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Mass Spectrometry — Sustaining Growth in a Challenging World

This article provides a focused outlook on how laboratory mass spectrometry (MS) techniques will fare this year compared to 2012. We look specifically at triple-quadrupole MS, Fourier transform MS, quadrupole time-of-flight MS, ion-trap MS, inductively coupled plasma-MS, matrix-assisted laser desorption-ionization time-of-flight MS, and magnetic sector instruments as well as the clinical market and government demand. Finally, we examine the competitive landscape in these areas.

Stuart M. Press

The worldwide market for laboratory mass spectrometry (MS) techniques in 2012 was worth in excess of $3.4 billion, which was up more than 4% versus 2011, despite increasing weakness in many areas toward the end of the year. Figure 1 provides estimated total revenues for the market from 2011 through 2014, which will average better than 5% annual growth. Triple-quadrupole instruments have become some of the most significant drivers of the market. High-end techniques including quadrupole time of flight (QTOF) and Fourier transform mass spectrometry (FT–MS) are also likely to be major contributors to the growth of the overall MS market, but are more sensitive to constrained capital spending because of their high price. Demand for ion-trap and magnetic sector technologies are among the most mature portions of the market, while matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF-MS) and inductively coupled plasma–mass spectrometry (ICP-MS) appear to have strong future potentials remaining. The clinical analysis sector is now becoming one of the strongest industrial drivers of demand for MS, but government and related demand will clearly present a challenge to the market in the short term. Despite the growth of the overall market, the barriers to entry have resulted in a significant concentration of vendor share among a handful of companies.

Triple-Quadrupole Technologies

Triple-quadrupole MS, often referred to as tandem MS, is currently the hottest technology in the MS market. Triple-quadrupole gas chromatography–mass spectrometry (GC–MS), liquid chromatography–mass spectrometry (LC–MS), and ICP-MS grew at a double-digit pace in 2012, and now account for more than a quarter of total MS demand. Triple-quadrupole instruments have proven to be extremely good for quantitation of very low trace levels of compounds of interest, even in very complex samples. This has made such techniques extremely popular in a wide range of industries and applications, such as analyzing low-level contaminants in environmental water samples, detecting trace levels of pesticides and other health threats in foods, and quantitating key biological markers in the clinical analysis sector. The pace of new model introductions has been extremely high over the past three years, with each new system often claiming to offer an order of magnitude improvement in sensitivity or better, as well as other major performance gains. All four current competitors in the triple-quadrupole GC–MS market have introduced new models during this period, including segment leader Agilent, and recent market entrant Shimadzu. In the triple-quadrupole LC–MS area, there have been more than a dozen new instrument introductions since 2010, including the entrance of both Bruker and Shimadzu to the area. In 2012, Agilent introduced the first ever commercial triple-quadrupole ICP-MS system, which leverages the low-level quantitative capabilities of triple-quadrupole MS for a technique that is already established for trace-analysis applications. Despite a
somewhat challenging environment in 2013, triple-quadrupole–based MS technologies should still average high single-digit growth.

**FT–MS Versus QTOF**

At the high end of the mass spectrometry market in terms of both cost and performance are QTOF and FT–MS instruments. Although the two categories are drastically different in how they work, QTOF and orbital trap–based FT–MS instruments both offer very high resolution and compete vigorously against one another. Thermo Scientific is the one and only supplier of the orbital trap–based mass spectrometers (Orbitrap) that it targets to the same customers served by QTOF suppliers, which primarily include AB Sciex, Agilent, Bruker, and Waters. Bruker is the only other major supplier of FT–MS instruments now that Agilent has discontinued the IonSpec product line that came with the Varian acquisition. However, Bruker’s models are generally a level above Thermo’s orbital trap–based models in both price and performance, and do not generally compete with QTOF instruments. It is not clear which technology, if either, will win out in the long term among the advanced life science researchers that are the primary users of these instruments. All of the major competitors in this area of the market have continued to develop and introduce new models with significant performance improvements year after year, which has helped to drive what has been among the strongest growing segments of the MS market. However, 2012 was a weaker year, as it was for most other analytical technologies, although the impact was significantly stronger in the QTOF market than for FT–MS. Looming government budget cuts and great uncertainty in government spending had a large part to do with it, causing many research institutions to delay such large capital expenditures. Such impacts are likely to continue well into 2013, although demand should pick back up to a double-digit pace in 2014 because these instruments are now leading the way in many life science sectors, as well as becoming more common in applied markets.

**Ion-Trap Mass Spectrometry**

One class of MS that appears to be waning is ion-trap-based instruments. Traditionally, ion-trap instruments, including both LC–MS and GC–MS, were better at qualitative analyses and quadrupole systems were better for quantitative analyses. In addition, ion-trap systems have traditionally been less expensive. However, the majority of applications for which GC–MS is used tend to be more quantitative than qualitative, and the rise of triple-quadrupole GC–MS and GC–TOF–MS has eaten away at ion-trap GC–MS demand as well, resulting in the potential for fading demand in some applications. Thermo Scientific and the former Varian product line that is now owned by Agilent have long been the only two major suppliers of ion-trap GC–MS systems. In the LC–MS market, the development of various technologies, including QTOF and FT–MS, has negatively affected demand for conventional ion-trap LC–MS. However, the development of novel ion-trap designs, and the incorporation of electron-capture dissociation (ECD) has helped the technique remain relevant in certain applications and, therefore, demand is still expected to see modest growth in the foreseeable future, although not at the pace of other LC–MS technologies. Only Thermo Scientific and Bruker, which offer advanced ion-trap designs, remain significant vendors, with Hitachi, and more recently Agilent, bowing out of the area. The low cost and simplicity of ion-trap MS has been attractive to vendors of portable mass spectrometers, variations of which can be found in newer models. However, uncertain government demand will make for weak demand for the portable MS market in the near term.

**ICP-MS and Secondary Ion Mass Spectrometry**

Demand for ICP-MS has steadily grown to more than $360 million since the first commercial introduction 20 years ago, and is on course to rival the size of total demand for atomic absorption (AA) and inductively coupled plasma–optical emission spectrometry (ICP-OES) in a few more years. ICP-MS offers higher throughput and much lower levels of detection relative to these other elemental analysis techniques. Much of the demand for ICP-MS is related to water analysis in the en-
vironmental and the semiconductor and electronics industry, while secondary ion mass spectrometry (SIMS) is also common in semiconductor labs. Combined, ICP-MS and SIMS account for 14% of the total laboratory MS market, as shown in Figure 2. Demand from the environmental sector continues to be driven by regulatory requirements that have been set tighter and tighter, and therefore necessitate the use of ICP-MS, as other techniques do not offer the same level of sensitivity. Microchips and other electronics are incredibly sensitive to trace metal contamination, which can come from process water and chemicals used in the manufacturing process. ICP-MS has become the standard technique for monitoring the quality of such fluids. Demand for capital equipment in the semiconductor and electronics industry is notoriously cyclical, and the early part of 2013 marks a point of decline for demand, which will limit overall growth in ICP-MS demand to the low single-digits. However, the prospect for stronger overall growth in ICP-MS demand is much better in 2014 and beyond. SIMS demand, on the other hand, is even more reliant on the semiconductor and electronics industry, and will see a small decline in 2013 before rebounding.

**MALDI-TOF**

MALDI-TOF mass spectrometry includes several significantly different variations, such as simple low-cost linear systems, reflectron TOF systems, and tandem TOF (that is, TOF-TOF) instruments. There are also a number of ion-trap time-of-flight (IT-TOF) and QTOF variants on the market, most of which are based on existing LC–MS platforms. Demand for the various configurations of MALDI-TOF instruments has been somewhat variable over the past decade, leading to inconsistent year-to-year growth. MALDI-TOF-TOF systems, which are the highest performing, most capable, and most expensive of the variants are now seeing high single-digit growth, mostly because of demand from biomarker research laboratories. However, it is the lower-cost linear MALDI-TOF segment that is really driving the entire MALDI-TOF market. Linear models, which in some cases cost less than $100,000, offer a relatively simple alternative to LC–MS, and are now commonly used as the basis for a number of clinical diagnostics solutions, as well as for rapid screening in other industries.

**Magnetic Sector**

Magnetic sector MS has become somewhat of a niche technique, with most of the demand centered around geological research, environmental testing, and sports doping applications. It is a very mature market and is expected to see demand continue to diminish gradually as the capabilities of other types of mass spectrometers continue to improve, enabling more and more applications to be transferred away from magnetic sector instruments. Thermo Scientific has consolidated the majority of vendor share in the market, while all of the other competitors in the market, with the exception of Waters, are small niche vendors that do not compete in other areas of the MS market.

**The Clinical Market**

The clinical analysis market, including both research and diagnostics, has seen very strong growth for more than a decade and has been largely immune to the impact of weaker economic environments and recessions. There has long been considerable interest and attention paid to the potential for MS to become a commonplace tool in the sector, particularly for clinical diagnostics, but thus far the vast majority of demand has remained on the research side of the market. The ability of MS techniques to quantify very low levels of critical biomarkers of diseases...
makes the technology potentially attractive to the clinical market, as it offers a combination of sensitivity, selectivity, and speed that is often unmatched by currently used techniques. Also, MS can analyze multiple analytes simultaneously. Triple-quadrupole LC–MS has by far become the most common category of MS used in clinical analysis, particularly on the diagnostics side of the market, although MALDI-TOF, QTOF, and FT–MS are also significantly used. The limitations to the use of MS in diagnostics continues to be complexity, sample preparation, and lack of standardization; however, vendors have been working hard to address these issues. Because of these limitations, very few MS systems have yet been able to gain the Food and Drug Administration (FDA) 510(k) approval as medical devices in the US, although many clinical laboratory improvement amendment (CLIA)-certified clinical laboratories, have developed MS-based laboratory developed tests (LDTs) that do not require FDA certification. In Europe and Canada, however, there are now a number of regulatory approved MS-based clinical diagnostic tests. Over the last several years vendors have been putting increased resources into gaining regulatory approval for their MS solutions in all regions, as well as partnering with major clinical testing laboratories to help them develop their own tests. The most obvious part of this effort on the part of MS vendors has been the acquisition of leading vendors in the clinical diagnostics market. PerkinElmer was one of the first with its 2006 acquisition of NTLabs, which focuses on prenatal screening, and helped PerkinElmer develop LC–MS–MS-based neonatal screening solutions that were among the first regulatory approved MS-based clinical testing solutions. In 2011, Danaher acquired IRIS International, which specializes in urinalysis, and combined it with the Beckman Coulter Diagnostics group. Also taking place in 2012 were the acquisitions of One Lambda by Thermo Scientific, and Dako by Agilent, which accounted for more than $500 million in diagnostics revenues in 2012. Thus far, the clinical market, including both research and diagnostics, accounts for about 6% of the total MS market, but that percentage will grow significantly over the next several years.

Government Demand
There has recently been a heavy focus on the potential impact to various industries by cutbacks and uncertainty in government spending, and the MS market is certainly no exception in this regard. Whether it is austerity in Europe, the sequester in the US, or cutbacks elsewhere, it is undeniable that overall demand for MS from government laboratories and laboratories that receive significant government funding are likely to take at least a small hit in demand in 2013. It is not only directly funded government research and regulatory testing laboratories that are affected, but so too are many academic research laboratories whose projects are government funded. Contract environmental testing laboratories are also likely to see an impact, as all levels of government, from federal to local, are facing difficult times and are likely to cut back the volume of water and environmental samples sent out for testing, when possible. Such impacts could already be clearly felt in late 2012, as government and related demand for initial systems, particularly for higher ticket models, was flat or declined slightly as end-users delayed large capital investments. However, most of these laboratories continued to perform their activities, thereby leading to slight growth in aftermarket and service demand. The major MS vendors are none too optimistic that this situation will reverse itself significantly before the end of 2013.

The Competitive Landscape
Although the major vendors and their overall vendor shares have remained fairly consistent over the past half-decade, the competitive landscape has shifted considerably. The complexity involved in designing, manufacturing, marketing, and supporting mass spectrometers provide for very high barriers to entry; therefore, vendor share has always been relatively concentrated. The top four vendors, consisting of AB Sciex, Agilent, Thermo Scientific, and Waters, have remained the same throughout this time, and have consistently accounted for about two-thirds of the overall vendor share, combined, although Agilent has gradually improved its vendor share. Bruker, Shimadzu, and PerkinElmer are also considered major MS system vendors and have steadily improved their overall positions in the market in recent years. Of course, the one notable name that has vanished is Varian, with most of its MS business being broken up between Agilent and Bruker. During this period, the somewhat convoluted arrangement between MDS SCIEX, Applied Biosystems, and PerkinElmer was reshuffled into much more simplified businesses, with the vast majority of the operation now part of AB Sciex, which is owned by Danaher, and the ICP-MS operation now fully controlled by PerkinElmer. These top seven companies now account for well over 80% of the entire MS vendor share.

