GC Data Analysis
How does your GC generate data?
MACHEREY-NAGEL

Columns and supplies

Your solutions at www.mn-net.com
Looking at the Past to Understand the Future: Soxhlet Extraction
Douglas E. Raynie

For well over 100 years, Soxhlet extraction was considered the gold standard in the analytical extraction of solids. Regulatory agencies, trade organizations, and individual laboratories collected a wealth of data based on the technique. In this instalment, we look back at Soxhlet extraction so that analysts can better appreciate the lessons that are fundamental to today’s extractions.

Recent Application and Instrumental Trends in Comprehensive Two-Dimensional Gas Chromatography
Peter Q. Tranchida, Ivan Aloisi, and Luigi Mondello

This article critically reviews recent applications and instrumental trends in comprehensive two-dimensional gas chromatography (GC × GC), with a focus on the fact that the concept of GC × GC is simple, and with an eye on future developments in GC × GC.

Recovering from a COVID-19 Shutdown: Tips and Tricks for Starting Up, Part 2
Dwight R. Stoll and Tony Taylor

COVID-19-related laboratory shutdowns are sure to cause a myriad of problems with liquid chromatography (LC) instrumentation across the globe. Taking a systematic approach to restarting these systems will save money and time in the long run by preventing problems that may otherwise appear in days or weeks following startup.

Image credit: Courtney Soden
Comprehensive two-dimensional gas chromatography (GC×GC) is the most powerful GC approach available for the analysis of mixtures of volatile compounds. This multidimensional technique was first reported in 1991, and stemmed from the research work of J.B. Phillips (1). Considering that two-dimensional (2D)-GC has been on the analytical scene for nearly 30 years, it can now be defined as “well known”. For this reason, no explanations on the basics of GC×GC are provided in this article. The reader is directed to the many reviews present in the literature, if in-depth details are required (2–5). From consulting such literature, it can be deduced that GC×GC has undergone great hardware and software evolution over the years. For example, during the first 10 years of GC×GC use, flame ionization detection (FID) was, by far, the most common form of detection. After that period, a rapid increase in the use of mass spectrometry (MS) was observed. It was only in 1998 that the first forms of cryogenic modulation (CM) and flow modulation (FM) were introduced (6,7), while in 2003 the first commercial instrument with fully integrated software was proposed (8). Finally, the overall history of GC×GC has been characterized by illustrations of chromatograms highlighting its exceptional separation power. An example was provided by Phillips and Xu (dated 1995) (9), who showed a GC×GC–FID chromatogram of kerosene. The separation was performed on an apolar 10 m × 530 μm, 8-μm df column in the first dimension (1D), and a more polar longer one (25 m × 250 μm) in the second dimension (2D). Each column was housed in a separate GC oven, thus recognizing the importance of independent temperature optimization. A dual-stage thermal modulator linked the two analytical dimensions and was located between the two GC ovens. The transfer device was of simple construction, inasmuch that it was prepared by using a 40 cm × 250 μm, 0.25-μm df apolar column, coated with gold paint. The first
The outstanding potential of GC×GC was clearly evident in these early works (1,9,10), and certainly created great excitement amongst many GC practitioners. In 1996, an article appeared reporting on “sophisticated separation methods and the oil industry”, with the authors declaring that the “instrumentation required for GC×GC is beautifully simple and potentially inexpensive” (11). Such a statement, in itself related to the instrumentation initially developed by J.B. Phillips and collaborators, is certainly agreeable (1,9,10).

Recent Application and Instrumental Trends
As mentioned earlier, recent application and instrumental trends will relate to the combined period 2018 to 2019. To obtain such information, a search process was launched by using the Scopus database and the keywords: “comprehensive two-dimensional gas chromatography”. The database provided a list of 136 papers for 2018 and 121 papers for 2019. It is obvious that, even though some published data were most probably missed, a total number of 257 works do give an overall view on the current situation. It is noteworthy that a recent review entitled: “Comprehensive two-dimensional gas chromatography advances in technology and applications: biennial update” has been published (5). Overlap of the information herein provided with respect to that work will try to be avoided, while particular attention will be devoted to the fact that the concept of GC×GC is inherently simple.

With regard to the topics, a main one was defined for each publication, with a clear dominance of application research observed (77%), followed by reviews and perspectives (9%) (Figure 1). Among the applications (Figure 2), food and beverage (29%), energy (24%), environment (21%), and biological (9%) occupy the first four positions, with the first three being by far the most popular. With regard to reviews and perspectives, these involve manuscripts covering both a specific GC×GC aspect or application (for example, metabolomics [12], modulation [13], environment [14]) and those describing GC×GC as an option within a specific research field (for example, analysis of S-containing compounds in petroleum [15], solid-phase microextraction [SPME] [16]).
Considering the sum of application research and reviews and perspectives, these represent 86% of the published work across the period 2018–2019. A third topic relates to theory and optimization (for example, retention time prediction [17], FM model [18], solid-state modulation optimization [19]) and 2D linear retention index (LRI) calculation (20,21), represented by 6% of the published works. With regard to instrumental evolution and data analysis, these were characterized by only 4% each of the published work. Technological advances have involved, among others, a miniaturized GC×GC system (22), the use of a micro-reactor prior to FID detection (23), a silver-based ionic liquid 2D column (24), and different FM approaches (25,26).

Proceeding onto the utilization of modulation approaches, specific information was attained from the majority of the 257 publications (for some papers we had access only to the abstract). As expected, CM was by far the most common choice (87%), followed by FM (10%), and solid-state modulation (3%). Focus is herein devoted to FM, which is an area that has undergone evolution in recent years. For example, in 2018 Seeley et al. introduced a novel system based on the Deans switch principle and defined multi-mode modulator (MMM) (25). A scheme of the MMM GC×GC system is shown in Figure 3. As can be observed,
the MMM is of simple construction and is characterized by a deactivated metal joining capillary, connected on the \(1\)D side to a cross union and to a tee union on the \(2\)D side. The tips of the \(1\)D and \(2\)D columns are fixed near to one another within the joining capillary. Two metal capillaries link the unions to a solenoid valve (the normally opened [NO] port of the solenoid valve is connected to the tee union), which in turn receives a gas flow \(F_s\) from a pneumatic control module. The fourth port of the cross union is linked to an FID-connected restrictor (FID1). There are two flows entering the modulator, \(F_s\) and \(F_x\) (\(1\)D flow), and two flows exiting it, \(F_x\) (restrictor flow) and \(F_2\) (\(2\)D flow). When the valve is in the NO position, the \(1\)D flow is directed to the restrictor (low duty cycle) or stored in the joining capillary (high duty cycle); when the valve is in the normally closed (NC) position, effluent from the \(1\)D column is directed to the second one, and then subjected to FID monitoring (FID2). Depending on the gas flows involved, and on the proximity of the \(1\)D and \(2\)D column tips within the joining capillary, the MMM could be operated as a low or high duty cycle modulator (consult reference 25 for more information). Under low duty cycle conditions, a 6% cyanopropyl phenyl + 94% polydimethylsiloxane 30 m × 0.25 mm, 1.4-μm df column was used in the first dimension (flow: 1.0 mL/min), and a polyethylene glycol 0.5 m × 0.18 mm, 0.18-μm df column in the second (flow: 0.9 mL/min). The modulation period was 1000 ms with a 75 ms injection time, meaning that only a low percentage of the \(1\)D effluent reached the \(2\)D.

