Evaluation of an Induction-Based Fluidics System for Delivery of Low Volume (nL) Samples

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INTRODUCTION
Chemical warfare agents (CWAs) are highly toxic compounds that induce toxic responses at very small doses when delivered through percutaneous exposure. For studies employing small animals, manual digital pipettes and syringes have been shown to be neither accurate nor precise over the volume range required. A patent induction-based fluidics (IBF) sorting device (Figure 1) was evaluated as a method of accurately and precisely deliver nanoliter volumes of liquid to the epidermal surface of animals during percutaneous dosing studies. The device employs non-contact electromagnetic fields to impart micro-drops from a syringe (Figure 2, Source). To assess IBF system sample delivery accuracy and precision under experimental conditions, we developed a quantitative GC/MS method for methyl salicylate (MeS). A 5-glycidyl-silane, O$_2$/methyl sulfoxide (O$_2$/MeS) was used as internal standard.

METHODS
Sample Collection Method
- Sample volumes ranging from 5-500 nL were aspirated from the IBF device into microvials containing volumes quantities of methanol.
- Accuracy and precision - 5 replicates at each volume were collected 5 different times; 10 - 5 mL replicates were collected.
- Accuracy was determined using the % recovery results; precision was determined using the CV.
- Samples were stored at -80°C until analysis.

RESULTS
Analytical Method
- The initial calibration curve fit a quadratic expression over the dynamic range with correlation coefficients routinely exceeding 0.993. A representative calibration curve is illustrated in Figure 4.
- To meet validation criteria, calibration curve points routinely quantified within 2% of their assigned concentrations. Positive controls at all three sample levels were also analyzed with each sample batch and quantified within 3% of theoretical.
- Mean recovery rates for the IBF sample delivery varied across volumes but were relatively accurate, ranging from 92.5 - 101.7% with mean %CV ranging from 0.0 - 0.35% (Table 1).
- Not surprisingly, the most variable results were found at 5 nL, the lowest volume, where mean recovery rates ranged from 86.6 - 96.6% with mean %CVs between 2.2 - 6.6%. At the 500 nL sample volume, mean recovery ranged from 91.8 - 104.6%, with %CVs between 1.9 - 4.2%, indicating that method accuracy and precision were relatively good at the higher volumes.

Table 1. Compound properties

<table>
<thead>
<tr>
<th>Compound</th>
<th>LOQ (µg/mL)</th>
<th>MEQ (µg/mL)</th>
<th>O$_2$/MeS (µg/mL)</th>
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<tbody>
<tr>
<td>MeS</td>
<td>0.012</td>
<td>0.015</td>
<td>0.013</td>
</tr>
<tr>
<td>O$_2$/MeS</td>
<td>0.012</td>
<td>0.015</td>
<td>0.013</td>
</tr>
</tbody>
</table>

Table 2. Manual Syringe Delivery Accuracy & Precision

<table>
<thead>
<tr>
<th>Volume (nL)</th>
<th>Mean Recovery</th>
<th>%CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>96.6</td>
<td>4.4</td>
</tr>
<tr>
<td>50</td>
<td>97.8</td>
<td>1.9</td>
</tr>
<tr>
<td>250</td>
<td>98.5</td>
<td>3.1</td>
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</table>

Table 3. IIB Accuracy and Precision

<table>
<thead>
<tr>
<th>Volume (nL)</th>
<th>Mean Recovery</th>
<th>%CV</th>
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<td>98.5</td>
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DISCUSSION
Initial IBF recovery rates were low, 72.5 - 82.6%, leading to several changes in our method. First, the volume-to-amount ratio was visually verified. This ensured that the correct volume was being dispensed. Also, the distance from syringe tip to liquid in the primary vial was minimized and controlled, as was the sample delivery interval, both reducing evaporation losses. After the second set of the study, the syringe tip was noted to be jarred, which could have affected sample delivery; it was then given a clean out. Other confounding variables that could have contributed to lower recoveries and high %CVs included dilution error from the additional dilutions required to bring sample concentrations within the assay dynamic range. To minimize this, we aspirated some of the secondary vials method before completely dispensing the primary sample. All of these adjustments resulted in relatively higher accuracy and precision, especially for the larger nanoliter volumes.

The system could not be universally calibrated across liquids having diverse physical characteristics. A volume-to-amount ratio would need to be established for each individual compound to ensure that the correct volume is being delivered; in the future, the method will be evaluated using a less volatile, more viscous fluid to simulate IV agents.

CONCLUSIONS
- The IBF system could be a very useful method for delivering nL quantities, since the data indicate that it is both accurate and precise method.
- Further evaluations with compounds of different physical properties need to be conducted.

REFERENCES

Figure 2. IBF diagram

Analytical Method
- A 7890A GC/5973 Mass Selective Detector with a ZB-5 (0.25 mm x 30 m x 0.25um film thickness x 0.5 m icon) column, a splitter GC, and the mass spectrometer operated in SIM and MS modes was validated.
- Characteristic ions for MeS and O$_2$/MeS are depicted in the spectrum of Figure 3 and listed in Table 1.
- Calibration curve dynamic range was 40-8000 nL/mL.

Figure 3. Methacryl-siloxane and O$_2$/MeS TIC (top) and spectrum (bottom).
Nanoliter Programmable Wave

Beta Prototype*

General Description:

The Nanoliter Programmable Wave is a low volume non-contact liquid dispense system, drop-on-demand, using proprietary IBF (induction based fluidics). This is a single channel system based on a standard 10ul or 100uL syringe with a provided disposable tip. It can also use UPLC and other Gaussian surfaces, not shown.

IBF can dispense to most targets with high precision over distance of 1-10cm. Requires only minimal air disturbance, clear path without adjacent materials. Limitations on viscosity and surface tension do apply.

Intended for analytical chemistry, other applications with precise analyte or non-contact drop delivery.

