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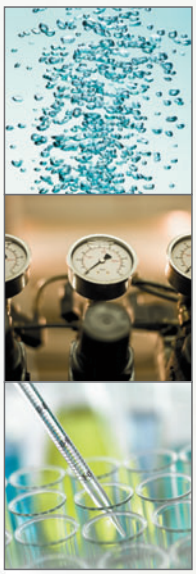
Recent Developments in Sample Preparation



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Solvent extraction is a sample preparation technique used to remove analytes from solid samples such as soil and tissue. This technique uses elevated temperature to accelerate the partitioning of analytes from the matrix and collects solvent extracts that can be analyzed by chromatographic techniques. Analytical laboratories often use solvent extraction prior to analysis by GC/GC-MS or LC/LC-MS. This technique has become an integral part of the complete laboratory workflow solution.

Solvent extraction is often used in the following industries:

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- Food and Beverage (Lipid Content, Oil Content, Vitamins, Pesticides)
- Chemical & Petrochemical (Consumer Products, Plastics & Electronics, Biofuels)
- Life Science (Herbal Supplements, Natural Products, Pharmaceuticals)

Solvent extraction can be automated to improve laboratory productivity. Automated extraction techniques such as Accelerated Solvent Extraction reduce the amount of solvent and time required to remove analytes from the matrix. Accelerated Solvent Extraction can reduce the extraction time to minutes per sample and uses a fraction of the solvent required for traditional techniques, such as Soxhlet. Traditional solvent extraction techniques may require hours to extract the analytes and use hundreds of milliliters of solvent. Table 1 shows a comparison of solvent use and extraction time per sample using the Thermo Scientific™ Dionex™ ASE™ 350 Accelerated Solvent Extractor system and several traditional solvent extraction techniques. It is clear that accelerated solvent extraction requires much less time solvent and time thereby increasing the productivity of the laboratory.

Table 1: Comparison of solvent extraction techniques (*per sample basis)

Technique	Solvent Usage*	Extraction Time*
Soxhlet	200 - 500 mL	4 - 48 hours
Automated Soxhlet	50 - 100 mL	1 - 4 hours
Sonication	150 - 200 mL	0.5 - 1 hour
Supercritical Fluid Extraction	5 - 50 mL	0.5 - 2 hours
Microwave	25 - 50 mL	0.5 - 1 hour
Accelerated Solvent Extraction	15 - 50 mL	0.2 - 0.3 hour

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Recent Developments in Sample Preparation

Ronald E. Majors, *LCGC Sample Preparation Perspectives Column Editor*

To keep readers informed of the latest developments and trends in sample preparation, *LCGC Europe* occasionally runs reader- and expert-surveys that provide information on overall technology and usage patterns. In addition, special issues on popular topics of the time bring together a host of experts who cover their specific areas of expertise in more depth than might be warranted in a monthly guest author column. In the past, we have assembled special issues on *Current*

Ronald E. Majors

Trends and Developments in Sample Preparation (1), *Sample Preparation of Solids* (2), and *Sample Preparation of Volatiles* (3). Since the last special issue, some of the sample preparation technologies that were new then have advanced further so it is time to re-visit those technologies to see what improvements have been made. In addition, we will look at some newer trends, particularly in the area of green sample preparation and automation.

Technologies for the extraction of solid materials have come a long way since the advent of the Soxhlet extractor, particularly in the automation of solid sample preparation techniques. In comparing this year's reader survey on sample preparation trends (4) to the last reader survey (5), the techniques of supercritical fluid extraction (SFE), pressurized-fluid extraction (PFE), and microwave-accelerated (or assisted) extraction (MAE) have all shown substantial growth. Of the readers surveyed, SFE usage went from 1.9% to 3.7%, PFE from 5.8% to 8.0%, and MAE from 2.6% to 8.0%. All three techniques haven't surpassed more popular techniques such as solid-phase extraction (SPE) and solid-phase microextraction (SPME) but have established themselves in the environmental, food safety, and natural products markets in particular. To bring us up-to-date in SFE, Professor Larry Taylor of the Department of Chemistry, Virginia Tech, Virginia, USA, provides a good perspective on why this extraction technique, so popular in the 1990s, is coming "back to the future" by providing a safer, greener, and rapid method for not only non-polar compounds but also for polar compounds. Part of the answer lies with the introduction of the latest generation of supercritical fluid chromatography (SFC) instrumentation. On the PFE front (also referred to as accelerated solvent extraction [ASE]), Aaron Kettle from Thermo Fisher Scientific reviews the latest instrumentation developments as well as selected applications of this rapid extraction technique. For MAE, Greg LeBlanc and coworkers from the CEM Corporation provide an in-depth update on greatly improved instrumentation in the field as well as some novel applications.

Since the last special issue, the movement towards green chemistry has been a driving force in chemistry in general and analytical chemistry and sample preparation in particular. Professor Douglas Raynie and his graduate student Victor Essel from the Department of Chemistry and Biochemistry, South Dakota State University, South Dakota, USA, discuss green chemistry perspectives on analytical extractions. Reduced solvent consumption, safer solvent alternatives, and reasonable energy demands all contribute to a greener environment. In that respect, Professor Roger Smith of the Chemistry Department of Loughborough University in the United Kingdom has a lot of experience in the use of superheated water as an extraction solvent and it is amazing the dissolution power that this entirely safe solvent can provide for organic compounds when heated well above its boiling point. He will provide a nice overview of the role of this novel extraction solvent in sample preparation.

Finally, if sample loads eventually increase enough, safety concerns arise, or labour costs become an issue, automation becomes a necessary part of the sample preparation portion of the analytical cycle. Most chromatography laboratories use autosamplers as the standard form of sample injection but modern instrumentation permits automation beyond injection. Edward Pfannkoch from Gerstel USA has a good handle on the role of sample preparation beyond the autosampler and will discuss the various steps prior to injection that are now automatable, freeing up the user to perform less routine tasks.

If you are faced with sample preparation challenges in your own laboratory, I hope you find something of interest in this special issue of *LCGC Europe*.

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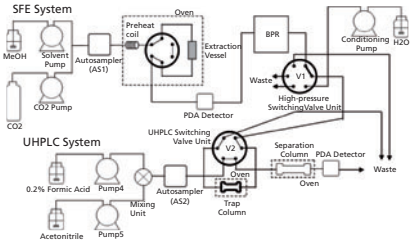


Analytical Supercritical Fluid Extraction Goes **Back to the Future**

Larry T. Taylor, Department of Chemistry, Virginia Tech, Blacksburg, Virginia, USA.

The past and future development of analytical supercritical fluid extraction (SFE) is traced in terms of experimental strategies, applications, vendor support, and timely acceptance of the existing technology. The current state of the art is compared with research activity in the 1980s. New vendor activity, in terms of automation and hyphenation with chromatographic separations, is discussed.

For someone who has between 1980 and 2000 (i) published hundreds of peer-reviewed manuscripts on analytical supercritical fluid extraction (SFE) for chromatographic analysis and isolation of additives, neutraceuticals, pollutants, and so on; (ii) co-taught short courses on SFE in Europe, South America, North America, and Asia; and (iii) guest lectured numerous times on an ACS short course entitled "Sample Preparation for Chromatography" organized by Harold McNair, the current state of SFE does sometimes sound as if we are going back to the future.

A sample preparation survey in 2002 suggested that SFE was being used by less than 2% of respondents. A similar survey repeated in 2012 showed that the use of SFE had only doubled, despite the obvious overall advantages of supercritical carbon dioxide-based fluids (1).

The situation now is different from the 1980s because instrumentation is more robust and engineering applications in food, pharmaceuticals, bioanalytical, and materials are more plentiful. On the other hand, analytical SFE has not changed very much because there is a lack of support from major vendors and newcomers to the technology are forced to re-learn SFE history and protocols that have been around for years.

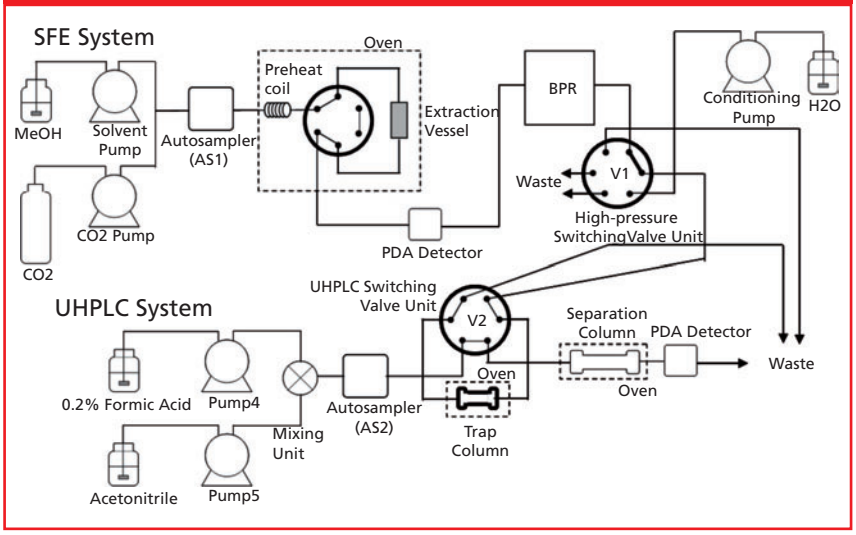
To put it in perspective, consider a report of the 9th Annual Waste Testing and Quality Assurance Symposium held in Crystal City, Virginia (USA) dated 23 July 1993 by Robert Stevenson, entitled "SFE in Purgatory". He writes: "SFE generates high interest in surveys, but low sales. All the necessary groundwork seems to be there, but where are the orders? Where are the users?"

Parenthetically, at about this same time, it was reported by experts in the field that "SFE is over the hump and that it is rapidly developing into the extraction method of choice for the 21st century with more and more laboratories around the U.S. and the world embracing it for sample cleanup and sample preparation" (2). No doubt this assessment was partly based upon the fact that between 1987 and 1989 more than 100 papers were published concerning the use of supercritical fluids for extraction (3).

Possible explanations for the perceived current lack of enthusiastic growth among analytical SFE practitioners are the limited number of suppliers that market the technology today and the exodus of vital vendors such as Hewlett Packard, Suprex, Dionex, ISCO, and Lee Scientific from the field around the turn of the century. A somewhat similar vendor exodus occurred slightly earlier in time in the field of SFC but, thanks to the efforts of Terry Berger and associates, the drought was not as long-lived, and SFC has survived to "live" again. Hopefully many of the developments that benefited the SFC community will now foster further development of analytical SFE. Currently, there appears to be a small core of established vendors, as well as relatively new vendors, that are committed to making analytical SFE a viable method for sample preparation prior to chromatographic analysis. Today these vendors are Jasco, Inc., Supercritical Fluid Technologies, Inc., Waters Corp., Applied Separations, and Taiwan Supercritical Technology.

The problem of long-term analytical SFE exploitation may also be in part an education issue as evidenced by the failure of academicians to both learn, explore, and apply the technology in junior-senior chemistry laboratory courses at the university level – even when new instrumentation is made available. Furthermore, the perceived remarkable speed and versatility of SFE often tempts beginners to look for shortcuts rather than to examine the application systematically (4). A hasty approach to method development often leads to unexpected problems and analytical SFE is a sophisticated technology that is best mastered by adhering to the rigour required when developing any analytical method. It should be remembered that while there has been and continues to be a plethora of applications in engineering, SFE was only developed as an analytical technique in the mid-1980s. To gain some appreciation for past developments (5–10), the reader is encouraged to read the numerous reviews that have appeared recently as well as in the older literature. Look for details concerning the pros and cons of: (a) On-line or off-line coupling with a variety of separation techniques; (b) dynamic versus static extraction protocols; (c) solid-phase or liquid-phase extract trapping; (d) modifier addition to the matrix

Figure 1: Schematic of a SFE–UHPLC system.



versus modifier addition to the fluid; (e) adsorbent in the extraction thimble to retain unwanted compounds such as water; (f) experimental strategies for extracting analytes from solids, gels, creams, sludges, liquids, and so on; and (g) polar-modified, high density CO_2.

