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Miniature Sensors for Biological Warfare Agents using Fatty Acid Profiles: LDRD 10775 Final Report

Curtis D. Mowry, Catherine H. Morgan, Gregory C. Frye-Mason, Lisa A. Theisen, Daniel E. Trudell, Quentin J. Baca, W. Clayton Chambers, and Jesus I. Martinez

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Abstract

Rapid detection and identification of bacteria and other pathogens is important for many civilian and military applications. The taxonomic significance, or the ability to differentiate one microorganism from another, using fatty acid content and distribution is well known. For analysis fatty acids are usually converted to fatty acid methyl esters (FAMEs). Bench-top methods are commercially available and recent publications have demonstrated that FAMEs can be obtained from whole bacterial cells in an *in situ* single-step pyrolysis/methylation analysis.

This report documents the progress made during a three year Laboratory Directed Research and Development (LDRD) program funded to investigate the use of microfabricated components (developed for other sensing applications) for the rapid identification of bioorganisms based upon pyrolysis and FAME analysis. Components investigated include a micropyrolyzer, a microGC, and a surface acoustic wave (SAW) array detector. Results demonstrate that the micropyrolyzer can pyrolyze whole cell bacteria samples using only milliwatts of power to produce FAMEs from bacterial samples. The microGC is shown to separate FAMEs of biological interest, and the SAW array is shown to detect volatile FAMEs. Results for each component and their capabilities and limitations are presented and discussed. This project has produced the first published work showing successful pyrolysis/methylation of fatty acids and related analytes using a microfabricated pyrolysis device.

Acknowledgments

The authors would like to acknowledge the team that provided this project with components, without their work this LDRD would not have existed: Ron Manginell, Sara Sokolowski, James Sanchez, Alex Robinson, Sherry Zmuda, and Matt Blain. In addition the authors would like to thank the following for their contributions to the success of this LDRD: Doug Adkins, Patrick Lewis, Stephen Meserole, and Diane Kozelka.

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Executive Summary

Rapid detection and identification of bacteria and other pathogens is important for many civilian and military applications. The profiles of biological markers such as fatty acids can be used to characterize biological samples or to distinguish bacteria at the gram-type, genera, and even species level. The taxonomic significance (or the ability to differentiate one microorganism from another) using fatty acid content and distribution is well known. Bench-top methods of extracting, derivatizing, and analyzing fatty acid content are commercially available. These methods chemically derivatize fatty acids to produce more volatile fatty acid methyl esters (FAMEs). More recent publications have demonstrated that FAMEs can be obtained from whole bacterial cells in an *in situ*, single-step pyrolysis/methylation analysis. Bacteria including *Bacillus anthracis, Brucella melitensis*, *Yersinia pestis*, and *Francisella tularensis* have been differentiated. This method can also detect dipicolinic acid (a biomarker for sporulated *Bacillus anthracis*, the bacterium that causes the illlness anthrax), amino acids, and oligopeptides.

The goal of this LDRD program was to investigate the use of microfabricated components for the rapid identification of bioorganisms using the same pyrolysis/methylation procedure. When fully developed, a sensor based on this technology could provide a unique miniaturized capability for biological warfare (BW) agent detection. The envisioned system consists of three microfabricated components, each utilized in chemical agent detection programs at Sandia. The first component, a microfabricated membrane (2x2 mm), has been shown to pyrolyze whole cell bacteria samples using only milliwatts of power. This pyrolysis simultaneously vaporizes and methylates bacterial fatty acids to produce FAMEs. The second component, a microfabricated gas chromatographic (GC) column (1.2 cm^2), has been shown to separate the FAMEs produced. The third component, an array of surface acoustic wave (SAW) sensors (9x7 mm) has been shown to detect some FAMEs as they elute from the GC column, however improvements are needed. The capabilities of each stage are demonstrated and limitations discussed in this report.

It should be emphasized that this research was not directed at aerosol collection or fluidic issues – both necessary for a fully operational sensor. A wide range of collectors exist commercially, and the focus was on determining the capabilities and proof of concept using microfabricated devices. While the sensor envisioned will be less specific than DNA/RNA-based methods for BW agent detection, it should be faster and cheaper and more appropriate for first responder applications. This project has produced the first published work showing successful pyrolysis/methylation of fatty acids and related analytes using a microfabricated pyrolysis device.

Acronyms and Abbreviations

Ab	antibody
ABO	agents of biological origin
ACPLA	agent containing particles per liter of air
BSP3	fluorinated polyol
b.p.	boiling point
BW	biological warfare
°C	degrees Celsius
CAS	Chemical Abstracts Service
DPA	dipicolinic acid
DRIE	deep reactive ion etched
FAME	fatty acid methyl ester
GC	gas chromatography
GC/MS	gas chromatography / mass spectrometry
i.d.	internal diameter
IMS	ion mobility spectrometry
IR	infrared
KOH	potassium hydroxide
mA	milliamp
MALDI	Matrix Assisted Laser Desorption-Ionization
mDPA	methylated dipicolinic acid
m.p.	melting point
mPA	methyl picolinate
MS	mass spectrometry
mV	millivolt
m.w.	molecular weight
NaOH	sodium hydroxide
ng	nanogram
nmol	nanomole
PA	picolinic acid
PECH	poly-epichlorohydrin
PEEK	Poly(ether ether ketone)
PDMS	polydimethylsiloxane
pg	picogram
ppm	parts per million
psi	pounds per square inch
PUFA	polyunsaturated fatty acids
SAW	surface acoustic wave
SPME	solid phase micro-extraction
TMAH	tetramethylammonium hydroxide
W	watts

1. Introduction

Fatty acids have long been molecules of environmental, biomedical, agricultural, and industrial importance. They are also components of cell membranes and the taxonomic significance, or the ability to differentiate one microorganism from another, using fatty acid content and distribution is well known [1]. Because of their high molecular weight and low volatility, they have always been a challenge for the analytical chemist. A common solution has been the use of derivatization reagents to create a more volatile analog. The most widely utilized derivatization for fatty acids creates fatty acid methyl esters (FAMEs). One method practiced since 1963 uses the derivatizing reagent tetramethylammonium hydroxide (TMAH) followed by pyrolysis or rapid heating of the mixture [2]. To effect the reaction, a derivatizing agent and heat are required. In this case, tetramethylammonium-hydroxide, a strong base, is mixed with the sample. The first reaction occurs over a matter of seconds at room temperature and yields a salt of the fatty acid and derivatizing reagent. Rapid heating completes the conversion to the fatty acid methyl ester. This method has been shown to work for triglycerides as well.

A benchtop commercial method of extracting, methylating, and analyzing fatty acid content has been available for some years [3]. The FAME analysis is performed by gas chromatography and the results compared with existing computer databases to identify possible matches. The analysis takes 15-20 minutes per run, however, not including the extraction and preparation time. The extraction and preparation time can range from one hour to one day.

The use of pyrolysis (rapid heating) to effect a reaction (derivatization) producing species more amenable to gas chromatographic analysis is also well known, with a review of useful reagents and example analytes published in 1979 [4]. To effect methylation reactions, pyrolysis has been performed using the injection ports of commercial GC instruments and Curie-point pyrolyzers. In Curie-point pyrolysis, the sample, including biological sample and methylation reagent, is coated on a metallic wire that is heated using a powerful (up to 1 kW) radio frequency generator. The wire heats until a characteristic Curie-point temperature is reached, at which point the wire is no longer magnetic and ceases to heat. Curie-point pyrolysis coupled with methylation of whole bacterial cells and GC analysis was performed as early as 1991 [5]. Typically the pyrolysis reaction is carried out in an inert gas such as helium or nitrogen, however it has also been demonstrated in air. The FAME profiles obtained in air were still sufficient to differentiate the bacteria tested [6].

Portable instrumentation being developed for bacterial detection uses direct pyrolysis (for biomarkers) or pyrolysis/methylation (for FAMEs). Direct pyrolysis produces limited biomarker peaks, reducing the ability to differentiate bacteria [7]. These instruments use either infrared or resistive heating pyrolysis and use large amounts of power in the pyrolysis step, and the infrared technique is slow and not

suitable for chromatographic sample introduction [8]. The availability of a rapid and low-power pyrolyzer could reduce the size and power required of existing instrumentation. The same can be said for a portable chromatograph. The goal of this work has been to determine whether microfabricated components could facilitate a pyrolysis/methylation reaction and therefore demonstrate the potential for a hand-held FAME sensor.

The performance results of the miniature pyrolyzer and miniature GC demonstrate that the potential exists for a microfabricated sensor to perform a FAME analysis similar to that performed by commercial instrumentation. Such a sensor could find many applications in the environmental, biomedical, agricultural, industrial, and military arenas. The advantages offered by a miniaturized system using microfabricated elements include the possibility of producing a detector that is low power, low cost, hand-held, and lightweight. In addition, the selectivity of these device elements and other components taken from Sandia's μChemLab system is tunable, allowing selectivity against many interferants. This project has produced the first published work showing successful pyrolysis/methylation of fatty acids and related analytes using a microfabricated pyrolysis device.

1.1. μChemLab

The μChemLab program at Sandia was initially an LDRD funded program to develop a portable autonomous gas phase detection system based on microfabricated components. Figure 1 shows a schematic of the system concept, which has been documented and described elsewhere [9, 10]. Briefly, the system draws air across a preconcentrator membrane which collects the sample into a selective sorbent material. The membrane is heated rapidly to vaporize the analytes and introduce them into a microfabricated gas chromatographic column which separates the collected analytes. The separated analytes then travel across a surface acoustic wave (SAW) detector which also has a sorbent coating. Each analyte interacts with the coating to produce a mass change on the detector surface which is detected and converted to a signal. The success of the program and the individual devices has led to many other applications.

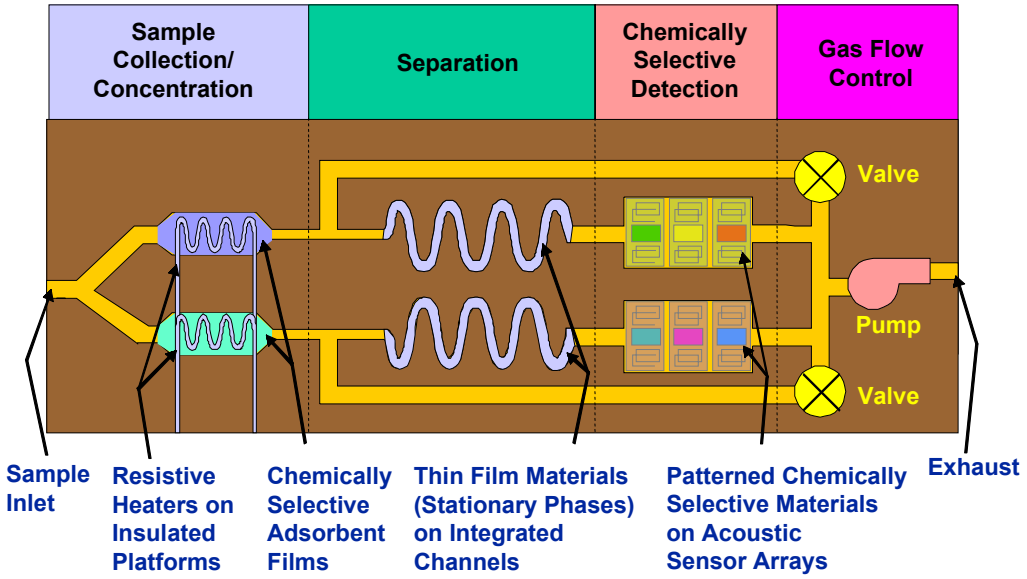


Figure 1: Schematic of µChemlab system.

The capabilities of the (preconcentrator) device used for sample collection/concentration led to the concept that is the subject of this report, a "Miniature Sensor for BW Agents using Fatty Acid Profiles". This concept is illustrated in Figure 2. Because the preconcentrator device could be rapidly heated with a small amount of power, it was hypothesized that it could also be used as a device for low power pyrolysis. The investigation of the feasibility of this concept was funded as a three year LDRD.

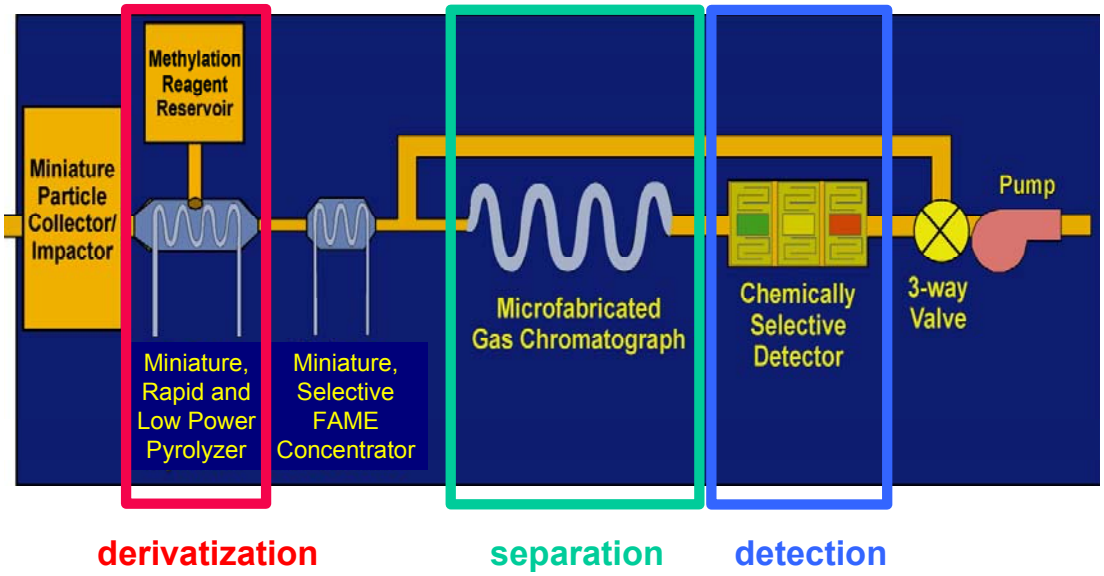


Figure 2: Schematic of concept for a biological sensor based upon microfabricated components.

Images of the microfabricated components used in the µChemLab and in this LDRD are shown below. The images are not to scale, but are included to show the state of the devices during and at the conclusion of this LDRD.

Figure 3: Images of the microfabricated components used in this work.

The use of these devices for this concept hinges on the importance of fatty acids and the use of pyrolysis to prepare and introduce fatty acids for analysis. These topics are discussed in the following section followed by a background of the use of pyrolysis and/or fatty acids for the detection and identification of BW agents and other bacteria.

1.2. Importance of Fatty Acids

Fatty acids are found in all living systems. The biological classification system is divided into the following groups: Domain, Kingdom, Phylum, Class, Order, Family, Genus, and Species. The Family designated bacterium is the focus of this work as it contains the BW agents. For BW agents and other living systems, fatty acids are found as a component of cell membranes as shown in Figure 4 and also individually within the cells.

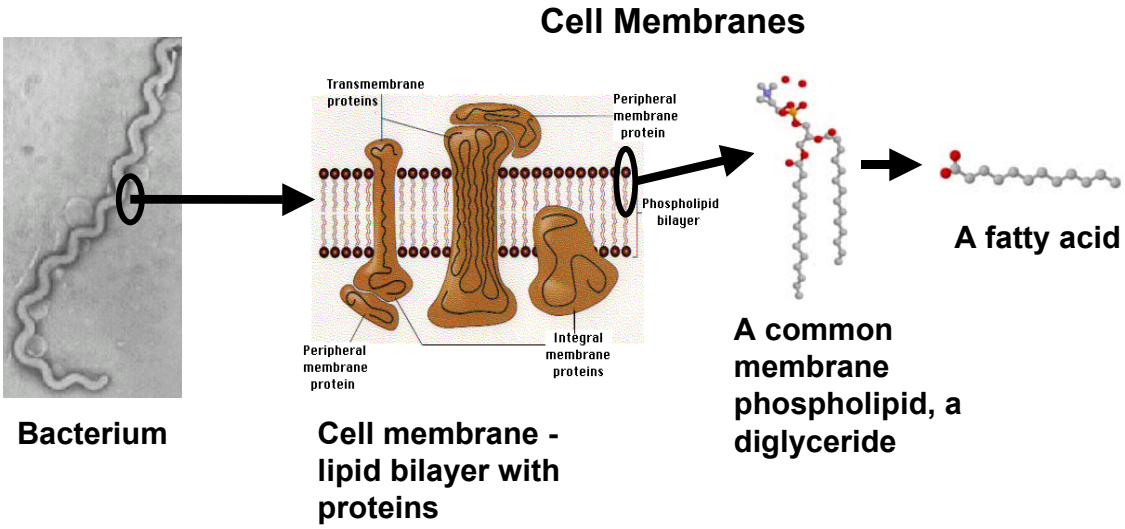


Figure 4: Fatty acids are present in cell walls.

For bacteria, fatty acids content can be found as phospholipids (6-80% total lipids) or free fatty acids (16-20%) [11].

Various published documents identify a wide range of pathogens, toxins, and other biologicals that are of particular threat if used as a bioterrorist weapon; among

these (listed in Table 1) are the most well known bacterial pathogens as given in "First Responder Chem-Bio Handbook, a Practical Manual for First Responders" [12].

Table 1: Diseases and the bacteria that cause them.

Disease	Source/Causal Agent
anthrax	*Bacillus anthracis*
brucellosis	*Brucella melitensis*
cholera	*Vibrio cholerae*
plague	*Yersinia pestis*
tularemia	*Francisella tularensis*
Q fever	*Coxiella burnetii*

Fatty acids are also important as a component of food and oils, and can be an industrial health hazard. For example, in the vegetable oil industry, monitoring and detection needs include quality assurance, shelf life, and impurity (or fraudulent replacement/adulteration) detection.

Table 2 illustrates the diversity of fatty acids (analyzed as fatty acid methyl esters) found in various food items or consumer items, along with references.

Table 2: Fatty acids found in food and other items.

Sample	FAMEs	reference
tuna lipids	16:0, 18:1 n9, 22:6 n3, most abundant (14:0-18:0), (16:1-24:1), 18:x, 22:x	[13] (extraction methods only)
bovine milk	180 different FAs	[14] extraction
infant formula(s), human milk	triglycerides, phospholipids, free FA: focus on 18:x and 20:4 n-6, 20:5 n-3, 22:6 n-3, sum 4:0-10:0, 12:0-14:0, 16:0 (18:1 n-9 and 16:0 most abundant)	[15] extraction
bleached beeswax, lanolin, yellow carnauba wax	beeswax: mix of (even)16-34, hydroxy FA became methylated FAMEs. lanolin: 52 compounds, odd- and even FA, methylated alcohols, sterols (incl. cholesterol). carnauba 16-24 (even only), aromatic acids.	[16] py-gc-ms
objects of art (wax seals)		[17]sfc extraction
soybean oil, sardine oil	FAs and PUFAs up to C18:3 and C22:6	[18] py-gc-ms
wood pulp / extracts	ratio of free to esterified FA	[19] py-gc-ms
edible oils (sesame, perilla, soybean, corn germ, canola, rapeseed, olive, coconut)	contain mainly C16:0, C18:0, C18:1,2,3	[20] soap, methylate, extract, gc/ms
kraft mill effluent / bioreactor wastewater treatment system	C12-C19 (roughly) many cy, l,a, several hydroxy- also 18:2(9c,12c) – wood based non-microbial (biomarker for wood?)	[21]
edible oils, butter, margarine	triglycerides, potassium methylate	[22]
wastewater; 2% milk	SPME deriv.: (C1-C5); C10	[23]
beeswax		[24]

1.3. Pyrolysis for the Identification of Biologicals

There exists a large range of organisms and constituents that have been differentiated using pyrolysis methods. These methods are introduced briefly based upon the target biological category: bacterial, viral, or sporulated (bacterial). This introduction is meant to serve as an illustration of the potential markets or applications for the miniature sensor system developed in this LDRD.

1.3.1. Bacterial

Differentiation of several gram-positive and gram-negative organisms based upon gram-type was achieved using pyrolysis/methylation/MS [25]. The organisms, including five Bacillus strains, 2 Staphylococcus strains, and 5 Pseudomonas strains, and the differentiation was based upon FAMEs between C12 and C19 without chromatography. Table 3 summarizes comparative results for *B. cereus* and *B. fluorescens*, showing a clear difference in signatures for the two bacteria; B. cereus is type gram positive and B. fluorescens is type gram negative. Full proof-of- concept development will consider signatures of the most common BW agents, their simulants, and less toxic bacteria as test platform samples, as well as background and interferant signals.

Table 3: Relative amounts of fatty acid constituents, detected by pyrolysis/mass spectrometry[26].

Fatty Acid	% by MS Analysis	
	Pseudomonas fluorescens	*Bacillus cereus*
C12:0	10.33	0.44
C13:0	0.09	11.2
C15:0	0.16	39.0
C16:0	28.8	3.2
C16:1	22.25	9.0
C17:0	17.7	7.0
C17:1	0.1	10.7
C18:1	9.0	not detected

Error! Not a valid bookmark self-reference. summarizes some of the fatty acids detected using pyrolysis methods for other BW agents and simulants.

Table 4: Fatty acids detected for bacteria using pyrolysis methods.

Bacteria	Fatty Acids	reference
E. coli (ATCC 9637)	12:0, 14:0, 16:1, 16:0, 17:0cy, 18:1, 18:0, 19:0cy	[5]
B. subtilis (ATCC 6633) whole cell, 5ug wet +tmah	13:0i,ai, 14:0i,n, 15:0i,ai, 16:0i,n, 16:1, 17:0i,ai, 18:1	[27]
B. anthracis (armed forces inst. of pathology)	14:0, 15:0, 16:0, 17:0 16:1	[28]
E. coli (ATCC 9647) whole cell, pyro loses 14:0 3-OH	12:0, 14:0, 16:1, 16:0, 17:0cy, 18:1, 18:0, 19:0cy	[27]
Bacillus subtilis var. niger	2Me-DPA 15:0, 16:0, 17:0	[8] (CBMS) [29] (CBMS)
Erwinia herbicola	16:0, 16:1, 18:1	[29]
"gram negative No.1"	10:0, 14:0, 16:0, 18:0, 20:0, 22:0, 24:0, 18:1, 24:1	[29]
"gram negative No. 2"	16:0, 18:0, cyclo-19:0	[29]
M. tuberculosis (H3820) whole cells, 5ug wet+tmah	14:0, 15:0, 16:1, 16:0, 17:0, 18:1, 18:0, 10-Me-18:0, 20:0, 22:0, 24:0, 26:0	[27]
Pediococcus damnosus, *P. dextrinicus*, and *Lactobacillus brevis*	C16:0, C18:1, cyC19:0, C18:1 ME, C16:0, C19:0	[30]
Coxiella Burnetti stage I and stage II	diff. profile due to growth factors	[8]

1.3.2. Viral

In addition to bacterial agents, there are viral BW agents of concern including yellow fever, adenovirus type 2, smallpox, and the virus-like bacterium Coxiella burnetii which causes Q-fever. These agents must be grown and propagated using eukaryotic host cells. The growth medium for these cells contains ingredients, such as nutrients, vitamins, electrolytes, antibiotics, and blood serum, that contain hormones and lipids. Chicken egg embryos, which contain cholesterol and free fatty acids, are also sometimes used. To harvest viral agents the host cells are ruptured, and it is at this stage that purification occurs. It has been shown that lipids from growth media, including cholesterol, dominate the high-mass range of the pyrolysis mass spectra of both purified and unpurified viral preparations [31]. Because cholesterol is not generally found in bacterial culture media, it can be thought of as a biomarker for the presence of viral aerosols. In another study using the same pyrolysis-methylation reaction demonstrated at

Sandia, methylated cholesterol and fatty acids were detected for purified Coxiella burnetii and yellow fever [8]. In the case of unpurified agents, the lipids from the host cells would also be detected by pyrolysis-methylation.

Table 5: Fatty acids detected (as FAMEs) for viral-related agents using pyrolysis methods.

Bacteria	FAMEs	reference
Coxiella burnetii 9-mile (phase 1,2)	iC14:0, 14:0, a15:0, 16:1, 16:0, cholesterol	[8]
yellow fever 17-D	C14:0i, 14:0, 15:0a, 16:1, 16:0, cholesterol	[8]
adenovirus type 2	C16:0	[8]
		[31]

1.3.3. Sporulated

For bacillus anthracis, the causal agent of anthrax, it has been shown using pyrolysis-methylation that the sporulated form can be detected using dimethylated dipicolinic acid [8, 32]. The sporulated form can contain from 5-15% by weight dipicolinic acid (DPA or 2,6-pyridinedicarboxylic acid) which can be methylated to 2Me-DPA (Dimethyl 2,6-pyridinedicarboxylate), and will be abbreviated in this report as mDPA.

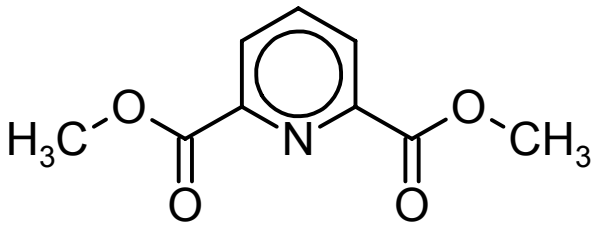


Figure 5: Chemical structure of dimethylated Dipicolinic Acid (mDPA).

DPA is present in all spores, and therefore does not provide species-specific information. It is also a useful biomarker for *B. cereus* which causes food poisoning and is found in rice and other products.

DPA pyrolyzes (in vacuo) to give picolinic acid (see Figure 6) and also pyridine which can be used to differentiate vegetative versus sporulated cells [33].

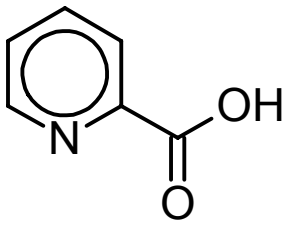


Figure 6: Chemical structure of picolinic acid.

Table 6: Species detected in spores using pyrolysis methods.

Bacteria	detected	reference
B. anthracis sterne, thuringensis (atcc 10792), lichenformis (atcc 14580), cereus (atcc14579), globigi var. Niger, subtilis (atcc 6051)	fatty acids: C14, C15 glycerides: C14, 15, 16 poly(3-hydroxybutyrate), pyranose compounds	[34] probe pyro, no TMAH
35 strains of Bacillus including anthracis	dipicolinic acid, pyridine (no methylation)	[33]

2. Background

Typical bacterium can be described as consisting of 70% protein, 6% lipid, 5% polysaccharide, 5% DNA, and 10% RNA [35]. Any of these molecules that are specific to a particular bacterium can be considered a biomarker – a unique molecule that can be used for identification or differentiation.

Other biomarkers that could be used for certain bacteria include teichoic acids, present in only some gram-positive bacteria and generally absent from gram-negative, and gamma-glutamyl polypeptide – present in the capsule of bacillus anthracis.

The following section describes methods for detecting bacteria, both in the laboratory and in the field, and is meant to illustrate where the technique of pyrolysis fits in the broad spectrum of techniques.

2.1. Methods for Detecting Biological Warfare Agents

There are many methods for the detection of BW agents, and they can be divided into laboratory methods (samples are taken back to a lab) or field or portable methods. A common technique for demonstrating a method is to use simulant bacteria rather than actual BW agents. These include *Bacillus globigi* (*B. subtilis var. niger*) for gram positive sporulating and *Erwinia herbicola* for gram negative. *Erwinia herbicola* is now known as *Pseudomonas agglomerans* [7].

2.1.1. Laboratory Methods

Many laboratory methods for biological detection/identification include culture methods, mass spectrometry (MS) methods, and pyrolysis methods. A good review of laboratory methods that utilize DNA or immunoassay techniques can be found in Iqbal et al [36]. A well known commercial method based upon fatty acid composition is sold by MIDI, Inc. (Microbial Identification, Inc., Newark, DE), which uses culture followed by extraction and GC analysis and computer database matching. Extraction and GC analysis can also be used for characterize biofilm populations [21]. Methods and conditions for FAME analysis listed in supplier catalogs are listed in Table 7.

Table 7: GC columns used for fatty acids.

Column	Conditions
Supelco: bonded; poly(ethylene glycol) 30m, 0.25µm	Temp. Limits: 50°C to 280°C
Hewlett Packard hp-225	med. to high polarity
Perkin-Elmer: PE-225	70ºC 1 min., 70-180ºC @20ºC/min., 180-220ºC @ 3ºC/min., hold 220ºC for 15 min.
Alltech catalog: 1. AT-225 (25% phenyl, 25% cyanopropyl-methyl silicone) 2. DB-5 (5% phenyl, 95% methyl silicone, 3. Heliflex AT-1 (100% methyl silicone,	1. up to C22:1, 200ºC 2. 150ºC 4min.,- 250ºC at 4ºC/min.) up to C20:0 3. 40-100ºC, 5ºC/min.) upto C:6
J&W DB-23	90ºC for 6 min., 90-210ºC @ 10ºC/min
A. Polar example 68% bixcyanopropyl-32%dimethylsiloxane, 50m, B. Intermediate example: wax, 15m [37]	A. 90ºC 1 min., 30ºC/min to 160ºC, 15ºC/min. to 200, slower ramps to 225ºC. separate C10-C24 less than 12 minutes @2mL/min. B. 160ºC 1 min., 5ºC/min. to 185ºC, 8ºC/min. to 240, @50cm/sec.
any polarity, depends on FAMEs, many prefer PEG (intermediate); nonpolar are more thermally stable.[38]	

Matrix assisted laser desorption-ionization (MALDI) bacterial analysis has been performed on whole cells as early as 1996, and has been used to characterize/differentiate microorganisms at the species and strain levels [39, 40]. MALDI has also been used to determine edible oil composition [41].

Another MS method is a laser ablation / ion trap MS system under development at Oak Ridge funded by the CBNP program [42]. This MS system should provide effective detection of BW agents.

2.1.2. Portable Biological Detectors

Methods that have been adapted to portable systems can be divided into three types: liquid based, pyrolysis based, and optical based.

2.1.2.1. Liquid Based Detection

Liquid based detection of pathogens relies on antibody-based or immunochemical assay chemistries. Antibody (Ab) based detectors are the best performing technology to date for high sensitivity/specificity detection and identification of BW agents. There are two detectors of this type that have been fielded, the Interim Biological Agent Detector (IBAD) and the Biological Detector, a component of Biological Integrated Detection System (BIDS). These detectors rely on the fluorescent signal of an Ab/fluorescent tag/BW agent complex for identification. Specific antibodies must be developed for each agent and existing devices have demonstrated systems that can detect 4-8 agents. Simultaneous coverage of the full spectrum of BW threats is limited by the development of effective antibodies and the possibility that developed antibodies will not detect engineered BW agents. The high specificity that can be achieved via antibody-based detection is balanced by the limited robustness of biological systems which are susceptible to fouling and have finite lifetimes and regenerability. Reaction times for identification range from 15 minutes (BIDS) to 45 minutes (IBAD).

Also, false positive results may be generated by non-specific binding to materials in the sample stream or by unforeseen cross-reactivity with sample materials, and the Ab-coated surface has limited regenerability once a positive sample is encountered.

2.1.2.2. Pyrolysis Based Detection

There are portable instruments being fielded and/or developed for biological detection, based on pyrolysis of the collected aerosol sample. None are autonomous (can be battery operated).