Features:

- status LED
- integrated motor controller
- dispense frequency max 5Hz
- wired or wireless (Android Bluetooth) control using ASCII terminal

Application:

- Analytical Chemistry: Mass Spectrometer Sample Introduction LC, MALDI, ESI, and variants
- Diagnostics: Assays, High-Throughput Screening, Chip Insert
- R&D applications: Manufacturing, 3D Printing
- Sample Input: Syringe, LC column, Capillary
Droplet Based Sampling of RNA Hydrolysates by Induction Based Fluidics

Overview
The goal of this study was to couple an inductive charging device to a liquid chromatography separation with a focus on lowering the LOD for standard RNA nucleoside analysis. As such, a synthetic test mix comprised of cytidine, uridine, 5-methylcytidine, adenosine and 2'-O-methyladenosine was separated by means of capillary chromatography and delivered into the mass spectrometer by using a modified inductive charging source powered by a modified inductor coupled to a digital programmed energy and polarity pulsed DC source.

Introduction
Post-transcriptional chemical covalent modification of adenosine, guanosine, uridine and cytidine occurs frequently in all types of ribonucleic acids (RNAs). In ribosomal RNA (rRNA) and transfer RNA (tRNA) these modifications make important contributions to RNA structure and stability and to the accuracy and efficiency of protein translation. These modifications can be present at very low levels and their analysis can be challenging. This work builds on previous work where the utility of Inductive Based Fluidics (IBF) as a sample introduction method is examined when coupled to an UPLC platform. Because IBF creates inductively charged droplets instead of an electrospray, theoretically, a droplet sampling method would allow for greater sensitivity as more sample would enter the mass spectrometer.

Methods
An equimolar RNA hydrolysate mixture was separated on a porous graphitic carbon packed capillary column inserted into an in-house inductive charging tube with capillary positioned 2.4 mm from inlet orifice. Mass spectra were recorded in positive polarity on a Thermon Fisher LTQ-XL. A capillary temperature of 275 °C, spray voltage of 0 kV, capillary voltage of 0 kV, and tube lens at 0 kV. IBF device was set to 2000V and pulsed + and - with 2 s intervals over a 40 min acquisition. Data acquisition was through the Thermo Fisher Xcalibur software.

Results and Discussion
Five RNA nucleoside standards, cytidine, uridine, 5-methyluridine, adenosine, and 2'-O-methyladenosine were separated and sampled using the IBF device. Extracted ion chromatograms of the analytes are shown in Figure 2.

Figure 1. Schematic of inductor employed in this work, inspired by R.W.Kiser1
A: Insulated copper tube (90 by 6 mm) B: Inductive charging device C: Capillary tubing (360 X 50 μm) with flow from column D: Field lines E: Inlet to mass spectrometer.

Figure 2. Extracted ion chromatograms of nucleosides cytidine, uridine, 5-methyluridine, adenosine, and 2'-O-methyladenosine separated on a PGC capillary column and introduced into the mass spectrometer by inductive charging.

Droplets were delivered with a 2 s interval over a total run time of 40 min. The total ion chromatogram (TIC) showed steady reproducible droplet peaks throughout the gradient. Each peak in the TIC corresponds to a single droplet delivered via IBF. Figure 3.

Figure 3. IEF-LC-MS/MS data of droplet introduction over a one minute acquisition window.

The intervals between droplet arrival in the mass analyzer are characterized by no background, which can be reflected in the summed mass spectral data. More importantly, the ion abundances present within a single droplet are similar to the integrated peak values as previously shown by Groenwold, et al.3

Conclusions
A programmable IBF droplet source appears suited for nucleoside UPLC sample introduction and mass spectrometric analysis. Preliminary results show this droplet based approach is equivalent to or may exceed nESI. Work to determine LOD's and more is continuing.

References
1. US patent numbers 5,100,107, 5,327,398, 7, 746,447 and pending patents

Acknowledgments
The presenting author would like to thank the members of the Limbach Research Group for their assistance. Financial support for this work was provided by the National Science Foundation (CHE#1216282). The nanoLITER Programmable Wave device was acquired under license from nanoLITER LLC.
Single droplet analysis of Li+ battery electrolyte w/fire retardants via IBF.


- Coin cells: dependent on Li+ transport in carbonate electrolyte – weakly coordinating solvents
  - EMC
  - EC
- Breakdown can lead to H₂ production, flammability issues

Lithium ion battery w/fire retardants

(Ethoxy)₂(2,2,2-trifluorethoxy)phosphazene cyclotrimers

(x,y) short hand nomenclature

Droplets Into An ESI!

HRMS of nanoLiter droplets!
Induction based fluidics (IBF) for droplet-based mass spectrometric analysis of oligonucleotides

Robert L. Ross, Andrew D. Sauter Jr., and Patrick A. Limbach

Here, we report the utility of induction-based fluidics (IBF) for the introduction of oligonucleotides to a mass spectrometer via charged droplets. The device produces nanoliter-sized droplets, which are field transported with minimal concerns related to source plugging or sampling loss. The IBF source enabled detection of oligonucleotides at the nanomolar concentration level. Importantly, analysis of individual droplets revealed that oligonucleotide mixtures could be detected with ion abundance ratios that closely match the initial concentration ratios within the sample. Copyright © 2015 John Wiley & Sons, Ltd.

Additional supporting information may be found in the online version of this article at the publisher’s website.

Keywords: droplet mass spectrometry; nanospray; RNA; DNA; ionization methods

Introduction

Electrospray ionization (ESI) is one of the most popular ionization sources for mass spectrometry analysis of oligonucleotides. However, oligonucleotides can be challenging to analyze by ESI because of the extremely polar phosphodiester backbone. This backbone leads to cation adduction and challenges in analyte desolvation. The addition of organic modifiers to the mobile phase as well as volatile salt precipitation increases signal response, yet salt adducts can limit mass spectral response and lead to sample clogging when low flow ESI sources are used. Nano-electrospray (nESI) offers the benefit of analyzing smaller sample sizes and can be coupled to a capillary liquid chromatography platform for oligonucleotide analysis. nESI requires pulled capillary tips for spraying where the internal diameters can range from 50 to 10 μm and less. As noted, however, the presence of cations in oligonucleotide samples can lead to salting out at the nESI orifice, which results in capillary plugging and poor analytical performance.

An alternative to ESI or nESI sample introduction is the use of inductive charging. One implementation of inductive charging is through induction-based fluids (IBF), a 2010 patented and pending ionization source that enables a liquid droplet initiated and directed by means of an inductive electric field to be transported into the orifice of a mass spectrometer. IBF can function by forming a nanoliter droplet on the end of a Gaussian surface, such as a nonconducting capillary. The capillary is connected to an electrical source, and application of a pulse results in induction of a charge on the surface of the droplet. The magnitude of the induced charge is correlated with surface area of the droplet per Gauss's law. With a sufficiently energetic pulse, the droplet is launched traversing the distance between the capillary and a target, in this case, the mass spectrometer.