A nice example on the various effects of a low duty cycle MMM process on a highly volatile compound (\(n\)-pentane) can be observed in Figure 4(a–f): the FID2 result when the valve is in the NO position is shown in (a), highlighting the fact that no effluent from the first dimension reaches the second; the FID1 result when the valve is in the NO position is shown in...
(b); the FID2 result when the valve is in the NC position (transfer mode) is shown in (c); the FID1 result when the valve is in the NC position is shown in (d), highlighting the fact that a small amount of effluent from the first dimension reaches the restrictor; the effects of modulation on n-pentane are shown in (e) (FID2 result) and in (f) (FID1 result). Very narrow peak widths at half height were reported for modulated n-octane (53 ms). Such MMM conditions were characterized by a duty cycle of 0.054 (ratio of the total area of the modulated pulses to that of the unmodulated peak), and were suitable for high-speed separations on short segments of microbore (≤ 0.18 mm internal diameter [i.d.]) column, and for MS detection (if used).

Under high duty cycle conditions, an apolar 40 m × 0.18 mm, 0.18-μm df column was used in the first dimension (flow: 0.50 mL/min), and a polyethylene glycol 5 m × 0.25 mm, 0.25-μm df column in the second (flow: 10.0 mL/min). The modulation period was 1500 ms, with a 150 ms injection time. A fuel sample was subjected to analysis with peak widths at half height of approximately 80 ms. Such MMM conditions were characterized by a duty cycle of 1, and were suitable for high-speed separations on medium-length (5–8 m) segments of columns with an i.d. ≥ 0.25 mm. A flow of 10.0 mL/min can be considered as rather high for mass spectrometry, and possibly requires splitting prior to the ion source depending on the MS system used.

The 100% transfer FM approach described in reference 26 is characterized by increased simplicity (compared to reference 25): a six-port valve modulator (without a sample loop) was used, with connections (in an anticlockwise manner) to the injector, to the inlet and outlet of the ¹D, to the inlet of the ²D, and to an additional gas source (the sixth port was blocked). Different samples were subjected to FM GC×GC–FID analyses under a variety of conditions.


**FIGURE 8:** Graph reporting the specific types of detection in GC×GC research published across the combined period 2018–2019. Refer to the text for the significance of the abbreviations. The % values have been rounded to the nearest integer.
of operational conditions: for example, a sample of diesel was subjected to a D separation on an apolar 15 m × 0.25 mm, 0.25-μm df column, and a D separation on a polyethylene glycol 5 m × 0.25 mm, 0.25-μm df column. The flow rates were 10 mL/min during the transfer process (0.4 s) and 30 mL/min for the remaining part of the separation on the D (14.6 s). During the analysis, the valve was maintained at a temperature of 300 °C. It is noteworthy that during the main part of the modulation period (14.6 s), there was a condition of stop-flow in the D, leading to an increase in the analysis time but also to a non-interdependence between the D and D separations. In general, the reported D gas flows were rather high (≥ 10.0 mL/min).

An even more simple FM approach of high interest (defined dynamic pressure gradient modulation (DPGM)) was described by Trinklein et al. at the beginning of 2020 (27): the D and D columns were linked through a tee union and connected to a pulse valve (Figure 5). An auxiliary gas source fed the valve (Paux). Under suitable conditions of injector pressure (Paux), Paux (both inlet and auxiliary pressures are ramped during the analysis), and valve open and close times, a 100% transfer GC×GC analysis could be achieved. When the valve was closed, a fraction of the D effluent was transferred onto the D, while when it was opened, D elution was interrupted (stop flow) and the D separation proceeded. For example, a 90-compound mixture was subjected to a DPGM GC×GC–FID analysis by using an apolar 10 m × 0.18 mm, 0.18-μm df D column, and a polar 1 m × 0.18 mm, 0.10-μm df D column. The modulation period was only 750 ms, with a 60 ms valve close time. The applied Paux generated a D gas flow of 22.9 mL/min at the beginning of the analysis. Peak widths were narrow in time and variable, being in the range 20–180 ms (Figure 6). In a further DPGM GC×GC–FID analysis on diesel fuel, the use of a longer D column (2 m × 0.18 mm, 0.18-μm df) enabled the reduction of the D flow to 8 mL/min. In this instance, a modulation period of 2000 ms with a 150 ms valve close time was used. Very recently, DPGM GC×GC was combined with low-resolution time-of-flight (LR TOF)-MS, using a D gas flow of 4 mL/min (28).

A major concern when using FM is the lack of analyte band compression; this is a typical feature of CM leading to very high peak capacities. It was within such a context that Aloisi et al. attempted to determine an equivalent standard column set between CM and FM GC×GC–MS (29). Cryogenic modulation was performed by using a loop-type system, while FM was performed by using a seven-port wafer chip equipped with an external sample loop and was a 100% transfer device (29). Very similar chromatography performances were attained when using an apolar 30 m × 0.25 mm, 0.25-μm df + polar 1.5 m × 0.25 mm, 0.25-μm df CM column set and an apolar 20 m × 0.18 mm, 0.18-μm df + polar 5 m × 0.32 mm, 0.25-μm df FM set (obviously the same types of stationary phases were used). The CM and FM results, attained on a sample of coconut bio-oil, are shown in Figure 7. This study provided an idea of the potential of the FM approach used compared with CM.

Considering detection (again, specific information was attained from the majority of the publications, not from all), obviously mass spectrometry confirms its dominant role (Figure 8). The first four positions are occupied by LR TOF-MS (52%), FID (16%), single quadrupole (QMS) (15%), and high-resolution (HR) TOF-MS (9%). The use of both LR TOF-MS and rapid-scanning QMS are now well-established. With regards to HR TOF-MS, the definition of “high resolution” is rather vague. A powerful HR TOF-MS system used in the GC×GC field is capable of exceeding a resolution of 25 000 (full width half maximum [fwhm]), operating with a normal GC mass range and a high acquisition frequency (200 Hz) (30). Even though there has been an increasing use of QTOF-MS, it should be noted that its MS/MS capabilities are usually not exploited. In fact, the quadrupole is normally operated as a fly-through zone, with high-resolution mass spectra generated by the TOF analyzer. For example, Bowman et al. used GC×GC–QTOF-MS with atmospheric-pressure chemical ionization (APCI) in an environmental study involving the Athabasca oil sands (31). The mass spectrometer was operated in the TOF mode, at a resolution of 20 000 (fwhm) and at an acquisition frequency of 30 Hz. If one sums up the use of HR TOF-MS and QTOF-MS during the period 2018–2019, then HR MS is used at a similar level with respect to QMS. Considering ionization processes, electron ionization (EI) is by far the most popular approach. The use of soft forms of ionization, such as APCI (31), is reported rather rarely. The use of an LR TOF-MS system with the capability to perform hard (70 eV) and soft (14 eV) EI, in a rapid alternate manner and with a satisfactory analytical response, has started to appear (32). The use of triple quadrupole (QqQ) MS was found in only 2% of the published research. Such a trend can be related to the targeted nature of QqQMS analyses, whereas the power of GC×GC stands out in untargeted analyses. However, the use of QqQMS, with the capability to generate both untargeted (scan) and targeted (multiple reaction monitoring) data in a rapid alternate manner, has been reported (33). The use of FID has declined greatly and is now comparable to that of QMS. Forms of selective detection, such as nitrogen and sulphur chemiluminescence detection (NCD, SCD), and electron capture detection (ECD), find little current use (Figure 8). In the authors’ opinion, among several reasons for such a trend is the fact that GC×GC separations are
generally characterized by many more peaks compared to conventional GC ones, increasing the need for mass spectrometry.

