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Single Multipoint Calibration Curve for Discovery Bioanalysis

Compensation for divergence during an analytical batch is a primary motive for including duplicate calibration curves that bracket the unknown samples being measured. The drawback is that this adds time to the analysis. A single calibration curve run with staggered calibrants bracketing the unknowns was compared to running complete duplicate calibration curves, one at the beginning and one at the end of unknown sample analysis in an effort to accelerate discovery bioanalysis. The data, in terms of correlation coefficient ($r^2$) and the weighted residuals plot, show that a single calibration curve, with staggered calibrants bracketing the unknown samples, can be used successfully to accelerate discovery (nonregulated) bioanalysis.

Benjamin Begley and Michael Koletto

Designing a robust and reproducible generic liquid chromatography–tandem mass spectrometry (LC–MS-MS) method that can support discovery (nonregulated) bioanalysis of different molecules on a daily basis requires balancing scientific rigor with adequacy. Data integrity remains the critical objective. The only assumptions made in the discovery environment are in regard to extraction efficiency, stability, and response function. In essence, the generic method has to be good, or “adequate,” enough such that reliable information can be quickly returned to facilitate the decision making process.

The minimum acceptance criteria are a measure of systematic bias, or accuracy, and a measure of random error, or precision, the combination of which is a measure of total assay error (1). In addition to these requirements, reproducibility, sensitivity, and selectivity are the other key factors needed to cover the typical 1–10,000 ng/mL range commonly required in discovery pharmacokinetic (PK) bioanalysis. These factors are constrained by typical chromatographic run times of 2 min or less and a minimal sample cleanup step that is usually protein precipitation–based for in-vivo applications. All of this adds to the challenge of developing robust generic LC–MS-MS methods.

The Calibration Curve — Regression Analysis Considerations

The main role of LC–MS-MS bioanalysis PK studies is to provide drug concentration data for an accurate indication of the new chemical entity (NCE) exposure time profiles. The standard curve serves as a reference line against which the concentrations of the unknown samples are calculated. The standard curve itself is a result of regression analysis that takes into consideration the mean values of all $x$ (concentration) and $Y$ (area ratio) and the line of best fit through each data point to attempt to account for random error.

The most important consideration for bioanalysis spanning a broad range (1–10,000 ng/mL) is the choice of weighting factor. This is because LC–MS-MS bioanalytical data in almost all cases is heteroscedastic, a statistical term used for data in which the standard deviation changes within the range of measurements. In other words, the error is more or less proportional to concentration, and without proper weighting, the regression is inherently biased by the highest concentration (2). This is especially relevant in discovery bioanalysis where the high point on the curve can be as great as four orders of magnitude away, inherently introducing bias at the critical low end, near the limit of quantitation (LOQ), of the curve. Additionally, the coefficient of determination, or $r^2$ (regression coefficient), actually can be misleading and often is a poor measure of curve fit quality because it fundamentally assumes constant error at all concentrations, which is never the case in bioanalysis (3).

It is important to determine the best weighting factor, especially when covering the wide dynamic range typical in discovery bioanalysis. Statistically, the $1/x^2$ weighting provides the best fit because it most correctly approximates the variance at the low end of the curve, and by doing so, normalizes the error across the range. This effectively allows for the best fit (2). Because unknown samples are bracketed between two standard curves (one run at the front and one run at the back), the weighting factor chosen also has to account for divergence. The divergence is especially important in LC–MS-MS as factors affecting response are continually changing. At this stage, stability of the NCE is still undetermined, column performance can degrade over time, and ion-source efficiency can be affected as the source becomes coated with sample components. All of these are typical considerations in discovery bioanalysis.

Running two curves to test for divergence, commonly referred to as standard technique (Figure 1b), takes time — and time is an
important factor in discovery bioanalysis. Another approach to estimate divergence is to run calibrants across the same range in a scattered manner at the beginning and the end of the sample analysis, referred to as staggered technique (Figure 1a). In this case, calibrants at concentrations 1, 5, 50, 500, and 5000 ng/mL are run at the beginning and then 1, 2.5, 10, 100, 1000, and 10,000 ng/mL are run at the end. The 1-ng/mL calibrant is run twice because it is most susceptible to divergence (exceeding the ±20% CV criteria at the LOQ). This article attempts to correlate regression data obtained from the use of a single staggered calibration curve and compare it to the data obtained from the standard approach of running two full calibration curves, one at the beginning and one at the end of sample analysis. The goal of the experiment is to accelerate discovery bioanalysis without compromising data integrity.

**Experimental**

Diphenhydramine (C₁₇H₂₁NO, FW: 256.2) was used as the test compound, with reserpine (C₃₃H₄₀N₂O₉, FW: 609.3; 10 ng/mL) as the analog internal standard (IS). Calibrants were diluted using K₂-EDTA rat plasma, and the calibration standards were serially diluted using a Hamilton Starlet Liquid Handler (Hamilton Co., Reno, Nevada).

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**Figure 1:** (a) Staggered multipoint calibration curve set-up. Calibrants are prepared from a single stock solution and no QCs are used. The 1 ng/mL sample is repeated twice to ensure the LOQ criteria are met. (b) Standard method using duplicate standard (STD) curves and quality control samples (QCs). The QCs and STDs are from two independent stock solutions to ensure the integrity of the regression.
Plate 1 (Figure 1a) contained the multipoint calibration standards. Calibrants at 1, 5, 50, 500, and 5000 ng/mL were placed in the front of the plate, plasma blanks were placed in the middle, and calibrants 1, 2.5, 10, 50, 100, 1000, and 10,000 ng/mL were placed at the end. Plate 2 (Figure 1b) contained duplicate sets of the calibrants in a low-to-high series, bracketing 50 plasma blanks in the middle, with interspersed quality control samples (QCs) at low, mid, and high concentrations. Both plates were repeated 10 times and the calibration curves were compared for divergence.

A model 1100 LC pump (Agilent Technologies, Santa Clara, California) was used to perform the chromatography with a CTC PAL autosampler (CTC, Basel, Switzerland). Mobile phase A was water (0.2% formic acid) and mobile phase B was acetonitrile (0.2% formic acid). A gradient was structured as follows, with starting conditions set at 5% B at 0 min and 45% B in 0.01 min with a flow rate of 0.2 mL/min. The % B was ramped to 95% in 1.4 min; the flow rate was increased to 1.3 mL/min. After holding these conditions for 0.2 min, the gradient was ramped back down to the starting conditions. The total run time was 1.8 min. The column was used as an analytical technique for divergence. Plate 2 (Figure 1b) contained duplicate calibration curves. The data with interspersed quality control samples (QCs) at low, mid, and high concentrations. No practical difference in the divergence was observed using the staggered technique in terms of $r^2$ (0.998 vs. 0.999), slope (1.089 vs. 1.077), or during the comparison of the weighted residuals versus the concentrations (Figures 3a and 3b). This indicates that running a single calibration curve using the staggered technique may be a viable alternative to running duplicate calibration curves.

**Results and Discussion**

Figures 2a and 2b show the results for the staggered and standard multi-point calibration curves, respectively. Both techniques use linear $r^2$ (0.998 vs. 0.999) regression coefficients (coefficients of determination) and thus can be considered comparable. Although reporting $r^2$ is conventional practice, one should take into consideration that the regression coefficient assumes that error is fairly constant across the bioanalytical range, which is seldom the case in bioanalysis. A better way to compare the techniques is to look at the plot of the weighted residuals versus the concentrations (Figures 3a and 3b), which is useful in determining divergence as a direct consequence of analytical sensitivity. Based on these graphs, the single calibration curve run in a staggered manner is comparable to duplicate calibration curves. The data thus indicate that the information content required for rigorous quantitation is relatively unaffected and that running a single calibration curve in a staggered setup can be used in discovery bioanalysis without compromising data integrity. One can almost argue that using a single calibration curve in a staggered manner is perhaps more rigorous in terms of adhering to guidelines that require simple statistical models (4). The larger question remains whether running a duplicate curve at the end of the sample analysis adds any significant value to the experimental end-point, which often determines bioavailability for an NCE.

**Conclusion**

Statistically, the $1/\chi^2$ weighting provides the best fit because it most correctly approximates the variance at the low end of the curve and by doing so, normalizes the error at the high, low, and mid levels. No practical difference in the divergence was observed using the staggered technique in terms of $r^2$ (0.998 vs. 0.999), slope (1.089 vs. 1.077), or during the comparison of the weighted residuals versus the concentrations (Figures 3a and 3b). This indicates that running a single calibration curve using the staggered technique may be a viable alternative to running duplicate calibration curves.

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**References**


Benjamin Begley is Analytical Chemist and Michael Koletto is Associate Director with PharmaNet in Princeton, New Jersey.
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High-Throughput Quantitative Analysis of Vitamin D Using a Multiple Parallel LC–MS System Combined with Integrated On-Line SPE

Recently, liquid chromatography–tandem mass spectrometry (LC–MS-MS) has emerged as a reliable method to perform 25-OH-vitamin D₃ and 25-OH-vitamin D₂ analysis. Because sample preparation and LC separation are usually the most time consuming steps in a typical quantitative LC–MS-MS analysis, we have developed a high-throughput method for the quantitation of vitamin D using both multiplexed LC and on-line SPE. The overlapping LC runs and targeted data acquisition, combined with online sample preparation, result in a higher overall throughput for the system. The method described here is for the routine analysis of 25-OH-vitamin D₃ and D₂ (25-hydroxycholecalciferol and 25-hydroxyergocalciferol) in human serum and plasma samples. Calibration is performed using a lyophilized, multilevel plasma calibrator set of known concentration. Lyophilized plasma control samples at clinically relevant low and high concentrations serve to establish the target analyte range. The method uses deuterated 25-OH-vitamin D₃ as an internal standard to correct for sample and instrument variability. Samples are analyzed using the atmospheric-pressure chemical ionization (APCI) mode for maximum sensitivity.

Adrian M. Taylor and Michael J. Y. Jarvis

Vitamin D comprises a group of fat-soluble vitamins involved in the regulation of calcium and phosphorus levels in the body (1). Research has shown that vitamin D deficiencies are linked to colon and breast cancers (2), impaired bone mineralization, rickets in children, and possibly osteoporosis in adults (3). The two major forms are vitamin D₃ (cholecalciferol) and vitamin D₂ (ergocalciferol). Vitamin D₃ is produced in
the skin upon exposure to ultraviolet B radiation from sunlight, or may be obtained from nutritional sources, especially fatty fish. Vitamin D$_2$ is not synthesized by the human body and must be obtained from plant and other nutritional sources. Vitamin D$_3$ is converted to 25-OH-vitamin D$_3$ in the liver and then further hydroxylated to 1,25-OH-vitamin D$_3$, the major biologically active hormone, in the kidneys. Vitamin D is stored in the body as 25-OH-vitamin D$_3$, which is the most commonly measured metabolite for determining an individual’s vitamin D levels.

Traditional laboratory-based testing for 25-OH-vitamin D$_3$ and 25-OH-vitamin D$_2$ is performed using radioimmunoassay (RIA), chemiluminescence immunoassay, or high performance liquid chromatography (HPLC)–UV. Recently, liquid chromatography–tandem mass spectrometry (LC–MS-MS) has emerged as a reliable method to perform 25-OH-vitamin D$_3$ and 25-OH-vitamin D$_2$ analysis (4). To improve the throughput of LC–MS-MS analysis, we have developed a rapid method for the quantification of these analytes using a system that has been specifically designed to significantly increase the throughput of routine assays by accelerating both the sample preparation and the LC analysis. The system synchronizes autosampler injection ports, LC pumps, selector valve,

**Figure 1:** Integrated multiplex LC–MS-MS system configuration and module arrangement used in the analysis of 25-OH-vitamin D$_3$ and 25-OH-vitamin D$_2$.
Figure 2: Plumbing diagrams and solvent flow paths at typical states of a multiplex run. Upper left: loading stream 1, eluting stream 2, acquiring stream 2; upper right: loading stream 2, eluting stream 1, acquiring stream 1; lower left: eluting stream 1, eluting stream 2, acquiring stream 1; lower right: eluting stream 1, eluting stream 2, acquiring stream 2.

Figure 3: Method pane of the MPX Driver software.

and a mass spectrometer, enabling the autosampler to inject samples into two parallel LC streams using a system-defined timing schedule. The mass spectrometer acquires data throughout a specified retention time window in each LC sample run. The system also controls a series of switching valves, which are used for on-line solid-phase extraction (SPE) and the subsequent liquid chromatography analysis. The overlapping LC runs and targeted data acquisition, combined with online sample preparation, result in a higher overall throughput for the system. This application demonstrates how the multiplex system with integrated online sample cleanup can be used to double the throughput of the vitamin D analysis by LC–MS-MS.

Experimental

An MPX-2 SP High-Throughput System (AB Sciex, Foster City, California) with on-line SPE was used for the analysis of 25-OH-vitamin D₃ and D₂. The integrated multiplex LC–MS-MS system consisted of a 4000 QTRAP mass spectrometer (AB Sciex), two Prominence XR LC systems (Shimadzu, Kyoto, Japan), a CTC PAL autosampler (CTC Analytics, Zwingen, Switzerland) with DLW option, a pump containing a four-solvent selection valve for sample loading and cleanup, and five switching valves for flow path control (Figure 1). The two chromato-
graphic channels in the multiplex system share a single high-pressure loading pump, which provides additional flexibility for injection and wash solvent composition. All hardware modules are controlled by Analyst 1.5.1 software (AB Sciex) with the MPX Driver Software 1.1 (AB Sciex). Throughout the analysis, precise timing for the switching valves allowed each LC stream to perform interleaved injection, on-line sample cleanup, and LC gradient elution. Targeted MS data acquisition was enabled by the selection of a retention time window around the chromatographic peaks of interest using the MPX Method Editor. Figure 2 shows common flow paths for a typical on-line sample cleanup and LC–MS-MS analysis.

