High Throughput Analysis for On-site Sampling

by

German Augusto Gómez-Ríos

A thesis

presented to the University of Waterloo

in fulfillment of the

thesis requirement for the degree of

Master of Science

in

Chemistry

Waterloo, Ontario, Canada, 2012

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

Until recently, multiple SPME fibres could not be automatically evaluated in a single sequence without manual intervention. This drawback had been a critical issue until recently, particularly during the analysis of numerous on-site samples. Recently, GERSTEL® has developed and commercialized a Multi-Fibre Exchanger (MFX) system designed to overcome this drawback. In this research, a critical evaluation of the MFX performance in terms of storage stability and long term operation is presented. It was established in the course of our research that the MFX can operate continuously and precisely for over 200 extraction/injection cycles. However, when the effect of residence time of commercial fibres on the MFX tray was evaluated, the results have shown that amongst the evaluated fibre coatings, carboxen/polydimethylsiloxane (CAR/PDMS) was the only coating capable of efficient storage on the MFX tray for up to 24 hours after field sampling without suffering significant loss of analytes. Additionally, the MFX system capability for high-throughput analysis was demonstrated by the unattended desorption of multiple fibres after on-site sampling of two different systems, indoor air and biogenic emissions. Subsequently, a protocol based on a new, fast, reproducible, reusable and completely automated method that enables quick assessment of SPME coatings was developed. The protocol consists of an innovative in-vial standard generator containing vacuum pump oil doped with McReynolds probes and subsequently mixed with a polystyrene-divinylbenzene resin. According to our results, the protocol has proven to be a useful tool for the quick assessment of inter-fibre reproducibility prior to their application in on-site analysis. The implications of such protocols include, but are not limited to: time-saving,

assurance of reliable and reproducible data, and a dependable guide for novice users of the technique.

Finally, an innovative, reusable and readily deployable pen-like diffusive sampler for needle traps (PDS-NT) is proposed. Results have shown that the new PDS-NT is effective for air analysis of benzene, toluene, and o-xylene (BTX). In addition, no statistically significant effects of pen geometry on the uptake of analytes were found.

Acknowledgements

I would like to take this opportunity to express my appreciation to all who have supported and assisted me in the completion of my project. First of all, I would like to thank my supervisor, Dr. Janusz Pawliszyn, for offering me the opportunity to work on this project. Second, I would like to thank my advisory committee members, Dr. Michael Chong, Dr. Mario Gauthier and Dr. Tadeusz Górecki for their valuable advice during my work and critical evaluation of my research.

Second, I would like to express my appreciation and admiration to my colleagues for their support during my work period, especially to Nathaly Reyes, Erica Silva, Erasmus Cudjoe, Ruifen Jiang and Emanuela Gionfriddo who are not only excellent scientists but also great friends that make this step of my life easier. Thanks for your valuable advice throughout my work and their important input to my thesis.

Third, I would like to express my gratitude to the Science Shop of the University of Waterloo, especially to Andrew Dube, Harmen Vander Heide, Krunomir Dvorski, Hiruy Haile and Michael Lang. Thanks for providing opportune help when I was in need.

Finally, I would like also to thank other colleagues at University of Waterloo for the selfless sharing of their knowledge and life experience, particularly to Walter Zhang, Anabella Bai, Oana Goli, Heide Flatt, Dr. David Donkor, Dr. Ezel Boyaci, Dr. Rodrigo Simoes, Dr. Angel Rodriguez, Dr. Sanja Risticevic and Dr. Paul Togunde.

Dedication

I dedicate this thesis to my beloved parents, my sister, my friends and my future wife Nathaly for their love, understanding and support.

Always keep your dreams alive, because the best things in life take time.

Table of contents

List of Tables

List of Figures

List of Abbreviations

BP	Boiling Point
BTEX	Benzene, Toluene, Ethylbenzene, Xylene
BTX	Benzene, Toluene, Xylene
BVOCs	Biogenic Volatile Organic Compounds
CAR	Carboxen/polydimethylsiloxane
CAS	Chemical Abstracts Service
DFH	Diffusive Fibre Holder
DVB	Divinylbenzene
EPA	US Environmental Protection Agency
FFA	Fast Fit Fibre Assembly
FID	Flame Ionization Detector
GC	Gas Chromatography
GC/MS	Gas Chromatography/Mass Spectrometry
GC/qMS	Gas Chromatograph/quadrupole Mass Spectrometer
GC/TMS	Gas Chromatograph/Toroidal Mass Spectrometer
HPLC	High Performance Liquid Chromatography
Log P	Logarithm of partition coefficient
MF	Molecular Formula
MFX	Multi-Fibre Exchanger
MS	Mass Spectrometry/Mass Spectrometer
MW	Molecular Weight

NIOSH	National Institute for Occupational Safety and Health
NT	Needle trap
OSHA	Occupational Safety and Health Act
PDAS	Portable Diffusive Air Sampler
PDMS	Polydimethylsiloxane
PDS	Pen-like diffusive sampler
ppb	Parts per billion
ppt	Parts per trillion
PTFE	Polytetrafluoroethylene (Teflon®)
Q mass	Quantitation mass
RH	Relative Humidity
SDVB	Styrene-divinylbenzene
SPME	Solid Phase Microextraction
t_R	Retention time
TWA	Time Weighted Average
VOCs	Volatile Organic Compounds
XAD	Trade mark for styrene-divinylbenzene particles
μ	Dipole moment

Chapter 1 – Introduction

1.1 The relevance of the sampling step

The analytical process typically consists of several steps such as sampling, sample preparation, separation, quantitation, statistical evaluation, and decision making.[1] The sampling step is critical in order to obtain correct and informative results; in this step, the analyst should choose a technique that acquires samples in accurate amounts, as well as decide optimal times and location to conduct sampling, so as to properly characterize the problem under study. [1] Ideally, all samples should be analyzed on-site to avoid losing sample integrity. [1] However, in most cases only preliminary assessments are taken on-site, in order to determine the number of samples and the location needed for further analysis. In cases where on-site analysis is not possible, simple sampling/sample preparation techniques for field applications are required. [2,3,4,5]

1.2 On-site sampling

Sampler devices for field sampling should be simple and reliable, since sampling sites are generally located far from the laboratory. Consequently, the device requires easy method deployment, one which allows technicians or operators with limited knowledge of the particulars of the mechanism to easily operate the sampler. Moreover, the production of the device should be uncomplicated and inexpensive. Additionally, during its transportation and storage, contamination, decomposition, and/or loss of the analytes should be negligible.[6,7] Finally, the device should be sensitive to the substances under study, insensitive to interfering matrix components, and not require in-laboratory sample pre-treatment. [8]

Several environmental and physicochemical parameters, such as humidity, temperature, and air velocity can affect the analytical results obtained using passive sampling devices; [6,8,9,10]

therefore, exposure chambers are used to calibrate and test samplers under simulated field conditions in order to estimate the effect of these parameters on their performance. Furthermore, analytical recovery, storage stability, sampling capacity, uptake rate, reverse diffusion, accuracy, and precision of the field sampler device must be evaluated and validated against conventional methods. [11,12] Since most conventional methods require costly equipment, considerable sampling expertise, and complicated cleaning and extraction procedures, Solid Phase Microextraction (SPME) and Needle Trap (NT) devices have shown to be suitable techniques to address this concern. [4, 13]

1.3 SPME

SPME is a green sampling/sample preparation technique comprehensively described in the literature. [1,13,14] SPME has been widely accepted in different fields of analytical chemistry, due to its easy handling, minimization of organic solvent consumption and short sample preparation. [15-23] The achievements of SPME during the last decades are the result of its multiple advantages; [15] first, the variety of fibres commercially available covers a comprehensive range of analytes (from VOCs to SVOCs). Secondly, the elimination of matrix interference by sampling directly from the liquid sample or from the headspace improves the results. Finally, but unquestionably the most important feature: SPME is easily automated.

The SPME device consists of a fibre assembly that is sheltered in a fibre holder. Figure 1.1 shows a representation of the internal view of a manual fibre assembly, as well as the recently commercialized fast fit fibre assembly (FFA). This assembly consists of a piercing needle with a solid support (core) inside that is coated with a thin layer of a suitable polymeric stationary

phase, which enables extraction and enrichment of the analytes by concentrating them during absorption and/or adsorption processes from the sample matrix.

Since SPME can extract a wide variety of analytes, the selection of an appropriate coating is a critical step in method development. In brief, the selection is based on four prominent criteria: 1) analyte polarity; 2) analyte concentration and range; 3) molecular weight (MW), shape and size of analyte, and 4) complexity of the sample.[1] Table 1.1 summarizes the main features of commercial coatings used in this research.

1.3.1 SPME equilibrium based calibration approach

Several calibration approaches have been developed for SPME; equilibrium extraction is the most frequently used method. After a certain amount of extraction time, concentration equilibrium is established between the sample matrix and the extraction phase; consequently, exposing the fibre for longer periods does not result in the accumulation of more analytes. Under equilibrium conditions, the number of moles of analyte extracted (n) by the coating at equilibrium can be expressed by Equation 1.1

$$n = \frac{K_{fs} V_e V_s C_o}{K_{fs} V_e + V_s} \qquad \text{Equation 1.1}$$

where V_e is the fibre-coating volume, V_s the sample volume, C_o the initial concentration of a given analyte in the sample and K_{fs} is the extracting phase/sample matrix distribution constant. Moreover, Equation 1 indicates that the amount of analyte extracted in the coating (n) is linearly proportional to the analyte concentration in the sample (C_o), which is the analytical basis for quantification using SPME. Additionally, when the sample volume is very large, $V_s >> K_{fs}V_e$, Equation 1.1 can be simplified to

$$n = K_{fs} V_e C_o \qquad \text{Equation 1.2}$$

Therefore, the amount of analyte extracted will correspond directly to its concentration in the matrix, independently on the sample volume, which points the advantage of SPME for field applications.

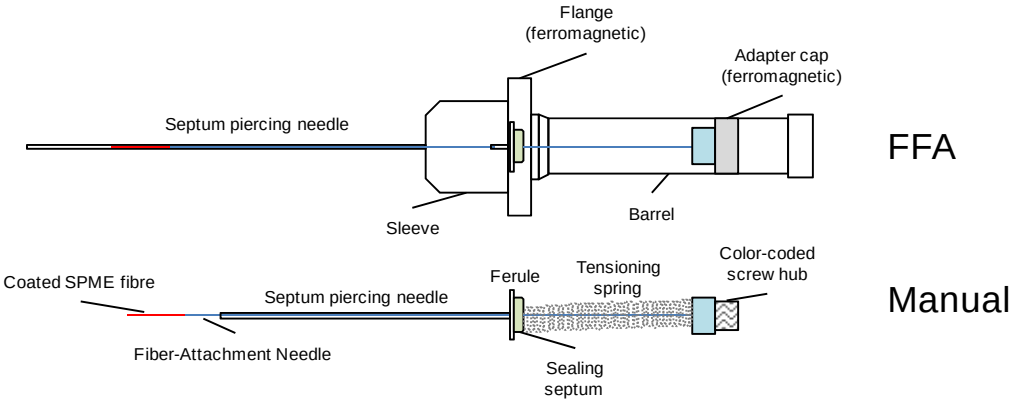


Figure 1.1 Schematic view of SPME manual fibre assembly and a fast fit fibre assembly (FFA)[1,24]

Table 1.1 Commercial SPME fibre coatings used in this research.[1,25] PDMS, polydimethylsiloxane; PDMS/DVB, polydimethylsiloxane/divinylbenzene; CAR/PDMS, carboxen/polydimethylsiloxane; DVB/CAR/PDMS, divinylbenzene/carboxen/polydimethylsiloxane.

SPME fibre coating	Coating thickness (μm)	Desorption temperature range (ºC)	Conditioning procedure	Polarity	Mechanism of extraction	MW range	Analytical application
PDMS	100	200-280	30 min, 250 ºC	nonpolar	absorption	60-275	nonpolar analytes, volatiles
PDMS/DVB	65	200-270	30 min, 250 ºC	bi-polar	adsorption	50-300	polar volatiles, amines, nitroaromatic compounds
CAR/PDMS	85	250-310	60 min, 300 ºC	bi-polar	adsorption	30-225	gases and volatiles
DVB/CAR/PDMS	50/30	230-270	60 min, 270 ºC	bi-polar	adsorption	40-275	volatiles and semivolatiles, broad range of polarities

It is important to emphasize that this equation is strictly valid for partitioning equilibrium involving liquid polymeric phases. In the case of solid sorbents the method is analogous only for low analyte concentrations, since at high concentrations competitive interference can displace the target analyte from the surface of the sorbent.[1]

1.3.2 SPME diffusion based calibration approach

Significant research with SPME has focused on applications for air sampling and analysis. As aforementioned, the theory behind the equilibrium and non-equilibrium extractions for liquid coatings is well understood and described in the literature [1]. Despite the sensitivity of solid coatings for the extraction of VOCs being higher when compared to PDMS, competitive adsorption and displacement effects make mass calibration and quantification particularly challenging. In order to solve this issue, Koziel *et al.* proposed an approach that relies on diffusion-controlled extraction [26]. This method has two critical restrictions: short sampling times and non-equilibrium conditions. Following these rules, the effects of competitive adsorption are diminished, and the coating can be calibrated on the initial linear extraction region. If the concentration of the analyte is assumed to be constant for a very short sampling time, the concentration can be estimated from the following equation

$$C_g = \frac{n \ln((b+\delta)/b)}{2\pi D_g L t} \qquad \text{Equation 1.3}$$

where n is the mass of extracted analyte in nanograms over sampling time t; D_g is the gas-phase molecular diffusion coefficient (cm^2/s); b is the outside radius of the fibre coating (cm); L is the length of the coated rod (cm); δ is the thickness of the boundary layer surrounding the fibre coating (cm); t is the sampling time (s), and C_g is analyte concentration in the bulk air (ng/mL). L and b are constant for each type of fibre coating, and n can be estimated from the detector

response. The diffusion coefficients of the analytes can be estimated from physicochemical properties by the method proposed by Fuller, Schettler, and Giddings (FSG) [27]

$$D_g = \frac{0.001 \times T^{1.75}\sqrt{1/M_{air} + 1/M_{VOC}}}{p\,[(\sum V_{air})^{1/3} + (\sum V_{VOC})^{1/3}]^2} \qquad \text{Equation 1.4}$$

where D_g is expressed in cm^2/s, T is the absolute temperature (K), M_{air} and M_{VOC} are molecular weights for air and the VOC of interest, p is the absolute pressure (atm), and V_{air} and V_{VOC} are the molar volumes of air and the VOC of interest (cm^3/mol). Additionally, based on Koziel and collaborators,[26] the effective thickness of the boundary layer can be estimated using the following equation

$$\delta = 9.52\,(b/Re^{0.62}Sc^{0.38}) \qquad \text{Equation 1.5}$$

where Re is the Reynolds number, $Re = 2ub/v$, where u is the linear air velocity (cm/s), v is the kinematic viscosity for air (cm^2/s), Sc is the Schmidt number, and $Sc = v/D_g$. Particular attention should be paid to control convection conditions during extraction in order to maintain a constant boundary layer, and hence avoid variations in the extracted amounts of analyte. In addition, by using forced air, the sensitivity of the solid coatings is enhanced. In order to facilitate control of convection conditions, Augusto *et al.* designed a Portable Dynamic Air Sampler (PDAS) for SPME.[28] Figure 1.2 presents the schematic of the device built using a hair-dryer.[28] This instrument was modified to revert the air flow direction and to disable the internal heating coil. When compared to standard methodologies, the authors demonstrated that a 30 s sampling time using PDAS-SPME allowed measurement of VOC concentrations that were not detected by the NIOSH standard method, even after several hours of extraction using expensive and non-reusable materials. [28]

It should be also emphasized that short sampling times are important so that the effect of relative humidity on the adsorption of VOCs on the solid coatings is minimized. Indeed, to obtain accurate and precise concentrations, the sampling time should be properly measured. One interesting advantage of this method is that external calibration is not needed since sampling conditions and constants are known.[26,28] Also, since the method depends on the dimensional parameters of the fibres, its dimensions, and integrity should be checked prior to sampling.

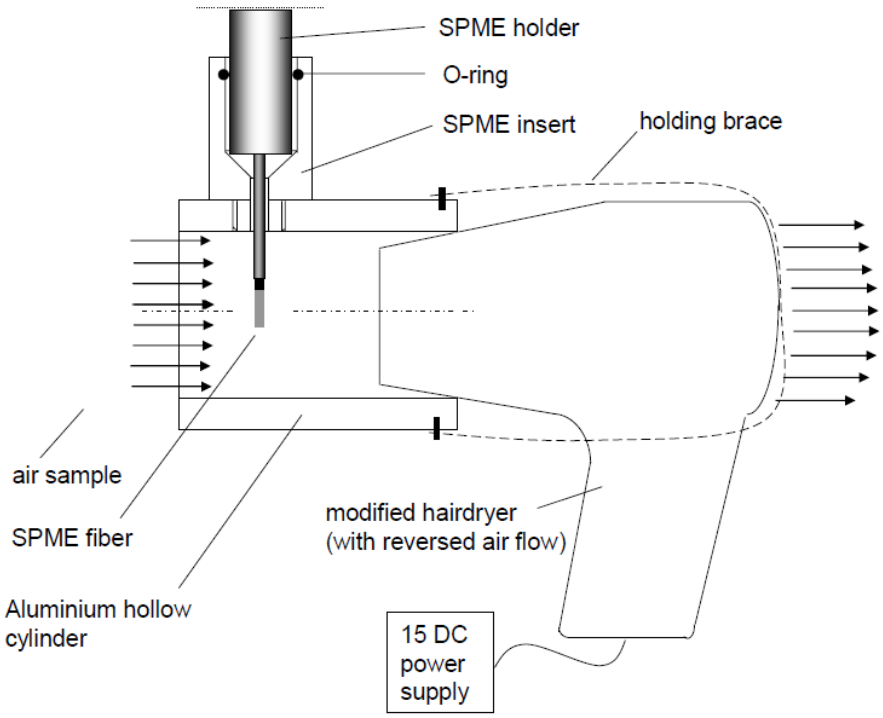


Figure 1.2 Schematic of the portable dynamic air sampling device for SPME developed by Augusto *et al.* [1,28]

1.4 Needle trap (NT)

A needle trap (NT) is an extraction trap that contains a sorbent inside of a needle. Indeed, a NT combines sampling, sample preparation, and sample introduction as SPME does. However, NT, as an active sampler, is an exhaustive technique that allows particle trapping. Hence, as

shown in Equation 1.6, total concentration of the analyte could be easily obtained by controlling the sampled volume (v) and determining the amount extracted (n) in an analytical instrument. [4,5]

$$n = C_o v \qquad \text{Equation 1.6}$$

Several factors, such as the pore size and shape, surface area, and particle size, can affect the ability of the analyte to access and interact with the surface of the adsorbent; therefore, these parameters must be contemplated and controlled.[5] Moreover, because of the special shape of the needle, sorbents used for NT must have the appropriate physical characteristics in size, hardness, shape (spherical), and mechanical and thermal stability.

The first practical and successful application of NT, suitable for automation and on-site application, was a 23 gauge stainless steel needle, 40 mm long, containing 5 mm of quartz wool packing.[29,30] Since then, several groups have worked on the development of sorbent-packed needles or similar devices.[4] Some of the sorbents that have been used for the analysis of VOCs are Porapak Q™ and Carbopack X™.[4]

According to previous developments,[5,29,31] the design of a NT must guarantee several factors: exhaustive extraction (active sampling), negligible breakthrough during sampling, and efficient desorption. Indeed, desorption assisted by inert gas flushing of the sorbent bed seems to be the most suitable method to achieve efficient desorption of the analytes. [4]

Figure 1.3 shows a schematic of a NT in which the sorbent was packed near the blunt tip of the needle. This NT was designed with a side-hole (I.D. 0.4064 cm) located 3 cm from the tip of the needle. This design allows the carrier gas to pass through the sorbent and aids the delivery of desorbed analytes into the GC column. Carry-over and memory effects were not detected using this design.[5,31] The procedure used to prepare this type of NT is described elsewhere. [4,5,31] The

most engaging characteristic of a NTD, demonstrated by Koziel and co-workers,[29] is that it is able to extract simultaneously both free and particle bound molecules.

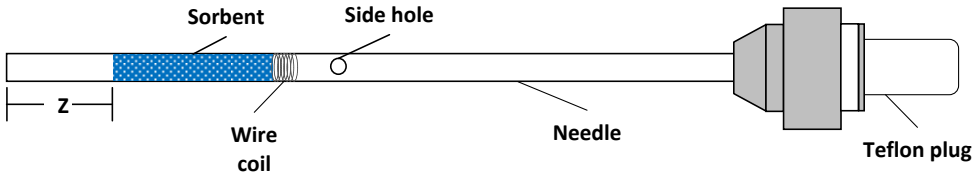


Figure 1.3 Schematic view of the side-hole NTD with inert spring to retain sorbent[4]

1.5 Passive sampling with SPME and NT

The basic principle of passive sampling is the free circulation of analyte molecules from the sampled medium to the sampling device, as a result of the difference in chemical potential between them. [6] Permeation through a membrane or diffusion through a barrier are the common mechanisms used by passive sampling devices. [7]

TWA sampling using SPME can be performed by withdrawing the fibre a defined distance Z inside the needle from the opening of fixed area A; thus, a diminutive tube-type diffusive sampler is created. In the case of NT, if a strong sorbent is packed at a defined distance Z from the needle opening of fixed area A, the device could be recognized as a very simple array for passive sampling as well.[4]

As shown in Figure 1.4, during the process of diffusion there exists a linear concentration gradient across Z. Therefore, by using Fick's law of diffusion, it is possible to determine the amount of analyte loaded on the fibre coating, *n*, during the sampling time, *t*. [2,32-34] The equations that describe the TWA analyte uptake in SPME and NT are summarized in Table 1.2. [1,6,7,11,14]

Three main conjectures should be achieved during the passive sampling with SPME and NT. First, the device should respond proportionally to changing analyte concentration at the face of the needle.[2,33] Secondly, the concentration of the gas system must be equal to the analyte concentration at the face of the opening.[2,33] And third, the sorbent should be a zero sink for the target analytes.[2,33]

An important feature of any passive sampler is whether it has the ability to integrate high peak concentrations. This function is directly related to the response time of the sampler. As shown in Table 1.2, according to Fick's law the response time is proportional to the square of the diffusion path length and inversely proportional to the diffusion coefficient. Using a Z of 0.5 cm for a compound such as hexane, a response time of 2 seconds can be achieved.[2] This short response time of SPME and NT in passive mode enables the integration of fluctuating concentration profiles and assures that the sample acquired represents an accurate TWA concentration.[1,2]

It has been proved by several authors that SPME and NT, different from tube type-samplers,[2,11] can be used for passive sampling without considering face velocity problems due to the extremely small inner diameter of the needle.[1,35-39] The fact that air velocity does not affect the measured concentration implies that there is no external resistance to mass transfer, even at very low air velocities. [1] Consequently, all the resistance to analyte transport is contained within the stagnant air layer inside the small tubing, and the concentration at the face of the sorbent is equal to the bulk analyte concentration. [2]

Studies have shown that the amount of analyte loaded on the fibre or sorbent during passive sampling should be smaller than 10% of the equilibrium amount in order to avoid altering the

mass-rate uptake of the device.[2,4,33] Also, the analyte concentration in the vicinity of the sorbent bed or coating comes close to zero ("zero" sink) only for high extraction phase/matrix distribution constants (K_{es}). Therefore, the best approach for volatile compounds is to use coatings/sorbents characterized by large K_{es}, such as Carboxen. [4]

In addition to the three pre-requisites of passive sampling with SPME and NT that were described before, passive sampler devices should be reliable under fluctuating environmental conditions such as humidity and temperature. Otherwise, corrections to the uptake rate of analytes must be applied.[2,9,11]

Regarding humidity effects, Chen and Pawliszyn,[2] using a CAR-PDMS 75 μm fibre (zero sink) to sample BTEX in passive sampling mode, found no remarkable effects on sampling rates when humidity was increased. This finding seems to be related to the hydrophobicity of Carboxen and the absence of competition between water and analyte molecules for active sites at their experimental conditions.[2] Similar results were reported by Gong *et al.* using Carboxen as a packed sorbent in a NTD to sample BTEX in passive mode.[5]

These findings are supported by other researchers that also concluded that humidity does not have a significant effect on the properties of the SPME/NT samplers.[37-40] On the other hand, according to some other authors,[35,36] it would appear that relative humidity changes coating properties as well as the occupied active adsorption sites, and consequently, fibre selectivity might be affected.

Based on these observations, it can be explained that sampling rates of compounds such as PEG, perchloroethylene, furfural and halothane were reduced when compared to the corresponding theoretical values.[35,41-43]

Table 1.2 Equations that describe passive sampling analyte uptake in SPME and NTD. n: mass of analyte loaded on the fibre or NTD during the sampling time t; Dg: diffusion coefficient of the target analyte; A: area of the cross-section of the diffusion barrier; Cs: gas-phase analyte concentration at the coating position (sorbent bed); C_F: concentration of the analyte at the needle opening; SR: sampling rate; $SR_{(Z)}$: sampling rate at the position Z. $SR_{(Z')}$: sampling rate at the position Z'; Dg: Diffusion coefficient at 298 K; D_T: Diffusion coefficient at a different temperature, T (K); T: temperature

Applications of Fick's Law	$n = \frac{D_g A}{Z} \Delta C_{(S-F)} t$
Sampling rate	$SR = D_g \frac{A}{Z}$
TWA concentration determination	$\overline{C_F} = \frac{nZ}{ADt}$
Sampling rate at different path	$SR_{(Z')} = SR_{(Z)} \frac{Z'}{Z}$
Sampler response time	$R = \frac{Z^2}{2D_g}$
Diffusion coefficient correction	$D_T = D_g \left(\frac{T}{298}\right)^{3/2}$

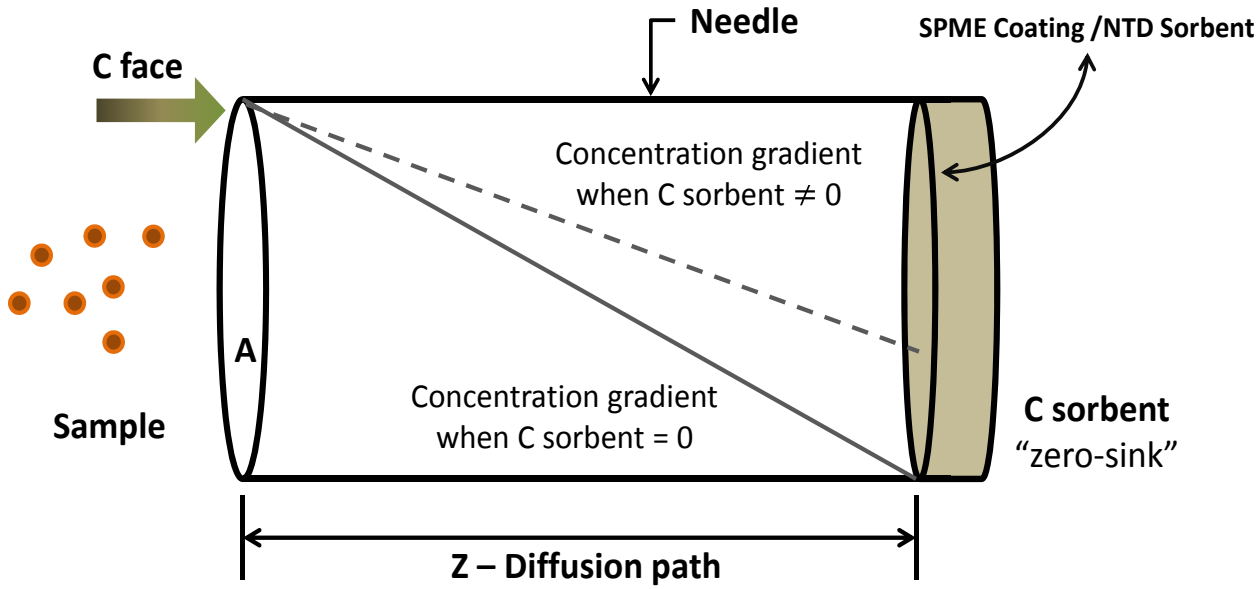


Figure 1.4 Concentration gradient of an analyte produced between the opening of the needle and position of the sorbent Z. Z: diffusion path; $C_{sorbent}$: Concentration near the sorbent interface; C_{face}: time dependent concentration of the analyte at the needle opening; A: area of the cross-section of the diffusion barrier.

Thus, it would seem that this behavior is not similar for all analytes, and that the effect of relative humidity on sampling rate is dependent on the volatility and polarity of a given analyte.[36,37]

The effect of temperature on passive sampling using SPME and NT has also been investigated. Chen *et al.* and Gong *et al.* found that there is an increase in the uptake of BTEX and n-alkanes that is proportional to temperature.[2,11] This is expected since the diffusion coefficient is a function of temperature ($T \alpha D_g^{1.75}$, see Equation 1.4). However, for heavier alkanes (C >11), it was observed that the uptake rates at the beginning of the sampling are higher than theoretical values, as a consequence of adsorption on the needle walls. Similar results were found by Shih *et al.* in the analysis of poly(ethylene glycol) ethers and by Zare *et al.* in the analysis of perchloroethylene.[36,39]

Several studies found that adsorption on the needle walls is not easily predictable, and seems to depend on the concentration at which the device is exposed.[2,37] In addition, at long exposure times, amounts of analytes collected on the sorbent are considerably higher than amounts adsorbed on needle walls, and consequently, under these conditions, the needle adsorption effect on uptake rates can be neglected. It has also been observed that if the sampling temperature increases, the adsorption of the compound on the needle diminishes, and the experimental value of the sampling rates is closer to the theoretical value.

Other authors, however, have suggested that the adsorption on the needle walls is not an issue only observed in relation to less volatile compounds. Chen and Hsiech reported that the experimental sampling rates of dichloromethane at very short sampling times were higher than rates obtained with long sampling exposures[37]. However, similarly to observations reported by

Chen and Pawliszyn, the values become constant as the sampling time increases.[37] In summary, in order to eliminate the effect of needle adsorption, Chen *et al.* proposed the use of deactivated needles for TWA samplers, such as Silicosteel-coated needles.[1,2,34]

SPME devices have been successfully used for TWA sampling of alkanes,[2,38,44] VOCs,[2] toluene,[3] volatile sulphur compounds (VSCs),[38] hydrocarbons and other analytes in air.[1] As an example, Martos and Pawliszyn (1999) have proven the usefulness of SPME-TWA sampling using on-fibre derivatization of formaldehyde.[33] The results were in good agreement with those obtained using the NIOSH Method 2541.[26,33]

Gong *et al.* have developed and validated a simple, easy to deploy and cost-effective NTD method for the TWA analysis of VOCs.[5] The evaluation showed that NTD packed with Carboxen 1000 is not only effective for air analysis of BTEX, but also has good storage stability for these compounds. Good agreement was observed between theoretical and experimental sampling rates, and results obtained using NTD active sampling, SPME, NIOSH method 1501 and NTD passive sampling compared well among them.[5]

As mentioned earlier, when developing a passive sampling method several environmental parameters might affect the uptake of the analytes. Figure 1.5 presents a modified protocol for the development of time-weighted average air sampling method with SPME or NT.[20,36] This procedure is an adaptation made by Zare *et al.* of the "protocol for solid-phase microextraction method development" established by Risticevic *et al.*[20] Detailed steps for the development of diffusive sampling methods are described elsewhere.[3,45]

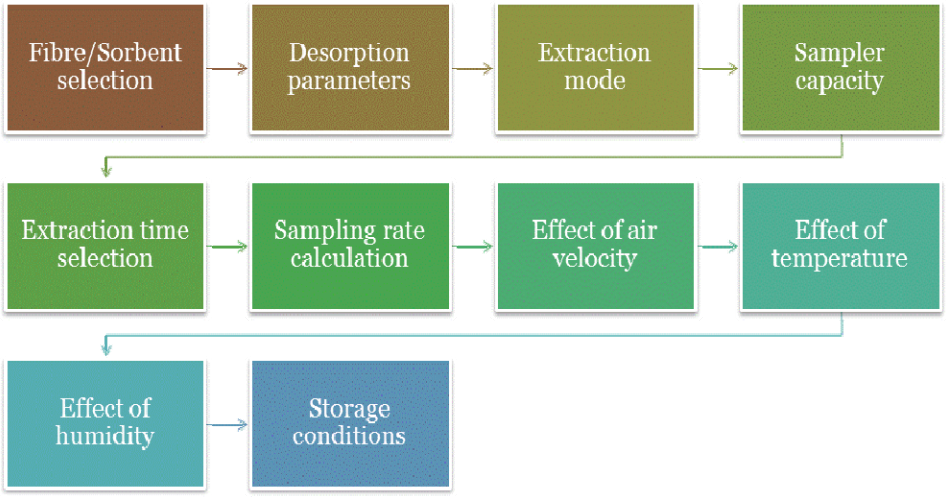


Figure 1.5 Typical parameters and steps that should be evaluated for development of a time-weighted average air sampling method with SPME or NT. [36]

Finally, it is important to highlight the flexibility SPME demonstrates in selecting different sampling times or concentrations ranges, since the fibre coating can be adjusted at different diffusion path lengths; this is an exceptional advantage that is not feasible with NTD or conventional methods.[3]

1.6 High-throughput SPME analysis

The automation of an analytical technique has several advantages, such as greater reproducibility, faster sample throughput, and reduced analyst time for both method development and routine analysis. Currently, when multiple analyses have to be conducted in a laboratory in a short period of time, traditional sample preparation methods are the bottleneck in the throughput of the analysis. In most cases, due to large sample sizes or high consumption of organic solvents, it is difficult to develop a method that integrates sample preparation with separation/detection for automation purposes.[46,47]

When the principles of SPME were originally developed, it was discovered that the fibre arrangement of SPME was suitable for automation with GC, due to its similarity to traditional GC syringes used for liquid injection.[48] SPME automation eliminates the drawbacks of the traditional techniques and is an efficient approach toward integration of sample preparation with GC or LC.[20,21] Indeed, method development with automated SPME has several advantages over manual SPME methodology. For instance, superior extraction time reproducibility allows the development of faster non-equilibrium extractions, which is otherwise unattainable when using a non-automated method[47]. Also, since the autosampler can run 24 h a day, a manual SPME method can be considered inconvenient when a considerable volume of samples needs to be processed.[20]

An SPME autosampler is basically a device equipped with a robotic arm, which enables manoeuvring for sampling and injecting in the gas chromatograph to be performed mechanically.[1,48] Several autosamplers with different features have been developed for SPME since 1992.[20] Autosamplers introduced by Varian in 1993 provided analysts the first opportunity to explore the potential of automated methods with SPME.[20,49,50] Six years later, with the release of the CTC autosampler, added functionality became available, which greatly expanded the variety of SPME sample preparation processes that were possible prior to its release.[1,49,50] Recently, GERSTEL® launched the MultiPurpose Sampler 2 (MPS2), with the remarkably simplified and user-friendly interface designed for method development.[1] An image of the commercial autosampler MPS 2 can be found in Figure 1.6. As can be observed, the robotic arm is equipped with several components, such as a sample tray, agitator tray, sample preparation/injection arm, and fibre conditioning station. The agitator tray provides temperature control and agitation during the incubation and extraction processes. The autosampler arm is able

to transport the vials from the sample tray to the sample preparation station and expose/withdraw the fibre coating. The fibre conditioning station, in this case the front injection port, is designed to clean the fibre coating after each extraction/injection cycle in order to prevent carry over.[20]

An interesting feature of the current commercialized autosamplers is the ability to start the sample preparation procedures for the sample sequence, while a different sample is still undergoing GC analysis,[20] thus achieving high sample throughput.