Such an IBF source has been used previously in mass spectrometric analysis. Tu and co-workers initially used an IBF device for spotting samples onto matrix-assisted laser desorption ionization targets prior to analysis. The nanoliter-sized deposition of analyte, delivered by the IBF device, resulted in a signal enhancement of up to a factor of 20 versus normal micro spotting techniques. Likewise, for SIMS, major increases in sensitivity for cocaine and RDX were reported by Brewer and co-workers when nanoliter volumes were used to spot as compared to microliter quantities of sample, otherwise acquired identically. Hilkier also reported enhanced analyte response for polymers, and he verified that IBF produced droplets in agreement with Gauss's law using a Faraday cup and varying concentrations of electrolytic solutions. Cody showed that DART analysis of drugs of abuse was more sensitive when nanoliter volumes of sample were employed for analysis as compared with microliter-sized samples.

Most recently, Groenewold and coworkers have used IBF droplet introduction to analyze an ethanolic solution of nitroglycerine. The IBF capillary was oriented to the entrance of the mass spectrometer allowing droplets to be generated at an infusion rate of ~1 μl/min. This previous demonstration of sample introduction of salt-containing solutions suggested that IBF may provide an attractive ionization method for oligonucleotide analysis. Here, we have examined the operating characteristics and performance of a commercial IBF source for oligonucleotide mass spectrometry. IBF and sample conditions were examined, with the goal of identifying those conditions conducive to sensitive analysis of oligonucleotides. Our results show that IBF is a simple, robust and low-cost ionization method quite compatible with analyzing oligonucleotides from a variety of solvent conditions in negative ion polarity.

Experimental

Materials

Oligonucleotides, dT15 (MW 4498.7 Da), dT30 (MW 9059.4 Da), ACATCTCCCTACCCGTATA (20-mer-LG, MW 5954.0 Da) and...
Mixing in Colliding, Ultrasonically Levitated Drops

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Supporting Information

ABSTRACT: Lab-in-a-drop, using ultrasonic levitation, has been actively investigated for the last two decades. Benefits include lack of contact between solutions and an apparatus and a lack of sample cross-contamination. Understanding and controlling mixing in the levitated drop is necessary for using an acoustically levitated drop as a microreactor, particularly for studying kinetics. A pulsed electrostatic delivery system enables addition and mixing of a desired-volume droplet with the levitated drop. Measurement of mixing kinetics is obtained by high-speed video monitoring of a titration reaction. Drop heterogeneity is visualized as 370 nl of 0.25 M KOH (pH = 13.4) was added to 3.7 µL of 0.058 M HCl (pH = 1.24). Spontaneous mixing time is about 2 s. Following droplet impact, the mixed drop orbits the levitator axis at about 5 Hz during homogenization. The video’s green channel (maximum response near 540 nm) shows the color change due to phenolphthalein absorption. While mixing is at least an order of magnitude faster in the levitated drop compared with three-dimensional diffusion, modulation of the acoustic waveform near the surface acoustic wave resonance frequency of the levitated drop does not substantially reduce mixing time.

Microfluidics has become popular due to the small sample volume needed for each experiment. In addition, microdevices are portable and produce small waste volume. Microfluidic systems are commonly made of glass or quartz, polydimethylsiloxane (PDMS), polymethylmethacrylate (PMMA), or moldable polymers such as cyclic olefin copolymer (COC) for injection molding for mass fabrication with reasonable optical and mechanical properties. An attractive use of microfluidics is as a lab on a chip (LOC), with microvolume mixing of chemical and biological samples allowing investigation of biochemical reactions using several, often simultaneous, techniques such as electrochemistry, optical absorption, chemiluminescence, fluorescence, and mass spectrometry. With the popularity of microfluidics research in chemistry and biology, it is becoming evident that biofueling is an important problem, because microchannels may clog, and as little as 20% of cells from a suspension may pass a biofouled capillary inlet. After ~2 h of use, typical cell culture microfluidics channels clog or foul and cannot be reused. In addition, one worries that free radicals in the channel may diffuse to the walls and annihilate. An ultrasonically levitated droplet may allow the use of micrometer-sized nozzle orifices to create smaller drops. Levitated drops consume limited reactant solution volume in a manner analogous to microfluidics. Not only do they save time and material, but biofueling cannot be an issue, because there is no liquid–solid interface. Following the work of Santesson and Nilsson at Lund University, Weis and others, we have been developing the use of ultrasonically levitated drops to study reaction kinetics. A number of physical constraints complicate diagnostics of such drops. The large refractive index difference between air (1.00) and water/buffer (~1.33 in the visible, but wavelength dependent) distort optical measurements inside a drop. Unless humidity is controlled, drops initially have microliters in volume may evaporate in about 20 min.

A prerequisite for carrying out biochemical reactions is the ability to initiate such reactions in a controlled manner and to rapidly generate homogeneous solutions. The focus of the current paper is on mixing behavior when submicroliter droplets are injected into microliter droplets. We describe our efforts to accelerate such mixing. Even more remote is the additional question of kinetics measurements. Once mixing is better understood, the apparatus can be exploited to study reaction kinetics. A concern for biochemical studies is that enzyme proteins commonly denature at gas–liquid interfaces. Fortunately, this process is not instantaneous. Although we have no data specifically on levitated droplets, Weis obtained data on alkaline phosphatase kinetics at ~6°C, and the enzyme of greatest interest to us, myeloperoxidase, has a pI of 10, so that at biological pH (~8), the ionized enzyme should be sufficiently hydrophilic to avoid the interface over relevant time scales.