**Future Prospects**

Comprehensive 2D-GC is becoming a mature technology, as can be concluded by observing recent literature, which is dominated by application research and with less space devoted to technological evolution. Such a tendency will probably continue, also in part as a result of the continuous evolution of MS. In fact, the availability of (powerful) MS reduces the requirements of an improved separation performance on the GC×GC side.

What are the future prospects of GC×GC? There will probably be an increasing development of smaller, less-energy consuming GC×GC(–MS) devices. It is anticipated that modulation will be part of such a downscaling, involving the use of robust, compact, simple, and effective devices. Modulation approaches that could potentially fit such a description are present both in past (1,10) and in present research (25–29).

**References**

15) Y. Han, Y. Zhang, C.Xu, and C.S. Hsu, Fuel 221, 144–158 (2018).

**Peter Q. Tranchida** is Associate Professor in food chemistry in the Department of Chemistry, Biological, Pharmaceutical and Environmental Sciences at the University of Messina, in Italy. His research activities are focused mainly on the study of complex food samples by using advanced chromatograph processes. In particular, he has performed a great deal of research work in the field of classical multidimensional gas chromatography, multidimensional liquid–gas chromatography, and comprehensive 2D-gas chromatography (GC×GC).

**Ivan Aloisi** received his master’s degree in pharmaceutical chemistry and technology in 2017 (University of Messina), discussing a thesis entitled “Qualitative analysis of the unsaponifiable fraction of vegetable oils using comprehensive two-dimensional gas chromatography coupled with high resolution mass spectrometry.” He is now a Ph.D. student in chemical sciences at the University of Messina.

**Luigi Mondello** is Full Professor of Analytical Chemistry at the University of Messina. His research is focused on the development of multidimensional chromatographic instrumentation and software, coupled to state-of-the-art MS, for the study of complex matrices constituents and contaminants. He is the author of around 500 scientific papers and 1000 conference presentations, with an H-index of 64 (Google Scholar) and a total impact factor of more than 1000. He has been awarded with the HTC Award, COLACRO Medal, Silver Jubilee Medal, Liberti Medal, TASIAS, IFEAT Medal, GC×GC Lifetime Achievement, Golay Award, Robert Kellner Lecture, and the Herbert J. Dutton Award.
Recovering from a COVID-19 Shutdown: Tips and Tricks for Starting Up, Part 2

Dwight R. Stoll¹ and Tony Taylor², ¹LC Troubleshooting Editor, ²Chief Science Officer of Arch Sciences Group and Technical Director of CHROMacademy

COVID-19-related laboratory shutdowns are sure to cause a myriad of problems with liquid chromatography (LC) instrumentation across the globe. Taking a systematic approach to restarting these systems will save money and time in the long run by preventing problems that may otherwise appear in days or weeks following startup.

In March of this year, many organizations took unprecedented steps to halt the spread of COVID-19, including severely restricting work in laboratories, or even shutting down entire laboratories, buildings, and worksites for weeks at a time. While some of these shutdowns were planned days in advance and executed well, I have heard many stories from scientists indicating that the shutdowns were sudden, and did not allow time to properly prepare their analytical instrumentation to be idle for weeks or months at a time. Unfortunately, this means that these scientists are going to encounter many challenges when they return to the laboratory that will necessarily include a lot of troubleshooting to figure out why their systems are not working properly before they can return to their normal experience of producing high quality data. For the August/September 2020 instalment of “LC Troubleshooting”, I asked Tony Taylor to join me in pulling together advice for starting up liquid chromatography (LC) systems after they have been idled for weeks or months at a time (1). In that instalment, we mainly addressed challenges with microbial growth in solvent bottles and different parts of the LC system itself, and obstruction of the LC flow path that can occur as a result of precipitation of buffer salts or other debris. For this month’s instalment, I’ve asked Tony to join me again, this time sharing advice related to the health of columns, qualifying system performance, and a little about restarting work with LC-mass spectrometry (MS) systems in particular. I hope that these suggestions are helpful as you return to the laboratory, but I am sure many strange things will be observed after so many LC users have been away from the laboratory for prolonged periods. If you’ve encountered a problem and gained some troubleshooting experience that you think others might be able to learn from, please don’t hesitate to send your story my way.

The Column: Is It Still Okay?

Last month, we noted that the column is the heart of any chromatography system, and as such we need to ensure that our columns are in a healthy condition prior to performing analyses. It is possible that the shutdown period may lead to mechanical or chemical problems, and the solutions to these problems will be different. Until you know that the column has been properly flushed, the column outlet should not be connected to the rest of the system. Disconnecting the column outlet from the flow path will avoid any unwanted compounds or debris that may come out of the column from causing problems with any other components in the system. You can either attach a waste line to the column outlet to collect the effluent, or simply let it drip into a beaker or similar container.

Mechanical Problems

If your column was left on the system when the laboratory was shut down, there is a possibility that
it will now be full of air, because the mobile phase solvent may have evaporated over time. Similarly, if the column was removed from the system, but not plugged at both ends, it will again be full of air, which can lead to problems for many types of columns. We need to carefully guard against applying high pressures to columns that are dry, as this may cause mechanical reorganization of the packed bed of particles, leading to unwanted voids, channelling, and significantly reduced column performance. In the following discussion, please bear in mind that the pressure applied to the column inlet should be increased gradually when first turning the flow back on (in steps of <10 bar where possible); this can be achieved by starting the pump at a very low flow rate (for example, 10 µL/min. for 2.1 to 4.6 mm i.d. columns), and increasing the flow rate in steps of 10 µL/min (larger steps can be used provided the corresponding pressure increase is not much more than 10 bar per step). Some newer systems also provide the option to specify a flow rate ramp rate that is used when the pump is turned on.

It may be possible that, upon starting the column flushing procedure, a high back pressure is encountered due to blockages in either the column inlet frit (pressure increase will be immediately noticeable) or the outlet frit (pressure will build more gradually over time). In the case of the former, it may be possible to reverse the direction of the column prior to turning on the flow, in order to back-flush the debris from the frit. Overall, this may have the longer-term effect of slightly reducing the efficiency of the packed bed, but the column should be usable for your application, unless it was heavily voided prior to the instrument shutdown.

Chemical Problems

In most cases, the following column flushing routine can be applied to reversed-phase, silica-based stationary phases as a kind of generic column cleanup step. Approximate column volumes for several different dimensions of columns in common use are given in Table 1.
• Set the column thermostat compartment to 60 °C.
• Flush with the following solvents in the order shown. For 2.1 mm i.d. columns use a flow rate of about 0.1 mL/min; for 4.6 mm i.d. columns use a flow rate of about 0.5 mL/min.
• 10:90 methanol:water for 20 column volumes (to remove any precipitated buffers)
• Increase organic composition to 100% methanol, and flush for 20 column volumes.
• Flush with 20 column volumes of 75:25 acetonitrile:isopropyl alcohol (IPA). Flush with 20 column volumes of the starting mobile phase of your method.

The rationale behind this series of flushing solvents is to cover a wide range of solvent polarities that give the best chance of dissolving and eluting anything that has adsorbed to the stationary phase. Only after these flushing steps should the column be reconnected to the detector. If the column flow direction has been reversed as discussed above, switch back to the original orientation prior to reconnecting the column to the system.

