SAMPLE PREPARATION/CONCENTRATION FOR TRACE ANALYSIS IN GC/MS

(A study of solid phase microextraction and headspace sampling)

by

Yuwen Wang

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APPROVED:

Dr. Harold M. McNair, Chairman
Dr. John G. Dillard
Dr. James O. Glanville
Dr. Gary L. Long
Dr. Mark R. Anderson

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(ABSTRACT)

Solid Phase Microextraction (SPME) associated with Microwave Assisted Extraction (MAE) [1-3], on-line headspace derivatization [4-6] and the selectivity of different SPME coatings[7] were studied. Trace amounts of Veltol®, Veltol Plus® and short chain fatty acids in food samples were analyzed by GC/MS.

Since SPME is not directly applicable to solid samples, SPME associated with MAE techniques was studied for solids, primarily food samples. The efficiency of classical solvent extraction and MAE was compared. The parameters which affect SPME, were optimized for the determination of Veltol® and Veltol Plus® in food products such as potato chips and coffee. The technique gave a detection limit of 2 ppb for Veltol Plus® which is 200 times more sensitive than conventional GC technique.

Headspace injection is characterized by simple and easy handling of complicated solid and solution matrices. Headspace injection, however, is not suitable for high molecular weight substances or non-volatile compounds. An on-line derivatization headspace technique was studied for short chain fatty acids. These samples are difficult to do by classical GC. The developed technique simplified the conventional derivatization procedures and combined the sample preparation and GC/MS analysis into one step. The thermostatting temperature,

time, solvent and matrix effects were investigated. Low calorie fat and some agricultural samples were analyzed. The detection limit for acetic acid is 8 ppb.

SPME is a novel sample introduction technique. The behavior of di(methylsiloxane), polyacrylate and Carbowax coatings on SPME fibers for compounds having different functional groups were investigated. The selectivities of the coating, sample pH and the sample temperature were investigated.

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CHAPTER I

GENERAL INTRODUCTION

With the development of advanced analytical techniques, trace and ultra trace analysis have been a major challenge for analytical chemists. Analytical chemistry involves the separation, identification, and quantitation of target compounds in complex samples. In analytical chemistry, chromatography is the most widely used separation technique. Modern chromatographic techniques have an excellent separation power. They are versatile and allow the use of a variety of detection techniques. Sensitive detectors have been well developed and are commonly applied. However, due to the increasing requirements of environmental and toxiological regulations, the current detection limits cannot meet all needs, so sample enrichment is frequently required before introduction into the chromatographic system. As a result, sample preparation is most time consuming and costly part of many analyses.

The goal of any sample preparation step is to yield the analytes of interest in a form and concentration that can be readily analyzed. Extractions using liquids, such as Soxhlet extraction and liquid-liquid extraction, are routinely used in laboratories throughout the world. Unfortunately, these methods are generally time consuming and sometimes require large amounts of toxic and expensive organic solvents. Supercritical Fluid Extraction (SFE) is an advanced extraction technique. It is characterized by the use of a non-toxic, easily removed supercritical fluid such as carbon dioxide. However, the expensive equipment and the high cost of ultra pure carbon dioxide limits its usage. For a successful chromatographic analysis, both the sample preparation step and the chromatographic process should be optimized carefully. The continuous search for rapid, efficient, cost effective and environment-friendly means of analytical extractions has prompted the introduction of Solid Phase Microextraction (SPME) into the field of analytical chemistry. The technique is fast, simple, sensitive and

does not require an extracting solvent. During the past five years, the application of SPME has experienced a rapid advance. Since the preferred matrix for SPME is water, the technique has been mainly used for trace organics in drinking, ground and waste water.

In order to expand the technique to solid samples, a headspace-SPME technique has also been developed. The combination of SPME and headspace GC combines the advantages of both techniques.

Static headspace sampling is a common technique for gas chromatography. Headspace sampling is characterized by using a simple sample preparation procedure for complicated liquid and solid matrices. Since the sample has to be heated and the analytes passed through a transfer line, it can only be used for volatile and thermally stable samples.

The primarily goal of this thesis has been to extend the applicability of trace and ultra trace analysis by using different sampling techniques including SPME and headspace with some modifications to overcome their natural limitations.

The thesis consists of six chapters. Chapter I is a general introduction. Chapter II introduces the techniques used and describes their development during recent years. The techniques involved include SPME, headspace, SPME-headspace and microwave assisted extraction (MAE). Chapters III, IV and V deal with studies of these respective techniques.

In order to efficiently use SPME techniques and explore the use of SPME for solid samples, Chapter III discusses the association of SPME techniques with microwave assisted extraction (MAE). Trace amounts of Veltol® and Veltol Plus® (flavor enhancers in food) were concentrated and analyzed by GC/MS.

Headspace-GC is not suitable for some polar compounds such as organic acids and bases due to their limited volatility or thermal stability. The analysis of these compounds are also difficult in both GC and HPLC because of their strong adsorption in GC columns and their lack of chromophores for UV detection in HPLC. Chapter IV discusses an on-line derivatization technique for headspace GC-MS. The trace analysis of short chain fatty acids was carried out using this technique.

Chapter V is a fundamental study of the selectivities of SPME fiber coatings with different functional groups . The purpose of this study was to attempt to understand the absorption mechanism and develop general guidelines for the SPME technique.

Chapter VI contains the conclusions for the research.

CHAPTER II

LITERATURE REVIEW

2.1 Solid Phase Microextraction(SPME)

SPME is a new sample enrichment technique which can easily transfer the analytes to the GC inlet. Since the invention of the technique in 1989 by J. Pawliszyn [8] its applications have dramatically increased. It has been used mainly for environmental water analysis[9-36].

The basic equipment of SPME is simple. As shown in Fig 1, a fused-silica rod is connected to a stainless steel tube that can be withdrawn inside a syringe needle, after sampling, for protection and transfer to GC inlets. The fused silica is coated with a thin film of hydrophoic usually dimethylsiloxane. During SPME sampling, the fiber is lowered from the syringe needle and inserted into the aqueous sample solution. The fiber coating is exposed to the stirred sample and the analytes are absorbed into the fiber coating. When the sampling is complete, the fiber is first withdrawn into the syringe needle and then transferred to a heated GC inlet. The analytes are then thermally desorbed and transferred into the GC column.

SPME preserves all the advantages of Solid Phase Extraction (SPE) such as simplicity, low cost, easy automation and on-site sampling. It also eliminates one disadvantages of SPE, the use of organic solvents. No special thermal desorption equipment is used and no modification of the GC inlet are required. SPME integrates the sample preparation and GC injection into one step. Because SPME is a static extraction process, the surface area is not as critical as in SPE and detection limits as low as ppt are often reported [37].

The basic principle of SPME sampling is the partitioning of the analytes between the fiber coating and the sample matrix. When equilibration of the analyte between the fiber coating and sample is reached, the partition coefficient can be defined as:

$$K_{SPME} = C_f/C_s \qquad (1)$$

Where K_{SPME} is the partition coefficient, C_f is the concentration of analyte in the fiber coating and C_s is the concentration of analyte in the sample. If the mass of the analyte is used, equation (1) can be rewritten as:

$$K_{SPME} = (n/V_f)/(C^o - n/V_s) \qquad (2)$$

Where n and V_f are the mass absorbed by the fiber and the volume of the fiber coating; C^o is the original concentration of the analyte in the sample and V_s is the volume of the sample. By rearranging equation (2), the equation can be written as:

$$n = K_{SPME}V_fC^oV_s/(K_{SPME}V_f + V_s) \qquad (3)$$

equation (3) describes the mass absorbed by the polymeric coating after equilibrium has been reached. Because the volume of the fiber coating is so small (~ 0.36-0.66 μL) compared with the volume of sample (usually ~ 5 mL), equation (3) can be simplified as:

$$n = K_{SPME}V_fC^o \qquad (4)$$

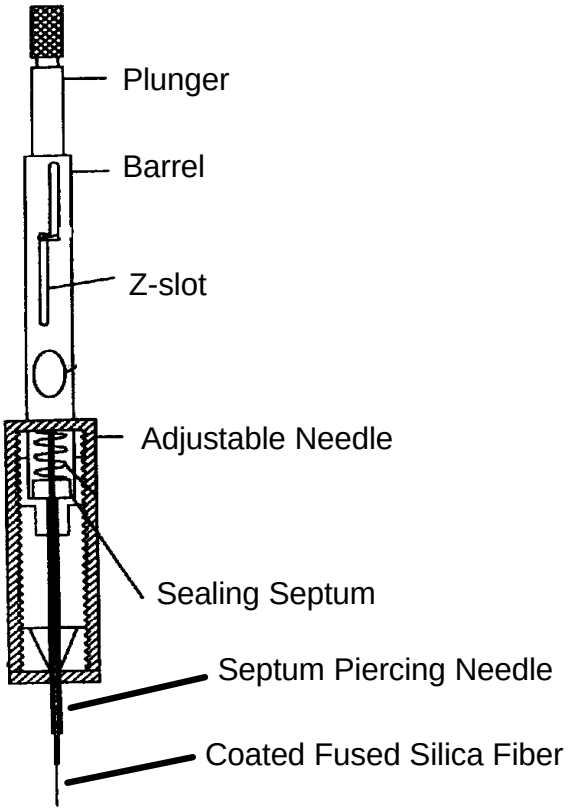


Fig 1. Schematic diagram of SPME assembly

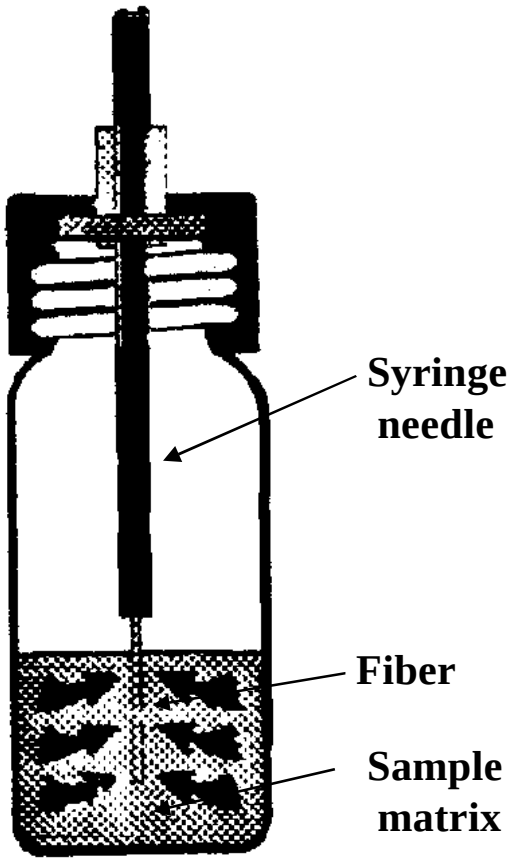


Fig. 2 Schematic diagram of SPME absorption

For large sample volumes the amount extracted is directly proportional to the initial concentration C^o in the sample. It should be noted that for compounds with a high K_{SPME} value, the sample volume V_s significantly contributes to the amount extracted. From equation (4), the sensitivity of the SPME technique is seen to be also affected by the partition coefficient and the volume of the fiber. Increasing the volume of the fiber is difficult because of the limited coating technique and also the fiber has to fit inside a syringe needle for easy injection into a GC. The best way to increase the sensitivity of the SPME extraction is to increase the partition coefficient K_{SPME}. This can be done by: (1) changing the chemical nature of the fiber coating; and (2) modifying the sample matrix by adjusting pH and/or adding salt.

2.2 SPME-Headspace

The SPME technique was originally invented for water samples. To accommodate solid samples, the technique has been developed as SPME-headspace[38], which combines both headspace and SPME techniques.

Since the introduction of the SPME-Headspace, many applications such as pharmaceutical, food and environmental samples have been studied [55-75]. In SPME-Headspace, a solid sample is put into a headspace vial and sealed. The vial is heated to increase the vapor pressure of the target compounds from the sample. Chemical equilibrium is allowed to establish between the solid sample and the vapor headspace. A SPME fiber is inserted into the headspace without contacting the sample. The fiber coating absorbs vapors of analytes from the headspace (Fig. 3).

In SPME-Headspace, volatile analytes are easily concentrated in headspace. For semivolatiles, their low volatility may slow the mass transfer from the matrix to the headspace. In some cases, the kinetically controlled desorption of volatiles from solid samples can limit the speed of extraction. In these cases, longer extraction times are required. In the case of solid samples, diffusion is much slower. This can been seen by comparing the order of magnitude of the diffusion coefficient D: it is around 10^{-6} in liquids and 10^{-8} to 10^{-11} in solids, while it is 10^{-1} in gases [76].

There are three phases (fiber coating, headspace and sample matrix) involved in the headspace SPME. The analytes first diffuse to the headspace from sample matrix and then to the fiber coating. According to Zhang and et. al. [38], the amount absorbed by the coating in SPME-headspace is

$$n = K_{SPME}V_fC^oV_s/(K_{SPME}V_f + K_{HS}V_{HS} + V_s) \quad (5)$$

where K_{HS} is the partition coefficient of the sample between headspace and sample which is defined as $K_{HS} = Cs/Cg$. V_{HS} is the volume of headspace. Other notations are same as in equation (3). Comparing equation (3) with equation (5), it is apparent that the amount absorbed by SPME-headspace is affected by the term $K_{HS}V_{HS}$. According to the two equations, the sensitivity of SPME-headspace can never be higher than that of the corresponding liquid sampling method, because generally $K_{HS}V_{HS} > 0$. This term inversely influences the amount absorbed or, in other words, the detection limit obtained. For volatile compounds, K_{HS} is relatively small so the sensitivity is not greatly affected. For semivolatiles, K_{HS} is large and this dramatically decreases the sensitivity of the technique. Thus the SPME-Headspace technique for solid samples loses both speed and detection limit when compared to classical SPME.

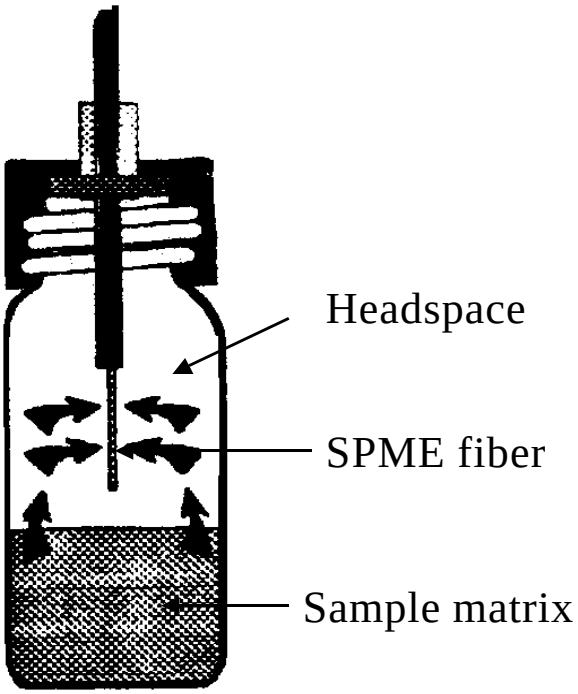


Fig. 3 Schematic diagram of SPME-headspace

2.3 Headspace sampling

Headspace analysis analyzes a gas in contact with a liquid or solid sample. The first documented combination of GC with headspace sampling was reported by Bovijn and co-workers at the 1958 Amsterdam Symposium [39], on continuous monitoring of the hydrogen content in the water at power stations. In 1960, W. H. Stahl and co-workers also used headspace sampling for the gas chromatographic analysis of the gas in sealed cans for oxygen content[40].

Since the commercial introduction of the technique, headspace sampling has been widely used for analysis for volatile compounds[41-52]. Static headspace analysis which is probably the simplest solvent-free sample preparation technique, has been used for decades to analyze volatile organic compounds. The sample (liquid or solid) is placed in a vial and the vial is sealed. The vial is then heated and the volatile compounds are driven into the headspace. An equilibrium between the headspace and the sample matrix is reached (Fig. 4). A portion of the vapor from the headspace is injected into a GC.

Among the several headspace sampling systems, balanced-pressure sampling systems is the one of the most popular injection systems. In balanced-pressure systems, an aliquot of the headspace of the vial is not withdrawn by suction as the normal syringe injection. Instead, after equilibrium has been reached, the vial is pressurized by the carrier gas to a pressure which can be chosen manually. Next the pressurized gas in the vial expands onto the GC column, resulting in a flow of the mixed headspace gas from the vial to the column. Since both the pressure that builds up in the vial and the time of transfer can be set, the transferred volume of the headspace gas can be accurately controlled. An automated system of balanced-pressure headspace was first introduced in 1967[53]. The schematic diagram of modified balanced pressure

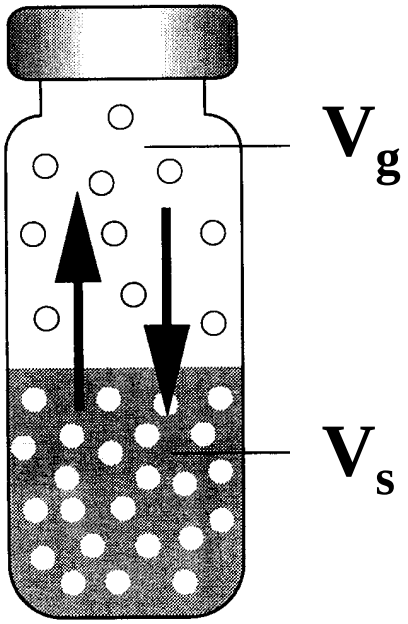


Fig. 4 Schematic diagram of headspace vial containing a liquid sample
V_g = volume of gas phase, V_s = volume of liquid phase

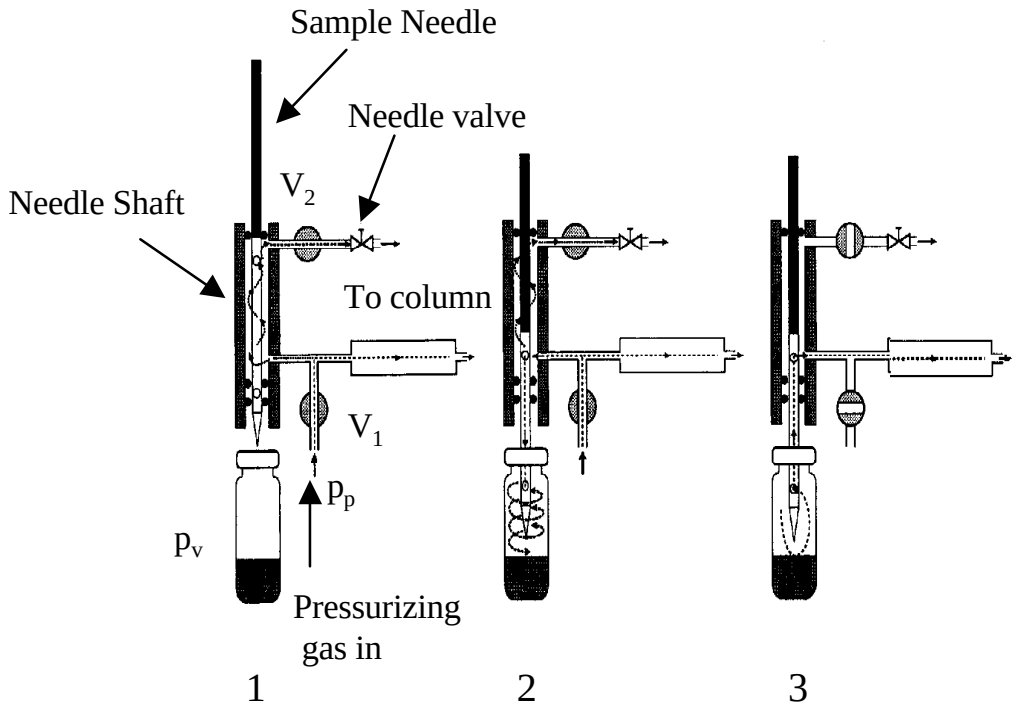


Fig. 5 Schematic diagram of the automatic balanced pressure system [(54)]. (1)equilibration (stand-by), (2) pressurization, (3) sample transfer. V = on/off solenoid valves, p_p = pressurizing pressure, p_v = original headspace pressure in the vial.

system is show in Fig. 5[54]. A heated needle made of either stainless steel or platinum, which has a hollow part permitting flow in either direction, moves in a heated shaft that is continuously swept by a small purge gas flow to avoid contamination. In the stand-by position (Step 1, Fig 5) the needle is sealed against atmosphere by an O-ring. After the equilibration has been reached, the needle penetrates the septum of the vial (Step 2, Fig. 5) and part of the carrier gas flows into the vial to build up the pressure. After a few minutes (pressurization time) the carrier gas is temporarily disconnected by closing valve V1 (Step 3, Fig. 5). Since the sample vial is connected to the GC inlet by a heated transfer line, an aliquot of the gas from the vial is transferred into the GC injector. The volume of the aliquot is set by controlling the time of transfer.