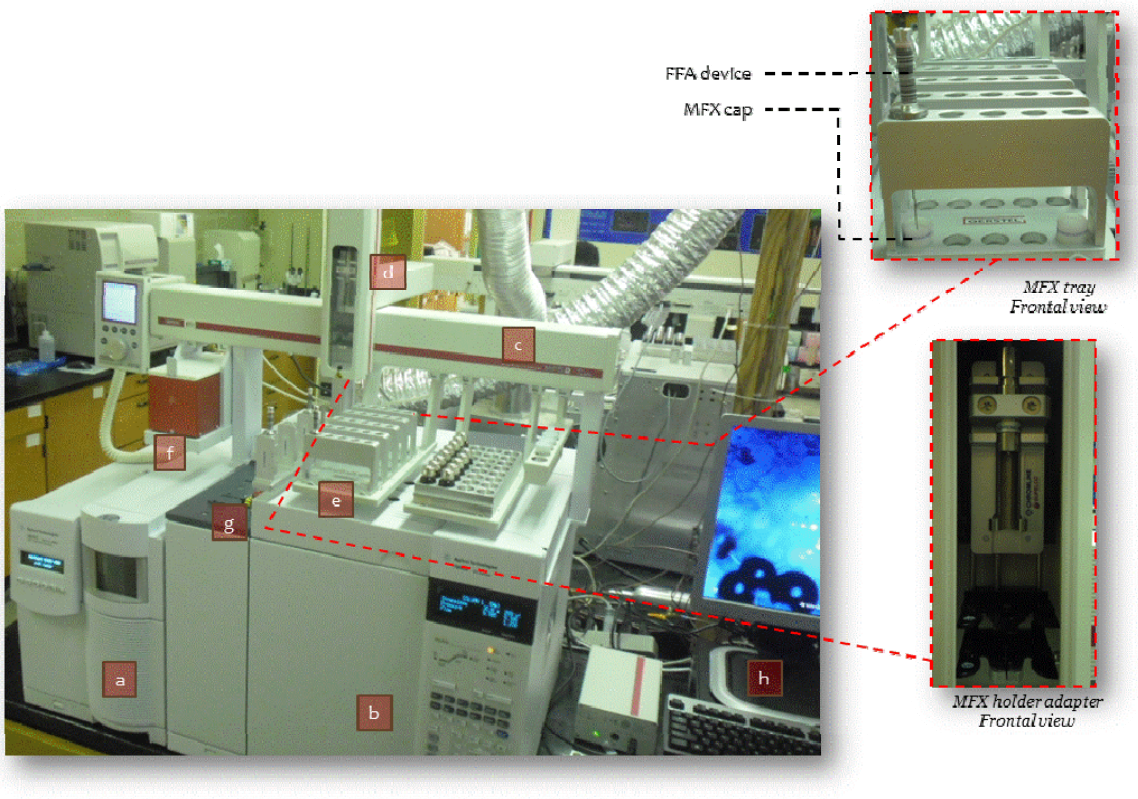


Figure 1.6 An image of the commercially available MPS2 autosampler for the performance of automated SPME processes. Shown in the image are: a) qMS; b) GC; c) MPS2; d) autosampler arm; e) MFX tray; f) agitator; g) Injection port; h) Workstation.

Despite significant advances made towards the automation of SPME-GC analysis, multiple fibre devices cannot be automatically injected in a single sequence. This is a critical issue, mainly because when running analysis of numerous on-site samples taken with various fibre assemblies, the operator has to stop the system and manually replace the fibre devices whenever needed. Thus, the development of a completely automated SPME sequence is limited by the number and type of fibres used by the analyst.[24]

Consequently, the ability to automatically exchange fibre devices during the analysis sequence is a highly desirable feature for SPME users. The new SPME Fast Fit Fibre Assembly (FFA, developed by CHROMLINE® and SUPELCO®) together with the Multi-Fibre Exchanger (MFX) system (designed for the GERSTEL® MPS2 autosampler) made this feature plausible. This innovative system offers SPME users the possibility to run in a single sequence multiple fibre assemblies previously employed for sampling (e.g. multiple probes used in *in-vivo* analysis). In addition, this system allows the users to program extractions from the same vial using different fibres without any manual intervention between runs.[24,51]

The original FFA invention[24] is comprised of a commercial SPME fibre assembly attached, via a hub, to an adapter cap made of a ferromagnetic material (refer to Figure 1.1). Next, the needle is assembled in a flange, also made of ferromagnetic material, which is screwed internally and externally with a sleeve in the lower part of the fibre assembly body, while a tubular piece is assembled in the upper part of this device (Figure 1.1). The original concept of the device allowed interchange of different fibres through a radial discontinuity in the flange. However, existing commercial FFA devices are manufactured without this radial gap and sold only for the use of one type of coating.[53,54]

Since the MFX system and the Fast Fit Fibre Assemblies (FFA) were developed and commercialized recently, few applications using this system have been published.[24,50] Indeed, all of them are mainly focused on the development of a specific application; however, to date no publications have focused on a critical evaluation of the system.[24,42,51,52] For instance, Pacenti *et al.* developed a method for the Time Weighted Average (TWA) sampling and analysis of 2-chloroacetophenone (CA), a tear gas broadly used by law enforcement agencies. The statistical evaluation of the method showed that neither air flow, relative humidity nor temperature affect the adsorption efficiency. According to the data, experimental sampling rates using a PDMS/DVB 65 µm fibre were in agreement with theoretical values.[52] Similarly, Pacenti and collaborators developed a SPME/GC-MS method for the determination of airborne peracetic acid (PAA). This technique was used to determine rapid and TWA concentrations of PAA in a hospital; good relative standard deviations were found for both forms of sampling (among 8-11%).[51]

1.7 Objectives of the project

The main objective of this research is to perform a critical evaluation of the MFX system and develop high-throughput SPME applications which are focused on on-site analysis. In order to achieve this objective, our study was developed in several steps that are briefly described below.

In applications where large numbers of samples need to be analyzed, such as food, or on-site analysis, robust and reliable fibre assemblies are required. Thus, in cases where the fibre used for analysis is broken or partially damaged, in order to continue the throughput of the analysis, it

should be replaced by a new fibre assembly that exhibits the same performance characteristics as the old one.[20,55] Consequently, an initial assessment of the fibres being used is required prior to their application. One of the aims of this research was to develop a protocol for the quick assessment of commercial SPME fibre reproducibility. The procedure consists of comparing the extraction efficiency of multiple probes towards a number of standards present in a vial head-space. However, since methods frequently used to release standards in the vial head-space are not all equally suitable for all types of coatings due to limited reusability, in this study, a new and innovative standard generator vial was developed and used as the source of standards for multiple fibre evaluation. Once reliable fibres were found, the performance of the MFX system was evaluated in terms of storage stability and long term operation. Finally, two on-site applications were developed: determination of indoor air contaminants in a polymer synthesis laboratory, and biogenic emissions in Pine tree using *in-vivo* analysis. Another application of the new in-vial standard gas generator, the determination of experimental sampling rates in passive sampling mode, was also explored.

An additional objective of this research was to design and evaluate a cost-effective passive sampler for NT. Based on the personal diffusive sampler developed by Gong *et al.*,[5] a new pen-like diffusive sampler (PDS), which guarantees the integrity of the sample, and in which NT is easily installed, was developed and evaluated. Unlike previous work, a sampling chamber was successfully designed and built for the evaluation of the sampler device under a controlled environment.

Chapter 2 – Development of a new in-vial standard gas system for calibration of SPME in high throughput applications

2.1 Introduction

At present, several calibration methods have been developed for both kinetic and equilibrium modes of SPME. Common calibration methods include pre-equilibrium extraction, diffusion-controlled calibration, equilibrium extraction and kinetic calibration[17]. The last method, henceforth named "in-fibre calibration", is based on the simultaneous desorption of an internal standard previously loaded on the coating and extraction of the target analyte from the sample matrix.[55] Placing an internal standard on the fibre coating prior to exposing the fibre to the analyte is a powerful approach; it reduces uncertainty, improves precision, increases the analysis throughput and corrects for measurement response drift, matrix effects, and sample loss.[21,56] Theoretical considerations for in-fibre calibration have been extensively described in previous studies and are well-supported by experimental findings.[55,57,58] The kinetic calibration approach is especially useful for on-site and *in vivo* investigations where there are difficulties adding the standard to the sample matrix or controlling environmental conditions.[21,56,59]

A critical parameter that needs to be controlled is the amount of internal standard loaded onto the fibre: when using the kinetic calibration method, the amount of standard loaded onto the fibre coating should be at a level not as low as to cause detection problems, or as high as to overload the detector.[55,60] It has been previously described that even for extremely short extraction times, large amounts of standard are loaded onto the fibre coating by headspace extraction of pure standards in a vial. On the other hand, if standards are diluted in water, loading by headspace extraction is reduced to a satisfactory level.[19] However, since the mass of the standard withdrawn on each loading step makes for a significant percentage of its total,

reusability on automated applications is restricted.[19,60] Similar procedures were also evaluated in previous studies: a vial containing either a PDMS membrane or Tenax particles, loaded with the standard; results showed that these approaches are not suitable due to the extensive amount of analyte loaded onto the fibre coating.[60] A plausible solution was first proposed by Wang *et al.*[60], an approach which consists of spiking a few milligrams of standard into a predetermined amount of pump oil placed in a sealed vial. It has been proved that this experimental set-up provides an excellent standard generator for over a 100 extraction/injection cycles using PDMS fibres, an essential feature when processing a large number of samples.[19-21,60] Due to the low distribution coefficient that exists between headspace and the pump oil, a considerable decrease in headspace concentration of standards can be obtained.[55]

Since SPME extraction efficiency is influenced by the type of coating used, the application of this method to fibre coatings like DVB-CAR-PDMS and CAR-PDMS, both which have higher extraction efficiencies towards volatile compounds than PDMS, might result in fast depletion of the concentration of the standards in the vial headspace. Consequently, vial reusability is limited.[59] It has also been observed that when higher amounts of standards are spiked in pump oil, the masses of the standards extracted by the fibre coating can either go above the linear dynamic range, or overload the mass spectrometry detector. Conversely, spiking smaller amounts of analyte in the pump oil to reach lower concentrations in the headspace can be a challenging task, and oftentimes produces inaccurate results. Yet another important limitation of pump oil is its liquid consistency, which hinders its applicability for on-site implementation, since the fibre can be easily splashed.[61] Recently, *Lee et al.*[61] developed a simple, reusable vial consisting of granular PDMS spiked with standards that surpass the transportation issue. This solvent-less standard was developed for on-site calibration of retention time, mass and

concentration, all in a single injection using a portable GC-TMS. However, headspace concentration is highly dependent on three factors: amount of calibrant spiked on PDMS particles, sampling time, and temperature. Therefore, its applicability using solid coatings in mass spectrometry detectors with a small linear dynamic range might be constrained. Due to its granular composition, as an added measure, a piece of glass wool should be used on top of the particles during transportation to avoid the spread of particles inside the vial.[61]

In gas chromatography, columns are classified based on selectivity and polarity, where polarity denotes the characterization of the interaction between the stationary phase and the solute on the basis of its structure. Therefore, polarity can be described as the sum of intermolecular interactions.[62] The dipole moment is often used as a symbol of polarity; however, chromatographic interactions are hardly described by a single measure.[62] As a result, several empirical measures for polarity and/or selectivity parameters of the stationary phases have been proposed[62]. The well-known and widely used Rohrschneider-McReynolds constants were developed to characterize stationary phases based on several different interaction parameters; numerous studies have shown that McReynolds' test molecules are adequate to characterize the polarity of GC columns.[62,63] The most representative of these probes are benzene, 1-butanol, 2-pentanone, nitropropane and pyridine; their ability to participate in various types of interactions with the stationary phases through inductive, donor-acceptor forces or H-bonding (H+ donor and acceptor) was used as the criterion to select these compounds for column characterization.[63-68] Listed on Table 2.1 are some of their physicochemical properties, as well as the analytical parameters used for the identification and quantification of the McReynolds probes used on our study. McReynolds standards were selected for this research based on the broad range of interactions aforementioned.

The term "adsorptive macroporous resin" is used to describe highly cross-linked, non-ionic (non-functionalized) resins with a large number of permanent pores, such as styrene-divinylbenzene (SDVB) copolymers. The copolymerization of DVB with styrene was first reported to yield gel resins that would swell, but not dissolve in solvents.[69] Due to its high DVB content, the resin has a stable and rigid structure that can tolerate rigorous conditions.[69,70] Several reviews associated with the fundamental aspects of macroporous resins, and history of their development can be found in the literature.[69-72] Amberlite® XAD, a type of SDVB resin, was specifically used in our study. XAD resins have been extensively used in the last decades for several applications such as removal and recovery of organic compounds in clinical and environmental analysis, separation and enrichment of pharmacologically-active natural products, and adsorption of gases. XAD-4, particularly, is a non-ionic and non-polar adsorbent and, as such, adsorbs compounds principally by Van der Waal's forces.[70,71,73] However, polar or ionic compounds having sufficiently large non-polar moieties can also be adsorbed.[70] Key parameters that characterize XAD-4 particles such as pore diameter, internal surface area and surface polarity are listed on Table 2.2.

In this chapter an innovative standard generator vial is introduced, consisting of vacuum-pump oil spiked with pure standards and subsequently mixed with a SDVB resin. By using XAD-4 particles, headspace concentration of the analytes is diminished and the pump oil capacity towards the analyte is enhanced. Hence, the applicability of the in-vial standard to all commercial coatings is plausible. Also, due to the compacted granular appearance of the new calibration solution, it is easy to transport. The new in-vial standard system is an ideal calibration standard not only for bench and field instruments but also for green sample preparation techniques such as SPME and needle trap.

Table 2.1 Physicochemical properties of the McReynolds probes solutions used for this study. CAS, CAS registry numbers; MF, molecular formula; MW, molecular weight; BP, boiling point, Q. mass, quantitation mass; Log P, logarithm of the partition coefficient; μ, dipole moment; t_R, retention time.[63-68]

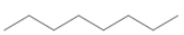
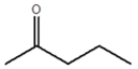
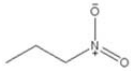
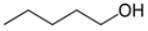
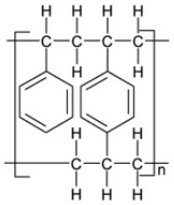
Analyte	CAS	MF	MW (g/mol)	BP (°C)	Density (g/cm^3)	Q. mass (*m/z*)	Log P	μ (10^3 cm)	t_R (min)	Structure	Chemical group represented/ Interaction measured
Benzene	71-43-2	C_6H_6	78.11	80	0.878	78	2.13	0.0	2.868		Aromatics/Primarily dispersion with some weak proton acceptor properties
Octane	111-65-9	C_8H_{18}	114.23	125	0.703	43	4.78	0.0	5.118		Alkanes/Weak induced dipole-induced dipole forces
2-Pentanone	107-87-9	$C_5H_{10}O$	86.13	102	0.807	43	0.98	2.7	3.134		Ketones, ethers, aldehydes, and esters, epoxides/Orientation properties with proton acceptor but not proton donor capabilities
1-Nitropropane	108-03-2	$C_3H_7NO_2$	89.09	117	0.998	43	0.94	13.0	3.969		Nitro and Nitrile derivatives/Dipole orientation properties. Weak proton acceptor.
Pyridine	110-86-1	C_5H_5N	79.10	115	0.978	79	0.84	7.9	4.030		Bases and N-heterocyclic compounds/Weak dipole orientation with strong proton acceptor capabilities Proton donor properties are absent
1-Pentanol	71-41-0	$C_5H_{12}O$	88.15	138	0.815	55	1.35	1.7	4.580		Alcohols, phenols and acids/Orientation properties with both proton donor and acceptor capabilities

Table 2.2 Physical characteristics of XAD-4 resin. CAS, CAS registry numbers; MS, mesh size; PS, particle size; MPD, mean pore diameter, PV, pore volume; MOT, maximum operational temperature; SA, surface area; μ, dipole moment.[70,73]

Analyte	CAS	MS	PS (mm)	MPD (Å)	PV (cm^3/g)	MOT (°C)	SA (m^2/g)	μ (10^3 cm)	Structure	Description/Applications
Amberlite® XAD-4	37380-30-2	20-60	0.49-0.69	78.11	0.98	150	750	0.3		Styrene-divinyl benzene copolymer, non-polar, hydrophobic resin Removal/recovery of organic pollutants/compounds from water and polar solvents

2.2 Experimental section

2.2.1 Materials and reagents

HPLC grade methanol was obtained from Caledon laboratories Ltd (Georgetown, ON, Canada). Benzene, 2-pentanone, pyridine, nitropropane, 1-pentanol, n-octane, and styrene-divinylbenzene particles (Amberlite® XAD-4) were purchased from Sigma-Aldrich (Mississauga, ON, Canada). The vacuum-pump oil, General Purpose (GP) mechanical pump oil, was supplied by Varian Vacuum Technologies (Lexington, MA). Vials, screw top, 20 mL size and caps with 20 mm polytetrafluoroethylene (PTFE)/silicone septa were purchased from Canada Life Sciences (Peterborough, ON, Canada). Vials, screw top, 40 mL size and caps with 22 mm PTFE/silicone septa were purchased from Sigma-Aldrich (Mississauga, ON, Canada). Pure water was obtained using a Barnstead/Thermodyne NANO-pure ultrapure water system (Dubuque, IA, USA). Helium of ultra-high purity was supplied by Praxair (Kitchener, ON, Canada). Drierite (anhydrous desiccant), was purchased from W. A. Hammond DRIERITE Co. (Xenia, OH, USA). The desiccator and magnetic bars were supplied by Fisher Scientific (Ottawa, ON, Canada). Temperature controller and thermocouples were obtained from Omega Engineering (Stamford, CT, USA). The hot plate stirrer (Catalog number 97042-642) was obtained from VWR Scientific (Mississauga, ON, Canada). The commercial SPME-Fast Fit Fibre Assembly (FFA) fibres polydimethylsiloxane (PDMS, 100 μm) and divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS, 50/30 μm) were also provided by Sigma-Aldrich. Fibres were conditioned according to the manufacturer's recommendation prior to their use. All preparations were carried out in a ventilated fume hood.

2.2.2 Instrumentation

Both the GERSTEL® MPS 2 autosampler, endowed with a Multi-Fibre Exchanger (MFX) system for 25 SPME-FFA devices (GERSTEL, Mülheim an der Ruhr, Germany), and the Agilent 6890 gas chromatograph coupled with 5973 MSD quadrupole mass spectrometer (Agilent Technologies, Mississauga, ON, Canada) were used in this study. Chromatographic separations were performed using a SLBTM-5MB (30 m x 0.25 mm x 0.25 μm) fused silica column from Sigma–Aldrich with helium as the carrier gas, at a flow rate of 1 mL min^{-1}. The oven temperature was initially held at 40 ºC for 1 min, gradually increased to 50 ºC at a rate of 5 ºC min^{-1}, then to 70 ºC at a rate of 6 ºC min^{-1}, and finally held for 0.47 min. The injector temperature was held at 250 and 260 ºC for PDMS and DVB/CAR/PDMS fibres, respectively. During analysis, the transfer line, MS Quad and MS source were set at 280 ºC, 150 ºC and 230 ºC, respectively, with MS being operated in electron ionization mode. Full scan mode (40–250 m/z) was used for all the compounds and quantitation was done using extracted ion chromatograms. The ions used for the quantitative analysis of compounds are listed in Table 2.1.

2.2.3 Conditioning of styrene-divinylbenzene (SDVB) particles

The adequate removal of impurities from the resin such as naphthalene, styrene, hydrocarbons, and phthalates is a critical step to be untaken; their presence was previously described in the literature as the main drawbacks of XAD resins.[70] In order to achieve this, approximately 50 grams of Amberlite® XAD-4 particles were placed in a 400 mL beaker and manually agitated with 250 mL of Nano-pure water for 10 minutes. Immediately after, particles were filtrated, and then washed twice more following the same procedure. Following this, the SDVB particles were transferred to another 400 mL beaker and washed with 150 mL of methanol, using constant manual agitation for 5 min. Next, the particles were decanted and the

methanol was removed. This procedure was repeated once more with another 150 ml of methanol. After these cleaning steps, the XAD-4 particles were filtrated and placed on four petri dishes previously covered with aluminum foil, and then dried under a constant nitrogen flow of 10 ml min^{-1} in a desiccator, for minimum 24 h. To ensure the elimination of any remaining impurities from the particles, the petri dishes were then placed in a vacuum/nitrogen oven at 60 ºC for 24 h. It should be noted that extreme heating should be avoided in order to prevent bead rupture and release of resin impurities.[70] Finally, XAD-4 particles were removed from the oven and kept in a desiccator with a continuous nitrogen flow, so as to avoid cross contamination before mixing them with pump oil.

2.2.4 Preparation of the vial standard solution

Approximately 200 grams of vacuum pump oil were weighted and placed in a 400 mL beaker. In order to remove possible impurities the pump oil was heated at 120 °C under constant agitation (120 rpm) and maintained under nitrogen flow (10 ml min^{-1}) for 24 hours[21]. Then, with the pump oil at room temperature, ca. 32 grams were placed in a 40 mL screw-top vial. To minimize evaporation, approximately 2 to 10 µL of pure standards were spiked in the vial below the level of the solution.[60] Once all the standards were added, the vial was capped and kept under continuous agitation at 1500 rpm for at least 48 hours using a 0.25 inch stir bar. Then, approximately 1.5 g of previously cleaned SDVB particles were weighted in a 20 mL screw top vial and mixed with approximately 3 g of the prepared pump oil. Immediately after, the vial was capped and sealed with Parafilm®, then kept for a couple of days to equilibrate before analysis on the autosampler. Figure 2.1 shows the overall schematic diagram for manual and automated extraction of the new standard gas system.

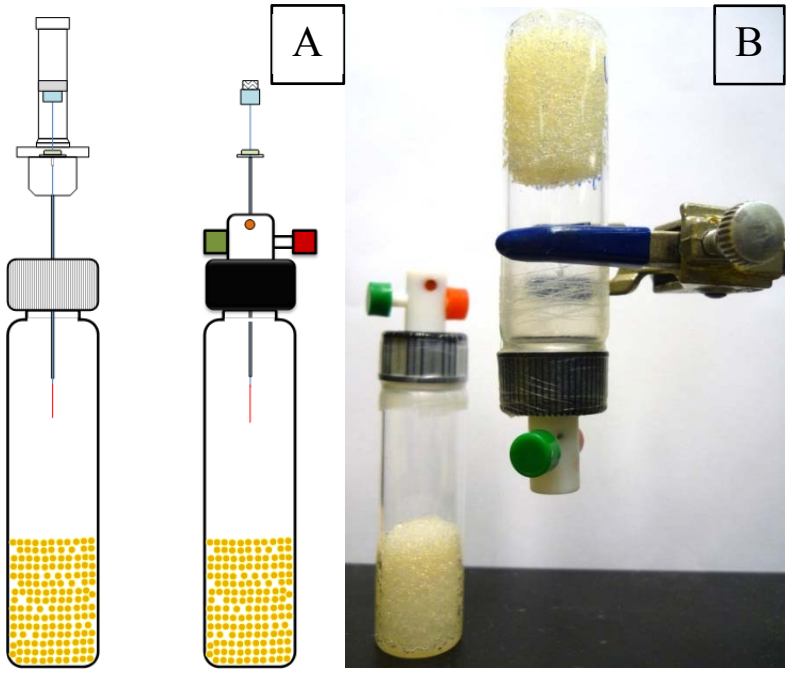


Figure 2.1 Schematic diagram of the new standard generator vial for automated and manual standard loading. On the left, automated extraction using the SPME-FFA device from a screw-top 20 mL vial. On the right, manual extraction using a conventional SPME fibre from a vial with a Miniert Valve® used to reduce contact between sample and atmosphere. B) Picture of an actual vial up-side down.

2.3 Results and discussion

2.3.1 Evaluation of vial reusability

The development of a calibration solution that can be re-used several times is critical, especially for high-throughput applications such as determination of food authenticity or SPME fibre aging evaluation, in which over 100 analyses must be carried out. Consequently, in order to determine standard gas generator reusability, 160 cycles of 1 min headspace extraction were performed using a 50/30 µm FFA-DVB/CAR/PDMS stableflex fibre. Automated SPME incubation/extraction/desorption/fibre bake-out cycles, together with the programmed GC-MS analysis, did not exceed 13 minutes, with GC separation of analytes completed within 7 minutes. Additionally, in order to ensure that variations observed in the amount extracted were unrelated

to fluctuations in the detector response and/or fibre aging, control tests for both factors were run in parallel to one another at 5 and 10 injections, respectively.

As can be seen in Figure 2.2, our findings showed that relative standard deviations for all compounds were smaller than 4%, up to 160 extraction/injection cycles. Although a decrease in the amount extracted for octane and 1-pentanol was observed, this decrease can be accounted by modeling the relationship between the amount spiked and the amount extracted. In order to evaluate if a relationship among the amount of analyte spiked in the solution and the amount extracted by the fibre exists, three solutions with different concentrations of McReynolds probes were evaluated (Figure 2.3).

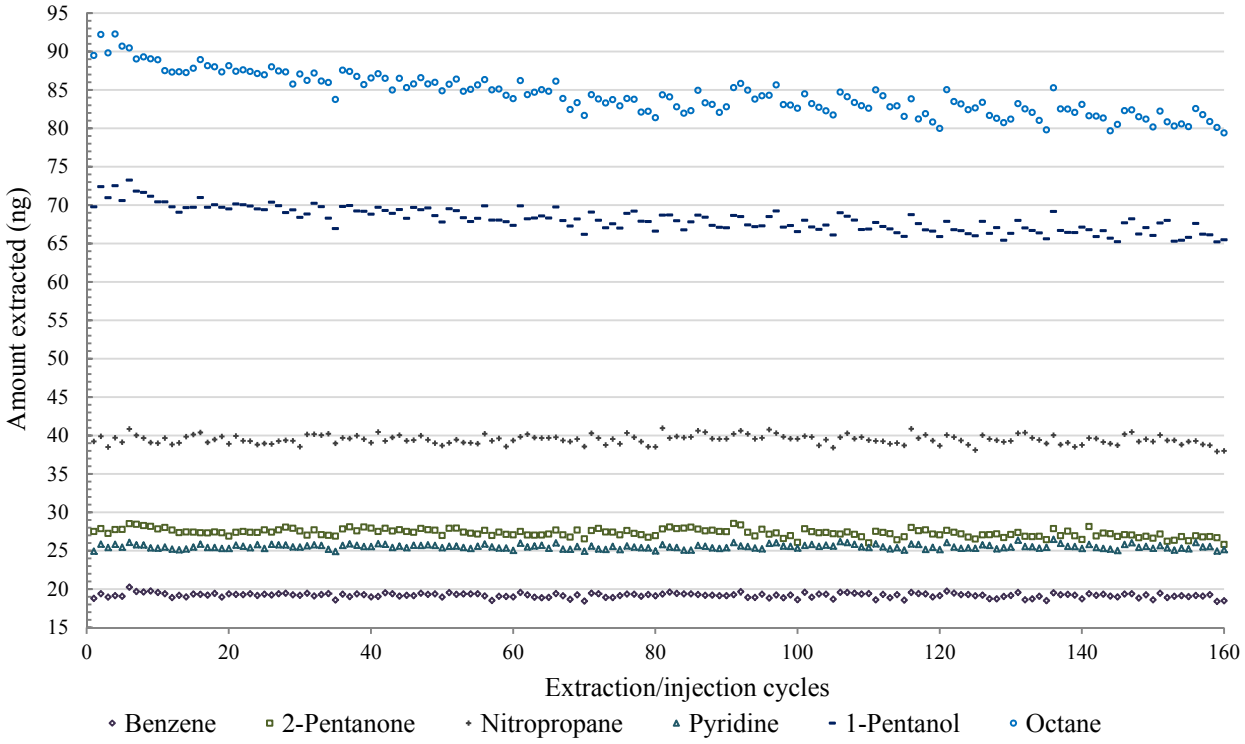


Figure 2.2 Durability of the new standard generator vial; amount extracted of McReynolds probes using a FFA-DVB/CAR/PDMS 50/30 µm stableflex fibre with 1 minute of extraction from the vial headspace. No agitation was used and vial temperature was kept constant at 35ºC. Analyses were performed automatically by a MPS-2 GERSTEL autosampler endowed with a MFX system.

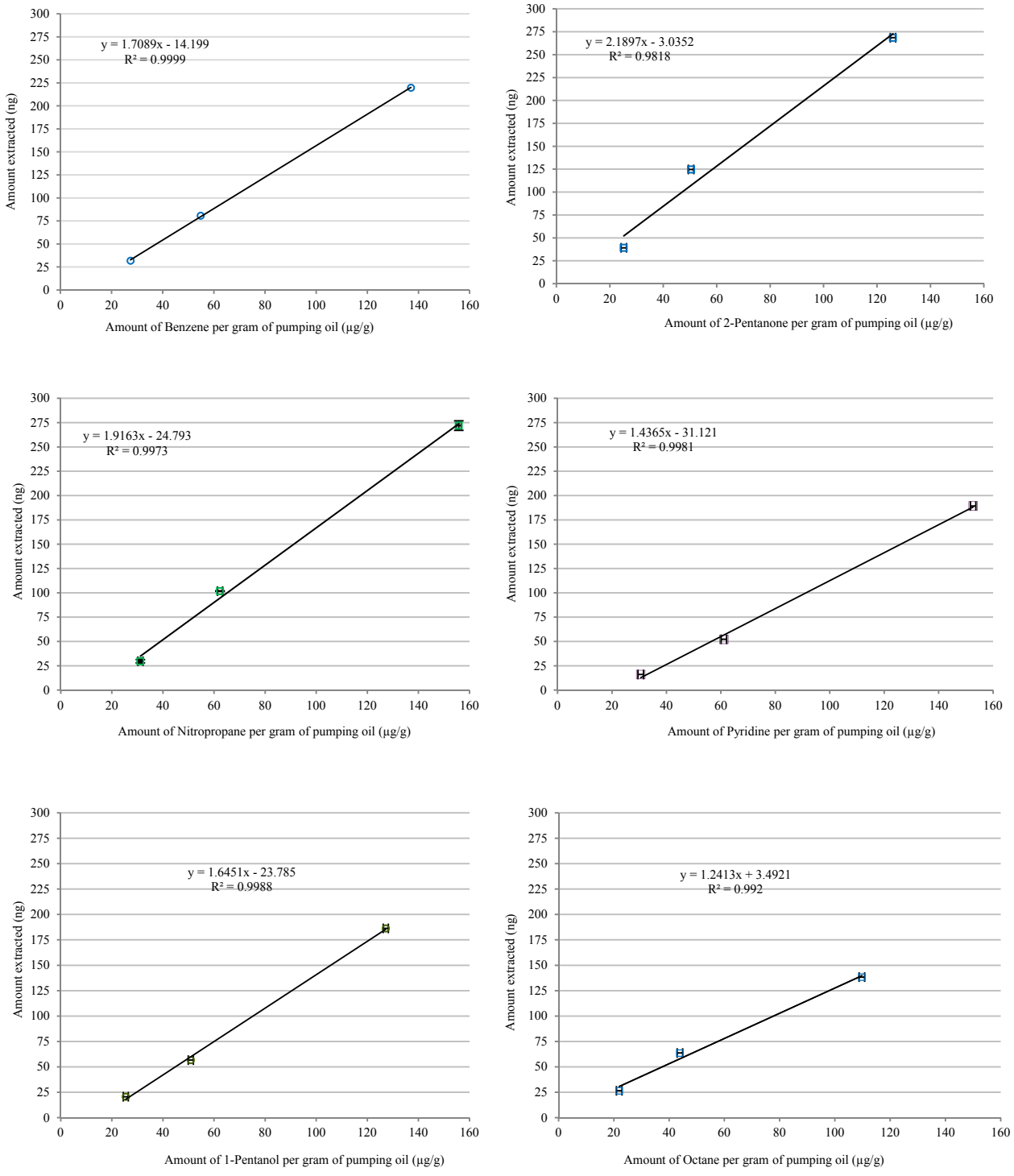


Figure 2.3 Evaluation of effect of analyte amount spiked on pump oil-XAD-4 mixture versus amount extracted by a 50/30 µm DVB/CAR/PDMS fibre (n=8), using an of 1 minute extraction time without agitation at 35 ºC.

As can be seen in Figure 2.3, the results showed a linear relationship among the mass of analyte spiked on the calibration vial and the amount extracted by the fibre for all McReynolds probes. Thus, it can be established that the concentration in the headspace is proportional to the total amount of analyte in the calibration vial. Accordingly, corrections can be applied if a decrease in fibre extraction is observed as a result of analyte reduction in the vial after several extractions; thus, vial reusability is limited to the total amount of standard initially spiked.

Similarly, as mentioned in previous studies,[60] the amount of standard loaded onto the fibre can also be adjusted by manipulating the extraction time (before equilibrium is reached). Therefore, as the extraction time is shortened, a larger number of extraction/injection cycles can be accomplished. Since this methodology was evaluated for a coating with high affinity towards volatile compounds, it can be determined that acceptable and reproducible loading on different fibre coating types can be achieved.

2.3.2 Evaluation of inter-vial repeatability

As mentioned before, calibration solution reusability is limited to a number of extraction/injection cycles which are in turn depending on the coating, final analyte concentration, and extraction time used before removal of 1% of the vial content.[23] In addition to the use of standards dissolved in pump oil solutions for loading onto fibre coating, they can also be used in the evaluation of new fibre assemblies, estimation of coating aging, and intra- and inter-fibre reproducibility measurements.[19-21] Having a replaceable standard gas generator vial results certainly helpful in applications where multiple loadings are necessary and periodical evaluation of performance of the fibre of interest is required to guarantee reliable results over

time. To determine the feasibility of using several vials indistinctly, the inter-vial repeatability was also evaluated. For this purpose, a randomized block design was carried out where injection order and position of vials in the agitator tray were blocked. As a result, effects related to instrumental signal drifts as well as variability in heating homogeneity were eliminated.[74] Results of the evaluation of inter-vial repeatability are presented in Table 2.3 and Figure 2.4.

ANOVA analysis of the data showed no significant effect for randomized injection, with random error probabilities between 28 and 91 % for all the compounds studied. Additionally, as can be seen in Table 2.3, at a 95 % level of confidence, the vials are statistically identical, with only a slight exception for pyridine. Based on these findings, it can be concluded that different vials prepared from the same pump oil are repeatable among themselves, and as a result, can replace one another after a specific number of extractions (which is dependent on the coating and extraction time used).

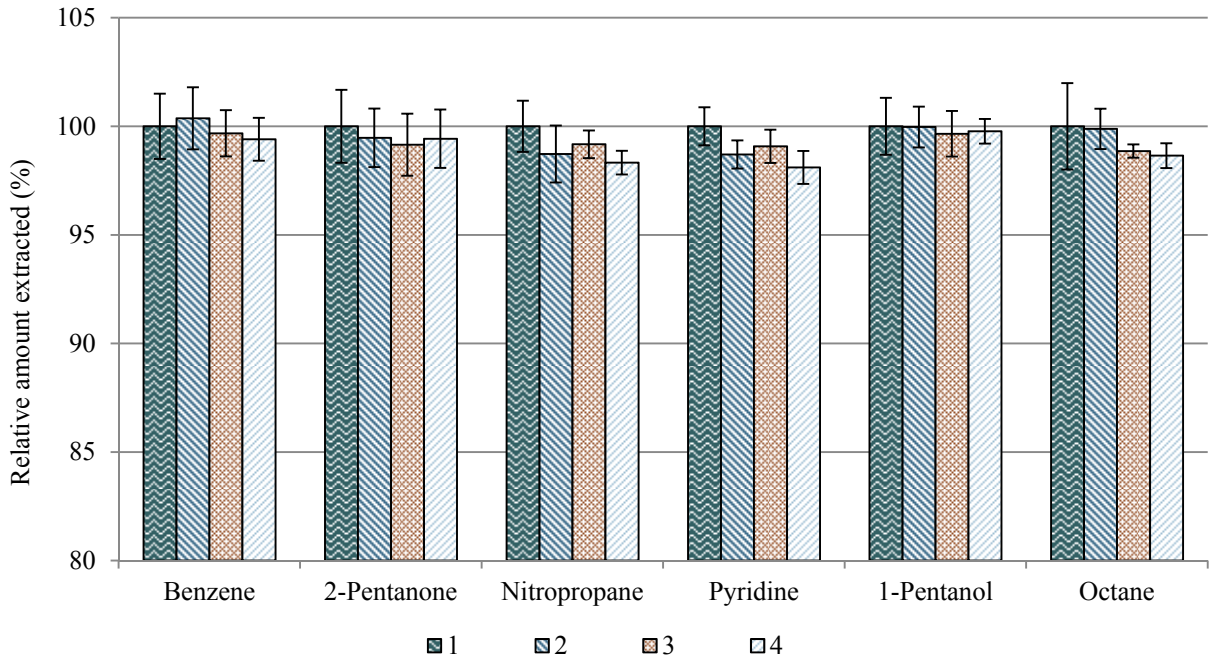


Figure 2.4 Evaluation of inter-vial repeatability of four vials using a randomized block design. Error bars represent the standard deviation of the mean (n = 5). Analyses were performed automatically by the MFX system using a single SPME-FFA 50/30 μm DVB/CAR/PDMS fibre.