Impinging a droplet on a stably levitated drop not only increases levitated mass but also transfers momentum from the impinging droplet to the merged drop. If impinging momentum is sufficiently great to displace the droplet far from the levitator axis, acoustic pressure is insufficient to maintain levitation, and the combined droplets fall. Low impinging velocity and accurate targeting are both requisite if droplets are to be

Received: December 6, 2013
Accepted: January 24, 2014
Rapid Analysis of Single Droplets of Lanthanide–Ligand Solutions by Electrospray Ionization Mass Spectrometry Using an Induction-Based Fluidics Source

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Supporting Information

ABSTRACT: Electrospray ionization mass spectra of lanthanide coordination complexes were measured by launching nanoliter-sized droplets directly into the aperture of an electrospray ionization mass spectrometer. Droplets ranged in size from 102 nL to 17 nL, while metal concentrations were 293 μM. The sample solution was delivered to a source capillary by a nanoliter dispenser at a rate of 21 nL/s, and droplets were ejected from the capillary by pulsing a potential onto the capillary. The end of the capillary was situated in front of the mass spectrometer and aimed directly at the aperture. The period and power of the electrical pulse was controlled by a digital energy source. The intensity of the extracted ion time profiles from the experiment showed reproducible production of lanthanide nitrate-anion complexes (Ce, Tb, and Lu). The integrated ion intensities of the complexes were reproducible, having relative standard deviations on the order 10% for anions, and 10–30% for cations. The integrated ion intensities were proportional to the droplet size, and the response was linear from about 100 to 650 pmol. However, the intercept is not zero, indicating a nonlinear response at lower analyte quantities or droplet sizes. Cation complexes were generated in separate experiments that corresponded to lanthanide nitrate ion pairs coordinated with the separations ligand octylphenyl(N,N-diolisobutyrylamino)methylphosphine oxide (CMPO). Experiments showed a preference for formation of CMPO complexes with Ln+, having larger ionic radii. The relative standard deviation values of the cation abundance measurements were somewhat higher for the more highly coordinated complexes, which are also less stable. The mass spectral quality was high enough to measure the ratios of the minor isotopic ions to a high degree of accuracy. The approach suggests that the methodology has utility for analysis of solutions where the sample quantity is limited, or where the sampling efficiency of a normal ESI source is limiting on account of hazards derived from the sample solution.

Over the past 10–15 years, droplets have found increasing utilization in analytical chemistry, particularly for sample collection. A nanoliter-sized droplet of a nonmiscible fluid formed on the end of a syringe or capillary can be inserted into a sample liquid or headspace, whereupon partitioning of analyte into the droplet will occur. The droplet can be retracted and then analyzed directly using GC or a comparable technique. Recently, even smaller droplets have been used for accurately and reproducibly forming samples for MALDI analyses. A technique referred to as induction-based fluidics (IBF) has been used to form and shoot droplets of ionic liquids having volumes as small as 20 nL at MALDI targets, and subsequent analysis of these targets resulted in higher sensitivity and better reproducibility compared to direct deposition of microliter volumes. IBF functions by forming a nanoliter droplet on the end of a Gaussian surface, such as a nonconducting capillary. The capillary is connected to an electrical source, and application of a pulse results in induction of surface charge on both the capillary and on the surface of the droplet. The magnitude of the induced charge is correlated with surface area of the droplet and causes repulsion between the capillary tip and the droplet, launching the droplet with sufficient energy to traverse the distance between the capillary and a target. Related approaches were first reported by Lord Raleigh and commercialized in the 1990s. In the MALDI analysis of bradykinin, nanoliter targets generated using IBF displayed significantly improved response. Similarly, MALDI analyses of polymer targets made using IBF had notably enhanced intensity and signal-to-noise, likely on account of increased surface concentration of polymer analyte compared to microliter droplet deposition and possibly because of field-enhanced crystallization. The improved sensitivity and precision motivated the use of IBF to prepare samples of single neurons.

Received: March 22, 2013
Accepted: June 6, 2013
Published: June 6, 2013
Method for improved secondary ion yields in cluster secondary ion mass spectrometry

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Received 23 October 2009; Revised 10 December 2009; Accepted 14 December 2009

A method to increase useful yields of organic molecules is investigated by cluster secondary ion mass spectrometry (SIMS). Glycerol drops were deposited onto various inkjet-printed arrays and the organic molecules in the film were rapidly incorporated into the drop. The resulting glycerol/analyte drops were then probed with fullerene primary ions under dynamic SIMS conditions. High primary ion beam currents were shown to aid in the mixing of the glycerol drop, thus replenishing the probed area and sustaining high secondary ion yields. Integrated secondary ion signals for tetrabutylationammonium iodide and cocaine in the glycerol drops were enhanced by more than a factor of 100 compared with an analogous area on the surface, and a factor of 1000 over the lifetime of the glycerol drop. Once the analyte of interest is incorporated into the glycerol microdrop, the solution chemistry can be tailored for enhanced secondary ion yields, with examples shown for cyclotrimethylene-trinitramine (RDX) chloride adduct formation. In addition, depositing localized glycerol drops may enhance analyte secondary ion count rates to high enough levels to allow for site-specific chemical maps of molecules in complex matrices such as biological tissues. Published in 2010 by John Wiley & Sons, Ltd.

Recent advances in molecular imaging mass spectrometry have led to an increased interest in the spatially resolved analysis of organic compounds. Specifically, techniques utilizing imaging mass spectrometry are resulting in important biochemical applications, with secondary ion mass spectrometry (SIMS) playing a vital role for imaging and identifying chemical signatures of organic compounds. Although there has been considerable progress in the field of organic SIMS in recent years, significant limitations remain in the applicability of organic SIMS to analyze site-specific molecular heterogeneity within complex organic matrices (e.g. samples such as brain tissue). Most notable is the inefficiency of SIMS-based techniques to generate enough ions and effectively detect them after primary particle impact with the target surface. Depending on experimental conditions and the degree of ionization of molecules, it can be estimated that only one in 10⁶ molecules reaches the detector as a detectable secondary ion. In addition, recent work in this group has shown that useful yields can vary by several orders of magnitude (10⁻² to 10⁻⁵). Therefore, for site-specific analysis where limited amounts of analyte material are available for detection, the overall secondary ion signals need to be increased to allow the detection of organic molecules without compromising sample integrity through degradation. Based on the current instrumentation (not including post-ionization techniques), there are two approaches to increase useful yields: sample modification and the use of cluster projectiles. An example of sample modification to enhance ion yields is through metal-assisted secondary ion mass spectrometry (ME-SIMS). In this methodology, secondary ion yields are enhanced by deposition of a small quantity of metal on the sample surface. The second method to improve organic ion yields is through the use of polyatomic primary ion projectiles such as Au³⁺, Bi³⁺, SF₆⁺ and C₆H₆⁺, resulting in several orders of magnitude improvement in secondary ion yields, enhanced sensitivities to surface-bound molecules, increased sputter yields, and lower beam-induced damage for the sputtering of molecules. These advantages allow for increases in useful information for organic and biological material analysis. However, even with cluster beams, limitations remain such as maintaining signal longevity, secondary ion yields that are too low for molecular imaging purposes, and the need for higher mass range (>m/z 500) ion signals. As a result, a strategy for both increased ionization and increased sample longevity of secondary ion signals is needed.