The basic equation for static headspace analysis can be derived as follows. When the vapor phase and the sample phase are equilibrated, the partition coefficient is:

$$K_{HS} = C_s/C_g \qquad (6)$$

Where K_{HS} is the partition coefficient, C_s is the concentration of the analyte in the sample and C_g is the concentration of the analyte in the vapor.
The total mass of the analyte is the sum of the mass in the sample and vapor phases:

$$C^oV_s = C_sV_s + C_gV_g \qquad (7)$$

where C^o is the original concentration of the sample, V_s is the volume of the sample and V_g is the volume of the headspace. According to this equation 7 can be rewritten as:

$$C^o V_s = K C_g V_s + C_g V_g \qquad (8)$$

$$C^o = C_g \ (K + V_g/V_s) \qquad (9)$$

where V_g/V_s is the phase ratio, defined as:

$$\beta = V_g/V_s \qquad (10)$$

Then, the concentration of the analyte in the sample is:

$$C^o = C_g(K+\beta) \qquad (11)$$

Equation (11) is the basic equation for quantitative analysis in headspace methods. The concentration of the analyte in the original sample is proportional to its concentration in the vapor phase which is injected into GC for analysis.

For trace analysis, the sensitivity of headspace methods need to be enhanced. From equation (11), we know that the headspace sensitivity is directly proportional to C_g. By rewriting equation (11), we get:

$$C_g = C^o/(K_{HS} + \beta) \qquad (12)$$

The sensitivity of headspace injection is influenced by the concentration of the sample, the partition coefficient and the phase ratio. C^o is directly proportional to the headspace sensitivity. If it is necessary to increase C^o to improve the sensitivity, a larger sample or a further sample enrichment step has

to be made before headspace analysis. K_{HS} is inversely proportional to the sensitivity. That means decreasing K_{HS} would increase the headspace sensitivity. K_{HS} is a characteristic of the compound analyzed and the type of the sample matrix. By modifying the functional group of the analyte by derivatization, and/or by improving the matrix, the sensitivity can also be increased.

2.4 Microwave Assisted Extraction (MAE)

MAE is a novel method of extracting soluble products into a fluid from a wide range of materials using microwave energy. It provides a technique whereby intact organic compounds can be extracted more rapidly with similar or better recoveries, when compared to conventional extraction processes [77]. MAE uses the ability of some liquids or solids to transform electromagnetic energy into heat. The *in situ* mode of energy conversion has many attractions for chemists, because its magnitude depends only on the dielectric properties of the processed material. This allows the selection of target specific molecules and deposition of the energy into the whole of the sample, without the usual limitations of heat conduction and convection. The solvent used in MAE is important since it determines: (1) the speed of heating; and (2) the selectivity of the extraction. The heating speed is proportional to the dipole rotation or dielectric constant of the solvent. For most extractions, the solvent is an organic compound. Many organic compounds have a low dielectric constant (Table 1) and this could lead to long extraction times. Water has a high dielectric constant (80.1) and it is easily heated by MAE. Since MAE can be done at both high temperature and pressure (see Fig. 6), the extraction efficiency must be higher than traditional Soxhlet and shake-flask extraction methods. This has been verified by Ganzler and et. al. [78-79].

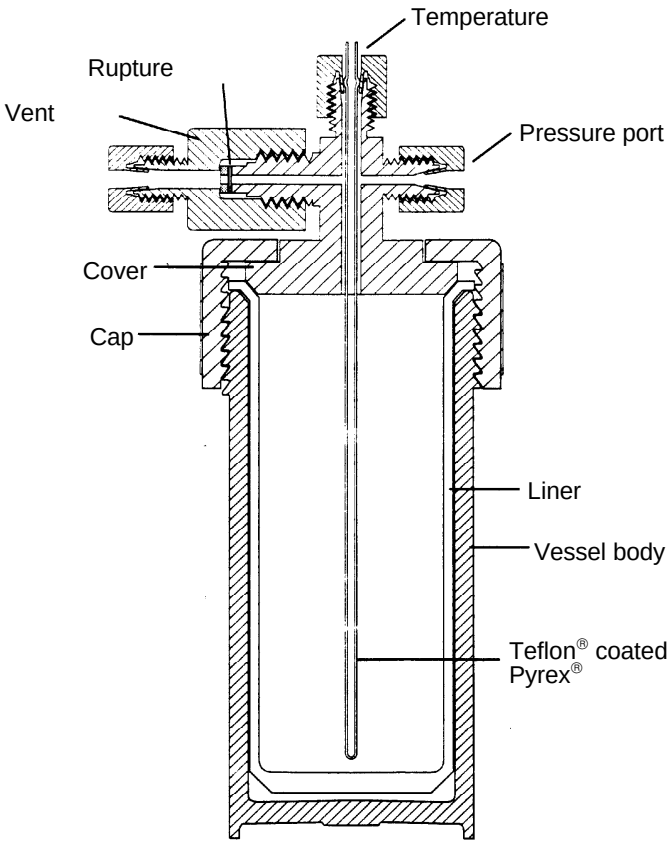


Fig. 6 Schematic diagram of a microwave assisted extraction vessel

Table 1 Dielectric constant of common solvents *

SOLVENTS	Dielectric constant (ε)
Water	80.1
Ethanol	25.3
Acetone	21.01
Methylene chloride	8.93
Benzene	2.2825
Chloroform	2.2379
Hexane	1.8865

* From Handbook of Chemistry and Physics (75th edition), CRC Press, Inc., Cleveland, Ohio, 1994

CHAPTER III

SPME ASSOCIATED WITH MICROWAVE ASSISTED EXTRACTION

3.1 Introduction

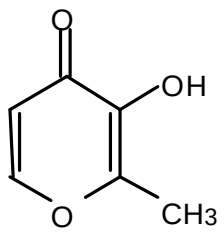
Sample preparation techniques based on adsorption have been widely used to pre-concentrate analytes for trace analysis. SPME developed by Pawliszyn and co-workers[8] is a new variation of these adsorption techniques which has been used mainly for the analysis of pollutants in environmental water samples [9, 10].

The hydrophobic poly(dimethylsiloxane) fiber coating has limited SPME, to date, to an aqueous sample matrix. Organic solvents if used would compete with the target compounds for the sorption sites on the fiber and, probably, saturate the fiber. This saturation would dramatically decrease the recovery of target compounds. On the other hand, Microwave Assisted Extraction (MAE) is an extraction technique which uses polar solvents, such as water, to extract target compounds primarily from solid matrices. Water absorbs the microwave energy, and when temperature and pressure are increased, the polar compounds are more rapidly desorbed from the matrix. The basic mechanism of this extraction is discussed in reference (77). SPME can then be used to concentrate these compounds and introduce them into a GC/MS. MAE followed by SPME is a useful combination which combines extraction speed with sample concentration. The combined use of the two techniques has not been described before this work.

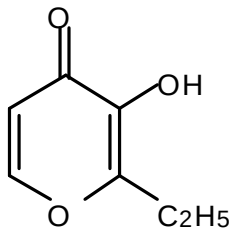
Veltol® (3-hydroxy 2-methyl 4-pyrone) and Veltol-Plus® (3-hydroxy 2-ethyl 4-pyrone) (Fig. 7) are flavor ingredients patented by Cultor Ltd (Ardsley, NY). Since its first isolation from larch tree bark in 1861, Veltol® has been a

useful component in food. It has many functions in food chemistry including: (1) flavor enhancement, by which flavors especially sweet and fruit flavors are modified and their strength is increased; (2) sweetness enhancement, by which the concentration of a high intensity sweetener can be reduced; (3) creaminess enrichment, in which the creamy taste of many foods will be enhanced; (4) bitterness reduction, which make products more palatable by masking bitterness; and (5) acid modification which enhances overall taste by muting or reducing acidity. Veltol® is found naturally; Veltol Plus® is a synthetic organic compound. Both synthetic Veltol® and Veltol-Plus® are frequently added to food products.

Because of the importance of Veltol® and Veltol-Plus®, trace analysis of these compounds is required. To quantify these compounds in food, a solvent extraction, packed column GC method was developed by Gunner, et. al. [80]. The tedious solvent extraction, low sensitivity and the requirement of derivatization make this method impractical. A GC/MS method was developed by Wang, et. al.[2] recently. It can determine Veltol-Plus® down to 200 ppb and Veltol® down to 400 ppb. These detection limits however are not adequate to effectively monitor the use of these compounds in some food products. For this reason, SPME for beverages and SPME in combination with MAE for solid food samples were studied.



Veltol ®



Veltol-Plus®

Fig. 7 Chemical structures of Veltol® and Veltol-Plus®

3.2 Experimental

3.2.1 SPME /MAE extractions

The SPME device was purchased from Supelco Inc. (Bellefonte, PA). The fibers used were 100 µm poly(dimethylsiloxane) coating. For liquid sampling, the SPME fiber was inserted directly into a 10 mL vial containing 4 mL of aqueous sample. The fiber remained inside the sample for 10 minutes with magnetic stirring of the solution. For solid samples, MAE was performed on a MES Model 1000 system (CEM, Matthews, NC) before SPME extraction. This system was equipped with an inboard pressure and fiberoptic temperature control system for regulating sample extraction conditions via magnetron power output control. The instrument controlled either pressure or temperature, depending on which parameter reached its control set point first. Teflon™ lined extraction vessels (110 mL) were used for extractions (Fig 6). The outer body and cap consisted of microwave-transparent Ultem™ poly (ether imide). The removable inner cover, and the safety rupture membrane were made of Teflon™ PFA. Gases could escape through the exhaust port if the vessel were hand vented by turning the vent fitting. The liner cover of the control vessel had Teflon™ PFA fittings to allow for pressure tubing connections and for the insertion of a Pyrex™ tube that ran through the cap into the vessel and ended close to the bottom of the vessel. This Pyrex™ tube, which housed the fiberoptic probe, provided a seal in the cap and protected the fiberoptic probe from solvent attack.

The extraction temperature was set to 100^{o}C and the pressure was 100 psi. After 10 minutes, the vessel was cooled to room temperature and pressure

released before it was opened. The extract was filtered under low vacuum through a GF/A binder-free glass fiber filter (Fisher Scientific, Pittsburgh, PA).

3.2.2 Analysis of food products

3.2.2.1 Beverages

In a 10 mL vial, 1.5 g of Na_2SO_4 was dissolved in 4 mL of beverage such as Cola. The pH was adjusted to 2 by adding 0.5 mL of 0.1 M HCl. SPME was performed at ambient temperature for 10 min. The SPME fiber was then introduced into GC/MS inlet in the splitless mode.

3.2.2.2 Solid food products

Solid food products such as potato chips were ground before weighing. One gram of sample and 10 mL of water (HPLC grade, Mallinckrodt Chemical, Inc., Paris, KY) were placed in a MAE vessel. A 4 mL aliquot from the filtered MAE extract was placed into a 10 mL SPME vial for SPME concentration.

3.2.3 GC/MS analysis

A HP model 5971 GC/MS system (Hewlett Packard, Palo Alto, CA) was used. The inlet temperature was set to 260^{o}C. A 30 m, 0.25 mm I.D., 0.5 µm film thickness Rtx-20 (80% methyl and 20% phenyl polysiloxane) (Restek, Bellefonte, PA) capillary column was used. The column temperature was held initially at 70^{o}C for 1 min, increased to 240^{o}C at 15^{o}C/min and held there for 1 min. A 30 m, 0.25 mm i. d., 0.25 µm film Stabilwax (100% bonded polyethylene glycol) (Restek, Bellefonte, PA) column was also used for some sample analyses. For this column, the oven temperature was programmed as follows:

40^{o}C for 1 min, increased to 230^{o}C at 15^{o}C/min, then 30^{o}C/min to 250^{o}C. For thermal desorption, the SPME fiber was left in the injector for 1 min at 260^{o}C. For direct injection, 1 µL of the sample solution was injected using a HP Model 7673 auto sampler (Hewlett Packard, Palo Alto, CA). A splitless mode was used for both the SPME and direct injection with the purge valve closed for 1 min. Column flow rate was helium at 1 mL/min.

3.3 Results and discussion

3.3.1 Optimization of SPME absorption conditions

The initial step was to optimize the SPME extraction conditions. The parameters investigated were pH, ionic strength, absorption time, GC inlet conditions and fiber conditioning.

3.3.1.1 pH

Veltol® and Veltol-Plus® are both weak acids. At pH 7, only a fraction of the molecules will be ionized. This does not favor their absorption by the hydrophobic fiber. A series of pH buffer solutions (pH 2, 0.1 M HCl/0.38 M NaCl; pH 3, 0.1 M H_3PO_4/ 0.7 M NaH_2PO_4 pH 4, 0.1M acetic acid/0.017 M sodium acetate; pH 6, 0.1 M KH_2PO_4/0.01 M NaOH.) were prepared and 1 mL added to 4 mL of a 1.5 ppm Veltol® and Veltol-Plus® solution. SPME extractions were made from each solution. Fig. 8 shows the results. The peak areas increase with decreasing pH. At pH 2, the absorption efficiency is 4 times that at pH 7. Veltol-Plus® has a higher absorption efficiency due to the increased hydrophobicity of the ethyl group relative to the methyl group in Veltol®.

3.3.1.2 Ionic strength

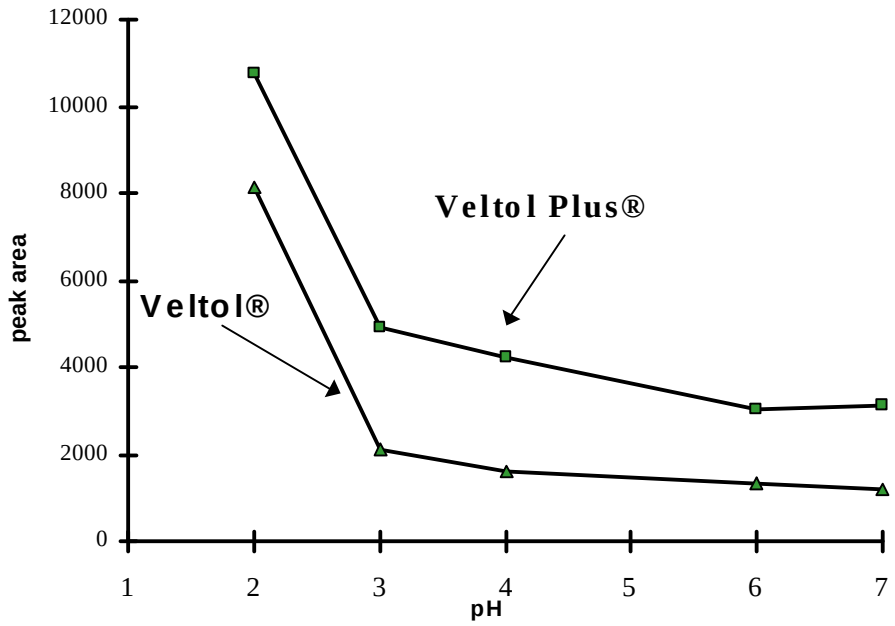


Fig. 8 Influence of pH on absorption by SPME fiber. One mL of pH buffer was added into a 4-mL aqueous sample of 1.5 ppm Veltol® and Veltol Plus®. The pH buffers used were : pH 2, 0.1 M HCl/0.38 M NaCl; pH 3, 0.1 M H_3PO_4/ 0.7 M NaH_2PO_4 pH 4, 0.1M acetic acid/0.017 M sodium acetate; pH 6, 0.1 M KH_2PO_4/0.01 M NaOH. For pH 7, HPLC water was used as solvent without adding buffer.

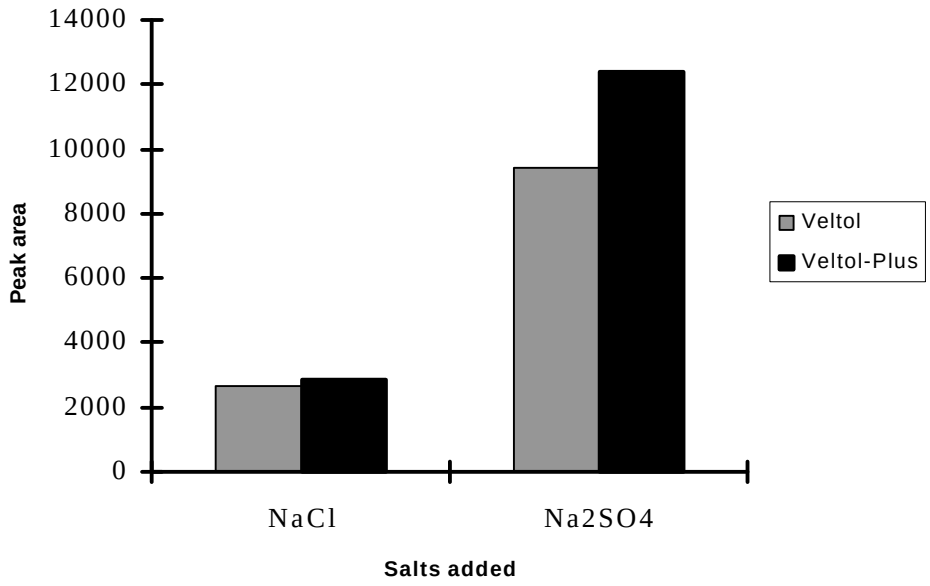


Fig. 9 Effect of salt on SPME extraction. Four mL of 30 ppb Veltol® and Veltol Plus® aqueous sample was used and 1 mL pH 2 buffer was added. The solution was saturated with salt in both cases.

The effect of decreasing solubility of organic compounds with the addition of salt is known as a “salting out” effect [81]. By adding a salt to the aqueous samples, the ionic strength of water can be increased, thereby increasing the partitioning of organic compounds (but not ions) into the polymer coating. The presence of sodium sulfate decreases the solubility of both Veltol® and Veltol-Plus® in water and enhances their absorption onto the SPME fiber. In this study, a saturated solution of sodium sulfate was made by adding 1.5 gram of sodium sulfate to the sample solution with magnetic agitation. The saturation was ensured by observing that there was still a small amount of sodium sulfate particle in the solution after ten minute agitation. As can be seen in Table 5, the detection limit for Veltol at pH 2 without Na_2SO_4 was 50 ppb while with Na_2SO_4 a limit of detection of 10 ppb was obtained. A 5 fold decrease in the detection limit is obtained for Veltol® by adding Na_2SO_4· For Veltol-Plus®, limits of detection of 30 and 2 ppb are obtained without and with Na_2SO_4. In this case, a 15 fold increase in sensitivity is obtained for the “salting out” effect. Fast stirring must accompany the addition of this salt to prevent recrystallization. Because dissolving of sodium sulfate is an endothermic process, the temperature of the sample solution decreased. This also may enhance the absorption efficiency of the fiber. Sodium chloride was also evaluated, but the extraction efficiency was less than with Na_2SO_4 (see Fig. 9).