**Sample Preparation**
Lyophilized calibrators and controls were obtained from Chromsystems (Munich, Germany), and were reconstituted in distilled water. A 100-µL volume of each sample-calibrator-control was pipetted into a 1.5-mL reaction vial. Next, 25 µL of a precipitation reagent and 200 µL of an internal standard solution were added, and the samples were mixed using a vortex mixer for at least 20 s to precipitate the proteins. Samples were then incubated for 10 min at 4 °C in a water–ice bath, and centrifuged at 15,000 g for 10 min. The clean, precipitate-free supernatant was transferred to an HPLC vial equipped with a 200-µL insert for analysis by LC–MS-MS.

**HPLC**
Chromatographic separation was achieved using the analytical column included in the Chromsystems MassChrom reagent kit for the LC–MS-MS analysis of 25-OH-vitamin D₃/D₂ in serum or plasma. Mobile phases A and B were used as provided in the kit. The injected sample was initially loaded onto a trap column and rinsed for 1 min using mobile phase A. After 1 min, the sample was eluted from the trap column onto the analytical column.

**Table I: Accuracy and precision for analysis of 25-OH-vitamin D₃**

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>%CV (n = 6)</th>
<th>Accuracy %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stream 1</td>
<td>Stream 2</td>
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<tr>
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</tr>
<tr>
<td>QC 2</td>
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<td>5.6</td>
</tr>
</tbody>
</table>
using mobile phase B. The duration of the entire run was 5 min.

MS-MS

MS detection was carried out using the integrated multiplex LC–MS-MS system operating in multiple reaction monitoring (MRM) mode. Atmospheric-pressure chemical ionization (APCI) was used, in positive polarity mode, for maximum sensitivity.

Results and Discussion

A multiplex LC method was created using the hardware module control software, which synchronizes sample injections in staggered LC runs and enables targeted MS data acquisition in a predetermined retention time window on both of the parallel LC streams. The retention time window to be used for MS data acquisition was specified by highlighting the chromatographic region of interest (Figure 3).

A conventional singleplex LC–MS-MS analysis of 25-OH-vitamin D$_3$ and 25-OH-vitamin D$_2$ was performed, consisting of 92 consecutive injections, and the run time was then compared to that of the equivalent multiplex LC–MS-MS analysis of 92 samples using the integrated multiplex LC–MS-MS system. The sequence of timed events during the multiplex run is illustrated in Figure 4. The use of Cliquid software (AB Sciex) enabled automated sample analysis, processing of acquired data, and reporting of results. The total run time for the singleplex analysis was 8 h 26 min. In comparison, the run time for the multiplex analysis was 4 h 19 min, thus saving 4 h 17 min and increasing overall throughput 1.9 times.
The quality of the results was in no way compromised when the analysis was performed on parallel channels using the integrated multiplex LC–MS-MS system. Representative chromatograms for Calibrator 1 and Control 1 are shown in Figure 5, and the %CVs and accuracies obtained for the calibrators and controls (analyzed on both streams) are summarized in Tables I and II. The coefficient of variation obtained for the analysis of the calibrators and controls, using on-line sample clean-up, were all less than 10%, and the accuracies ranged from 85.9% to 107.6% for both 25-OH vitamin D₃ and 25-OH vitamin D₂ on both stream 1 and stream 2.

The calibration curves in Figure 6 were generated using the Chromsystems plasma calibrators, with the low- and high-level controls. The calibration curves were produced on both LC streams, for both 25-OH vitamin D₃ and 25-OH vitamin D₂, and demonstrate the range and linearity obtained for the assay. These results demonstrate that the data integrity was maintained when adapting the singleplex on-line SPE LC–MS-MS method to the integrated multiplex LC–MS-MS system.

**Conclusions**

The ability to multiplex two LC systems, synchronized to a single tandem mass spectrometer, allows laboratories to increase throughput to handle increasing numbers of samples. The dual channel multiplex LC–MS-MS system, with integrated and automated on-line sample cleanup, achieved a twofold increase in throughput for the analysis of 25-OH-vitamin D₂ and 25-OH-vitamin D₃ when compared to the same analysis performed on a conventional LC–MS-MS system with on-line SPE. This allows for considerable savings in both time and cost. No compromise in data quality, accuracy, or reproducibility was observed when the identical analysis was transferred from a singleplex to a multiplex system.

**References**


Adrian M. Taylor and Michael J. Y. Jarvis are with AB Sciex, 71 Four Valley Drive, Concord, Canada L4K 4V8. □

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High performance time-of-flight mass spectrometry (TOF-MS) is applied to the analysis and characterization of complex biological samples. High mass accuracy, high resolution, and accurate relative isotope abundance are all applied to the determination of analytes covering a range of concentrations in complex matrices including plasma and urine. Qualitative and quantitative evaluations are provided and include demonstrations of the impact of high-performance MS on sensitivity and selectivity. The ability to leverage high-performance MS in conjunction with a broad dynamic range in rapid ultrahigh-pressure liquid chromatography (UHPLC) analyses to identify unknowns and to propose putative metabolic biomarkers is demonstrated. The impact of speed of analysis and selectivity to the depth of coverage and accuracy of the analyses are discussed.

Jeffrey S. Patrick, Kevin Siek, Joe Binkley, Viatcheslav Artaev, and Michael Mason

Demands on modern analytical chemistry are driven by enhanced information content, faster analyses, faster data processing, and higher throughput. Tools in separation science continue to increase the speed and information content of traditional analyses as demonstrated by the growing popularity of ultrahigh-pressure liquid chromatography (UHPLC) (1), fast gas chromatography (GC) (2), and GC×GC (3). This creates demands for faster data acquisition and higher fidelity data, which in mass spectrometry (MS) translate to mass accuracy and resolution. To address these demands, the mass resolution, mass accuracy, and data acquisition speeds of mass spectrometry have been pushed to improve instrument capabilities. A historical overview of mass analyzers is provided in Table I with the representative performance attributes provided.
The utility and value of high-resolution, high mass accuracy MS has been emphasized in numerous recently reported applications of the technology (4–9). High-resolution MS has been the domain of Fourier-transform (FT) MS and magnetic-sector instruments for decades. The paradigm is shifting away from just high-resolution MS to high-performance MS that includes mass resolving power, mass accuracy, isotope abundance, and acquisition speed in its considerations. New developments in high-performance mass spectrometers have shown significant impact. Time-of-flight (TOF) instruments with higher resolution and enhanced performance are a significant portion of the efforts. Recent advances in TOF mass spectrometers have enabled these instruments to provide high resolution and mass accuracy with speed, simplicity, and convenience. The measurement of mass-to-charge ratio (m/z) in a TOF analyzer is described by the following equation:

\[ t = D(m/2z)^{1/2} \]

in which \( t \) is time, \( D \) is the flight distance, \( m \) is the mass, and \( z \) is the charge on the ion. This provides the fundamental relationship that \( t \) is proportional to \( D \), and in turn, resolution (defined as \([m/\delta m] \) or \([t/2\delta t]\)) is also a function of \( D \) and \( t \). This has been one of the challenges to TOF — to achieve long flight paths in reasonable physical space and provide high performance. A number of key advances have occurred over the past two decades that have improved the performance of TOF mass analyzers and have included orthogonal acceleration (10), high-speed electronics, delayed extraction (10), and pulsed ionization (11), which permit the most effective utilization of the flight path available. In spite of these advances, TOF mass analyzers are highly sensitive to the initial conditions of the ions as they enter the mass analyzer. This includes distributions in velocity, time, and space during ion generation and at entry to the mass analyzer. To provide an extended flight path and address initial ion dispersion, electrostatic time-focusing elements such as reflectrons (12) have been implemented, improving resolution significantly.

However, these approaches are limited by dispersions and higher order aberrations, as well as flight lengths on the order of a few meters. Other efforts have attempted to extend the flight path using cyclotron and toroidal analyzers (13). Duty cycle and effective mass range are typical compromises to high levels of mass measurement performance. With these advances, TOF has achieved a position in the realm of mass analyzers in which it provides sensitivity, mass accuracy and resolution that surpass some magnetic sector systems and encroach on the analyses historically reserved for FTMS systems. The principle advantage of a TOF system is the absence of scanning. This provides a mass measurement system well suited to analyze surveys rich in information content and high in duty cycle. These advances have opened the world of high resolution accurate mass analysis, historically the domain of FTMS and magnetic sectors, to TOF mass analyzers with the added benefit of simplicity and speed. The significant advances of TOF mass analyzers toward high-performance mass spectrometers continue in the approach discussed below.

### The New Technology

In one of the most recent advances in TOF-MS, Verenchikov and colleagues (14,15) have introduced the multireflecting time-of-flight analyzer that uses a Folded Flight Path (FFP). This is depicted in Figure 1, which shows how the ions effectively pass between each of two planar ion mirrors and through an Einzel lens array. A major challenge in the multireflecting systems is poor transmission because of defocusing of ion trajectories. In the FFP configuration, transmission is improved by using nonlinear electrostatic fields in the gridless mirrors. The ions are constantly refocused as they traverse the flight path and create the extended flight path needed to enhance the resolution. The gridless design minimizes ion loss during the flight. The mass analyzer offers three effective flight paths of approximately 2-, 20-, and 40-m lengths with operations in the same planar flight “tube.” These operational options are depicted in Figure 2. The number of passes or reflections determines the effective pathlength, and by inference, the available resolving power. The performance capabilities of this system are provided in Table I.

<table>
<thead>
<tr>
<th>Platform</th>
<th>Resolving Power</th>
<th>Mass Accuracy</th>
<th>Acquisition Rate</th>
<th>Isotopic Abundance</th>
<th>Mass Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magnetic sector</td>
<td>&gt;50,000</td>
<td>&lt;100 ppb</td>
<td>&lt;10 spectra/s</td>
<td>Excellent</td>
<td>Limited</td>
</tr>
<tr>
<td>Fourier transform (includes both magnetic and electrostatic traps)</td>
<td>&gt;10⁶</td>
<td>&lt;10 ppb (trade-off with acquisition rate and magnet size)</td>
<td></td>
<td>Poor (space charge)</td>
<td>Limited by duration, field strength, and experiment</td>
</tr>
<tr>
<td>Time of flight (includes TOF and Q-TOF configurations)</td>
<td>&gt;10⁴ (new systems approaching 10⁵)</td>
<td>1–5 ppm (lower values achievable with special consideration)</td>
<td>Typically trade-off with resolving power</td>
<td>Good (limited by dynamic range)</td>
<td>Limited by pulse frequency and analyzer attributes</td>
</tr>
</tbody>
</table>

### Table I: Overview of high-performance mass spectrometers

In the form of UHPLC coupled to atmospheric pressure ionization with TOF-MS. The matrices include plasma and urine, and the analytes include compounds of pharmaceutical interest and naturally occurring...
biological analytes associated with disease states. The applications discussed include the identification and relative quantitation of metabolites in plasma from three strains of Zucker rats, an example of the study of animal models of disease (16), and the analysis for metabolites of common cold medications in human urine.

**Experimental**
The following represent the basic conditions used in the analyses described in the results and discussion which follow. Chromatographic separation was achieved using an Agilent Technologies (Santa Clara, California) 1290 UHPLC system with mobile phases consisting of water with 0.1% (v/v) formic acid (A) and acetonitrile with 0.1% (v/v) formic acid (B). Mobile phase was delivered at 0.1–0.2 mL/min and with gradients covering 0–80% B between 3 and 30 min. Injection volumes varied between 1 and 5 µL. The column used was a 50 mm × 1 mm Hypersil Gold C18 AQ (Thermo Fisher Scientific, West Palm Beach, Florida). MS was achieved on a LECO Citius LC-HRT system equipped with a LECO electrospray ionization (ESI) source (LECO Corporation, St. Joseph, Michigan) and operated in high resolution mode ($R = 50,000$ [FWHM]). Data were processed using ChromaTOF-HRT software (LECO). Acquisition rates of 2–40 spectra/s were used both with and without in-source collision-induced dissociation. Calibration was achieved using external calibration with Agilent Tune Mix.

Rat plasma samples were obtained from Bioreclamation (Hicksville, New York) and were from lean, fatty, and obese Zucker animals. The plasma was from terminal bleeds with the rats being 7–9 weeks of age. The plasma samples were deproteinated using Microcon (Billerica, Massachusetts) centrifugal devices with a 5K cutoff. After protein removal, samples were diluted 5× into aqueous 0.2% heptafluorobutyric acid before UHPLC analysis.

Urine was obtained from a healthy male volunteer before and after a single dose of cold medicine containing 6.5 mg doxylamine, 15 mg dextromethorphan, and unspecified amounts of polyethylene glycol (PEG) and other excipients. To remove salt and protein from each sample, a 0.15-mL aliquot was diluted with 1.05 mL methanol and centrifuged. A 1.0-mL aliquot of the clear supernatant was evaporated, reconstituted with 0.025 mL methanol and 0.10 mL water with 0.1% formic acid, and analyzed by UHPLC–TOF-MS under high resolution conditions. All other chemicals used were obtained from Fisher Scientific (Fairlawn, New Jersey) or Sigma-Aldrich (St. Louis, Missouri).