Table 2.3 Statistical evaluation of intra-vial repeatability using a randomized block design. F*injection* is the F-ratio for randomization of injections. F*vial* is the F-ratio for different treatments evaluated (different vials) and F*crit* is the critical value of F for twenty experiments at a 95% level of confidence. RSD is the relative standard deviation for inter-vial repeatability of four vials (n=5).

Compound	Benzene	2-Pentanone	Nitropropane	Pyridine	1-Pentanol	Octane
F*injection*	0.38	0.64	1.14	0.96	1.31	1.01
F*vial*	0.25	2.30	1.71	4.07	0.43	1.17
F*crit*			3.36			
RSD	1.16	1.67	1.15	0.99	1.12	1.15

2.4 Conclusions

A new in-vial standard gas system for calibration of SPME in high-throughput applications was presented in this study. The loading technique is fast and reproducible, and the same standard generation vial can be used for more than a hundred analyses, which is essential when processing a large number of samples. Both intra- and inter-vial repeatability were evaluated and results showed no statistical differences for the compounds used as internal standard models. The analyses performed were fully automated by a MPS-2 GERSTEL autosampler endowed with a MFX system, which offers SPME users the ability to use several fibres to extract from the same or different samples without manual intervention. In addition, due to the compacted granular appearance of the new calibration solution, previous issues related to spills or fibre contaminations with pump oil are not a concern. Similarly, the vial can be easily transported and it is an ideal calibration standard for both bench and field instruments and devices. Application of the in-vial standard gas system to the evaluation of commercial SPME coatings, as well as determination of experimental sampling rates for TWA-SPME are presented in the next chapter. Loading of derivatization reagents for the analyses of different compounds such as aldehydes or steroids in environmental and biological complex matrices, will be further evaluated in our group.

Chapter 3 – Applications of the in-vial standard gas system

3.1 Protocol for quick evaluation of commercial coatings

3.1.1 Introduction

While SPME has matured as a solvent-free sample preparation technique, there is still debate in the literature regarding the variations in reproducibility encountered between fibres. Indeed, several publications have described dissimilarities in the response of commercial coatings. For instance, both Natera *et al.* and Castro *et al.*, [75,76] conducted validation studies of a method developed to analyse aromatic compounds of vinegar by HS-SPME, and found considerable differences between the amounts extracted by three different Carboxen-PDMS (CAR/PDMS) fibres. Similarly, Paschke *et al.* reported questionable fibre-to-fibre reproducibility for determined mass uptake rates when implementing diffusion-based calibration for extraction of BTEX and chlorobenzenes from aqueous samples using DVB/PDMS fibres.[40]

As a result, two intrinsic and indispensable conditions must be satisfied when using SPME in laboratory and field applications: the repeatability of a fibre over time, and the reproducibility of fibres within or between lots. It is found in the literature that some authors, subsequently to fibre selection, reported the evaluation of multiple fibres with the same type of coating with the aims to account for inter-fibre reproducibility.[39] To the best of our knowledge, it is the first time that a standardized protocol for the initial assessment of inter-fibre reproducibility is reported.

In this body of research, a fast, reproducible, reusable and completely automated new method that allows rapid assessment of multiple SPME coatings is presented. In this protocol, a single standard generator vial, containing vacuum pump oil doped with McReynolds probes and subsequently mixed with XAD-4 particles (previously described in chapter 2), was used as the

source of standards for the evaluation of fibres. Because several extractions/injection cycles with multiple fibres were performed employing the same vial, the effect of the injection order had to be considered. Hence, a randomized block design was used for this experimental evaluation. Additionally, since multiple treatments and variables were evaluated, the results were graphically represented using PCA plots. The proposed protocol was used to evaluate four commercial coatings namely: PDMS, DVB/PDMS, CAR/PDMS and DVB/CAR/PDMS. The complete analysis of each coating is presented in the results and discussion section.

3.1.1.1 Advantages of randomized block designs and Latin square designs

In measurements made over a period of time, variations in uncontrolled factors such as temperature, pressure, and instrument response may affect the obtained results;[77] if the experiments are not properly randomized, known hazards and biases can randomly occur. Nevertheless, bare randomization is not sensitive enough. A randomized block design, on the other hand, is a design in which the experimental runs are arranged in groups (called blocks), which are similar to one another.[74] Generally, blocking factors are sources of variability that are not of primary interest to the analyst. Indeed, research shows that in comparisons between blocked and unblocked experiments with the same measurements, blocked designs are proven to be more sensitive and yield more information.[77] In fact, at times, more than one source of disturbance can be eliminated by blocking. In cases when there are equal numbers of treatments and blocks, it is possible to use an experimental design that allows the separation of an additional factor.[77] This powerful block design, in which each treatment is present once in each column and once in each row, is known as a Latin square. The Latin square design separates several variations that may be present: between-treatment, between-blocks, and random experimental

error components.[77] In the following chapters, randomized and Latin square designs were used to evaluate the intra-fibre and inter-fibre reproducibility.

3.1.1.2 Fundamentals of principal component analysis

In the last decades, the application of principal component analysis (PCA) in analytical chemistry has become widespread due to its ability to provide information otherwise barely accessible.[78] PCA is especially useful for dimension reduction, cluster identification, and pattern recognition.[79] Additionally, PCA can disclose numerous critical components that generally explain the vast majority of variance found in data.[74] The aim of this tool is to characterize each case, not by analyzing every variable, but by projecting the data in a smaller subset of new variables or principal components.[78] The principal components, also called factors, are linear combinations of the initial variables; factors remove variable redundancy and highlight variances within the dataset.[74] The coefficients between old and new variables are named variable contributions or loadings; they explain how new factors are composed from the original variables.[77] Usually, only relevant portions of information are supported by few principal components.[72] Furthermore, the standardization of variables in PCA is necessary if variables are measured on different scales. An additional reason for standardizing would be when one variable has a much larger variance than the others, and as a result dominates the first principal component; standardizing avoids this issue by making all variables carry equal weight.[77] Thus, due to its specific qualities, PCA was applied in this study to assist in better graphical representation of the results, and the identification of trends otherwise not observable. In order for data to be standardized, it was processed based on correlations rather than covariances.

3.1.2 Experimental section

3.1.2.1 Materials and reagents

All standards and solvents were obtained in the same way as in the previous chapter. The commercial SPME-FFA fibres used in this study: PDMS (100 μm), DVB/PDMS (65 μm), DVB/CAR/PDMS (50/30 μm), and CAR/PDMS (85 μm), were provided by Sigma-Aldrich. All fibres were conditioned according to the manufacturer's recommendation prior to their use. Parameters used for qualitative and quantitative analysis of each compound are listed in Table 2.1.

3.1.2.2 Instrumentation

Both the GERSTEL® MPS 2 autosampler, equipped with a GERSTEL Cooled Injection System (CIS4), and a Multi-Fibre Exchanger (MFX) system for 25 SPME-FFA devices (GERSTEL, Mülheim an der Ruhr, Germany), as well as the Agilent 6890 gas chromatograph coupled to a 5973 MSD quadrupole mass spectrometer (Agilent Technologies, Mississauga, ON, Canada) were used in this study. The GC/MS parameters were the same as in Section 2.2.2. The parameters selected on the Maestro software for the evaluation of different coatings being analyzed were the following: A) injection temperature (ºC) with extraction times (min): 260/1, 260/1, 250/2 and 300/0.5 for DVB/CAR/PDMS, DVB/PDMS, PDMS and CAR/PDMS, respectively; B) agitator temperature: 35ºC; C) vial penetration: 30 mm; D) injector penetration (CIS4): 54 mm E) desorption time: 180 s; F) fibre bake-out time: 1.5 min and bake-out penetration (front injector): 45 mm. An Olympus SZX10 microscope coupled with an Olympus SC30 camera (Olympus, Tokyo, Japan) was used for physical inspection of the fibres. The software analySIS getIT (Olympus, version 5.1) was used to process the resulting images.

3.1.3 Results and discussion

3.1.3.1 Randomized block design

In this study, different randomized block designs were performed according to the number of fibres available for each coating type. Table 3.1 illustrates an example of the randomized design used to evaluate seven PDMS fibres. As mentioned previously, the treatments (fibres) were not only randomized on each experimental set, but also blocked for extraction/injection order. As a result, each fibre extracted from the standard vial in a specific order position once only. Consequently, both fibre effect and standard vial effect (after multiple extractions) can be evaluated.

Table 3.1 Randomized block design used for the evaluation of seven 100 µm PDMS fibres. The nomenclature F*i* represents the fibre evaluated on each experimental block.

Experiment block	Extraction/Injection order						
	1	2	3	4	5	6	7
1	F1	F2	F3	F4	F5	F6	F7
2	F6	F5	F4	F1	F2	F7	F3
3	F5	F4	F2	F3	F7	F1	F6
4	F7	F6	F1	F5	F3	F4	F2
5	F3	F1	F5	F7	F6	F2	F4
6	F4	F7	F6	F2	F1	F3	F5
7	F2	F3	F7	F6	F4	F5	F1

3.1.3.2 Evaluation of seven commercial 100 µm PDMS fibres

Seven FFA 100 µm PDMS fibres from three different lots were evaluated using a randomized block design, as shown in Table 3.1. Fibres 1 to 3 belong to lot A, fibres 4 to 6 belong to lot B, and fibre 7 belongs to lot C. As can be seen from Table 3.2, the non-statistical effect of the randomized extractions from the vial, using multiple PDMS fibres, was observed for all of the McReynolds probes. Moreover, as shown in Table 3.3, intra-fibre reproducibilities, expressed as RSDs (%, n=7), were far below 5%, in most cases.

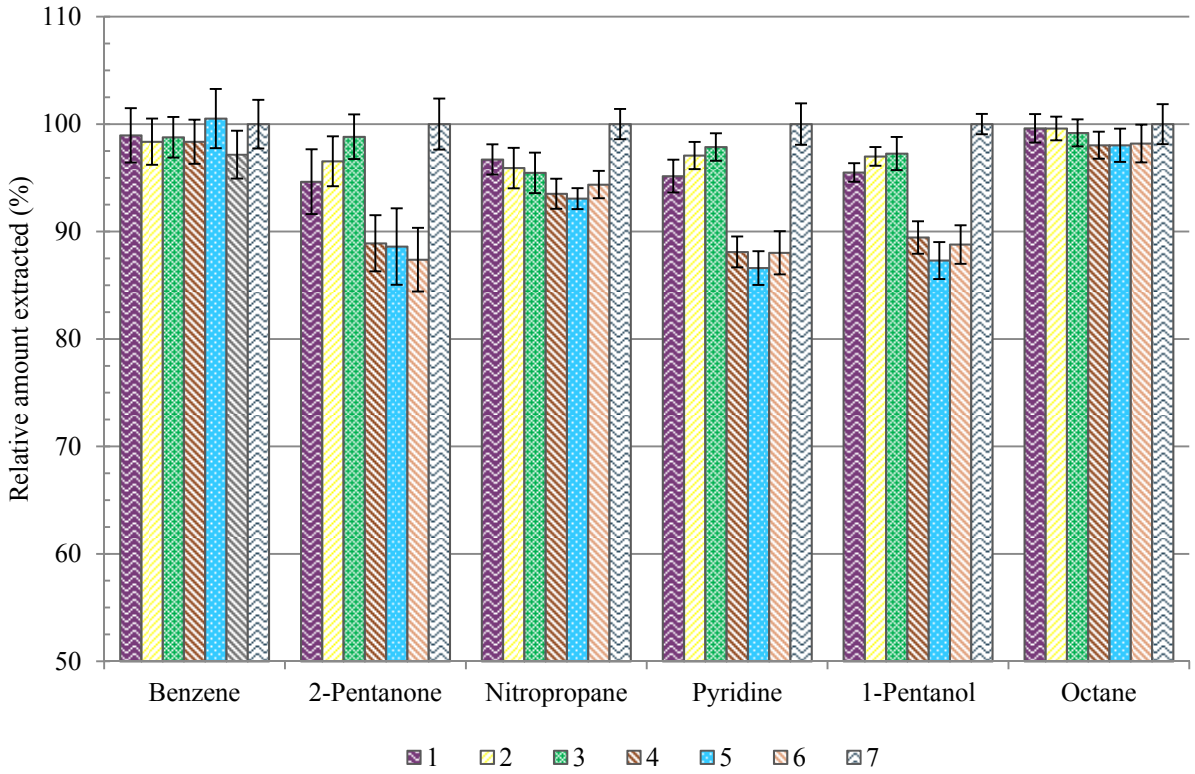


Figure 3.1 Evaluation of intra- and inter-fibre repeatability of seven 100 µm PDMS fibres using a randomized block design. Error bars represent the standard deviation of the mean (n = 7).

Table 3.2 Statistical evaluation of the inter-fibre repeatability of 7 PDMS fibres using a randomized block design. F*injection* is the F-ratio for the randomization of the injection. F*fibre* is the F-ratio for the different treatments evaluated (different fibres) and F*crit* is the critical value of F for 49 experiments at a 95% level of confidence. RSD is the relative standard deviation for the inter-fibre repeatability of seven fibres (n=7).

Compounds	Benzene	2-Pentanone	Nitropropane	Pyridine	1-Pentanol	Octane
F*injection*	1.62	1.10	1.32	0.38	0.95	0.47
F*fibre*	1.41	24.61	18.39	74.45	90.94	1.86
F*crit*			2.36			
RSD	2.4	5.7	2.7	5.7	5.1	1.6

Table 3.3 Intra-fibre and inter-fibre repeatability of seven 100 µm PDMS fibres evaluated using a randomized block design. RSD is the relative standard deviation for the inter-fibre repeatability of seven fibres (n=7).

Compounds	Intra-fibre repeatability (RSD)							RSD
	1	2	3	4	5	6	7	
Benzene	2.6	2.2	1.9	2.1	2.7	2.3	2.3	2.4
2-Pentanone	3.2	2.4	2.1	2.9	4.0	3.1	2.4	5.7
Nitropropane	1.5	2.0	2.0	1.4	1.0	1.3	1.4	2.7
Pyridine	1.6	1.3	1.3	1.6	1.8	2.3	1.9	5.7
1-Pentanol	1.0	1.0	1.6	1.7	2.0	1.9	0.9	5.1
Octane	1.3	1.1	1.3	1.3	1.6	1.8	1.9	1.6

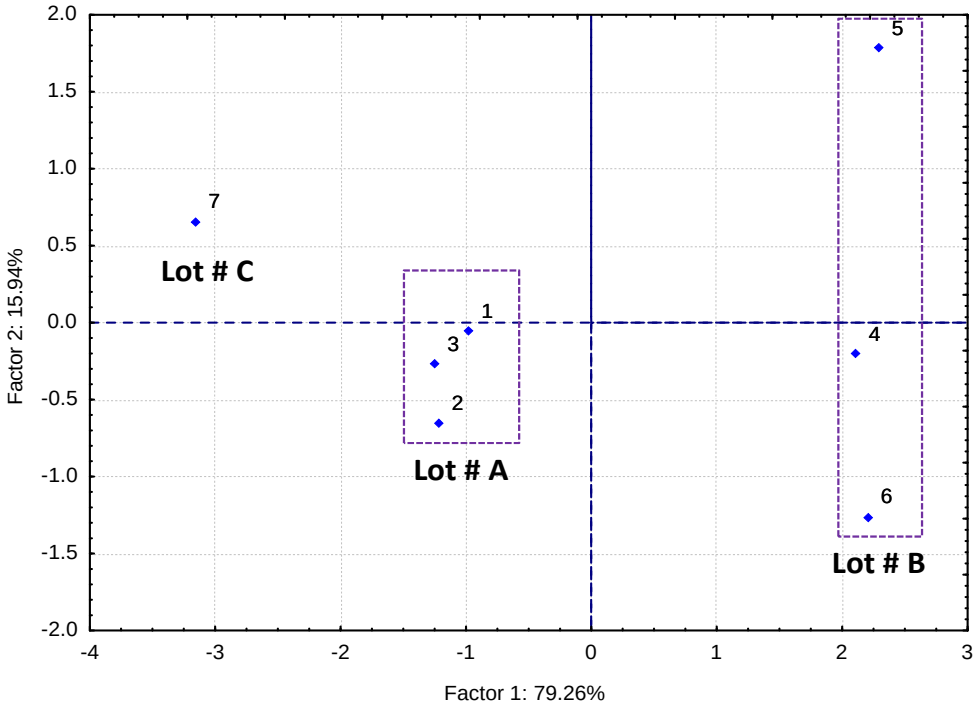


Figure 3.2 Principal component analysis of the average amount extracted by seven 100 μm PDMS fibres using a randomized block design.

Despite the inter-fibre reproducibility for all the compounds being lower than 5.8%, the F test showed statistically significant differences for all compounds, with the exception of benzene and octane (Table 3.2). The same trend can also be observed in Figure 3.1. In order to guarantee that data was normalized prior to PCA, the relative amount extracted for all the McReynolds probes was calculated. Therefore, the fibre that performed best for the majority of the analytes was set at 100% (fibre number 7).

Following the procedure described by Heberger and coworkers,[78] it was determined that 2 principal components explained more than 95% of the total variance in the data; this means the data can be represented in only 2 dimensions. The PCA analysis, as represented in Figure 3.2, showed that fibres can be gathered according to fabrication lot. The first principal component was well correlated to 2-pentanone, nitropropane, pyridine, 1-pentanol, and octane, in order of contribution respectively.

The loadings of all the compounds to factor one were approximately the same. Comparing Figure 3.1 and Figure 3.2, it can be observed that as the extracted amounts increase (Figure 3.1), the further the treatment will be located on the left of the graph on Figure 3.2. Additionally, it was determined that the second component was only significantly correlated with benzene. Therefore, the differences observed for lot B in the y-axis (factor two) are due to dissimilar amounts of benzene extracted for each fibre, which skews the graph upwards. These results are consistent with the one-way ANOVA test performed for lot B fibres: fibres 4 to 6 are statically identical, with slight variations observed for benzene. At a 95% level of confidence, it was found that there is a 4.7% probability that differences observed in the amounts of benzene extracted for fibres of lot B are not due to random errors. Conversely, a one-way ANOVA evaluation of lot A showed statistically significant differences among fibres 1 to 3, while Student's t test of fibres 2 and 3 demonstrated that these fibres are statically identical. Analysis between Figure 3.1 and Figure 3.2 shows that differences between fibres 1, 2 and 3 are due to amounts extracted of the compounds that characterize the first principal component.

Table 3.4 summarizes the intra-lot reproducibility, as well as the one-way ANOVA evaluation for lots A and B. Despite the statistical results obtained by one-way ANOVA for lot A presenting apparent differences, the intra-lot reproducibility for all compounds was below 3%. Also, as shown in Table 3.3, the intra-fibre reproducibility of fibres from lot A is lower than 4% for all the McReynolds probes. Since the intra-fibre and the intra-lot reproducibility for lot A and B are significantly lower than their corresponding inter-fibre repeatability (all the fibres), it can be established that variations found using the randomized block design are related to variations on the coating manufacturing from lot to lot. It was also observed that the differences between fibres are larger for polar compounds such as 1-pentanol.

Table 3.4 Intra-lot repeatability lots A and B. RSD is the relative standard deviation for the intra-lot repeatability of three fibres (n=7). One-way ANOVA; F*cal* is the F-ratio for the different treatments evaluated (different fibres) and F*crit* is the critical value of F for 21 experiments at a 95% level of confidence.

Compounds	LOT A		LOT B		F_{crit}
	RSD	F_{cal}	RSD	F_{cal}	
Benzene	0.3	0.13	1.7	3.63	3.55
2-Pentanone	2.2	4.90	0.9	0.48	
Nitropropane	0.7	0.91	0.7	2.03	
Pyridine	1.4	7.28	1.0	1.74	
1-Pentanol	1.0	4.87	1.2	3.01	
Octane	0.2	0.37	0.1	0.02	

In addition, smaller variations were observed for non-polar compounds as well. Since PDMS is the most common non-polar absorbent in SPME, it has affinity towards non-polar compounds. Hence, small variations on polymer coatings from lot to lot would have a larger impact on the amounts extracted from compounds with high polarity. However, it is important to note that results described in this study are only valid for these specific lots; it cannot be generalized that the same effects would be observed from any lot to lot. Thus, it was established that although randomized block designs and ANOVA are very powerful tools to detect small variations on the results, careful observation of results must follow before rejecting any fibre.

For instance, most EPA methods consider that the maximum acceptable level for RSD falls within 15-25%;[1] selecting the fibres with smaller inter-fibre repeatability is desirable in order to guarantee that scattering observed is mostly due to variations on the sample, and not to the sampling device[22].

3.1.3.3 Evaluation of six commercial 50/30µm DVB/CAR/PDMS fibres

Six 50/30 µm DVB/CAR/PDMS fibres from the same lot were evaluated using a randomized block design (refer to Figure 3.3). Although all 6 fibres were from the same batch, they were acquired in two different boxes and labeled: 1 to 3 for box 1, and 4 to 6 for box 2.

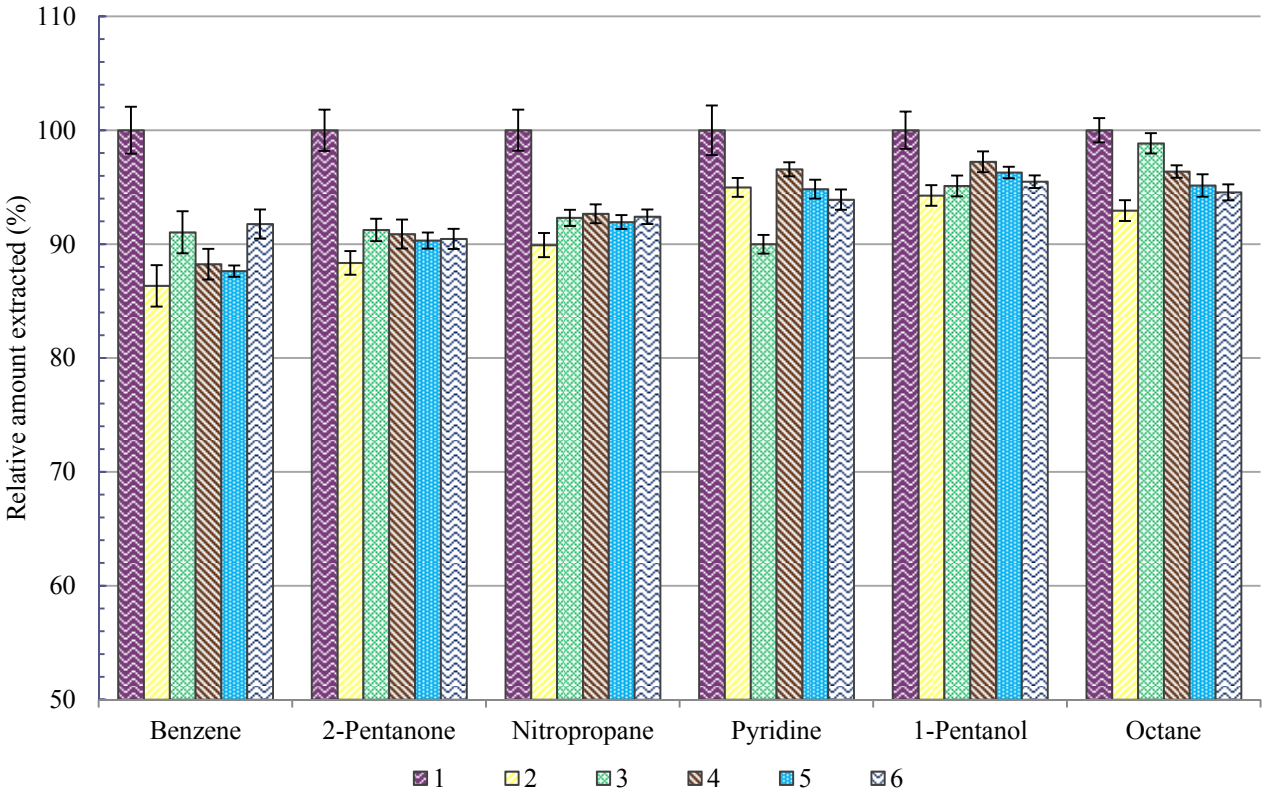


Figure 3.3 Evaluation of the intra- and inter-fibre repeatability of six 50/60 µm DVB/CAR/PDMS fibres using a randomized block design. Error bars represent the standard deviation of the mean (n = 6).

Table 3.5 Statistical evaluation of the inter-fibre repeatability of 6 DVB/CAR/PDMS fibres using a randomized block design. F*injection* is the F-ratio for the randomization of the injection. F*fibre* is the F-ratio for the different treatments evaluated (different fibres) and F*crit* is the critical value of F for 36 experiments at a 95% level of confidence. RSD is the relative standard deviation for the inter-fibre repeatability of six fibres (n=6).

Compounds	Benzene	2-Pentanone	Nitropropane	Pyridine	1-Pentanol	Octane
F*injection*	0.11	1.36	0.17	0.62	1.35	0.68
F*fibre*	62.81	80.55	57.32	50.75	28.85	58.15
F*crit*			2.60			
RSD	5.2	4.3	3.6	3.4	2.2	2.7

Table 3.6 Intra-fibre and inter-fibre repeatability of six 50/60 µm DVB/CAR/PDMS fibres evaluated using a randomized block design. RSD is the relative standard deviation for the inter-fibre repeatability of six fibres (n=6).

Compounds	Intra-fibre repeatability (RSD)						RSD
	1	2	3	4	5	6	
Benzene	2.1	2.1	2.0	0.8	0.6	1.0	5.2
2-Pentanone	1.8	1.2	1.1	1.8	0.8	1.0	4.3
Nitropropane	1.8	1.2	0.8	1.1	0.7	0.7	3.6
Pyridine	2.2	0.9	0.9	0.7	0.9	1.0	3.4
1-Pentanol	1.6	1.0	1.0	1.0	0.5	0.6	2.2
Octane	1.1	1.0	0.9	0.7	1.0	0.7	2.7

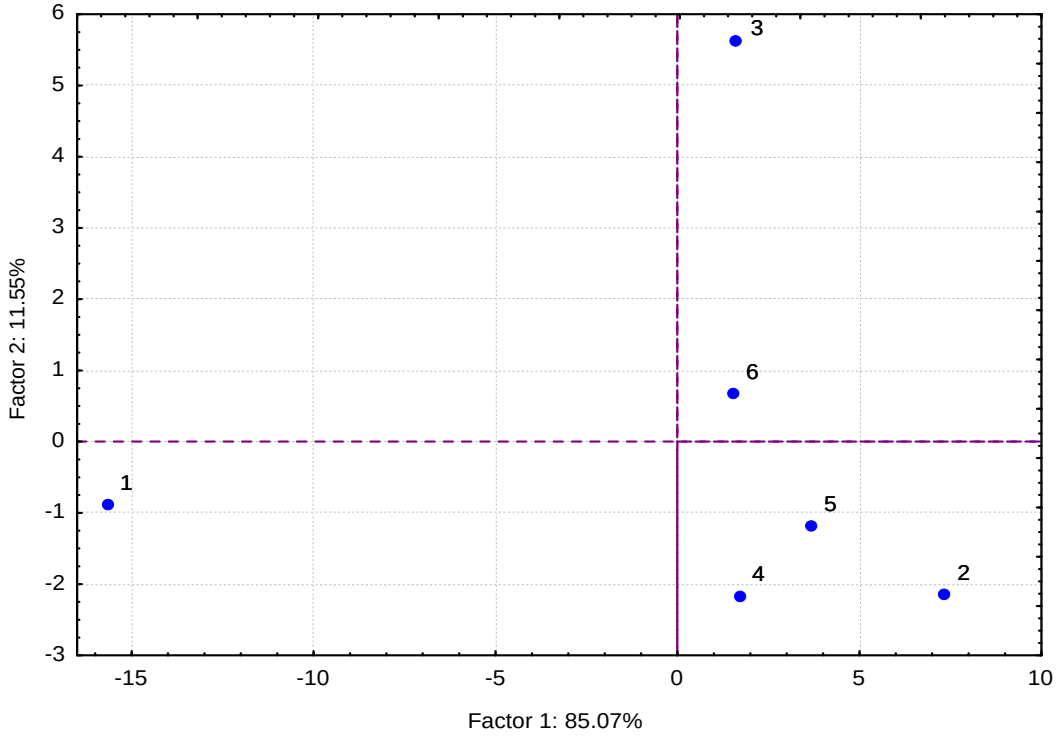


Figure 3.4 Principal component analysis of the average amount extracted by six 50/60 μm DVB/CAR/PDMS fibres using a randomized block design.

As can be seen from Table 3.5, statistical evaluation of the data showed no effect for the randomized extractions from the vial. Moreover, as shown in Table 3.6, the intra-fibre reproducibilities, expressed as RSDs (%, n=6), were below 2.2% in all cases. Similarly, the inter-fibre RSD was lower than 5.2%. Nevertheless, statistical differences were found in amounts extracted for each probe (for each fibre) using a randomized block design (refer to Table 3.5). Likewise, in the analysis of data obtained for PDMS fibres, the randomized design distinguished that fibres were statistically different due to the low intra-fibre RSD.

It was determined that two principal components explained more than 96% of the total variance in the data[78]. Factor one is well correlated to benzene, 2-pentanone and nitropropane. The loading of benzene to factor one is approximately double of that of nitropropane, while

slightly larger than 2-pentanone. By comparing Figure 3.3 and Figure 3.4, it can be observed that as the extracted amount increases, the treatment is located further towards the left on Figure 3.4. Factor two, on the other hand, is well correlated to octane and pyridine, which has the major loading to factor two. Therefore, differences observed in the y-axis of the PCA plot are primarily due to different pyridine amounts extracted by each fibre. This trend increases downwards in the graph (Figure 3.4). According to our results, 1-pentanol and octane do not explain variability among fibres. Looking at Figure 3.3, it can be seen that smaller differences among fibres occur for 1-pentanol and octane. This agrees with the observations found by PCA; the low inter-fibre reproducibility for these compounds can be seen on Table 3.6.

Although fibres 4, 5 and 6 seemed to be similar, a one-way ANOVA evaluation showed that statistically, these fibres do not extract the same amount of analytes, with the exception of nitropropane and 2-pentanone. Student's t test demonstrated that fibres 4 and 5 are statistically similar, with slight variations for pyridine and octane. On the other hand, Student's t test of Fibres 4 and 6 only showed similarity in amounts of nitropropane and 2-pentanone. Other arrangements among fibres from both boxes did not yield any agreement from a statistical point of view.

Moreover, intra-fibre reproducibilities in the range of 1.2 to 2.6% can be achieved by eliminating fibre one from the set of fibres (refer to Table 3.7). Despite the fact that statistical tools showed "significant" differences for the remaining 5 fibres, the intra-fibre reproducibility is acceptable. As shown in Table 3.7, fibres from the second box are more reproducible. Indeed, a contrast of fibre 1 and fibre 2 provided intra-fibres reproducibilities up to 10.4% for benzene.

Table 3.7 Intra-box repeatability for boxes 1 and 2. RSD is the relative standard deviation for the intra-box repeatability of three fibres (n=6). RSD_{2-6} is the intra-fibre reproducibility for all the fibres but fibre 1 (n=6). RSD_{1-2} is the intra-fibre reproducibility for fibres 1 and 2.

Compounds	RSD_1	RSD_2	RSD_{2-6}	RSD_{1-2}
Benzene	7.5	2.5	2.6	10.4
2-Pentanone	6.5	0.3	1.3	8.8
Nitropropane	5.6	0.4	1.2	7.5
Pyridine	5.3	1.4	2.6	3.6
1-Pentanol	3.2	0.9	1.2	4.2
Octane	3.9	1.0	2.3	5.2

In summary, major dissimilarities were observed in more volatile compounds. This trend may be associated with differences in the manufacturing process of the fibres evaluated. Since the affinity of carboxen towards volatile compounds is higher than DVB, the differences observed might be related to variations in the layer ratio of the fibres. However, a clear correlation between the irregularities found on the physical inspection of the fibres (mainly non-uniform coating) and the data obtained in this study was not found. Nevertheless, it should be noted that physical inspection allows the user to identify common manufacturing problems on fibres such as fibre cracks and improperly attached fibres.

3.1.3.4 Evaluation of seven commercial 65 µm DVB/PDMS fibres

A randomized block design was used to compare seven 65 µm DVB/PDMS fibres from three different lots (refer to Figure 3.5). Table 3.8 presents the statistical evaluation of the data. Although an effect (variance) for randomized extraction from the vial was not found, significant differences among fibres were observed as inter-fibre reproducibility varied between 5.2 and 9.6 % (refer to Table 3.9). As can be seen from Table 3.10, these results are in agreement with the low intra-lot reproducibility observed for lot A. Moreover, as shown in Table 3.9, intra-fibre reproducibilities, expressed as RSDs (%, n=5) in this case, were below 3.5% in nearly all the cases.

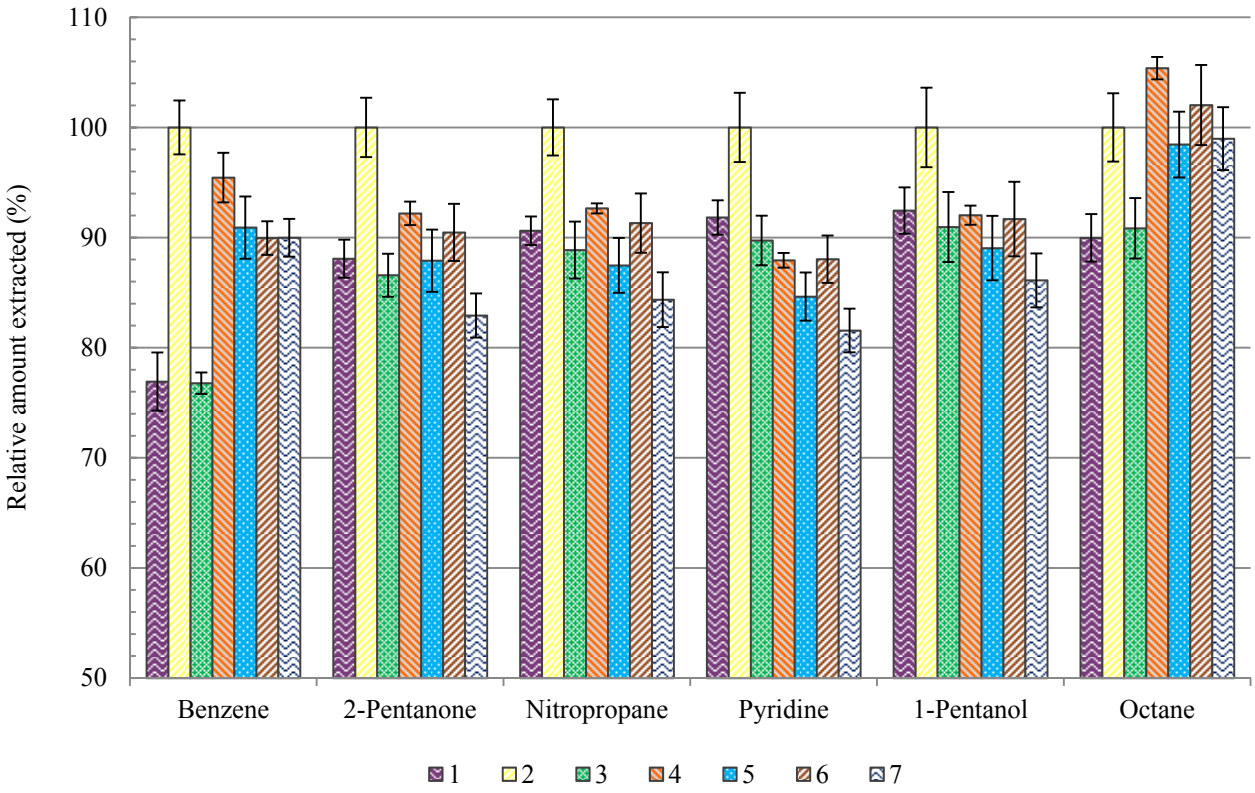


Figure 3.5 Evaluation of the intra- and inter-fibre repeatability of seven 65 µm DVB/PDMS fibres using a randomized block design. Error bars represent the standard deviation of the mean (n = 5).