The use of SFE in modern process engineering applications was initiated in Germany during the late 1960s. These early studies showed that SFE was a viable alternative to distillation and solvent extraction processes. Furthermore, it allowed the processing of substances whose extraction could be adversely affected by high temperatures and the presence of solvent residuals. SFE using CO_2 is now an established industrial process for the production of high-value natural products such as hops, decaffeinated tea and coffee, herbs and spices, medicinal herbs, seeds, and marine oils. Further examples include extraction processes where an undesirable component is removed from the matrix, such as pesticides from medicinal herbs. In ancillary fashion, SFE processing continues to find applications as shown by the variety of lipophilic extracts available as commercial products such as polyunsaturated fatty acid esters derived from fish oils, neat and roasted sesame seed oil, cranberry seed-based oils, oils high in n-3 and n-6 fatty acid content, and pumpkin seed extracts coupled with more traditional SFE-derived products such as decaffeinated coffee (11).

The scope of this report is therefore limited to advances concerning analytical SFE. The goals of this article are to both describe the current

state of the art in relation to new instrumentation and to address unique extraction strategies, theories, and applications. In addition, interesting hyphenations of analytical SFE with chromatography and spectroscopy that have been reported during the past three years, as well as new aids for achieving successful extraction with CO_2 will be considered.

Vendor Activity

The development of second generation SFC instrumentation by several major vendors over the past four to five years introducing improvements to mobile phase delivery, reproducible modifier introduction, and greater analytical sensitivity appears to have facilitated analogous selective advances in SFE. While these developments are encouraging, they will not take away, for example, the critical need for quantitative trapping that must occur when modified fluids are used to extract polar analytes that have marginal solubility in pure CO_2. Furthermore, a greater understanding of matrix effects and the availability of universal SFE methodologies that are not specifically matrix and analyte sensitive and dependent is also desired.

Waters Corp. introduced at Pittcon 2013 an accelerated analytical supercritical fluid extraction system that provides unattended operation for method development, multiple samples, and multiple collection vessels. The unit utilizes ChromScope software to achieve better selectivity and specificity with no organic solvent exposure and no disposal cost. The system accommodates 10 sample vessels and 12 collection vessels that can be the same or different in vessels that range in volume from 5-mL to 15-mL. The key parameters of CO_2 pressure, vessel temperature, percent co-solvent, and use of dynamic and static conditions, as well as collecting extracts at individual user criteria can all be programmed and run unattended. The unit may use up to six different co-solvents to develop new methods for multiple samples while enhancing the extraction process.

A second product line is pre-assembled and cart-mounted that varies in size from 100 mL to 2 × 5 L. Based upon the control software chosen and the application-specific component options used, the system offers the ability to do everything from sophisticated studies to routine scale up. Complementary techniques such as counter current chromatography, particle formation (for example, RESS and SAS), phase monitoring, and reaction engineering options are available.

Two years earlier Jasco, Inc. introduced a supercritical fluid extraction coupled to ultrahigh-pressure liquid chromatography (SFE–UHPLC) system (Figure 1). The vendor noted that hyphenating multiple methods in an analysis system has many advantages such as decreasing the analysis time, reducing cost, simplifying sample pre-treatment, and increasing sensitivity and selectivity. In addition to hyphenation of SFE with SFC, gas chromatography (GC) and HPLC have been reported in the open literature (12). Hyphenation of SFE and UHPLC by Jasco, Inc. has only been reported in a vendor application note. The on-line SFE–UHPLC employs valve-switching techniques. Piperine in peppers was extracted effectively in the SFE system and then trapped and concentrated on the trap column that was then connected in the high-pressure switching valve unit section (13). After the extraction, a conditioning solvent was delivered from the conditioning pump to remove the gaseous CO_2 in the trap column. The extract was then automatically introduced into the UHPLC system and subjected to rapid separation and photodiode array detection with high sensitivity. The overall system is reported to offer very high speed and extremely sensitive analysis without complicated pre-treatment. RSD for retention time was listed as 0.36% and for peak area was 1.91%. Overall piperine recoveries were 96.0%.

Several other vendors are quite active and supportive of advances in SFE. Applied Separations, Inc. offers a four vessel simultaneous oven-based extraction system with modifier addition capability. Temperatures up to 240 °C, pressures up to 680 bar, and flow rates up to 400 mL/min can be accommodated.

PIC Solution, Inc. has recently reported an useful technique to improve the quality of preparative separations called “injection by extraction” as a substitute for traditional mixed stream injection and modifier stream injection (14,15). Often one is able to load significantly more sample using this technique because of better solubility and less peak distortion. In addition, the vendor notes that the procedure avoids many of the problems associated with more conventional injection techniques. In particular sample precipitation, which is a problem often met in preparative SFC, is eliminated by dissolution of the sample in the mobile phase. The technique is also useful for purification of samples with insoluble impurities or that require extraction from a matrix before separation.

Hyphenation of SFE

Historically, the development of analytical SFE has been associated with a form of chromatography. The research of Stahl in Germany combined SFE with thin layer chromatography (16). In 1989, Anderson and colleagues reviewed some of the theoretical considerations surrounding the use of SFE as the means of introducing samples into chromatographic systems (17). The data presented indicated that SFE could be fine-tuned into a selective sample preparation or injection method for chromatography. The practice of analytical SFE is divided between “off-line” and “on-line” methods. Such definitions refer to the mechanism of conducting the extraction. The on-line methods are usually combinations of SFE with ancillary techniques such as GC, SFC, and HPLC. Off-line SFE offers more flexibility with respect to extracting different sample sizes and types, as well as in the choice of the final analytical method. The bottom line is that the selection of an analytical SFE method should be based on the problem faced. The design of the interface is a crucial aspect in developing a hyphenated technique involving the direct coupling of SFE to a destructive or non-destructive analytical detector.

More current studies concerning hyphenation continue to be published in the open literature. A direct aqueous

SFE system designed to extract water samples contained in vials has been coupled with a reversed-phase LC–MS–MS system using a single 10-port valve (18). An SFE trap system using a C1 stationary phase connected to a C18 analytical HPLC column enabled the SFE–LC–MS–MS analysis of three polyether ionophore antibiotics in water. In another study, a new hyphenated technique for the extraction and determination of isoflavones in sea and freshwater algae and cyanobacteria with sonication sample pretreatment has been developed (19). A third reported study concerns the development of an analytical strategy based on rapid extraction techniques coupled to comprehensive bi-dimensional gas chromatography (GC×GC) for the characterization of the volatile fraction of tobaccos. The high peak capacity of GC×GC allowed global extraction techniques that do not focus on restricted chemical families to be considered (20). Finally, a novel, sensitive, and selective method for the separation and quantification of a group of nonpolar heterocyclic amines in commercial meat samples has been developed (21). The method is based on the combination of a SFE procedure followed by analysis of the extracted plug by capillary electrophoresis under fluorimetric detection.

Applications of SFE

The bioanalytical applications of supercritical fluid techniques such as SFE are of increasing interest. The main role of this technique is in the sample preparation and separation of biologically active compounds such as drugs and their metabolites, as well as endogenous compounds (22). The selection of the operating conditions depends on the specific compound or compound family to be extracted. Molecular weight and polarity have to be taken on a case-by-case basis. Some recently published applications have been selected to demonstrate the great variety of applications and to indicate the diversity of journals in which SFE is presented: (a) SFE of triterpenic acids from *Eucalyptus globulus* bark (23); (b) acrylamide from coffee (24); (c) monoterpenes from coniferous needles (25); (d) lycopene from tomatoes (26); (e) amphenicols from shrimp (27); (f) cannabinoids from marihuana (28); (g) abuse drugs in biological media (29); and (h) benign resolution of trans-1,2-cyclohexanediol by two-step SFE (30).

In summary, there is not only interest in SFE as an analytical tool but also for process development. It is clear from the recent literature that SFE for processing commands a greater interest at this time in a variety of areas such as food science, natural products, pharmaceuticals, bioprocessing (31), and environmental science. Advances in analytical SFE appear to have waned during the past decade as vendor commitment to the technology has been sporadic. Yet, mathematical modelling appears to be on the rise (32). Improved instrumentation for analytical applications such as automation, coupling to chromatographic and spectroscopic techniques, and sample throughput would benefit the technology. The time seems right for analytical SFE to expand on the gains made over the past 25 years as a rapid and efficient sample preparation tool. The technological developments first made in fast liquid chromatography and now supercritical fluid chromatography will no doubt bring greater emphasis and applications to analytical SFE.

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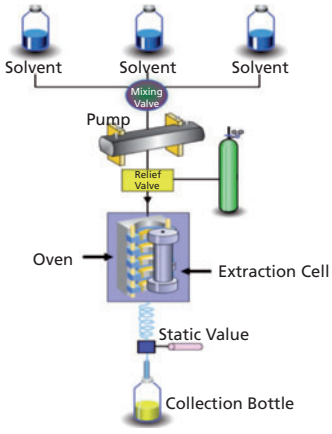


Recent Advances in **Pressurized-Fluid Extraction**

Aaron Kettle, Thermo Scientific, Sunnyvale, California, USA.

Pressurized-fluid extraction (PFE) is an automated extraction technique that uses elevated temperature and pressure to increase the rate and efficiency of the extraction process. PFE reduces the amount of time and solvent required, while significantly improving laboratory throughput relative to traditional extraction techniques such as Soxhlet. This article discusses current advances in commercial PFE instrumentation and shows how these systems can benefit scientists in analytical laboratories who wish to perform automated extraction.

Pressurized-fluid extraction (PFE) is a technique performed to extract solid or semi-solid samples using organic solvents. Elevated temperatures (up to 200 °C) are used to increase the kinetics of the extraction process while applying high pressures (for example, 1500 psi) to maintain the organic solvents in the liquid state. PFE is unique in that extractions are performed rapidly with reduced solvent use, compared with traditional extraction techniques. For example, PFE can reduce the extraction time down to 20 min per sample versus hours using Soxhlet and reduce solvent consumption to 30 mL per sample. The physiochemical processes influenced by PFE are described in Table 1.

PFE instrumentation follows a common pathway to produce extracts. An extraction cell containing the sample is loaded into an oven and a pump transfers extracting solvent into the cell from a reservoir. The cell is then pressurized and heated to a preset temperature. The temperature and pressure in the extraction cells rises above ambient levels and the hot solvent enhances the extraction rate of the analytes from the matrix. PFE systems are designed so that solvent will flow through the extraction cell and be collected into a bottle or tube at the end of the flow path. Once the extraction is complete, the extraction cell is purged with nitrogen gas to remove residual solvent and the collected extract is ready for concentration and analysis. PFE systems are currently manufactured by three vendors: Thermo Scientific (the accelerated solvent extraction [ASE] system); Fluid Management Systems, Inc. (FMS) (the pressurized liquid extraction [PLE] system); and Büchi (the SpeedExtractor system). Each system offers automation capabilities for the analytical laboratory to reduce the amount of time spent on sample preparation. These systems use elevated temperature and pressure to improve extraction efficiency and productivity compared with traditional extraction techniques such as Soxhlet. A summary of each system along with significant features is provided in this article.