**Results and Discussion**

**Rat Plasma Metabolomics:** Plasma samples from lean ($n = 10$), fatty ($n = 9$) and obese ($n = 10$) Zucker rats...
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were analyzed by LC–MS using high-performance TOF-MS. The analysis of the data was achieved by means of two mechanisms. In one, a list of established analytes was used to search the data for these analytes using an accurate mass targeted approach. In these instances, the exact \( m/z \) value was searched against the existing data to within 0.001 \( m/z \) unit. In the other, the accurate \( m/z \) values obtained from the analyses were used to determine molecular formulas, and the formulas or the accurate \( m/z \) values were searched in ChemSpider (17) or KEGG (18) or other similar metabolite databases to facilitate the identification of the analyte in question. In both of these instances, the ratio of area responses in the samples from the different phenotypes were then averaged and compared to each other for differences as three ratios. Specifically, the Lean:Fatty, Lean:Obese, and Fatty:Obese response ratios were generated, and the averages for each phenotype are provided graphically in Figure 3 for representative analytes. The plot shows several selected or readily identified analytes that show positive changes in the Lean:Fatty and Lean:Obese ratios relative to the Fatty:Obese ratio. These include leucine/isoleucine, hippurate, dimethylarginine, kynurenine, and urate. The nearly sixfold change in leucine/isoleucine is the most striking and largest proportional positive change for the “fatty” phenotype, while the change in kynurenine is the highest magnitude change negatively correlated with the “fatty” state. Both kynurenine and leucine/isoleucine have been previously associated with diabetes and obesity (19–23).

At the beginning of this study, standards were not available for confirmation of identity. As such, experiments including the fragmentation of “precursor” ions before the mass analyzer (so-called in source CID) with accurate mass measurement of fragment ions and the use of relative isotope abundance were used to clarify or support identification. One analyte identified in these studies was butyryl carnitine. In this case, an ion at \( m/z = 232.1554 \) was observed to change in intensity between lean and fatty/obese states. The formula search for this \( m/z \) provided two formulas within 5 ppm of the target \( m/z \): \( C_{11}H_{21}N_1O_4 \) (0.77 ppm error) and \( C_{12}H_{17}N_5 \) (1.3 ppm error). To provide additional information on the structures of the analyte, the fragment ions were extracted into a separate data channel and evaluated. Figure 4 shows an extracted ion chromatogram for both low (lean) and high (obese/fatty) samples along with the fragment ion spectrum from a lean sample. The two prominent fragment ions at \( m/z \) 173.082 and 85.03 match the loss of trimethylamine from 232 and the loss of butyric acid group and a trimethylamino group from 232, respectively. These are consistent with the identification of 232.1554 as butyryl carnitine. To provide yet another piece of supporting evidence, the relative isotope abundance was generated for the two formula above and these were compared to what was observed in high (fatty/obese) and low (lean) intensity samples. These findings are provided in Table II.
Table II: Mass accuracy in the assignment of identities to doxylamine metabolites

<table>
<thead>
<tr>
<th>Assignment</th>
<th>Expected m/z</th>
<th>Observed m/z</th>
<th>Relative Error (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Didesmethyldoxylamine</td>
<td>243.149190</td>
<td>243.14925</td>
<td>0.2</td>
</tr>
<tr>
<td>Desmethyldoxylamine</td>
<td>257.164840</td>
<td>257.16497</td>
<td>0.5</td>
</tr>
<tr>
<td>Doxylamine</td>
<td>271.180490</td>
<td>271.18049</td>
<td>0.0</td>
</tr>
<tr>
<td>Doxylamine + O</td>
<td>287.175404</td>
<td>287.17543</td>
<td>−0.1</td>
</tr>
</tbody>
</table>

For the monoisotopic peak and first isotope the agreement with the theoretical distribution is better than ±5% (relative) in all cases versus Option 1 (butyryl carnitine). For the second isotope (2\(^{13}\)C), the agreement is still better than ±17% (relative) for four of the five samples, with the fifth being of very low signal intensity. In all cases, the relative isotope abundance defines which of the two formula options best fits the data. This is most clearly seen in the first isotope data shown in Figure 5. This combination of accurate mass precursor analysis, accurate mass fragment ion analysis, and high integrity relative isotope evaluation is a clear demonstration of the impact that high-performance MS can have in metabolomic analysis and the characterization of analytes.

Of additional importance in metabolomic analysis is the dynamic range of the instrument. To effectively interrogate both abundant and trace level analytes simultaneously, the linearity of response across several orders of magnitude is necessary. To explore this and the limit of detection of the accurate mass system, a standard curve of butyryl carnitine was created that covered the range of 0.16 through >500 nM. The plots of response versus concentration are shown in Figure 6 (Figure 6a: 0.16–500 nM; Figure 6b: 0.16–20 nM) and demonstrate that the dynamic range achievable here is clearly in excess of the 500 nM point shown (2500 nM not shown). This is equivalent to a linear dynamic range greater than 3000-fold (15,000-fold at 2500 nM). The experiment has its lowest detectable level at 0.16 nM or approximately 200 fg of butyrylcarnitine on-column. This translates to the ability to examine either dramatic differences in concentrations of a single analyte or a range of analytes at substantially different concentrations in the same analysis.

**Analysis and Characterization of Drug Metabolites in Urine:** One of the most complex, but also most readily available, biological fluids is urine. It is difficult to standardize and is affected by diet, physiological state, disease, and other hard-to-control factors.
With all of this said, urine is still able to indicate the health of individuals and to provide insights into what the body has been exposed to, as it does give up its metabolites easily through renal clearance mechanisms. Here, urine from a healthy donor was analyzed by UHPLC with high-performance detection. A minimal level of cleanup was performed with the objective of providing a global survey for metabolites of doxylamine and dextromethorphan. Figure 7 shows a representative set of chromatograms for actives and metabolites detected in urine. The extraction window of ±2 ppm shows the necessary stability of the mass accuracy across the entire peak. This facilitates accurate integration of signal with high resolution and accuracy relative to other peaks. Although there are known compounds which should be present from the dosing, urine is a complex matrix, and as such, the identity of the analytes was confirmed by accurate mass analysis (Table II), which showed less than 0.5 ppm mass errors for the proposed doxylamine-related peaks. Similar to what was achieved with the butyryl carnitine peak, relative isotope abundance for the doxylamine provided highly accurate agreement with the theoretical isotope abundance. This is shown in the top of Table III. In addition, the application of “in-source CID” to generate fragment ions provided both accurate mass fragment identification and accurate relative isotope abundance of the fragment ions. These findings are shown in the bottom of Table III. All but the lowest of signals provide better than 5% relative difference versus the theoretical isotope abundance. Furthermore, the measured resolving power was in excess of 40,000 at \( m/z \) 271, and even at \( m/z \) 90, the resolving power was a robust 35,000.

**Conclusions**

The above applications of high-performance MS to the analysis of physiological analytes and metabolites in complex biological matrices provide a representative overview of the impact that this type of experimentation can have on analyte identification and quantitation. High mass accuracy, mass resolving power, and relative isotope abundance prove to be invaluable tools for analyte identification in the experiments discussed and facilitated the identification of analytes as potential biomarkers for obesity and as metabolic byproducts of pharmaceutical agents. Having high mass accuracy, resolving power, and isotope measures available in both precursor and fragment ions make the confidence an analyst might have in the identification of analytes extremely high. As this can be achieved on femtogram amounts of material using high acquisition rates with analysis times of a few minutes and provide high performance under all acquisition conditions, high-performance MS is both fast and of high information content. These findings clearly define the ability of high-performance MS to provide a positive impact in the advancement of metabolomic and metabonomic analyses.

**Acknowledgments**

The authors wish to thank Celine Aguer and Mary-Ellen Harper from the University of Ottawa (Ottawa, Canada), Oliver Fiehn from UC Davis (Davis, California), and Sean Adams from the Western Human Nutrition Research Center (WHNRC) at UC Davis for butyryl carnitine standards through work funded by the Na-
tional Institute of Health (DK 078328, Bethesda, Maryland).

References

Table III: Mass accuracy and relative isotope abundance for doxylamine using “in source CID.” Parent ion data are provided at the top of the table and product ion data at the bottom.

<table>
<thead>
<tr>
<th>Assignment</th>
<th>Observed m/z</th>
<th>Mass Resolving Power</th>
<th>m/z Error (ppm)</th>
<th>Expected Relative Abundance</th>
<th>Observed Relative Abundance</th>
<th>Relative Isotopic Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>[C17H23N2O]+</td>
<td>271.18049</td>
<td>49409</td>
<td>0.0</td>
<td>0.1942</td>
<td>0.1854</td>
<td>−4.5%</td>
</tr>
<tr>
<td>M+1</td>
<td>272.18398</td>
<td>49044</td>
<td>0.1942</td>
<td>0.1854</td>
<td>−4.5%</td>
<td></td>
</tr>
<tr>
<td>M+2</td>
<td>273.18671</td>
<td>41602</td>
<td>0.0180</td>
<td>0.0173</td>
<td>−3.8%</td>
<td></td>
</tr>
<tr>
<td>[C17H23N2O]+</td>
<td>271.18049</td>
<td>49409</td>
<td>0.0</td>
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</tbody>
</table>

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Introduction

The U. S. Environmental Protection Agency (EPA) has been investigating and regulating PFOA, PFOS and other fluorinated telomers, because a number of recent studies have indicated serious health effects in various animal models. Perfluorinated compounds (PFCs) have been manufactured for more than 50 years, and have been widely used as surfactants and surface protectors in carpets and fabric, and as performance chemicals in products such as fire-fighting foams, floor polishes and shampoos.

Sampling methods utilizing LC/MS/MS have been recommended in EPA Method 537 to analyze the concentration of PFCs in ground water as a source of the hazardous contact. To improve the detection limit of the PFCs with this equipment, scientists have tried to replace flow lines containing fluoropolymers (such as PTFE, FEP) with PEEK™ or stainless steel to eliminate interference effects. Membrane degassers, which are generally manufactured from TFE/PDD copolymer or from PTFE. These fluorinated polymer membranes slowly release PFCs into the solvent flow which can cause changes in MS background, and can significantly affect the accuracy of the analysis.

As a major supplier of degassers, IDEX Health & Science introduces Systec™ Interference-Free Degassing for Mass Spectrometry applications such as the measurement of trace PFOA. These premium degassers have the same degassing capability as standard degassers, but are free of PFOA and offer a significant reduction of other PFCs. When coupled with a fluorinated-polymer-free flow path, this degassing technology enables much more accurate analysis of PFCs.

Why Do We Need Degassing in HPLC?

In low-pressure-mixing HPLC, two or more solvents are mixed together prior to introduction into the HPLC pump. The seminal engineering study in 1976 by Tokunaga, which analyzed the concentration of air in alcohols and in water and their mixtures, clearly demonstrated the need to remove air from solvents prior to mixing in order to eliminate bubbles. Simply put: alcohol contains nearly seven times as much air as water, but mixtures of water and alcohol cannot contain the total amount of air each brings to the mix —between 30% and 70% methanol. Tokunaga found that at atmospheric pressure, water-methanol mixtures can contain only 38% of the amount of air each solvent brings to the mixture. Tokunaga found that at atmospheric pressure, water-methanol mixtures can contain only 38% of the amount of air each solvent brings to the mix—between 30% and 70% methanol. Studies of other solvent-solvent interactions also demonstrated a similar reduction of solubility along a mixing curve.

In Figure 1, the excess air in the mixture forms bubbles in the low-pressure, gradient-forming portion of the HPLC pumping system. Various other solvents (where one solvent dissolves more air than another with which it will be mixed) will produce similar outgassing and bubble formation.

Figure 2 shows the difference in HPLC chromatography performed with and without a degasser. The retention times for the peaks are very consistent when using a degasser, yet vary significantly when not using a degasser. Also bubble formation can cause unstable back pressure, and detector baseline shift.

1. 65:35 MeOH/WATER, Column: Agilent Zorbax SB-C18 length: 4.6 X 100 mm, 3.5 µm, Flow Rate: 2.00 mL/min, Wavelength: 254 nm, Injection Volume: 0.5 µL, Temperature: Not Controlled.
2. Vacuum Degassing Conditions: 50 mm Hg, Part Number: 9000-1385.
3. Vacuum Degassing Conditions: 760 mm Hg, other conditions the same as in 2.
**Limitation of Existing Degasser on PFC Detection**

There are a number of limitations to instrumental analyses of PFCs. One of the most severe limitations is high levels of background. The background comes from the presence of PFCs in a number of the instrumental components. This contamination can come from everything from the septa used in autosampler vials, from pump seals, or even tubing.

To conduct the analysis of PFCs with sufficiently high sensitivity and reproducibility, it has been recommended to substitute stainless steel or PEEK tubing for Teflon™ in all parts of the instrument. It has also been recommended that the instrument should be operated without the degasser. Unfortunately, this will cause the unstable chromatographic result demonstrated in Figure 2. Other researchers have tried to flush the entire HPLC system with solvent for upwards of several days to decrease or eliminate the contaminants from the instrument.