In this brief discussion, we have focused on suggestions for restarting work with reversed-phase columns, and in a very generic way. Readers interested in a more detailed discussion of cleaning procedures for reversed-phase columns are referred to the excellent “Column Watch” article on this topic by Ron Majors (2).

Is It Safe to Collect Data Again?
At a minimum, before collecting important data again, one should run the system suitability test that is appropriate to the analysis at hand. The topics of system suitability and operational qualification have been discussed in prior instalments of “LC Troubleshooting” by John Dolan, and readers interested in more detail are referred to these articles (3,4).

In general terms, system suitability tests are designed to provide data that indicate an LC system is functioning in a way that it can be expected to produce reliable data for a particular analysis. In other words, it is application- or method-specific. In many situations, these data are sufficient to give the user confidence that the system is “ready to go”. However, in other situations, under certain regulatory frameworks, it may be necessary to carry out a more extensive operational qualification (OQ) of the instrument, which will verify instrument performance regardless of the methods being run. While doing OQ tests will undoubtedly take more time when restarting an LC system, we feel strongly that this time spent at startup can save a lot of time in the long term by catching problems early and addressing them before data acquisition resumes. In other words, a little extra effort now will decrease the likelihood that we encounter unwelcome surprises down the line!

A typical OQ verification routine will include some or all of the following tests, which we have annotated briefly to give an explanation of what the test does, and the information it provides toward an assessment of operational performance. Wherever a “reference compound” is referred to in these descriptions, caffeine is very commonly used, particularly for systems with ultraviolet (UV) detectors.

**Pump Flow Rate Accuracy and Precision**
Typically, a number of flow rate measurements are made at different flow rates using a digital flow meter. Flow rate accuracy is important for transfer of methods between systems and retention time agreement with standard methods of test. Flow rate precision has a direct impact on the repeatability of peak height and area.

<table>
<thead>
<tr>
<th>Column Dimensions (mm × mm i.d.)</th>
<th>Column (Void) Volume (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>250 × 4.6</td>
<td>2.5</td>
</tr>
<tr>
<td>150 × 4.6</td>
<td>1.5</td>
</tr>
<tr>
<td>150 × 3.0</td>
<td>0.64</td>
</tr>
<tr>
<td>150 × 2.1</td>
<td>0.28</td>
</tr>
<tr>
<td>50 × 4.6</td>
<td>0.50</td>
</tr>
<tr>
<td>30 × 4.6</td>
<td>0.30</td>
</tr>
<tr>
<td>15 × 4.6</td>
<td>0.15</td>
</tr>
</tbody>
</table>
Column Temperature
Accuracy and Stability
Typically, a temperature sensor is used to measure either the column compartment temperature, or the temperature of the column effluent. Temperature is measured over time at two different setpoints, and the absolute difference between them, as well as the variability, are compared to manufacturers or regulatory criteria. Temperature accuracy can be critical for chromatographic selectivity, especially when separating ionizable analytes (5), and temperature stability has a strong influence on the repeatability of retention times.

UV Wavelength Accuracy
Typically, a caffeine or holmium oxide standard is used under conditions of no mobile phase flow, and the absorbance is recorded at several wavelengths across the range of the detector. The measured positions for the peaks (maxima) and troughs (minima) of the standard are compared to the known (expected) values for the standard. The difference between the measured and expected wavelengths of maximum and minimum absorption is compared to manufacturers specifications. Wavelength accuracy is vital for both qualitative and quantitative work, and transferability of methods between systems.

Detector Noise and Drift
Short- and long-term variation in the detector signal is determined with a fixed eluent composition (typically water). The detector signal is measured over a certain period of time and at a particular frequency to determine the short-term variation in the signal, referred to as the noise. The slope of the detector signal over a longer time period (tens of minutes) is also measured, and this is referred to as the detector drift. The magnitudes of the noise and drift directly influence the ability to differentiate between real peaks for low concentration analytes and random variation in the detector signal.

Signal-to-Noise Ratio (S/N)
The detector sensitivity is also evaluated using a reference compound under specific conditions and compared to a target specification. There are many different manufacturer and regulatory recommendations on the topic of signal-to-noise ratio (S/N), and you should follow the guidance most appropriate for your situation.

Injection Precision
Using a reference standard, the peak height and area are measured for several replicate injections, sometimes for both small and large injection volumes. The absolute values for peak height and area, as well as the relative standard deviation of each value, are compared to manufacturers specifications. Injection precision is particularly important for quantitative analysis; the better the injection precision is (that is, smaller standard deviation), the better is the ability of the method to differentiate between samples having similar analyte concentrations.

Detector Response Linearity
Typically a reference standard is injected multiple times in a range of concentrations that cover the normal operating range of the detector (for example, up to 1.5 AU for a UV detector). Statistical assessment of linearity is performed using a combination of linear regression, residuals analysis, F-tests, and relative standard deviation of detector response for each analyte concentration, as well as ratios of signals. Linearity of detector response is critical for quantitative analysis, and has a direct impact on the accuracy of analyte concentrations reported based on use of calibration curves.

Solvent Gradient Composition
Typically, a tracer compound such as acetone is added to one of the mobile phase solvents (usually the “B solvent”), and a method is used that steps through different mixtures of two solvents, one of which contains the tracer compound that can be observed by the detector (for example, acetone absorbs well at 265 nm). For example, a method may start at 0% B, and increase in steps of 5% B until 100% B is reached. The signal due to the presence of the tracer compound is used as an indirect measure of the ratio of the volumes of the A and B solvent that are combined by the pump to make the mobile phase mixture of A and B. The average detector signal at each % B level is compared to the expected value, and the short-term variation at each step may also be evaluated. Finally, some OQ routines call for the analysis of a linear gradient profile using the same tracer compound. The accuracy and repeatability of the gradient profile are critical for both qualitative and quantitative analysis, transfer of methods between instruments, and repeatability of retention times.
Suggestions for LC Systems with Mass Spectrometric (MS) Detectors

MS detectors can be particularly susceptible to problems on startup following extended periods in standby mode or shutdown, and one needs to pay particular attention to these detectors prior to restarting work with them. As there is a lot of variation in maintenance protocols for different MS manufacturers and instrument types, it is essential that you carefully follow the manufacturer’s guidance when considering the following steps.

First, thoroughly clean the ionization source, preferably when the instrument is not under vacuum. However, it is not necessary to vent the instrument if it is under vacuum at the time of cleaning. In any case, follow the manufacturer’s recommended procedure for cleaning the source. The emitter (that is, sprayer or nebulizer) should also be checked carefully prior to re-establishing flow from the LC system, as residual eluent solvents evaporate during extended storage periods, often leaving residues or even blocking the flow path entirely, and can be difficult to remove. If significant residue or an obstruction is observed, remove the nebulizer and sonicate for 10 min (first in water, and then in IPA). Be careful to suspend the nebulizer tip in the cleaning solvent in such a way that it does not contact the bottom or walls of the container, as this could damage the tip itself, and affect spray performance.

If the instrument has been vented, carefully monitor the vacuum levels in the instrument when pumping it back down, and check the vacuum levels against the manufacturer’s specifications.