One instrument called the "Block II CBMS", uses pyrolysis / methylation to create detectable species from biologicals [29, 43]. The system, however, weighs approximately 130 lbs. and uses on the order of 500 W (average) power for operation, including aerosol collection, pyrolysis and mass spectrometric analysis. Pyrolysis is performed at 550°C for 16 seconds in a quartz tube. The slow pyrolysis causes transport effects where all fatty acids do not arrive at the detector simultaneously, complicating identification. The instrument has been demonstrated in field trials and can detect biological aerosol concentrations less than 50 agent containing particles per liter of air (APCLA). They state that "there is no significant interference from other cellular products of the thermolysis-methylation", although they do see diketopiperizines when they pyrolyze albumin.

Another instrument uses pyrolysis (but without methylation) coupled with an IMS detector [44, 45]. A laptop computer was required, however, for the signal processing and the instrument weighed approximately 30 lbs. (without the aerosol collector). The instrument performed well in biological aerosol trials, but

identification may suffer in the future from the small number of peaks detected for biologicals. Pyrolysis is performed using a 0.015" diameter nichrom wire 65 mm long (3.3ohm) for 4-10 seconds at temperatures estimated by the researchers at 700-900°C. The approximate power required for pyrolysis alone appears to be <60 W. They also perform temperature ramp chromatography with air carrier gas, and have spore detection limits of 3300 spores using picolinic acid as the biomarker.

2.1.2.3. Optical Based Detection

A fluorescent-based detector, called the CIBADS II, has been fielded that detects the fluorescent signal from live organisms [46]. The detection is rapid (15 sec.), can discriminate between non-biological and biological aerosols, and detected viral species contaminated with live bacteria. Because the detector uses nonspecific NADH fluorescence, identification will be difficult; and therefore this system is currently only used in an “alarm” mode which indicates a biological particle count “above baseline". This system also has an IMS detector incorporated for simultaneous detection of CW agents.

2.2. Anthrax Detection

2.2.1. Cells

The following table contains basic information on *B. anthracis* cells [35].

Table 8: Characteristics of *B. anthracis* cells.

cell size	1.0-1.2 by 3-5 μm
biovolume	2.36 to 5.65 $x10^{-13}$ m^3 (236-565 picoliters)
cell dry weight	0.7- $1.7x10^{-12}$ g (0.7-1.7 picograms)

Four strains (vollum, sternes, ames, zimbabwe) of *B. anthracis* grown on two different media, both vegetative cells and spores, were pyrolyzed [47]. Fatty acids that were important in the detection included the following chain lengths: saturated 12, 14-22, and 24; monounsaturated 16, 18, 24; cyclic 17 and 19; iso-15, 17; and anteiso 15. In other work using chemical ionization MS, B. anthracis was grown and profiled 2 months apart and the following fatty acids were detected: saturated 15, 16, 17 and monounsaturated 16 and 17 [48].

The fatty acid composition of *B. anthracis* (Sterne) and *B.cereus* cells grown on two different media is shown below [35].

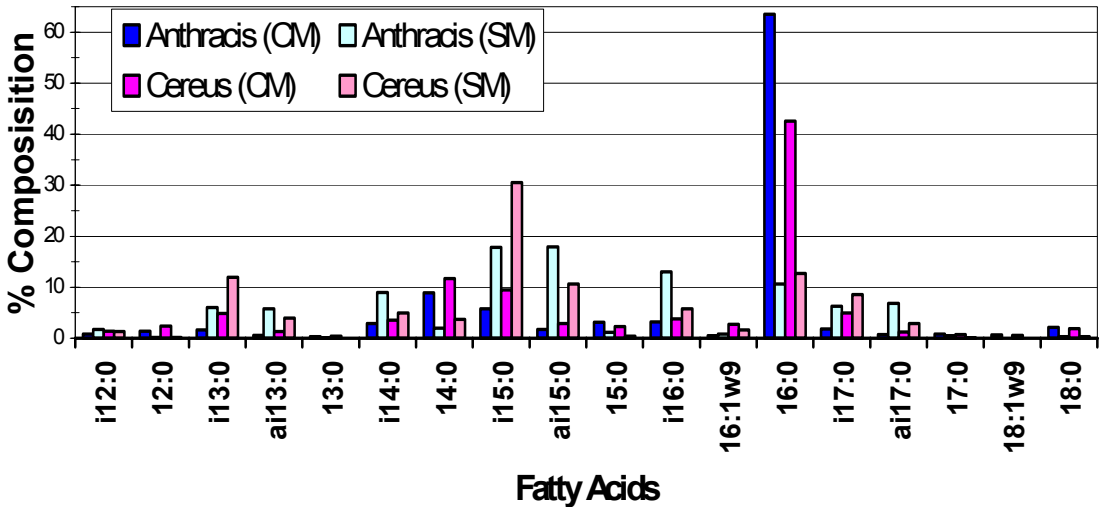


Figure 7: Vegetative cell fatty acid composition for *B. anthracis* and *B. cereus* grown on Complex (CM) or Synthetic (SM) medium.

Figures 8-11 illustrate the diversity of the fatty acid composition for several *Bacillus*, *Clostiridum*, and *Pseudomonas* species [11]. Trace components are not shown.

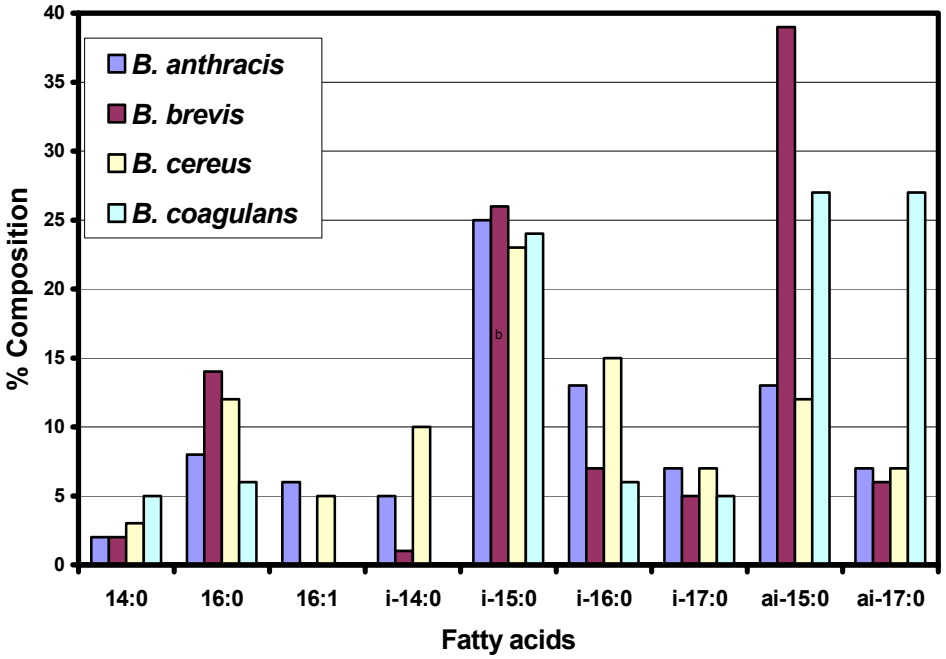


Figure 8: Comparison of fatty acid composition for several *Bacillus* species.

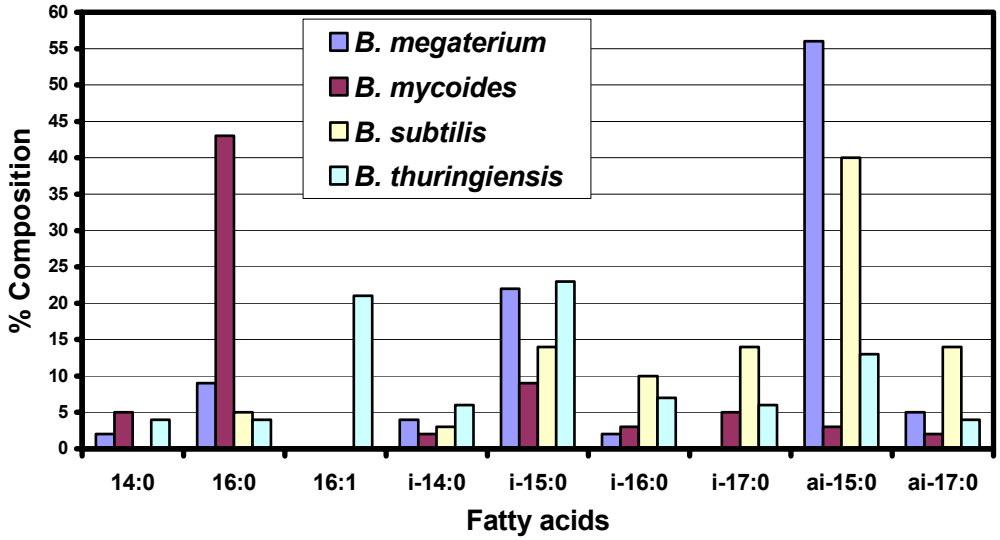


Figure 9: Comparison of fatty acid compositions of additional *Bacillus* species.

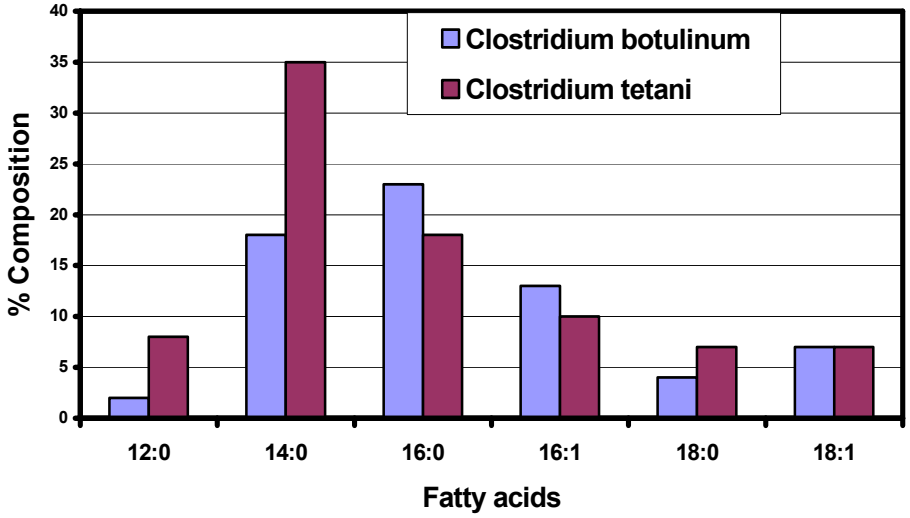


Figure 10: Comparison of fatty acid content of two Clostridium species.

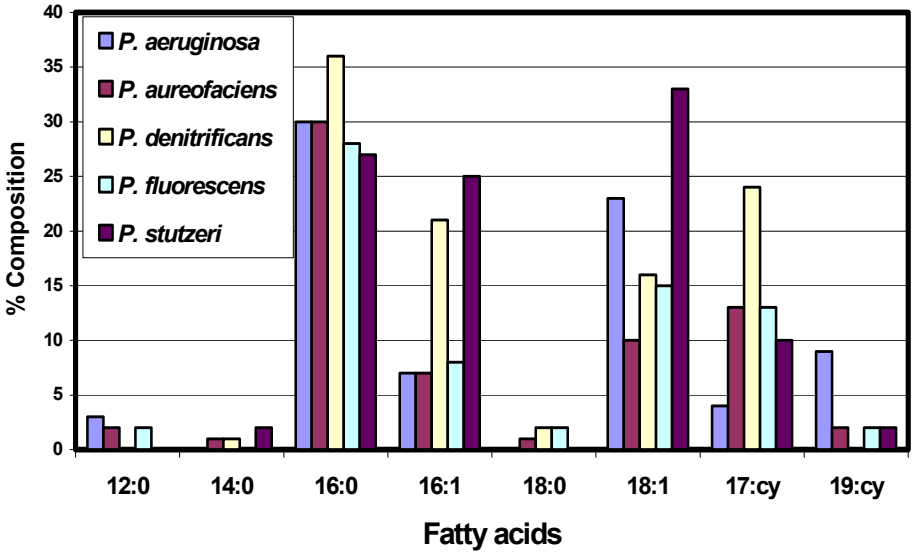


Figure 11: Comparison of fatty acid content for pseudomonas species.

2.2.2. Spores

The following table contains basic information on *B. anthracis* spores.

Table 9: General characteristics of *B. anthracis* spores.

dry weight	approx. 0.08µg/8,000 spores = 10 pg/spore [49]
dry weight	5pg/spore [50]
volume/density	0.52 femtoliters (1µm diameter sphere), therefore density = 10pg/0.52fL = 19.1 g/mL
volume/density	14.14 femtoliters based upon 6pg/3µm dia. spore [51] = 0.42 g/mL density

It is known that *Bacillus* spores contain between 5-15% by weight dipicolinic acid (DPA) [52]. The structure of DPA is shown below.

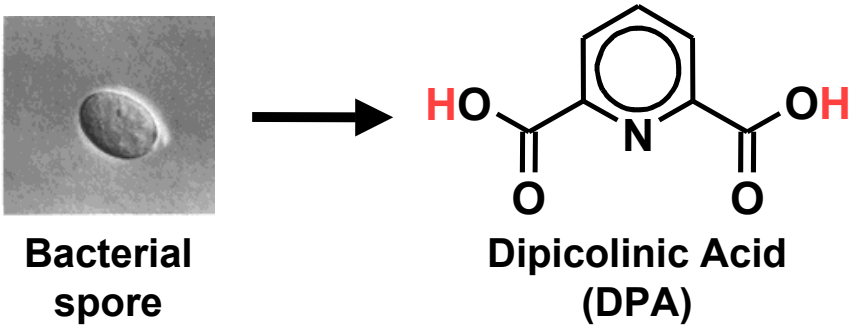


Figure 12: Spores contain DPA.

Based upon the values given in Table 9, the detection limits required of a detection method based solely on DPA are calculated below.

Equation 1: Calculation of detection limits based on DPA.

from (0.08ug x 0.15) to (0.08ug x 0.05)
=0.012 – 0.004 µg DPA in 8,000 spores
= 4-12 ng/ 8,000 spores
= **0.5-1.5 pg DPA/spore**
DPA detection limit of:
100 ng requires 200,000 spores (2 micrograms)
100 pg requires 200 spores (2 nanograms)

Beverly et al pyrolyzed whole Bacillus spores of several species (anthracis sterne, thuringensis atcc10792, lichenformis atcc 14580, cereus atcc14579, globigi var. niger, and subtilis atcc6051) and obtained very similar EI spectra for all except for cereus and subtilis [34]. The spectra contained peaks for C14:0, c15:0 free fatty acids and C14,15,16:0 glyceride peaks.

Other potential biomarkers for spores that have not been utilized but are known spore constituents include poly(3-hydroxybutyrate), found in the cell walls of spores, and muramic acid and N-acetylglucosamine [52].

2.3. Aerosol Collectors

An important component of an fieldable detection method for BW agents must include an aerosol collector. Several types have been used in the literature: 1) a 330 L/min. from MSP corporation (Minneapolis, MN) [29], 2) a 600-to-1 liter collector that concentrates into 5mL of liquid from Dycor [46], and 3) a 1000 L/min. collector from SCP Dynamics [45]. A compilation of additional companies and their products is included in Appendix F: Commercial Aerosol Collectors/Samplers.

2.4. Chemistry Background

This section is intended to provide information on the chemical reactions that convert fatty acids into FAMEs in order to provide a context for the simplicity and speed at which pyrolysis reactions can be performed.

2.4.1.1. Chemical Reactions

Transesterification, or the "swapping" of constituents on an ester bond, can be performed simply by using a solvent. Below is an example of this using a TMAH / alcohol equilibrium [53]. This type of transesterification can also be used to convert triglycerides into FAMEs [54].

Equation 2: General representation of an equilibrium transesterification reaction.

$$N(CH_3)_4OH + ROH \leftrightarrow N(CH_3)_4OR + H_2O$$

Another reaction commonly used for fatty acids and glycerides is called saponification – the conversion of an ester into a carboxylic acid and alcohol:

Equation 3: General representation of a saponification reaction.

$$R_1\text{-}(CO)\text{-}O\text{-}R_2 \rightarrow R_1\text{-}(CO)\text{-}OH + HO\text{-}R_2$$

2.4.1.2. Conventional Saponification, methylation, extraction

To produce FAMEs from both fatty acids and glycerides, the conventional wet chemistry method is as follows [55]. Saponification (30 min. @100C) with methanolic sodium hydroxide (3N in 50% MeOH) is followed by methylation (10 min. @ 80C) using 3N HCl in 40% aqueous methanol which is followed by extraction with diethyl ether/hexane (1:1 vol/vol). The aqueous phase is removed and the extract is washed with a mildly basic solution of NaOH in water. Remove organic layer for analysis. The labor intensive nature and time involved is clear.

2.4.1.3. General methylation

For free fatty acids a number of methylation reactions have been performed. In some cases such as short-chain fatty acids, the FAMEs produced are volatile and water-soluble; this can be overcome by producing higher molecular weight isopropyl ester derivatives. The following table contains several of the common reagents used along with references.

Table 10: Derivatizing reagents used for general methylation.

derivatizing reagent	sample / results	reference
1% H2SO4:MeOH	marine (albacore tuna) lipids	[13]
5% HCl: MeOH		[13]
14% BF3: MeOH	(pierce catalog – strong Lewis acid, doesn't work well with <C8 FA) "works well with heating"	[13]
0.5 M NaOCH3: MeOH	not good for free FA, but performed at room temp.	[13], [15]
(1:4) 1,1,3,3-Tetramethylguanidine :MeOH		[13]
acetyl chloride: MeOH (creates anhydrous HCl:MeOH)	[75ºC 15 min.]	[15] [p484]
0.1 M potassium methylate + ascorbic acid to eliminate α-tocopherol degradation	1 min. 70ºC, triglycerides	[22]
PFB-Br	55ºC, 2 hr., SPME extract	[23]
PFPDE	room temp, 10 min. SPME extract	[56]
diazomethane in diethyl ether/methanol	milk FA microbial FA	[15],[14]
"methyl-8" N,N-Dimethylformamide dimethyl acetal	in solvent pyridine	Pierce catalog

2.4.1.4. Pyrolysis / methylation

Pyrolysis/methylation is a two step process a shown in Figure 13. The first step is saponification which yields a salt. During the heating step there is a nucleophilic attack of salt anion to methyl group which yields the FAME.

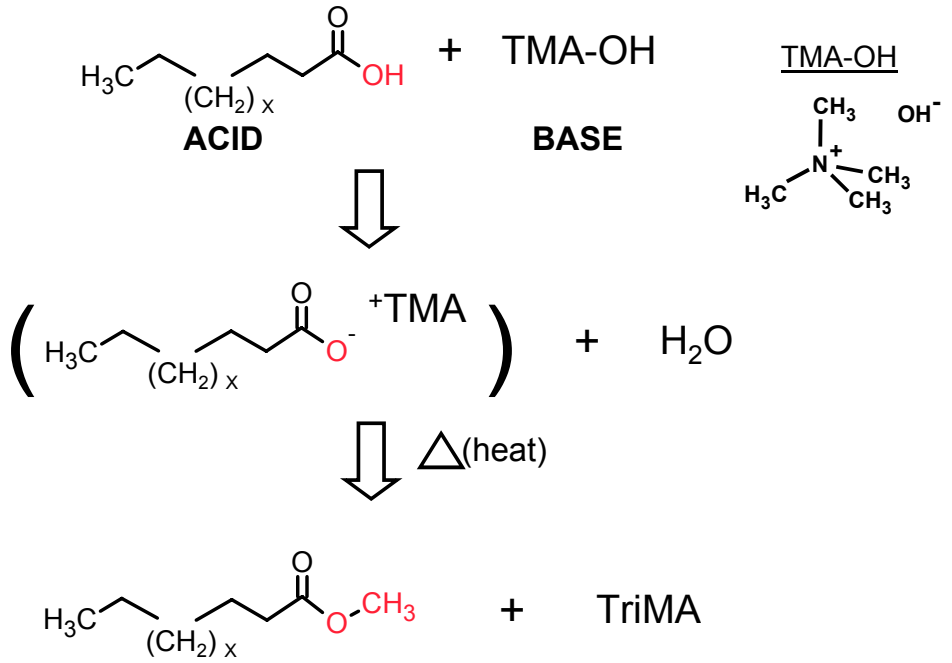


Figure 13: Pyrolysis/methylation is two-step process.

This derivatization reaction is well known, simple and versatile and has also been used for barbiturates, phenols, purines, pyrimidines. The reaction also works with glycerides (the example of a triglyceride) and also with spore biomarker DPA shown in the following figures.

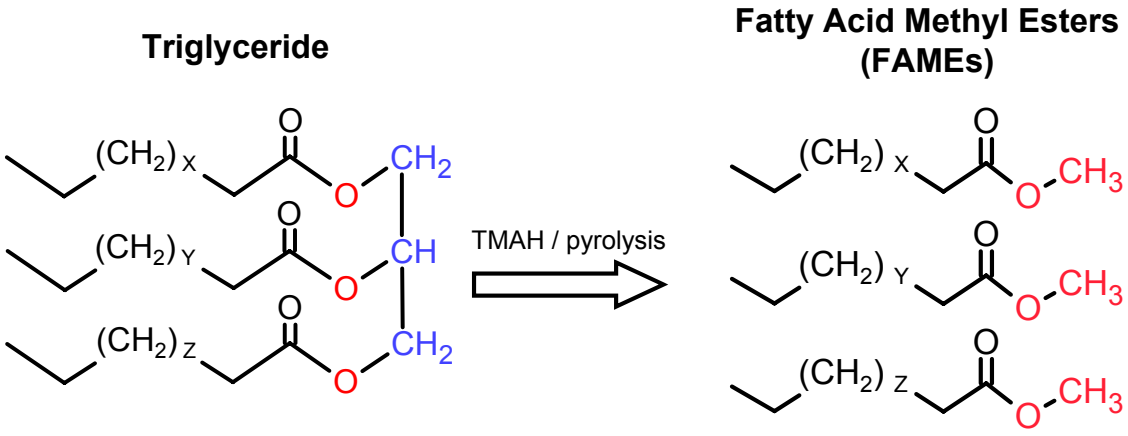
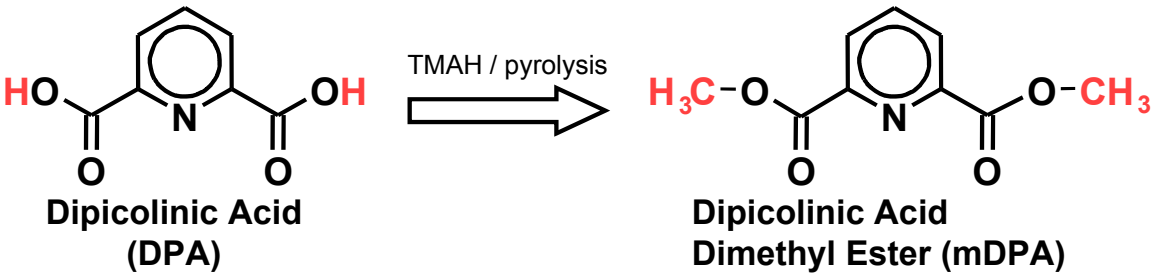


Figure 14: Conversion of triglyceride to individual fatty acid methyl esters by pyrolysis/methylation.



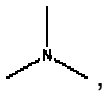
Dipicolinic Acid (DPA) **Dipicolinic Acid Dimethyl Ester (mDPA)**

Figure 15: Conversion of DPA to methylated DPA by pyrolysis/methylation.

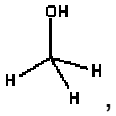
A drawback that is sometimes observed is that TMAH can cause isomerization/degradation of polyunsaturated fatty acids and requires an optimum amount of TMAH [57]. Pyrolysis/methylation can also be used to analyze for amino acids or oligopeptides [58]. The byproducts of the use of TMAH must be considered in any detection scheme and are tabulated here:

Table 11: Byproducts of TMAH derivatization [5].

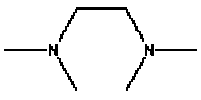
trimethylamine [75-50-3] m/z 59



methanol [67-56-1] m/z 32



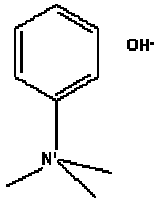
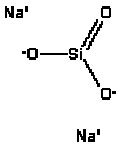
N,N'-tetramethyl-ethylenediamine [110-18-9] (m/z 116)



N,N'-dimethyldiazine (m/z 114)

The reagents used for pyrolysis/methylation vary on the application; several are compiled along with references in Table 12.

Table 12: Derivatizing reagents used for pyrolysis methylation.

derivatizing reagent(s)	CAS#	reference	results / notes
tetramethyl ammonium hydroxide TMAH	75-59-2	[23, 27]	
TMA-HSO4		[23]	saw more FAMEs than w/ TMA-OH
(pentafluorophenyl)diazoethane (PFPDE)		[23]	advantage that it reacts with FA directly in aqueous soln. (ref. only analyzed <C6 FA)
Trimethylphenylammonium hydroxide; Trimethylanilinium hydroxide PTMA-OH 	1899-02-1	[55, 59]	[“MethElute” Pierce, USA – will methylate phenols, degrade GC column in in-column inj.]
sodium metasilicate 	6834-92-0	[5]	
trimethyl (trifluoro-m-tolyl) ammonium hydroxide (TMTFTH)		[60]	
trimethylammonium acetate (TMAAc) Buffer solution 1 M pH 6.5-7.5 (volatile)	6850-27-7	first use: [19, 59]	methylates free FA in presence of esterified FA. 10% aqueous soln., dry before pyro

derivatizing reagent(s)	CAS#	reference	results / notes
trimethylsulfonium cyanide (TMSu-CN)		[59]	potential toxicity, selective, not stable in O2
phenyl-trimethylammonium acetate (PTMA-OAc)		[59]	selective, stable
phenyl-trimethyl ammonium fluoride (PTMA-F)		[59, 61]	NEUTRAL! no column degradation
“methprep II”, 3-trifluoromethyl phenyl-trimethyl ammonium hydroxide			Supelco 2000
trimethylsulfonium hydroxide (TMSH) 	17287-03-5	[18], [59]	0.2 M in MeOH, 350ºC – keeps PUFA ratios intact (no isomerization), degrades column Hazard Symbol: Highly flammable, Very toxic Storage Temp: 4°C
benzylation: 3,5 bis (trifluormethyl) benzyl trimetylammonium fluoride (BTBTA-F)		[61]	**benzylates** phenols, cresols, organic acids, FA
3,5 bis(trifluoromethyl) benzyl dimethylphenyl-ammonium fluoride (BTBDMA-F)		[61]	**benzylates** phenols, cresols, organic acids, FA

2.5. Pyrolysis

The information in this section is intended to provide context with respect to the small size and low power of the microfabricated pyrolyzer or micropyrolyzer demonstrated in this LDRD.

Pyrolysis has been used to detect and differentiate gram-negative bacteria such as *Brucella melitensis, Yersinia pestis, and Francisella tularensis* and gram-positive bacteria such as *Bacillus anthracis* (the causal agent of anthrax). It can be performed either in an inert atmosphere (gas) or in an oxidative atmosphere

such as air. There have been few investigations, however, that compare the pyrolysis results of these two atmospheres [62].

There are several types of instrumentation used to perform pyrolysis including gas chromatographic inlet, infrared, Curie-point, and resistive pyrolyzers. The characteristics of each type are summarized below, including a brief history of pyrolysis/methylation, and are listed in Table 13.

2.5.1. Gas Chromatographic inlet pyrolysis.

In this method the sample of interest is injected in liquid form into the inlet, which is simply a heated glass tube, of a commercial gas chromatograph. The inlet temperature cannot exceed about 250ºC and is kept constant. Depending on the volume, the liquid is vaporized within 0.5 seconds. A portion of the sample is swept by an inert carrier gas into the gas chromatographic separation column. Because of the limitations of the upper temperature, this method is not practiced widely.

2.5.2. Infrared pyrolysis.

In this method, infrared laser radiation heats the sample. Various lasers are available that can be used for this purpose. Their emission is usually pulsed, and the heating rate depends upon the irradiance or energy per unit area focused upon the sample during the pulse. As a chromatographic introduction technique, this technique is rarely used. It is more often used as a sample introduction for a mass spectrometer.

2.5.3. Curie-point pyrolysis

For this type of pyrolysis, a magnetic metal foil or wire of particular alloy composition is excited by radio frequency energy. The metal heats until the characteristic Curie-point temperature of the alloy is reached, at which point the metal is no longer magnetic and ceases to heat. In this way temperatures from 300 to nearly 1000ºC can be achieved in a matter of 10-20 milliseconds. The major limitation of the method is that particular alloys are required, limiting the pyrolysis to discrete temperatures. Available temperatures include 220, 358, 423, 500, 670, 920, and others. The alloys are somewhat specialized, which increases the cost per sample. There are three manufacturers currently offering Curie-point pyrolysis instrumentation, GSG Analytical Instruments Ltd. (UK), Japan Analytical (Japan), and Horizon Instruments (UK). For solids analysis the foil must be crimped to enclose the sample. Curie point can take 100W (0.5Mhz) to produce a 1-2 second rise to 358, 510, or 610°C [27].

2.5.4. Resistive pyrolysis

This type of pyrolysis is perhaps the simplest, requiring only a metal filament (often platinum) and a capacitive power supply capable of sending a large current rapidly through the filament. The filament heats due to its electrical resistance. This method is more flexible than Curie-point because it is not limited to discrete

temperatures. The sample is either deposited onto the filament or onto a quartz substrate that is placed within a resistive coil. Temperatures as high as 1400ºC are possible in less than 70 milliseconds, corresponding to a heating rate of 20ºC/msec. Manufacturers of resistive pyrolyzers include SGE (Houston, Texas), Pyrola AB (Lund, Sweden), and CDS Analytical (Oxford, Pennsylvania).

While the filament(s) themselves are small (35mm x 1.5mm x 0.0127mm) and can reach 1000ºC in 17 msec. (ribbon) or 1000ºC in 1200 msec. The supply and control electronics are large, however [63].

Table 13: Instruments used for pyrolysis.

pyrolyzer / manufacturer	rise-time, temp.	reference
PYROLA-85, Pyrol AB, Lund, Sweden www. pyrolab.com	rise?, 400-600ºC, 2-4sec total, 8 ms to 1400ºC platinum filament, measure temp. by resistance/light emission	[19]
CDS pyroprobe 1000	15ºC/ms, resistively heated Pt-filament, 300-600ºC in He	[64], web
Gerstel PM1	rise?, 500-1000°C, weight 0.24 kg, 16 W	Gerstel flyer
GSG Analytical Instruments Ltd, UK	curie-point autosampler	web
SGE, Inc., Houston, TX	resistive, $5600	web
Japan Analytical Industry Co., Ltd. (us patent no. 3,879,181), dist. by Dychrom (Santa Clara, CA – 800-439-2476)	curie-point JHP-3: 225 watts RF, 16-18 kg, 6 amp max. JHP-2: 48 watts RF, 100V, 7 amp max.	JAI literature
Horizon Instruments RAPyD-400 or PYMS-200X (Ghyll Industrial Estate, Heathfield, East Sussex)	curie point	[33]
non-commercial	100W curie point	[27]
CBMS	5 kg instrument, IR 580°C, 30 sec. pyro, 25x10x18"	[8]

2.5.5. Additional Thermally-based Analysis Methods

2.5.5.1. Heated chemistry – Desorption / Vaporization

There is no technology currently commercially available that can perform both the function of the heated reaction and the desorption/vaporization. Reaction or derivative chemistry is usually performed separately from the analysis instrumentation, and only a small volume of sample or extract is then used for the analysis.

