS; the emission current was 10 µA for MS and 80 µA for MS/MS. For MS/MS, the AGC pre-scan ionization time was 1500 µs, and the isolation window 3m/z (except for diniconazole, where a 5m/s window was used). All specific MS/MS conditions for the studied triazole pesticides are listed below.

Table 2-1 Optimum MS/MS parameters for the selected target compounds.

Compound	Start time (min)	m/z Range	Parent ion (m/z)	Quantitation ion (m/z)	Excitation storage level (m/z)	Excitation amplitude (volts)
Triadimefon	8.3	125-300	208	144+180	75	63
Penconazole	8.7	130-300	248	157+192+206	100	84
Triadimenol	8.9	65-300	168	70	48	38
Hexaconazole	9.5	100-325	231	175+213	100	70
Diniconazole	10.5	200-340	268	232:234	118	86

For BTEX analysis, the column was initially set at 40°C, held for 4 min at this temperature, ramped at 15°C/min to 130°C, and held at this temperature for 5 min. In both cases, automated analysis was performed

using a CTC CombiPal autosampler (Zwingen, Switzerland) using the associated Cycle Composer software (Version 1.4.0). The PAL was equipped with a SPME fiber holder, a temperature controlled six-vial agitator tray, and a fiber-conditioning device.

2.2.4 SPME Procedure

2.2.4.1 Triazoles analysis in grapes

Different types of commercial SPME fibers were evaluated using DI-SPME and HS-SPME modes in order to ensure the best extraction efficiency for all triazole pesticides. For this purpose, uncontaminated white grapes, purchased at local markets in Waterloo (ON Canada), were manually stemmed, washed with deionized water, dried, and crushed using a blender.

For HS-SPME extraction mode, a sample aliquot (4g) was weighed in a 10 mL vial, fortified at 500 ng/g. Spiked analytes in grape matrix were pre-incubated at room temperature for 60 min prior to extraction to allow for the analytes-matrix binding to occur. A 5 min incubation of the sample was performed in the agitation unit at 500 rpm at 70°C, followed by a 60 min extraction at 70ºC, while stirring at 500rpm. Following extraction, the fiber was placed in the GC injection port for desorption for 7 min at temperatures 10ºC below the maximum operational temperature recommend by the manufacturer.

For DI-SPME extraction mode, a sample aliquot (9g) was weighed in a 10 mL vial, fortified at 100µg/g. A 5 min incubation of the sample was performed in the agitation unit at 500 rpm and at 30°C, followed by a 30 min extraction at

30ºC, while stirring at 500rpm. Following extraction, the fiber was desorbed using the same above-mentioned conditions.

2.2.4.2 BTEX analysis in water

The kinetics of extraction of BTEX by the PDMS-modified fiber were studied and compared with the extraction kinetics obtained with the commercial fiber PDMS/DVB 65µm. Aqueous solutions containing 100 µg/L of each solute (BTEX) were daily prepared. The extractions were performed with the fiber exposed to the headspace of a 10 mL vial filled with 3 mL of solution (7 mL of headspace volume). A 5 min pre-extraction equilibration of the sample was performed in the agitation unit at 500 rpm and at 30°C. Varying extraction times, between 15 sec and 20 min, were applied. All experiments were performed in triplicate.

2.2.4.3 Triazoles analysis in water

This approach was also employed to compare the extraction kinetics between the commercial fiber PDMS/DVB and the PDMS-modified fiber. An aliquot of 18 mL of an aqueous solution containing c.a. 5.5 µg/L of each triazole was placed into a 20-mL vial. A 5 min incubation of the sample was performed in the stir plate while stirring at 1200 rpm and at 30°C. Extraction time ranged from 5 to 1440 min. Following extraction, the fiber was placed in the GC injection port for desorption for 7 min at 260ºC. All extraction time points were performed in duplicate.

2.3 Results and Discussion

2.3.1 Evaluation of commercial SPME coatings

Generally, the selection of fiber coating is the first step in SPME method development. For this reason, various types of commercial coating fibers, namely PDMS 100 μm, PDMS/DVB 65 μm, DVB/Car/PDMS 50/30 μm, Car/PDMS 75 μm and PA 85 μm, were compared in terms of extraction efficiency, while extraction (30 min) and desorption times (7 min), and sample temperature (30 ºC) were kept constant.

Another crucial parameter to be optimized in SPME method development is the extraction mode. Conventionally, HS-SPME mode is used for the extraction of analytes from complex samples in order to protect the fiber coating from damage by high molecular masses and other non-volatile interferences present in the sample matrix. Conversely, DI-SPME mode should be used for the extraction of compounds with low-to-medium volatility. In the present study, a difficult system comprised of a highly complex matrix (grapes) and analytes of low volatility was studied, as seen in *Table 2-2*.

Table 2-2 Summary of target triazole physicochemical properties.

Analyte	Molecular Weight g/mol	Solubility in Water mg/L	Log P (at pH 7, 20°C)	Vapour Pressure (at 25°C, mPa)	Henry's Law Constant ($Pa.m^3.mol^{-1)}$
Triadimefon	293.8	70	3.18	0.02	$9x10^{-05}$
Penconazole	284.18	73	3.72	0.366	$6.6x10^{-04}$
Triadimenol	295.76	72	3.18	0.0005	$3.5x10^{-06}$
Hexaconazole	314.21	18	3.9	0.018	$3.33x10^{-04}$
Diniconazole	326.2	4	4.3	2.96	$4x10^{-02}$

Figure 2-1 shows the extraction efficiency obtained for triplicate extractions of triazole pesticides performed on each fiber. As expected, the obtained extraction of triazole pesticides was much lower in HS-SPME mode, which indicated that the larger molecular weight and the low Henry's law constants of triazoles caused them to fail to be transported through the barrier of air. Conversely, all compounds had very higher extraction efficiencies with DI-SPME for all the fiber coatings assayed relative to HS-SPME mode. Best results were obtained using the PDMS/DVB, where an approximately 100-fold improvement was observed when using DI-SPME compared to HS-SPME.

It is worth noting that after only three extractions in DI mode, an inspection of the fibers on an optical microscope showed dark little spots suggestive of fiber fouling (especially for the solid coatings PDMS/DVB and DVB/Car/PDMS).

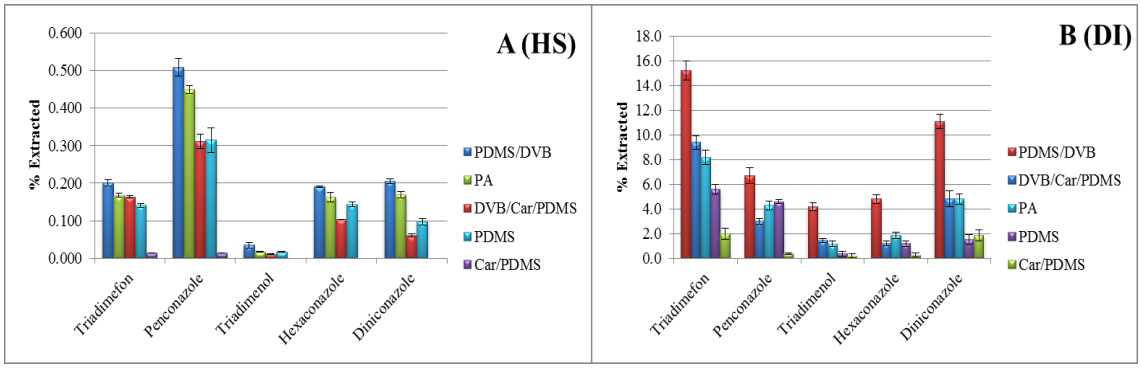


Figure 2-1 Extraction efficiency of commercial fibers in grape matrix. (A) HS-SPME at 70 ºC for 60 min, 4 g grape pulp spiked at a concentration of 500 ng/g; (B) DI-SPME at 30 ºC for 30 min, 9 g grape pulp at a concentration of 100 ng/g. Values are mean (%) of extraction ± standard deviation (n=3).

2.3.2 PDMS/DVB repeatability in grape matrix

A stableflex PDMS/DVB coating was submitted to direct immersion extraction in 20 grape samples. The results obtained showed a steady decrease in extraction efficiency throughout the sequence. More importantly, the PDMS/DVB coating was completely blacked, as shown in the photo presented in *Figure 2-2*.

Fiber fouling is one of the most commonly encountered problems with existing commercial SPME coatings applied to direct extraction in food matrices. Fouling can be very problematic, as it can change the chemistry of the coating, affecting the uptake of the analyte and the reproducibility of extraction, resulting in poor accuracy and decreasing fiber extraction efficiency upon repeated use [150].

Figure 2-2 Microphotography of PDMS/DVB coating after 20 direct immersion extractions in grape slurry.

In an attempt to overcome this problem, a rapid rinsing of the fiber in water after extraction and prior to desorption was evaluated. Nine mililiters of nanopure water spiked with triazole pesticides was submitted to DI-SPME using PDMS/DVB fiber (all other extraction parameters kept the same). Rinsing times, varying from 20 s to 60 s, were tested and compared to extractions without fiber rinsing in order to account for any substantial analyte loss. It was observed that no significant loss of analyte occurred up to 50 s rinsing; thus, 50 s was chosen for further experiments (data not shown).

Subsequently, grape samples were subjected to extraction with the PDMS/DVB coating, applying a 50 s rinsing in DI water between the steps of extraction and desorption. Overall, the obtained results demonstrate an ineffectual improvement in the fiber lifetime. In agreement with De Jager *et al* [145], after 20 extraction/desorption cycles in grape matrix, the PDMS/DVB fiber was blackened, and a substantial decrease in extraction efficiency was observed,

resulting in very irreproducible results. After 10 extractions, the extraction efficiency had decreased by 33-41% and by the 20th extraction, the efficiency had dropped by 83-89%.

The same experimental set up was repeated for the PDMS fiber to evaluate the performance of the PDMS coating, since the ability of PDMS to withstand complex matrices without changes in its sorptive properties has been subject to study [149]. The results obtained for both sets of experiments are shown in *Figure 2-3*.

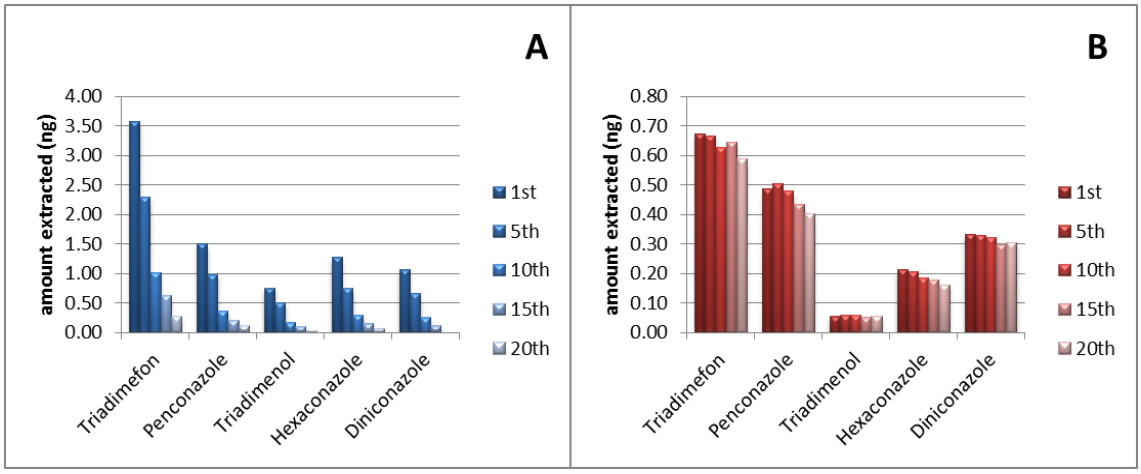


Figure 2-3 Repeatability of commercial fibers in grape matrix, as represented by amount of analyte extracted after multiple extraction-desorption cycles. (A) PDMS/DVB 65 µm; (B) PDMS 100 µm. Extractions were peformed for 30 min at 30ºC, from 9 g grape pulp with triazole pesticides at a concentration of 100 ng/g.

The results for the PDMS coating show that after 10 extractions, the extraction efficiency had decreased by 1-14 % and by the 20th extraction, the efficiency had dropped by 2-24 %. In agreement with the literature [149,151], the

PDMS coating offered higher repeatability and robustness to directly extract from a complex matrix. At this point, the question of sacrificing sensitivity for robustness and vice-versa arises; in the present work, a new approach is attempted, in which the benefits of the high sensitivity exhibited by the PDMS/DVB and the robustness of PDMS are combined together by applying a thin layer of PDMS over the PDMS/DVB coating surface.

2.3.3 PDMS-modified coating preparation and characterization

The first steps in optimization involved the choice of coating method (spraying or dipping) and overall parameters such as addition of solvent, rate of pulling, and aperture diameter. The spraying method resulted in highly irregular coatings; thus, the work proceeded using dip-coating. The optimized procedure for dip coating is presented in the experimental section.

An optimum PDMS outer layer was obtained for two layers of PDMS, which resulted in optimum surface coverage of the original coating. The study showed that thinner coatings (1 layer) did not ensure total surface coverage, resulting in a coating that still exhibited a porous surface. In addition, thicker coatings (3 layers) resulted in non-uniform surface coverage in terms of thickness throughout the coating length, rendering a weaker physical stability due to excessive thickness, which could led to stripping of the coating when withdrawn inside the fiber needle. After curing and conditioning, the modified coatings were inspected using an electronic microscope to ensure that a thin layer of smooth surface was achieved.

Electronic microphotographs of the PDMS/DVB fiber before and after adding the PDMS external layer are shown in *Figure 2-4*. The microphotographs show the formation of a very thin PDMS film on the surface of the PDMS/DVB fiber. The image shows a uniform, non-porous, and smooth surface throughout the coating.

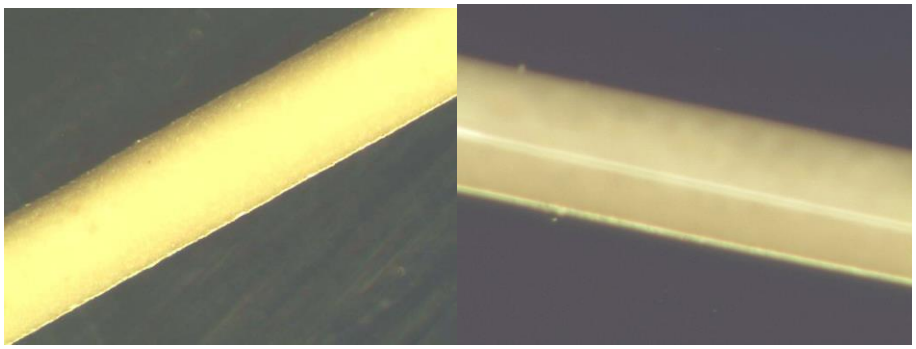


Figure 2-4. Microphotographs of a PDMS/DVB fiber: (A) as commercially available; (B) the same fiber coated with an external PDMS layer.

To verify the topography of the coating surface, as well as the thickness of the PDMS outer layer, scanning electron microscopy (SEM) images were acquired using a LEO 1530 field emission scanning electron microscope (Carl Zeiss NTS GmbH, Germany). *Figure 2-5* shows the SEM images of the coatings after application of a 10nm layer of gold on its surface. The estimate PDMS outer layer thickness for the optimized coating was estimated to be around 25-30 µm.

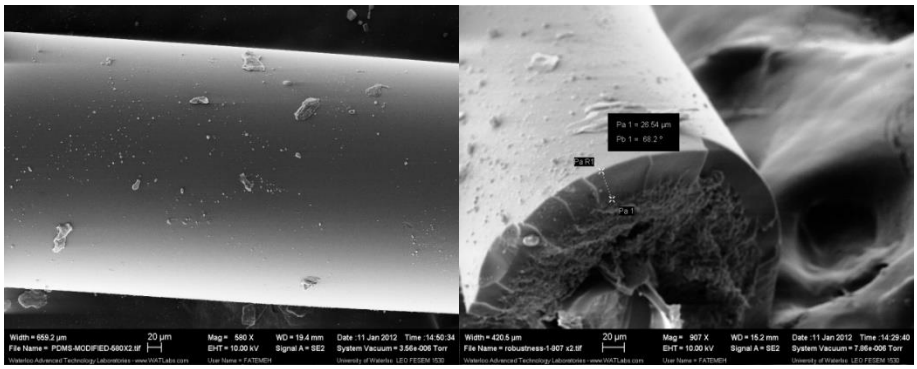


Figure 2-5. SEM images of the PDMS-modified coating (2 layers): (A) Surface morphology using 580x magnification; (B) Estimation of coating thickness using 900x magnification.

2.3.4 Intra-fiber and Inter-fiber Reproducibility

In order to investigate the reproducibility of the PDMS overcoating procedure, the extraction reproducibility was evaluated by performing the extraction of triazole pesticides from water and grape pulp matrices. The results obtained are summarized in Table 3. For all triazole analytes, the intra-fiber (n=4), as well as the inter-fiber reproducibility (three fibers prepared in the same day; 3 replicates each) in both matrices was found to be very good, as indicated by R.S.D values ranging from 0.1 to 11.4%.

Table 2-3 Intra-fiber and inter-fiber reproducibility of PDMS-modified fibers for extraction of triazole pesticides from water and grape pulp.

	Intra-fiber (*n* = 4)	**Inter-fiber (3 fibers, *n* = 9)**
Water (% R.S.D.)	0.1-2.4	0.1-8.5
Grapes (% R.S.D**.)**	0.3-7.6	4.0-11.4

2.3.5 BTEX Extraction

At first, HS-SPME analysis of BTEX compounds was used to evaluate the effect of the external PDMS layer on the extraction capabilities of the PDMS/DVB coating by comparing its extraction time profiles with those obtained with a commercial PDMS/DVB 65 µm fiber.

As expected, the slopes of the initial stage of the adsorption profile were slightly decreased, indicating that the kinetics of extraction for those analytes were, even if in a small proportion, influenced by the additional barrier. In this new configuration, the analytes must first diffuse through the PDMS interface prior to the adsorption in the solid DVB coating. Since this in-between phase is a liquid polymer where analytes have low diffusion coefficients, the mass transfer is slowed down and the extraction process is kinetically limited.

Additionally, as can be seen in *Figure 2-6*, the amount of analyte extracted at equilibrium or near equilibrium by the PDMS-modified coating is higher than the amount extracted by the commercial PDMS/DVB. In fact, the PDMS-modified coating presents a higher total volume when compared to the original fiber. The additional PDMS layers has a volume of about 0.260µL (assuming average PDMS layer thickness of 27.5µm), rendering the coating a total volume of about 0.700 µL against the 0.440 µL presented by the commercial PDMS/DVB stableflex fiber. Despite the above-mentioned calculations being only rough approximations, an estimate of how the performance of the modified coatings compare to commercial one based on different dimensions can be a useful tool. In

this new configuration, the PDMS layer does not only plays a role as a barrier slowing down the kinetics of extractions, but also acts as an additional extraction phase for analyte concentration, hence, changing the total analyte capacity of the coating. In such configuration, the total equilibrium amount of the analyte extracted is the sum of its amounts in both layers. In contrast to standard membrane techniques, analytes in the PDMS outer layer phase were also transferred to the gas chromatograph injection port, since PDMS is part of the probe. Moreover, the obtained results suggest that no blockage of the extraction sites occurred on the surface of the original PDMS/DVB coating due to the additional PDMS layer, thus, without any impairment occurring on the extraction capabilities of the original coating.

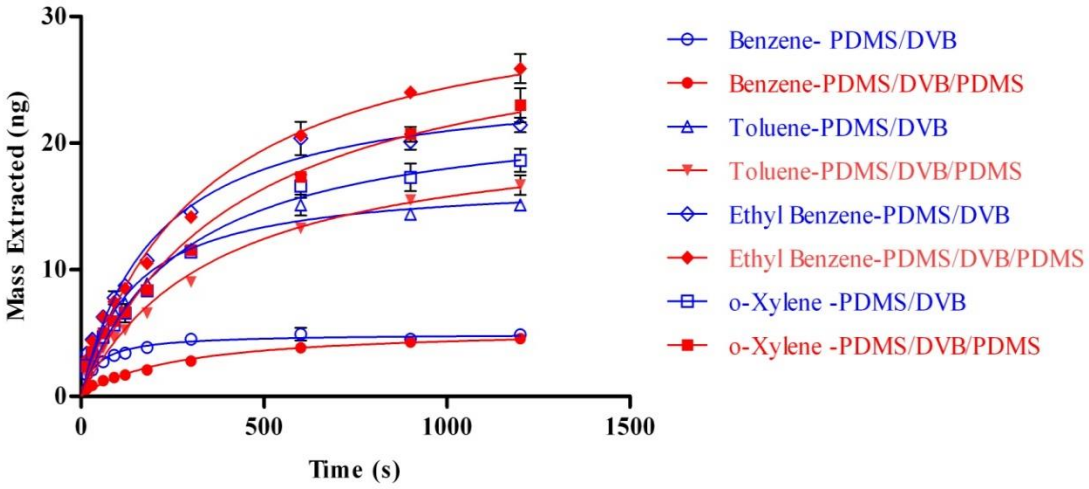


Figure 2-6. Comparative extraction equilibration for BTEX obtained with the commercial PDMS/DVB and modified PDMS/DVB/PDMS fiber.

2.3.6 Triazoles Extraction

If the additional layer of PDMS had substantially affected the extraction of triazoles pesticides in the overall extraction process, then different extraction profiles would be derived from the comparison of the commercial PDMS/DVB fiber and the modified PDMS/DVB/PDMS.

Extraction time profiles for triazoles pesticides in water obtained with the commercial and modified PDMS/DVB fibers are shown in *Figure 2-6* to *Figure 2-11*. As expected, in comparison to the commercial PDMS/DVB, the profile of extraction obtained with the PDMS/DVB/PDMS fiber resulted in a very similar profile for all the triazoles pesticides studied. In corroboration with the findings for BTEX extractions, some differences in extraction efficiency could be observed at shorter extraction times due to the additional step of diffusion of the analytes through the thin PDMS outer layer. Additionally, the amount of analyte extracted at equilibrium or near equilibrium by the PDMS-modified coating is either the same or slightly higher than the amount extracted by the commercial PDMS/DVB.

In the present study, it seems that the PDMS layer did not substantially change neither the kinetic nor the thermodynamic parameters associated to the original coating. Hence, in the present studied matrix-analytes-coating system, it can be understood that the diffusion through the PDMS outer layer dictates the rate of mass transfer at short pre-equilibrium extraction times. Conversely to our findings, Kloskoeski *et al.* [152] presented a system comprised of a polyethylene

glycol (PEG) coating restricted within a PDMS outer layer. The authors referred to the system as a membrane-SPME, where the external layer of PDMS of 25µm significantly slowed down the diffusion of the polar phenol analytes across the PDMS membrane, which could serve as a physical barrier as well as a concentrating medium, analogous to the extraction phase.

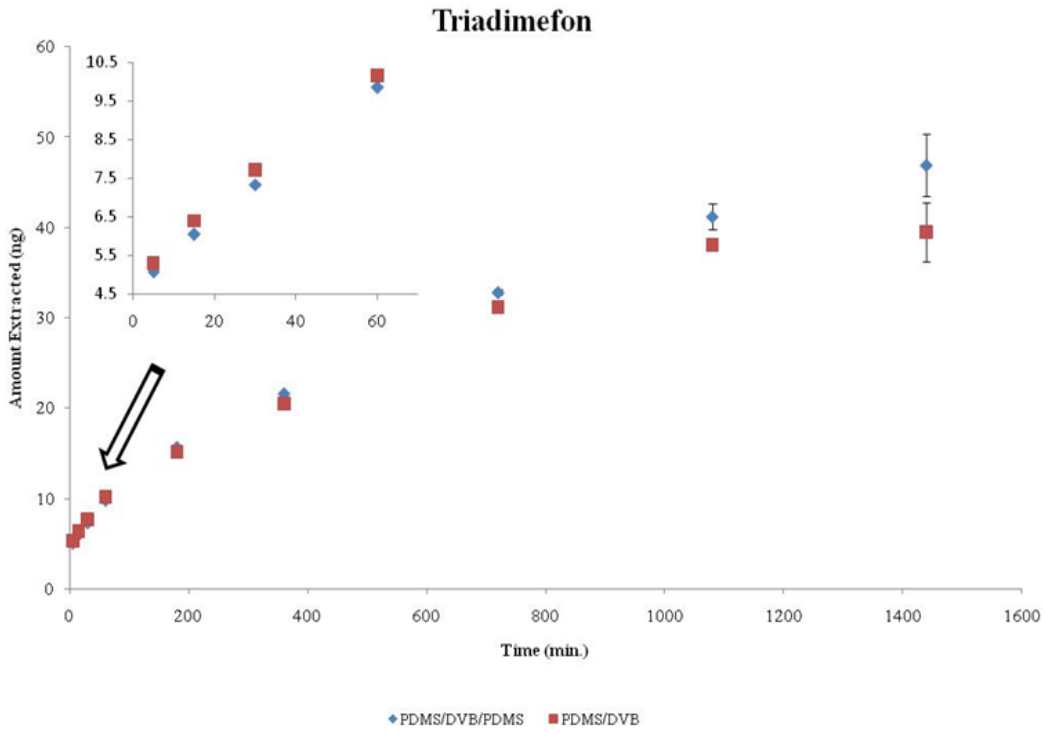


Figure 2-7 Comparative extraction time profiles for triadimefon pesticide using commercial PDMS/DVB fiber and PDMS/DVB/PDMS fiber. Extractions were performed in 18 mL of water spiked at 5.5 ng/mL at 23 ºC (± 2 ºC) and 1200 rpm.

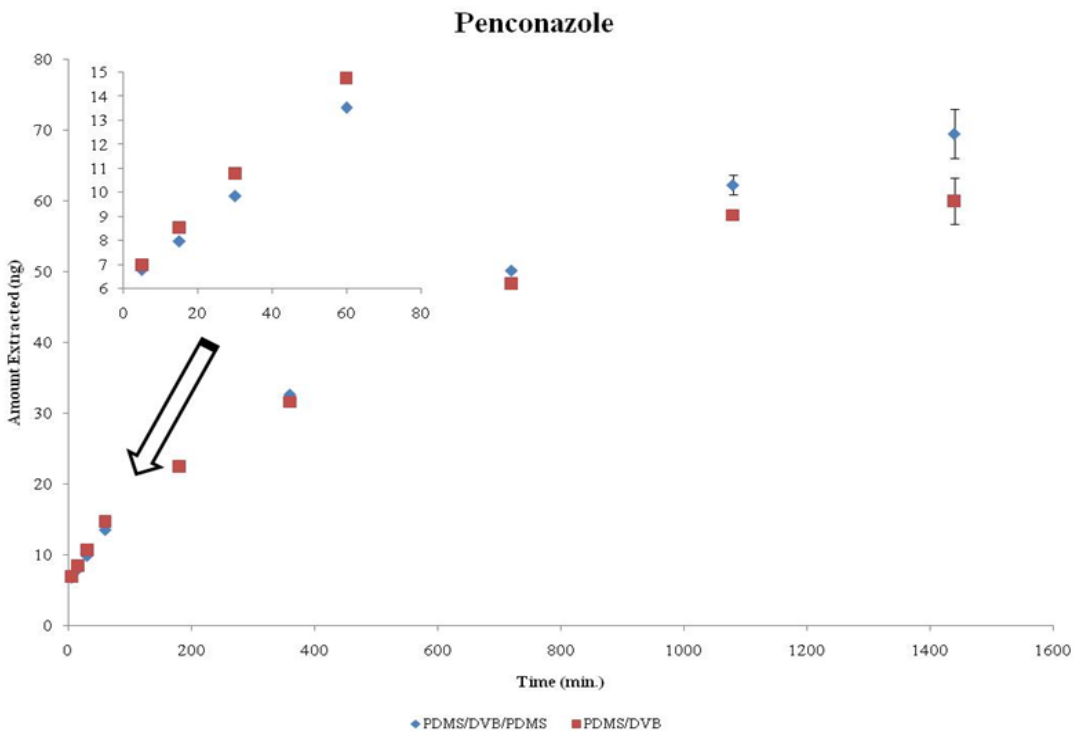


Figure 2-8 Comparative extraction time profiles for penconazole pesticide using commercial PDMS/DVB fiber and PDMS/DVB/PDMS fiber. Extractions were performed in 18 mL of water spiked at 5.5 ng/mL at 23 ºC (± 2 ºC) and 1200 rpm.

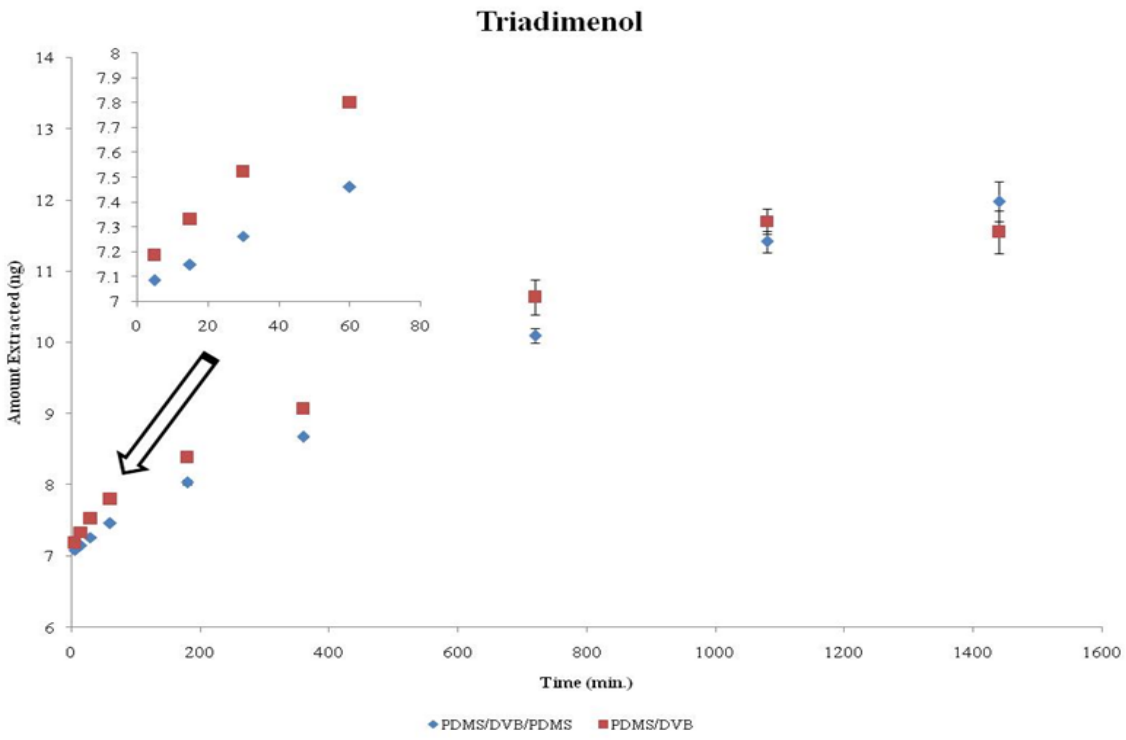


Figure 2-9 Comparative extraction time profiles for triadimenol pesticide using commercial PDMS/DVB fiber and PDMS/DVB/PDMS fiber. Extractions were performed in 18 mL of water spiked at 5.5 ng/mL at 23 ºC (± 2 ºC) and 1200 rpm.

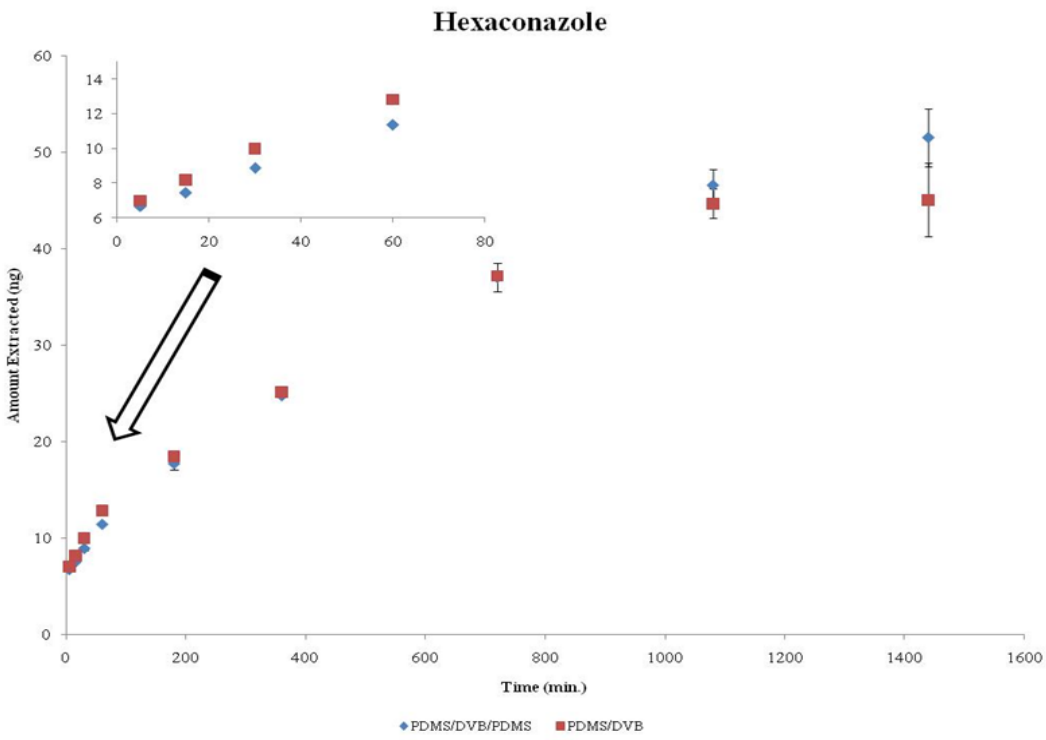


Figure 2-10 Comparative extraction time profiles for hexaconazole pesticide using commercial PDMS/DVB fiber and PDMS/DVB/PDMS fiber. Extractions were performed in 18 mL of water spiked at 5.5 ng/mL at 23 ºC (± 2 ºC) and 1200 rpm.

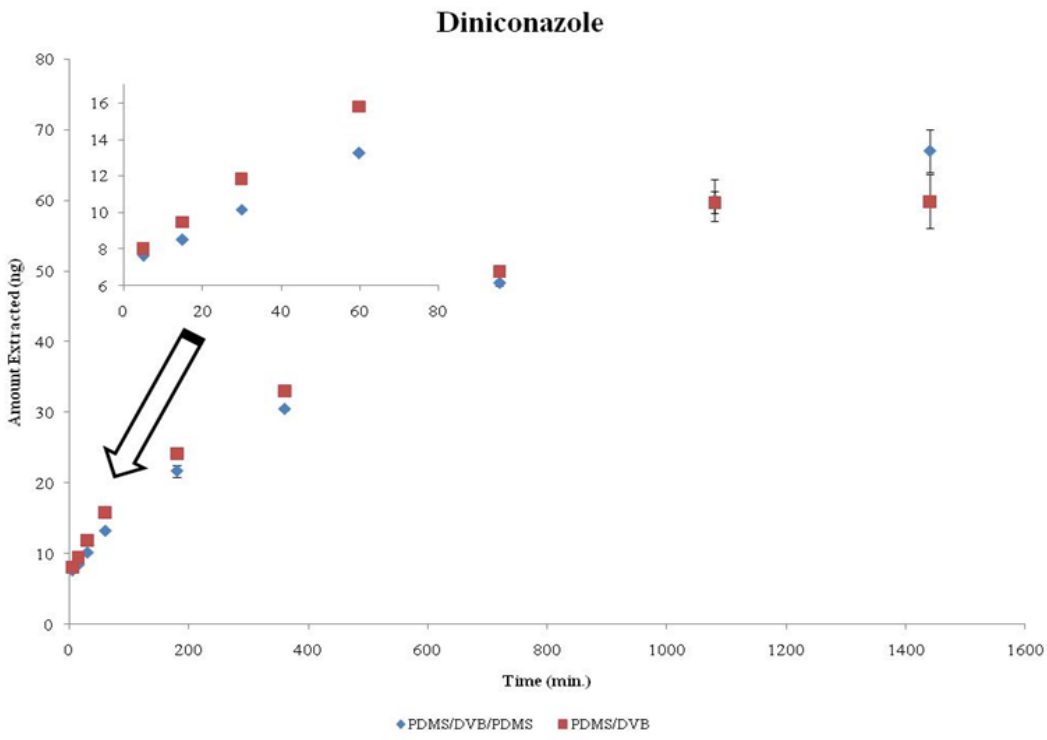


Figure 2-11 Comparative extraction time profiles for diniconazole pesticide using commercial PDMS/DVB fiber and PDMS/DVB/PDMS fiber. Extractions were performed in 18 mL of water spiked at 5.5 ng/mL at 23 ºC (± 2 ºC) and 1200 rpm.

2.3.7 Fiber long-term reusability

The stability of the coatings over the time is yet another practical parameter of top importance in SPME methodology. One of the problems commonly encountered with existing commercial SPME coatings applied in food analysis is the significant fouling of these coatings on exposure to highly complex matrices.

To determine endurance and reusability, the modified PDMS/DVB/PDMS fiber was subjected to a series of 130 successive direct immersion SPME cycles in

whole grape pulp. Each cycle consisted of a 15 min extraction at 30ºC; 50 s rinsing in de-ionized water prior to desorption; 7 min desorption at 260º; post-desorption washing in de-ionized water for 2.5 min; and 2.5 min fiber conditioning at 250º in the autosampler conditioning station device. In the present experiment, the fiber was constantly inspected under an electronic microscope (every 10 cycles) and, when needed, manually freed of any possible debris attached to its surface by simply using a KimWipe® tissue. No irreversible damage on the surface was observed. Moreover, QCs consisting of water samples spiked with triazole pesticides were distributed along the batch to ensure that the fiber performance was not altered.

As presented in *Figure 2-12*, the fiber endurance measured as amount of analyte extracted throughout the experiment presented RSDs below 20%. Taking into account the complexity of the studied matrix, this is an impressive achievement, with a performance highly superior to that exhibited by the original commercial fiber. Only one triazole pesticide (triadimefon) exhibited pronounced variations throughout this study. One possible explanation for this behaviour could be the fact that triadimefon can undergo biotransformation in rich organic matter media [153,154].

It is also worth noting that the amount of analyte extracted by SPME is proportional to the free (unbound) concentration of the analyte in the sample matrix. In addition, in the present study, a short pre-equilibrium extraction time was employed, which would explain the small amounts of absolute recoveries

observed for all analytes. If sensitivity is an issue, this can be overcome by applying longer extractions time.

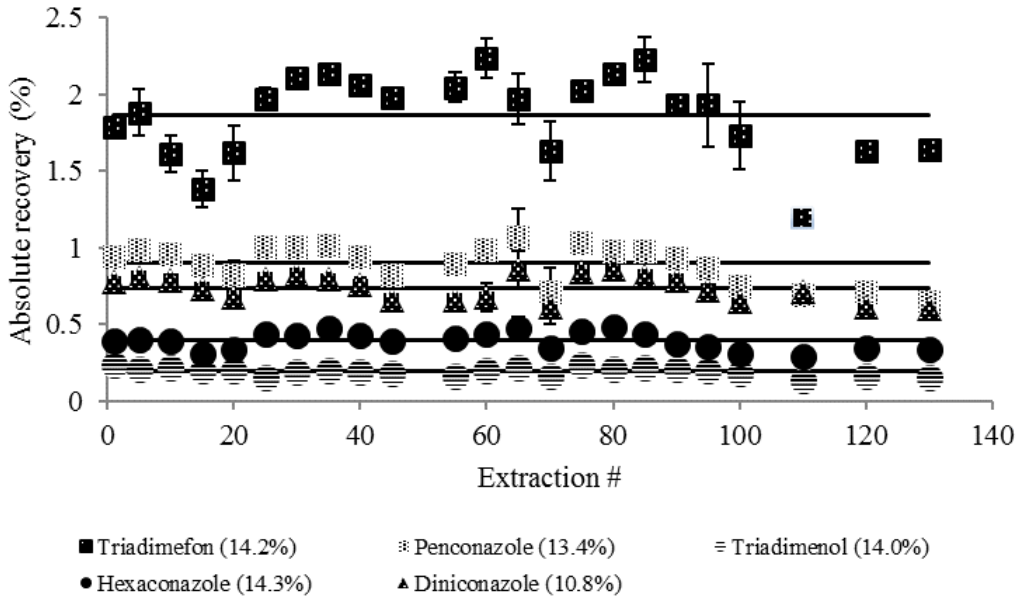


Figure 2-12 Reusability of the PDMS/DVB/PDMS fiber in DI-SPME mode in grape pulp for studied triazole pesticides. Numbers in brackets and solid lines represent the pooled R.S.D. (%) values and average values over the 130 extractions, respectively.

In terms of reusability, due to the very complex matrix, there was a drop in the amount extracted after the 90th extraction, but the amount extraction remained reproducible up to the 130th extraction. Despite that, the reusability of this fiber coating over 50 extractions in such a complex food matrix is alone an exceptional achievement. For example, De Jager et al. reported a 65% drop in signal by the 10th extraction when analyzing food samples diluted in water using a PDMS/DVB fiber in DI-SPME mode [155]. Moreover, one could expect that by employing the

right calibration technique, i.e. the use of internal standard to compensate for any possible variation in the method, the coating could be easily reusable for over 100 extractions in complex food matrices such as pure grape pulp.

It should also be noted that an accumulation of high molecular pigment compounds on the inner DVB phase, causing discoloration of this phase, was observed along the slight downwards trend on extraction efficiency.

Finally, the improvement achieved by the newly modified coating is illustrated in *Figure 2-13*, where microscope pictures of the surface morphologies for the commercial PDMS/DVB coating fiber and the PDMS-modified coating fiber are presented. The extent of fouling on the surface of the coating was dramatically reduced by the application of the PDMS outer layer.

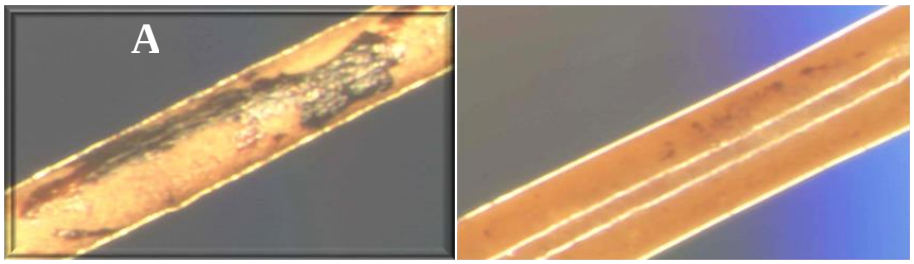


Figure 2-13 Microphotographs of the PDMS/DVB coating after 20 extractions cycles in grape (A); and PDMS/DVB/PDMS coating after over 130 extractions cycles in grape (B).

2.4 Summary

In the present study, a DI-SPME-GC/MS method to analyze triazole pesticides in grape matrix using a commercially available fiber was attempted. However, it is a known fact that DI-SPME is a poor choice for complex matrices analysis when using solid sorbents. To address the previously established limitations of DI-SPME analysis in complex food matrices, a preliminary study of a new concept of modified SPME fiber coating suitable for direct extraction from highly complex food matrices is presented. A procedure for preparing the newly modified fiber was developed. Subsequently, its extraction capabilities were proven similar to those exhibited by the original commercial PDMS/DVB coating.

Results suggested that the PDMS layer did not substantially change the extraction efficiency associated to the original coating. The results showed that the modified PDMS-DVB/PDMS coating provided enhanced robustness in highly complex food matrices such as grape pulp when compared to the original commercially available PDMS/DVB.

The developed coating enabled the performance of direct immersion SPME in a complex food matrix in the worst-case scenario, i.e., without the use of any sample pre-treatment.

The practical aspects of the PDMS-modified coating create new opportunities for SPME applied in food analysis. The creation of perfectly smooth, uniform, non-fouling surfaces is one of the major prerequisites for food applications, and to date, no commercially available fiber is suitable for such applications.

To expand the understanding of the kinetics and thermodynamics parameters associated with the addition of a PDMS outer layer to the coating, additional research investigating the PDMS-modified coating capabilities towards analytes bearing different physical-chemical properties is presented in Chapters 4 and 5 of this thesis.

2.5 Addendum

The text of this chapter was rewritten in comparison to the published research article.

3 Simple and Ultra-sensitive Method for Determination of Triazole Fungicides in Fruits

3.1 Preamble and introduction

3.1.1 Preamble

This chapter has been published as a paper: Souza Silva, E A., Lopez-Avila, V. & Pawliszyn, J., Fast and robust direct immersion solid phase microextraction coupled with gas chromatography-time-of-flight mass spectrometry method employing a matrix compatible fiber for determination of triazole fungicides in fruits. *J. Chromatog. A*, 1313, 139-146 (2103). The figures and tables are reprinted from this publication with permission from Elsevier (Copyright Elsevier 2012). The contribution of co-author Viorica Lopez-Avila was technical advice and scientific discussion. All of the work reported within this chapter has been performed solely by the author.

3.1.2 Introduction

The use of pesticides in agricultural practices is necessary to guarantee the production of quality, disease-free produces. However, ever increasing is the concern related to the rightful application of pesticides and the possible threats posed by their residues found in food commodities. Triazole pesticides are part of the new generation of pesticides, considered “less” harmful than the older pesticides, yet as efficient. Triazoles represent the most important category of

fungicides that have excellent protective, curative and eradicant power towards a wide spectrum of crop diseases. Triazoles are widely used in fruit crops, especially in vineyards. Their characteristics, such as high chemical and photochemical stability, and moderate lipophilicity, make them persistent in the environment. Besides their antifungal activity, triazoles are endocrine disruptors and considered harmful to human health.

Therefore, developing a simple, fast and reliable method to monitor the residues of triazoles in food commodities is of utmost importance. Nevertheless, this is not a simple task, as foodstuffs are well known by their complex nature. Due to the complex nature of food matrices, efficient sample preparation is crucial for trace-level detection and identification of contaminants.

Various analytical methods have been reported in the literature for the determination of triazoles in water and food products [61,156–168].

The most recent methods have focused on the employment of more environmentally friendly sample preparation techniques such as QuEChERS [169,170], microwave assisted extraction (MAE) [170,171], liquid phase microextraction (LPME), dispersive liquid-liquid microextraction (DLLME) [172,173], air-agitated liquid-liquid microextraction (AALLME) [172], solid phase microextraction (SPME) [163–165], and a combination of methods such as stir bar sorptive extraction/dispersive liquid-liquid microextraction [167], and solid phase extraction/hollow fiber solid phase microextraction (SPE-HF) [160]. Nonetheless, the development of automated methodology to analyze pesticides in food matrices

can present an important advantage of high sample throughput, with the entire analysis being completely automated.

In this context, SPME coupled to GC analysis addresses the need to facilitate rapid sample preparation and integrate sampling, extraction, concentration and sample introduction to an analytical instrument into one solvent-free step. However, the suitability of the fiber coating for the analysis of less volatile analytes such as triazoles, which requires direct immersion of the fiber into the matrix, has been a major impediment to the development of fast and simple SPME-GC methods for the determination of triazoles in food.

Fortunately, all aforementioned limitations of SPME have been successfully addressed based on the results obtained in Chapter 2 during the development of a GC-based food matrix compatible SPME. Therefore, the work presented in Chapter 3 focused on the development of a fast and robust DI-SPME-GC-ToFMS method for the simultaneous extraction and determination of ten triazole fungicides in grapes and strawberries without any sample pre-treatment, employing the developed PDMS-overcoated PDMS/DVB coating. Furthermore, the performance of the DI-SPME method was compared to results obtained using the well-stablished QuEChERS method.

To the best of this author's knowledge, this was the first time that such analytical methodology employing the newly developed matrix-compatible SPME coating was documented in the literature.

3.2 Materials and Methods

3.2.1 Chemicals and Materials

Triazole pesticides standards were Pestanal grade from Sigma-Aldrich (Oakville, ON, Canada), with the exception of tebuconazole-d_6 (100 ng/µL in acetone), which was obtained from EQ Labs (Atlanta, GA, USA). Individual stock solutions (ca. 20 mg/mL) of each pesticide were prepared in methanol. A mixture standard stock solution at 2000 mg/L of each pesticide was prepared in methanol from individual stock solutions and stored at -30°C. Various working standards at 2-200 ng/µL for each pesticide were prepared by serial dilution in methanol for SPME experiments and acetonitrile for QuEChERS experiments, and stored in a refrigerator (4°C) for up to 2 months.

The commercial SPME fiber PDMS/DVB 65 µm was purchased from Supelco (Bellefonte, PA, USA). Sylgard 184® (PDMS pre-polymer and curing agent) was purchased from Dow Corning (Midland, MI, USA).

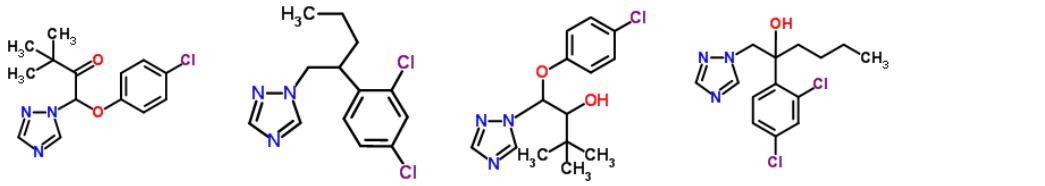
QuEChERS kits were purchased from Supelco (Bellefonte, PA, USA). They consisted of 50-mL centrifuge tubes containing 6 g of anhydrous $MgSO_4$ and 1.5 g sodium acetate, and 15-mL centrifuge tubes for dispersive solid-phase extraction (d-SPE) containing 400 mg of primary secondary amine (PSA) and 1200 mg of anhydrous $MgSO_4$.

The physical-chemical properties of triazole pesticides targeted in this study can be seen in *Table 3-1*, as well as their respective structures depicted in Figure 3-1.

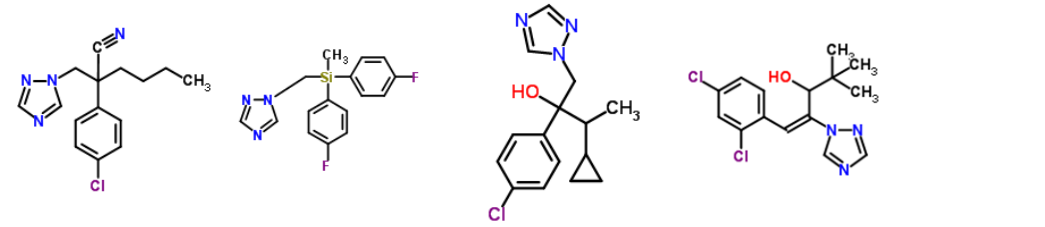
Table 3-1 Physical-chemical properties of the target triazole pesticides studied.