It has long been known that vacuum-compatible liquids, such as glycerol, can be used as matrices to facilitate the production of sample ions in high abundance for relatively long periods in fast atom bombardment (FAB) and liquid SIMS experiments. Previous work using liquid SIMS with a glycerol matrix has yielded molecular information from many molecules formerly considered unsuitable for mass spectral analysis. Glycerol is commonly used as a matrix for liquid SIMS because it allows for the study of fragile and thermally labile organic compounds since the beam-induced damage is mitigated and its low volatility ensures relatively long matrix lifetimes. One critical
Mapping Neuropeptide Expression by Mass Spectrometry in Single Dissected Identified Neurons from the Dorsal Ganglion of the Nematode *Ascaris suum*

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**Abstract**

We have developed a method for dissecting single neurons from the nematode *Ascaris suum*, in order to determine their peptide content by mass spectrometry (MS). In this paper, we use MALDI-TOF MS and tandem MS to enumerate and sequence the peptides present in the two neurons, ALA and RID, that comprise the dorsal ganglion. We compare the peptide content determined by MS with the results of immunocytochemistry and *in situ* hybridization of previously isolated peptides AF2, AF8, and six peptides encoded by the *afp-1* transcript. We find complete agreement among the three techniques, which validates single neuron MS as a method for peptide localization. We also discovered and sequenced six novel peptides in the ALA neuron. Cloning of cDNAs and database searching of Genomic Survey Sequences showed that transcript *afp-12* encodes peptide AF36 (VPSAADMIRFamide) and *afp-13* encodes AF19 (AEGLSPLPLRFamide), AF34 (DSKLMDPLIRFamide), AF35 (DPQPQIVTDETVRFamide), and three nonamidated peptides (PepTT, PepTL, and PepGE). We have found no similarities with reported peptide expression in the nematode *Caenorhabditis elegans*. This method promises to be ideally suited for determining the peptide content of each of the 298 neurons in the nervous system of this nematode.

**Keywords:** Neuropeptide, MALDI-TOF, nematode, *Ascaris suum*, single neuron, *de novo* sequencing

Peptides have long been known to be important intercellular signaling molecules in a wide variety of species. Their role as hormones was established many years ago, yet new peptide hormones continue to be discovered (e.g., kisspeptin *(1)*). In the nervous system, peptides can act as primary neurotransmitters *(2)*. More commonly, they act as neuromodulators, affecting multiple aspects of neuronal activity. Their effects include modulation of the ion channels involved in action potential propagation and synaptic transmission and modulation of the molecular machinery of transmitter release *(3)*. In most well-studied systems, the number of peptide signaling molecules is impressively large, and as with peptide hormones, new neuropeptides are still being discovered. The details of their activity and sites of action are intricate and particular, so a full description of the role of peptides in a neuronal circuit can be complex, but it must be complete if we are to understand how neuronal circuits work.

The nervous system of the parasitic nematode *Ascaris suum* contains only 298 neurons *(4)*. This numerical simplicity makes it an attractive system for the study of the role of peptides in neuronal function. We have already shown in *A. suum* that neuropeptides are numerous, widespread, and varied and that they have potent effects on muscle and subsets of neurons *(5–8)*. Most of the peptides we have identified in *A. suum* are FMRFamide-like peptides (FLPs), and are named AF peptides *(Ascaris* FMRFamide-like peptides). The transcripts and the genes that encode the AF peptides are named *afp*’s *(Ascaris* FMRFamide-like precursor proteins).

In the related, free-living nematode *Caenorhabditis elegans*, three large families of peptides have been predicted by genome searches or sequenced directly *(9–12)*: FLPs encoded by 34 *fhp* genes, NLPs (neuropeptide-like proteins) encoded by 42 *nlp* genes, and insulin-like
The Measurement of Charge for Induction-Based Fluidic MALDI Dispense Event and Nanoliter Volume Verification in Real Time

Brent Hilker,† Kevin J. Clifford,† Andrew D. Sauter Jr.,§ Andrew D. Sauter 3rd,§ and Julie P. Harmon†
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This study preliminarily investigates whether nanoliter volumes of concentrated polar liquids and organic monomers launched to targets using induction based fluidics (IBF) can be verified through the real time charge measurements. We show that using a nanoliter IBF dispensing device and nanocoulomb meter, charge measurements made on nanoliter drops in real time are correlated with surface area following Gauss’s Law. We infer the “induction only” formation of the double layer showing the ability to determine nanoliter volumes, nearly instantaneously, in real time. We discuss the implications that these observations may have for on improving/monitoring MALDI quantitation and its quality control. (J Am Soc Mass Spectrom 2009, 20, 1064-1067) © 2009 American Society for Mass Spectrometry

Herein we used an induction based fluidics (IBF) nanoliter, microliter syringe [1]. In IBF, a charge is induced on the liquid by passing the fluid through an electric field [2, 3], inductively, not conductively as in electrospray ionization (ESI). In IBF there are no Faradaic processes, only capcitance based ones, unlike ESI. The physics behind IBF reveals [2, 3] that, unlike piezoelectric, sound, or any other technologies that are applied to transport liquids at low volumes, IBF kinetically launches drops to targets, as it dynamically directs the liquids to targets, and—as we show here—measures them on arrival, in real time.