3.3.1.3 Absorption time

SPME extraction is a dynamic partitioning process of the target compounds between the SPME fiber and the sample solution.
With stirring, diffusion of the target compound to the fiber is increased, and 10 minutes was necessary to reach equilibrium (see Fig. 10).

3.3.1.4 Fiber Conditioning

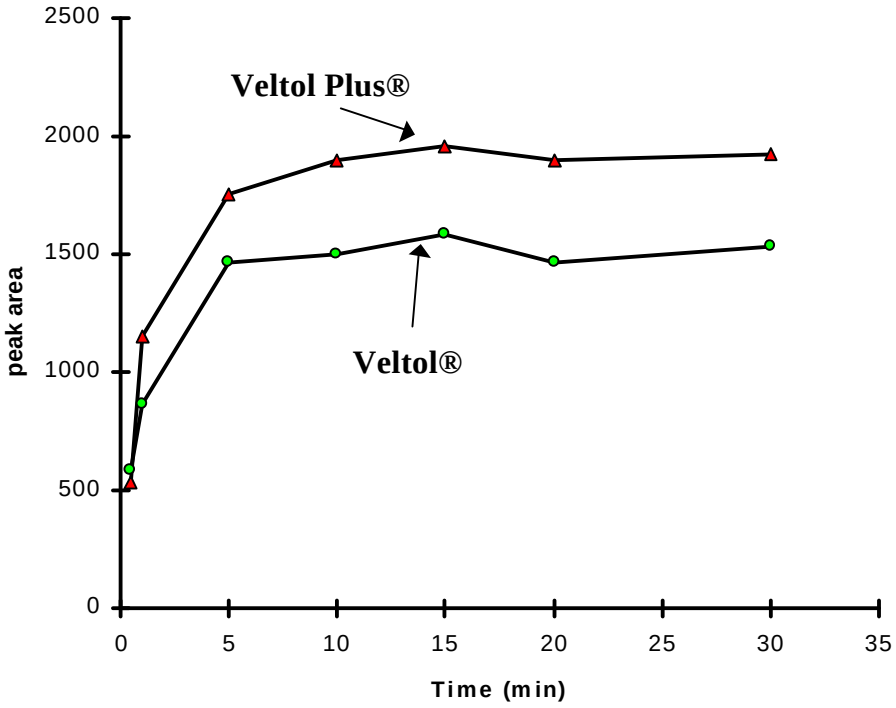


Fig. 10 Influence of absorption time on extraction efficiencies of Veltol® and Veltol-Plus®. Four mL of 15 ppb of each of Veltol® and Veltol-Plus® was used and 1 mL of pH 2 buffer was added. The solution was saturated with sodium sulfate.

Replicate absorption and desorption trials were carried out by successively absorbing six fresh samples using the same fiber and desorbing in the GC injector. The results show an increase in peak area with each consecutive injection; the maximum peak area was reached after the 5th absorption. This means the fiber needs to be conditioned in the sample matrix before use. The conditioning can also be achieved by rinsing the fiber in the sample for 20 to 30 minutes and then thermally desorbing the fiber in the heated injection port.

3.3.1.5 GC inlet.

A major benefit of SPME for GC/MS is the absence of an extracting solvent. This advantage eliminates: 1) the peak area variations with solvent type or inlet temperature; and 2) the solvent delay time necessary for MS. SPME thus allows detection of early eluting volatile compounds not possible with direct injection. When the splitless injection mode with a 1 minute purge time off is used, peak areas decreased dramatically with an increase in the inlet temperature when conventional syringe injection was employed. This is probably due to sample loss through the septum purge with a fast solvent evaporation. The higher the inlet temperature, the greater the volume of the vaporized analytes, the greater the sample loss through the septum purge. Using SPME no significant changes in peak areas were observed (see Table 2).

3.3.2 SPME associated with MAE

As discussed in Chapter 2, the present technique, SPME headspace, for solid samples lost the sensitivity and extraction speed which were originally the major advantages of SPME. To seek a better technique for solid samples which can maintain the advantages of SPME, a new technique, SPME combined with

MAE was studied. The technique joins the advantages of SPME and MAE, and was successfully applied to several solid food samples (potato chips, canned food and chewing gum). This work is the first published combination of SPME and MAE[(1)].

3.3.2.1 MAE with water as solvent for hydrophobic sample matrices

With a high dielectric constant (80.1), water is a preferred solvent in MAE. It is characterized by its high heating rate and high affinity for polar compounds. MAE can be operated at high temperatures, thus increasing the diffusion rates of analytes from solid sample into solvent. To investigate the potential of MAE by using water as solvent, MAE was compared with conventional solvent extraction.

Table 2. Effect of injector temperature on peak area

	Direct injection		SPME	
Injector temperature (^oC)	Veltol® ($X10^5$)	Veltol-Plus® ($X10^5$)	Veltol® ($X10^5$)	Veltol-Plus® ($X10^5$)
250	84	78	0.53	1.7
300	64	59	0.55	1.8

In this study, one gram of potato chips was used as the sample. The sample was ground before extraction. For solvent extraction, one gram of sample and 10 mL of ethanol (ethanol is the best solvent for these compounds) [83] was put into a 22 mL vial. The vial was crimped with a Teflon coated septum to avoid evaporation. The vial was shaken and sonicated for 10 min.

For MAE, the sample was put into a 110 mL MAE vessel and 10 mL of HPLC grade water (J.T. Baker, Phillipsburg, NJ) was added. The extraction time was 10 min. A one μL sample of each extraction was injected in the GC. Table 3 shows the results. At 100^{o}C, the recovery by MAE is 4 times that of conventional solvent extraction. At 150^{o}C, the recovery is about 4.6 times of that of solvent extraction. MAE is more efficient than solvent extraction for these solid samples.

3.3.2.2 MAE with water as solvent for water soluble sample matrices

In this study, ground coffee beans were extracted by MAE. The solvent for solvent extraction was acetone and for MAE was HPLC grade water (acetone is a better solvent than water for caffeine, so we used acetone as a standard. Acetone cannot be used for SPME therefore in this case the water had to be used). The procedure and the extraction conditions were the same as for potato chips (part 3.3.2.1). The extraction results are shown in Table 4. The efficiency of the water MAE extraction is higher than the acetone solvent extraction.

3.3.3 Comparison of SPME with conventional GC syringe injection

Evaluation of this SPME technique was conducted by comparing the detection limits and reproducibility with the conventional syringe injection.

3.3.3.1 Detection limits

The detection limits and were calculated as that concentration corresponding to 3 times the noise level. Duplicate injections were made for each trial and the average value used. For direct injection, 1 µL of Veltol® and

Table 3. Comparison of extraction efficiency of solvent with MAE (1)*
(Integrator area counts)

Compounds	Solvent extraction	MAE (100°C) 10 min	MAE (150°C) 10 min
Veltol®	3.7×10^5	15×10^5	17×10^5

* For solvent extraction, one gram of sample was extracted with ten mL of ethanol and 1 µL was injected into GC. For MAE extraction, one gram of sample was extracted with 10 mL of water and 1 µL was injected.

Veltol-Plus® standard solutions in ethanol were injected into the GC/MS. SPME of the original aqueous standard solution is represented by SPME (1) (see Table 5). SPME (2) is the original solution at pH 2. SPME (3) represents the original solution at pH 2 and saturated with sodium sulfate. The combination of salt and lower pH increased the sensitivity of SPME ten fold for Veltol® and twenty five fold for Veltol-Plus®. This method can be used to determine trace amounts of Veltol® and Veltol-Plus® in food products, such as coffee where trace amounts of Veltol® are present. A comparison of the sensitivity of both direct GC injection and SPME injection is shown in Fig. 11. Since the same attenuation was used the SPME technique shows abount a fifty fold increase in signal.

3.3.3.2 Precision

The precision of the method was determined by conducting six replicate injections for both the SPME and direct GC injection. The relative standard deviations (%RSDs) from both GC area counts and retention times were determined for each analyte. For conventional syringe injections, concentrations of 6 ppm for Veltol® and 5.1 ppm for Veltol-Plus® were investigated. For SPME, 1.5 ppm of Veltol® and Veltol-Plus® were tested. The %RSD of both the peak area and retention times are listed in Table 6.

Direct syringe injection has better reproducibility for both peak area and retention time. Poor precision for SPME peak areas resulted from the variation of the adsorption and the desorption of the target compounds. It should be kept in mind that the precision of these results represents the entire method, including the sampling stage, not only the chromatographic separation itself. Peak area

precision by SPME is not as good as direct injection, but it is still reasonable for trace level analyses. The variation of the retention time makes the identification

Table 4. Comparison of extraction efficiency of solvent with MAE (2)*
(Integrator area counts)

Compounds	Solvent extraction	MAE (100^{o}C) 10 min	MAE (150^{o}C) 10 min
Caffeine	3.7×10^{7}	9.3×10^{7}	13×10^{7}

* For solvent extraction, one gram of sample was extracted with ten mL of acetone and 1 μL was injected into GC. For MAE extraction, one gram of sample was extracted with 10 mL of water and 1 μL was injected.

of the target compounds difficult based solely on retention time. Use of a mass spectrometer is recommended for confirmation.

3.3.4 Application of SPME in food products

These SPME and MAE-SPME techniques were applied to several food products including coffee, cola drinks, potato chips, canned food and chewing gum.

3.3.4.1 Beverages

Most of the beverages are in aqueous solutions which make direct SPME extraction easy. Fig. 12 shows a comparison of gas chromatograms obtained from Gevalia® coffee by conventional GC direct injection and SPME. As shown, the SPME method shows higher sensitivity. Direct injection shows no peak for Veltol®. These results were confirmed by mass spectroscopy. These results also demonstrate the selectivity of this SPME method. The unidentified peak at retention time 7.85 min has a peak height of 1000 for direct injection and only 150 for SPME. SPME minimized the interference of this peak with Veltol®, which has a retention time at 7.80 min. An additional advantage of SPME sampling is that it prevents water from entering the GC, avoiding possible damage to the GC column.

3.3.4.2 Chips and corn

Chips and corns are solid samples. Instead of performing headspace SPME [82], a MAE technique was examined. Water is a good solvent for MAE as it possesses a high dielectric constant, hence it is characterized by a high ability to absorb microwave energy. Therefore, MAE is a complementary method to

combine with SPME for solid samples. Fig. 13 shows the chromatograms of potato chips with MAE-direct injection and MAE-SPME injection. Obviously MAE-SPME exhibits higher sensitivity for Veltol®. The concentration of Veltol®

Table 5. Comparison of the detection limit

Compounds	Direct injection. (1 μL)	SPME (1) HPLC water	SPME (2) pH 2 (0.1 N HCl/NaCl)	SPME (3) pH 2 (0.1 N HCl/NaCl) and saturated with Na_2SO_4
Veltol® (ppb)	200	100	50	10
Veltol-Plus® (ppb)	400	50	30	2

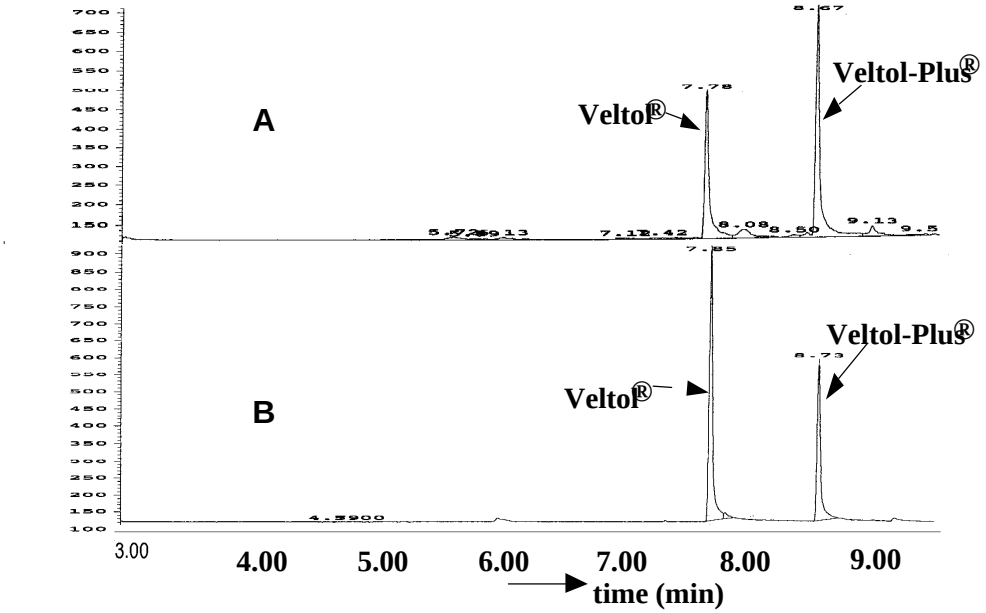


Fig. 11 Comparison of SPME injection and direct GC.
A: 30 ppb Veltol® and 30 ppb Veltol Plus® by SPME; B: 1.5 x 10^3 ppb Veltol® and 1.5 x 10^3 ppb Veltol Plus®by direct injection. A Rtx-20, 30 m x 0.25 mm capillary column was used for the separation. Same attenuation is used in the chromatograms.

in this sample was found 0.3 ppm by using a external standard calibration and Veltol-Plus® was not detected.

The significant peak height of Veltol® in MAE-SPME compared with other unidentified components having m/z 126 and 140 in MAE-direct injection shows the selectivity of this SPME technique. The drawback of the MAE-SPME technique for solid foods is that the particulate matter in the sample solution could coat the extraction fiber and interfere with the extraction. Further sample clean-up prior to extraction may help in these cases.

Table 6 Precision of direct GC injection and SPME*

(n=6)

	Direct injection (1 μL)		SPME 10 min absorption	
Compounds	Area (%RSD)	t_R (%RSD)	Area (%RSD)	t_R (%RSD)
Veltol®	2	0.005	10	0.1
Veltol-Plus®	2	0.005	10	0.05

* For direct injection, 6 ppm of Veltol® and 5.1 ppm Veltol-Plus® solution were used. For SPME, 1.5 ppm of Veltol® and 1.5 ppm Veltol-Plus® solution were used. The solution was adjusted to pH 2 and saturated with sodium sulfate.

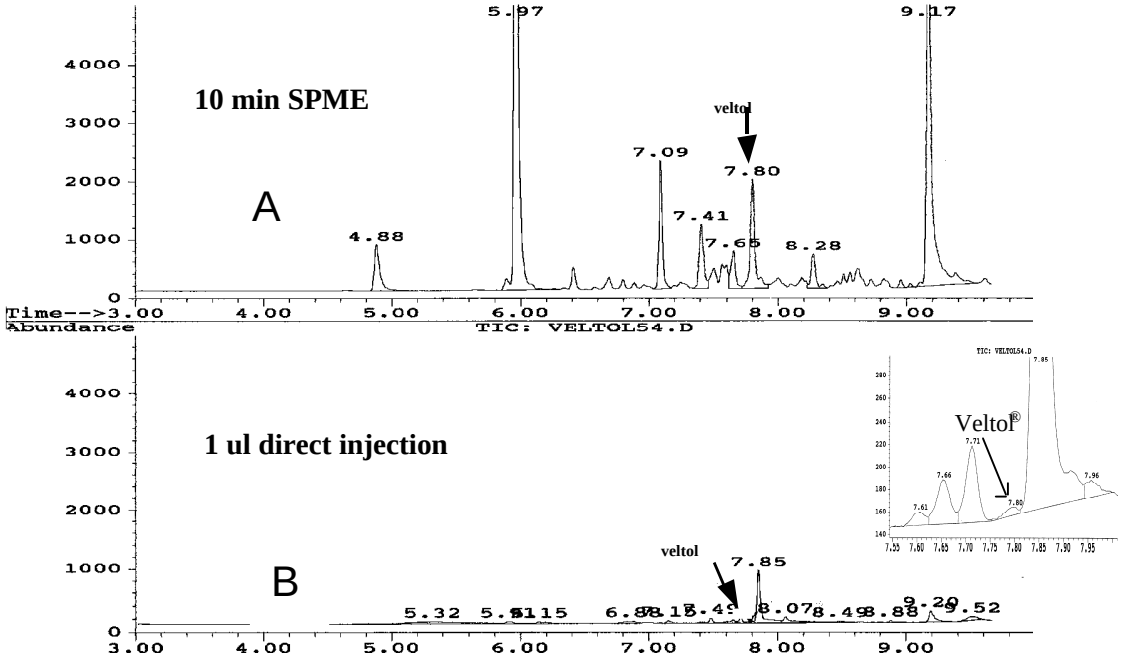


Fig. 12 Chromatograms of coffee by both SPME (A) and direct GC injection (B). Gevalia@ coffee (0.1 gram) was dissolved in 10 mL hot water. A Rtx-20, 30 m x 0.25 mm x 0.25 μm capillary column was used for the separation and ions 126 and 140 amu in SIM mass spectroscopy were used for the detection.

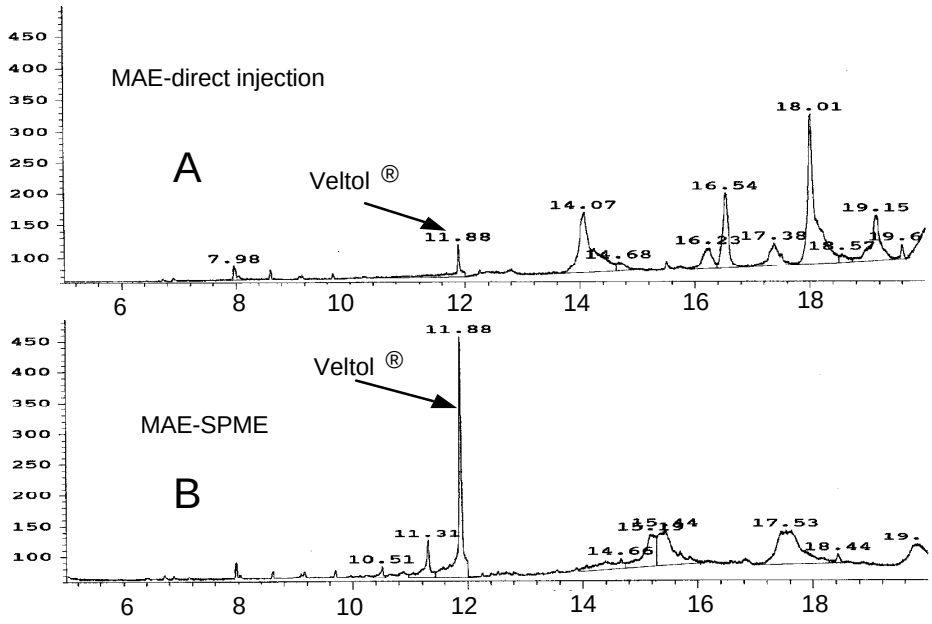


Fig. 13 Chromatograms of potato chips by direct MAE-injection (A) and MAE-SPME (B). Potato chips (1 gram) were extracted by MAE with 10 mL water. Four mL of sample with pH at 2 and saturated with sodium sulfate was used for SPME. One uL of MAE extract was used for direct injection. A Stabilwax column, 30 x 0.25 mm x 0.25 μm was used for the separation and ions 126 and 140 amu in SIM mass spectroscopy were used for the detection.