Pesticide	**CAS #**	**MW (g/mol)**	**Boiling Point (ºC)**	**Solubility in water at 20ºC (mg/L)**	**LogP (pH7, 20ºC)**	**pKa (25ºC)**	**Vapour Pressure at 25º C (mPa)**	**Henry's law constant at 25ºC (Pa.m3.mol-1)**
Cyproconazole	94361-06-5	291.78	479	93	3.09	n.a.	2.60E-02	5.00E-05
Diniconazole	83657-24-3	326.22	501	4	4.34	n.a.	2.96E+00	4.00E-02
Flusilazole	85509-19-9	315.39	392	41.9	3.87	2.5	3.87E-02	2.70E-04
Hexaconazole	79983-71-4	314.21	490	18	3.9	2.3	1.80E-02	3.33E-04
Myclobutanil	88671-89-0	288.78	465	132	2.89	2.3	1.98E-01	4.33E-04
Penconazole	66246-88-6	284.18	415	73	3.72	1.51	3.66E-01	6.60E-04
Propiconazole	60207-90-1	342.22	480	150	3.72	1.09	5.60E-02	9.20E-05
Tebuconazole	107534-96-3	307.82	477	36	3.7	n.a.	1.30E-03	1.00E-05
Triadimefon	43121-43-3	293.8	442	70	3.18	n.a.	2.00E-02	9.00E-05
Triadimenol	55219-65-3	295.76	465	72	3.18	n.a.	5.00E-04	3.50E-06

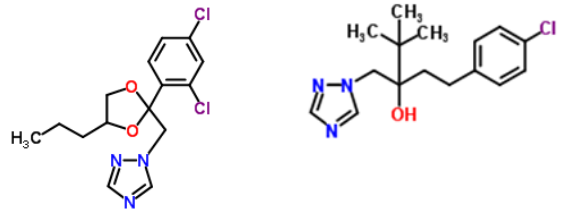
n.a. data not available



Triadimefon **Penconazole** **Triadimenol** **Hexaconazole**



Myclobutanil **Flusilazole** **Cyproconazole** **Diniconazole**



Propiconazole **Tebuconazole**

Figure 3-1 Chemical structure of the target triazole pesticides studied.

3.2.2 Preparation of PDMS-modified coating

PDMS-modified coatings were prepared *in-house* employing the procedure described in Chapter 2. All coatings were prepared at least in triplicate. Prior to their usage, each coating was conditioned at 250ºC for one hour, and visually evaluated for uniformity and smooth surface coverage. If any defect was noted, coatings were discarded and new coatings were prepared.

3.2.3 Instrumentation

A Pegasus 4D instrument consisting of an Agilent 6890N gas chromatograph equipped with a split/splitless injector (Agilent Technologies, Palo Alto, CA, USA), a MPS2 autosampler for automated SPME (Gerstel, Mülheim and der Ruhr, Germany), and a Pegasus III high speed time-of-flight mass spectrometer (Leco Corp., St. Joseph, MI, USA) were used for all experiments reported here.

Chromatographic separation was performed in a Restek Rxi®-5Sil MS capillary (30 m, 0.25 mm i.d., 0.25 µm film thickness) with a 5 m integra-guard column®. Helium was used as carrier gas at 1.5 mL/min. The oven temperature was initially set at 70 °C for 2 min, ramped at 40 °C/min to 200 °C, then ramped at 10 ºC/min to 280 ºC, where it was held for 1.75 min, resulting in a total run time of 15 min. The injector was maintained at 260 ºC (0.75mm I.D. insert used for SPME, and 4mm I.D. insert packed with glass wool at the bottom used for QuEChERS).

MS operational conditions were: electron ionization (EI) at 70eV; ion source temperature: 240 °C; transfer line temperature: 260 °C; mass range: *m/z* 55-350; acquisition rate: 20Hz; detector voltage: -1700V. ChromaTOF (Leco Corp.) software (v. 4.24) was used for instrument control, data acquisition, data processing and peak deconvolution. Library searching was performed using the commercial NIST library. For quantitative purposes, the following ions were selected: m/z 208 for triadimefon; m/z 159 for penconazole; m/z 112 for triadimenol; m/z 214 for hexaconazole; m/z 179 for myclobutanil; m/z 233 for flusilazole; m/z 222 for cyproconazole; m/z 268 for diniconazole; m/z 173 for propiconazole; m/z 250 for tebuconazole; and m/z 256 for tebuconazole-d_6 (internal standard).

3.2.4 Samples

Grapes and strawberries, purchased at local markets in Waterloo (ON, Canada), were manually stemmed, washed with nanopure water, and dried. Samples were crushed and homogenized using a blender, transferred to 200 mL amber glass flasks, then stored in a freezer at -30 °C until analysis. Organically grown fruits were obtained for method development and validation steps (previously analyzed for the absence of the target pesticides); while conventionally grown fruits were obtained for real sample analysis.

3.2.5 SPME Procedure

During SPME method optimization, a 9-g aliquot of whole fruit pulp was weighed in a 10-mL vial and fortified with 5 µL of a specific working standard to

achieve the appropriate analyte concentration, followed by addition of 5 µL of 100 ng/µL tebuconazole-d_6 acetone solution. Pre-incubation for at least 60 min prior to extraction was conducted to allow for the binding of the analyte to the matrix to occur.

The extraction procedure consisted of a 5-min pre-extraction incubation of the sample performed in the agitation unit of the autosampler at 500 rpm and at 50 ºC, followed by a 15-min extraction in direct immersion mode at 50 ºC, while stirring at 500 rpm. Following extraction, the fiber was rinsed in deionized water, and then desorbed for 5 min at 260 °C (+ 2 min fiber bake-out at 260 °C).

Quantification was performed by means of matrix-matched calibration curves, for each matrix. Method validation was performed as to establish the linear dynamic range, limits of quantitation objective (LOQs), and method precision and accuracy.

Throughout the entire study, fibers were submitted to quality control (QC) checks to assure that extraction efficiency remained the same. It is also worth mentioning that the method validation was completed with a single fiber.

3.2.6 QuEChERS Procedure

A modified version of the QuEChERS AOAC Official Method 2007.01 [33] was used in this study. Namely, no solvent exchange/pre-concentration was performed, and only 1 µL of the final acetonitrile extract was injected in the GC system since no PTV injector was available.

Fifteen grams of homogenized fruit were weighed in a 50-mL centrifuge tube and mixed with 15 mL of acetonitrile (1% acetic acid). For recovery studies, samples were spiked with appropriate standards and left to equilibrate for at least 60 min prior to acetonitrile addition. After shaking sample until sufficiently mixed, 1.5 g of sodium acetate and 6 g of $MgSO_4$ were added. Next, the mixture was vortexed for 1 min, followed by 2 min centrifugation at 3000 rpm. Eight milliliters of the extract were transferred to a 15-mL centrifuge tube containing 400 mg of PSA and 1200 mg of $MgSO_4$. The mixture was then vortexed for 1 min, followed by 2 min centrifugation at 3000 rpm. Subsequently, 1 mL of the final extract was transferred to the appropriate GC vials for GC-ToFMS analysis. For analyte quantification, matrix-matched calibration curves were generated for each matrix by adding 100 μL of each respective standard solution to 900 μL of the final extract.

3.3 Results and Discussion

3.3.1 SPME optimization

Optimization of SPME parameters was performed using the one-factor-at-the-time approach. Extraction temperature and time, ionic strength, and desorption time parameters were investigated using a PDMS-modified PDMS/DVB fiber coating. For this purpose, grape samples were spiked at 100 µg/kg.

Extraction temperature was the first parameter to be considered, as it could have opposite effects on the extraction efficiency. Extractions were performed at 30, 40, 50, 60 and 70 °C, while keeping extraction time at 30 min, desorption time at 10 min, and desorption temperature at 260 °C. Figure 3-2 shows that the extraction efficiency for most of the triazole pesticides increased with temperature.

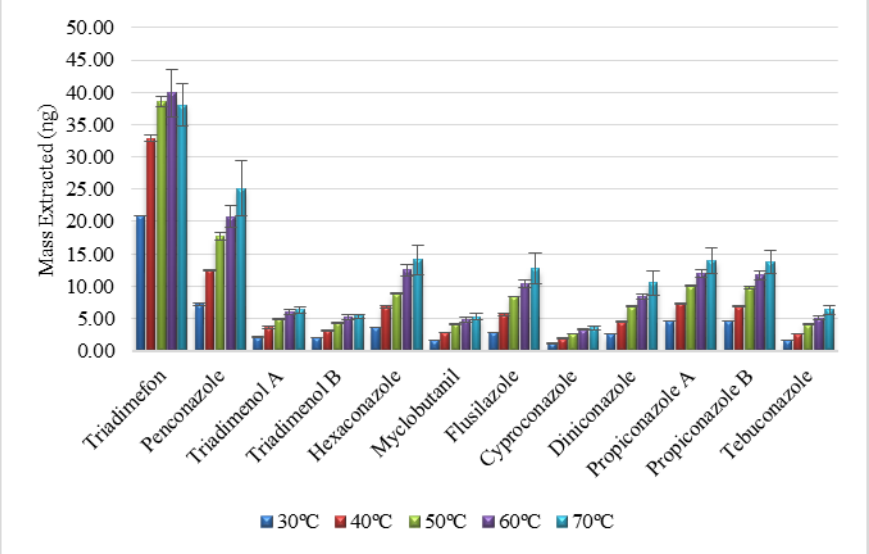


Figure 3-2 Effect of sample temperature on extraction efficiency of triazole pesticides in grape pulp. Error bars represent standard deviation (n = 3).

The increase in extraction efficiency with increasing temperature indicates that the temperature favored the release of analytes from the matrix (organic matter). The release, in turn, transferred analytes to the liquid phase, facilitating their diffusion from the sample matrix to the fiber coating. Although higher extraction efficiencies were observed as temperature increased, method repeatability (RSD %) decreased as temperatures above 50 °C were used. Thus, 50 °C was chosen as the optimal working temperature.

Next, the amount of analyte extracted by the PDMS-modified coating, as a function of the extraction time, was studied by varying the extraction time between 5 to 120 min. Figure 2 shows that the amount of mass extracted increased with extraction time. For most analytes, equilibrium was not reached even after 120 min. As the amount of analyte extracted into the fiber is proportional to the initial concentration in the sample matrix, provided that mass transport conditions and sampling time are strictly controlled, an extraction time of 15 min was selected as a compromise between method sensitivity and practicality of method throughput.

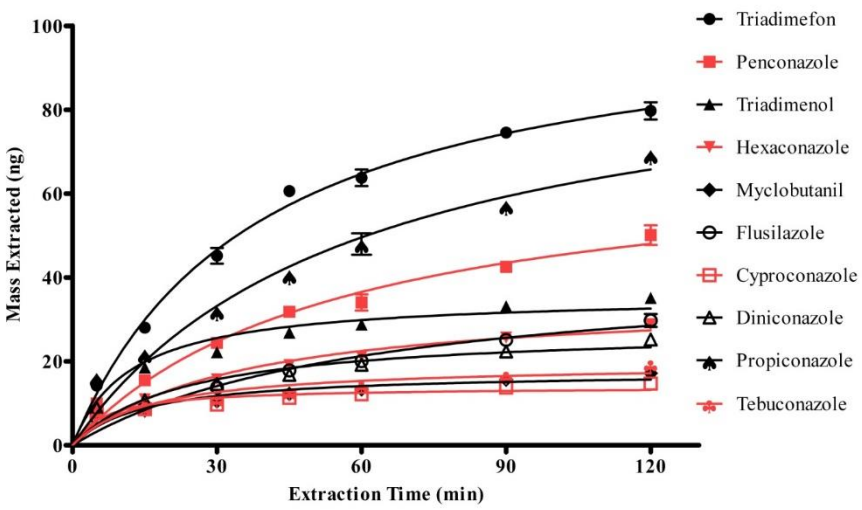


Figure 3-3 DI-SPME extraction time profiles obtained for the studied triazole pesticides in grape pulp. Error bars representing the standard deviation (n=3). Triadimenol and propiconazole data are presented as sum of both isomers.

The effect of ionic strength on extraction efficiency was studied by varying the amount of sodium chloride from 0 to 30 % (w/w). As shown in *Figure 3-4*, for some analytes, mainly for those bearing polar functionalities, the extraction efficiency did not change by more than 10% (see Figure 3-1 for the chemical structures of studied triazoles). For all other triazole pesticides, either no significant positive effect was observed, or a negative effect was observed when NaCl was added.

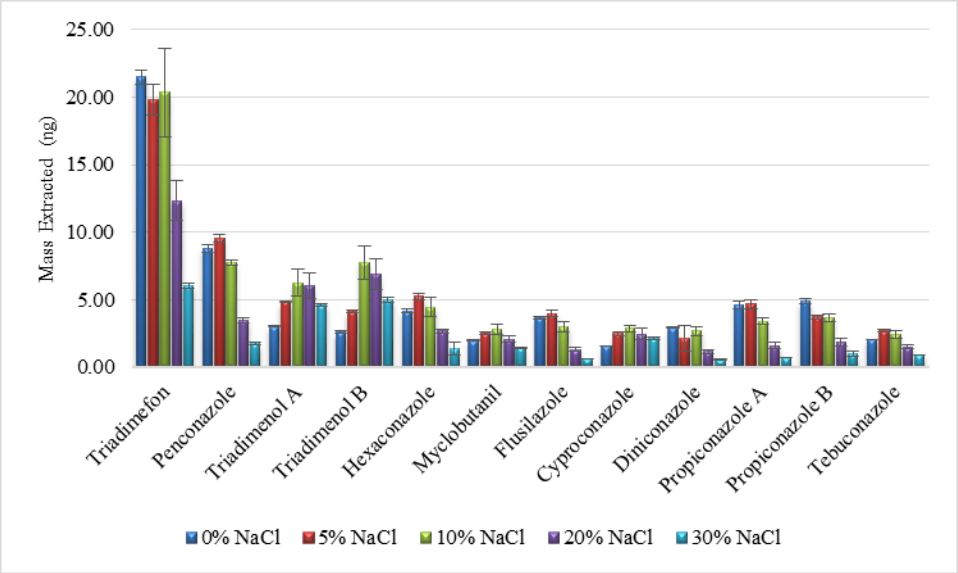


Figure 3-4 Effect of ionic strength on the extraction efficiency of triazole pesticides in grape pulp. Error bars representing the standard deviation (n=3).

Generally, the addition of NaCl causes the "*salting-out*" effect, which decreases the analyte solubility in water, facilitating extraction of analytes by the fiber coating. However, assuming that the pesticides are distributed in the sample in both free-form in the liquid phase of the sample, and bound-form within the sample matrix components (organic matter), salting-out could lead to a negative effect of equilibrium shift towards the bound-form of the analyte. As a result, the amount of analyte extracted by the fiber coating would decrease. Another possible reason leading to such a negative effect could be the deposition of salt on the surface of the fiber, impairing its extraction performance. Therefore, the addition of salt to the sample was not implemented.

Last, desorption time was optimized to prevent carryover of analytes from sample to sample. Desorption temperature was set at 260 °C, 10 °C below the maximum temperature recommended by the manufacturer of the DVB coatings. This temperature was chosen as a good compromise between two factors: a higher temperature would avoid carryover in the fiber coating, not only for the triazole pesticides, but also for the less volatile endogenous matrix compounds co-extracted. However, the temperature should not be so high as to trigger the collapse of the DVB particle pores due to the extensive exposure of DVB to high temperatures, as such collapse causes irreversible damage to the fiber coating. Desorption times were set at 3, 5, 7 and 10 min. Although the data shown in *Figure 3-5* indicated that a 5-min desorption time yielded no significant carryover, when the total ion chromatograms were compared, the observed background was very noisy for shorter desorption times, indicating that the fiber had not been properly cleaned, and that less volatile matrix compounds were still present even after the second carryover run (data not shown). For this reason, a 7-min desorption at 260 °C was chosen for subsequent experiments.

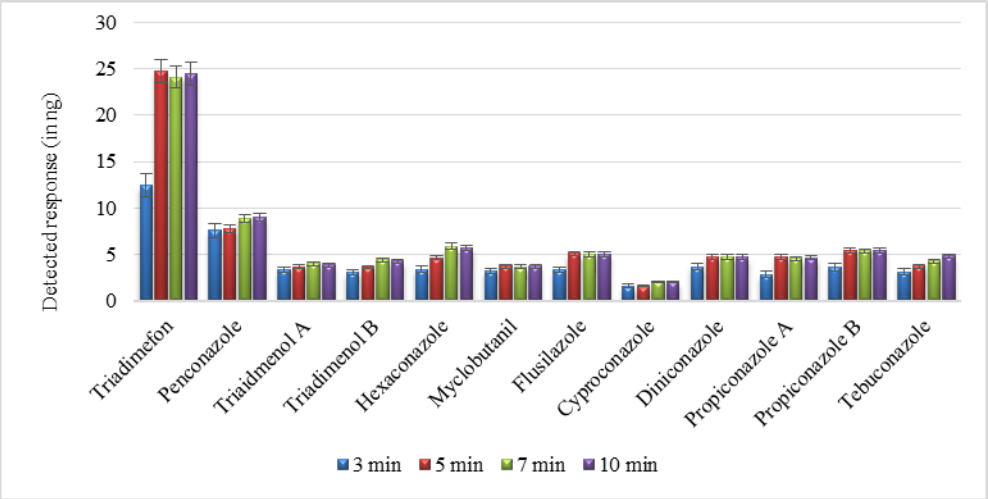


Figure 3-5 Comparison of desorption efficiency at 3, 5, 7 and 10 min (at 260 ºC). Extractions were performed for 15 min at 50 ºC from grape pulp spiked at 100 ng/g.

Other parameters, such as agitation speed (250, 500 and 750 rpm) and sample pH (natural and 8) were also evaluated (data not shown). As agitation speed increased, extraction efficiency also increased due to a decrease in the boundary layer thickness, leading to a faster uptake of analytes by the fiber coating. However, faster agitation (750 rpm) speeds could cause extra stress in the fiber needle, leading to needle bending. For practical reasons, an agitation speed of 500 rpm was chosen as a good compromise between extraction efficiency and fiber longevity. Regarding matrix pH, there was no significant effect on the extraction efficiency. This result was expected given the typical pKa values of the studied analytes (see *Table 3-1* for physical-chemical properties of the studied triazole pesticides). For this reason, fruit matrices were studied at their natural pH (~ 3.6 for grapes and ~ 3.5 for strawberries).

3.3.2 Method Validation

3.3.2.1 Selectivity and Linearity

Selectivity is an important parameter of method validation. The method to be validated must be able to quantify the analytes in the presence of endogenous compounds found in the matrices under study. In the present study, selectivity was assessed by subjecting seven matrix blank samples to the developed SPME procedure. No co-extracted compounds peaks were observed that could interfere with the identification and quantitation of the target analytes. Moreover, using the deconvolution feature of the ChromaTOF® software, it was possible to obtain pure mass spectrum and identification in case of co-elutions, given that complete overlap between peaks did not take place.

The linear range of the proposed method was investigated for all ten triazole pesticides in both whole grape and strawberry pulps. Matrix-matched calibration curves were obtained for all pesticides by spiking blank fruit pulp samples at 12 concentration levels, ranging from 0.005 to 1000 μg/kg. As seen in *Table 3-2*, the concentration range over which the method was linear was not the same for all test compounds. Calibration ranges were chosen to include concentration levels bracketing the MRL. However, MRLs were "relatively high" for some compounds, and the proposed method did not meet the linearity requirement at the MRL concentration for triadimefon (sum with triadimenol) and tebuconazole in grapes (MRL 2,000 μg/kg). Despite these findings, under the proposed analytical conditions, the method showed good linearity for all the

compounds in the entire calibration range selected for each analyte, with $R^2 > 0.9968$ and $R^2 > 0.9983$, for grapes and strawberries, respectively.

Table 3-2 Comparison of performance characteristics of the DI-SPME and QuEChERS method applied to triazole pesticides determination in grapes and strawberries.

Analyte	DI-SPME-GC-TOFMS								QuEChERS GC-TOFMS							
	Grapes				Strawberry				Grapes				Strawberry			
	Linearity (R^2)	Linearity range (μg/kg)	LOQs (μg/kg)	R.S.D (%)	Linearity (R^2)	Linearity range (μg/kg)	LOQs (μg/kg)	R.S.D (%)	Linearity (R^2)	Linearity range (μg/kg)	LOQs (μg/kg)	R.S.D (%)	Linearity (R^2)	Linearity range (μg/kg)	LOQs (μg/kg)	R.S.D (%)
Triadimefon	0.9992	0.25-1000	0.25	14.1	0.9986	0.5-1000	0.5	5.9	0.9989	50-1000	50	11.4	0.9998	50-1000	50	5.2
Penconazole	0.9991	0.25-1000	0.25	13.6	0.9999	1-1000	1	6.1	0.9994	50-1000	50	14.3	0.9977	50-1000	50	9.4
Triadimenol[a]	0.9999	2.5-1000	2.5	8.3	0.9999	5-1000	5	6.5	0.9999	50-1000	50	8.5	0.9986	50-1000	50	10.2
Hexaconazole	0.9994	1-1000	1	14.5	0.9993	2.5-1000	2.5	6.5	0.9999	50-1000	50	15.2	0.9987	50-1000	50	10.8
Myclobutanil	0.9968	1-1000	1	8.1	0.9983	2.5-1000	2.5	7.2	0.9985	50-1000	50	3.7	0.9976	50-1000	50	1.9
Flusilazole	0.9970	0.5-1000	0.5	10.0	0.9990	0.5-1000	0.5	7.6	0.9983	50-1000	50	8.2	0.9971	50-1000	50	3.2
Cyproconazole	0.9999	5-1000	5	11.4	0.9995	5-1000	5	6.8	0.9996	50-1000	50	4.8	0.9966	50-1000	50	6.0
Diniconazole	0.9998	1-1000	1	10.9	0.9988	2.5-1000	2.5	12.9	0.9998	50-1000	50	20.1	0.9986	50-1000	50	2.9
Propiconazole[b]	0.9999	1-1000	1	16.8	0.9988	2.5-1000	2.5	8.1	0.9990	50-1000	50	20.1	0.9991	50-1000	50	15.8
Tebuconazole	0.9999	5-1000	5	8.4	0.9999	5-1000	5	4.2	0.9985	50-1000	50	7.1	0.9990	50-1000	50	8.5

Linearity ranges are shown together with appropriate regression coefficient (R^2), objective LOQ defined as the lowest concentration which meets SANCO/12495/2011 validation criteria , and repeatability (R.S.D) at concentration level 100 (μg/kg) (n=5).
[a] Sum of isomers.
[b] Sum of isomers.

3.3.2.2 Limits of Detection and Quantification

LOQ objective is defined in the present study as the lowest assessed concentration of an analyte that gives a reproducible response that is both accurate (according to the expected value using the linear regression equation) and precise (≤ 20 % RSD) [140,174]. Excellent LOQs were achieved using pre-equilibrium extraction times as short as 15 min. For instance, LOQ values ranged between 0.25 μg/kg for triadimefon in grapes, and 5 μg/kg for cyproconazole in strawberries. Moreover, if sensitivity is of concern, method sensitivity can be further improved by increasing the extraction time, as the maximum sensitivity in SPME is achieved when extraction is performed at equilibrium. However, considering that the goal of this work was to propose a rapid automated method for high throughput, this option was not further pursued. An extraction time of 15 min used in this study gave reproducible results and adequate sensitivity to enable compliance with food legislation [174,175].

3.3.2.3 Precision

Intra-day repeatability of the proposed method was assessed using blank samples spiked with pesticides at three different spiking levels (*n=5,* each level). The results, expressed as relative standard deviations (RSD, %), were < 20 %, and meet the requirements according to SANCO/12495/2011 [174].

As seen in *Table 3-1*, the intermediate precision was assessed on three different days using blank samples spiked at three different concentration levels (*n= 9,* each level). RSDs ranged from 8 to 20 % and 4 to 13 %, for grapes and strawberries, respectively.

3.3.2.4 Accuracy

Method accuracy is presented here as the mean recovery of the analyte from the spiked matrix. As expected, all triazole pesticides exhibited low absolute recoveries in both matrices. As previously published by SANCO12495/2011, a pesticide residue method should demonstrate mean recovery values within the range of 70-120 %. However, the same organization also states that an exception applies where recoveries are low but consistent (demonstrating good precision), and the basis for this low recovery is well established. The present method fulfills both requirements, as SPME is an equilibrium technique, and the pesticides are distributed between the complex fruit matrix and the fiber coating. In addition, as mentioned before, the precision results show that the method is highly reproducible.

Martins et al. [140] developed an SPME-GC-MS/MS method that yielded low absolute recoveries for pesticides in wines. They were able to overcome the problem of low recovery values by working with "*estimated concentration values*".

In order to check the accuracy of the method in our study, recovery by means of "*estimated concentration values*" was assessed by fortifying blank samples of both whole grape and strawberry pulps at three levels corresponding to low, medium, and high ($n=5$ intra-day, and $n=9$ inter-day) concentrations. Recovery of triazole pesticides from spiked grape samples ranged from 86 to 117 %. For spiked strawberry samples, the recoveries for all pesticides ranged from 91 to 117 %. A summary of the results for method precision and accuracy are presented in *Table 3-3*.

Table 3-3 DI-SPME-ToFMS method precision under optimized conditions.

Pesticide	Grapes					Strawberry				
	Conc . (μg kg^{-1})	Intra-day[a]		Inter-day[b]		Conc . (μg kg^{-1})	Intra-day[a]		Inter-day[b]	
		Recovery[c] (%)	R.S.D[d] (%)	Recovery[c] (%)	R.S.D[d] (%)		Recovery[c] (%)	R.S.D[d] (%)	Recovery[c] (%)	R.S.D[d](%)
Triadimefon	1.02	88	8.1	89	10.1	1.02	95	8.6	94	7.3
	10.18	108	6.8	103	7.7	10.18	108	6.8	104	6.7
	101.85	101	3.2	102	5.3	101.85	101	3.2	102	5.3
Penconazole	0.98	104	11.6	103	14.4	0.98	109	10.4	101	12.7
	9.77	97	7.2	106	10.9	9.77	97	7.2	100	6.0
	97.68	103	2.3	105	2.7	97.68	103	2.3	105	2.7
Triadimenol	4.84	86	7.4	95	8.5	4.84	102	13.3	101	7.5
	48.38	105	7.2	102	8.7	48.38	105	3.2	104	5.0
	483.79	100	3.7	101	2.3	483.79	109	6.3	99	8.3
Hexaconazole	0.97	84	14.6	92	9.5	0.97	97	14.2	96	7.7
	9.72	95	3.9	91	5.9	9.72	95	3.9	91	5.9
	97.22	92	2.5	91	3.9	97.22	90	2.0	93	5.7
Myclobutanil	0.97	104	14.6	108	9.9	4.84	104	14.3	108	9.9
	9.67	91	3.0	86	7.5	48.38	98	5.3	98	4.2
	96.76	98	2.1	99	3.3	483.8	95	4.1	95	3.4
Flusilazole	1.01	113	6.3	106	7.8	1.01	105	7.0	103	6.0
	10.14	113	5.8	100	10.8	10.14	113	5.8	102	8.7
	101.39	105	5.1	100	5.8	101.39	105	5.1	98	8.0
Cyproconazole	4.87	113	8.4	113	5.2	4.87	113	8.4	113	5.2
	48.62	100	3.7	100	5.8	48.62	100	3.7	100	5.8
	486.15	99	4.1	98	5.4	486.15	89	5.1	95	7.2
Diniconazole	1.03	108	8.3	110	6.2	1.03	96	13.8	106	10.6

	10.28	111	8.2	103	9.1	10.28	111	8.2	103	9.1
	102.77	96	7.9	92	7.4	102.77	96	7.9	92	7.4
Propiconazole	0.99	110	7.0	117	7.3	5	117	5.1	117	4.1
	9.99	89	3.3	99	11.1	50	89	3.3	99	8.7
	99.99	114	8.0	115	4.6	500	111	3.3	114	3.5
Tebuconazole	4.92	107	10.6	108	6.3	4.92	98	4.7	102	4.8
	49.18	103	4.9	104	5.1	49.18	104	7.2	103	6.1
	491.8	100	1.7	101	5.1	491.8	105	3.9	108	5.7

[a] *n = 5*
[b] *n = 9*
[c] Recovery calculated using "*estimated concentration values*"
[d] Relative standard deviation

3.3.3 Comparison of DI-SPME with QuEChERS method

As part of this study, comparisons between the performances of the SPME and QuEChERS methods were drawn. Among the different options that could be modified in the AOAC QuEChERS method, the option that uses only PSA (primary-secondary amine) in the clean-up step was chosen, as grapes and strawberries have a low lipid content. According to SANCO/12495/2011 guidelines, both methods meet the criteria for method performance of mean recoveries in the range 70-120%, and $RSD_r \leq 20$ %.

As shown in *Table 3-2*, the LOQs objectives obtained with the DI-SPME method were substantially lower than those obtained by QuEChERS method, owing to the enrichment achieved by the SPME technique. However, it is important to note that LOQ objectives for the QuEChERS method could be improved by using larger injection volumes (10 µL) together with a PTV injector, or, by performing a solvent exchange/pre-concentration of the extracts. Regarding method precision in grapes, the RSD_r values at the level 100 µg/ kg were in the range of 4.2 to 16.8 % and 2.9 to 20.1 %, for the DI-SPME and QuEChERS methods, respectively. In this context, the results obtained in this study demonstrate the potential of the DI-SPME method to detect and quantify all triazole pesticides in grape and strawberry samples at low µg/kg levels.

Moreover, some advantages of the DI-SPME procedure over the QuEChERS strategy are clearly documented in *Figure 3-6*, as an example of improved determination of triadimefon in spiked grapes at 100 ng/g level.

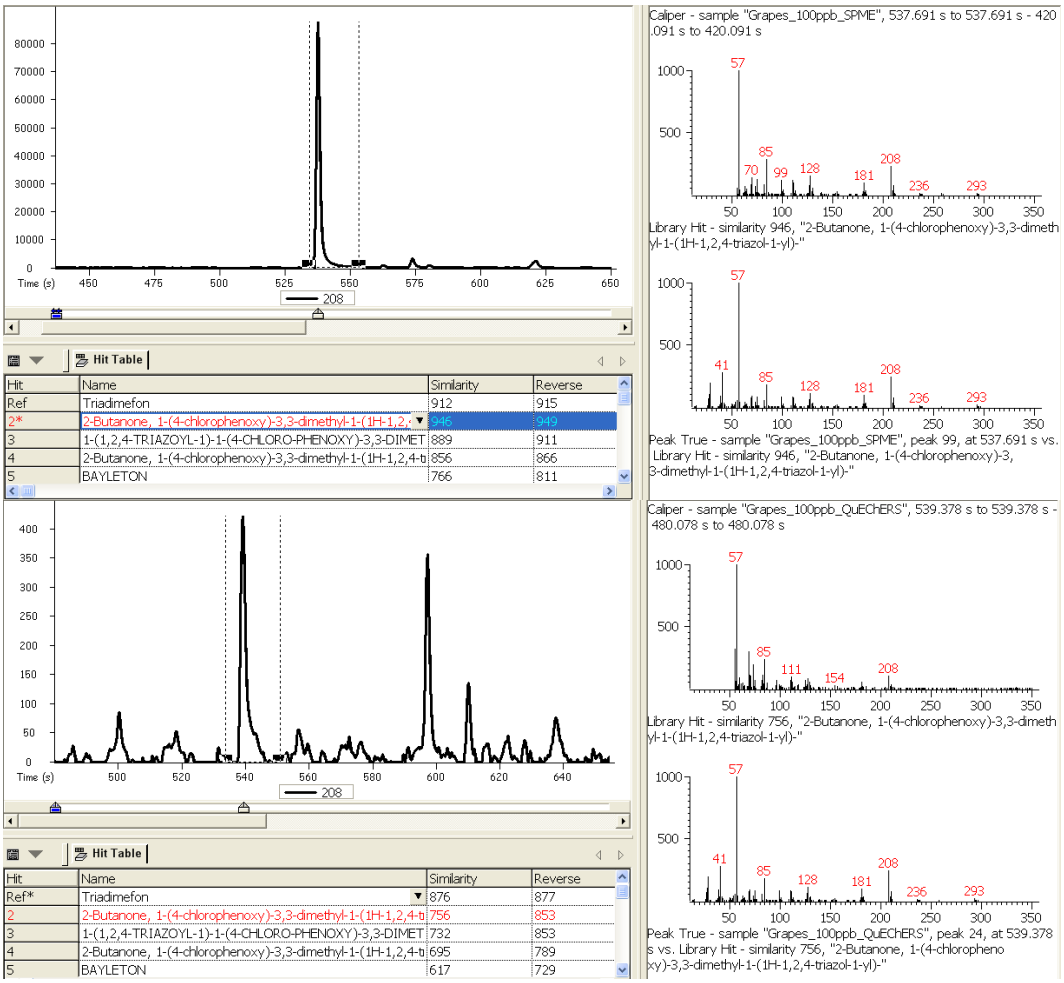
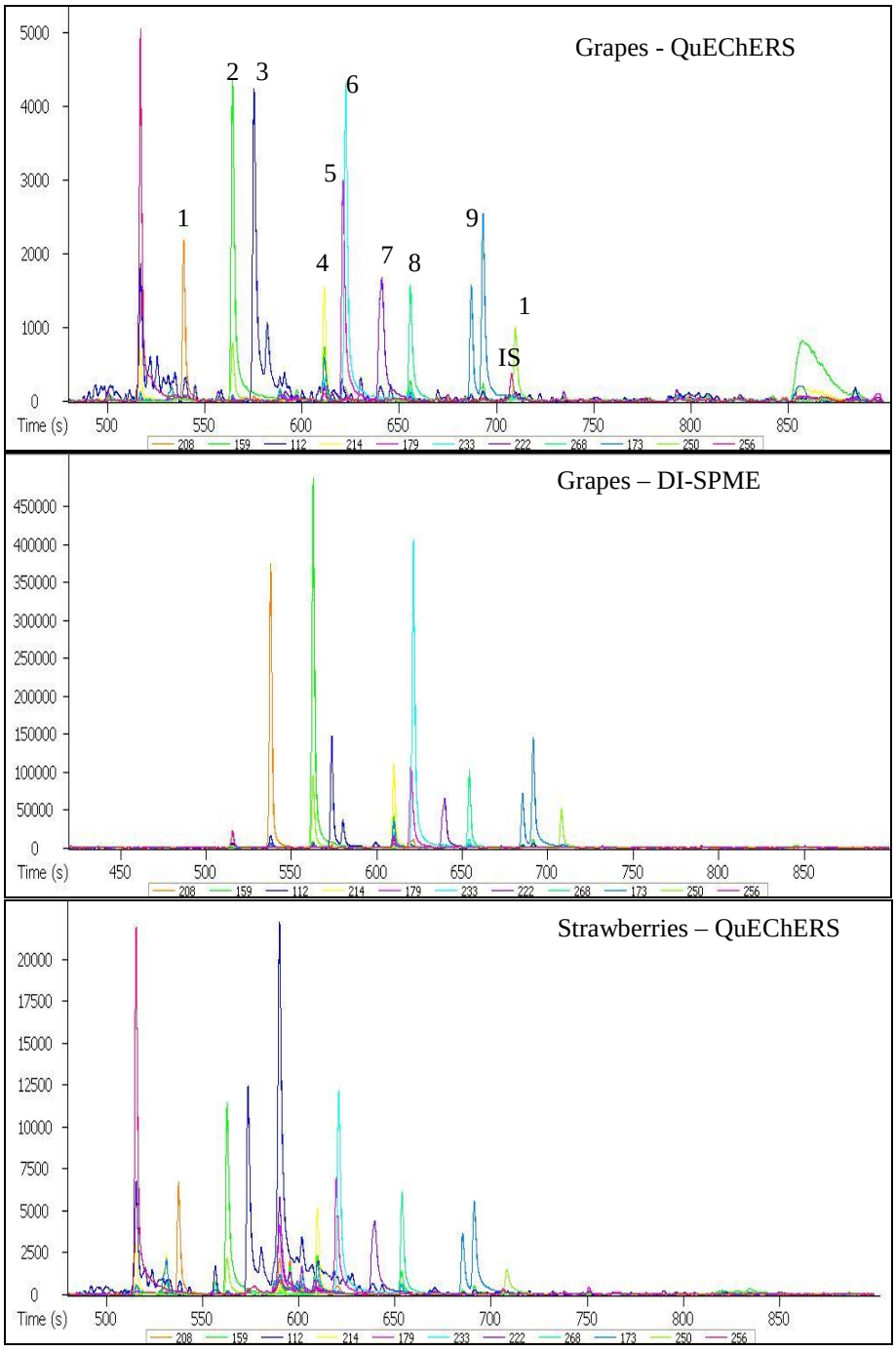


Figure 3-6 Potential of DI-SPME sampling vs.QuEChERS sample preparation approach in detection of target analyte triadimefon ;(A) Spiked grape pulp sampled by DI-SPME; (B) QuEChERS extracted of spiked grape pulp. Sample concentration of 100 ng/g.

In spite of the clean-up step employing PSA, the QuEChERS methodology yields a complex extract containing many interfering matrix components, including non-volatiles that could lead to build-up deposits in the injector and front part of the capillary column. Conversely, the DI-SPME method reduces interferences, translating into relatively simpler chromatograms with

fewer extraneous peaks. In addition to increased selectivity, an increase in sensitivity is also achieved; in DI-SPME, the totality of the analytes extracted is transferred to the GC injection port, whereas in the QuEChERS method employed in the present study, only 1 μL of the final extract is injected. When analyzing the QuEChERS extracts, at an acquisition rate of 20 Hz, the deconvolution software failed to identify triadimefon within the library similarity match ≥ 800, mainly due to high chemical noise. Conversely, a high library similarity match for this analyte was obtained when using DI-SPME for sampling, due to a less noisy background and the superior enrichment factor achieved by the SPME method. Typical extracted ion chromatograms obtained with both methods for grapes and strawberries spiked at 500 ng/g, as well as a total ion chromatogram obtained by SPME are presented in Figure 3-7.


Grapes - QuEChERS
1
2
3
4
5
6
7
8
9
1
IS
Time (s)
Grapes – DI-SPME
Time (s)
Strawberries – QuEChERS
Time (s)

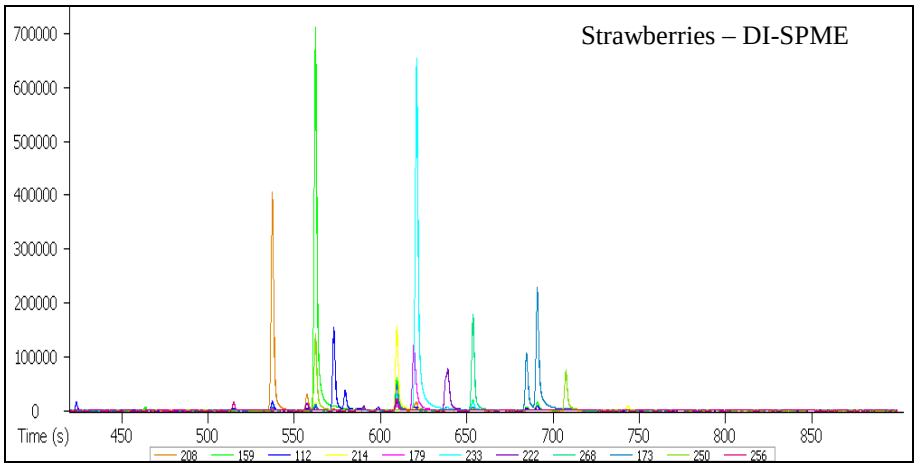


Figure 3-7 Typical extracted ion chromatograms obtained with QuEChERS and DI-SPME methods for grapes and strawberries samples spiked at 500 ng/g. Peak labels: 1. Triadimefon (m/z 208); 2. Penconazole (m/z 159); 3.Triadimenol isomers(m/z 112); 4. Hexaconazole (m/z 214); 5. Myclobutanil (m/z 179); 6. Flusilazole (m/z 233); 7. Cyproconazole (m/z 222); 8. Diniconazole (m/z 268); 9. Propiconazole isomers (m/z 173); IS. Tebuconazole-d_6 (m/z 256); 10. Tebuconazole (m/z 250).

3.3.4 Analysis of blind samples

As certified reference materials were not available for the present study, in order to confirm the accuracy of the SPME method and compare it with the QuEChERS method, analyses of blind samples were carried out. Blind samples of strawberries and grapes spiked at unknown concentrations were provided. These blind samples were processed according to the workflow presented in the experimental section.

As can be seen in *Figure 3-8*, good accuracy was obtained by the SPME method, as well as good agreement between results obtained by the SPME and QuEChERS methods.

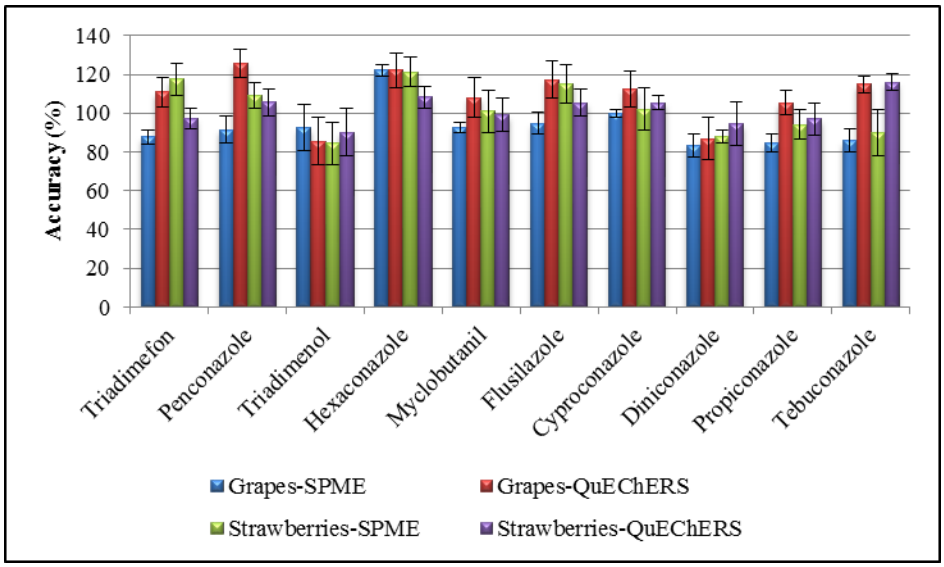


Figure 3-8 Accuracy results for blind samples obtained by DI-SPME and QuEChERS methods in grapes and strawberry. Error bars representing standard deviation (n=5).

The importance of such results lies in the confirmation of SPME as a rightful tool for quantitative trace-level studies. Owing to the equilibrium nature of the microextraction technique, the amount extracted by SPME is proportional to the free concentration of the analytes in the samples. This principle often causes misconceptions regarding the quantification capabilities of SPME, especially when dealing with complex matrices, where the absolute recovery of analytes may lie within few percent of the total amount. In such cases, the achievement of an accurate quantification method demands the choice of a proper

calibration technique. There are a number of calibration methods for SPME reported in the literature. In this study, the method of choice was a matrix-matched calibration curve to account for possible matrix effects. As well, an isotopically labeled internal standard (tebuconazole-d_6) was used, as it closely reassembles the analytes of interest in terms of its affinity for the extraction phase and any competing phase in the matrix, thus mimicking the behavior of the analytes during the extraction process. This internal standard offers enhanced accuracy and precision to the developed method, as it corrects for instrumental response drifts, and for variabilities that often occur when dealing with biological matrices.

3.3.5 Analysis of commercial samples

To ascertain its applicability, the validated DI-SPME-GC-TOFMS method was applied to the analysis of real grape and strawberry samples (described in experimental section of this chapter). The automated method allowed for the incubation and extraction of the next sample while the previous one was submitted to separation and detection. This automation resulted in a desirable high throughput of samples. Eight samples of each fruit, cultivated according to conventional agricultural procedures, were purchased from different retailers in the city of Waterloo, Ontario, Canada, and immediately processed according to the procedure described in experimental section.

Three samples of grapes revealed the presence of myclobutanil (9.2 ± 0.2 ng/g, 18.2 ± 0.5 ng/g, 15.7 ± 0.7 ng/g), and one contained tebuconazole (36.5 ±

0.9 ng/g). Regarding strawberries, three samples tested positive for myclobutanil (43 ± 4 ng/g, 119 ± 7 ng/g, 24 ± 1 ng/g), and two samples tested positive for propiconazole (500 ± 4 ng/g; 54 ± 5 ng/g). However, taking into account EU MRLs, only one strawberry sample was non-compliant, for propiconazole, exhibiting a concentration above 50 ng/g.

3.4 Summary

The DI-SPME-GC-ToFMS method developed in this chapter demonstrates potential for the automated screening of triazole pesticides in fruit samples with no sample pre-treatment needed. The use of a PDMS-modified fiber coating allows for the analysis of less-volatile analytes in direct immersion mode. This automated method for screening of triazole pesticides in fruits requires minimal sample handling, and shows a promising analytical performance. The sample preparation protocol is simple, fast and automated; therefore, it significantly reduces the average time required per sample, increasing precision and minimizing human mistakes.

As the LOQs achieved by this method are well below the maximum residue levels (MRLs) allowed for those compounds in both matrices, the DI-SPME method can be conveniently used as a rapid screening method to test for contamination of the considered samples, i.e., grapes and strawberries. More importantly, the DI-SPME-GC-TOFMS method developed in this study meets several standard parameters of analytical data quality, as established by SANCO/12495/2011. It shows potential as a suitable qualitative (screening) and quantitative (confirming) alternative to the more complex and time-consuming conventional existing methods.

Moreover, the SPME method provides improved sample clean-up, which can be translated to cleaner chromatograms and the achievement of lower limits of quantification. Since the fiber coating could be easily used for over 50 analysis,

the cost per analysis is competitive with the QuEChERS method utilizing commercially available kits. In terms of instrumentation, it is also worth mentioning that no sophisticated GC injector, such as PTV for large solvent injection (LVI), nor the implementation of column back-flush are needed. The cost/benefit of the SPME method can also be extended to the fact that due to its better clean-up capabilities, less down time for instrumental maintenance and clean up is also achieved.

Continuous efforts are currently underway in order to enable the analysis of multiclass pesticides in produces. In the Chapter 6 of this thesis, a SPME method for the determination of pesticides covering a broad range of physical-chemical properties and classes is presented. Even though the application of SPME method for analysis of semivolatile pesticides in food is still in its infancy, it is expected that in the near future, many more qualitative and quantitative applications in the field of pesticide residue analysis in food matrices that are based on DI-SPME methods will be reported in the literature.

3.5 Addendum

The text of this chapter was rewritten in comparison to the published research article. The author expresses sincere gratitude to Dr. Emanuela Gionfriddo for providing the blind samples.

4 Insights into the Effect of the PDMS-layer on the Kinetics and Thermodynamics of Analytes Sorption onto the PDMS-DVB/PDMS Coating

4.1 Preamble and introduction

4.1.1 Preamble

The data presented in this chapter has not yet been published.

4.1.2 Introduction

In any DI-SPME method dealing with highly complex matrices, it is important to assure that matrix components do not impair the performance of the method due to non specific attachment of matrix components onto the coating surface. As presented in Chapter 2, the implementation of a thin layer outerlayer of PDMS onto the commercial PDMS/DVB coating has led to the achievement of a matrix-compatible coating surface. The developed configuration can be seen a a built-in membrane, utilyzing a non-porous membrane, i.e. PDMS, placed between the sample and the DVB coating. This arrangement provided a highly effective clean up as well as high enrichement.

In fact, PDMS appears to be highly suitable for sampling in complex and challenging matrices, and problems resulting from surface-catalyzed analyte transformation and from analyte competition for adsorptive sites, which can occasionally been observed for adsorbents, can be circumvented [149].

Moreover, PDMS-based materials have been used in wide range of applications because they are nontoxic, relatively inert, easy to fabricate, and its characteristics are well known [149,176–179].

In the present thesis work, one of the main premises behind the choice of PDMS as an overcoating to create the matrix-compatible fiber for food analyzes is attributed to its hydrophobicity, which lessens the attachment of sugars and charged macromolecules to its surface. This process, in turn, significantly decreases the formation of fouling, especially for hot desorption, due to reactions between carbohydrates and other matrix components.

PDMS materials are widely regarded as hydrophobic, therefore, the ability of given compound to permeate through PDMS must be carefuly investigated. Understanding the role of the PDMS layer in the extraction process employing the PDMS-overcoated solid coatings is particularly important when considering the following questions: (1) how does the PDMS layer affect the uptake of analytes for kinetic extractions (under non-equilibrium conditions)? (2) Would the PDMS layer impose a bias on the representativeness of sampling (polar vs non-polar analytes)? (3) Does the PDMS layer affect the coating capacity towards target analytes as compared to the original coating?

To address these questions, eleven analytes were selected, from various application classes (pesticides, industrial chemicals and pharmaceuticals) and with a wide range of log P values (ranging from 1.43 to 6), to model and discuss the mass transfer of analytes within the PDMS-modified coating during the mass

uptake process. In addition, the thermodynamic parameters, here associated with the fiber constant $K_{fs}V_f$, were also investigated.

4.2 Materials and Methods

4.2.1 Chemicals and Materials

All contaminants standards used in this study were Pestanal grade and kindly provided by Supelco (Bellefonte, PA, U.S.A.). PDMS/DVB Stableflex fibers were purchased from Supelco. Sylgard 184® (PDMS prepolymer and curing agent) was purchased from Dow Corning (Midland, MI, USA). Deionized water used was from a Barnstead/Thermodyne NANO-pure ultra-water system (Dubuque, IA, U.S.A.).

4.2.2 Standards and Samples Preparation

Individual solutions of standards were prepared in methanol at 1 or 2 mg/mL, with the exception of chlorothalonil, which was prepared in dichloromethane. A working standard mixture was prepared containing each contaminant in the range of 2.5 to 150 µg/mL. The concentration of each analyte was carefully chosen in order to guarantee enough sensitivity for all analytes with all coatings tested. A detailed list of chemical structures, log P values, concentrations, and structures for analytes in the working mixture is described on Table 4-1 and Figure 5-1. To evaluate the amounts extracted for each analyte, a stock standard mixture was prepared at 100 ng/µL in methanol. This stock solution was used for sucessive dilutions in order to obtain calibration solutions ranging from 0.5 to 80 ng/µL (8 levels). Liquid injections of calibration solutions were carried out in quadruplicates.

Table 4-1 - Model analytes in standard mixture for coating evaluation.