**Test Result of Systec Interference-Free Degassers**

In cooperation with Thermo-Fisher Scientific, IDEX Health & Science provided samples of the new Systec Interference Free degassers for their evaluation. The Interference-Free degasser (referenced as the “pre-cleaned PFC-free” degasser in Thermo Scientific notes), incorporating PEEK tubing from IDEX Health & Science rather than Teflon tubing, enabled Thermo Scientific to develop a new UHPLC/MS method for sensitive, precise, and reproducible trace-level analysis of PFCs. Other PFCs, including PFBS, PFHxS, PFHpA, PFUnA, and PFDoA are also analyzed. Excellent linearity in detector response was observed over the range of 0.04-2.5 ppb. Figure 4 shows the separation and detection of 10 ppt PFBS and 10 ppt PFDS at different SRM transitions, and the corresponding blanks as comparisons.

The sensitivity of the method is dependent on the levels of interferences that are present in the blank and in the solvent used. Limits of detection (LODs) and limits of quantitation (LOQs), defined as S/N ratio of 3 and 10, respectively, are shown in Table 1.

The result shows that by using the Systec Interference-Free degasser, very low background (very low interference) could be achieved, thus a highly sensitive, accurate and reproducible HPLC/MS/MS method for PFC analysis could be developed.

More details about the tests can be found in the Thermo Scientific Application Note 51 36 – “Sensitive and Accurate Quantitation of Perfl uorinated Compounds in Human Breast Milk using Selected Reaction Monitoring Assays by LC/MS/MS”.

**Conclusion**

The Systec Interference-Free Degasser, with the PFC-free pump and flow path, enables our customer to reliably achieve the maximum sensitivity and repeatable accuracy of their instrument for PFC detection and quantitation.

**Acknowledgements:** The authors thank Mark Joiner and Joe Rotter of IDEX Health & Science, and Gufeng Jiang and Robert Szilasie of Thermo-Fisher Scientific for their assistance with this work.

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Why Use Signal-To-Noise As a Measure of MS Performance When It Is Often Meaningless?

In the past, the signal-to-noise ratio of a chromatographic peak determined from a single measurement served as a convenient figure of merit used to compare the performance of two different mass spectrometry (MS) systems. The evolution in the design of MS instrumentation has resulted in very low noise systems that have made the comparison of performance based upon signal-to-noise increasingly difficult, and in some modes of operation, impossible. This is especially true when using ultralow-noise modes, such as high-resolution MS or tandem MS, where there often are no ions in the background and the noise is essentially zero. This occurs when analyzing clean standards used to establish the instrument specifications. Statistical methodology that is commonly used to establish method detection limits for trace analysis in complex matrices is a means of characterizing instrument performance that is rigorously valid for both high and low background noise conditions. Instrument manufacturers should begin to provide customers an alternative performance metric, in the form of instrument detection limits based on the relative standard deviation of replicate injections, to allow analysts a practical means of evaluating an MS system.

Greg Wells, Harry Prest, and Charles William Russ IV

For decades, signal-to-noise ratio (S/N) has been a primary standard for comparing the performance of chromatography systems including gas chromatography–mass spectrometry (GC–MS) and liquid chromatography (LC)–MS. Specific methods of calculating S/N have been codified in the U. S., European, and Japanese Pharmacopeia (1–3) to produce a uniform means of estimating instrument detection limits (IDL) and method detection limits (MDL). The use of S/N as a measure of IDL and MDL has been useful, and is still in use, for optical-based detectors for LC, and flame-based detectors for GC. As MS instrument design has evolved, the ability to accurately compare performance based on S/N has become increasingly difficult. This is especially true for trace analysis by MS using ultralow-noise modes, such as high-resolution mass spectrometry (HRMS) or tandem MS (MS-MS). S/N is still a useful parameter, particularly for full scan (EI) MS, but the comparison of high-performance MS analyzers should be based upon a metric that is applicable to all types of MS instruments and all operating modes. Statistical means have long been used to establish IDL and MDL (4–7) and are suitable to all operating modes for mass spectrometers.

Evolution of Instrumentation

Many sources of noise have been reduced by changes to the MS design such as low noise electronics, faster electronics allowing longer ion sampling (signal averaging), modified ion paths to reduce metastable helium (neutral noise), and signal processing (digital filtering). HRMS and MS-MS are also effective means to reduce chemical background noise, particularly in a complex sample matrix. For the signal, a longer list of improvements to the source, analyzer, and detect-
tor components have resulted in more ions produced per amount of sample. In combination, the improvements in S/N and the increase in sensitivity have resulted in significant and real lowering of the IDL and MDL.

**Lack of Guidelines for S/N Measurements**

The measurement of the “signal” is generally accepted to be the height of the maximum of the chromatographic signal above the baseline (Figure 1). However, some of the changes in GC–MS S/N specifications have been artificial. The industry standard for GC–electron ionization (EI) MS has changed from methyl stearate, which fragments extensively to many lower intensity ions, to compounds that generate fewer, more intense ions, such as hexachlorobenzene (HCB) and octafluoronaphthalene (OFN). The change to OFN held a secondary benefit for noise: the m/z 272 molecular ion is less susceptible to baseline noise from column bleed ions (an isotope of the monoisotopic peak of polysiloxane at m/z 281 increased baseline noise for HCB at m/z 282).

Improvements in instrument design and changes to test compounds were accompanied by a number of different approaches to measuring the noise. In the days of strip chart recorders and rulers, a common standard for noise was to measure the peak-to-peak (minimum to maximum) value of baseline noise, away from the peak tails, for 60 s before the peak (Figure 1) or 30 s before and after the peak. As integrator and data systems replaced rulers, the baseline segments for estimation of noise were autoselected and noise was calculated as the standard deviation (STD) or root-mean-square (RMS) of the baseline over the selected time window.

Automation of the calculation holds many conveniences for a busy laboratory, but the noise measurement criteria have become less controlled. In some cases, vendors have estimated noise based on a very narrow window (as short as 5 s) and location that can be many peak widths away from the peak used to calculate signal. These variable “hand-picked” noise windows make it easy for a vendor to claim a higher S/N by judiciously selecting the region of baseline where the noise is lowest. Generally, the location in the baseline where the noise is calculated is now automatically selected to be where the noise is a minimum. Figure 2 shows three different values of RMS noise calculated at different regions of the baseline. The value of the noise at positions a, b, and c is 54, 6, and 120, and the factor of 20 variation in S/N is exclusively because of where in the baseline the noise is measured. However, these S/N values will not correlate to the practical IDL for automated analyses that the laboratory performs. Therefore, the use of signal-to-noise as an estimate of the detection limit will clearly fail to produce usable values when there is low and highly variable ion noise and the choice of where to measure it is subjective.

The situation becomes even more indeterminate when the background noise is zero, as shown in the MS-MS
chromatographic peak in Figure 3. In this case, the noise is zero and S/N then becomes infinite. The only “noise” observed in Figure 3 is because of the electronic noise, which is several orders of magnitude lower than noise because of the presence of ions in the background. This situation can be made more severe by increasing the threshold for ion detection. Under these circumstances, it is possible to increase the ion detector gain, and hence the signal level, without increasing the background noise. The signal of the analyte increases, but the noise does not. This is obviously misleading since the signal increased, but there was no increase in the number of ions detected, and therefore, no change to the real detection limit. This allows S/N to be adjusted to any arbitrary value without changing the real detection limit.

Two decades ago, instrument specifications often described the detailed analytical conditions that affected the signal or noise value, such as chromatographic peak width, data rate, and time constant. Today, those parameters are missing or hard to find, even though a novice chromatographer realizes a narrow peak will have a larger peak height than a broad peak. In many cases, the GC conditions are selected to make the chromatographic peak extremely narrow and high, with the result that the peak is under-sampled (only one or two data points across a chromatographic peak). This may increase the calculated S/N, but the under-sampling degrades the precision and would not be acceptable for most quantitative methods. Once again, a false perception of performance has been provided. Accurate, meaningful comparison of instrument performance for practical, analytical use based on published specifications is increasingly difficult, if not impossible. Alternative means are required to determine the instrument performance and detection limit, which is generally applicable to all modes of MS operation.

**What Is the Alternative for S/N?**

S/N is still useful and represents a good first estimate to guide other statistically based estimates of performance. Every analytical laboratory should understand and routinely use statistics to evaluate and validate their results. The analytical literature has numerous articles that utilize a more statistical approach for estimating an IDL or MDL. The U.S. EPA has dictated a statistical approach to MDL, and can be found in the recommended EPA “Guidelines Establishing Test Procedures for the Analysis of Pollutants” (6). The European Union also has supported this position. A commonly used standard in Europe is found in “The Official Journal of the European Communities,” Commission Decision of 12 August 2002; Implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results (8).

Both of these methods are generally similar and require injecting multiple replicate standards to assess the uncertainty in the measuring system. A small number of identical samples having a concentration near the expected limit of detection (within 5–10) are measured, along with a comparable number of blanks. Because of the specificity of MS detection, the contribution from the blank is negligible and is often excluded after the significance of the contribution has been confirmed. The mean value \( \bar{X} \) and standard deviation (STD) of the set of measured analyte signals, \( X_n \) (that is, integrated areas of the baseline subtracted chromatographic peaks), is then determined. Even for a sensitivity test sample, the “sampling” process is actually a complex series of steps that involves drawing an aliquot of analyte solution into a syringe, injecting into a GC system, and detecting by MS. Each step in the sampling process can introduce variations in the final measured value of the chromatographic peak area, resulting in a sample-to-sample variation or “sampling noise.” These variances will generally limit the practical IDL and MDL that can be achieved.

The variance in the measured peak areas includes the analyte signal variations, the background noise, and the variance from injection to injection. Distinguishing the statistical significance of the mean value of the set of measured analyte signals from the combined system and sampling noise can then be established with a known confidence level. The IDL is then the smallest signal or amount of analyte that is statistically greater than zero within a specified probability of being correct. The IDL (or MDL) is related (9,10) to the standard deviation STD of the measured area responses of the replicate injections and a statistical confidence factor \( t_{\alpha} \) by

![Figure 3: EI MS-MS extracted ion chromatogram of m/z 222.00 from 1 pg OFN exhibiting no chemical ion noise.](image)
IDL = (tα)(STD), where the STD and IDL are expressed in area counts.

Alternately, many data systems report relative standard deviation (RSD = STD/Mean Value). In this case, the IDL can be determined in units of the amount of the standard (ng, pg, or fg) injected by

IDL = (tα)(RSD)(amount standard)/100%

When the number of measurements n is small (n < 30), the one-sided Student t-distribution (11) is used to determine the confidence factor tα. The value of tα comes from a table of the Student t-distribution using n-1 (number of measurements minus one) as the degrees of freedom, and 1–α is the probability that the measurement is greater than zero. The larger the number of measurements n, the smaller is the value of tα, and less the uncertainty in the estimate of the IDL or MDL. Unlike the statistical method of determining IDL and MDL, the use of signal-to-noise from a single sample measurement does not capture the “sampling noise” that causes multiple measurements of the same analyte to be somewhat different.

As an example, for the eight replicate injections in Figure 4 (n = 7 degrees of freedom) and a 99% (1–α = 0.99) confidence level, the value of the test statistic from the t-table is tα = 2.998. For the eight 200-fg samples, the mean value of the area is 810 counts, the standard deviation is 41.31 counts, and the value of the IDL is: IDL = (2.998)(41.31) = 123.85 counts. Since the calibration standard was 200 fg and had a measured mean value of 810 counts, the IDL in femtograms is: (123.85 counts)(200 fg)/(810 counts) = 30.6 fg. Alternately in terms of RSD, the IDL = (2.998)(5.1%)(200 fg)/100 = 30.6 fg. Thus, an amount of analyte greater or equal to 30.6 fg is detectable and distinguishable from the background with a 99% probability. In contrast, S/N measured on one single chromatogram (Figure 5) would give an IDL of 1.1 fg, assuming that IDL = 3 × rms noise (first entry in Table I). The high S/N is the result of a commonly

### Table I: Comparison of S/N for eight injections

<table>
<thead>
<tr>
<th>Injection Number</th>
<th>Signal</th>
<th>RMS Noise</th>
<th>S/N</th>
<th>IDL = 3×RMS (fg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>795</td>
<td>1.4</td>
<td>568</td>
<td>1.1</td>
</tr>
<tr>
<td>2</td>
<td>821</td>
<td>2.5</td>
<td>328</td>
<td>1.8</td>
</tr>
<tr>
<td>3</td>
<td>835</td>
<td>10.9</td>
<td>77</td>
<td>7.8</td>
</tr>
<tr>
<td>4</td>
<td>854</td>
<td>2.8</td>
<td>305</td>
<td>2.0</td>
</tr>
<tr>
<td>5</td>
<td>818</td>
<td>2.9</td>
<td>282</td>
<td>2.1</td>
</tr>
<tr>
<td>6</td>
<td>776</td>
<td>26.6</td>
<td>29</td>
<td>20.6</td>
</tr>
<tr>
<td>7</td>
<td>853</td>
<td>2.6</td>
<td>328</td>
<td>1.8</td>
</tr>
<tr>
<td>8</td>
<td>735</td>
<td>2.7</td>
<td>272</td>
<td>2.2</td>
</tr>
</tbody>
</table>

Figure 4: EI full-scan extracted ion chromatograms of m/z = 272 from 200 fg OFN, eight replicate injections, 3.3 Hz data rate.