3.4 Conclusion

SPME is a useful tool for trace analysis of organic compounds like Veltol® and Veltol-Plus® in food products and can be considered superior to commonly used methods such as solvent extraction. SPME is simple to perform; it takes only a few minutes to complete and uses no extracting organic solvent. It has been applied successfully to the determination of trace amount of Veltol® and Veltol-Plus® in coffee, Coca, potato chips, canned food and chewing gums. The combination of MAE and SPME make solid sample SPME possible. It compensates for the disadvantage of headspace SPME that can only detect volatile compounds [84].

CHAPTER IV

HEADSPACE SAMPLING - ON LINE DERIVATIZATION

4.1 Introduction

Short chain fatty acids are common components in agricultural products. It is well know that these compounds directly contribute to the aroma and taste of many consumer products[85-86]. Therefore, it is often necessary to determine their concentrations accurately and at low levels.

Direct determination of short chain fatty acids by GC is often unsatisfactory due to their high polarity and low vapor pressure. Therefore, a derivatization step is often carried out to provide the fatty acids with better chromatographic characteristics. Several derivatization techniques have been developed for the analysis of short chain fatty acids[87-95]. By modifying the functional groups, most acids can be well separated by GC. Methyl esterification has been widely used for the GC analysis of fatty acids. However, due to the high sample volatility [4, 89,], peak broadening and poor recoveries were obtained. Several other procedures for the preparation of less volatile derivatives have been used for the analysis of the short chain fatty acids [91-95].

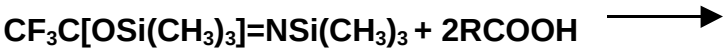
Although the available determination methods provide accurate and precise data for sample standards, most are not applicable to complex matrices such as agricultural products. Furthermore, the procedures involved in the derivatization of fatty acids are usually lengthy. They usually entail a great deal of sample handling which often leads to errors.

Headspace gas chromatography offers a quick and simple solution to many of these problems. A short chain fatty acid methyl ester headspace GC

technique has been developed by Wang, et. al. [4]. The method is simple, sensitive and reproducible. However, formic and acetic acid, both of which are important components in many agricultural products cannot be detected due to their coelution with the methanol which is a component of the derivatizing reagent.

Because of these problems, this thesis presents a headspace GC procedure for the determination of free short chain fatty acids by on-line derivatization with bis(trimethylsilyl)trifluoroacetamide (BSTFA). The entire process is carried out in the injection vial used in the headspace device. Under these conditions, a three-fold advantage can be obtained: (1) losses of volatiles and errors caused by sample preparation are minimized; (2) chromatographic properties of fatty acids are improved, and formic and acetic acid can be detected; (3) tedious procedures in conventional derivatization including derivation, separation, and concentration are eliminated.

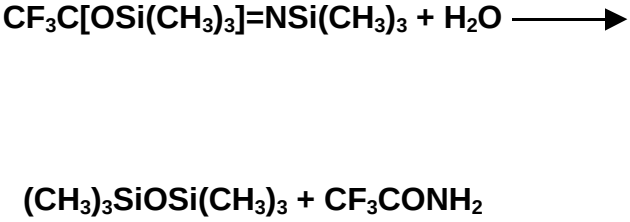
BSTFA is the most widely used reagent for trimethylsilylation. The reagent was first prepared by Stalling et al [96]. It is very versatile, reacting with all the common protic sites present in organic compounds. The reaction with short chain fatty acids can be expressed as following:



$$CF_3C[OSi(CH_3)_3]=NSi(CH_3)_3 + 2RCOOH \longrightarrow 2(CH_3)_3SiOOCR + CF_3CONH_2$$

BSTFA + Acid = Acid ester + Trifluoroacetamide

BSTFA also can react with water:



Large amounts of water should be avoided to minimize the quantity of BSTFA required.

4.2 Experimental

4.2.1 Chemicals

Acetonitrile, benzene and hexane were purchased from Fisher Scientific (Fair Lawn, NJ), and chloroform and methylene chloride were obtained from EM Science (Gibbstown, NJ). All solvents were used without further purification. The following reference standards were purchased from Ultra Scientific (North Kingstown, RI): acetic, propionic, butyric, pentanoic, hexanoic, heptanoic, and octanoic acid. BSTFA with 1% trimethylchlorosilane was obtained from Pierce Chemical Co. (Rockford, IL).

4.2.2 Instrumentation

A Model 5970 GC/MS system (Hewlett Packard, Palo Alto, CA) was used for the analysis. A PE Model HS-40 static headspace injector (Perkin Elmer, Norwalk, CT) was directly connected to the GC system. The column used was a 30 m x 0.25 mm x 1 um HP-5 (Hewlett Packard, Palo Alto, CA). The chromatographic conditions are listed in Table 7.

4.2.3 Sample analysis

Samples of tea, coffee, cigarette and tobacco (ground to a powder) and low calorie fat were directly put into 22 mL headspace vials. One mL of acetonitrile was added to each vial and the vial was sealed immediately. Fifty µL BSTFA was injected to the vial. The vial was manually shaken before headspace sampling.

Table 7. GC/MS and headspace conditions

Sample thermostatting	80^{o}C, 10 min
Needle temperature	120^{o}C
Transfer line temperature	120^{o}C
Pressurizing time	2 min
Injection time	0.25 min
Head pressure	15 psi
GC oven programming	40^{o}C (2 min), 15^{o}C/min to 250^{o}C (hold for1 min)
Injector temperature	280^{o}C
MS mode	SIM 75
Electron Ionization	70 eV
Electron Multiplier Voltage	2200 V

4.3 Results

4.3.1. Optimization of the method

To maximize the sensitivity of the method, the volume of the derivatizing agent, the headspace thermostatting time and the sample vial temperature, the solvent and the inorganic salts were investigated.

4.3.1.1 Derivatization

To ensure the separation of the derivatized acids on a HP-5 column, an acid mixture of formic, acetic propionic, butyric, pentanoic, hexanoic, heptanoic and octanoic acids was derivatized and headspaced at 80^{o}C for 10 minute. The trimethyl silyl esters of the eight acids were well separated (Fig 14). The amount of BSTFA used affects the yield of the acid esters. This has been well studied for the derivatization in solution [97]. According to Blau[97], the recommended molar ratio for driving the reaction to completion is 2:1 for silylating reagent: acids. To study the effect of the amount of derivatizing reagent on the yield, different amount of BSTFA with 1 mL of 100 ppm of each acid standard was headspaced (see Fig. 15). The maximum response was obtained when 50 µL of BSTFA was added which is six times the amount of acid. The excess amount of BSTFA could compensate for the trace amount of water already in the sample.

4.3.1.2 Thermostatting temperature and time

The formation of the trimethylsilyl esters depends on both temperature and time of the reaction, both parameters also affect the equilibrium between

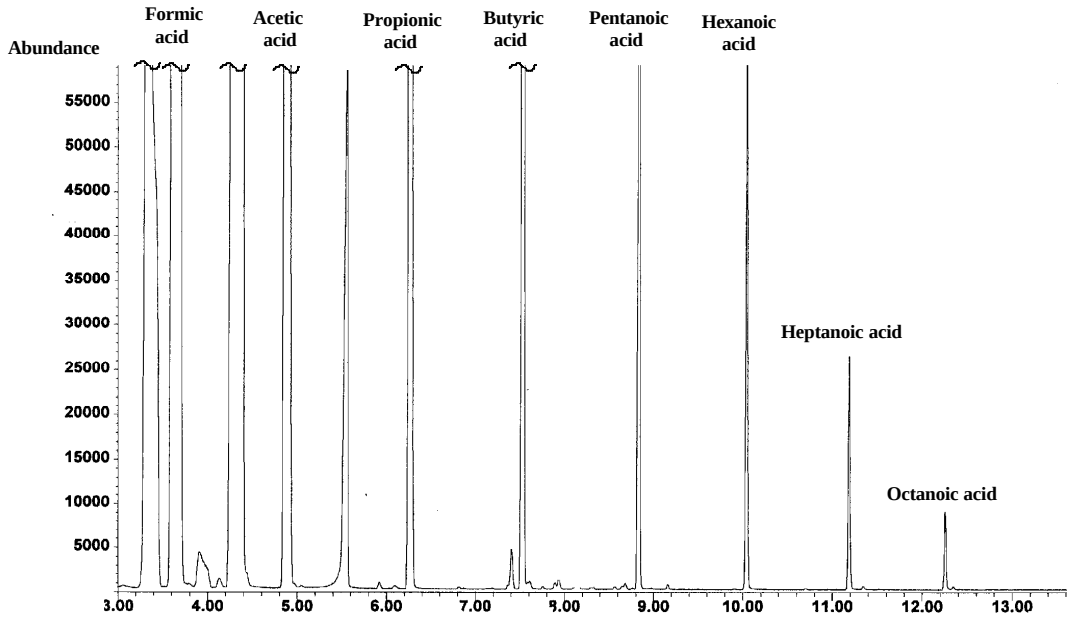


Fig. 14 Headspace of fatty acid trimethylsilyl esters (10 ppm). One mL of a 10 ppm acid mixture was placed in a headspace vial and 50 μL BSTFA was added. The vial was thermostatted at 80°C for 10 minute. GC/MS conditions are given in Table 7.

the liquid and vapor phases in the headspace injection.

The thermostatting temperature affects both the reaction yield and vapor phase distribution coefficient. In order to isolate the two parameters, a 10 minute at room temperature reaction was made and a 1 μL reaction product was injected onto a capillary Carbowax column. No free acid peaks were found. This means the reaction is complete at room temperature and the only effect of higher temperature on the headspace peak area is the vapor phase distribution.

The effect of temperature on headspace vapor phase distribution is shown in Fig. 16 and 17. The peak area, which corresponds to the vapor phase distribution, increases with the increase of thermostatting temperature. At 80^{o}C, the peak area of acetic acid is about 10 times as that at 30^{o}C. In order to avoid the destruction of the glass vial at high temperatures. Higher temperatures were not possible with the experimental set-up.

Essentially the time needed for headspace equilibration depends on the diffusion of the volatile sample components into and from the sample matrix. In this case, the equilibration time depends on both the reaction rate and the diffusion rate of the derivatized product. To ensure the maximum derivatization and the vapor equilibrium of the fatty acids, the thermostatting time was varied from 1 to 25 minutes. The peak area became constant after 5 min at 80^{o}C (Fig. 18 and 19). This suggests that ten minutes were adequate for all of the fatty acids studied here.

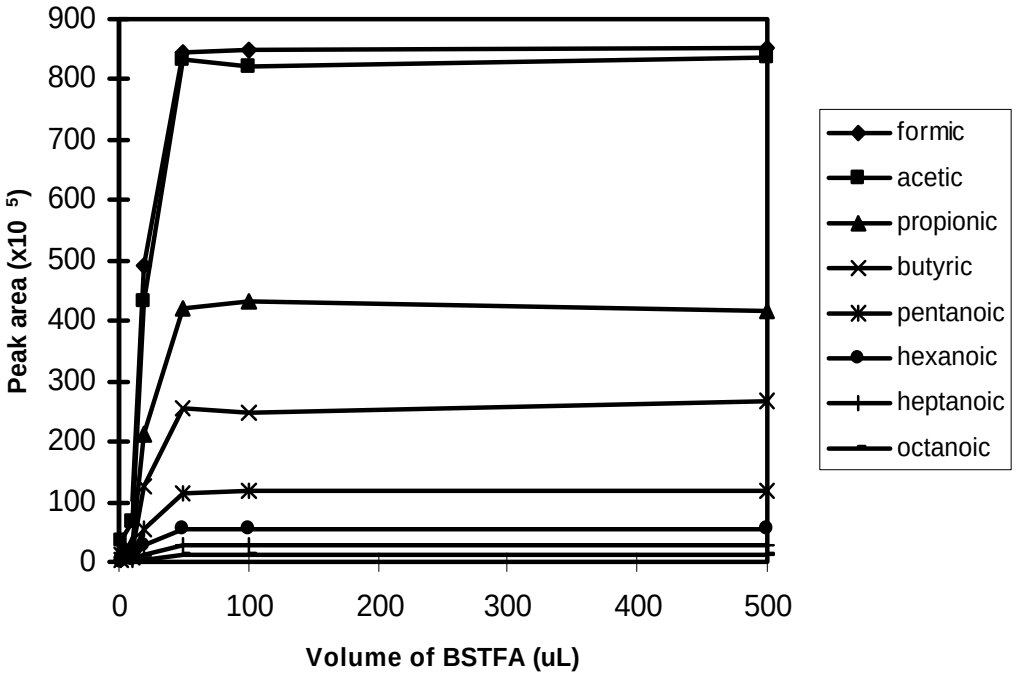


Fig. 15 Effect of volume of BSTFA. One mL of 100 ppm acid mixture was placed into a headspace vial and thermostatted at 80°C for 10 minute. Peak areas are for ion 75 AMU in the SIM mode of GC/MS (see Table 7 for more details).

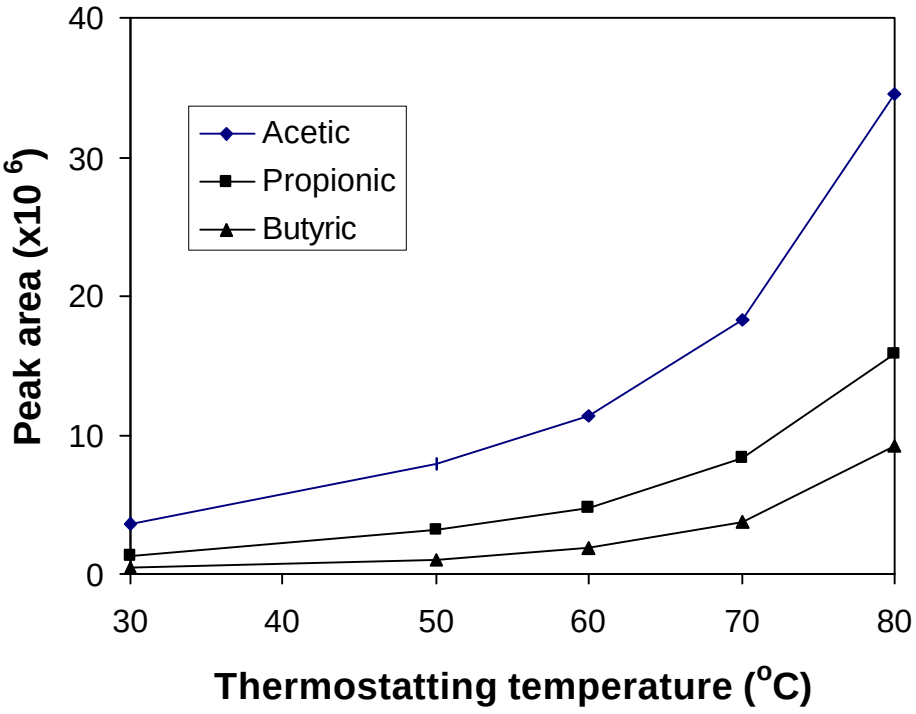


Fig. 16 Effect of thermostatting temperature (1). One mL of 50 ppm acid mixture was used and thermostatting time was 10 minute. GC/MS conditions are same as in Fig. 15.

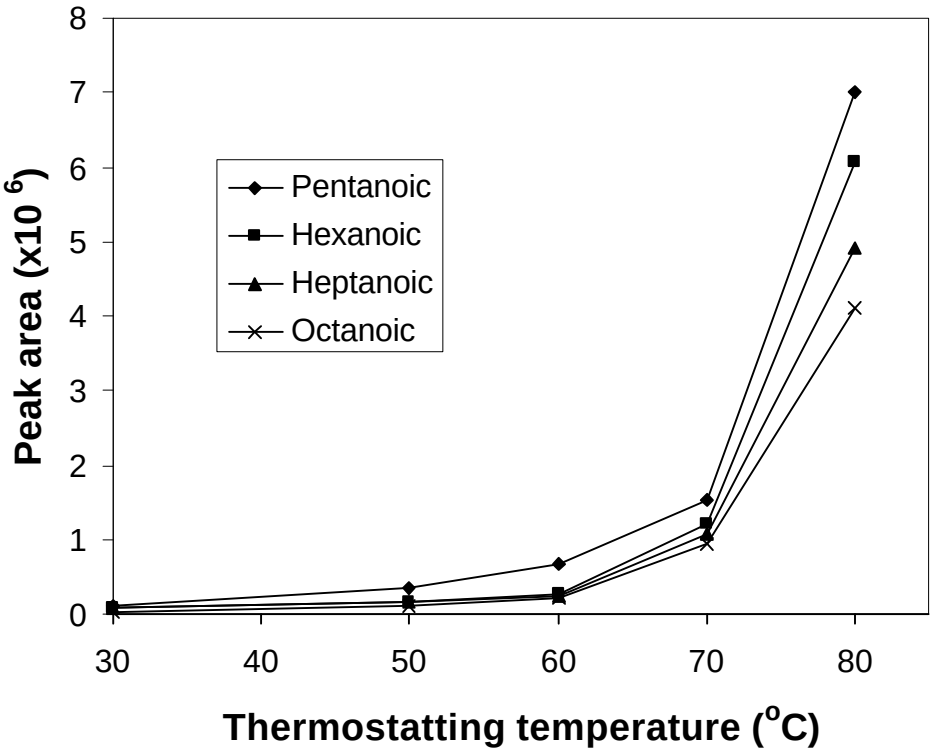


Fig. 17 Effect of thermostatting temperature (2). One mL of 50 ppm acid mixture was used and thermostatting time was 10 minute. GC/MS conditions as in Fig. 15.

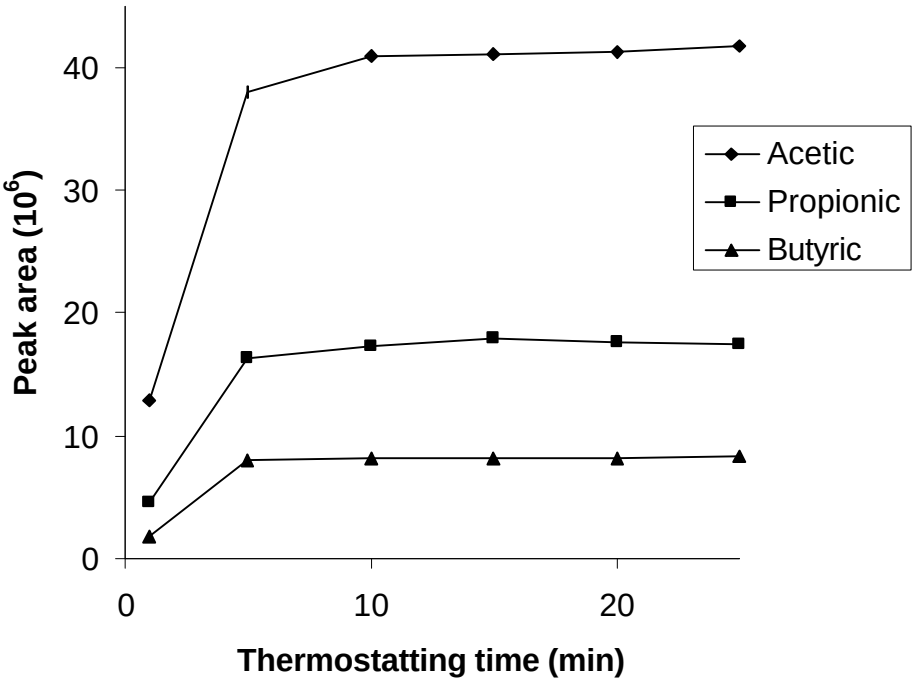


Fig. 18 Effect of headspace equilibration time on mass spectrometry peak area (1). One mL of 50 ppm acid mixture was used and thermostatting temperature was 80^{o}C.