	Working Mixture (µg/mL)	Log P (pH 7)	MW (g/mole)	Quant. Ion (*m/z*)
Nitrobenzene	50	1.90	123	77
1,3-Dinitrobenzene	150	1.43	168	168
2,6-Dinitrotoluene	75	2.42	182	165
Trifluralin	5	5.07	325	306
4-Phenylphenol	20	3.20	170	170
Diazinon	7.5	3.40	304	304
Chlorothalonil	15	2.94	266	266
Parathion	5	3.83	291	291
Pendimethalin	5	5.18	281	252
p,p'-DDE	2.5	6.00	318	318
Diazepam	100	2.80	284	256

4.2.3 Preparation of PDMS-modified coating

PDMS-modified coatings were prepared *in-house* employing the procedure described in Chapter 2. The only difference from the previous procedure is that the Sylgard 184® mixture was left to stand for 1h to start the cross-linking, allowing it to gain more viscosity before the coating procedure started. This modification allowed for thinner and more homogenous coatings to be attained with only one immersion into the Sylgard 184 solution. PDMS-modified coatings were prepared by coating once (~ 10 µm PDMS layer) or twice (~ 30 µm PDMS layer). All coatings were prepared at least in triplicate. Prior to their usage, each coating was conditioned at 250 ºC for one hour, and visually evaluated for uniformity and smooth surface coverage. If any defects were noted, coatings were discarded and new coatings were prepared.

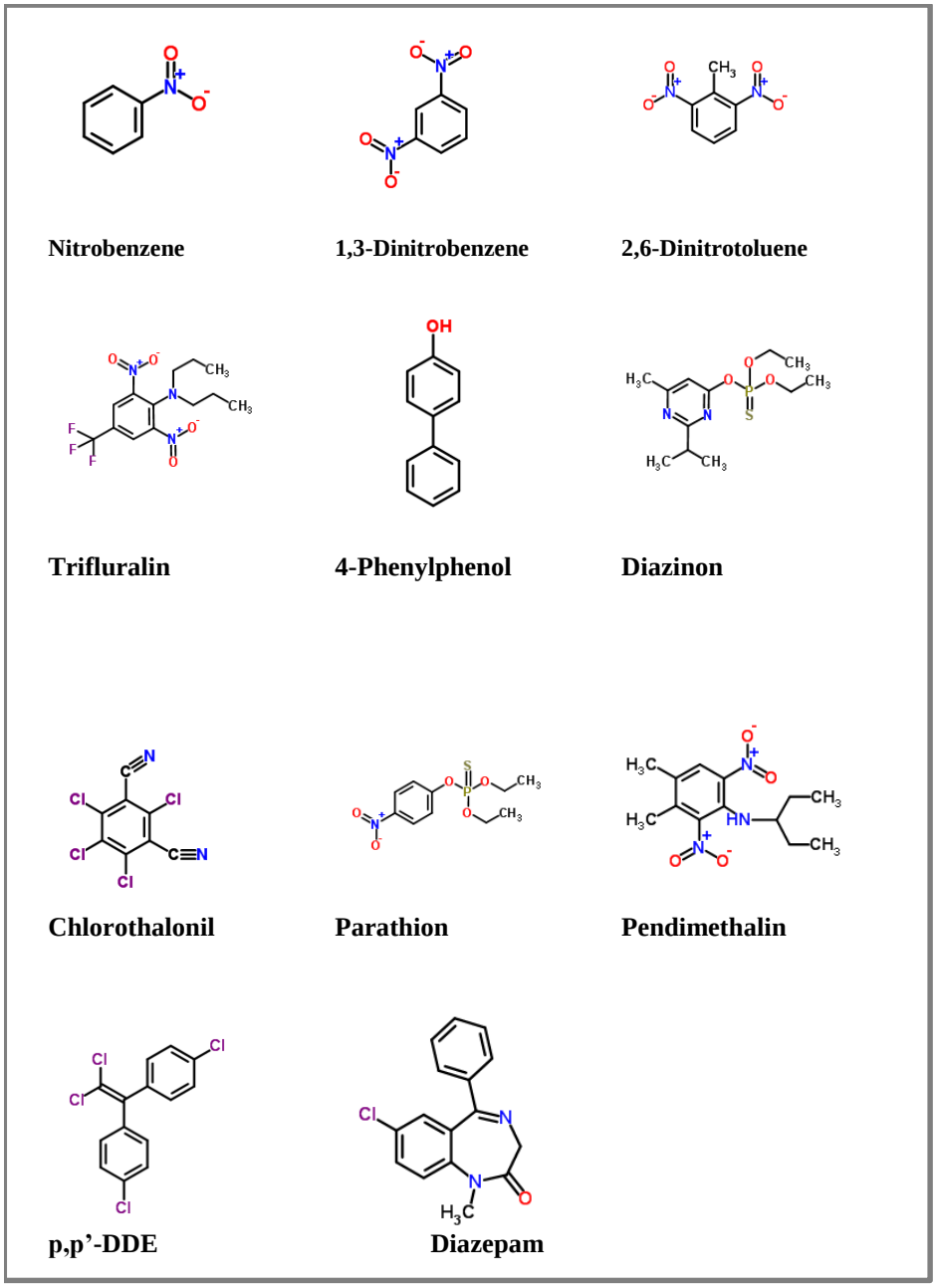


Figure 4-1 Structures of model analytes employed in the current coating evaluation.

4.2.4 Instrumentation

Analyses were performed on an Agilent 6890 gas chromatograph (GC) and a 5973 quadrupole mass spectrometer (MS) (Agilent Technologies, CA, USA), coupled with a GERSTEL® cooled injection system (CIS) (GERSTEL GmbH, Mullheim, GE). Helium as the carrier gas was set to 1.5 mL/min. The injector was set at a temperature of 270 °C. Chromatographic separation was performed using a SLB^{TM}-5MS (30 m × 0.25 mm I.D, 0.25 µm) fused silica column (Sigma–Aldrich, Mississauga, ON, CA). The column temperature program was initially set at 40 °C for 2 min, ramped at 10°C/min to 180°C, then ramped at 20ºC/min to 300ºC and held for 5 min, for a total run time of 25 min. The MSD transfer line temperature was set at 280◦C, while the MS Quad and MS source temperatures were set at 150◦C and 230◦C, respectively. The MS system was operated in electron ionization (EI) mode, and ion fragments were collected in the m/z 70–340 range. The quadrupole analyzer was operated in full scan mode: electron ionization (EI) at 70 eV; temperatures of 280, 150 and 230 °C for transfer line, quadrupoles, and ion source, respectively; a mass range of 70-340 *m/z* was scanned; a minimum of three ions were chosen for identification of each analyte. Automated analysis was performed with a Gerstel multipurpose (MPS 2) autosampler (GERSTEL GmbH, Mullheim, GE) using the software Chemstation (Agilent Technologies, CA, USA). The MPS 2 autosampler was equipped with the option of performing agitation using stir bar.

4.2.5 SPME Procedure

This first approach was employed to compare the extraction kinetics between the commercial fiber PDMS/DVB and the PDMS-modified fibers. An aliquot of 500 mL of nanopure water was spiked with 500 µL of working standard mixture and stirred for 5 min to ensure homogeneous distribution of analytes in the solution. Subsequently, an aliquot of 18 mL was tranferred into a 20-mL vial containing a 0.5-inch teflon-coated stir bar. Concentrations of the individual compounds in the water sample ranged from 2.5 to 150 ng/mL. For the SPME procedure, a 1-min incubation of the sample at 35 ºC was performed using the stirring feature of the MPS2 Gerstel autosampler instead of the normally employed agitation feature; in the agitation feature, the vial moves in relation to the fiber, causing a more turbulent flow, which would complicate the calculations presented further in this chapter. Automated extraction using stirring was set up so that the fiber pierced the vial cap septum 0.2 cm off-center. This arrangement ensured a tangential flow direction of the sample to the fiber was, thus enabling the use of semi-empirical relationships previously reported in the literature [71]. Extraction times ranged from 1 min to 120 min. Stirring velocities of 500 and 1500 rpm were investigated. Following extraction, fibers were placed in the GC injection port for desorption for 2 min at 270 ºC. All extraction time points were performed at least in duplicate.

The second approach aimed to compare the thermodynamics parameter between the original PDMS/DVB fiber and the analogous PDMS-modified ones. This was accomplished by investigating the capacities of these fibers by

calculating the fiber constants, namely the product of the partitioning coefficient (K_{fs}) by the fiber volume (V_f). For this purpose, equilibrium extractions were performed at room temperature (25 ± 2 ºC) with 35 mL of water spiked with 20 µL of standard working mixture. Samples were placed in a 40-mL amber vial, and a special aluminum insert between the hollow plastic cap and the septum was used to ensure accurate fiber positioning. Each sample was agitated using a teflon-coated stir bar (1 inch in length) with a magnetic stirrer (VWR 7x7" ceramic hot plate/stirrer, 120V Pro). Following extraction, fibers were placed in the GC injection port for desorption for 2 min at 270 ºC. All extraction time points were performed in duplicate.

4.3 Results and Discussion

4.3.1 Kinetic Considerations

The previous findings reported on Chapter 2 investigated the PDMS-overcoated coating properties towards a single class of hydrophobic analytes, triazoles, where the PDMS outerlayer did not substantially change neither the kinetic nor the thermodynamic parameters associated to the original coating. In this chapter, the investigation is extended to the behavior of the PDMS outerlayer on the extraction efficiency of this coating towards compounds with a wide range of polarities and diverse chemical functionalities.

To better exemplify the PDMS-overcoated fiber system herein studied, the diagram shown in *Figure 4-2* illustrates the expected mass transport process undertaken by analytes during extraction using a PDMS-overcoated fiber., where δ_w, δ_{PDMS}, and δ_D are the thickness of the aqueous boundary layer, PDMS layer, and DVB sorbent, respectively; C_0 is the analyte's concentration in the bulk of the sample; C_1 is the concentration of analyte in the sample at the interface of PDMS and the sample boundary layer, C_2 is the concentration of analyte in the PDMS side at the interface of PDMS and the sample boundary layer; C_3 is the concentration of analyte in the PDMS side at the interface of PDMS and DVB sorbent; C_4 is the concentration of analyte at DVB side at the interface of PDMS and DVB sorbent; C_5 is the concentration of analyte at DVB inner-side at the fiber core interface.

In this discussion the $K_{DVB,\ PDMS}$ is expected to be large for all compounds studied. Furthermore, the experimental design consisted of quite low water concentration for all analytes (from 2.5 to 150 ng/mL) which make it unlikely that localized displacement effects would occur. Based on targeted analytes functionalities it is expected DVB's affinity to be quite strong (high $K_{DVB,PDMS}$) due to benzene rings in addition to nitrogenated and oxygenated groups that offer great π-π interaction between analytes and DVB.

For a given sample velocity, smaller polar molecules (dashed black lines), such as 1,3-dinitrobenzene, are expected to diffuse through the aqueous boundary layer faster, since diffusion coefficient depend on molecule size, which will result in higher C_1. However, due to the limited affinity of these polar molecules for PDMS (low $K_{PDMS,water}$) lower C_2 is then expected. Since DVB is expectd to behave as a zero sink sorbent under the experimental conditions herein used, the concentration of analytes in the PDMS is expected to be zero at the PDMS/DVB interface, and a lower C_2 gives a smaller concentration gradient between C_2-C_3, thus resulting in decreased permeability for these compounds in the PDMS. The overall effect is that the PDMS layer becomes then the rate-limiting step in the mass uptake.

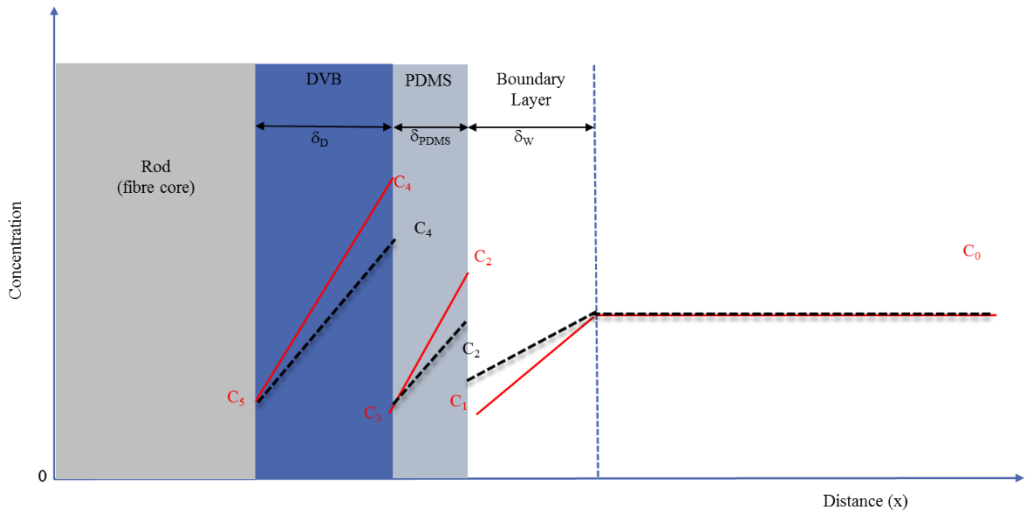


Figure 4-2 Process and concentration profile during analytes uptake using a PDMS-overcoated fiber (dashed black line – polar analytes; red line – non-polar analytes).

The red lines in *Figure 4-2* shows the diagram for larger and more hydrophobic compounds, such as p,p-DDE. P,p'-DDE size leads to low diffusivity in water and the aqueous boundary layer controls the uptake rate, resulting in a lower value of C_1. Conversely, these compounds have very high $K_{PDMS,water}$ values, which increases their permeability through the PDMS layer to an extent that the resistance imposed by the aqueous boundary layer thickness is concealed by their accumulation on the PDMS/sample interface (C_2). Moreover, given the very high $K_{PDMS,water}$ values, the PDMS acts also as a concentration medium.

According to the discussion above, $K_{pdms,water}$ plays an important role in determining the effect of the PDMS outerlayer on the analyte's uptake rate. In the

PDMS-modified system, the movement of chemicals occurs through two contiguous layers, aqueous boundary layer and PDMS layer, and each layer offers its own impedance to the mass transfer. The resistance imposed by the aqueous boundary layer to different analytes will be dependent on each analyte's diffusion coefficient in water, which in turn depends on each molecule's size. In the PDMS layer, the resistance is controlled by each analyte's permeation through the PDMS layer. The permeability of analytes through the PDMS layer is a product of analyte's diffusion coefficient (D_{PDMS}) and analyte's partitioning coefficient ($K_{PDMS,sample}$). The permeation process occurs due to the difference between the concentrations of analytes in each side of the PDMS layer. In the system herein studied, on one side, there is the interface sample/PDMS, on the other side the interface PDMS/DVB. And again, since DVB is a strong sorbent and under the conditions used in this experiment it behaves as a zero sink for the target analytes, the concentration in the PDMS layer at this interface is kept at zero.

It is important to note that since PDMS has a low diffusivity selectivity, the differences in permeability through the PDMS layer are mostly governed by their partition coefficients rather than the diffusivities in the polymer [179,180]. In fact, analyte's diffusion coefficients in PDMS are smaller than the corresponding water coefficients by a factor of 5-6 [71]. Accordingly, as $K_{pdms,water}$ increases, the contribution of the rate-limiting barrier associated with the aqueous boundary layer is expected to become more pronounced.

To investigate the rate-limiting barrier in the mass transfer of the analytes through the aqueous boundary layer and the PDMS outer layer, extractions ranging from 1 to 120 min were performed for each coating. Subsequently, each profile was inspected at a range of short extraction times to identify the linear section of mass uptake. By obtaining the linear section of mass uptake, the diffusion-based calibration model stated by Sukola et al. could be applied [181].

As the thickness of the boundary layer is determined by both the rate of agitation in the sample and by the diffusion coefficient of the analytes, in the same extraction process, the boundary layer will be different for different analytes. The experimental set up employed (fiber exposed off-centre of the vial) provided a tangential flow direction of the sample to the fiber, which allowed for the estimation of the effective thickness of the boundary layer (δ) using equation 4.1, adapted from the heat transfer theory:

$$\delta = 9.52 \left({}^{b}/_{Re^{0.62}\, Sc^{0.38}} \right) \qquad \textit{Equation 4-1}$$

Where *b* is the radius of the fiber (135 µm for PDMS/DVB; 145 µm for 10 µm PDMS layer; and 165 µm for 30 µm PDMS layer), *Re* is the Reynolds number ($Re = 2ub/v$; *u* is the linear velocity of the sample (10.6 cm/s for 500 rpm; and 31.9 cm/s for 1500 rpm) and *v* is the kinematic viscosity of the matrix medium, here water), and *Sc* is the Schmidt number ($Sc = v/D_W$; with D_W as diffusion coefficient of the analytes in the sample matrix, here water). D_w values

(cm^2/s) calculated using the Hayduk and Laurie method [182] are presented in Table 4-2 below.

Table 4-2 Calculated values of diffusion in water (Dw) at 30 ºC.

	Dw
Nitrobenzene	1.01E-05
1,3-Dinitrobenzene	8.99E-06
2,6-Dinitrotoluene	8.29E-06
Trifluralin	5.51E-06
4-Phenylphenol	7.76E-06
Diazinon	5.75E-06
Chlorothalonil	6.92E-06
Parathion	6.38E-06
Pendimethalin	5.77E-06
4,4'-DDE	5.90E-06
Diazepam	5.87E-06

To estimate the linear velocity, *u*, the following equation was used:

$$u(r) = 1.05\pi Nr\left[2 - \left(\frac{r}{0.74R}\right)^2\right] \qquad \textit{Equation 4-2}$$

where *N* is the magnetic stirrer speed in revolutions per second (8.33 for 500 rpm, and 25 for 1500 rpm), *r* is the distance between the fiber and the centre of the vial (0.1965 cm), and *R* is the radius of the stirring bar (0.635 cm).

The calculated boundary layer thicknesses are presented in Table 4-3. As predicted by the mass transfer theory, an increase in agitation conditions

significantly decreased the aqueous boundary layer, as the Reynolds number, used in Equation 4.2, decreased. In fact, a 3-fold increase in agitation speed led to an approximate 2-fold decrease in aqueous boundary layer thickness for all tested compounds. Conversely, since the additional PDMS layer is quite thin in both cases, the overall surface area does not change substantially and only marginal differences were observed between fibers exposed to same agitation conditions. Regarding the analytes, as expected, larger analytes have thinner aqueous boundary layers than smaller molecules such as nitrobenzene. This interplay exists since larger molecules have lower diffusion coefficients in water (see Table 4-2), and will take longer to cross the boundary layer and reach the coating surface.

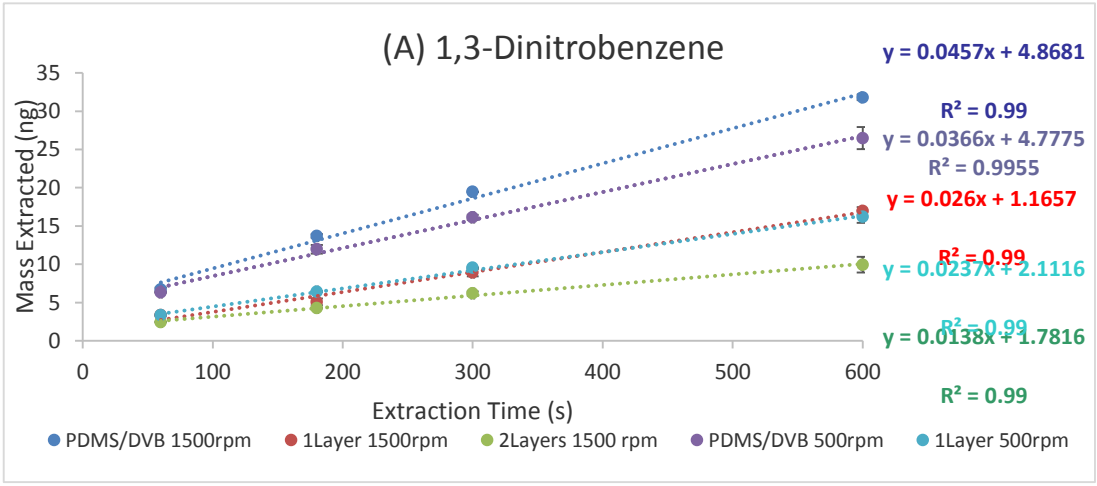
Table 4-3 Calculated values of aqueous bondary layer thicknesses (in µm) for different sample agitation speed (in rpm)

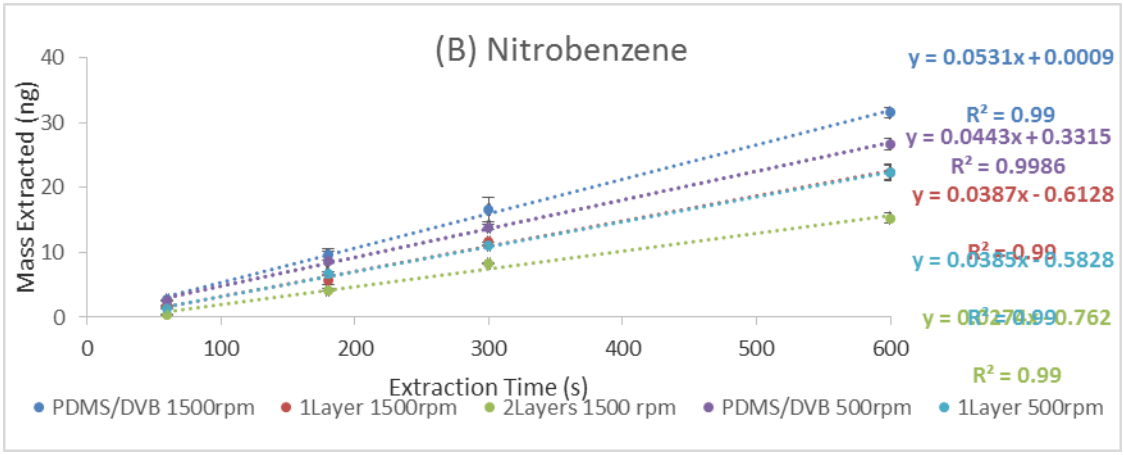
	PDMS/DVB		1 Layer PDMS		2 Layers PDMS	
	$\delta_{500\ PTIM}$	$\delta_{1500\ PTIM}$	$\delta_{500\ PTIM}$	$\delta_{1500\ PTIM}$	$\delta_{500\ PTIM}$	$\delta_{1500\ PTIM}$
Nitrobenzene	10.98	5.55	11.28	5.71	11.85	5.99
1,3-Dinitrobenzene	10.5	5.31	10.79	5.46	11.33	5.74
2,6-Dinitrotoluene	10.18	5.15	10.46	5.29	10.99	5.56
Trifluralin	8.72	4.41	8.96	4.53	9.41	4.76
4-Phenylphenol	9.93	5.03	10.2	5.16	10.72	5.42
Diazinon	8.86	4.48	9.11	4.61	9.56	4.84
Chlorothalonil	9.51	4.81	9.77	4.94	10.26	5.19
Parathion	9.22	4.66	9.47	4.79	9.95	5.03
Pendimethalin	8.87	4.49	9.12	4.61	9.58	4.85
4,4'-DDE	8.95	4.53	9.19	4.65	9.66	4.89
Diazepam	8.93	4.52	9.18	4.64	9.64	4.88

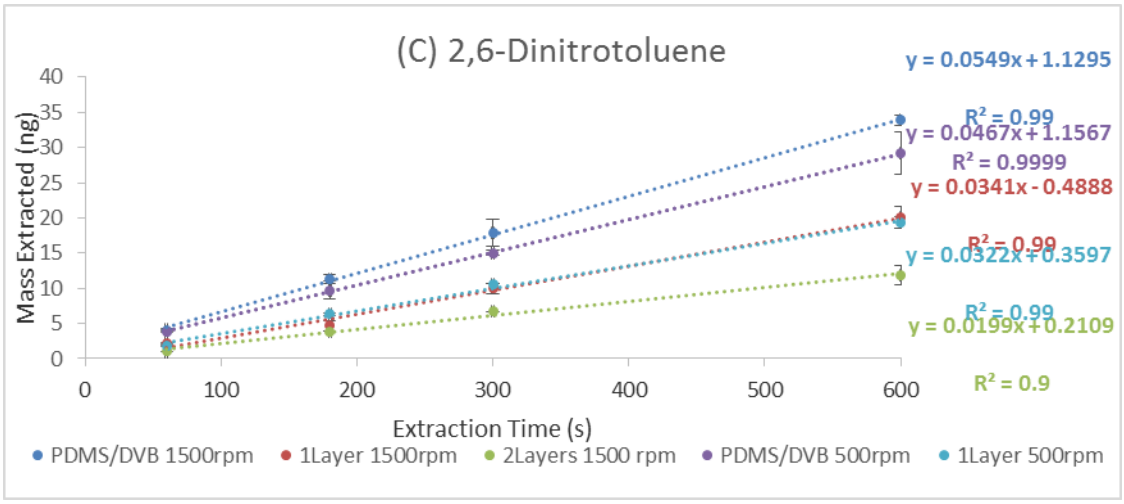
Initial mass uptake rates were calculated for all cases using least-square approximation, using data obtained from extractions between 1 and 10 min; the data is presented in Figure 4-3 to 4-5. All correlation coefficients (R^2) were > 0.99, showing good fit.

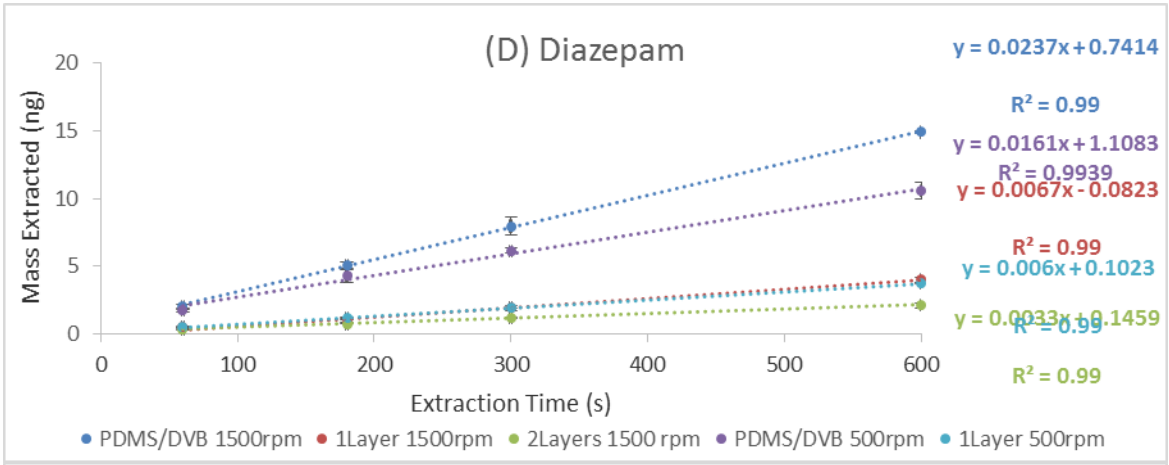
For most polar analytes – namely, 1,3-dinitrobenzene (log K_{ow} 1.43), nitrobenzene (log K_{ow} 1.9), 2,6-dinitrotoluene (log K_{ow} 2.42), diazepam (log K_{ow} 2.8), chlorothalonil (log K_{ow} 2.94), and 4-phenylphenol (log K_{ow} 3.2) – the effect of the aqueous boundary layer, noticed by comparing the curves for PDMS/DVB acquired at sample velocities of 10.6 (purple curve) and 31.9 cm/s (blue curve), gradually increased as the size of the analytes (molecule volume) increased (Figure 4-3 (A-F)). However, for both curves acquired using the fiber with the 10 µm PDMS layer (1 layer), mass transport through the PDMS barrier becomes the rate-limiting step (red curve for sample velocity of 31.9 cm/s, and light-blue curve for sample velocity of 10.6 cm/s). In fact, when comparing the curves obtained at the same sample velocity of 31.9 cm/s with the 10 µm PDMS outer layer (1 layer) and with the 30 µm PDMS outer layer (2 layers), it is evident that the increase in PDMS layer thickness significantly hampers the mass uptake of these more polar analytes. For larger polar molecules such as diazepam, given the low diffusivity in water (D_w $5.87x10^{-6}$ cm^2/s), one would expect the effect of the aqueous boundary layer to play an important role in the resistance to mass uptake; however, this effect is concealed by the effect of the low $K_{PDMS,water}$, and the outcome profiles are similar to smaller polar molecules. Therefore, for polar molecules, the thickness of PDMS (δ_{PDMS}) will exert an accentuated effect on the uptake rates.

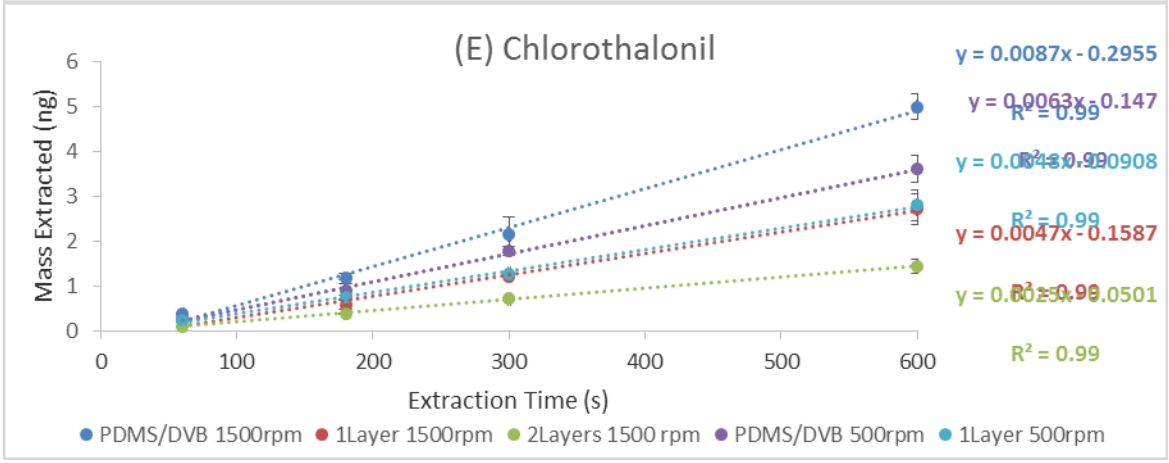
Additionally, the slower uptake of polar analytes extracted by PDMS-overcoated fibers could be attributed to the fumed silica fillers contained within the Sylgard 184 PDMS. These hydrophilic regions in the Sylgard 184 PDMS would allow for the immobilization of polar molecules via Langmuir type adsorption. Such entrapment would slow down even further the permeability of these molecules, already characterized by low $K_{PDMS,water}$ values [177].






(C) 2,6-Dinitrotoluene
Mass Extracted (ng)
Extraction Time (s)
y = 0.0549x + 1.1295
R² = 0.99
y = 0.0467x + 1.1567
R² = 0.9999
y = 0.0341x - 0.4888
R² = 0.99
y = 0.0322x + 0.3597
R² = 0.99
y = 0.0199x + 0.2109
R² = 0.9
PDMS/DVB 1500rpm
1Layer 1500rpm
2Layers 1500 rpm
PDMS/DVB 500rpm
1Layer 500rpm


(D) Diazepam
Mass Extracted (ng)
Extraction Time (s)
y = 0.0237x + 0.7414
R² = 0.99
y = 0.0161x + 1.1083
R² = 0.9939
y = 0.0067x - 0.0823
R² = 0.99
y = 0.006x + 0.1023
R² = 0.99
y = 0.0033x + 0.1459
R² = 0.99
PDMS/DVB 1500rpm
1Layer 1500rpm
2Layers 1500 rpm
PDMS/DVB 500rpm
1Layer 500rpm


(E) Chlorothalonil
Mass Extracted (ng)
Extraction Time (s)
y = 0.0087x - 0.2955
R² = 0.99
y = 0.0063x - 0.147
R² = 0.99
y = 0.0047x - 0.1587
R² = 0.99
y = 0.0023x - 0.0501
R² = 0.99
PDMS/DVB 1500rpm
1Layer 1500rpm
2Layers 1500 rpm
PDMS/DVB 500rpm
1Layer 500rpm

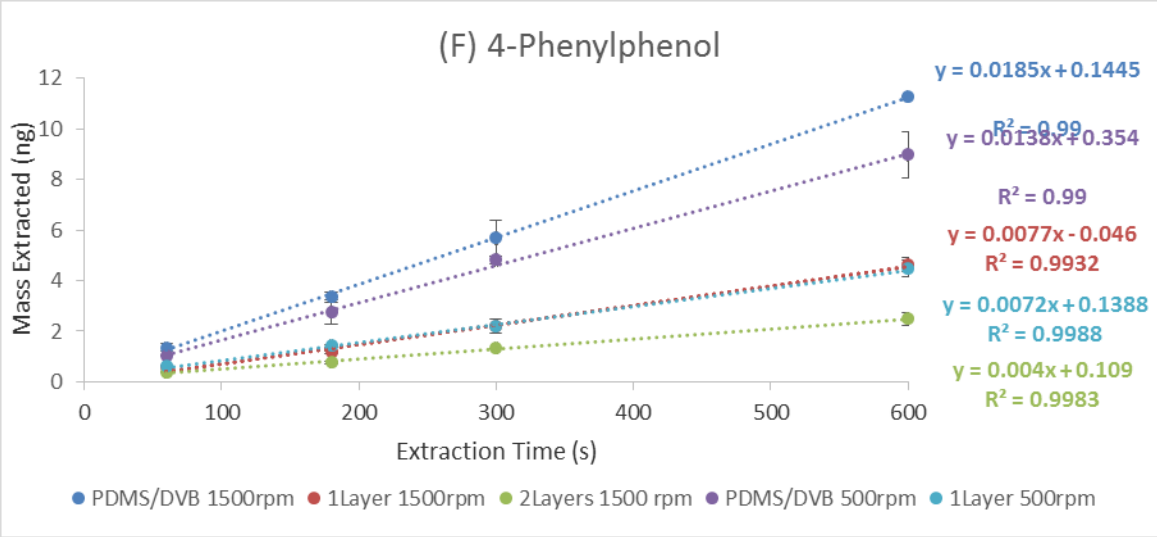


Figure 4-3 Mass uptake profiles of most polar target analytes obtained from aqueous solutions at 30 ºC.

As shown in Figure 4-4 (A-B), for medium polarity analytes such as diazinon (log K_{ow} 3.4) and parathion (log K_{ow} 3.83), the effect of the aqueous boundary layer is seen between both curves for PDMS/DVB (blue and purple curves), as well as between both curves acquired using the fiber with the 10 µm PDMS outer layer (red and light-blue curves). For parathion, the contribution from the thickness of the aqueous boundary layer in the mass uptake was clearly diminished, as can be seen by comparing both curves obtained with the fiber with the 10 µm PDMS outer layer, as opposite to both curves obtained with the PDMS/DVB fiber. As expected according to their molecule volumes, the effect of the aqueous boundary layer thickness is more evident for diazinon (D_w $5.75x10^{-6}$) than for parathion (D_w $6.38x10^{-6}$). A closer inspection of the curves obtained at the same sample velocity of 31.9 cm/s with the 10 µm PDMS outer layer (1 layer)

and the 30 μm PDMDS outer layer (2 layers) reveals the effect of PDMS layer thickness; the thicker the layer, the slower the mass uptake. For these analytes, a trade-off exist between the aqueous boundary layer and PDMS layer resistances, with the overall mass uptake depending on the magnitude of resistance imposed by these two barriers. Since both layers play a significant role in slowing down the mass uptake, it becomes somehow difficult to deconvolute the contribution of each layer in the mass transfer process in order to identify the rate-limiting step in the mass transfer process.

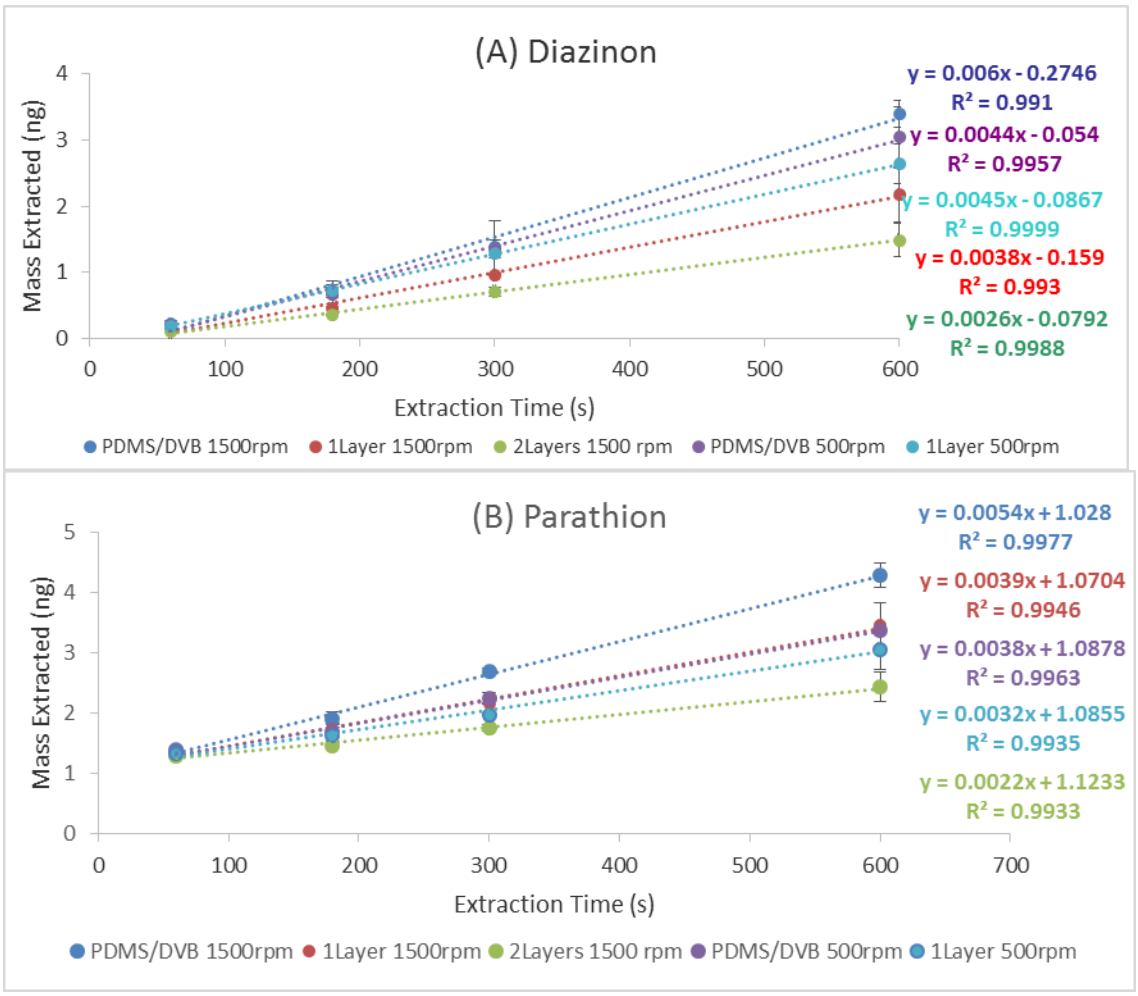


Figure 4-4 Mass uptake profiles of medium polarity target analytes obtained from aqueous solutions at 30 ºC.

For the most hydrophobic analytes, namely, trifluralin (log K_{ow} 5.07), pendimethalin (log K_{ow} 5.18), and p,p'-DDE (log K_{ow} 6), the interpretation of the plots takes another direction, as presented in Figure 4-5. Examination of all curves obtained for trifluralin revealed the effect of the aqueous boundary layer as the rate-limiting step in all cases, which is expected given the low diffusivity of trifluralin in water. Interestingly, all curves pertaining to PDMS overcoated fibers exhibited faster uptake rates than PDMS/DVB. Given the high hydrophobicity of trifluralin, it can be understood that the accumulation of trifluralin on the sample/PDMS interface was facilitated (high $K_{pdms,water}$); for the absorbent and hydrophobic PDMS extraction phase, the values of $K_{pdms,water}$ correlate quite well with the hydrophobicity (i.e. K_{ow}) of the analytes [183,184].

For the most hydrophobic compound, p,p'-DDE, the boundary layer effect is clearly depicted when comparing both curves obtained with PDMS/DVB. Similarly to trifluralin, all curves obtained with the PDMS overcoated fibers have steeper curves regardless of sample velocity. The higher surface area for all PDMS-overcoated fibers, as compared to the non-modified PDMS/DVB, added to the very high value of $K_{pdms,water}$ for p,p'-DDE, may be a plausible explanation for higher uptake rates obtained with PDMS-modified fibers. Indeed, it is well reported in the literature that amongst all commercially available SPME coatings, the PDMS coating has the highest affinity towards organochlorine pesticides, including p,p'-DDE [185,186].

In the case of pendimethalin, the curves obtained at the same sample velocity of 31.9 cm/s for all fibers displayed the same uptake rates, which clearly evidences the aqueous boundary layer thickness as the rate-limiting step

controlling the mass uptake of this large compound. Moreover, it is important to note the differences in uptake rates when comparing the PDMS/DVB and 10 µm PDMS outer layers at lower sample velocities; the additional PDMS layer seems to favor the uptake of pendimethalin by the fiber, which could also be explained by the high $K_{pdms,water}$ of pendimethalin.

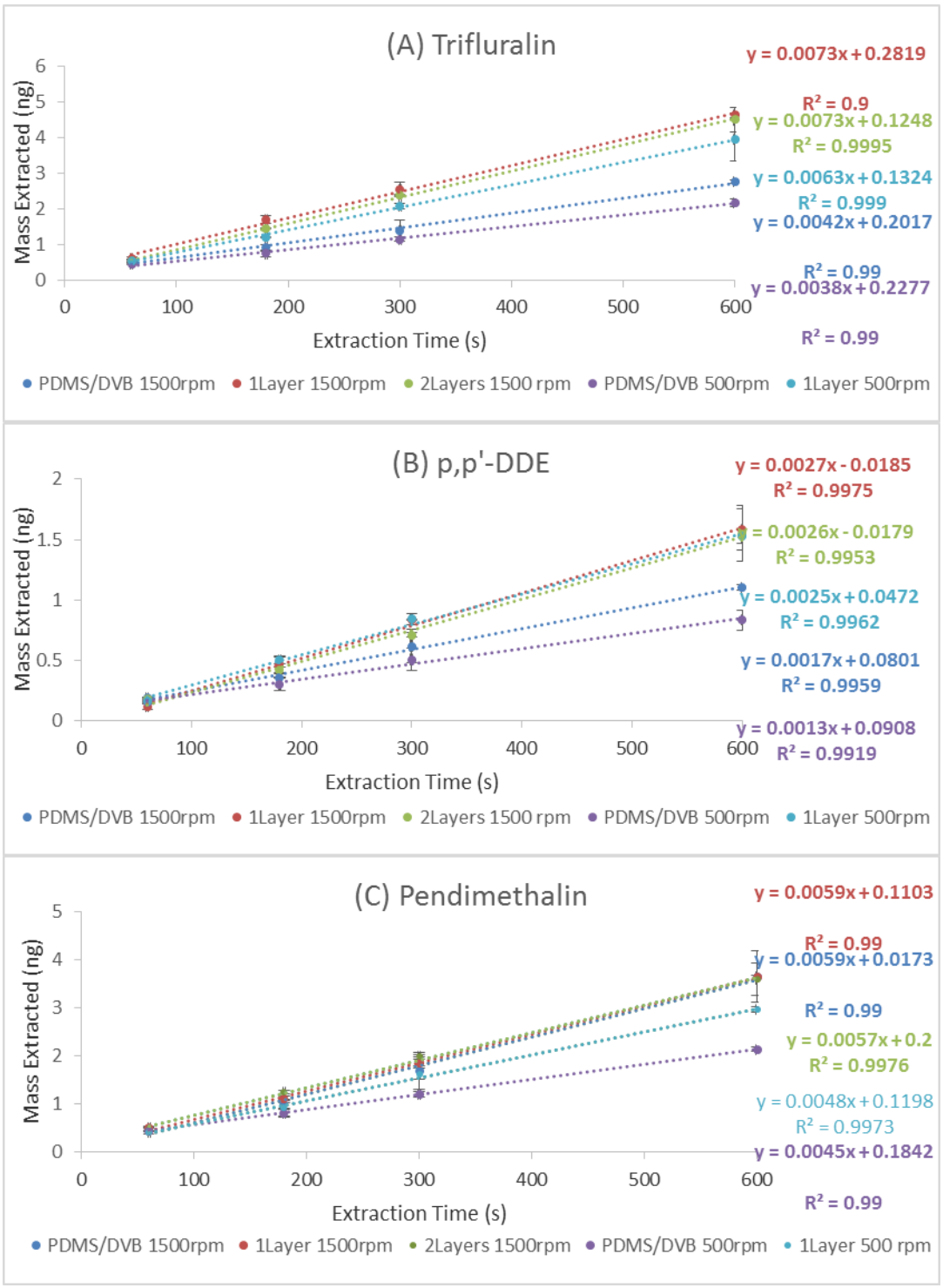


Figure 4-5 Mass uptake profiles of non-polar target analytes obtained from aqueous solutions at 30 ºC.

A closer look into the parameters and functionalities of molecules could shed some light into explaining the sources of differences obtained for pendimethalin uptake rates as compared to trifluralin and p,p'-DDE. Frequently, a strong correlation is observed between analyte size and transport properties in PDMS. In general, as analyte size increases, permeability in PDMS increases [180]. However, the difference in molecule size is not very large, and according to the poor size selectivity of PDMS, it is quite unlikely that such differences rise from molecule size. A second possibility is associated with the plasticization effect that depends on the magnitude of the $K_{PDMS,water}$ value for each analyte. Here, the rationale is that as the analyte concentration in the PDMS layer increases, there is an increase in PDMS local segmental motion, which in turn would increase the analyte permeability in PDMS [180]. According to this approach, p,p-DDE would have enhanced permeability in the PDMS, given its larger log K_{ow} value. Therefore, this hypothesis still does not explain the effect seen experimentally.

However, the boundary layer-controlled uptake observed for pendimethalin, and to a smaller degree for trifluralin, might be explained by taking into consideration the contribution of DVB in the overall mass transfer process. Based on the structure of these analytes, high $K_{DVB,PDMS}$ values are expected due to nitrogenated groups, in addition to the benzene ring that offers great π-π interactions between molecules and DVB sorbent. If the diffusion through the aqueous boundary layer is too slow to supply analytes to the PDMS layer, as compared to the rate of mass transfer from the PDMS layer to the DVB phase, a starvation effect takes place. As the starvation effect increases, the uptake

rates become more aqueous-boundary layer controlled [179]. It is very important to note that based solely on the experiments performed and the data discussed, none of these hypotheses can be empirically confirmed at this point.

4.3.2 Thermodynamic Considerations

PDMS is well known to be an absorbent media; in light of this, the modification of solid SPME sorbents by applying a further outer PDMS layer can potentially affect the overall coating capacity. To address this point, the study of thermodynamic parameters characterizing the coating, such as fiber constants, need to be carried out. For this purpose, overnight extractions (12h) were performed from aqueous samples, assuming the establishment of equilibrium conditions. The obtained results are presented in Figure 4-6. As can be seen, similar amounts were extracted by both fibers (original PDMS/DVB and overcoated with 10 µm PDMS) for most analytes. By means of t-test paired two samples for means, it has been confirmed that only 2,6-dinitrotoluene, trifluralin and p,p'-DDE resulted in statistically different amounts extracted ($p > 0.05$). In agreement with the results obtained in the previous subsection, an increase in the extracted amounts of trifluralin and p,p'-DDE was observed for the PDMS-overcoated fiber in comparison to PDMS/DVB, indicating that the PDMS layer also acts as a concentrating medium for analytes bearing high $K_{PDMS,water}$ values. Regarding the decreased amount of 2,6-dinitrotoluene extracted by the PDMS-overcoated fiber, it is worth mentioning the high variation in response obtained between the first and second replicate, 31 and 43 ng, respectively. Despite this

finding, overall, the results obtained are in good agreement with the initial observations made for triazole analytes in Chapter 2.

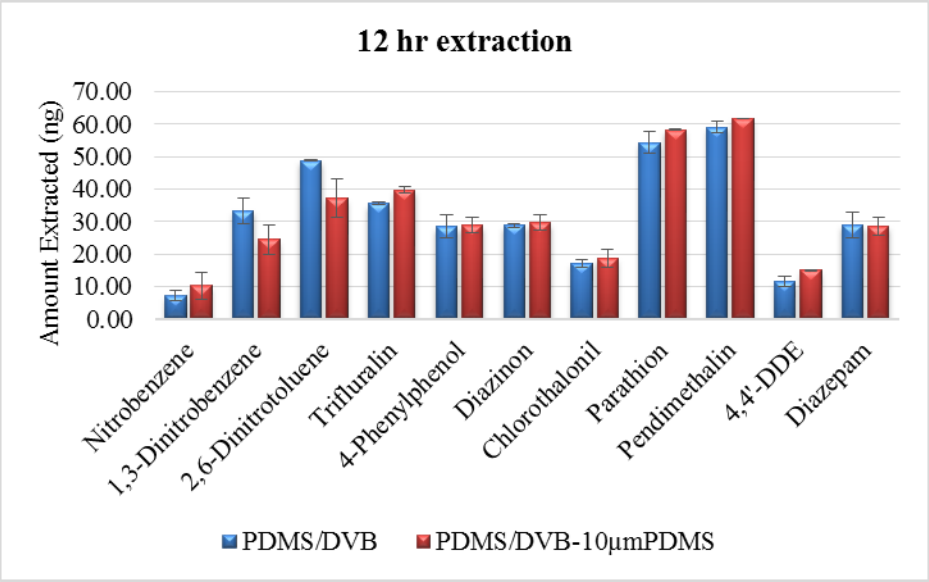


Figure 4-6 Comparison of the extraction efficiency of PDMS/DVB, PDMS/DVB-10 µm PDMS, and PDMS/DVB-30 µm PDMS (n=2, each coating) for the extraction of various analytes. Extraction conditions: 35 mL of aqueous samples (concentrations ranging from 1.4 to 85.7 ng/mL), 12 h extraction under 1000 rpm agitation at room temperature (23-25 ºC).

The fundamental thermodynamic principle of SPME involves the distribution of analytes between the sample medium and the extraction phase. The distribution constant defines the maximum enrichment factor achievable by a given extraction phase. At equilibrium, the amount extracted by a given coating can be expressed by Equation 1.1. However, for solid adsorptive coatings such as DVB, the active volume available for adsorption is not easily calculated, as it

depends on the porosity displayed by the sorbent. In such cases, the coating capacity towards a given analyte can be measured by calculating the fiber constant, which is the product of the partitioning coefficient of an analyte between the sample and the solid coating (K_{fs}), and the volume of the coating (V_f). The fiber constant can be calculated as follows:

$$K_{fs}V_f = \frac{n_e V_s}{(C_0 V_s) - n_e} \qquad \text{Equation 4-3}$$

Where n_e is the amount of analyte extracted at equilibrium, C_0 is the analyte concentration in the sample, and V_s is the sample volume. Using Equation 4.3, fiber constants were calculated for all analytes with both coatings, and the results are presented in Table 4-4.