Table 3.8 Statistical evaluation of the inter-fibre repeatability of 7 DVB/PDMS fibres using a randomized block design. F*injection* is the F-ratio for the randomization of the injection. F*fibre* is the F-ratio for the different treatments evaluated (different fibres) and F*crit* is the critical value of F for 35 experiments at a 95% level of confidence. RSD is the relative standard deviation for the inter-fibre repeatability of six fibres (n=5).

Compounds	Benzene	2-Pentanone	Nitropropane	Pyridine	1-Pentanol	Octane
F*injection*	2.06	2.71	2.67	2.24	2.01	0.29
F*fibre*	76.94	28.05	22.28	28.70	13.07	27.50
F*crit*			2.74			
RSD	9.6	6.1	5.6	6.5	5.2	6.00

Table 3.9 Intra-fibre and inter-fibre repeatability of seven 65 µm DVB/PDMS fibres evaluated using a randomized block design. RSD is the relative standard deviation for the inter-fibre repeatability of six fibres (n=5).

Compounds	Intra-fibre repeatability (RSD)							RSD
	1	2	3	4	5	6	7	
Benzene	3.4	2.4	1.3	2.4	3.1	1.7	1.9	9.6
2-Pentanone	2.0	2.7	2.3	1.2	3.2	2.9	2.4	6.1
Nitropropane	1.4	2.6	2.9	0.5	2.9	3.0	3.0	5.6
Pyridine	1.7	3.2	2.5	0.8	2.6	2.4	2.4	6.5
1-Pentanol	2.3	3.6	3.5	1.0	3.3	3.7	2.8	5.2
Octane	2.4	3.1	3.0	1.0	3.0	3.6	2.9	6.0

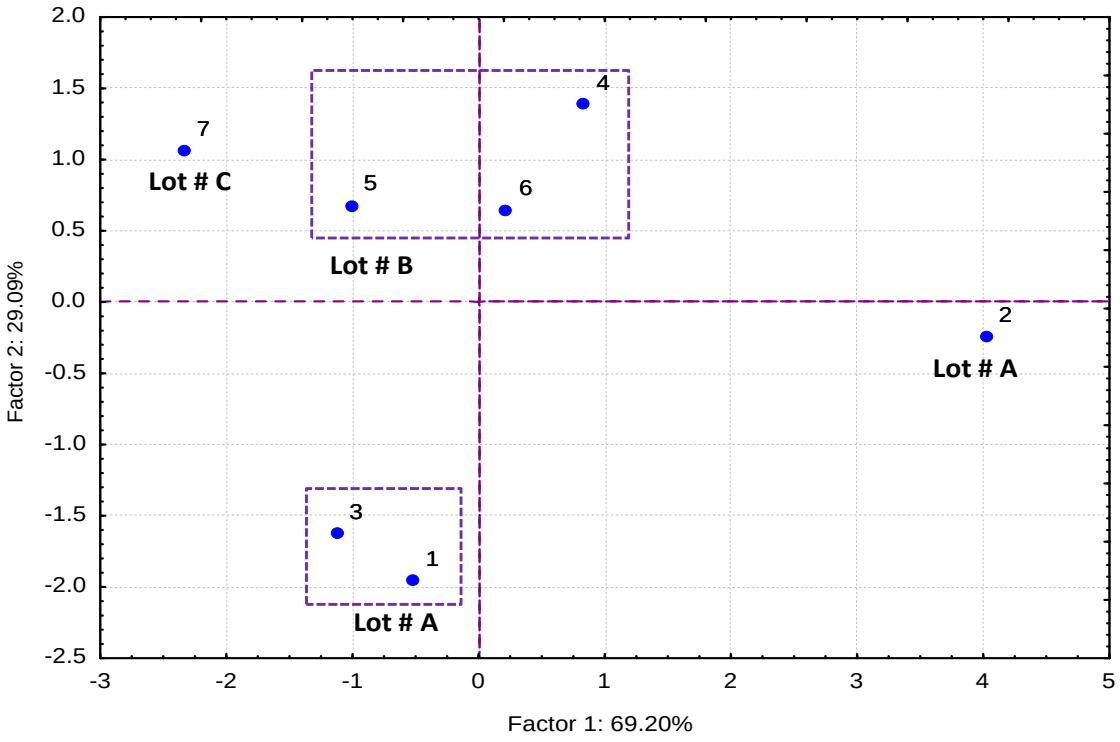


Figure 3.6 Principal component analysis of the average amount extracted by seven 65 µm DVB/PDMS fibres using a randomized block design.

The PCA analysis, presented in Figure 3.6, showed that only fibres from lot B could be grouped. Following the procedure described by Heberger *et al.*,[78] it was determined that 2 principal components explained more than 98% of the total variance in the data. The first factor is well correlated to 2-pentanone, nitropropane, 1-pentanol and pyridine. The loadings of all the compounds to factor one were approximately the same. By comparing Figure 3.5 and Figure 3.6, it can be observed that as extracted compound amounts increase, the further the treatment will be located at the right on the PCA plot. Furthermore, the second principal component is well correlated to the amount extracted of octane and benzene. Therefore, the differences observed on the y-axis of the PCA plot are mainly due to different amounts of non-polar compounds extracted by each fibre. As can be seen, this trend increases upwards in the graph; as previously mentioned, a PCA plot allows us to easy perceive differences on lot A.

Table 3.10 Intra-lot repeatability of lots A and B. RSD is the relative standard deviation for the intra-lot repeatability of three fibres (n=5). One-way ANOVA; F*cal* is the F-ratio for the different treatments evaluated (different fibres) and F*crit* is the critical value of F for 15 experiments at a 95% level of confidence. RSD_{-2} is the relative standard deviation of fibres 1 and 3. RSD_{-4} is the relative standard deviation of fibres 5 and 6; t_{cal} is the Student's t calculated for two fibres assuming equal variance (n=5); t_{crit} is the two tail *t* critical value for 8 degrees of freedom at a 95% level of confidence.

Compounds	LOT A				LOT B				F_{crit}	t_{crit}
	RSD	RSD_{-2}	F_{cal}	t_{cal}	RSD	RSD_{-4}	F*cal*	t_{cal}		
Benzene	15.8	0.1	192.28	0.11	3.2	0.8	8.39	0.66	3.89	2.31
2-Pentanone	8.0	1.2	57.68	1.29	2.4	2.0	4.43	1.50		
Nitropropane	6.3	1.4	36.20	1.36	3.0	3.0	7.91	2.34		
Pyridine	5.8	1.6	25.32	1.70	2.2	2.8	5.68	2.47		
1-Pentanol	5.1	1.2	12.77	0.87	1.8	2.1	1.93	1.32		
Octane	5.9	0.7	21.23	1.50	3.4	2.5	7.81	1.71		

A thorough assessment of the data is presented on Table 3.10. As can be observed, a one-way ANOVA analysis and the Student's t test analysis both established that the major source of variation on lot A is fibre number 2. By excluding fibre two, it was found that fibres 1 and 3 perform statistically alike. Likewise, an ANOVA analysis performed on lot B also exposed statistical differences among fibres, although these differences were less significant than the ones observed in lot A. When comparing fibres 5 and 6, the calculated *t* value is s higher for nitropropane and pyridine when compared to the critical value (t_{crit}). Since no statistically significant differences were observed for the remaining compounds, it can be suggested that these fibres are statistically similar. In summary, DVB/PDMS fibres gave the worst inter-fibre reproducibility when compared to other coatings evaluated in this study. Additionally, significant differences in non-polar compounds were observed among lots.

3.1.3.5 Evaluation of six commercial 85 µm CAR/PDMS fibres

Six FFA 85 µm CAR/PDMS fibres from three different lots were evaluated using a randomized block design. Fibres 1 to 3 belong to lot A, fibre 4 belongs to lot C, and fibres 5 and 6 belong to lot B.

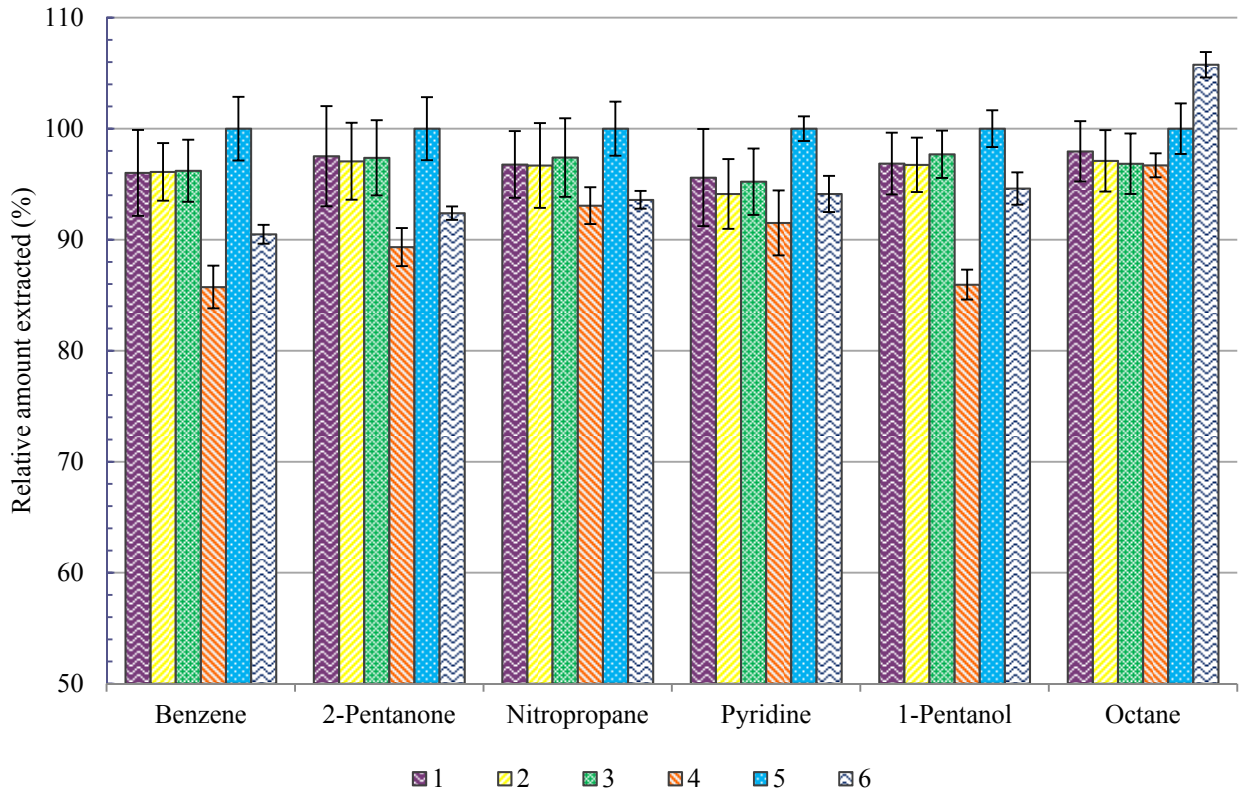


Figure 3.7 Evaluation of the intra- and inter-fibre repeatability of seven 65 µm DVB/PDMS fibres using a randomized block design. Error bars represent the standard deviation of the mean (n = 5).

Table 3.11 Statistical evaluation of the inter-fibre repeatability of 85 µm CAR/PDMS fibres using a randomized block design. F*injection* is the F-ratio for the randomization of the injection. F*fibre* is the F-ratio for the different treatments evaluated (different fibres) and F*crit* is the critical value of F for 30 experiments at a 95% level of confidence. RSD is the relative standard deviation for the inter-fibre repeatability of six fibres (n=5).

Compounds	Benzene	2-Pentanone	Nitropropane	Pyridine	1-Pentanol	Octane
F*injection*	1.58	1.38	0.74	1.25	2.80	1.15
F*fibre*	20.05	10.14	5.13	6.27	31.63	13.94
F*crit*			2.84			
RSD	5.7	4.8	3.7	3.9	5.2	3.9

Table 3.12 Intra-fibre and inter-fibre repeatability of six 85 µm CAR/PDMS fibres evaluated using a randomized block design. RSD is the relative standard deviation for the inter-fibre repeatability of six fibres (n=5).

Compounds	Intra-fibre repeatability (RSD)						RSD
	1	2	3	4	5	6	
Benzene	4.0	2.7	2.9	2.2	2.9	0.9	5.7
2-Pentanone	4.6	3.6	3.5	1.9	2.8	0.7	4.8
Nitropropane	3.1	4.0	3.6	1.8	2.4	2.4	3.7
Pyridine	4.6	3.3	3.1	3.2	1.1	1.7	3.9
1-Pentanol	2.9	2.5	2.2	1.6	1.7	1.5	5.2
Octane	2.8	2.9	2.8	1.1	2.3	1.1	3.9

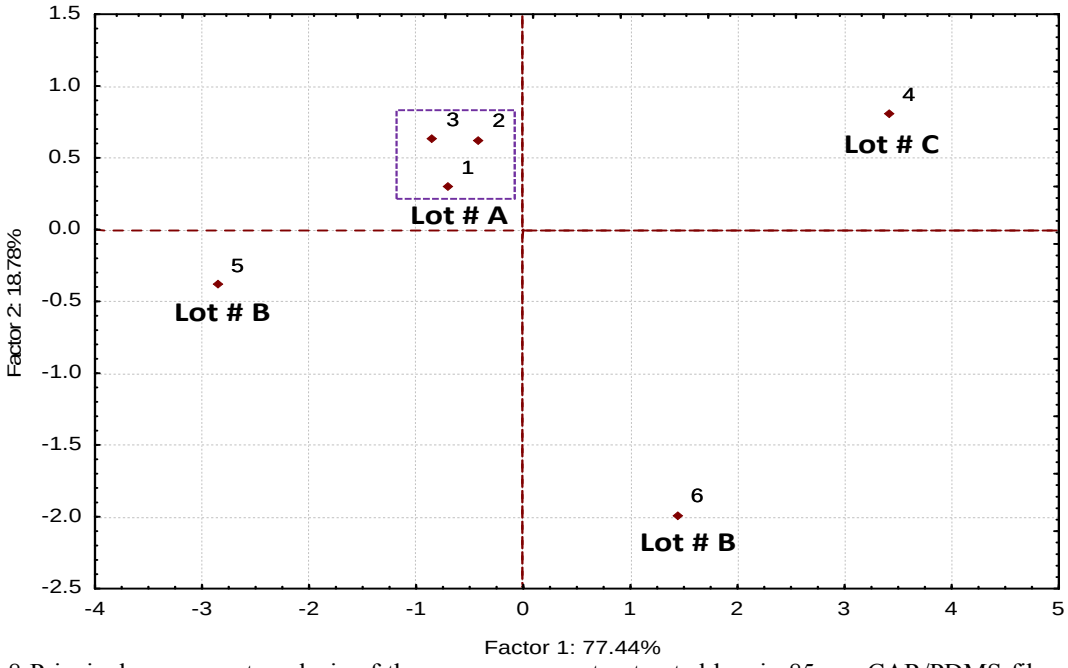


Figure 3.8 Principal component analysis of the average amount extracted by six 85 µm CAR/PDMS fibres using a randomized block design.

As can be seen from Table 3.11, no significant variations of the vial headspace concentration were found during evaluation of the fibres for all the McReynolds probes. Also, as shown in Table 3.12, intra-fibre reproducibilities, expressed as RSDs (%, n=5), were significantly below 5% for most cases. In addition, inter-fibre reproducibilities lower than 5.7% were obtained. In this experiment, fibres 4 and 6 were found to be the major source of variability; by rejecting these fibres from the set, inter-fibres reproducibilities lower than 2.7% can be achieved. These findings are well supported by the one-way ANOVA of the data presented on Table 3.13.

The PCA analysis led to the computation of two principal components having the initial eigenvalues >1, which contributed to 96.18% of the total variance of the data set (refer to Figure 3.8). The first principal component, identified as a linear combination of all the compounds, with the exception of octane, accounted for 77.44 % of the variance.

Table 3.13 Intra-lot repeatability of lots A and B. RSD is the relative standard deviation for the intra-lot repeatability of three fibres for lot A and 2 fibres for lot B (n=5). One-way ANOVA; F*cal* is the F-ratio for different treatments evaluated (different fibres) and F*critA* is the critical value of F for 15 experiments at a 95% level of confidence. t_{cal} is the Student's t calculated for two fibres assuming equal variance (n=5); t_{critB} is the two tail *t* critical value for 8 degrees of freedom at a 95% level of confidence; RSD_g is the relative standard deviation for inter-fibre repeatability after rejecting fibres 4 and 6. F*calg* is the F-ratio for the different treatments evaluated (4 different fibres, lot A plus fibre 5) and F*critg* is the critical value of F for 20 experiments at a 95% level of confidence.

Compounds	LOT A			LOT B			RSD_g	F_{calg}	F_{critg}
	RSD	F_{cal}	F_{critA}	RSD	t_{cal}	t_{critB}			
Benzene	0.1	0.01	0.01	7.1	7.10	2.31	2.0	2.00	3.24
2-Pentanone	0.2	0.02	0.02	5.6	5.86		1.4	0.70	
Nitropropane	0.4	0.06	0.06	4.7	-6.58		1.6	1.15	
Pyridine	0.8	0.23	0.23	4.3	6.66		2.7	3.42	
1-Pentanol	0.5	0.22	0.22	3.9	5.46		1.5	2.17	
Octane	0.6	0.23	0.23	4.0	-5.06		1.5	1.48	

The loadings of all the compounds to factor one were approximately the same. By comparing Figure 3.7 and Figure 3.8, it can be observed that as compound amounts increase, the treatment is located further to the left on the PCA plot. Conversely, the second principal component explained 18.8% of the variance and, was mainly represented by octane. Consequently, differences observed on the y-axis of the PCA plot can be mainly attributed to different amounts of octane extracted by each fibre. This observation clarifies why fibres 1 to 5 are statistically identical in regards to octane amounts extracted.

Although lot A demonstrated excellent intra-lot repeatability, inter-lot and intra-lot variability were also observed for the six CAR/PDMS fibres evaluated on this study. These findings are in agreement with those reported by Paschke *et al.* and Setkova *et al.* [19,40] It is well known that fibres containing carboxen have a higher affinity towards volatile compounds as compared to other coatings, and that affinity decreases as the analyte boiling point increases. [1,59] Besides, absorbent coatings, such as PDMS, display better behaviour towards heavier compounds.[1,79-81] A plausible explanation for the differences observed in the extracted amount of heavier compounds could be due to a different ratio of PDMS-CAR particles in fibre 6 as

compared to the others fibres studied. However, the differences presented in the present study are significantly less pronounced when compared to previous studies.[1,82]

3.1.3.6 Protocol for quick assessment of commercial SPME coatings

Based on the results obtained in this chapter, a protocol for the quick assessment of commercial SPME coatings was developed. Figure 3.9 summarizes the five steps that should be taken whenever the need to assess SPME coatings is present: a) physical inspection of the fibre coating and mechanical inspection of the FFA device; b) Fibre conditioning and stabilization; c) Fibre evaluation; d) Statistical evaluation, and e) Decision.

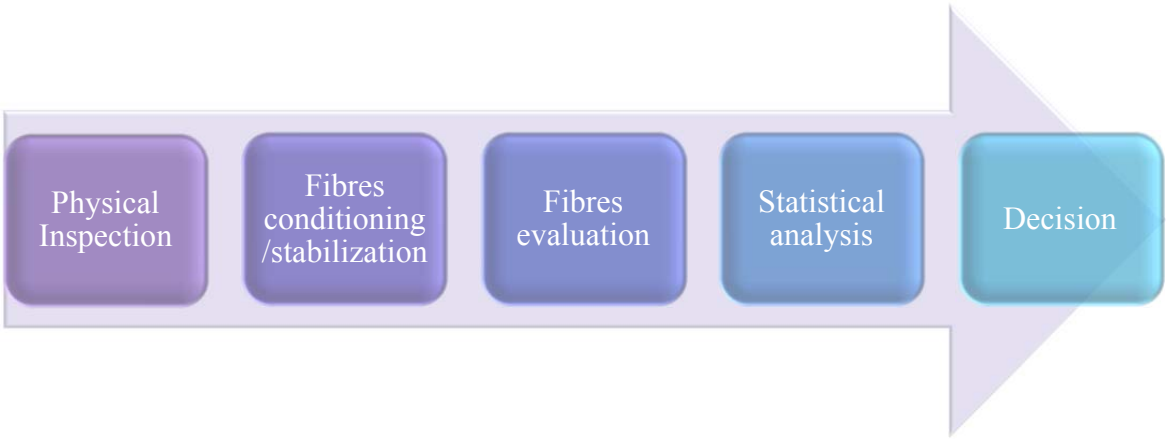


Figure 3.9 Scheme of the protocol for quick assessment of commercial coatings

A. Physical Inspection of the fibre coating and mechanical inspection of the FFA device

Prior to conditioning the fibres, it is important to check the mechanical quality of the FFA devices (see Figure 1.1). Use a magnetic plunger as shown in Figure 3.12 to move up and down the fibre-attachment needle. If any resistance is observed, avoid using this device and contact the manufacturer.

i) Place the FFA device under the microscope and observe if any anomalies are present on the surface of the fibre coating. Since diverse types of irregularities might be found on DVB/CAR/PDMS fibres, this type of coating was chosen as a model. Typically, anomalies can be defined as: a) fibre cracking; b) non-uniform coating (including surfaces irregularities due to over coating or lack of coating), and c) fibre misalignment. Note that other irregularities may also present themselves on the fibre coating after fibre analysis starts, even if such was not observed at first. Thus, it is important to highlight that fibre coating should be observed under a microscope regularly throughout analysis to ensure it is still viable. Figure 3.10 shows an ideal DVB/CAR/PDMS coating schematic, while Figure 3.11 demonstrates typical coating inspection procedure being observed. Coating examination procedure should follow these steps: a) inspect the entire fibre coating surface by rotating the FFA body 360º (inset A and B from Figure 3.11); b) observe the fibre tip (inset C from Figure 3.11); and c) observe the fibre bottom (inset D and E from Figure 3.11). The first step allows the analyst to discover frequent anomalies such as non-uniform coating (inset A and D) as well as coating cracks. Second and third stages are useful to find tip scratches (inset C) and fibre misalignment (inset E), respectively.

However, although the anomalies described might be correlated to bad inter-fibre reproducibility, it is important to emphasize that physical inspection can only identify possible trends in the results. Thus, fibres should not be excluded at this point. Consequently, fibre evaluation using the in-vial standard is needed prior to making any decision regarding its usability.

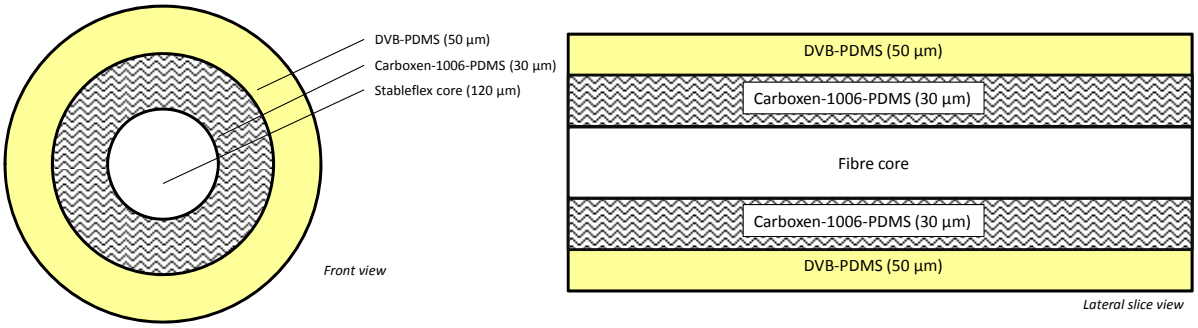


Figure 3.10 Schematic of an ideal 50/30 µm DVB/CAR/PDMS SPME fibre

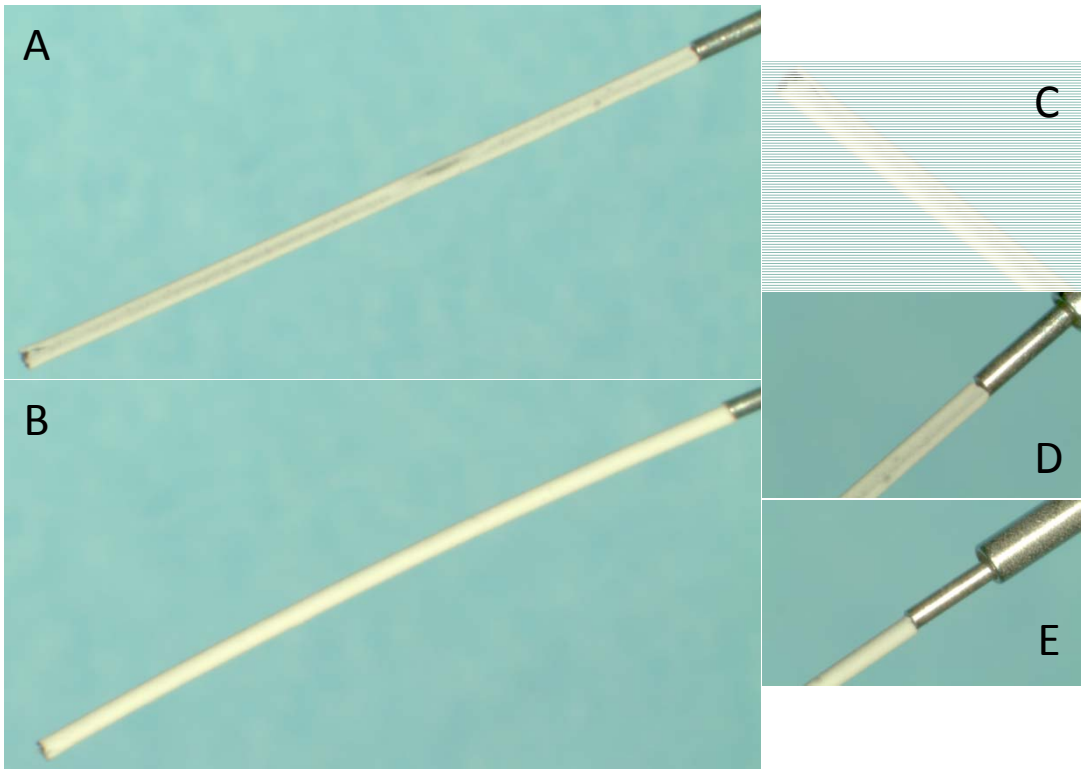


Figure 3.11 Physical inspection of a 50/30 µm DVB/CAR/PDMS. A. Entire fibre; B. Entire fibre (rotated 180º); C. Tip of the fibre; D. Bottom of the fibre; E. Bottom of the fibre (rotated 90º).

B. Fibre conditioning and stabilization

i) Condition the fibres according to manufacturer specifications. Table 1.1 lists the temperatures recommended for each coating.

It is recommended that at least ten extraction/injection cycles are performed from an alternative standard vial prior to fibre evaluation; it has been reported that fibre stability, in terms of amount extracted, is reached after 5-10 SPME cycles.[19]

C. Fibre evaluation

i) Place the in-vial standard generator on the autosampler agitator and adjust the temperature to 35 ºC.

ii) Together with the in-vial standard generator, place an additional in-vial standard generator in a different position in the agitator, and use this for the evaluation of the instrument performance evaluation. This vial should be labeled as the QC vial (quality control vial). This step must be completed in conjunction with the previous stage, and at least 2 hours prior to the next step.

iii) Write your method in the Maestro software of the GERSTEL MPS2 autosampler. Follow the parameters listed on section 3.1.2.2.

iv) Create the GC-MS sequence in the Maestro software of the GERSTEL MPS2 autosampler. When creating the sequence, 5 QC tests (extractions from the QC vial of 30 seconds using a 100 μm PDMS fibre) must be included at the beginning and at the end of the sequence, in order to account for instrument response drifts. To assure that differences observed among fibres are not due to variations in the in-vial standard gas system, a randomized block design must be used. Additionally, a QC test should be run between experimental blocks.

v) Randomize fibres on the MFX-25 tray and verify that positions created on the sequence are correlated with those on the tray.

vi) Confirm that the GC/MS and the autosampler are ready. Then start the sequence.

vii) Once the sequence is finished, review the results and verify if instrument drifts occurred. If so, use a QC test between experimental blocks and use this data to correct for the results of each fibre.

viii) Proceed to the statistical analysis of the results.

D. Statistical analysis

i) Calculate amounts extracted of each analyte for each fibre on every experimental run. Determine the average amounts extracted for each fibre, and calculate the standard deviation and relative standard deviation for each fibre.

ii) Compute the intra-fibre and inter-fibre reproducibility.

iii) Evaluate the intra-lot and inter-lot reproducibility.

iv) Plot the relative amounts extracted from every probe for each coating on a single graph.

v) Use the randomized block design data to perform an ANOVA and determine with a 95% level of confidence whether differences observed among fibres are statistically significant.

vi) Use PCA to determine possible clusters and result trends. Compare the results obtained by PCA with those obtained on step number three.

E. Decision

i) Select fibres that were found to be statistically similar on the previous step. Proceed to develop your application.

ii) If any fibre was found to have initial serious surface abnormalities (as described on physical evaluation), and the statistical evaluation shows significant differences, contact the manufacturer.

3.1.4 Conclusions

It must be emphasized that inter-fibre variability is a serious limitation in the future development and application of SPME in areas where multiple fibres are required, such as food, environmental, forensic analysis, metabolomics and *in vivo* applications.[80,83-86] Thus, a standardized protocol consisting of a new standard vial gas generator for quick assessment of commercial SPME fibre reproducibility is presented. This approach allows the user to determine whether a number of fibres can assure the acquisition of reliable and reproducible results for applications where analysis of compounds with different physicochemical properties is required. Since fibre-to-fibre variability was observed for almost all the coatings, use of this methodology by SPME users prior to the development of an application is imperative. Otherwise, each fibre needs to be considered a particular sampling device, and be characterised individually depending on the required accuracy. Additionally, it is highly recommended that, as a standard, manufacturers perform this evaluation preceding shipments. Thus, the inclusion of a certification test for fibres emitted by the manufacturer, similar to the procedure adopted by manufacturers of chromatographic columns, should be considered.

3.2 Determination of the experimental sampling rates of SPME for passive sampling using the in-vial standard gas generator

3.2.1 Introduction

Due to the growing number of SPME applications being developed for air sampling, a new gas standard generation system needs to be considered. In order to satisfy current market needs, it should be easy to manufacture, inexpensive, and contain a reliable calibration method for the sampling device at different temperatures.[87,88] There has been prior exploration on the use of viscous liquids, such as pump oil or silicone oil doped with chemical substances, being used as a mechanism to load standards on a SPME fibre. [19,20,60,89,90] Recently, Sheehan *et al.* used silicone oil for the passive release of chlorinated volatile organics (cVOCs); the aim of this study was determining the experimental sampling rates of these compounds using a CAR/PDMS fibre.[89] However, the method developed has limitations; for instance, silicone oil is a non-polar solvent. Consequently, a low concentration of polar compounds cannot be easily achieved in the headspace. In addition to the limited capacity of silicone oil, splashing of oil onto the fibre may occur if the vial containing pump oil is not handled carefully. In this study, a new in-vial standard gas generator, consisting of vacuum pump oil doped with standards and subsequently mixed with SDVB particles, is presented for the determination of experimental sampling rates. By using XAD-4 particles, not only a decrease in the headspace analyte concentration can be achieved, but the total analyte capacity of the pump oil is enhanced as well. As a general rule, the in-vial standard gas generator buffers the analyte concentration in the headspace. For this study, BTEX were used as model compounds in this proof-of-concept evaluation since, as shown in Chapters 2 and 3, in-vial applicability to analytes with a wide range of functionalities, such as McReynolds probes, is plausible.

3.2.2 Experimental section

3.2.2.1 Materials and reagents

HPLC grade methanol was obtained from Caledon laboratories LTD (Georgetown, ON, Canada). Benzene, ethylbenzene, toluene, o-xylene and the styrene-divinylbenzene particles (Amberlite® XAD4) were purchased from Sigma-Aldrich (Mississauga, ON, Canada). The vacuum-pump oil, General Purpose (GP) mechanical pump oil, was supplied by Varian Vacuum Technologies (Lexington, MA). Vials, screw top, 40 mL size and caps with 22 mm PTFE/silicone septa were purchased from Sigma-Aldrich (Mississauga, ON, Canada). Mininert® Valve and screw thread, for use with 18/400 mm thread, and 15 mL clear glass vials with screw top were also obtained from Sigma-Aldrich. Pure water was obtained using a Barnstead/Thermodyne NANO-pure ultrapure water system (Dubuque, IA, USA). Helium of ultra-high purity was supplied by Praxair (Kitchener, ON, Canada). The desiccator and magnetic bars were supplied by Fisher Scientific (Ottawa, ON, Canada). A temperature controller and thermocouples were obtained from Omega Engineering (Stamford, CT, USA). A hot plate stirrer was obtained from VWR Scientific (Mississauga, ON, Canada). Commercial SPME-Fast Fit Fibre Assembly (FFA) fibres used in this study polydimethylsiloxane (PDMS, 100 μm) and carboxen/polydimethylsiloxane (CAR/PDMS, 85 μm) were also provided by Sigma-Aldrich. Both types of fibres were conditioned according to manufacturer's recommendation prior to their use. All preparations were carried out in a ventilated fume hood. A magnetic plunger was built at the University of Waterloo machine shop in order to control the diffusion path of the FFA fibre. Rare-earth magnets employed in the manufacturing of the plunger were acquired at Lee Valley (Waterloo, Ontario, Canada). Gas tight syringes (1 and 5 mL) were purchased from Hamilton Company (Reno, NE, USA)

3.2.2.2 Instrumentation

An Acme 6100 series gas chromatograph (Young Lin Instruments, Anyang, Korea) equipped with a flame ionization detector (FID), and a capillary column (RTX-5, 30 m × 0.25 mm I.D., 0.25 μm film thickness) was used for the separation and detection of the BTEX. The oven temperature was initially held at 40 ºC for 1 min, gradually increased to 180 ºC at a rate of 25 ºC min^{-1}, then held again for 2 min. An ATAS GL Optic 3 injection port (ATAS GL, Eindhoven, Netherlands) was used for liquid and SPME injections. The injector temperature was held at 250 and 300 ºC for PDMS and CAR/PDMS fibres, respectively. A custom-made heater block was manufactured by the University of Waterloo electronic shop in order to guarantee homogenous heating of the vial headspace (refer to Figure 3.12).