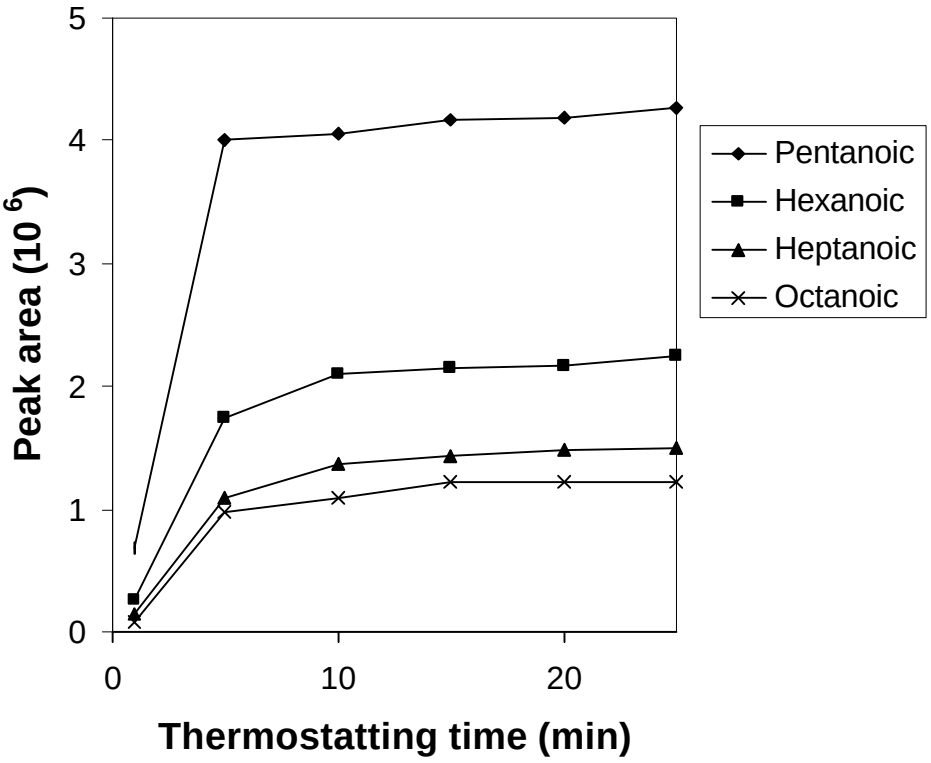


Fig. 19 Effect of thermostatting temperature on mass spectrometry peak area (2). One mL of 50 ppm acid mixture was used and thermostatting temperature was 80°C.

4.3.1.3 Solvents

Hexane, acetonitrile, chloroform and toluene were investigated as solvents to optimize the yield and vapor equilibration (see Fig. 20). The solvents had a significant influence on the headspace peak area (see Fig 20). The reaction yield was determined by direct syringe injections (not headspace) of the products. The reaction yields did not show significant differences. However, the headspace results shown a dramatic peak area change as a function of reaction solvent. The peaks produced by hexane and acetonitrile are about 2.5 times that produced by chloroform and toluene. Acetonitrile was used for all future studies.

4.3.1.4 Salt effect

The effect of salts in aqueous extractions has been well studied [(76)]. Even though the salt has a limited solubility in acetonitrile, it still influences the distribution of acid esters between the liquid and vapor phases. This was studied by saturating the solution with salts including NaCl, KCl, Na_2SO_4 and $MgSO_4$. The salts did not increase the vapor phase distribution as indicated by peak areas (Fig. 21). A salt free solution was the best choice and used in all future studies.

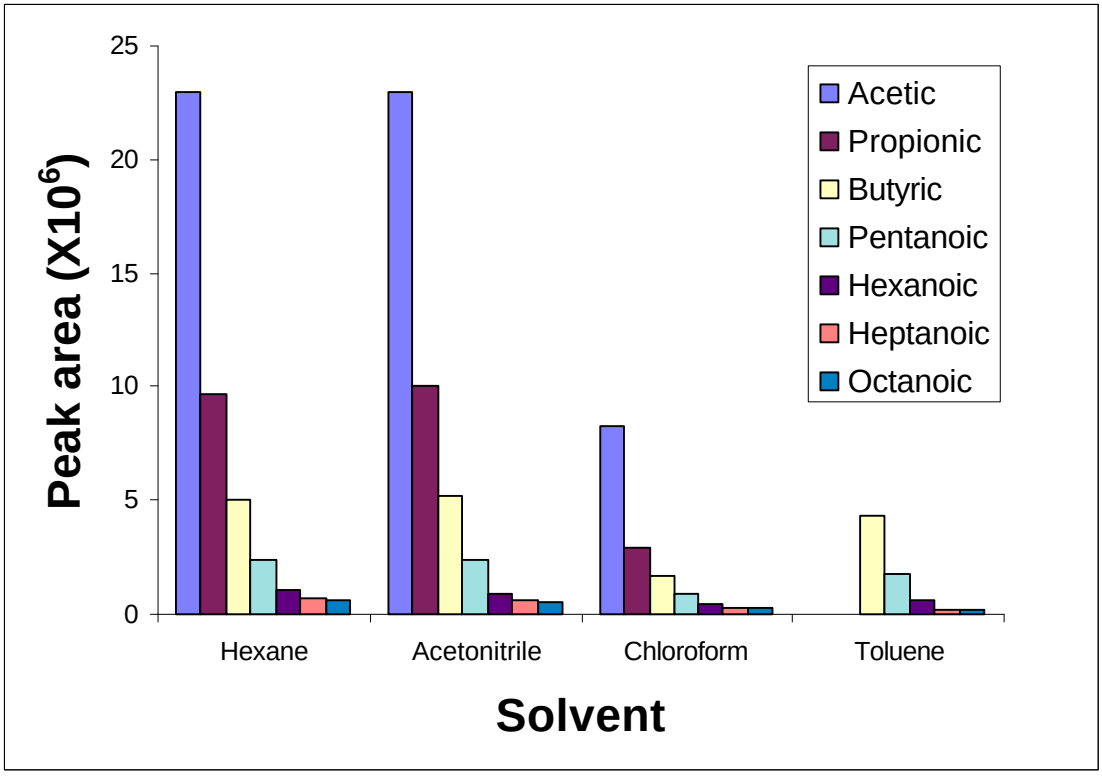


Fig. 20 Effect of solvents. One mL acid mixture was on-line derivatized and headspaced at 80^{o}C for 10 minute. GC/MS conditions same as Fig. 15.

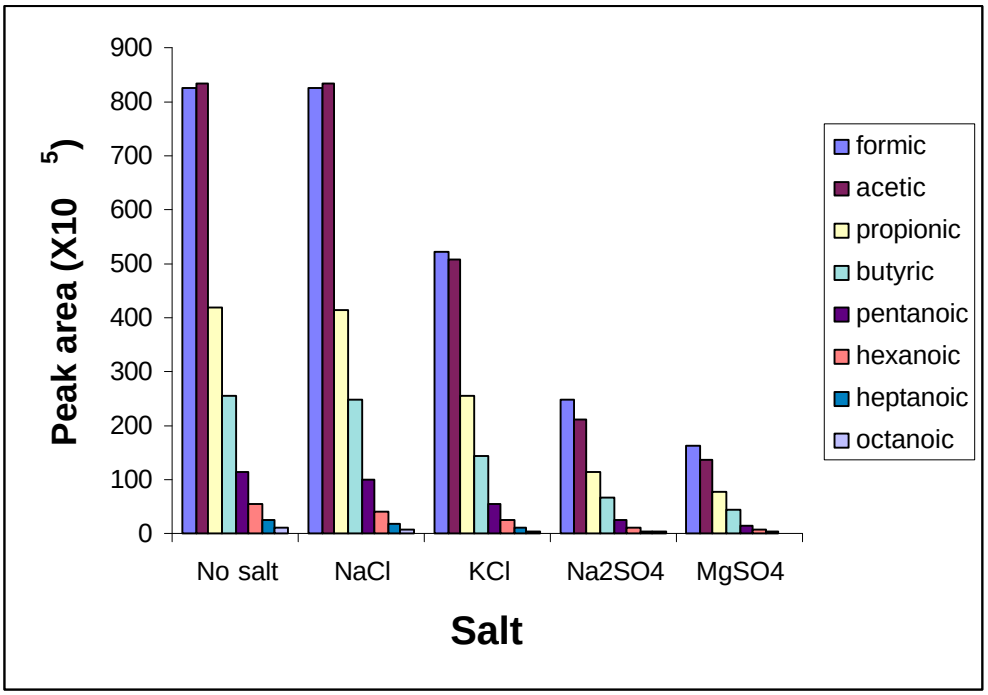


Fig. 21 Salt effects. One mL acid mixture was on-line derivatized and headspaced at 80°C for 10 minute. GC/MS conditions same as Fig. 15.

4.3.2 Precision

The precision was evaluated by a series of replicate analysis (n=6) of standard acid sample with a concentration of 20 ppm. Table 8 shows the results. The relative standard deviations range between 4 and 7%.

4.3.3 Sample matrix effect

The described headspace derivatization sampling involves two or three phase equilibration (for low calorie fat, there are two phases: sample solution and headspace; for tobacco, there are three phases: solid tobacco matrix, solution and headspace.) To investigate the matrix effect, the target compounds in the sample were removed using the following procedure: 0.2 gram of sample (low calorie fat or tobacco) was placed into a headspace vial. One mL of acetonitrile was added and the vial was sonicated for 2 min. Then 50 μL BSTFA was added and the vial was sonicated for another 2 min. Finally, the vial was put in a 80^{o}C oven with the the vial open to evaporate the solvent and the formed acid trimethylsilyl esters. To ensure that the matrix does not have a detectable amount of short chain acids and their derivatized trimethylsilyl esters, it was analyzed following the procedure described in 4.2.3. No acid ester was found. One mL of 1 ppm acid standard was spiked onto the matrix and the results of analysis are shown in Fig. 22. A significant peak area change was found. The effect of the tobacco matrix is bigger than that of low calorie fat. For example the butyric acid peak area was reduced about 50% when tobacco matrix was present and only 34% when low calorie fat matrix was used. This study indicates that a standard external calibration using pure solvent as matrix is not feasible in this case.

Table 8. Reproducibility of fatty acid trimethylsilyl esters in headspace GC/MS

Sample: one mL of 20 ppm acid mixture (n=6)

Acids	%RSD (peak area)
Formic acid	4
Acetic acid	4
Propionic acid	4
Butyric acid	6
Pentanoic acid	4
Hexanoic acid	5
Heptanoic acid	5
Octanoic acid	7

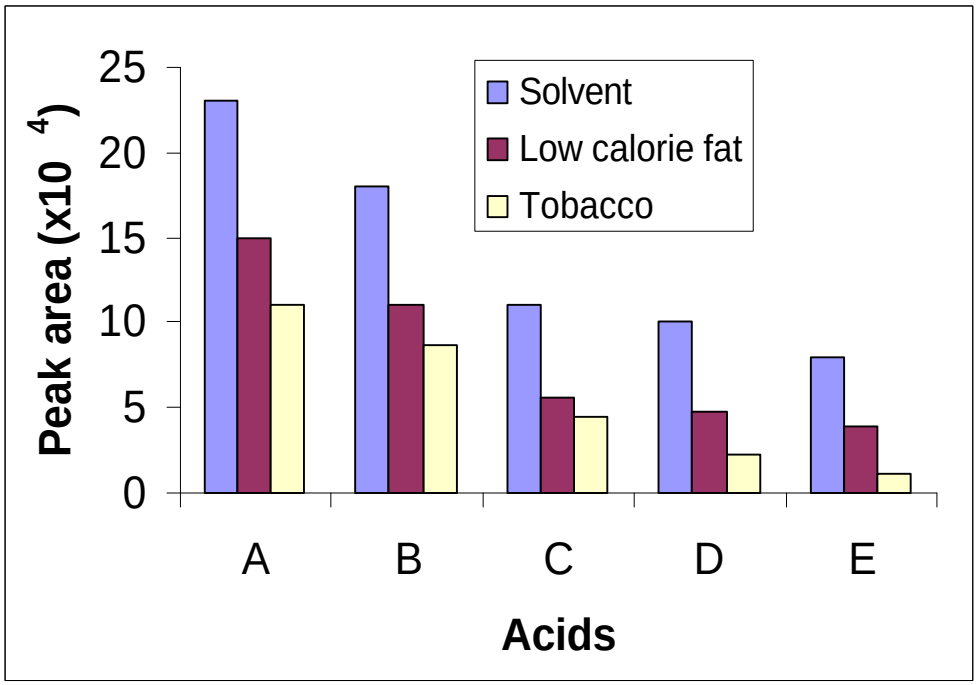


Fig. 22 Effect of sample matrix on headspace sampling. One mL of 1 ppm acid mixture was spiked into the matrix and headspaced at 80^{o}C for 10 minute. Trimethylsilyl esters of A. butyric acid, B. pentanoic acid, C. hexanoic acid, D. heptanoic acid and E. octanoic acid.

4.3.4 Calibration

Concerning the matrix effect, all the calibrations were carried out using the matrix described in 4.3.3. Standard solutions of different concentrations of the fatty acids were spiked on to the matrix. Each standard was analyzed three times. The data were plotted by regression analysis using a linear model (Table 9). Detection and quantitation limits were evaluated for signal/noise (S/N) ratios of 3 and 10 respectively. Figures 23 and 24 show the calibration curves by using low calorie fat matrix.

4.3.5 Sample analyses

To demonstrate the feasibility of this technique, different food and consumer products including low calorie fat, coffee, tea, and tobacco were analyzed. Table 10 shows the results. Free fatty acids were found in all six samples. The concentration ranges from 0.45-38 ppm. Tobacco contains all the eight acids investigated at a low level. Coffee contains significant levels of acetic acid (38 ppm). Two typical chromatograms are shown in Figures 25 and 26.

Table 9 Results of regression analysis

Acids	Acetic	Propionic	Butyric	Pentanoic
Calibration range (ppm)	0.1-10	0.1-10	0.1-10	0.1-10
Correlation coefficient	0.9993	1.0000	0.9993	0.9998
Detection limit (ppm)*	0.008	0.01	0.05	0.1
Quantification limit (ppm)*	0.027	0.033	0.17	0.33

- Maria P. Llompart-Vizoso, et. al., J. High Resol. Chromatogr., 19, 209(1996). The detection limit was evaluated for signal/noise ratio (S/N) of 3. By injecting low concentrations of standards, the detection limits were corresponding to the concentration of three times noise level and the quantification limits were ten times noise level.

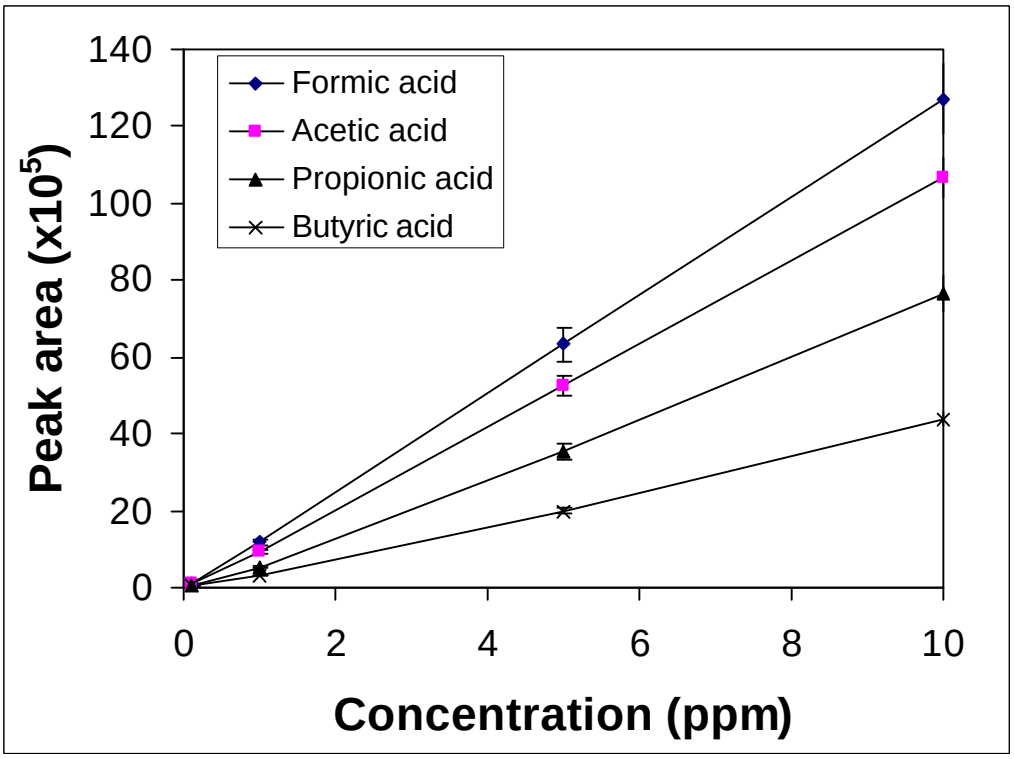


Fig. 23 Calibration curves of formic, acetic, propionic, and butyric acid trimethylsilyl esters using low calorie fat matrix. GC/MS conditions are same as in Fig. 15.

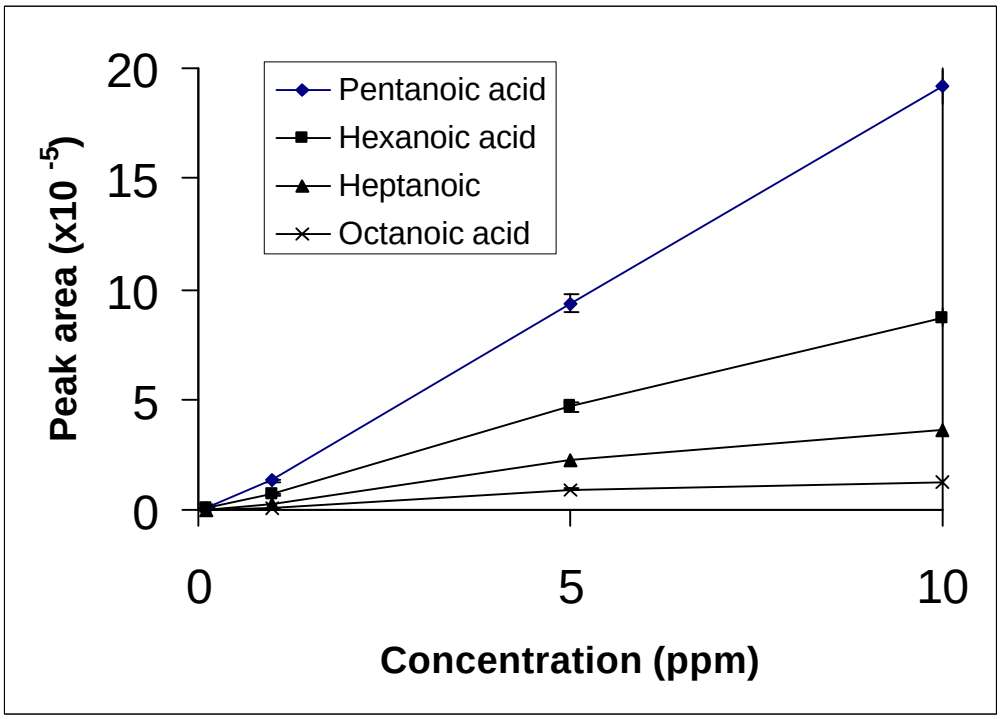


Fig. 24 Calibration curves of pentanoic, hexanoic, heptanoic and octanoic acid trimethylsilyl esters using low calorie fat matrix. GC/MS conditions are same as in Fig. 15.