Accelerated Solvent Extraction (ASE)

Accelerated solvent extraction (ASE) was first introduced at Pittcon in 1995 by Dionex (now part of Thermo Fisher Scientific). ASE increases extraction efficiency by using elevated temperatures of up to 200 °C with a fixed pressure of 1500 psi using both stainless steel and zirconium extraction cells (1–100 mL). These latter cells are especially resistant to low concentrations of mineral acids and strong bases. Currently, two ASE instruments are available from Thermo Fisher: The ASE 150 Accelerated Solvent Extraction instrument, a single-cell system for the extraction of solid and semisolid samples; and the multiple cell ASE 350 instrument. The ASE 350 instrument can process up to 24 samples in a batch and store up to 24 extraction methods. Extraction methods can be preprogrammed and multiple extraction methods run in a single batch, providing sequence control. Each sample is processed sequentially under the same programmed method conditions. The total extraction time is usually less than 20 min and the amount of solvent used is approximately 1.5 times the volume of the sample cell (for example, 15 mL for a 10-mL cell). Extracts are delivered to the collection vessels through a filter inserted at the bottom of the cell, and in many cases do not need any additional preparation prior to analysis. The schematic shown in Figure 1 demonstrates the operating principles of this system.

The features of the ASE method are summarized in Table 2. The ASE system is capable of performing in-line clean up through use of adsorbents to the extraction cell. These absorbents are layered at the bottom of the cell prior to sample introduction and selectively remove interfering compounds during the extraction. For example, adding activated alumina oxide eliminates the co-extraction of lipids when extracting organic compounds. Several different absorbents can be used and the correct selection is documented in reference 1. Once the extraction is complete, the collection vial can be interfaced with a Rocket Evaporator (Genevac Ltd.) for automated sample concentration and evaporation. This method significantly improves the sample preparation process by eliminating the need for off-line clean-up steps and by enabling the direct transfer of samples from the system to an automated evaporator. With the use of in-cell adsorbents and the Rocket Evaporator, ASE is able to combine sample filtration, cleanup, and evaporation into one workflow.

Pressurized-Liquid Extraction (PLE)

Pressurized-liquid extraction (PLE) is widely used in persistent organic pollutants (POPs) extraction from environmental matrices such as soil, and from biological matrices such as tissue. The FMS PLE instrument makes use of elevated temperature and pressure to increase the rate and efficiency of the extraction process, producing extracts in as little as 20 min. Up to six samples can be extracted simultaneously using the systems parallel extraction mechanism. The system is modular, can be expanded from one to six channels, and uses stainless steel extraction cells (5–250-mL) with end caps that contain Teflon filters. DMS-6000 Editor software is used to control the instrument, store multiple extraction methods, and plot temperature and pressure data for each channel used. The operating principles of the PLE instrument with in-line cleanup is shown in Figure 2.

The PLE instrument can be interfaced with both in-line clean columns and an evaporator that combine the extraction, cleanup, and concentration procedures into one step. In-line cleanup of extracts is accomplished with the use of in-cell packing materials (for example, silica or carbon), and by interfacing with columns that are packed with adsorbents (carbon, silica, and alumina). A flow-through design is used to move solvent downward through a heated and pressurized extraction cell and through the packing material that is located at the bottom of the cell. An additional column clean-up module can be added to the output of the extraction cell for cleaning the samples prior to analysis. In addition, PLE instruments can be interfaced directly with a concentrator to automate the entire sample preparation workflow. This configuration helps to automate the sample preparation process and improve productivity prior to analysis. Table 3 lists the key features for the FMS PLE system.

Parallel Sample Extraction

The Büchi Labortechnik AG SpeedExtractor is a parallel extraction instrument that can process up to six samples simultaneously. For six samples, a total of 20–40 min is required for extraction, and 5–60 mL of solvent is required per sample, depending on the application. Stainless steel extraction

Figure 1: Accelerated solvent extraction (ASE) system schematic.

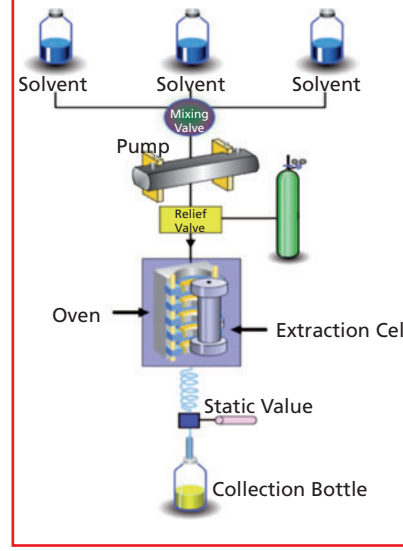


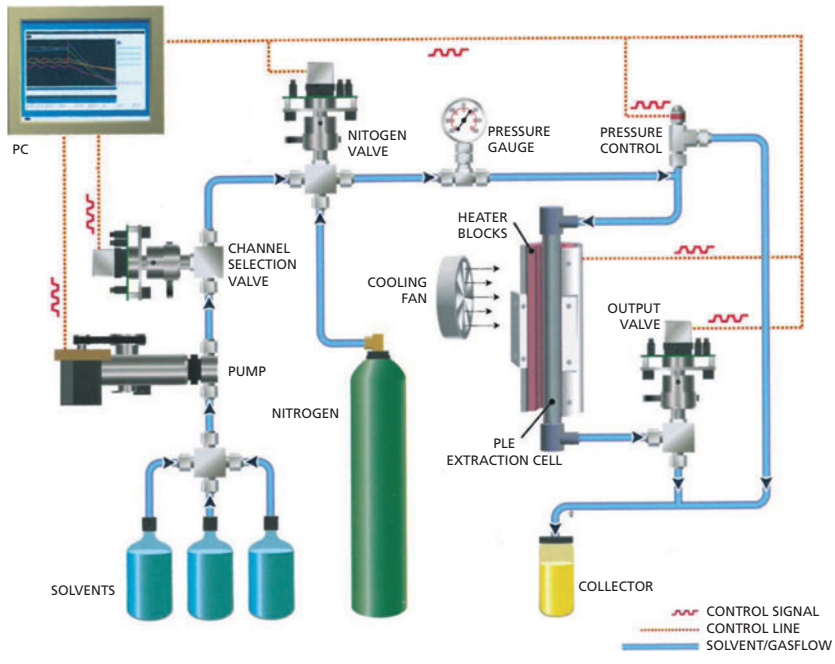
Table 1: Physiochemical processes influenced by pressurized-fluid extraction (PFE).

Parameter	Effect on the Extraction Process
Temperature	Elevated temperature increases analyte diffusion from the matrix and improves analyte solubility in the extraction solvent.
Pressure	Increased pressure enables liquid solvents to be used at high temperature.
Analyte solubility	Increases as temperature increases to improve extraction efficiency (for example, solubility of anthracene increases 13-fold in DCM [50 °C to 150 °C]).
Solvent viscosity	Decreases as temperature increases. Improves solvent migration through the matrix to increase extraction efficiency.
Solvent surface tension	Decreases as temperature increases. Allows solvents to better coat the matrix and helps improve analyte diffusion.

Table 2: Summary of Thermo Scientific accelerated solvent extraction (ASE) features.

Feature	Description	Use
Static extraction cycles	Extraction cells filled with solvent are held at elevated temperature and pressure without a continuous flow of solvent.	Maximizes analyte diffusion from the matrix while reducing the amount of solvent required for the extraction.
In-line filtration	Disposable filters (cellulose or fibre glass).	Automatically removes solid particulates from the extract.
In-line cleanup	Adsorbents (for example, silica, activated alumina).	Automatically removes interfering molecules (for example, lipids) from the extract.
Rocket evaporator	Centrifugal system that evaporates up to 18 accelerated solvent extractions.	Automates the evaporation process for high-throughput laboratories.
pH-hardened pathways	Zirconium extraction cells.	Allows the extraction of acid or alkaline pretreated samples (for example, total fat from foods).
Integrated solvent controller	Internal proportioning valve that allows solvent mixing with user-defined ratios.	Automatically mixes up to three solvents for complex extractions.
Solvent saver modes	Programmable modes in the extraction method that further reduce the amount of solvent required for the extraction.	Reduces the amount of solvent required to 5 mL per sample.
Chromeleon 7.2 chromatography data system software control	Software to standardize control of the instruments involved in the analytical workflow.	Controls the ASE 350 instrument and chromatographic systems involved in the workflow.
Sequence processing control	Increases flexibility by allowing multiple methods to be run in a single batch.	Multiple extraction methods can be run on the same sample or series of samples within a single batch.

Figure 2: FMS PLE system schematic.



cells (10–120-mL) are added to a pressurized heating block for operation up to 200 °C and 2000 psi. Extraction cells are automatically sealed once entered into the oven permitting the use of up to four solvents. Software control is available to record method parameters and run preprogrammed extraction methods. The operating principles of the SpeedExtractor instrument are shown in Figure 3.

Similar to ASE and PLE instruments, the SpeedExtractor instrument offers a parallel evaporation option that further automates the sample preparation workflow. Once the extraction is complete, bottles containing the extracts can be placed into the Multivapor evaporator for concentration. Up to six samples can be simultaneously concentrated improving sample throughput prior to analysis. Features of the SpeedExtractor are listed in Table 4.

Table 3: Summary of FMS pressurized-liquid extraction (PLE) instrument features.

Feature	Description	Use
In-line filtration	Extraction cell end caps contain Teflon filters.	Automatically removes solid particulates from the extract.
In-line cleanup	Adsorbents added to extraction cell and in-line columns.	Automatically removes interfering molecules (for example, lipids) from the extract.
In-line concentration	Evaporator that processes up to six samples simultaneously.	Automates the evaporation process for high-throughput laboratories.
Modular design	One to six channels are available to meet changing throughput needs.	Additional channels can be added to the system as laboratory workload increases.
Parallel extraction	Processes multiple samples simultaneously.	Improves throughput by extracting up to six samples in 20 min.
DMS-6000 Editor software control	Software that controls the extraction process and allowing the laboratory to edit extraction methods and monitor and store extraction data.	Enables multiple extraction methods to be run and plots temperature and pressure in real time for each channel.
Solvent selection valve	Valve that permits the use of up to five solvents.	Enables the use of a different solvent for each extraction channel.

Table 4: Summary of Büchi SpeedExtractor instrument features.

Feature	Description	Use
Sealed extraction cells	Extraction cell caps are automatically sealed when placed in the oven.	Cell caps do not need to be manually tightened as part of the sample preparation.
Multivapor concentrator	Parallel concentration system that processes up to six extracts.	Samples can be added to the concentrator immediately following extraction and concentrated simultaneously.
Parallel extraction	Processes multiple samples simultaneously.	Improves throughput by extracting up to six samples in 20 min.
SpeedExtractorRecord software	Software that controls and records method parameters from the extraction process.	Method parameters and external intervention are recorded for reporting.
Multiple collection bottle sizes	60-, 150-, 220-, or 240-mL collection bottles.	Flat-bottom vials that are compatible with the Multivapor concentrator.
Integrated solvent mixer	Two or four-port mixer for multiple extraction solvents.	Solvent mixtures can be prepared to handle complex extractions.

Table 5: Representative applications using pressurized-fluid extraction (PFE).