Figure 5: EI full-scan extracted ion chromatograms of m/z = 272 from 200 fg OFN, first entry in Table I, 3.3 Hz data rate.
used algorithm that calculates the noise using the lowest noise sections of the baseline adjacent to the peak where the noise is unusually low. The individual S/N and IDL values measured for each injection in Figure 4 are tabulated in Table I. The IDL values range from 1.1 fg to 20.5 fg, and even the largest value is significantly smaller than the IDL determined by statistical means, which is more realistic.

**Estimating Relative Sensitivity**

A secondary benefit to specifying detection limits in statistical terms is to use the RSD as an indirect measure of the relative number of ions on a chromatographic peak. It is known (12) that if a constant flux of ions impinge on a detector and the mean number of ions detected in a particular time interval averaged over many replicate measurements is $N$, then the RSD in the number of ions detected is

$$\frac{1}{\sqrt{N}}$$

Hence, decreasing the number of ions in a chromatographic peak will increase the area RSD. This effect can be seen by comparing the RSD in Figure 6 and Figure 4 where the analyte amounts are 1 pg and 200 fg, respectively. The increased RSD at the lower sample amount is, in large part, because of ion statistics. Five times lower number of ions means

$$\sqrt{5} = 2.24$$

times increase in RSD. It follows that the 2.1% RSD at 1 pg will become 4.7% at 200 fg just from ion statistics. It is not unexpected that, at lower sample amounts, there also will be some additional variance from the chromatography. Thus, RSD measurements of the same amount of analyte on two different instruments can be used to indicate the relative difference in sensitivity near the detection limit, assuming that other contributions to the total variance of the signals are small. The more sensitive instrument will have the smaller RSD if all other factors are the same (that is, peak width and data rate). This avoids the uncertainty of inferring sensitivity from the measurement of peak areas where there is no baseline noise.

**Impact of Changing from S/N to RSD**

For MS vendors, there is complexity and cost in providing customers this valuable information. If the GC is configured with an autosampler and a splitless inlet, it should be fairly simple to add 7–10 replicate injections. This might add a little time to the installation, but the use of an autosampler should keep this at a minimum. But how do you test a system that does not have an autosampler or the appropriate inlet? Manual injections will add operator-dependent imprecision to measuring the peak area. Reproducible injection using a manually operated injector device like the Merlin MicroShot Injector (Merlin Instrument Co., Half Moon Bay, California), are necessary to reduce the sampling noise. If the method uses headspace or gas analysis, it could be costly to test as a liquid injection and then reconfigure for the final analysis. These costs must be managed appropriately, but factors like cost and complexity for some configurations should not prevent a transition to a better, statistically based standard such as %RSD for the majority of the systems that do not have these inlet limitations.

From the customer’s perspective, however, there is a significant benefit to having a system level test of performance that offers a realistic estimate of the IDL and the system precision near the limits of detection where it is the most critical. The purchase of a mass spectrometer is a significant capital expenditure, and often purchasing decisions are based on a customer’s specific application need. Instrument manufacturers do not have the resources to demonstrate their product’s performance for every application. It would be valuable to have a simple, but realistic, means of evaluating a particular MS instrument’s performance in the form of the IDL and the system precision. These will form lower bounds to the MDL and method precision for specific applications.

**Summary**

In the past, the S/N of a chromatographic peak determined from a single measurement has served as a convenient figure of merit used to compare the performance of two different MS systems. However, as we have seen, this parameter can no longer be universally applied and often fails to provide meaningful estimates of the IDL. A more practical means of comparing instrument performance is to use the multi-injection statistical
methodology that is commonly used to establish MDLs for trace analysis in complex matrices. Using the mean value and RSD of replicate injections provides a way to estimate the statistical significance of differences between low level analyte responses and the combined uncertainties of the analyte and background measurement, and the uncertainties in the analyte sampling process. This is especially true for modern mass spectrometers for which the background noise is often nearly zero. The RSD method of characterizing instrument performance is rigorously and statistically valid for both high and low background noise conditions. Instrument manufacturers should start to provide customers with an alternative performance metric in the form of RSD-based instrument detection limits to allow them a practical means of evaluating an MS system for their intended application.

References
(1) European Pharmacopoeia 1, 7th Edition.
(2) United States Pharmacopeia, XX Revision (USP Rockville, Maryland, 1988).
(4) ASTM: Section E 682–93, Annual Book of ASTM Standards, 14(1).

Greg Wells, Harry Prest, and Charles William Russ IV are with Agilent Technologies, Inc., Santa Clara, California.

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Food Metabolomics: Fact or Fiction?

Comprehensive analysis of both volatile and nonvolatile metabolites in food, combined with information on sensory properties and multivariate statistics, can be a valuable tool in understanding and improving the taste of food. However, performing food metabolomics studies is challenging and requires the analytical measurements to be of a very high quality. Although in some cases more-targeted approaches are adequate, it is expected that new developments in analytical chemistry will increase the value of food metabolomics in the future.

Leon Coulier, Albert Tas, and Uwe Thissen

Metabolomics plays an important role in systems biology. It enables better understanding of complex interactions in biological systems. There are many definitions and interpretations of metabolomics, but generally, it aims to analyze as many — if possible, all — small molecules (<1500 Da) in a biological system. The idea is that the biochemical level of the metabolome is closest to that of the daily function of a cell. It is an emerging tool in many disciplines such as plant physiology, drug discovery, human disease and nutrition, and others. Developments in analytical chemistry such as high-resolution mass spectrometry (HRMS), ultrahigh-pressure liquid chromatography (UHPLC), and software programs for fast processing of large analytical data sets have been responsible for the popularity and evolution of metabolomics.

Metabolomics has also found its place in food science, as recently reviewed by Wishart (1) and Cevallos-Cevallos and colleagues (2). Within the area of food science, three main applications of metabolomics can be distinguished:

• Food consumption monitoring and physiological monitoring of diet and nutrition focuses mainly on analyzing nutrients and their metabolites in biofluids or health effects of food on an organism. Although this is a very exciting application, concomitant analysis is performed on biofluids or tissue of humans or animals and not on the food itself.
• Food authenticity. This type of analysis is also performed on food itself and the main purpose is to classify samples according to, for example, origin and age. The advantage of this approach is that one is only interested in accurate classification and that can, in many cases, be achieved by a single method like nuclear magnetic resonance (NMR) spectroscopy covering a limited number of metabolites. Furthermore, understanding the reason why different samples are separated and thus identifying discriminating metabolites is not of primary interest.
• Food safety or quality improvement where the analyst wants to correlate a specific property to metabolite patterns using biostatistics. Taking taste as an example, the main goal is often to understand the taste of food in terms of chemical composition and physical properties so it can be optimized by processing and growth conditions, or to find markers that can be measured routinely and easier than taste itself. This puts strong demands on the experimental design. First, the specific property, such as sensory scores, should be quantifiable and the variance herein should be well represented by the different samples. Furthermore, the coverage of the analytical methods used should be high to detect all relevant metabolites that lead to understanding and optimizing the specific property. Depending on the research question, in some cases the relevant metabolites can be analyzed by a target analytical platform, whereas in other cases a more holistic view (that is, multiple platforms) is required.

Sample Preparation

The typical workflow of a metabolomics experiment is shown in Figure 1. Every aspect of this workflow has to be optimized to make metabolomics studies a success. With respect to the analytical chemistry involved in metabolomics, sample work-up, analysis of the samples, and data-
Figure 1: Schematic diagram of the different steps in a food metabolomics experiment.

preprocessing are items that have to be dealt with. The sample preparation procedures strongly depend on the type of matrix and compounds of interest. In the case of volatiles, food samples are preferably analyzed directly by headspace, solid-phase microextraction (SPME), or stir-bar sorptive extraction (SBSE) in combination with gas chromatography–mass spectrometry (GC–MS). Choices should be made with respect to adding agents to stop enzymatic reactions, like salt-solutions. This can have both positive and negative effects on the response, depending on the specific compound.

For less volatile and nonvolatile compounds, the sample extraction is determined by the polarity range of interest. In the case of metabolomics, where all metabolites can be of importance, extraction using chloroform–methanol–water mixtures are most often used to extract both polar and nonpolar compounds. Samples should be quickly frozen before extraction to stop any further reactions. Extraction should always be performed at low temperature to prevent loss of volatile compounds or thermal degradation.

Usually, a single analytical method will not be sufficient to analyze all relevant metabolites and thus a combination of different methods should be applied to increase the coverage. In the case of taste, it is essential to cover both volatile and nonvolatile compounds. Hence, the combination of methods for very volatile components, like SPME–GC–MS, and less or nonvolatile components, like GC–MS and LC–MS, is essential but can hardly be found in literature.

In this article, an example of food metabolomics in food quality will be described using tomato sensory properties as a demonstration case. Some highlights of this study will be shown; an extensive paper on this study can be found elsewhere (3). The challenges of this approach will be described, especially from an analytical chemical point of view, and recommendations will be made for future developments in food metabolomics with respect to analytical chemical requirements.

**Experimental**

**Sample generation and work-up:** A total of 19 different tomato samples from different origins were used. From each sample, 22 sensory attributes were quantified in duplicate by a sensory panel consisting of nine assessors.

For SPME–GC–MS, tomatoes were cut into equal parts and stored for 20 min to allow enzymatic reactions to take place. Next, the samples were pulped on ice and liquid nitrogen was added to stop enzymatic reactions. Before analysis, an equal weight of saturated calcium chloride solution was added.

The sample extraction method applied for GC–MS and LC–MS was adapted from a procedure developed for microbial metabolomics samples where it is essential to quench samples as quickly as possible (4). For oximation silylation–GC–MS (OS–GC–MS) and LC–MS, tomatoes were freeze-dried and ground followed by extraction using a 20:11:9 chloroform–methanol–water mixture. After extraction for 30 min at −40 °C, the extracts were centrifuged. The aqueous phase was pipetted off and freeze-dried. Before analysis, the freeze-dried samples were redissolved in 3:1 (v/v) water–methanol for LC–MS or derivatized using a solution of ethoxyamine hydrochloride in pyridine followed by silylation with MSTFA (5).

**Instrumentation and operational conditions:** GC–MS was performed on an Agilent 6890 gas chromatograph with an Agilent 5973 mass selective detector (Agilent Technologies, Palo Alto, California). Detection was performed using MS detection in electron ionization mode. For SPME–GC–MS, volatiles from mixed tomato–CaCl₂ samples were absorbed on a 50/30 μm DVB/Car/PDMS fiber (Supelco, Bellefonte, Pennsylvania) for 15 min at 50 °C. Desorption was performed at 250 °C for 10 min followed by splitless injection on a 30 m × 0.25 mm, 1-μm d₁ HP-5–MS column (Agilent) with a 0–320 °C temperature gradient at a rate of 10 °C/min. For OS–GC–MS, derivatized samples were analyzed by 1-μL PTV injection on a 30 m × 0.25 mm, 0.25-μm d₁ HP-5–MS column (Agilent) with a 0–320 °C temperature gradient at a rate of 10 °C/min. LC–MS was performed on an LTQ linear ion-trap system consisting of a Surveyor AS autosampler, Surveyor MS pump, and LTQ LT–10000 mass detector with an Optron ESI probe (Thermo Fisher Scientific, San Jose, California). Separation of tomato extracts (10 μL) was performed on a 100 mm × 2.1 mm
Extreme volatile metabolites are of course difficult to analyze by techniques like SPME–GC–MS, and more-specific techniques have to be used when it is expected that these types of compounds might be of interest.

A common method used in metabolomics in many application areas is GC–MS, in which metabolites are first oximated and silylated (5). With this method, various classes of small polar compounds can be analyzed by GC–MS, including organic acids, amino acids, sugars, sugar monophosphates, alcohols, aldehydes, amines, and acyl monophosphates. These classes of compounds are present in almost every biofluid or food sample, which explains the popularity of this method. As a result, this method can be easily applied to a new (food) matrix without extensive optimization. Moreover, large databases exist of mass spectra of reference compounds, facilitating identification. Derivatized water–methanol extracts of tomato analyzed by OS-GC–MS resulted in the detection of 112 metabolites, including aldehydes, alcohols, and thiazoles. Various SPME fibers were tested but the fiber used here showed, on average, the best results for a broad range of volatile compounds. In general, SPME shows better sensitivity and coverage than headspace GC–MS but requires more method optimization with respect to adsorption conditions and the type of SPME fiber. Other techniques, like SBSE, seem to have superior performance over SPME. Coupling of these techniques to GC×GC–time of flight (TOF)-MS will increase the sensitivity and coverage significantly but nontargeted metabolite quantification of GC×GC–TOF-MS data is still a challenge and very time-consuming (6).

Results and Discussion

Analysis of the samples: SPME–GC–MS was chosen as the method for volatiles for the tomato samples (Figure 2a) and resulted in the analysis of 101 metabolites, including aldehydes, alcohols, and thiazoles. Various SPME fibers were tested but the fiber used here showed, on average, the best results for a broad range of volatile compounds. In general, SPME shows better sensitivity and coverage than headspace GC–MS but requires more method optimization with respect to adsorption conditions and the type of SPME fiber. Other techniques, like SBSE, seem to have superior performance over SPME. Coupling of these techniques to GC×GC–time of flight (TOF)-MS will increase the sensitivity and coverage significantly but nontargeted metabolite quantification of GC×GC–TOF-MS data is still a challenge and very time-consuming (6).