Before using the MS for data acquisition, a verification of its performance will be required. At a minimum this will include a full tune (autotune), and this is typically achieved using the manufacturer’s recommended tuning compound or solution and performance criteria. If your instrument includes an on-board tuning solution that can be activated using the control software, make sure there is enough tuning solution in the reservoir prior to starting the autotune routine. The autotune routine will tune the electrostatic lenses within the instrument as well as the voltages applied to the mass filtering device in order to optimize and verify, amongst other things, mass accuracy across a wide range of values, instrument sensitivity, and response profile.

While the on-board autotune is very useful to set the detector parameters and check them against the manufacturer’s performance requirements, one may also need to carry out further “whole-system” performance checks with a typical set of performance criteria involving: 1) response linearity (or response profile if it is expected to be non-linear based on previous experience); 2) injection precision; 3) carryover; 4) signal-to-noise ratio; and 5) minimum detection limits.

Summary

Given the variety of ways different laboratories were shutdown early on in the global COVID-19 outbreak, it is likely that LC users will encounter a wide variety of problems with their instruments when they return to their laboratories and resume work with their instruments. In this instalment of “LC Troubleshooting”, we have provided suggestions specific to handling LC columns and LC systems with MS detectors when restarting work after a long time away. We have also discussed the value of running system suitability and operational qualification tests before starting to collect important data again. These tests will be helpful for identifying problems that may have been caused by the shutdown, so that they can be resolved before causing trouble later on.

References
1) D.R. Stoll and T. Taylor, LCGC Asia Pacific 23(3), 13–16 (2020).

Dwight R. Stoll is the editor of “LC Troubleshooting.” Stoll is a professor and the co-chair of chemistry at Gustavus Adolphus College in St. Peter, Minnesota, USA. His primary research focus is on the development of 2D-LC for both targeted and untargeted analyses. He has authored or coauthored more than 60 peer-reviewed publications, four book chapters in separation science, and more than 100 conference presentations. He is also a member of LCGC’s editorial advisory board. Tony Taylor is the Chief Science Officer of Arch Sciences Group and the Technical Director of CHROMacademy. Direct correspondence to: amatheson@mjlifesciences.com.
From Detector to Decision: How Does the GC Generate Your Data?

Nicholas H. Snow, Department of Chemistry and Biochemistry, Seton Hall University, New Jersey, USA

Gas chromatographs today are easy to use. With modern web-based controls and data analysis, you don’t even have to be in the laboratory to run the instrument and collect the data. In this first instalment on how this magic happens, we discuss signal generation and processing from a classical flame ionization detector (FID), so that you can use the data to make decisions. The fundamental operation and chemistry of signal generation in an FID is unchanged since the 1960s, yet the data are accessed, processed, and stored much more easily today. We will discuss analogue signal generation in the FID using historical references, analogue-to-digital conversion, and the storage and processing of digital data that happens with today’s instruments. In future instalments, we discuss how the magic of controlling today’s “smart” and remote controlled GC’s works, more detail on how the analogue signal is converted to digital data for the computer, and more on best practices and tools that our data systems can do with chromatographic data.

I write this column in my socially-distanced home office on a laptop computer, wirelessly connected to the internet, along with my cell phone streaming music in the background and playing it on a Bluetooth-connected wireless speaker. I cannot help but marvel at how easy these tasks have become since my first desktop computer in 1983, a TRS-80 Model III from now-defunct Radio Shack. I also cannot help but think about how far gas chromatographs and their data and control systems have come since I performed my first manual injection in 1985 with the chromatogram recorded on a strip-chart recorder. Some knowledge of electronics and circuits was necessary just to assemble and operate most instruments. In a recent blog post, Jim Grinias discusses the “lost art of electronics” in chromatography and analytical chemistry (1). He is correct in that today’s “plug and play” systems have moved much of this into the background. However, the same electronic principles, and sometimes the same electronics as in the distant past, still form the heart of modern instrument control and data analysis systems.

Inside a gas chromatograph (GC), however, the chemistry and the fundamental electronics needed to produce an electrical signal at a detector when an analyte passes through it are not much different today than when most of our detectors were invented in the 1950s and 1960s. GC is unique among instrumental methods in that most of the classical detectors were invented or adapted to the specific needs of detection in GC, which, in this case, is high sensitivity and selectivity in a rapidly moving, vapour phase eluent stream. Using the flame ionization detector (FID) as an example, we will explore how the detector generates a signal, what that signal is, and how it is processed into the chromatograms and other information that is stored and provided by a modern data system. We will do this by walking through the evolution of data processing in GC from the early days to today, examining how the various components work and how they were ultimately integrated into the instrument.

A classical schematic of a flame ionization detector is shown in Figure 1, adapted from early works (2–4). There were several early designs, including single and multi-jet. Today’s FIDs use a single jet design, as seen in Figure 1. In short, the column effluent is mixed with hydrogen and air (or, in the case of some of the early work, hydrogen and nitrogen, the most common carrier gas back then), and ignited, generating a flame between two electrodes. The flame temperature of about 2000 °C is not sufficient to ionize water vapour, the product of hydrogen combustion, but is sufficient to ionize a small portion of the carbon dioxide produced by the combustion of organic compounds.
The ionized \( \text{CO}_2 \) present in the flame then allows the circuit to be completed and electrical current to flow. The amount of current (amperes) is proportional to the mass of \( \text{CO}_2 \) generated in the flame. Variations in the chemistry of combustion reactions in the flame lead to the need to determine response factors, and provides the selectivity of the FID for organic (carbon-containing) analytes (5). A complete description of how to operate an FID can be found on LCGC’s learning platform, CHROMacademy (6).

While the techniques and electronics we use to measure and analyze the signal have changed over the decades, the fundamental combustion chemistry that generates it has not changed.

The electrical current produced by the FID is usually measured in picoamperes (pA) by an electrometer that may also convert the current into a voltage for output to a data system. The output of an FID is an analogue signal, in which the output, an electrical current, varies continuously with the input, the mass of carbon entering the detector. This signal must be further processed in order to produce a chromatogram, perform calculations and store the data. Figure 2 shows a simplified block diagram of the data processing steps in GC over the years. First, the current is amplified (think about an old stereo with an amplifier) and then may be converted to a voltage. The electrometer output (volts or amperes) is represented by the third block in the middle of Figure 2. In predigital-age GC, shown by the green box in Figure 2, the voltage was plotted against time on a continuous roll of paper using a strip-chart recorder that was connected to the electrometer by a cable. The voltage signal (y-axis) and time (x-axis) scales could be adjusted to obtain a proper appearance for the chromatogram, but there was no data storage capability. If you wanted to make the peaks appear larger or smaller to fit on scale, you usually had to rerun the sample. Quantitation was most often done using peak height (again, the scale was limited to what would fit on the paper), which was much simpler than peak area. To measure the peak area, one could count the little square blocks on the paper under the peak, carefully cut the peak out with scissors and weigh it, or use a challenging device called a planimeter (7).

The analogue output and strip chart recorder combination was the most common means of data collection in GC until the 1980s, when microprocessors became available in desktop or bench top computers. In the 1980s and 1990s, digital electronic integrators, specialized small computers, were commonly used for data collection and analysis. Like a strip chart recorder, these devices printed chromatograms on rolls or sheets of paper. In addition to printing out the chromatogram, the raw data could be digitized and stored for later processing or analysis in computer memory within the integrator.