The obtained $K_{fs}V_f$ data shows the insignificant effect of the PDMS overcoating on the equilibrium extraction of polar and mid-polar analytes when compared to the original PDMS/DVB coating. In fact, for polar and mid-polar analytes, fiber constants obtained with the PDMS-overcoated fiber were statistically similar to the ones obtained with the original PDMS/DVB coating, except for 2,6-dinitrotoluene. In contrast, for the most hydrophobic compounds, there was an increase in the fiber constant for the PDMS-overcoated fiber, associated with the enhanced hydrophobic partitioning of these compounds into PDMS. In fact, the additional 10 µm of PDMS also acts as a concentrating medium for analytes bearing high $K_{PDMS,water}$ values, which adds to the overall coating capacity.

Table 4-4 Calculated fiber constants (KfsVf) for unmodified and PDMS-overcoated (10 µm) PDMS/DVB coatings.

		PDMS/DVB		PDMS 10 µm Layer	
	log K_{ow}	K_{fs} x V_f	± C. V	K_{fs} x V_f	± C. V.
1,3-Dinitrobenzene	*1.43*	0.39	0.05	0.29	0.05
Nitrobenzene	*1.9*	0.25	0.06	0.36	0.15
2,6-Dinitrotoluene	*2.42*	1.174	0.004	0.89	0.14
Diazepam	*2.8*	0.51	0.07	0.51	0.05
Chlorothalonil	*2.94*	2.11	0.15	2.32	0.36
4-Phenylphenol	*3.2*	2.68	0.32	2.71	0.22
Diazinon	*3.4*	8.32	0.17	8.60	0.67
Parathion	*3.83*	41.65	2.59	48.84	0.06
Trifluralin*	*5.07*	19.26	0.24	23.04	0.58
Pendimethalin*	*5.18*	50.27	1.57	56.65	0.04
4,4'-DDE*	*6*	10.42	1.36	14.86	0.12

* non-equilibrium conditions

The information now acquired answers questions raised during the work developed using triazole pesticides as models (Chapter 2), in the sense that it corroborates and validates the hypothesis that the extraction capabilities of the original coating towards a wide range of analyte sizes and polarities is not significantly affected by the addition of the PDMS outer layer.

4.4 Summary

The research presented in this chapter investigated the performance of PDMS-overcoated fibers as compared to the unmodified PDMS/DVB fiber using aqueous samples, and employing a wide range of analyte polarities, molecular weights, and functionalities.

In the first part of the work, taking a kinetic approach, the effect of the PDMS outer layer in the rate of uptake of analytes during the mass transfer process was studied. In short, the results can be simplified in two models: (1) rate-limiting step is the diffusion through the coating, and (2) rate-limiting step is the diffusion through the aqueous boundary layer. For polar compound, according to theoretical discussion, the rate-limiting step is the diffusion through the coating, therefore, the overcoated PDMS layer affects the kinetic uptake. On the other hand, for non-polar compounds, the rate-limiting step is the diffusion through the aqueous boundary layer, therefore, the overcoated PDMS should not affect the kinetic uptake rate. Since the PDMS-overcoated fibers have been proven to be robust and compatible for use in fruit pulp (Chapter 2), it is important to have in mind such kinetics considerations if a method aiming at simultaneous determinations of both polar and non-polar analytes is to be developed, such as is the case in multiclass pesticide analysis. As previously stated, when employing adsorbent SPME coatings, short extractions are preferred as a mean to extend the linearity range of the method, as well as to avoid inter-analyte displacement effects.

However, if PDMS-overcoated fibers are employed, the sensitivity of the method at too short extraction times might not be enough for most polar analytes.

In the second part, from a thermodynamic point of view, the objective was to determine changes in distribution constants, and thus, the extraction efficiency of these fibers. The calculated fiber constants further corroborate the hypothesis that the additional PDMS layer does not substantially change the extraction phase capacity. A positive effect, though, was observed for most hydrophobic analytes, where the additional PDMS layer also acted as a concentrating phase, increasing the coating capacity towards these more hydrophobic analytes.

4.5 Addendum

The author thanks Supelco for the provision of analytical standards used in this study.

5 Evaluation of Different PDMS Types on the Performance of Matrix-Compatible Coatings for Direct Immersion Solid Phase Microextraction in Food Analysis

5.1 Preamble and Introduction

5.1.1 Premable

The data presented in this chapter has not yet been published.

5.1.2 Introduction

Since its introduction in early 1990s, SPME has been expanding to different areas such as biological, clinical, pharmaceutical, environmental and food studies [118,126,135,138,187–193]. Successful and reliable utilization of SPME for different applications relies on addressing the requirements for each individual study. Unquestionably, the main quest for the implemantation of SPME to complex matrices has been the development of competent coatings that present compatibility towards the matrices being investigated while providing enough sensitivity towards the analytes being targeted [86,127,150,194–197]. In this sense, the results reported in Chapters 2 and 3 shows that the PDMS-modified coating has exhibited great compatibility performance in complex matrix such as grape pulp, as well as satisfactory extraction capabilities towards triazole pesticides.

Nothwithstanding the rewarding results obtained for triazole pesticides, another great challenge associated with the development of an SPME method increases when not oly the matrix to be investigated is complex, but also the study

requires the analysis of compounds from different classes bearing a wide diversity of physicochemical properties. The results presented in Chapter 4, for water samples, showed that the extraction capabilities of the the PDMS-modified coating was similar to that presented by the original PDMS/DVB coating towards compounds in a wide range of polarity, except that for polar compounds the overcoated PDMS layer affects the kinetic uptake.

So far, Sylgard ®184 has been used for the fabrication of PDMS-overcoated fibers. Sylgard ®184 is one amongst the plethora of commercial blends of PDMS available. In fact, a different type of PDMS is used to fabricate the commercially available PDMS fibers. Therefore, it is important to study the matrix compatibility and sorption properties of these two PDMS formulation independently rather than assuming that their performances will be the same.

Therefore, the focus of this chapter is the thorough evaluation of PDMS-overcoated fibers capability to simultaneously extract analytes from different polarities while maintaining adequate matrix compatibility. Types of PDMS, as well as other intrinsic factors associated with the PDMS-overcoated fiber fabrication are studied. For this, the mixture of analytes employed in Chapter 4 is used, and the model matrix is commercial Concord grape juice, which is rich in pigments such as anthocyanins, and contains approximately 20 % of sugar (w/w) and is rich.

5.2 Materials and Methods

5.2.1 Chemicals and Materials

All contaminant standards used in this study were Pestanal grade and kindly provided by Supelco (Bellefonte, PA, U.S.A.). PDMS/DVB 65µm Stableflex fibers were purchased from Supelco, and PDMS-modified prototype coatings using proprietary PDMS and procedure were also kindly provided by Supelco. Sylgard 184 ® (PDMS prepolymer and curing agent) was purchased from Dow Corning (Midland, MI, USA).Concord grape juice was purchased at a local market in Waterloo (ON Canada). Deionized water came from a Barnstead/Thermodyne NANO-pure ultra-water system (Dubuque, IA, U.S.A.).

5.2.2 Standards and Samples Preparation

Individual solutions of standards were prepared in methanol at 1 or 2 mg/mL (except chlorothalonil, which was prepared in dichloromethane). A working standard mixture was prepared containing each contaminant within a range of 2.5 to 150 µg/mL. The concentration of each analyte was carefully chosen in order to guarantee enough sensitivity for all analytes with all coatings tested. A detailed list of chemical structures, log P values, and concentrations for each analyte in the working mixture is described in Chapter 4. To evaluate the amounts extracted for each analyte, a stock standard mixture containing all analytes of interest was prepared at 100 ng/µL in methanol. This stock solution was used for successive dilutions in order to obtain calibration solutions ranging from 0.5 to 80 ng/µL (8 levels). Liquid injections of calibration solutions were carried out in quadruplicates.

5.2.3 Preparation of PDMS-modified coating

PDMS-modified coatings were prepared *in-house* employing the procedure described in Chapter 2. The only difference from the previous procedure is that the Sylgard 184® mixture was let to stand for at least 1h to start the cross-linking, allowing it to gain more viscosity before the coating procedure started. This modification allowed for thinner and more homogeneous coatings to be attained with only one immersion into the Sylgard 184 solution. The PDMS-modified coatings prepared at Supelco followed the same procedure, with the exception of minor modifications explained during the discussion of the results. All coatings were prepared at least in triplicate. Prior to their usage, each coating was conditioned at 250ºC for one hour, and visually evaluated for uniformity and smooth surface coverage. If any defect was noted, coatings were discarded and new coatings were prepared.

5.2.4 SPME Procedure

5.2.4.1 Analysis of Grape Juice

Different types of PDMS-modified DVB/PDMS coatings were evaluated using DI-SPME mode. An aliquot (200g) of Concord grape juice was weighed into a 250-mL jar and spiked with 200 µL of working standard mixture. Proper seal of the jar was ensured, and the spiked matrix was pre-incubated at room temperature for 60 min prior to extraction to allow for the binding analytes-matrix to occur. Subsequently, aliquots of 7 g of spiked grape juice were weighted into 10-mL amber glass vials for SPME procedure. A 1 min incubation of the sample was performed in the agitation unit at 300 rpm and at 35 °C, followed by a 40 min

extraction at 35 ºC, while stirring at 300 rpm. Following extraction, fibers were rinsed in water for 30 sec, followed by desorption for 2 min at 270 ºC.

The experimental setup during grape juice analysis involved the analysis of instrumental quality control (QC) samples, which were used to correct for any inter-day instrumental drift.

The coating longevity experiment sets were divided in batches of 20 grape juice extractions. Fiber QCs were analyzed at the beginning and at the end of each batch. Following each batch of 20 extractions, the fiber was taken out of the autosampler and microscope pictures were taken in order to track the build up of fouling onto the coating surface.

5.2.4.2 Analysis of Water Samples

This first approach was employed to compare the extraction capabilities of the commercial fiber PDMS/DVB and the PDMS-modified fibers (fiber QCs). An aliquot of 7 mL nanopure water was transferred into a 10-mL amber vial, spiked with 7 µL of working standard mixture, and vortexed to ensure the homogeneous distribution of analytes in the solution. The same SPME procedure was employed for the grape juice matrix, except that the pre-desorption rinsing step was omitted. All extraction time points were performed in triplicate. Figure 5-1 shows a typical chromatogram obtained during daily QC checks.

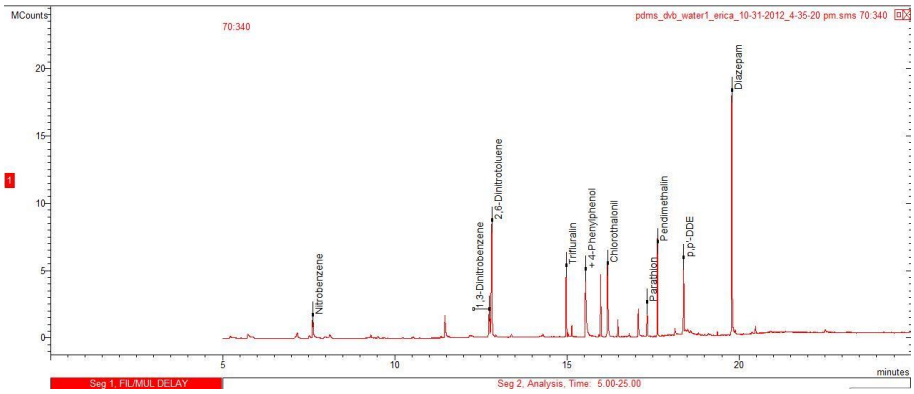


Figure 5-1 Representative chromatogram: analytes from standard mixture extracted from water using commercial PDMS/DVB fiber.

5.2.5 Instrumentation

Analyses of grape juice samples were performed using a Varian 3800 GC/4000 IT-MS system equipped with a SLB-5MS column (30 m, 0.25 mm i.d., 0.25 µm film thickness) Helium as the carrier gas was set to 1.5 mL/min. The 1079 injector was set at a temperature of 270 °C (unless otherwise specified). The column temperature program was initially set at 40 °C for 2 min, ramped at 10°C/min to 180°C, then ramped at 20ºC/min to 300ºC and held for 5 min, for a total run time of 25 min. The ion trap analyzer was operated in full scan mode: electron ionization (EI) at 70eV; temperatures of 200, 50 and 280 °C for the trap, manifold and transfer line respectively; a mass range of 70-340 *m/z* was scanned; a minimum of three ions were chosen for identification of each analyte. Automatic gain control (AGC) was turned on with an AGC target value of 25000 counts; the emission current was 10 µA. Automated analysis was performed using a CTC CombiPal autosampler (Zwingen, Switzerland) using the associated Cycle Composer software (Version 1.4.0). The CombiPal autosampler was equipped

with a SPME fiber holder, a temperature controlled six-vial agitator tray, and a fiber-conditioning device.

5.3 Results and Discussion

5.3.1 Effect of outer layer thickness & PDMS type on extraction efficiency and coating reusability

Initially, the extraction efficiency of the tested coatings was investigated by evaluating the influence of matrix modification, namely pH and ionic strength (NaCl, %). As presented in Figure 5-2 and Figure 5-3, the effect of pH was insignificant for most analytes in both water and grape juice matrices, with the exception of those analytes that exhibit their ionized forms, or might undergo degradation such as chlorothalonil. The addition of NaCl caused a decrease in the amounts extracted from grape juice samples for most of the analytes. Conversely, for the water matrix, the extraction efficiency for the most polar analytes such as nitrobenzene, 1,3-dinitrobenzene, 2,6-dinitrotoluene, 4-phenylphenol and diazepam was improved by adding NaCl. It is possible that a change in ionic strength, as a result of *salting-out*, enhanced binding of the analytes to the hydrophobic matrix components.

Based on the results, further experiments were conducted without any matrix modification. Hence, the grape juice was analyzed at its natural pH (~3.5), and no salt was added. It is important to emphasize that the aim of this work is to evaluate coating reproducibility, longevity and robustness, rather than a complete optimization of the SPME method. For this reason, all factors within the experimental design, from the concentration of the analytes to SPME parameters,

were set as to ensure appropriate sensitivity in order to guarantee a meaningful comparison between coatings.

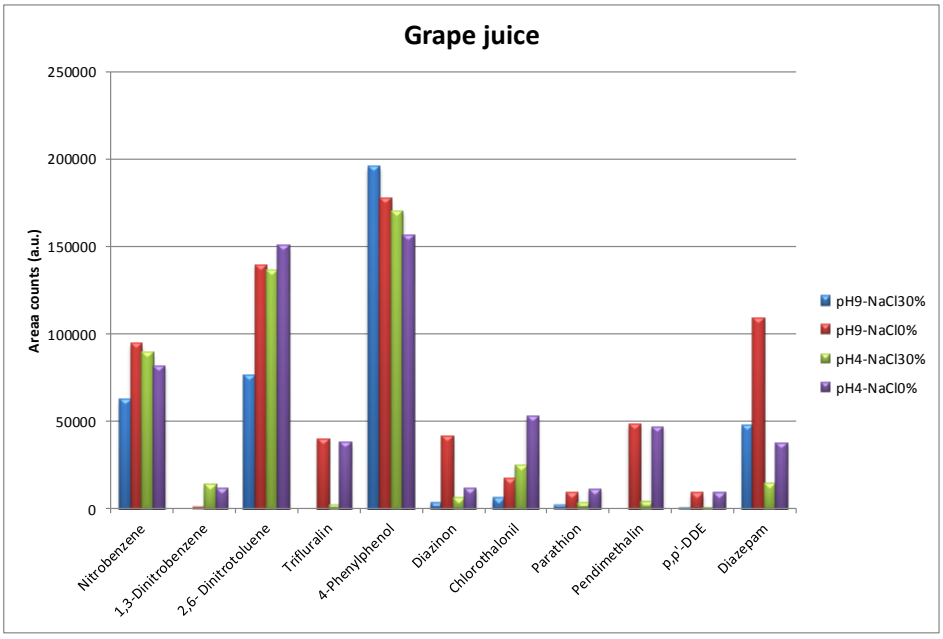


Figure 5-2 pH and salt (%) dependence of the amount extracted by a PDMS/DVB fiber from grape juice samples.

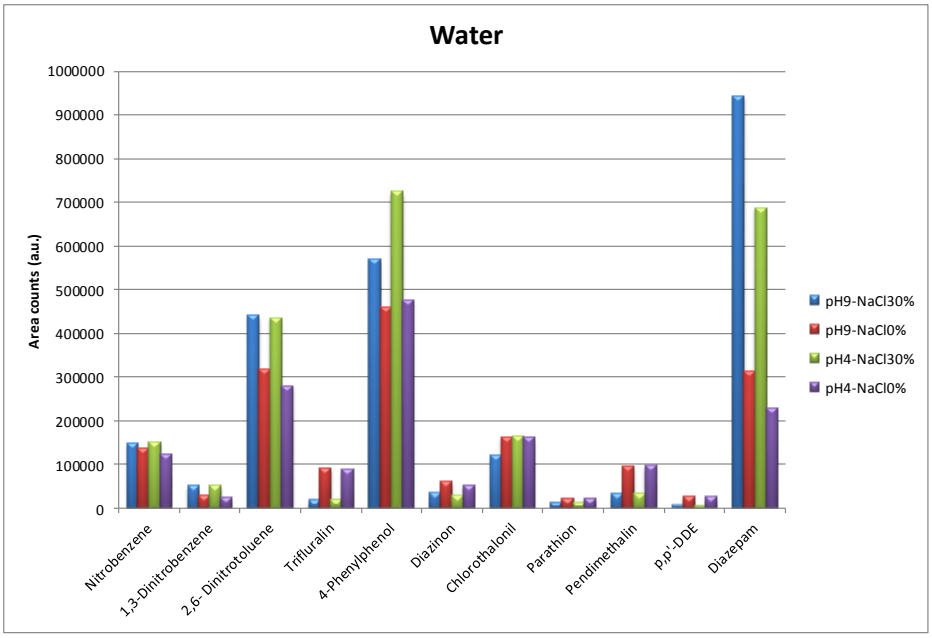


Figure 5-3 pH and salt (%) dependence of the amount extracted by a PDMS/DVB fiber from water samples.

The first batch of fibers analyzed comprised of (i) unmodified PDMS/DVB Stableflex, (ii) home-made PDMS-modified PDMS/DVB utilizing Sylgard 184® (~ 30 µm PDMS layer), (iii and iv) Supelco's PDMS-modified PDMS/DVB utilizing proprietary PDMS (10 and 30 µm PDMS layers).

At first, the performance of each coating type was evaluated through extractions from spiked grape juice and water samples. Three water samples were extracted followed by 20 grape juice samples, and then another triplicate of water samples. The water samples at the beginning and at the end worked as fiber QCs in order to evaluate any variations in extraction capabilities due to coating degradation by fouling.

In agreement with the findings reported on Chapter 4, the preliminary investigation presented in

Figure *5-4* through Figure 5-7 clearly demonstrates the necessity of investigating the behavior of the PDMS outer layer on the extraction efficiency of compounds with a wide range of polarities and diverse chemical functionalities. Indeed, as can be seen in the figures below, the additional layer of PDMS caused a decrease in the amounts extracted (in water) for some of the more polar analytes as compared to the amounts extracted by the unmodified PDMS/DVB coating due to an additional barrier slowing down the kinetics of extraction. For example, in the first set of extractions from water samples, compared to the unmodified PDMS/DVB for 1,3-dinitrobenzene, the amounts extracted were decreased by 14.6% (Supelco's PDMS 10μm), 23.4 % (Supelco's PDMS 30 μm), and 17.5 % (lab-made Sylgard 184® PDMS 30 μm). Similar trends were also observed for diazepam and 4-phenylphenol, except that the amount extracted by the Supelco's PDMS 10μm overcoated coating was not statistically different from the amount extracted by the PDMS/DVB coating.

However, when investigating the effect of the PDMS layer on extractions performed in grape juices samples, a decrease in extraction efficiency was only observed for diazepam for Supelco's PDMS 30 μm (28.2%) and the lab-made Sylgard's 184 PDMS 30 μm (20%). Once again, the amount of diazepam extracted from grape juice by Supelco's PDMS 10μm overcoated coating was not statistically different from the amount extracted by the PDMS/DVB coating. In

the overcoated configuration, the polar analytes must first diffuse through the PDMS interface prior to adsorption in the solid DVB coating. Since this in-between phase is a liquid polymer and the analytes have low diffusion coefficients in it, the mass transfer is slowed down and the extraction process is kinetically limited. Moreover, owing to the hydrophobicity of this outer film, the concentration of these polar analytes on the interface sample/PDMS is diminished, thus, also decreasing the partitioning of analytes between sample and coating. In fact, comparing both Supelco's PDMS overcoated coatings with different outer layer thicknesses, namely 10 µm and 30 µm, the results clearly demonstrate that, within this extraction time of 40 min, a thicker PDMS outer layer leads to a more prominent decrease in the amount extracted for these polar analytes as compared to unmodified PDMS/DVB.

In agreement to these findings, Kloskoeski *et al*. presented a system comprised of a polyethylene glycol (PEG) coating restricted within a PDMS outer layer. The authors referred to the system as a membrane-SPME, where an external layer of PDMS of 25µm significantly slowed down the diffusion of the polar phenol analytes across the PDMS membrane, which could serve as a physical barrier as well as a concentrating medium, analogous to the extraction phase [152].

It is also worth noting the opposite effect of the PDMS overcoating on the extraction efficiency of more hydrophobic compounds, such as trifluralin, pendimethalin, and p,p'-DDE. For instance, the amount of trifluralin extracted from water samples increased in comparison to the amount extracted by

commercial PDMS/DVB coating by 37 % (Supelco's PDMS 10μm), 19.1 % (Supelco's PDMS 30 μm), and 48.4 % (lab-made Sylgard 184® PDMS 30 μm). A similar trend was also observed for grape juice sample extractions. Differently from what is seen for polar analytes, these hydrophobic compounds have a higher affinity for the PDMS polymer, and even though their diffusion is also impaired by this additional barrier, their accumulation on the interface sample/PDMS is greatly enhanced as compared to their accumulation on the sample/DVB interface when employing unmodified PDMS/DVB fibers. As a result, the liquid matrix boundary layer, rather than the PDMS layer, is the major contributor as the rate limiting step for the diffusion of these analytes from the matrix to the overall coating. Additionally, for these hydrophobic compounds, the PDMS layer acts also as a concentrating media, adding capacity to the overall sorbent; thus, increased amounts extracted are obtained in comparison to amounts obtained by the unmodified PDMS/DVB coating.

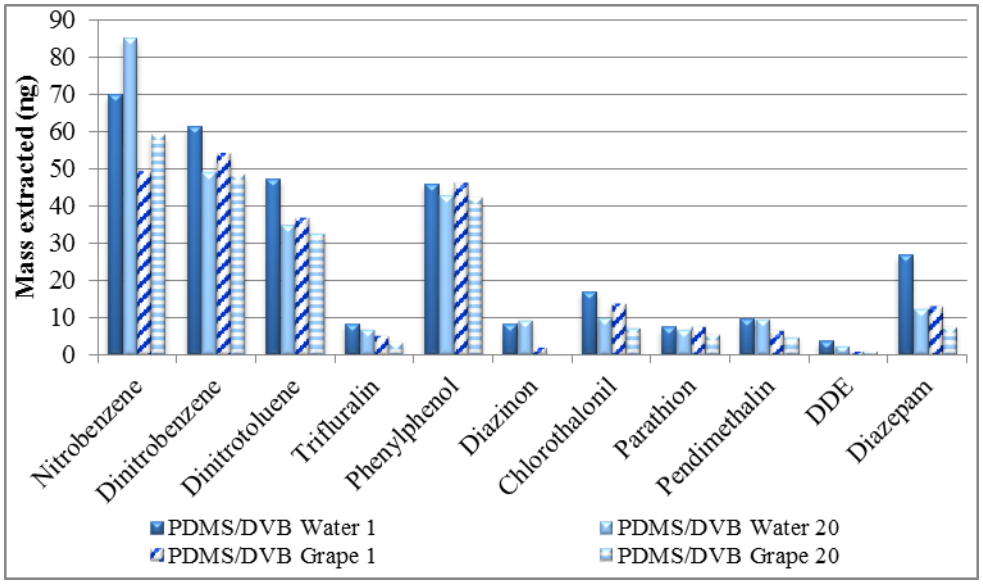


Figure 5-4 Comparison of analyte response from grape juice (1st and 20th extractions) and water (before and after grape juice extractions) using commercial PDMS/DVB fiber.

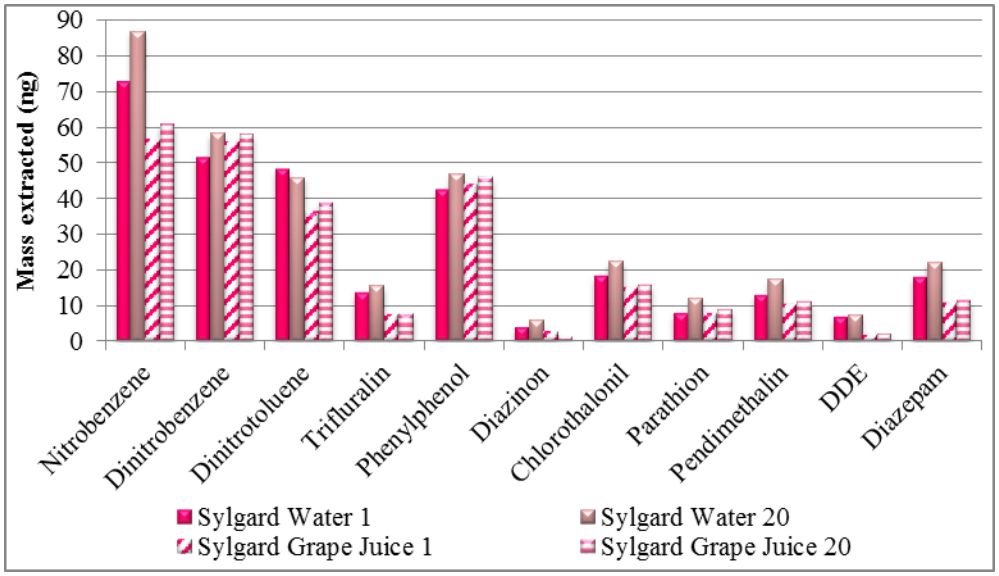


Figure 5-5 Comparison of analyte response from grape juice (1st and 20th extractions) and water (before and after grape juice extractions) using PDMS/DVB with Sylgard 30 µm overcoat.

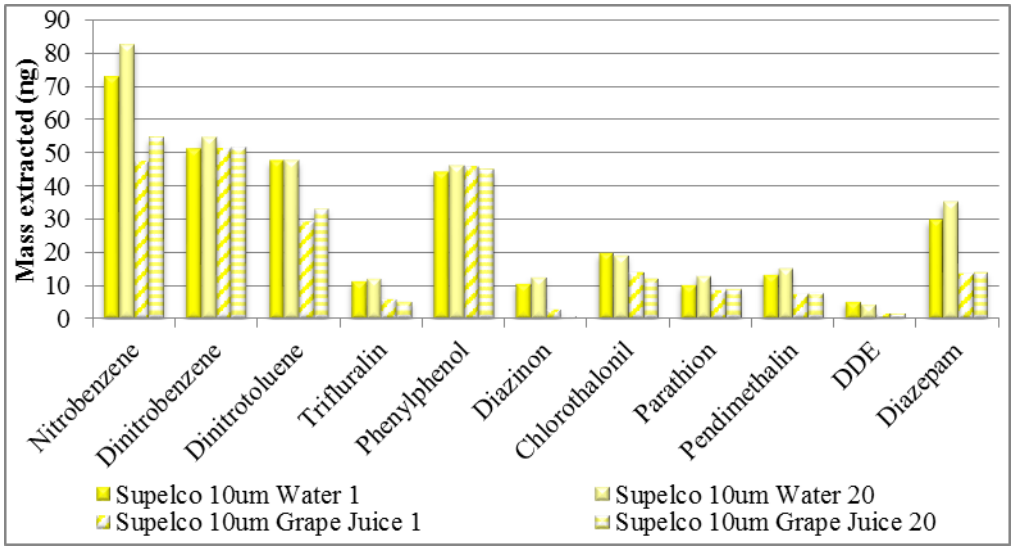


Figure 5-6 Comparison of analyte response from grape juice (1st and 20th extractions) and water (before and after grape juice extractions) using PDMS/DVB with a 10µm PDMS overcoat (Supelco).

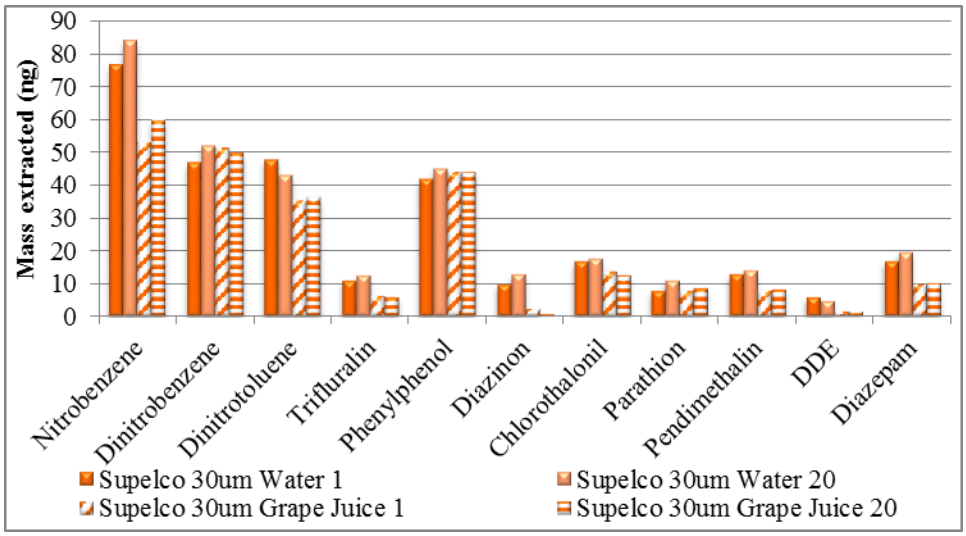


Figure 5-7 Comparison of analyte response from grape juice (1st and 20th extractions) and water (before and after grape juice extractions) using PDMS/DVB with a 30µm PDMS overcoat (Supelco).

Besides extraction efficiency, the most sought after feature in this new type of matrix-compatible coating is its ability to perform upwards to tens of extractions with the same coating from complex matrices, not only overcoming typical drawbacks of commercial coatings such as precocious deterioration by fouling and limited reproducibility, but also enhancing method throughput in an automated fashion. In the context of food analysis, solid SPME coatings are the most commonly used; PDMS/DVB for pesticides, and DVB/Car/PDMS for a wider range of compounds. Giving the need to overcome the limitations associated with these coatings in regards to DI-SPME extractions from complex matrices, a compromise between extraction efficiency and matrix-compatibility would still be advantageous for the area of food analysis.

To investigate fiber reusability, each coating was subjected to 40 extractions of Concord grape juice samples, divided into 2 sets of 20 extractions. After each set of extractions, the fiber was submitted to an extraction in water (QC), and microscope pictures of the coatings were taken to assess the extent of fouling. As can be seen in Figure 5-8 to Figure 5-18, regardless of PDMS type or overcoating thickness, the addition of PDMS clearly improved the response obtained in the 40^{th} extraction in grape juice, as compared to the 1^{st} extraction. The lower and upper lines in the plots denote a ± 20% error interval. In terms of extracted amounts, the curve obtained for Supelco's PDMS 10 µm overlayer is the one that mostly resembles the profile exhibited by the non-modified fiber.

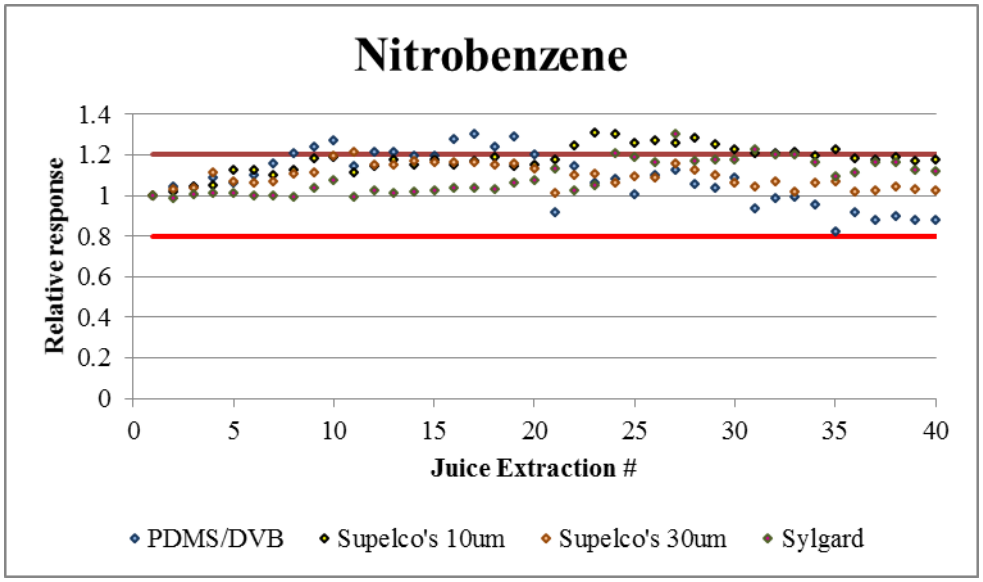


Figure 5-8 Nitrobenzene: reusability profile of coatings subjected to 40 DI-SPME in Concord grape juice.

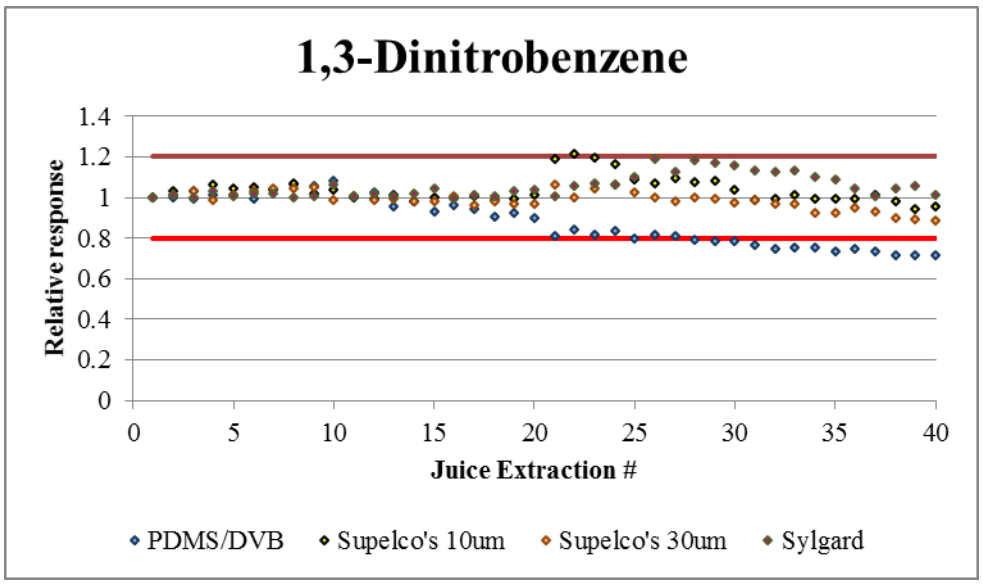


Figure 5-9 1,3-Dinitrobenzene: reusability profile of coatings subjected to 40 DI-SPME in Concord grape juice.

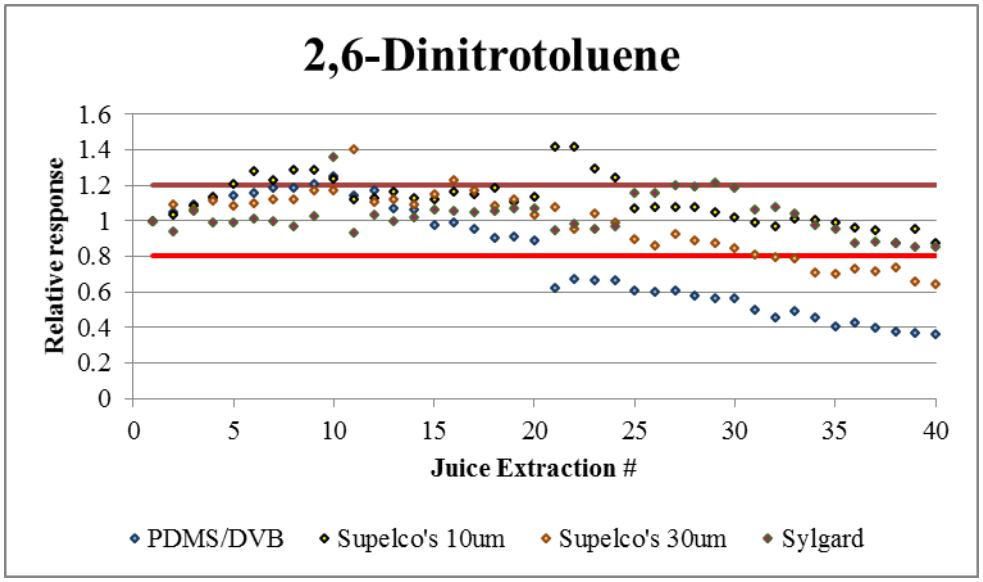


Figure 5-10 2,6-Dinitrotoluene: reusability profile of coatings subjected to 40 DI-SPME in Concord grape juice.

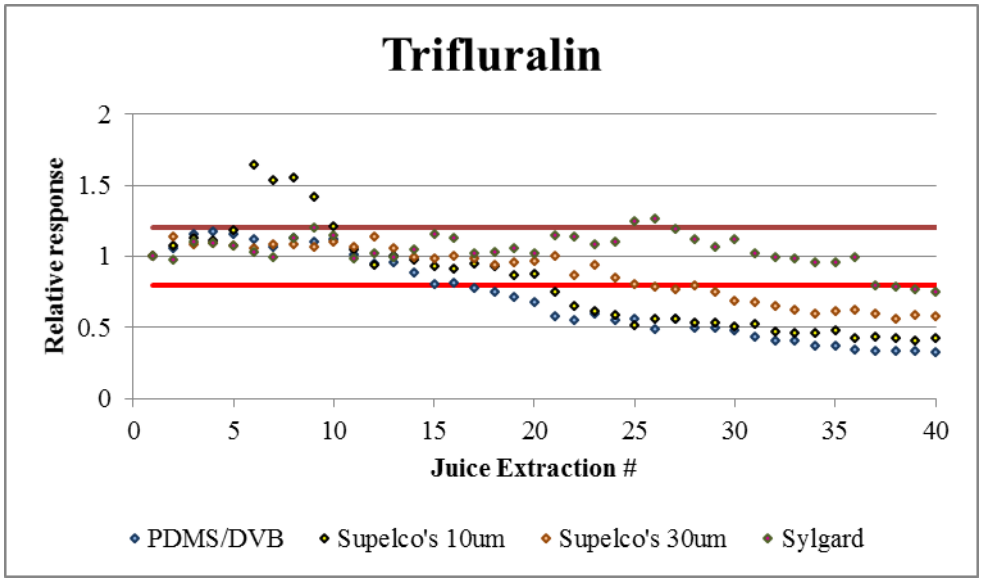


Figure 5-11 Trifluralin: reusability profile of coatings subjected to 40 DI-SPME in Concord grape juice.

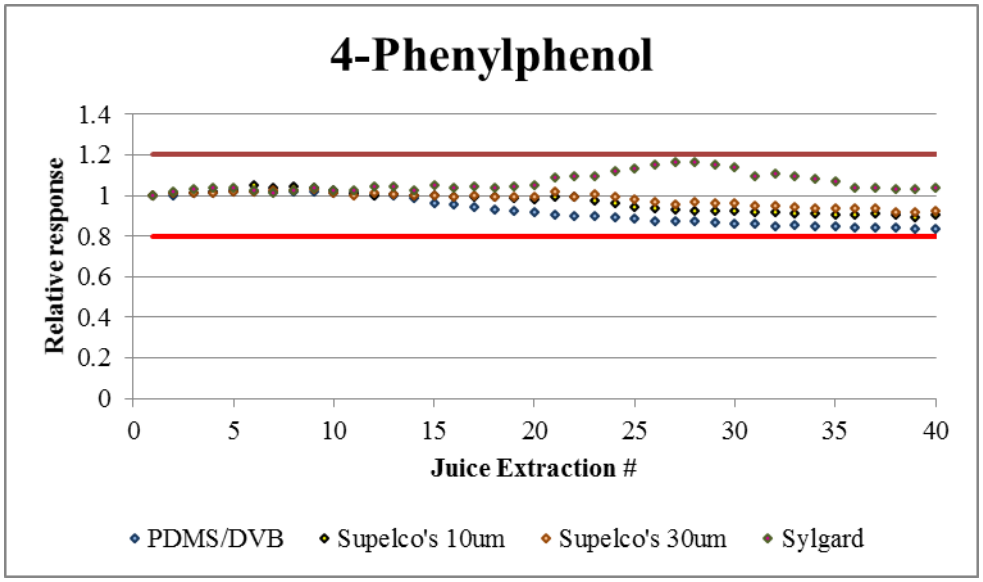


Figure 5-12 4-Phenylphenol: reusability profile of coatings subjected to 40 DI-SPME in Concord grape juice.

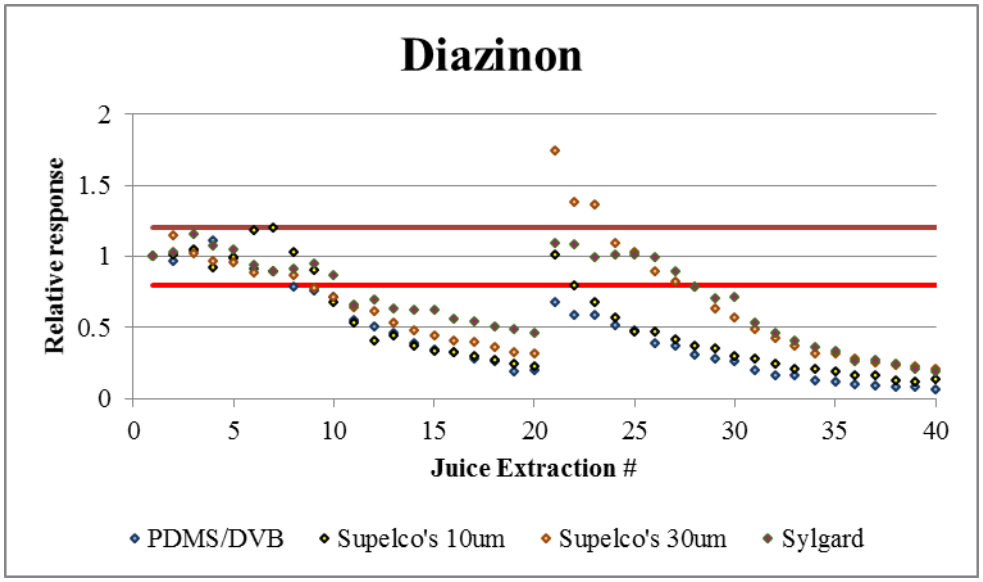


Figure 5-13 Diazinon: reusability profile of coatings subjected to 40 DI-SPME in Concord grape juice.

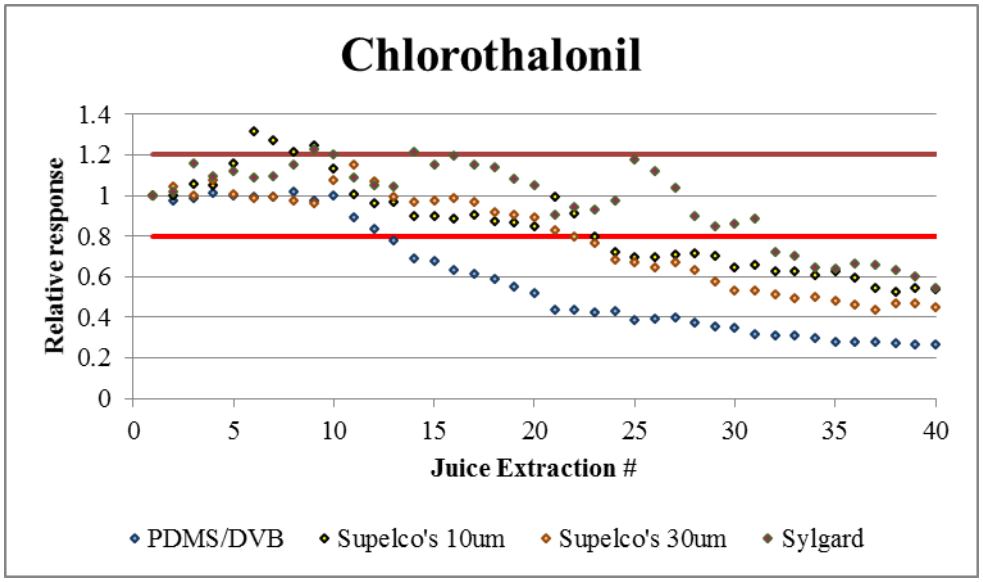


Figure 5-14 Chlorothalonil: reusability profile of coatings subjected to 40 DI-SPME in Concord grape juice.

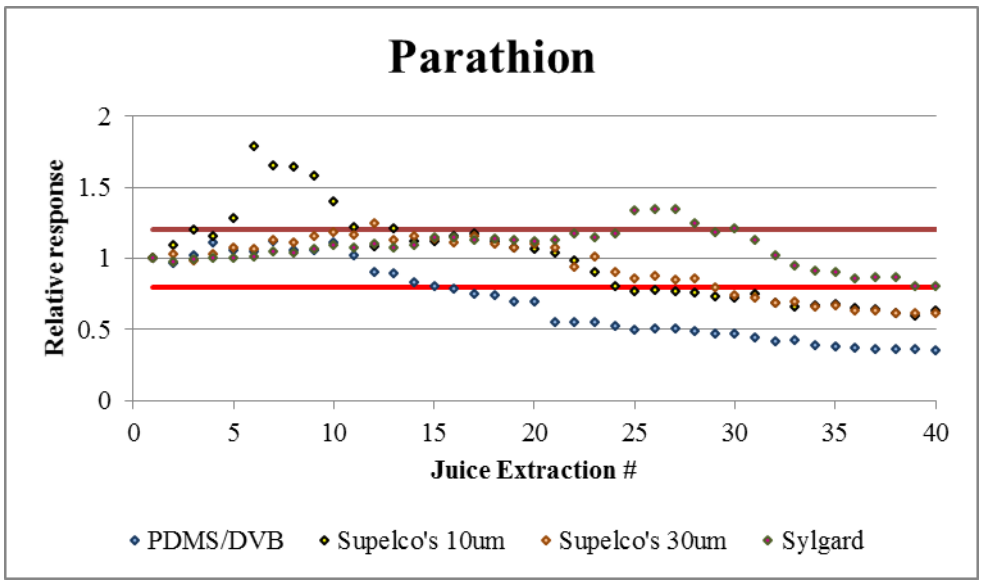


Figure 5-15 Parathion: reusability profile of coatings subjected to 40 DI-SPME in Concord grape juice.

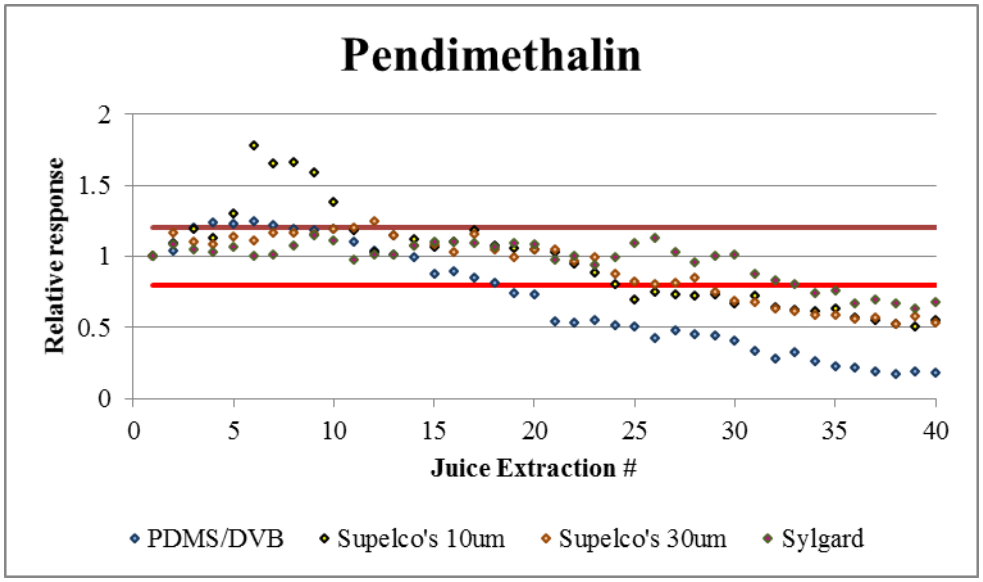


Figure 5-16 Pendimethalin: reusability profile of coatings subjected to 40 DI-SPME in Concord grape juice.

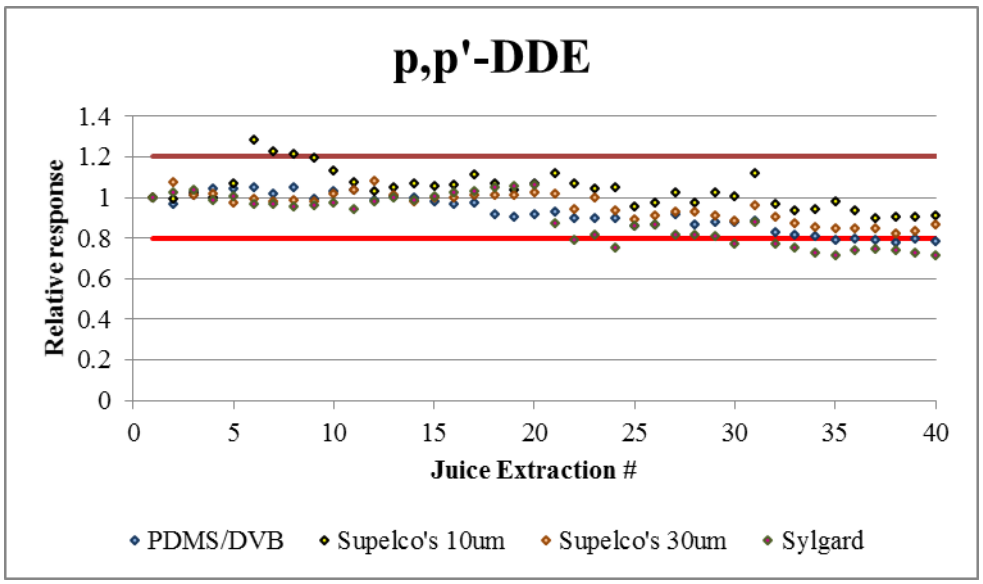


Figure 5-17 p,p'-DDE: reusability profile of coatings subjected to 40 DI-SPME in Concord grape juice.