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using GC×GC–TOF-MS, as has been demonstrated for other matrices (7). However, as mentioned before, non-targeted metabolite quantification of GC×GC–TOF-MS data in large scale studies is mainly hampered by the limitation of existing data preprocessing tools.

Nonvolatile metabolites are usually analyzed by LC–MS. Reversed-phase LC–MS methods using a C18 column with a mobile phase gradient from water to acetonitrile or methanol are most commonly used for aqueous extracts of food and plant material. Tomato extracts were analyzed by reversed-phase LC–MS in both the positive ionization (PI) and negative ionization (NI) mode (Figure 2c). Data preprocessing finally resulted in 1394 features that were used for further data analysis. Compounds identified were mainly secondary metabolites like (poly)phenolic compounds and derivatives thereof.

Small differences exist between methods used in literature and usually include additives used for the mobile phases and different types and suppliers of C18 columns. Untargeted screening is performed using ion-trap or TOF mass spectrometers, preferably in the PI and NI mode to increase the coverage. These methods are especially capable of analyzing larger, nonvolatile compounds such as phenolics and peptides. These classes of compounds do exist in many different foods and plant materials, but the exact structures are very type-specific. Because of this and the fact that many LC–MS methods exist with somewhat different experimental conditions, no databases are available that can be used for identification. Databases have been set up, but primarily starting from a specific matrix, such as tomato (8). However, these are often of little use for other matrices. As a result, many peaks can often be detected by LC–MS, but many remain unidentified. This is a key problem in the success of LC–MS in metabolomics studies. Consequently, standardization of LC–MS methods, in combination with proper databases and successful identification strategies, are essential for the future of the use of LC–MS in (food) metabolomics.

Recent developments in LC, such as columns with sub-2-μm particles in UHPLC, have led to increased throughput, better separation, and higher sensitivity in metabolomics applications (9). The issue with respect to the large number of unknown peaks and their identification will then become even more urgent. Other developments, such as robust hydrophilic interaction chromatography (HILIC) and mixed-mode columns or ion-pair methods, may lead to the use of more than one LC–MS method in metabolomics studies, resulting in increased coverage (10). Ideally, these methods will be used for high-throughput detection of small polar metabolites without derivatization, in addition to the larger nonpolar metabolites detected with reversed-phase LC–MS. Some examples do exist in literature, especially in the field of microbial metabolomics, and have shown that it is possible to detect a wide range of underivatized small polar compounds in complex matrices with LC-based methods (11–13). One limitation of these LC–MS methods is their separation efficiency compared with GC–MS, especially when dealing with isomers like monosaccharides and sugar-phosphates. However, the speed, sensitivity, and sample preparation of the LC–MS methods can be superior to GC–MS (for example, UHPLC coupled to high-resolution MS or MS–MS in HILIC or ion-pair mode). Quantification of many metabolites in a complex matrix is challenging for both types of methods. Although one always has to deal with ion-suppression in LC–MS, the GC–MS method with oximation and silylation shows strongly deviating analytical performance for different classes of compounds, mainly because of the characteristics of the derivatization step (5). Furthermore, with the GC–MS method, the results obtained for some metabolites in calibration solutions are very different from that in a complex matrix (5). As a result, the use of labeled standards for LC–MS methods might be sufficient for quantification but not for the GC–MS method, although each metabolite will require its own labeled standard. Therefore, these profiling methods are unlikely to be used for absolute quantification in the near future. Future developments in both GC–MS and LC–MS will determine whether LC–MS methods might replace the existing GC–MS methods used in metabolomics.

**Data Preprocessing**

After collection of the data, the next step in the metabolomics workflow is data preprocessing (that is, converting the raw data to reliable peak areas and peak lists). Various programs exist for the different analytical techniques. For GC–MS methods, either peak picking or deconvolution is applied. Because of the nature of EI, peak-picking routines usually lead to enormous peak lists, represented by a mass and retention time, in which a metabolite is represented by many entries, which is not ideal for statistical analysis. Therefore, deconvolution
TOF equipment, the mass spectrum of the soft ionization in ion-trap and Align, XCMS, and MzMine. Because software packages (for example, Metally) are applied using different freeware LC–MS data, deconvolution is under the consideration of the mass spectra. For DECO (14) and resulted in 101 and 112 metabolites, respectively. For the LC–MS data of the tomato samples, in-house developed alignment and peak-picking software was used, resulting in 1181 peaks excluding isobutyl and adducts for as much as possible. These are typical numbers of entries obtained with LC–MS in combination with peak-picking algorithms, but so far, it is not known what percentage of the number of entries are real metabolites as no one has annotated these peak lists.

Many different analytical methods have been developed and applied in metabolomics-related research areas. Interestingly, attention has only very recently been paid to the data quality of these methods using quality control (QC) samples, such as pooled study samples (Figure 3). These samples reflect the average metabolite concentrations and the performance of the analytical platform can be assessed using the relative standard deviation (RSD) of the metabolites in these samples. This approach has only been applied in a qualitative way — by visual inspection — and no attempt was made to really improve the data quality in a quantitative way. However, an explicit way of reducing the analytical error is better (15). Its workflow first consists of data normalization using internal standards (IS), which reduces differences in sample extraction, derivatization, and injection volume. With the use of the RSDs of the analyte response in the QC samples to quantify the amount of analytical variation, the best IS is the one that gives a minimum RSD. Adjustments of the analytical instrument between batches of samples can be the cause of analytical errors. This behavior results in different response factors between, and even within, batches. The data quality can be improved further using two types of QC samples. The first type, calibration QC samples, is used to perform a one-point calibration, and the second type, validation QC samples, is used to assess how well the calibration procedure improved the data quality in a quantitative way. Figure 4 shows the result of the batch calibration procedure on both the study samples and QC samples. It can be seen that both the intra- and inter-batch trends are corrected for by the procedures. Table I shows the RSDs of the analyte response in the QC samples to quantify the amount of analytical variation, the best IS is the one that gives a minimum RSD. Adjustments of the analytical instrument between batches of samples can be the cause of analytical errors.

Table I: Relative standard deviation (RSD) of 118 metabolites in QC tomato samples in raw data and after calibration procedures.

<table>
<thead>
<tr>
<th>RSD (%)</th>
<th>Raw Data</th>
<th>After Calibration</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;10</td>
<td>96</td>
<td>110</td>
</tr>
<tr>
<td>10–20</td>
<td>12</td>
<td>5</td>
</tr>
<tr>
<td>20–30</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>&gt;30</td>
<td>6</td>
<td>2</td>
</tr>
</tbody>
</table>

Figure 4: Improvement of the analytical data quality by (a) internal standard (IS) normalization and (b) batch correction.
tomato data. It can be seen that the raw data are already of good quality and that the calibration procedures lead to further improvement of the data quality.

It is essential to integrate the analytical data coming from the different analytical platforms before statistical analysis to find better correlations with the taste attributes. This so-called data fusion procedure (16) has been applied to the three different data sets obtained for the tomato samples.

**Data Analysis**

Multivariate data, such as metabolomics data, can be analyzed best by multivariate statistics. In contrast to univariate statistics, which looks at one metabolite at a time, multivariate statistics analyzes all metabolites simultaneously. This approach has shown to be more powerful and sensitive because it exploits existing relations between metabolites. Principal component analysis (PCA) is a common multivariate statistical method that is often used to summarize and to visualize the relations that exist within variables, such as metabolites or sensory scores, within samples, and between samples and variables. Methods like regression analysis that build models on both metabolomics and sensory data are much more suitable for food metabolomics studies because they explicitly model the relation between two data sets (that is, multivariate metabolomics data and a selected sensory attribute). The quality of the regression model determines if the model is good enough for further interpretation. For that, the correlation coefficient ($R^2$) between the predicted and the true outcome is used. Double cross validation is used to determine the $R^2$ in an unbiased way.

Both the sensory data and (fused) analytical data were analyzed by typical multivariate techniques such as PCA and partial least squares (PLS). Figure 5a shows a so-called PCA biplot displaying both the tomato samples and the sensory attributes. In this plot, it can be directly seen which tomato samples score high or low on specific taste attributes. For example, samples 1 and 3 score high on sour, while samples 8 and 12 score high on sweet. It is also clear that tomatoes 16 and 17 are clearly different from the other tomatoes. A similar PCA plot can be made of the fused analytical data (Figure 5b). Again, it can be seen that tomato samples 16 and 17 are different from the others. From these plots, it can be concluded that these samples have a significantly different taste and a significantly different metabolite pattern. However, this is not true for all samples. Samples 4, 14, and 19 cluster together in Figure 5a, but in Figure 5b, sample 4 is separated...
Table II: Double cross validated correlation coefficients ($R^2$) for PLS models obtained for several taste attributes

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Analytical Method</th>
<th>Correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavor intensity</td>
<td></td>
<td>0.72</td>
</tr>
<tr>
<td>Taste intensity</td>
<td></td>
<td>0.89</td>
</tr>
<tr>
<td>Sweetness</td>
<td></td>
<td>0.87</td>
</tr>
<tr>
<td>Soursness</td>
<td></td>
<td>0.92</td>
</tr>
<tr>
<td>Aftertaste duration</td>
<td></td>
<td>0.82</td>
</tr>
</tbody>
</table>

Table III: Selected metabolites on basis of PLS regression on metabolomics data and sensory data on soursness

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Analytical Method</th>
<th>Correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Terpineol</td>
<td>SPME–GC–MS</td>
<td>Negative</td>
</tr>
<tr>
<td>Cysteine</td>
<td>OS–GC–MS</td>
<td>Positive</td>
</tr>
<tr>
<td>Coumaroyl-quinic acid</td>
<td>LC–MS</td>
<td>Negative</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>LC–MS</td>
<td>Positive</td>
</tr>
<tr>
<td>Dimethyl-3-cyclohexene-1-aldehyde</td>
<td>SPME–GC–MS</td>
<td>Negative</td>
</tr>
</tbody>
</table>

significantly from samples 19 and 14. In this case, the taste of these three samples is similar but the metabolite patterns show significant differences. One other striking example is samples 8 and 10. In Figure 5b, these samples are almost on the same spot, indicating very similar metabolite patterns. However, in Figure 5a, samples 8 and 10 are clearly separated, sample 8 scoring high on sweet and sample 10 high on bitter. From this, it can be concluded that, despite the large differences in taste, the metabolite patterns are very similar, indicating that taste differences can be caused by very subtle differences in metabolites. Summarizing, the relative positions of the tomato samples in the sensory PCA plot and metabolomics PCA plot are very different. Hence, it can be concluded that PCA analysis does not suffice in correlating tomato sensory data and instrumental metabolomics data. Nevertheless, from the overview of Cevallos-Cevallos (2), it is seen that methods like PCA are still used.

PLS was carried out for different sensory attributes. For sourness, $R^2 = 0.92$ as can be seen in Table II, which indicates that at least 92% of the sensory attribute variation is independently described by the metabolomics data. For other taste attributes, similar high $R^2$ values were found after variable selection (see Table II). From these models, the most important metabolites can be determined that contribute most to the model (i.e., explain the most variation in the sensory attribute). Table III shows an example of such important metabolites for the PLS model of sourness, including the analytical platform they were derived from and whether the metabolites showed a positive or negative correlation with the sensory score. The results in Table III demonstrate the added value of applying and integrating different analytical platforms while all methods used in this study are represented. It should be mentioned that part of the important metabolites found for the different taste attributes could not be identified and these were mostly metabolites detected by LC–MS. It was discussed earlier that identification of metabolites, especially those detected by LC–MS, is one of the critical issues in metabolomics that should be solved in the near future to make metabolomics a more successful technology. It should be noted that the metabolites themselves do not necessarily lead to the sensory experience with which they correlate. They can also be precursor molecules, cause synergetic or masking effects with other metabolites, or have a regulatory role. In this study, most of the important metabolites could be identified, and of those 29 compounds, 20 were reported earlier to occur in tomatoes.

The next step in the whole process is the biological interpretation of the results (that is, understanding the observed relation between the identified metabolites and the specific taste attribute). This of course needs profound knowledge of the chemistry of taste in the specific matrix.

Validation is another key step in the metabolomics workflow. In order to demonstrate the validity of the results obtained, a separate second study with new samples and sensory data should be conducted and treated in the same way as described here. With respect to the analytical chemistry involved in metabolomics, this requires good reproducibility of the analytical methods used. For bioanalytical methods, it is standard procedure to validate analytical methods according to FDA guidelines including reproducibility. This part has been largely overlooked in metabolomics and only recently has more attention been paid to the stability of analytical methods over long time periods for, as an example, large-scale metabolomics studies (17).

After positive validation, the results obtained can be used to optimize or improve the taste of food products by approaches such as adapting growth or food processing conditions.

Conclusions

The different aspects of food metabolomics were described using tomato taste as an example. It is shown that the different parts of the metabolomics workflow should be of high quality in order to be successful. With respect to analytical chemistry, it is essential to have as much metabolite coverage as possible. Therefore, different complementary analytical techniques should be used and the data should be integrated. Also, aspects like data quality, data preprocessing, and metabolite identification are essential within a food metabolomics study. This makes food metabolomics studies often laborious, tedious, and costly. However, in cases where targeted approaches using existing
knowledge are not sufficient, the food metabolomics approach can be a valuable tool in food science. New technological developments in analytical chemistry and statistics will increase the value of food metabolomics even further.