2.5.5.2. Thermal Desorption from Solids

Several companies sell laboratory-scale instruments for this purpose, including Perkin-Elmer and Dynatherm. The sample is heated and those chemical species released are usually trapped for further analysis. There are no portable or field systems sold for this purpose.

2.5.6. History of pyrolysis / methylation

Table 14: History or pyrolysis / methylation.

Procedure	Reference
conversion of TMAH salt of carboxylic acids to methyl esters in GC inlet	1963 [2]
methanolic solution of quat. amm. hydroxide to produce methyl ester from triglyceride via transesterification (must remain anhydrous to prevent saponification)	1982 [53]
whole cell + (not) tmah but Trimethylphenylammonium hydroxide + curie point pyro of whole cells – dubbed on-line derivatization “OLD”	1989 [55]
whole cell, demonstration of py-gc-ms produces same FAME pattern as extraction (lose hydroxy-substituted FA) and similar repeatability	1990 [27]
whole cell (or phospholipid) + tmah + curie point pyro of whole cells	1991 [5]
first SPME deriv. in GC inj.	1997 [23]

3. Experimental Details

3.1. Micropyrolysis Devices / Testing

There have been several micropyrolysis devices tested during the course of this LDRD. The devices are etched to reveal a thin silicon nitride membrane which is the working surface. There are round or square membranes in a variety of configurations with respect to heater layout and resistance. As an example, one design has a typical resistance of 120 or 240 ohms. At 120 ohms, a bias on the order of 12 V was required to reach 350 to 400°C. Many of the devices used have 4 electrical pads with a heater and a thin resistor that can be used as a temperature measurement (these are not used for field portable applications).

The tetramethyl ammonium hydroxide (TMAH) solution is a known etchant of silicon, but does not etch the nitride very rapidly. A ballpark cost of the devices would be somewhere around $1200/wafer, which is approximately 200 devices. This is an upper limit which would decrease as more were made.

A schematic of the experimental setup used for testing micropyrolysis devices is shown in Figure 16. The gas chromatograph (GC) and mass spectrometer (MS) are commercially available. A commercial GC column (J&W DB-23, 0.25um film, 0.25mm x 15 m) is used which has a high polarity 50%-cyanopropyl-methylpolysiloxane stationary phase. This phase, bonded and cross-linked, is designed for separation of FAMEs and has excellent resolution for cis- and trans-isomers.

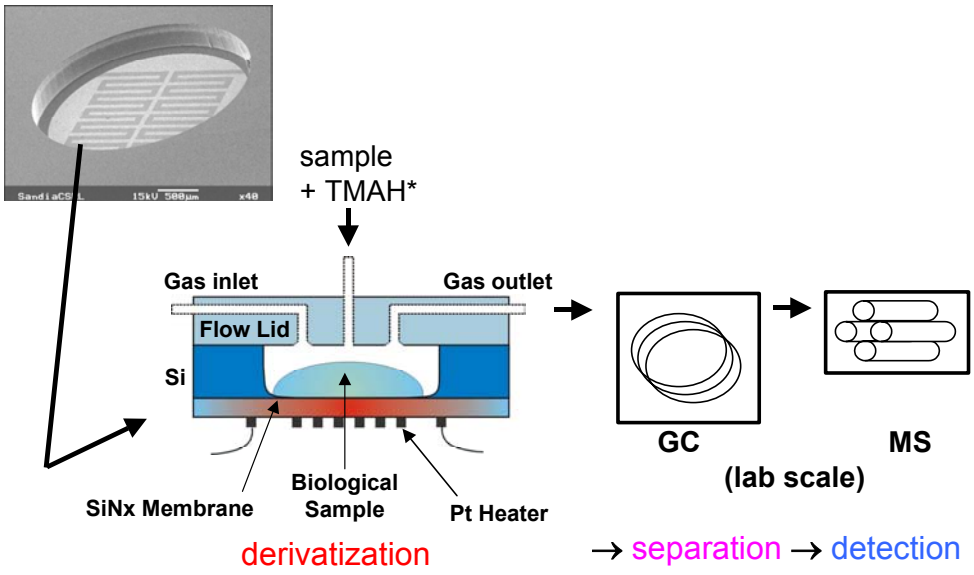


Figure 16: Schematic of the instrumental configuration used for micropyrolyzer testing.

A picture of the commercial instrument with the transfer line, power supply, and the test fixture in included below. The gas flow is controlled through a toggle valve (at right) and the transfer line temperature is monitored via thermocouple.

Figure 17: Picture of the Hewlett Packard GC/MS instrument with the micropyrolyzer and transfer line.

3.2. MicroGC columns / testing

In order to test the microfabricated GC (microGC) columns as a single device, they are installed into a commercial GC oven as shown below. The commercial system has a liquid sample injection port and a flame ionization detector.

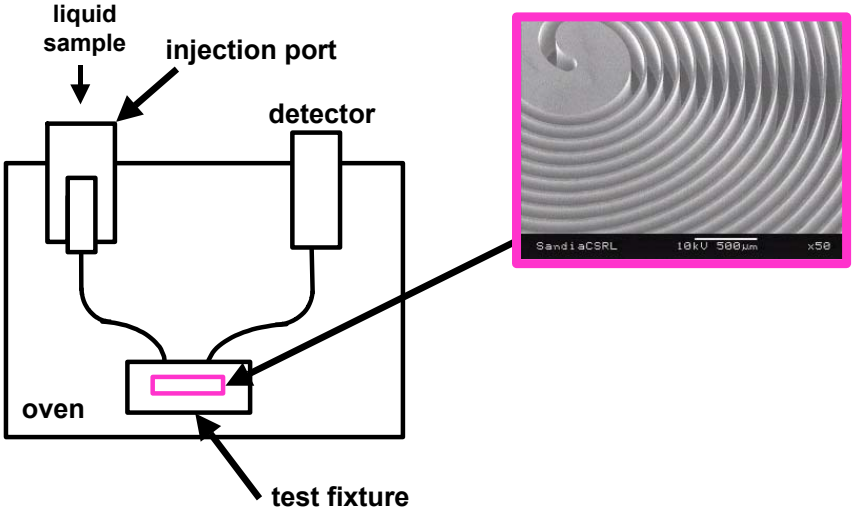


Figure 18: Schematic of test setup for testing microGC columns.

To connect the injection port to the microGC, fused silica connectors (Supelco part no. 23628) are used in conjunction with uncoated, deactivated fused silica capillary "pigtails". The most common microGC column used has the nominal

dimensions of 100 microns wide by 400 microns and 86 cm long. A variety of coatings are used to tailor the separations. Most temperature ramp chromatography presented here has been performed using microGC column #079 which is a "standard" size and is coated with OV101 (polydimethylsiloxane) stationary phase.

3.3. Surface Acoustic Wave (SAW) Detectors

Several different SAW detectors were utilized. In most tests a four channel SAW (one reference channel) was used, with coatings that included polyepichlorohydrin (PECH), a fluorinated polyol (BSP3), or polyisobutylene. These detectors utilized DC power, and the data was in the form of a DC signal and was collected. There were three types of SAW detector tests: 1) SAW with vapor introduction, 2) SAW with accompanying preconcentrator/microGC system with vapor introduction, and 3) SAW with micropyrolyzer sample introduction.

For the FAME vapor tests, a gravimetric vapor system was utilized. This system is controlled by a commercial GC oven and has glass flow-through tubes in which the desired chemical is placed. The tube is weighed over time to get a chemical flux rate which is then used to calculate the concentration of the chemical in the stream. An example of this calculation for the C12 methyl ester tests is shown below.

Equation 4: Calculations for the quantity of methyl laurate (M.L.) detected using µChemLab gas phase system.

$$\left(\frac{1m^3\ \text{methyl laurate}}{10^6 m^3\ \text{air}}\right)\left(\frac{214.34kg\ \text{M.L.}}{24.47m^3\ \text{M.L.}}\right)\left(\frac{10^6 mg\ \text{M.L.}}{1kg\ \text{M.L.}}\right)=8.76\frac{\text{mg}}{\text{m}^3}\ \text{M.L.}$$

$$\left(\frac{8.76mg\ \text{M.L.}}{m^3}\right)\left(\frac{1m^3}{1000L}\right)\left(\frac{1L}{1000cc}\right)\left(\frac{200cc}{\text{min}.collect}\right)(1\text{min}.collect)\left(\frac{1000ug}{1mg}\right)=1.75ug\ \text{M.L.}$$

$$1.75ug\ \text{M.L.}\left(\frac{1mol}{214.34g}\right)\left(\frac{1g}{10^6 ug}\right)=8.17x10^{-9}mol\ \text{M.L.}=8.17nmol\ \text{M.L.}$$

The SAW with accompanying preconcentrator/microGC system with vapor introduction tests utilized a "full" µChemLab system. Gas flow into the system was 200cc/min. and the vapor was collected for 1 minute. The temperature of the GC was approximately 120°C and was cooled to 80°C during the analysis, but the temperature is not precisely known.

In the experiments in which the micropyrolyzer introduced the sample, a heated transfer line connected the micropyrolyzer test fixture with the PEEK SAW detector fixture. The transfer line was kept at approximately 105°C, while the SAW was at room temperature and the micropyrolyzer test fixture temperature was varied between 60 and 100°C. The procedure for the data shown with respect to air flow

was as follows: t=0 scan start, t= 1 min. flow on, t= 1min 10 sec. fire micropyrolyzer, and t= 1 min. 15 sec. micropyrolyzer off. For the C16 FAME tests presented, the micropyrolyzer test fixture temperature was 90°C.

3.4. Colorado School of Mines (CSM)

The schematic below illustrates the instrumental setup for micropyrolyzer testing at CSM. The micropyrolyzer housing was supplied by Sandia. Note that the 1.4 meter capillary transfer line goes directly from the test fixture to the inside of the ion trap mass spectrometer.

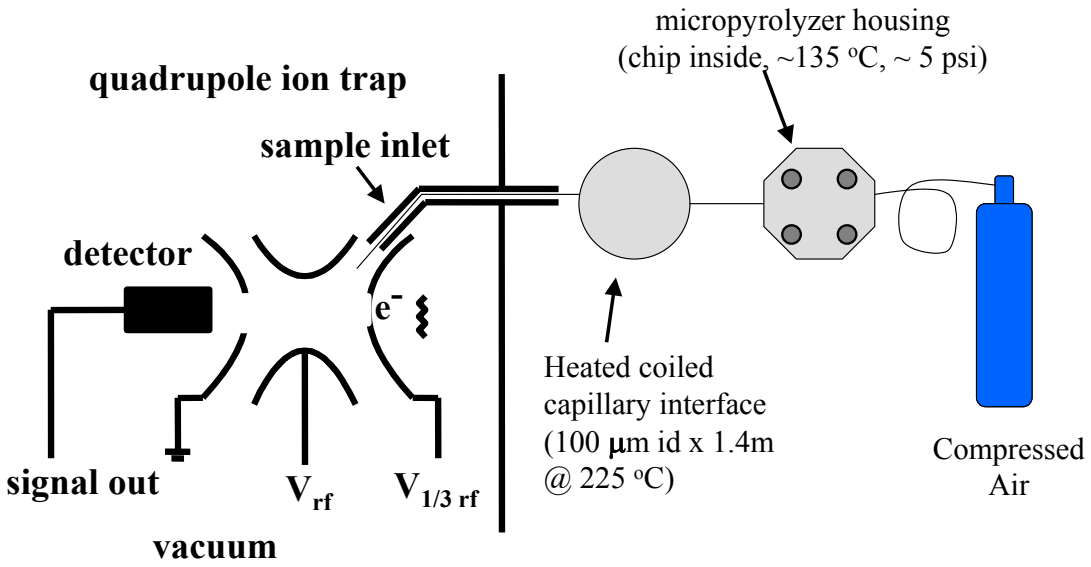


Figure 19: Schematic of instrumental setup for CSM tests.

A photo of the instrumentation shows the relative sizes of the components. The mass spectrometer is a modified Bruker (Billerica, MA) instrument. Not shown are the vacuum pumps and electronics that operate the mass spectrometer.

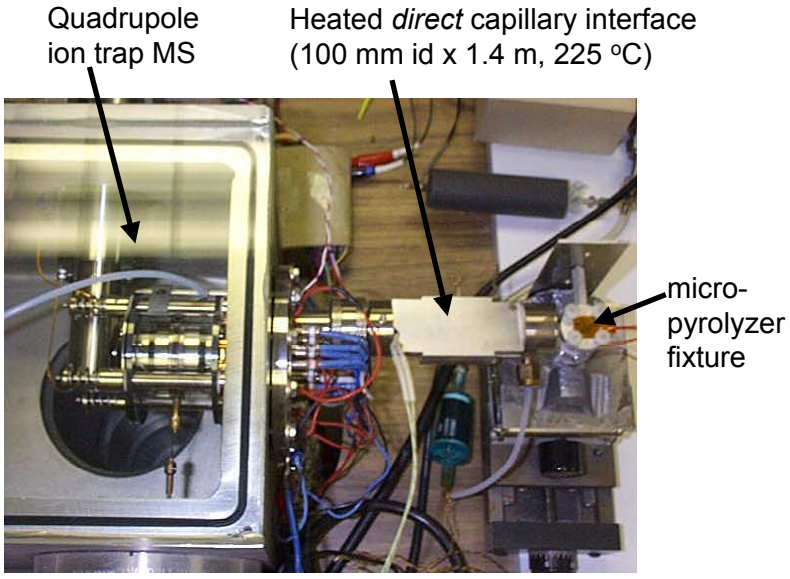


Figure 20: Photo of CSM instrumentation.

3.5. Chemicals

3.5.1. Fatty acids and fatty acid methyl esters

Chemical were used as received from Supelco, Aldrich, and Pierce. The following table lists the individual fatty acids and FAMEs used in the course of the LDRD.

Table 15: Standards used evaluation of microfabricated devices.

Fatty Acids	Fatty Acid Methyl Esters
individuals: C13, 15, 18, 19, 20, 22, 21, 18:1trans9	**individuals:** C20, 22, 24
	mixtures: GLC-40 C16, 18, 20, 22 2x100mg GLC-70 C8-12 100mg GLC-10 C16, 18 18:1,2,3 2x100mg GLC-90 C13, 15, 17, 19, 21 100mg RM-1 C16, 18:0,1,2,3, 20 2x100mg C16:0 (palmitate m.e.), 6.0% C18:0 (stearate m.e.), 3.0% C18:1 (oleate m.e.), 35.0% C18:2 (linoleate m.e.), 50.0% C18:3 (linolenate m.e.)**, 3.0% C20:0 (arachidate m.e.), 3.0% RM-4 16, 18:0,1,2 100mg RM-5 C8, 10, 12, 14, 16, 18:0,1,2 100mg 189-8 C13-17 6x100mg

3.5.2. Freeze-dried Bacteria

Two bacterial samples were ordered from the American Type Culture Collection (ATCC, Manassas, VA), with details of each provided in the following table.

Table 16: Freeze-dried bacteria obtained from ATCC.

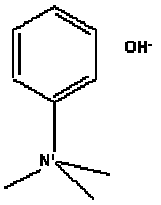
ATCC Number:	**23059**	**13525**
Organism:	*Bacillus subtilis* (Ehrenberg) Cohn	*Pseudomonas fluorescens* Migula
Designation:	W23	NCTC 10038 [28/5; CCEB 546; DSM 50090; NCIB 9046; NCPPB 1964; PJ239; R. Hugh 818; R.Y. Stanier 192, Biotype A]
Depositors:	K.F. Bott	NCTC
History:	ATCC <<-- Depositor <<-- C. Thorne	ATCC <<-- Depositor <<-- M.E. Rhodes 28/5
Isolation:	soil	Pre-filter tanks, England
Type Strain:		type strain [RF4738] [RF16740]
Applications:	produces: isoprene [RF17235]	assay of: antimicrobial preservatives [RF31876]
Descriptions:	bacteriophage host	
Comments:		This strain is recommended by ATCC for use in the tests described in RF31876 where only the taxon is specified.
References:	RF17235: Kuzma J et al. Bacteria produce the volatile hydrocarbon isoprene. Curr. Microbiol. 30: 97-103, 1995 PubMed: 95187061	RF4738: Skerman VB et al. Approved lists of bacterial names. Int. J. Syst. Bacteriol. 30: 225-420, 1980 RF15605: Stanier RY et al. The aerobic pseudomonads: a taxonomic study. J. Gen. Microbiol. 43: 159-271, 1966 PubMed: 67128131 RF16740: Opinion 37. Designation of strain ATCC 13525 as the neotype strain of Pseudomonas fluorescens Migula. Int. J. Syst. Bacteriol. 20: 17-18, 1970 RF17504: J. Gen. Microbiol. 21: 221-263, 1959 RF19775: Int. Bull. Bacteriol. Nomencl. Taxon. 14: 145-155, 1964 RF31876: Standard test method for evaluation of antimicrobial agents as preservatives for invert emulsion and other water containing hydraulic fluids. ASTM Standard Test Method E979-91
Propagation:	ATCC medium: 273 Nutrient broth salts medium	ATCC medium: 3 Nutrient agar (Difco 0001) or nutrient broth (Difco 0003)
Temperature:	37C	26C
BioSafety Level:	1	1
Shipped:	freeze-dried	freeze-dried

ATCC Number:	23059	13525
Price:	$20.00	$20.00
Price Note:	Preceptrol Non-profit discounts do not apply	Preceptrol Non-profit discounts do not apply
Revised :	Jan 02, 2001	Jan 02, 2001

3.5.3. Alternative Methylating Agents

The following table details methylating agents other than TMAH that were ordered or available by synthesis or in the Sandia inventory.

Table 17: Alternative methylating agents.

derivatizing reagent(s)	source	notes
Meth-Prep II 0.2N methanolic (m-trifluoro-methylphenyl) trimethylammonium hydroxide	Alltech 800-255-8324 10x1mL vials part no. 18007 page 347 (web catalog)	
BF_3-methanol 14% BF3 [7637-07-2] 86% MeOH [67-56-1]	Pierce 800-874-3723 100 mL product #49370 price $42 page 508 (2000)	strong Lewis acid pungent odor!
MethElute™ Reagent Trimethylphenyl-ammonium hydroxide; Trimethylanilinium hydroxide [1899-02-1] PTMA-OH or TMPAH	Pierce 800-874-3723 10 mL product #49300 price $55 page 509 (2000)	
Methyl-8® Reagent N,N-Dimethylformamide dimethyl acetal [4637-24-5]	Pierce 800-874-3723 10 x 1mL ampules product #49356 price $68, page 509 (2000)	
trimethylammonium acetate (TMAAc) [6850-27-7] Buffer solution 1 M pH 6.5-7.5 (volatile)	[19] methylates free FA in presence of esterified FA. 10% aqueous soln., dry before pyro	
phenyl-trimethyl ammonium fluoride (PTMA-F)	[59, 61]	NEUTRAL! no column degradation

4. Results and Discussion

During the three years of this LDRD much has been learned about the devices (micropyrolyzer, micro gas chromatographic (microGC) column, and the surface acoustic wave (SAW) detector in the context of rapid biological agent detection. The results have demonstrated that the micropyrolyzer is capable of performing the desired pyrolysis reaction, that the microGC is capable of separating FAMEs, and the SAW detector is capable of reversible response to low molecular weight FAMEs. Each component is discussed separately.

4.1. Micropyrolysis

4.1.1. Device characteristics

The wide range of commercial pyrolysis instruments discussed in the Background section of this document illustrate that there is no strict definition of pyrolysis that defines temperature ramp rate or final temperature. The initial target here was 500°C in less than 1 second with sample load. Devices used in the course of this work (shown in Figure 21) included deep reactive ion etched (DRIE, round) and potassium hydroxide (KOH) etched devices (square). The platinum heater is not visible on the KOH device.

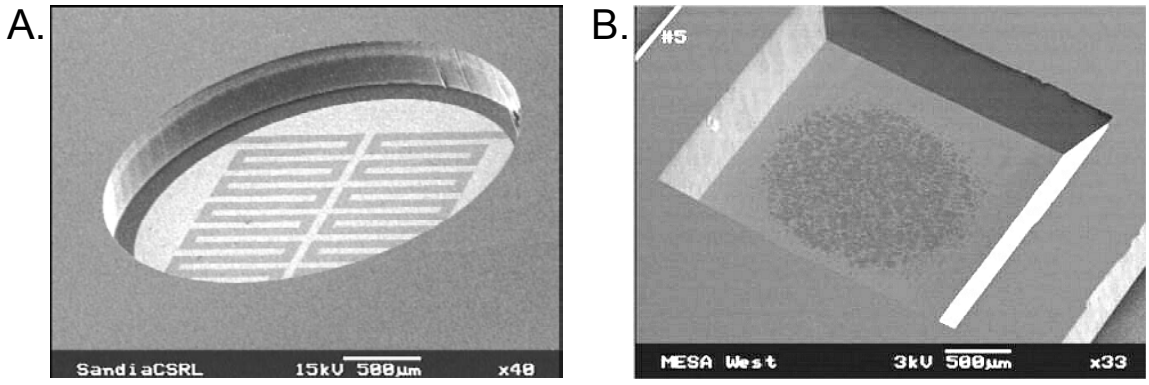


Figure 21: Scanning electron micrographs of micropyrolyzer devices A) DRIE and B) KOH-etched.

Device membranes were more than capable of being heated adequately, both in temperature (>500°C) and response time. Figure 22 shows the temperature profile for a device with a FAME sample load. The upper limit measured in this case is only about 270°C, however this was a limitation of the infrared camera used to collect the data. It is clear that only a few milliseconds are required to ramp from 80 to 270°C using only 130 mW of power (6.65V at 18.84 mA). A ramp rate of approximately 70°C/ms was achieved. Additional IR camera analyses demonstrated that both the round and rectangular micropyrolyzers exhibited a significant temperature gradient from edge to center which was more pronounced in the rectangular device. Variations in heating rates were observed dependent upon presentation of sample load, the type of sample (e.g. fatty acids in methanol versus straight canola oil), mass load, power level and sequence, and on

micropyrolyzer design. It is unknown how the observed gradients or heating rate variations might affect the pyrolysis reactions.

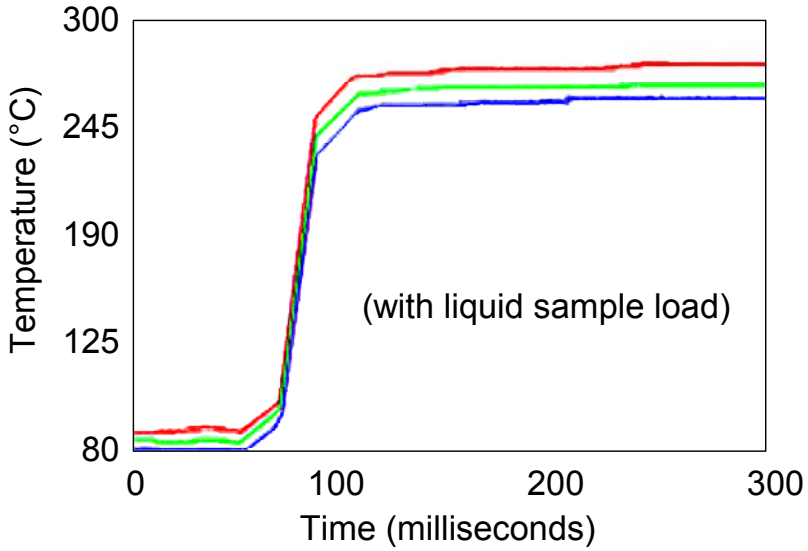


Figure 22: Temperature profile of micro-pyrolyzer device using infrared camera.

The membranes have been robust under pyrolysis conditions, however they are not without lifetime issues. These issues include delamination of the metal layer, apparent vaporization of the metal, hot spots, and degradation due to the methylating agent used in the reaction, tetramethylammonium hydroxide (TMAH). Some devices would begin to delaminate yet retain their resistance value so that the only diagnostic was visual inspection. An example of this is shown in Figure 23. Delamination was more prevalent on the KOH devices. Also apparent is a "greying" circle near the center of the device. This appeared to be slight vaporization of the metal, which would occur during the first few high temperature runs (affecting the resistance) and then stabilize.

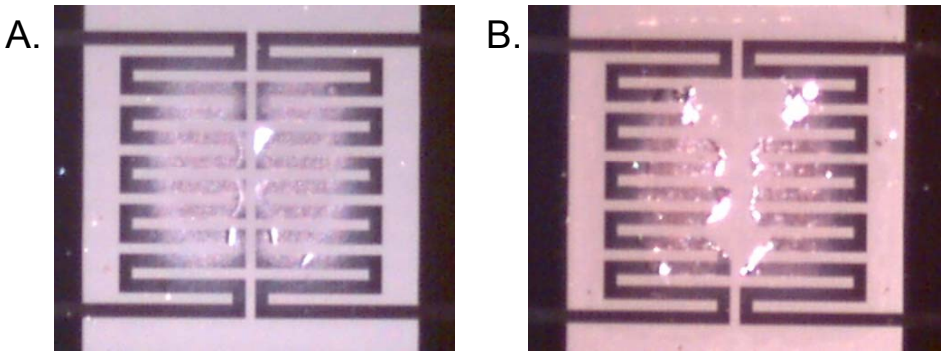


Figure 23: Platinum delamination after A) initial observation and B) 20 pulses later.

The TMAH degradation (and eventual destruction) that was observed was only near the beginning of the project when higher concentrations and volumes of reagent were utilized. This effect was not a significant factor in the loss of devices later in the project.

A significant concern in the use of the micropyrolyzer for bacterial detection is the issue of multiple use. Ideally the device should be capable of many analyses. Except for the issues just discussed, it is true that devices could be used over and over again and perform adequately. In the case of bacteria, however, there is non-volatile residue that remains on the surface of the device after pyrolysis. Commercial pyrolysis instrumentation uses disposable media to solve this problem. It is conceivable that an engineering solution could be achieved to "swap" micropyrolysis devices as they become contaminated. Alternate solutions were investigated in the absence of an engineering solution. The residue problem and one advance toward a solution is illustrated in Figure 24, which shows the residue after pyrolysis of whole-cell bacteria. On "bare" devices, aqueous samples tend to spread and sometime wick to the edge of the device, where pyrolysis is incomplete. A solution that has shown promise is to coat the edge of the device with a hydrophobic coating such as polydimethylsiloxane (PDMS). This keeps the sample near the center of the device for more complete pyrolysis, yet does not solve the residue problem completely. The sample in Figure 24B is presumed to be overloaded, and experiments to determine whether smaller samples could be pyrolyzed completely were not completed.

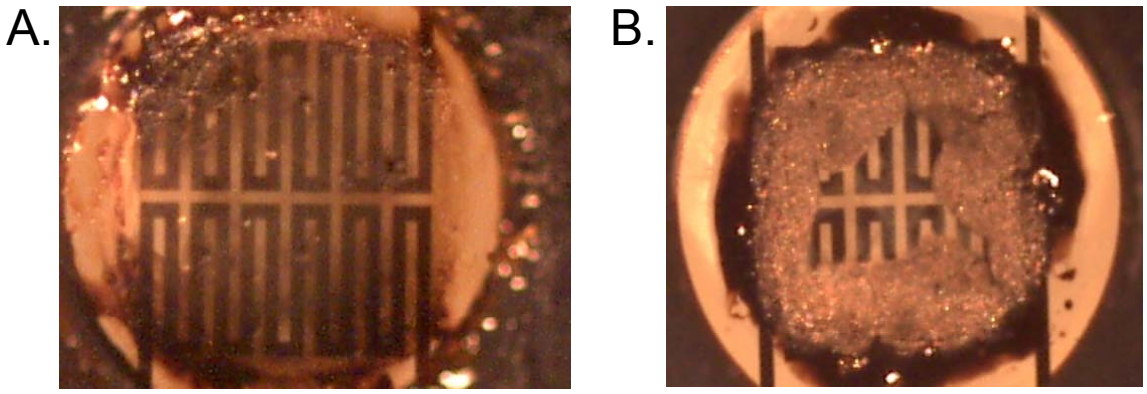


Figure 24: Comparison of bacterial residue after pyrolysis on an A) bare and B) PDMS-coated device.

4.1.2. Test fixtures

Several different test fixtures were used during this LDRD. The test fixture holds the micropyrolyzer in place and provides electrical and plumbing connections. The first fixture was fabricated from PEEK. While easily machineable this material is difficult to heat and some degradation either due to heat or the TMAH was observed. A second generation stainless steel fixture (see Figure 25) was designed and fabricated to allow solvent rinsing/cleaning of the membrane, better gas transfer of pyrolysis products, and more reproducible sample deposition (using a needle/septum introduction). Reproducibility of samples deposited via needle through the lid of the fixture was very poor, and sample material was observed on the fixture and outside the membrane area of the micropyrolyzer.

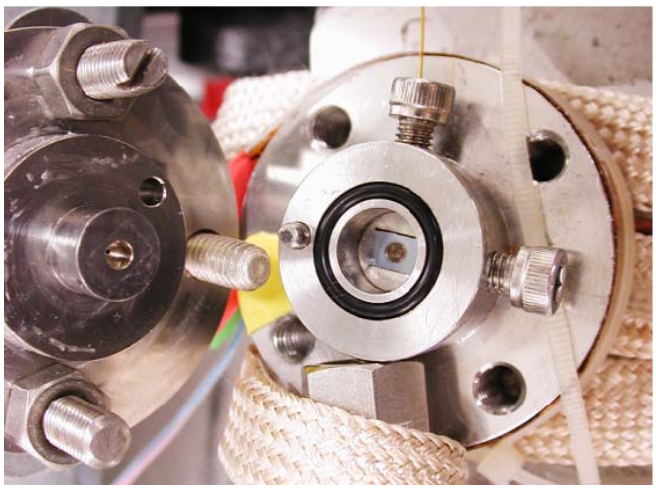


Figure 25: Second generation micropyrolyzer test fixture.

A third generation fixture (see Figure 26) was designed and fabricated without the capability to deposit liquid on the micropyrolyzer while in the fixture. This fixture has the improvements of smaller size (easier to heat and lower power) and luer-lock fittings that are easier to plumb than the septa connections used in the first two generation fixtures. To deposit sample the lower portion of the device is lowered and the micropyrolyzer removed. This procedure proved cumbersome however compared to the "removable lid access" of the previous design.

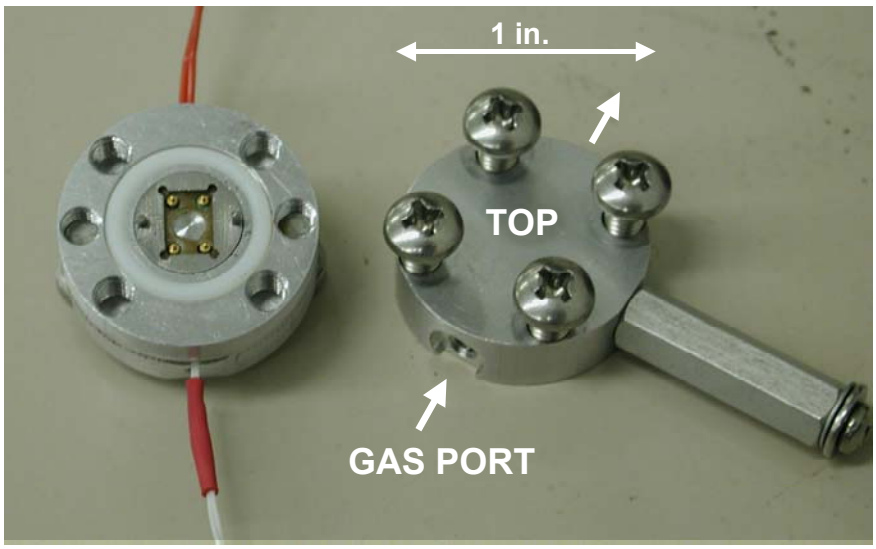


Figure 26: Third generation test fixture.