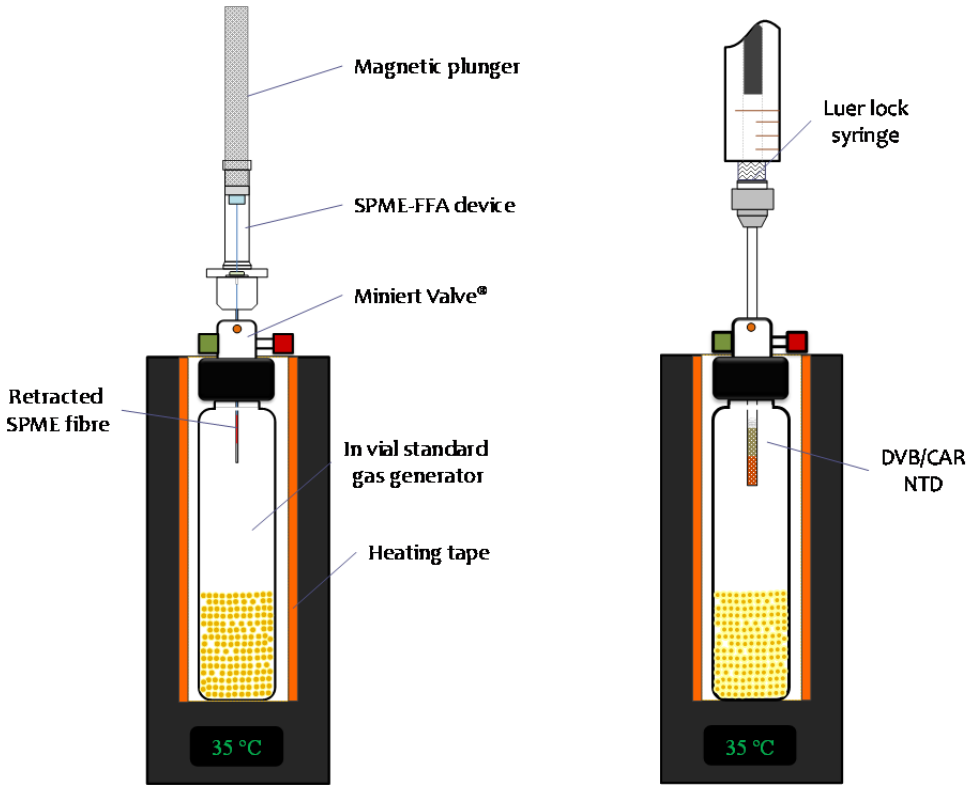


Figure 3.12 Schematic diagram of the set-up used for the determination of experimental sampling rates with the new in vial standard gas generator (left). Experimental set-up to determine the concentration on the vial headspace (right)

3.2.2.3 Preparation of the in-vial standard gas generator

A quantity of approximately 150 grams of vacuum pump oil was weighted into a 400 mL beaker. To remove possible impurities, the pump oil was heated at 120 °C under constant agitation (120 rpm), and maintained under nitrogen flow (10 ml min^{-1}) during 24 hours[21]. Then, with the pump oil at room temperature, ca. 32 grams were placed into a 40 mL screw top vial. To minimize evaporation, 2 µL of pure BTEX were spiked below the level of the solution present in the vial[60]. Once all the standards were added, the vial was capped, and then kept under continuous agitation at 1500 rpm for 48 hours, using a 0.25 inch long stir bar. Next, approximately 1.5 g of cleaned DVB/STY particles were weighted on a 15 mL screw top vial and mixed with approximately 3 g of the previously prepared pump oil containing the standards. Immediately, the Mininert® valve was capped, sealed with Parafilm® and then kept for a couple of days to equilibrate before its analysis. Four vials containing BTEX were developed for the current study. Finally, the prepared vials were placed in the heating block for a minimum of 3 hours before the start of the experiments.

3.2.2.4 Determination of experimental sampling rates

An 85 µm CAR/PDMS FFA-SPME fibre with a diffusion length adjusted at 0.147 cm was manually exposed to BTEX in the in-vial standard gas generator. Four diffusive sampling times, ranging from 15 to 60 min, were used to examine the effect of sampling duration on mass loading rates of BTEX. For these experiments, the heating block temperature was adjusted to 35ºC.

3.2.2.5 Determination of standard gas generator concentration

A multi-bed NTD, without side-hole and packed with 1 cm of carboxen 1000 (60/80 mesh) and 1 cm of DVB Haysep Q (100/120 mesh), was used to determine the headspace concentration of the in-vial standard gas generator. One mL of the headspace was withdrawn using a gas-tight syringe, and subsequently injected on the GC/FID. Figure 3.12 presents a schematic of the devices used for this purpose. As for SPME, the temperature of the heating block was maintained at 35 ºC.

3.2.3 Results and discussion

3.2.3.1 Determination of the headspace concentration in the in-vial standard gas generator

As previously mentioned in Chapter one, analytes in air samples can be collected by drawing air across a NT. The mass loading of the compounds can be controlled by adjusting the volume of air pulled through the NT. However, if the sample volume is small, for instance, the headspace of a 15 mL vial, using an exhaustive technique to determine the headspace concentration might result in significant depletion of the standards present in the vial. As a result, vial reusability becomes limited for methods such as the in-vial standard gas generator. Previously however, in Section 2.3.2 it was established that vials prepared from the same pump oil are reproducible among each other, therefore they can be exchanged without affecting results. Subsequently, in order to determine the concentration of the headspace, two vials containing the same amount of BTEX were prepared. Then, for the purpose of demonstrating that concentrations in both vials were equivalent, a CAR/PDMS fibre, with a diffusion path of approximately 0.147 cm, was exposed for 30 min to the headspace of both vials. The results are presented in Tables 3.14 and 3.15. As can be seen from Table 3.16, the statistical evaluation of

the results showed that, at a 95% level of confidence, the prepared vials were identical. Based on these results, three extractions of 1 mL using a gas-tight syringe were performed from the second vial to determine the headspace concentration. Table 3.17 displays the headspace concentrations of each BTEX.

Table 3.14 Amount collected in ng of BTEX from the in-vial standard gas generator # 1 using an 85 µm CAR/PDMS fibre. SD, standard deviation; Intra-vial reproducibility, expressed as RSDs (%, n=5).

Compounds	1	2	3	4	5	Average	SD	RSD
Benzene	48.3	50.8	48.9	49.2	48.3	49.1	1.1	2.1
Toluene	10.6	11.6	11.1	11.0	10.9	11.0	0.3	3.1
Ethylbenzene	6.0	6.7	6.2	6.0	6.1	6.2	0.3	4.6
Xylene	5.2	5.6	5.1	5.2	5.3	5.3	0.2	3.8

Table 3.15 Amount collected in ng of BTEX from the in-vial standard gas generator # 2 using an 85 µm CAR/PDMS fibre. SD, standard deviation; Intra-vial reproducibility, expressed as RSDs (%, n=4).

Compounds	1	2	3	4	Average	SD	RSD
Benzene	47.1	51.1	49.9	46.7	48.7	2.1	4.3
Toluene	10.4	11.3	11.2	11.1	11.0	0.4	3.8
Ethylbenzene	5.8	6.3	6.3	6.0	6.1	0.3	4.2
Xylene	4.7	5.4	5.4	5.2	5.2	0.3	5.9

Table 3.16 Comparison of the passive sampling extraction from two different in-vial standard gas generators containing BTEX. t_{stat} is the Student's t calculated for two vials assuming equal variance; t_{crit} is the two tail *t* critical value for 8 degrees of freedom at a 95% level of confidence.

Compounds	t_{stat}	*p*-value	t_{crit}
Benzene	0.37	0.71	2.36
Toluene	0.03	0.98	
Ethylbenzene	0.49	0.64	
Xylene	0.74	0.48	

Table 3.17 Concentrations in nanograms per millilitre (ng/mL) of the in vial standard gas generator doped with BTEX and used for the determination of the sampling rates (n=3).

Compounds	[ ] (ng/mL)	SD	RSD (%)
Benzene	28.5	1.0	3.4
Toluene	7.3	0.2	2.1
Ethylbenzene	4.3	0.2	5.5
Xylene	4.2	0.1	1.8

3.2.3.2 Evaluation of experimental sampling rates versus sampling duration

In order to use the in-vial standard gas generator for the determination of experimental sampling rates of VOC, it is important to guarantee that the vial can be used as a continuous source of standards. The results, shown in Figure 3.13, illustrate that the adsorbed mass of BTEX on the CAR/PDMS fibre increased linearly (R^2 ranged from 0.992 to 0.999) within a sampling duration of 15-60 min. These results indicate that the CAR/PDMS fibre acts as zero sink for BTEX during this sampling duration, as well as demonstrates that a single in-vial standard gas generator can release the analytes of interest continuously. However, a slight decrease in the collected amounts of benzene over the time was found. This singularity may be related to coating saturation at the tip of the fibre, and will be discussed in the following section.

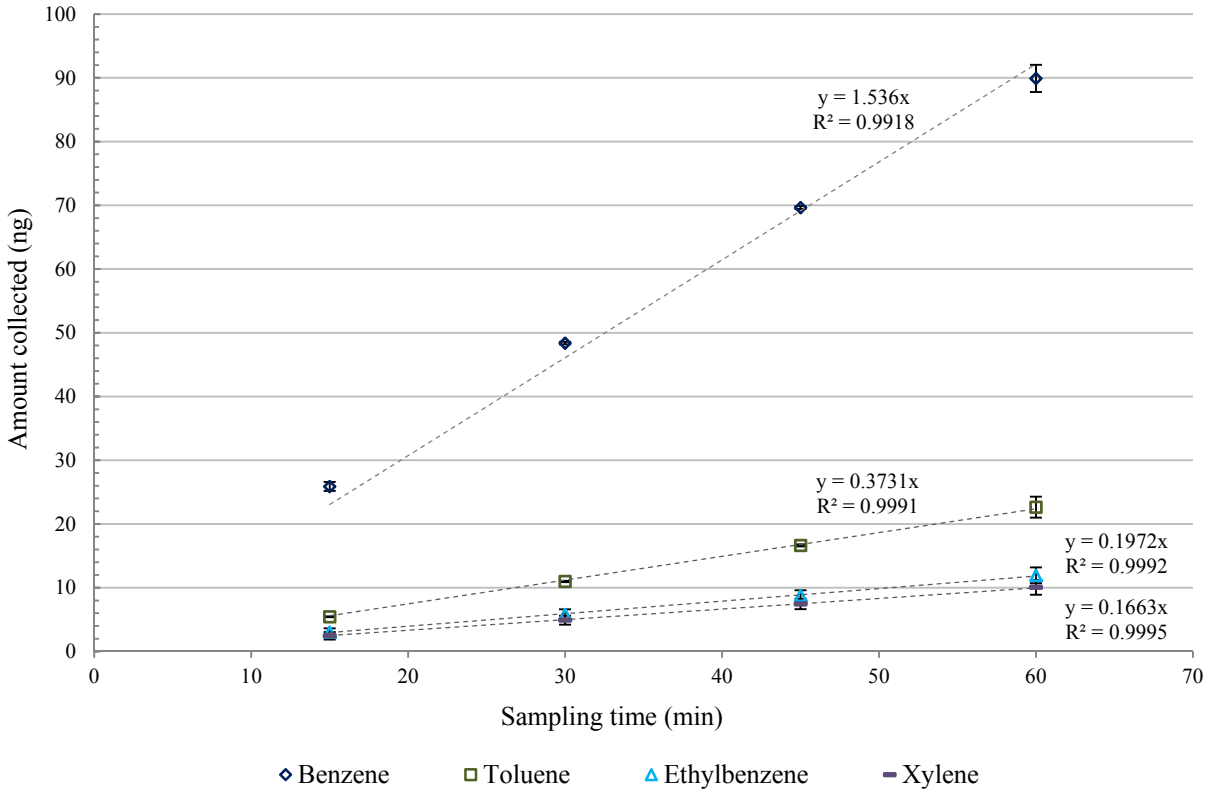


Figure 3.13 Mass of BTEX collected on a 85 µm CAR/PDMS FFA-SPME fibre with a diffusion path of 0.147 cm during sampling duration from 15 to 60 min (n=4).

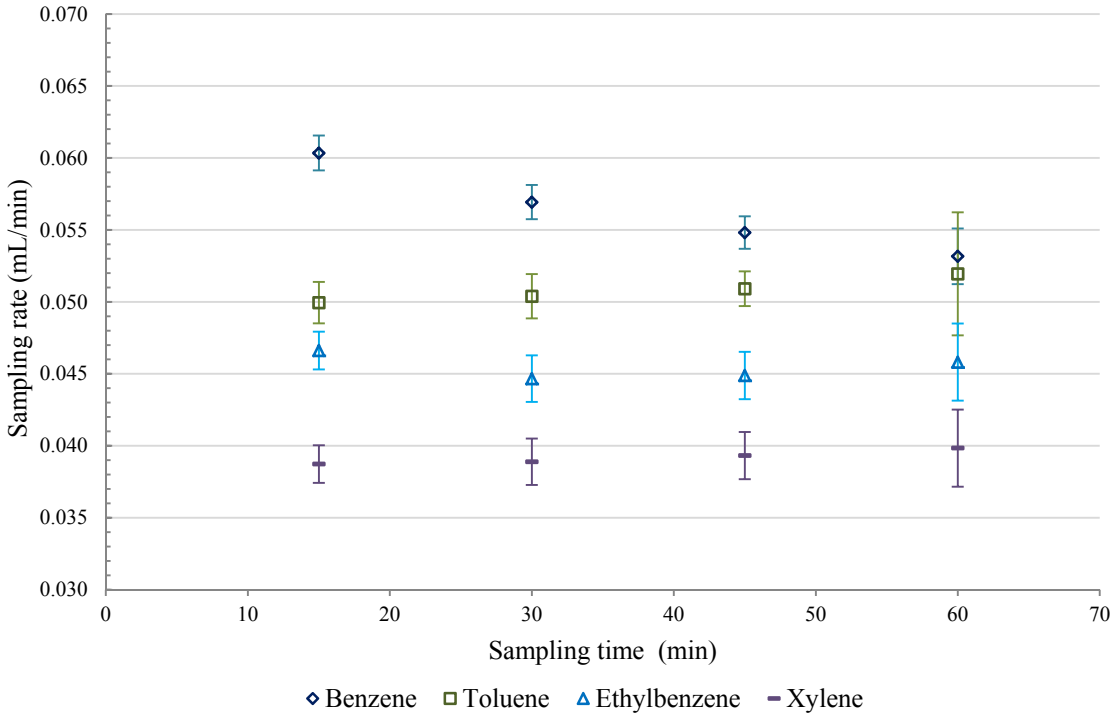


Figure 3.14 Sampling rate of BTEX (ml/min) versus sampling time (min) using the in-vial standard gas generator.

Table 3.18 Comparison of the theoretical sampling rates versus the experimental sampling rates at different sampling times using a diffusion path of 0.147 cm and an 85 µm CAR/PDMS FFA-SPME fibre.

Parameter	Sampling time (min)/Sampling rate (mL/min)			
	15	30	45	60
Theoretical Benzene	0.056	0.056	0.056	0.056
Theoretical Toluene	0.051	0.051	0.051	0.051
Theoretical Ethylbenzene	0.046	0.046	0.046	0.046
Theoretical Xylene	0.046	0.046	0.046	0.046
Experimental Benzene	0.060	0.057	0.055	0.053
Experimental Toluene	0.050	0.050	0.051	0.052
Experimental Ethylbenzene	0.047	0.045	0.045	0.046
Experimental Xylene	0.039	0.039	0.039	0.040

3.2.3.3 Comparison of experimental sampling rates versus theoretical sampling rates

A comparison between the theoretical and the experimental sampling rates at different times is shown in Table 3.18. A statistical comparison of the experimental mean value with the true value (theoretical value), calculated using the equations presented on Table 1.2, showed no differences for toluene and ethylbenzene at any sampling time (Figure 3.14). Similarly, using

sampling times of 30 and 45 minutes, no statistical differences between the theoretical and experimental sampling rates for benzene were observed. Conversely, differences in sampling rate using sampling times longer than 45 minutes were found. Observed differences on the sampling rate of benzene may be related to several factors: for instance, since the initial sampling rate (0.060 mL/min) was slightly higher than the theoretical value (0.056 mL/min), it is then possible that a few nanograms of benzene were adsorbed on the needle walls. As a result, amounts collected and the resulting sampling rates marginally exceeded theoretical values. When sampling duration was longer than 15 minutes, the mass of benzene adsorbed on the needle walls was not significant compared to the amount collected on the CAR/PDMS fibre. Consequently, the amount of analyte adsorbed on the fibre is statistically similar to the value predicted by the theoretical model. Despite the same amount of standards being spiked in the pumping oil-SDVB mixture (approximately 170 μg), benzene has a higher Henry's law constant and, accordingly, its concentration in the headspace was also higher. As shown in Figure 3.13, benzene amounts collected were about 4-times greater than amounts of toluene, which exhibited good agreement with the theoretical model. Furthermore, for sampling times larger than 45 minutes, the collected amount of benzene (approximately 91 ng) may be out of the linear uptake range of the CAR/PDMS fibre for this analyte, thus, the collected amount is below the predicted value. In other words, the fibre is not fulfilling the 'zero-sink' requirement.[57] Further evaluations at longer extraction times from the in-vial standard gas system are required in order to further understand this behaviour.

Additionally, it is worth emphasizing that amounts of benzene extracted after the first 8 experimental runs (approximately 300 nanograms, using sampling durations of 15 and 30 minutes) correspond to less than 0.2 percent of the total concentration in the vial. Therefore,

depletion of standard concentration in the vial may not be the reason for the observed variations on the sampling rate of benzene.[19,60] Generally, good agreement between experimental and theoretical sampling rates was observed. Deviations from the theoretical values, such in xylene, could be explained in terms of short equilibration times between extractions, errors in the diffusion length measurement, or inaccurate estimation of the diffusion coefficients.

3.3 Conclusions

In this study, a simple and inexpensive in-vial standard gas system for the initial evaluation of experimental sampling rates by SPME was presented. BTEX were used as model VOCs in this proof-of-concept evaluation, and it has been proved that the in-vial standard gas system can be used as a continuous source of standards. Good agreement was observed between the theoretical and experimental sampling rates using the method proposed. Intra-vial and inter-vial repeatability were also evaluated, and results showed no statistically significant differences for any of the compounds used as models. These results agree with results presented in Chapter two.

A common criticism of SPME is a lack of published experimental sampling rates values. [82,91] In this sense, since most of the variables involved in SPME passive sampling can be controlled or calculated (such as sampling time, diffusion path, cross sectional area, and vial concentration), the vial approach could be further pursued with the aim to build a comprehensive database of experimental diffusion coefficients of VOCs.

Chapter 4 – Evaluation and application of the Multi Fibre Exchanger (MFX) system for on-site and *in vivo* sampling

4.1 Introduction

4.1.1 On-site sampling of indoor air

Indoor air quality is a vital issue in occupational health. Factors such as ventilation system deficiencies, microbiological contamination, and off-gassing from building materials can cause poor indoor air quality.[92] Since an average person in a developed country spends up to 90% of their time indoors, there has been growing concern over the past decades in regards to indoor pollutants, and methods used in their analysis.[93] SPME has become an attractive technique for indoor air sampling due to its accuracy, cost, simplicity and speed.[38] In addition, SPME can be indistinctively used for either active or passive sampling.

In passive sampling mode, the fibre is retracted a known distance into its needle housing. Because of the flexibility of selecting a wide range of sampling times in passive mode (from less than 1 min to days), several SPME-TWA applications designed to test a broad range of analytes have been developed to date, such as propylene glycol esters,[39] chlorinated organic volatiles, [36,37,89] volatile sulfur compounds,[32] alkanes,[1] pesticides,[94] inhalation anaesthetics,[35,41,42] BTEX,[95] and aldehydes.[43]

Conversely, in active mode, the fibre is only exposed for spot measurements. For instance, SPME dynamic air samplers (PDAS) were designed for rapid field-air sampling under non-equilibrium and dynamic conditions.[28] When compared to conventional SPME extraction in static air, a greater VOC mass is adsorbed and an increase in the number of detected compounds is achieved. Augusto *et al.* proved that the PDAS-SPME is a powerful tool for both qualitative

and quantitative analysis of occupational air.[28] Using a similar approach, Tollback and collaborators[96] found detection limits below 1 ng/m^3 when analyzing triphenyl phosphate. In this work, multiple CAR/PDMS fibres were used in both modes to evaluate the indoor air quality of a polymer chemistry laboratory. A diffusive fibre holder (DFH), recently commercialized by Supelco, and PDAS-SPME were used for passive and active sampling, respectively. Subsequently, samples collected were analyzed using a multi-fibre exchange (MFX) instrument; the results were compared to active sampling using a multi-bed needle trap. To the best of our knowledge, few methods for indoor air have been developed using multiple fibres,[35,36,41] and only one method involving MFX has been reported to date.[51] Thus, a critical evaluation of MFX performance was performed prior to the development of the applications included in this chapter.

4.1.2 On-site and *in vivo* sampling of plants

Volatile and semi-volatile compounds produced by plants are collectively known as biogenic volatile organic compounds (BVOC).[96] They comprise a wide variety of organic substances, such as alcohols, terpenes, alkanes and esters. These compounds are usually present in the atmosphere at concentrations in the parts-per-trillion (ppt) to the parts-per-billion (ppb) range. Since BVOCs are responsible for multiple interactions between plants and other organisms, and also play a key role in atmospheric chemistry, their identification, characterization and quantification are of great relevance.[97,98]

Generally, *in vivo* research is best suited to observe overall effects when compared to *in vitro* research.[97,99] Since the experimental system is a complex biological system, a better indication of what is happening in the real world can be observed.[100,101] An ideal *in vivo* sampling technique should be solvent-free, portable, and offer integration of the sampling, sample preparation and

analysis steps. With SPME, both *in vivo* sampling and sample preparation are accomplished by placing the fibre in the area surrounding the system under study.[98,100] Consequently, the plant tissue being analyzed does not have to be destroyed. *In vivo* analysis using SPME is gaining ground in metabolomics studies[59,80,86] because of its unique characteristics: on-site sampling, easy extraction, and analysis of whole extracted amounts.[102] Until now, numerous applications for the analysis of BVOCs have been developed with SPME.[98] For instance, circadian BVOC emission profiles and phytoremediation properties of plants were explored by Zini *et al.* and Sheehan *et al.*, respectively.[89,97] However, just as observed in air quality studies, only a handful of these studies have included the use of multiple devices.[98,99]

In real applications, numerous fibres are required in order to obtain a better spectrum of the emissions being studied.[97] For that reason, the application of multiple SPME-FFA DVB/PDMS fibres used in the identification and quantification of BVOCs emitted by a Pine tree is also presented in this chapter. The selection of DVB/PDMS fibres was based on previous studies conducted in BVOCs analysis.[96,97,103,104]

4.2 Experimental

4.2.1 Materials and reagents

HPLC grade methanol was obtained from Caledon laboratories LTD (Georgetown, ON, Canada). BTEX, limonene and decane were purchased from Sigma-Aldrich (Mississauga, ON, Canada). Pure water was obtained using a Barnstead/Thermodyne NANO-pure ultrapure water system (Dubuque, IA, USA). Helium of ultra-high purity was supplied by Praxair (Kitchener, ON, Canada). Commercial SPME-Fast Fit Fibre Assembly (FFA) fibres used in this study,

namely divinylbenzene/polydimethylsiloxane (DVB/PDMS, 65 μm) and carboxen/polydimethylsiloxane (CAR/PDMS) were also provided by Sigma-Aldrich. Fibres were conditioned prior to their use according to the manufacturer recommendation. Gas tight syringes (1 and 5 mL) were purchased from Hamilton Company (Reno, NE, USA). All preparations were carried out in a ventilated fume hood. CAR particles (surface area: 1200 m^2/g) of 60/80 mesh were purchased from Sigma-Aldrich (Bellefonte, PA, USA). DVB particles (surface area: 582 m^2/g) of 100/120 mesh were purchased from Ohio Valley (Marietta, OH, USA). The purchase of 3.5 inch long 22-gauge blunt needles (I.D. 0.41 mm, O.D. 0.71 mm) was made from Dyna Medical Corporation (London, ON, Canada). Stainless steel wires (O.D. 100 μm) were purchased from Small Parts (Lexington, KY, US). A 5-min epoxy glue was purchased from Henkel Canada (Mississauga, Ontario, Canada). An ADM 1000 flow-meter was purchased from Agilent Technologies (Mississauga, ON, Canada)

4.2.2 Preparation of the needle traps

In the preparation of NTs, first, a stainless wire was pressed by two steel guides and fixed into the desired position as a spring plug. Then, sorbent particles were aspirated into the needle by a tap-water aspirator and held by the spring plug. After packing the desired length of sorbent bed, a small amount of epoxy glue was used to immobilize the sorbent in the opening end. During the packing process, the aspirator was kept running until the epoxy glue was cured so as to avoid the blockage of the NT by the epoxy glue. The sorbent beds packed inside the needles for this work were 1 cm 100/120 mesh DVB plus 1 cm 60/80 mesh CAR. After packing, the NTs were conditioned in a GC injector for 3 hours with helium gas continuously flowing through the

needle. The conditioning temperature was 260 °C. This process was similar to previous works[105-108].

4.2.3 Instrumentation

Both the GERSTEL® MPS 2 autosampler, equipped with a GERSTEL Cooled Injection System (CIS4), and a Multi-Fibre Exchanger (MFX) system for 25 SPME-FFA devices (GERSTEL, Mülheim an der Ruhr, Germany), as well as the Agilent 6890 gas chromatograph coupled to a 5973 MSD quadrupole mass spectrometer (Agilent Technologies, Mississauga, ON, Canada) were used in this study. For the analysis of biogenic emissions, the chromatographic separations were performed using a SLBTM-5MB (30 m x 0.25 mm x 0.25 μm) fused silica column from Sigma–Aldrich with helium as the carrier gas at a flow rate of 1.5 mL min^{-1}. The oven temperature was initially held at 50 ºC, gradually increased to 60 ºC at a rate of 1 ºC min^{-1}, then increased to 280 ºC at a rate of 30 ºC min^{-1} and finally held for 0.67 min. The chromatographic separations for indoor air analysis were performed using an Rxi®-624Sil MS (30 m x 0.32 mm x 1.80 μm) column from Restek with helium as the carrier gas at a flow rate of 1.5 mL min^{-1}. The oven temperature was initially held at 40 ºC for 2 min, gradually increased to 55 ºC at a rate of 3 ºC min^{-1}, then increased to 250 ºC at a rate of 20 ºC min^{-1}, and finally held for 3.25 min. During the analysis, the transfer line, MS Quad and MS source were set at 280 ºC, 150 ºC and 230 ºC, respectively, with the MS operated in electron ionization mode. Full scan mode (40–250 m/z) was used for all compounds, and quantitation was done using extracted ion chromatograms. The ion *m/z* 93 was used for quantitative analysis of α-pinene, β-pinene and limonene, while the ion *m/z* 91 was used for quantitative analysis of toluene. The following parameters were selected on

the Maestro software for both analyses: A) injection temperature (ºC), 260 and 300 for DVB/PDMS, and CAR/PDMS, respectively. B) injector penetration (CIS4): 54 mm C) desorption time: 180 s; D) fibre bake-out time: 1.5 min and bake-out penetration (front injector): 45 mm. Chromatographic peak identification was made by library matching using the 2002 NIST MS Library (V.2.0 NIST MS Search software). Sampling with the NTDs was made using a bi-directional syringe pump purchased from Kloehn (Las Vegas, NE, USA).

4.2.4 Standard Gas Mixture and permeation tubes

Permeation tubes for BTEX, limonene and decane were made by encapsulating pure analyte inside a 100 mm long (1/4 in.) Teflon™ tubing capped with 20 mm long solid Teflon™ plugs and (1/4) in. Swagelok caps. Emission rates for each permeation tube were verified by periodic monitoring of weight loss of individual analyte tubes. A standard gas generator (model 491 MB, Kin-Tech Laboratories, LaMarque, TX, USA) was used to generate the standard gases with desired concentrations. The permeation tubes made in our lab were placed inside a glass chamber held in a temperature-controlled oven, and swept with a controllable, constant flow of compressed air. Different concentrations of the analytes were obtained by adjusting both the permeation chamber temperature and air flow rate.

4.2.5 Sampling chambers

For the extraction of BVOCs emitted by live pine trees, a glass chamber design by Zini *et al.* was used.[97] It consisted of a Pyrex glass cylinder (120 mm wide, Ø = 60 mm), where pine needles from a pine tree can be inserted through a hole in one of its ends (Figure 4.15). After the

introduction of the small branch, this hole can be sealed using Teflon tape. A round glass lid secured by clamps closes the other end of the chamber. This lid has several 5-mm holes sealed by Thermogreen LB-2 predrilled septa (Supelco), into which a SPME fibre can be introduced to sample the air inside. To maintain a constant convection during the SPME sampling of biogenic compounds, a mini-fan connected to a 12 V battery set was used throughout the experiment. When powered, this fan produces a constant air stream directed towards the front of the sampling holes. The velocity of the stream, measured using a HHF51 digital wire anemometer (Omega Engineering, Stanford, CT), is approximately 61 cm/s. All glass parts of the container were silanized prior to their use. In order to prevent the presence of artifacts and contamination from previous analyses, the container was cleaned with methanol and dried with a constant nitrogen flow in a fume hood between samplings. For the analysis of VOCs and semi-VOCs, a sampling chamber, designed by Koziel *et al.*,[88] was installed downstream from the standard gas generators. A schematic of the sampling chamber is provided in Figure 4.6.[88] This sampling chamber facilitated a steady-state mass flow of all the standards. The sampling chamber consisted of a custom made 1.5 L glass bulb with several sampling ports that were plugged with Thermogreen LB-2 predrilled septa. Omega 120 W heating tape was wrapped around the glass bulb to control temperature inside the bulb. An Omega K-type thermocouple was attached to the outside surface of the glass bulb in order to control its internal temperature. Both heating tape and thermocouple were connected to an electronic heat control device constructed by the Electronic Science Shop at the University of Waterloo (UW). Air temperatures in the vicinity of the SPME fibres were maintained within ±1.2% of the adjusted temperature. Standard glass flow rates ranged from 50 to 3000 mL/min, resulting in mean air velocities similar to those encountered in indoor air

environments. Standard gas generators and sampling chambers were validated using a multi-bed needle trap.

4.2.6 *On-site* and *in vivo* sampling of Pine trees

A pine tree branch was sealed in the glass sampling chamber, and air inside the chamber was extracted for 30s using a 65 μm SPME-FFA DVB/PDMS fibre. This procedure was performed every 3 hours between 8 am and 8 pm. The micro-fan was powered during the extractions. For each sampling period, four replicates using independent fibres were run. The fibres were selected following the protocol described in Section 3.1.3.6. Blank analyses of the fibres and glass chamber were run before the start of each sampling. After sampling, fibres were sealed with Teflon caps and kept under dry ice while transported to the laboratory.[3] Time elapsed between sampling and analysis never exceeded two hours; under these conditions the loss of extracted analytes is expected to be insignificant, as proved by Chen *et al.*[3] The concentration of the target analytes was calculated using the diffusion-based SPME quantitative model defined by Equation 1.3 in Section 1.3.2. A complete description of the parameters used in these calculations is listed in Table 4.5.

4.2.7 Indoors time-weighted average sampling

A diffusive fibre holder (DFH) recently commercialized by Supelco, as well as bare 85 μm FFA CAR/PDMS fibres were used for passive sampling. A magnetic plunger was built at the machine shop of the University of Waterloo to control the diffusion path of the bare FFA fibre. Rare-earth magnets employed to manufacture this plunger were acquired at Lee Valley (Waterloo, Ontario, Canada). Fibres were selected following the procedure described in Section 4.3.3. Samplings

were performed over a period of 8 hours, between 9 am and 5 pm, during three different days in the same week. For DFH evaluation, as described in Section 4.3.4, one of the fibres was installed on the diffusive fibre holder while another two were used as controls.

4.2.8 Indoors air sampling in active mode

A portable dynamic air sampling device (PDAS) for SPME, previously described in Section 1.3.2, was employed in the quantification of indoor contaminants present in the air of a polymer synthesis laboratory at University of Waterloo. A magnetic plunger was used to expose the fibre coating; measurements exposing a SPME-FFA CAR/PDMS fibre for 30 s were performed four times during a day, using independent fibres. The fibres were selected following the protocol described in Section 3.1.3.6. Concentrations of the analytes were calculated using Equation 1.3 in Section 1.3.2.

4.2.9 Sampling and desorption of needle traps

For indoor air sampling and verification of concentrations in the exposure chamber, the NTD was connected to the sampling pump while a volume of the gaseous sample was pumped from the gas standard generator through the needle, at a flow rate of 5 mL/min. After sampling, the NTD was connected to a 1 mL gas-tight syringe filled with helium, and then introduced into a GC injector for desorption. Helium was consistently pushed out to assist desorption throughout the whole desorption period. For NTDs packed with DVB/CAR, the needle was injected into the hot GC injector at a temperature of 260 °C for 1 min, assisted by 0.3 mL of helium.

4.3 Results and Discussion

4.3.1 Initial assessment of the multi-fibre exchange system (MFX)

To the best of our knowledge, few publications exist in the literature related to the use of a multi-fibre exchange system. To date, research in this area seems to mainly focus on development of a specific application, rather than a critical evaluation of the system.[24,42,50,51] In the initial experiments that were performed with the MFX system, two main issues were observed. First, it was found that the holder adapter was not able to remain attached to the autosampler arm after several extractions. This is a critical issue; if the holder were to fall while the fibre is being transported, the piercing needle may bend, consequently damaging the FFA device. This problem was solved by installing a small screw at the back of the holder adapter. As shown in Chapter two, after adding the screw, no further issues were observed for up to 240 continuous extraction/injection cycles.

Secondly, Bisphenol A was found accumulated on fibres while these were in storage for times typical of a standard analysis. We determined that Bisphenol A originated from the plastic body of the sealing caps (refer to Figure 4.5). A procedure to clean the caps of the MFX tray was developed to eliminate the possibility of fibre contamination. This procedure consists in sonicating the Teflon piece of the cap with three different solvents (methanol, acetone and hexane). Next, the caps were dried in a fume hood by applying a flow of nitrogen. Finally, to eliminate residues of any volatile compounds remaining in the caps, they were placed in an oven at 200°C for 30 minutes. We have found it suitable to store the cleaned fibre caps in a beaker with a cover on the top (aluminum foil or Parafilm) to avoid any back-contamination. Based on our experience, it is recommended to run a blank of a fibre that has been stored on the tray for at least 1 hour to verify that new contaminants were not adsorbed on the cap.

4.3.2 Evaluation of the multi-fibre exchange system storage stability

An important ability of the MFX system is the unattended desorption/analysis of multiple FFA fibres after field sampling (active or passive). Up to date, the integrity of the samples on the MFX tray after several hours of residence has not been reported by the manufacturer in an application note. The integrity of a sample depends on two main factors: the sealing efficiency of the cap and the sorption efficiency of the sorbent. It has been established that the CAR/PDMS fibre is the best option for volatiles analysis,[1] and consequently is the recommended coating in the manufacturer's brochure for VOCs analysis.[54] However, SPME users may also wish to select the DVB/CAR/PDMS fibre if their sample contains semi-volatiles. Thus, 3 different fibre coatings (PDMS, CAR/PDMS and DVB/CAR/PDMS) were used to evaluate the integrity of samples/fibres while they were left in storage at the workstation. Since 1 hour is a relatively common GC runtime, and the tray can store up to 25 fibres, the sealing efficiency was evaluated for a period up to 24 hours, assuming that the tray was full. Figures 4.1, 4.2 and 4.3 present the results for CAR/PDMS, DVB/CAR/PDMS and PDMS, respectively.

In summary, the results show that sealing efficiency of commercial caps is insufficient to prevent the loss of volatile compounds (<C10) for both PDMS (~90% loss) and DVB/CAR/PMDS (~20% loss) fibres. As expected, only CAR/PDMS fibres prevent excessive loss of volatiles during 24 hours of storage. Thus, it was identified that for field sampling of samples containing both high- and low-boiling compounds, where the use of a DVB/CAR/PDMS fibre is preferred, modifications to the sealing process need to be implemented if sample integrity is to be preserved. In order to address this issue, a new cap with a more efficient sealing mechanism was designed and tested at the University of Waterloo (UW). The proposed cap is presented on Figure 4.5.