Table 10 Content of free acids in commercial products*

Acids	Low calorie fat µg/g	Tea 1 µg/g	Tea 2 µg/g	Coffee µg/g	Cigarette µg/g	Tobacoo µg/g
Formic	0.62	2.8	2.0	5.9	0.62	1.2
Acetic	1.9	0.45	2.7	38	1.1	4.3
Propionic	0.96	ND	0.89	3.3	ND	0.99
Butyric	1.2	ND	1.3	1.5	1.6	1.5
Pentanoic	ND	ND	1.5	1.6	1.7	1.7
Hexanoic	1.1	ND	2.4	1.4	1.4	5.2
Heptanoic	ND	ND	ND	ND	3.7	14
Octanoic	ND	ND	ND	ND	2.6	7.4

*ND: not detected. Average values were used (n=3)

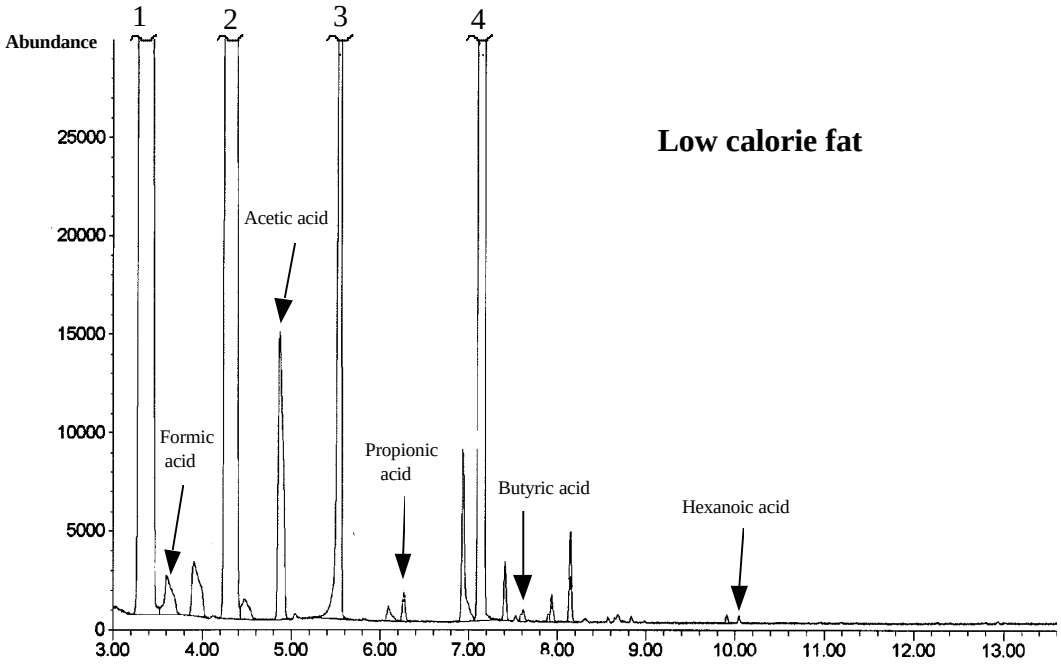


Fig. 25 Typical sample chromatogram of low calorie fat. Peak 1. Trifluoroacetamide; 2. bis(trimethylsilyl) ether; 3. mono(trimethylsilyl)trifluroacetamide, 4. BSTFA. Sample was on-line derivatized and thermostatted at 80^{o}C for 10 minute and detected by SIM GC/MS at 75 amu.

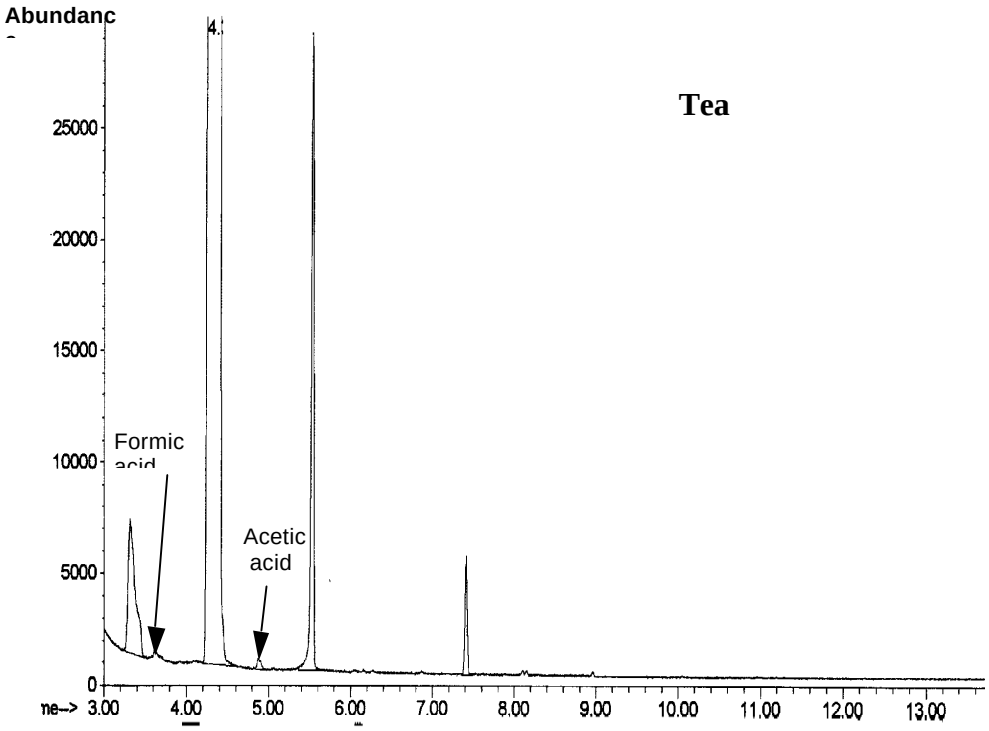


Fig. 26 Typical sample chromatogram of tea. Sample was on-line derivatized and thermostatted at 80^{o}C for 10 minute and detected by SIM GC/MS at 75 amu.

4.4 Discussion

Reliable quantitative analysis of short chain free acids is needed in the food industry, especially in the production of low calorie fat in which acetic, propionic and butyric acid are important. Although these acids have been determined by GC, most methods use a solvent extraction procedure followed by GC. Extraction with organic solvents usually results in errors from the loss of volatile acids such as formic and acetic acid. With in line derivatized headspace analysis described here, little sample manipulation is required and losses are minimized. Errors associated with vapor sampling and injection into the GC are reduced, and batch analysis is possible. Since vapor equilibration occurs above an organic solution, partition into the vapor phase is not enhanced by the addition of salts (no salting out effect). Direct headspace of short chain fatty acids has been studied [98]. However, the introduction of serious ghosting peaks and peak tailing, due to adsorption on the headspace transfer line and the GC inlet liner, make this technique complicated. Formic acid cannot be detected with direct headspace analyses[98]. With BSTFA on-line derivatization, all eight acids can be detected and no system deactivation is needed. The derivatized acids can be detected by using a routine headspace GC or GC/MS.

All the acid trimethylsilyl esters investigated have a base ion at m/z 75. Therefore, using single ion monitoring (SIM 75), sensitivity and selectivity are enhanced. The derivatizing reagent (BSTFA) and its by-products (e.g. mono(trimethylsilyl)trifluoroacetamide and trifluoroacetamide) do not have a strong ion at m/z 75 so they show only minor interference. Only trimethylchlorosilane has a relatively strong ion at m/z 75, but fortunately it has a retention time of 3.32 min and elutes before any of the acids.

On-line derivatization of low calorie fat, tobacco, tea, coffee and cigarette with BSTFA provided an excellent method for extraction and conversion

of fatty acids to their volatile trimethylsilyl esters. Therefore this technique could be used to evaluate the freshness and the taste of these products.

4.5 Conclusions

In summary, on-line silylation of short chain acids with BSTFA followed by GC/MS provide an excellent substitute to the existing techniques for the analysis of free short chain acids. This technique is rapid, simple, quantitative and requires only limited sample preparation. It can be applied to samples with complicated matrices such as low calorie fat, tobacco, tea and coffee.

CHAPTER V
BEHAVIOR OF SPME FIBER COATINGS

5.1 Introduction

Solid Phase Microextraction (SPME) is a new sample enrichment technique. It is a fast, simple, sensitive and solventless technique which can be used for analysis in a variety of fields including water [9-36], air [99-102], soil [103-105], human fluids [106-111] and food [1, 112-118]. In SPME, a fused silica fiber coated with a thin hydrophobic stationary phase is immersed in a stirred aqueous solution. The organic analytes partition from the water into the stationary phase. Then, the fiber is removed from the solution and inserted into a gas chromatograph (GC) inlet. The analytes are thermally desorbed in the hot GC inlet and transferred to the column. The most commonly used stationary phase for the SPME fiber is poly(dimethylsiloxane). There is a linear relationship between the amount absorbed by the fiber and the concentration in solution [49,113] . The SPME technique is used for quantitative trace analysis. The technique is extremely sensitive and ppt levels can be achieved in some cases[120].

Several SPME fiber coatings which have different chemical properties have been investigated. These include poly(dimethysiloxane), polyacrylate and Carbowax. Among the advantages of the SPME technique, fiber selectivity for a target compound is an important parameter. By choosing a proper fiber, the sensitivity can be enhanced and the resolution required for a specific sample separation can be increased. Until now, a systematic study of the selectivities of the fiber coatings has not been published.

In this study, a study of the selectivity of fiber coatings has been carried out. The behavior of test compounds which represent five different functional groups with three SPME fibers. The effect of temperature on the absorption and the modification of the matrix were also investigated.

5.2 Experimental

5.2.1 Fiber coatings

The SPME fibers used in this study were purchased from Supelco Inc. (Bellefonte, PA). They were fused silica fiber, 110 um diameter and 1 cm length, coated with poly(dimethylsiloxane), polyacrylate and Carbowax. The fibers used and their calculated coating volumes are listed in Table11.

Ten chemicals which represent different functional groups were used. Decane was obtained from Fisher Scientific Company (Fair Lawn, NJ). Naphthalene, anthracene and tetradecane were from Aldrich Chemicals (Milwaukee, WI). Decylamine, tetradecylamine, decyl alcohol, tetradecanol, decanoic acid, and tetradecanoic acid were from Chem Service Chemicals (West Chester, PA). They were used without further purification. A stock solution of 100 ppm of each of the 10 chemicals was prepared by dissolving 10 mg of each in a small amount of ethanol and diluting to 100 mL with water. For the experiments studying selectivities and the temperature influence, a 1 mL stock solution was diluted to 100 mL with deionized water. The concentration of the working solution was 1 μg/mL. The concentration of the compounds for the investigation of pH was 0.9 μg/mL. The pH was adjusted with HCl/NaCl (pH 2) and sodium tetraborate/HCl (pH 9.2) Sodium chloride and sodium tetraborate were purchased from Fisher Scientific Company (Fair Lawn, NJ).

Table 11 Parameters of fiber coating

Trade name	Coating	Coating thickness (um)	Calculated coating volume (mL)
PDMS	Poly(dimethylsiloxane)	100	6.6×10^{-4}
PA	Polyacrylate	85	5.2×10^{-4}
Carbowax	Polyethylene Glycol-divinylbenzene	65	3.6×10^{-4}

5.2.2 SPME extraction

Four milliliters of the test solution was placed in a 10 mL vial. The vial was capped with a Teflon® coated septum. The solution was agitated with a Thermix® Model 120 MR magnetic stirrer (Fisher Scientific, Springfield, NJ) at the spinning rate of 6 (division). The SPME needle pierced the septum and the fiber was directly inserted into the aqueous solution. The absorption time was 20 minutes. After each sampling, the aqueous solution was replaced with a fresh one.

5.2.3 Temperature study

For the temperature studies, the temperature was controlled with a water bath. For the temperatures lower than room temperature, the water bath was filled with salted ice water. For the temperatures higher than room temperature, a PC-351 Hot Plate Stirrer was used (Corning, Inc. Coring, NY). A thermometer was inserted to the water bath to monitor the temperature changes. The variation of the temperature was $\pm 2^{o}$C. The pH was adjusted with 0.1N HCl/NaCl and 0.1 N sodium tetraborate/HCl.

5.2.4 GC/MS

A GC/MS was used as the detection device. A HP Model 5890 Gas Chromatography was interfaced with a HP Model 5970 Mass Selective Detector (Hewlett-Packard, Palo Alto, CA). A 30 m x 0.25 mm with 0.25 μm film HP-5 column (Hewlett Packard, Little Falls, PA) was used as the analytical column. The injection port temperature which was also the desorption temperature for the SPME fiber was 250^{o}C, and the desorption time was 4 min. The GC split valve was set to open after the 4 min desorption time. The GC injector liner was a quartz liner with an internal diameter of 0.75 mm (Supelco Inc. Bellefonte, PA).

The GC column temperature program was 40^{o}C (hold for 4 min), 15^{o}C/min to 250^{o}C (hold for 1 min). A mass scan from 35 to 250 amu was acquired in the electron impact ionization mode (70 ev). The amount that was thermally desorbed from the fibers during injection was calibrated using standards.

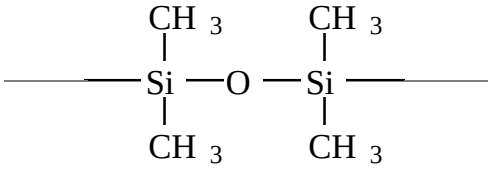
5.3 Results and discussion

5.3.1 SPME coatings

The early work of the SPME technique was carried out by Hawthorne and et al [8] using an uncoated silica fiber to determine caffeine in beverage samples. However, much effort has been directed toward searching for more capable adsorbents that can improve the performance of SPME [8]. SPME is a single batch method, and the surface available for adsorption is very limited (typically less than 10 mm^2). Therefore, trace compounds in a sample must have large distribution coefficients in order to be first highly enriched on the fiber surface and then later successfully analyzed by subsequent separation and detection methods. This situation means that a useful SPME fiber must have a efficient coating. So far, the most frequently used fiber coatings have been poly(dimethylsiloxane) with a thickness of 100 um, polyacrylate with 85 um thickness and Carbowax/PDV with a film thickness of 65 um. Fig. 27 shows the structures of these three polymers. The methyl groups of poly(dimethylsiloxane) make this fiber coating relatively apolar, whereas polyacrylate and carbowax are more polar because of the carboxyl and hydroxy groups respectively. Poly(dimethylsiloxane) is used as a stationary phase in apolar GC columns. Polyacrylate is used to make Amberlite XAD resins such as XAD-7 and XAD–8. Carbowax is a polyethylene glycol used as a GC stationary phase for polar compounds. The use of these polymer to enrich compounds should be selective depending on their chemical properties.

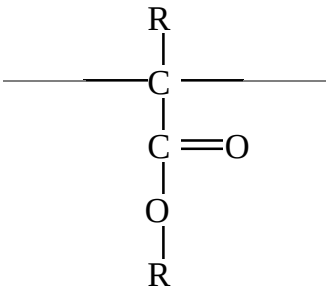
5.3.2 Degrees of absorption on SPME coatings

In a standard aqueous solution containing 1 ppm each of the ten investigated compounds, the amount absorbed by the three coatings is shown in



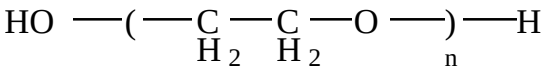
PDMS

Poly(dimethylsiloxane)



PA

Polyacrylate



Carbowax

Fig. 27 Schematic chemical structures of fiber coatings

Tables 12 and 13. For the ten-carbon compounds (Table 12), the PDMS coating shows good selectivity for decane and naphthalene and a fair selectivity for decanol. Both decane and naphthalene are nonpolar and volatile compared with the other three compounds. Since polyacrylate is polar, it shows high selectivity for both decanol and decanoic acid but not decylamine. Interestingly, Carbowax shows low selectivity for all five test compounds. It does show more selectivity for decylamine than either PDMS or polyacrylate. In this case, SPME is not efficient for direct extraction of short chain amines. A modification of the solution or derivatization step may be required for short chain amines [60].

For longer carbon chain compounds (Table 13), all three coatings show better selectivity compared to the ten carbon compounds. This is expected since all three coatings have a hydrophobic moiety. For the longer chain carbon compounds, the hydrophobic moiety dominates the absorption. For tetradecylamine, the PDMS coating is the best of the three coatings.

5.3.3 Modifications of the sample matrices

In an aqueous solution, different forms of weak acids or bases may coexist. Because the dissociation of weak acids or bases is pH dependent, the distribution coefficients will be greatly influenced by pH. The ionic form of an acid or a base is too hydrophilic (large hydration enthalpy) to be adsorbed by the hydrophobic surface of the fiber. By adjusting the solution pH and suppressing the ionization, the absorption efficiency can be increased. Fig. 28 shows the effect of pH on absorption of two acids on a PDMS coating. By adjusting the pH to 2, a eleven-fold increase of the amount of decanoic acid and a 15% increase for the amount of tetradecanoic acid. Lower pH significantly improved the absorption efficiency for decanoic acid.

Table 12. Amount absorbed (ng) by different fiber coatings for compounds having ten carbons

n=3

Fibers	Decane	Naphtha-lene	Decanol	Decanoic acid	Decyl amine
PDMS	220	310	440	36	0.29
PA	21	280	460	550	1.1
CW	17	66	55	30	7.3

Table 13. Amount absorbed (ng) by different fiber coatings for compounds having fourteen carbons

n=3

Fibers	Tetra-decane	Anthracene	Tetra-decanol	Tetra-decanoic acid	Tetradecyl amine
PDMS	380	610	640	450	630
PA	260	710	760	870	370
CW	59	590	290	450	160

For amines (Fig. 29), increasing the pH to nine, a 1000 fold increase of the amount of decylamine absorbed was obtained. It was almost impossible to determine decylamine at pH 7. The pK_a of decylamine is 10.64 (25^oC)[121], a higher pH is preferred to suppress the ionization. However, due to the deterioration of the fiber at high pH, pHs higher than 9 were not investigated.

Compared to PDMS and Carbowax coatings, PA is the best coating for acids. By adjusting the sample pH, the amounts absorbed for both decanoic acid and tetradecanoic acid are increased (see Fig. 30). The amount of decanoic acid absorbed by PA is 2.8 folds of that absorbed by PDMS and 5.3 folds of that absorbed by Carbowax fiber. For amines (see Fig. 31), as expected, the amount absorbed increased by increasing pH but it is not as significant as the case of PDMS coating.

The pH effect on acids on the Carbowax coating is shown in Fig. 32. The amount absorbed increased as the pH was adjusted to 2. However, this coating is not as good as PDMS or PA coating for decanoic acid. For decylamine (Fig. 33) at pH nine, the amount absorbed is less than that of PDMS coating, but more than that of the PA coating.