Acknowledgments
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Leon Coulier is a research scientist and project manager analytical research at TNO. His current research interests include development and application of MS-based metabolomics technology for food, microbial, and medical applications.

Albert Tas is a consultant in analytical research and statistics at TNO. He has been active in the field of mass spectrometry, NMR, and multivariate statistics for more than 30 years.

Uwe Thissen is a research scientist and project manager life sciences at TNO Triskelion BV. His main interests regard the development and application of analytical technologies for innovations in food.

Leon Coulier

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Determining High-Molecular-Weight Phthalates in Sediments Using GC–APCI-TOF-MS

The environmental analysis of phthalates in environmental samples is an important application because some phthalates are considered to be potential endocrine-disrupting chemicals (EDCs) (1). Most classical environmental methods focus on the analysis of the dialkyl esters of phthalic acid ranging from dimethylphthalate (DMP) to dioctylphthalate (DOP) (2), among which di-isobutylphthalate (DiBP), dibutylphthalate (DBP), and bis(2-ethylhexyl) phthalate (DEHP) are the most important single isomer phthalates found in environmental samples (3,4). These solutes are single isomer compounds and relatively easy to analyze by gas chromatography–mass spectrometry (GC–MS) because only one peak for each compound is obtained by chromatographic analysis (5).

Using electron ionization (EI), the phthalic acid diesters strongly fragment, the most abundant ion being a phthalic anhydride ion at \( m/z = 149 \). Consequently, quantification of DiBP using the most abundant ion is impossible and the same sensitivity as for the single isomer phthalates cannot be reached. GC, combined with atmospheric-pressure chemical ionization (APCI), was used to analyze the high-molecular-weight phthalates. DiBP was successfully ionized in proton transfer mode yielding spectra that contained the molecular ion as the base peak. The method was also applied to measure DiNP and DiDP in a sediment extract at relevant levels for environment samples.

Frank David, Pat Sandra, and Peter Hancock

More recently, attention has been paid to the analysis of di-isononylphthalate (DiNP) and di-isodecylphthalate (DiDP). These compounds are synthesized from phthalic acid and a mixture of \( C_9 \) or \( C_{10} \) alcohols, respectively. Consequently, the GC analysis of these solutes results in a cluster of peaks corresponding to different branched isomers.

In the case of DiNP and DiDP determination, the \( C_9 \) isomers present in commercial DiNP samples partially overlap with the \( C_{10} \) isomers present in DiDP. No column or temperature program conditions could be found to completely separate \( C_9 \) from \( C_{10} \) isomers, making quantification of DiNP and DiDP using the most abundant ion at \( m/z =149 \) impossible. To differentiate DiNP and DiDP, another ion should be measured. This can be done using ions 293 and 307, respectively, corresponding to the M-alkyl ion (5). However, these ions are relatively low in abundance, and consequently, the same sensitivity as for the single isomer phthalates cannot be reached.
Possible solutions are offered by the use of soft ionization techniques, such as chemical ionization (using ammonia), or by liquid chromatography (LC)–MS. Recently, the combination of GC with atmospheric-pressure chemical ionization (APCI) became commercially available (6) and this technique was tested for the analysis of the high-molecular-weight phthalates. The method was also applied to measure DiNP and DiDP in a sediment extract from the Netherlands.

**Experimental**

Reference samples of DiNP \((C_{26}H_{42}O_4)\) and DiDP \((C_{28}H_{46}O_4)\) were obtained from BASF (Ludwigshafen, Germany). Individual stock solutions at 1000 ppm were prepared in cyclohexane. Calibration solutions containing DiNP and DiDP at the 1–10 ppm level were prepared by dilution in cyclohexane.

A sediment sample, obtained during an environmental study (4), was dried by lyophilization and a 10-g sample was extracted with 50 mL of cyclohexane in an ultrasonic bath. The extract was concentrated to 10 mL in a Zymark Turbovap system (Caliper Life Sciences, Teralfene, Belgium).

Analysis was performed on an Agilent 7890 GC (Palo Alto, California) coupled to a Waters Xevo QTOF MS system (Waters, Manchester, United Kingdom). Sample introduction was performed using a CTC GC-Pal autosampler (Basel, Switzerland) by means of a split–splitless injector. The MS system was operated in GC–MS mode using an atmospheric-pressure GC (APGC) source (Waters). Water was continually bled into the source during acquisition to promote proton transfer conditions.

The analysis used a 1-µL splitless injection at 300 °C with a 1.05-min purge delay. A 30 m × 0.25 mm, 0.25-µm df DB-5MS column was used (J & W Scientific, Folsom, California). The carrier gas was helium at a constant flow of 2 mL/min. The oven temperature program was as follows: 50 °C for 1 min, then 20 °C/min to 320 °C, hold for 2.5 min.

The instrument was tuned and calibrated using perfluorotripentylamine (FC 70) before acquisition, so that the resolution of the instrument was greater than 10,000 full width half maximum (FWHM). Exact-mass spectra were obtained (5 Hz) using a single-point lock mass (column bleed, \(m/z = 281.0517\)) infused into the source continuously during the temperature program.

The MS system had a corona current of 4.0 µA, a sampling cone voltage of 10 V, and a source temperature of 140 °C. The cone gas was nitrogen at 30 L/h, the source auxiliary gas was nitrogen at 300 L/h, and the make-up gas was nitrogen at 300 mL/min. The GC interface temperature was 310 °C.

**Results and Discussion**

**GC–EI-MS (Single-quadrupole system):** A typical total ion chromatogram (TIC) obtained for the 10 ppm standard mixture of DiNP and DiDP using electron impact ionization (performed on a single quadrupole instrument) is shown in Figure 1. A complex cluster of...
DiNP and DiDP isomers is observed. The spectrum taken at 25.6 min, corresponding to the most abundant DiDP isomer, is given in Figure 2. The molecular ion at $m/z = 446$ ($C_{28}H_{46}O_4$) cannot be detected and the main ions are at $m/z = 149$ (phthalic anhydride, $C_8H_5O_3^+$) and 307 ($M-C_{10}H_{19}$).

Differentiation of the two high-molecular-weight phthalates can only be completed by monitoring the ions at 293 (DiNP) and 307 (DiDP). However, because of the low abundance of these ions compared to the base peak, lower sensitivity is obtained, and, moreover, confirmation by a second ion is not possible.

**GC–APCI-TOF:** The analysis of the 10 ppm standard mixture was also performed using the conditions described in the experimental section. The TIC and the extracted ion chromatograms (XIC) at $m/z = 419.316$ (DiNP) and

![Figure 3: GC–APCI-TOF-MS analysis of a 10-ppm standard mixture containing DiNP and DiDP. (a) TIC; (b) XIC at 419.316 (DiNP); (c) XIC at 447.347 (DiDP).](image)

![Figure 4: Proton-transfer APCI spectrum for DiDP ($m/z = 447.3494 = [C_{28}H_{46}O_4 + H]^+$).](image)
447.347 (DiDP) are shown in Figure 3. The mass spectrum at 13.7 min (DiDP fraction) is shown in Figure 4. DiNP and DiDP give strong pseudomolecular ions, [M + H]^+ with no fragmentation compared with EI ionization. The main ion in the spectrum of DiDP is at m/z = 447.3494, a deviation of 4.5 ppm from the theoretical value for [C_{28}H_{44}O_4 + H]^+ The XICs for DiNP and DiDP, included in Figure 3, show that partial chromatographic separation is possible between these two compounds. However, the XICs are needed to allow quantification. The exact-mass detection enhances selectivity and allows confirmation of the presence of the individual solutes. Interestingly, the spectrum for DiDP shown in Figure 4 also contains m/z = 461.3615, −3.5 ppm from the calculated mass of C_{29}H_{49}O_4. This can probably be explained by the presence of mixed undecyl–decyl phthalates in the DiDP standard. If traces of C_{11} alcohols were present in the C_{10} alcohols used in DiDP synthesis, this would result in these impurities. Next, a sediment extract was also analyzed by GC–APCI-time of flight (TOF)-MS. The extracted ion chromatograms at 419.316 (DiNP) and 447.347 (DiDP) are given in Figure 5. Both solutes can be detected easily. It is, however, interesting to observe that the isomer distribution of DiNP and DiDP detected in the sediment sample are different from the standard mixture. Moreover, by using XICs at 433.332 and 461.363, additional phthalate solutes are detected. The masses correspond to C_{27}H_{44}O_4 and C_{29}H_{48}O_4, respectively, and are probably mixed C_{9}/C_{10} and C_{10}/C_{11} phthalates. At the end of each phthalate elution window, a single abundant peak is detected (for instance, at 13.7 min for DiNP and at 14.41 min for DiDP). These probably correspond to the linear di-C_{9} and di-C_{10} phthalates, respectively. The calculated concentrations of DiNP and DiDP in this sediment sample are 0.2 and 2 ppm, respectively.

**Conclusions**

DiNP and DiDP were successfully ionized in proton transfer APCI, yielding spectra that contained the molecular ion as the base peak. These high-molecular-weight phthalates could be detected below 1 ppm in sediment samples using APCI by extracting exact-mass chromatograms. At least two additional phthalate moieties were also identified in the extracted sediment sample using exact-mass chromatograms. The calculated formulas C_{27}H_{44}O_4 and C_{29}H_{48}O_4 suggest mixed C_{9}/C_{10} and C_{10}/C_{11} phthalates, respectively. Good exact-mass calculation was obtained for all solutes, with mass errors less than 5 ppm. GC–APCI-TOF-MS therefore offers interesting perspectives for detailed characterization of high-molecular-weight phthalates and for their sensitive and selective detection in environmental samples. The APGC source used allows all of the acquisition modes of tandem quadrupole and QTOF spectrometers to be used with a GC inlet combined with a soft ionization mechanism.

**References**

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**Frank David** is R&D Manager at RIC, Kortrijk, Belgium and visiting professor at Ghent University, Belgium.

**Pat Sandra** is director of the Research Institute for Chromatography (RIC), Kortrijk, Belgium, professor in Separation Science at Ghent University, Belgium, and director of the Pfizer Analytical Research Centre-UGent, Belgium.

**Peter Hancock** is Technical Manager in the Chemical Analysis Business Operations team working for Waters Corporation, Manchester, United Kingdom, where he and his team are actively involved in developing analytical methods on new MS technologies in application areas such as food and environmental testing and chemical materials.

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Preview of the 59th Annual ASMS Conference

We present a brief overview of this year’s ASMS conference, which takes place June 5–9, 2011, in Denver, Colorado.

Megan Evans, Spectroscopy Managing Editor

A popular theme song from the 1980s comes to mind when thinking about the 59th Annual ASMS Conference on Mass Spectrometry and Allied Topics — “Sometimes you want to go where everybody knows your name, and they’re always glad you came.” While the conference floor is a long way from the Boston-based Cheers bar, the sentiment of welcome and familiarity among friends and colleagues is always present at this show.

This year’s conference will be held at the Colorado Convention Center in Denver, Colorado, June 5–9, 2011. All oral sessions, poster sessions, and exhibit booths will be located in the convention center. Although the conference does not officially kick off until Sunday, there will be several two-day short courses starting on Saturday, June 4 but attendees must preregister for those. On-site registration for the conference only will be from 2:00 to 5:00 pm on Saturday, 10:00 am to 8:00 pm on Sunday, and 7:30 am to 5:00 pm on Monday through Thursday.

Sunday will mark the opening of the conference with short courses going on during the day, tutorial lectures from 5:00 to 6:30 pm, the plenary lecture from 6:45 to 7:45 pm, and the welcome reception from 7:45 to 9:30 pm. The tutorial lectures will be given by Mark W. Duncan of the University of Colorado and James Jorgenson of the University of North Carolina. Duncan’s lecture topic will focus on good mass spectrometry (MS) and its place in good science. Jorgenson, who is winner of the LCGC Lifetime Achievement award this year, will discuss liquid chromatography (LC) and MS. This year’s opening plenary lecture, entitled, “Our Stellar Origins Revealed by Stardust Grains,” will be given by Ernst Zinner of Washington University. The lecture will address the MS analysis of tiny stardust grains from primitive meteorites and what that analysis reveals about stellar production of the elements.

Program Schedule

Staying consistent with previous year’s schedule, Monday through Thursday will consist of oral sessions, poster sessions, workshops, plenary lectures, and corporate hospitality suites. Oral sessions will take place daily from 8:30 to 10:30 am and 2:30 to 4:30 pm. Poster sessions, exhibits, and lunch breaks will be from 10:30 am to 2:30 pm.
There are so many interesting sessions to attend at this year’s conference that it can often be difficult to choose which ones to attend. Some of LCGC and *Spectroscopy*’s Editorial Advisory Board members offered recommendations to help our readers narrow it down.