One major IBF application, nanoliter (nL) depositions for the production of matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) plates, has been shown to increase 10-fold in signal-to-noise ratios for both bradykinin measurements and analysis precision [4]. Nanoliter-IBF depositions also produce a major increase in MALDI sensitivity and reproducibility for synthetic polymers [5] (PMMA, PEG, and PS), even with polymers with Mn values greater than 90,000 units. Yergay has also observed up to a 100-fold increase in analysis sensitivity [6] for a major class of proteins, tubulins. These and similar observations on the analytes in the Applied Biosystems (ABI) 4700 Standard [4] show significant improvement (increases of around 5-, 10-, to 100-fold) in sensitivity and a 3 to 20% increase in reproducibility using nanoliter IBF depositions for proteins, peptides, and synthetic polymers. These enhancements in a wide range of molecules and mass ranges in both positive-ion reflectron mode and negative-ion linear mode indicate that nL-IBF deposition improves sensitivity across many MALDI applications. The ability to verify a dispense event and its magnitude in real time may aid quantitative quality control (QC) on MALDI measurements, irrespective of whether they are produced on a one-at-a-time basis or via high-throughput applications on robotic systems.

IBF has many MS and non-MS applications because the simple technique can be appended to common laboratory devices that are used for routine nanoliter sample handling from syringes, pipettes, chips, pumps, and other fluid movement devices including tissue and liquid chromatography (LC)/MALDI devices. The ability to accurately measure and to verify the volume deposited per event by IBF in real time could further aid the ability to QC and to improve MALDI quantitation. The ability to easily manipulate small-volume solvents or solutes to targets also has applications in the areas of green chemistry and in biological MALDI with limited sample sizes [4]. Applications of nL-IBF exist in non-touch dispensing (micro-, nano-, and picoliter samples), in parallel LC/MALDI, in defense homeland security [7], chemistry, for desorption electrospray ionization/direct analysis in real time (DESI/DART) standardization and applications [8], DNA/RNA sample preparation [9].
Sample Preparation and Sample Presentation for Direct Analysis in Real Time (DART)

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"Less is More"

Introduction: The DART [1,2] ionization mechanism is dominated by gas-phase ion-molecule reactions [3]. Like atmospheric-pressure chemical ionization (APCI), matrix effects in positive-ion mode are related to the relative proton affinities of compounds present in a mixture. Condensed-phase phenomena that cause suppression in electrospray ionization (e.g., competition for the surface of a drying droplet) do not play a role in DART ionization. Nevertheless, suppression can occur with the DART ion source if a trace analyte with a relatively low proton affinity is associated with a large excess of a compound with a relatively high proton affinity. Smith and coworkers have shown the suppression of oxazepam in the presence of a large excess of creatinine [4].

Liquid samples have commonly been analyzed by DART ionization by dipping the sealed end of a glass rod, such as a 2 mm-diameter melting point tube, into a liquid sample. Alternatively, several microtubes of sample are deposited onto the outside of the glass rod which is then positioned within a few millimeters of the DART ion source exit. Here we show that by presenting nanoliter droplets directly in front of the DART ion source, suppression effects are drastically reduced, resulting in better detection limits and a more uniform response for mixture components. The ultimate goal is to achieve efficient ionization and transmission into the API interface of the entire sample.

Experimental: An atmospheric-pressure ionization (api-of-flight mass spectrometer (JEOL-AccuTOF™) equipped with a DART ion source (DART-SVP™) from biorad™) was used for all measurements. The DART ion source was operated with helium gas at a flow rate of approximately 1.2 liters per minute. The DART gas heater was set to 300°C and the DART exit gas was set to +250 V. The mass spectrometer atmospheric pressure interface was operated with the following potentials: orifice 1 = 20V, orifice 2 and ring lens = 5 V. The Rp ion guide voltage was set to 1000 V to transmit and detect ions of m/z 100 and greater.

Aqueous solutions containing 200 ng/mL (200 ng/mL) of creatinine were spiked with oxazepam at levels of 100 ng/mL, 1 ng/mL, 10 ng/mL, 100 ng/mL, and 1000 ng/mL. DART-exempt solutions (Albachi) of a 1 mg/mL methanol solution of 5 stimulant drugs were analyzed without further dilution. Approximately 50 nanoliter droplets for each solution were manually deposited within 1-2 mm in front of the exit grid of the DART ion source by using Nanoliter LLC's patent-pending nanoliter Cool Wave® pipette. For comparison, 2 µL aliquots of the solutions were pipetted onto the outside of the sealed end of a 2 mm Pyrex melting point tubes, which were then positioned within 2 mm of the DART exit grid for analyses using the DART-SVP linear rail. All measurements were made in triplicate to average out variations in signal intensity due to variations in sample placement.

Results: We observed that signal suppression for low-z components in the stimulant mixture was clearly reduced for 50 nl sample introduction compared to 2 µL volumes by a factor of approximately 50 (Figure 1). This led us to wonder if this was a general phenomenon that could be reproduced for the documented example of oxazepam in the presence of excess creatinine. The results for 50 nl droplets were compared to the results for 2 µL deposited on melting point tube. Suppression of oxazepam by creatinine was significantly reduced by using nanoliter droplets, resulting in better detection limits. Oxazepam could barely be detected in an actual urine sample at the 1 ng/mL level (not shown) but the ability to detect oxazepam in the presence of other chemical interferences from urine was detected by the mass spectrometer (Figure 3). Improving signal by reducing sample quantity is a well-known phenomenon in mass spectrometry and has been shown for FAB, ESI and other ionization methods. A significant improvement in MALDI sensitivity was observed for 50 nl sample deposition [6].

Conclusions:

• Sample introduction as nanoliter droplets results in a significant reduction in sample suppression.
• Nanoliter droplets permit better detection limits than the commonly-used melting-point tube method, despite the smaller sample volumes used.
• Detection limits reported here are 10 to 100 times lower than previously reported values for the same solution concentrations.
• The working hypothesis is that an excess of reagent ions in the DART gas stream results in complete ionization of the all of the components in the nanoliter droplets. In contrast, if larger samples volumes are used, the number of analyte molecules can exceed the number of reagent ions and charge competition can occur.
• Shorter desorption times for the nanoliter droplets result in temporal focusing of the signal, perhaps reducing discrimination of volatile components and improving the signal-to-background.