To understand how analogue signals are transferred to a digital electronic computer or integrator, we need some definitions of the language and standards for digital data storage and transmission. Digital signals use binary (or base-2) numbers and logic. A binary number is code for a simple switch that may be either ON (1 or one) or OFF (0 or zero). A single binary data point is called a bit. For example, when we discuss internet service provider speeds at 100 Mbps, they mean that you can transmit up to 100 megabits (100 million bits) per second. A string of eight bits is termed a byte. A byte of data can be thought of as the equivalent of a single alphanumeric character (a letter or a number). A memory card with one GB of storage space can hold one gigabyte or approximately one billion characters of information.

To standardize the use of alphanumeric characters in digital storage, nearly all computers use ASCII or the American Standard Code for Information Interchange, which provides a seven-bit representation...
of all the letters, numbers, and characters on a standard United States keyboard plus representations for various control functions such as carriage returns and line feeds. When eight-bit microprocessors, such as the Z80 and 8088, the precursors to the microprocessors in today’s personal computers, were developed in the 1980s, ASCII was extended to eight bits, allowing for additional special characters. ASCII is still in use today, included as the first 128 characters in the Unicode standard that now includes over 140 000 different characters and symbols (8). The Unicode standard is how your cell phone knows which emoji is which, so your smiley face emoji does not turn into a frown (or worse) on someone else’s phone.

The second aspect of communicating between a data system and an instrument, besides having a standard code or rubric for converting letters, numbers, and symbols into bits and bytes, is a common standard for transferring the actual electronic signals. This is usually accomplished using a serial port on the computer. There have been many standards used over the decades, but the most common for GCs are RS-232, GPIB, USB, and Ethernet. RS-232 uses a 9- or 32-wire connector, and a ribbon cable to transfer...
the signals with one of the wires actually carrying the signal and the others related to a “handshake” between the devices in that both would have to trade the correct separate signals to demonstrate that they were ready to send or receive data. RS-232 is the classical serial port used in personal computers, but that port is slow by today’s standards as the single signal wire means that one bit is transferred at a time, hence the term “serial”. With the other control lines available, instrument manufacturers often modified the standard RS-232 connections to make their instrument and data system connections proprietary. GPIB, or General Purpose Interface Bus (also called HPIB, Hewlett Packard Interface Bus and IEEE-488) took serial communications one step further, with eight signal lines, allowing the transfer of a byte of data instead of a bit at one time. In the 1980s and 1990s, GCs with less requirements for fast data transfer often used RS-232. Meanwhile, many GC–mass spectrometry (MS) systems with need for higher throughout used GPIB. Today, most instrument communications are based on USB and Ethernet, which provide much greater speed and more strict standardization, so there is much better connectivity of instruments and data systems between vendors. While Ethernet and USB are much faster RS-232 and GPIB, the same basic principles apply. Both instrument and data system must be ready to send and receive data, the connection must be working, and the data must be transferred and stored according to industry standards.

The third necessary component is an analogue to digital converter that converts the analogue signal into the binary digital numbers for the computer to store and process. An analogue to digital converter may be included in the GC itself, or it may be added as a separate converter box. External converters were common in the 1990s and 2000s, with an example shown in Figure 3, showing a common data system interface box of 1990s vintage. The right-side image is the rear of the box where all the connections are shown. The left-side image shows a side view of the box with the cover removed to show the electronic circuitry. The back shows several types of connectors that made this interface mostly universal in that it worked with almost any GC on the market. The inputs on the top left are analogue detector inputs. These could be connected directly to the analogue detector outputs on the GC. Below these are connectors for remote starting and stopping the instrument, controlling an autosampler, and several connectors for activating valves or switches on the GC. To the right are both types of serial connectors, RS-232 and GPIB, that connect to a personal computer data system.

Looking at the left side of Figure 3, we see an electronic circuit board showing the various components allowing this box to function. Some key components include the analogue-to-digital converter circuitry in the top right, within the silver rectangle. The large square microprocessor in the bottom middle

---

**FIGURE 4:** Modern USB and ethernet connectors. (Source: Photo image courtesy of the author)

**FIGURE 5:** Photograph of the remote control port on a 1990s-era GC showing connections for several basic control functions. (Source: Photo image courtesy of the author)
is a Zilog Z80 microprocessor, already mentioned in this column. This was the microprocessor used in my first computer, revealed at the beginning of the article. There were no graphics, the display was monochrome, and a separate modem allowed me to communicate with other computers over the phone lines at a whopping 300 bits per second. In the 1990s and 2000s, while no longer used in personal computers, the Z80 was commonly used in digital electronic integrators and today it is still used in many devices in the “internet of things”, such as appliances. The white chip to the left of the Z80 contains the box manufacturer’s own firmware. The large chips between the Z80 and the cable connectors on the right provide the interface between the microprocessor and the communication cables to the PC. Finally, the rest of the chips provide memory. In short, these control boxes were computers in their own right that provided an interface between the GC and the data system.

Figure 4 shows the back panel of a new GC purchased in 2019, showing the connections and capabilities that are now inside the cabinet of a modern GC. In this case, all of the functions of the control box shown in Figure 3 are now internal to the GC. Several control ports, along with input and output ports, are shown at the top of the panel. These allow the GC to send and receive signals from other devices such as a headspace sampler. This GC has a specialized port for an auto-sampler and seen with the cable attached. This port uses Ethernet to communicate with the computer, which is also seen with the cable attached. The analogue-to-digital converter is now contained within the GC. If needed, a classical analogue output is still available. Note that these functions are all very similar to those shown on the control box in Figure 3.

Looking back at Figures 3 and 4, we saw several additional connectors for control lines used to send and receive various commands to or from the instrument. Most commonly, these are based on transistor to transistor logic (TTL) that allows each to act as a switch that is either “on” or “off” or “1” or “0”, respectively. Each of these lines represents an opportunity for the user to activate or deactivate an electronically actuated switch or valve in the instrument or to start or stop an external device. This logic is also used to send the “start” signal between the data system and the instrument to signal the start of a run or a “stop” signal in either direction to indicate the end of a run. These lines are connected to the GC through a remote control port, such as the one shown in Figure 5, from a 1990s era GC. Each pin on the control port activates or deactivates a certain function on the GC, such as “start” and “stop”. There are multiple lines for each command to allow multiple devices to communicate. This port has lines for ready; they indicate that the instrument or device is ready to run, that it can both send and receive start signals, that it can send out information on its configuration, and ground.

In this instalment, we have discussed the basics of how a GC generates signals and transfers them to a data system to generate your data. By looking “inside the box” of a data transfer device, we have seen how the analogue signal converts to a digital signal and transfers to the data system using standardized digital communications protocols. In future instalments, we will look more closely at the processes for controlling the GC from the data system or from anywhere in the world and how “smart GCs” work, the process of analogue to digital conversion and at best practices and tips for data systems and analysis in GC.

Remember that even with all the new technology in the foreground, a GC is still performing the same basic functions that it has done for decades: injection, separation, and detection. Fundamentally, an inlet, column oven (with a column in it), and detector have not changed. The basic digital and analogue electronics that provide our ability to collect data accurately, precisely and conveniently are still there under the covers and require our understanding.