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For more information on this topic, please visit our homepage at: www.spectroscopyonline.com
Sample Preparation Guide for Mass Spectrometry–Based Proteomics

Sample preparation is an integral, but sometimes neglected, part of a successful mass spectrometry (MS)-based proteomics experiment. Good sample preparation techniques require a profound understanding of the biological samples to be analyzed and of the liquid chromatography (LC)–MS process. Depending on the experiment, biological samples often contain components (buffers and salts, detergents, polyethylene glycols, lipids, chromatins, antibodies, and streptavidin) that are not necessarily compatible with the LC–MS-MS analysis. Thus, successful sample preparation starts with a proper experimental design. Whenever possible, electrospray ionization-MS incompatible components should be systematically replaced with compatible components, such as volatile salts and MS-friendly detergents. In addition, the removal of incompatible components should be advised when they cannot be avoided (for example, an insoluble membrane protein requires detergent for solubilization or streptavidin resin is needed to enrich biotin-labeled proteins and peptides). This review article summarizes successful sample preparation strategies that led to some of the highest peptide and protein identification rates reported in the literature.

Sonja Hess

Mass spectrometry (MS)-based proteomics is playing an increasingly important role in fundamental and applied biology. As such, it attracts many newcomers to the field who want to apply this powerful technique to their specific biological question. To ensure that MS-based proteomics delivers the expected outstanding results, it is important to communicate the requirement on the sample preparation. It is also crucial to understand the unique aspects of MS measurements, next to optimizing data acquisition and interpretation. First, unlike any other biophysical technique, such as nuclear magnetic resonance (NMR) spectroscopy or microscopy and imaging, MS consumes its samples by ionizing them in the gas phase. Thus, ionizable contaminants in the sample will compete to be detected. Furthermore, complex biological matrices may not only be rich in proteins but also rich in metabolites, lipids, nucleic acids, sugars, and other molecules. If not removed, they will also compete with the peptides for ionization. In addition, complex buffer and detergents systems commonly used in biological samples can also lead to ion competition and ion suppression. Therefore, one goal should be to eliminate or at least reduce contamination and increase the number of peptides in a sample. In addition, in discovery-based proteomics operated in the data-dependent mode, where the most abundant ions are successively subjected to fragmentation, a bias toward the most abundant species exists. When one considers all these factors, it is apparent that a high demand is to be placed on sample preparation techniques when optimized MS results are sought. This review addresses some general considerations and gives practical advice for complex proteome analyses to achieve overall higher peptide and protein identification rates (1,2).
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General Guiding Principles

Although it should be noted that every proteomics sample is different and likely requires individual optimization, some general guiding principles apply to all proteomics samples. To achieve optimized results, the entire pipeline from initial sample preparation, liquid chromatography–tandem mass spectrometry (LC–MS-MS) operation, and bioinformatics analyses need to be taken into account. Systematic preventive elimination of contaminants is preferable over retrospective reduction of contaminants, although this cannot always be achieved in every setup.

To reduce the number of unwanted keratin contaminations, as a general rule, the use of a laminar air flow hood, protective clothes, and gloves is strongly encouraged for any proteomics experiments. This also means that a gel, for instance, should not be carried around uncovered when a subsequent MS investigation is intended.

Efficient cell lysis (or organelle isolation and lysis) is a prerequisite for subsequent comprehensive proteome analysis. Mechanical cell lysis is preferred over detergent-based lysis. If a detergent-based lysis is used, the detergent should be removed, either using a gel-based method or filter-assisted sample preparation (FASP) (3,4). Equally important is an effective digestion protocol that produces few missed cleavages, few unspecific cleavages, and few undesired side reactions during disulfide reduction and alkylation. Standard database searches rely on specific enzymatic cleavages, although missed cleavages and semispecific cleavages can be specified, enabling their identification as well. Nevertheless, if a peptide is present in properly cleaved and missed cleaved form, its signal intensity will be distributed into the number of forms present and the sample complexity (that is, number of detectable peptide ions) will be increased. This consideration is particularly important when very complex samples are analyzed in the smallest possible time frame.

For bottom-up proteomics approaches, trypsin is the most commonly used enzyme. Specifically, when complex proteomes are digested in solution, tryptic digestion is usually done after a predigestion with Lys-C, which is more tolerable to urea. This ensures proper digestion of proteins that would otherwise be inaccessible to trypsin. The use of urea is not without its own disadvantages and can cause carboxymyllations (via its decomposition to...
ammonium cyanate), particularly when aged solutions (that is, not prepared fresh before use) are used or when used at elevated temperatures (above 25 °C) (5–7).

While commonly used to solubilize proteins, the use of polyethylene glycol (PEG)-based detergents (NP-40, TritonX) is discouraged. As shown in Figure 1, PEG can be easily recognized in a spectrum by its equidistant spacing of 44.026 Da due to its ethoxy groups. In a worst-case scenario, this dominates a spectrum over an entire chromatogram, making the identification of peptides difficult, if not impossible. Fundamental analyses by Ogorzalek-Loo showed a limited effect of nonionic detergents such as n-dodecyl-β-D-maltoside (DDM) on MS ion suppression (8). Experience from my laboratory shows that DDM or 5-cyclohexyl-1-pentyl-β-D-maltoside (CYMAL-5) can be used instead of NP-40 or TritonX if a detergent is needed to keep proteins in solution.

Before LC–MS-MS analysis, samples need to be desalted. Depending on the sample size, commercial traps with bed volumes of as little as 0.5 µL can be used in an offline high performance liquid chromatography (HPLC) setup. When combined with UV detection, this approach is advantageous over cartridge or StageTips (9) usage, in which no quantitative data are obtained during the desalting steps.

In addition to in-solution digestion, in-gel digestion is still a common way to purify proteins from otherwise hard to remove contaminants. Direct comparison of in-gel and in-solution digestion protocols in the Mann laboratory show that the sensitivity is comparable (10). Gel digestion seems to be slightly more preferential for membrane proteins, whereas in-solution digestion seems to favor soluble proteins.

The most challenging proteomics studies are those that try to identify and quantify global proteomes of prokaryotes (such as Mycobacterium tuberculosis [11]) or eukaryotes (such as yeast [1,10,12] or HeLa lysates [13–15]). To reduce sample complexity, many experiments are focused on the subproteome of an organelle (for example, mitochondria [16]) using classical subcellular fractionation techniques. In addition, sample complexity can also be effectively reduced when enrichment techniques are used. For instance, the enrichment could be achieved using immunoprecipitations (17,18) or activity- or affinity-based pharmacoproteomic approaches (19). Enrichment could also be directed toward a specific characteristic of proteins — for example, toward newly synthesized proteins.

Figure 2: LC–MS (top) and LC–MS-MS (bottom) chromatogram of a global tryptic digest of a bacterium. The aim is to optimize the LC gradient so that peptides are distributed equally and are available for MS and MS-MS analysis throughout the chromatogram.
proteins (20,21) or glycoproteins (22). For these experimental setups, additional considerations are necessary:

- In the case of organelar proteomics, all possible precautions should be taken to isolate the desired organelle. It should nevertheless be clear that all biochemical methods used to do so will remain crude methods and contaminating proteins from other cytosol or other organelles should be expected. Repeated analyses with strong statistical tests are often needed to differentiate contaminating proteins from true organelar proteins. Another (undesired) organelle can serve as a good negative control.

- In the case of enrichment proteomics, two main approaches have emerged, either immobilizing the bait or the proteins of interest. Many approaches take advantage of the strong noncovalent interactions of biotin and streptavidin using streptavidin or neutravidin beads and biotin-tagged proteins. Other commonly exploited affinity tools are the FLAG-tag, HA tag, or GFP tag used in combination with an antibody towards either of these tags.

Driven by the desire to achieve the highest sensitivity, it was not too uncommon in the past to digest bound proteins directly on beads. In our experience, this has two distinct disadvantages. First, the beads themselves may introduce polymeric background ions that lead to ion suppression and may persist in the LC and MS system that could in extreme cases require the replacement of all tubing and cleaning of the ion optics. Thorough washing of the beads (before and after immobilizing the protein and without denaturing the immobilized protein) is highly recommended. Secondly, peptides generated from the digested antibody, streptavidin, or neutravidin may dominate the chromatograms and spectra, preventing lower abundance proteins to be detected. To address this disadvantage, we recommend to either elute all proteins with biotin and separate them on a gel omitting the inevitably eluting streptavidin or neutravidin band or to carefully denaturing the protein so that the bound proteins can be released predominately, followed by in-solution digestion (17). Using click-based chemistry, in which alkyne-activated agarose resins are reacted with azidohomoalanine (AHA) containing newly synthesized proteins, Eichelbaum and colleagues (23) developed a method that entirely circumvents the streptavidin contamination. The alkyne-activated agarose resins can and must be much more rigorously washed before and after binding, further reducing contamination. In our hands, so far the FLAG tag achieved best results (17,18). In all cases, these steps have to be carefully optimized in terms of bait and sample load with appropriate positive and negative controls.

Figure 3: The MS ion injection time over retention time indicates stable spray conditions. Only at the beginning of the chromatogram where few peptides were eluted, MS ion injection times reach 100 ms and more. The median ion injection time was 3.5 ms.
old for triggering MS-MS events, the number of MS-MS events, the automatic gain control target value (ion population) for MS and MS-MS, the maximum ion injection time for MS-MS, when to choose rapid and normal scan rate, monoisotopic precursor selection criteria, and prediction of ion injection time for a selected mass resolving power (1,2).

**Bioinformatics**

Part of our integrated approach is the use of bioinformatic quality control tools. To assess data quality and performance of an individual experiment or instrument, the freely available visualization tools LogViewer (pel.caltech.edu/software) and RawMeat (http://vastscientific.com/rawmeat/) are indispensable (24). By displaying useful instrument statistics (such as ion current fluctuation indicates problems with the spray), these tools guide users towards better sample preparation and data acquisition. Figure 3 shows the MS ion current of a 90-min analysis indicating good spray conditions with a median injection time of 3.5 ms. Only at the beginning, when no ions are eluted, higher injection times of around 100 ms are observed.

Preview (http://www.proteinmetrics.com) is used to diagnose unusual modifications or incomplete digestion in a peptide mixture (25).

To convert raw files into a searchable file format, my laboratory uses ReAdw4Mascot2 because of its superior monoisotopic peak picking. To identify peptide spectrum matches, my laboratory has developed a freely available second generation ROCIT search engine (rocit.caltech.edu), which is among the first search engines that take into account known modifications listed in Uniprot (2). The sophisticated signal processing tools for Thermo raw files make MaxQuant a premier choice for quantitative bottom-up proteomics (1,2,26).

**What to Expect?**

To optimize our workflow and for quality control, we use global tryptic digests of yeast lysates that we analyze in 160-in analyses. In an optimized analysis using an Orbitrap Elite, from a yeast lysate (200 ng sample load), we expect 5500 MS scans and 41,000 MS-MS scans yielding 17,000 peptide spectrum matches and identifying 12,800 unique peptides (or 2100 proteins with more than two peptides) (2). This equals 80 unique peptides and 13 proteins/min analysis and is among the highest identification rates reported in the literature (2).

**Conclusion**

While there is no magic bullet, knowledge in the sample requirements and careful sample preparation enables high identification rates of complex biological samples. Sample preparation is an integral and often underestimated part of the success of a proteomics experiment and is as important as optimizing the LC, MS, and bioinformatics analyses. When all parts of a proteomics experiment are optimized, outstanding results can be obtained.

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For more information on this topic, please visit our homepage at: www.spectroscopyonline.com
Rapid and Specific Determination of Contaminants, By-Products, and Additives in Insulating Mineral Oils with Tandem Mass Spectrometry

Insulating oils are often considered the life blood of electrical devices such as transformers. Proper functioning of these devices is essential for uninterrupted electrical power over long periods, often stretching into decades. To ensure longevity of insulating oils and improve certain characteristics additives are often added to the oils. On the other hand, the presence of contaminants can deteriorate the performance of the oils and can cause extensive damage to the electrical devices. To ensure optimal operation of electrical devices, additive and contaminant levels should be periodically monitored. Because of the complex chemical composition of insulating mineral oils, the determination of additives and contaminants is a difficult and often laborious task. However, the task can be made manageable with the use of current state-of-the-art analytical instrumentation. This article demonstrates the use of electrospray ionization–mass spectrometry for rapid and specific determination of widely used metal deactivators Irgamet 30 and Irgamet 39. The article also presents specific quantitative determination of a highly corrosive sulfur compound dibenzyl disulfide and its principle nonsulfur by-product bibenzyl with gas chromatography and tandem mass spectrometry (GC–MS–MS).

Carlo Roggero, Jinyu Du, R. Seemamahannop, S. Kapila, V. Tumiatti, and Michela Tumiatti

Corrosion of metals, such as copper, resulting from chemical reactions with sulfur and other chemical species in insulating liquids has been a matter of concern for decades. The presence of corrosive species in general and in particular corrosive sulfur has been linked to failures of electrical equipment used in generation, transmission, and distribution of electrical energy. For this reason, the International Electrotechnical Commission (IEC) standard for mineral insulating oils states that corrosive sulfur compounds shall not be present in unused and used insulating liquids (IEC 60296 clause 6.10) (1).