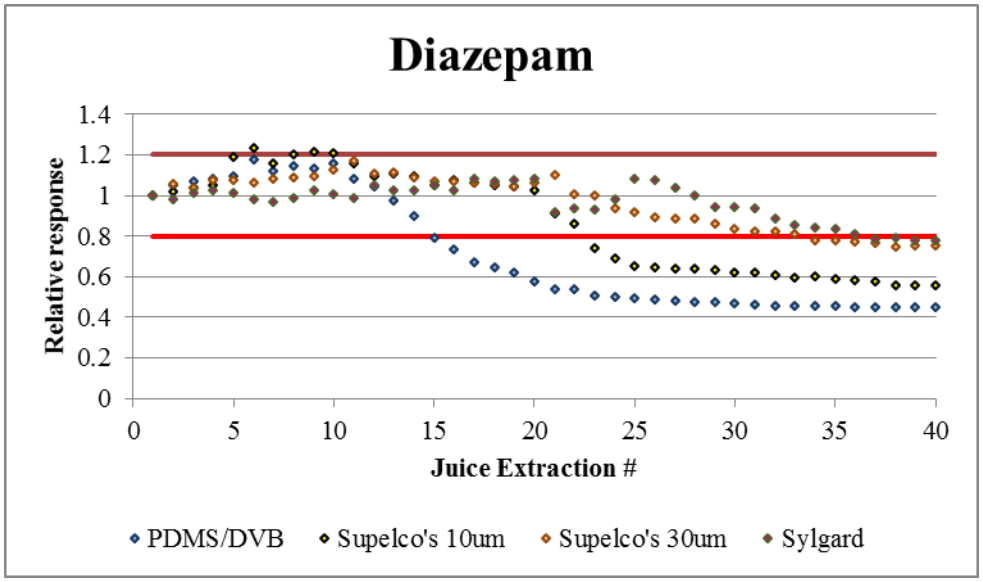


Figure 5-18 Diazepam: reusability profile of coatings subjected to 40 DI-SPME in Concord grape juice.

The pronounced decrease in extracted amounts exhibited by Diazinon for all fibers was closely investigated. Spiked juice samples were allowed to stand overnight, and the extraction results showed the same decreasing trend. For this reason, one possible explanation would be that given the relatively high Henry's constant of diazinon and the headspace volume in the vial of approximately 3 mL, diazinon could have been transferred to the headspace, which would account for the decrease on the amount extracted over time (time of vial sitting on the autosampler tray prior to extraction). To evaluate this behaviour, 10 water samples were analyzed. Six samples were spiked and run immediately after spiking, two samples were run 12h after spiking, and another two samples were

extracted 24 h after spiking. The results can be better visualized in Figure 5-19; as denoted by the graph, no decreased extraction amounts were observed, even after 24 h of samples sitting on the autosampler tray. Therefore, the observed behaviour may be attributed to degradation and/or interaction of diazinon with ascorbic acid present in grape juice, rather than a binding effect. In fact, previous data acquired for diazinon in pure grape pulp did not show such effect. As no meaningful data regarding coating longevity and reproducibility could be obtained with such data, the results from diazinon will be excluded from further discussion.

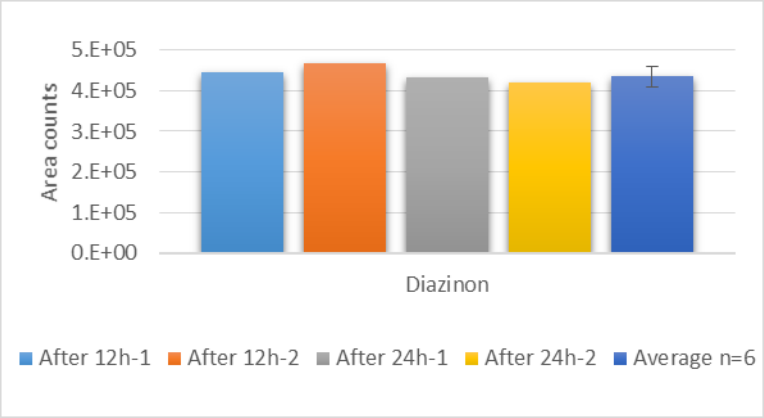


Figure 5-19 Diazinon stability test in water matrix.

The results summarized in Figure 5-20 and Figure 5-21 allow for a better visualization of the effect of the PDMS overcoating on the quality of data obtained from a batch of 40 grape juice samples. Based on a comparison between the amounts of analyte extracted in the 40th sample, as compared to the first sample, the extraction efficiencies also changed according to the type of PDMS used for overcoating, although it is evident that an improvement in reusability by the PDMS overcoating took place for all analytes. As shown by the experimental

data, the Sylgard ® 184 was shown to outperform Supelco's PDMS when comparing both coatings with the same overcoating layer of 30 µm. Sylgard 184 ® also offered the best reproducibility, by means of RSD (%) for *n=40* samples; it provided RSDs < 20% for nearly all analytes, except for chlorothalonil, which yielded an RSD of 20.8 % (*Figure 5-21*).

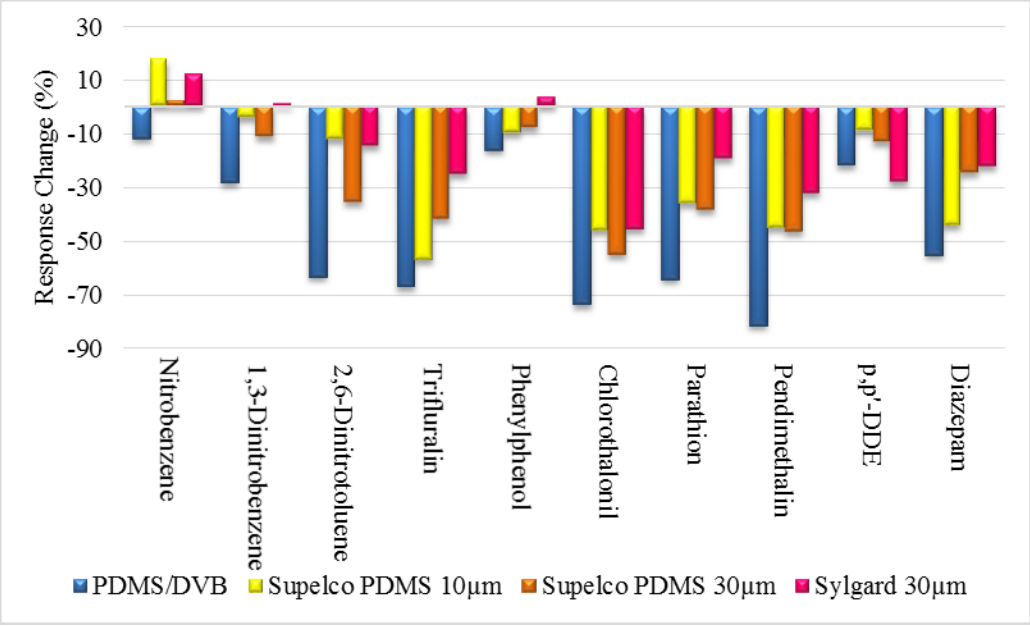


Figure 5-20 Comparison of changes in extracted amounts between 1st and 40th extractions in grape juice.

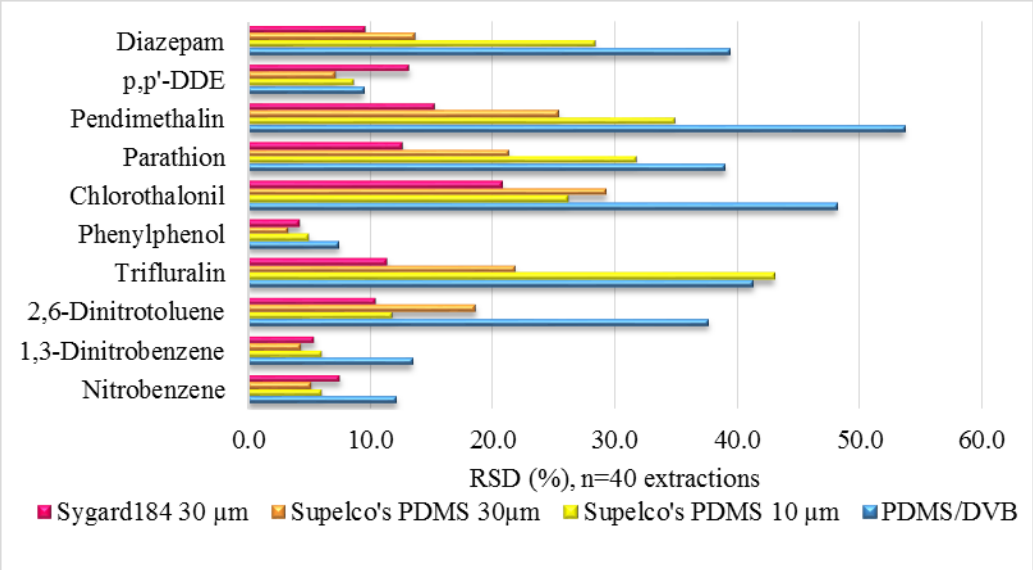


Figure 5-21 Comparison of RSDs throughout 40 extractions in grape juice sample.

As stated in Chapter 2, the observed matrix effect in DI-SPME-GC analysis of fruits and some vegetables leads to a decrease in extraction capabilities. This can be mainly attributed to the attachment of sugars onto the coating surface; the attached sugars caramelize once brought into the hot injector for desorption, forming a layer of fouling. The extent of this fouling is a critical parameter in the acquisition of reliable analytical data and in the determination of the coating lifetime.

In order to test the longevity of the coatings, all coatings were further studied with another set of fibers throughout 100 extractions (except PDMS/DVB, n=60) in grape juice, divided in sets of 20 extractions. After each set of extractions, the fiber was submitted to an extraction in water (QCs), and

coating pictures were taken. In agreement with the previously discussed results, the longevity studies have shown that the introduction of a PDMS outer layer slowed down the damaging process in the coating compared to the non-modified fiber (Figure 5-22 to Figure 5-26). In fact, the unmodified PDMS/DVB coating could not be used beyond 60 extractions due to extensive fouling of the coating.

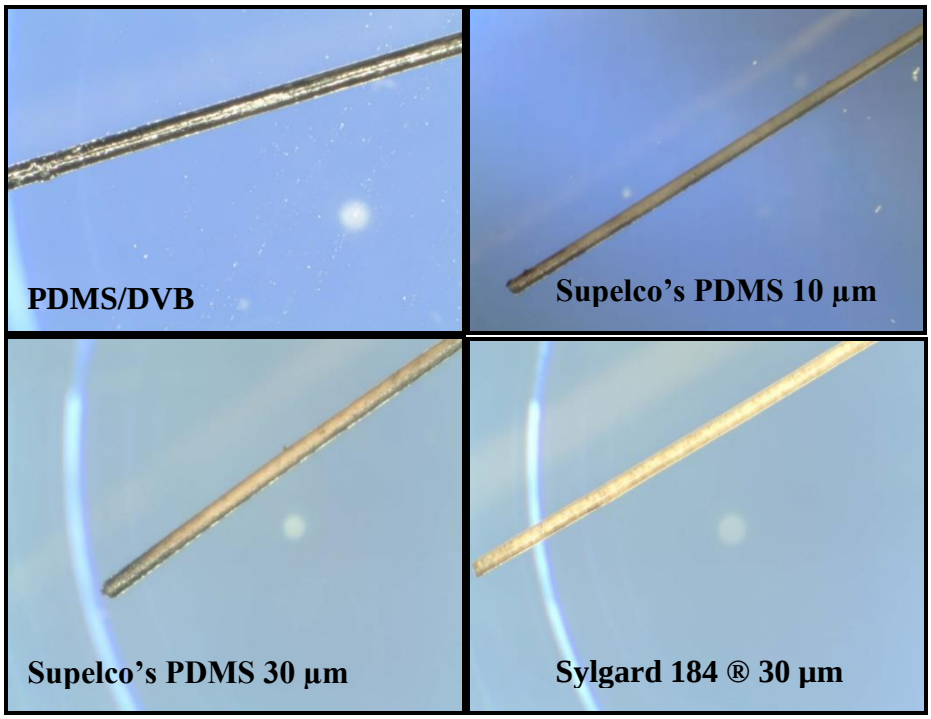


Figure 5-22 Microscopic pictures of coatings after 60 extractions in grape juice.

The behaviour exhibited by the Sylgard 184® overcoated fibers is worthy of note; after each fiber QC, the coating presented a higher response than the one obtained for the last extractions from the previous set of samples. For instance, after the 21st extraction, the extraction efficiency of this coating steadily decreased until the end of that set of samples (40th extraction). After extraction in water

(QC), it once again recovered its extraction capability for the 41st extraction. The same declining behaviour was observed from the 41st to the 60th extraction, with a detected recovery again at the 61st extraction. One of the possible explanations to this behaviour is that the water QC extraction (40min) acted as a cleaning step, hence, restoring the extraction capabilities of the fiber. The effect of this water extraction was not as pronounced in the Supelco's PDMS 10 µm and Supelco's PDMS 30 µm overcoatings because both extremities of these fibers had an exposed DVB phase (not coated with PDMS – see coating pictures in Figure 5-27). Conversely, in the case of the Sylgard 184® fibers, both extremities were overcoated with the polymer. This difference in fiber configuration is mainly due to the coating process that implemented for each fiber: Supelco's PDMS fibers were prepared by overcoating DVB/PDMS fibers strands of ~ 60 cm, which was then cut into 1 cm segments that were subsequently assembled into the commercial SPME assembly. On the other hand, the Sylgard ® 184 overcoat was performed by dipping commercial PDMS/DVB fibers (with their assembly) into the liquid polymer, thus assuring a complete seal of fiber's extremities. The following data, including the water QC results and coating pictures, add valuable information to the longevity studies, as well as also help to corroborate the aforementioned hypothesis.

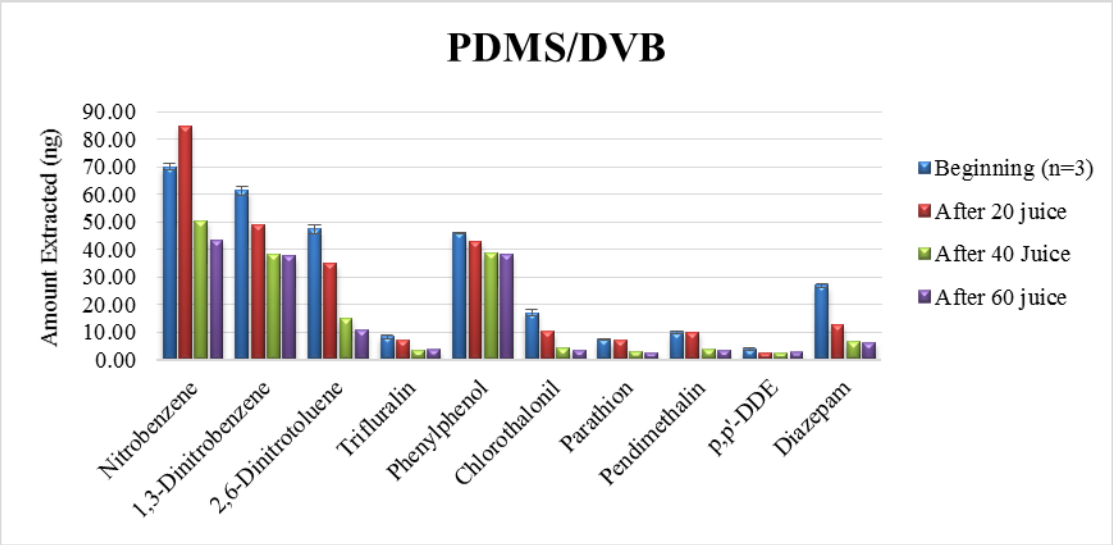


Figure 5-23 Comparison of analytes responses from extractions in water with PDMS/DVB (fiber QCs).

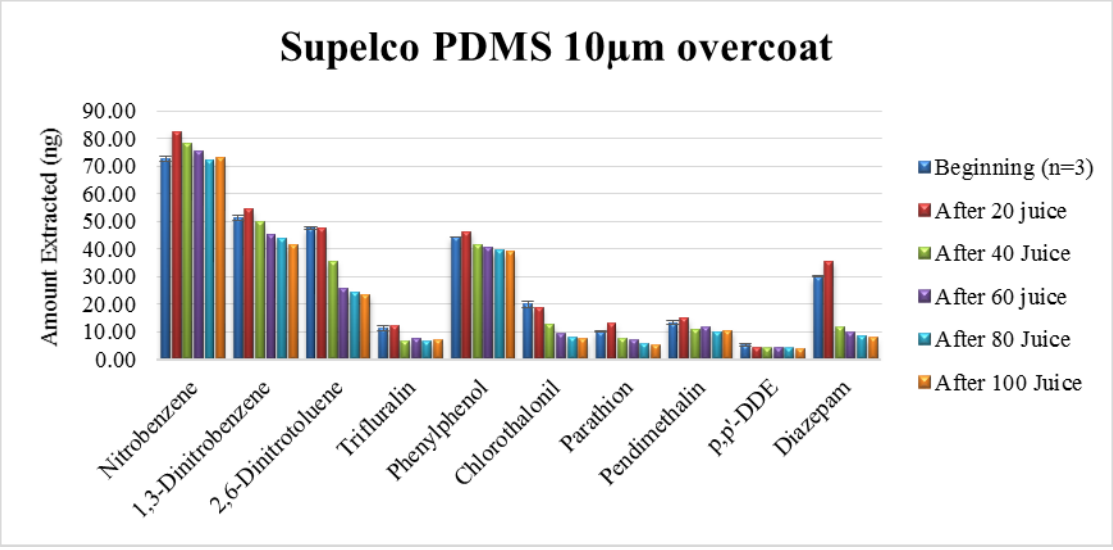


Figure 5-24 Comparison of analytes responses from extractions in water with Supelco PDMS 10 µm overcoat (fiber QCs).

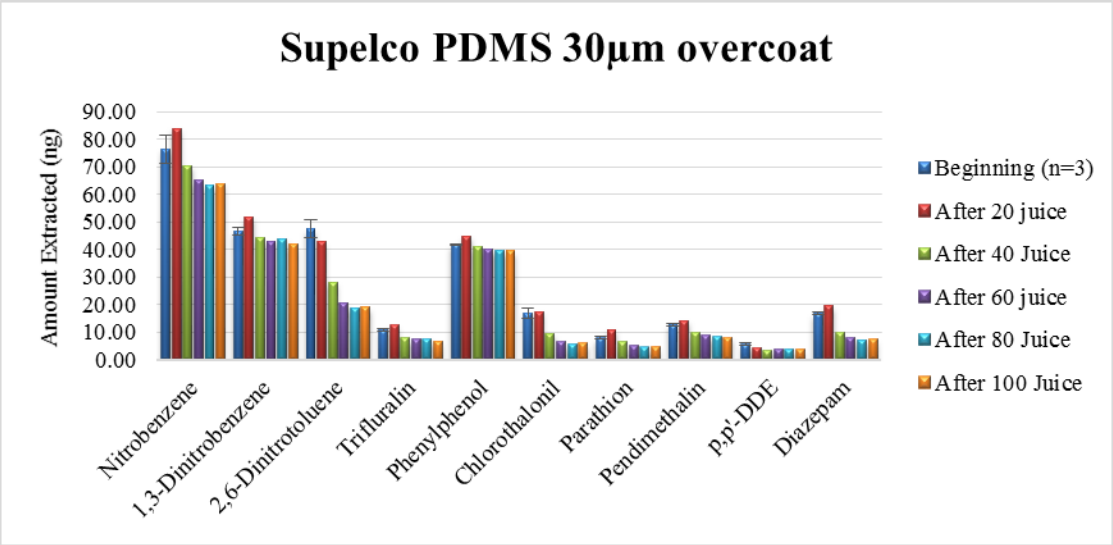


Figure 5-25 Comparison of analytes responses from extractions in water with Supelco PDMS 30 µm overcoat (fiber QCs).

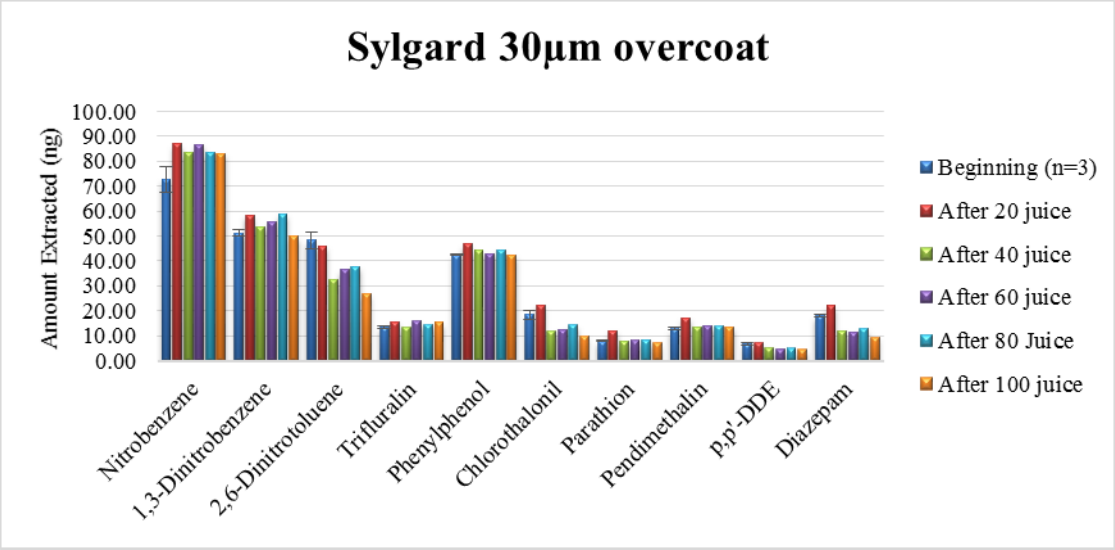


Figure 5-26 Comparison of analytes responses from extractions in water with Sylgard 184® 30 µm overcoat (fiber QCs).

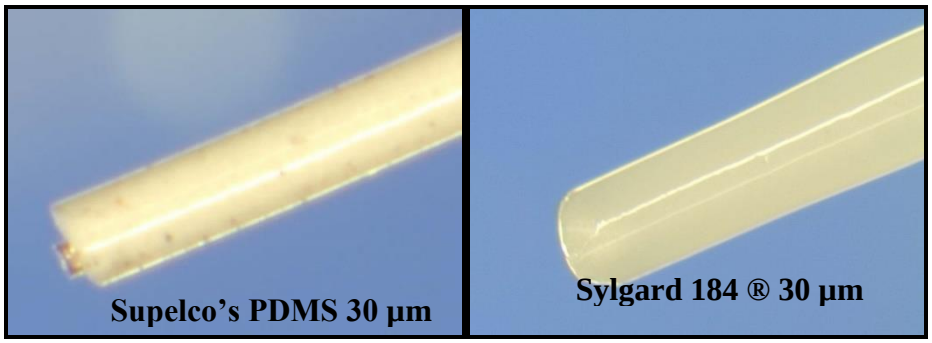


Figure 5-27 Overcoated fiber tips.

5.3.2 Statistical Analyses

A statistical evaluation by means of Student's t-test (at 95% confidence level) was performed for each pair of fibers, as follows: Table 5-1 shows intra-fiber paired two samples for means (for each fiber, comparing the first 20 and last 20 extractions), and Table 5-2 shows inter-fiber: two-sample assuming unequal or equal variances (comparing each pair of fibers throughout the entire longevity study). The results are summarized below.

Table 5-1 Intra-fiber: paired two samples for means (for each fiber, comparing the first 20 and last 20 extractions). ***Yes*** *means statistically significant difference, whereas,* ***No*** *means not statistically significant difference.*

Paired t-test for means				
	PDMS/DVB	***Supelco's 30µm***	***Supelco's 10 µm***	***Sylgard 30 µm***
Nitrobenzene	No	No	Yes	Yes
1,3-Dinitrobenzene	No	No	No	Yes
2,6-Dinitrotoluene	No	No	No	Yes
Trifluralin	No	No	No	Yes
4-Phenylphenol	No	No	No	Yes
Chlorothalonil	No	No	No	No
Parathion	No	No	No	No
Pendimethalin	No	No	No	No
p,p'-DDE	No	Yes	Yes	No
Diazepam	No	No	No	No

Table 5-2 Inter-fiber: two-sample assuming unequal or equal variances (n=100, except PDMS/DVB n=60)). ***Yes*** *means statistically significant difference, whereas,* ***No*** *means not statistically significant difference.*

Two sample t-test						
	Syl/ Sup30µm	***Syl/ Sup10µm***	***Syl/ PDMS/DVB***	***Sup30µm/ PDMS/DVB***	***Sup10µm/ PDMS/DVB***	***Sup30µm/ Sup10µm***
Nitrobenzene	No	No	No	No	No	Yes
1,3-Dinitrobenzene	No	No	No	No	No	No
2,6-Dinitrotoluene	No	No	No	Yes	Yes	Yes
Trifluralin	No	No	No	No	No	Yes
4-Phenylphenol	No	No	No	Yes	Yes	Yes
Chlorothalonil	No	No	No	No	No	Yes
Parathion	No	No	No	No	No	No
Pendimethalin	No	No	No	No	No	Yes
p,p'-DDE	No	No	No	No	No	No
Diazepam	Yes	No	No	No	Yes	No

According to the tables above, statistically, the Sylgard 184 ® overcoat extracted the same amount (at 95% confidence level) between the first 20 and last

20 extractions (averages) for nearly half of the analytes studied. Interestingly, the best performance was shown towards the early eluting, more polar analytes.

In terms of inter-fiber performance, it can be seen that Supelco's 10μm and 30μm were statistically equal throughout the 100 extractions performed. However, none of these Supelco overcoat fibers performed statistically the same as Sylgard 184 ®. Therefore, given the same type of PDMS, the thickness of the overcoat did not display a significant effect on the fiber longevity. Conversely, the type of polymer used may have had a significant influence on the fiber lifetime; an inspection of the results obtained for different types of PDMS overcoated at the same layer thickness (30 μm), demonstrated that Sylgard 184 ® and Supelco's PDMS behaved differently towards the investigated analytes. Indeed, considering the observed differences between the commercial blends of PDMS, it becomes impossible to blanket the sorption properties of these materials under just one name (i.e., PDMS); rather, these results corroborate previous findings, which indicate that each formulation needs to be independently assessed [177].

To better understand the performance differences observed within this work between these two distinct PDMS coatings, the specific attributes of each need to be further examined. While a thorough examination of the particulars of these fibers may fall beyond the scope of the current work, a cursory overview of their distinctive fabrication processes can help shed some light into possible reasons for the observed discrepancies in performance.

The PDMS overcoated fibers manufactured and provided by Supelco employed a proprietary PDMS formulation, which limited the information available about this material. In short, the PDMS used is a high molecular weight plot adhesive that employs a peroxyde catalyst. After overcoating, the PDMS sets fast by the use of a heat gun, which is applied for a few seconds. Subsequent curing is then performed for 10 h at 280 ºC, in order to ensure minimal siloxanes bleeding. The coupling groups on this polymer are more reactive than coupling groups more commonly used with PDMS; thus, it crosslinks to a greater extent. As such, the highly crosslinked PDMS layer obtained has great mechanical properties and decreased fluidity.

Sylgard 184 ® from Dow Corning ® was employed for *in-laboratory* preparation of PDMS overcoated fibers. The Sylgard 184® silicone elastomer is widely used in bioanalytical applications due to its many useful characteristics, including transparency to ultraviolet light, gas permeability, toughness, flexibility, and non-stick properties. It has a composition comprising of vinyl end-capped oligomeric dimethyl siloxane chains cross-linked with methyl hydrosilane reinforced with trimethylated silica, and a platinum catalyst. Sylgard 184® comes as a two-part epoxy consisting of a base or resin, and a curing agent, mixed by weight in a ratio of 10:1 to form the resulting silicone-based elastomer material. The formulation chemistry of Sylgard ® 184 was intentionally designed with a stoichometrically large excess of silane groups in order to achieve faster curing at mild temperatures [177].

In the present study, after degassing, the polymer was used for overcoating PDMS/DVB fibers in their original commercial assembly (as detailed in Chapter 2). In this study, curing of the Sylgard 184® was carried out in a vacuum oven at 50 ºC for 12 h. It has been reported in the literature that both temperature and time of curing affects the properties of the final polymer [178,198]. Indeed, Johnston *et al.* reported that the hardness of the Sylgard 184® increased linearly as curing temperature was increased [178]. Another feature of the Sylgard 184® worth mentioning is the presence of fumed silica (SiO_2), which functions as a filler to increase its mechanical strength. However, the effect of SiO_2 nanoparticles in the polymer's properties towards analytes, such as permeability, has been a source of debate. One school of thought believes that SiO_2 nanoparticles somewhat induce the disruption of polymer chains, by means of less crosslinking, which increases the size of the free volume within this membrane through which molecular transport can occur, thus enhancing polymer permeability [177,179,199]. Regardless, the mechanical properties of the polymer are not compromised, since SiO_2 glassy nanoparticles add significant stiffness to the polymer. Furthermore, it has also been reported that the fumed silica nanoparticles in the Sylgard 184 do not alter the surface morphology, and as such, a smooth and uniform coverage can still be attained (Palchesko, Zhang, Sun, & Feinberg, 2012).

In a retrospective fashion, taking into account the abovementioned characteristics of both types of PDMS used for overcoating and the results obtained, it is evident that Sylgard ® 184 provides better results compared to Supelco's high density PDMS. The exact mechanism that leads to this

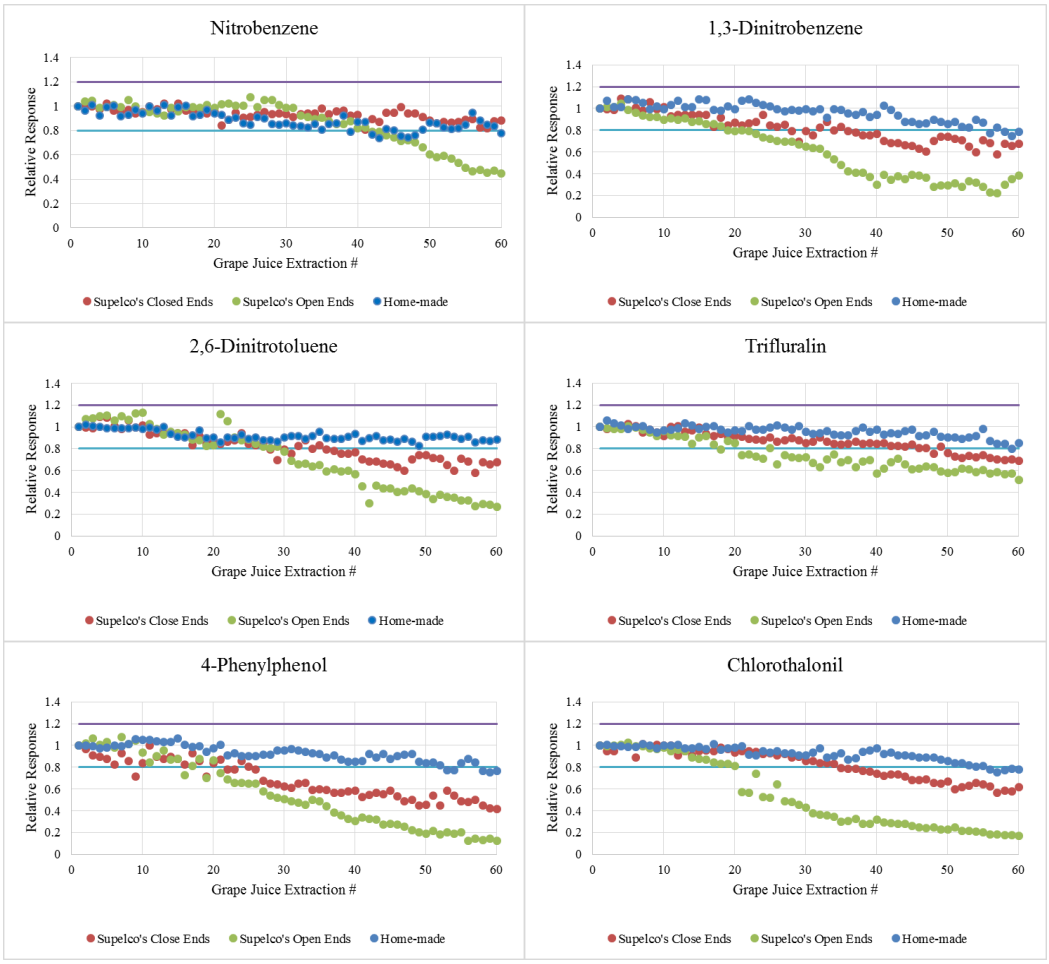
improvement is not fully understood; however, one can hypothesize that the silica fillers could render the final polymer hydrophilic regions. In addition, an excess of silane groups, which readily hydrolyze upon exposure to water (resulting in hydroxyl groups within the material), would offer Sylgard ® 184 superior interactions with aqueous media, resulting in improved cleaning of the fiber during the rinsing steps (and perhaps, QC water samples).

5.3.3 Importance of sealed extremities

As previously mentioned, one possible explanation for Sylgard's enhanced performance could be attributed to the sealed extremities obtained during the Sylgard 184 ® overcoating process. Sealed extremities would ensure that no porous surface (e.g., DVB) is exposed to the matrix, thus, avoiding easier attachment of matrix constituents that induce irreversible fouling.

To continue this investigation towards the most robust coating, Sylgard ® 184 overcoated fibers were fabricated with sealed and unsealed ends. To prepare the sealed fibers, after assembly of the overcoated segments, each fiber was dipped into a diluted Sylgard ® 184 solution (since this process was performed by Supelco, the identity of the diluting solvent is unknown). It should be noted that for this investigation, a post-desorption fiber-washing step was added throughout the experiment for a lifetime evaluation, similarly to the step implemented in Chapter 2. After desorption, fibers were washed for 5 minutes in a water:methanol (50:50, v/v) solution.

The results obtained for the overcoated fibers prepared at Supelco's facilities (unsealed = open ends, and sealed= closed ends), as well as the results obtained with the home-made overcoated fiber (all prepared using Sylgard ® 184) are shown in Figure 5-28. Significant differences can be observed between sealed and unsealed fibers. For instance, RSD%,$_{n=60}$ obtained for 1,3- dinitrobenzene were 43%, 16% and 9% for open ends, closed ends and home-made, respectively. Indeed, the highest values for RSD% obtained with the unsealed fibers were observed for the most polar analytes.



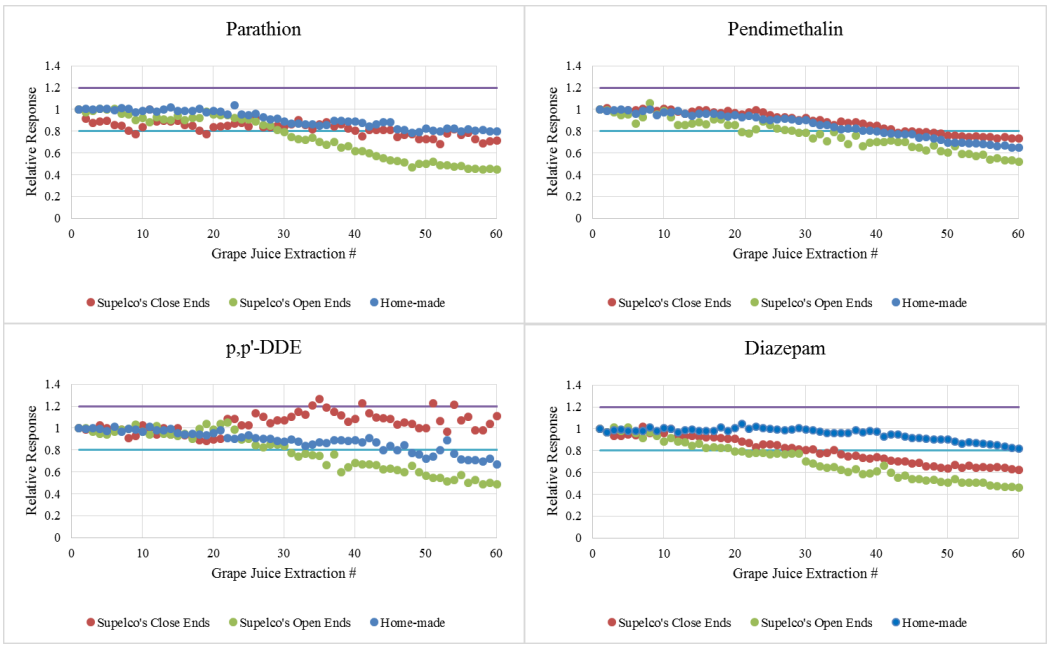


Figure 5-28 Reusability profile of coatings subjected to 60 DI-SPME in Concord grape juice.

Sealed fibers yielded acceptable results with all compounds, having RSD%, n=60 < 20% (except for 4-phenylphenol, RSD = 25%). Even though minimal, some differences were observed between sealed and home-made fibers, most likely related to the finishing used during the sealing of the fiber. By using the diluted Sylgard ® 184 solution, DVB particles were only superficially covered by the PDMS film. In this scenario, the presence of rugosity at the tip of the coating still allowed for the attachment of matrix particulate that would be eventually brought into the hot injector, thus, leading to fouling. The SEM pictures depicted in Figure 5-29 evidences that such overcoating would not deter matrix particulates from entering the void volume between the coating and the fused-silica core, as is the case for the unsealed fibers. In brief, it can be

concluded that ensuring total coverage of DVB particles and effectively sealing the fibers can lessen the extent of fouling, and as such, increase the longevity of the fiber.

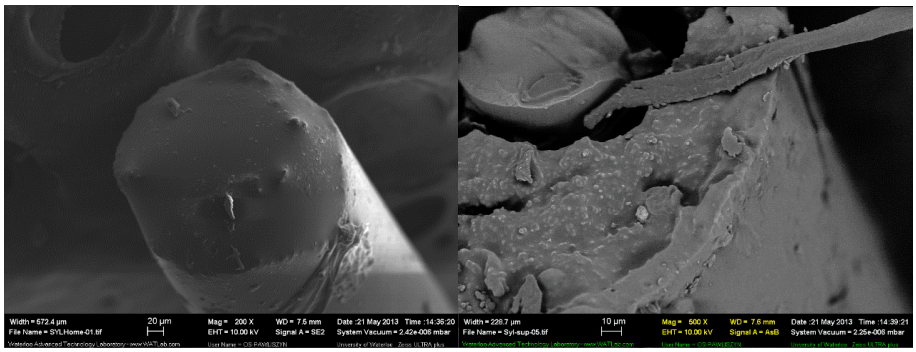


Figure 5-29 SEM images of the PDMS-modified coatings: (A) home-made 200x magnification; (B) Sealed end (Supelco) using 500x magnification.

5.4 Summary

In this chapter, a critical evaluation of the PDMS-overcoated fiber for food-matrix analysis employing a wide range of analyte polarities, molecular weights, and functionalities, was performed. Although the evaluation was performed in one matrix, Concord grape juice, due to its high sugar content (~ 20%), this matrix can be assumed to be an appropriate model for high water and high sugar content fruits and vegetables. The evaluation showed that the PDMS-overcoated fibers considerably slowed down the damaging process onto the coating surface during direct immersion in complex matrices of high sugar content. Noteworthy differences could be seen between the two types of PDMS tested, with Sylgard ® 184 presenting a superior performance due to the presence of excess reactive groups and fillers. Conversely, the thickness of the outer layer did not seem to have a significant effect on the fiber lifetime. However, the results outline significant differences in the rate of uptake (sorption mechanism) associated with the addition of the PDMS outer layer, especially towards the more polar analytes. Moreover, it has been shown that the uniformity of the overcoated PDMS layer, as well as proper sealing of both extremities, are paramount to the achievement of reliable data and extended fiber lifetime. It can be concluded that fiber lifetime can be prolonged if both ends are also coated with PDMS to avoid exposure to porosity (DVB particles), which decreases the likelihood of fouling. Additional improvement might be attainable by introducing washing of the coatings after desorption, so that any carbon build-up (fouling) left on the coating surface after desorption can be removed.

Even though the matrix-compatibility of the PDMS-overcoated fiber has been already demonstrated in Chapter 2, the present evaluation spans the utilization of such coatings over a wider range of analyte properties. Overall, the evaluation protocol herein presented can be further expanded for the analysis of other food matrices. Therefore, upon completion of the present evaluation, it has been proven so far that the PDMS-overcoated fiber is robust and compatible for use in fruit pulp and juice (high sugar content). Adding to it the thermodynamics and kinetics considerations studied in chapter 4, the next step aims at the simultaneous determination of both polar and non-polar analytes, and such is the case in multiclass pesticide analysis.

5.5 Addendum

The authors thanks Supelco for the provision of prototype fiber overcoated with proprietary PDMS blend.

6 Multiresidue Pesticide Determination in Grapes by Means of DI-SPME-GC-ToFMS

6.1 Preamble and introduction

6.1.1 Preamble

Part of the experiments described in this chapter are currently submitted for publication: Souza-Silva, E. A., Pawliszyn J., Ultrasensitive DI-SPME-GC-TOFMS method employing matrix-compatible fiber coating for multiresidue pesticides determination in grapes, (2015).

6.1.2 Introduction

Analyses of pesticide residues in food and environmental samples have been performed for over 40 years. Increasing concern related to pesticide applications and subsequent residues found in food commodities has led to the establishment of strict legislation regulating the maximum residue limit (MRL) allowed for any given pesticide in different types of food commodities. As a result, analysts are required to develop analytical procedures that are less labor-consuming, faster, greener, and more accurate in order to perform multiclass, multiresidue analyses capable of detecting a wide variety of pesticides in a variety of food matrices.

Ideally, a multiresidue method for pesticides analysis in food commodities should be rapid and easy to perform, require a minimum amount of chemicals (especially solvents), provide a certain degree of selectivity, and cover a wide

scope of analyte–matrix combinations. If sample handling can be minimized by use of simple sample preparation procedures, reproducibility (precision) and accuracy can be improved. The development of automated methodologies to analyze pesticides in food matrices can present an important advantage of high sample throughput, in addition to a decrease in errors associated to human mistakes.

In the early 2000s, Anastassiades and co-workers introduced the QuEChERS extraction procedure. The QuEChERS method effectively covers a wide analyte scope; as such, it is extensively applied in multiresidue analysis of pesticides in fruits and vegetables [34,35,38,41,200,201]. Conversely, the main drawback of QuEChERS is that the pre-concentration capability obtained by this method per initial gram of sample is lower than those achieved by the use of most conventional procedures. Hence, the final extract must be concentrated to a larger extent to provide high sensitivity and obtain acceptable limits of quantification (LOQ). In addition, QuEChERS is not easily automated.

SPME is able to address the drawbacks aforementioned, and as a result, in the last several years, extensive applications of SPME in the extraction of different classes of pesticide residues in a wide variety of food matrices have been reported in literature [16,86,88,111,113,114,117,118,137,139,140,143,202–207]. For instance, Zambonin et al. [158] developed a SPME-GC-MS method for determination of organophosphorous pesticides in fruit juices employing centrifugation and water dilution of the sample before performing SPME. Similarly, Viñas et al. [208]

developed and validated a SPME method for analysis of strobilurin fungicides in baby food by diluting the raw matrix in phosphate buffer. Menezes Filho et al. [113] carried out pre-extraction of the analytes from mango matrix using isopropyl alcohol:water mixtures, and then subjected the obtained solution to DI-SPME. Interestingly, Aguado et al. [116] developed a SPME-based screening method for the determination at parts per trillion (ppt) levels of multiclass pesticides residues in vegetables employing a solvent extraction with ethyl acetate prior to SPME step. The proposed method was accredited by the Spanish Accreditation Body and participated in a proficiency test with adequate results.

6.1.2.1 SPME method development

The acquisition of reliable data via SPME method requires careful investigation of a number of experimental parameters that affect extraction efficiency; namely, extraction phase chemistry, extraction mode, agitation method, sample modification (pH, ionic strength, organic solvent content), sample temperature, extraction time and desorption conditions [122]. Traditionally, the effects of these parameters in the extraction efficiency of a given system are investigated utilizing univariate optimization design, which involves the performance of one-factor-at-the-time (OFAT) experiments, thus offering the possibility to examine the effects of each variable at a time, while all other variables are kept constant during a particular optimization experiment. Even though univariate approaches have been used in the development of SPME methods for the determination of pesticides in fruits and vegetables [114,116,143,158], the main drawback, apart from the large number of experiments required, is that

this tradional method overlooks interaction between variables, since it assumes that factors are independent [78,209]. Particularly, in the case of systems dealing with the determination of multiple analytes bearing a wide range of physicochemical characteristics, the utilization of the univariate approach is not recommended.

Multivariate designs offer the possibility to study simultaneously the variation of several variables, consequently reducing the number of experiments to be performed during SPME method development. More importantly, multivariate designs offer the possibility to visualize interactions between variables that would not be detected by classical univariate design [78]. SPME method development by multivariate approaches are often performed in two steps. First, variables to be investigated are screened in order to identify those that may affect significantly the response of a particular SPME procedure. In addition to detecting highly influential variables, some types of screening design are also capable of detecting interactions between variables. Once the influential variables are selected, the next step involves the performance of a multivariate design capable of locating the optimum set of conditions for these extraction parameters [78].For this purpose, a two-level fractional or fractional factorial designs are usually employed.

6.1.2.1.1 Screening the influential factors

When there is a need to screen a large number of variables, Plackett-Burman (two-level fractional design) can be employed. Plackett-Burman (P-B) designs are used in order to obtain qualitative information about the system

studied, since it does not offer information on interactions between factors. The most important feature of PB designs is that they all involve 4n experiments, where n = 1, 2, 3… In each case, the maximum number of factors that can be studied is 4n -1, so a 12-experiment PB design can study no more than 11 factors[210,211]. This means that if eight variables are to be studied, a 12-experiment design is to be used, and the later three variable will be *dummy* factors. Dummy factors are fictitious factors with no chemical meaning. However, the apparent effects of these dummy factors can be used to estimate the random measurement error. In PB designs, each variable is studied at two levels, high (+) and low (-), as shown in *Table 6-1*.

Table 6-1 Plackett-Burman design for 11 variables at two levels (+, -), total of 12 runs. For any two X_i each combination (- -, - +, + -, + +) appears three times, i.e. the same number of times.

Run	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}
1	+	+	+	+	+	+	+	+	+	+	+
2	-	+	-	+	-	+	-	+	-	+	-
3	-	-	+	-	+	+	+	-	-	-	+
4	+	-	-	+	-	+	+	+	-	-	-
5	-	+	-	-	+	-	+	+	+	-	-
6	-	-	+	-	-	+	-	+	+	+	-
7	-	-	-	+	-	-	+	-	+	+	+
8	+	-	-		+	-	-	+	-	+	+
9	+	+	-	-	-	+	-	-	+	-	+
10	+	+	+	-	-	-	+	-	-	+	-
11	-	+	+	+	-	-	-	+	-	-	+
12	+	-	+	+	+	-	-	-	+	-	-

The results obtained are confirmed using ANOVA, and their visualization is often accomplished via a Pareto chart of effects, which is a useful plot for identifying the factors that are important. In the Pareto chart shown in , bar lengths are proportional to the absolute value of the estimated effect, helping to compare their relative importance. Working at a 95% confidence interval, the chart will present a vertical line as a critical P value ($\alpha = 0.05$); any factor yielding a bar with a length that surpasses this vertical line is deemed statistically significant to the outcome response. Negative values mean that passing from a lower level (-) to higher level (+) of that given factor caused the overall response to decrease.

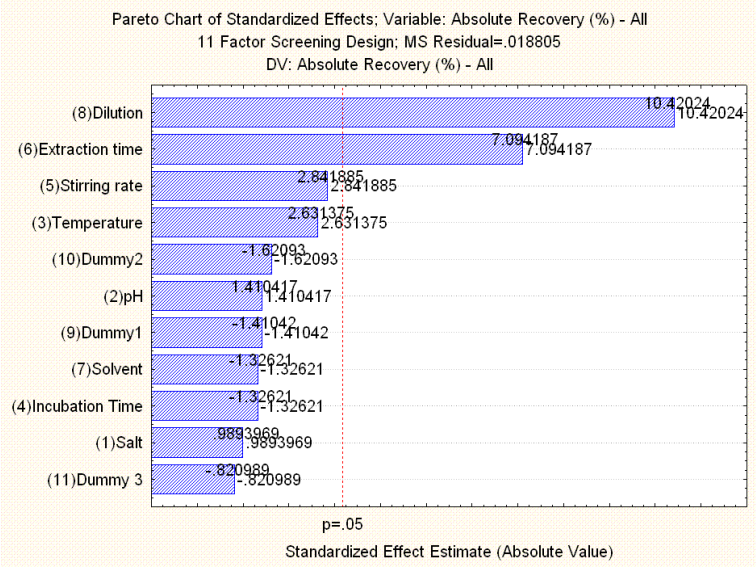


Figure 6-1 Example of Pareto chart of effects for screening of seven variables using a 12-experiments PB. In this case only dilution and extraction time are deemed statistically significant.

6.1.2.1.2 Optimization of influential factors

Once the significant variables have been identified, the next step is the optimization of these variables in order to find the optimum values that can lead to the maximum signal. For this purpose, there are several reports in the literature that employ Central Composite Design as a tool to optimize SPME extraction efficiency [212–216]. Overall, the objective of CCD optimization is the estimation of the factor coordinates (levels) that maximize the response, in this case, extraction efficiency. In addition to the interactions between variables, CCD also allows for the estimation of the quadratic contribution of each variable[217].

A graphical representation of a CCD is presented in *Figure 6-2*. In general, a CCD for k factors has three groups of design points: (1) two-level full factorial design points, containing a total of $n_{factors} = 2^k$ with coordinates $x_i = -1$ or $x_i = 1$, for $i = 1, \ldots, k$; (2) axial points ("star" points), formed by $n_{axial} = 2k$, with all factors set to coded level zero (the midpoint) and the value $+\alpha$, and (3) central points, which are points with all levels set to coded level zero, the midpoint for each factor range. These points at the centre serve two purposes: they provide an estimate of experimental error, and determine the precision of an estimated response at and in the proximity of the central point [210,211,217].