References

Nicholas H. Snow is the Founding Endowed Professor in the Department of Chemistry and Biochemistry at Seton Hall University, USA, and an Adjunct Professor of Medical Science. During his 30 years as a chromatographer, he has published more than 70 refereed articles and book chapters and has given more than 200 presentations and short courses. Direct correspondence to: amatheson@mjhlifesciences.com
Looking at the Past to Understand the Future: Soxhlet Extraction

Douglas E. Raynie, Sample Preparation Perspectives Editor

Franz Ritter von Soxhlet is credited with inventing the extraction technique that bears his name in 1879. The method was a significant advance in the isolation of solutes from difficult-to-extract solid samples. For well over 100 years, Soxhlet extraction was considered the gold standard in the analytical extraction of solids. Regulatory agencies, trade organizations, and individual laboratories collected a wealth of data based on the technique. While still widely used, it is being replaced by a number of other techniques, such as ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), and pressurized solvent extraction (PSE). The application of temperature, continuous flow, and preference for flowable samples that make these techniques advantageous were taken from the lessons from Soxhlet extraction, which still has much to teach us. In this instalment, we look back at Soxhlet extraction so that analysts can better appreciate the lessons that are fundamental to today’s extractions.

A highly significant advance in extraction technology, perhaps the most significant occurrence in the field for the century that followed, occurred 140 years ago with the first report of the Soxhlet extractor, a unique continuous–discontinuous device (1). This was the same year, 1879, that a 31-year-old Franz Ritter von Soxhlet (Figure 1) became a professor of agricultural chemistry at the Technical University of Munich, Germany. Jensen’s history on the origins of the device credits a laboratory glassblower, known only as “Herr Szombathy,” as a significant contributor to this creation (2). Soxhlet is discontinuous in that it relies on a number of solvent siphon cycles. It is continuous in the sense that solvent is continuously evaporated from the solvent reservoir flask and condensed. This continuous–discontinuous process minimizes channelling of solvent through the sample, as can occur with continuous flow methods. So, Soxhlet is more correctly a batch process. The key to this solvent cycling is the constant-level siphon, based on a Pythagoras Cup.

Soxhlet’s original paper (1) concerned itself with the determination of milk fat. Since then, this extraction technique is widely used for fats and oils, as well as other mostly nonpolar solutes, from solid and semi-solid matrices. Because its efficiency of extraction is greater than that of other manual techniques, especially at the time, it gained broad acceptance. For example, one unsubstantiated story I’ve heard is about a major food company that performs 20,000 Soxhlet extractions per year across the company, as the confirmatory method after placing a modern instrumental extraction method in each of their plants. Personally, about 20 years ago, a food science colleague showed me his laboratory where about 20 Soxhlet extractors were connected to run in parallel. (Of course, he was talking with me about developing a supercritical fluid extraction method to replace his extensive use of Soxhlet.) The wide use of the Soxhlet technique for fats and oils can be gleaned from Table 1, which shows selected standard methods using this approach. Because of its wide acceptance and the considerable time before suitable alternatives emerged, Soxhlet extraction is considered the benchmark to which other techniques are compared; several organizations have volumes of historic data based on Soxhlet approaches.

Operation of Soxhlet Extraction

The Soxhlet apparatus is shown in Figure 2, and the middle third
of the device is the actual Soxhlet extractor. In practice, the solid sample is placed into a porous (typically cellulose) extraction thimble, positioned into the Soxhlet extractor, and the device is assembled. Solvent (shown in green in Figure 2) is added to the solvent reservoir flask, and mounted onto a heating mantle. As it boils, the solvent vapours rise through the solvent vapour tube sidearm. As the solvent vapours hit the condenser (cooled with running water or other circulating fluid), the now liquid solvent flows down onto the sample, where it may permeate into the sample matrix and dissolve the target analytes. When the liquid solvent level reaches the top of the siphon tube, the solvent–solute mixture (the extract), is syphoned away from the extractor, and back into the solvent flask. This is one extraction cycle. The solvent re-evaporates (boils), and the solutes concentrate as they are left behind in the solvent flask.

More specific to the performance of a chemical extraction using the Soxhlet approach, details are presented in Table 2 (with information from references 4–9). Many of these attributes can be applied to the extraction of solids by nearly any approach, as presented later.

With this understanding of how to perform Soxhlet extraction, the advantages and disadvantages of the technique become apparent. Perhaps the most important, and underappreciated, advantage, as mentioned previously, is the historical legacy. Not only are newly developed extraction methods for solid samples typically compared with Soxhlet for acceptance, but regulatory agencies, contract laboratories, and industrial laboratories potentially have over a century of data collected based on Soxhlet extraction, and decisions are made based on these data. Related, and as suggested in Table 1, several official methods issued by regulatory agencies and trade associations worldwide are based on Soxhlet extraction. Procedurally, the sample is continually exposed to fresh solvent (via the reflux) at some elevated temperature that is less than the normal boiling point. Increasing solvent temperature typically increases solute solubility, and accelerates diffusion into and out of the sample matrix. The Soxhlet apparatus is widely available from glassware and laboratory supply vendors and is inexpensive, only US$200–300. The cellulose extraction thimble acts as a filter to separate the extract from the solid residue, and, with the extractor, is available in a variety of sizes to accommodate a range of sample sizes. The technique is simple to perform; any laboratory personnel can set up the apparatus, and allow the extraction to run in an unattended manner.

Despite the plethora of advantages of Soxhlet extraction, the technique has a few drawbacks that can be limiting. A Soxhlet extraction can easily take 6–48 h, and the technique is manual, though the procedure runs
unattended. Even more important are the disadvantages associated with solvent use. Depending on sample size, hundreds of millilitres of solvent are used in a single extraction. This magnifies each of the solvent attributes, including safety (such as flammability) and environmental and human health concerns. As a result, Soxhlet extractors are often set up in a laboratory fume hood. Increased solvent use means increased solvent waste disposal. Both the solvent acquisition and disposal carry a significant cost. In fact, despite the low cost of the apparatus, the solvent-related expenses boost the cost per extraction up to 1.5–2 times that of alternative methods, including methods derived from Soxhlet extraction, as well as supercritical fluid extraction (SFE), microwave-assisted extraction (MAE), pressurized solvent extraction (PSE), and ultrasound-assisted extraction (UAE). The large amount of solvent generally requires evaporation, costing time, manpower, and energy, to obtain the desired extract concentration. Extraction selectivity (that is, the ability to extract the analyte of

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Characteristic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Particle size</td>
<td>Solid samples should be ground to a flowable powder. That is, the particle size should generally be no larger than typical coffee grounds or sand. For fine particles, about the size of flour or talcum powder, consider mixing with an inert material like sand to disperse the sample. This approach to particle size holds for any extraction method applied to solid samples.</td>
</tr>
<tr>
<td>Solvent selection</td>
<td>General solubility guidelines, including the “like dissolves like” principle, should be considered. Equally important are considerations of human health and environmental impact. Often, mixed solvents provide better results than a single solvent. While one general guideline when performing chemical extractions is to consider compatibility with the subsequent liquid chromatography (LC) mobile phase, keep in mind that, in many instances, solvent evaporation and extract clean-up are needed after Soxhlet extraction. This approach to solvent selection holds for any extraction method applied to solid samples. Water is not often used in Soxhlet extraction.</td>
</tr>
<tr>
<td>Sample moisture</td>
<td>Generally, samples should be dried to about 10% water content. For wet samples, up to about 30% moisture, the sample can be mixed with a suitable adsorbent such as silica gel, alumina, diatomaceous earth, or sodium sulfate. The extraction efficiency of visibly moist samples can be enhanced by the addition of a miscible, polar solvent to the extraction solvent. This approach to sample moisture holds for any extraction method applied to solid samples.</td>
</tr>
<tr>
<td>Extraction cycles</td>
<td>The rate of extraction cycles is predominantly governed by the rate of heating of the solvent reservoir flask. The heating mantle should be adjusted such that the Soxhlet apparatus cycles at four to six cycles per hour. A more frequent cycling rate risks channelling and inefficient extraction, so the solvent siphoning must occur as a distinct event. The solvent must be allowed the opportunity to diffuse into the sample matrix for a more complete extraction.</td>
</tr>
<tr>
<td>Extraction time</td>
<td>Method development guidelines for the amount of time appear to be lost to history. Except for easy-to-extract applications, one generally assembles the extractor at the beginning of the work day, and shuts it down before leaving for the day (an approximately six- to eight-h extraction). If that doesn't work, the extraction is allowed to continue over one night (24-h extraction) or two (48-h extraction).</td>
</tr>
</tbody>
</table>