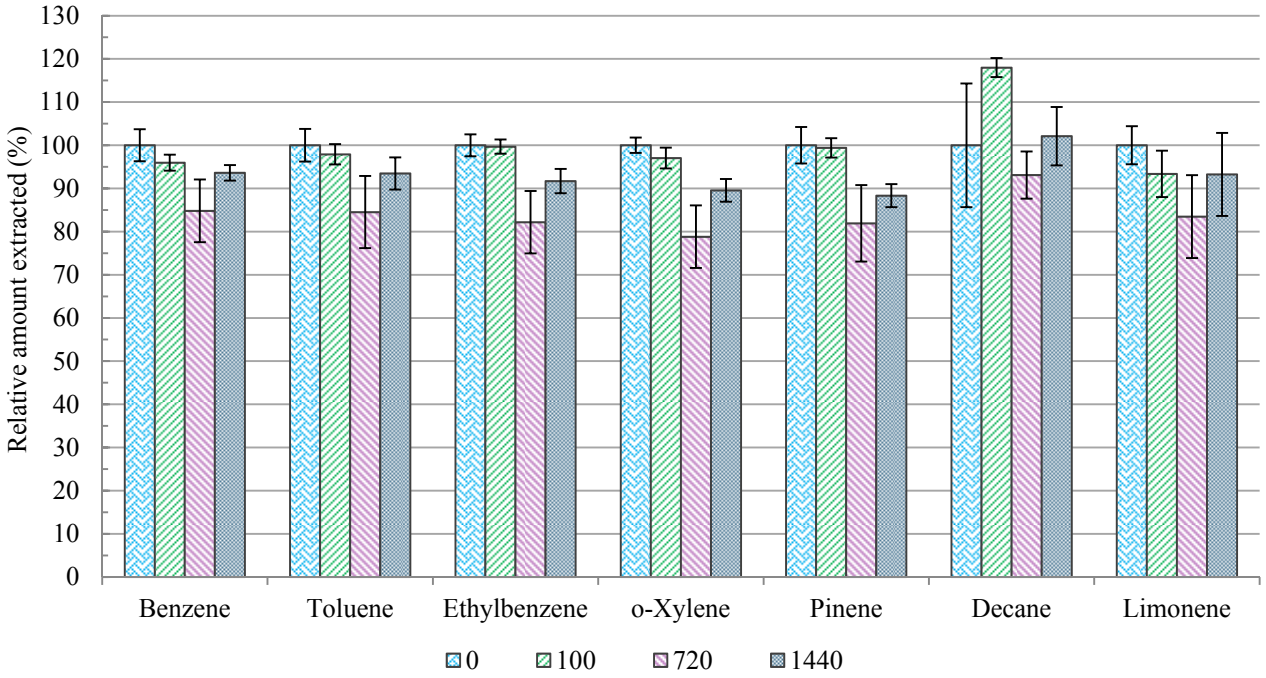


Figure 4.1 Evaluation of residence time effect on the MFX tray using CAR/PDMS fibres after 0, 100, 720 and 1440 minutes (n=3).

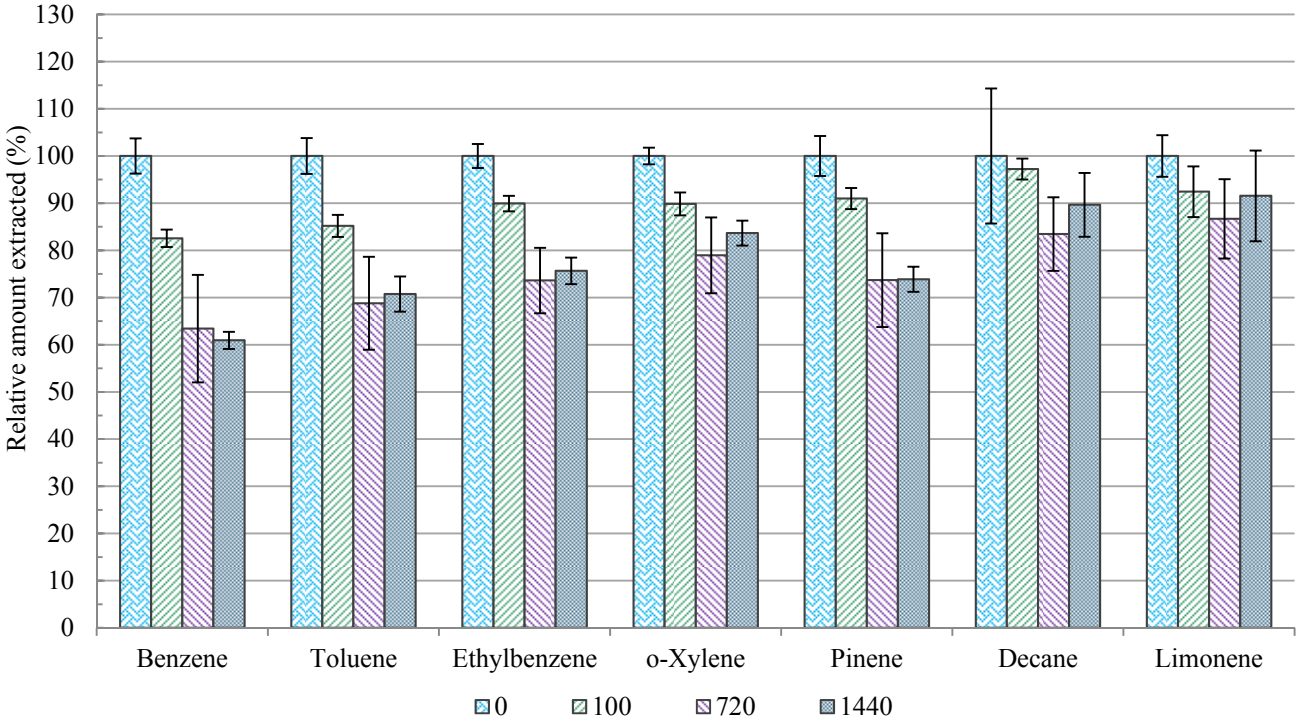


Figure 4.2 Evaluation of residence time effect on the MFX tray using 50/30 µm DVB/CAR/PDMS fibres after 0, 100, 720 and 1440 minutes (n=3).

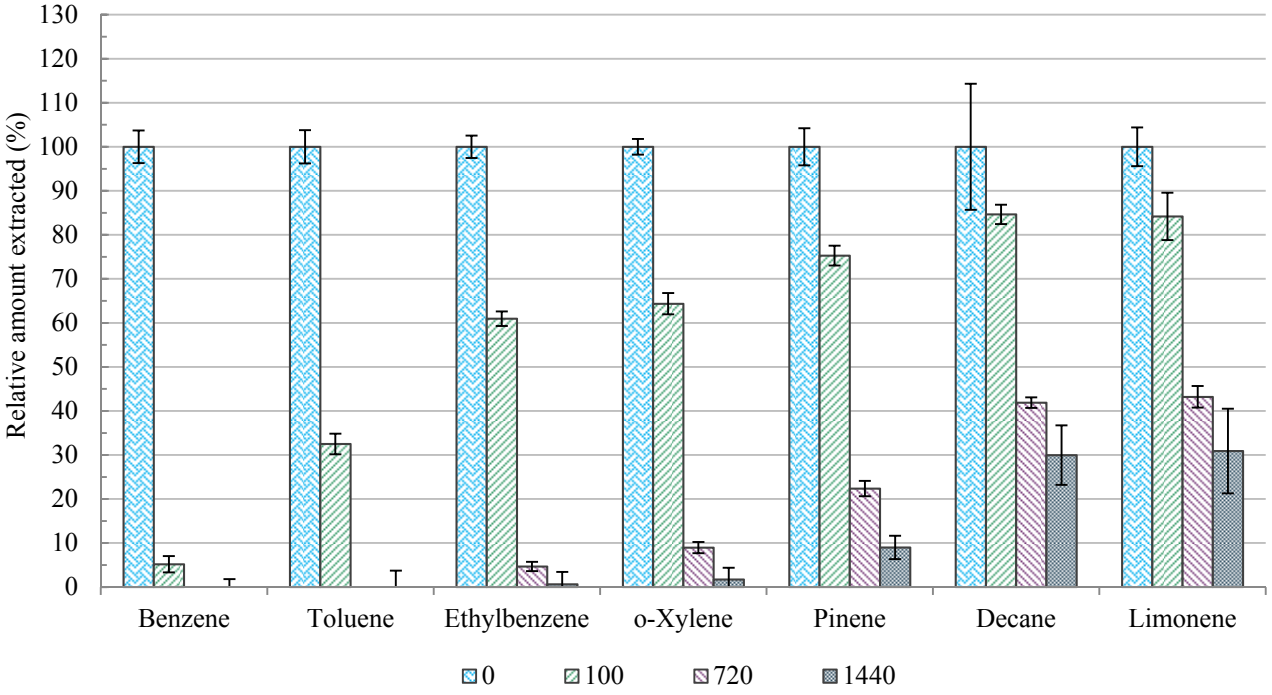


Figure 4.3 Evaluation of residence time effect on the MFX tray using 100 µm PDMS fibres after 0, 100, 720 and 1440 minutes (n=3).

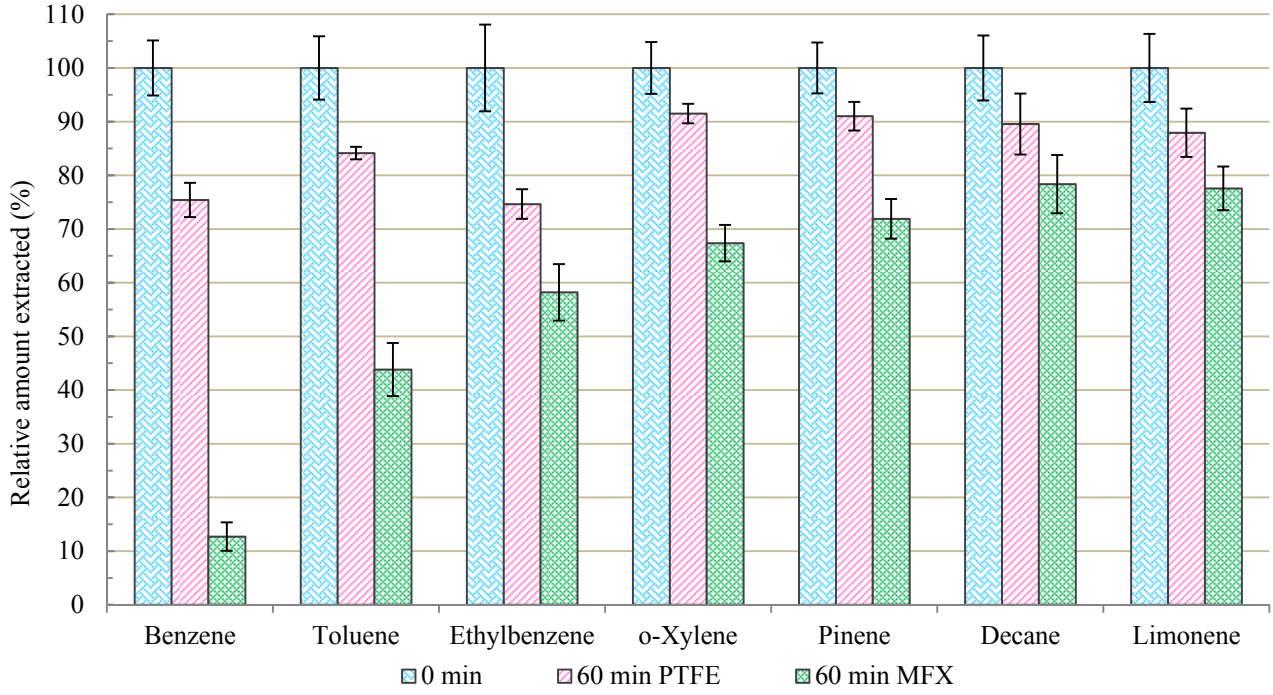


Figure 4.4 Comparison of the effectiveness of the commercial cap for a residence time of 60 minutes on the MFX tray using 100 µm PDMS fibres (n=3). PTFE, house made Teflon cap; MFX, commercial cap.

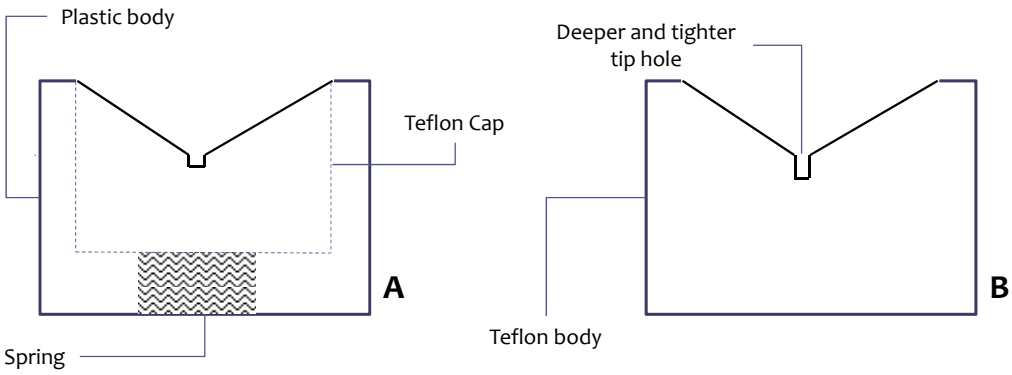


Figure 4.5 Comparison of the commercial cap versus the proposed cap developed in this study. A. MFX commercial cap; B. Teflon cap manufactured at the University of Waterloo machine shop.

Compared to the commercial version of the cap, the new Teflon-only cap has a tighter and deeper hole (refer to Figure 4.5). Automatic system operation using this custom-made cap would require modifications of both tray and software. However, since the Maestro software being used cannot be customized, evaluation of the UW-caps could not be performed with complete system automation; due to the new design of the caps, the system was unable to automatically lift the fibre from the tray. Therefore, in order to prove the advantages of the new design, fibres were manually uncapped by the analyst prior to the injection.

The sealing efficiency of commercial and custom-made caps was evaluated by exposing the fibre to the standard gas generator, followed by immediate storage using both devices. PDMS was the chosen fibre in this study since it is not a very efficient fibre in the retention of VOCs, and in this case, we were interested in studying a worst case scenario. Results were compared to a fibre injected right after the extraction. As can be seen in Figure 4.4, it was demonstrated that a tighter hole prevents considerable loss of most volatile compounds. When the PDMS fibre is stored on the MFX tray, desorption of the standards from the fibre occurs until an equilibrium is reached between standards in the fibre and the ambient air inside the needle. However, if the

sealing around the needle is not tight enough, analytes in the ambient air inside the needle escape, causing further desorption of analytes from the fibre. In summary, the currently used configuration of MFX caps allows only for high-throughput and unattended analysis of *on-site* samples taken with CAR/PDMS fibres based on the strong adsorption capability exhibited by this coating.

4.3.3 Evaluation of MFX suitability in the analysis of multiple fibres

As shown in Figure 4.1, when analyzing a single CAR/PDMS fibre, no noteworthy losses were found up to approximately 24 hours of residence on the tray. However, the effect of the spatial position on the tray was not evaluated. Since the tray is located a few centimeters above the GC oven, areas hotter than the one assessed in the previous study may exist on the tray. As a result, considerable loss of analytes may occur when fibres are stored in a specific position. In order to evaluate the suitability of the MFX system for the analysis of multiple fibres, a Latin-square design was proposed. The two factors that can affect the measurement of the amount collected by the SPME fibre were blocked, namely the position on the sampling chamber (see Figure 4.6) and the position on the MFX tray. Table 4.1 presents the design used for this evaluation.

Table 4.1 Latin-square design used for the evaluation of 5 different 85 μm CAR/PDMS fibres. The position on the MFX tray and the sampling chamber were both randomized. F#Xx, where # is the number of the fibre, X is the position on the chamber, and x is the position on the MFX tray. a, position 1; b, position 5; c, position 21; d, position, 25, and e, position 13.

Replicate	Fibre Position (F#Xx)				
1	F1Aa	F5Bd	F4Cd	F3De	F2Ec
2	F2Ab	F1Be	F5Cc	F4Da	F3Ed
3	F3Ac	F2Ba	F1Cd	F5Db	F4Ee
3	F4Ad	F3Bd	F2Ce	F1Dc	F5Ea
5	F5Ae	F4Bc	F3Ca	F2Dd	F1Eb

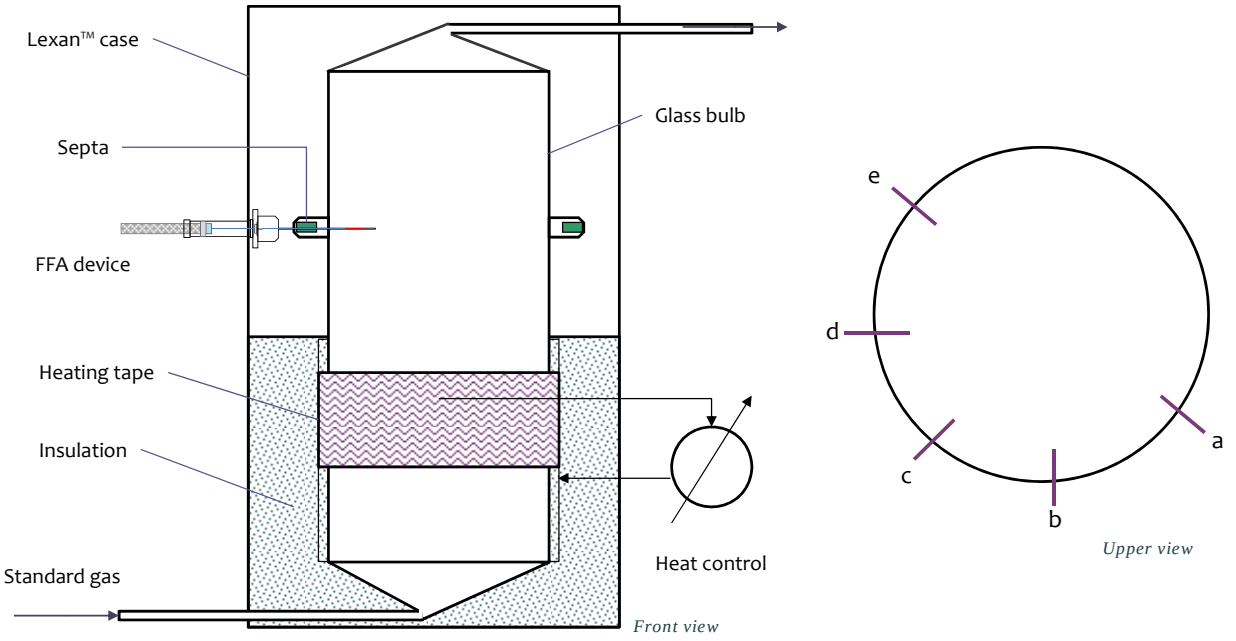


Figure 4.6 Schematic of the sampling chamber for SPME extractions

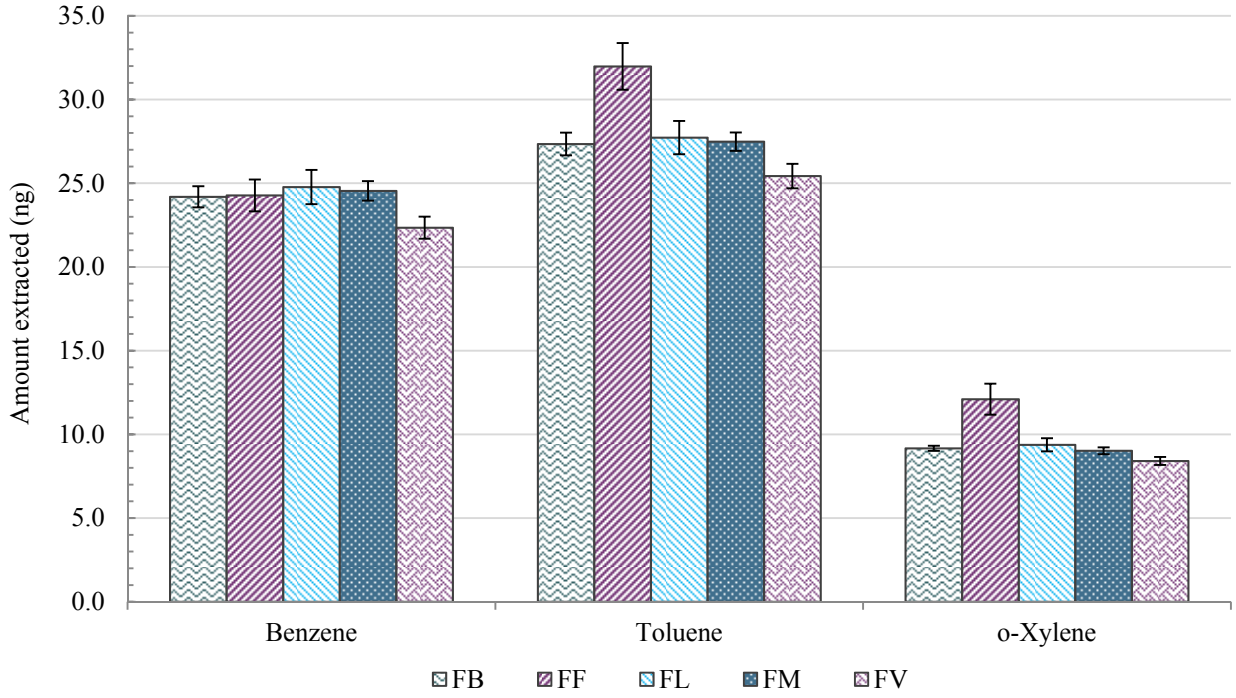


Figure 4.7 Amount extracted of BTX in nanograms using passive sampling with 5 different CAR/PDMS fibres (n=5). Samples were taken from a standard gas generator using SPME in passive sampling mode (Z=0.2 cm, t = 15 minutes).

Table 4.2 Statistical evaluation of the inter-fibre repeatability of five 85 µm CAR/PDMS fibres using a Latin-square design. F*tray* is the F-ratio for the randomization of position on the MFX tray; F*chamber* is the F-ratio for the randomization of position on the sampling chamber; F*fibre* is the F-ratio for the different treatments evaluated (different fibres) and F*crit* is the critical value of F for 25 experiments at a 95% level of confidence. RSD is the relative standard deviation for the inter-fibre repeatability of five fibres (n=5).

Compound	Benzene	Toluene	Xylene
F*tray*	1.2	1.5	1.3
F*chamber*	1.6	2.0	1.5
F*fibre*	7.6	45.8	54.6
F*crit*		3.3	
RSD	4.0	8.6	15.0

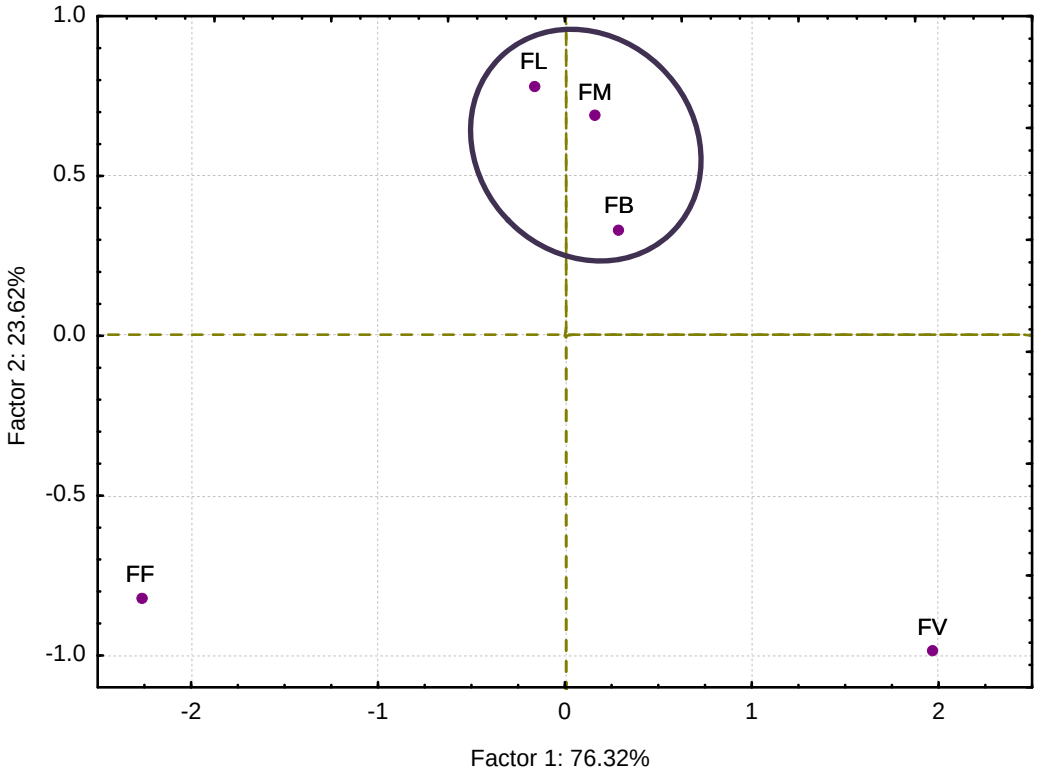


Figure 4.8 Principal component analyses of 5 CAR/PDMS SPME-FFA fibres. Samples were taken from a standard gas generator using SPME in passive sampling mode (Z=0.2 cm, t = 15 minutes).

In order to assess the contamination of a given pollutant with on-site sampling, a significant number of sampling points are required to get a better understanding of the problem. SPME has been extensively used to determine TWA concentrations of a broad range of analytes. Although inter-fibre reproducibilities of multiple fibres have already been evaluated preceding their application on real samples in a few studies,[35] complementary to the evaluation of the

storage stability of the MFX tray, in this study the intra and inter-fibre reproducibilities of five CAR/PDMS fibres were also evaluated using passive sampling. As aforementioned, CAR/PDMS have been shown to be the most suitable coating towards volatile compounds. Therefore, BTX were selected as the target compound for this evaluation.

As can be seen in Figure 4.7, statistical differences among fibres were found. Statistical evaluation of the data (refer to Table 4.2), using the Latin-square design, showed no effects for the position on the sampling chamber; this means that the experiments reported here were conducted under ideal conditions for air circulation in the exposure chamber.[10,12] Furthermore, as shown in Table 4.2, at a 95% level of confidence, it was proven that the MFX tray does not have an effect on the amount of analytes measured by multiple fibres. This is a clear advantage of this system, particularly when numerous probes are required, such as in environmental on-site analysis.

Although the factors blocked do not appear to have any effect, it is important to note that relative standard deviations up to 15 percent were obtained. The PCA analysis, shown in Figure 4.8, allows us to easily observe which fibres are performing similarly to one another. By selecting only the fibres enclosed on the ellipse in Figure 4.8, relative standard deviations lower than 2 percent were obtained for all analytes. As shown in Table 4.2, the largest differences were observed for xylene. This might be related to its smaller diffusion coefficient. Thus, differences on the diffusion distance (Z) are more significant for xylene than benzene or toluene. Such dissimilarities might be associated with measurement inaccuracy of the diffusion distance, or irregularities on the tip of the fibre. For further studies, only fibres that yielded an inter-fibre RSD lower than 5 percent for all analytes were selected.

4.3.4 Evaluation of the Diffusive Fibre Holder (DFH)

To determine TWA concentrations, a diffusive sampling fibre holder (DFH), developed by CHROMLINE and commercialized by SUPELCO, was combined with an FFA. According to its design (sees Figure 4.9), the DFH seems to achieve the three requirements of a field sampler: adequate needle sealing and protection, as well as a user-friendly interface. Additionally, this device allows adjusting Z distances from 0 to 35 mm with intervals of 1 mm, using a magnetic plunger. As well, the sealing cap of the DFH is a PTFE cup inserted into a spring loaded mechanism that seals and protects the needle tip once it is installed. To the best of our knowledge, this device has not been evaluated to date, and publications reporting its use in determining TWA concentrations, with the exception of the company brochures available, were not found.

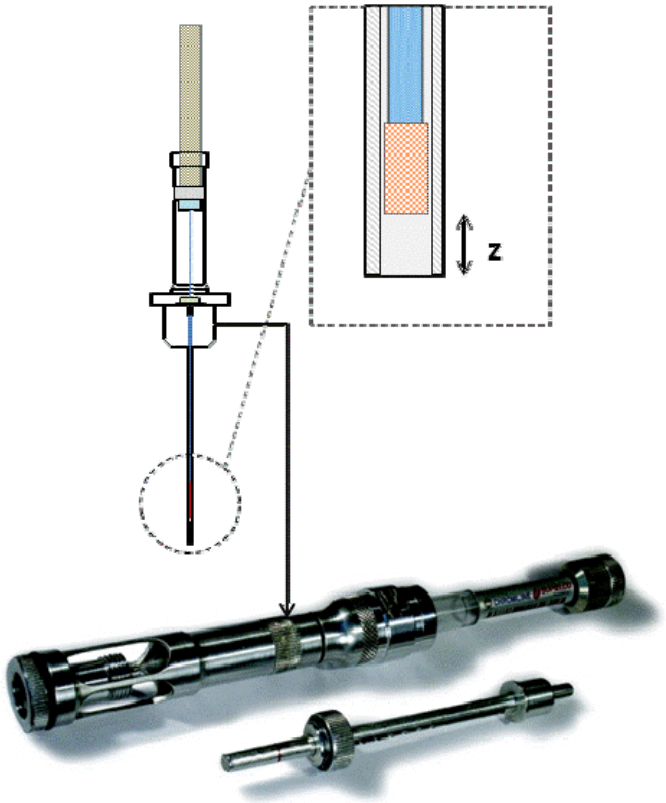


Figure 4.9 Schematic drawing of the Diffusive Sampling Fibre Holder (DFH)[53]

Two critical parameters of the DFH were evaluated: its storage stability for up to 12 hours at room temperature, and the effect of the sampler device on the uptake rate of the analytes. Since a special exposure chamber to evaluate the DFH was not available at the laboratory, the uptake rate of the analytes was studied by carrying out 8-hour samplings in passive mode at a polymer synthesis laboratory in the University of Waterloo.[109] Three 85 µm CAR/PDMS fibres, previously assessed on section 4.3.3, were used for this purpose. Since only one DFH was available for this study, one of the fibres was placed inside the holder and the other two were used as controls. These experiments were performed on three different days in order to demonstrate that the sampler does not have an effect on the rate of analyte collection. As shown in Figure 4.10, No differences in toluene concentration were found among the bare FFA devices and the FFA device inside the DFH. The RSDs for multiple devices agreed with those reported by Zare *et al.* [35,41] However, it would seem that turbulent currents in the workplace lead to much higher variation in the results compared to those obtained under controlled conditions. [36,109]

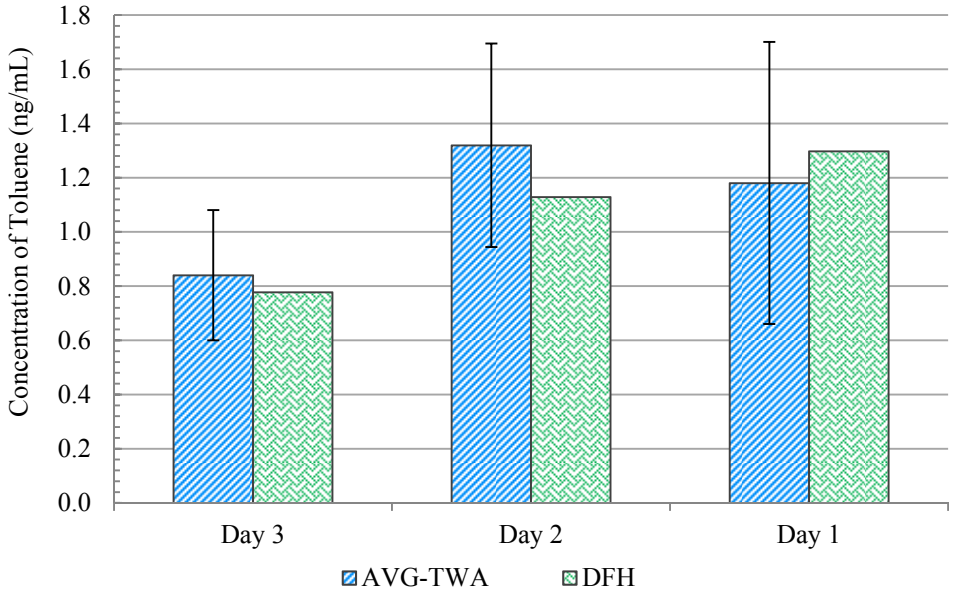


Figure 4.10 Evaluation of the diffusive fibre holder (DFH) versus conventional FFA-SPME devices using 85µm CAR/PDMS (Z=0.147 cm, t = 8 hours). AVG-TWA is the mean value obtained with two fibres without holder. DFH is the value obtained using a single FFA device placed on the DFH.

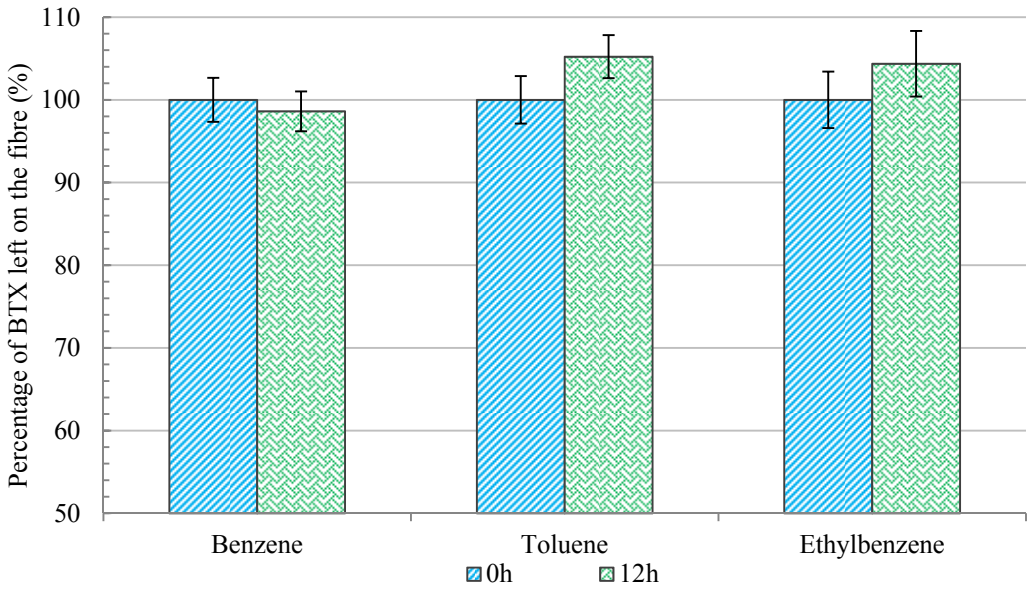


Figure 4.11 Evaluation of the storage stability up to 12 hours of the diffusive fibre holder (DFH) using 85 µm CAR/PDMS (Z=0.147 cm, t = 8 hours, n=5).

Preservation of sample integrity can be achieved in different ways, such as using a system that perfectly seals the fibres, as well as storing fibres at sub-ambient temperatures. The last was proven to be an efficient solution to enhance preservation of sample integrity by Chen *et al.*, and it is commonly used after on-site sampling.[3]

In order to evaluate the sealing capacity of the PTFE cup used on the DFH, two fibres of 85 µm CAR/PMDS were exposed to a gas generator in passive sampling mode (Z=0.147 cm, t=15 min) followed by either immediate desorption, or storage at room temperature for a period of 12 hours. As shown in Figure 4.11, no statistical differences were found between the fibres that were sealed at room temperature for 12 hours and those injected immediately. These results agree with previous studies where PTFE was used as the sealing material.[3] Therefore, since the caps accomplished the minimum requirements for a field sampler, this device was used for the studies presented on the following section.

4.3.5 Evaluation of indoor air contaminants

Indoor air was analyzed at a polymer synthesis laboratory at the University of Waterloo. Several samples were collected in the span of a workday (8 h) to determine variations in the air contamination profile within this time limit.[92] Active sampling through a multi-bed NTD was carried out every hour to observe the intra-day variations. Spot sampling, using the diffusion based calibration approach with an 85 µm CAR/PDMS fibre, was performed four times during the day to corroborate the results obtained with the NTD. Passive sampling over a period of 8 hours, using three 85 µm CAR/PDMS fibres, was used to determine the average concentration of toluene that workers were exposed to. Table 4.3 summarizes the parameters used to calculate the concentrations of toluene with spot sampling. As can be seen in Figure 4.12, good agreement was observed between passive and active techniques.