Automated reagent deposition will be a necessary component of any user-friendly or autonomous instrument, and therefore further solutions to this goal should be pursued.

4.1.3. Sample vaporization

It was necessary to show that the micropyrolyzer could heat rapidly enough to vaporize chemicals, and to compare the vaporization characteristics with other

techniques. Because the product of the target pyrolysis/methylation reactions are FAMEs, it was desirable to show that FAMEs could be vaporized intact and without degradation. This is demonstrated in Figure 27 which shows a GC/MS analysis of a mixture of FAMEs with chain lengths from 13 to 17 carbons that have been vaporized intact using a micropyrolyzer. The ratio of the peaks reflects the original composition of the mixture. The instrumentation and conditions are described in the Experimental Details section.

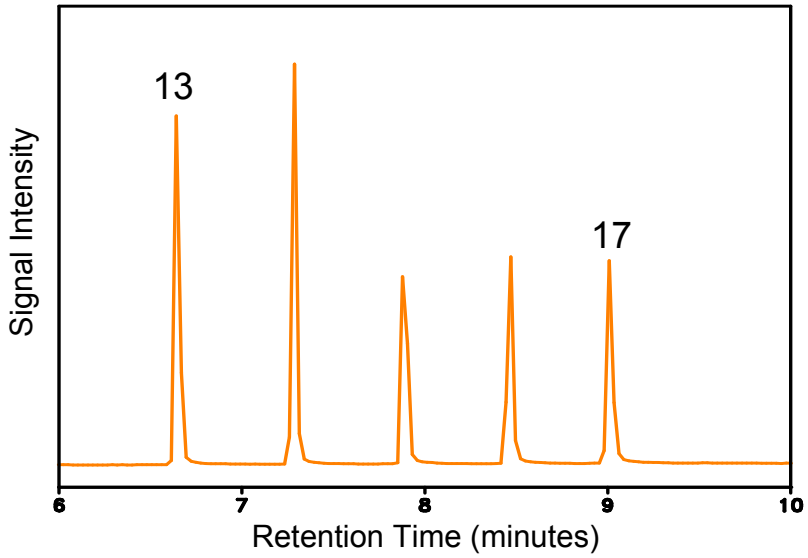


Figure 27: GC/MS separation/detection of micropyrolyzer-vaporized FAMEs C13:0 (13) to C17:0 (17).

It is also useful to investigate the vaporization of other compounds such as biomarkers, including dipicolinic acid (DPA) which is found in the spores of *Bacillus* species. The following two figures demonstrate that methylated picolinic acid (mPA, Figure 28) and methylated dipicolinic acid (mDPA, Figure 29) can be vaporized intact. The accompanying mass spectrum in each figure is used to confirm the identity of the peak in the chromatogram using library spectra (see Appendix A: Reference mass spectra).

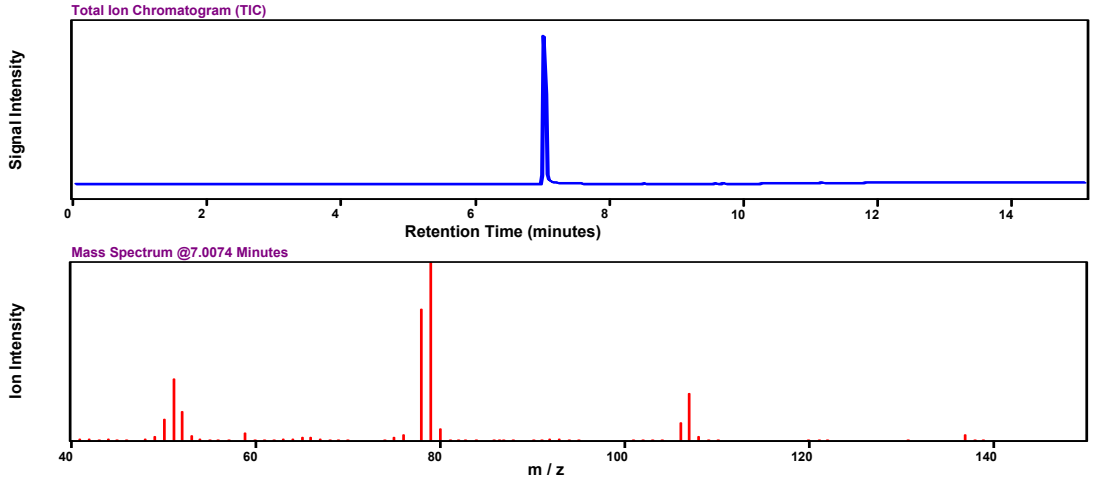


Figure 28: Micropyrolysis vaporization of methyl picolinic acid (mPA).

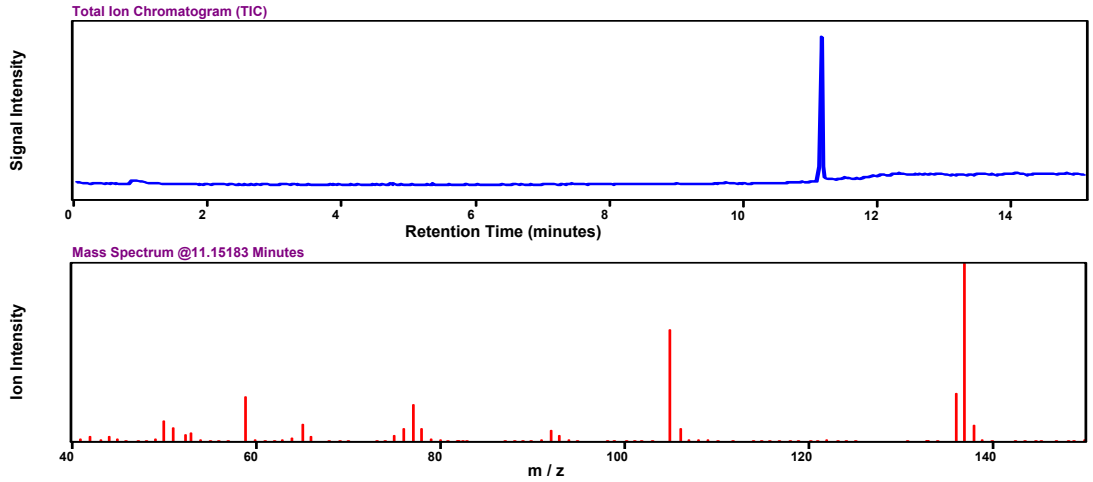


Figure 29: Micropyrolysis vaporization of methyl dipicolinic acid (mDPA).

In the following chromatogram, picolinic acid (PA) is vaporized and detected. The broad peak is characteristic of an acid.

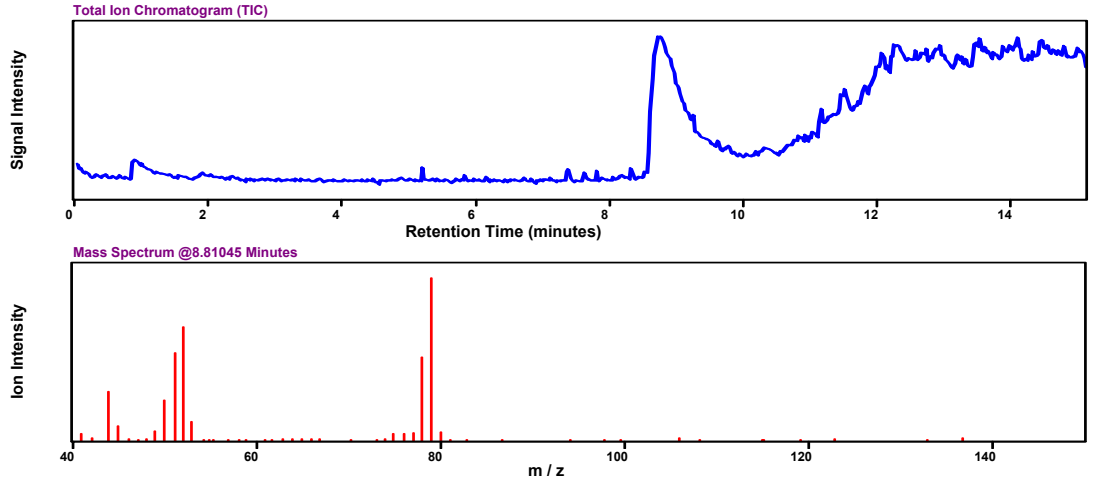


Figure 30: Micropyrolysis vaporization of picolinic acid (PA).

Thermal degradation of DPA upon pyrolysis to pyridine and picolinic acid has also been observed [65]. These degradation products have not been observed using a micropyrolyzer. In contrast, the products usually observed (as shown in Figure 31) are a small amount of methylated DPA and a second peak that has the mass spectrum characteristic of methylated PA but a slightly different retention time (7.35 minutes in Figure 31, versus 7.00 minutes in Figure 28). Intact DPA was not detected probably due to the chromatographic conditions. further investigation of the peak at 7.35 minutes and lack of intact DPA was not warranted in the scope of this work.

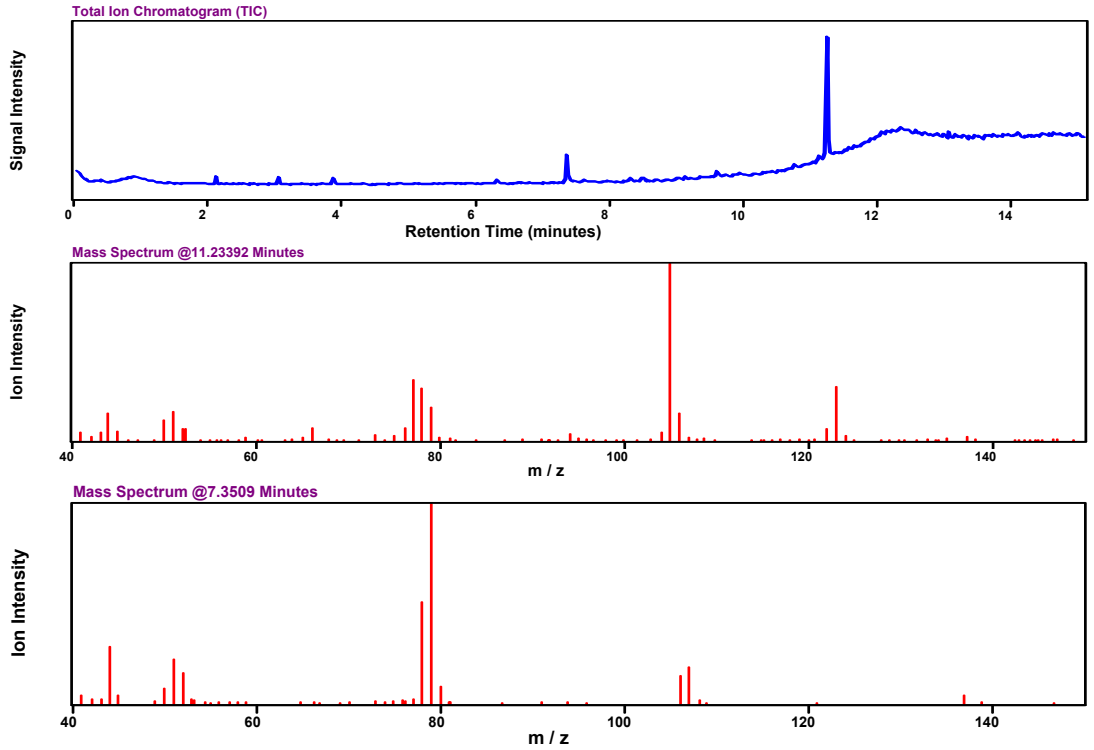


Figure 31: Micropyrolysis vaporization of DPA (no TMAH).

4.1.4. Micropyrolysis / methylation of chemicals

The ability of the micropyrolyzer to perform a pyrolysis/methylation reaction is even more important than the ability to perform simple vaporization. Figure 32 shows data from the micropyrolysis/methylation of a mix of purified fatty acids (C13:0-C20:0 and C18:1, not in equal amounts)) using the microfabricated pyrolyzer device and commercial GC/MS equipment. Micropyrolysis/methylation was accomplished in a few seconds followed by separation and detection as described in the Experimental Details section. The chromatogram in Figure 32 shows the 2 minute period of peak elution which on this column corresponds to a column temperature range of approximately 160^{o}C-210^{o}C. Key points demonstrated by this result include an original demonstration of pyrolysis/methylation on a microfabricated membrane, a representative FAME profile reflecting the relative quantities of fatty acids, and the quick separation possible even on a relatively long column. The spectra in Figure 33 compare the spectrum of the peak labeled C14:0 in Figure 32 and the library spectrum of the C14:0 methyl ester confirming that methylation did indeed occur.

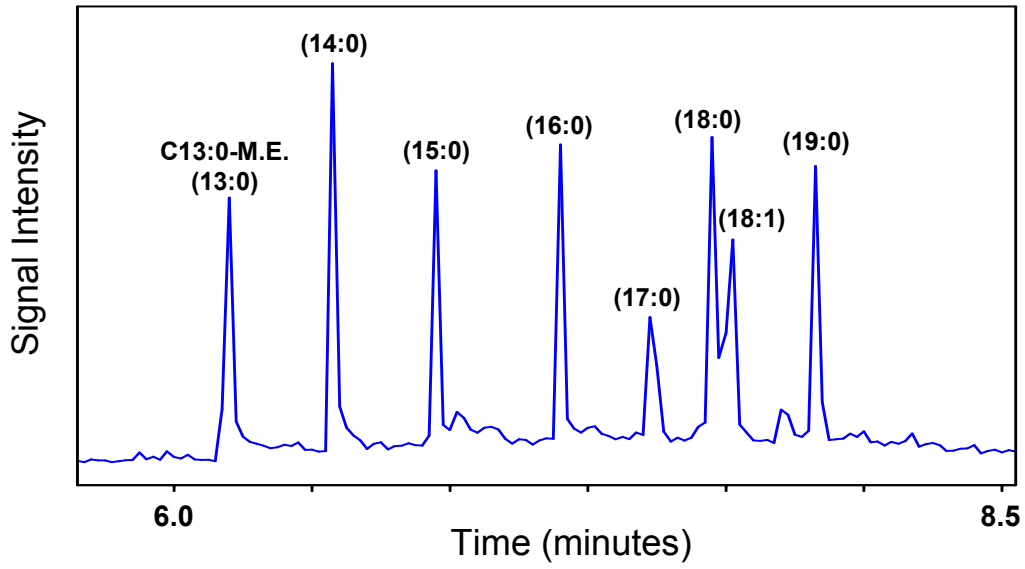


Figure 32: Micropyrolysis/methylation of fatty acid mixture.

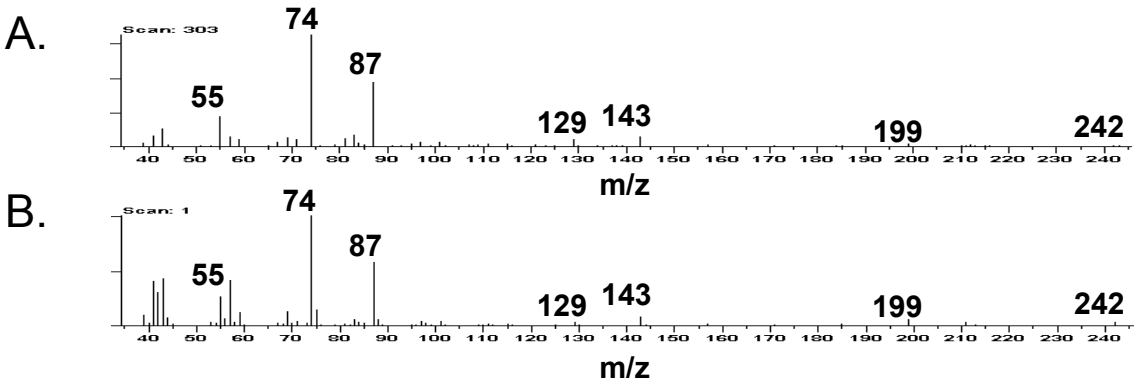


Figure 33: Comparison of (A) C14 peak created by micropyrolysis/methylation to (B) library mass spectrum of C14:0-ME.

For micropyrolysis to be useful in the identification of bacteria, the conversion from fatty acids to FAMEs should be quantitative as has been shown with laboratory-scale pyrolysis. To investigate this, two mixtures (A, B) of fatty acids with varying composition were micropyrolyzed with TMAH. The corresponding chromatogram of FAMEs produced is shown in Figure 34.

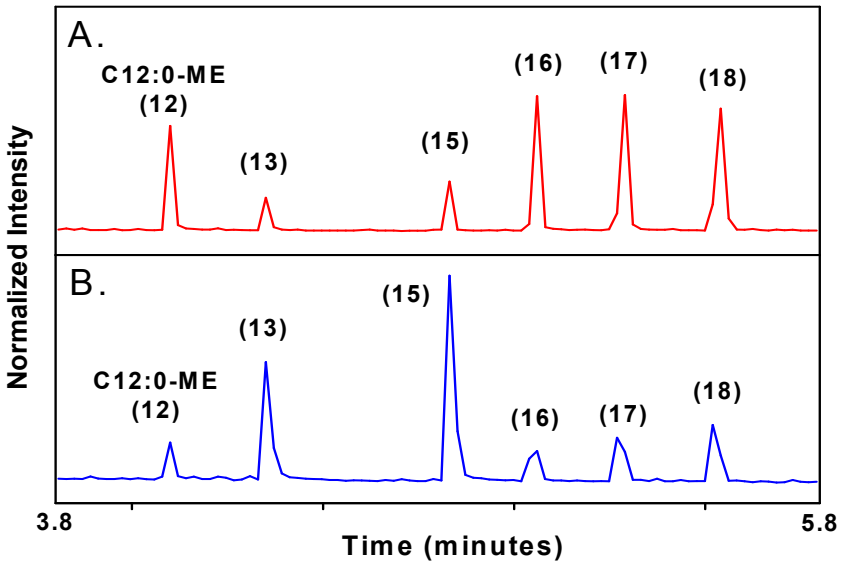


Figure 34: Micropyrolysis/methylation of fatty acid mixtures A and B.

The relative mass percent of the mixtures shown in Figure 34 as well as the relative peak areas for the FAMEs detected are shown in the following table.

Table 18: Composition of fatty acid mixtures compared to FAME peak areas.

Fatty Acid	Fatty Acid Composition (mix A)	FAME peak area	Fatty Acid Composition (mix B)	FAME peak area
C12	22	16.9	8	5.6
C13	6	5.5	33	23.1
C15	6	7.5	33	39.7
C16	22	21.7	8	7.9
C17	22	24.0	8	11.1
C18	22	24.4	8	12.7

To further demonstrate that the micropyrolyzer used in this LDRD could perform the same reactions as commercial laboratory-scale pyrolyzers, a triglyceride mixture was tested. This mixture contained equal amounts of the five triglycerides: tricaprylin, tricaprin, trilaurin, trimyristin, and tripalmitin. The micropyrolysis/methylation reaction converted the triglycerides into the methyl esters of their component fatty acids as shown in Figure 35. All the peaks were confirmed by library matching, and the figure contains the mass spectrum of one peak to demonstrate that it is indeed the C12:0 methyl ester peak.

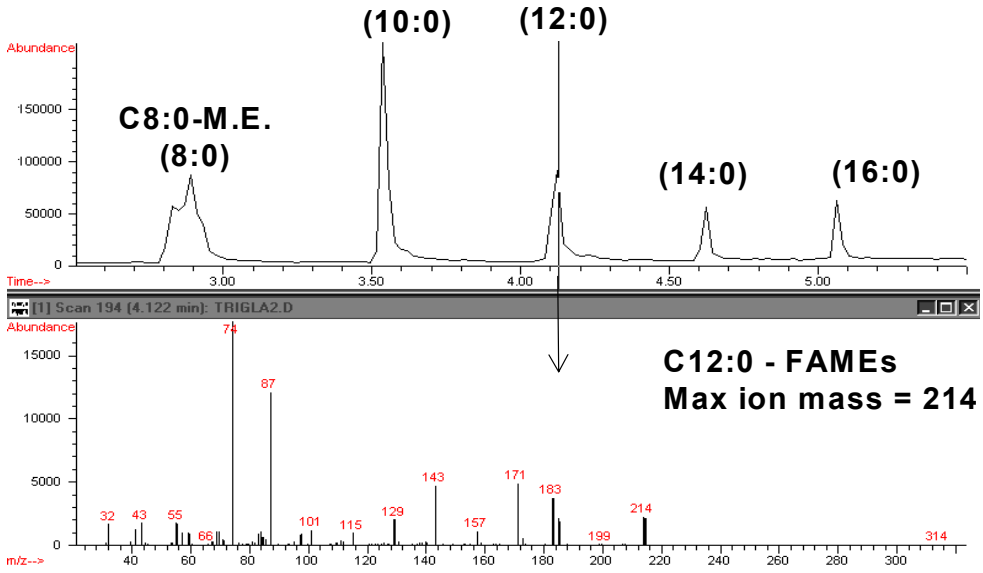


Figure 35: Micro-pyrolysis/methylation of triglyceride mixture.

Fatty acids are commercially valuable chemicals and in fact are the main component of edible oils. The demonstration of the micropyrolyzer for the analysis of an edible oil would be of commercial interest. Figure 36 shows the GC/MS analysis of a micropyrolysis/methylation reaction of canola oil as well as an oil sample without the methylating reagent. Analysis of the component acids would be very difficult with a portable system, however the conversion to FAMEs by the micropyrolyzer makes portable analysis an achievable goal.

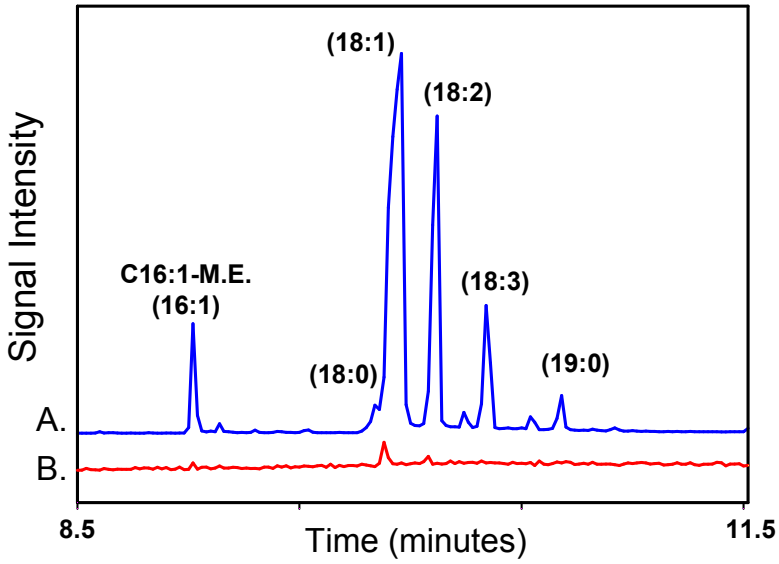


Figure 36: Micro-pyrolysis/methylation of canola oil sample, (A) with methylating reagent and (B) without reagent.

For the micropyrolyzer to be used in the detection of BW agents, the methylation of biomarker compounds such as DPA is also necessary. The successful conversion of DPA to mDPA is confirmed by GC/MS analysis (see Figure 37).

The large peak at the beginning of the chromatogram is the byproducts of the methylation reagent.

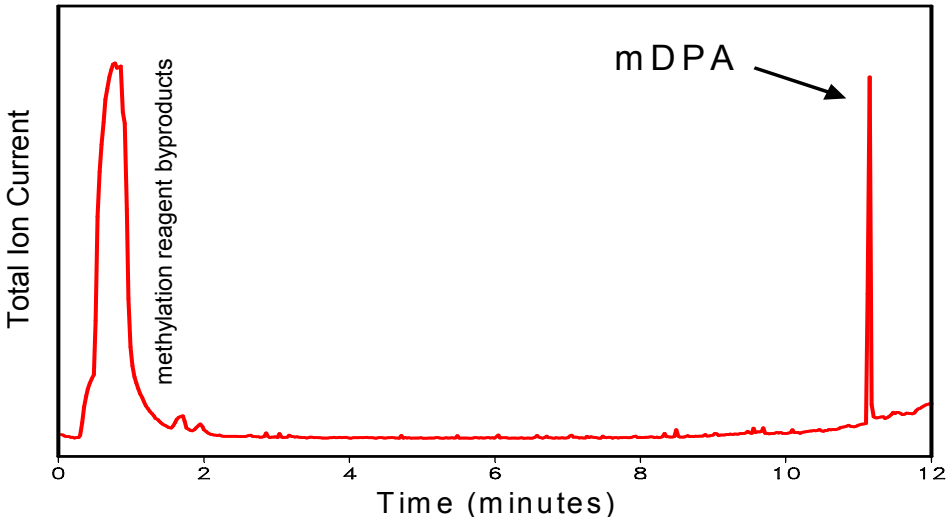


Figure 37: Micropyrolysis/methylation of DPA to mDPA.

In some cases it was possible to detect mDPA conversion from DPA without the large methylation byproducts seen in Figure 37. An example of this is shown in Figure 38 and was usually the result of multiple pyrolysis runs with a single sample. The first pyrolysis would only yield a small amount of methylated product, whereas a second pyrolysis would appear as in Figure 38. Experimental conditions have not been characterized, however. Further investigation is warranted because a procedure that eliminates the byproducts would be beneficial to the chromatography.

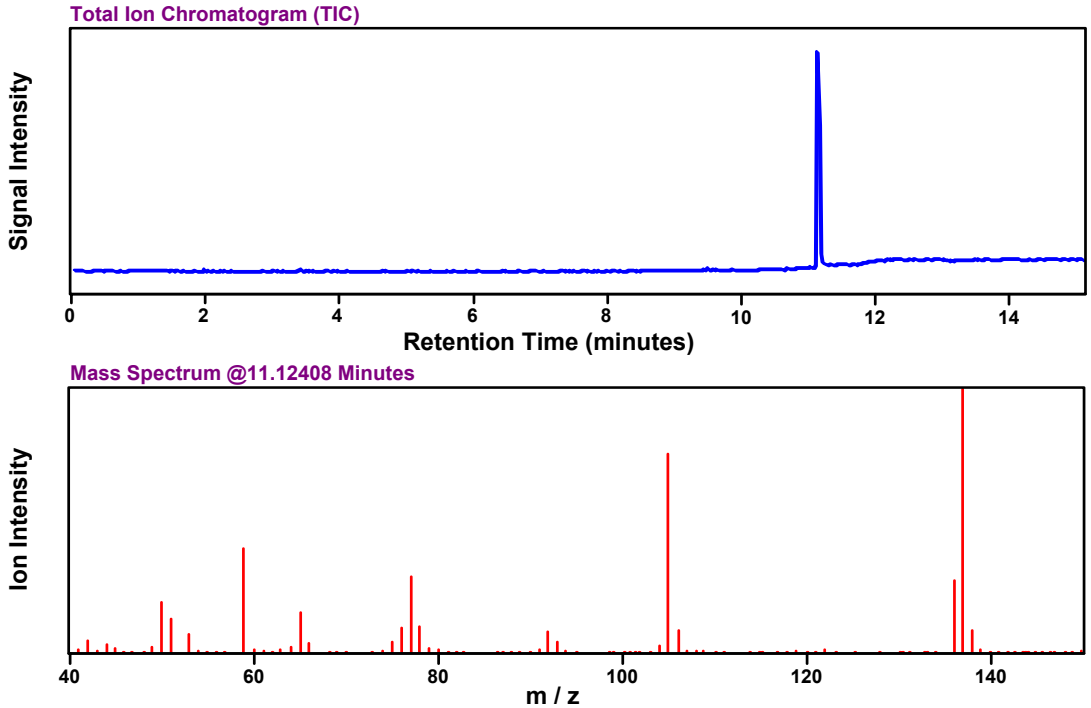


Figure 38: Pyrolysis/methylation of DPA.

4.1.5. Micropyrolysis / methylation of whole cells / spores

Field pyrolysis of bacteria has been demonstrated using a variety of pyrolyzers, and in a field situation these bacteria would be whole cells. Whole cell bacteria have more mass than the chemicals discussed in the previous sections, and the utility of the micropyrolyzer for field detection lies in the ability to perform pyrolysis/methylation of whole cell bacteria. Results show that the micropyrolyzer is indeed capable of whole cell pyrolysis/methylation. Figure 39 compares the pyrolysis of whole cell *Bacillus subtilis* with and without methylation reagent. In the upper chromatogram (without reagent) a large amount of low molecular weight fragments/species are observed below 2 minutes retention time. A few other products are observed below 6 minutes. In the lower chromatogram (with TMAH) there is also a large peak below 2 minutes, most of which however are the byproducts from the TMAH. What can also be observed is a number of higher molecular weight species with retention times greater than 6 minutes. In this total ion chromatogram two FAMEs (indicated by arrows) were observed. This demonstrates that the micropyrolyzer is capable of transforming whole cells into FAMEs for analysis.

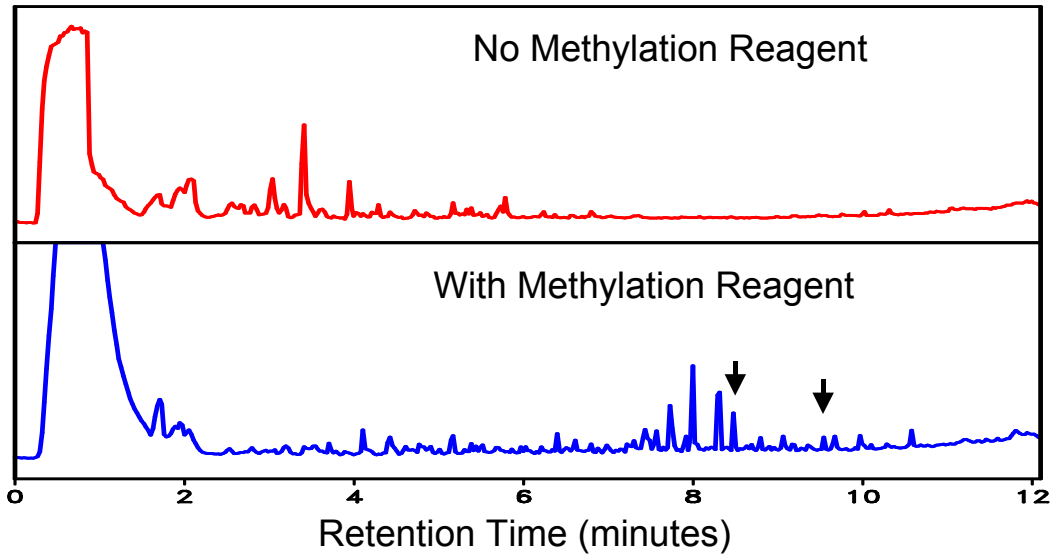


Figure 39: Total ion chromatogram of *Bacillus subtilis* micropyrolysis with and without methylation reagent (same y-axis scale).

It is important that this transformation is reproducible, and the following figure plots several total ion chromatograms each for Pseudomonas fluorescens and Bacillus subtilis. While there are a couple minor differences, the replicates are fairly similar. The large peak at the beginning of each chromatogram is primarily byproducts from the TMAH.