Feature	Description	Use
Total fat in food	Total fat (bound and unbound) extraction from foods.	Enables compounds to be extracted from acid/base pretreated food matrices.
Acid pretreated biomass	Use of zirconium extraction cells to extract water and ethanol soluble components from acid pretreated biomass.	Enables extraction of sugars from acid treated biomass to expand the versatility of PFE.
Persistent organic pollutants	PAHs, PCBs, brominated flame-retardants, dioxins/furans, pesticides from soil, sediment, and tissue.	Rapid extraction of a broad class of compounds from environmental matrices. Extraction times as low as 20 min and 30 mL of solvent per sample.
Plasticizers in polymers	Extraction and determination of plasticizers in polymeric material for manufacturing quality control.	Important step in assuring the quality of the end product prior to release. PFE can reduce extraction time to 12 min per sample vs. 6 h with Soxhlet and typically requires 30 mL of solvent per sample.
Active ingredients in pharmaceuticals and natural products	Extraction and determination of active ingredients from herbal supplements (for example, St. Johns Wort, goldenseal root).	Verifies label claim and ensures quality control for production. Reduces extraction time to as low as 14 min per sample with 15 mL of solvent used.
Pesticides and herbicides in foods	Extraction of pesticides and herbicides from multiple different types of food including fruits and vegetables.	Helps ensure food safety by allowing rapid extraction of multiple pesticides from food products. Compounds can be extracted in as little as 12 min per sample using 15 mL of solvent. PFE can work with high water content, highly pigmented, and high fat content samples.

Figure 3: Büchi SpeedExtractor instrument schematic.

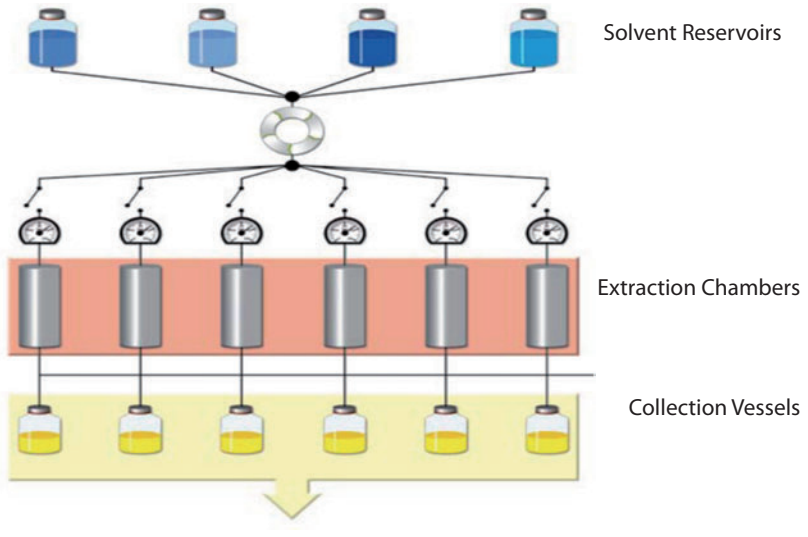


Table 6: Average recovery of pesticides from three soil types.

Pesticide	Average Recovery*	Average RSD (%)		
Heptachlor	88.0	Matrix	ASE	Soxhlet
Aldrin	94.9	Clay	5	9.7
Gamma chlordane	99.5	Loam	7.8	6.2
Alpha chlordane	102.2	Sand	12	10.1
Dieldrin	101.2	*% of Soxhlet, n = 6		
Endrin	97.2			
p,p'-DDT	74.9			

New Applications Using Pressurized-Fluid Extraction

PFE is versatile and can be applied to numerous solid matrices, as shown in Table 5. PFE has been used for new applications across a wide spectrum of industries including the environmental, food, chemical, petrochemical, and pharmaceutical industries. Most applications now require only 20 min of extraction time and approximately 30 mL of solvent per sample to extract myriad compounds for analysis.

Examples of key PFE applications include POPs and dioxin extraction from a diverse range of matrices. In one study, the performance of ASE was compared with Soxhlet for the extraction of pesticides, PCBs, and dioxins (polychlorinated dibenzodioxins [PCDDs] and polychlorinated dibenzofurans [PCDFs]) in soil, river sediment, and other solid matrices (2). The extraction efficiency was measured by the average percent recovery for six samples and reproducibility was measured by % relative standard deviation (% RSD). Tables 6–8 show the results of the ASE extraction as a proportion (%) of Soxhlet. This example illustrates the effectiveness of the ASE technique in obtaining recoveries of analytes equivalent to the Soxhlet approach.

Another study used a Büchi SpeedExtractor instrument and an ASE instrument for dioxin and furan extraction from soil (3). The extraction efficiency of both instruments was compared and it was demonstrated that both could meet the recovery requirements of EPA Method 3454A (4). The extraction efficiency was measured through the mean quantitated amount by performing gas chromatography coupled with high resolution mass spectrometry (GC–HRMS). Table 4 lists the comparison of both instruments for several PCDDs and PCDFs and demonstrates that the SpeedExtractor Model E-916 instrument

delivers extractions equivalent to those obtained with the ASE.

Summary

The principle of pressurized-fluid extraction has not changed significantly over the last 10 years; however, the level of automation provided by this technique has expanded greatly. The biggest advance in PFE instrumentation has been the addition of in-line cleanup techniques and direct transfer of samples to concentrators. The advance has markedly improved sample preparation throughput by combining what were once separate procedures. Over the last 10 years the continuing of these combined techniques has improved the productivity of sample preparation and reduced bottlenecks in sample preparation. With the advances made in these techniques, laboratories are now able to able to remove analytes from the original matrix and place them into a gas chromatography (GC) or liquid chromatography (LC) autosampler vial for analysis with minimal analyst intervention.

Table 7: Average polychlorinated biphenyls (PCB) recovery from river sediment (SRM 1939).

PCB Congener	Average Recovery*	RSD (%)
PCB 101	89.2	3.7
PCB 153	62.3	4.1
PCB 138	122.1	2.3
PCB 180	111.5	5.9

*% of Soxhlet, n = 6

Table 8: Total polychlorinated dibenzo-p-dioxins.

Sample Matrix	Soxhlet (ng/kg)	ASE (ng/kg)
Chimney brick	8040	8170
Urban dust	1110	1159
Fly ash	93,000	107,900
Hamilton Harbor sediment	4283	4119
Parrots Bay sediment	2836	2444

References

(1) Thermo Scientific Technical Note 210 [Available from: http://www.dionex.com/en-us/webdocs/57304-TN210-ASE-In-Line-Interferences-Removal-05Oct2012-TN70242_E.pdf].

(2) Thermo Scientific Application Note 352 [Available from: http://www.dionex.com/en-us/webdocs/40970-AN352-ASE-Persistent-Organic-Pollutants-23Jun2011-LPN1676-03.pdf].

(3) Büchi Application Note 012/2009 [Available from: http://www.buchi.com/fileadmin/user_upload/01_products/10_extraction/pdf/SN%20012-2009%20Dioxin_Furan%20Soil_p%20Version%20B.pdf].

(4) EPA Method 3454A: Pressurized Fluid Extraction (PFE), January 1998 [Available from: http://www.epa.gov/sam/pdfs/EPA-3545a.pdf].

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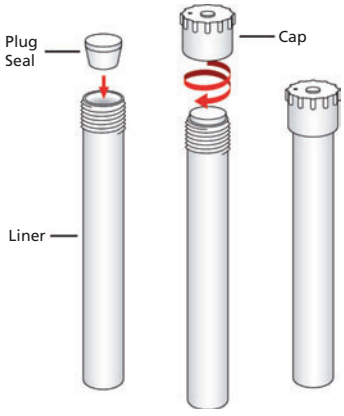
Table 9: Mean values in pg/g and % relative standard deviation (%RSD) for dioxins and furans.

	Buchi SpeedExtractor		Thermo Fisher Acelerated Solvent Extraction	
	Content (pg/g) (n = 3)	%RSD	Content (pg/g) (n = 2)	%RSD
2378-TCDF	154	17	158	11
2378-TCDD	7	29	7	5
12378-PeCDF	504	2	439	6
23478-PeCDF	370	15	325	18
12378-PeCDD	47	4	51	5
123478-HxCDF	657	9	557	9
123678-HxCDF	311	14	265	15
234678-HxCDF	276	21	208	2
123789-HxCDF	190	10	201	1
123478-HxCDD	35	14	32	12
123678-HxCDD	58	5	54	3
123789-HxCDD	33	9	39	3
1234678-HpCDF	2807	11	2068	13
1234789-HpCDF	529	5	484	15
1234678-HpCDD	672	5	519	7
OCDF	2006	2	1191	23
OCDD	2003	10	1509	17
WHO-TEQ	476	6	424	4



Microwave-Accelerated Extraction — SW-846 Method 3546 and Beyond

Bobbie McManus, Michelle Horn, Steve Smith, Bob Lockerman, and Greg LeBlanc, CEM Corporation, Matthews, North Carolina, USA.

Microwave-accelerated extraction (MAE) was evaluated by the authors of this review in 1999 (1). Since this time, the United States Environmental Protection Agency (USEPA) has promoted the use of SW-846 Method 3546 using MAE for the extraction of organic compounds from solid matrices. Applications for this technique have increased and MAE equipment has been streamlined to dramatically increase throughput and ease of use. This article reviews the latest enhancements to this technology from a hardware and applications perspective.

In 1999, microwave-accelerated extraction (MAE) was a relatively new technique for solvent extraction, and approvals were only obtained when the technique became mainstream. The first approval was from the state of California under its California Environmental Technology Certification programme for the extraction of semivolatile organic compounds in soil, sediment, and sludge (2). As the technique substantially reduces sample extraction time and solvent consumption, the sample turnaround time for data generation improved significantly. The certification was intended to encourage use of the technique where data quality objectives could be met by its use.

The United States Environmental Protection Agency's (USEPA's) Test Methods for Evaluating Solid Waste (SW-846) provides a comprehensive source of information on sampling, sample preparation, analysis, and reporting for compliance with the Resource Conservation and Recovery Act (RCRA). SW-846 outlines test procedures used to characterize solid waste in accordance with 40 CFR Part 261, Identification and Listing of Hazardous Waste. The sample preparation and analytical procedures (or determinative steps) are categorized by the analyte, which can be inorganic or organic.

The sample matrix and analytes define the SW-846 3500 series sample extraction methods. The matrix is either aqueous, solid, an air sampling train, or non-aqueous soluble. Analytes are characterized as either nonvolatile or semivolatile organic compounds. All samples analyzed for non-volatile or semi-volatile organic compounds require a solvent extraction step, with the exception of non-aqueous solvent soluble samples.

Microwave extraction (Method 3546) was formally included in SW-846 in Final Update IV of the Third Edition of the manual in 2008 (3). Microwave extraction is the process of heating solid sample–solvent mixtures in a sealed (closed) vessel with microwave energy under temperature-controlled conditions. The temperature is elevated significantly above the atmospheric boiling point of the solvent, accelerating extraction while giving performance comparable to the standard Soxhlet method. Solvent consumption is only 25–50 mL per sample.