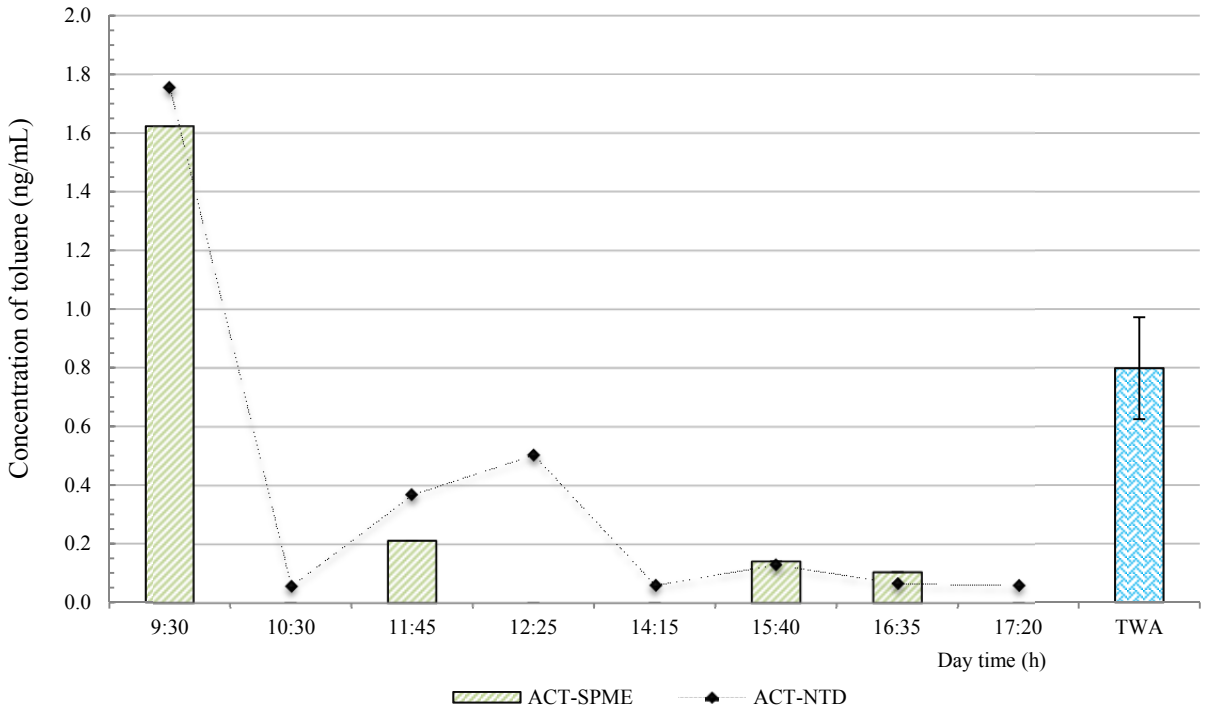


Figure 4.12 Evaluation of the concentration of toluene at different hours in a polymer chemistry laboratory at University of Waterloo. TWA sampling using three 85µm CAR/PDMS (Z=0.147 cm, t = 8 hours); SPME active sampling using four 85µm CAR/PDMS (t=30 s, Avg. T=22.4ºC); NTD sampling using a multi-bed DVB/CAR (100 mL at 2mL/min).

Table 4.3 Experimental parameters used to determine the concentration of toluene in indoors air at a polymer synthesis laboratory using the SPME diffusion based calibration

Parameters/Day time (h)	10:30	11:45	15:30	16:35	Units
Concentration	1.62	0.21	0.14	0.10	ng/mL
Amount extracted	37	5	3	2	ng
Temperature	295.6	295.6	295.3	295.2	K
Diffusion coefficient (D_g)	0.0793	0.0793	0.0791	0.0791	cm^2/s
Boundary layer thickness	0.0135	0.0135	0.0135	0.0135	cm
Reynolds number	28.339	28.339	28.391	28.407	-
Air kinematic viscosity (v)	0.1535	0.1535	0.1532	0.1531	cm^2/s
Schmidt number	1.937	1.937	1.937	1.937	-
Fibre radio (b)	0.0145				cm
Fibre length (L)	1				cm
Sampling time (t)	30				s
Pressure (P)	1				atm
Mass air	28.97				g/mol
Volume air	20.1				cm^3/mol
Mr	0.045				mol/g
Mass toluene	92.14				g/mol
Volume toluene	111.14				cm^3/mol
Linear velocity of the air (u)	150				cm/s

The increase in the concentration of toluene, observed at two different times during the day, at 9:30 am and 12:25 pm, was directly correlated to the use of a rotary evaporator by the workers. It is important to highlight that the sampling devices were located at approximately 2.5 meters from the rotavap. This means that average concentrations of toluene closer to the rotavap may be even higher than the ones reported here.

The NTD concentration can be considered as a time-weighted average sample over a short sampling period (approximately 20 min). In contrast, the PDAS-SPME concentrations can be associated with spot 30-s sampling. However, both techniques only allow the analyst to see a specific fragment of the day rather than the entire day variation. This explains why the average of the concentrations calculated using NTD and spot SPME (0.37 and 0.26 ng/mL, respectively)

were lower than the one obtained with SPME in passive sampling mode (0.80 ± 0.17 ng/mL, n=3). Other contaminants, such as tetrahydrofuran, chloroform and cyclohexane were also quantified using the techniques mentioned above (data not shown). Similarly to toluene, by means of NTD and the PDAS-SPME, intra-day variations in the concentrations of these solvents were tracked. All contaminants mentioned above were not found to be present in higher concentrations than the regulatory quantities established by the National Institute for Occupational Safety and Health (NIOSH) at all times. For instance, the highest concentration of toluene found during the sampling was 1.8 ng/mL, whereas the established 10 hour Threshold Limit Value (TLV) and the short-time exposure limit (STEL) of toluene are 377 and 565 ng/mL, respectively. The results presented in this study highlight the applicability of these techniques in the monitoring of more toxic compounds such benzene, which have lower thresholds (0.32 ng/L TLV and 8 ng/L STEL).[38,92]

4.3.6 Determination of Biogenic emissions in pine trees using *in vivo* SPME

The BVOCs emission profiles of a pine tree branch were evaluated in a time span of 12 hours during the third week of July 2012. The typical chromatographic profile after *in vivo* sampling and the peak identity are presented in Figure 4.13 and Table 4.4. Three major compounds found at any time of the day were selected for quantitation: limonene, α-pinene and β-pinene.[110] Figure 4.14 presents the concentrations determined for each compound every 3 hours from 8 am to 8 pm. Error bars represent the standard deviation of the mean calculated with four independent SPME-FFA 65 μm DVB/PDMS. The experimental parameters used to determine the concentration of these compounds are presented in Table 4.5.

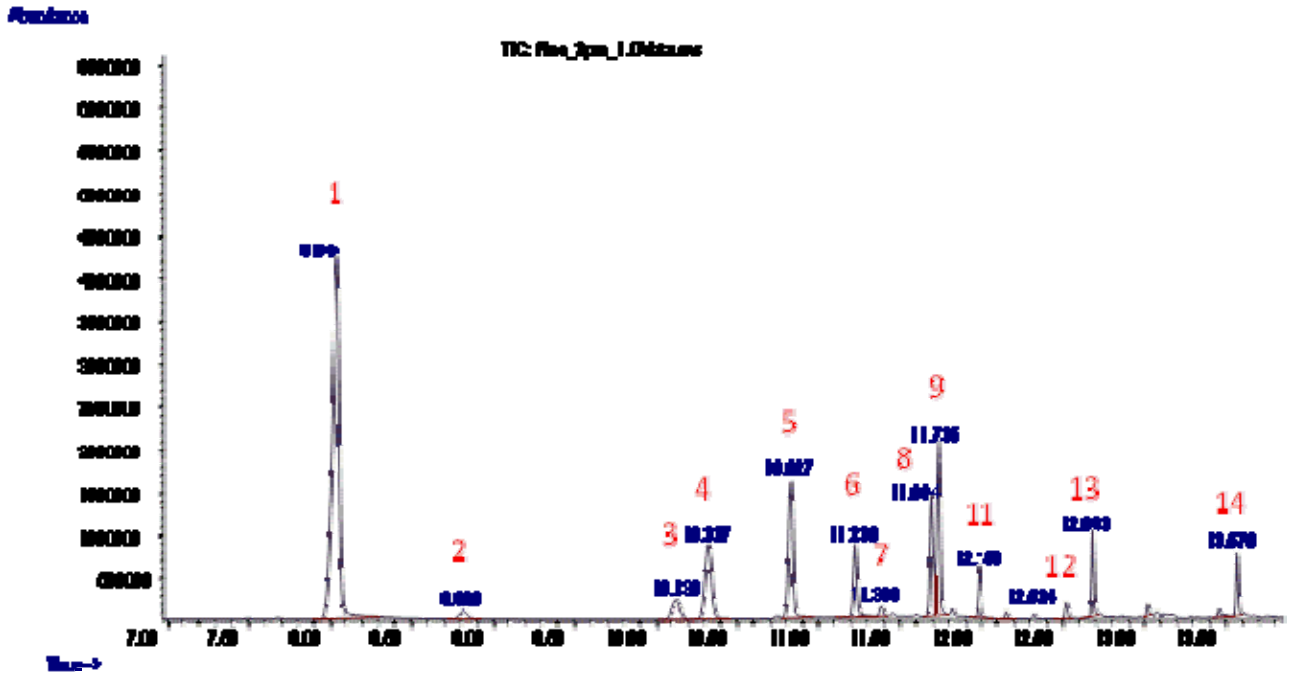


Figure 4.13 Typical GC-qMS profile of Pine tree BVOC after 30-s *in vivo* sampling with a SPME-FFA 65 µm PDMS/DVB. Peak identity was included on Table 4.4.

Table 4.4 Experimental parameters used to determine the concentration α-pinene, β-pinene and limonene at different hours in pine trees. t_R, retention time (min); $I_{(calc)}$, retention index calculated; $I_{(lit)}$, retention index reported on the literature; CAS, CAS registry numbers.

Peak	t_R	$I_{(calc)}$	$I_{(lit)}$	Compound	CAS
1	8.045	933	936	α-pinene	7785-26-4
2	8.820	950	947	Camphene	79-92-5
3	10.130	978	978	Sabinene	3387-41-5
4	10.327	983	981	β-pinene	127-91-3
5	10.827	993	992	β-Myrcene	123-35-3
6	11.230	1007	1005	3-Hexen-1-ol, acetate, (E)-	3681-71-8
7	11.389	1017	1011	n-Hexyl acetate	142-92-7
8	11.694	1036	1032	Limonene	138-86-3
9	11.735	1039	1035	Eucalyptol	470-82-6
10	11.988	1055	1047	β-(E)-ocimene	3779-61-1
11	12.159	1066	1062	γ-terpinene	99-85-4
12	12.524	1090	1086	Terpinolene	586-62-9
13	12.683	1100	1098	Linalool	78-70-6
14	13.570	1200	1190	α-terpineol	98-55-5
15	14.503	1356	1351	α-terpineol acetate	80-26-2
16	14.753	1405	1405	Methyleugenol	93-15-2
17	14.947	1447	1419	Caryophyllene-E	87-44-5
18	15.227	1508	1480	Germacrene D	23986-74-5

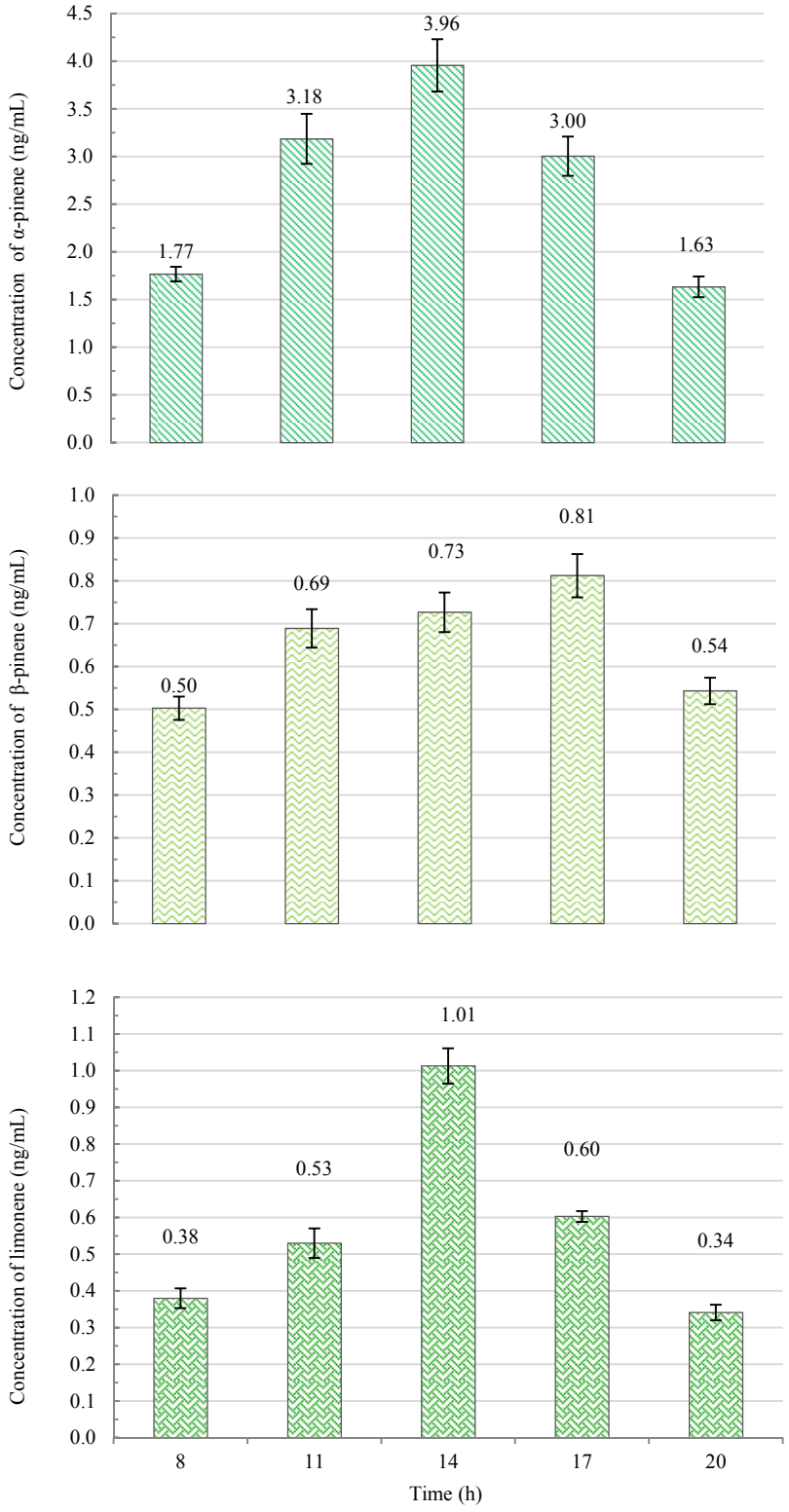


Figure 4.14 Evaluation of the concentration of α-pinene, β-pinene and limonene emitted at different hours by a pine tree at University of Waterloo. Spot sampling using four 65 µm DVB/PDMS (t=30 s, Avg. T=26.1°C, n=4).

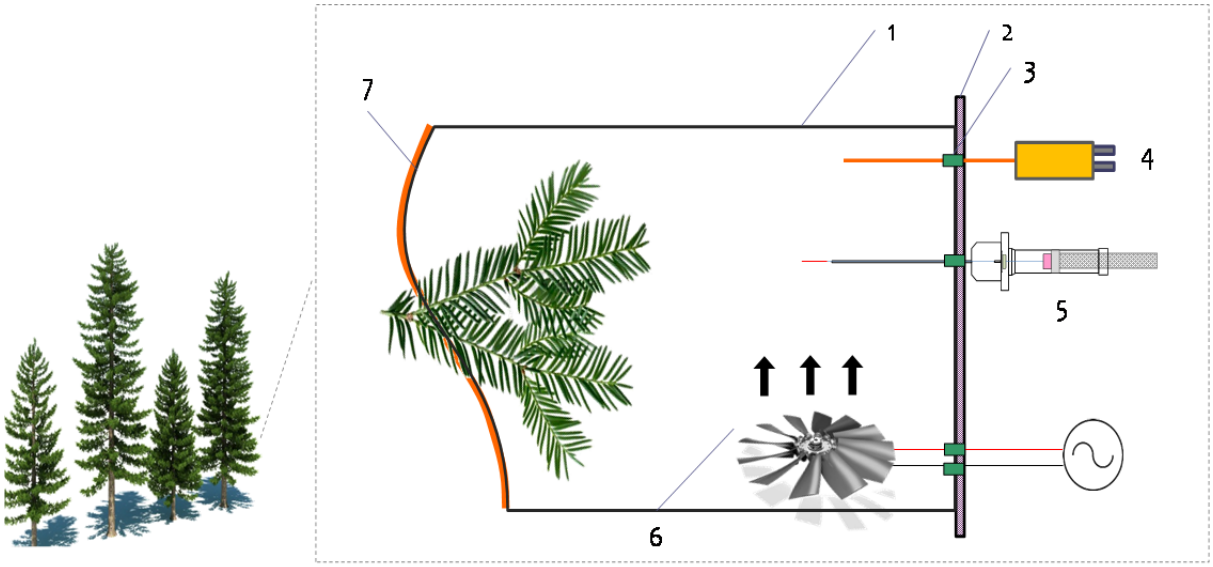


Figure 4.15 Glass container for live plants BVOC extraction: 1, silanized glass cylindrical body (120 mm x 60 mm); 2, silanized glass lid; 3, sampling holes topped with Thermogreen LB-2 septa; 4, thermocouple; 5, SPME-FFA 65µm DVB/PDMS; 6, microfan (40 mm x 40 mm x 6 mm); and 7, Teflon tape seal.

Table 4.5 Experimental parameters used to determine the concentration α-pinene, β-pinene and limonene at different hours in pine trees at University of Waterloo using the SPME diffusion based calibration approach.

Parameter	Time (h)					Units
	8	11	14	17	20	
Concentration α-pinene	1.77	3.18	3.96	3.00	1.63	ng/mL
Concentration β-pinene	0.50	0.69	0.73	0.81	0.54	ng/mL
Concentration limonene	0.38	0.53	1.01	0.60	0.34	ng/mL
Temperature	296.3	297.6	300.6	301.6	299.3	K
Diffusion coefficient (D_g)	0.0595	0.0599	0.0610	0.0613	0.0605	cm^2/s
Boundary layer thickness	0.0197	0.0198	0.0200	0.0201	0.0199	cm
Reynolds number	9.492	9.418	9.252	9.198	9.323	-
Air kinematic viscosity (v)	0.1541	0.1553	0.1581	0.1591	0.1569	$cm^{2/}s$
Schmidt number	2.592	2.593	2.593	2.593	2.593	-
Fibre radios (Outside) (b)			0.0120			cm
Fibre length (L)			1			cm
Sampling time (t)			30			s
Pressure (P)			1			atm
Mass air			28.97			g/mol
Volume air			20.1			cm^3/mol
Mass analyte			136.23			g/mol
Volume analyte			196.68			cm^3/mol
Linear velocity of the air (u)			60.96			cm/s

In summary, 18 compounds were completely identified by their linear retention indices and comparison of mass spectra with those found in the NIST database and literature.[111] The concentration of the target analytes showed a similar trend over the duration of the experiment: the highest concentration for the target compounds was obtained at 2 pm with 0.73, 1.01 and 3.96 ng/mL for β-pinene, limonene and α-pinene, respectively. All the concentrations were in the range of hundreds of nanograms per liter, which are within the typical range for forest atmospheric environments. Good inter-fibre repeatability for 4 FFA-SPME fibres was found and the RSD values were within the range of 4 to 8 percent in all the cases. Circadian variations observed in the concentrations of the target analytes can be a reflex to the variations of temperature and illumination conditions during the sampling cycle. Similar trends have been previously reported for isoprene in the analysis of *Eucalyptus citriodora,* and eucalyptol in the analysis of *Brugmansia suaveolens* flowers.[97,100]

4.4 Conclusions

A critical evaluation of the MFX system performance was accomplished. Storage stability as well as continuous operation over 200 extraction/injection cycles was studied. It was found that the CAR/PDMS fibre is the only coating that can be stored in the tray for up to 24 hours after field sampling without significant losses. With the current set up of the MFX system, other coatings may present significant losses (>10%) depending on the volatility of the analyte and its affinity for the fibre coating. This should be emphasized by the manufacturer in order to avoid potential customer disappointment. In order to solve this problem a new cap was designed and built at the University of Waterloo machine shop. The results here reported demonstrated that this cap is an effective solution to prevent analyte losses during the storage. However, some modifications in the software and the tray are required to properly implement the use of the

modified cap. Suitable modifications of the tray and software were proposed to the manufacturer for their evaluation and implementation. It is expected in the near future that with these modifications, a broader range of compounds can be analyzed after field sampling without concerns in regards to storage time on the tray.

The capabilities of the MFX system for high-throughput analysis were demonstrated by doing active and passive sampling applications. Indoor air was analyzed at a polymer synthesis laboratory at University of Waterloo using a total of seven CAR/PDMS fibres. Both, TWA and short-term exposure concentrations of toluene were found to be below threshold limits. The results obtained by SPME were in good agreement with those obtained using active sampling with NTDs. Also, a BVOCs emission profile of a pine tree was evaluated over a period of 12 hours. Outstanding inter-fibre repeatability (≤8%) was found using 4 FFA-DVB/PDMS fibres. Thus, it can be predicted that SPME, in conjunction with FFA devices, will have a significant contribution as a sampling technique for living plants or similar biological systems where multiple fibres are required. In summary, this study proved the feasibility of the MFX system to analyze multiple fibres after on-site sampling without manual intervention.

A new diffusive fibre holder (DFH) was also evaluated. No effects from the holder case on the uptake rate of analytes were observed. Moreover, good storage stability of the sealing system was found. Despite the DFH ability to achieve the basic requirements of a field sampler, it has been observed that special care must be taken when adjusting the diffusion path distance (Z); as reported by several authors,[36,37,43] small variations or erroneous measurement of Z can considerably affect results. A plausible solution is to install an electronic digital display, similar to a low-cost digital caliper, on which the accurate retracted distance of the fibre is shown.

Chapter 5 – Development and preliminary evaluation of a new Pen-like Diffusive Sampler (PDS) for NT-TWA sampling

5.1 Introduction

Several SPME field samplers have been developed, such as the SPME field sampler with a two-leaf closure, the gastight valve syringe modified for SPME field applications, the disposable field sampler with a Teflon cap, and the Supelco field sampler.[1,3,112-114] Nevertheless, the majority of these devices do not integrate these important factors: preservation of samples, ease of deployment, storage, and transportation.

The field sampler developed by Chen and Pawliszyn[3] was designed to use and interchange commercial fibre assemblies, making this sampler more universal. Moreover, this device achieved three of the four design requirements of an SPME field sampler, namely proper sealing of the needle, needle protection, and a user-friendly interface. However, despite its small size and simple movements required to operate this sampler, this fibre assembly is not suitable for use in a multiple device exchanger, such as one presented in chapter 4 of this thesis.[51]

Recently, Zare and *et al.* developed a SPME pen-shaped holder for passive sampling of anaesthetics in operating rooms.[35] According to the authors, it is 11 cm in length and weighs approximately 25 g. Depending on the concentration of the analyte being studied, the sampler sensitivity can also be modified by adjusting the diffusion path, via the screw-like end of the holder.[35] However, a serious drawback of this device is that storage features, such as a Teflon cap, were not included in its design.

The Pawliszyn group found that Teflon is an appropriate sealing material with negligible memory effects, and that it appropriately isolates the fibre coating from the ambient

environment, thus avoiding contamination and protecting sample integrity.[3,114] This group also demonstrated the effectiveness of the Teflon cap when it was used with a high efficient sorbent like Carboxen, since it can retain VOCs for up to two weeks without significant losses.[3] Cross contamination may only be an issue when Teflon caps are used repeatedly. Nonetheless, simple solutions, such as Teflon cap conditioning at high temperatures, can diminish the potential for cross contamination.[3]

Up to date, only two portable personal diffusive samplers have been developed for NT; the badge-like sampler (Figure 5.1, inset A) consists of two components, a sampler holder and a NT. The sampler holder is a metal plate with four Teflon chips. A hole in the centre of each chip allows sealing of the side hole and tip of the needle, so as to preserve sample integrity. An advantage of this device is that it could be fixed either to the front pocket of the operator or under a shirt collar during the sampling process.[95]

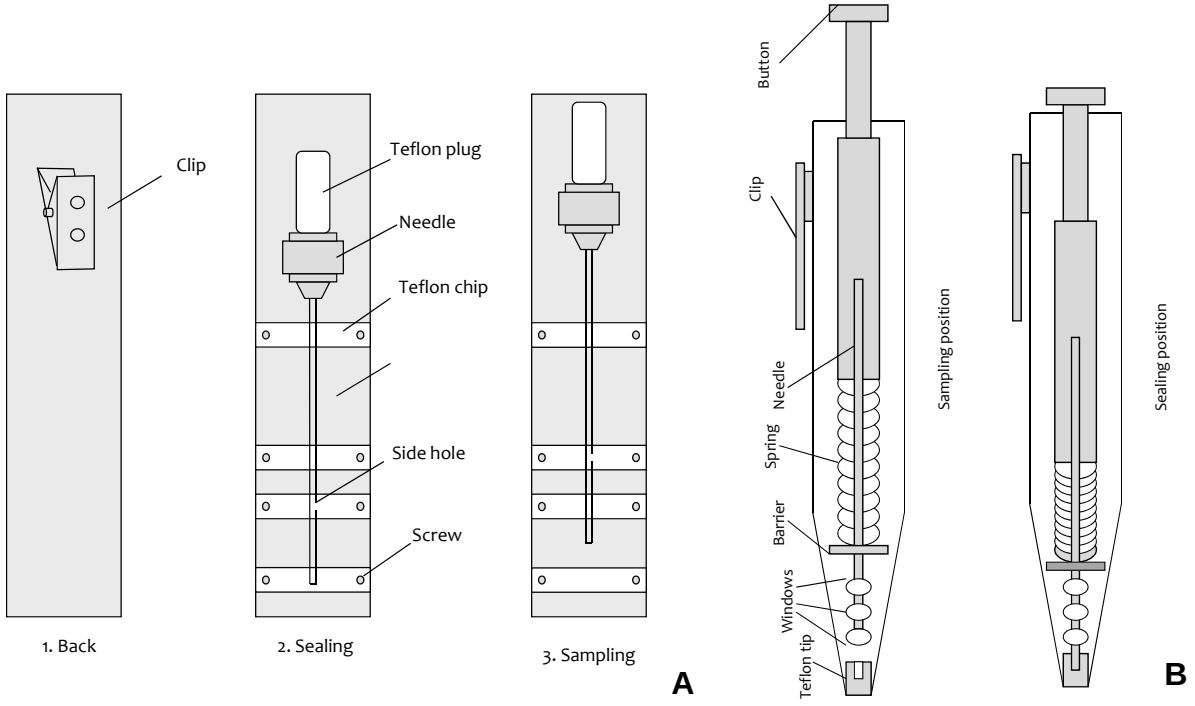


Figure 5.1 Schematic drawing of field samplers developed for NTD in passive mode. A, badge like sampler; B, pen-like sampler.

Conversely, the pen-like device (Figure 5.1, inset B) is lighter and more user-friendly than the former.[95] However, because of its design, it is complicated not only to load the NTD into the holder, but also in the tray of the autosampler. This device operates in two positions, the sealing position and the sampling position. When the button at the end of the pen is pressed, the tip of the needle is sealed by a Teflon cap found in the tip of the pen. Alternatively, when the needle is retracted by pressing the button, the tip of the needle is exposed to air that moves in and out through the elliptical windows on each side of the pen. In this study, a new diffusive sampler is presented. It has a similar manoeuvre mechanism to the one described by Gong *et al.*[95] However, in contrast to the previous design, the loading of the NT on the holder is simpler and can be accomplished in few seconds. Also, a clever clicking exposure system placed the NT automatically in the sampling position when it was fixed in a pocket. Unlike previous works, a sampling chamber was successfully designed and built for the evaluation of the sampler device under a controlled environment. Moreover, the new PDS-NT can be used for either manual desorption with the holder, or automated unattended NT desorption using the Concept autosampler commercialized by PAS Technologies.[4]

5.2 Experimental

5.2.1 Materials and reagent

HPLC grade methanol was obtained from Caledon laboratories LTD (Georgetown, ON, Canada). BTX was purchased from Sigma-Aldrich (Mississauga, ON, Canada). Helium of ultra-high purity was supplied by Praxair (Kitchener, ON, Canada). Gas tight syringes (1 and 5 mL) were purchased from Hamilton Company (Reno, NE, USA). All the preparations were carried out in a ventilated fume hood. CAR particles (surface area: 1200 m^2/g) of 60/80 mesh were

purchased from Sigma-Aldrich (Bellefonte, PA, USA). DVB particles (surface area: 582 m^2/g) of 100/120 mesh were purchased from Ohio Valley (Marietta, OH, USA). The 3.5 inch long 22-gauge blunt needles (I.D. 0.41 mm, O.D. 0.71 mm) were purchased from Dyna Medical Corporation (London, ON, Canada). Stainless steel wires (O.D. 100 μm) were purchased from Small Parts (Lexington, KY, US). The 5-min epoxy glue was purchased from Henkel Canada (Mississauga, Ontario, Canada). The ADM 1000 flow-meter was purchased from Agilent Technologies (Mississauga, ON, Canada)

5.2.2 Instrumentation

An Acme 6100 series gas chromatograph (Young Lin Instruments, Anyang, Korea) equipped with a flame ionization detector (FID), and a capillary column (RTX-5, 30 m × 0.25 mm I.D., 0.25 μm film thickness) was used for the separation and detection of BTX. The oven temperature was initially held at 40 ºC for 1 min, gradually increased to 180 ºC at a rate of 25 ºC per min, and then held for 2 min. An ATAS GL Optic 3 injection port (ATAS GL, Eindhoven, Netherlands) was used for liquid, needle trap and SPME injections.

5.2.3 Preparation of the needle traps

The packing process for each needle was the same as the procedure described in Section 4.2.2. The sorbent beds used were 1 NT packed with 1 cm 100/120 mesh DVB plus 1 cm 60/80 mesh CAR, and several NT packed with 1 cm 60/80 mesh CAR. After packing, the NTDs were conditioned in a GC injector for 3 hours with helium gas continuously flowing through the needle. The conditioning temperature was 260 °C for DVB/CAR NT and 300 ºC for CAR NT. This process was similar to previous works.[105-108]

5.2.4 Standard Gas Mixture and permeation tubes

Permeation tubes for BTX were made by encapsulating pure analyte inside a 100 mm long (1/4 in.) Teflon™ tubing capped with 20 mm long solid Teflon™ plugs and (1/4) in. Swagelok caps. Emission rates for each permeation tube were verified by periodic monitoring of weight loss of individual analyte tubes. A standard gas generator (model 491 MB, Kin-Tech Laboratories, LaMarque, TX, USA) was used to generate standard gases with desired concentrations. The permeation tubes made in our laboratory were placed inside a glass chamber, held in a temperature-controlled oven and swept with a controllable constant flow of compressed air. Different concentrations of the analytes were obtained by adjusting both the permeation chamber temperature and the air flow rate.

5.2.5 Desorption of needle traps

After sampling, the NTD was connected to a 1 mL gas-tight syringe filled with a certain volume of helium, and then introduced into a GC injector for desorption. The helium was consistently pushed out to assist the desorption during the whole desorption period. NTDs packed with CAR were injected into the hot GC injector at 300 °C for 1 min, with the assistance of 0.3 mL helium.

5.2.6 Sampling Chamber for the evaluation of the new pen-like device

For the evaluation of the new pen-like device with BTX, a new sampling chamber was built at the glass blowing shop of the University of Waterloo, based on a previous system designed by

Koziel *et al.*[88]. A schematic of the sampling chamber is provided in Figure 5.2. This sampling chamber facilitated a steady-state mass flow of all the standards; it consisted of a custom made 1.5 L glass bulb with 4 sampling ports that were plugged with Thermogreen LB-2 predrilled septa. As shown in Figure 5.2, for sampling of the pen-like device, a special sampling port was built; this sampling port has a clever system that circumvents the release of contaminants in the laboratory atmosphere. It consists of two Teflon O-rings embedded within a predrilled Teflon stopper that presses the pen-device and seals the system. When the pen is not sampling, a Teflon plug of the same outside diameter was used to seal the system. Custom-made glass restrictions, as well as a special Thermogreen washer were built to hold the Teflon stopper in position, by tightening its cap. In order to evaluate multiple devices, 4 ports were constructed following the same design. Standard gas generators and sampling chambers were validated using a multi-bed needle trap.

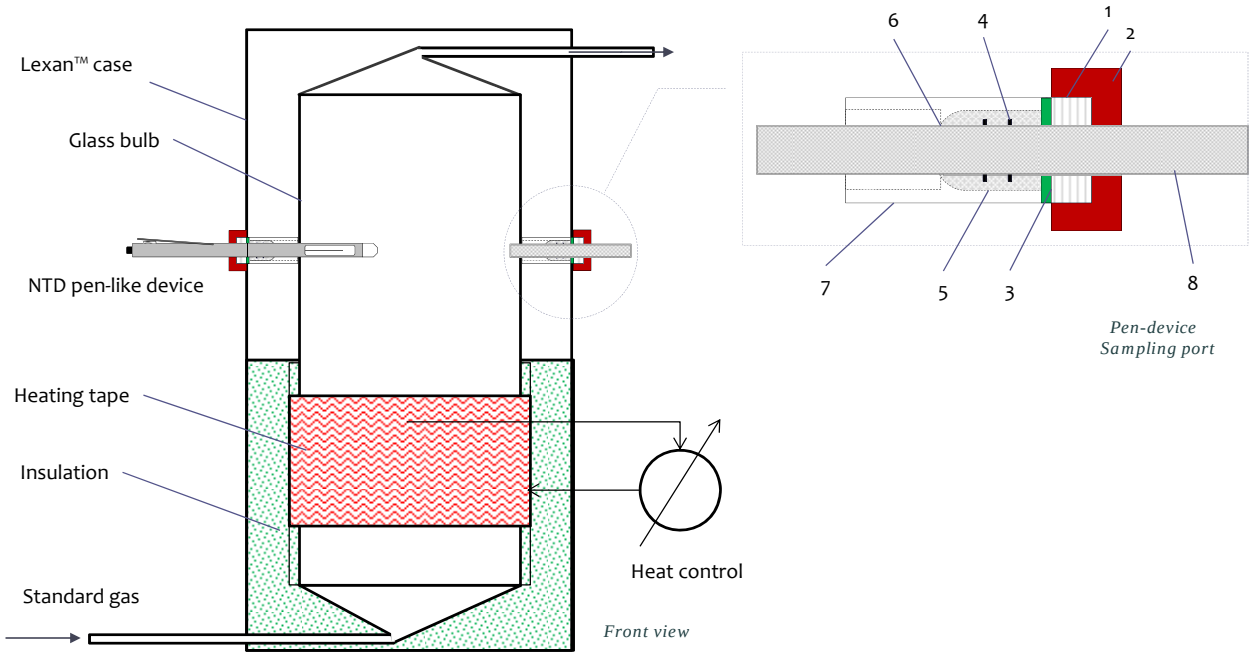


Figure 5.2 Schematic of the sampling chamber used for the evaluation of the pen-like NT diffusive sampler. 1, GL thread; 2, chamber cap; 3, Thermogreen washer; 4, Teflon O-ring; 5, Pre-drilled Teflon stopper; 6, glass restriction; 7, glass tubing; 8, Teflon plug that seals the chamber when the pen is not sampling.

5.3 Results and Discussion

5.3.1 Design of the new pen-like diffusive sampler

Figure 5.3 and 5.4 summarize the main features of the new PDS. One of the most important characteristic of the new device is its versatility; most commercial needle traps can be installed. Because of the plug-screw system designed for the top of the needle, it can be easily fitted to the upper part of the holder. This feature allows the analyst to do a manual injection whenever a needle trap with a side-hole is used[106-108]. Another remarkable characteristic is the automatic exposure system. By placing the PDS on a shirt pocket (Figure 5.4) the needle is moved automatically to the sampling position. Finally, the screw-type Teflon tip not only guarantees sample preservation during its transportation/storage but can also be easily disassembled for cleaning purposes[3].