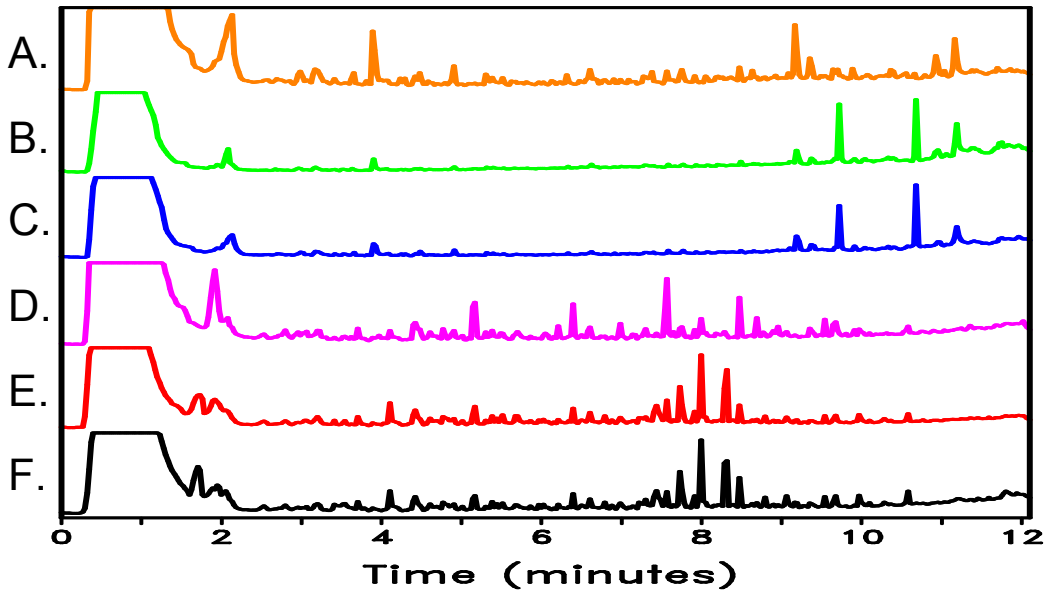


Figure 40: Total ion chromatograms of micropyrolysis/methylation products of *Pseudomonas fluorescens* (A-C) and *Bacillus subtilis* (D-F).

A total ion chromatogram contains a full mass spectrum at each point in time. An alternative display and mode of data analysis is to plot extracted ion chromatograms in which a single mass is plotted versus retention time. This is often used for the detection of known species within a complex chromatogram. For FAMEs the indicative ions are the fragment peaks at m/z 74 and 87. An extracted ion chromatogram of the micropyrolysis/methylation of *Bacillus subtilis* (chromatogram F Figure 40) is plotted in Figure 41. A FAME is confirmed at each point where both m/z 74 and 87 are detected, this assignment is supported by the use of standards such as in Figure 27.

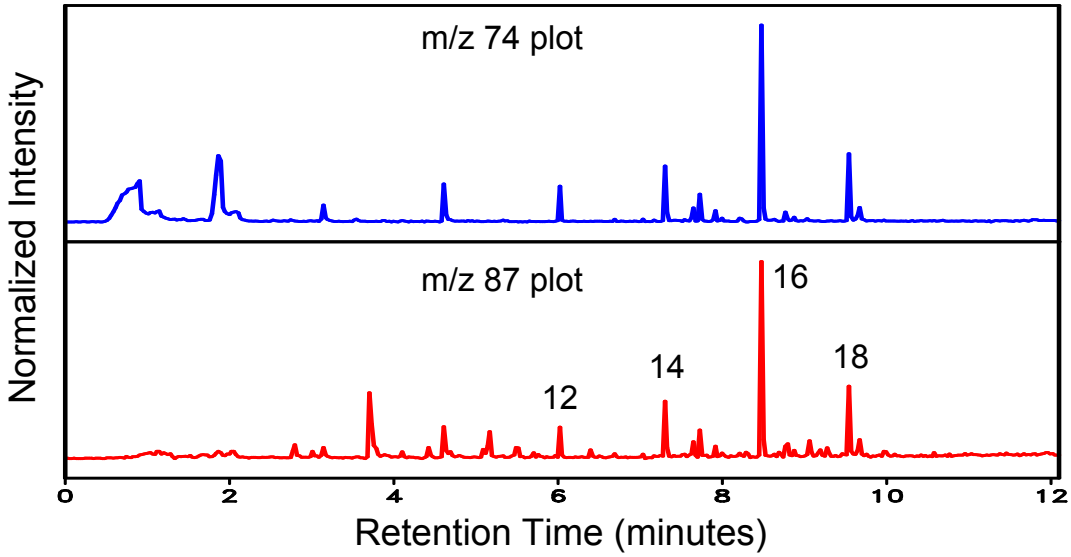


Figure 41: Extracted ion chromatograms (m/z 74, 87) from the micropyrolysis/methylation of *Bacillus subtilis*.

In order to compare results from the pyrolysis/methylation of different bacteria, which is visually difficult using total ion chromatograms, it is convenient to compare a single extracted ion chromatogram. Such a comparison is shown below for the two bacteria analyzed in this work. The FAMEs detected in each case are labeled. Since these bacteria are unrelated taxonomically, they should and indeed do, have very different FAMEs. This illustrates to a first degree how a field portable unit would operate – using the particular FAMEs and their quantities to crosscheck library values for identification purposes.

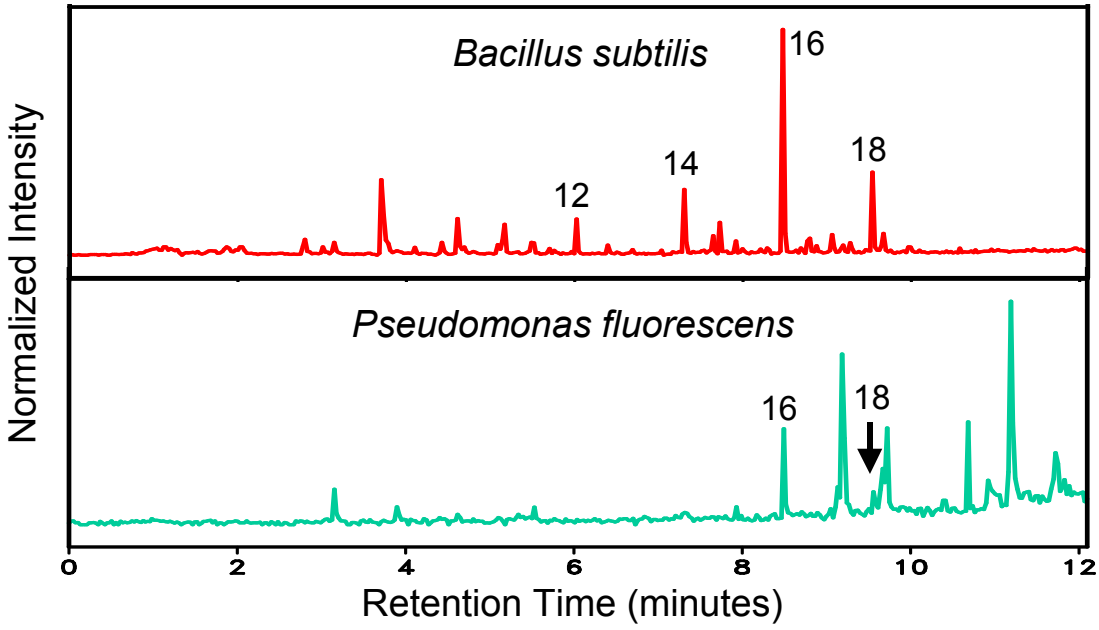


Figure 42: Comparison of m/z 87 extracted ion chromatograms from the micropyrolysis/methylation of *B. subtilis* and *P. fluorescens*.

4.1.6. Alternative Reagents

Because of degradation of the micropyrolyzer membrane at the onset of the LDRD, and to demonstrate a wider performance range, alternative reagents (non-TMAH) were tested. Several commercially available methylating reagents were obtained, including Meth Prep II (m-trifluoro-methylphenyl trimethylammonium hydroxide), MethElute™ (trimethylphenylammonium hydroxide). These reagents react just as the TMAH reagent does, producing FAMEs from fatty acids. In the data shown below, emphasis was placed not on the chromatography but on whether the reagent tested was producing FAMEs. The pyrolysis products were detected by mass spectrometry so that the mass spectrum of peaks detected could be compared to library spectra as in previous tests. Figure 43 and Figure 44 show the total ion chromatograms of successful pyrolysis/methylation using the Meth Elute and Meth Prep II reagents, respectively. The mass spectrum of the C16 FAME peak, which elutes just after 5 minutes, is also shown. The C18 FAME peak is asymmetric due to the unoptimized chromatographic conditions.

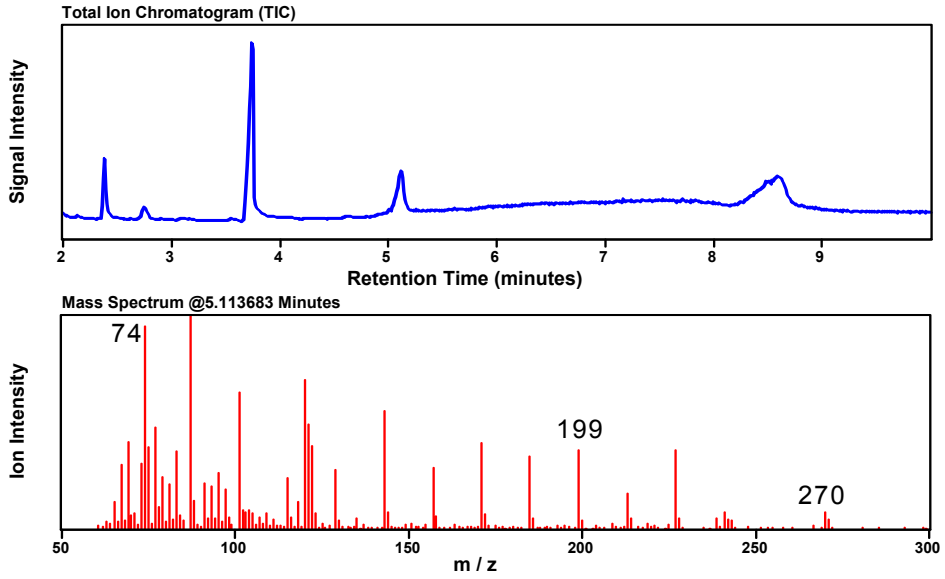


Figure 43: TIC and mass spectrum of micropyrolysis/methylation products of C14, 16, 18 saturated fatty acids using Meth Elute reagent.

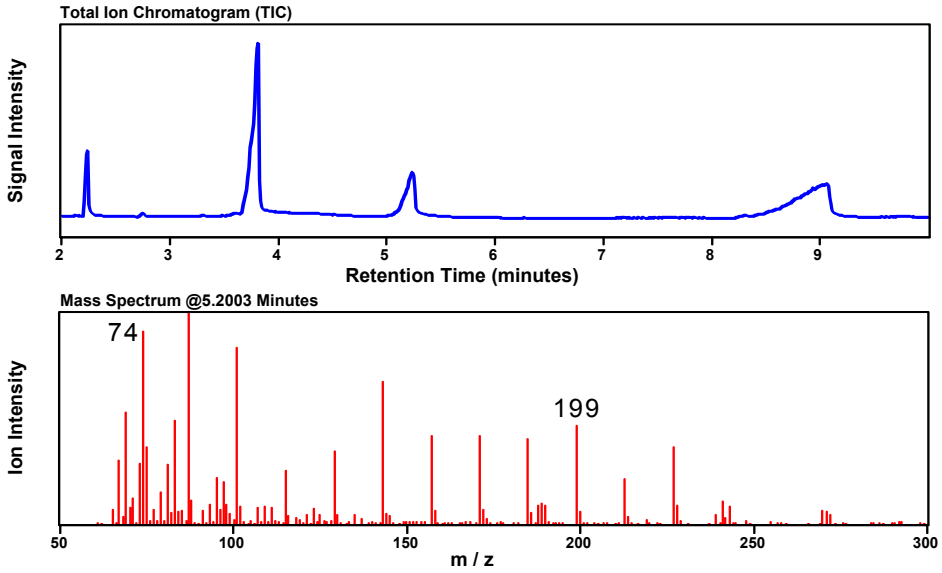


Figure 44: TIC and mass spectrum of micropyrolysis/methylation products of C14, 16, 18 saturated fatty acids using the Meth Prep II reagent.

These results demonstrate that other reagents can be used for methylation, which is important for tailoring the reactions for other detection modes such as electron capture or optical. These alternative detection modes may provide advantages over the SAW detector discussed later in this document.

4.1.7. Colorado School of Mines micropyrolysis

Micropyrolysis and micropyrolysis/methylation experiments were performed at the Colorado School of Mines as described in the

Experimental Details section. The goal was to determine if the micropyrolyzer could perform the pyrolysis/methylation reaction on whole cells and/or spores. Initially micropyrolysis and micropyrolysis/methylation tests were performed on DPA. A mass spectrum of each test is shown below. In the pyrolysis-only experiment (upper spectrum), only the pyridine degradation product of DPA was detected. In the SNL work described earlier this product was not detected, only a small amount of self-methylated DPA. The transfer line and fixture temperature in the CSM work is higher and might explain the full degradation to pyridine. In the micropyrolysis/methylation results (lower spectrum), mDPA was detected as expected. These experiments formed a baseline for the bacterial and spore tests that follow.

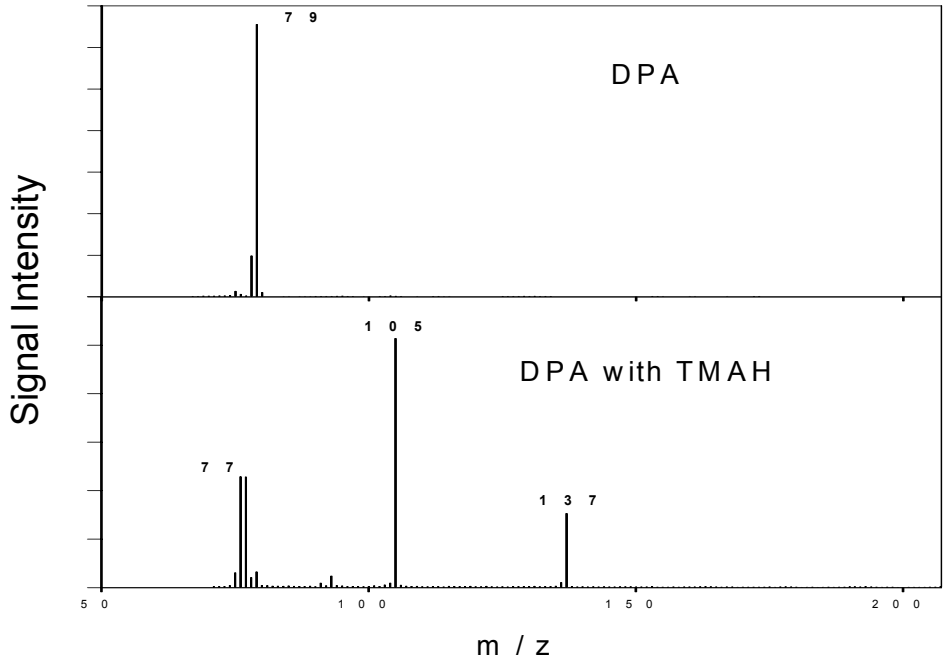


Figure 45: Mass spectra of products detected in micropyrolysis (upper) and micropyrolysis/methylation (lower) of DPA with TMAH.

Viable *Bacillus anthracis* sterne spores were micropyrolyzed with and without the methylating reagent TMAH. Mass spectra of the primary product peak detected in the instrument in each case are shown in Figure 46. The major mass peaks detected in the micropyrolysis test (Figure 46A), m/z 117, 91, and 79 are thought to originate from an unknown, aromatic amino acids and protein degradation, and pyridine (product of DPA breakdown), respectively. No mass peaks were observed above m/z 200. This is a typical result from pyrolysis-only.

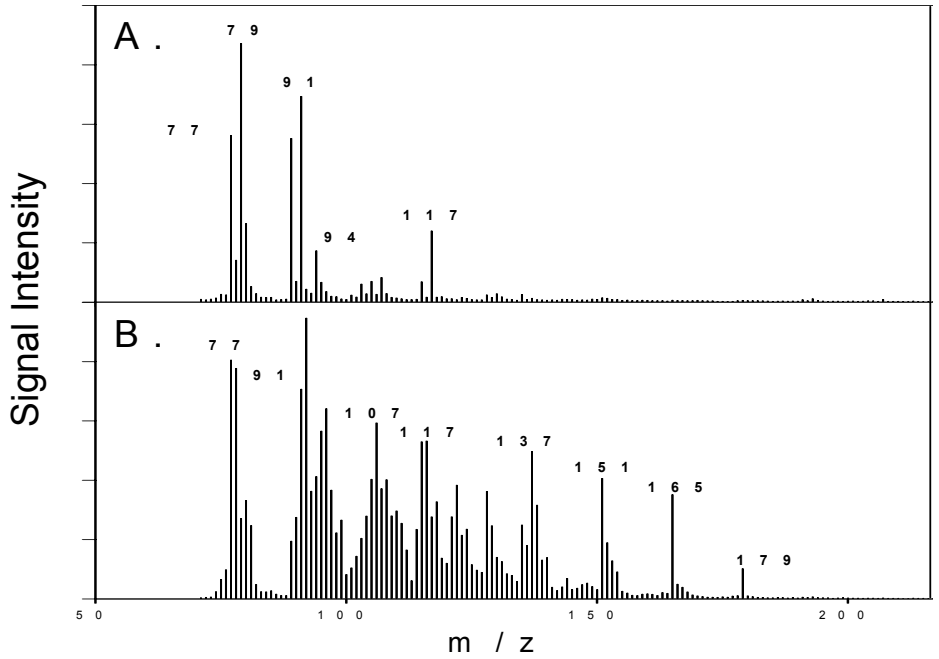


Figure 46: Micropyrolysis (A) and micropyrolysis/methylation (B) of viable *Bacillus anthracis* sterne spores.

The mass spectrum of spore micropyrolysis/methylation (Figure 46B) shows more peaks and higher mass peaks. The molecular ion peak for mDPA is observed at m/z 137. The peaks at m/z 165 and 179 are thought to originate from methylated guanine, a methylated DNA product. Representative FAME peaks were not observed as expected from bench-top pyrolysis experiments. It is not known why FAME peaks were absent, however one possibility is that they were retained by the transfer line and did not elute in a sharp pulse due to the slow transfer flow rate required by the instrument.

Similar results were observed for *Bacillus globigii* spores (gamma-killed, lyophilized) and for vegetative cells of *Bacillus anthracis* vollum. Results for *Bacillus anthracis* zimbabwe spores (gamma-killed, lyophilized) showed fewer mass peaks and no peaks indicative of DPA. Again, the lack of FAME peaks was unexpected and could not be conclusively determined.

It is unknown why the micropyrolyzer did not produce results similar to the larger and less portable bench-top pyrolyzer used by the CSM laboratory. Further tests are warranted.

4.1.8. Summary

The use of the micropyrolyzer for several tasks was successfully demonstrated. These tasks included A) vaporizing chemicals from mDPA to FAMEs, B) performing pyrolysis/methylation reactions on fatty acids and biomarker compounds (using TMAH or other reagents), and C) performing TMAH pyrolysis/methylation reactions on whole cell bacteria, including spores. The task

of pyrolysis/methylation of spores was only partially successful, creating mDPA but no FAME species.

The micropyrolyzer heated rapidly (500°C in less than 100 milliseconds), was capable of heating with a bacterial sample load, and required less than 150 milliamps (at between 8-15 volts) to effect the reactions. These voltage and power requirements are easily fulfilled in a field portable instrumentation concept.

4.2. Microfabricated GC Column

In the original concept for a fieldable pyrolysis instrument for bacterial detection, separation of the pyrolysis products would be achieved using a microfabricated gas chromatography (GC) column. In comparison to other work using Sandia microGC columns, the separation of FAMEs requires higher temperatures. This is a challenge in the context of field use because portability requires the use of air carrier gas, and typical GC stationary phases are not stable at high temperatures in air. Air degradation has not been observed in other µChemlab work, which operate at lower temperatures. It is a key point to be addressed for a fieldable instrument.

From GC manufacturer catalogs, column phases for use with bacterial FAMEs range from nonpolar to high polarity, depending on the analysis emphasis. The column used in the bench-top GC/MS for pyrolyzer testing (a DB-23 phase) has high polarity, chosen to gain excellent separation (on a longer column) for both saturated and unsaturated FAMEs. For the microGC, a lower polarity phase represents an appropriate starting point since nonpolar columns tend to be more temperature stable. The µChemLab program has fabricated five different types of microGC columns, from nonpolar to high polarity, offering a full range of options. A summary of the microGC columns that have been tested for FAME separation performance is shown in the following table.

Table 19: Summary of microGC columns tested using FAMEs or biomarkers.

column	phase, i.d., length	data/notes
154	OV225, 100um, 86cm	c8-17, mDPA, mixed fames
158	OV101	
079	OV101	temperature ramps
181	ov-3 poly(phenylmethyldimethyl) siloxane (10% phenyl	surface etch label = 44
182	ov-3	surface etch label = 36

These columns were tested under a variety of conditions including isothermal or temperature ramped operation and a variety of carrier gases including helium, nitrogen, and air.

4.2.1. Isothermal

Isothermal operation can require less power consumption than temperature ramping, and can be easier to implement in a field system. The FAMEs and biomarkers necessary for bacterial detection span a large range of volatilities and molecular weights. With such a large range, optimizing the separation is difficult under isothermal conditions. Pressure/flow ramping can be used to compensate, but also requires more sophisticated or complicated equipment and power and was not considered.

Some examples of the separations possible using a microGC under isothermal conditions are shown in Figure 47. This particular microGC (#026, OV-17 phase) is narrower and longer than the typical column used in the µChemLab program – having a width of 52 microns and a length of 150 cm. A helium carrier gas was used which gives better performance than air. The resolution is excellent at 5 psi. and 100°C (upper chromatogram), but analysis time is very long in the context of portable analysis. The analysis time is significantly reduced at a higher temperature (120°C, lower trace) with acceptable resolution, however this analysis only covers up to C12 FAME. Biologically relevant fatty acids range up to C24, and the reader should note the separation in time increases between subsequently larger FAMEs. Even at 120°C the analysis would be too lengthy. At higher pressures the lower FAMEs would overlap.

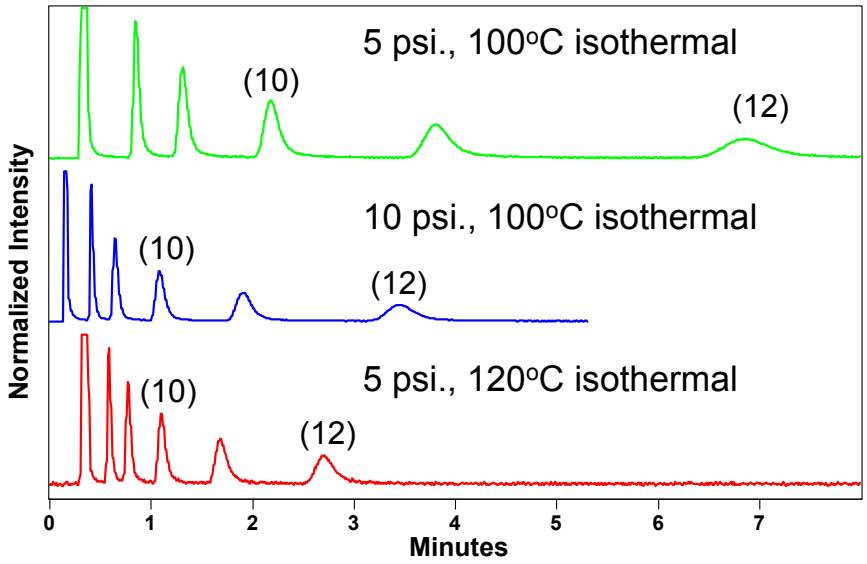


Figure 47: Separation of C8-10 FAMEs using a microGC column (for details see text).

The effect on retention times of an air carrier gas (as compared to nitrogen) was briefly evaluated under isothermal conditions. The results of this comparison (see Figure 48) demonstrate only a small difference in the separation of C8-12 FAMEs.

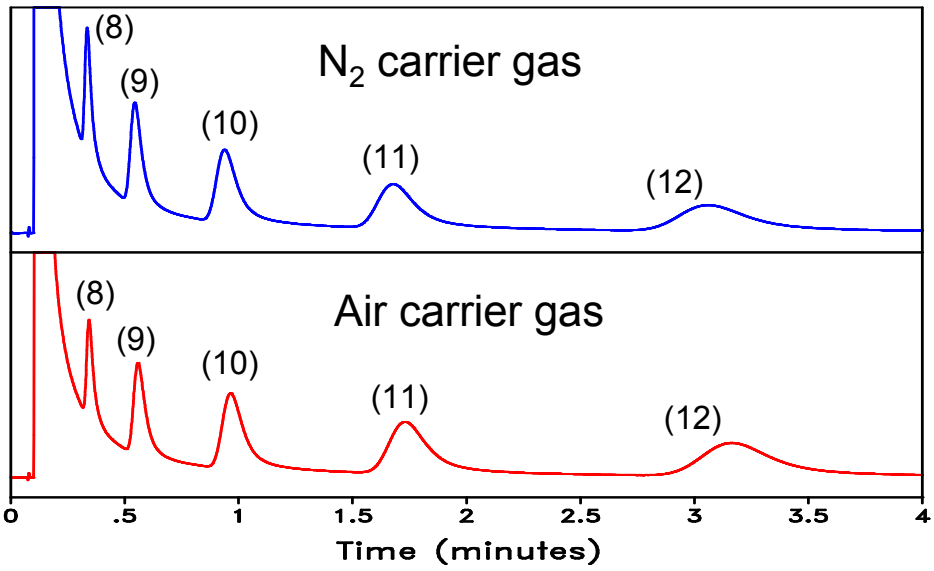


Figure 48: Comparison of FAME separation using a microGC column (#026) and nitrogen or air carrier.

At the current state-of-the-art of microGC columns, the resolution does not allow for isothermal analysis of these analytes. There may be an application, however, that utilizes a narrower range of FAMEs, and in this case the current microGCs would be acceptable.

4.2.2. Temperature ramped

One common solution to lengthy chromatography is to perform a temperature ramp during the analysis. Figure 49 shows separation of an equal mix of FAMEs from C8-17 on a one meter long microGC column developed for µChemLab; the temperature was ramped from 60-150°C and the column phase was equivalent to the OV1, a nonpolar column phase; the total GC process time was less than 5 minutes. The key points demonstrated by these results are that high molecular weight FAMEs can be separated using a microGC column in a rapid analysis. Figure 49 shows a rise in baseline at the higher temperatures, indicating column bleed, or loss in polymer from the column; the more the bleed, the shorter the lifetime of the column.

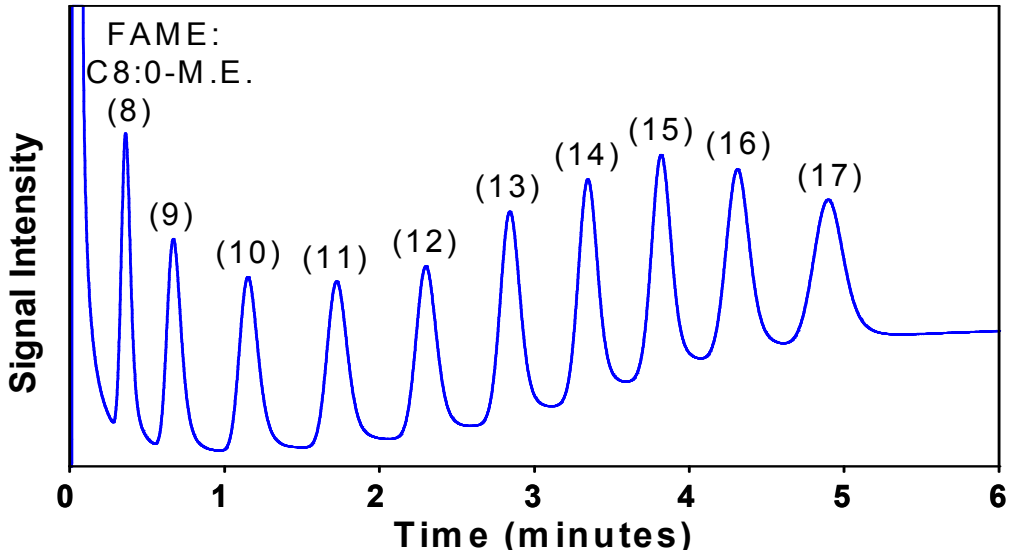


Figure 49: FAME separation using temperature ramped microGC column.

The upper temperature limit on the current columns is due to the fixtures – the epoxies and interconnect fittings used at the inlet and outlet of the microfabricated devices. The polymers used for GC column phases can easily tolerate temperatures up to 210°C and higher, subject to issues of column lifetime.

Current columns have been tested up to 20 psi, and higher temperature epoxies are being used to enable higher temperature usage. An example of this is shown in Figure 50, showing rapid (less than one minute) separation of FAMEs (C8-12) using high pressure and rapid temperature ramping of 40°C per minute from 100 to 140°C. This microGC column (#079) is coated with OV101 and is one meter long.

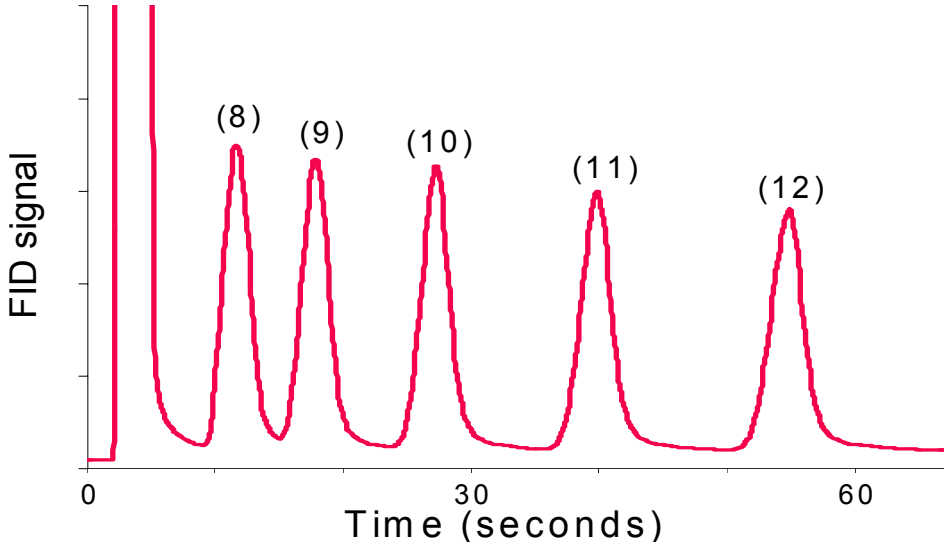


Figure 50: Rapid (less than 1 minute) microGC chromatography of C8-12 FAMEs.

Temperature ramping can improve the analysis time for biomarker compounds as well. The following chromatograms illustrate the effect of different ramp rates on the separation of mDPA and a mixture which includes saturated and unsaturated of FAMEs. Note that the elution order does not change with the ramp that starts at a higher initial temperature. The retention time of mDPA is between the C12 and C13 FAME peaks. Note also that this column does not separate the saturated from the unsaturated FAMEs. This could be a limitation in some applications including bacterial detection.