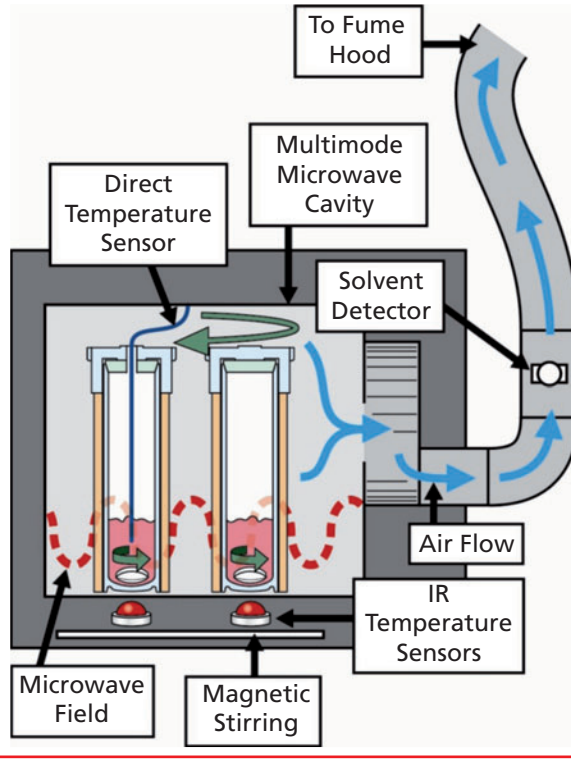
The American Society for Testing and Materials (ASTM) is an international standards organization that publishes voluntary consensus technical standards, including test methods that define how a method is performed and the accuracy of the result. Test results may be used to assess compliance with a Standard Specification. The ASTM has three test methods that incorporate the MAE technique:

- ASTM 5765 – 05 (2010) is the Standard Practice for Solvent Extraction of Total Petroleum Hydrocarbons from Soils and Sediments Using Closed Vessel Microwave Heating (4). The soil or sediment sample is extracted with acetone or hexane in a sealed microwave transparent vessel using microwave heating to an internal temperature of 150 °C, producing an extract suitable for analysis by gas chromatography (GC) or gravimetric techniques.
- ASTM D6010-12 is the Standard Practice for Closed Vessel Microwave Solvent Extraction of Organic Compounds from Solid Matrices. The soil, sediment, sludge, or waste sample is extracted in an acetone-hexane mixture at 115 °C producing an extract suitable for analysis of semi-volatile or volatile organic compounds by GC or gas chromatography–mass spectrometry (GC–MS) (5). This standard practice reduces sample preparation time, solvent consumption, and operating costs.
- ASTM D7210-13 is the Standard Practice for Extraction of Additives in Polyolefin Plastics. This provides guidelines for extracting phenolic antioxidants, phosphite antioxidants, UV stabilizers, antistatic agents, and slip additives from milled polyolefin plastics (6). It now includes an MAE technique for subsequent analysis of the

Table 1: Approved microwave accelerated extraction techniques.

Governing Body	Method	Sample	Solvent	Analytes
United States Environmental Protection Agency	SW 846 - 3546	Soils, sediments, and sludges	Acetone:Hexane	Semivolatile organic compounds, organophosphorus pesticides, organochlorine pesticides, chlorinated herbicides, phenoxyacid herbicides, substituted phenols, PCBs, and PCDDs or PCDFs
American Society of Testing and Materials	D 5765 - 05(2010)	Soils and sediments	Acetone:Hexane	Total petroleum hydrocarbons
American Society of Testing and Materials	D 6010 - 12	Soil, sediments, sludges, or waste samples	Acetone:Hexane	Semi-volatile and volatile organic compounds
American Society of Testing and Materials	D 7210 - 13	Polyolefins	Sample dependent	Antioxidants, UV stabilizers, slip, and anti-static agents
Consumer Product Safety Commission	CPSC-CH-C1001-09.3	Plastics (children's toys or childcare articles)	Acetone:Hexane	Phthalates

Figure 1: Representation of a batch (multimode) microwave extraction system.



extract by chromatographic techniques.

Phthalates are mainly used as plasticizers to increase flexibility and durability, but they are linked to health issues and are being phased out of use. The Consumer Product Safety Commission (CPSC) issued Test Method: CPSC-CH-C1001-09.3, a Standard Operating Procedure for Determination of Phthalates in April 2010 (7). Phthalate content analysis in children's toys and childcare articles was the result of standards passed in the Consumer Product Safety Improvement Act Section 108. The test method uses a microwave-extraction technique based upon EPA Method 3546 as an acceptable method of extraction.

Advances In Hardware And Instrumentation

MAE involves heating solid sample–solvent mixtures with microwave energy to separate compounds from the sample to the solvent. The extraction is most commonly performed in a sealed vessel under temperature-controlled conditions. This provides a significant temperature elevation above the atmospheric boiling point of the solvent and accelerates the extraction process.

Batch Microwave Systems: The components of the batch style microwave system remain the same as when reviewed in 1999 (1) in terms of microwave energy generation, application of microwave energy to the sample load, safety concerns, sample stirring, and indirect heating. However, there have been significant enhancements in terms of its "intelligence", vessel technology, and temperature control. This review focuses attention on these areas of the batch microwave system for solvent extraction applications (see Figure 1).

Recent systems have a level of "intelligence" not present in earlier versions. The latest systems possess libraries of predefined methods loaded into software with the capability to count the number of samples and recognize the vessel type loaded — this enables "one-touch" operation. A method is selected by the user based on the sample matrix. The sample is then weighed into the vessel, solvent is added, and the vessel is sealed. The turntable with the vessels is then placed into the system cavity. On pressing the start button, the system can then determine the vessel type and number of vessels,

Figure 2: Microwave extraction vessel components and assembly.

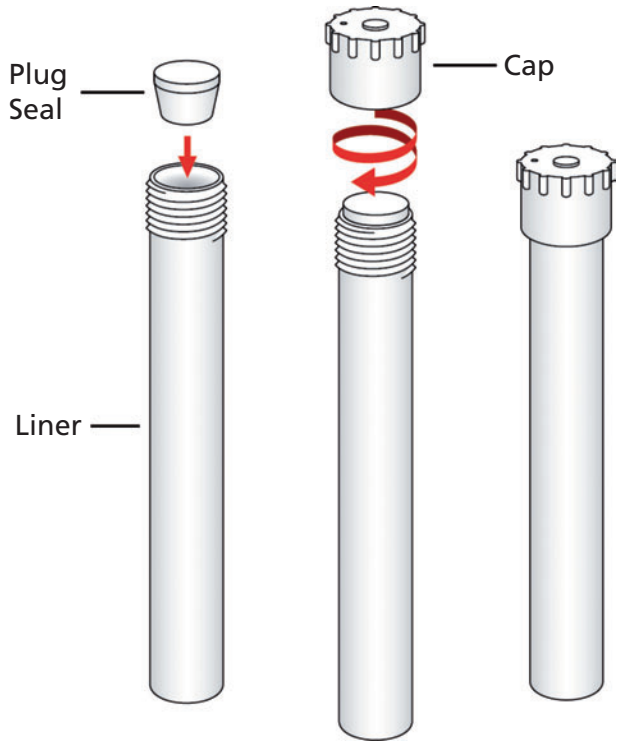
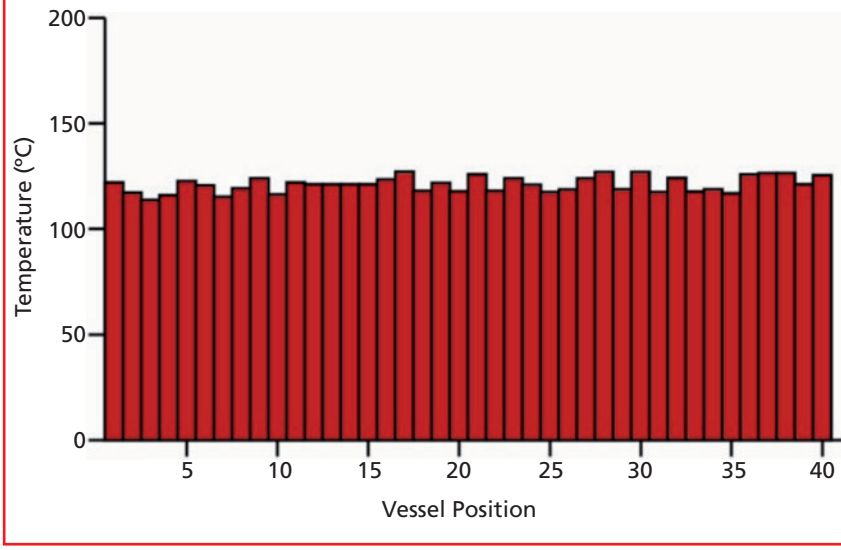


Figure 3: Graphical representation of temperature versus vessel using "all vessel" temperature control.



subsequently applying the correct level of microwave energy to meet the method-defined temperature for its hold time to complete extraction. This minimizes operator error as it automatically defines the input power levels needed for different numbers of samples in the system and the conditions for the extraction.

Past generation vessels were assemblies containing six components that were rather cumbersome, and only 12–14 samples could be loaded into the system at one time. To properly seal vessels, a torque wrench was required. The latest vessel technology now only has three components — a vessel body, a plug seal, and a cap — simplifying assembly. Figure 2 represents an example of this design.

With the reduction in the number of components and size, up to 41 samples can be processed for each batch using 75-mL vessel assemblies. When processing larger scale samples, 100-mL vessel assemblies are available that have a throughput of 24 samples for each batch. Both vessel sizes have a built-in pressure relief mechanism for safety purposes. If the pressure inside the vessel exceeds its operating limitations, the vessel will automatically vent to relieve the internal pressure.

The 100-mL vessel assemblies can be used with an optional disposable glass liner inserted inside the vessel liner. This component insertion eliminates the need to clean the vessel liner in between runs, eliminating issues associated with carry over contamination. Table 2 compares modern features of currently available batch-type MAE systems on the market.

Previous generation systems used a temperature sensor inserted into one of the vessel assemblies containing a sample with solvent to create a feedback control loop regulating microwave input power to maintain target temperature. This assumed equivalent absorbtion for all samples in the system. This temperature technology has since been replaced with a floor-mounted temperature sensor that measures the temperature of all samples as they rotate inside the system. The system then takes an average of all the sample temperatures measured to regulate the microwave input power for the temperature control. The enhancement improves control over the whole extraction process and removes the need for an operator to insert a temperature probe into one of the sample vessel assemblies. Figure 3 shows vessel-to-vessel temperature variation and is typical for all vessel temperature controlled systems.

The discussion up until this point has related to batch style microwave systems or systems that extract a batch of samples at one time. These systems are well-suited to the

Table 2: Batch, closed vessel microwave accelerated extraction (MAE) system comparison.

Supplier	Anton Paar	CEM Corporation	Milestone	PerkinElmer
Model	Multiwave Pro	MARS 6	Ethos EX	Titan MPS
Feature				
Power output (Watts)	1500	1800	1200	1500
Direct, in-vessel temperature control	Yes (Gas bulb)	Yes (Fibreoptic)	Yes (Fibreoptic)	No
Contactless, all vessel temperature control	Yes (Measures only)	Yes	Yes	Yes
Direct pressure control	Yes	Yes	Yes	No (Indirect optical technique)
Stirring	Yes	Yes	Yes	No
Time to parameter software control	No	Yes	Yes	No
Vessel recognition and counting capability	No	Yes	No	No
User interface	Integrated touchscreen	Integrated touchscreen	External touchscreen	Integrated touchscreen
Viewing window	Yes	Yes	Yes	No
Exhaust solvent sensor	Yes	Yes	Yes	No
Number of vessels or batches	16	40/24	41/24	16
Volume of vessel (mL)	100	75/100	70/75	75
Vessel liner material of construction	Teflon	Teflon/optional glass	Teflon/optional glass	Teflon
Indirect heating	Silicon carbide inserts	Carboflon	Weflon	No
Instrument safety approval (ETL/UL/NRTL)	Yes	Yes	Yes	No

needs of laboratories processing environmental samples.