References:
1. DART is a trademark of JEOL USA, Inc.
Electric field enhanced sample preparation for synthetic polymer MALDI-TOF mass spectrometry via Induction Based Fluidics (IBF)

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ABSTRACT
MALDI-TOF mass spectrometry is used in the characterization of synthetic polymers. MALDI allows for determination of: modal, most probable peak (Mn), molecular number average (Mn), molecular weight average (Mw), polydispersity (PD), and polymer spread (Psp). We evaluate a new sample preparation method using Induction Based Fluidics (IBF) to kinetically launch and direct nanoliter volumes to a target without contact. IBF offers signal improvement via field enhanced crystallization. This is the first paper to discuss field enhanced crystallization in MALDI sample preparation. IBF can increase signal/noise (S/N) and signal intensity for polystyrene (PS), poly(methyl methacrylate) (PMMA), and poly(ethylene glycol) (PEG) across a mass range of 2500–92,000 Da showing more accurate Psp increases in S/N range up to: 27% for PS, 140% for PMMA, and 650% for PEG. Signal intensities increased up to: 438% for PS, 115% for PMMA, and 160% for PEG. Cross-polarization microscopy indicates dramatic morphology differences between IBF and micropipette. Finally, we speculate as to why IBF nanoliter depositions afford higher S/N values in experiments conducted in different instrumental configurations even without optimization.

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1. Introduction
Matrix assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry is currently widely used in the structural characterization of synthetic polymers. Polymer characterization by MALDI allows for the rapid determination of: modal, most probable peak (Mn), molecular number average (Mn) (Eq. (1)), molecular weight average (Mw) (Eq. (2)), polydispersity (PD) (Eq. (3)), and polymer spread (Psp) (Eq. (4)) [1–4].

\[ M_N = \sum \frac{N_x M_x}{N_x} \]  

(1)

and

\[ M_W = \sum \frac{N_x M_x^2}{N_x M_x} \]  

(2)

where \( M_x \) is the molecular weight of a molecule corresponding to a degree of polymerization \( x \), \( N_x \) the total number of molecules of length \( x \), \( M_n \) the number average molecular weight, and \( M_w \) is the weight average molecular weight.

\[ PD = \frac{M_W}{M_N} \]  

(3)

\[ P_{sp} = \frac{\Delta M_w}{M_M} \]  

(4)

PD is used to estimate the breadth of the distribution by the ratio of molecular weight average to molecular number average. \( P_{sp} \) defined by Tatro et al. is the width of the spectrum without bias being caused by the magnitude of the molecular weight of the polymer [3]. To calculate \( P_{sp} \), a Gaussian distribution is formed by aggregating the observed peaks about the \( M_w \) where \( \Delta M_w/2 \) is the absolute value difference of the width at half height. \( M_M \) is the molar mass of the monomer repeat unit. This allows for the determination of the number of monomer units within one standard deviation from the \( M_w \) which can make classification and comparison simpler.

Preparation of the MALDI target is a crucial step in obtaining optimum spectra. In 2006, G. Montaud et al. reviewed advances in sample preparation techniques that improved high mass resolution, end group identification, and sequence analysis [1]. These
FOCUS: DESORPTION IONIZATION AND MACROMOLECULAR MS
SHORT COMMUNICATION

Improving the Signal Intensity and Sensitivity of MALDI Mass Spectrometry by Using Nanoliter Spots Deposited by Induction-Based Fluidics

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A new contact-free, small droplet deposition method using an induction-based fluidics (IBF) technique to disperse nanoliter drops is described and evaluated for sample preparation in matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS). The signal intensities available when using nanoliter spots are greater than those obtained with normal, microliter spots when the same amount of analyte is used. When using an ionic-liquid matrix, the improvement in sensitivity is equal to the concentration enhancement that was achieved by using smaller quantities of matrix. When using a conventional solid matrix, however, the increase in signal intensity shows a more complicated relationship to concentration. The approach of nanoliter deposition also supports multiple spotting to increase sample concentration and, thus, sample signal intensity. Nanoliter spotting not only improves the signal intensity and sensitivity achieved by MALDI-MS but also allows a major fraction of trace samples to be saved for other experiments, thus expanding the application of MALDI-MS to biological studies where sample quantity is limited. (J Am Soc Mass Spectrom 2008, 19, 1086–1090) © 2008 American Society for Mass Spectrometry

Although matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) has wide applicability to biological and synthetic polymers, obtaining high-quality MS signals when the quantity of sample is small is difficult—a challenge that is endemic in biological studies. A limited sample amount gives rise to poor signal intensity, hampering spectra interpretation, unknown identification, and quantification, thus limiting the application in biological studies. One way to increase the signal intensity without increasing the sample amount is to make MALDI spots smaller and more concentrated.

Small-scale sample preparation on a MALDI target has been achieved in several ways. One approach minimizes dispersion of sample materials by etching or drilling small channels on the target [1, 2] or using hydrophobic materials as MALDI plate surfaces [3–6]. A second approach uses microdispensing, including use of capillary tubes [7–10], piezoelectric techniques [11–14], electrodynamic methods [15], and acoustic ejection [16]. This general approach improves MALDI for imaging [14, 16], on-line analysis [13, 17], and concentration enhancement by microextraction [10]. Common in both approaches [1–12, 15, 17] is the concept that the sensitivity of MALDI-MS increases when small sample spots are used and that the search for “hot spots” within the sample area becomes more productive when solid matrices are used. Our approach of using small volumes of concentrated analytes is motivated by the concept of purposefully creating a “hot spot.”

Herein, we describe an approach by which a nanoliter syringe and induction-based fluidics (IBF) deposit small-volume droplets (in the nanoliter range) in a noncontact, flight-controlled way. Using this technique, we first investigated the relationship between the spot size and signal intensity with an ionic-liquid matrix (ILM), taking advantage of the homogeneity of ILM and subsequent ion-signal reproducibility that they permit [18–20]. This is the first report that quantitatively compares the signal intensity enhancement achieved by nanoliter spotting with that by normal spotting using both ILM and solid matrices. Furthermore, we introduce concentration enhancement of nanoliter spots by using multiple spotting, whereby small amounts of sample and matrix are built up into a single spot of relatively high

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© 2008 American Society for Mass Spectrometry. Published by Elsevier Inc.
1044-0305/08/$32.00
doi:10.1016/j.jasms.2008.03.017

Published online April 8, 2008
Received January 21, 2008
Revised March 27, 2008
Accepted March 28, 2008
Counterintuitive, real! 20 nLs best!!! 50 nLs = higher S/N!