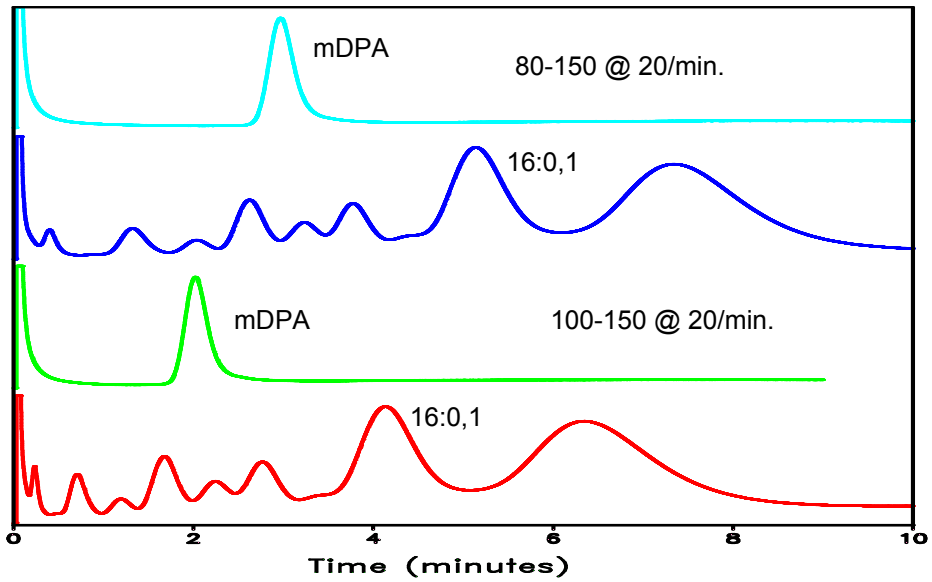


Figure 51: Temperature ramped separation of mDPA and FAMEs using microGC (#158).

A different column coating can be used to change the separation characteristics of the mDPA and FAMEs. Using the same temperature ramp conditions on a column with an OV225 coating moves the relative retention of mDPA as shown in Figure 52. The mDPA peak overlaps partially with the C16:0/C16:1 peak. The lack of separation between the saturated and unsaturated FAMEs is also observed with this column.

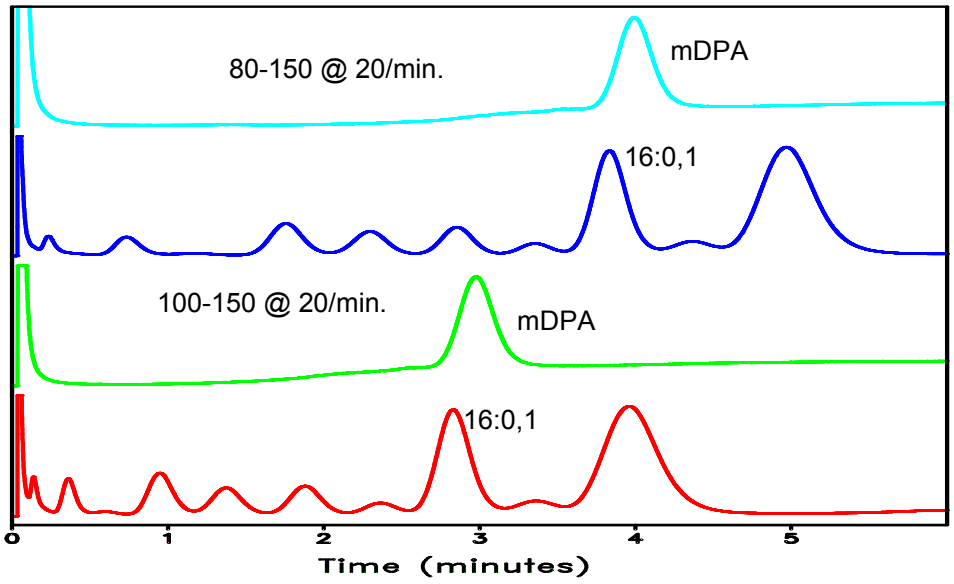


Figure 52: Temperature ramped separation of mDPA and FAMEs using microGC (#154).

4.2.3. Summary

The microGC experiments performed under this LDRD have demonstrated successful separation of saturated FAMEs using both isothermal and temperature ramp profiles. Temperature ramping provides a more rapid separation as expected. There is moderate column bleed at elevated temperatures, verifying need for modest improvements in phase stability.

There is proof in the literature and in column catalogs, however, that a number of column phases can be made with long lifetimes even when used the moderately high temperatures, as in this project, and with air as the carrier gas. Stability of column phases is dependent on a number of fabrication issues, including the degree of column coverage, the strength of the polymerization, and surface adhesion. Continuing research into more stable coatings should continue.

4.3. Surface Acoustic Wave Detection

The surface acoustic wave (SAW) detector developed for the µChemLab program was the primary detector in the original LDRD proposal for the pyrolysis/microGC biological detection concept. The challenge was understood that FAMEs are much larger and less volatile compounds that those normally detected with SAW detectors. Since the µChemLab SAW uses coatings in order to interact with the analyte, there would be potential difficulty with these coatings being reversible with respect to FAMEs.

4.3.1. FAMEs

Normally the SAW array is used for smaller molecules – detection of FAMEs is not considered straightforward. The SAW coating must bind the FAME in a reversible

manner to achieve proper detection. Figure 53 demonstrates a significant achievement in the development of a portable biodetector – the detection of a 32 ppm vapor of C6 FAME by a 2 sensor SAW array. SAW sensor signal is plotted versus time. Rapid signal rise and fall is observed as the FAME is introduced/removed from the gas stream, indicating a rapidly reversible interaction.

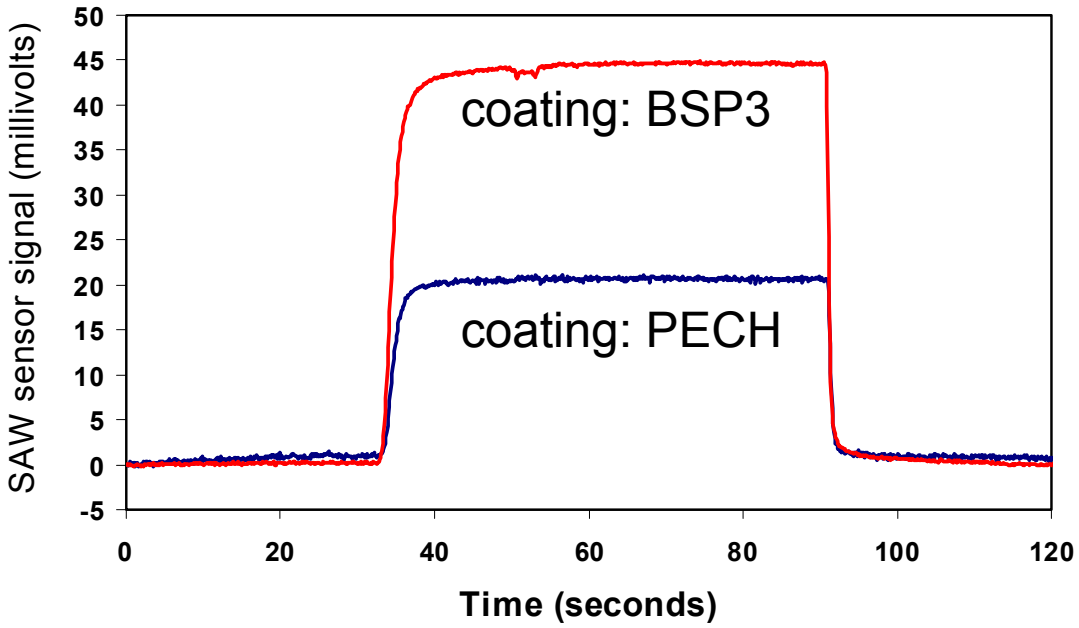


Figure 53: SAW detection of C6 FAME vapor.

One device was coated with a fluoropolyol polymer designated “BSP3” [66] while the other was coated with polyepichlorohydrin (PECH). The signal level for each SAW demonstrates that the response factor for methyl caproate varies for each SAW. This type of response is desirable because the signal ratio would give an additional factor of confidence (in addition to retention time) in the measurement. While this FAME is more volatile and less indicative for the detection of bacteria, it is encouraging that the SAW response was both rapid and reversible. The difficulty in testing higher molecular weight FAMEs is in producing a constant vapor source. In addition, the signal ratio between the poly-epichlorohydrin (PECH) coated and BSP3-coated sensors could be used to differentiate a FAME analyte versus a coeluting interferant – decreasing the probability of a false positive detection. The chemical selectivity of the coatings also minimizes interference from high background signals such as diesel.

A second test was performed, as described in the

Experimental Details section, using a 1 ppm vapor stream of C12:0 methyl ester (methyl laurate). A "full" µChemLab system was utilized – consisting of a preconcentrator, microGC, and SAW detector. A mass calculated at 1.75 µg of FAME (8.17 nmol) was collected, desorbed, sent through the microGC and across the SAW detector. SAW response is plotted in Figure 54.

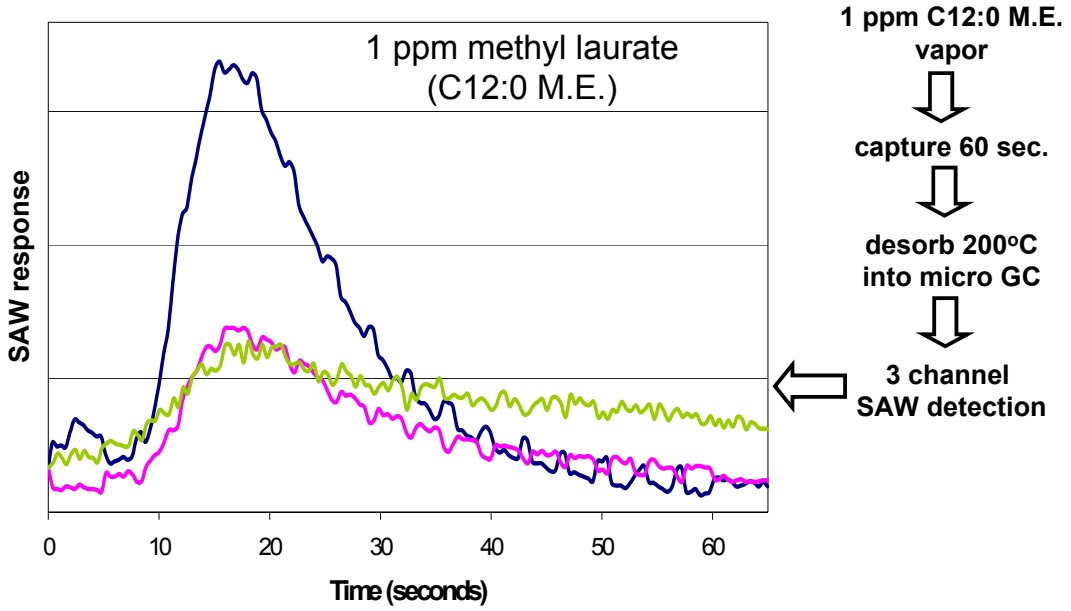


Figure 54: µChemlab system detection of C12:0 FAME vapor.

Additional tests were performed with a single channel SAW detector with micropyrolyzer introduction of C16 FAME. Data from several tests is plotted in Figure 55. The SAW response is complicated by the fact that the airflow was heated to keep the FAME in the gas phase and the SAW is temperature sensitive. The initial response was proportional, however, to the amount of FAME introduced. This result is encouraging but illustrates the lack of reversibility for this higher molecular weight FAME.

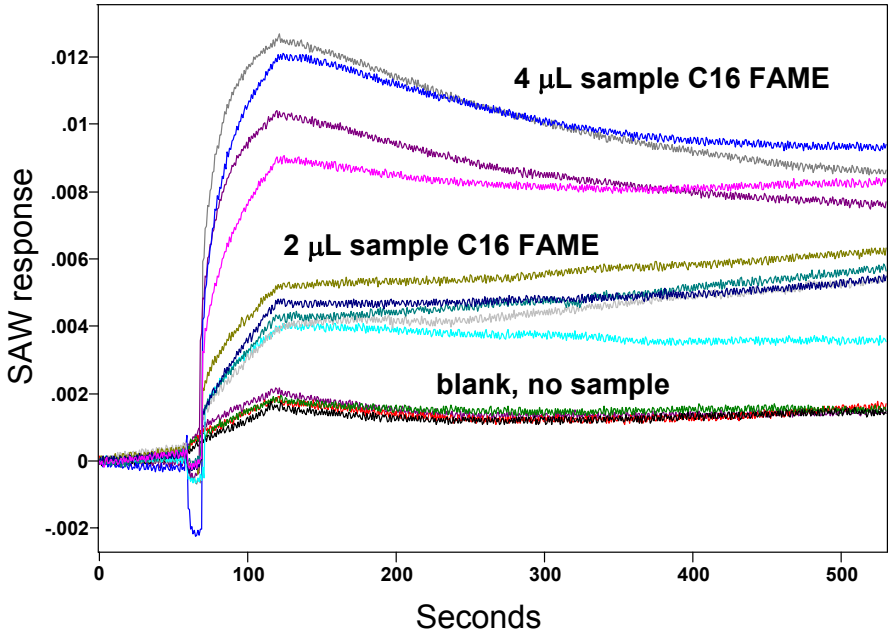


Figure 55: SAW detection of C16 FAME.

Looking more closely at the SAW response, the initial rapid rise when the micropyrolyzer is energized (fired) and the FAME interacts with the SAW can be observed in Figure 56. Following that response is an increase in the signal caused by the hot airflow that slowly decreases once the flow is turned off. A return of the signal to baseline does not occur for many minutes – suggesting that the C16 FAME is only slowly desorbing out of the SAW coating.

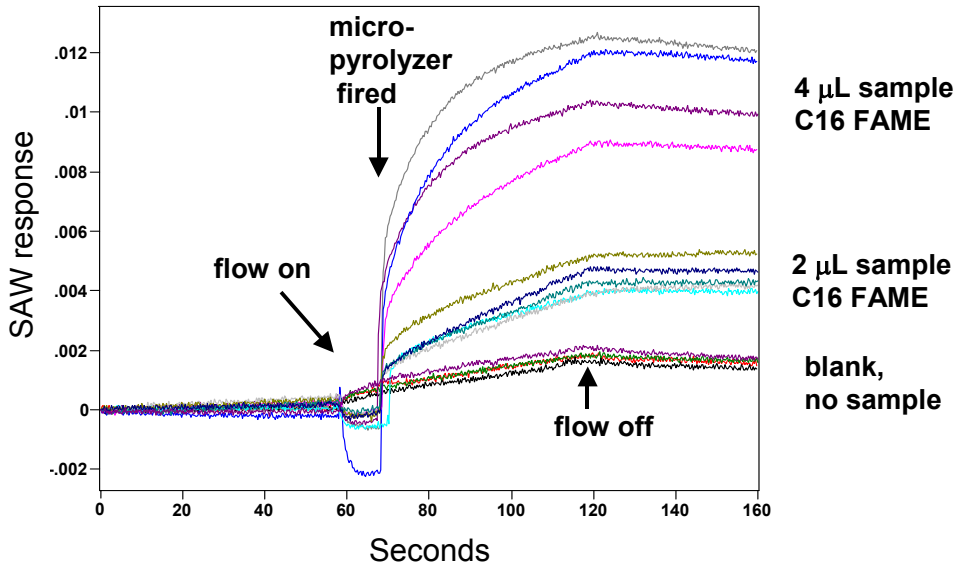


Figure 56: Zoomed view of SAW response to C16 FAME.

4.3.2. Dipicolinic acid

Experiments with mDPA were inconclusive on the single SAW detector used for C16 FAME detection. Some response was observed but was not reproducible. It is not known whether this was caused by an absorption problem or temperature issues with the test fixture.

4.3.3. Summary

Current SAW coatings showed reversible response for relatively volatile FAMEs, and slowly reversible response to C16 FAME. While some results were encouraging for the use of a SAW detector in the original biological detection concept – additional work is necessary to increase the operating temperature and/or produce SAW coatings that are more reversible for FAMEs up to the approximate C22 range that would be useful for biological detection.

4.4. Issues to Resolve

SAW array detectors have been used extensively with the microGC technology at Sandia and have many benefits such as small size and low power requirements. Other miniaturized detectors under development by Sandia include an ion mobility spectrometer and flame ionization detector. These (non-SAW) detectors have not reached the stage of development at which FAME tests could be conducted,

however these detectors could provide a significant advantage over SAW technology and FAME tests should be performed as these detectors become available.

Another issue that has not been fully addressed is the difference between micropyrolysis in helium versus nitrogen versus air. Most of the work presented here was under helium or nitrogen, whereas a field instrument would use only air. This will likely have a greater impact on the chromatography rather than the pyrolysis, but the issue should be addressed.

5. Conclusions

In conclusion, major advancements have been made toward the goal of a miniature sensor for biological warfare agents. It has been demonstrated that Sandia's microfabricated devices can perform the tasks of pyrolysis and separation needed to achieve this goal. Low power and small size of these devices are significant advantages toward portable biological detection. While SAW detection was less successful, limitations and future improvements were identified.

Also, it should be noted that the results of this LDRD have brought further research and funding into the laboratories and yielded one technical advance that is in process for patenting[67]. Results have been presented at several conferences and included in conference proceedings [68-73].

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7. Appendix A: Reference mass spectra

7.1. Dipicolinic- and picolinic-related compounds.

All of the following mass spectra were obtained from a commercial mass spectral library database [74].

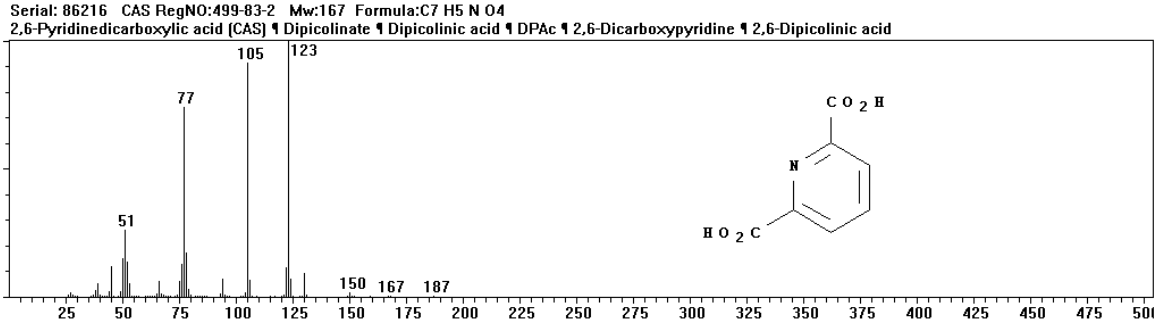


Figure 57: Library mass spectrum of dipicolinic acid (DPA).

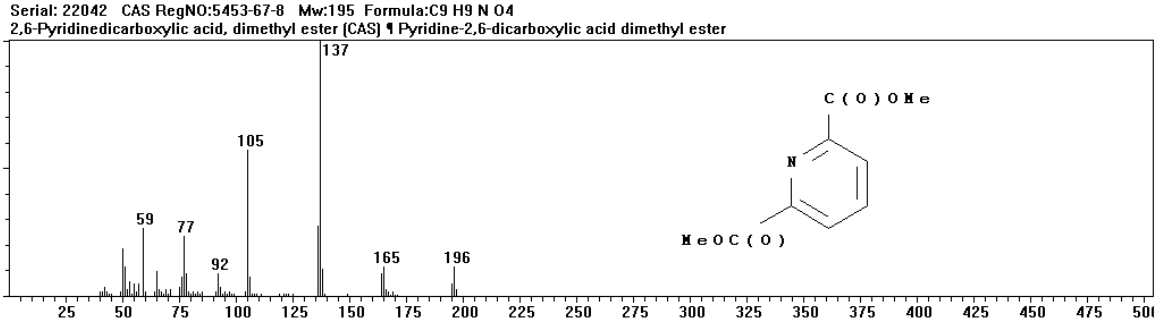


Figure 58: Library mass spectrum of dimethylated dipicolinic acid (mDPA).

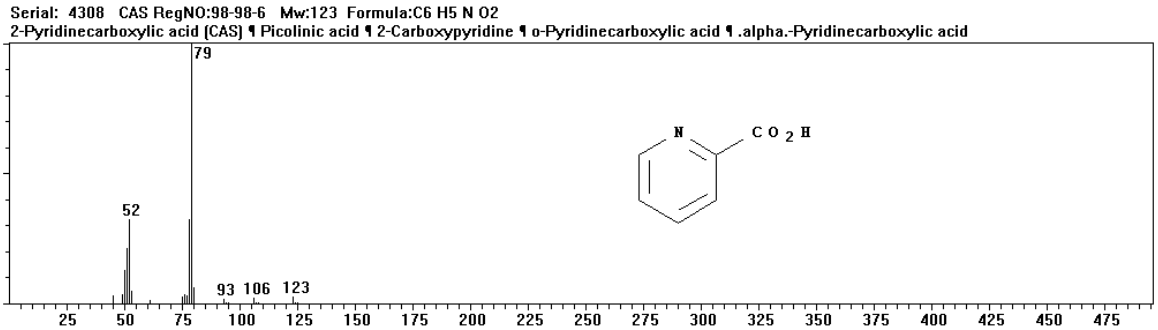


Figure 59: Library mass spectrum of picolinic acid (PA).

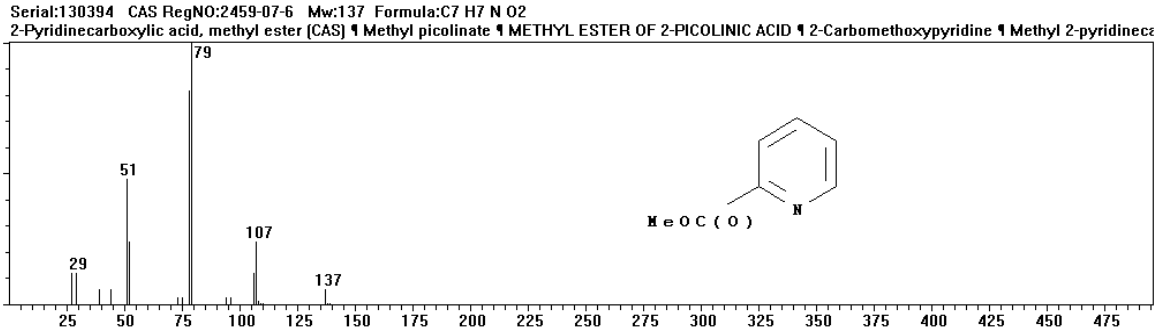


Figure 60: Library mass spectrum of methyl picolinate (mPA).

7.2. Saturated fatty acid methyl esters

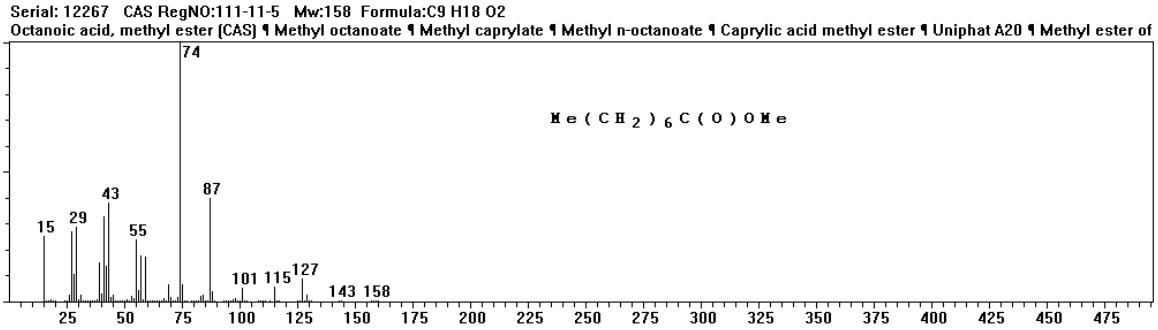


Figure 61: Library mass spectrum of octanoic acid methyl ester (C8:0 ME).

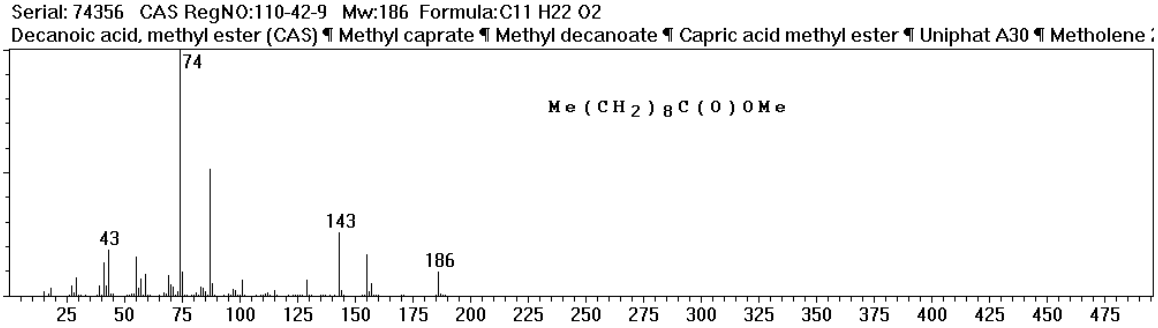


Figure 62: Library mass spectrum of decanoic acid methyl ester (C10:0 ME).

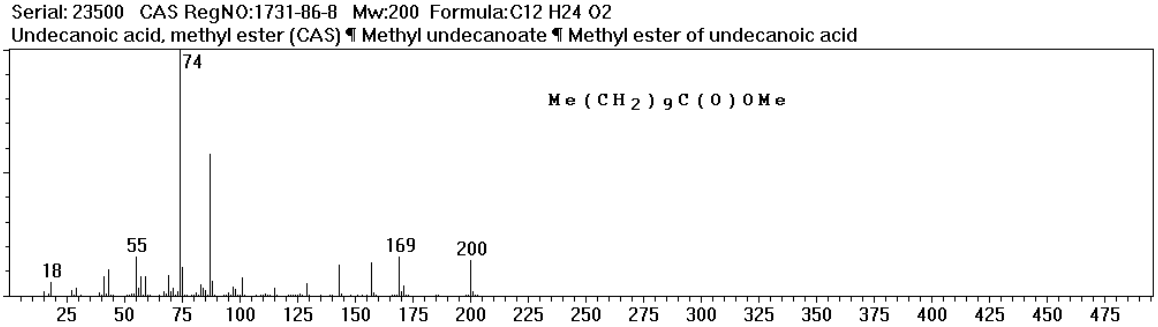


Figure 63: Library mass spectrum of undecanoic acid methyl ester (C11:0 ME).

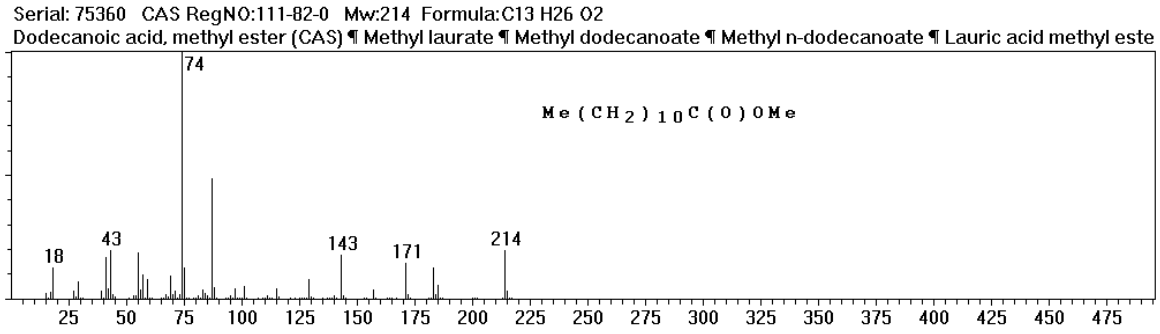


Figure 64: Library mass spectrum of dodecanoic acid methyl ester (C12:0 ME).

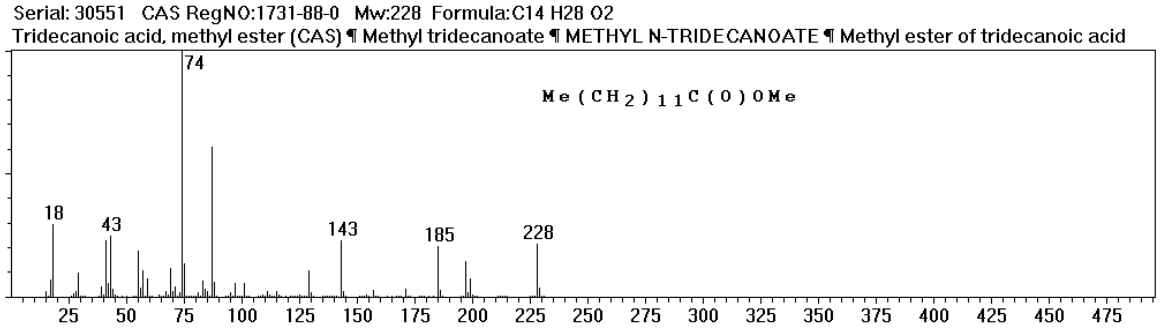


Figure 65: Library mass spectrum of tridecanoic acid methyl ester (C13:0 ME).

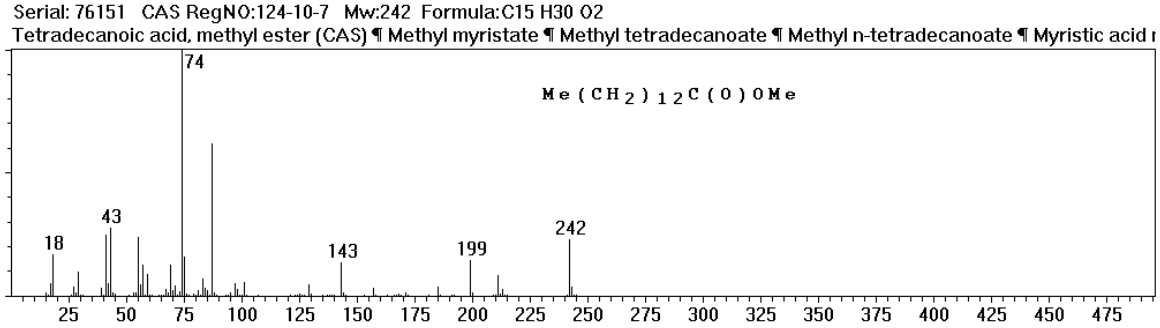


Figure 66: Library mass spectrum of tetradecanoic acid methyl ester (C14:0 ME).

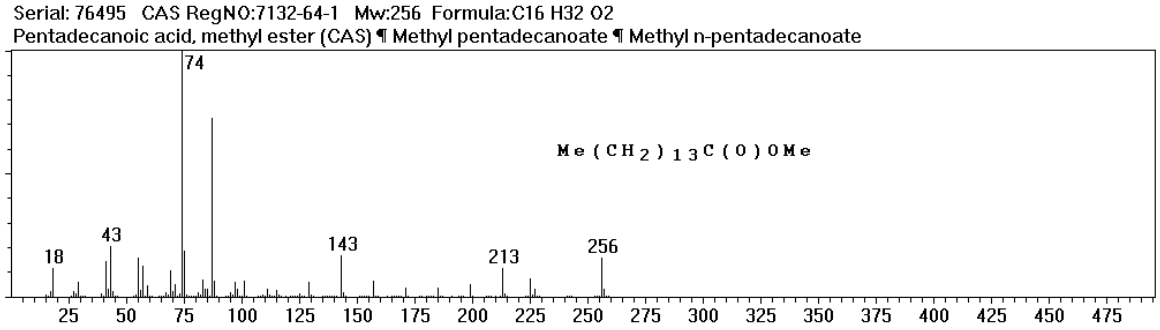


Figure 67: Library mass spectrum of pentadecanoic acid methyl ester (C15:0 ME).