Sequential Closed Vessel MAE Systems: Microwave systems that process samples sequentially have recently been introduced for extraction applications. The systems consist of a microwave component with a relatively small "focused" cavity, and an automated vessel handling component that feeds and removes vessels containing sample to and from the microwave system cavity.

When samples are introduced into the microwave system cavity, vessels are sealed so samples can achieve elevated temperatures and pressures during the microwave irradiation cycle. The maximum operating conditions for vessels are up to 300 °C and 450 psi (28 bar) — easily meeting conditions needed for extraction applications.

Temperature is monitored and measured within each vessel using an infrared sensor mounted in the cavity. Temperature data is used to regulate the microwave input power and control the temperature in the same way as the batch systems. Sample pressure is measured using a sensor in the component that "seals" the vessel into the microwave system cavity. The component measures the deflection of the vessel cap as pressure is generated during the irradiation cycles causing the cap to move outward. The system stirs the sample solvent mixture in the vessel using a magnetic component located under the cavity floor and a magnetic spinbar placed in the extraction vessel. At the end of the irradiation cycle, the sample is automatically cooled using compressed air, rapidly decreasing the temperature and pressure of the vessel. The "cool down" time is reduced resulting in turnaround times as low as five min per sample. Figure 4 depicts a typical design of a sequential style MAE system.

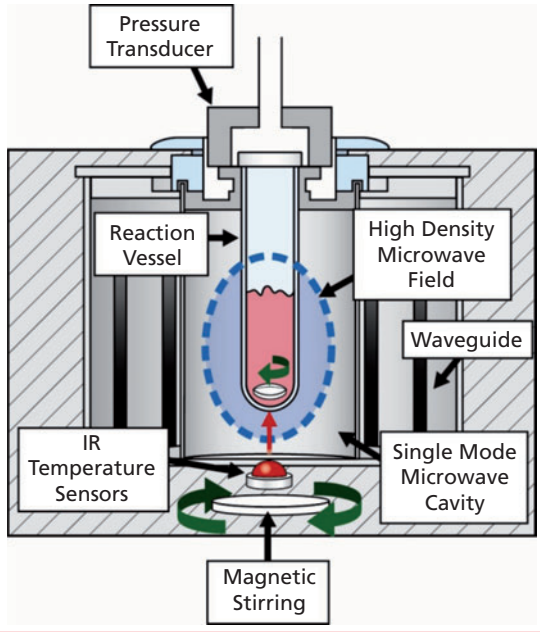
Vessels used with sequential style systems are available in sizes from 5–80 mL with a working volume range of 2–50 mL respectively. The vessel material is normally glass, with quartz as an option. Caps have a Teflon sealing surface between the cap and vessel lip to minimize contamination issues. The rack size, or the number of vessels, that can be held for automated processing is variable — most commonly 48 or 60 of 10-mL size vessels and 24 for 80-mL size vessels. Table 3 provides a comparison of the important features of the sequential MAE systems on the market.

The potential benefits of a sequential versus a batch style microwave system are:

- The option to use unique extracting conditions for each sample.
- Selection of precise temperature control for every sample.
- Different sample types can be processed within a "rack" of samples.
- Samples can be processed with different extracting solvents within a "rack" of samples.
- A sample can be immediately removed after completion for post extraction handling.
- Small sample sizes/solvent volumes can be processed (where feasible) with the 10-mL vessel.

This sequential approach provides more flexibility than with a batch-style system. Both systems apply microwave

Figure 4: Representation of a focused (single-mode) microwave extraction system.



energy to sample–solvent mixtures at elevated temperature and pressure under controlled conditions with sample stirring to accelerate the extraction process. For laboratory scientists, the sample variety, throughput, and objectives will define which system is better suited for their needs.

Solvent-Free MAE System: Milestone recently introduced a system capable of performing solvent-free microwave extraction following a collaboration with the Université d'Avignon et des Pays de Vaucluse (Avignon, France). The system uses a technique of microwave hydrodiffusion (a type of steam distillation) followed by gravity separation to produce essential oils in a concentrate form from raw aromatic plant materials. The technique is performed at atmospheric conditions and does not use any solvent or added water. Internal heating of the water within the plant material causes cell disruption and release of the extract (water and essential oils). The extract is then gravity fed into a collection system underneath the microwave system to separate the essential oil from the water. Samples are processed one at a time in a 1- to 2-L proprietary glass vessel using a microwave power and time- or temperature-based method. Key benefits are enhanced yield, quicker isolation times, and an environmentally friendly approach.

New Applications Areas

The introductory section of this review discussed approvals granted for the MAE technique. For a technique to be approved for a specific application, it must have records documenting its performance for a wide-variety of matrices. The primary use of MAE is in environment-related applications. However, scientists typically explore other applications for existing technologies once they have been proven to work. This section looks at other applications for the MAE technique.

Mercury Speciation: The total mercury concentration in a sample does not provide an accurate picture for "cause–effect" relationships. The distribution of mercury species can provide an improved understanding of data relationships leading to more accurate conclusions. In 2013, Leng and co-workers published an article titled "Speciation Analysis of Mercury in Sediments by HPLC Following Microwave-Assisted Extraction" (7). The authors proposed the combination of microwave-assisted extraction of samples with HPLC–VGAFS determination. It provided a sufficiently low detection limit to determine $MeHg^+$, $EtHg^+$, and $Hg2^+$ in sediment samples. The limits of detection were 0.013 ug/L, 0.022 ug/L, and 0.011 ug/L for mercury species using the microwave technique. The technique was validated by applying it to two certified reference materials — IAEA-405 and ERM-CC580 — that gave good agreement with the certified value.

Antioxidants: There are potential health advantages to diets rich in antioxidant compounds that include lowering the risk of cardiovascular disease and certain cancers. In 2011, Mathur and co-workers investigated MAE versus traditional solvent extraction techniques for screening plant extracts for antioxidant activity (9). The MAE technique was optimized on 20 g of plant samples using methanol as the extracting solvent at 80 °C with a 20 min hold time. Extracts were screened for in vitro antioxidant activity. The results indicated that extracts prepared by the MAE technique showed potent antioxidant activity relative to the traditional method of extraction.

Active Pharmaceutical Ingredients: The extraction of active pharmaceutical ingredients (APIs) from solid dosage forms is critical to validate concentrations within formulations. Brannegan published a chapter titled "Extraction Techniques Leveraging Elevated Temperature and Pressure" within *Sample Preparation of Pharmaceutical Dosage Forms: Challenges and Strategies* (10). The application of MAE can facilitate rapid troubleshooting of low potency results and help identify the source of these issues. MAE can be performed in parallel, samples can be stirred during the extraction step, and rapid heating of the sample is provided, therefore providing solubilization and extraction of APIs from the solid dosage forms. The chapter provides case studies documenting the benefits of MAE to troubleshoot low potency results.

RoHS/WEEE: The adoption of the "Restriction of Hazardous Substances (RoHS) and Waste from Electrical and Electronic Equipment (WEEE) Directives" by the European Union (EU) created a problem. There were no reliable, cost-effective

Table 3: Sequential, closed vessel microwave accelerate extraction system comparison.

Supplier	Anton Paar	Biotage	CEM
Model	Monowave 300 with MAS 24	Initiator Robot 60	Discover SPX
Feature			
Power output (Watts)	850	400	300
Infrared temperature control	Yes	Yes	Yes
Direct pressure control	Yes	Yes	Yes
Stirring	Yes	Yes	Yes
Post reaction cooling	Yes	Yes	Yes
Time to parameter software control	Yes	No	Yes
User interface	Integrated touchscreen	Integrated touchscreen	External controller
Volume of vessel (mL)	4, 10, or 30	5, 10, and 20	10, 35, and 80
Vessel material of construction	Glass/Quartz	Glass	Glass/quartz with optional Teflon liners
Autosampler capacity (Number of vessels)	24	60/30	72/36/24
Indirect heating	Silicon carbide inserts	No	Carboflon
Instrument safety approval (ETL/UL/NRTL)	Yes	Yes	Yes

methods of testing for the presence of high-mass unit additives such as polybrominated biphenyls (PBBs) and polybrominated diphenyl ethers (PBDEs) in polymer samples. NSL Analytical resolved the problem by developing an MAE technique for the extraction of PBB and PBDE from polymers and subsequent analysis by GC–MS (11). The technique provided savings in time and cost (labour, solvent cost, and disposal) while providing 99% recovery of the additives from sample sizes of only 0.5 g.

Food Safety: Dicyandiamide is a white crystalline compound primarily used for the production of melamine. The detection of dicyandiamide in milk-based products can be indicative of melamine, responsible for kidney stones. Shen and coworkers developed a MAE method in combination with liquid chromatography tandem mass spectrometry (LC–MS–MS) for the determination of dicyandiamide residue in infant formula (12). The extraction was performed in 5% formic acid with recovery in the range of 83–96% at levels of 0.25 mg/kg.

Essential Oils: Essential oils are highly concentrated, volatile oils that can be extracted from aromatic plants. The traditional approaches to extract the essential oils use a chemical process of steam distillation or solvent extraction, or a mechanical process using a cold press technique. Vian and associates developed a microwave-assisted technique for the extraction of essential oils without the use of added solvents or water — microwave hydrodiffusion and gravity, a new technique for extraction of essential oils (13). The microwave extracted essential oils were produced with yields and aromatic profiles similar to standard methods but in one-sixth of the time.

Future

Over the past decade, MAE has become a mainstream sample preparation method, because of advances in vessel technology, software control, and throughput. The remaining obstacle for the batch-style approach is sample–solvent separation after the extraction step and before the analytical step. The majority of future application areas are likely to be focused on developing flexibility of sequential systems. The ability to control conditions for each sample extraction will enable scientists to rapidly develop methods for a variety of sample types and analytes. This is especially true for those compounds that might degrade based upon temperature variability in a batch process.

Current research being performed on sequential systems includes extraction of fatty acid methyl esters (FAMEs) in a wide variety of food samples, as well as amino acid hydrolysis for adulteration testing in products — such as skimmed milk and infant formula. Work is also being conducted to test the possibility of performing simultaneous hydrolysis and extraction of total fats in foods. With the increasing levels of food safety and fraud concerns, it is likely that this technique will expand significantly in the food testing arena in the coming years. Other areas of research include the extraction of AZO dyes from textiles that have been banned in Europe since 2003, as well as metabolite extractions in the biological field of study.

Summary

Over the past 14 years, MAE has become well-accepted for environmental testing. Regulatory approval by the EPA, ASTM, CPSCs, and others validate the technique as an analytical method. The

combination of high-throughput, solvent reduction, and ease of use make it an excellent alternative to other extraction techniques. Advances in temperature measurement, software control, vessel technology, and automation have allowed expansion of the technique into the regulatory environmental arena, a trend that should continue.

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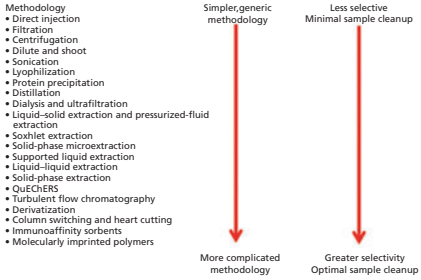

Green Chemistry Perspectives on Analytical Extractions

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The growing interest in green chemistry requires fresh perspectives on analytical extractions. Reduced solvent consumption, alternative safer solvents, and reasonable energy demands must be balanced with traditional analytical considerations such as extraction yield and selectively. This article introduces some of the concepts behind green chemistry, and discusses green solvent selection and extraction techniques. An overview of alternatives to conventional solvents, new green solvents, ionic liquids, and other solvent options are also described.