It is generally accepted that the presence of corrosive sulfur species in mineral oil leads to cuprous sulfide (Cu$_2$S) deposits on the surface of copper conductors. Cuprous sulfide is a semiconductor, and its buildup and subsequent migration into insulating paper over time disrupts the integrity of the insulating paper, which leads to short-circuit faults, sometimes accompanied by windings deformation (2–4). Efforts have been made to assess Cu$_2$S formation resulting from the presence of corrosive sulfur compounds in mineral insulating oils for more than 60 years. Clark and Raab (5) developed a qualitative method for detecting corrosive sulfur in mineral insulating oil during the 1940s. Their approach is still being used with some modifications as standard test methods; for examples, see ASTM D-130 (6), ASTM D-1275 06 (7), DIN 51353 (8), and IEC 62535 (9). However, the standard test methods yield only qualitative results for whether oil contains corrosive sulfur compounds or not under test conditions. Furthermore, the IEC standard test method has been shown to yield false positive results with aged insulating oils and false negative results with oils containing metal deactivators or passivators (10). To overcome the limitations of
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the current standard test methods for corrosive and potentially corrosive sulfur species, the IEC initiated the development of a quantitative method for a highly corrosive sulfur compound dibenzyl disulfide (DBDS) as well as the development of methods for the quantitative assessment of total corrosive sulfur and specific corrosive species in insulating liquids (11).

A part of this article deals with the specific method for the determination of DBDS and its principle nonsulfur by-product bibenzyl in mineral insulating oils.

This article also deals with the determination of metal deactivators in insulating mineral oils. Metal deactivators or passivators are corrosion-inhibiting chemicals (additives) that interact with metal surfaces and form 0.5–1 nm thick monolayers that limit the access of the corrosive chemical species to the metal surface (12). Common metal passivators added to insulating mineral oils are triazole and benzotriazole derivatives; the two surface passivators in mineral insulating oils are triazole-1-methyl amine. Irgamet 30 consists of N,N-bis(2-ethylhexyl)-1-triazole-1-methyl amine and N,N-bis(2-ethylhexyl) 5-methyl-1-benzotriazole-1-methyl amine. Irgamet 30 contains two 2-ethylhexyl chains attached to the triazole or the tolyltriazole moieties. The presence of the 2-ethylhexyl chains in the structure enhances solubility of these compounds in mineral oils. However, the addition also makes the molecules highly unstable and extremely difficult to detect and quantify with methodologies that involve the use of gas chromatography (GC) or liquid chromatography (LC). In fact a well-accepted reversed-phase LC–UV absorption-based method for determination of Irgamet 39 in insulating mineral oil, quantifies only toluyl triazole and not the intact Irgamet 39 (14). However, reversed-phase LC coupled with electrospray ionization (ESI) and tandem mass spectrometry (MS-MS) has been used for quantitative determination of triazole and benzotriazole derivatives in aqueous environmental samples at very low concentrations (15). An LC–ESI-MS-MS method for determination of Irgamet 39 and Irgamet 30 has been reported (16). The method makes use of fragment ions resulting from precursor ion m/z 242 for quantification of both Irgamet 39 and Irgamet 30. The authors assumed that intact Irgamet 30 and Irgamet 39 molecules are separated with reversed-phase LC and the ion m/z 242 results from fragmentation of the parent ion in the ESI source. The ion m/z 242 results from protonation of di 2-ethylhexyl amine, which is present in both metal deactivators. Results obtained in our laboratory indicate that di 2-ethylhexyl amine is released from metal deactivators before their introduction into the LC column. Thus, the approach as described is unlikely to yield results that can be used for specific quantification of Irgamet 30 and Irgamet 39. Therefore, it is apparent that current methods for the detection of these widely used metal deactivators or passivators in mineral insulating oils are unsatisfactory. This article reports a rapid and specific method for the determination of Irgamet 39 and Irgamet 30 in mineral insulating oils with direct infusion ESI-MS, detection limits of the method for both metal deactivators were lower than 1 mg/kg.

**Experimental**

**Materials**

Dibenzyl disulfide (DBDS), diphenyl disulfide (DPDS), and bibenzyl (99% purity) were purchased from Sigma Aldrich. White mineral oil was purchased from a local vendor. Insulating mineral oil samples were kindly provided by Sea Marconi. Acetone, acetonitrile, hexanes, iso-octane, and methanol (all Optima grade) were purchased from Thermo Fisher Scientific. Irgamet 30 and Irgamet 39 were kindly provided by BASF. The GC column was purchased from P.J. Cobert Assoc.

**Reaction of DBDS with Metallic Copper**

Mineral oil was fortified with DBDS at concentrations ranging from 50 to 500 mg/kg. Aliquots of the fortified oil weighing 10 g were transferred to 20-mL borosilicate glass vials, and 1 g of copper granules (~425 µm) was put in the vials along with a 13 × 8 mm PTFE-coated magnetic bar. Vials were purged with argon for 20 min and then were sealed with PTFE-lined crimp caps. The sealed vials were inserted in a thermostated aluminum heating block with 15 cylindrical bores (23 × 56 mm). The size of the bores permitted insertion of the vials in the aluminum block up to their necks. The crimp caps were thus maintained at near-ambient temperature and remained gas tight. The magnetic bars were activated with a magnetic stirrer. The block temperature was maintained at 150 °C. The vials were removed from the heating block after reaction periods varying from 30 min to 8 h.

**Quantification of DBDS and Bibenzyl**

The concentration of DBDS in mineral oils before and after reaction with copper granules was determined with a gas chromatograph interfaced to a triple-quadrupole mass spectrometer (GC system model 3800; MS system model 320, Varian,
Electrospray Ionization–Mass Spectrometry of Irgamet 30 and Irgamet 39

These experiments were carried out with a triple-quadrupole mass spectrometer (model 320, Varian [Agilent Technologies]) equipped with an ESI source. The mass spectrometer was operated in the single-quadrupole mode, whereby ion analysis was performed only with the first quadrupole and the second and third quadrupoles were operated in the pass all mode. Ions were monitored in the positive ion scan mode with nitrogen as the nebulizing and drying gas. The scan range was 50–450 Da. The ESI source and the analyzer manifolds were maintained at 40 °C. The drying gas temperature was varied from 50 to 150 °C. The needle voltage was varied from 2000 to 5000 V. The drying gas flow rate was maintained at 4 L/min.

To investigate dissociation of Irgamet 30 and Irgamet 39, standard solutions were prepared by dissolving known amounts of commercial formulations as received from BASF in methanol (CH3OH), water (H2O), and deuterium oxide (D2O) followed by equilibration with copper granules at 150 °C.

After optimization of the ESI-MS parameters, the system was used for quantitative determination of Irgamet 30 and Irgamet 39 in mineral oil. Calibration standards were prepared by fortifying white mineral oil with Irgamet 30 or Irgamet 39 as described earlier. For quantification of the metal deactivators in insulating mineral oils, samples (0.5 g – 0.01 g) of the oils were taken and mixed with 5 mL of n-pentane, and the solutions were passed through silica gel solid-phase extraction cartridges. Irgamet 30 or Irgamet 39 adsorbed on the silica gel was desorbed with methanol. Then, 5 µL of the extract was introduced into the ESI-MS system. The procedure was used for determining Irgamet 30 in mineral insulating oil samples and a blind Irgamet 30 fortified mineral insulating oil sample obtained from Sea Marconi Technologies. Irgamet 30 or Irgamet 39 in oil was also recovered through liquid–liquid extraction by extracting oil samples diluted in hexanes and extracted with methanol. The oil and hexane layer was decanted, and 200 µL of decafluoropentane (DFP)
was added to the methanol extract to facilitate separation of residual hexane and oil from methanol. Aliquots (5 µL) of the methanol layer were introduced into the ESI-MS system.

Results and Discussion

Specific Determination of DBDS and its Byproduct Bibenzyl
Since the discovery of DBDS in commercially available insulating mineral oil, this highly corrosive compound has been found in many unused and used mineral insulating oil samples, particularly in oils from transformers in South America. This sulfur compound has been implicated in a number of transformer failures in that region (17). It has been shown that DBDS is quantitatively converted to cuprous sulfide (Cu₂S), which was monitored as sulfate (SO₄²⁻) with ion chromatography or turbidity measurements. Determination confirmed that 1 mol of DBDS forms Cu₂S, and the major nonsulfur by-product is bibenzyl. The overall results of these determinations are shown in Figure 3. Because Cu₂S formed in an operating transformer cannot be determined, the assessment of DBDS reaction with the copper conductor in closed systems can be made by monitoring bibenzyl in insulating oils.

Quantitative Determination of Irgamet 30 and Irgamet 39 by Direct Infusion ESI-MS
Experiments carried out for quantification of Irgamet 30 and Irgamet 39 showed that both the triazole-derived Irgamet 30 and tolyl triazole-derived Irgamet 39 are very unstable in protic solvents. As a result protonated pseudomolecular ions for the two molecules at m/z 323 and m/z 387 were not observed in the ESI spectra. The likely reason for the absence of these ions is rapid dissociation of Irgamet 30 and Irgamet 39 through the retro Mannich reaction (19). Irgamet 30 dissociates into triazole and N,N-bis(2-ethylhexyl) amine when it comes in contact with water or other protic solvents (Figure 4). Similarly, Irgamet 39 dissociates into tolyl triazole and N,N-bis(2-ethylhexyl) amine.

The dissociation of Irgamet 39 into tolyl triazole and N,N-bis(2-ethylhexyl) amine was supported by its ESI-MS spectrum. The spectrum showed two dominant ions at m/z 134 and m/z 242, resulting from protonation of tolyl triazole and protonation of N,N-bis(2-ethylhexyl) amine, respectively. The ratio of ion m/z 135 and ion m/z 134 was in agreement with the expected isotopic ratio for ions containing seven carbon and three nitrogen atoms and the ratio of ion m/z 243 and ion m/z 242 was found to be in...
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The results of experiments carried out during the study clearly demonstrate that sensitive and specific quantification of both the corrosive sulfur compound DBDS and its principal nonsulfur by-product bibenzyl in insulating mineral oils can be achieved with GC–MS-MS with minimal sample preparation. Under suitable conditions bibenzyl can be used as diagnostic tool for assessing DBDS-related Cu$_2$S formation and potential risk of transformer malfunction.
Results also show that commonly used triazole and tolyl triazole derived metal deactivator Irgamet 30 and Irgamet 39 can be readily determined in mineral insulating oils with ESI-MS through direct infusion of the oils extracts. Quantification is rapid, sensitive, and specific over a wide range of concentrations.

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For more information on this topic, please visit our homepage at: www.spectroscopyonline.com
Using GC–MS to Observe Changes in the Essential Oil Concentrations in Cedar Tree (Juniperus virginiana) Leaves During a Drought Year

Essential oils were extracted from the leaves of cedar trees using ~50 mL of methylene chloride with an internal standard, m-xylene. The extract was then concentrated to ~2.0 mL by boiling off the solvent and analyzed by gas chromatography–mass spectrometry (GC–MS). Samples were collected monthly and the results were compared to weather data for an entire year, during a drought. By far, the compound in greatest abundance was β-pinene, comprising 25 – 9% of the total essential oils. Total essential oils were found to comprise 0.7 – 0.3% of the mass of the cedar leaves. It was found that during the summer, the concentration of essential oils was five times that of the concentration in the winter with sufficient rainfall. The concentration of essential oils in the cedar leaves increases with temperature, but is inversely proportional to the rainfall.

Norman E. Schmidt and Nikol M. Sandoval

The red cedar tree (Juniperus virginiana) is commonly found over most of North America and has needle-like leaves (1). Both the wood and leaves of the red cedar contain essential oils. The wood is most commonly used for making chests and dressers to keep moths and other insects away from valuables and clothing. Cedar essential oils serve many functions, such as insect and fungus defense (2). The concentration of these essential oils in cedar trees fluctuate throughout the year depending on the season (2). Essential oils are also used in disinfectants, soaps, and candles because of their concentrated aroma (3). This study was performed to identify the essential oils found in cedar leaves and to determine how the concentrations of these essential oils varied through an entire year. This study was also performed during a period of drought and the results are interesting to determine how the lack of rainfall affected these concentrations.

The red cedar tree (Juniperus virginiana) is commonly found over most of North America and has needle-like leaves (1). Both the wood and leaves of the red cedar contain essential oils. The wood is most commonly used for making chests and dressers to keep moths and other insects away from valuables and clothing. Cedar essential oils serve many functions, such as insect and fungus defense (2). The concentration of these essential oils in cedar trees fluctuate throughout the year depending on the season (2). Essential oils are also used in disinfectants, soaps, and candles because of their concentrated aroma (3). This study was performed to identify the essential oils found in cedar leaves and to determine how the concentrations of these essential oils varied through an entire year. This study was also performed during a period of drought and the results are interesting to determine how the lack of rainfall affected these concentrations.

The most common method for extracting essential oils from cedar is by gathering a wood sample, grinding it up, and distilling the sample using steam distillation. There are other methods using supercritical carbon dioxide, liquid carbon dioxide, and pressurized water (1). In this study, the solvent methylene chloride (CH₂Cl₂) was used to extract oils from cedar leaf samples. There are a large number of essential oils that are found in cedar wood. Some of these chemicals include: α-pinene, β-pinene, limonene, safrole, α-cedrene, β-cedrene, thujopsene, cuparene, cedrol, widdrol, sabinene, caryophyllene oxide, and many other oils (3,4). These oils are also expected to be found in cedar leaves.

Different seasons bring a different amount of insects as well as different temperatures. The warmer seasons, spring and summer, are expected to present warmer temperatures and more insects. In comparison to the warmer seasons, the colder seasons, winter and fall, bring cooler temperatures and fewer insects. When the weather is warmer and the insects are present, it is expected that essential oil concentrations will increase as op-
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posed to cooler weather and the absence of insects.