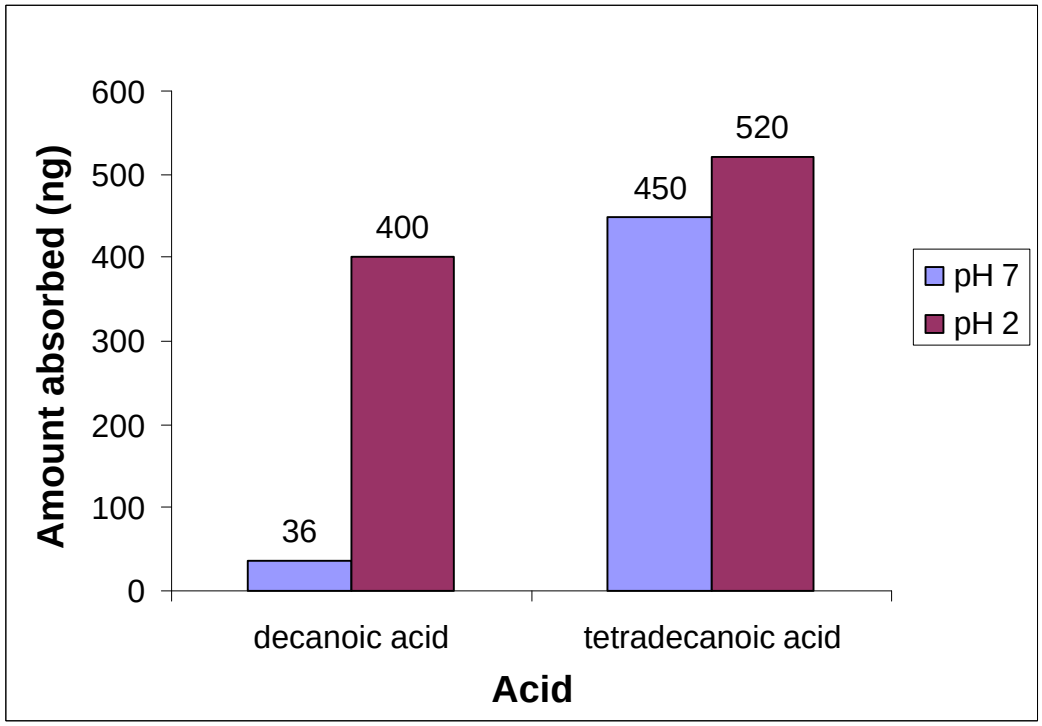


Fig. 28 pH effect on acid absorption in PDMS coating. Four mL of 1 ppm sample mixture was extracted by PDMS at different pH for 20 minute.

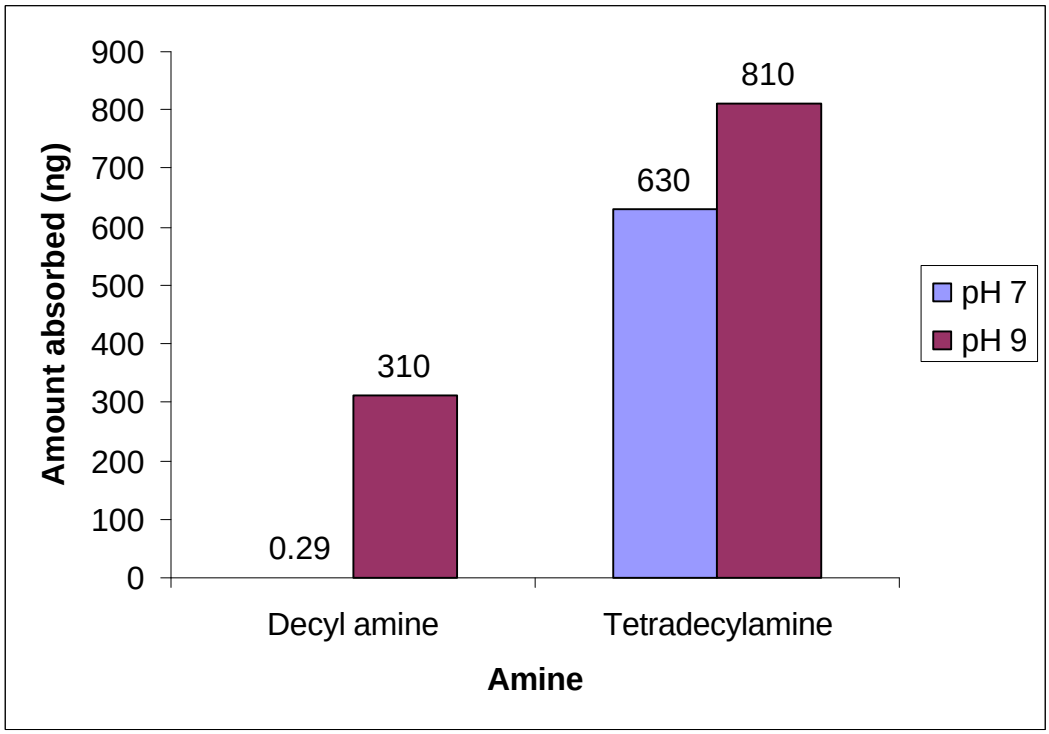


Fig. 29 pH effect on amine absorption in PDMS coating. Four mL of 1 ppm sample mixture was extracted by PDMS at different pH for 20 minute.

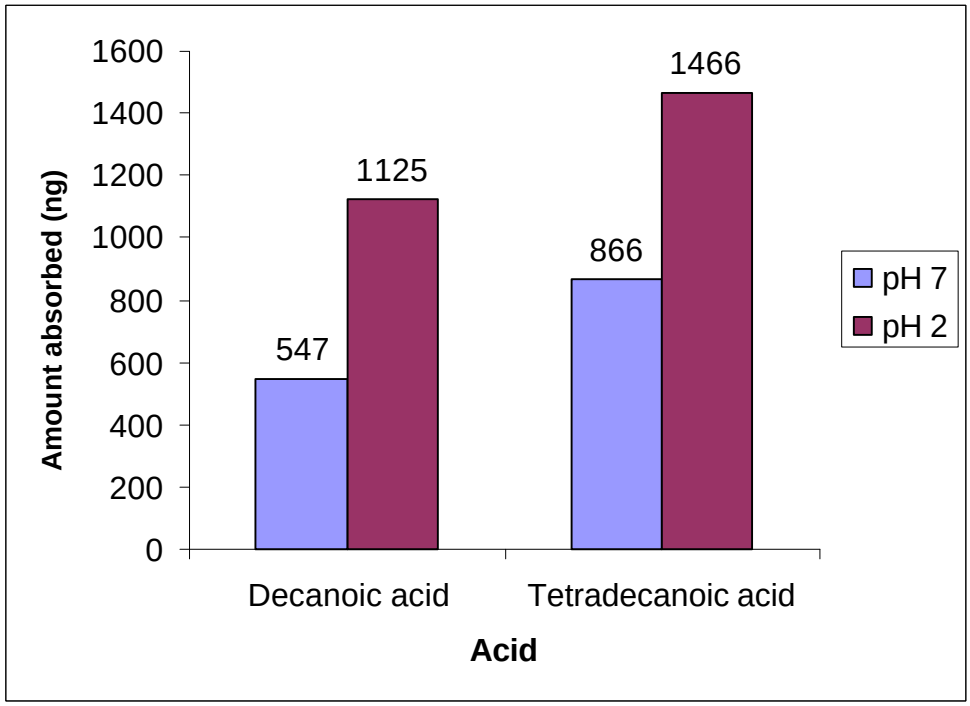


Fig. 30 pH effect on acid absorption in PA coating. Four mL of 1 ppm sample mixture was extracted by PA at different pH for 20 minute.

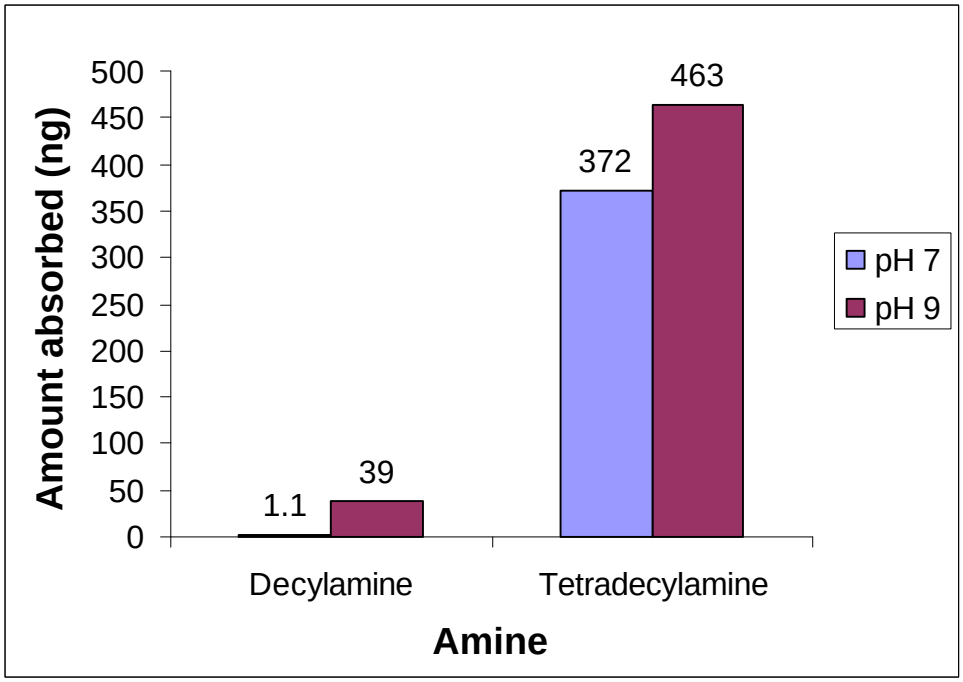


Fig. 31 pH effect on amine absorption in PA coating. Four mL of 1 ppm sample mixture was extracted by PA at different pH for 20 minute.

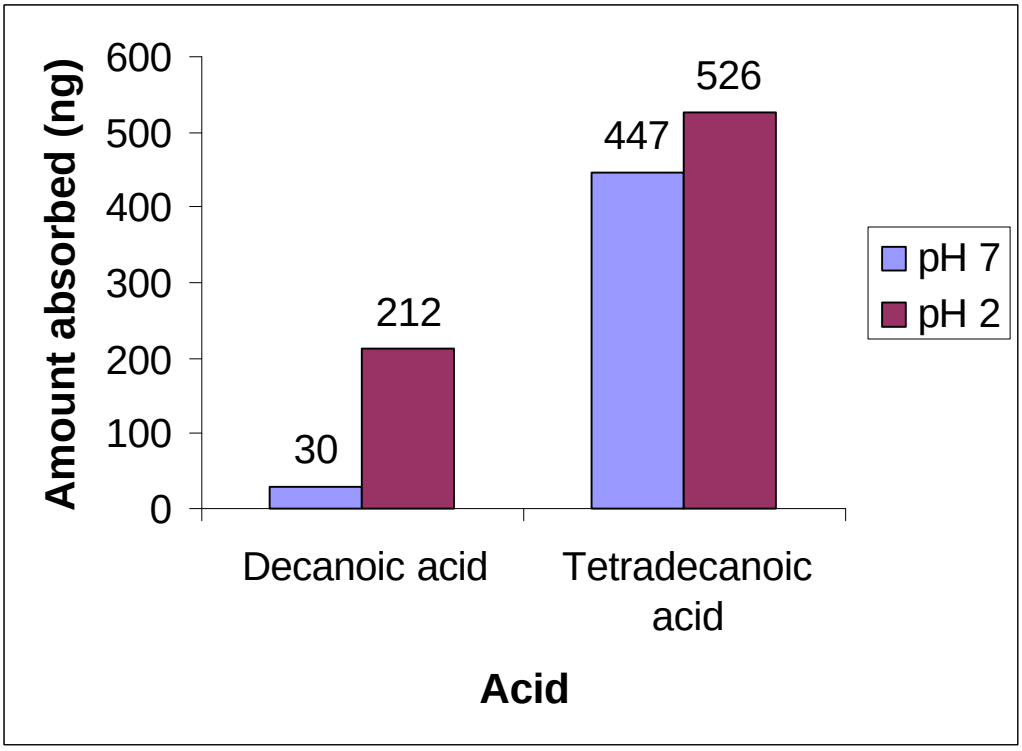


Fig. 32 pH effect on acid absorption on Carbowax coating. Four mL of 1 ppm sample mixture was extracted by Carbowax at different pH for 20 minute.

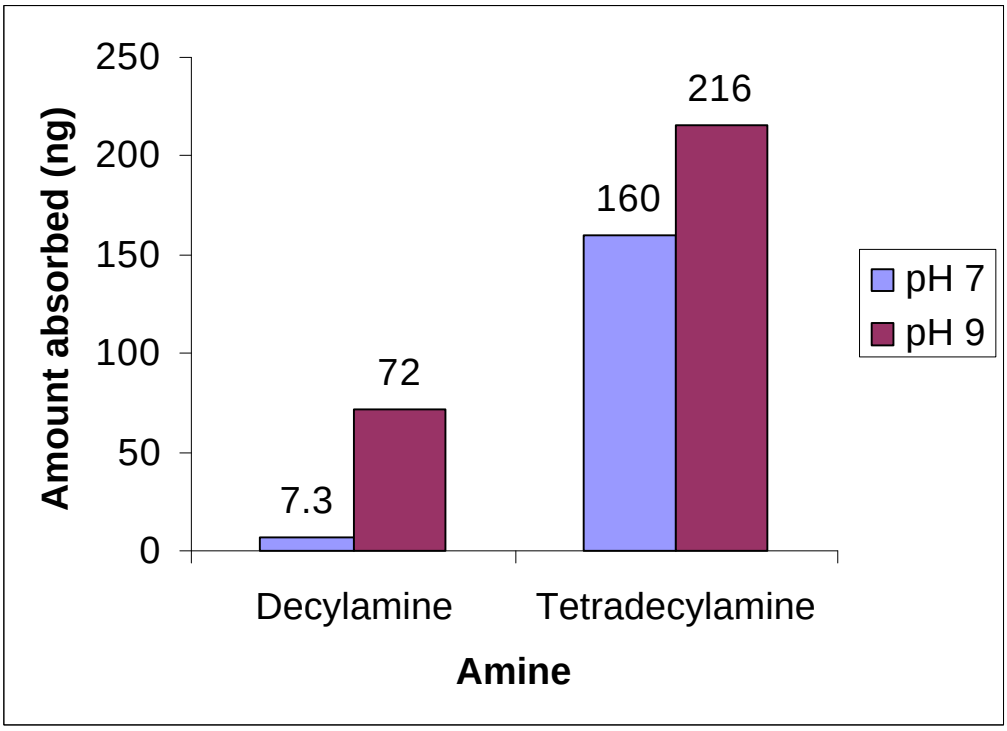


Fig. 33 pH effect on amine absorption in Carbowax coating. Four mL of 1 ppm sample mixture was extracted by Carbowax at different pH for 20 minute.

5.3.4 Temperature effect

Diffusion coefficients of analytes can be increased by increasing the sample temperature. Even though magnetic stirring was applied to the solution, for some compounds, diffusion rate still a limiting parameter for SPME extraction[119]. To investigate the influence of sample temperature, the ten compounds were extracted at 4, 15, 25, 40, 52, and 72^{o}C for 20 minutes. The results are listed in Table 14 for the PDMS coating, Table 15 for the PA coating and Table 16 for the Carbowax coating. The highest temperature investigated was 72^{o}C and the lowest temperature was 4^{o}C.

It can be seen from Table 14 that the effect of temperature on SPME extraction of the ten compounds is complicated. For some compounds such as amines, the extraction recovery increases slowly with temperature. This result suggests that the diffusion of those compounds dominates the extraction. For other compounds the extraction recovery initially increased with temperature, but later decreased with temperature. The maximum efficiency was obtained at 25^{o}C in most cases, but 15^{o}C for decanoic acid; 52^{o}C for anthracene; 40^{o}C for tetradecanol and 52^{o}C for tetradecylamine. Apparently, at low temperature the rate limiting parameter is diffusion of analytes in the sample solution and the polymer coating. At higher temperatures, diffusion will be enhanced. However, at same time, the distribution coefficient will decrease. Above a certain temperature, diffusion of the analytes in the solution and coating are relatively fast while at the same time the distribution coefficient is low. Now the solubility of the compounds in the fiber becomes the rate limiting parameter. Solute molecules diffusing to the fiber surface can no longer be absorbed because of a less favorable distribution coefficient.

In PA coating (Table 15), the amount absorbed increases with the increase of the solution temperature. This indicates that a 20 min absorption is

not enough for equilibrium. Increasing the solution temperature speeds up the diffusion of the compounds, so more sample can be absorbed to the coating at a constant absorption time. As described by Ai [119], some compounds (e.g. 2, 4-dimethyl phenol) did not reach equilibrium after one hour absorption with magnetic stirring in a PA coating. In this case, increasing solution temperature can increase the amount absorbed and increase the sensitivity.

Carbowax coating shows the similar trend as PDMS coating except for the low absorption efficiency for ten carbon compounds (Table 16).

5.4 Conclusion

In summary, the PDMS coating is the most selective for volatile nonpolar compounds; polyacrylate is good for polar compounds and Carbowax shows a slight but better selectivity for short chain amines than the other two fibers. Matrix pH has a significant effect on the absorption of weak acids and bases on SPME coatings. By suppressing the ionization of acids and bases, the sensitivity of the SPME technique can be greatly increased. For some compounds, diffusion in the sample solution and the fiber coating is slow. Especially for polyacrylate coating, a twenty minute extraction time is not adequate for the investigated compounds at room temperature. In some cases, by increasing the temperature of the solution, the extraction speed and efficiency can be increased.

Table 14 Temperature effect on PDMS absorption*

(n=3)

Temperature (^{o}C)	Decane	Naphthalene	Decanol	Decanoic acid	Decylamine
4	11	200	230	30	0
15	170	280	420	42	1
25	220	310	440	36	0
40	200	160	440	19	4
52	160	140	330	12	4
72	110	140	250	10	4
Temperature (^{o}C)	Tetradecane	Anthracene	Tetra-decanol	Tetra-decanoic acid	Tetradecyl amine
4	170	290	310	180	330
15	370	430	570	250	580
25	380	610	640	450	630
40	370	610	800	440	660
52	180	670	700	430	890
72	100	490	560	380	820

*Represented by amount absorbed (ng)

Table 15 Temperature effect on PA absorption*

(n=3)

Temperature (^{o}C)	Decane	Naphthalene	Decanol	Decanoic acid	Decylamine
4	6	160	170	63	0
15	19	280	370	200	0
25	21	280	460	550	1
40	24	290	640	320	1
52	96	290	680	220	1
72	85	340	710	170	1
Temperature (^{o}C)	Tetradecane	Anthracene	Tetradeca-nol	Tetra-decanoic acid	Tetradecyl amine
4	86	300	290	260	260
15	190	520	560	390	560
25	260	710	760	870	370
40	270	930	760	950	300
52	330	960	890	990	270
72	383	1054	1100	1000	260

*Represented by amount absorbed (ng)

Table 16 Temperature effect on Carbowax absorption*

(n=3)

Temperature (^{o}C)	Decane	Naphthalene	Dodecanol	Decanoic acid	Decylamine
4	1	45	32	33	1
15	13	42	33	30	4
25	17	66	55	30	7
40	22	84	84	33	8
52	29	86	80	32	13
72	32	81	54	24	13
Temperature (^{o}C)	Tetradecane	Anthracene	Tetra-decanol	Tetra-decanoic acid	Tetradecyl amine
4	27	230	140	220	39
15	48	270	120	340	56
25	59	590	290	450	160
40	70	720	320	500	360
52	76	960	410	880	870
72	88	940	360	880	810

*Represented by amount absorbed (ng)

CHAPTER VI
GENERAL CONCLUSIONS

The application of SPME for trace organic analysis has experienced exciting developments during the past 5 years and the interest continues to grow. Compared to conventional extraction methods, SPME has many advantages, such as minimized usage of toxic organic solvents, high concentrating efficiency, no transfer of organic solvents to chromatographic systems, low cost and the ability for easy on-line combination with GC.