The history of modern analytical extractions mirrors the development of green chemistry. For nearly a century Soxhlet extraction, combined with shake-flask methods, was the standard method for the isolation of analytes from solid samples, while multiple liquid–liquid extractions (LLEs) using separatory funnels was the method of choice for liquid samples. In the mid-1980s, new forms of analytical extractions were developed and popularized — such as supercritical fluid extraction (SFE), pressurized-fluid extraction (PFE), solid-phase extraction (SPE), solid-phase microextraction (SPME), microwave-assisted extraction (MAE), single-drop microextraction (SDME), and ultrasonic extraction. These new extraction techniques had benefits such as faster times, lower cost for each extraction, improved yield and reproducibility, and lower solvent volumes. Lower solvent volumes provide significant environmental advantages. Extraction solvents generally provide the bulk of the waste encountered in any analytical method and often have health and safety concerns, such as toxicity or flammability.

In parallel with these developments, the concept of green chemistry emerged. This culminated in the statement of the 12 principles of green chemistry in 1998, which are shown in Table 1 (1). The goals of green chemistry are to address environmental, health, and safety concerns when planning a chemical process rather than after it has been performed. Despite the fact that modern extraction technologies and green chemistry are contemporaries, the two fields have advanced independently of each other. The green advantages of newer extraction methods are widely promoted, but they are rarely placed in the context of the green chemistry principles.

Green chemistry in the chromatography laboratory was recently reviewed and this article described ways to save on solvent consumption, alternatives to using acetonitrile in liquid chromatography (LC), and how to assess the "greenness" of analytical methods (2). These topics will not be repeated in this review.

Green Chemistry in the Planning Stages

A somewhat tongue in cheek, though serious, adage is that "the 'greenest' analytical procedure is that which you do not perform"— but well-executed analytical methods can inherently be green. Following correct sampling theory (as outlined in several undergraduate textbooks), the minimum number of samples and minimum sample size is desired. Samples collected should also support the problem-solving efforts behind the analysis.

One disturbing trend of many modern extraction technologies, that runs counter to the planning of an analysis, is the trend toward miniaturization. Miniaturization is advantageous when used properly, but it seems that many analysts embrace miniaturization because they can, rather than following appropriate sampling theory. Miniaturization may reduce solvent use and waste generation, but it does not address, for example, solvent toxicity.

R.E. Majors recently reported on developments in an article entitled '"Just Enough" Sample Preparation: A Proven Trend in Sample Analysis' (3). Following the examples of "just-in-time" inventory control in total quality management systems, "just-enough" sample preparation balances analytical considerations such as selectivity and quantitative needs with labour, time, and solvent intensiveness. Important "just-in-time" methods are presented in Figure 1. This approach reiterates the importance of understanding the purpose of a given analysis:

"*Minimizing the number of sample handling steps in any analytical technique is desirable since the more times the sample is transferred, the greater the chance of analyte loss (or modification), thereby resulting in poor analytical precision and accuracy,*" — R.E. Majors (3).

Similarly, the appropriate choice of the subsequent analytical characterization technique can drive sample preparation and the overall "greenness" of an analytical procedure. For example: Desorption electrospray ionization (DESI) and

Table 1: Principles of green chemistry. Adapted from reference (1).

1. **Prevention** It is better to prevent waste than to treat or clean up waste after it has been created.
2. **Atom Economy** Synthetic methods should be designed to maximize the incorporation of all materials used in the process into the final product.
3. **Less Hazardous Chemical Syntheses** Wherever practicable, synthetic methods should be designed to use and generate substances that possess little or no toxicity to human health and the environment.
4. **Designing Safer Chemicals** Chemical products should be designed to affect their desired function while minimizing their toxicity.
5. **Safer Solvents and Auxiliaries** The use of auxiliary substances (for example solvents, separation agents) should be made unnecessary wherever possible and innocuous when used.
6. **Design for Energy Efficiency** Energy requirements of chemical processes should be recognized for their environmental and economic impacts and should be minimized. If possible, synthetic methods should be conducted at ambient temperature and pressure.
7. **Use of Renewable Feedstocks** A raw material or feedstock should be renewable rather than depleting whenever technically and economically practicable.
8. **Reduce Derivatives** Unnecessary derivatization (use of blocking groups, protection/deprotection, temporary modification of physical/chemical processes) should be minimized or avoided if possible, because such steps require additional reagents and can generate waste.
9. **Catalysis** Catalytic reagents (as selective as possible) are superior to stoichiometric reagents.
10. **Design for Degradation** Chemical products should be designed so that at the end of their function they break down into innocuous degradation products and do not persist in the environment.
11. **Real-time Analysis for Pollution Prevention** Analytical methodologies need to be further developed to allow for real-time, in-process monitoring and control prior to the formation of hazardous substances.
12.**Inherently Safer Chemistry for Accident Prevention** Substances and the form of a substance used in a chemical process should be chosen to minimize the potential for chemical accidents, including releases, explosions, and fires.

direct analysis in real time (DART) approaches to mass spectrometry (MS) eliminate or minimize the need for sample preparation (4,5,6). In both approaches, the sample (often in solid form) is bombarded by energetic species to create ions of interest. Further development and maturation of these techniques (and those that they have inspired), will contribute to the overall "greenness" of the analytical endeavour.

The use of the biodegradable chelant ethylene diamine disuccinate (EDDS) has been investigated as a substitute for the common ethylene diaminetetraacetic acid (EDTA) in analytical applications (7). Table 2 displays the quantitative results for the isolation of aqueous divalent cations. Although this review focuses on the sample preparation of organics for subsequent chromatographic analysis, these results demonstrate the application of green chemistry principles across a broad spectrum of chemical analysis.

Green Sample Preparation Methods

As emphasized, nearly all of the extraction technologies developed within the past twenty years tend to have green advantages, regardless of whether that was the initial intent. SFE, MAE, accelerated-solvent extraction (ASE), and the use of superheated water for analytical extractions are all discussed elsewhere in this supplement. Table 3 shows a comparison of the green attributes of these, and similar, sample preparation methods (8).

Table 2: Isolation of divalent cations from water with EDDS (7).

Cation	Recovery with EDDS relative to Recovery with EDTA (%RSD)
Ca^{2+}	102.3% (0.32)
Mg^{2+}	96.7% (0.62)
Mn^{2+}	115.3% (0.44)
Zn^{2+}	101.1% (0.19)
Pb^{2+}	110.0% (1.11)

Here, we present a brief overview of analytical extraction technologies with green attributes.

Micro-Liquid–Liquid Extraction: In a review of classical extraction procedures, Murray reported an approach for extracting large liquid sample volumes with a minimal amount of solvent, mixing 980 mL of aqueous sample and 200 µL of hydrocarbon (9). The addition of water through a side-arm forces the lighter organic phase into a capillary tube where it is sampled with a syringe. With three extractions recoveries greater than 90% are obtained. Of course, a highly favourable solute partition coefficient and complete water immiscibility of the extracting solvent are vital to the success of this approach.

Single-Drop (10) and Suspended Microdroplet (11): These related techniques also rely on favourable partition coefficients. However, the single-drop approach may also be used in the headspace mode. Once optimized, these techniques can provide good analytical results.

Sorptive-Based Extractions: Since its introduction in the 1970s, SPE has become almost routine in many laboratories, especially in the pharmaceutical industry. In addition to the extraction selectivity offered by the stationary phase, substantial solvent savings may be made. Refinements and developments of other extraction technologies based on sorbents provide even greater green chemistry advantages. Among the most established sorptive methods that apply these advantages are the use of molecular recognition sorbents (12), SPME (13), stir-bar sorptive extraction (SBSE) (14), and supported liquid–liquid extraction (15).

Membrane Methods: Supported liquid membranes (16) and microdialysis also consume small volumes of extracting solvents for specialized applications.

Extraction Solvent Considerations

Regardless of extraction technique, one key green attribute is solvent use, as directly addressed in the fifth green chemistry principle. There are two approaches to addressing green chemistry concerns from the solvent perspective — minimization of solvent use and the use of "greener" solvents. With the exception of superheated water, each of the extraction techniques discussed in this supplement approaches this by reducing solvent use. However, solvent selection considerations are also important.

Solvent Selection: In addition to the physical-chemical properties of solvents, cumulative energy demand, toxicity, flash point, and similar characteristics should be addressed. For example, hexane is widely used as a nonpolar organic extraction solvent; yet it is the most neurotoxic of the n-alkanes (17) and hexane–acetone mixtures have even greater toxicological effects. Heptane is preferable as a nonpolar solvent. Many major pharmaceutical companies have led efforts to replace solvents. For example: Pfizer recommends the substitution of ethyl acetate, methyl-t-butyl ether, toluene,

Figure 1: Summary of just-in-time sample preparation methodologies and their attributes (3).

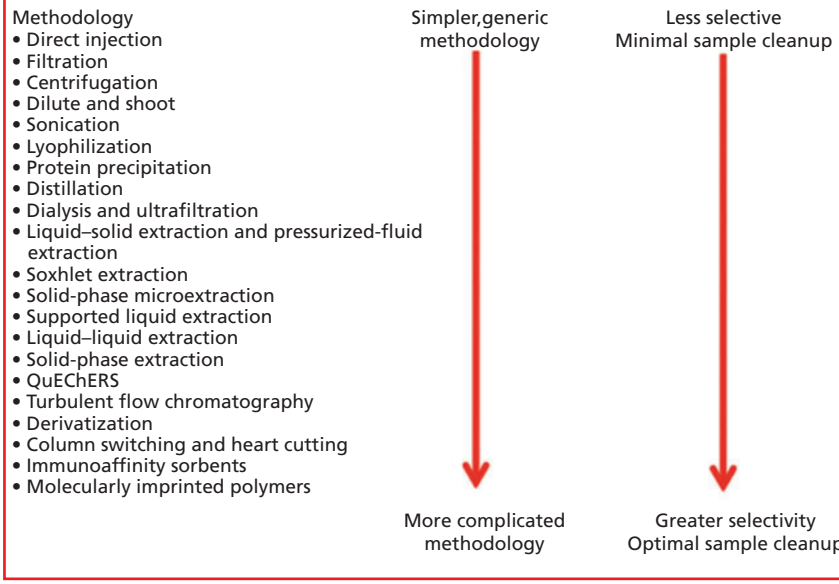

Table 3: Comparison of greenness issues of typical sample pre-treatment techniques used for trace analysis C. Adapted and reproduced with permission from Elsevier (8).

Treatment Method			"Green Issues"	
	Time	**Energy**	**Safety**	**Solvents**
Inorganic				
Dry ashing	6-8 h	High consumption	Very safe	Use of small volumes of diluted acids to solubilize the ash
Wet digestion (open vessel)	Several hours	High consumption	Risk of explosion and spills	Use of large volumes of concentrated mineral acids
Microwave-assisted digestion (closed vessel)	<1 h	Moderate consumption	Risk of explosion, safety at the expense of expensive instrumental equipments	Use of small volumes of concentrated mineral acids
Organic				
Soxhlet	6–24 h	High consumption	Exposure risk to organic vapours	150–500 mL of organic solvents
Microwave-assisted extraction	10–30 min	Moderate consumption	Potential explosion risk with closed vessels	10–40 mL of organic solvents
Supercritical fluid extraction	10–60 min	Moderate consumption	Very safe (high pressure and temperatures)	2–5 mL (solid trap); supercritical CO_2 used as extractant fluid
Accelerated solvent extraction	10–20 min	Moderate consumption	Very safe (high pressure and temperatures)	10–40 mL of organic solvents
Organic and inorganic				
Ultrasound extraction with a bath sonicator	<1 h	Moderate consumption	Very safe, extractions performed at atmospheric pressure and room temperature	Use of moderate volumes of concentrated mineral acids and organic solvents for inorganic and organic analysis, respectively
Ultrasound extraction with an ultrasonic probe	<1 h	Moderate consumption	Very safe, extractions performed at atmospheric pressure and room temperature	Use of small volumes of diluted mineral acids and organic solvents for inorganic and organic analysis, respectively

or 2-methyltetrahydrofuran for dichloromethane during extractions. These industrial efforts have led to the development of several methods to characterize "greenness" of solvents (18,19,20) and solvent guides. These reports also include summaries of computer-aided solvent selection and property tools.