This work is a preliminary study of the effect of the seasons, temperature, and rainfall on the essential oils found in red cedar leaves. Data have been collected over the period of a year. During that time the cedar trees under study experienced abnormally warm weather and less rainfall than normal. The study is continuing to this day to determine what the essential oil concentrations are in a “normal” year.

**Experimental Methods**

A random selection of 12 cedar trees were chosen on the campus of Tabor College, in Hillsboro, Kansas. On a monthly basis, 3.0-g leaf samples were collected from the outer leaves of each tree. The leaves were then placed in ~50 mL of methylene chloride for a month. Next, each sample was filtered through filter paper and concentrated to a total volume of ~2.0 mL by boiling off the solvent on a hot plate and placing it in an autosampler vial. If samples were not analyzed immediately they were stored in a freezer at -20 °C. Finally, the extract was analyzed using a Shimadzu GC-17A gas chromatograph and GCMS-QP5000 mass spectrometer. The analysis conditions were as follows:

- GC column: 30 m × 0.25 mm, 0.25-µm df ZB-5
- Initial oven temperature: 40 °C (initial hold time of 1.00 min)
- Initial rate: 10 °C/min
- Final temperature: 300 °C (final hold time of 5.00 min)
- Injector temperature: 280 °C
- Split ratio: 1:50
- Interface temperature: 300 °C
- Helium flow: 0.29 mL/min

Masses from 50 to 250 Da were recorded. Each sample analysis took approximately 45 min. A similarity search of mass spectra was done to identify the peaks using a National Institute of Standards and Technology (NIST) library. Peak identities were confirmed using Kovats indexes and authentic samples where possible.

**Results and Discussion**

Figure 1 shows a typical total ion chromatogram (TIC) obtained in this study. Approximately 90 different peaks could be observed in a typical chromatogram. However, in most cases, 75% of the essential oils were found in the 10 most abundant chemicals. Some of the major peaks observed include: 3,3-dimethylpentene, 3,3-dimethylbutene, α-pinene, β-pinene, myrcene, limonene, safrole, α-cedrene, β-cedrene, thujaolene, and cuparene. The majority of peaks were very minor, but because of the sensitivity of the instrument and method they could be quantitated as well as the major peaks.

One of the first peaks in the chromatogram is that of the internal standard, m-xylene. The largest peak in most samples was β-pinene and, on average, it comprised 25% of the total essential
oils extracted from the leaves. This is in contrast to other literature that mentions β-pinene as a minor component monoterpene (5). Many other terpenes were found in the leaves, but in much smaller concentrations than β-pinene. The next three major peaks shown in Figure 1 are safrole, α-cedrene, and cuparene. As shown in Figure 1, there were numerous smaller peaks, and most of these comprised less than 1% of the total area and were primarily essential oils. Some of these compounds include camphene, myrcene, p-cymene, citronellol, aromadendrene, and many unidentified compounds. Some trees tended to consistently have a certain distribution of essential oils and other trees had a rather different distribution of oils. For instance, some trees consistently had a fairly high percentage of safrole, but others had no detectable safrole.

Figure 2 shows the mass percent of essential oils in the leaves as a function of month. Depicted are the average mass percent of all 12 samples for each month. The error bars in the figure represent one standard deviation of the results. The x-axis is the month in 2012, and zero represents December 2011. The y-axis represents the mass percent of the compound. In many cases, the standard deviations of the data points are as great as 50% of the values of the data points. We very much wish that we had started this study a few months earlier so that we could have seen what the mass percent of the essential oils was before the drought.

It was expected that the concentration of essential oils in the leaves would increase in the summer with the temperature, then decrease as the temperature cools. However this was not what was observed. Unexpectedly, the mass percent of essential oils increased almost linearly throughout the entire study. A maximum occurred in July, the hottest month of the year, and it would then be expected that as the temperature cooled off that the mass percentage of essential oils would drop. That was observed for August, but at that point the total mass percentage basically held constant, and then abruptly increased in November. By itself this data seems very contradictory. However, Table I shows the average temperature and measured rainfall for Wichita, Kansas, during the time at which samples were collected. (Wichita is the closest major weather station where continuous measurements are recorded.) Although the temperature follows a periodic cycle of warm weather in summer and cooler weather in winter, the amount of rainfall per month seems almost random with some months having more than 5 in. of rain while other months have less than one-tenth that amount. The average annual rainfall in Wichita, Kansas, is about 32 in. (6). At the beginning of the study, rainfall was close to that average. However, by the end of the study the annual rainfall was as much as 8 in. (20 cm) below average. It is believed that the cumulative lack of water is primarily responsible for the increased concentration in essential oils. Reductions in rainfall have been shown to have a positive effect on the mass percentage of essential oils in plants (7).

The very last data point for November 2012 is unusual in two respects. First, the mass percentage of essential oils in those samples was higher than any other in the study. This is somewhat expected because there had been almost no rain for two months. Second, the standard deviation of those samples was much higher than any other samples. This tends to imply that the drought affected some plants more than others.

Figure 3 shows how the mass percentage of three different compounds changed as the study was conducted. As in Figure 2, the error bars represent one standard deviation in the data. Figure 3a shows a plot of β-pinene concentration as a function of month. As the study was conducted the concentration of β-pinene dropped to one-half of its initial value. Figure 3b shows a plot of safrone concentration as a function of month. This concentration also decreased, but not as much as the β-pinene. Finally, Figure 3c shows a plot of cuparene as a function of month. In this case, the concentration increased with time and almost doubled the initial concentration.

A very interesting, minor inflection in Figure 3a is that there is a dip in the graph in February 2012 which follows directly from the relatively wet November and December, a dry January, and then a relatively wet February and March. Normally, January is the coldest month in Kansas. However, as shown in Table I, December was the coldest month of the entire study and the temperature was abnormally warm for the state and continued to rise until July 2012. In hindsight, it is rather serendipitous that we happened to start our study in the coldest month of the winter. As the temperature rose, the % area of β-pinene declined. However, after July the temperature dropped, and the % area of β-pinene continued to decline. This decrease is attributed to insufficient rainfall, especially at the end of the study, and the cedar trees were producing other essential oils instead of β-pinene.

Holzinger and colleagues showed that monoterpene emissions from pine trees varied dramatically with the seasons (8).

### Table I: Total monthly rainfall and average temperatures for Wichita, Kansas, during the study period

<table>
<thead>
<tr>
<th>Month</th>
<th>Temperature (°F)</th>
<th>Temperature (°C)</th>
<th>Total Monthly Rainfall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oct 2011</td>
<td>60.2</td>
<td>15.7</td>
<td>1.83</td>
</tr>
<tr>
<td>November 2011</td>
<td>45.1</td>
<td>7.3</td>
<td>3.32</td>
</tr>
<tr>
<td>December 2011</td>
<td>36.7</td>
<td>2.6</td>
<td>3.69</td>
</tr>
<tr>
<td>January 2012</td>
<td>37.9</td>
<td>3.3</td>
<td>0.03</td>
</tr>
<tr>
<td>February 2012</td>
<td>40.5</td>
<td>4.7</td>
<td>3.57</td>
</tr>
<tr>
<td>March 2012</td>
<td>57.9</td>
<td>14.4</td>
<td>4.03</td>
</tr>
<tr>
<td>April 2012</td>
<td>62.4</td>
<td>16.9</td>
<td>5.24</td>
</tr>
<tr>
<td>May 2012</td>
<td>72.8</td>
<td>22.7</td>
<td>2.1</td>
</tr>
<tr>
<td>June 2012</td>
<td>79.7</td>
<td>26.5</td>
<td>2.55</td>
</tr>
<tr>
<td>July 2012</td>
<td>88.2</td>
<td>31.2</td>
<td>0.26</td>
</tr>
<tr>
<td>August 2012</td>
<td>80.1</td>
<td>26.7</td>
<td>3.38</td>
</tr>
<tr>
<td>September 2012</td>
<td>71.8</td>
<td>22.1</td>
<td>2.64</td>
</tr>
<tr>
<td>October 2012</td>
<td>58.4</td>
<td>14.7</td>
<td>0.32</td>
</tr>
<tr>
<td>November 2012</td>
<td>49.9</td>
<td>9.9</td>
<td>0.55</td>
</tr>
</tbody>
</table>
In the summer, emissions were 4–5 times greater than in the fall. Therefore, it is easy to explain decreases in $\beta$-pinene concentrations during the summer. However, at this point the continual decrease in $\beta$-pinene concentration under the conditions of lower temperatures and lower rainfall are harder to explain.

Safrole generally follows the same trends as $\beta$-pinene as shown in Figure 3b. Over the course of the study the safrole concentration decreased. However, the decrease was not as pronounced as that of $\beta$-pinene. While the area percent of $\beta$-pinene decreased to less than 50% of the initial amount, the area percent of safrole decreased to about 30%. Also, after a dry month when the area percent of $\beta$-pinene decreased, so did the area percent of safrole. However from July to the end of the study the area percent of safrole was essentially unchanged. Safrole is a larger molecule than $\beta$-pinene with a higher boiling point, a higher heat of vaporization, and lower vapor pressures. Therefore, higher temperatures are expected to have less effect on safrole concentrations than $\beta$-pinene concentrations. Apparently, reduced rainfall had little effect upon the area percent of safrole produced as shown at the end of the study.

In contrast to the $\beta$-pinene and safrole which decreased as the study progressed, the area percent of cuparene increased (Figure 3c). Cuparene is a larger molecule than $\beta$-pinene or safrole. Therefore, higher temperatures would be expected to have less effect upon cuparene vaporizing from the leaves than smaller molecules. If the tree is then suffering from lack of water, it is therefore not surprising for the concentration of cuparene to increase because of evaporation of water, but not cuparene.

In summary, these results show that $\alpha$-pinene and safrole concentrations decrease with water stress while cuparene concentrations increase with water stress. These results are similar to those found by Bahr and colleagues when they induced water stress upon plants of *Satureja hortensis* L. (7). In their results, carvacrol concentrations increased with moderate water stress while $\gamma$-terpinene concentrations decreased. In another study by McGimpsey and colleagues, the essential oil yield and composition of *Thymus vulgaris* L. changed with the seasons (9). During the winter, some essential oils increased in concentration and some decreased. The opposite effect was seen in the summer.

Safrole concentrations varied widely from tree to tree. A few trees would consistently have more than 10% of the total.

---

**Figure 3:** The area percent of total ion chromatogram due to (a) $\beta$-pinene, (b) safrole, and (c) cuparene as a function of month.
peak area due to safrole, and a few other trees would consistently have no safrole at all or an extremely small peak. Therefore, the effects seen in Figure 3b are aggregate effects with standard deviations in these values as large as the values themselves. The standard deviations in Figure 3c are smaller, comprising about 50% of the average values and the standard deviations in Figure 3a are even smaller, comprising about 30% of the average values.

In a final analysis of the data, the authors attempted to see if a model could be determined to fit the data relating mass percent of essential oils with the temperature and total rainfall. Initial efforts to fit only temperature or monthly rainfall to the mass percent of essential oils were unsuccessful. A reasonable fit was obtained by combining both temperature and cumulative rainfall over the previous two months into a weather index which is defined as follows:

\[
\text{Weather Index} = \frac{\text{average monthly temperature \times cumulative rainfall all over the two previous months}}{1}
\]

At this point the logarithm of the weather index is taken and a reasonably good fit was obtained with the data (Figure 4). Although the weather index is an odd function, it does make sense. As the temperature increases, the stress on the tree also increases. Likewise, as the rainfall increases, the stress on the tree decreases. The best fit to the data occurred with the sum of two months of rainfall. Using one month or three months gave much worse fits to the data. This sum of two months of rainfall probably is indicative of the ability of the tree to store water, the ability of the ground around the tree to store water, or probably a combination of the two. All of the trees in the study were approximately the same size and in ground that is relatively the same. If the trees were of different size, then it could be reasoned that size could affect the ability of the tree to store water. Also, if the trees were in sandy soil that quickly drained compared to soil with a lot of clay that could hold water, then this would affect the water stress of the trees. The data shown in Figure 4 are quite interesting. However, the number of data points is small and the true test of the model is to see if it holds during a time of abundant rain. Future research will also compare trees of different size or trees planted in different soil types.

**Conclusions**

Throughout this study the total concentration of the essential oils in the cedar trees increased. This effect primarily occurred through an increase in concentration and the number of higher molecular weight essential oils. Less rainfall appears to have a greater effect on essential oil concentrations than temperature. The effects of temperature and rainfall were combined into a weather index, which is a reasonable predictor of the total mass percentage of essential oils in the cedar leaves. Further study needs to determine whether this same model works in a year with higher rainfall.

**Acknowledgments**

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**References**


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Ultrahigh-Performance Liquid Chromatography–Mass Spectrometry in Lipidomics

This article describes an analytical method for the global profiling of molecular lipids in biological samples, with particular emphasis on the plasmalogen lipids. The global profiling method is based on ultrahigh-performance liquid chromatography combined with quadrupole time-of-flight-mass spectrometry (UHPLC–QTOF-MS). The profiling approach is complemented by UHPLC–orbital trap MS in MS^n mode for de novo lipid identification.