**TABLE 2: Characteristics in the performance of a Soxhlet extraction**
interest in exclusion of anything else) with the Soxhlet approach is solely due to the solvent used. Consequently, some type of post-extraction clean-up, such as using selective adsorbents, is often required. While the elevated temperature of extraction provides some advantages, one must also be concerned with potential thermal degradation of analytes. Lastly, while Soxhlet data have driven analytical chemistry for the past 140 years, the rise of modern instrumental approaches to extraction (SFE, MAE, PSE, and UAE) has shown, at least in some cases, that the results obtained from Soxhlet extraction are sometimes incomplete quantitatively.

Derivations of Soxhlet Extraction

With the significance of the solvent and time disadvantages of Soxhlet extraction, there have been reports of addressing these concerns while maintaining the advantages (7–9). One of these is the high-pressure approach developed by Jennings, Wohleb, and Wolters (7) and used, for example, initially as a screening tool in the development of SFE. In this approach, the entire apparatus, with some modification, is mounted inside a high-pressure vessel. A volatile solvent, like carbon dioxide, is used, and the high pressure allows the liquid–vapour phase transition vital to creating the extraction cycles key to Soxhlet extraction. Ankom has commercialized a similar approach, using petroleum ether for fat determination.

Another derivation of Soxhlet extraction is based on the Randall method (7–9), and is commercialized by Buchi, C. Gerhardt, and Foss, going by the names automated Soxhlet, hot Soxhlet, Soxtherm, and Soxtec. The key feature of this approach is that, during the initial portion of the extraction, the extraction thimble containing the sample is immersed directly into the boiling extraction solvent. Then, the more conventional Soxhlet approach functions more as a rinse function. With this approach, solvent use decreases from hundreds of millilitres to about 50 mL, and the time from several hours to 30–60 min.

The final approaches take advantage of more intense energy sources, ultrasound or microwave, to enhance the extraction. These approaches have yet to more completely catch the attention of researchers for two reasons. First, especially with ultrasonic energy, is concerned with loss of energy intensity as it is transferred to the sample. Second is the advent of the other modern instrumental approaches (SFE, MAE, PSE, and UAE) previously discussed. However, as the technical concerns are alleviated, focused microwave and ultrasound approaches to Soxhlet extraction should become increasingly viable and competitive.

Conclusions

Soxhlet extraction is approaching a century and a half of use. During this time, it has firmly established itself as the comparison benchmark as new extraction methodologies are developed. While serious concerns exist associated with the large volumes (hundreds of millilitres) of organic solvent used and the extraction time (several hours), lessons learned from the development of Soxhlet in terms of treating samples through grinding and dispersing with adsorbents, as well as solvent selection, have guided the modern approaches to analytical extraction.

References


Douglas E. Raynie “Sample Prep Perspectives” editor Douglas E. Raynie is a Department Head and Associate Professor at South Dakota State University, USA. His research interests include green chemistry, alternative solvents, sample preparation, high-resolution chromatography, and bioprocessing in supercritical fluids. He earned his PhD in 1990 at Brigham Young University under the direction of Milton L. Lee. Raynie is a member of LCGC’s editorial advisory board. Direct correspondence to: amatheson@mjhlifesciences.com
**Ion Chromatography System**
Shimadzu’s IC system with anion suppressor reduces band spreading and achieves high sensitivity and reliable performance for the quantitative determination of anions. According to the company, the suppressor provides stable functionality over long periods of operation. The system features a compact design and integrates with Shimadzu’s LabSolutions.

www.shimadzu.eu
Shimadzu Europa GmbH, Duisburg, Germany.

**Crimp Closure**
Extremely tight sealing and excellent analytical purity are of great importance, especially in the headspace area. A new 20-mm crimp closure with Silicone/PTFE liner (Pharma-Fix) from Macherey-Nagel meets these demands. This closure is characterized by high analytical purity and high temperature resistance, good penetration properties and excellent sealing.

www.mn-net.com
Macherey-Nagel GmbH & Co. KG, Düren, Germany.

**EAF4 System**
Postnova’s simultaneous electrical and asymmetrical flow field-flow fractionation (EAF4) system is designed to enhance the separation and characterization of biopharmaceutical, environmental, and nanomaterials. In an EAF2000 system electrical and cross flow fields are applied simultaneously to enable separations by particle size and particle charge based on electrophoretic mobility to characterize complex proteins, antibodies, and viruses as well as environmental and charged nanoparticles or polymers.

www.postnova.com
Postnova Analytics GmbH, Landberg, Germany.

**Column Selection App**
Columns are the heart of any GPC/SEC system. However, it can be a challenge to find the best matching column from the wide range of options. PSS has therefore developed the Column Selection App to support users. The app offers the following choices: How to Replace Existing Columns, Column Recommendations, USP methods/EP methods, and Application Searches.

www.psscolumnselector.com
PSS GmbH, Mainz, Germany.

**Degasser**
The novel Flat Film Degasser reportedly offers a wide flow range and chemical compatibility, while reducing flow resistance. A variable vacuum control enables the degassing process to be fully optimized for users’ needs. The Flat Film Degasser will soon be available as an OEM solution or as a stand-alone version in the DEGASi series.

www.biotechfluidics.com
Biotech AB, Onsala, Sweden.

**Multi-Angle Static Light Scattering**
The DAWN is an advanced multi-angle static light scattering (MALS) detector for absolute characterization of the molar mass and size of macromolecules and nanoparticles in solution. DAWN offers high sensitivity, a wide range of molecular weight, size, and concentration, and a large selection of configurations and optional modules for enhanced capabilities.

www.wyatt.com/dawn
Wyatt Technology, Santa Barbara, California, USA.
Adeno-associated viruses (AAVs) are increasingly used for gene therapy due to their versatility and safety. One of the biggest concerns for manufacturing a uniform AAV suspension is the presence of viral aggregates, which can create problems with transduction efficiency, biodistribution, and immunogenicity. These large AAV aggregates are challenging to separate and characterize by traditional column-based chromatography techniques such as size exclusion chromatography (SEC).

Asymmetrical Flow Field-Flow Fractionation with Multi Angle Light Scattering (AF4-MALS) can separate and size large AAV aggregates, and discern a difference in aggregate concentration due to the stressing protocol. Some or all of the large aggregates would be filtered out by SEC, resulting in incorrect determination of the aggregate content or the false conclusion that no aggregates are present.

For more details visit www.postnova.com and search for ‘virus’.
A breakthrough in sample automation and concentration for GC–MS

**Centri®** – Multi-technique sample extraction and enrichment platform, delivering enhanced analytical sensitivity and flexibility.

Discover more – Deliver more

For more information visit [chem.markes.com/LCGC/Centi](http://chem.markes.com/LCGC/Centi)