The extension of SPME to solid samples by combining SPME and MAE has been demonstrated for the first time in this work. The parameters (salt content, pH, and extraction time) which affect extraction efficiency, have to be optimized. Both the temperature of the GC inlet, injection mode (split or splitless) affect the desorption and the transfer of the compounds from fiber to the GC column. Veltol® and Veltol Plus® from food products were successfully analyzed by MAE/SPME and the detection limit was decreased 200 fold compared to conventional solvent extraction for Veltol Plus®.

Headspace analysis is an excellent GC technique for volatile compounds and can be used for “dirty” matrices (both liquids and solids). The technique is characterized by simple sample preparation, and analytes can be transferred directly to a GC. The limitations of the technique are: (1) not suitable for polar compounds because of adsorption on the long transfer line; and (2) low sensitivity for semivolatiles because of their low vapor pressure. Short chain fatty acids are typical examples for samples with high polarity and low volatility. Trace levels of these acids in foods and agricultural products usually affect the taste and odor of these products. The method developed in this work (on-line silylation followed headspace GC/MS analysis) reduces the tedious sample preparation procedures, such as derivatization, separation and concentration for trace analysis. Since all the sample processing occurs in an enclosed vial, sample loss

is minimized. All the fatty acid trimethyl esters have a base ion at 75 amu which makes the detection by GC/MS both simple and sensitive. Low ppb levels of formic to octanoic acids were obtained in this work.

The selectivity and capacity of the fiber coating used in SPME are important in matching fibers with analyte type. Poly(dimethylsiloxane) is a good fiber coating for non-polar and semipolar volatile compounds. It also shows selectivity for different chain lengths. It is not good, however, for short chain acids or amines. The Polyacrylate coating shows high affinity for semipolar and polar compounds, but low affinity for decane and short chain amines. Polyacrylate would be good for decanol, decanoic acid, anthracene, tetradecanol and tetradecanoic acid. Carbowax does not show good selectivity for any short chain compounds, but does show a good recovery for long chain alcohols and acids.

The sample pH has a significant effect on both acid and amine recoveries. By adjusting the pH to suppress ionization, the sensitivity can be increased as the analytes partition better into the hydrophobic fiber. Solution temperature affects both the distribution coefficient into the fiber and the diffusion coefficient of the analytes in solution. At low temperatures, the dominating factor is the molecular diffusion. At a constant extraction time, low temperature results in a low recovery. At higher temperatures, the distribution coefficient of the compounds between the fiber coating and the sample solution dominates the SPME recovery. At even higher temperatures, the distribution coefficient is small which results in a low recovery. For real samples, the chosen solution temperature is a compromise between the diffusion coefficient and the distribution coefficient.

LITERATURE CITED

Abstract

1 Wang, Y., Khaled, M., Bonilla, M. and McNair, H. M., *J. High Resol. Chromatogr*. , 20, 213 (1997).

2 Wang, Y., Khaled, M. and McNair, H. M., *34th Eastern Analytical Symposium and Exhibition*, Sommerset, NJ, Nov. 12-17, 1995.

3 Wang, Y., Khaled, M., Bonilla, M. and McNair, H. M., *18th International Symposium on Capillary Chromatography*, Riva del Garda, Italy, May 20-24, 1996.

4 Wang, Y., Khaled, M. and McNair, H. M., *35th Eastern Analytical Symposium and Exhibition*, Sommerset, NJ, Nov. 17-22, 1996.

5 Wang, Y., Khaled, M. and McNair, H. M, *The Pittsburgh Conference*, Atlanta, Georgia, March 16-21, 1996.

6 Wang, Y., Khaled, M. and McNair, H. M., *19th International Symposium on Capillary Chromatography*, Wintergreen, VA, May 18-22, 1997.

7 Wang, Y. and McNair, M. H., submitted to The Pittsburgh Conference New Orleans, Louisiana, March 1-6, 1998.

Chapter II

8 Arthur, C.L., and . Pawliszyn, J. *Anal. Chem*., 62, 2145 (1990).

9 Arthur,C. L., Killam, M., . Motlagh, S., Lim, M., Potter, D. W. and Pawliszyn, J., *Environ. Sci. Technol*. 26, 979 (1992).

10 Arthur, C. L., Potter, D. W., . Buchholz, K. D., Motlagh, S. and Pawliszyn, J., *LC-GC*, 10, 656 (1992).

11 Arthur, C.L., Pawliszyn, J., *Anal. Chem*., 62, 2145 (1990).

12 Louch, D., Motlagh, S., Pawliszyn, J. *Anal. Chem*., 64, 1187 (1992).

13 Arthur, C. L., Killam, L., Motlagh, S., Lim, M, Potter, D., Pawliszyn, J. *J. Environ. Sci. Technol.*, 26, 979 (1992).

14 Hawthorne, S., Miller, D. J., Pawliszyn, J., Arthur, C. L., *J. Chromatogr*., 603, 185 (1992).

15 Arthur, C. L., Killam, L. M., Buchholtz, K. D., Pawliszyn, J., Burg, J. R., *Anal. Chem*., 64, 1960 (1992).

16 Potter, D. W., Pawliszyn, J., *J. Chromatogr*., 625, 247 (1992).

17 Arthur, C. L., Potter, D. W., Buchholz, K. D., Motlagh, S., Pawliszyn, J. *LC-GC*, 10, 656 (1992).

18 Hassan, A. A. M., Benfenati, E., Facchini, G. and Fanelli, R., *Toxicol. Environ. Chem*., 55, 73 (1996).

19 Huang, S., Yi Ting, C, Lin, C., *J. Chromatogr*., 769, 239 (1997).

20 Huang, S., Cheng, C., Sung, Y., *Anal. Chim. Acta*, 343, 101 (1997).

21 Valor, I., Molto, J.C., Apraiz, D., Font, G., *J. Chromatogr., A*, 767, 195 (1997).

22 Saraullo, A, Martos, P. A., Pawliszyn, J., *Anal. Chem*., 69, 1992 (1997).

23 Lakso, H., Ng, W.F., *Anal. Chem*., 69, 1866 (1997).

24 Wittkamp, B.L., Hawthorne, S. B., Tilotta, D.C*., Anal. Chem*., 69, 1197 (1997).

25 Sng, M.T., Lee, F.K., Laks, H.A., J. Chromatogr., A, 759, 225 (1997).

26 Johansen, S. S., Pawliszyn, J., *J. High Resolut. Chromatogr*., 19, 627 (1996).

27 Valor, I., Cortada, C., Molto, J. C., *J. High Resolut. Chromatogr*., 19, 472 (1996).

28 Santos, F.J., Galceran, M.T., Fraisse, D., *J. Chromatogr*., A, 742, 181 (1996).

29 Choudhury, T. K., Gerhardt, K. O., Mawhinney, T. P*., Environ. Sci. Technol.,* 30, 3259 (1996).

30 Boyd-Boland, A. A., Magdic, S., Pawliszyn, J., *Analyst,* 121, 929 (1996).

31 Young, R., Lopez-Avila, V., Beckert, W. F., *J. High Resolut. Chromatogr.,* 19, 247 (1996).

32 Eisert, R., Levsen, K., *J. Chromatogr*., A, 733, 143 (1996).

33 Nilsson, T., Pelusio, F., Montanarella, L., Larsen, B., Facchetti, S., Madsen, J. O., *J. High Resolut. Chromatogr*., 18, 617 (1995).

34 Langenfeld, J. J., Hawthorne, S. B., Miller, D. J., *Anal. Chem*., 68, 144 (1996).

35 Wittkamp, b. L., Tilotta, D. C*., Anal. Chem*., 67, 600 (1995).

36 Sarna, L. P., Webster, G. R. B., Friesen-Fisher, M. R., Ranjan, R. S., *J. Chromatogr*., A, 677, 201 (1994).

37 Potter, D. W. And Pawliszyn, *J., Environ. Sci. Technol.*28, 298 (1994).

38 Zhang, Z. and Pawliszyn, J., *Anal. Chem*., 65, 1843 (1993).

39 Bovijn, L., Pirotte, J. And Berger, A., in Desty, D. H. (editor), Gas Chromatography 1958 (Amsterdam Symposium), Butterworths, London, 1958; p 310-320.

40 Stahl, W. H., Voelker, W. A., and Sullivan, J. H., Food Technol., 14, 14 (1960).

41 Vitenberg, A. G., Novikaite, N.V., Kostkina, M.I., *Chromatographia,* 35, 661 (1993).

42 Seto, Y., Tsunoda, N., Ohta, H., Shinohara, T., *Anal. Chim. Acta*, 276, 247 (1993).

43 Leblanc., Y., Gilbert, R., Duval, M., Hubert, J., *J. Chromatogr*., 633, 185 (1993).

44 Zhang, A. Q., Mitchell, S. C., Ayesh, R., Smith, R. L., *J. Chromatogr., Biomed. Appl.*, 584, 141 (1992).

45 Rizzolo, A., Polesello, A., Polesello, S., *J. High Resolut. Chromatogr*., 15, 472 (1992).

46 Gangrade, N., Price, J. C., *J. Pharm. Sci*., 81, 201 (1992).

47 Barcarolo, R., Casson, P., Tutta, C., *J. High Resolut. Chromatogr.,* 15, 307 (1992).

48 Pavlostathis, S. G., Mathavan, G. N., *Environ. Technol*., 13, 23 (1992).

49 Lansens, P., Casais Laino, C., Meuleman, C., Baeyens, W., *J. Chromatogr*., 586, 329 (1991).

50 Kintz, P., Baccino, E., Tracqui, A., Mangin, O., Forensic Sci. Int., 82, 171 (1996).

51 Pinnel, V., Vandegans, J., J. High Resolut. Chromatogr., 19, 263 (1996).

52 Kolb, Bruno, LC-GC, 14, 44 (1996).

53 Jentzsch, D., Kruger, H., Lebrecht, G., Dencks, G. and Gut, *J., Fresenius Z. Anal. Chem.*, 236, 96 (1968).

54 Closta,W., Klemm, H., Pospisil, P., Riegger, R., Siess, G., and Kolb, B., *Chromatogr. Newslt*. 11, 13 (1983).

55 Ai, Jiu, *Anal. Chem*. 69, 3260 (1997).

56 Struppe, C., Schafer, B., Engewald, W., *Chromatographia*, 45, 138 (1997).

57 Gandini, N., Riguzzi, R., *J. Agric. Food Chem*. 45, 3092 (1997).

58 Schafer, B., Hennig, P., Engewald, W., *J. High Resol. Chromatogr*., 20, 217 (1997).

59 Nishikawa, M., Seno, H., Ishii, A., Suzuki, O., Kumazawa, T., Watanabe, K., and Hattori, H., *J. Chromatogr. Sci*. 35, 275 (1997).

60 Okeyo, P., Rentz, S. M., and Snow, N. H., *J. High Resol. Chromatogr*., 20, 171 (1997).

61 Ishii, A.; Seno, H.; Kumazawa, T.; Watanabe, K.; Hattori, H.; Suzuki, O. *Chromatographia*, 43, 331 (1996).

62 Furton, Kenneth G.; Almirall, Jose R.; Bruna, Juan C., *J. Forensic Sci*. 41, 12 (1996).

63 Field, J. A., Nickerson, G., James, D. D., and Heider, C., *J. Agric. Food Chem*. 44, 1768 (1996).

64 Page, B. D., and Lacroix, G., *J. Chromatogr*. 648, 199 (1993).

65 Santos, F. J., Sarrion, M.N., Galceran, M.T., *J. Chromatogr*., 771, 181 (1997).

66 Centini, F., Masti, A., Comparini, I. Barni, *Forensic Sci. Int.,* 83, 161(1996).

67 Fromberg, A., Nilsson, T., Larsen, B. R., *J. Chromatogr*., 746, 71(1996).

68 Nilsson, T., Larsen, T. O., Montanarella, L., Madsen, J. Oe. *J. Microbiol. Methods,* 25, 245, (1996).

69 Steffen, A., Pawliszyn, J., *J. Agric. Food Chem*., 44, 2187, (1996).

70 Steffen, A., Pawliszyn, *J. Anal. Commun*., 33, 129 (1996).

71 Lee, X.-P., Kumazawa, T., Sato, K., Suzuki, O., *Chromatographia,* 42, 135, (1996).

72 Pelusio, Fabio, Nilsson, T., Montanarella, L., Tillo, R., Larsen, B., Facchetti, S., Madsen, J., *J. Agric. Food Chem*., 43, 2138 (1995).

73 MacGillivray, B., Pawlizyn, J., Fowlie, P., Sagara, C., *J. Chromatogr. Sci*., 32, 317 (1994).

74 Zhang, Z., Pawlizyn, J. *J. High Resolut. Chromatogr*. 16, 689 (1993).

75 Page, B. D., Lacroix, G., *J. Chromatogr*., 648, 199 (1993).

76 Kolb, Bruno and Ettre, Leslie S., eds., *Static Headspace-Gas Chromatography. Theory and Practice*, p. 31 Wiley-VCH, Inc., New York, NY (1997).

77 Pare, J.R. Jocelyn and Belanger, Jacqueline M.R. , *Trends in Analytical Chemistry*, 13, 176 (1994).

78 Ganzler, K., Salgo, A. And Valko, K., J. Chromatogr. 371, 299 (1986).

79 Ganzler, K., Szinai, I. And Salgo, A, J. Chromatogr. 520, 257 (1990).

CHAPTER III

80 Gunner, S.W., Haxd, B. and Sahasrabudhe, M., *J. Assoc. Off. Anal. Chem*., 51, 959 (1968)

81 Fessenden, R. and Fessenden, J. (eds), "Organic Laboratory Techniques", Brooks/Cole Publishing, Monterey, CA, (1984).

82 Zhang, Z. and Pawliszyn, J.*, Anal. Chem*., 65, 1843 (1993).

83 Internal Document. Not be published.

84 Pelusio, F., Nilsson, T., Montanarella, L., Tilio, R. , . Larsen, B., Facchetti, S. and Madsen, J., *J. Agric. Food Chem*., 43, 2138 (1995).

CHAPTER IV

85 Smith, E. R., Finley, W. J. and Leveille, A. G., *J. Agric. Food Chem*., 42, 432-434 (1994).

86 Leffingwell, J. C., *Tob. Sci*. 18, 55 (1974)

87 Mccalley, D.V., Thomas, C.W. and Leveson, L.L., *Chromatographia* 18, 309 (1984).

88 Burke, D.G. and Halpern,. B. *Anal. Chem*. 55, 822-26 (1983).

89 Moffat, C.F., McGill, A.S. and Anderson, R.S., *J. High Resol. Chromatogr. Chromatogr. Commun*. 14, 322-26 (1991).

90 Mulligan, K.J., *J. Microcolumn Separations* 7, 567-73 (1995).

91 Sjow, C.A.R. and Bickling, M.K.L., *Chromatographia,* 21, 157-60 (1986).

92 Chauhan, J. and Darbre, A., *J. Chromatogr*. 240, 107-115 (1982).

93 Bahre, F. and Maier,. H.G., *Fresenius J. Anal.Chem*. 355, 190-93 (1996).

94 Kim, K., Zlatkis, A. E., Horning, C. and Middleditch, B.S., *J. High Resol. Chromatogr. Chromatogr. Commun.*, 10, 522-23 (1987).

95 Molnar-Perl, I. and Szakacs-Pinter, M., *Chromatographia* , 17, 493-96 (1986).

96 Stalling, D. L., Gehrke, C.W. and Zumwalt, R.W., *Biochem. Biophys. Res. Commun.*, 31, 616 (1968)

97 Blau, K. and Halket, J. Meds. , Handbook of Derivatives for Chromatography (2nd ed.), John Wiley & Sons (1993).

98 Stansbridge, E.M., Mills, G. A., and Walker, V. , *Journal of Chromatography*, 621, 7 (1993).

CHAPTER V

99 Pan, L., Chong, J. M., Pawliszyn, J., *J. Chromatogr*., A, 773, 249 (1997).

100 Martos, P. A., Pawliszyn, J., *Anal. Chem*., 69, 206 (1997).

101 Robacker, D. C., Bartelt, R. J., *J. Agric. Food Chem*., 44, 3554 (1996).

102 Czerwinski, J., Zygmunt, B., Namiesnik, J*., Fresenius Environ. Bull*., 5, 55 (1996).

103 Santos, F. J., Sarrion, M. N., Galceran, M. T., *J. Chromatogr*., 771, 181 (1997).

104 James, K. J., Stack, M. A., *J. High Resolut. Chromatogr*., 19, 515, (1996).

105 Fromberg, A., Nilsson, T., Larsen, B. R., *J. Chromatogr*., 746, 71 (1996).

106 Chiarotti, M., Strano-Rossi, S., Marsili, R., *J. Microcolumn Sep*., 9, 249 (1997).

107 Nishikawa, M., Seno, H., Ishii, A., Suzuki, O., Kumazawa, T., Watanabe, K. and Hattori, H., *J. Chromatogr. Sci*., 35, 275 (1997).

108 Okeyo, P., Rentz, S. M. and Snow, N. H., *J. High Resolut. Chromatogr*., 20, 171 (1997).

109 Penton, Z., *J. –Can. Soc. Forensic Sci.,* 30, 7 (1997).

110 Seno, H., Kumazawa, T., Ishii, A., Watanabe, K., Hattori, H. and Suzuki, O., *Jpn. J. Forensic Toxicol*., 15, 16 (1997).

111 Centini, F., Masti, A., Comparni, I. B., *Forensic Sci. Int.,* 83, 161 (1996).

112 Gandini, N. and Riguzzi, R., *J. Agric. Food Chem*., 45, 3092 (1997).

113 Fischer, C. Fischer, U., *J. Agric. Food Chem*., 45, 1995 (1997).

114 Harmon, A. D., *Food Sci. Technol. (N.Y.)* 79, 81 (1997).

115 Steffen, A. and Pawliszyn, J., *J. Agric. Food Chem*., 44, 2187 (1996).

116 Ferreira, V., Sharman, M, Cacho, J. F. and Dennis, J., *J. Chromatogr*., 731, 247 (1996).

117 Ng, L., Hupe, M., Harnois, J. and Moccia, D., *J. Sci. Food Agric*. 70, 380 (1996).

118 Yang, X. and Peppard, T., *J. Agric. Food Chem*., 42, 1925 (1994).

119 Ai, Jiu, *Anal. Chem*., 69, 1230 (1997).

120 Karen D. Buchhoiz and Janusz Pawliszyn, *Anal. Chem*., 66, 160 (1994).

121 Lide, R.D, Editor-in-Chief, *Handbook of Chemistry and Physics (75th edition),* CRC Press, Inc., Cleveland, Ohio, 1994

VITA

Yuwen Wang was born in Hebei Province, China. He received his Masters degree in 1985 in Chemistry from Hebei University in Baoding, China. He has worked for Hebei Academy of Sciences as Assistant, and then Associate Research Fellow since his graduations. He entered the graduate program at Virginia Polytechnic Institute and State University in August, 1993 and studied Chemistry under the direction of Professor Harold M. McNair.