Heli Nygren, Päivi Pöhö, Tuulikki Seppänen-Laakso, Ulla Lahtinen, Matej Orešic, and Tuulia Hyötyläinen

Lipids are an important class of essential metabolites and have many key biological functions. They are structural components of cell membranes, energy storage sources, and intermediates in signalling pathways (1,2). For example, tight control of membrane lipid composition is of central importance to maintain normal cellular physiology, and its dysregulation may affect membrane fluidity as well as topology, mobility, or activity of membrane-bound proteins. Lipids originate entirely or in part from two distinct types of building blocks: ketoacyl and isoprene groups. They are both functionally and structurally a very diverse group of compounds, partly because of the many possible variations of the lipid building blocks and the different ways of noncovalent linkage. The structural diversity of lipids is demonstrated by the huge number of molecular lipid species found in biological systems, which is estimated to be in the order of hundreds of thousands (3).

Plasmalogens are a specific group of ether phospholipids. Structurally, they are glycerophospholipids which mainly consist of ethanolamine and choline, that is, phosphatidylcholines (PCs) and phosphatidylethanolamines (PEs) characterized by an alk-1ʹ-enylether bond in position sn-1 where aliphatic moieties C16:0, C18:0, or C18:1 carbon chains are incorporated preferentially; the sn-2 position is specifically occupied by polyunsaturated fatty acids (Figure 1) (1–3). Plasmalogens are abundant lipid species representing 20% of the total pool of phospholipids in cells. They contribute to membrane structural integrity, and are also involved in multiple cellular functions, such as vesicle formation and membrane fusion (4–6), ion transport (7,8), and generation of secondary signal mediators, such as platelet activating factor (PAF) (9). The presence of the vinyl ether bond gives antioxidant properties to these molecules which diminishes free radical based cellular damage (10,11) and thus protects cells against oxidative stress. Indeed, plasmalogens have been shown to play an important role in human health as a factor involved in ageing, obesity, diabetes, and diseases of the central nervous system (12–14).

Global characterization of lipids in biological samples — lipidomics — is a challenging task because of the high diversity of lipids. These approaches include shotgun lipidomics, which uses direct infusion of lipid extracts into the mass spectrometer (15,16), as well as liquid chromatography coupled to mass spectrometry (LC–MS) (17). While the shotgun approach is relatively simple and rapid, it suffers from matrix effects and ion suppression, and it is not very well suited for global profiling of previously unknown lipids. The advantage of LC–MS-based methods over the shotgun approach is the higher sensitivity as well as the ability, by using a nontargeted strategy, to detect and identify novel lipids. However, matrix effects cannot be fully avoided in LC–MS either, and careful optimization of eluent composition as well as cleanup steps is required to avoid carry-over and contamination.

Here we describe an ultrahigh-performance liquid chromatography–mass spectrometry (UHPLC–MS)-based global lipidomics platform and identification workflow for the characterization of plasmalogens from biological samples. In the identification stage, an orbital trap MS system is used as the detector as this system provides outstanding mass accuracy and mass resolution. The ability to detect accurate masses allows the
unequivocal compositional and structural elucidation of the compounds.

**Experimental**

**Sample Preparation**
A standard mixture 1 (20 μL) containing PC(17:0/0:0), PC(17:0/17:0), PE(17:0/17:0), and Cer(d18:1/17:0), (Avanti Polar Lipids, Inc.) and TG(17:0/17:0/17:0) (Larodan Fine Chemicals) was added to 10 μL of serum samples. HPLC-grade chloroform and methanol (2:1; 100 μL) were added to the samples, which were then vortexed for 2 min and allowed to stand for 30 min. Subsequently, samples were centrifuged and the lower phase (60 μL) was collected and 10 μL of internal standard mixture 2 was added. The internal standard mixture 2 contained the labeled lipids PC(16:1/0:0-D3), PC(16:1/16:1-D6), and TG(16:0/16:0/16:0-13C3).

The internal standard mixture contains compounds from several different lipid classes: PCs, PE, ceramide (Cer), phosphatidylserine (PS), phosphatidic acid (PA), as well as mono-, di- and triacylglycerols (MG, DG, and TG, respectively). Lipids are denoted by their molecular composition as follows: <Lipid class> <Number of carbon atoms in the first fatty acid moiety>:<Number of double bonds in the first fatty acid moiety> / < Number of carbon atoms in the second fatty acid moiety>:<Number of double bonds in the second fatty acid moiety>. For example, the abbreviation PC(17:0/17:0) indicates a phosphatidylcholine comprising two C17 fatty acids with no double bonds.

**Instrumental Conditions**

The extracts were analyzed on a Q-TOF Premier mass spectrometer (Waters, Milford) combined with an Acquity UPLC system (Waters) for the analysis of the whole data sets. For structural identification, UHPLC combined with TriVersa Nanomate (Advion Biosciences) electrospray ionization and LTQ-Orbitrap orbital trap MS (Thermo Fisher Scientific) systems was used, with the same analytical conditions for the UHPLC analysis. Separations were obtained using an 100 mm × 2.1 mm, 1.7-dp Acquity UPLC BEH C18 column (Waters) at a temperature of 50 °C. The solvent system included (A) ultrapure water (1% 1 M ammonium acetate, 0.1% formic acid) and (B) LC–MS-grade acetonitrile–isopropanol (1:1, 1% 1 M ammonium acetate, 0.1% formic acid). The gradient started from 65% A and 35% B, reached 80% B in 2 min, 100% B in 7 min, and remained there for 7 min. The flow rate was 0.400 mL/min and the injected volume was 2.0 μL (Acquity Sample Organizer, at 10 °C). Reserpine was used as the lock spray reference compound. The lipid profiling was performed using electrospray ionization (ESI) in positive mode and the data were collected at a mass range of m/z = 300–1200 with a scan duration of 0.2 s.

For the structural characterization with UHPLC–orbital trap MS, the following procedure was applied: Fractions collected from the UHPLC run were injected into the orbital trap mass spectrometer from the TriVersa Nanomate system using chip-based nanoelectrospray in positive and negative ion modes. Identifications were based on the exact mass (with target mass resolution of R = 60,000) and MS^n spectra (LTQ). Plasmalogens and O-alkyl ethers were identified with the LTQ system in negative ion mode using normalized collision energy of 30%.

**Data Processing**

The data processing used MZmine 2 software (an open source software) (18) included alignment of peaks, peak integration, normalization, and peak identification. Lipids were identified using an internal spectral library. The data format specifies the identification number of the peaks, the average mass-to-charge ratio (m/z) value (average m/z), and the average retention time (RT) in seconds (average
The average values are obtained as the average value of the specific compound in the aligned dataset. The name of the compound is specified based on the search of the database.

**Results**

**Lipidomic Analysis**
The UHPLC–TOF-MS method covers the main classes of lipids, namely, cholesteryl esters (ChoE), PC, PE, Cer, MG, DG, TG, sphingomyelins (SM), and lysophosphatidylcholines (lysoPC). The sample preparation was based on modified Folch extraction, that is, liquid extraction using a mixture of chloroform:methanol (2:1), and these lipid classes are efficiently extracted by the method. For more acidic lipids, such as phosphatidylethanolamines, adjustment of the pH in the aqueous phase is required. The solvent system was chosen to be compatible with MS and to allow the ionization of a wide range of lipids. In addition, the solvent composition was optimized to contain a relatively strong solvent (acetonitrile–isopropanol) to obtain good peak shapes for late eluted TGs and also to avoid any significant carry-over as a result of complex biological samples. Elevated temperature (50 °C) was also used to enhance the elution of the late eluted lipids. No significant carry-over was noticed, proved by blank analyses after real samples. The total analysis time for UHPLC–MS analysis was 12 min, thus allowing analysis of approximately 100 samples a day.

**Figure 3:** ESI+ identification of two unknown lipids. (a) Fragmentation of [M+H]+ ion of a phosphatidylcholine produce an abundant ion at m/z = 184 corresponding to phosphocholine head group. (b) Neutral loss of phosphoethanolamine head group (141 u) is observed when [M+H]+ ion of a phosphatidylethanolamine is fragmented.

**Figure 4:** Fatty acid composition phospholipids can be characterized using ESI– and fragmentation in ion trap. Fragmentation of PC(17:0/17:0) is shown as an example.
Typically, around 800 lipid species can be detected from human plasma and serum samples.

A typical LC–MS total ion current (TIC) chromatogram of a blood plasma sample obtained in positive ionization mode is shown in Figure 2. In the UHPLC separation, phospholipids are separated based on their fatty acid composition and degree of desaturation. Thus, lysophospholipids are eluted early, with lysoPCs containing one fatty acid attached to the glycerol backbone eluted before diacylphospholipids, which have two fatty acids. Phospholipids and sphingomyelins (SMs) are eluted in the same region while ceramides that do not contain a phosphatidylcholine (PC) head group as their sphingomyelin counterparts are eluted after PCs and SMs. In addition, DGs are eluted close to PCs and SMs. Cholesterol esters and TGs are eluted last, in the isocratic part of the gradient (100% of organic solvent), and the peaks are therefore slightly broader.

The quantitation of lipids was done by using group-specific standards for the normalization of the data. All monoacyl lipids except cholesterol esters, such as monoacylglycerols and monoacylglycerophospholipids, were normalized with PC(17:0/0:0); all diacyl lipids except ethanolamine phospholipids were normalized with PC(17:0/17:0); all ceramides with Cer(d18:1/17:0); all diacyl ethanolamine phospholipids with PE(17:0/17:0); and TG and cholesterol esters were normalized with TG(17:0/17:0). Other (unidentified) molecular species were normalized with PC(17:0/0:0) for retention time <300 s, PC(17:0/17:0) for RT between 300 s and 410 s, and TG(17:0/17:0/17:0) for higher retention times.

The method was also linear over a large concentration range for most of the lipid classes (Table I). To control the quality of the lipid analyses, a set of control serum samples, standards extracted with the lipid analyses, a set of control serum samples, standards extracted with the lipid analyses.

### Table I: The calibration range and linearity of selected lipids

<table>
<thead>
<tr>
<th>Lipid</th>
<th>R²</th>
<th>Range (µmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PE17:0</td>
<td>0.9712</td>
<td>0.5–1700</td>
</tr>
<tr>
<td>PC17:0</td>
<td>0.9868</td>
<td>0.5–800</td>
</tr>
<tr>
<td>LPC 17:0</td>
<td>0.9933</td>
<td>0.5–1000</td>
</tr>
<tr>
<td>CE 19:0</td>
<td>0.9985</td>
<td>0.002–15</td>
</tr>
<tr>
<td>SPH</td>
<td>0.9958</td>
<td>0.02–10</td>
</tr>
<tr>
<td>CL14:0</td>
<td>0.9805</td>
<td>20–750</td>
</tr>
<tr>
<td>TG 17:0/17:0/17:0</td>
<td>0.9821</td>
<td>50–2200</td>
</tr>
</tbody>
</table>

### Table II: Relative standard deviations (RSDs) of internal standards added to samples (n = 401), and in standards that have been extracted or analyzed directly

<table>
<thead>
<tr>
<th>ISTD</th>
<th>RSD Samples</th>
<th>RSD Extracted Standards</th>
<th>RSD Standards</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cer(d18:1/17:0)</td>
<td>10.9</td>
<td>12.4</td>
<td>9.9</td>
</tr>
<tr>
<td>LysoPC(17:0)</td>
<td>11.2</td>
<td>13.7</td>
<td>9.3</td>
</tr>
<tr>
<td>PC(34:0)</td>
<td>8.3</td>
<td>11.4</td>
<td>12.4</td>
</tr>
<tr>
<td>PE(34:0)</td>
<td>12.6</td>
<td>14.8</td>
<td>14.1</td>
</tr>
<tr>
<td>TG(16:0/18:0/17:0)</td>
<td>9.7</td>
<td>9.6</td>
<td>9.2</td>
</tr>
</tbody>
</table>

### Plasmalogen Identification

In the UHPLC separation, plasmalogens were also easily separated from their acyl-lipid counterparts in the LC. A comprehensive set of MS³ studies was performed for the identification of fatty acid composition in lipids and to differentiate between O-alkyl ethers and plasmalogens. In particular, ES⁺ was used for the identification of specific product ions for PE and PC plasmalogens. A spectral database, containing RT information as well, has been constructed based on these studies, using both standard compounds (limited availability), and the identification of specific plasmalogens and alkylethers in a variety of biological samples (serum/plasma and tissues).

In the identification of lipids, and in particular plasmalogens, MS⁰ was utilized, using both ES⁺ and ES⁻. In ES⁺, only limited information could be obtained (Figure 3), mainly from the polar head group of the lipid (19). Therefore, ES⁻ mode is also needed (Figure 4) for detailed identification of the lipid structure and of the fatty acid side chains (20,21). In negative ion mode, PCs are detected mainly as formate adducts [M+COO⁻], which will fragment to yield abundant [M–15]⁻ ion. Fragmentation of [M–15]⁻ ion (MS³) reveals information about the fatty acid composition of the PC lipids. The most abundant negative ions in MS³ spectra correspond to carboxylate anion from sn-2 and sn-1 position (R₂ and R₁ in Figure 4). In addition, MS³ spectra show a complementary ion which comprises fatty acyl (from sn-1 position) and glycerophosphocholine head group [M–15–R₂COOH⁻]. MS⁴ analysis of [M–15–R₂COOH⁻] ions reveals critical information regarding the two etherlipid subclasses, plasmalogens, and O-alkyl ethers (Figure 5). While the plasmalogen PC species, for example, produces a class specific ion at m/z 224, the O-alkyl-ethers instead yield a phosphatidic acid anion [M–15–R₂COOH–89]⁻ (as a result of the neutral loss of dimethylamino ethylene).