Similar to P-B designs, upon acquisition, the results are conferred for statistical significance via Pareto chart of effects. Furthermore, the results are fitted into empirical models in order to find the operating conditions that

maximize the system response. For this, response surface is usually used (see *Figure 6-3*).

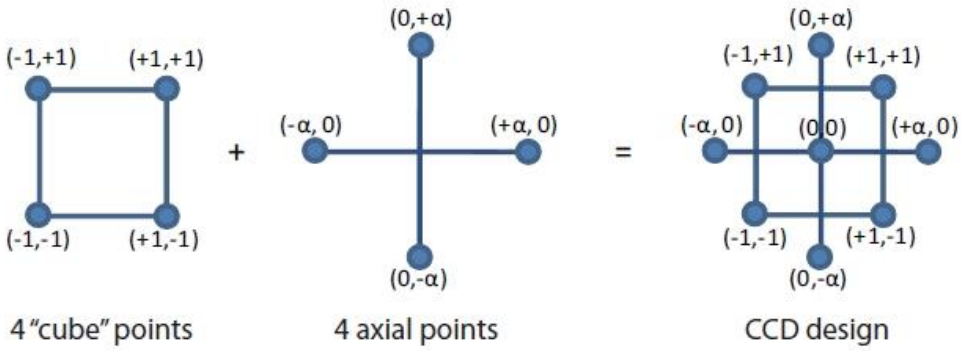


Figure 6-2 Representation of generation of a Central Composite Design for two factors

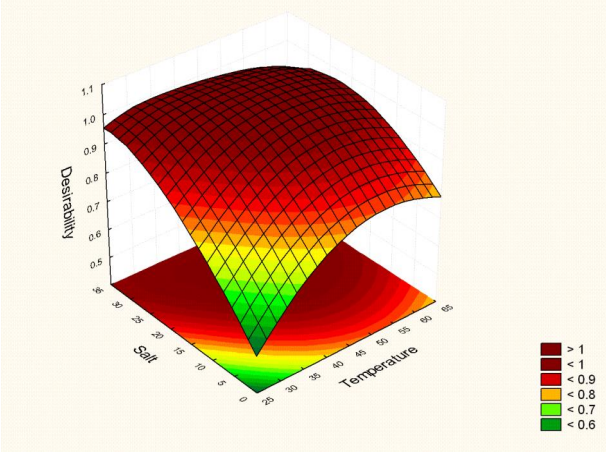


Figure 6-3Example of surface response for allocation of optima obtained for a central composite design.

In this chapter, the practical aspects of the PDMS-modified coating demonstrated so far were put to test to analyze a broad range of pesticide residues in a grapes. The multiresidue SPME method was optimized via multivariate

designs, and a thorough investigation on the use of internal standards to compensate for any possible variation was also presented. Advantages and limitations of SPME applied to multiresidue pesticides in grapes were sagaciously discussed.

6.2 Materials and Methods

6.2.1 Solvents and Materials

All solvents used were HPLC grade. Acetonitrile (MeCN), acetone, methanol (MeOH), dichloromethane (DCM), and isopropryl alcohol were purchased from Fisher Scientific. Glacial acetic acid, sodium sulfate, and sodium chloride were purchased from Supelco (Bellefonte, PA, U.S.A.). PDMS/DVB 65µm Stableflex fibers were purchased from Supelco. Sylgard 184 ® (PDMS pre-polymer and curing agent) was purchased from Dow Corning (Midland, MI, USA). Green seedless grapes were purchased at local markets in Waterloo (ON, Canada). Deionized water was obtained from a Barnstead/Thermodyne NANO-pure ultra-water system (Dubuque, IA, U.S.A.).

6.2.2 Standards

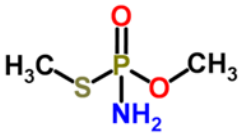
Pesticide standards (see Table 1 for the list of all analytes) of purity of 95% or higher were purchased from Supelco, Accustandard, C/D/N Isotopes, and Restek. Details of the chemical structures and physicochemical properties for each analyte used in this work are described below in *Figure 6-4* and *Table 6-2*.

Individual solutions of standards were prepared in acetone, methanol, dichloromethane and acetonitrile at 10 mg/mL (except chlorothalonil,which was prepared in dichloromethane, and folpet, w/hich was prepared in toluene). A composite mixture was prepared, containing each contaminant at 100 µg/mL in acetonitrile (0.1% acetic acid).

Internal standard (quintozene, triphenylphosphate, trifluralin-d_{14}, terphenyl-d_{14}, 2-fluorobiphenyl, 2,4-dichlorophenol, phenol-d_5 and nitrobenzene-d_5) solutions were prepared at 1 mg/mL, except for parathion-d_{10} and α-HCH-d_6, which were purchased from Restek as a 40 µg/mL solution in acetonitrile. All standards solutions were stored in freezer at -30 ºC.

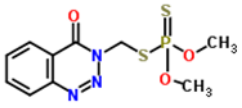
During method development, spiking standard mixtures containing target analytes at either 20 or 100 µg/mL, and internal standards at 50 µg/mL were prepared. The concentration of each analyte was carefully chosen in order to guarantee enough sensitivity for all analytes (final sample concentrations of 20, 100, and 50 ng/g).

To calculate the amount extracted by SPME for each analyte, a stock standard mixture containing all analytes of interest was prepared at 100 ng/µL in acetonitrile. This stock solution was used for successive dilutions in order to obtain calibration solutions ranging from 0.25 to 50 ng/µL (8 levels). Liquid injections of calibration solutions were carried out in quadruplicates.

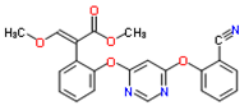


Acephate

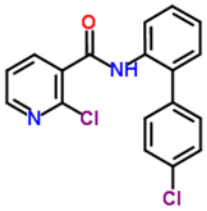
Atrazine



Azinphos-methyl

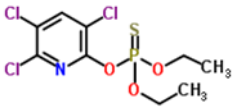


Azoxystrobin

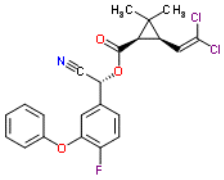


Boscalid

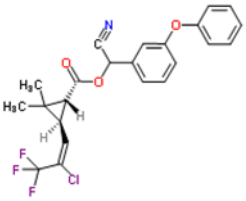
Chlorothalonil



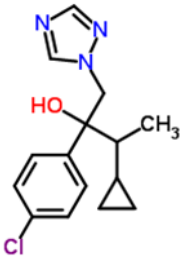
Chlorpyrifos



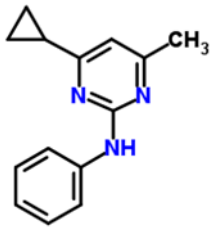
Cyfluthrin[a]



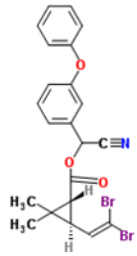
Cyhalothin[a]



Cyproconazole[a]



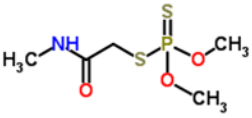
Cyprodinil



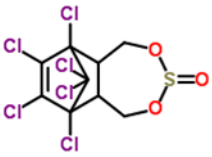
Deltamethrin[a]

Diazinon

Difenoconazole[a]

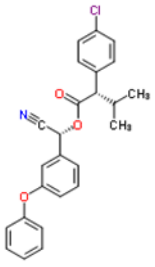


Dimethoate

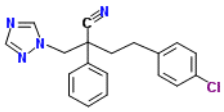


Endosulfan I

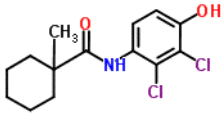
Endosulfan II



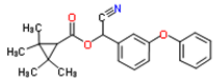
Esfenvalerate



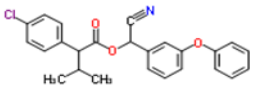
Fenbuconazole



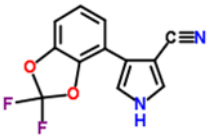
Fenhexamide



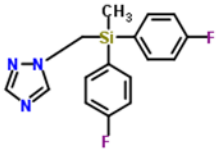
Fenproprathrin



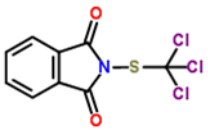
Fenvalerate



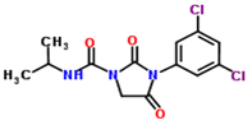
Fludioxonil



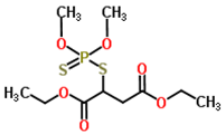
Flusilazole



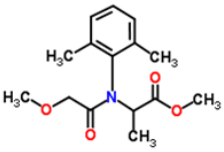
Folpet



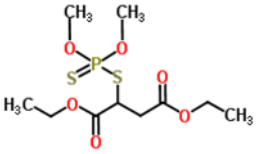
Iprodione



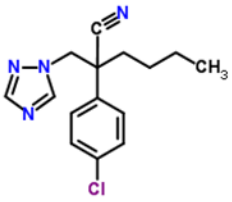
Malathion



Metalaxyl



Methidathion



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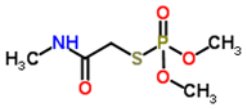
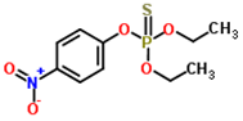
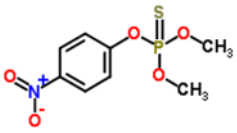


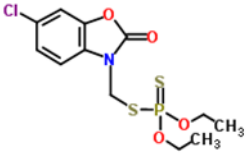
Omethoate



Parathion



Parathion-methyl



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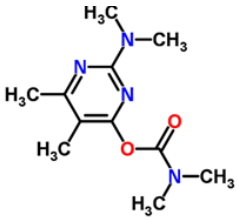

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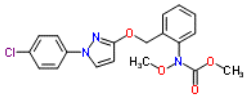
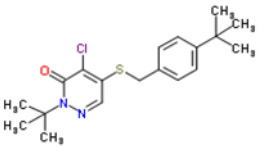


Pyraclostrobin



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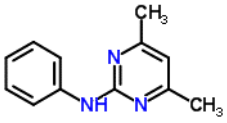

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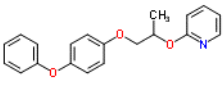

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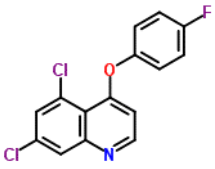

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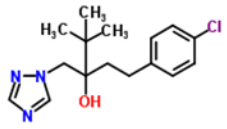

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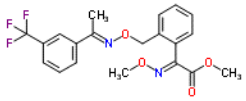
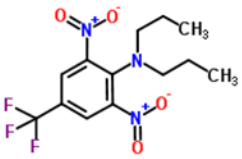
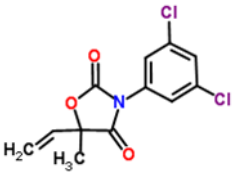


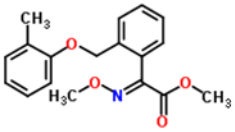
Trifloxystrobin



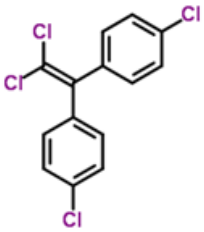
Trifluralin



Vinclozolin



Kresoxim-methyl



4,4'-DDE

Figure 6-4 Chemical structure of studied analytes ([a]more than one isomer).

Table 6-2 Classification and physicochemical properties of target analytes.

Pesticide	Class (application)	CAS #	MW (g/mol)	Solubility in water 20ºC (mg/L)	Log P (pH 7, 20ºC)	pK_a (25ºC)	Vapor Pressure at 25º C (mPa)
Acephate	Organophosphate (I)	30560-19-1	183.17	790000	-0.85	8.35	2.26E-01
Omethate	Organophosphate (I, A)	1113-02-6	213.2	10000	-0.74	n.a.	3.30E+00
Dimethoate	Organophosphate (I, A)	60-51-5	229.26	39800	0.704	n.a.	2.47E-01
Pirimicarb	Carbamate (I)	23103-98-2	238.1	3100	1.55	4.4	4.30E-01
Metalaxyl	Phenylamide (F)	57837-19-1	279.33	7100	1.65	0	7.50E-01
Azoxystrobin	Strobilurin (F)	131860-33-8	403.4	6.7	2.5	n.a.	1.10E-07
Methidathion	Organophosphate (I, A)	950-37-8	302.3	240	2.57	n.a.	2.50E-01
Atrazine	Triazine (H)	1912-24-9	215.68	35	2.7	1.7	3.90E-02
Malathion	Organophosphate (I, A)	121-75-5	330.36	148	2.75	n.a.	3.10E+00
Pyrimethanil	Anilinopyrimidine (F)	53112-28-0	199.11	121	2.84	3.52	1.10E+00
Myclobutanil	Triazole (F)	88671-89-0	288.78	132	2.89	2.3	1.98E-01
Chlorothalonil	Chloronitrile (F)	1897-45-6	265.91	0.81	2.94	n.a.	7.60E-02
Azinphos-methyl	Organophosphate (I, A)	86-50-0	317.32	28	2.96	5	5.00E-04
Boscalid	Carboxamide (F)	188425-85-6	343.21	4.6	2.96	n.a.	7.20E-04
Parathion-methyl	Organophosphate (I, A)	298-00-0	263.21	55	3	n.a.	2.00E-01
Folpet	Phthalimide (F)	133-07-3	296.56	0.8	3.02	n.a.	2.10E-02
Vinclozolin	Oxazole (F)	50471-44-8	286.11	3.4	3.02	n.a.	1.60E-02

Pesticide	Class (application)	CAS #	MW (g/mol)	Solubility in water 20ºC (mg/L)	Log P (pH 7, 20ºC)	pK_a (25ºC)	Vapor Pressure at 25º C (mPa)
Cyproconazole	Triazole (F)	94361-06-5	291.78	93	3.09	n.a.	2.60E-02
Iprodione	Dicarboximide (F)	36734-19-7	330.17	12.2	3.1	n.a.	5.00E-04
Kresoxim-methyl	Strobilurin (F, B)	143390-89-0	313.35	2	3.4	n.a.	2.30E-03
Fenhexamid	Hydroxyanilide (F)	126833-17-8	302.2	20	3.51	n.a.	4.00E-04
Diazinon	Organophosphate (I, A)	333-41-5	304.35	60	3.69	2.6	1.20E+01
Tebuconazole	Triazole (F)	107534-96-3	307.82	36	3.7	n.a.	1.30E-03
Fenbuconazole	Triazole (F)	114369-43-6	336.82	2.47	3.79	n.a.	3.40E-04
Parathion	Organophosphate (I, A)	56-38-2	291.26	12.4	3.83	n.a.	8.90E-01
Flusilazole	Triazole (F)	85509-19-9	315.39	41.9	3.87	2.5	3.87E-02
Cyprodinil	Anilinopyrimidine (F)	121552-61-2	225.29	13	4	4.44	5.10E-01
Phosalone	Organophosphate (I, A)	2310-17-0	367.8	1.4	4.01	n.a.	1.56E-02
Fludioxonil	Phenylpyrrole (F)	131341-86-1	248.19	1.8	4.12	14.1	3.90E-04
Pyraclostrobin	Strobilurin (F)	175013-18-0	387.8	1.9	4.25	n.a.	2.60E-05
Dicofol	Organochlorine (A)	115-32-2	370.49	0.8	4.3	n.a.	2.50E-01
Difenoconazole	Triazole (F)	119446-68-3	406.26	15	4.36	1.07	3.33E-05
Trifloxystrobin	Strobilurin (F)	141517-21-7	408.37	0.61	4.5	n.a.	3.40E-03
Deltamethrin	Pyrethroid (I)	52918-63-5	505.2	0.0002	4.6	n.a.	1.24E-05
Quinoxyfen	Quinoline (F)	124495-18-7	308.13	0.047	4.66	n.a.	1.20E-02
Chlorpyrifos	Organophosphate (I)	2921-88-2	350.89	1.05	4.7	n.a.	1.43E+00

Pesticide	Class (application)	CAS #	MW (g/mol)	Solubility in water 20ºC (mg/L)	Log P (pH 7, 20ºC)	pK_a (25ºC)	Vapor Pressure at 25º C (mPa)
Endosulfan-isomer mix	Organochlorine (I, A)	115-29-7	406.93	0.32	4.75	n.a.	8.30E-01
Fenvalerate	Pyrethroid (I)	51630-58-1	419.9	0.001	5.01	n.a.	1.92E-02
Trifluralin	Dinitroaniline (H)	1582-09-8	335.28	0.221	5.27	n.a.	9.50E+00
Pyriproxyfen	Unclassified (I)	95737-68-1	321.37	0.37	5.37	6.87	1.33E-02
Cyfluthrin	Pyrethroid (I)	68359-37-5	434.29	0.0066	6	n.a.	3.00E-04
Fenproprathrin	Pyrethroid (I)	39515-41-8/ 64257-84-7	349.42	0.33	6.04	n.a.	7.60E-01
Esfenvalerate	Pyrethroid (I)	66230-04-4	419.9	0.001	6.24	n.a.	1.20E-06
Pyridaben	Pyridazinone (I, A)	96489-71-3	364.93	0.022	6.37	n.a.	1.00E-03
4,4'-DDE	Organochlorine (I)	72-55-9	318.02	0.12	6.51	n.a.	n.a.
Cyhalothrin	Pyrethroid (I)	68085-85-8	449.85	0.004	6.8	9	1.00E-09

Data obtained from Refs [218,219].
A – acaricide
B – bactericide
F – fungicide
I – insecticide
H – herbicide
n.a. – not available

6.2.3 Preparation of PDMS-modified coating

PDMS-modified coatings were prepared *in-house* employing the procedure described in Chapter 4. All coatings were prepared at least in triplicate. Prior to their usage, each coating was conditioned at 250ºC for one hour, and visually evaluated for uniformity and smooth surface coverage. If any defect was noted, coatings were discarded and new coatings were prepared.

6.2.4 SPME Procedure

Green seedless grapes, purchased at local markets in Waterloo (ON, Canada), were manually stemmed, washed with nanopure water, and dried. Next, grapes were crushed, homogenized using a blender, transferred to 200 mL amber glass flasks, then stored in a freezer at -30 ºC until analysis. Organically grown fruits were obtained for method development and validation steps (previously analyzed for the absence of the target pesticides), while conventionally grown fruits were obtained for real samples analysis.

During initial SPME method development, 8 g of whole fruit pulp was weighed in a 10-mL vial, fortified with 8 µL of a spiking standard mixture to achieve the appropriate analyte concentration, and pre-incubated for at least 60 min prior to extraction in order to allow for the binding of the analyte to the matrix to occur. Once dilution occurred, the samples were thoroughly vortexed, and let to stand for 1 h prior to extraction. A 5 min pre-extraction incubation of the sample was performed in the agitation unit at 500 rpm and at 30C, followed by a 30-min extraction in direct immersion mode at 30 ºC, while stirring at 500

rpm. Following extraction, the fiber was rinsed in deionized water, and then desorbed for 10 min at 260 °C.

For Plackett-Burman experiments, 200 g of grape pulp was weighed into a 250-mL jar and spiked with an appropriate amount of standard mix. The sample was then let to stand in the agitation table for 1 h. Subsequently, appropriate amounts of salt (Na_2SO_4), followed by spiked grape pulp amounts, were weighed into each vial. Next, appropriate amounts of DI water and isopropyl alcohol were added to each vial, corresponding to each experiment, as defined by the Plackett-Burman design matrix.

For the Central Composite Design experiments, sample preparation was similar to that performed for Plackett-Burman, except that no isopropyl alcohol was added.

For the final SPME method, 4 g of whole fruit pulp was weighed in a 10-mL vial, followed by 4 mL of Na_2SO_4 aqueous solution at 15% (w/w) (1.2 g of Na_2SO_4/vial). The mixture was thoroughly vortexed to ensure homogeneity. A 5 min pre-extraction incubation of the sample was performed in the agitation unit at 750 rpm and at 55C, followed by a 30-min extraction in direct immersion mode at 55 ºC, while stirring at 750 rpm. Following extraction, the fiber was rinsed in deionized water, and then desorbed for 10 min at 260 °C.

SPME matrix-matched calibration curves were obtained in grape matrix to establish the linear dynamic range, limits of quantitation objective (LOQs), and method precision and accuracy.

6.2.4.1 Quality Control (QC) Samples

In addition to daily instrumental checking, such as tuning and leak checks, this approach was employed to monitor system suitability during the duration of the present study. An aliquot of 8 mL nanopure water was transferred into a 10-mL amber vial and then spiked with 5 µL of QC standard mixture, resulting in a concentration of analytes in water at 10 ng/mL. A box of PDMS/DVB Stableflex fibers (3 fibers from the same lot #) was dedicated to QC samples. SPME conditions comprised of 30 min extraction at 30 ºC and desorption for 10 min at 260 ºC. In a similar fashion, the same experimental set up, including monitoring of analytes, was employed to monitor the suitability of the fiber coating throughout this study.

In both cases, five analytes were selected as monitoring analytes, namely, atrazine, tebuconazole, trifloxystrobin, 4,4'-DDE, and azoxystrobin, as these analytes cover well a range of polarities, as well as chromatographic behaviors. All daily extractions were performed in triplicate, and plotted with their respective standard deviations. *Figure 6-5* shows a typical control chart plot of daily QC checks for fungicide tebuconazole. Horizontal dashed lines refer to average 3 standard deviation (red), and average 2 standard deviation (blue).

In the case of system monitoring, if non-compliant responses were observed for all traced analytes, QC extractions were repeated (if an outlier was observed during QCs runs), or system was inspected and submited to cleaning and

service if necessary. In the case of fiber coating QCs, if extraction efficiency was non-complaint, a new fiber would be used.

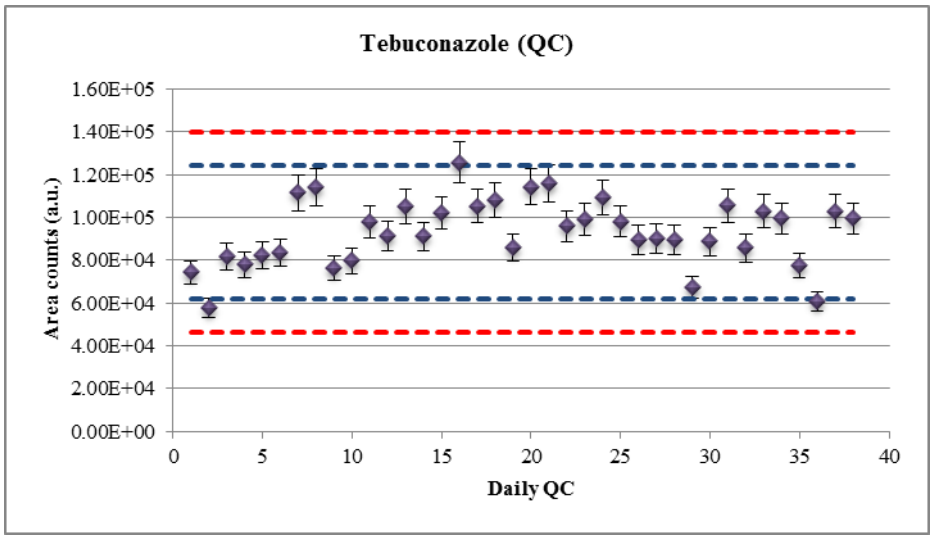


Figure 6-5 Representative control chart plot for daily QC obtained for Tebuconazole. SPME conditions: 7-mL water spiked at 10 ng/mL; 30 min extraction at 30ºC; PDMS/DVB stableflex fiber.

6.2.5 Instrumentation

Sample pH was measured by a Metter Toledo MP220 (Schwerzenbach, Switzerland) pH meter. For centrifugation of samples, a Thermo Jouan BR4i (Waltham, MA, USA) centrifuge was used.

GC-ToFMS analyses were carried out using a Pegasus 4D instrument consisting of an Agilent 6890N gas chromatograph equipped with a split/splitless injector (Agilent Technologies, Palo Alto, CA, USA), an MPS2 autosampler for automated SPME (Gerstel, Mülheim and der Ruhr, Germany), and a Pegasus III high speed time-of-flight mass spectrometer (Leco Corp., St. Joseph, MI, USA).

Chromatographic separation was performed in a Restek Rxi®-5Sil MS capillary (30 m, 0.25 mm i.d., 0.25 μm film thickness) with a 5 m integra-guard column®. Helium was used as carrier gas at 1.2 mL/min. Oven temperature was initially set at 70 °C for 1.5 min, ramped at 40 °C/min to 210 °C for 1 min, then ramped at 10 ºC/min to 280 ºC, where it was held for 10 min, resulting in a total run time of 23 min. The injector was maintained at 250 ºC (0.75mm I.D. insert used for SPME, and 4mm I.D. insert packed with glass wool at the bottom used for liquid injections) in splitless mode (split was opened for SPME method after 10 min; for liquid injections, the split was opened after 1.5 min). MS operational conditions were: electron ionization (EI) at 70eV; ion source temperature: 240 °C; transfer line temperature: 280 °C; mass range: *m/z* 35-550; acquisition rate: 50Hz; detector voltage: -1700V.

Retention times, as well as the selected quantifier and qualifier ions are presented in *Table 6-3*.

Table 6-3 GC-ToFMS retention times and quantifier ions (m/z) for all targeted pesticides, degradation products, and internal standards candidates.

Analyte	R. T. (s)	Quantifier (m/z)	Type
Nitrobenzene-D_5	251.2	82	IS
2,4-Dichlorophenol	274.6	165	IS
1,1'-Biphenyl, 2-fluoro-	324.2	172	IS
Acephate	338.0	136	Analyte
Omethoate	344.6	110	Analyte
1H-Isoindole-1,3(2H)-dione	349.9	147	DP
Trifluralin-d14	394.3	315	IS
Trifluralin	397.1	264	Analyte
2,4,6-Tribromophenol	403.7	62	IS
alpha-HCH-d_6	417.8	224	IS
Dimethoate	426.1	125	Analyte
Atrazine	430.1	200	Analyte
Diazinon	441.1	137	Analyte
Quintozene	441.5	237	IS
Pyrimethanil	449.6	198	Analyte
Chlorothalonil	455.3	266	Analyte
Pirimicarb	460.7	72	Analyte
Vinclozoline	484.3	124	Analyte
Methyl parathion	487.5	109	Analyte
Metalaxyl	492.2	206	Analyte
Malathion	510.5	173	Analyte
Chlorpyriphos	520.4	197	Analyte
Parathion-d_{10}	522.2	301	IS
Parathion	526.7	109	Analyte
2,4'-Dichlorobenzophenone	536.4	139	DP
Cyprodinil	551.2	224	Analyte
Folpet	576.4	76	Analyte
Methidathion	579.9	145	Analyte
Endosulfan I	601.0	195	Analyte
Fludioxonil	605.0	248	Analyte
Enilconazole	605.3	215	Analyte
4,4'-DDE	616.2	246	Analyte
Myclobutanil	617.3	179	Analyte

Analyte	R. T. (s)	Quantifier (m/z)	Type
Kresoxim-methyl	617.6	116	Analyte
Flusilazole	619.0	233	Analyte
p-Terphenyl-d_{14}	621.9	244	IS
Cyproconazol, R*,S* / R*,S*	638.4	222	Analyte
Endosulfan II	657.5	195	Analyte
Trifloxystrobin	677.8	116	Analyte
Quinoxyfen	690.5	237	Analyte
Fenhexamide	698.7	97	Analyte
Tebuconazole	710.3	125	Analyte
Triphenylphosphate	712.1	326	IS
Iprodione	733.1	314	Analyte
Fenpropathrin	751.9	181	Analyte
Dicofol	759.4	139	Analyte
Phosalone	782.0	182	Analyte
lambda-Cyhalothrin I	786.0	181	Analyte
Azinphos-methyl	787.1	160	Analyte
Pyriproxyfen	789.0	136	Analyte
lambda-Cyhalothrin II	797.1	181	Analyte
Pyridaben	866.4	147	Analyte
Cyfluthrin I	892.9	163	Analyte
Fenbuconazole	894.8	129	Analyte
Cyfluthrin II	900.8	163	Analyte
Cyfluthrin III	906.7	163	Analyte
Cyfluthrin IV	910.3	163	Analyte
Boscalid	931.2	140	Analyte
Esfenvalerate I	1014.5	125	Analyte
Pyraclostrobin	1024.9	132	Analyte
Esfenvalerate II	1039.0	125	Analyte
Difenoconazole I	1069.3	265	Analyte
Difenoconazole II	1077.9	265	Analyte
Deltamethrin I	1082.9	181	Analyte
Deltamethrin II	1110.7	181	Analyte
Azoxystrobin	1134.6	344	Analyte

IS = Internal Standard Candidate; DP = Degradation Product

6.2.6 Data Processing

ChromaTOF (Leco Corp.) software (v. 4.24) was used for GC-ToFMS instrument control, data acquisition, peak deconvolution and data pre-processing. Library searching was performed using the commercial NIST library. Data was further processed using Microsoft Excel (2010) and GraphPad Prism 5 (Version 5.01, 2007, GraphPad Softwate, San Diego, CA, USA). The Statistica 8.0 (2007, StatSoft, Tulsa, OK, USA) program was used to construct experimental design matrices and evaluation of results.

6.3 Results and Discussion

6.3.1 GC-ToFMS Method Development

The GC-ToFMS method was optimized in order to achieve chromatographic separation of the target pesticides with good peak shape, minimum matrix interferences and increased sensitivity (S/N) within minimum possible run time.

6.3.1.1 Oven Temperature Programming

For optimization of the GC oven temperature program, the following conditions were kept: injector at 260 ºC, splitless mode (split opens at 90 s); carrier gas (He) flow at 1.2 mL/min; acquisition rate of 10 Hz; ion source at 220 ºC; transferline at 280 ºC; and detector voltage at -1700 V. Standard mixture in acetonitrile was injected (1µL).

Initially, the simplest programming was tested, with an initial oven temperature of 40 ºC, held for 3 min, then ramped at 5 ºC/min to 300 ºC, and held for 5 min. This method resulted in a 60 min run, which proved to be excessively long for the separation of all analytes. Moreover, the slow ramping rate resulted in broader peaks for some compounds, which in turn reduced peak resolution, possibly rendering compound identification at trace levels difficult and ambiguous. Making use of the advantage presented by the TOFMS, which enables deconvolution of spectra, after thorough evaluation, the GC oven temperature program was set as follows: initial oven temperature of 70 ºC, held for 1.5 min, then ramped at 40 ºC/min to 200 ºC, then ramped at 10ºC/min to 280 ºC, and held for 8 min.

Grape has a number of natural compounds that could inhibit the analysis of several compounds through co-elution. Since the GC oven temperature program was optimized using a standard solution, at this point, in order to evaluate possible interference of co-extrated grape matrix compounds, the method was tested for SPME extractions from grape pulp. For this, all conditions were kept as mentioned before, except that for the SPME method, the split was opened at 10 min (in agreement with desorption time).

Indeed, when analyzing the chromatogram obtained for SPME in spiked grape pulp, it became evident that major matrix co-extrated components interfered with the cluster of peaks in the middle of the chromatogram, and that heavier matrix components were eluting at the very end of the chromatogram. The major

matrix interfering compounds were fatty acids such as hexadecanoic acid and octadecanoic acid, as well as vitamin E (α-tocoferol). These compounds had considerable tailing that masked signals from analytes of interest. In addition, these interfering compounds had fragment ions (*m/z*) common to the target analytes, which could lead to over-estimation or false negatives. To solve this, the GC oven temperature program was modified as follows: initial oven temperature of 70 ºC, held for 1.5 min (this ensures enough column focusing for the ealier eluting analytes), then ramped at 40 ºC/min to 210 ºC, held for 1 min (in order to separate the cluster of analyte peaks from a major peak of matrix co-extracted compounds), then ramped at 10ºC/min to 280 ºC, and held for 10 min (final hold time prolonged in order to provide effective column cleaning from matrix components).

6.3.1.2 Acquisition rate

TOF-MS, with its spectral continuity and the use of automated peak discovery and spectral deconvolution algorithms, which are integrated to the LECO ChromaTOF software, allows for the employment of faster acquisition rates.

As seen in *Figure 6-6*, the optimized GC oven temperature program was not able to separate the pair of peaks comprised by myclobutanil (*m/z* 179) and kresoxim-methyl (*m/z* 116) with an acquitision rate of 10 Hz. Generally, peak heights decrease as the acquisition rate increases; however, the resolution is higher at faster acquisition rates, since a higher number of data points across the

peak is obtained. Therefore, an acquisition rate of 50 Hz was chosen as the optimal compromise between the ability to resolve the closely eluated analytes by means of spectral deconvolution and signal-to-noise ratio (S/N).

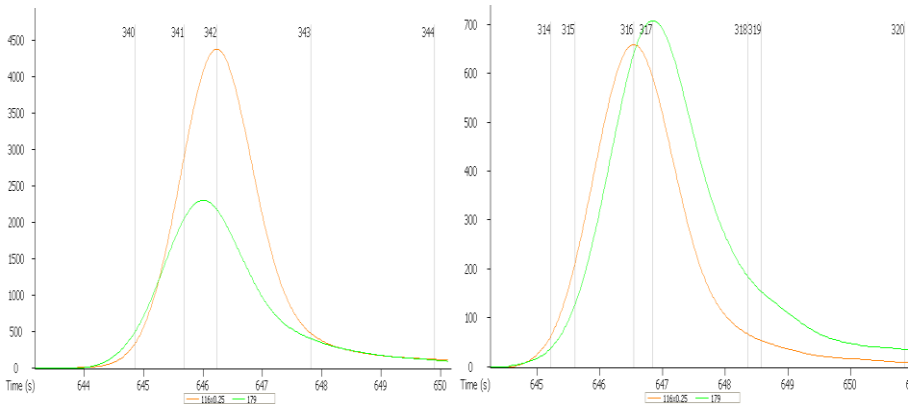


Figure 6-6 Comparison between acquisition rate of 10 Hz (A) and 50 Hz (B) to deconvolute the peaks of kresoxim-methyl (m/z 116, orange) and myclobutanyl (m/z 170, green).

6.3.1.3 Ion Souce Temperature

In electron impact ionization (EI), the ion source temperature is an important parameter that influences the extent of analyte ionization and fragmentation.

Ion source temperatures of 220, 220, 230, 240 and 250 ºC were evaluated for their impact on the response of analytes. In general, the S/N for most pesticides were lower for 210 and 220 ºC, and increased to 230 ºC (*Figure 6-7*).

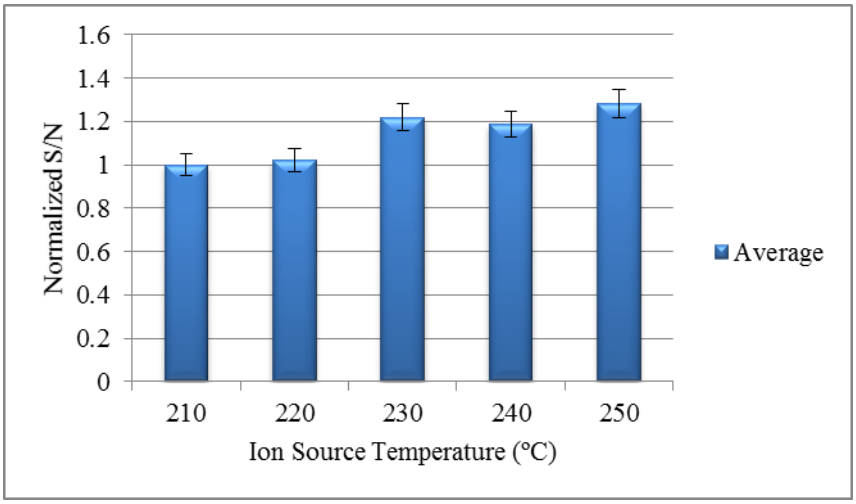


Figure 6-7 Effect of ion source temperature on average analyte response (S/N).

As demonstrated by Figure 6-4, at a first look, the average S/N seemingly increased between 210-230 ºC, but remained constant over 230-250 ºC. However, when looking at individuals S/N figures obtained for 230 ºC and 240 ºC, it can be seen that an increase of approximately 15% in S/N occurred when the ion source was increased from 230 ºC to 240 ºC (*Figure 6-8*). Since 250 ºC is the maximum ion source temperature recommended by the instrument manufacturer, experiments were conducted with the ion source at 240 ºC.

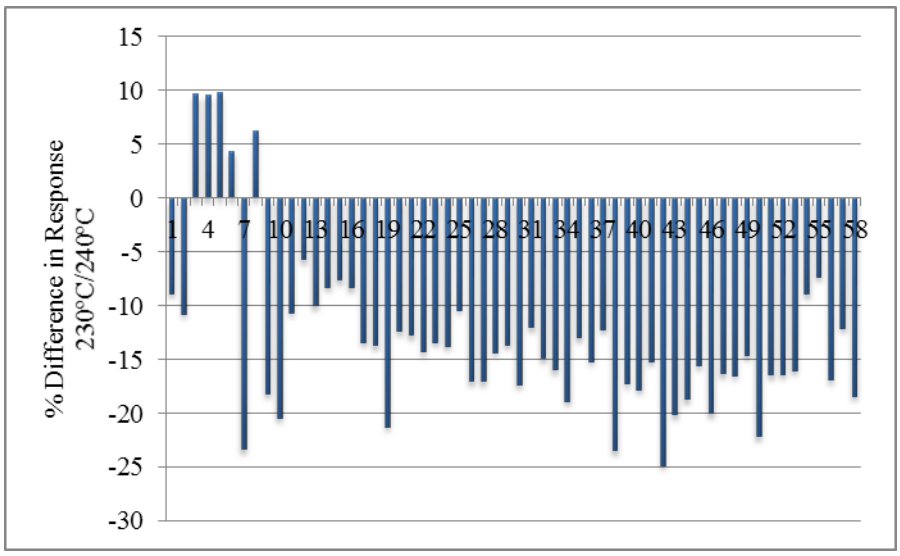


Figure 6-8 Difference in analyte response (S/N) for ion source temperatures of 230 ºC and 240 ºC. Results computed as percentages of S/N_{230^oC} *divided by* S/N_{240^oC}.

6.3.1.4 Injector Temperature

The utilization of instruments equipped with a PTV injector is a common practice used in the development of multiresidue methods that result in final solvent extracts being injected in the GC. This is often done to minimize the residency of analytes in the hot injector, therefore, minimizing thermal degradation. However, in the present study, the analytes extracted by SPME were submitted to isothermal desorption at a high temperature for a relatively long period of time (length of desorption time). As such, the injector temperature was optimized to minimize analyte degradation and carry-over, at a desorption time fixed at 10 min.

Desorptions at 240 ºC presented unacceptable carry-over, whereas all other temperatures presented carry-over values below 1 %. The results obtained as the total sum of all peak areas (normalized for responses at 240 ºC as 1) are presented in the figure below; as can be observed, responses for 250, 260 and 270 ºC were statistically equal when taking into account the standard deviation of triplicate measurements.

The stability of troublesome pesticides in function of injector temperature has been the focus of some studies reported in the literature [34,220,221]. For instance, Maštovská and Lehotay discussed the behavior of some pesticides prone to degradation and/or adsorption in the GC inlet system, such as dicofol and α-cyano substituted pyrethroids (deltamethrin, λ-cyhalothrin, and cyfluthrin) [221]. Accordingly, it is important to analyze the behavior of the well-known thermal labile pesticides mentioned previously, namely, dicofol, deltamethrin, λ-cyhalothrin, and cyfluthrin . As seen in *Figure 6-9*, dicofol response decreases for injector temperatures above 250 ºC, most likely due to thermal degradation. Dicofol readily degrades to 4,4'-dichlorobenzophenone when exposed to high temperatures, but only the parent compound is included in pesticide residue studies (Maštovská & Lehotay, 2004). Conversely, for the three pyrethroids investigated in the present study, the responses were statistically the same for injector temperatures above 250 ºC. The best result achieved for the pyrethroids may be due to the peak areas for all diasteroisomers being summed up; as such, potential isomerization (a change of peak ratios in function of thermal treatment) is not critical.

For further experiments, the injector temperature was kept at 260 ºC, with desorption for 10 min.

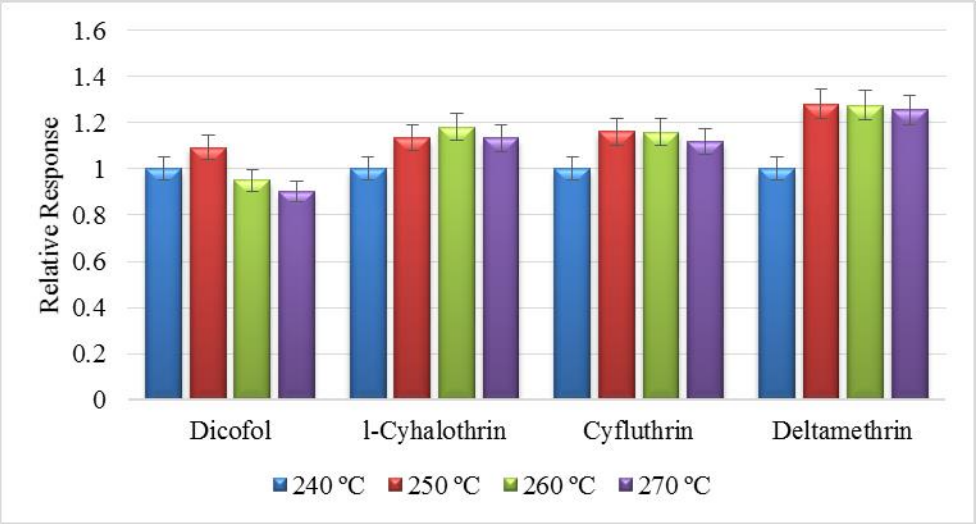


Figure 6-9 Effect of desorption temperature on the responses of thermo-labile pesticides (10 min desorption).

6.3.2 SPME Method Development

6.3.2.1 Preliminary Tests

Once the GC-ToFMS method was developed, some preliminary DI-SPME studies in spiked water and grapes were carried out. Due to the addition of new analytes to the method, the time used for rinsing the fiber in water before desorption was reassessed. The pre-determined time of 10 s did not cause any statistically significant loss of analytes as compared to non-rinsing (data not shown).

Next, preliminary extractions in spiked grape pulp were performed to assess the optimum concentration level to be used during SPME method development. In order to establish such level (analyte-dependent) the following factors were taken into account: (i) instrument response; (ii) SPME extraction efficiency; (iii) MRLs in grapes; and (iv) solubility of analytes. According to this assessment, pesticides were divided by low, medium and high levels (10, 50 and 100 ng/g). Internal standards were kept at 50 ng/g. Regardless of the increased sample concentration of 100 ng/g, more polar pesticides such as acephate lLog P -0.85), omethoate (log P -0.74), and dimethoate (log P 0.704) could not be detected.

Since a multiresidue method should competently analyze both polar and non-polar pesticides, some strategies were assessed to improve method sensitivity towards polar analytes (salting-out and water dilution), as well as heavier non-polar analytes characterized by high binding to the matrix (addition of modifier).

A pre-assessment of the *salting-out* effect on extraction efficiency, determined by literature review, together with this author's previous findings, was carried out for two salts, NaCl and Na_2SO_4, at 10% (w/w). In agreement with a previous study (chapter 3), the addition of NaCl did not yield satisfactory results. Interestingly, Na_2SO_4 not only provided better extraction efficiencies as compared to NaCl, it also provided better repeatability of the data (assessed as RSD%, n=3). The Figure below shows the obtained results for selected pesticides

(assigned according to their log P values). As expected, extraction efficiencies for pesticides bearing log P < 3 were positively affected by the addition of salt. It is important to note, however, that the addition of salt also improved the extraction of endogenous matrix compounds, such as organic acids.

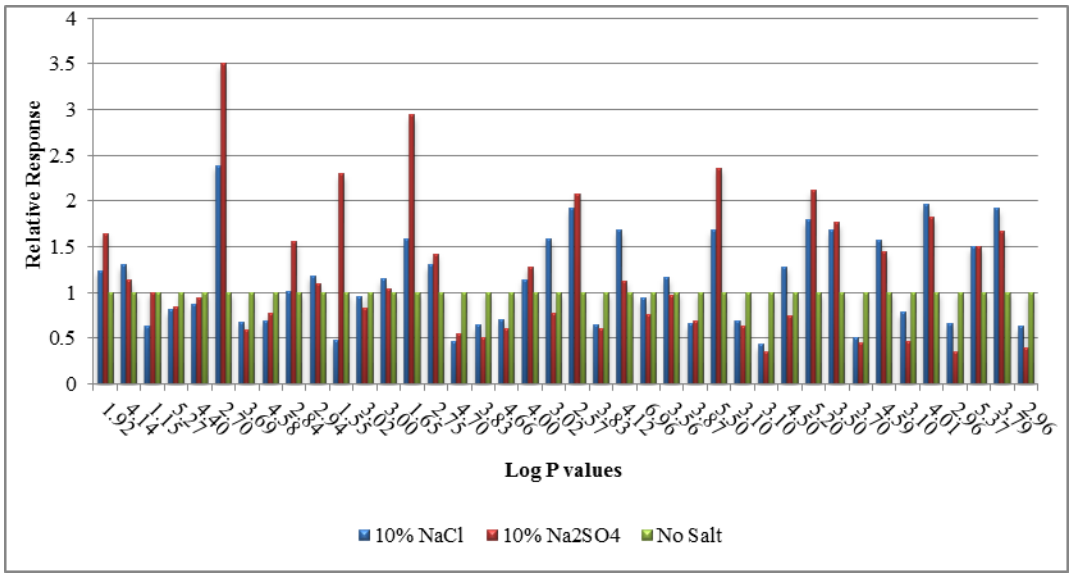


Figure 6-10 Influence of salt addition on extraction efficiency. Results normalized for the response obtained without salt addition.

Next, an investigation of modifier addition was undertaken. Methanol (1%), acetonitrile (1 %) and isopropylalcohol (1% and 10%) were assessed, and the extraction efficiencies compared to those obtained when only water was added (*n*=3, each). As can be seen in *Figure 6-11*, the addition of isopropyl alcohol considerably improved extraction efficiency in only one case. In all other cases, the addition of solvent to the matrix caused a significant decrease in the amounts extracted. This is most likely due to the decreased polarity of the matrix,

causing a decrease in the partitioning of these analytes from the sample to the fiber coating.

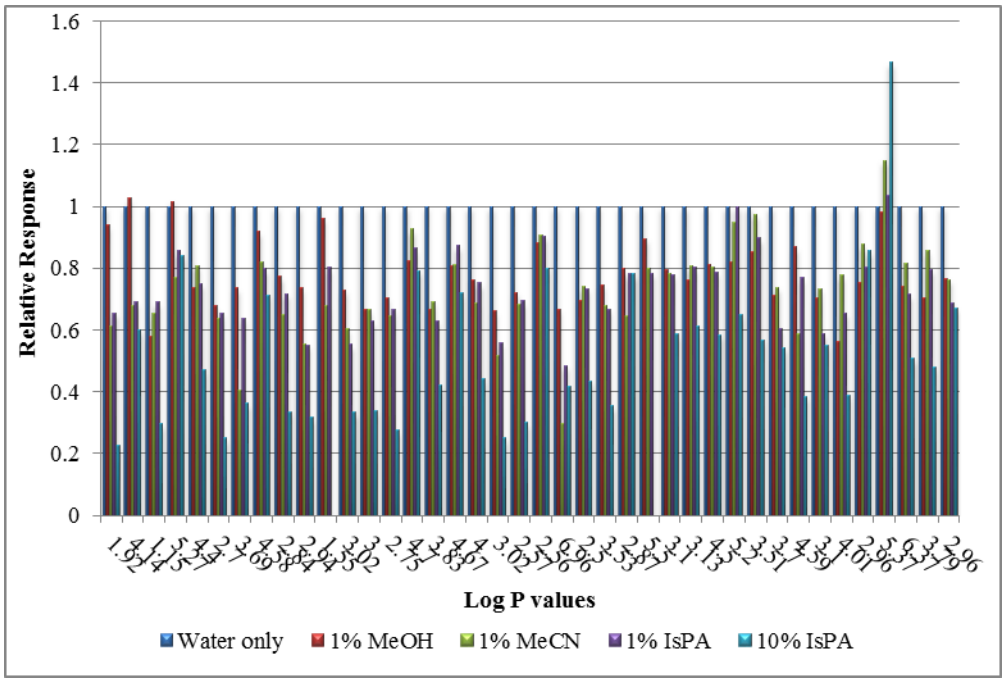


Figure 6-11 Comparison amongst SPME extraction efficiencies by addition of different types and amounts of solvents to sample. Results were normalized for those obtained with the addition of pure water.

6.3.2.2 Screening of influential factors: Plackett-Burman

Plackett-Burman designs are used to obtain qualitative information, such as to detect the factors with the greatest influence on extraction efficiency in SPME. However, this saturated design assumes that there are no interactions between the different extraction variables, with each variable being tested at two experimental levels: a high level (+) and a low level (-).

In the present study, eight factors were studied, namely: (i) Extraction time (15 and 60 min); (ii) Sample temperature (30 and 60 ºC); (iii) Incubation time (5 and 15 min); (iv) Na_2SO_4 addition (0 and 10 %, w/w); (v) Isopropyl alcohol addition (0 and 10 %, v/v); (vi) Sample dilution (0 and 50 %, w/w); (vii) Stirring rate (250 and 750 rpm); and, (viii) pH (natural grape pulp pH levels of 3.8 and 9). The ranges of the considered factors were chosen according to preliminary tests. The rationale behind the selection of these factors was: (i) Extraction time affects the extraction efficiency; when extraction time increases, extraction efficiency also increases, until a maximum is reached (equilibrium). (ii) An increase in sample temperature decreases sample viscosity, increasing analyte diffusivity in the medium, which in turn increases the amounts extracted in pre-equilibrium conditions. Increasing sample temperature may also favor the release of matrix-bounded analytes into their free-form in aqueous media. Conversely, an increase in temperature negatively affects the K_{fs}; however, this will only be an issue for equilibrium extractions. (iii) Sample pre-incubation is closely related to sample temperature, although this parameter is more relevant in HS extraction than in DI; in DI mode, in order to avoid reproducibility problems, pre-incubation time must be enough to ensure uniform temperature throughout the whole sample. (iv) The addition of salt modifies the ionic strength of the medium, and can improve the extraction of more polar analytes via salting-out effect. However, as a possible adverse effect of salt addition, free-form analytes may be driven into matrix binding (as explained in Chapter 3 for the triazoles method). When dealing with biological matrices, the addition of salt may aid in the natural normalization

of occurring variations in salt content. (v) An addition of modifier (organic solvent) should be avoided, or kept at minimum, not exceeding 1-5 % [71]. However, in some cases, the addition of organic solvent may help release matrix-bound analytes. (vi) In general, dilution with water improves method performance in several ways: it minimizes the effect of the matrix, augmenting the release of analytes bound to matrix components to the aqueous phase (free-concentration). Water addition may also minimize the attachment of matrix macromolecules onto the coating and decrease matrix viscosity, which increases the diffusion coefficients of the analytes, allowing higher extraction efficiencies in pre-equilibrium conditions. (vii) Increasing the stirring rate, i.e. agitation, assists the mass transport of the analytes between the sample and the fiber coating, which improves pre-equilibrium extraction, and can shorten equilibration time. (viii) Sample pH is important for analytes possessing pH-dependent ionizable groups, as only the non-ionized form of the analyte is extracted by the coating.