**Sunday, June 5:**
- Tutorial Lecture by James Jorgenson, University of North Carolina, “LC and MS: A Match Made in Heaven”

**Monday morning, June 6:**
- Integrated Qualitative and Quantitative LC–MS for Elucidation of PK/PD
- Protein Therapeutics in Drug Discovery and Development: LC–MS Quantification
- Imaging MS: Instrumentation and Ionization Sources

**Monday afternoon, June 6:**
- Challenges in Quantitative Bioanalysis in a Regulated Environment for Biomarkers

**Tuesday morning, June 7:**
- Dried Blood Spot for Drug and Metabolite Analysis
- Clinical Chemistry: Advances in Separation Technologies

**Tuesday afternoon, June 7:**
- Small Molecule Drug Discovery: Advances in Micro- and Nano-flow Separations

**Wednesday morning, June 8:**
- PK Assays: Novel Approaches to Increase LC–MS Throughput

**Thursday Afternoon, June 9:**
- Biomarkers of Drug/Metabolite Toxicity: LC–MS Methods

**Workshops and Interest Group Meetings**

Workshops and interest group meetings will be held Monday through Wednesday from 5:45 to 7:00 pm. Topics include MS fundamentals, polymer mass spectrometry, mass spectrometry in drug target identification, MS–MS based protein identification and characterization strategies, LC–MS libraries, career development in MS, and a discussion to generate content to stimulate undergraduate student interest in MS.

**Awards**

Two awards will be presented at this year’s conference: the award for a Distinguished Contribution in Mass Spectrometry and the Biemann Medal. The award for a Distinguished Contribution in Mass Spectrometry will be presented on Monday, June 6, during the plenary lecture scheduled from 4:45 to 5:30 pm. The Biemann Medal will be presented on Tuesday, June 7, during the plenary lecture scheduled from 4:45 to 5:30 pm. The award recipients were not announced as of press time.

**Conference Finale**

The conference will conclude on Thursday, June 9, with a plenary lecture from Arthur Shapiro of American University. Shapiro’s presentation, titled, “Why Are We Surprised by Only Some of the Things that We See? Visual Illusions, the Brain, and Baseball,” will present recently developed visual illusions and discuss the implications of our perceptive (in)abilities. After the lecture there will be a closing toast, officially ending the 59th ASMS Conference.

**Plan Your Visit to Denver**

Discounted pricing for ASMS attendees is available for travel from the airport to downtown hotels. Rates from the ASMS website are $32 round trip on the SuperShuttle and $70 one-way on the ExecuCar from the Denver International Airport; reservations are required. The ASMS room block features all major downtown hotels with very attractive conference rates, including Crowne Plaza ($150), The Curtis ($169), Denver Marriott ($169), Embassy Suites ($179), Grand Hyatt ($174), Hilton Garden Inn ($169), Hyatt Regency ($189 single, $199 double), and the Sheraton ($157).

**Beer, Biking, and Baseball**

Denver features several enjoyable attractions for attendees looking to do some sightseeing in their free time. The Denver area is often referred to as the “Napa Valley of Beer” and there are plenty of breweries and microbreweries for visitors to explore. For outdoor enthusiasts, Denver offers more than 850 miles of paved off-road trails that connect to hundreds of miles of dirt trails for hiking and biking. There is also Coors Field, home of the Colorado Rockies who will be facing the Los Angeles Dodgers toward the end of this conference.

**Registration and Further Information**

Advance conference registration fees are $150 for ASMS members, $300 for nonmembers, $75 for ASMS students, and $120 for student nonmembers. One-day registration is available onsite for $175 and includes a printed program, but no conference proceedings or access to webcasting.

Advance registration for the conference and short courses will close April 30. Those who have not registered by April 30 may register onsite at the convention center beginning Saturday, June 4, 2:00–5:00 pm, Sunday, June 5, 10:00 am–8:00 pm, and Monday through Thursday 7:30 am–5:00 pm. An additional $50 will be charged for onsite registration. There is no onsite registration for short courses.

For further information about the conference please contact: ASMS, Tel. (505) 989-4517, fax (505) 989-1073, email: office@asms.org, website: www.asms.org.
**PRODUCT RESOURCES**

**MS system**
Bruker Daltonics’ maXis 4G UHR-QTOF MS system is designed to offer full sensitivity resolution greater than 60,000 and mass accuracy greater than 600 ppb. The system reportedly maintains maximum sensitivity at full mass resolution, and can acquire data at speeds of up to 30 Hz during UHPLC separations. According to the company, the system can discover, identify, and quantify low-level compounds and metabolites in a single LC–MS-MS run.

Bruker Daltonics, Billerica, MA; www.bdal.com

**Portable GC–MS system**
Torion’s Guardian GC-TMS system is designed to be fast and portable for field GC applications. According to the company, the system combines a high-speed, EPC-controlled capillary chromatograph with an amplitude-scanning toroidal ion trap mass spectrometer. The system reportedly is supported by the company’s SPME syringes, calibration standards, and data processing software.

Torion, American Fork, UT; www.torion.com

**Column selection resource**
The Reversed Phase Column Screener from Phenomenex is a website-based resource designed to help chromatographers select the best HPLC or UHPLC column for their applications from the company’s product line. With the column-screening resource, users reportedly can search by compound characteristics, pharmacopeia classification, column, or application, and access application notes. According to the company, the resource gives primary and secondary recommendations for fully porous particle columns, complementary selectivities, and core-shell particle options, and recommends mobile phase starting conditions. The resource is available online at www.ColumnMatch.com.

Phenomenex, Torrance, CA; www.phenomenex.com

**Ion-trap GC–MS systems**
Agilent Technologies’ model 220 and 240 Ion Trap GC–MS systems are designed with the multiple ionization and scan modes of an ion-trap mass spectrometer and the capillary flow technology of a gas chromatograph. According to the company, the model 240 has MS–MS and MS² capabilities and can perform EI, CI, and MS–MS in the same run. The model 220 reportedly is designed for educational laboratories and laboratories needing rugged operation for high-throughput routine testing.

Agilent Technologies, Santa Clara, CA; www.agilent.com

**Mass spectrometer**
The BenchTOF-dx mass spectrometer from Almsco International is designed to identify, quantify, and differentiate the nature of known and unknown compounds at trace levels. According to the company, the instrument can screen for these compounds at lower levels with a higher degree of definition and clarity. The instrument reportedly provides full-scan sensitivity at typical GC–quad SIM levels and can integrate with existing gas chromatographs and data handling and control software.

Almsco International, Llantrisant, United Kingdom; www.almsco.com

**Capillary inlet tubes**
Photonis USA’s capillary inlet tubes, manufactured with resistive glass, are designed to control the speed and direction of ions, significantly increasing their transfer efficiency. Resistive glass utilizes lead silicate, which acts as a semiconductor, reportedly creating uniform electric fields within the capillary inlet tubes. When voltage is applied, positive or negative ions are preferentially attracted to more effectively draw them into the mass spectrometer. According to the company, the resistive glass prevents collisions between the ions and the tube walls, reducing ion loss and increasing transfer efficiency. The capillary inlet tubes are available as single-channel tubes or as six-channel multicapillary arrays.

Photonis USA, Sturbridge, MA; www.photonis.com

**Multipurpose sampler**
Gerstel’s MPS multipurpose sampler is designed to perform sample preparation and introduction techniques such as SPE, dynamic headspace, stir-bar sorptive extraction, thermal desorption, and SPME. According to the company, the sampler, matrix residues can be eliminated, standards or reagents can be added, dilution series can be created, and analytes can be concentrated. The system reportedly can operate independently as a workstation or can be integrated with GC–MS or LC–MS.

Gerstel, Linthicum, MD; www.gerstelus.com

**TOF mass spectrometer**
The Waters Xevo G2 time-of-flight (TOF) mass spectrometer is designed to identify and quantify unknown compounds in complex samples. According to the company, the bench-top mass spectrometer incorporates features from research-grade mass spectrometers. The mass spectrometer reportedly uses the company’s UPLC-MS² method of data acquisition, which provides both mass precursor and fragment ion data from a single data set.

Waters Corporation, Milford, MA; www.waters.com
Mass spectrometer
Shimadzu’s LCMS-8030 triple-quadrupole mass spectrometer is designed to complement UHPLC systems, offering power and speed in the detection of target analytes. According to the company, the system features multiple reaction monitoring (MRM) transitions that enable data acquisition of as many as 500 channels/s, 15-ms polarity switching, and mass spectrum measurement speeds of 15,000 u/s. The instrument reportedly accelerates ions out of the collision cell by forming a pseudo-potential surface, producing high-efficiency collision-induced dissociation (CID) and high-speed ion transport. Shimadzu Scientific Instruments, Inc., Columbia, MD; www.shimadzu.com

Microplates
MicroLitter’s microplates for chromatography are available in 2-mL square, deep well, round, and conical bottom; 1.3-mL round well round bottom; and 0.75-mL round medium well round bottom designs. According to the company, the microplates feature clear polypropylene for visual reference of samples and have pierceable covers in either EVA or silicone with sprayed on PTFE barriers. Microliter Analytical Supplies, Inc., Suwanee, GA; www.microliter.com

Autosampler
The PAL-xt autosampler from CTC Analytics includes the company’s XCHANGE option for automatic syringe exchange. The system is designed for adding internal standards without cross contamination, injection of small and large volumes in one sample sequence, and preventative exchange of syringes when using harmful media. The autosampler’s coupling system reportedly comprises a mating mechanical piece that captures the syringe and a magnet that automatically aligns. CTC Analytics AG, Zwingen, Switzerland; www.palsystem.com

Concentration system
Fluid Management Systems’ PowerVap Automated Direct to Vial Concentration system is designed to automate the manual steps in the sample evaporation and concentration process. According to the company, the compact, stand-alone system can be used for concentrating samples such as the by-products of pesticides, herbicides, persistent organic pollutants, polychlorinated biphenyls, polycyclic aromatic hydrocarbons, pharmaceuticals, and personal care products. The system reportedly uses no water, can concentrate up to six samples simultaneously, and provides method documentation. Fluid Management Systems, Watertown, MA; www.fmnsenvironmental.com

Trap columns
Optimize Technologies’ hand-tight OPTI-TRAP EXP trap columns are sample purification and preconcentration products designed for use at pressures as high as 20,000 psi (1400 bar). According to the company, the columns can connect directly to any injection valve (with 10-32 threads) or in-line with the company’s OPTI-LOK EXP fittings. Optimize Technologies, Inc., Oregon City, OR; www.optimizetech.com

Chromatography vials
Verex chromatography vials, caps, and inserts from Phenomenex are designed to meet specifications for performance and recoveries and to exceed industry standards. The vials’ borosilicate glass reportedly has the lowest ion content possible. According to the company, the vials can be supplied as kits and with mix-and-match cap choices, including crimp, snap, or screw, with septa available in preslit or nonslit formats. The vial kits reportedly are provided in dispenser packages made from recycled materials. Phenomenex, Inc., Torrance, CA; www.phenomenex.com

MS system
AB Sciex’s TripleTOF 5600 mass spectrometry system is designed for high-performance qualitative and quantitative analysis. According to the company, the system features Smart-Speed 100-Hz acquisition; Accelerator TOF Analyzer for high-resolution data at high speed; and EasyMass Accuracy to achieve stable ~1 ppm mass accuracy without continuous user calibration. AB Sciex, Foster City, CA; www.absciex.com

Mass spectrometer
The Waters SYNAPT G2 mass spectrometer is designed for use in biopharmaceutical, metabolite identification, metabolomics, proteomics, biomarker studies, and food and environmental applications. According to the company, the quadrupole time-of-flight system features greater than 40,000 FWHM resolution, high sensitivity, a data acquisition rate of 20 spectra/s, exact mass (1 ppm RMS) information, and a dynamic range of up to five orders of magnitude. Waters Corporation, Milford, MA; www.waters.com
Call for Papers

Current Trends in Mass Spectrometry
2011 Supplements to LCGC and Spectroscopy

LCGC and Spectroscopy magazines are seeking contributed manuscripts for the 2011 supplement series, Current Trends in Mass Spectrometry. These issues will be published in July and October 2011.

These supplements will examine developments in mass spectrometry and MS-hyphenated methods and their application to analytical problems in many fields, including but not limited to pharmaceutical and biopharmaceutical discovery, development, and manufacturing, biology and medicine, energy, the environment, forensics, defense, and food safety.

Manuscripts should be approximately 3500 words long, plus figures and tables as needed, and follow a standard experimental article format, including an abstract of approximately 150–200 words. Figures and tables, along with their captions, should appear at the end of the manuscript, and figures also must be sent as separate files, preferably in JPG, TIF, PNG, or XLS format. References should be called out using numbers in parentheses and listed at the end of the manuscript in numerical order.

Submissions from equipment manufacturers will be considered but must be devoid of promotional content.

Submission Deadlines
October 2011 issue:
Abstracts (150-200 words): July 8, 2011
Completed manuscripts: August 8, 2011

Where to Submit
Send all proposals and completed articles to Editor Laura Bush, at lbush@advanstar.com (tel. +1.732.346.3020).

About LCGC
For nearly 30 years, LCGC has been the gold standard relied upon by chromatographers across all technique areas for objective, unbiased, nuts-and-bolts technical information they can use in their labs every day. LCGC’s columns and peer-reviewed articles continue to bring readers technical advice from the most respected, veteran industry experts in the areas of UHPLC, HPLC, GC, LC–MS, GC–MS, CE, SFC, and more.

About Spectroscopy
For more than 25 years, Spectroscopy has been providing information to enhance productivity, efficiency, and the overall value of spectroscopic instruments and methods as a practical analytical technology across a variety of fields. Scientists, technicians, and laboratory managers gain proficiency and competitive advantage for the real-world issues they face through unbiased, peer-reviewed technical articles, trusted troubleshooting advice, best-practice application solutions, and educational web seminars.

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