Therefore, all three subclasses of important membrane lipids, plasmalogens, and O-alkyl ethers as well as diacyl PCs, can readily be distinguished using ES⁻ and MS⁰ when the components are chromatographically separated. In a similar manner, the PE ether lipid subclasses can also be characterized based on unique fragments in MS³ spectra (22).

### Conclusions

A UHPLC–MS-based lipidomics platform that uses high-resolution MS with both ES⁺ and ES⁻ analysis and MS⁰ fragmentation provides a powerful tool to characterize lipid subclasses. Phospholipids with ester linkages (diacyl components) can be identified based on accurate mass and chromatographic retention time, and the fatty acyl composi-
tion can be revealed based on MS\textsuperscript{n} fragmentation of negative ions. In addition, MS\textsuperscript{n} analysis in negative ion mode can readily distinguish the two etherlipid subclasses of phospholipids, namely plasmalogens and O-alkyl ethers. Plasmalogens produce a class specific ion at m/z 224 while the other ether phospholipids yield a phosphatidic acid.

References

| Table III: Relative standard deviations (RSDs) in control samples of the identified lipids (n = 26) |
|-----------------------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Name                          | RSD (%)       | Name                          | RSD (%)       | Name                          | RSD (%)       |
| Cer(d18:1/16:0)               | 11.4          | PC(36:3)                       | 7.5           | PE(38:4e)                       | 12.4          |
| Cer(d18:1/18:0)               | 18.3          | PC(36:3e)                      | 9             | PE(38:5)                        | 11.1          |
| Cer(d18:1/20:0)               | 13.4          | PC(36:4)                       | 7.2           | PE(38:5e)                       | 16.2          |
| Cer(d18:1/22:1)               | 13.8          | PC(36:4e)                      | 9.1           | PE(38:6)                        | 9.4           |
| ChoE(16:0)                    | 16.6          | PC(36:4e)                      | 9             | PE(40:3)                        | 10.8          |
| ChoE(16:1)                    | 19.7          | PC(36:5)                       | 7.4           | PE(40:4)                        | 14.3          |
| ChoE(18:1)                    | 17.5          | PC(37:2)                       | 8.7           | PE(40:4)                        | 10.7          |
| ChoE(18:2)                    | 15.4          | PC(38:2)                       | 11.4          | PE(40:4e)                       | 13.6          |
| ChoE(20:3)                    | 20.8          | PC(38:2e)                      | 15.9          | SM(d18:1/14:0)                  | 9.3           |
| ChoE(20:4)                    | 20            | PC(38:3)                       | 9.5           | SM(d18:1/16:0)                  | 10            |
| LysoPC(16:0)                  | 8.1           | PC(38:3e)                      | 9.7           | SM(d18:1/18:0)                  | 11.4          |
| LysoPC(18:0)                  | 8.3           | PC(38:4)                       | 13.1          | SM(d18:1/18:2)                  | 9.4           |
| LysoPC(18:1)                  | 12.8          | PC(38:4)                       | 5.5           | SM(d18:1/18:3)                  | 16.1          |
| LysoPC(18:2)                  | 8.1           | PC(38:5)                       | 8.1           | SM(d18:1/20:0)                  | 10.3          |
| LysoPC(20:3)                  | 10.2          | PC(38:5)                       | 7.7           | SM(d18:1/21:0)                  | 9.9           |
| LysoPC(20:4)                  | 6.2           | PC(38:5e)                      | 8.8           | SM(d18:1/22:1)                  | 8.3           |
| LysoPC(22:6)                  | 11.3          | PC(38:5e)                      | 11.2          | SM(d18:1/23:1)                  | 9.1           |
| LysoPE(18:2)                  | 9.1           | PC(38:6)                       | 7.5           | SM(d18:1/26:2)                  | 14            |
| LysoPE(20:0)                  | 7.7           | PC(38:7e)                      | 14            | TG(16:0/18:1/16:0)              | 18            |
| LysoPE(20:1)                  | 7.9           | PC(40:3e)                      | 12.3          | TG(42:0)                        | 29.2          |
| LysoPE(20:1)                  | 9.4           | PC(40:4)                       | 8.2           | TG(44:0)                        | 20.5          |
| PC(30:3e)                     | 13.5          | PC(40:4e)                      | 14.2          | TG(44:1)                        | 16.9          |
| PC(32:0)                      | 8.9           | PC(40:4e)                      | 12.1          | TG(44:2)                        | 11.4          |
| PC(32:0)                      | 8.9           | PC(40:5)                       | 8             | TG(46:0)                        | 20.4          |
| PC(32:0e)                     | 9.5           | PC(40:5)                       | 6.8           | TG(46:1)                        | 11.9          |
| PC(32:1)                      | 7.2           | PC(40:5e)                      | 7.1           | TG(46:2)                        | 13.7          |
| PC(32:2)                      | 11.8          | PC(40:6)                       | 17.1          | TG(48:0)                        | 12.3          |
| PC(32:4e)                     | 10.5          | PC(40:8)                       | 10.2          | TG(48:1)                        | 9.5           |
| PC(34:1)                      | 6             | PE(30:2)                       | 14.9          | TG(48:2)                        | 9.6           |
| PC(34:2)                      | 5.6           | PE(34:1)                       | 8.3           | TG(48:3)                        | 7.3           |
| PC(34:2e)                     | 6.7           | PE(34:3e)                      | 15.1          | TG(49:1)                        | 10.4          |
| PC(34:3)                      | 8.1           | PE(36:1)                       | 13            | TG(49:2)                        | 13.1          |
| PC(34:3)                      | 7.1           | PE(36:2)                       | 9.3           | TG(49:3)                        | 11.9          |
| PC(35:2)                      | 8.1           | PE(36:3)                       | 12            | TG(50:0)                        | 14.7          |
| PC(36:0)                      | 9.8           | PE(36:6e)                      | 17.9          | TG(50:1)                        | 9.9           |
| PC(36:1)                      | 5.8           | PE(38:1)                       | 11.3          | TG(50:2)                        | 11.9          |
| PC(36:2)                      | 6.5           | PE(38:2)                       | 14.7          | TG(50:2)                        | 18            |
| PE(38:3)                      | 12.3          | TG(50:3)                       | 12.8          | TG(56:6)                        | 8.7           |
| PE(38:4)                      | 16.9          | TG(51:1)                       | 13.5          | TG(56:7)                        | 10.5          |
Heli Nygren, PhD, is a senior scientist in the metabolomics laboratory at VTT Technical Research Centre of Finland. Her research activities focus on the development and use of advanced chromatographic and mass spectrometric methods for metabolomics.

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For more information on this topic, please visit our homepage at: www.spectroscopyonline.com
Applying Comprehensive Analysis to EPA Method 1613B Samples — Discover Those Compounds Usually Discounted in Environmental Samples

Polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) are ubiquitous environmental pollutants that are persistent and toxic. The historical quantitative analysis of these compounds has been achieved with high-resolution targeted analysis using magnetic sector instruments, with subsequent lower resolution analysis to identify other contaminants. Advances in technology have led to comprehensive time-of-flight (TOF) mass spectrometers that can quantify PCDDs or PCDFs while simultaneously acquiring data on other contaminants in the samples. Samples that had been analyzed using EPA Method 1613B conditions on a sector instrument were analyzed with a high performance TOF mass spectrometer and a low-resolution comprehensive two-dimensional gas chromatography (GC×GC) TOF instrument. The quantitative results from the sector, the high-resolution TOF, and the GC×GC-TOF systems are compared in this article.

Jayne de Vos, Jack Cochran, Eric J. Reiner, and Peter Gorst-Allman
mercial standards and confirmed that the TOF systems could successfully achieve or surpass (4) detection at the mandatory lower limit required by EPA Method 1613 (TCDD at 500 fg) (3). (Note: Throughout this article the relevant PCDD and PCDF will be described using the following abbreviations: XCDD and XCDF, where X = T [tetra], P [penta], Hx [hexa], Hp [hepta], and O [octa]). The quantitative results obtained from the GC–high-resolution TOF (GC–HRTOF-MS) system and the GC×GC–TOF-MS systems were correlated with those obtained under EPA Method 1613B conditions.

More significantly, while providing comparable quantitative results, the TOF systems simultaneously allowed for the identification of a diverse set of other POPs not detected under the highly selective, selected ion monitoring (SIM) conditions defined in EPA Method 1613B. SIM analyses require prior knowledge of the compounds to be analyzed. The retention times and windows for mass-to-charge are highly constrained to limit interference and provide selective detection with correlation to labeled standards. Because of the constrained conditions for mass analysis under Method 1613B, other analytes would not be detected, while the TOF systems, not suffering the same constraints, always yield full range mass spectra and provide considerable additional sample information. The absence of constraints (that is, comprehensive analysis) enables a single experiment to obtain good quantitative correlation with regulated methods while data for prospective or retrospective analysis of unknowns is also acquired. The isotopic abundance of the analyte ions, mass accuracy, and resolving power achieved during the comprehensive experiment on the GC–HRTOF-MS system provide

### Table I: GC–MS conditions for PCDD and PCDF analyses

<table>
<thead>
<tr>
<th>Parameter</th>
<th>GC–HRMS</th>
<th>GC–HRTOF-MS</th>
<th>GC×GC–TOF-MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amount injected (µL)</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Inlet temperature (°C)</td>
<td>280</td>
<td>280</td>
<td>250</td>
</tr>
<tr>
<td>Helium flow (mL/min)</td>
<td>0.8</td>
<td>1</td>
<td>1.4</td>
</tr>
<tr>
<td>Column 1</td>
<td>DB-5 (40 m × 0.18 mm, 0.18 µm d.)</td>
<td>Rtx-Dioxin 2 (40 m × 0.18 mm, 0.18 µm d.)</td>
<td>Rtx-Dioxin 2 (40 m × 0.18 mm, 0.18 µm d.)</td>
</tr>
<tr>
<td>Oven 1 (°C)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Oven 2 (°C)</td>
<td>—</td>
<td>—</td>
<td>125 (hold 2 min to 205 (20/min) to 325 (5/min) (hold 3 min)</td>
</tr>
<tr>
<td>Transfer line (°C)</td>
<td>—</td>
<td>280</td>
<td>320</td>
</tr>
<tr>
<td>Modulation period (s)</td>
<td>—</td>
<td>—</td>
<td>2</td>
</tr>
<tr>
<td>Ion source (°C)</td>
<td>280</td>
<td>280</td>
<td>250</td>
</tr>
<tr>
<td>Start mass (amu)</td>
<td>SIM</td>
<td>140</td>
<td>45</td>
</tr>
<tr>
<td>End mass (amu)</td>
<td>SIM</td>
<td>520</td>
<td>750</td>
</tr>
<tr>
<td>Acquisition rate (spec/s)</td>
<td>—</td>
<td>3</td>
<td>100</td>
</tr>
<tr>
<td>Electron energy (eV)</td>
<td>35</td>
<td>50</td>
<td>70</td>
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<tr>
<td>Calibration</td>
<td>PFK</td>
<td>PFTBA</td>
<td>—</td>
</tr>
</tbody>
</table>

SIM = single ion monitoring. See method for details.

![Figure 1: Selected ion chromatogram (m/z 321.893) for TCDD at 500 fg/µL (CSI standard) on the HRT system.](image-url)
for both robust quantitative information and confident analyte identification. In the case of the GC×GC–TOF-MS system, the added chromatographic resolving power and cryofocusing effect of thermal modulation provides added sensitivity and selectivity.

**Legislative Considerations**

The analysis of PCDDs and PCDFs is regulated globally and concentrations are determined using a variety of EPA methods (such as 1613B and 8290A) (3,5), and by Council Directives in the European Union (6). In most other countries requiring dioxin analysis, modified forms of these methods govern testing protocols. These methods prescribe the use of GC coupled with high-resolution mass spectrometry (HRMS) and stipulate minimum quantifiable levels of the analyte (TCDD at 500 fg) for an analytical system and the testing to be deemed acceptable.

Any alternative technology should be able to achieve the levels of detection required by these methods and the capabilities must be verified through testing.

**Analytical Considerations**

The limitation of the sector HRMS approach lies in its targeted approach that provides accurate information on quantitative levels of PCDDs and PCDFs, but gives no information on the presence of other POPs in the sample. This has to be determined in separate runs, and is generally done using low-resolution mass spectrometry. To be able to do both quantification and sample component investigation in a single analysis holds obvious advantages.

**Samples**

Six samples were investigated in this study. These were sourced from the Ministry of the Environment Laboratory Services Branch in Toronto, Canada. All samples were prepared according to standard methods used for the preparation of samples for PCDD and PCDF analysis by HRMS (7).

**Instrumentation**

Conditions for EPA Method 1613B acquisition were achieved using a Waters Autospec magnetic sector mass spectrometer at a resolving power of 10,000 interfaced to an HP6890 gas chromatograph (Agilent Technologies) using a 40 m × 0.18 mm, 0.18-µm df DB-5 column (J&W Scientific).