0.5 uL Eppendorf pipette = Noise!

"Electric" Zip Tips™, Preliminary Results
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Nanoliter

ABSTRACT
Induction based fluidics (IBF), a new, simple patented approach for transporting liquids in the micro and the macro world, is discussed. Electric fields are shown to energize liquid/s in a container/s to execute an array of purposes. IBF is shown uniquely to energize N liquids in simple off the shelf devices, inductively. We discuss calibration and other issues, as we demonstrate how simple devices can dispense nanoliters and microliters with high precision and accuracy. Furthermore, we show preliminary single and eight channel data for the Zip Tip™ made by Millipore where the device transports liquids "electrically." We briefly consider how such new devices, "electric" Zip Tips™, might automate desalting and the placement of digests for MALDI TOF analysis.

INTRODUCTION
Recently we introduced the new, simple technique, Induction Based Fluidics, IBF.* The approach is discussed in the reference and at nanoliter.com where information, pictures and video are available from a wide array of experiments. In IBF, an electric field imparts energy inductively, into (i.e., through) a container such that the liquids take on a higher level of order. Subsequently, such energy can be released kinetically to a locale or a receiver be it a surface such as a MALDI target, a microtome plate, microscope slide or other surface or container. It has been shown* that when an electric field deposits energy into what amounts to an inanimate object containing a liquid, highly accurate and precise flows can result from inexpensive materials such as capillaries, Zip Tips™, microliter syringes and other "inanimate" or static objects. Moreover, such objects can transport one or more liquids when so energized simultaneously, and since such containers (e.g., cylinders) can have contents that perform functions (e.g., desalting media for MALDI TOF experiments, SPE, etc), composite inanimate objects can be energized to perform functions.

We have shown that energy, deposited inductively into static devices, can cause such objects to become dynamic and perform dispensation or other useful functions. As such, simple inexpensive devices which have no moving parts other than the liquid itself, offer the potential for high reliability and excellent accuracy and precision in, for example, liquid dispensing. For this and other reasons such devices can be operative across an enormous dynamic range from the μL/sec to pL/sec flow regimes facilitating interaction of the micro and macro world.

Consider the implications of this very simple arrangement shown in Figure 1. Objects such as Zip Tips™, glass microliter syringes, and capillaries (singular or plural) can be made to be "electric" simply by appropriately placing them in an electric field. Just as in an ion source of a mass spectrometer, where the potential of gas phase ions is accelerated to lower energy, an analogous approach can be employed to "fly" liquids to lower areas of energy through simple holding devices like pipette tips to appropriately targeted receivers (a beaker, vial, or plastic array). This simple approach is executed without using exotic and costly devices manufactured from silicon wafers in expensive clean rooms and sold at great premiums or as per chemistry labs on chips where the eventual device costs as much as a real lab to develop.

To illustrate the principle, we show five Gaussian surfaces, square tubes we imagine containing five liquids which may be the same or different (if these were cones, they would physically resemble Zip Tip™). Note the electric field pointing from positive to negative inductively couples this energy into the liquids with them not being physically electrically connected. This inductive coupling provides energy which eventually can be released

Figure 1. Electric field inductively coupling with five containers holding liquids.

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Fused Silica Capillary Column GC/MS for the Analysis of Priority Pollutants

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Key Words:
GC/MS
Capillary columns, fused silica
Priority pollutants
Quantitation

Summary
Operational characteristics have been determined for fused silica capillary column (FSCC) GC/MS as applied to "extractable" priority pollutants. Chromatographic data show excellent relative retention time (RRT) interlaboratory precision and interlaboratory accuracy when multiple internal standards are employed. Potential chromatographic problems, such as column overload and "double peakin", are addressed. Response factor relative standard deviations (RSD) at 50 ng for most of the extractable priority pollutants over the long term indicated precise determination (i.e., RSD generally <10%). Linearity was demonstrated over two orders of magnitude for FSCC GC/MS analysis of compounds with relatively low and high RF (response factor) values. Potential quantitative problems, such as saturation, are discussed. For certain aromatic priority pollutants, interlaboratory RF agreement was observed. This was noted as perhaps the most important property of FSCC GC/MS analysis when the multiple internal standard approach is utilized. Determinations of extractable priority pollutants are directly compared for packed column GC/MS and FSCC GC/MS analysis of separate and composited extracts. For six extracts analyzed in triplicate, the latter configuration was shown to produce more consistent results. In view of the advanced analysis logistics of composite extract FSCC GC/MS analysis, this approach was established as the preferred method for the analysis of priority pollutants classified as extractable.

1 Introduction
The U.S. Environmental Protection Agency (EPA) has proposed analytical protocols for the analysis of extractable priority pollutants. The EPA has been employing these protocols for the determination of priority pollutants in hazardous waste [2]. Sample preparation procedures utilized to isolate priority pollutants from aqueous and nonaqueous samples generally involve liquid extraction procedures which generate at least two extracts containing base/natural and acid extractable priority pollutants. Current analysis protocols require the separate GC/MS analysis of each extract using different packed GC columns.

We originally reported that fused silica capillary columns (FSCC) coupled directly to the ion source of the mass spectrometer could be employed for the simultaneous GC/MS analysis of acid, base-neutral, and pesticide priority pollutants [3, 4]. This analysis configuration affords a potential reduction of GC/MS acquisition time of approximately 80% as compared to existing packed column GC/MS methods and, therefore, should significantly lower the cost of priority pollutant analysis. As importantly, initial results indicated that the data were generally of better quality. The analysis time reduction is realized because extracts can be combined and analyzed in one injection rather than two. Compositing of extracts is possible because capillary columns provide higher resolution (more effective theoretical plates) per unit time. Moreover, direct coupling of FSCC to the ion source and the apparent inertness of fused silica provides for consistent chromatographic elution for reactive (e.g., hexachlorocyclopentadiene) and very polar analytes (e.g., isophorone); therefore, the extractable priority pollutants can be analyzed simultaneously. Analysis methods which reduce costs, and produce data of equivalent or better accuracy and precision, are of obvious benefit to the U.S. Environmental Protection Agency and to others testing for priority pollutants.