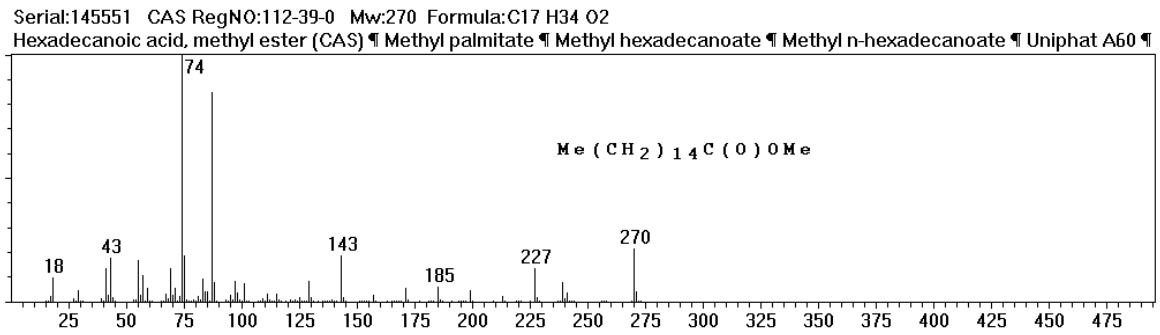


Figure 68: Library mass spectrum of hexadecanoic acid methyl ester (C16:0 ME).

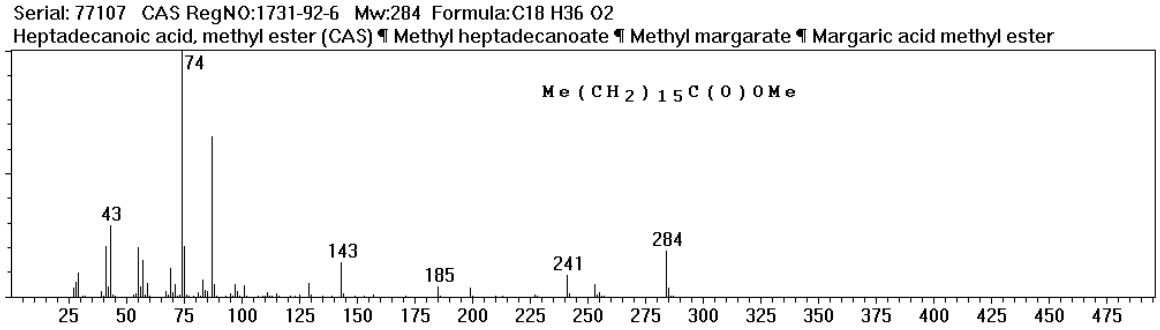


Figure 69: Library mass spectrum of heptadecanoic acid methyl ester (C17:0 ME).

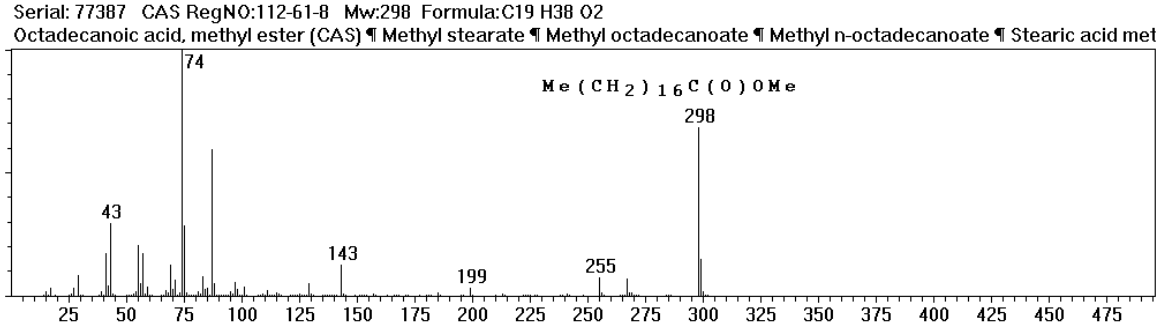


Figure 70: Library mass spectrum of octadecanoic acid methyl ester (C18:0 ME).

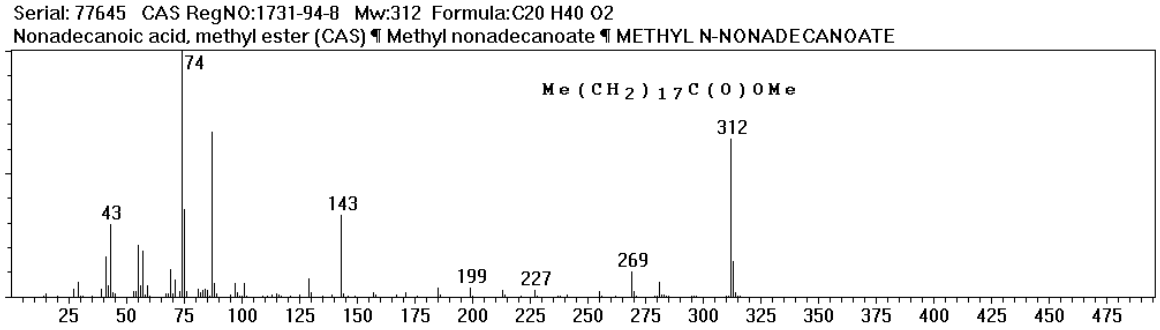


Figure 71: Library mass spectrum of nonadecanoic acid methyl ester (C19:0 ME).

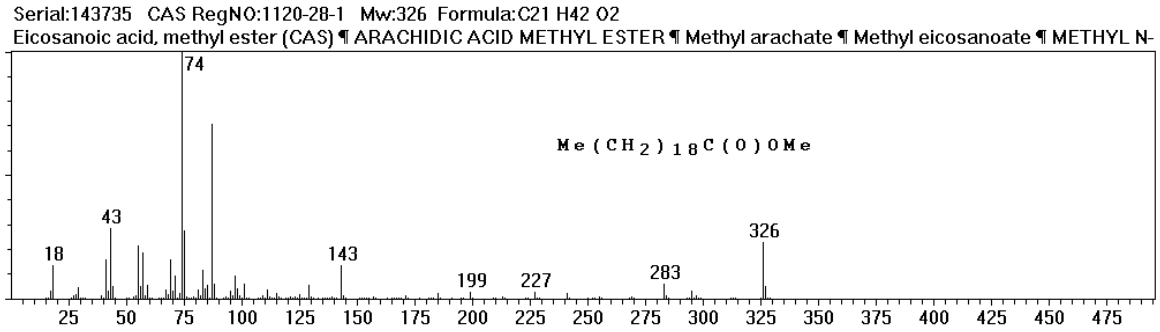


Figure 72: Library mass spectrum of eicosanoic acid methyl ester (C20:0 ME).

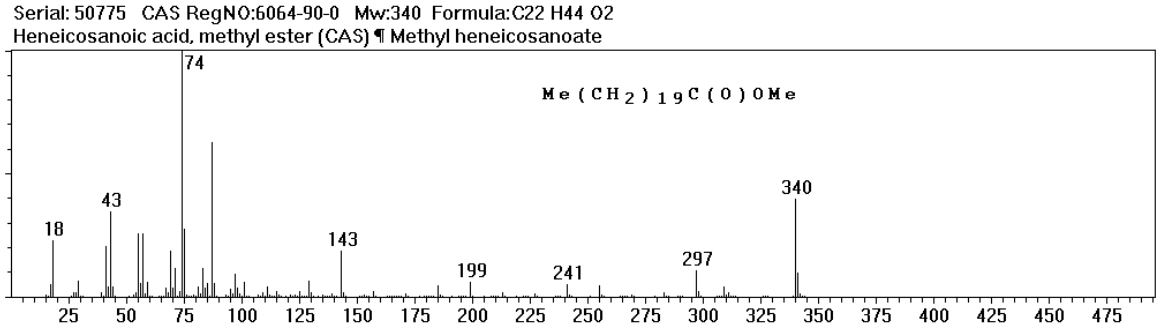


Figure 73: Library mass spectrum of heneicosanoic acid methyl ester (C21:0 ME).

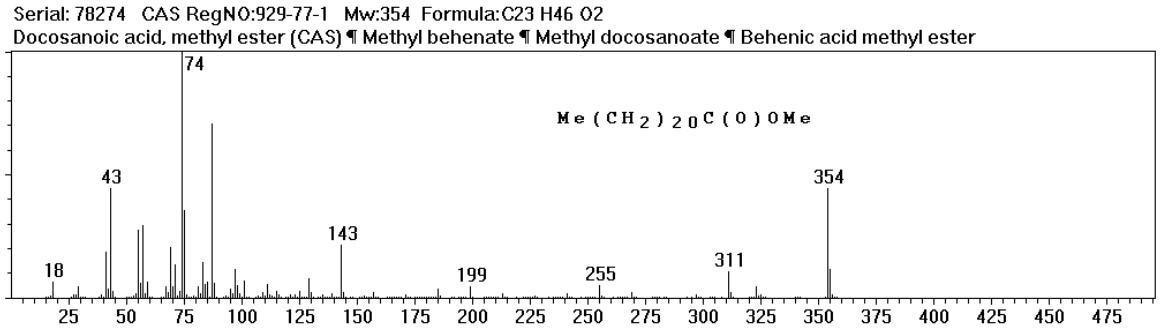


Figure 74: Library mass spectrum of docosanoic acid methyl ester (C22:0 ME).

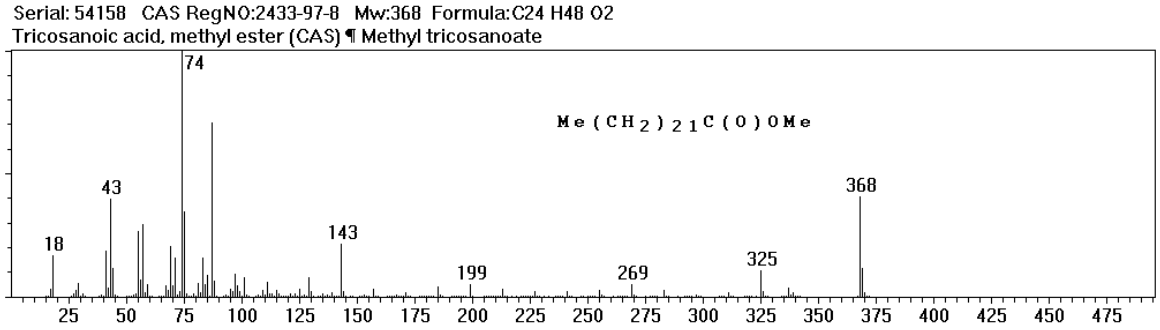


Figure 75: Library mass spectrum of tricosanoic acid methyl ester (C23:0 ME).

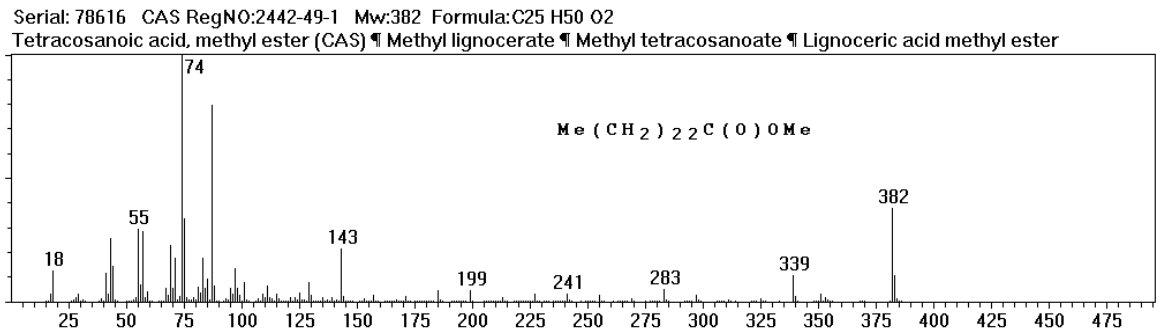


Figure 76: Library mass spectrum of tetracosanoic acid methyl ester (C24:0 ME).

7.3. Unsaturated fatty acid methyl esters

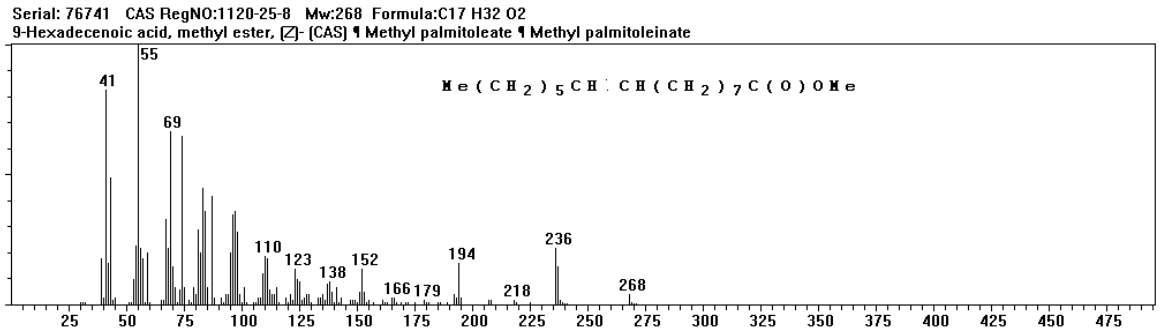


Figure 77: Library mass spectrum of cis-9-hexadecenoate (C16:1 cis-9 ME).

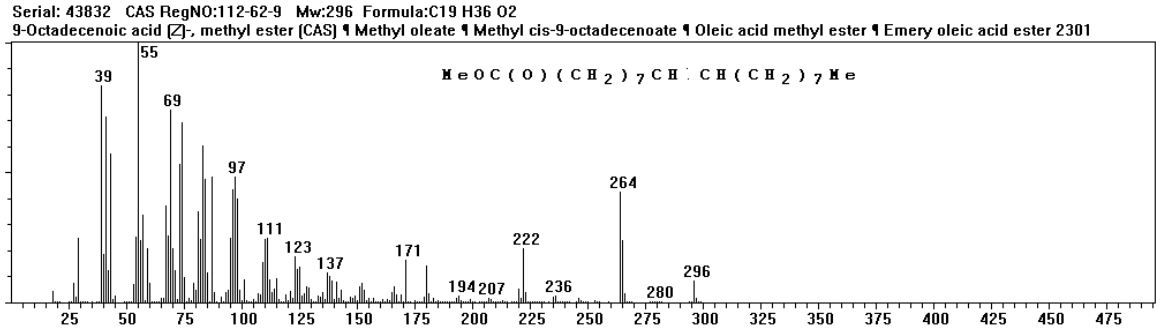


Figure 78: Library mass spectrum of methyl cis-9-octadecenoate (C18:1 cis-9 ME).

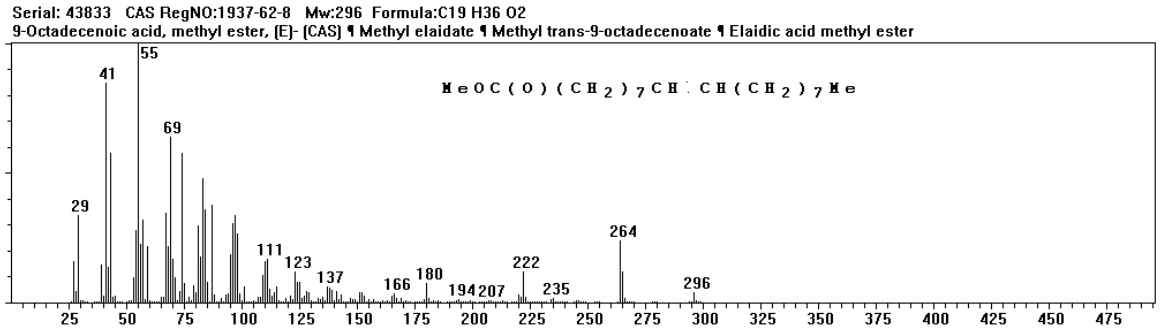


Figure 79: Library mass spectrum of methyl trans-9-octadecenoate (C18:1 trans-9 ME).

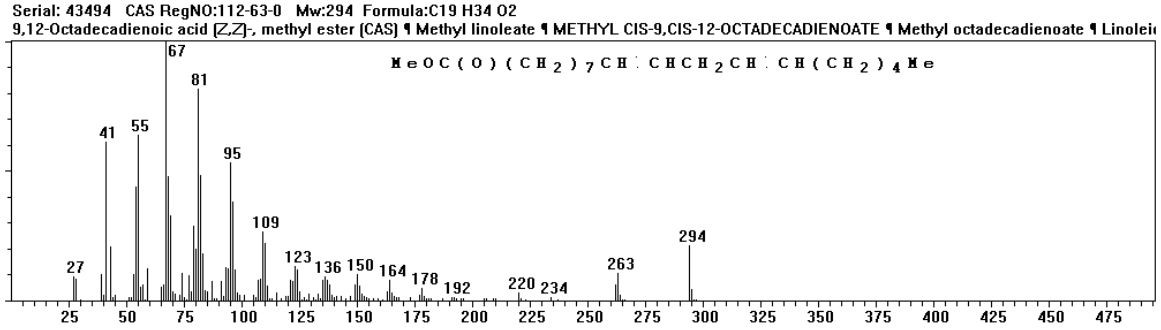


Figure 80: Library mass spectrum of methyl cis-9,12-octadecadienoate (C18:2 cis-9,12 ME).

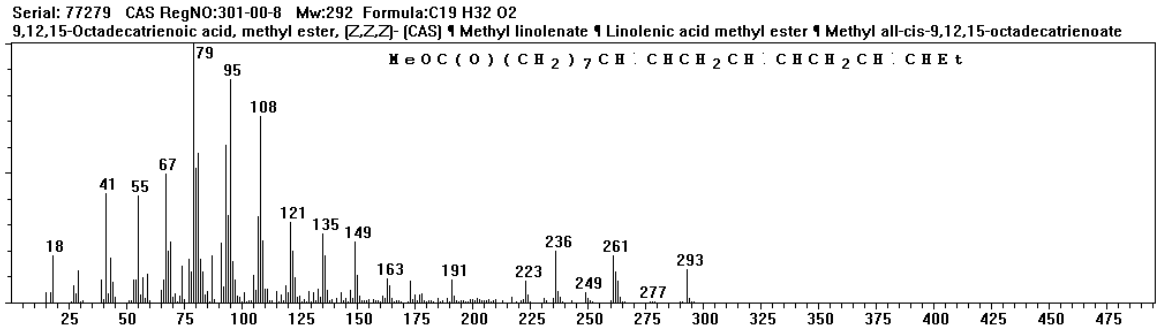


Figure 81: Library mass spectrum of methyl cis-9,12,15-octadecatrienoate (C18:3 cis-9,12,15 ME).

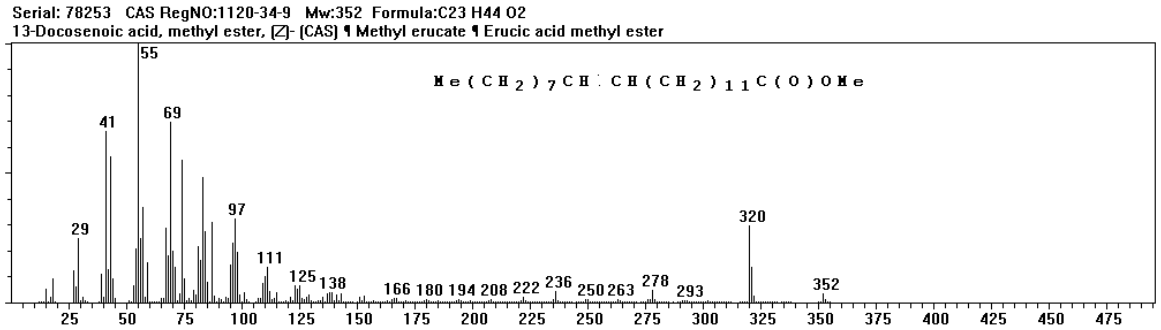


Figure 82: Library mass spectrum of cis-13 docosenoic acid methyl ester (C22:1 cis-13 ME).

7.4. FAME fragment ions

Table 20: Characteristic Ions for FAME detection.

C12:0	214
C14:0	242
C15:0	256,225
aC15:0	256,199
iC15:0	256,213
C16:0	270,239,241,227,213,199,185
C16:1	268,237,236,194
c17:0	284,253
iC17:0	284,241
cyC17:0	282,251,250,208
C18:0	298,267
C18:1	296,265,264,222
C19:0	312,281
cyC19:0	310,279,278,236
C20:0	326,295
C21:0	340,309
C22:0	354,323

8. Appendix B: Chemical reference and physical data.

Table 21: Chemical reference and physical data.

Chemical	Formula and CAS#	m.p. / b.p.	m.w.	Misc.
dimethyl pyridine dicarboxylate (mDPA)	$C_9H_9NO_4$ 5453-67-8	121 to 125 /	195.17	Aldrich 37,933-6
Methyl picolinate (mPA)	$C_7H_7NO_2$ 2459-07-6	liquid at room temp. density=1.137 b.p.=95 (1 mm Hg)	137.14	Aldrich 55,628-9 water solubility (25°C): 4.24E+004 mg/L v.p. 0.277 mm Hg
2,6-Pyridinedicarboxylic acid (dipicolinic acid – DPA)	$C_7H_5NO_4$ 499-83-2	248-250 / flash 188 v.p. 6.1E-006 mm Hg @25C	167.12	water solubility (25°C): 5000mg/L [75] v.p.: 6.1E-006 mm Hg
Pyridine-2-carboxylic Acid (picolinic acid - PA)	$C_6H_5NO_2$ 98-98-6	139-142	123.111	water solubility (25°C): 2.372E+004 mg/L v.p. 0.00789 mm Hg [75]

Table 22: FAME reference and physical data.

carbons in	double bonds	FAME name (-ic -acid methyl ester)	FAME m.w.	FAME b.p. (ºC)	FAME CAS #	FAME Formula	phys-prop data [75]	
1		formate						
2		acetate						
3		proprionate						
4		isobutyrate		90	547-63-7			
4		butyrate		102	623-42-7			
5		isovalerate		114	556-24-1			
5		valerate	116.16	126.5	624-24-8	$C_6H_{12}O_2$		
6		isocaproate	130.19	151 52@15 torr	106-70-7	$C_7H_{14}O_2$		
7		caproate	144.21	172.1	106-73-0	$C_8H_{16}O_2$		

carbons in	double bonds	FAME name (-ic -acid methyl ester)	FAME m.w.	FAME b.p. (ºC)	FAME CAS #	FAME Formula	phys-prop data [75]
8		octanoate	158.24	194.6 83@15 torr	111-11-5	$C_9H_{18}O_2$	192.9 deg C
9		nonanoate	172.27	213.6	1731-84-6	$C_{10}H_{20}O_2$	
10		decanoate	186.29	224.1 114.1@15 torr	110-42-9	$C_{11}H_{22}O_2$	
11		undecanoate	200.32	na	1731-86-8	$C_{12}H_{24}O_2$	
12		laurate	214.34	141.1@15 torr	111-82-0	$C_{13}H_{26}O_2$	0.00411 mm Hg
13		tridecanoate	228.37	131.1@3.7torr 92 [1 mmHg] (crc)	1731-88-0	$C_{14}H_{28}O_2$	
14		myristate	242.40	323.1 295 (crc)	124-10-7	$C_{15}H_{30}O_2$	
14	1	myristoleate (cis-9)	240.39		56219-06-8	$C_{15}H_{28}O_2$	
15		pentadecanoate	256.42	141.6@3 torr 153.5 (CRC)	7132-64-1	$C_{16}H_{32}O_2$	141.5 deg C at 3 mm Hg
16		palmitate	270.45	163.6 @ 3.7 torr 417 (CRC) 148 [2 mmHg] crc	112-39-0	$C_{17}H_{34}O_2$	211.5 deg C at 30 mm Hg
16	1	palmitoleate (cis-9)	268.44		1120-25-8 (cis-9) 10030-74-7 (?)	$C_{17}H_{32}O_2$	
17		heptadecanoate	284.48	185 [9 mmHg]	1731-92-6	$C_{18}H_{36}O_2$	
18		stearate	298.50	442 443 (crc) 215.1@15 torr 180@4 torr 215 [15 mmHg] crc	112-61-8	$C_{19}H_{38}O_2$	370 deg C v.p.1.36E-005 mm Hg
18	1	oleate (cis-9)	296.49	218.5 at 20 mm Hg	112-62-9		218.5 deg C at 20 mm Hg v.p. 6.29E-006 mm Hg

carbons in	double bonds	FAME name (-ic -acid methyl ester)	FAME m.w.	FAME b.p. (ºC)	FAME CAS #	FAME Formula	phys-prop data [75]	
18	1	elaidate (trans-9)	296.49		1937-62-8			
18	2	linoleate (cis-9,12)	294.26		112-63-0		215 deg C at 20 mm Hg (phsy prop.) v.p. 3.67E-006 mm Hg	
18	3	linoleanate						
19		nonadecanoate	312.53	190 [4 mmHg]	1731-94-8	$C_{20}H_{40}O_2$		
20		arachidate	326.56	488.7K @0.013bar 461.2K @0.003 bar 215 [10 mmHg] crc	1120-28-1	$C_{21}H_{42}O_2$		
20	1	eichosenoate				$C_{21}H_{40}O_2$		
20	4	arachidonate						
21		heneicosanoic	340.59		6064-90-0	$C_{22}H_{44}O_2$		
22		behenic	354.62		929-77-1	$C_{23}H_{46}O_2$		
22	1	erucate (cis-13)	352.33		1120-34-9	$C_{23}H_{44}O_2$		
23		tricosanoic	368.64		2433-97-8	$C_{24}H_{48}O_2$		
24	1	nervonate						
24		lignocerate	382.66		2442-49-1	$C_{25}H_{50}O_2$		

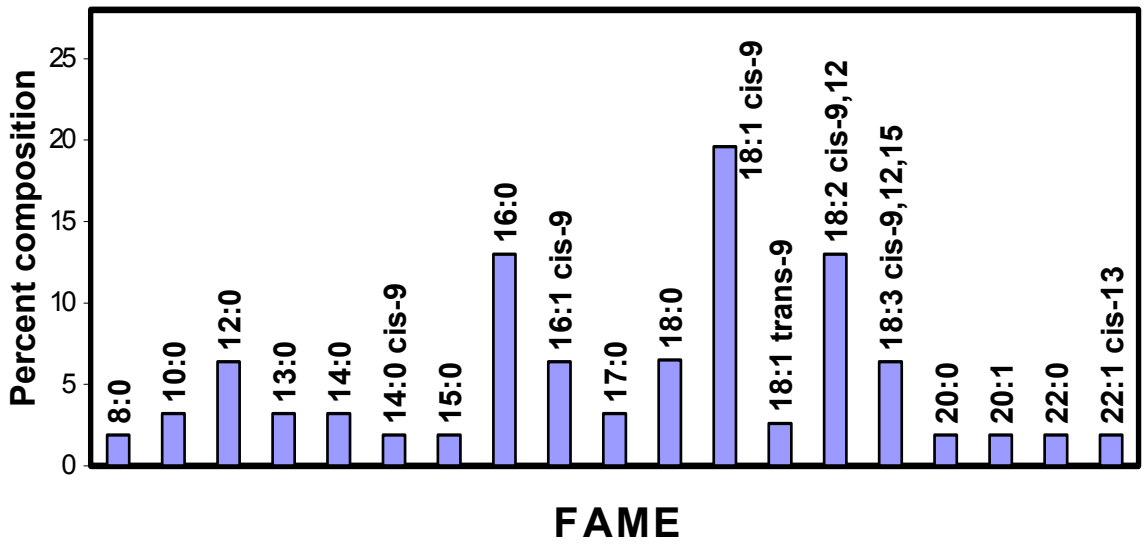
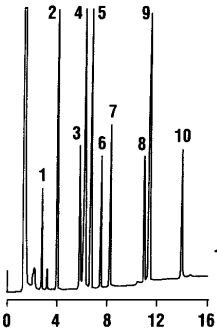


Figure 83: Composition of Supelco 18920-1 FAME mixture.

9. Appendix C: Commercial FAME chromatograms.

C14:0-C24:0 FAME

CHROMATOGRAM 1317

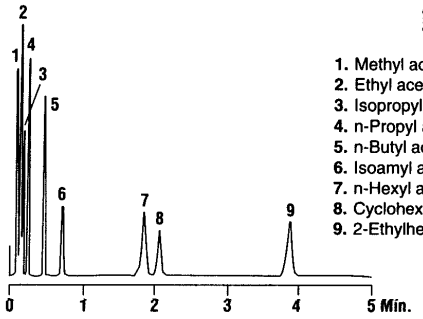


1. Methyl myristate (C14:0)
2. Methyl palmitate (C16:0)
3. Methyl stearate (C18:0)
4. Methyl oleate (C18:1)
5. Methyl linoleate (C18:2)
6. Methyl linolenate (C18:3)
7. Methyl arachidate (C20:0)
8. Methyl behenate (C22:0)
9. Methyl erucate (C22:1)
10. Methyl lignocerate (C24:0)

Column: Heliflex® AT™-WAX, 25m x 0.25mm x 0.2µm (Part No. **9925252**)
Temp: 180°C to 240°C at 5°C/min
Carrier Gas: Hydrogen, 0.8mL/min (FID)

Acetates

CHROMATOGRAM 1314

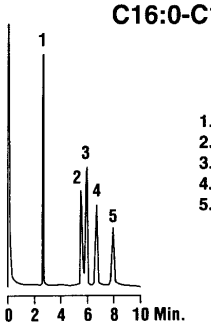


1. Methyl acetate
2. Ethyl acetate
3. Isopropyl acetate
4. n-Propyl acetate
5. n-Butyl acetate
6. Isoamyl acetate
7. n-Hexyl acetate
8. Cyclohexyl acetate
9. 2-Ethylhexyl acetate

Column: Heliflex AT-1, 10m x 0.53mm x 1.2µm (Part No. **935110**)
Temp: 60°C to 160°C at 5°C/min
Carrier Gas: Helium, 20mL/min (FID)

C16:0-C18:3 FAME

CHROMATOGRAM 1318

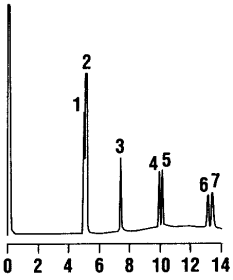


1. Methyl palmitate (C16:0)
2. Methyl stearate (C18:0)
3. Methyl oleate (C18:1)
4. Methyl linoleate (C18:2)
5. Methyl linolenate (C18:3)

Column: Heliflex AT-225, 10m x 0.53mm x 1.2µm (Part No. **915110**)
Temp: 170°C
Carrier Gas: Helium, 8mL/min (FID)

C18:0-C24:1 FAME

CHROMATOGRAM 1321

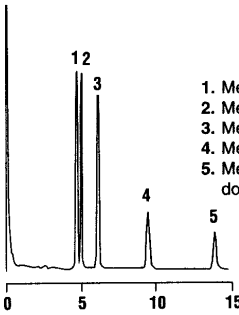


1. Methyl stearate (C18:0)
2. Methyl oleate (C18:1)
3. Methyl arachidate (C20:0)
4. Methyl behenate (C22:0)
5. Methyl erucate (C22:1)
6. Methyl lignocerate (C24:0)
7. Methyl nervonate (C24:1)

Column: Heliflex AT-225, 10m x 0.53mm x 1.2µm (Part No. **915110**)
Temp: 170°C to 220°C at 5°C/min
Carrier Gas: Helium, 5mL/min (FID)

C20:0-C22:6 FAME

CHROMATOGRAM 1319

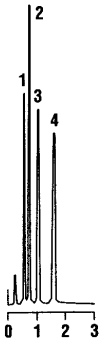


1. Methyl arachidate (C20:0)
2. Methyl cis-11-eicosenoate (C20:1)
3. Methyl arachidonate (C20:4)
4. Methyl erucate (C22:1)
5. Methyl 4,7,10,13,16,19-docosahexaenoate (C22:6)

Column: Heliflex AT-225, 10m x 0.53mm x 1.2µm (Part No. **915110**)
Temp: 200°C
Carrier Gas: Helium, 5mL/min (FID)

Dibasic Esters

CHROMATOGRAM 1322



1. Dimethyl malonate
2. Dimethyl succinate
3. Dimethyl glutarate
4. Dimethyl adipate

Column: Heliflex AT-WAX, 10m x 0.53mm x 1.2µm (Part No. **9951102**)
Temp: 75°C to 100°C at 10°C/min
Carrier Gas: Helium, 10mL/min (FID)

Figure 84: Alltech catalog FAME chromatograms 1314, 1317-1319, 1321, and 1322.