The GC×GC–TOF-MS system consisted of a Pegasus 4D time-of-flight mass spectrometer (LECO Corporation) coupled to an Agilent 7890 GC system equipped with an Agilent 7683B autosampler, a secondary oven and a dual-stage, quad-jet thermal modulator. Liquid nitrogen was used for the cold jets and

<table>
<thead>
<tr>
<th>Sample</th>
<th>Instrument</th>
<th>2378-TCDF</th>
<th>2378-TCDD</th>
<th>2378-PCDF</th>
<th>2378-PDCD</th>
<th>12378-HxCDF</th>
<th>12378-HxCDF</th>
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<td>2.7</td>
<td>4.5</td>
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<td>1.7</td>
<td>5.6</td>
<td>1.1</td>
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<td>ND</td>
<td>ND</td>
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<td>ND</td>
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<td>GC×GC–TOF-MS (&lt;5)</td>
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<td>6.6</td>
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<td>150</td>
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<td>16</td>
<td>17</td>
<td>5.2</td>
<td>71</td>
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<td>GC–HRMS</td>
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<td>19</td>
<td>17</td>
<td>2.5</td>
<td>73</td>
</tr>
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<td>21</td>
<td>21</td>
<td>18</td>
<td>ND</td>
<td>59</td>
</tr>
</tbody>
</table>

GC–HRTOF-MS = Pegasus HRT; GC–HRMS = AutoSpec (Waters); GC×GC–TOFMS = Pegasus 4D; GC×GC–TOFMS (<5) = Pegasus 4D with 5-µL injection

Table II: Quantitative values (pg/g) for PCDDs and PCDFs (T = tetra; P = penta; Hx = hexa; Hp = hepta; O = octa)
synthetic air for the hot jets. The liquid nitrogen levels were maintained using an AMI model 186 liquid level controller.

The HRTOF-MS system was a Pegasus HRT (LECO Corporation) using Folded Flight Path technology (8,9). The system included an Agilent 7890 GC system equipped with an Agilent 4513A autosampler.

Experimental

EPA Method 1613B calibration and verification solutions (EPA-1613CVS), labeled calibration solutions (EPA-1613LCS), internal standard spiking solution (EPA-1613ISS), and cleanup standard stock solution (EPA-1613CSS) were selected for spiking and calibration purposes. These solutions were purchased from Wellington Laboratories and contained the 17 native and corresponding mass-labeled PCDD and PCDF congeners in nonane.

All instrument functions and data processing for the GC×GC–TOF-MS were managed with the LECO ChromaTOF software (version 4.44). All instrument functions and data processing for the Pegasus HRT system were managed using LECO ChromaTOF software (version 1.61). Manual review of all peak identifications and integrations was performed using this software. Library searching was performed using a PCDD and PCDF user library compiled from the PCDD and PCDF standards. GC–HRMS data were obtained using conditions and processing as specified in EPA Method 1613B.

Experimental conditions used for the analysis of the samples are shown in Table I.

Results

Limits of Detection

The results obtained from the EPA Method 1613B (3) standard set provides an estimate of the limits of detection (LOD) possible using the GC×GC–TOF-MS system and GC with HRTOF-MS detection, with the understanding that matrix interference and sample preparation effectiveness will contribute significantly. The low-level standard (CS1), which contains 2,3,7,8-TCDD at 0.5 pg/µL, was used to provide an estimated LOD using GC×GC–TOF-MS. Using the methodology described in EPA Method 1613B, the signal-to-noise ratio (S/N) for the ion of m/z 322 for 2,3,7,8-TCDD was calculated as 20 (EPA Method 1613 requires this ratio to be >10 [3]). However, for GC–HRTOF-MS the prescribed approach is not applicable. Modern high-resolution mass spectrometers show little chemical noise on the plot of an exact mass ion owing to the elimination of noise during processing and acquisition. This is demonstrated in Figure 1, which shows a plot of the ion at m/z 321,893 for TCDD at

<table>
<thead>
<tr>
<th>123478-HxCDD</th>
<th>123678-HxCDD</th>
<th>234678-HxCDF</th>
<th>1234678-HpCDF</th>
<th>1234678-HpCDF</th>
<th>1234789-HpCDF</th>
<th>OCDF</th>
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<tr>
<td>ND</td>
<td>3.1</td>
<td>ND</td>
<td>12</td>
<td>33</td>
<td>ND</td>
<td>19</td>
<td>147</td>
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<td>0.8</td>
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<td>1.6</td>
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<td>31</td>
<td>16</td>
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<td>160</td>
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<td>ND</td>
<td>6.3</td>
<td>ND</td>
<td>ND</td>
<td>31</td>
<td>16</td>
<td>31</td>
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<td>3.7</td>
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<td>2.9</td>
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<td>4.7</td>
<td>420</td>
<td>75</td>
<td>79</td>
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<td>2.9</td>
<td>8.3</td>
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<td>250</td>
<td>130</td>
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<td>15</td>
<td>220</td>
<td>110</td>
<td>31</td>
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</tbody>
</table>
As can be seen, the compound is readily detected at the lowest level (500 fg/µL) required by EPA Method 1613B indicating a capability of achieving the minimum levels. It is, however, often desirable to detect lower in routine analyses to ensure compliance and analytical capability.

**Quantitative**

The quantitative values for the 17 PCDDs and PCDFs whose levels are regulated using Method 1613B are shown in Table I. Values obtained using GC×GC–TOF-MS and GC–HRTOF-MS are compared to the levels acquired using 1613B conditions. At the lowest levels, the GC×GC–TOF-MS system is not capable of detecting analytes, but this may be partially offset using the concurrent solvent recondensation technique of large-volume splitless injection (10). This technique, originally described by Magni and Porzano (10), can be done in an unmodified split–splitless injection port, provided that a guard column is used to protect the analytical column and that the matrix is clean to minimize fouling of the inlet and columns. For dioxin analysis in which the sample has been subjected to considerable cleanup, this latter condition is met, and the technique is advantageous when trying to analyze the trace-level PCDDs and PCDFs in the sample on the low-resolution system.

The data show strong correlation between Method 1613B results and those from GC×GC–TOF-MS analysis, suggesting its viability as a screening tool for dioxin levels in complex samples prepared using Method 1613B protocols.

Similarly, when compared to one another, the results from the two high-resolution systems are generally in excellent agreement, showing the high-resolution TOF system to provide quantitative results comparable to those obtained under Method 1613B criterion when concentrations are above a threshold near 500 fg/µL.

**Comprehensive**

Targeted dioxin analysis, as obtained with Method 1613B, fails to provide a comprehensive picture of the samples. Only the targeted PCDDs and PCDFs are detected, and additional POPs and analytes with dioxin-like properties present in the samples are transparent to detection. The capability of TOF instrumentation to generally reach or approach the required low levels, while still acquiring full-range mass spectra, means that these systems can be used not only to quantify the PCDD and PCDF components, but also to detect and identify additional components in the sample in the same run. The ability to detect and identify analytes not specifically monitored in Method 1613B is a feature demonstrated by both the GC×GC–TOF-MS and GC–HRTOF-MS systems.

For example, the GC×GC–TOF-MS chromatogram for sample 2 (Figure 2a) shows a high-boiling compound containing a prominent molecular ion cluster at m/z 360, not specified in the ions monitored in Method 1613B. The deconvoluted mass spectrum obtained for this compound can be library-searched to obtain the result shown in Figure 3. The compound is identified as 2,7-dibromo-pyrene, that can be confirmed against a standard to verify the congener, with a spectral match of 84% and would have gone undetected under Method 1613B conditions. Bromopyrenes have been shown to be irritating to the skin, eye, and respiratory systems (11) and while not the health concern of a dioxin, this compound represents what could be missed and may provide valuable insight should a contamination with dioxins occur.

Although the toxicity of this specific dibromopyrene is not known, it may well...
contribute to the overall sample toxicity and so its presence in the sample may be of importance.

This same compound can be located in the chromatogram of sample 2 obtained when using the GC–HRTOF-MS system (Figure 2b). In this case, the relevant mass is measured as 359.89648, which can be used to determine the formula $\text{C}_{16}\text{H}_8\text{Br}_2$ with a mass accuracy of -0.55 ppm. In the case of the GC×GC experiment, it is library match alone that provides identification. In the case of the GC–HRTOF-MS analyses, accurate mass provides a clear indication of the likely formula and confirms the library match.

Numerous other POPs, which would not have been detected in the sector data, have been found in the samples. Examples of these are shown in Table III. It should be noted that the compounds shown are a random selection among many. It is only possible to obtain a full picture of the sample when a comprehensive analysis is performed. By focusing just on the PCDDs and PCDFs, only a partial understanding of the total toxicity factor is obtained. It should also be kept in mind that the sample has undergone rigorous cleanup before analysis. It is likely that this cleanup would remove other potentially harmful components (for example, pesticides).

**Table III: A selection of other potentially harmful compounds contained in the samples analyzed, and detected by a comprehensive analysis**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Compound</th>
<th>Similarity</th>
<th>Mass Accuracy (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Tetrachlorobiphenyl</td>
<td>516</td>
<td>2.20</td>
</tr>
<tr>
<td>1</td>
<td>7H-Benz[de]anthracen-7-one</td>
<td>816</td>
<td>-0.66</td>
</tr>
<tr>
<td>2</td>
<td>Perylene</td>
<td>832</td>
<td>-0.71</td>
</tr>
<tr>
<td>2</td>
<td>Trichloropyrene</td>
<td>702</td>
<td>0.29</td>
</tr>
<tr>
<td>2</td>
<td>Trichloroterpheyl</td>
<td>608</td>
<td>-1.74</td>
</tr>
<tr>
<td>2</td>
<td>Benz[a]anthracene</td>
<td>942</td>
<td>-0.22</td>
</tr>
<tr>
<td>2</td>
<td>Dichloroantracene</td>
<td>958</td>
<td>-0.11</td>
</tr>
<tr>
<td>3</td>
<td>Tetrachlorobiphenyl</td>
<td>854</td>
<td>-0.15</td>
</tr>
<tr>
<td>4</td>
<td>Benzo[e]pyrene</td>
<td>824</td>
<td>0.23</td>
</tr>
<tr>
<td>5</td>
<td>Coronene</td>
<td>860</td>
<td>-0.16</td>
</tr>
<tr>
<td>5</td>
<td>Tetrachlorobiphenyl</td>
<td>750</td>
<td>1.57</td>
</tr>
<tr>
<td>5</td>
<td>Pentachlorobiphenyl</td>
<td>769</td>
<td>0.51</td>
</tr>
<tr>
<td>6</td>
<td>Pentachlorobiphenyl</td>
<td>664</td>
<td>0.84</td>
</tr>
<tr>
<td>6</td>
<td>Hexachlorobiphenyl</td>
<td>853</td>
<td>1.82</td>
</tr>
<tr>
<td>6</td>
<td>Benzyl butyl phthalate</td>
<td>927</td>
<td>-0.41</td>
</tr>
<tr>
<td>6</td>
<td>Tetrabromodiphenylether</td>
<td>653</td>
<td>0.62</td>
</tr>
</tbody>
</table>

**Figure 3:** Mass spectrum of high boiling compound from sample 2, which gives an 84.1% match with dibromopyrene.

Mass Accuracy and Isotope Abundance
An important part of the identification of unknown compounds is the measurement of accurate mass (see above) and the robust determination of isotope abundance. For example, one of the important POPs identified in sample 6 is tetrabromodiphenylether (TBDE), a member of a class of compounds known as brominated flame retardants (BFRs), with a chemical formula $\text{C}_{12}\text{H}_6\text{Br}_4\text{O}$, and a molecular mass of 482. The measured molecular ion was 481.71496 Da and the calculated value is 481.71455 Da, with a $\Delta m$ of 0.85 ppm leading to confident confirmation of its identity.

In the case of compounds with pronounced molecular ion clusters (arising from the presence of chlorine or bromine in the molecule), measurement of the relative isotopic abundance in the molecular ion cluster is also an important confirmation of molecular formula. In the case of the tetrabromodiphenylether, a high-resolution mass spectrum is shown in Figure 4 and the calculated values comparing measured with theoretical are provided in Table IV.
In general, differences of up to 30% are considered acceptable (Method 1613B) when working with PCDDs and PCDFs at low levels. If that criterion is applied to the spectrum of the low-level tetrabromodiphenylether then, as can be seen from Table IV, all of the ions fall within the acceptable level except for the low-level ion at 481.71 Da. These values, coupled with the excellent mass accuracy obtained on the molecular ion provide strong confirmatory evidence of the proposed identification.

**Conclusion**

Analysis of samples suspected to contain PCDDs and PCDFs using GC–MS with appropriate selectivity and sensitivity requires a targeted approach. EPA Method 1613B uses GC–HRMS using SIM analysis. This requirement compromises the ability to identify other priority POPs that may be present in the samples in the same run using a single analytical technique. Time-of-flight mass spectrometry, implemented either as a high-resolution, accurate mass instrument, or as a low-resolution instrument coupled with comprehensive two-dimensional GC, provides an alternative to the traditional, regulated approach. TOF systems have the sensitivity to achieve the low detection levels mandated by regulated methods while showing a strong correlation to results obtained using the regulated methods, and in addition provide the flexibility to detect and identify other priority pollutants in the same analytical run. Although not able to achieve detection in 100% of the instances, the benefit of providing a comprehensive result and detection of EPA Method 1613B analytes at or above the limits provides advantages. These capabilities lead to the opportunity for considerable savings of time and money. As such, these comprehensive technologies form a potent weapon in the hands of the environmental analyst.