Three "dummy" factors were also added to the design. These were fictitious factors, representing no procedural changes, and were used as a quality control measure for the design. If these factors appeared to influence the results, then the method was affected by variables other than the defined experimental parameters. The use of three central points also effectively accounted for experimental errors that could be associated with instrumental drifts.

Table 6-4 - Non-coded levels of the factors screened by Plackett-Burman design.

Run order	Salt %	pH	Temperature ºC	Incubation Time min	Stirring rate rpm	Extraction time min	Solvent %	Dilution %
5	10.0	9.0	30.0	15.0	750.0	15.0	10.0	0.0
2	10.0	9.0	30.0	15.0	250.0	15.0	0.0	50.0
12	0.0	4.0	30.0	5.0	250.0	15.0	0.0	0.0
11	0.0	9.0	30.0	5.0	250.0	60.0	10.0	50.0
3	0.0	9.0	60.0	5.0	750.0	15.0	0.0	0.0
1	10.0	4.0	60.0	5.0	250.0	15.0	10.0	50.0
9	0.0	4.0	30.0	15.0	750.0	60.0	0.0	50.0
13 (C)	5.0	6.5	45.0	10.0	500.0	37.5	5.0	25.0
14 (C)	5.0	6.5	45.0	10.0	500.0	37.5	5.0	25.0
8	0.0	4.0	60.0	15.0	750.0	15.0	10.0	50.0
4	10.0	4.0	60.0	15.0	250.0	60.0	0.0	0.0
15 (C)	5.0	6.5	45.0	10.0	500.0	37.5	5.0	25.0
7	0.0	9.0	60.0	15.0	250.0	60.0	10.0	0.0
10	10.0	4.0	30.0	5.0	750.0	60.0	10.0	0.0
6	10.0	9.0	60.0	5.0	750.0	60.0	0.0	50.0

Results were evaluated by calculating the amount extracted, then calculating the absolute recovery for each analyte in each experiment. Analysis of variance (ANOVA) was used to evaluate the results, and effects were deemed statistically significant at a 95% confidence level ($p < 0.05$). Because of the physical-chemical diversity of the analytes, results were analyzed as the average

absolute recovery for all analytes, as well as per range of log P values. Absolute recovery (%) was selected to evaluate the processes, since analytes were present in different concentrations, and different amounts of spiked matrix were used for the experiments.

The results presented below showed that responses obtained for all three "dummy" factors, representing no procedural changes, showed no significant effect on the recovery of analytes. If these factors had appeared to influence the outcome, the method would be affected by variables other than the defined experimental parameters, and should have been re-designed. The obtained data confirmed that this was not the case, indicating that all appropriate factors were considered.

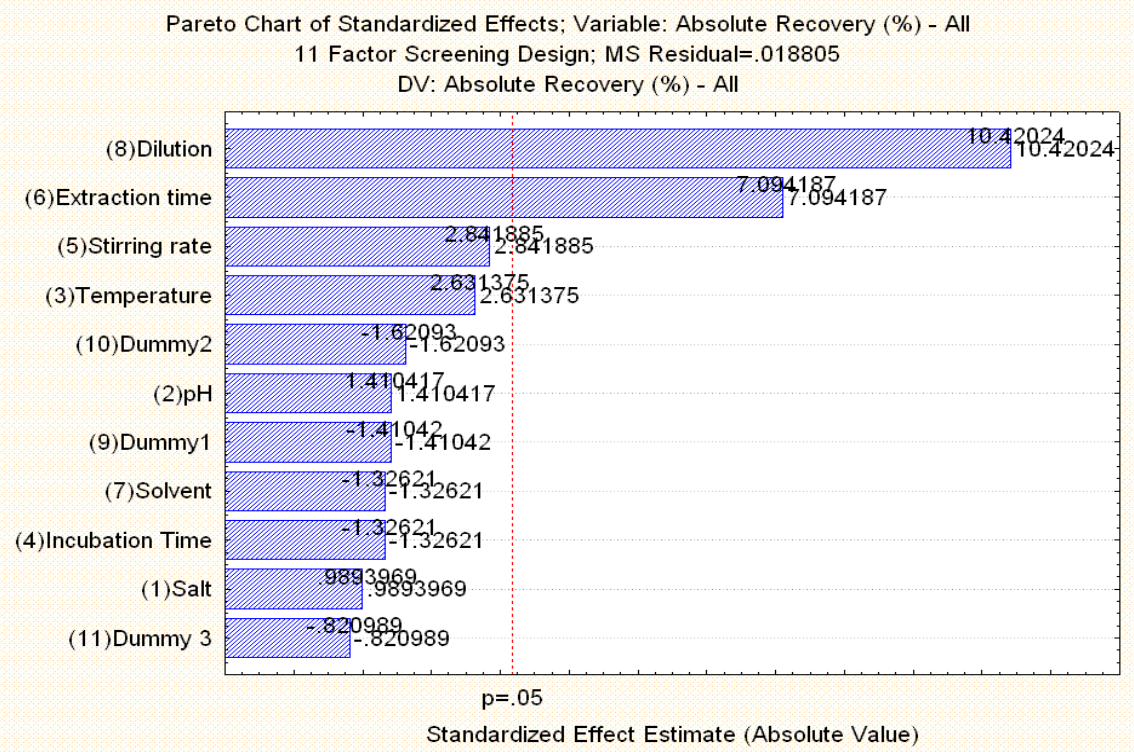


Figure 6-12 Pareto chart showing significant variables obtained in the Plackett-Burman design as a function of the average absolute recovery of all pesticides studied. Dilution and extraction time were significant variables ($p < 0.05$).

As can be seen in *Figure 6-12*, when taking into account the average absolute recovery for all pesticides, the factors dilution and extraction time were significant, in a positive direction. This means that increasing sample dilution from zero to 50 % (4 mL of water) caused an increase in the amount extracted. In SPME, when additional phases are present in the sample, partitioning of the analytes in complex matrices occur among all the phases present in the system. Therefore, in the case of complex matrices, such as grapes, sample dilution may improve the partitioning of analytes between the sample and the coating as the competition between the other phases for analytes is diminished. [71,222,223].

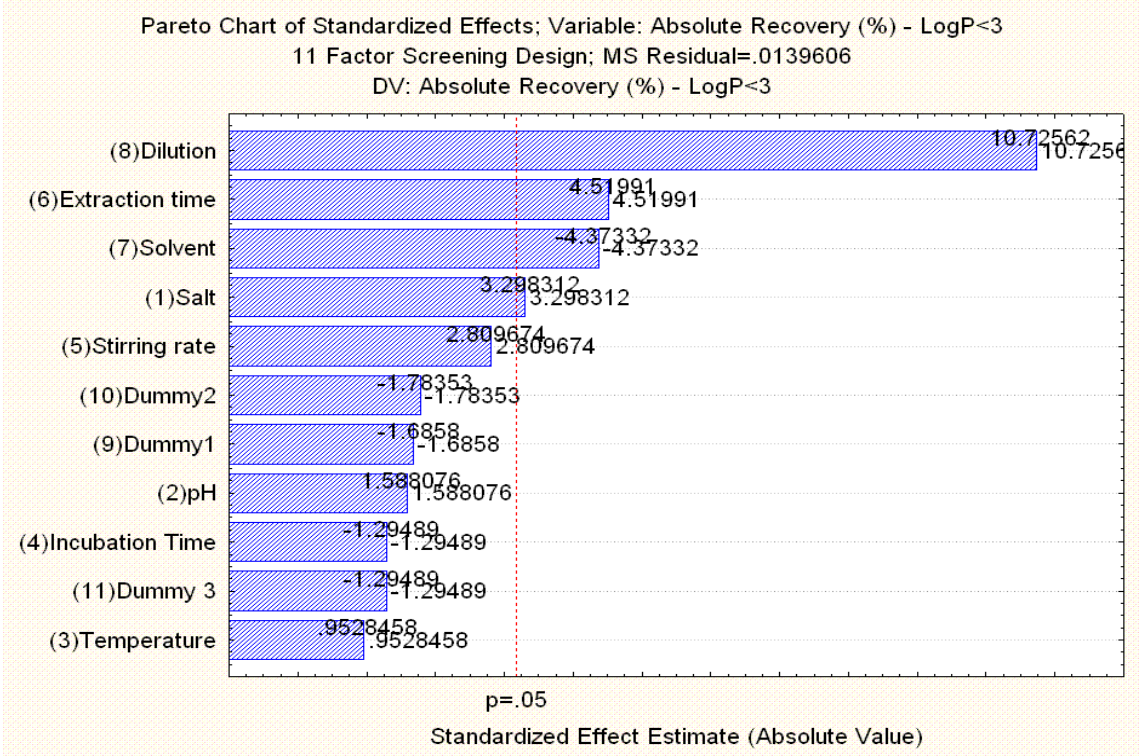


Figure 6-13 Pareto chart showing significant variables obtained in the Plackett-Burman design as a function of the average absolute recovery for the most polar pesticides (log P < 3). Dilution, extraction time, solvent addition (negative), and salt were significant variables ($p < 0.05$).

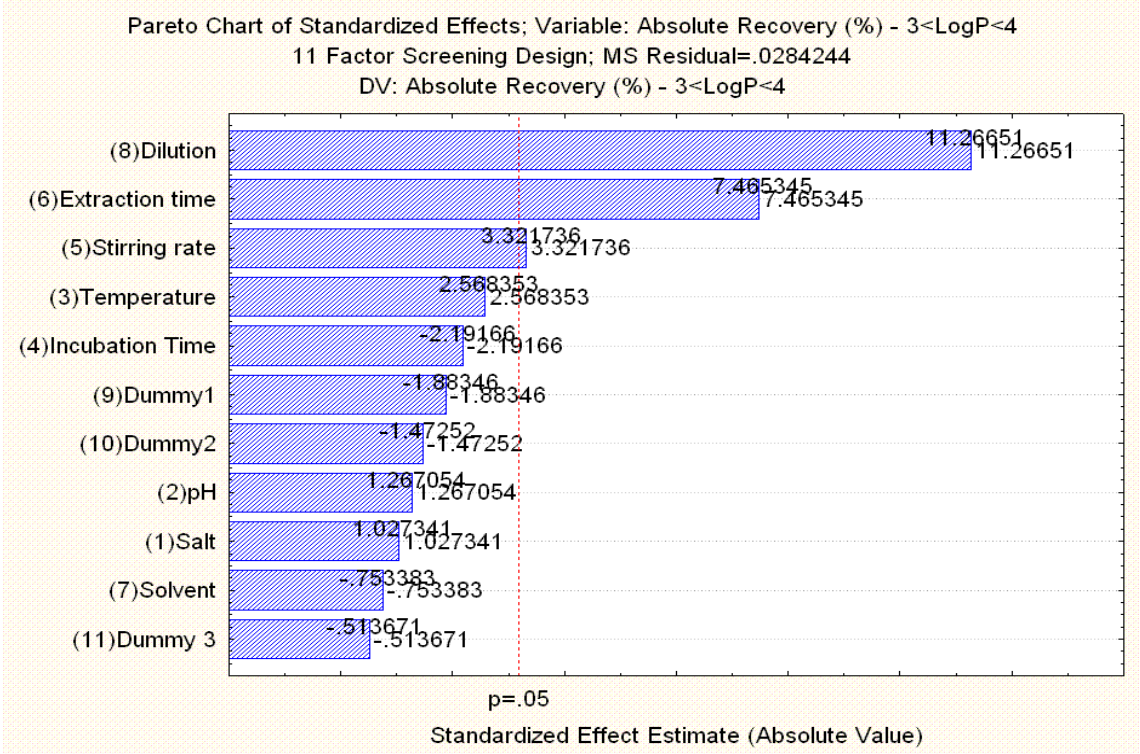


Figure 6-14 Pareto chart showing significant variables obtained in the Plackett-Burman design as a function of the average absolute recovery for the mid-polarity pesticides (3 < log P < 4). Dilution, extraction time, and stirring rate were significant variables ($p < 0.05$).

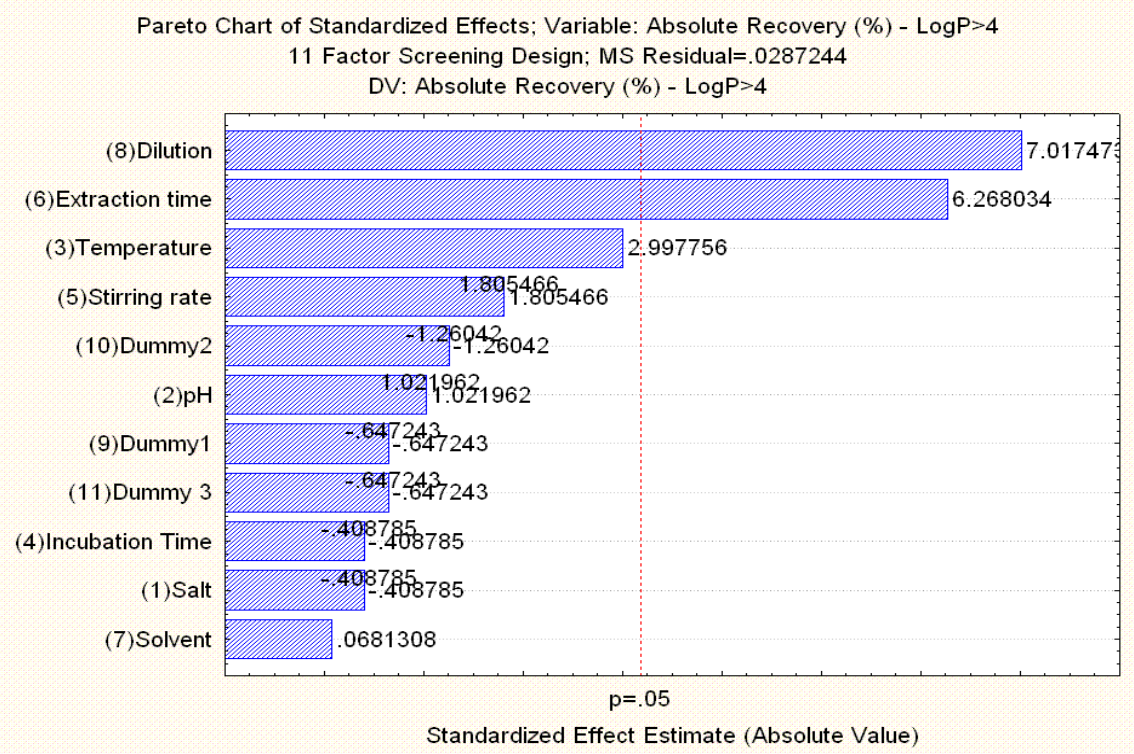


Figure 6-15 Pareto chart showing significant variables obtained in the Plackett-Burman design as a function of the average absolute recovery for the most hydrophobic pesticides (log P > 4). Dilution and extraction time were significant variables ($p < 0.05$).

An assessment of the results presented in *Figure 6-12* to *Figure 6-15* showed that extraction time and sample dilution were factors positively significant when examining all subgroups. The addition of organic solvent was negatively significant for most polar analytes (Log P < 3); the addition of isopropyl alcohol decreased the polarity of the aqueous medium, therefore, causing the equilibrium between the free-form and the matrix-bounded analytes to be shifted towards the matrix-binding, which in turn decreased the extraction

efficiency. The addition of salt was shown to be significant (positively) only for more polar analytes, as the *salting-out* effect increased their extraction efficiency. Stirring rate was only significant (positively) for mid-polarity pesticides ($3 < \text{Log } P < 4$), possibly due to the improved extraction efficiency obtained as a function of the enhanced mass transfer through a thinner boundary layer. It is important to note that this is only significant for this group of analytes, taking there molecular size together with diffusion through the PDMS overcoating. As discussed in Chapter 4, for more polar analytes, diffusion through the PDMS layer would still be the major limiting-step in mass transfer, which explains why the stirring rate does not play a significant role for these analytes. Conversely, according to the discussion in Chapter 4, the stirring rate would be expected to play a significant role in the pre-equilibrium extraction efficiency of more hydrophobic pesticides. A possible explanation for the result herein obtained could be associated with the high matrix binding of these compounds, and the inherent limitation of a Plackett-Burman design that only evaluates main factors, with all interactions being cofounded. Therefore, keeping in mind that faster agitations lead to enhanced mass transfers, 750 rpm was chosen for all subsequent experiments. Surprisingly, extraction temperature did not have a significant effect on any subgroup assessed, since it is known that some increase in the sample temperature may lead to an improved extraction efficiency, as explained before. Even though sample temperature presented no effect on the outcome, it was still chosen for the following optimization step due to another limitation of the Plackett-Burman design; it only evaluates two levels, and no information regarding the behavior of

a given factor in small increments comprising the range between these two levels can be acquired. To summarize, the factors chosen for optimization via surface response were: extraction time, sample temperature, salt addition and dilution.

6.3.2.3 Optimization of conditions: Central Composite Design

Subsequently, a Central Composite Design (CCD) was employed for optimization of dilution ratio (% of water added), extraction time (in min), extraction temperature (in ºC), and salt addition (% of Na_2SO_4). All other parameters were kept constant, as follows: stirring rate at 750 rpm; pre-incubation time of 5 min; and no pH modification (natural pH of ~ 3.8).

In this study, the CCD consisted of four factors, with rotatability $\alpha = 2$ (the choice of α value will determine the predictability of the model), and 3 central points. To summarize, all four factors were evaluated at 5 levels ($-\alpha$, -1, 0, +1, $+\alpha$), resulting in 27 experiments. The summarized conditions utilized for each experiment are presented in *Table 6-5*.

Table 6-5 – Non-coded levels of the factors analyzed by CCD.

Run order	Temperature ºC	Extraction Time min	Dilution %	Salt %	Mass water, g	Mass Grapes, g	Mass Salt, g
25 (C)	45.0	37.5	25.0	15.0	2.0	6.0	1.2
10	52.5	26.3	12.5	22.5	1.0	7.0	1.8
12	52.5	26.3	37.5	22.5	3.0	5.0	1.8
22	45.0	37.5	50.0	15.0	4.0	4.0	1.2
20	45.0	60.0	25.0	15.0	2.0	6.0	1.2
5	37.5	48.8	12.5	7.5	1.0	7.0	0.6
9	52.5	26.3	12.5	7.5	1.0	7.0	0.6
17	30.0	37.5	25.0	15.0	2.0	6.0	1.2
14	52.5	48.8	12.5	22.5	1.0	7.0	1.8
8	37.5	48.8	37.5	22.5	3.0	5.0	1.8
11	52.5	26.3	37.5	7.5	3.0	5.0	0.6
15	52.5	48.8	37.5	7.5	3.0	5.0	0.6
7	37.5	48.8	37.5	7.5	3.0	5.0	0.6
23	45.0	37.5	25.0	0.0	2.0	6.0	0.0
21	45.0	37.5	0.0	15.0	0.0	8.0	1.2
13	52.5	48.8	12.5	7.5	1.0	7.0	0.6
18	60.0	37.5	25.0	15.0	2.0	6.0	1.2
24	45.0	37.5	25.0	30.0	2.0	6.0	2.4
26 (C)	45.0	37.5	25.0	15.0	2.0	6.0	1.2
3	37.5	26.3	37.5	7.5	3.0	5.0	0.6
1	37.5	26.3	12.5	7.5	1.0	7.0	0.6
19	45.0	15.0	25.0	15.0	2.0	6.0	1.2
4	37.5	26.3	37.5	22.5	3.0	5.0	1.8
27 (C)	45.0	37.5	25.0	15.0	2.0	6.0	1.2
16	52.5	48.8	37.5	22.5	3.0	5.0	1.8
2	37.5	26.3	12.5	22.5	1.0	7.0	1.8
6	37.5	48.8	12.5	22.5	1.0	7.0	1.8

The data obtained for CCD experiments was also calculated as absolute recovery (%). The pareto charts of effects for all pesticides, as well as for subgroups, are presented below in *Figure 6-16* to *Figure 6-19*.

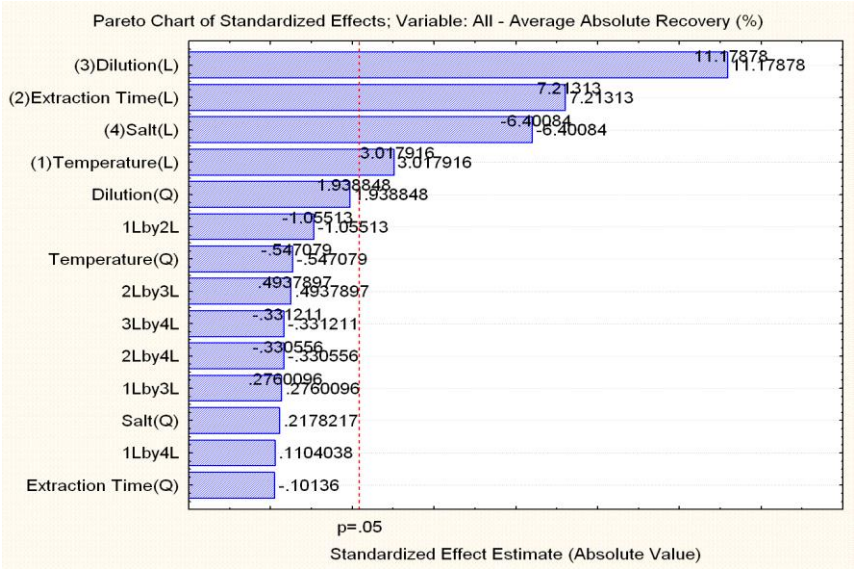


Figure 6-16 Pareto chart of effects showing significant variables obtained for Central Composite Design as a function of the average absolute recovery of all pesticides studied.

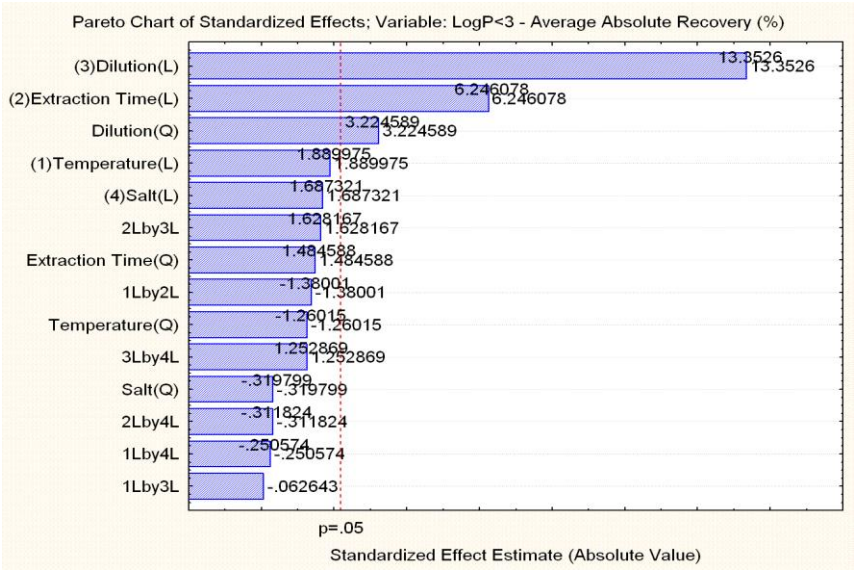


Figure 6-17 Pareto chart of effects showing significant variables obtained for Central Composite Design as a function of the average absolute recovery for the most polar pesticides (log P < 3).

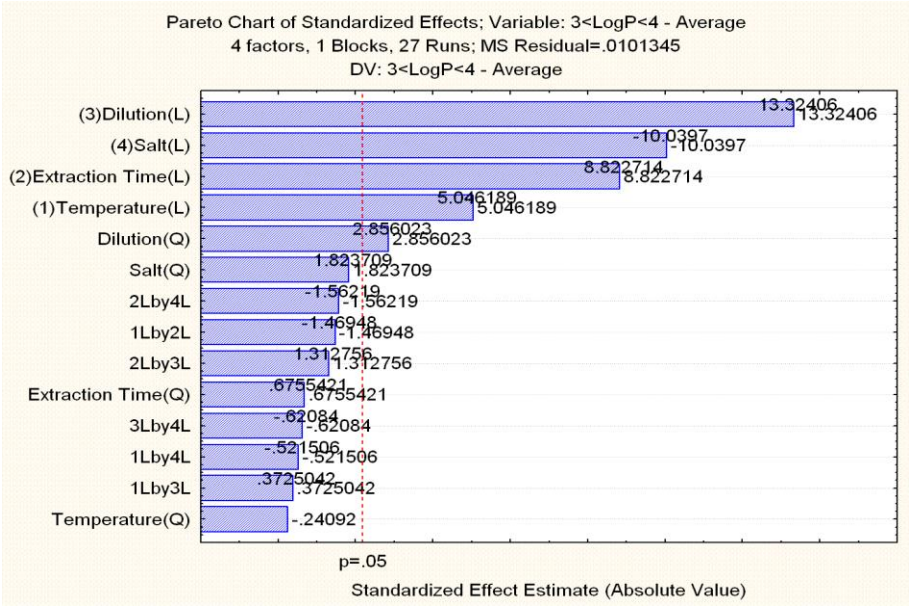


Figure 6-18 Pareto chart of effects showing significant variables obtained for Central Composite Design as a function of the average absolute recovery for the mid-polarity pesticides (3 < log P < 4).

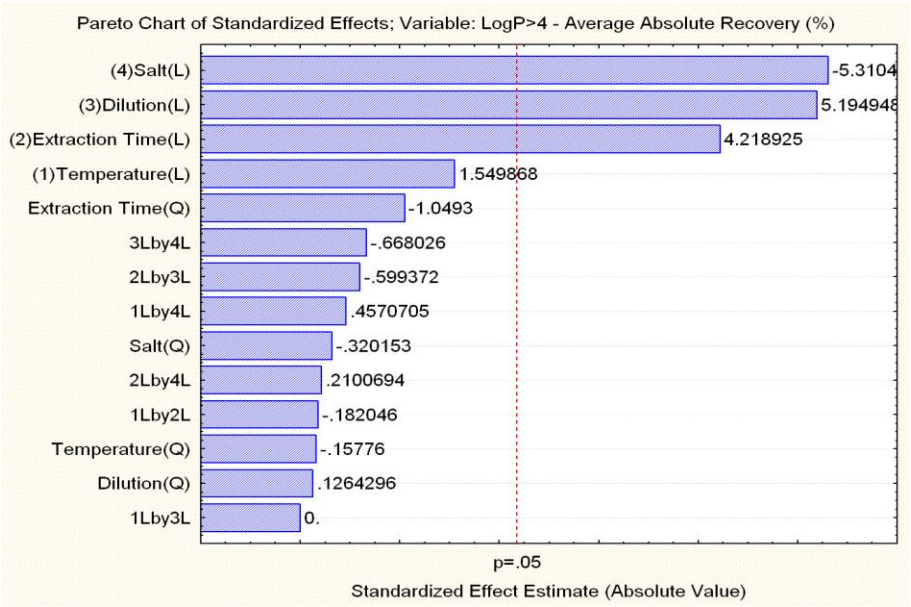


Figure 6-19 Pareto chart of effects showing significant variables obtained for Central Composite Design as a function of the average absolute recovery for the most hydrophobic pesticides (log P > 4).

By investigating the pareto charts of effects above together with the profiles for predicted values and desirability, it could be seen that overall, the most important factor leading to improved extraction efficiencies is the dilution of the matrix with water, with optimum results obtained at dilution percentages as high as 50 %. Similarly, the effect of extraction time also led to improved extraction efficiencies, as expected.

Sample temperature effects were only observed for mid-polarity analytes (*Figure 6-18*), and the temperature effect in the extraction efficiencies for these analytes have already been discussed. However, a closer inspection at surface responses generated between extraction time and sample temperature (*Figure 6-20* to *Figure 6-23*) shows that for a given extraction time, an improved extraction efficiency should be expected with an increase in sample temperature. Obviously, the effect varies amongst the subgroups, but in general, to differing extents, analyte recovery increases as temperature is increased (within the scope of the present study). Possible reasons are likely associated with decreased sample viscosity, as well as unbinding of analytes from the matrix.

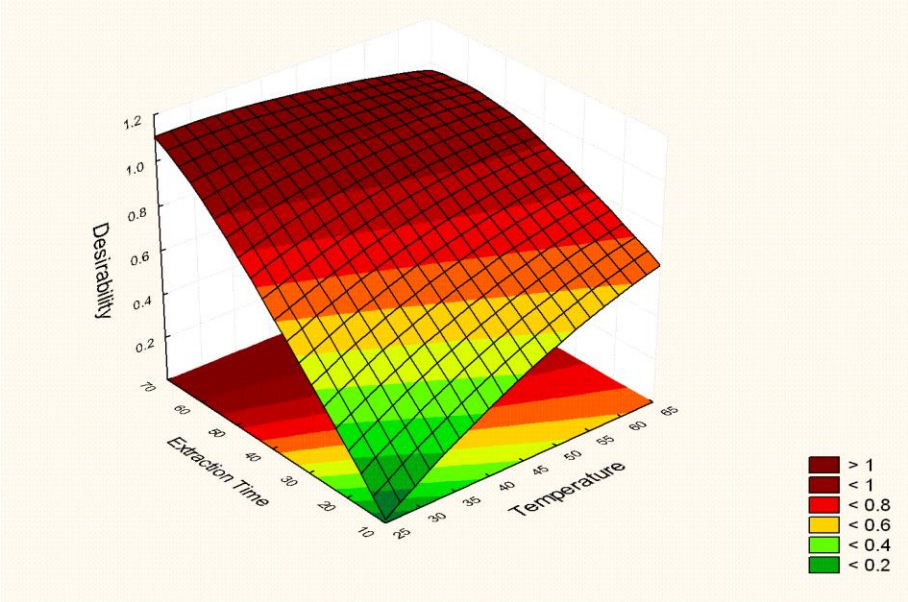


Figure 6-20 Response surface plot for desirability versus extraction time (min) and sample temperature (ºC) for all analytes.

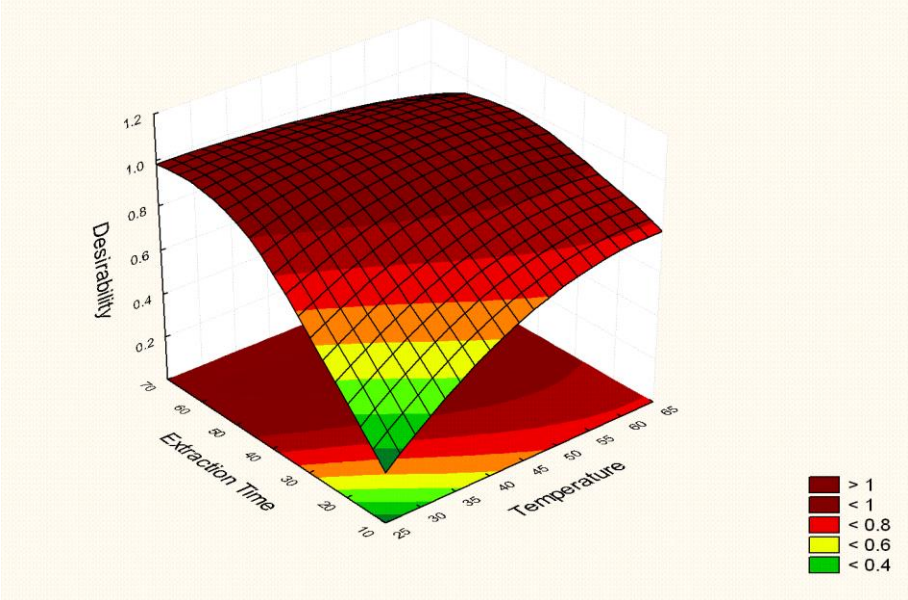


Figure 6-21 Response surface plot for desirability versus extraction time (min) and sample temperature (ºC) for most polar analytes.

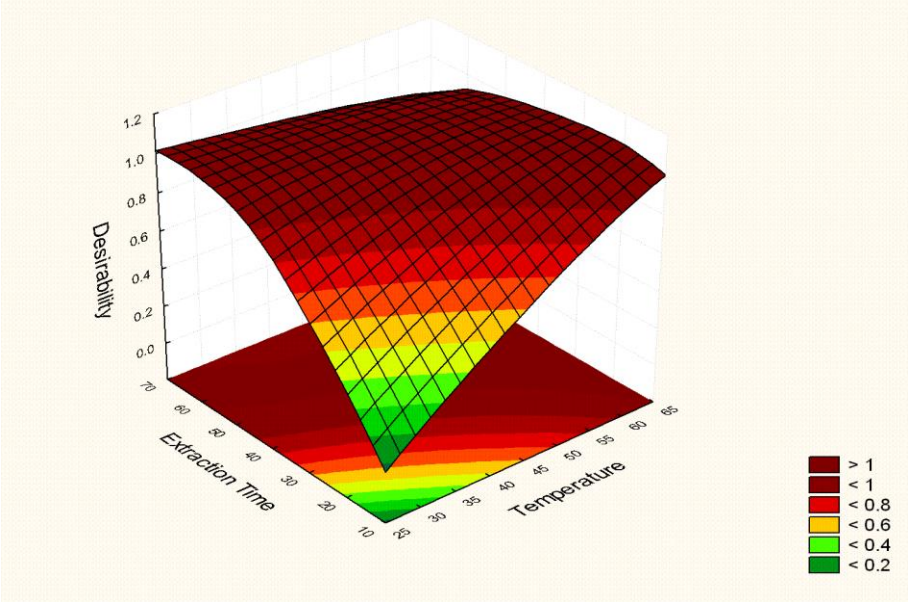


Figure 6-22 Response surface plot for desirability versus extraction time (min) and sample temperature (ºC) for mid-polarity analytes.

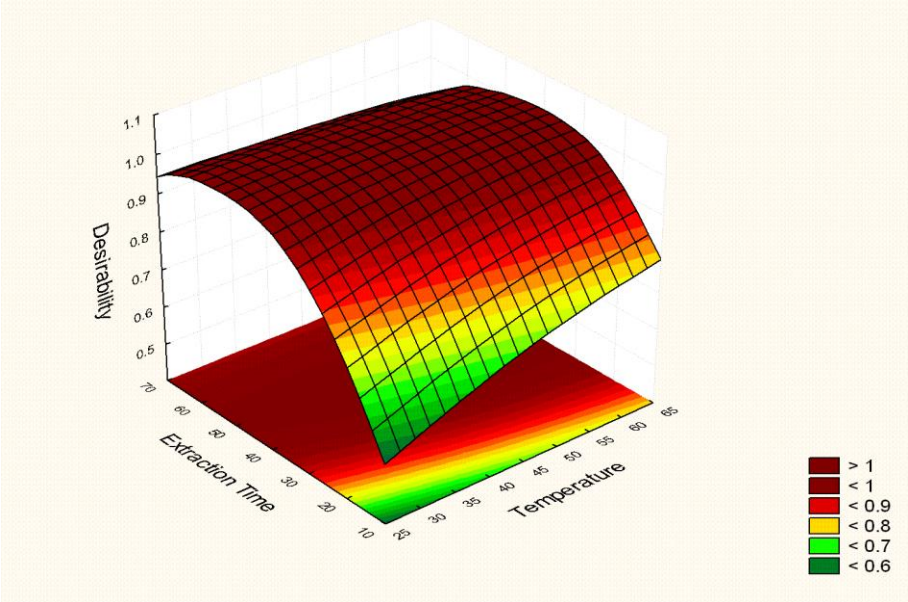


Figure 6-23 Response surface plot for desirability versus extraction time (min) and sample temperature (ºC) for most hydrophobic analytes.

The addition of salt seems to worsen analyte extraction, especially for mid-polarity and more hydrophobic pesticides. This result was expected; as previously stated, the addition of salt might drive these analytes into matrix binding. However, the most surprising outcome of salt addition was obtained for the most polar pesticides: according to the pareto chart of effects, salt addition had no significant effect in the recovery of these pesticides. Nonetheless, the surface responses generated between salt addition and sample temperature for these most polar analytes shows that for a given sample temperature, improved extraction efficiency should be expected when adding salt to the sample (*Figure 6-24*). Accordingly, due to the *salting-out* effect, water molecules would form hydration spheres around the ionic salt molecules, reducing the concentration of water available, thus driving analytes into the fiber coating. However, if the salt concentration is increased past a certain extent, then a concurrent effect that reduces the ability of the analytes to move into the fiber coating takes place, where the analytes participate in electrostatic interactions with the salt ions in solution.

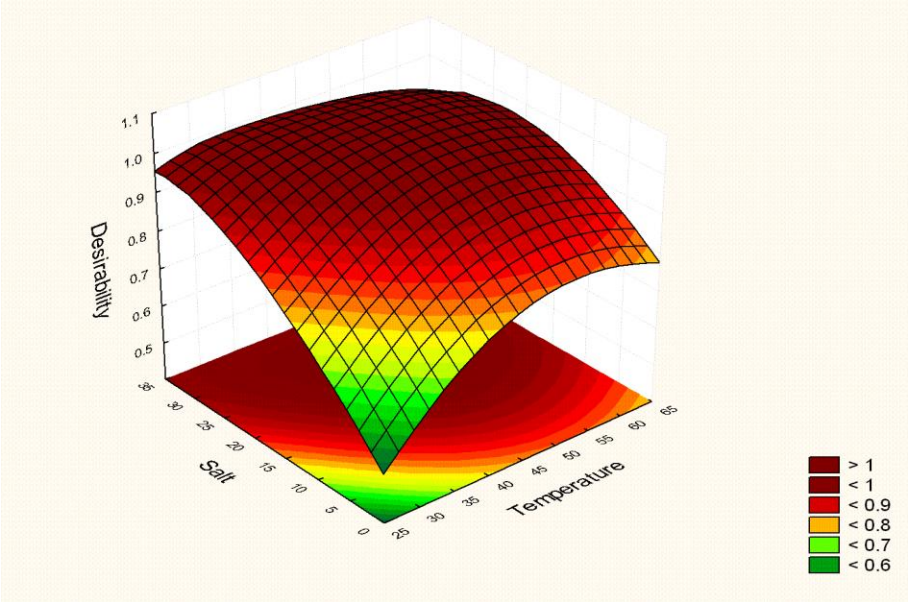


Figure 6-24 Response surface plot for desirability vs salt addition (%, w/w) and sample temperature (ºC) for most polar analytes.

To further investigate this effect, a closer look was taken at individual data for most polar analytes, which at this point may be considered the weak link in developing such a multi-analyte SPME method. Dimethoate (log P 0.704) displayed optimum results for sample temperatures above 43.5 ºC and salt (%, w/w) above 20 %. Similarly, pirimicarb (log P 1.55) displayed little sample temperature effect, and optimum extraction efficiency at values above 19.5% of salt added.

In summary, the effect of each factor in the extraction efficiency seen for each subgroup of analytes are as follows:

- For compounds characterized by log P < 3: Sample temperature and extraction time had little effect; Salt addition (%, w/w) yielded optimum results above 18 %; Sample dilution with water (%, w/w) yielded optimum results above 48 %.

- For compounds characterized by 3 < log P < 4: Extraction time had little effect; Sample temperature yielded optimum results above 54 ºC; Salt addition (%, w/w) yielded optimum results below 4.5 %; Sample dilution with water (%, w/w) yielded optimum results above 42.5%.

- For compounds characterized by log P > 4: Sample temperature and extraction time had little effect; Salt addition (%, w/w) yielded optimum results below 7.5 %; Sample dilution with water (%, w/w) yielded optimum results above 45 %.

The development of an SPME method aiming at simultaneous extraction of numerous analytes covering a broad range of polarities cannot succeed without a compromise in "optimum" values. Indeed, a multiresidue pesticide method, such as the one proposed here, must focus on the overall quality of data, keeping in mind method sensitivity. Therefore, rather than choosing the "optimum" values proposed by the CCD computation of all pesticide recoveries, the "optimized" method will have the following conditions:

- Extraction time of 30 min: Good compromise between good sample throughput with the GC run time and method sensitivity;

- Sample temperature of 55 ºC: Enhanced mass transfer;

- 50 % Dilution (4 mL of water): Also improves mass transfer, increased coating lifetime, and broadens method linear dynamic range;

- 15 % of Na_2SO_4 (1.2 g): Main compromise of this method. Even though it does not help the extraction efficiency of most hydrophobic analytes, it does provide better results for the most polar analytes. Since more hydrophobic analytes display good extraction efficiency by SPME, a sacrifice was made here to improve the extraction efficiency of the more polar analytes (which typically yield low GC responses, together with a low affinity by the SPME coating), while keeping the response for all other analyte groups' $\geq$ 75 % of the optimum value.

- Other parameters already defined are a stirring rate of 750 rpm; pre-incubation time of 5 min; desorption temperature of 260 ºC; desorption time of 10 min.

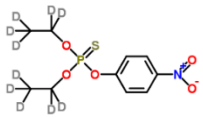
6.3.2.4 Internal Standards Selection

In quantitative determinations of multiresidue pesticides in food, the utilization of internal standards is of utmost importance, as they are used to correct for possible drifts in instrumental responses, as well as to account for losses during sample preparation steps.

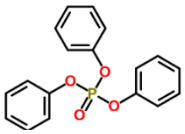
A literature review of the most recent publications regarding GC-based multiresidue analysis in food shows that the vast majority of methods employ a solvent extraction step, as is the case for QuEChERS and its modifications [31,35,37,38,54,224–229]. In such cases, most of the work published report an addition of internal standards (surrogates), such as parathion-d_{10} and α-HCH-d_6, before extraction to account for losses during sample preparation steps. Triphenylphospate (TTP) is then added to the final extract to correct for any instrumental drift. In short, given the exhaustive nature of such extraction methods, these three internal standards are successful in ensuring adequate quantitative data.

Having in mind the non-exhaustive nature of SPME, the application of these widely employed internal standards, generally used for exhaustive methods, would not be advisable without a previous investigation of their applicability. In SPME, internal standards should resemble, as closely as possible, the behaviour of the analyte in the system matrix/fiber coating; in other words, the IS should be able to mimic the partition of the analyte for the extraction phase and any competing phase.

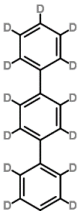
For this reason, ten compounds (*Figure 6-25*), widely used as internal standards and surrogates in pesticide determinations by various methods, were investigated for their applicability as internal standards in the SPME method. Employing the condition previously optimized, matrix-matched calibration curve ranging from 1 to 1000 ng/g (8 points, quadruplicate) was performed with the addition of the mixture of internal standard (each IS at 50 ng/g).



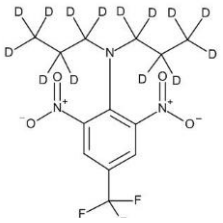
Parathion-d_{10} (3.84)



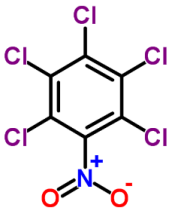
Triphenylphosphate (4.59)



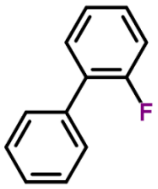
Terphenyl-d_{14} (5.5)



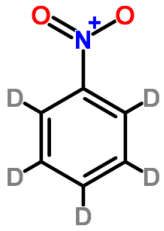
Trifluralin-d_{14} (4.56)



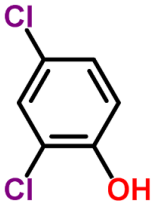
Quintozene (4.58)



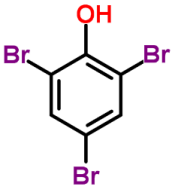
2-Fluorobiphenyl (4.14)



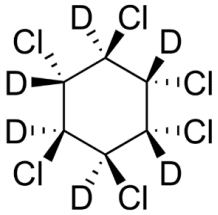
Nitrobenzene-d_5 (1.92)



2,4-Dichlorophenol (3.09)



2,4,6-Tribromophenol (4.4)



α-Hexachlorocyclohexane-d_6 (α-BHC-d_6) (3.99)

Figure 6-25 Chemical structure of potential internal standards investigated in this study (log P value).

The obtained data was processed by computing the ratio between the peak area of the analyte by that obtained for the internal standard (A_{an}/A_{IS}). This process was accomplished for each analyte and each internal standard candidate. For each pair analyte/IS, the data was analyzed according to a linear range that

yielded a correlation coefficient (R^2) $\geq$ 0.995. The data is summarized in *Table 6-6*.

Tribromophenol was excluded from the evaluation at the data processing stage due to its poor ionization in EI. Nitrobenzene-d_5, and 2,4-dichlorphenol were also eliminated from the IS selection because their extraction efficiency was far superior than those displayed by the targeted pesticides. These small polar molecules are less prone to matrix binding, and as such, are more available in their free form in the aqueous phase of the matrix. Due to mismatched extraction efficiency, 2-fluorobiphenyl was also eliminated from further assessment. The areas obtained by this IS were one order of magnitude higher than those obtained by the target analytes (at the same concentration of 50 ng/g).

In the analysis of complex matrices, where a significant and variable competing phase is present in the sample, the selection of internal standard is critical. If the internal standard has a different affinity for the competing phases than that of the analyte of interest, the amounts of internal standard and analyte bound to the matrix may not correlate linearly, and peak ratio analysis would not yield an adequate quantification.

The internal standards most used in the QuEChERS methods, parathion-d_{10} and TTP, resulted in very similar performances, as they both corrected well for approximately 70% of the analytes. Trifluralin-d_{14}, quintozene, and α-HCH-d_6 exhibited similar behaviors amongst themselves, and were able to correct well for

approximately 20% of the analytes. More interestingly, these three IS candidates corrected analytes that were not corrected well by parathion-d_{10} and TTP.

Table 6-6 Internal Standard Selection

In red: R^2 < 0.9; [a] Sum of Malathion and Malaoxon (DP); [b] Sum of Esfenvalerate and Fenvalerate; [c] Sum of Dicofol and 4,4'-dichlorobenzophenone (DP); [d] Sum of Folpet and phthalimide (DP); [e] Sum of two isomers; [f] Sum of four isomers; [g] Sum of two isomers; [h] Sum of two isomers.)