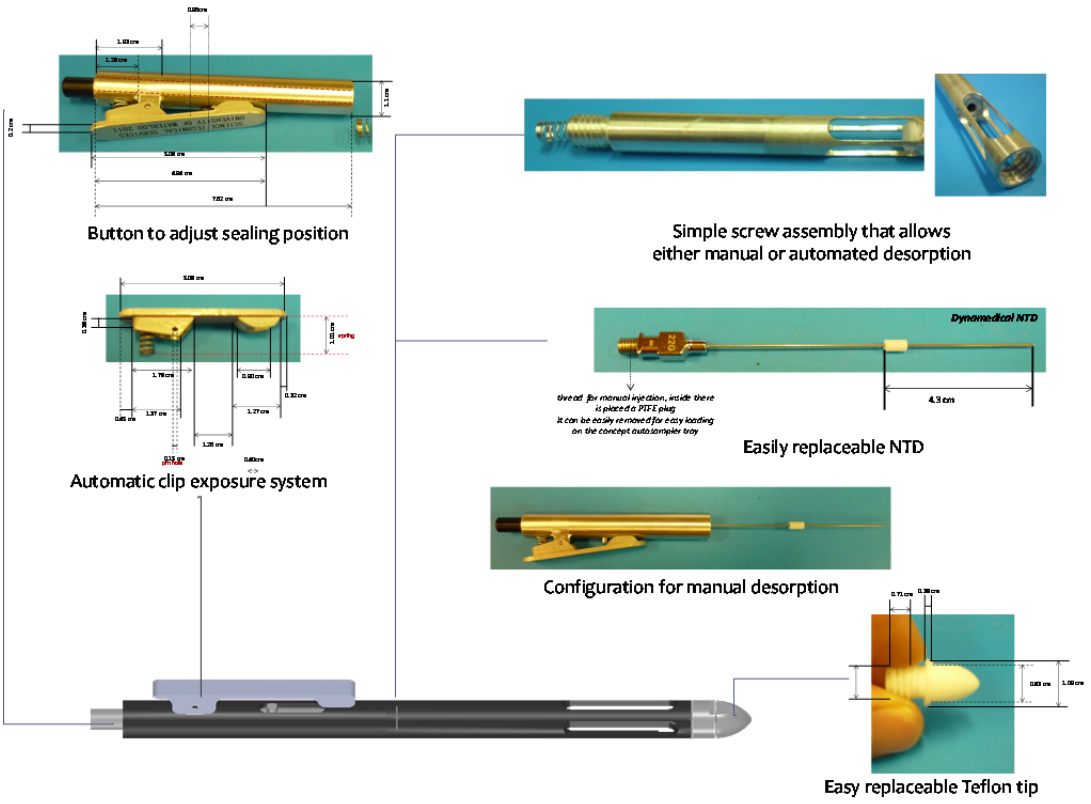


Figure 5.3 Schematic drawing of the new pen-like diffusive sampler for needle trap.

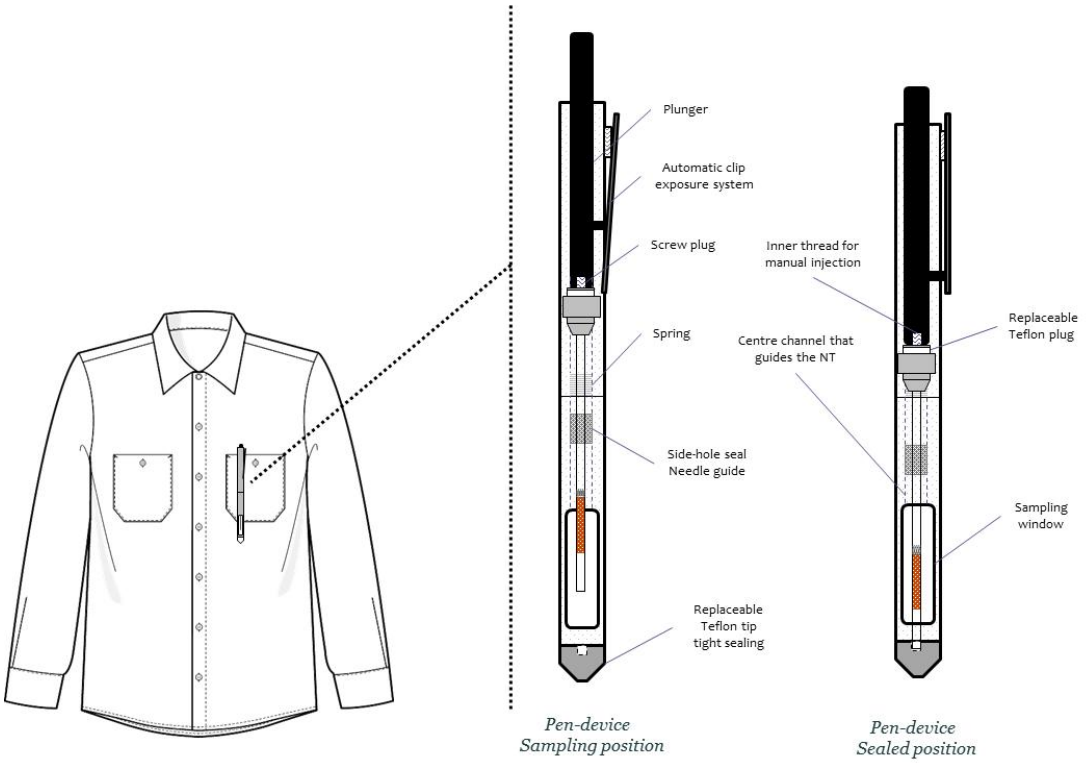


Figure 5.4 Schematic of the sampling and sealed positions of the PDS-NT.

5.3.2 Effect of the holder on the uptake rate

Two critical parameters of the pen-like diffusive sampler (PDS) were evaluated, specifically storage stability for up to 24 hours at room temperature, and possible effects of the sampler device on the uptake rate of the analytes. The former was evaluated by comparing the amount of BTX collected by a needle trap with and without the sampling holder. These compounds were selected based on data provided by *Gong et al.,*[5] who demonstrated that a NTD packed with Carboxen1000 is a successful diffusive sampler for monitoring TWA concentrations of BTEX under low relative humidity.[5] Figure 5.5 presents the comparison of two independent needle traps versus the same needle trap installed in the holder. No statistical differences were found for any of the needle traps. Thus, based on these experimental findings, it is possible to use the PDS with no concerns regarding possible holder effects on analyte uptake rates.

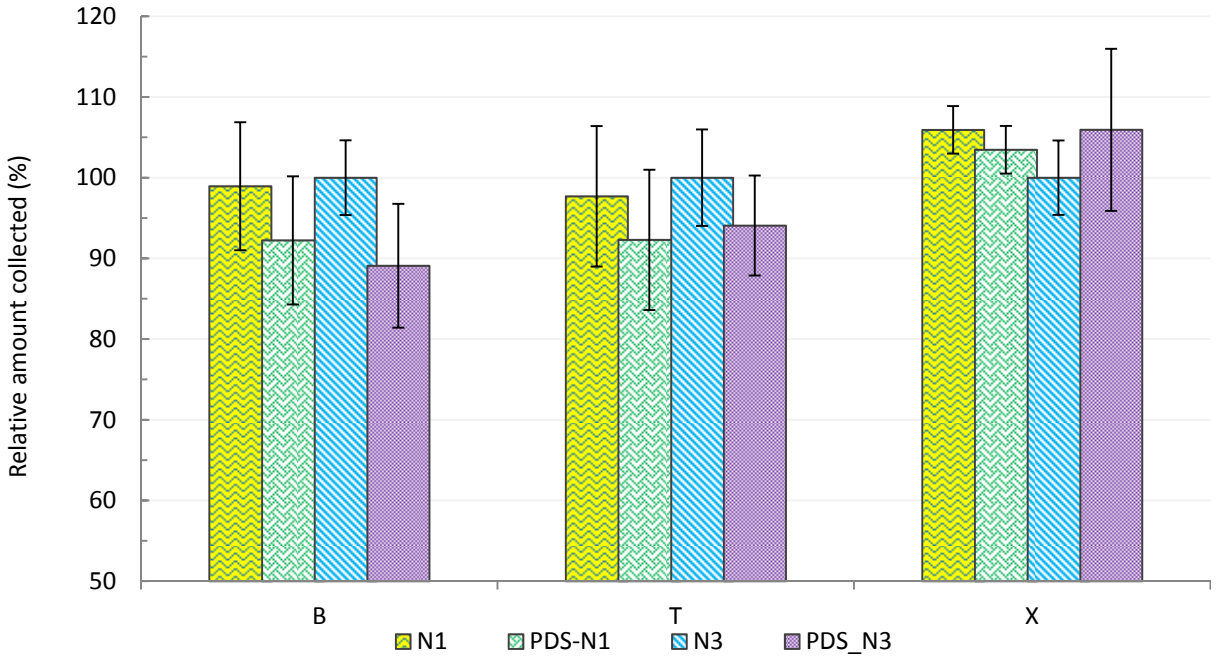


Figure 5.5 Evaluation of the effect of the pen-like diffusive sampler (PDS) on the uptake rate of two different NT pack with 1 cm of CAR (Z=0.25 cm, t = 30 min, T= 25ºC). Error bars represent the standard deviation of the mean (n = 4).

5.3.3 Evaluation of storage stability

Storage stability is critical for field TWA sampling. If storage is unstable, analytes adsorbed inside the sampler may be lost, introducing experimental error. The storage stability of the PDS containing a NTD packed with Carboxen1000 was evaluated. First, the PDS-NTD was used to passively sample BTX from the standard gas system, and then instantaneously injected into the GC/FID. Second, the same device was used to sample passively, and immediately after, the button on top of the PDS was pressed to seal the needle with the pen's tip (made of Teflon). Subsequently, the pen was wrapped with aluminum foil to prevent cross contamination, and stored for 24 h at 23.5°C; after a 24 hour period, the NT was injected into the GC/FID. The results from the analysis, presented on Figure 5.6, showed no significant losses after 24 hours of storage at room temperature. These results agreed with those reported by Gong *et al.*[5] However, in the future, storage duration of up to 2 weeks should be evaluated.[5,115]

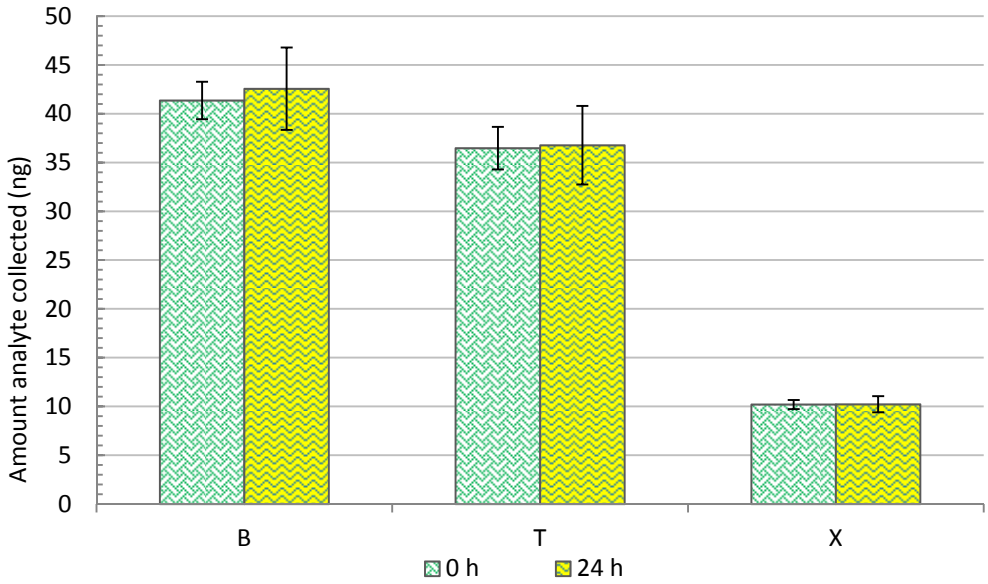


Figure 5.6 Storage stability of the pen-like diffusive sampler (PDS) containing a NT packed with 1 cm of CAR (Z=0.25 cm, t = 30 min, T= 25ºC). Error bars represent the standard deviation of the mean (n = 3). Storage temperature = 23.5 ºC (room temperature).

5.3.4 Comparison of two PDS-NT holders

Two PDS-NT were built at the University of Waterloo machine shop. Two needle traps found to be statistically similar, in terms of the amount of BTX collected, were selected for the evaluation of these PDS devices. As shown in Figure 5.7, statistical differences were not found when comparing two independent PDS devices (n=5). Inter-PDS repeatability was below 9 percent for all compounds. Therefore, it can be concluded that two independent PDS-NT devices have the same performance under the controlled conditions here described. In order to have a complete acceptance of the PDS-NT, other environmental conditions that critically affect diffusive passive samplers, such as temperature and humidity, should be studied.[36,115] Several studies have shown that these environmental parameters might affect the uptake rate of the analyte, depending on its molecular weight and polarity.[42] Consequently, a broader range of VOCs should be evaluated using the PDS-NT.

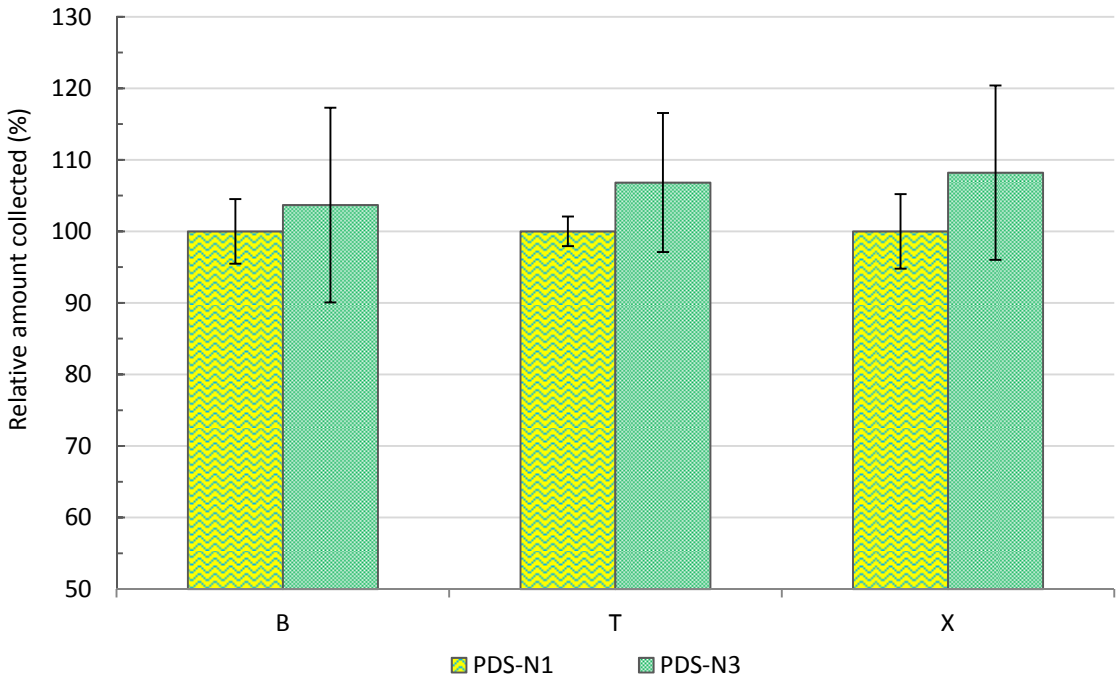


Figure 5.7 Evaluation of two pen-like diffusive samplers (PDS) using NT packed with 1 cm of CAR (Z=0.25 cm, t = 30 min, T= 25ºC). Error bars represent the standard deviation of the mean (n = 5).

5.4 Conclusions

In this work a new, easy to deploy, and reusable needle trap pen-like diffusive sampler (PDS-NT) was presented. Unlike previous designs, a clever clicking exposure system positioned the NT automatically in the sampling position when placed in a fixed position; in this case, a pocket was used. In addition, the loading of the NT on the pen is simpler, and the device can be used for both manual or automated unattended NT desorption. The design PDS-NT allows the installation of any of the commercial available needles such as Dynamedical, SGE and Shinwa.[116-120]

This study demonstrated that the new PDS-NT is effective for air analysis of benzene, toluene, and o-xylene (BTX). No effects based on pen geometry were observed in regards to the

uptake of analytes. Good storage stability of the target analytes was observed for up to 24 hours. Finally, the comparison of two independent PDS-NT devices showed no statistical differences.

Further efforts have to be made to conduct a series of experiments under different environmental conditions to monitor a greater range of VOCs. It can be predicted that the PDS-NT will be useful and convenient for monitoring both personal exposure in the occupational environment and ambient air quality.

Chapter 6 – Summary

The MFX system showed accurate performance over more than 200 extraction/injection cycles (2 days running nonstop) using multiple fibres, a result greatly desired for high-throughput applications. However, in terms of storage stability, it was found that CAR/PDMS fibres are the only coating type that can be stored in the MFX tray up to 24 hours after field sampling without incurring significant losses. Studies showed that commercial caps do not have tight enough sealing and, as a result, other coatings with weaker sorbents are prone to loss of analytes, depending on their volatility. However, if the sealing efficiency of the current caps is enhanced, a broader range of compounds could be analyzed after field sampling without concerns over storage time on the tray.

The capabilities of the MFX system for high-throughput analysis were demonstrated by the unattended desorption of samples taken on-site in two different systems where multiple fibres are required, named indoor air in a polymer synthesis laboratory and biogenic emission profile of a pine tree.

The evaluation of the diffusive fibre holder (DFH) for SPME showed no holder case effect on the uptake rate of the analytes, as well as good storage stability. Despite the ability of the DFH to achieve the basic requirements of a field sampler, special care must be taken when adjusting the diffusion path (Z). Small variations on Z can significantly affect the uptake of the analytes.

A new in-vial standard gas system for calibration of SPME in high-throughput applications was presented in this study. The loading technique is fast and reproducible, and the same standard generation vial can be used for more than a hundred analyses (RSD < 3%). It was found

that vials from the same batch are reproducible and interchangeable. Additionally, due to the physical characteristics of the new in-vial calibration solution, they can be easily transported, thus an ideal calibration standard for both bench and field instruments and devices.

Thus, a new in-vial gas generator was used in the development of a standardized protocol for a quick assessment of the reproducibility of commercial SPME fibres. This approach allows the user to determine whether a number of fibres can assure the acquisition of reliable and reproducible data prior to their application. The application of this protocol using the MFX system allows the complete evaluation of 7 fibres in less than 14 hours.

The in-vial standard gas system was also used for the determination of experimental sampling rates with SPME in passive mode. BTEX were selected as model compounds in this proof-of-concept evaluation. It was proven that the new in-vial standard gas system can be used as a continuous source of standards. Good agreement was observed among the theoretical and experimental sampling rates using the method here proposed for most of the compounds. Intra-vial and inter-vial repeatability were evaluated in passive mode, and results showed no statistical differences for any of the compounds used as a model. It should be emphasized that the application of the new in-vial standard gas generator in the determination of experimental sampling rates of common air contaminants should be done in the very first stages of method development, since other environmental factors such as humidity and air velocity might affect the results, depending on the polarity of the analyte.

Finally, an easily deployed and reusable needle trap pen-like diffusive sampler (PDS-NT) was presented. The loading of the NT on the pen is simpler, and allows the installation of different commercial NTs. Preliminary studies presented here have shown that the PDS-NT is

effective for air analysis of BTX. Additionally, no pen geometry effects were found on the uptake of the analytes. However, further experiments under different environmental conditions and for a broader range of analytes are recommended prior to its application in the monitoring of the occupational environment and ambient air quality.

References

1. J. Pawliszyn, Handbook of Solid Phase Microextraction, Chemical Industry Press, Beijing 2009.
2. Chen, Y.; Pawliszyn, J. Anal. Chem. 2003, 75, 2004.
3. Chen, Y.; Pawliszyn, J. Anal. Chem. 2004, 76, 6823.
4. Lord, H.; Zhan, W.; Pawliszyn, J. Anal. Chim. Acta 2010, 677, 3.
5. Gong, Y.; Eom, I.-Y.; Lou, D.-W.; Hein, D.; Pawliszyn, J. Anal. Chem. 2008, 80, 7275.
6. Górecki, T.; Namiesnik, J. Trends Anal. Chem. 2002, 21, 276.
7. Partyka, M.; Zabiegala, B.; Namiesnik, J.; Przyjasny, A. Crit. Rev. Anal. Chem. 2007, 37, 51.
8. Namiesnik, J.; Zabiegala, B.; Kot-Wasik, A.; Partyka, M.; Wasik. A. Anal. Bioanal. Chem. 2005, 381, 279.
9. Brown. R. J. Environ. Monit. 2000, 2, 1.
10. Seethapathy, S.; Górecki, T. J. Chromatogr., A 2011, 1218, 143.
11. Seethapathy, S.; Górecki, T. J. Chromatogr., A 2008, 1184, 234.
12. Seethapathy, S.; Górecki, T. J. Chromatogr., A 2010, 1217, 7907.
13. Pawliszyn, J. Solid Phase Microextraction: Theory and Practice. Wiley-VCH: New York, USA, 1997.
14. Pawliszyn, J. Anal. Chem. 2003, 75, 2543.
15. Handley, J.; Harris, C. Anal. Chem. 2001, 73, 660A.
16. Anastas, P.T.; Warner, J. C. Green Chemistry: Theory and Practice. Oxford University Press: Oxford, UK, 1998.
17. Pawliszyn, J. Anal. Chem. 2003, 75, 2543.
18. Risticevic, S.; Niri, V. H.; Vuckovic, D.; Pawliszyn, J. Anal. Bioanal. Chem. 2009, 393, 781.
19. Setkova, L.; Risticevic, S.; Linton, C. M.; Ouyang, G.; Bragg, L. M.; Pawliszyn, J. Anal. Chim. Acta. 2007, 581, 221.
20. Risticevic, S.; Lord, H.; Górecki, T.; Arthur, C. L.; Pawliszyn, J. Nat. Protoc. 2012, 5, 122.
21. Risticevic, S.; Chen, Y.; Kudlejova, L.; Vatinno, R.; Baltensperger, B.; Stuff, L.; Hein, D.; Pawliszyn, J. Nat. Protoc. 2010, 5, 162.

22. Souza-Silva, E. A.; Pawliszyn, J. Anal. Chem. 2012, 84, 6933.
23. Bojko, B.; Cudjoe, E.; Gómez-Ríos, G. A.; Krzysztof, G.; Jiang, R.; Reyes-Garcés, N.; Risticevic, S.; Souza-Silva, E. A; Togunde, O.; Vuckovic, D.; Pawliszyn, J. Anal. Chim. Acta. 2012, 750, 132.
24. Degli-Esposti, F.; Dugheri, V. "Automatic Solid Phase Microextraction (SPME) Sampling Apparatus" US Pat. 8,006,540 (issued October 22, 2009).
25. Risticevic, S.; Vuckovic, D.; Lord, H. L.; Pawliszyn, J. In Comprehensive Sampling and Sample Preparation Editor-in-Chief: Janusz Pawliszyn; Academic Press:Oxford, 2012; pp 419-460.
26. Koziel, J.; Jia, M.; Pawliszyn, J. Anal. Chem. 2000, 21, 5178.
27. Fuller E.N., Schettler, P.D., Giddings, J.C. Ind. Eng. Chem., 1966, 58, 18.
28. Augusto, F.; Koziel, J.; Pawliszyn, J. Anal. Chem. 2001, 73, 481.
29. Koziel, J.; Odziemkowski, M.; Pawliszyn, J. Anal. Chem. 2001, 73, 47.
30. Pawliszyn, J. "Needle Trap" US Pat. 6,481,301 (issued November 19, 2002).
31. Wang, A.; Fang, F.; Pawliszyn, J. J. Chromatogr., A 2005, 1072, 127.
32. Lestremau, F.; Andersson, F. A. T.; Desauziers, V.; Fanlo, J. Anal. Chem. 2003, 75, 2626.
33. Martos, P.; Pawliszyn, J. Anal Chem. 1999, 71, 1513.
34. Khaled, A.; Pawliszyn, J. J. Chromatogr. A 2000, 892, 455.
35. Zare Sakhvidi, M. J.; Bahrami, A.; Ghiasvand, A.; Mahjub, H.; Tuduri, L. Environ. Monit. Assess. 2012, 184, 6483.
36. Zare Sakhvidi, M. J.; Bahrami, A. R.; Ghiasvand, A.; Mahjub, H.; Tuduri, L. Environ. Monit. Assess. 2012, Article in press.
37. Chen, C.; Hsiech, C.; Lin, J. J. Chromatogr., A 2006, 1137, 138.
38. Batista, R. B.; Grimm, C. C.; Godshall, M. A. J. Chromatogr. Sci. 2002, 40, 122.
39. Bicchi, C.; Cordero, C.; Liberto, E.; Sgorbini, B.; Rubiolo, P. J. Chromatogr., A. 2007, 1152, 138.
40. Paschke, A.; Popp, P. J. Chromatogr. A. 2004, 1025, 11.
41. Zare-Sakhvidi, M. J.; Bahrami, A.; Ghiasvand, A.; Mahjub, H.; Tuduri, L. Anal. Lett. 2012, 45, 375.

42. Dugheri, S.; Pacenti, M.; Traldi, P.; Degli Esposti, F.; Perchiazzi, N.; Franchi, E.; Calamante, M.; Kikic, I.; Alessi, P.; Bonacchi, A.; Salvadori, E.; Arcangeli, G.; Cupelli, V. Journal of Automated Methods and Management in Chemistry. 2010, 1.
43. Lee, I.; Tsai, S. Anal.Chim.Acta. 2008, 610, 149.
44. Koziel, J.; Jia, M.; Khaled, A.; Noah, J.; Pawliszyn, J. Anal. Chim. Acta 1999, 400, 153.
45. ASTM Standard D6246-08, "Standard Practice for evaluating the performance of diffusive samplers", ASTM International, West Conshohocken, PA, 2008, 10.1520/D6246-08.
46. Lord, H. L.; Moder, M.; Popp, P.; Pawliszyn, J. B. Analyst. 2004, 129, 107.
47. Lord, H. L.; Pfannkoch, E. A. In Comprehensive Sampling and Sample Preparation Editor-in-Chief: Janusz Pawliszyn; Academic Press:Oxford, 2012; pp 597-612.
48. Arthur, C.; Killam, L.; Buchholz, K.; Pawliszyn, J.; Berg, J. Anal. Chem. 1992, 64, 1960.
49. O'Reilly, J.; Wang, Q.; Setkova, L.; Hutchinson, J.; Chen, Y.; Lord, H.; Linton, C. M.; Pawliszyn, J. J. Sep. Sci. 2005, 28, 2010.
50. Berg, J.R. Am. Lab. 1993, 25, 18.
51. Pacenti, M.; Dugheri, V.; Boccalon, P.; Arcangeli, G.; Dolara, P.; Cupelli, V. Industrial Health 2010, 48, 217.
52. Pacenti, M.; Dugheri, V.; Gagliano-Candela, R.; Struiullo, G.; Franchi, E.; Degli-Espoti, F.; Perchiazzi, N.; Boccalon, P.; Arcangeli, G.; Cupelli, V. Acta Chromatogr. 2009, 3, 379.
53. Vitkuske, D.; Peterson, K. Reporter US-Sigma-Aldrich, 2010, 28.1, 16.
54. SPME-Diffusive Sampling Fiber Holder (DFH) for TWA analysis, http://www.sigmaaldrich.com/etc/medialib/docs/Supelco/General_Information/1/dfh_manual_v1_6.Par.0001.File.tmp/dfh_manual_v1_6.pdf, Web, n.d. October, 2012.
55. Zhao, W.; Ouyang, G.; Pawliszyn, J. Analyst. 2007, 132, 256.
56. MacCrehan, W.; Moore, S.; Schantz, M. Anal. Chem. 2011, 83, 8560.
57. Chen, Y., Pawliszyn, J. Anal. Chem. 2004, 76, 5807-5815.
58. Xiong, G.; Chen, Y.; Pawliszyn, J. J. Chromatogr., A. 2003, 999, 43.
59. Risticevic, S.; DeEll, J. R.; Pawliszyn, J. J. Chromatogr., A. 2012, 1251, 208.
60. Wang, Y.; O'Reilly, J.; Chen, Y.; Pawliszyn, J. J. Chromatogr., A. 2005, 1072, 13.

61. Lee, E. D.; Xie, X.; Murray, J.; Contreras, J.E.; Tolley, H.D.; Lee, M.L.; Sadowski, C. "Simple device for calibration of hand-portable GC-MS systems for field applications" presented at The Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy (Pittcon), March 13-17, 2011.
62. Rajkó, R.; Körtvélyesi, T.; Sebők-Nagy, K.; Görgényi, M. Anal. Chim. Acta. 2005, 554, 163.
63. Hoffmann, E. A.; Fekete, Z. A.; Rajkó, R.; Palinko, I.; Körtvélyesi, T. J.Chromatogr.A. 2009, 1216, 2540.
64. Jiang, R.; Zhu, F.; Luan, T.; Tong, T.; Liu, H.; Ouyang, G.; Pawliszyn, J. J. Chromatogr., A. 2009, 1216, 4641.
65. Zhang, Z.; Pawliszyn J. J. Phys. Chem. 1996, 100, 17648.
66. Anderson, J.; Ding, J.; Welton, T.; Armstrong, D. J.Am.Chem.Soc. 2002, 124, 14247.
67. Armstrong, D.; He, L.; Liu, Y. Anal.Chem. 1999, 71, 3873.
68. Kersten, B.; Poole, C.; Furton, K. J. Chromatogr. 1987, 411, 43.
69. Li, J.; Chase, H. A. Nat. Prod. Rep. 2010, 27, 1493.
70. Daignault, S.A.; Noot, D.K.; Williams, D.T.; Huck, P.M. Wat. Res. 1988, 22, 803.
71. Camel, V.; Caude, M. J. Chromatogr. A. 1995, 710, 3.
72. Dettmer, K.; Engewald, W. Anal.Bioanal.Chem. 2002, 373, 490.
73. Rohm and Haas Amberlite XAD-4, Industrial grade polymeric adsorbent product data sheet, http://www.rohmhaas.com/ionexchange/pharmaceuticals/Bioprocessing_doc/english/xad4.PDF, Web, n.d. Web, October, 2012.
74. Box, G.E.P.; Hunter, J.S.; Hunter, W.G. Statistics for Experimenters: Design, Innovation and Discovery, 2005.
75. Natera Marín, R.; Castro Mejías, R.; De Valme García Moreno, M.; García Rowe, F.; García Barroso, C. J. Chromatogr., A. 2002, 967, 261.
76. Castro Mejías, R.; Natera Marín, R.; De Valme García Moreno, M.; García Barroso, C. J. Chromatogr., A. 2002, 953, 7.
77. Miller, J.C.; Miller, J.N.; Statistics and Chemometrics for Analytical Chemistry, 2005.
78. Heberger, K.; Milczewska, K.; Voelkel, A. J. Chromatogr. Sci. 2001, 39, 375.
79. Voelkel, A.; Milczewska, K.; Heberger, K. Anal. Chim. Acta. 2006, 559, 221.

80. Risticevic, S.; Carasek, E.; Pawliszyn, J. Anal. Chim. Acta. 2008, 617, 72.
81. Górecki, T.; Yu, X.; Pawliszyn, J. Analyst. 1999, 124, 643.
82. Tumbiolo, S.; Gal, J.F.; Maria, P-C; Zerbinati, O. Anal. Bioanal. Chem. 2004, 380, 824.
83. Nilsson, T.; Ferrari, R.; Facchetti, S. Anal.Chim.Acta. 1997, 356, 113.
84. Ferrari, R.; Nilsson, T.; Arena, R.; Arlati, P.; Bartolucci, G.; Basla, R.; Cioni, F.; Del Carlo, G.; Dellavedova, P.; Fattore, E.; Fungi, M.; Grote, C.; Guidotti, M.; Morgillo, S.; Müller, L.; Volante, M. J Chromatogr., A 1998, 795, 371.
85. Górecki, T.; Mindrup, R.; Pawliszyn, J. Analyst. 1996, 121, 1381.
86. Vuckovic, D.; Shirey, R.; Chen, Y.; Sidisky, L.; Aurand, C.; Stenerson, K.; Pawliszyn, J. Anal.Chim.Acta. 2009, 638, 175.
87. Namiesnik, J. J. Chromatogr., A. 1984, 300, 79.
88. Koziel, J. A.; Martos, P. A.; Pawliszyn, J. J. Chromatogr., A. 2004, 1025, 3.
89. Sheehan, E. M.; Limmer, M. A.; Mayer, P.; Karlson, U. G.; Burken, J. G. Environ. Sci. Technol. 2012, 46, 3319.
90. Legind, C. N.; Karlson, U.; Burken, J. G.; Reichenberg, F.; Mayer, P. Anal.Chem. 2007, 79, 2869.
91. Esteve-Turrillas, F. A.; Pastor, A; de la Guardia, M. In Comprehensive Sampling and Sample Preparation Editor-in-Chief: Janusz Pawliszyn; Academic Press: Oxford, 2012; pp 201-222.
92. Bocchini, P.; Dello Monaco, D.; Pozzi, R.; Pinelli, F.; Galletti, G. C. Microchim. Acta 2009, 165, 271.
93. Jia, M.; Koziel, J.; Pawliszyn, J. Field Anal. Chem. Technol. 2000, 4, 73.
94. Wang, J.; Tuduri, L.; Millet, M.; Briand, O.; Montury, M. J Chromatogr., A 2009, 1216, 3031.
95. Gong, Y.; Eom, I. -.; Lou, D. -.; Hein, D.; Pawliszyn, J. Anal.Chem. 2008, 80, 7275.
96. Tollbäck, J.; Isetun, S.; Colmsjö, A.; Nilsson, U. Anal. Bioanal. Chem. 2010, 396, 839.
97. Zini, C. A.; Augusto, F.; Christensen, E.; Smith, B. P.; Caramão, E. B.; Pawliszyn, J. Anal.Chem. 2001, 73, 4729.
98. Musteata, F. M.; Pawliszyn, J. J.Biochem.Biophys.Methods. 2007, 70, 181-193.
99. Lord, H.; Pawliszyn, J. LC-GC Europe. 2012, 25, 5.
100. Stashenko, E. E.; Martínez, J. R. J. Sep. Sci. 2008, 31, 2022.

101. Lord, H. L.; Möder, M.; Popp, P.; Pawliszyn, J. B. Analyst. 2004, 129, 107.
102. Liu, X.; Pawliszyn, R.; Wang, L.; Pawliszyn, J. Analyst. 2004, 129, 55.
103. Yassaa, N.; Custer, T.; Song, W.; Pech, F.; Kesselmeier, J.; Williams, J. Atmospheric Measurement Techniques 2010, 3, 1615.
104. Belliardo, F.; Bicchi, C.; Cordero, C.; Liberto, E.; Rubiolo, P.; Sgorbini, B. J.Chromatogr.Sci. 2006, 44, 416.
105. Wang, A.; Fang, F.; Pawliszyn, J. J Chromatogr., A 2005, 1072, 127.
106. Zhan, W.; Pawliszyn, J. J Chromatogr., A 2012, 1260, 54.
107. Lord, H. L.; Zhan, W.; Pawliszyn, J. Anal.Chim.Acta. 2010, 677, 3.
108. Warren, J. M.; Pawliszyn, J. J Chromatogr., A 2011, 1218, 8982.
109. Essah, E. A.; Sanders, C. H. International Journal of Ventilation 2010, 9, 115.
110. Chvíličková, I.; Kubáň, V. Anal. Bioanal. Chem. 2004, 378, 150.
111. Kovats, E. Helv. Chim. Acta 1958, 41, 1915.
112. Ouyang, G.; Zhao, W.; Bragg, L.; Qin, L.; Alee, M.; Pawliszyn, J.; Environ. Sci. Technol. 2007, 41, 4026.
113. Ouyang, G.; Chen, Y.; Pawliszyn, J.; Anal. Chem. 2005, 77, 7139.
114. Muller, L.; Górecki, T.; Pawliszyn, J. Fresenius' J. Anal. Chem. 1999, 364, 610.
115. Health and Safety Executive. Methods for the determination of Hazardous Substances: Protocol for assessing the performance of a diffusive sampler, MDHS 27, 1994, 1-10.
116. De Crom, J.; Claeys, S.; Godayol, A.; Alonso, M.; Anticó, E.; Sanchez, J. M. J. Sep. Sci. 2010, 33, 2833.
117. Alonso, M.; Godayol, A.; Antico, E.; Sanchez, J. M. J. Sep. Sci. 2011, 34, 2705.
118. Saito, Y.; Ueta, I.; Ogawa, M.; Jinno, K. In Comprehensive Sampling and Sample Preparation Editor-in-Chief: Janusz Pawliszyn; Academic Press:Oxford, 2012; pp 927-942.
119. Mieth, M.; Schubert, J.; Groger, T.; Sabel, B.; Kischkel, S.; Fuchs, P.; Hein, D.; Zimmermann, R.; Miekisch, W. Anal. Chem. 2010, 82, 2541.
120. Mieth, M.; Kischkel, S.; Schubert, J.; Hein, D.; Miekisch, W. Anal. Chem. 2009, 81, 5851.