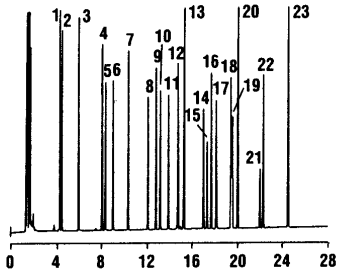
DB-5 and DB-5ht Fused Silica Capillaries

- **5% Phenyl, 95% Methyl Silicone**
- **Similar to SE-54, CP-Sil-8, Dexsil® 300, DC-200, Rtx™-5, Fluorolube, OV-73, DC-560, OV-3**

Bacterial Acid Methyl Esters

CHROMATOGRAM 1754

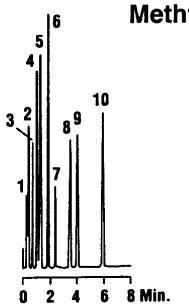
1.	C11:0	Methyl undecanoate
2.	2-OH C10:0	Methyl 2-hydroxydecanoate
3.	C12:0	Methyl laurate
4.	C13:0	Methyl tridecanoate
5.	2-OH C12:0	Methyl 2-hydroxydodecanoate
6.	3-OH C12:0	Methyl 3-hydroxydodecanoate
7.	C14:0	Methyl myristate
8.	12-Me C14:0	Methyl 12-methyltetradecanoate
9.	C15:0	Methyl pentadecanoate
10.	2-OH C14:0	Methyl 2-hydroxytetradecanoate
11.	3-OH C14:0	Methyl 3-hydroxytetradecanoate
12.	C16:1	Methyl palmitoleate
13.	C16:0	Methyl palmitate
14.	14-Me C16:0	Methyl 14-methylhexadecanoate
15.	9,10-diMe C16:0	Methyl cis-9,10-methyl hexadecanoate
16.	C17:0	Methyl heptadecanoate
17.	2-OH C16:0	Methyl 2-hydroxyhexadecanoate
18.	C18:1	Methyl stearate
19.	C18:1	Methyl elaidate
20.	C18:0	Methyl oleate
21.	9,10-diMe C18:0	Methyl cis-9,10-methyleneoctadecanoate
22.	C19:0	Methyl nonadecanoate
23.	C20:0	Methyl arachidate



Column: DB-5, 30m x 0.25mm x 0.25µm, (Part No. **9372**)
Temp: 150°C (4min) to 250°C at 4°C/min
Carrier Gas: Hydrogen, 42cm/sec (FID)

Methyl Esters

CHROMATOGRAM 1327

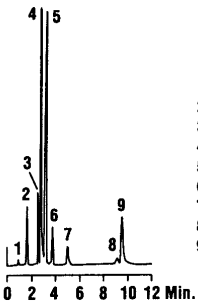


1. Methyl formate
2. Methyl acetate
3. Methyl proprionate
4. Methyl isobutyrate
5. Methyl butyrate
6. Methyl isovalerate
7. Methyl valerate
8. Methyl isocaproate
9. Methyl caproate
10. n-Decane

Column: Heliflex AT-1, 10m x 0.53mm x 1.2µm (Part No. **935110**)
Temp: 40°C to 100°C at 5°C/min
Carrier Gas: Helium, 5mL/min (FID)

C14:0-C22:1 FAME

CHROMATOGRAM 1316



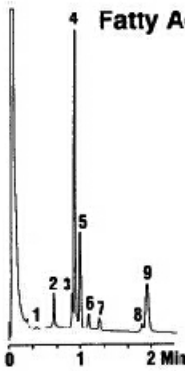
1. Methyl myristate (C14:0)
2. Methyl palmitate (C16:0)
3. Methyl stearate (C18:0)
4. Methyl oleate (C18:1)
5. Methyl linoleate (C18:2)
6. Methyl linolenate (C18:3)
7. Methyl arachidate (C20:0)
8. Methyl behenate (C22:0)
9. Methyl erucate (C22:1)

Column: Heliflex AT-225, 10m x 0.53mm x 1.2µm (Part No. **915110**)
Temp: 200°C
Carrier Gas: Helium, 5mL/min (FID)

Figure 85: Alltech catalog FAME chromatograms 1754, 1327, and 1316.

Fatty Acid Methyl Esters

CHROMATOGRAM 2140



1. C14:0 Methyl myristate
2. C16:0 Methyl palmitate
3. C18:0 Methyl stearate
4. C18:1 Methyl oleate
5. C18:2 Methyl linoleate
6. C18:3 Methyl linolenate
7. C20:0 Methyl arachidate
8. C22:0 Methyl behenate
9. C22:1 Methyl erucate

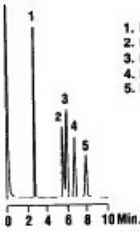
Column: MC-WAX 20M Multicapillary (Part No. **17061**)
Temp: 180°C (0.2min hold) to 210°C at 40°C/min
Carrier Gas: Helium, 150mL/min (0.4min hold) to 230mL/min
Make-up: None **Detector:** FID/275°C

Alltech AT-225

- **25% Phenyl, 25% Cyanopropyl-methyl Silicone Phase Similar to DB-225, Rtx-225, OV-225, RSL-500**

Fatty Acid Methyl Esters

CHROMATOGRAM 1318



1. Methyl palmitate
2. Methyl stearate
3. Methyl oleate
4. Methyl linoleate
5. Methyl linolenate

Column: Heliflex AT-225, 10m x 0.53mm x 1.2µm (Part No. 915110)
Temp: 170°C
Carrier Gas: Helium, 8mL/min (FID)

Figure 86: Alltech FAME chromatograms 2140, 1318.

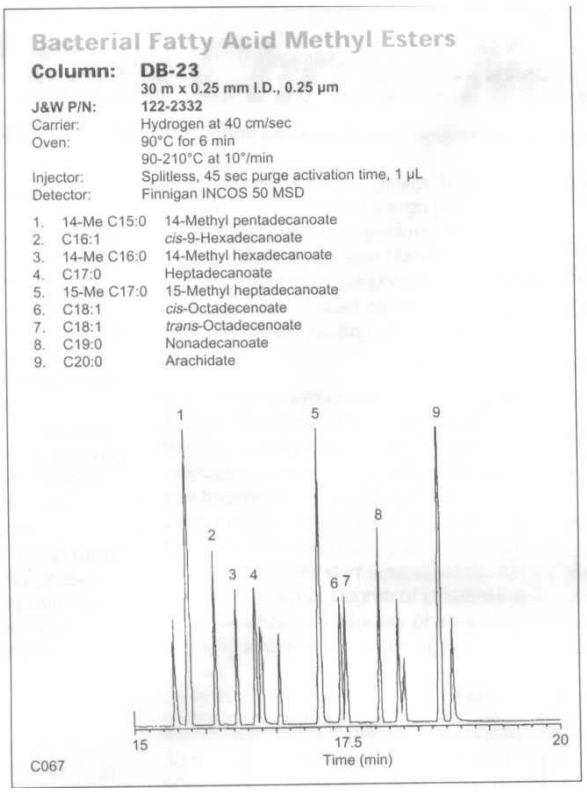


Figure 87: J&W DB-23 FAME chromatogram.

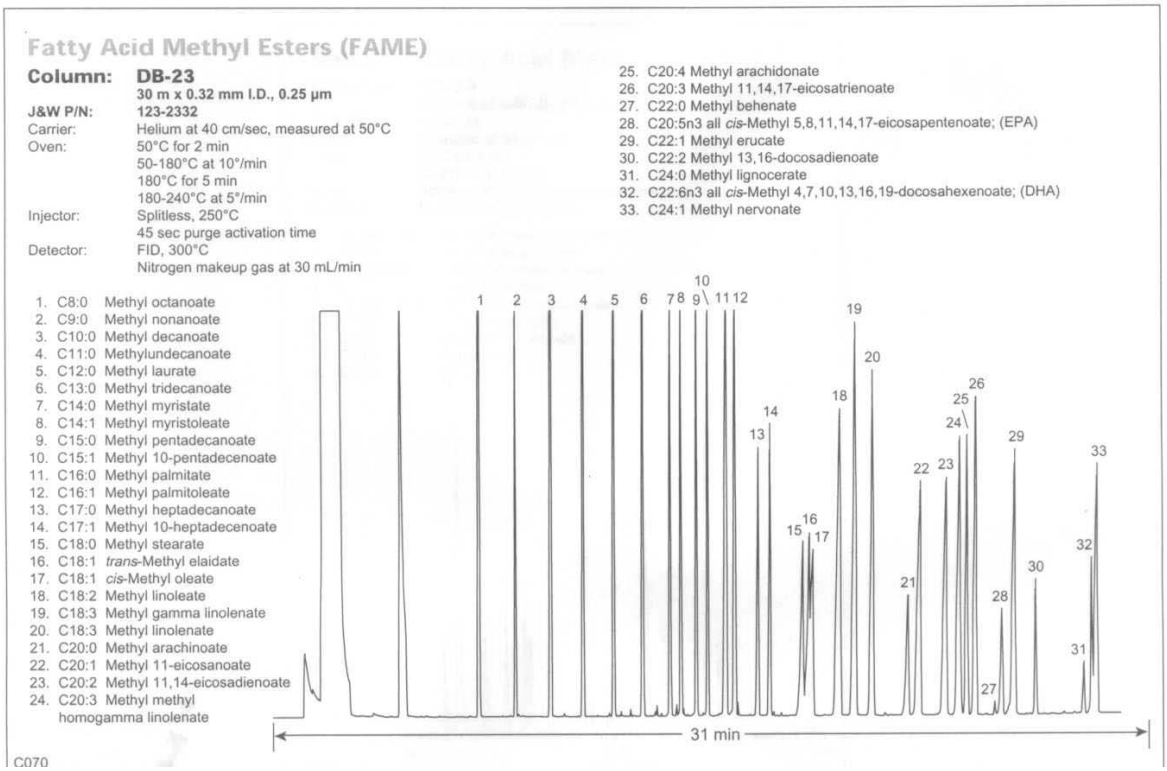


Figure 88: J&W DB-23 FAME chromatogram.

10. Appendix D: Canola oil Reference Information

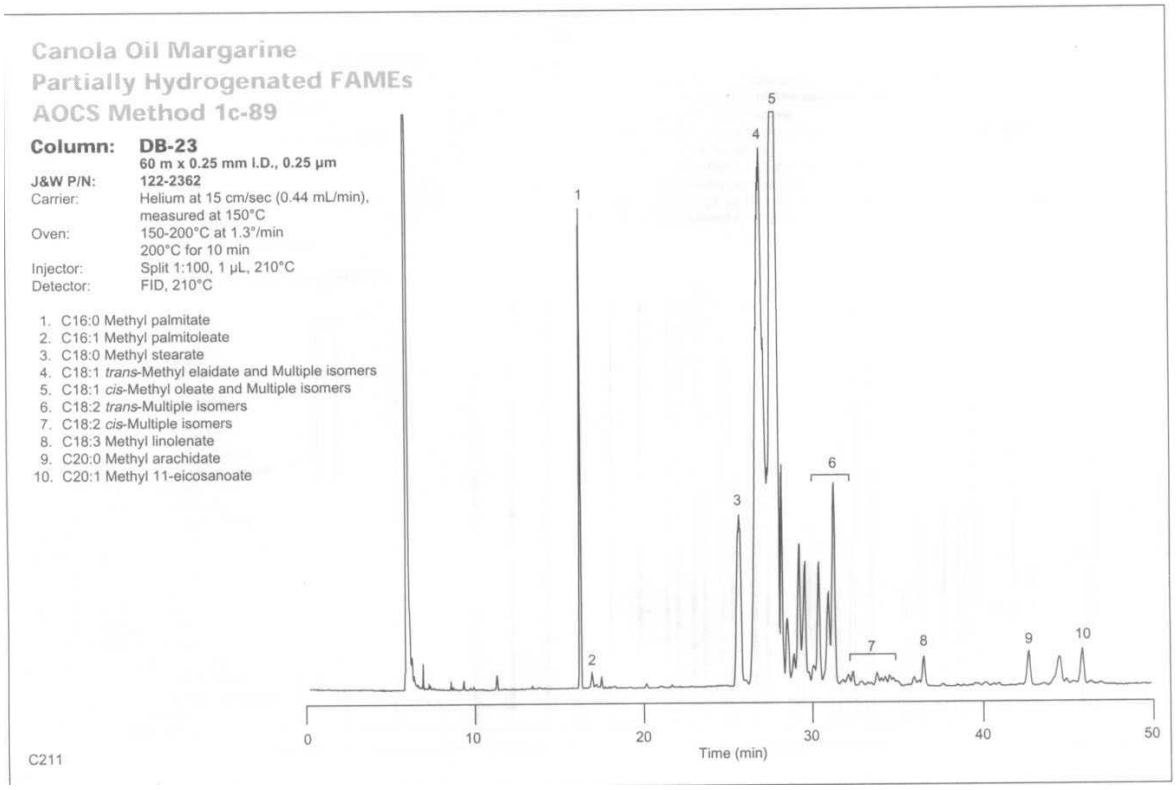


Figure 89: J&W canola chromatogram.

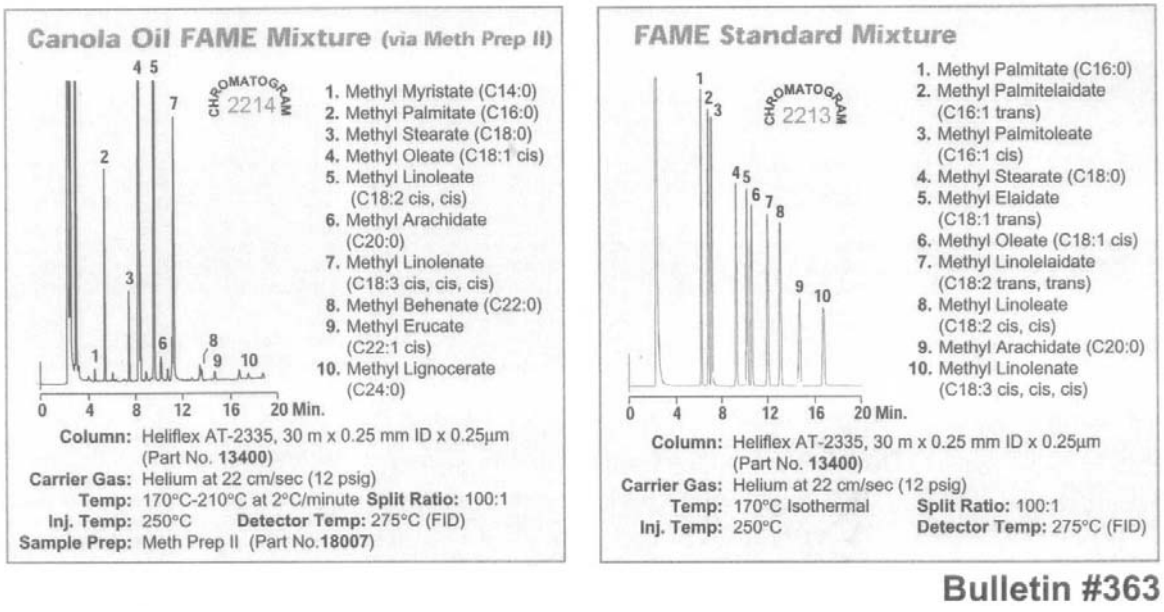


Figure 90: Alltech canola chromatograms 2214, 2213.

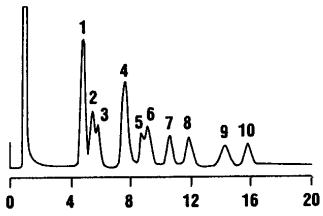
11. Appendix E: Packed column FAMEs

GC Packed Column Applications

Packed columns for gas chromatography are still widely used in many different analytical situations. While capillary GC columns have become commonplace in areas such as the biological sciences, packed columns are still the choice in gas analysis and analysis of low molecular weight species. The following chromatograms demonstrate applications where packed columns are still used effectively. If you have questions or need help choosing a column, please contact the Alltech Technical Service Department.

C_{16}-C_{18} FAME's

CHROMATOGRAM 1142

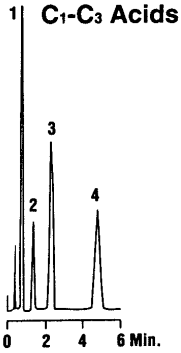


1. Methyl palmitate
2. Methyl palmitelaidate
3. Methyl palmitoleate
4. Methyl stearate
5. Methyl elaidate
6. Methyl oleate
7. Methyl linolaidate
8. Methyl linoleate
9. Methyl γ-linolenate
10. Methyl linolenate

Column: 6ft x 4mm ID, Glass
Packing: 10% Silar®-10C on Gas Chrom Q, 100/120 (Part No. **12428**)
Temp: 180°C
Flowrate: Nitrogen, 40mL/min (FID)

C_1-C_3 Acids (Aqueous)

CHROMATOGRAM 1072

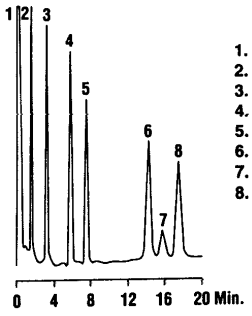


1. Water
2. Formic acid
3. Acetic acid
4. Propionic acid

Column: 6ft x 2mm ID, Glass
Packing: Porapak® QS, 80/100 (Part No. **2719**)
Temp: 175°C
Flowrate: Helium, 25mL/min (TCD)

Low Molecular Weight Acids

CHROMATOGRAM 1801

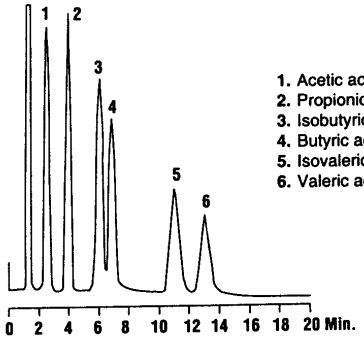


1. Formic acid
2. Acetic acid
3. Propionic acid
4. Isobutyric acid
5. Butyric acid
6. Isovaleric acid
7. Lactic acid
8. Valeric acid

Column: 2m x 2mm ID, Glass
Packing: 4% Carbowax®20M on Carbograph™ 1-DA, 80/120 (Part No. **1727**)
Temp: 180°C
Flowrate: Nitrogen, 20mL/min (FID)

Volatile Fatty Acids

CHROMATOGRAM 1074

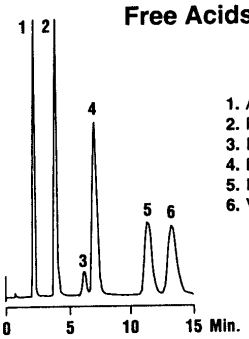


1. Acetic acid
2. Propionic acid
3. Isobutyric acid
4. Butyric acid
5. Isovaleric acid
6. Valeric acid

Column: 6ft x 2mm ID, Glass
Packing: Gas Chrom® 220, 80/100 (Part No. **2484**)
Temp: 175°C
Flowrate: Nitrogen, 20mL/min (FID)

Free Acids

CHROMATOGRAM 1439



1. Acetic acid
2. Propionic acid
3. Isobutyric acid
4. Butyric acid
5. Isovaleric acid
6. Valeric acid

Column: 6ft x 2mm ID, Glass
Packing: HayeSep® R, 80/100 (Part No. **2807**)
Temp: 220°C
Flowrate: Helium, 39.2mL/min (FID)

Figure 91: Alltech catalog FAME chromatograms (packed columns).

12. Appendix F: Commercial Aerosol Collectors/Samplers

collector-collects sample on media
sampler-collects sample on media & includes sampling system
analyzer-may include sampler/collector & analyzes the collected sample

	MANUFACTURER/builder	UNIT NAME/product	TYPE DEVICE
1	InnovaTek, Inc.	Bioguardian	sampler
4	MSP corporation 1313 Fifth Street S.E. Minneapolis, MN, U.S.A. 55414 Telephone: 612/379-3963 Fax: 612/379-3965 e-mail: *sales@mspcorp.com*	Model 150 Marple-Miller Impactor™ (Low Flow) Model 150P Model 160 Marple-Miller Impactor™ (High Flow) Model 100 MOUDI™ - Micro-Orifice Uniform Deposit Impactor (8 stage) Model 110 MOUDI™ - Micro-Orifice Uniform Deposit Impactor (10-stage) Model 200 PEM™ - Personal Environmental Monitor Model 210 PRS™ - Personal Respirable Sampler for Diesel/Coal Dusts Model 310 UAS™ - Universal Air Sampler Model 340 HVVI™ - High-Volume Virtual Impactor Model 400 MEM™ - Micro-Environmental Monitor Model 450 DDS™ - Diffusion Denuder Sampler	collector collector collector collector collector collector collector sampler collector sampler sampler
5	Dycor	XMX/2a XMX/2AL XMX/2AL-MIL	sampler LIQUID sampler LIQUID sampler
6	scp dynamics research paper: Snyder, A.P. et al. Field Analytical Chemistry and Technology, 4(2-3), 111-126 (2000)	XM-2	Dycor sampler
7	Lares div of Camber Phone: (800) 750-3990 Fax: (703) 465-4699 Email: spincon@camber.com	SPINCON	sampler-supports MS, GC, MS/MS, IC, LC, LC/MS, GCMS, and atomic spectroscopy. Biological techniques such as PCR assay, standard culturing, particle/ organism counting, microscopy, immunoassay, and flow cytometry are readily interfaced.
8	Met One Instruments, Inc. 1600 Washington Blvd., Grants Pass, Oregon 97526 Phone 541/471-7111, Fax 541/471-7116 Regional Service: 3206 Main St., Suite 106, Rowlett, Texas 75088 Phone 972/412-4747, Fax 972/412-4716	9012 ambient aerosol.. esampler beta-attenuation mass… SASS Super-SASS JP2000-multiple monitor E-BAM	collector/size analyzer collector/size analyzer collector/size analyzer sampler sampler uses es-640 particulate monitor sampler/mass analyzer
10	TSI Incorporated 500 Cardigan Road Shoreview, MN 55126-3996, U.S.A. Telephone: 651-483-0900	RESPICON DUSTTRAK SIDEPAK ATOFMS	sampler sampler/analyzer sampler/analyzer sampler/MASS & PARTICLE SIZE ANALYZER

	MANUFACTURER/builder	UNIT NAME/product	TYPE DEVICE
	Fax: 651-490-2748 E-mail: info@tsi.com		
11	Millipore	M Air T Tester M Air T Isolator	sampler sampler
13	Research International 18706 142nd Ave. Phone 425-486-7831 Fax 425-485-9137 East Coast office J. Tobelmann e-mail jtobelmann@compuserve.com	RAPTOR FAST 6000 SASS 2000 SASS 3000	sampler/analyzer sampler/analyzer sampler sampler
14	MesoSystems MesoSystems Technology, Inc. 1001 Menaul Blvd. NE, Suite A Albuquerque, NM 87107 (877) 692-2120	Biocapture BT-550 (latest model is BT-600)	sampler
17	Sartorius 131 Heartland Blvd. Edgewood, New York 11717 Phone: (1) 800 - 635 - 2906, (1) 631 - 254 - 4249 Fax: (1) 631 - 254 - 4253	AirPort MD 8	sampler
18	ALLERGENCO PO Box 8571 Wainwright Station San Antonio, TX 78208-0571	MK-3	sampler
19	SPECTREX 3580 Haven Ave. Redwood City, CA 94063 800-822-3940 or 650-365-6567 fax: 650-365-5845	PAS-500 PAS-1500 PAS-2000 PAS-3000	sampler sampler sampler sampler
20	Spiral Biotech Two Technology Way Norwood, MA 02062 1-800-554-1620 + US 781-320-9000 (Intl.) + US 781-320-8181 (fax) infosbi@spiralbiotech.com sales@spiralbiotech.com	MB2 Burkhard Portable Air Sampler Burkhard Personal Volumetric Air Sampler	sampler sampler sampler
21	Airmetrics 2121 Franklin Boulevard, #9 Eugene, Oregon 97403 (541) 683-5420 Fax (541) 683-1047 E-Mail Addresses Karene Gottfried - sales@airmetrics.com Sales, marketing, advertising, distributors	MiniVol Portable Air Sampler	sampler
22	Rupprecht & Patashnick Co., Inc. 25 Corporate Circle Albany, NY 12203 USA phone 518 452 0065 fax 518 452 0067 e-mail info@rpco.com	Partisol 2000 DustScan Sentinel 3030 DustScan Scout 3020 Mini-Partisol 2100	sampler sampler/analyzer sampler/analyzer sampler
23	PIXE International Corp. P.O. Box 2744 Tallahassee, FL 32316 USA Fax: 850-574-6469 email: info@pixeintl.com	Streaker Cascade Impactor	sampler collector
24	ECOTECH 12 Apollo Court Blackburn, Victoria 3130 Phone: (61 3) 9894 2399 Fax: (61 3) 9894 2445	Series 3000 MicroVol 1100	sampler sampler

	MANUFACTURER/builder	UNIT NAME/product	TYPE DEVICE
	Email: ecotech@ecotech.com.au		
25	General Oceanics Inc. 1295 N.W. 163th Street Miami, Florida 33169 USA Phone: (305) 621-2882 Fax: (305) 621-1710 e-mail: Sales@GeneralOceanics.com	Model 8001 Personal Air Quality Sampler	collector collector
26	mbv (MICROBIOLOGY AND BIOANALYTIC) distributed by VWR in the USA	MAS-100 MAS-100 Ex MAS-100 Eco MAS-100 Iso MAS-100 CGX	sampler sampler sampler sampler sampler
27	Total Air Care Ltd 14 Gloucester Park Road, Onehunga Auckland, New Zealand phone +64-9-636 0663, fax +64-9-636 0963 e-mail info@totalaircare.co.nz	P100 MicroPortable Air Sampler made by Veltek	sampler
28	GENEQ inc. 8047 Jarry E. Montreal, Que. Canada, H1J 1H6 Tel. : (514) 354-2511 • 1-800-463-4363 Fax. : (514) 354-6948 E-mail: info@geneq.com	MiniVol HVP-3000 Hi-Vol Air Sampler	 sampler
29	CALIFORNIA MEASUREMENTS, INC. 150 E. MONTECITO AVENUE SIERRA MADRE, CA 91024 TEL 1-626-355-3361 FAX 1-626-355-5320 E-MAIL calmeasure@earthlink.net	Model PC-2 Real-Time Air Particle Analyzer Model PC-2H Real-Time Air Particle Analyzer Model PC-2AS/SK76 Real-Time Pharmaceutical Aerosol Analyzer IMPAQ AS-6 Six-Stage Cascade Impactor Model MPS-4G1 Clean Room Microanalysis Particle Sampler Model MPS-3 Microanalysis Particle Sampler	sampler sampler sampler sampler sampler sampler
30	Particle Measuring Systems 5475 Airport Blvd Boulder, CO 80301	LASAIR LASAIR II AirNet HandiLaz 301	sampler-particle counter sampler-particle counter sampler-uses external vacuum source-particle counter sampler-particle counter
31	Biotest Diagnostics Corporation-HYCON Div. 66 Ford Road, Suite 131 Denville, New Jersey 07834 973.625.1300 800.522.0090 Fax: 973.625.9454	RCS-Standard RCS High Flow RCS Plus & Plus Explosion Proof	sampler sampler sampler
32	Parrett Technical Developments F.W. Parrett Limited 65 Rienfield Road London, SE9 2RA Phone 020-8853-3254 Fax 020-7504-3536	MB1 MB2	sampler sampler
35	M TRUNOV, S TRAKUMAS, K WILLEKE, SA GRINSHPUN, T REPONEN DEH-Ohio	Air-O-Cell sampling cassettes	collector
36	M TRUNOV, S TRAKUMAS, K WILLEKE, SA GRINSHPUN, T REPONEN same group and testing as ref 35-DEH-Ohio	Air-O-Cell sampling cassettes	collector
43	SKC Inc. 863 Valley View Road, Eighty Four, PA 15330 USA Phone: 724-941-9701	AirChek 2000 210 Pocket Pump AirCheck models 225-254 AirLite Pumps	pumps-hand held pumps-hand held pumps-hand held pumps

	MANUFACTURER/builder	UNIT NAME/product	TYPE DEVICE
	Phone: 800-752-8472 (USA Only) General email: skcinc@skcinc.com Tech Support: skctech@skcinc.com	222 Series SKC Universal Sampling Pumps AirCheck HiLite 30 AirCheck HV 30 Vac-U-Go Double Take Sampler	pumps pumps pumps sampler pumps sampler
44	A. P. Buck Inc. 7101 Presidents Drive Suite 110 Orlando, FL 32809 USA Phone: 407-851-8602 Fax: 407-851-8910 Phone Toll Free: 800-330-2825 (USA Only) E-mail: apbuck@apbuck.com	BioAire Pump MicroFlow 60 MicroFlow 90-for anthrax sampling B 6 impactor Buck-Genie VSS-1 Buck-Genie VSS-5 Buck-Genie VSS-12 LinEar 40 Buck-Basic-1 Buck-Basic-5 Buck-Basic-12	pump sampler sampler collector-hooks to vacuum source pump pump pump pump pump pump

13. Distribution

11	MS 0892	Curtis Mowry, 1764
1	MS 0892	Richard Cernosek, 1764
1	MS 0892	Lisa Theisen, 1764
1	MS 0892	Dan Trudell, 1764
1	MS 1073	Catherine Morgan, 1738
1	MS 0175	Jesus I. Martinez, 3132
1	MS 9018	Central Technical Files, 8945-1
2	MS 0899	Technical Library, 9616
1	MS 0612	Review and Approval Desk, 9612 for DOE/OSTI
1	MS 0161	Patent and Licensing Office, 11500
1	MS 0188	D. Chavez, LDRD Office, 1030