Analyte	***Internal Standard***						
	None	**Parathion-d_{10}**	**TPP**	**Terphenyl-d_{14}**	**Trifluralin-d_{14}**	**Quintozene**	**α-BHC-d_6**
Trifluralin	1-1000	50-5000	50-5000	1-100	1-1000	1-1000	1-1000
	R^2 0.9964	R^2 0.9986	R^2 0.9977	R^2 0.9958	R^2 0.9986	R^2 0.9995	R^2 0.9985
Dimethoate	50-500	50-500	50-500	50-500	50-1000	50-1000	50-1000
	R^2 0.9977	R^2 0.9993	R^2 0.9982	R^2 1	R^2 0.9997	R^2 0.9979	R^2 0.9957
Atrazine	1-500	1-1000	1-1000	1-250	1-500	1-500	1-500
	R^2 0.9955	R^2 0.9993	R^2 0.9982	R^2 0.9964	R^2 0.9957	R^2 0.9991	R^2 0.9992
Diazinon	1-500	1-1000	1-1000	1-250	1-250	1-500	1-500
	R^2 0.9981	R^2 0.9964	R^2 0.9963	R^2 0.9957	R^2 0.9976	R^2 0.9982	R^2 0.9992
Pyrimethanil	1-250	1-500	1-100	1-250	1-500	1-500	1-500
	R^2 0.9971	R^2 0.9954	R^2 0.9999	R^2 0.9962	R^2 0.9989	R^2 0.9997	R^2 0.9985
Chlorothalonil	25-500	1-1000	1-1000	1-250	50-500	1-500	50-500
	R^2 0.9962	R^2 0.9996	R^2 0.9991	R^2 0.9968	R^2 0.997	R^2 0.9968	R^2 1
Pirimicarb	1-500	1-1000	1-1000	1-250	50-500	1-500	1-500
	R^2 0.9988	R^2 0.9991	R^2 0.9980	R^2 0.9981	R^2 0.9984	R^2 0.9982	R^2 0.9981
Vinclozoline	1-500	1-1000	1-1000	1-250	50-500	1-500	1-500
	R^2 0.9965	R^2 0.9988	R^2 0.9979	R^2 0.9984	R^2 0.9972	R^2 0.998	R^2 0.9985
Parathion-methyl	1-500	1-1000	1-500	1-250	1-50	1-500	1-500
	R^2 0.9954	R^2 0.9953	R^2 0.9985	R^2 0.9967	R^2 0.9981	R^2 0.9977	R^2 0.9979
Metalaxyl	1-500	1-1000	1-1000	1-250	50-500	1-500	50-500

	R^2 0.9968	R^2 0.9997	R^2 1	R^2 0.9979	R^2 0.9958	R^2 0.9954	R^2 0.9998
Malathion[a]	1-250	1-500	1-100	1-250	1-50	1-500	1-100
	R^2 0.9982	R^2 0.9935	R^2 0.9970	R^2 0.9946	R^2 0.9972	R^2 0.9989	R^2 0.9970
Chlorpyrifos	5-1000	5-500	5-500	5-250	5-500	5-1000	5-1000
	R^2 0.9967	R^2 0.9996	R^2 0.9971	R^2 0.9992	R^2 0.9957	R^2 0.9991	R^2 0.996
Parathion	5-500	5-1000	5-1000	5-250	5-50	5-500	5-500
	R^2 0.9976	R^2 0.9995	R^2 0.999	R^2 0.9986	R^2 0.996	R^2 0.9952	R^2 0.9966
Cyprodinil	1-500	1-1000	1-500	1-250	50-500	1-500	1-500
	R^2 0.9951	R^2 0.9957	R^2 0.9976	R^2 0.998	R^2 0.9984	R^2 0.9987	R^2 0.9987
Methidathion	5-500	5-1000	5-1000	5-250	5-50	5-500	5-500
	R^2 0.9963	R^2 0.9988	R^2 0.9984	R^2 0.996	R^2 0.9959	R^2 0.9948	R^2 0.9958
Endosulfan I	5-1000	5-500	5-500	5-100	5-1000	5-1000	5-1000
	R^2 0.9983	R^2 0.993	R^2 0.9853	R^2 0.9969	R^2 0.9941	R^2 0.9977	R^2 0.9998
Fludioxonil	1-500	1-1000	1-1000	1-250	5-100	1-500	1-500
	R^2 0.9967	R^2 0.9997	R^2 0.9996	R^2 0.9988	R^2 0.9953	R^2 0.9955	R^2 0.9963
4,4'-DDE	1-100	1-50	1-50	1-100	1-1000	1-1000	1-500
	R^2 0.9996	R^2 0.9995	R^2 0.9970	R^2 0.997	R^2 0.9959	R^2 0.9931	R^2 0.9988
Myclobutanil	1-500	1-1000	1-1000	1-250	50-500	1-500	1-500
	R^2 0.997	R^2 0.9995	R^2 0.9992	R^2 0.9978	R^2 0.9952	R^2 0.9954	R^2 0.9966
Kresoxim-methyl	1-500	1-1000	1-1000	1-250	50-500	1-500	1-500
	R^2 0.997	R^2 0.9974	R^2 0.9963	R^2 0.9983	R^2 0.9961	R^2 0.9966	R^2 0.9974
Flusilazole	1-500	1-1000	1-1000	1-250	50-500	1-500	1-500
	R^2 0.9972	R^2 0.9993	R^2 0.9989	R^2 0.9983	R^2 0.995	R^2 0.9952	R^2 0.9963
Cyproconazole	5-500	5-1000	5-1000	5-250	50-500	5-500	5-500
	R^2 0.9967	R^2 0.9999	R^2 1	R^2 0.9981	R^2 0.9957	R^2 0.9958	R^2 0.9969
Endosulfan II	5-500	5-1000	5-1000	5-250	50-500	5-500	5-500
	R^2 0.997	R^2 0.9967	R^2 0.9973	R^2 0.9995	R^2 0.9958	R^2 0.9969	R^2 0.9979

Trifloxystrobin	5-500	5-1000	5-1000	5-250	5-500	5-500	5-500
	R^2 0.9939	R^2 0.9993	R^2 0.9991	R^2 0.999	R^2 0.9959	R^2 0.999	R^2 0.9988
Quinoxyfen	5-500	5-1000	5-1000	5-250	5-500	50-500	5-500
	R^2 0.9956	R^2 0.9989	R^2 0.9993	R^2 0.9978	R^2 0.9971	R^2 0.9986	R^2 0.9973
Fenhexamide	5-500	5-1000	5-1000	5-250	50-500	50-500	5-500
	R^2 0.9967	R^2 0.9999	R^2 0.9997	R^2 0.9985	R^2 0.9958	R^2 0.9989	R^2 0.9971
Tebuconazole	5-500	5-1000	5-1000	5-250	5-500	5-500	5-500
	R^2 0.9972	R^2 0.9997	R^2 1	R^2 0.9982	R^2 0.9952	R^2 0.9956	R^2 0.9969
Iprodione	5-500	5-1000	5-1000	5-250	5-500	5-500	5-500
	R^2 0.9969	R^2 0.9997	R^2 1	R^2 0.997	R^2 0.9954	R^2 0.9954	R^2 0.9961
Fenpropathrin	10-250	10-1000	10-1000	$R^2 < 0.9$	10-1000	25-1000	10-1000
	R^2 0.998	R^2 0.9707	R^2 0.9707		R^2 0.9999	R^2 0.9989	R^2 0.9976
Phosalone	10-500	10-1000	10-1000	10-1000	50-500	10-500	10-500
	R^2 0.9956	R^2 0.9998	R^2 0.9999	R^2 0.9999	R^2 0.9967	R^2 0.9967	R^2 0.9973
Azinphos-methyl	5-500	5-1000	5-1000	5-1000	5-500	5-500	5-500
	R^2 0.9961	R^2 0.9993	R^2 0.998	R^2 0.998	R^2 0.9963	R^2 0.999	R^2 0.9994
Pyriproxyfen	5-500	5-1000	5-1000	5-1000	10-500	5-500	5-500
	R^2 0.9977	R^2 0.9967	R^2 0.9974	R^2 0.9974	R^2 0.9975	R^2 0.998	R^2 0.9989
Pyridaben	25-1000	50-1000	25-1000	25-1000	50-1000	50-1000	25-1000
	R^2 0.9973	R^2 0.9855	R^2 0.9852	R^2 0.9852	R^2 0.9956	R^2 0.9981	R^2 0.9988
Fenbuconazole	10-500	5-1000	5-1000	5-1000	5-500	5-500	5-500
	R^2 0.9985	R^2 0.9994	R^2 0.9997	R^2 0.9997	R^2 0.9904	R^2 0.996	R^2 0.9977
Boscalid	1-500	1-1000	1-1000	1-1000	50-500	1-500	50-500
	R^2 0.9956	R^2 0.9993	R^2 0.9997	R^2 0.9997	R^2 0.9965	R^2 0.9952	R^2 0.9996
Pyraclostrobin	10-500	10-1000	10-1000	10-1000	10-250	10-500	10-500
	R^2 0.9891	R^2 0.9992	R^2 0.9981	R^2 0.9981	R^2 0.9975	R^2 0.999	R^2 0.9979
Es/Fenvalerate	$R^2 < 0.9$	$R^2 < 0.9$	$R^2 < 0.9$	$R^2 < 0.9$	$R^2 < 0.9$	$R^2 < 0.9$	$R^2 < 0.9$

Azoxystrobin[b]	25-500	5-1000	5-1000	5-250	50-500	5-500	5-500
	R^2 0.999	R^2 0.9984	R^2 0.9989	R^2 0.9983	R^2 0.9929	R^2 0.9948	R^2 0.9967
Dicofol[c]	10-1000	10-500	10-500	10-100	10-1000	10-1000	10-1000
	R^2 0.999	R^2 0.9977	R^2 0.9958	R^2 0.9984	R^2 0.9954	R^2 0.9984	R^2 0.9997
Folpet[d]	10-500	10-500	10-500	10-100	10-500	10-500	10-500
	R^2 0.9994	R^2 0.9986	R^2 0.9951	R^2 0.9962	R^2 0.9845	R^2 0.9907	R^2 0.9942
λ–Cyhalothrin[e]	$R^2 < 0.9$	$R^2 < 0.9$	$R^2 < 0.9$	$R^2 < 0.9$	$R^2 < 0.9$	$R^2 < 0.9$	$R^2 < 0.9$
Cyfluthrin[f]	$R^2 < 0.9$	$R^2 < 0.9$	$R^2 < 0.9$	$R^2 < 0.9$	$R^2 < 0.9$	$R^2 < 0.9$	$R^2 < 0.9$
Difenoconazole[g]	10-500	10-1000	10-1000	10-250	10-500	10-500	10-500
	R^2 0.9956	R^2 0.9999	R^2 0.9995	R^2 0.9976	R^2 0.9958	R^2 0.9991	R^2 0.9994
Deltamethrin[h]	$R^2 < 0.9$	$R^2 < 0.9$	$R^2 < 0.9$	$R^2 < 0.9$	$R^2 < 0.9$	$R^2 < 0.9$	$R^2 < 0.9$

While terphenyl-d_{14} showed good correlation, the linear range was significantly shortened for all corrected analytes. Terphenyl-d_{14} presented the lowest extraction efficiencies amongst all tested IS candidates, as it was expected to be more strongly bounded to the matrix. One hypothesis is that, as the concentration of more hydrophobic compounds increased (in the calibration) more competition took place for adsorption onto the solid matrix. As such, more terphenyl-d_{14} would be available in its free-form, which could account for the loss of linearity analyte/IS. This explanation is in line with the plot of instrumental response for terphenyl-d14 in function of calibration level. For comparison, and to assure that no mistakes were made during the spiking of the IS mixture, quintozene is also plotted in *Figure 6-26*.

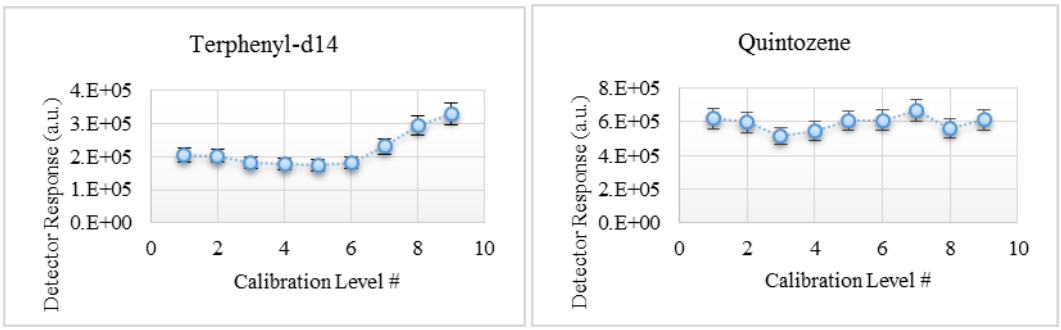


Figure 6-26 Detector response for IS candidates in function of calibration level. (A) Terphenyl-d_{14}; (B) Quintozene. Concentration = 50 ng/g.

The more hydrophobic pyrethroid pesticides did not yield linear responses throughout the evaluation. It is possible that due to their low solubility in water and high hydrophobicity, these compounds adsorbed onto the vial walls during the duration of the sequence run. However, this hypothesis must be further investigated. At this point, the proposed method will move towards the validation steps without the inclusion of these troublesome pesticides.

6.3.2.5 Method Validation

After establishing the extraction conditions, the method obtained was validated in fortified samples by evaluating linearity, sensitivity, accuracy and precision.

6.3.2.5.1 Linearity and limit of quantification

Method linearity was studied by means of matrix-matched calibration curves, using relative area versus adequate IS. Calibration levels were prepared at 1, 5, 10, 25, 50, 100, 250, 500, and 1000 ng/g. Linearity was assumed when the correlation coefficient (R^2) was higher than 0.995 with randomly distributed residuals lower than 20%.

For each pesticide, the results were analyzed for hetero or homoscedasticity using no weight, weight 1/X, and weight $1/X^2$ (X = concentration). In addition, as shown in *Figure 6-27*, the obtained curves were also inspected at the lower range to assure the validity of the regression model.

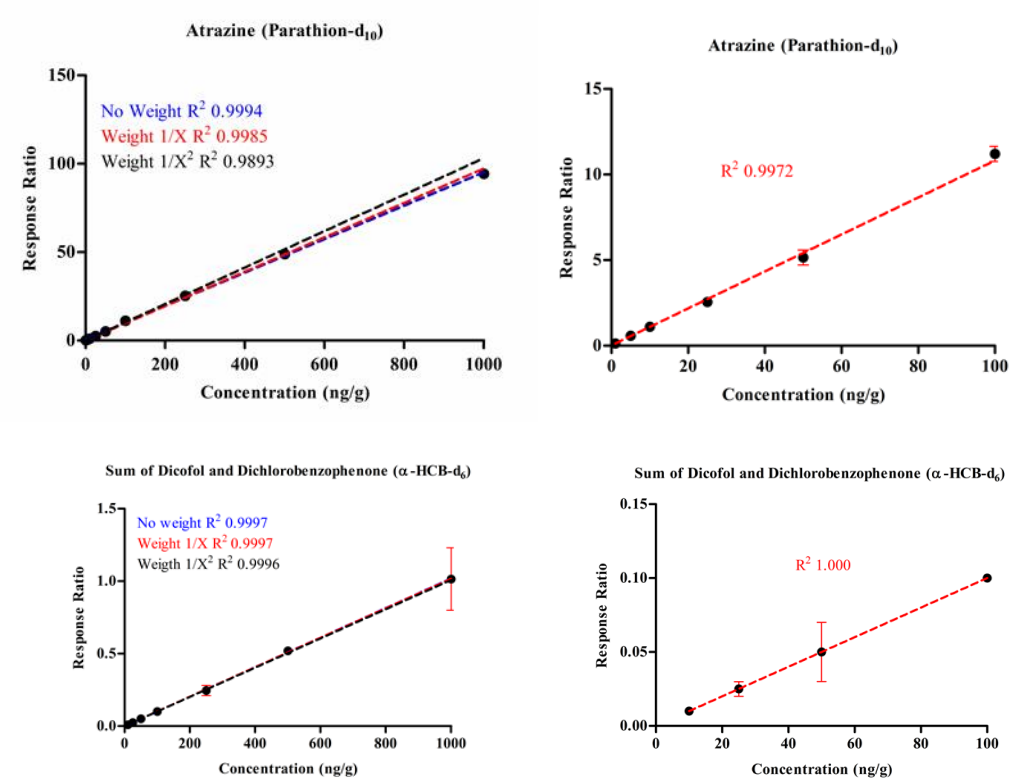


Figure 6-27 Representative calibration plots comparing different weights for regression curve (A, C), and inspection at lower concentration levels using weight 1/X (B, D), for atrazine (A, B) and Dicofol (C, D).

The results for method validation are summarized in

Table 6-7. As can be seen, the method showed good linearity for all 40 pesticides in the entire calibration range selected for each analyte, with $R^2 > 0.995$. Even though calibration ranges were chosen to include concentration levels bracketing the EU MRL, when "relatively high", the proposed method did not meet the linearity requirement at the MRL concentration. For example, as the MRL for boscalid was 5,000 µg/kg, the present method was validated and linear for boscalid in the range of 1-1,000 µg/kg. In effect, the greatest strength of the proposed method also leads to one disadvantage. On one hand, owing to the pre-concentration capabilities of this

method, admirably low limits of quantitation can be attained. On the other hand, the same pre-concentration power causes the proposed SPME method to have a "relatively low" upper limit of linearity. For example, for the QuEChERS method, without solvent exchange or large volume injection, 1 g of sample will generate 1 mL of extract, of which only 1-3 µL will be injected in the GC. Conversely, in the present method, the totality of analytes extracted by the fiber coating will be transferred to the GC. In the QuEChERS method, if the analyte is present in the sample in concentrations above the upper limit of linearity, the sample can be simply diluted so that a concentration within the linearity range is attained. In SPME, as discussed previously, the analyte will partition between the coating and all competing phases present in the sample. Dilution of the sample may cause significant differences in the content of these competing phases, which could lead to the unsuitability of the calibration data for the diluted sample. Matrix effects caused by further dilution of samples were not investigated in this work; however, it is an important point to be validated in future work.

In the present study, the LOQ objective is defined according to SANCO guidelines (SANCO/12495/2011) as the lowest assessed concentration of an analyte that yields a reproducible response that is both accurate (according to the expected value using the linear regression equation) and precise (≤ 20 % RSD). Excellent LOQs were achieved for 38 pesticides (LOQ ≤ 10 ng/g, except dimethoate (LOQ = 50 ng/g), and pyridaben (LOQ = 25 ng/g)). By comparing the LOQ values obtained with the proposed method with recent publications reported for GC-based multiresidue pesticides analysis in grapes, it is evident that the LOQs obtained in this study are in a similar range or better than those reported [29–31,37,230–232]. Furthermore, the LOQ values validated in this study are far below the MRL set for grapes [233]. The only exception is dimethoate, which has the MRL set as 20 ng/g, and a presented LOQ value of 50 ng/g.

Table 6-7 Method validation parameters for grape analysis at optimized SPME conditions.

Analyte	MRL[a] ng/g	LOQ (ng/g)	Linearity (ng/g)	R^2	Intra-day accuracy[b]			Intra-day precision[c]			Inter-day accuracy[d]			Inter-day precision[e]		
					10 ng/g	*50 ng/g*	*100 ng/g*	*10 ng/g*	*50 ng/g*	*100 ng/g*	*10 ng/g*	*50 ng/g*	*100 ng/g*	*10 ng/g*	*50 ng/g*	*100 ng/g*
Trifluralin	10	1	1-1000	0.9986	94	90	113	14	19	12	100	113	91	15	12	8
Dimethoate[f]	20	50	50-500	0.9993	-	102	85	-	20	2	-	117	82	-	13	8
Atrazine	50	1	1-1000	0.9993	111	109	109	16	20	12	109	120	120	15	20	12
Diazinon	10	1	1-1000	0.9964	102	114	95	19	17	2	83	117	109	17	17	11
Pyrimethanil	5000	1	1-500	0.9985	94	111	111	21	10	2	91	82	104	12	18	4
Chlorothalonil	1000	1	1-1000	0.9996	94	99	87	11	17	4	82	112	89	8	18	4
Pirimicarb	1000	1	1-1000	0.9991	91	97	105	20	5	4	99	115	82	20	13	9
Vinclozoline	50	1	1-1000	0.9988	105	105	108	9	7	7	85	110	100	18	18	8
Parathion-methyl	20	1	1-1000	0.9953	85	95	93	22	13	3	83	113	112	22	11	11
Metalaxyl	2000	1	1-1000	0.9997	101	100	99	17	7	9	97	86	90	20	7	11
Malathion	20	1	1-500	0.9989	101	94	103	10	15	9	91	112	115	20	15	3
Chlorpyrifos	500	5	5-1000	0.9991	115	109	113	21	12	6	110	97	92	15	19	11
Parathion	50	5	5-1000	0.9995	96	115	109	14	7	10	98	86	110	10	13	4
Cyprodinil	5000	1	1-1000	0.9957	106	91	93	14	15	5	103	91	94	19	18	7
Methidathion	20	5	5-1000	0.9988	108	109	103	9	9	12	94	110	112	21	17	11
Endosulfan I[g]	50	5	5-1000	0.9998	89	113	94	17	9	4	81	86	110	12	8	5
Fludioxonil	2000	1	1-1000	0.9997	104	112	112	13	8	12	81	94	105	19	5	2
4,4'-DDE	50	1	1-500	0.9988	102	95	87	16	20	5	112	89	107	15	7	9
Myclobutanil	1000	1	1-1000	0.9995	111	108	109	20	7	6	119	83	100	21	13	7
Kresoxim-methyl	1000	1	1-1000	0.9974	85	110	111	13	8	10	101	100	103	9	7	2
Flusilazole	50	1	1-1000	0.9993	107	112	103	14	13	2	116	111	102	20	18	8
Cyproconazole	200	5	5-1000	0.9999	110	94	98	12	12	8	98	113	86	13	15	6
Endosulfan II[g]	50	5	5-1000	0.9967	88	100	105	11	12	11	105	88	115	19	17	11

Analyte	MRL[a] ng/g	LOQ (ng/g)	Linearity (ng/g)	R^2	Intra-day accuracy[b]			Intra-day precision[c]			Inter-day accuracy[d]			Inter-day precision[e]		
					10 ng/g	*50 ng/g*	*100 ng/g*	*10 ng/g*	*50 ng/g*	*100 ng/g*	*10 ng/g*	*50 ng/g*	*100 ng/g*	*10 ng/g*	*50 ng/g*	*100 ng/g*
Trifloxystrobin	5000	5	5-1000	0.9993	102	115	91	11	7	9	97	94	115	11	15	11
Quinoxyfen	1000	5	5-1000	0.9989	102	106	96	22	18	4	91	86	103	12	8	7
Fenhexamide	5000	5	5-1000	0.9999	108	87	104	8	19	12	95	115	86	11	16	9
Tebuconazole	2000	5	5-1000	0.9997	90	96	110	10	5	9	107	83	120	19	17	7
Iprodione	10	5	5-1000	0.9997	86	94	86	16	11	2	87	112	92	15	11	12
Fenpropathrin	10	10	10-1000	0.9989	90	114	112	19	6	5	122	102	103	22	14	6
Phosalone	50	10	10-1000	0.9998	113	85	91	21	19	9	80	119	108	19	19	7
Azinphos-methyl	50	5	5-1000	0.9993	100	103	97	12	19	6	120	114	80	16	7	2
Pyriproxyfen	50	5	5-1000	0.9967	114	114	91	12	7	6	83	111	115	22	16	6
Pyridaben	500	25	25-1000	0.9988	-	99	96	-	19	8	-	102	102	-	16	12
Fenbuconazole	1000	10	5-1000	0.9994	117	90	103	16	8	3	82	89	96	17	18	10
Boscalid	5000	1	1-1000	0.9993	97	94	114	11	5	7	114	83	119	18	11	12
Pyraclostrobin	1000	10	10-1000	0.9992	82	106	110	20	9	11	119	93	111	17	20	7
Azoxystrobin	2000	5	5-1000	0.9984	86	102	108	19	12	7	120	107	113	15	16	2
Dicofol	20	10	10-1000	0.9997	112	115	85	21	20	7	83	102	96	20	6	6
Folpet	20	10	10-500	0.9986	111	97	93	19	13	10	106	118	112	23	7	11
Difenoconazole	500	10	10-1000	0.9999	94	95	97	17	17	2	83	83	97	21	8	4

[a] EU MRL Ref [233] ; [b] $n = 5$; [c] RSD, % ($n = 5$); [d] $n = 9$; [e] RSD, % ($n = 9$); [f] LOQ > MRL; [g] for mixture of both isomers;

6.3.2.5.2 Precision and Accuracy

Precision as intra-day repeatability were determined for grape samples spiked at three concentrations: 10, 50, and 500 ng/g. Data from five analyses for each concentration level performed in the same day were used for calculations (n =5), with the data expressed as relative standard deviations (RSD, %). As presented in

Table 6-7, good results were obtained for nearly all pesticides (RSD ≤ 20%).

Intermediate precision as inter-day reproducibility was also determined for grape samples spiked at three concentrations, 10, 50, and 500 ng/g. Data obtained from three analyses for each concentration level performed in three non-consecutive days (n = 9) was used for calculations, with the data expressed as relative standard deviations (RSD, %). As in the case of repeatability, good results were obtained for nearly all pesticides (RSD ≤ 20%).

Accuracy was assessed by fortifying blank samples of grapes at three concentration levels (n = 5 intra-day, and n = 9 inter-day). In the same fashion used for the triazoles method in chapter 3, results obtained were back calculated by means of "estimated concentration values" to estimate accuracy [234]. For all pesticides, accuracy ranged from 82 to 120 %.

It is worth highlighting the excellent performance obtained by the proposed method for pesticides such as chlorothalonil, dicofol and folpet, which are considered the weak link in QuEChERS-based multiresidue methods [34,221].

6.3.2.6 Real Samples

The validated method was applied for the analysis of 6 commercial samples of conventionally grown green seedless grapes purchased at local markets in Waterloo (ON, Canada).

Twelve pesticides, all pertaining to the so-called new generation of pesticides, were detected in the commercial samples (*Table 6-8*). These pesticides are preferred for applications in grape crops because they are considered somehow more innocuous, but just as effective as the older pesticides [235]. Pyrimethanil was detected in four of the six samples analyzed. The highest concentration was found for fenhexamide. Despite of these findings, all pesticides were present at concentrations much lower than their respective MRL.

Table 6-8 Target pesticides found in analyzed commercial grape samples.

Analyte	**Concentration Found in ng/g (± C.V.) (*n* = 3, each)**					
	Sample 1	**Sample 2**	**Sample 3**	**Sample 4**	**Sample 5**	**Sample 6**
Pyrimethanil	< LOQ	192 ± 12	< LOQ	n.d	n.d	< LOQ
Cyprodinil	n.d	83 ±8	n.d	73 ± 1	n.d	< LOQ
Fludioxonil	n.d	47 ± 8	n.d	n.d	n.d	n.d
Myclobutanil	n.d	1.1 ± 0.1	n.d	n.d	n.d	< LOQ
Kresoxim-methyl	n.d	n.d	n.d	< LOQ	< LOQ	1.4 ± 0.2
Trifloxystrobin	n.d	n.d	n.d	n.d	n.d	n.d
Quinoxyfen	n.d	36 ± 3	53 ± 19	n.d	n.d	n.d
Fenhexamide	n.d	269 ± 51	n.d	n.d	n.d	n.d
Tebuconazole	n.d	6.4 ± 0.1	5.8 ± 0.3	17 ± 1	n.d	n.d
Fenbuconazole	n.d	n.d	n.d	< LOQ	n.d	n.d
Boscalid	62 ± 2	n.d	n.d	19 ± 1	161 ± 12	n.d
Pyraclostrobin	12 ± 7	n.d	< LOQ	n.d	75 ± 4	n.d

n.d = non detected

In summary, the optimized and validated DI-SPME-GC-ToFMS method was suitable for analysis of grape samples with the required sensitivity, precision and accuracy. The method was successfully applied for multiresidue determination of pesticides in the incurred samples. Therefore, the present method could be satisfactorily applied in routine monitoring of pesticides in agricultural commodities, provided that proper optimization is carried out.

6.3.2.7 Re-visiting the troublesome Pesticides

Highly hydrophobic pyrethroid pesticides display low water solubility and are prone to adsorption onto labware, such as vial walls. As discussed previously, the quantitative performance of SPME-based approaches is very sensitive to all competing phases present in the system.

The determination of pyrethroids in food matrices is well documented in the literature, and successfully performed via solvent-based extraction methods, such as QuEChERS [31,33,37,40,54,229,231,236]. In these solvent extraction methods, regardless of the secondary interaction of the bound/free portion, all analytes extracted from the sample, as well as analytes eventually sorbed onto the glassware will be washed away, and exhaustive extraction takes place. In such cases, analyte adsorption onto glassware does not affect the quantification reliability of the method.

To evaluate the extent of non-specific adsorption onto glassware, 250-mL of water was spiked with a standard mix and placed in a 250-mL tightly closed jar. From this jar, 8 mL of fortified water was analyzed in triplicate immediately after preparation, and then after 24 and 48 h had elapsed (triplicate measurements). As shown in *Figure 6-28*, significant losses, probably due to glassware adsorption, were observed for all pyrethroids (Trifluralin-d_{14} is also depicted to degree of variability not associated to interaction to glassware).

Based on the aforementioned observation, experiments were performed to tentatively improve the SPME extraction efficiency of pyrethoids from grapes.

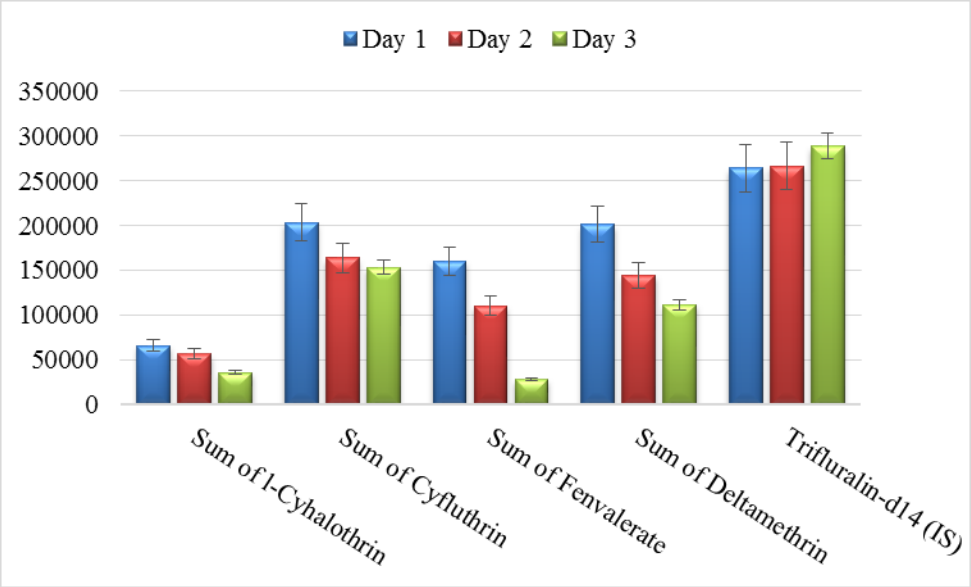


Figure 6-28 Evaluation of adsorption losses for pyrethroid pesticides. Conditions: 8 mL water, 30 min extraction at 55 ºC.

6.3.2.7.1 Solvent Pre-extraction

Regardless of the challenges faced so far in regards to pyrethroids, there have successful examples in the literature employing SPME for the determination of pyrethroids in food samples [113,114,141,203,237,238]. Common to all of those work is the employment of a solvent extraction step prior to SPME.

Based on the aforementioned observation, some experiments were performed to evaluate the improvement obtained by solvent extraction to the overall SPME extraction efficiency of pyrethoids from grapes. Taking advantage of the well-stablished QueChERS procedure, a pre-extraction with acetonitrile was implemented based on the solvent extraction step method of QuEChERS. For this, 100 g of grape pulp was spiked with the pesticide mix and allowed to

equilibrate under agitation for 1 h. Subsequently, 5 g of fortified grape pulp was weighed into a 15-mL centrifuge tube and fortified with the pesticides mix. After allowing 1h to elapse for analyte/matrix binding to occur, 0.5 g of sodium acetate and 5 mL of acetonitrile (1% acetic acid) were added. The mixture was vortexed well, and then centrifuged for 5 min at 4000 rpm. Four mililiters of the supernatant were transferred to a 10-mL vial, and 4 mL of DI water was added. The final pH of the SPME-ready extract was 4.88.

An observation worth mentioning is that when using the solvent extraction prior to SPME, the fiber coating became quite coloured by the matrix pigments co-extracted by acetonitrile. Although dilution with water helped to minimize this effect, the coating became quite yellowish, and a considerable increase in the number of peaks referent of endogenous matrix compounds could be observed in the chromatogram.

Further experiments investigated the extraction time, and extraction time profiles (5, 15, 30, 45, and 60 min) were obtained at 55 ºC and 30 ºC. As seen in Figure 6-29, at higher temperatures, the K_{fs} decreased, and smaller amounts were extracted at equilibrium; however, this translated into shorter equilibration times. For this reason, in order to take advantage of equilibrium extraction, the experiments were continued with the previous settings of 30 min extraction at 55 ºC.

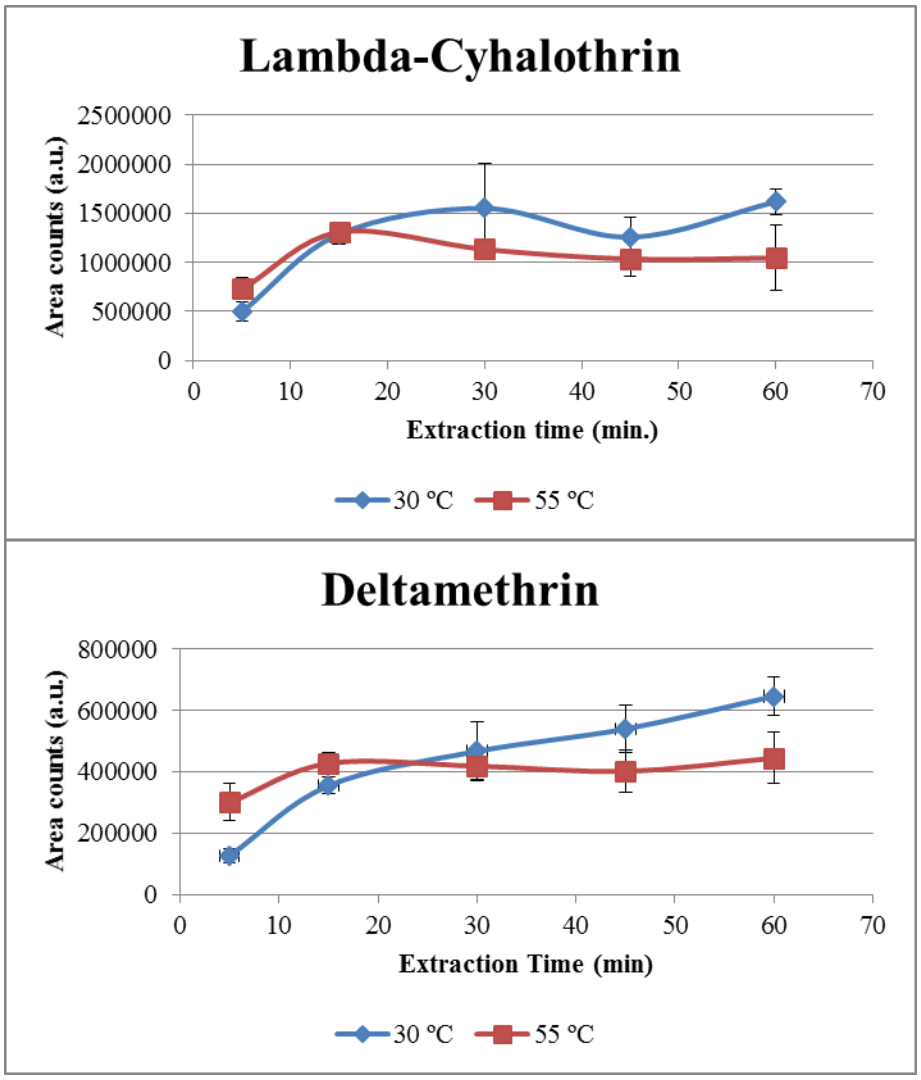


Figure 6-29 Extraction time profiles obtained for λ-Cyhalothrin and Deltamethrin at 30 ºC and 55 ºC (n=3, each point).

Next, a matrix-matched calibration curve was constructed at levels 1, 5, 10, 25, 50, 100, 250, 500, and 1000 ng/g. For all pyrethroids, the results obtained with this modified method were very satisfactory in terms of linearity, expressed as R^2, as well as in terms of LOQ obtained.

While a high content of organic solvent may cause negative effects for the least hydrophobic analytes, the positive effects upon the extraction efficiency of more hydrophobic pyrethroids is clearly depicted in Figure 6-30 to Figure *6-33*. Not only was the method linearity considerably improved, but a considerable decrease in the lowest calibration level to be precisely measured (RSD < 20%) was observed. For instance, the lowest calibration level for fenvalerate obtained with the previous SPME method was 50 ng/g, whereas the solvent/SPME method precisely reached 5 ng/g, meeting the MRL requirement of 20 ng/g. The results have been plotted as a function of the instrumental response (area counts), since the internal standards could not be detected, or had responses too low to be used reliably. From these findings, it can be concluded that it would be quite important to use a deuterated pyrethroid compound as an internal standard.

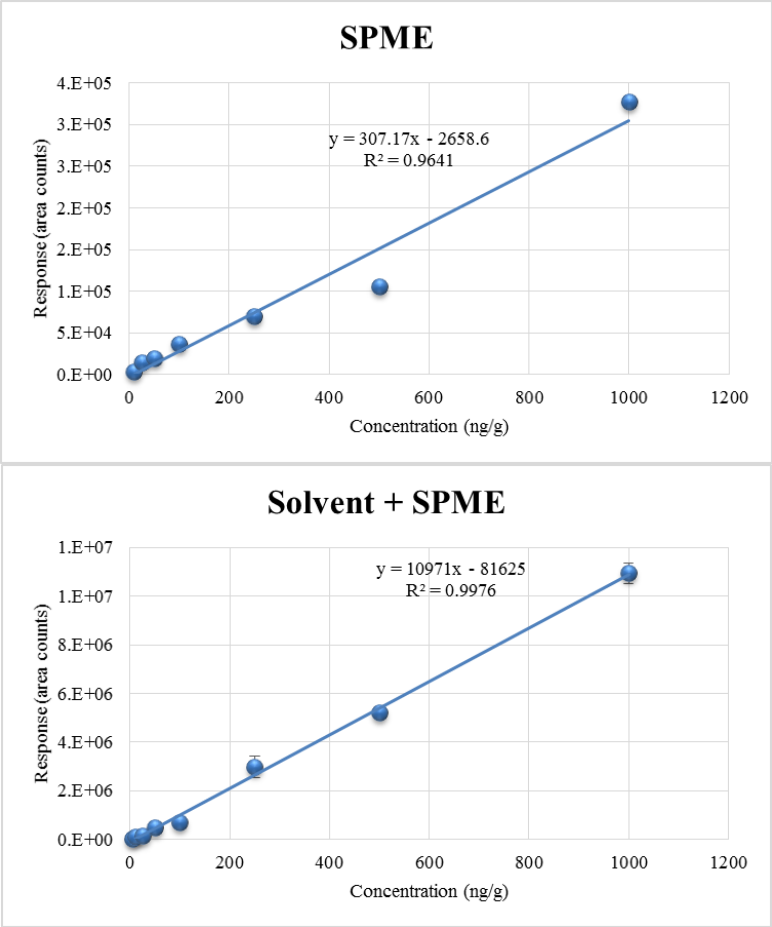


Figure 6-30 Matrix-matched calibration curve obtained for Cyhalothrin, comparison of the optimized SPME method and the pre-solvent extraction method.

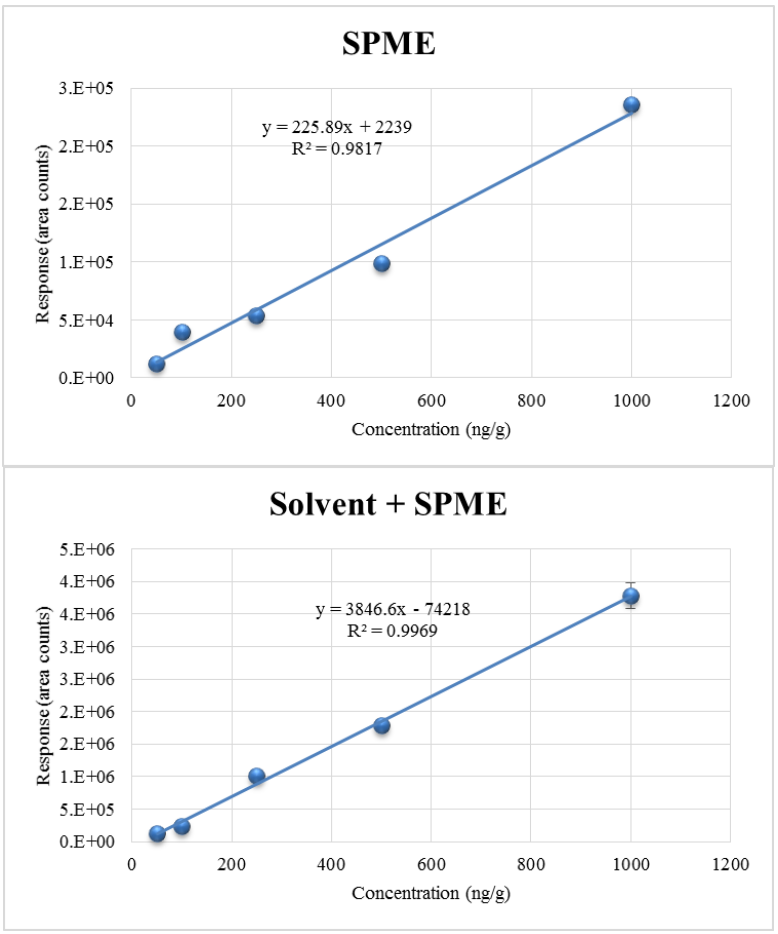


Figure 6-31 Matrix-matched calibration curve obtained for Cyfluthin, comparison of the optimized SPME method and the pre-solvent extraction method.

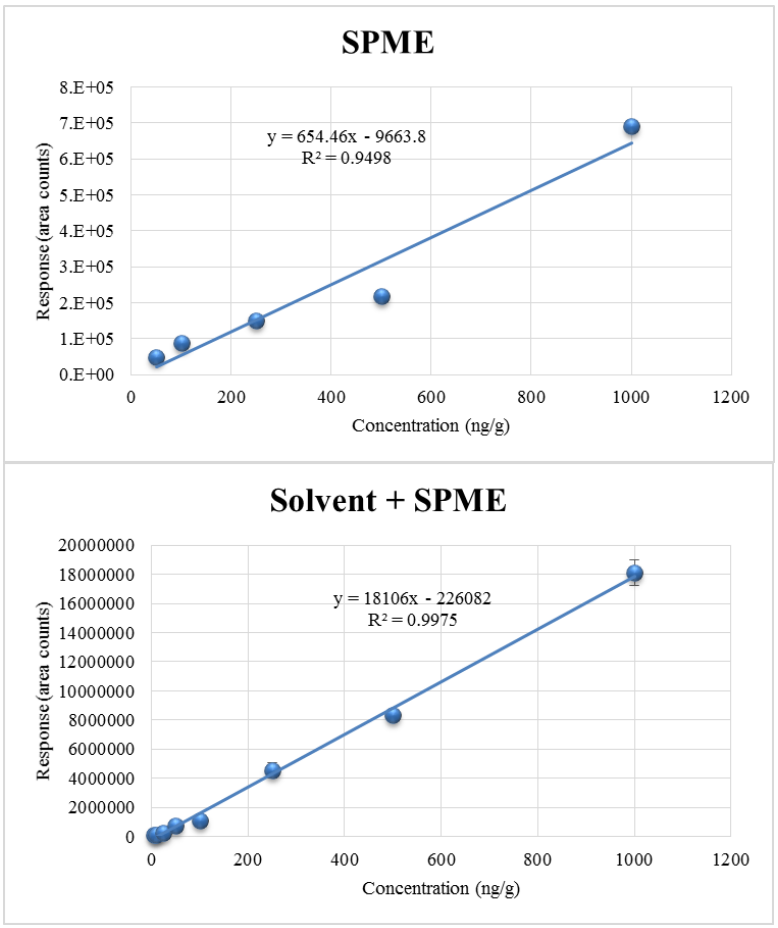


Figure 6-32 Matrix-matched calibration curve obtained for Es/Fenvalerate, comparison of the optimized SPME method and the pre-solvent extraction method.

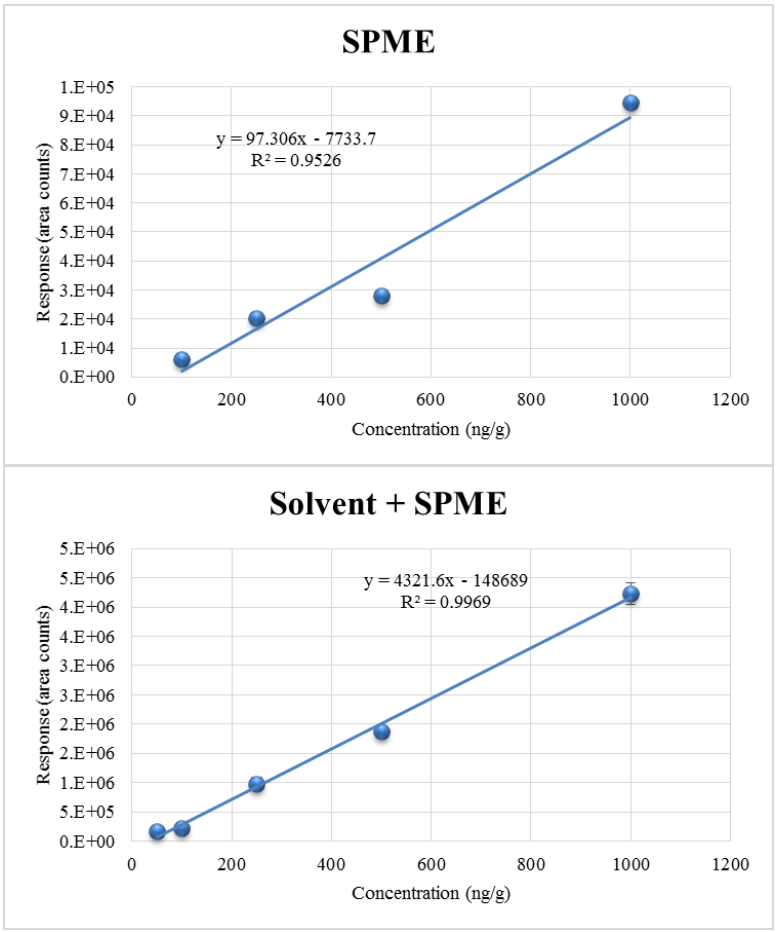


Figure 6-33 Matrix-matched calibration curve obtained for Deltamethrin, comparison of the optimized SPME method and the pre-solvent extraction method.

In short, this approach uses a QuEChERS solvent extraction in conjunction with SPME to meet regulatory[233] limits of quantitation for determination of these pyrethroids in grapes. Therefore, this approach can be considered a QuEChERS/SPME method. In the original QuEChERS procedure for grapes analysis, the acetonitrile extract is submitted to a d-SPE cleanup to remove organic acids, excess water, and other interfering components. However,

this cleanup step does not provide any additional concentration factor, making it difficult to obtain detection limits meeting the current MRLs unless the final extract is submitted to solvent exchange or large volume injection (which requires a PTV injector). The procedure described herein uses SPME as a combined cleanup and concentration step, thus, providing a substantial concentration factor to easily achieve the regulatory limits of quantitation.

Despite the fact that this assessment is only a preliminary investigation rather than a complete evaluation of method performance as per analytical figures of merit, it can be concluded that pre-extraction with acetonitrile and dilution with water prior to SPME (30 min, 55 ºC) yielded high sensitivity and compensated for the effect of adsorption to the glass walls of the extraction vial.

6.4 Summary

In this chapter, a multiresidue DI-SPME-GC-ToFMS method for determination of pesticides in grapes employing a PDMS-modified PDMS/DVB coating was developed utilizing multivariate approaches for optimization of the most important factors affecting SPME performance, thus assuring the acquisition of reliable data.

Furthermore, a comprehensive investigation of appropriate internal standards using a bottom-up approach led to the satisfactory selection of five suitable compounds that adequately covered a range of 40 pesticides pertaining to various classes. Most importantly, it has been determined that using only one pair of internal standards (Parathion-d_{10} or TPP + Quintozene or α-HCH-d_6) is sufficient to ensure reliable correction not only for instrumental drifts, but also for variations that could occur when dealing with natural matrices. Moreover, the choice of TPP/Quintozene as internal standards would render significant economic savings, since these compounds are much less expensive than the isotopically labelled ones.

The validated method yielded good accuracy, precision and sensitivity, and has been successfully applied to the analysis of commercial samples. Taking into account the method's performance by using an environmentally friendly and automated technique, the proposed method is considered appropriate for the determination of 40 pesticides, comprising 21 different classes, in grapes, with a

performance similar or superior to exhaustive extraction methods based on solvent extraction.

Regarding the limitations of the proposed method, the DI-SPME method did not meet a satisfactory performance towards more polar pesticides (e.g., acephate, omethoate, dimethoate) due to the low affinity of these compounds towards the fiber coating in conjunction with their impaired GC responses. Another limitation of this method was its inability to provide reliable results for highly hydrophobic pesticides, such as pyrethroids, which are prone to adsorption onto vial walls. In this case, a solvent pre-extraction step should be incorporated in order to avoid losses due to the interaction of these compounds with labware.

Despite the challenges and limitations encountered by this method, the practical aspects of the PDMS-modified coating demonstrated here create new opportunities for SPME applied in food analysis.

7 Summary and Future Directions

7.1 Summary

Since its introduction in 1990, the scope of SPME applications has broadened immensely as a result of the production of new SPME devices, commercialization of more robust and stable assemblies, development of fully automated systems, development of inter-laboratory protocols, as well as development of new extraction phases. Given the rising interest in the development of more environmentally friendly, yet very sensitive methods in the area of food analysis, SPME can be a perfect fit for the development of such methods. Nonetheless, the drawbacks associated with commercially-available extraction phases in the context of insufficient matrix-compatibility still persist when such coatings are employed in the analysis of complex matrices such as food. This limitation propels research in the field of design and development of novel extractive phases focusing on high quality, efficiency, and long-term reusability. This thesis addresses the necessity for the development of a suitable SPME coating for analysis of complex food samples in direct immersion mode. The development of a matrix-compatible SPME coating for GC-based analysis of food matrices was conducted in order to achieve a robust coating of improved longevity. The matrix-compatible coating was realized by incorporating a thin PDMS layer onto the surface of a solid SPME coating (PDMS/DVB). The resulting coating was evaluated for its capacity to withstand tens of extraction by direct immersion into food matrices, while maintaining appropriate extraction efficiency. In addition to the matrix-compatibility properties of this coating shown

in Chapters 2 and 5, the results presented in Chapters 3 and 6 clearly demonstrate the potential of using the PDMS-modified fiber for implementation of quantitative DI-SPME methods.

An imperative requirement in modern methods for food analysis is the capability of a given method to simultaneously determine multiple analytes with a broad range of polarities. From the results obtained in Chapters 4, 5, and 6, it can be concluded that the PDMS-modified fiber clearly demonstrates potential in determining analytes of various polarities in a single method. It has been shown in Chapter 4 that a PDMS-modified fiber exhibits excellent extraction efficiency, similar to the original PDMS/DVB, for most analytes, except that for polar analytes, the kinetic uptake rate is affected by the hydrophobic PDMS layer. Therefore, when employing the PDMS-modified for analysis of polar analytes, longer extraction times might be needed to achieve desired sensitivity.

Ideally, a multiresidue method for pesticides analysis in food should be rapid and easy to perform, automated, requiring a minimum amount of solvents, providing a certain degree of selectivity and covering a wide scope of analytes. The realization of a fully validated DI-SPME method for multiresidue pesticides analysis in grapes presented in Chapter 6 successfully addresses most of the requirements aforementioned. In addition to its easy implementation and cost/analysis, it is worth highlighting the excellent performance obtained by the proposed method for pesticides such as chlorothalonil, dicofol and folpet, which are considered the weak link in QuEChERS-based multiresidue methods.

Nonetheless, there are inherent challenges in the proposed method, especially if the method is intended for monitoring more polar pesticides (log P < 1). This limitation is intrinsic to the principles commanding SPME extraction. Increasing the polarity of the sorbent so that it would have increased affinity for more polar analytes would also increase its affinity for the sample matrix, which may lead to competitive adsorption issues. Another challenge is the extraction of highly hydrophobic compounds that tend to adsorb onto glassware. However, as proposed in Chapter 6, a possible approach to solve this issue would be the incorporation of a solvent extraction step prior to SPME analysis.

7.2 Future directions

Despite of the challenges and limitations encountered, the practical aspects of the PDMS-modified coating demonstrated in this thesis create new opportunities for SPME applied in food analysis. Other researchers are currently evaluating the PDMS-modified coating developed in this thesis in order to assure its compatibility towards diverse challenging matrices, such as spinach, avocado and soymilk.

The research presented herein supports the use of SPME for GC-based multiresidue pesticide determinations in food as a sensitive and cost-effective method. In future, this protocol can also be evaluated for the analysis of different food matrices posing different challenges, such as spinach (high chlorophyll content), avocado (high fat content), legumes (low water content), and dairy products (high fat and protein content). Furthermore, it would be very important

to extend the range of pesticides being analyzed in food by SPME. For this purpose, complementary platforms, namely SPME-GC and SPME-LC, should be implemented. Recent developments of SPME coatings for LC applications, with improved hydrophilic properties for better extraction of polar compounds are very promising towards such complementary platform[127,239]. Moreover, high-throughput simultaneous preparation of 96 samples utilizing the 96-blade SPME system can reduce time significantly without sacrificing precision or sensitivity.

Some of the research outcomes presented within this thesis are relevant not only for the use of SPME in the analysis of pesticides in food, but other type of analyses that required the simultaneous determination of a broad range of analytes. For instance, in a recent study of apples metabolomics comparing HS and DI SPME coupled to GCxGC[240], it has been found that DI-SPME resulted in a lower degree of discrimination toward high molecular weight and more polar metabolites, thus, attaining a more complete metabolite coverage. These new results will unquestionably benefit from advancements in developing matrix-compatible coatings and encourage the future facilitation of DI-SPME ex vivo and in vivo approaches in advanced metabolomics studies in fruit biology.

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Title: Optimization of Fiber Coating Structure Enables Direct Immersion Solid Phase Microextraction and High-Throughput Determination of Complex Samples

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