**References**


(5) EPA Method 8290A: Polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) by high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS).


(11) www.lookchem.com, Safety Data Sheet.

Jayne de Vos is with the National Metrology Institute of South Africa in Pretoria, South Africa.

Jack Cochran is with Restek Corporation in State College, Pennsylvania. Eric J. Reiner is with Ontario Ministry of the Environment in Ontario, Canada. Peter Gorst-Allman is with LECO Africa in Pretoria, South Africa. Direct correspondence to: peter@lecoafrica.co.za

For more information on this topic, please visit our homepage at: www.spectroscopyonline.com
The 61st Conference on Mass Spectrometry and Allied Topics took place this year June 9–13 at the Minneapolis Convention Center in Minneapolis, Minnesota. The Sunday opening got under way with short courses taking place throughout the day, a tutorial session from 5:30 p.m. to 6:30 p.m., and the conference opening lecture at 6:45 p.m. The tutorials were given by Andrew Hoofnagle of the University of Washington and John Engen of Northeastern University. Hoofnagle’s talk focused on the wide spectrum of clinical diagnostics for the masses. Engen spoke about the nuts and bolts of protein hydrogen exchange mass spectrometry.

The opening lecture was given by Michael L. Gross of Washington University of St. Louis, on “The First Fifty Years of MS: Building a Foundation.”

Three more plenary events took place during the week. The Monday and Tuesday plenary sessions were award lectures given by Richard D. Smith and Yinsheng Wang (details below). On Thursday, Peter Onyisi of the University of Texas at Austin gave a plenary talk on the discovery of the Higgs boson. And as in previous years, the conference also featured daily oral sessions, poster sessions, exhibits, and workshops.

**Awards**

Several awards were presented at this year’s conference. The Award for a Distinguished Contribution in Mass Spectrometry was presented on Monday afternoon to Richard D. Smith of the Pacific Northwest National Laboratory for the development of the electrodynamic ion funnel in his laboratory. With the ion funnel, Smith has provided a basis to greatly improve mass spectrometers, today allowing routine detection of low-concentration species that would have been undetectable 15 years ago.

The Biemann Medal went to Yinsheng Wang of the University of California, Riverside, for his research on discovering the biological consequences of DNA damage and on unraveling mechanisms of action for antitumor drugs and environmental toxicants. His laboratory developed a method that combined liquid chromatography–tandem mass spectrometry (LC–MS-MS) with a plasma-based shuttle vector approach to quantitatively assess how structurally defined DNA lesions alter the frequency and efficiency of DNA replication and transcription in cells, and to measure the types and frequencies of mutations induced by lesions.

Two other awards were given out at this year’s conference. Yu Xia of Purdue University and Matthew F. Bush of the University of Washington each received the ASMS Research Award, which was funded by Thermo Scientific and Waters Corporation. The Ron Hites Award for an Outstanding Research Publication in the Journal of the American Society for Mass Spectrometry was presented to Alexander W.G. Graham, Steven J. Ray, Christie G. Enke, Charles J. Barinaga, David W. Koppenaal, and Gary M. Hieftje for a paper titled “First Distance-of-Flight Instrument: Opening a New Paradigm in Mass Spectrometry.”

**ASMS 2014**

The 62nd Annual ASMS Conference will be held June 15–19, 2014, in Baltimore, Maryland. For more information, visit www.asms.org in the coming months.
Dioxins, PCBs and PBDEs in Human Serum Using Automated Pressurized Liquid Extraction, Multi-Column Cleanup and Concentration

Because of its complexity, human serum is one of the most challenging sample matrices encountered. Organic contaminants often exist at low lipid concentrations (~600 mg/dL) that require extremely low detection levels and ultra clean blanks. The manual sample preparation process for human serum consists of multiple, time-consuming steps that are messy, difficult to reproduce and yield inaccurate results.

By combining the analysis of multiple analytes into a single extraction, cleanup, and concentration method using the Pressurized Liquid Extraction (PLE) system, the PowerPrep system to fractionate analyte classes, and the SuperVap Concentrator, the sample preparation process can be streamlined into a rapid, reproducible method.

### Instrumentation
- FMS, Inc. PLE System
- FMS, Inc. PowerPrep System
- FMS, Inc. SuperVap Concentrator
- FMS, Inc. 250 mL direct-to-vial concentrator tubes
- FMS, Inc. 250 mL concentrator tubes (1 mL termination)
- Thermo Scientific Trace Ultra GC with Quantum TSQ
- Thermo Scientific Trace Ultra GC with DFS HRMS

### Consumables
- FMS, Inc. PBDE free ABN columns
- FMS, Inc. PBDE free Alumina columns
- FMS, Inc. PBDE free Carbon columns
- Fisher Optima* Toluene
- Fisher Optima* ETAC
- Fisher Optima* n-Hexane
- EMD Omni* Benzene
- Fisher Formic Acid
- NIST 1958 RM; Fortified Human Serum
- Cambridge Isotopes EDF-4143, Dioxin, Furan, & PCB in Tissue calibration Standards
- Cambridge Isotopes EDF-4144B, Spiking solution for Dioxin, Furan, and PCB in Tissue
- Cambridge Isotopes EDF-4145, Recovery Standard for Dioxin, Furan, and PCB in Tissue
- Cambridge Isotopes EC-5366, PCB Calibration Standards
- Cambridge Isotopes EC-5367, PCB Spiking Solution
- Cambridge Isotopes EO-5319, BFR calibration Standards
- Cambridge Isotopes EO-5158, BFR Spiking Solution
- Cambridge Isotopes EO-5169, Recovery Standard

### Conclusions
The results of the 20 sample study indicate that Pressurized Liquid Extraction (PLE) combined with the PowerPrep multi-column fractionation and SuperVap Concentration systems generated rapid, reproducible extractions. The relatively low deviation between recoveries demonstrates the robustness of the extraction process as well as the ability to deliver a high level of reproducibility across multiple samples. The need to perform an additional cholesterol removal step, typically required with traditional SPE extractions of human serum, was eliminated. The FMS PLE, Power Prep and SuperVap combine the extraction, cleanup, and concentration steps into one process, allowing sample prep and analysis to be done in 24 hours.

**Table I: Mean recoveries and deviations for labeled compounds over 20 samples.**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mean Rec.</th>
<th>STD DEV</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,7,8-TCDD</td>
<td>67.4%</td>
<td>8.5%</td>
</tr>
<tr>
<td>1,2,3,7,8-PeCDD</td>
<td>78.1%</td>
<td>9.8%</td>
</tr>
<tr>
<td>1,2,3,4,7,8-HxCDD</td>
<td>81.8%</td>
<td>10.0%</td>
</tr>
<tr>
<td>1,2,3,6,7,8-HxCDD</td>
<td>67.8%</td>
<td>8.0%</td>
</tr>
<tr>
<td>1,2,3,7,8,9-HxCDD</td>
<td>81.1%</td>
<td>9.9%</td>
</tr>
<tr>
<td>1,2,3,4,6,7,8-HpCDD</td>
<td>60.9%</td>
<td>7.0%</td>
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<tr>
<td>OCDD</td>
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<td>6.3%</td>
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<tr>
<td>2,3,7,8-TCDF</td>
<td>71.7%</td>
<td>8.8%</td>
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<tr>
<td>1,2,3,7,8-PeCDF</td>
<td>76.6%</td>
<td>9.2%</td>
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<td>2,3,4,7,8-PeCDF</td>
<td>78.2%</td>
<td>10.0%</td>
</tr>
<tr>
<td>1,2,3,4,7,8-HxCDF</td>
<td>75.5%</td>
<td>9.0%</td>
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<td>1,2,3,6,7,8-HxCDF</td>
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<td>PBDE-209</td>
<td>95.5%</td>
<td>25.7%</td>
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<td>PCB-28</td>
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<td>PCB-101</td>
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<td>7.5%</td>
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<td>PCB-126</td>
<td>78.9%</td>
<td>10.7%</td>
</tr>
<tr>
<td>PCB-169</td>
<td>101.3%</td>
<td>14.0%</td>
</tr>
</tbody>
</table>

**FMS, Inc.**
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tel. (617) 393-2396, fax (617) 393-0194
Website: fmsenvironmental.com
Complex proteomics sample identification is among the most challenging tasks of modern analysis. Identifying, quantifying, and characterizing proteins as comprehensively as possible from low-amount highly-complex samples requires the utmost speed, dynamic range, and sensitivity. The new maXis impact offers a no-compromise solution delivering full resolution at full sensitivity and mass accuracy. It is capable of high sensitivity acquisitions at rates up to 50 Hz in MS and MS-MS with excellent spectral quality obtained under dynamic control of MS-MS acquisition speed. Together with state-of-the-art data evaluation capabilities from Bruker’s ProteinScape software suite, this package proves to be a powerful tool to address the most challenging proteomic analyses. Here we will demonstrate the performance of the maxis impact using a quantified standard of complex proteins — the Protein Dynamic Standard set from Sigma (UPS-2).

**Experimental**

The Protein Dynamic Standard Set from Sigma (UPS-2) contains 48 proteins covering five orders of magnitude in concentration. Each concentration level is represented by eight proteins with various pI, molecular weight, and hydrophobicity.

240 ng of an UPS-2 digest, corresponding to on-column protein amounts ranging from 11 amol to 1.1 pmol have been injected for LC–MS-MS analysis. The sample was applied on a Dionex Ultimate 3000 nanoRSLC system. Separation was performed with a 120 min water/acetonitrile gradient on a Dionex PepMap C18 column (250 mm × 75 μm). Database search was performed using MASCOT against a dedicated database. Carbamidomethylation was considered as fixed modification whereas methionine oxida-

![Figure 1: Proteins identified after Mascot search with the corresponding amount on-column and the number of unique peptides per protein.](image-url)

![Figure 2: Display of the ProteinScape result table, showing the eight most abundant (1.1 pmol on column) and the eight least abundant proteins, covering four concentration levels. Mass accuracy stability is shown by the High Resolution XIC extracted ion chromatogram with a 0.001 Da filter. Such high-selectivity XIC traces enable accurate quantitation.](image-url)

![Figure 3: High MS-MS accuracy and spectral quality is key to reliable assignment: 28% of the fragmented peptides have been assigned. Here we show typical MS-MS spectra for two concentrations. Y ions are in blue, B ions in red. MS-MS tolerance is 0.01 Da.](image-url)
tion and N-Term protein acetylation were considered as variable modifications. All results were stored and further analyzed using Bruker’s ProteinScape 2.1.

Results
The 32 most abundant proteins of the USP-2 Mix have been identified over the Mascot threshold (p < 0.05) (Figure 2), thus fully covering the first four concentration levels of the UPS-2 (Figure 1). Such results could be obtained thanks to the system ability to perform high-speed MS-MS acquisition at high sensitivity.

Conclusions
The Maxis Impact combines several technical features to offer an industry first — a benchtop ESIq-TOF which simultaneously delivers maximum resolution (~40,000) at full sensitivity whilst maintaining full mass accuracy. This ensures that low abundance peptides can be detected in the presence of high abundance peptides. It is this unique performance capability which ensures reliable identification of minority proteins in complex mixtures. Combining the performance power of the maxis impact with the evaluation capabilities of ProteinScape addresses one of the most challenging biological sample analysis tasks; the detection, identification, and quantitation of proteins in complex samples.

For research use only. Not for use in diagnostic procedures.
**Product Resources**

**LC–MS system**
The Orbitrap Fusion Tri-brid LC–MS system from Thermo Fisher Scientific combines quadrupole, Orbitrap, and linear ion-trap mass analyzers and is designed for the analysis of complex biological samples. According to the company, users can choose between fragmentation modes at any stage of MS\(^n\) analysis and unknowns in small-molecule experiments can be identified conclusively as they are encountered.

**Thermo Fisher Scientific**, San Jose, CA; www.thermoscientific.com

**Mass spectrometer**
The LCMS-8040 triple-quadrupole mass spectrometer from Shimadzu is designed to provide polarity switching at 15 ms and a high speed-scanning rate of 15,000 u/s. According to the company, the instrument’s UFsweeper II collision cell design enables MRM transition speeds of up to 5555 MRM/s.

**Shimadzu Scientific Instruments, Inc.**, Columbia, MD; www.ssi.shimadzu.com

**PEEK fittings**
Cheminert high-pressure PEEK fittings from VICI Valco are rated at 5000 psi with fingertight nuts. According to the company, the fitting designs permit direct connection of 360-µm o.d. fused-silica, PEEK, stainless steel, or electroformed nickel tubing without having to use liners.

**Valco Instruments Co., Inc.**, Houston, TX; www.vici.com

**Portable GC–MS system**
The Tridion-9 portable GC–toroidal mass spectrometry system from Torion is designed for field GC–MS applications. The system reportedly combines an electronic pressure-controlled gas chromatograph with an amplitude scanning toroidal ion-trap mass spectrometer. According to the company, the system can be used with SPME syringes for sample introduction.

**Torion Technologies Inc.**, American Fork, UT; www.torion.com

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