Green Solvents: Several natural product-derived organic solvents are being explored for their green attributes. Jessop has called for the continued investigation into new solvents (21). Among emerging green solvents of interest are d-limonene (22), ethyl lactate (23), γ-valerolactone (24), 2-methyltetrahydrofuran (25), and cyclopentyl methyl ether (26).

Ionic Liquids and Deep Eutectic Solvents: The use of ionic liquids to extract solvents was recently reviewed (27). Emphasis was placed on single-drop microextraction, SPME, dispersive liquid–liquid microextraction (DLLME), hollow fibre-supported liquid membrane extraction, and SPE. While ionic liquids may be tuned for the application, have negligible vapour pressure, and possess high thermal stability, their high viscosity and toxicity (especially of methylimidazolium ionic liquids) render their widespread use somewhat limited. It will be interesting to see what developments occur over the next decade enabling utility of these intriguing solvents.

On the other hand, deep eutectic solvents (DESs) — formed by the interaction of, for example, hydrogen bond donors with quaternary ammonium compounds — offer several unique advantages compared with their ionic liquid cousins. DESs can be an order of magnitude, or more, less viscous, inexpensive, and nontoxic. Natural DES, made from sugars, water, and small acids have been reported as extraction media (28,29,30,31). Their high solubilization power for both polar and nonpolar compounds was demonstrated in the isolation of coumaroyls and other safflower compounds, non-water soluble bioactive natural products, gluten, starch, DNA, and quercetin. The properties of DES comprised of choline, chloride, or acetylcholine chloride with urea or glycerol have also been intensively studied (32). Solvatochromic studies indicated that these DES fall into the category of polar hydrogen bond-donating solvents because of their high $E_T(30)$ or E_T^N values. In addition, the effect of temperature and water content on polarity and Kamlet–Taft parameters of DESs was studied.

Summary

Modern sample preparation technologies grew up alongside, but separate from, green chemistry. However, the environmental aspects featured in these technologies parallel the 12 principles of green chemistry. Recent developments have tended to focus on reducing solvent usage in chemical extractions. Over the next decade or so, advances based on research into green solvents, ionic liquids, and deep eutectic solvents, as well as solvent cumulative energy demand and chemical toxicity will drive the green chemistry attributes of current and future sample preparation technologies.

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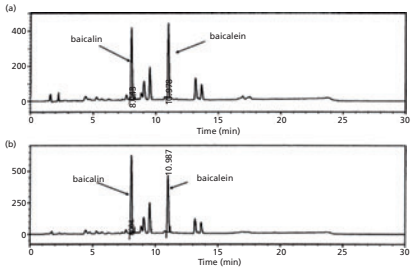

Superheated Water as an Extraction **Solvent in Sample Preparation**

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Pressurized high temperature or superheated water is a green extraction solvent used in food, environmental, and traditional medicine studies for the extraction of non-polar and polar analytes including essential oils and spices, agrochemicals, pharmaceuticals, and petrochemicals. The technique can be used on both the analytical and preparative scale to give a clean, solvent-free product for chromatographic analysis. This article reviews the current status of superheated water extraction, discussing extraction methods, applications, and, briefly, problems encountered with this approach.

Water at high temperature is often forgotten as a potential extraction solvent for sample preparation, yet it can be used in a wide range of applications, including the extraction of polycyclic aromatic hydrocarbons (PAHs) from soil and nutraceuticals from plant materials, for both non-polar and polar analytes. Unlike many other solvents, water is readily available in high purity at minimal cost, with minimal disposal costs and a negligible environmental impact. Its potential as a solvent is the result of it's unique properties when heated — the polarity changes from a polar solvent at room temperature with a specific permittivity of $\varepsilon = 80$ to $\varepsilon = 30$ at 220 °C in a pressurized system, which is comparable to many organic solvents at room temperature, such as methanol $\varepsilon = 33$. Mechanically it is easy to handle because only low pressures (15 bar at 200 °C) are required to maintain the liquid state. Unlike most organic solvents, it presents no danger from ignition or toxicity, and, although it is more volatile than ionic liquids, its vapours are environmentally benign.

Hot water is not often thought of as a typical extraction solvent but it is used every day at 100 °C to extract tea leaves and coffee beans. When we raise the temperature further, the polarity decreases and extraction power increases. From 100 °C up to around 300 °C under low pressures it is referred to as either superheated water, subcritical water, or pressurized hot water and has found application as an extraction solvent, as a chromatographic eluent, and as a solvent for organic synthesis. The recent interest in sample preparation and extraction was aroused in the 1990s by Hawthorne and co-workers who used superheated water as an extraction solvent in environmental studies for the extraction of PAHs from soils (1) and compared it to alternative extraction methods (2). They found that for non-polar analytes the change in extraction power with temperature can be dramatic. For example, the solubility of anthracene, chrysene, and perylene in water increased by 20,000-fold over the range 25–200 °C.

In later studies by a number of groups a wide range of analytes and matrices have been examined and these have been presents in reviews by Smith in 2002 (3); by Kronholm, Hartonen, and Riekkola in 2007 (4); and most recently in 2010 by Teo and co-workers (5) and Smith (6).

Extraction Methods

In common with other solvents, superheated water is most suitable for the extraction of solid samples such as soils, environmental solids, and dried plant material but can be difficult to apply to tissues, impervious food samples, or clinical samples. The equipment (shown in Figure 1) is fairly simple, consisting of a water supply, pump, an oven containing an extraction vessel, and post-oven restrictor to maintain the pressure. Typically, an old gas chromatograph (GC) oven can be used that will have good temperature control up to 300 °C. An empty column or similar configuration can act as the extraction vessel. Extraction can either be performed in a static or dynamic mode. In the latter case, a system as simple as a capillary or narrow-bore tube can provide sufficient restriction and back pressure to maintain the water in the liquid phase in the oven. The solvation power of high temperature water is effectively independent of the pressure, so an accurate control is usually not needed. Some recent extractions have used commercial accelerated solvent extraction systems but these are limited to 200 °C.

After the extraction, the eluent water is cooled to room temperature and if sufficiently concentrated can be analyzed directly either by high performance liquid chromatography (HPLC) or gas chromatography (GC). The extraction can also be linked directly to HPLC or superheated water chromatography, for example, to determine herbicides in compost (7). In that study, a single stream of water steam performed a sequential extraction, fractionation, and chromatographic separation by using temperature control.

For less concentrated samples, analytes can be extracted from the polar aqueous phase and concentrated before an assay using either a small volume of organic solvent, or a solid-phase microextraction (SPME) fibre,

Figure 1: Superheated water extraction system.

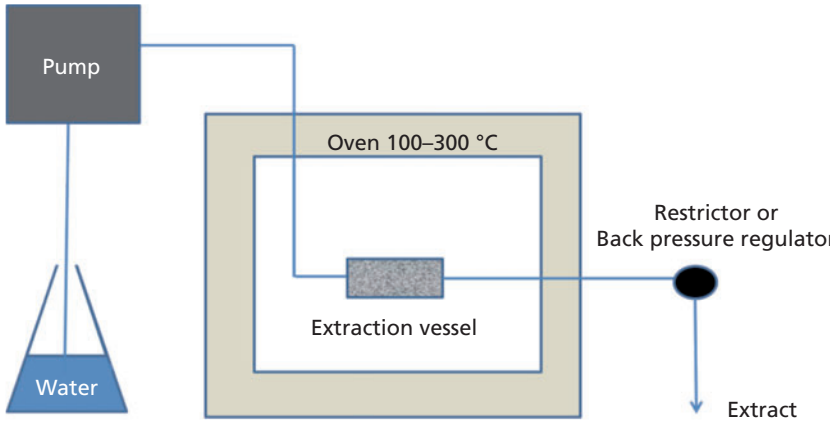
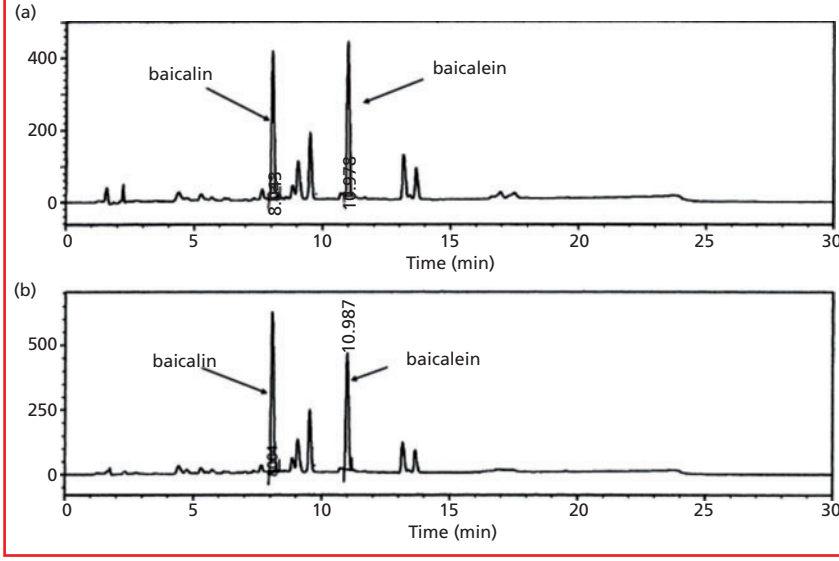


Table 1: Recent examples of the use of superheated water extraction for environmental analysis and related topics.

Analyte	Matrix	Conditions	Ref
Aliphatic hydrocarbons	Petroleum source rocks	250 °C	9
Hydrocarbons	Oil shale	260 °C	10
PAHs	Sediments	100–200 °C	11
Pesticides	Soil and sediment	120–180 °C	12
Neonicotinoid insecticides	Eels	120 °C	13
Parabens	House dust	80 °C	14
Hydroxylated metabolites of PAHs	Sediment samples	150 °C	15
Perfumes, personal care products, and pharmaceuticals	Marine sediments	200 °C	16

Figure 2: Comparison of high performance liquid chromatography (HPLC) chromatograms obtained for baicalin and baicalein in Scutellariae radix by: (a) Soxhlet extraction; and (b) pressurized hot water extraction. Adapted and reproduced with permission from Elsevier (36).



adsorbent disc, stir-bar, hollow fibre, or a solid-phase extraction (SPE) cartridge before assay.

Post extraction derivatization or modification can also be performed in the aqueous solution (8).

Applications

The wide scope of superheated water extraction is shown in the range of environmental, food, and herbal extracts that are listed in the many examples given in the earlier reviews (3–6). Some recent examples from the areas of environmental and petrochemical samples and from the rapidly growing area of food and plant materials illustrate the possible roles for the technique.

These studies include non-polar samples, such as aliphatic hydrocarbons from petroleum source rocks (9) and hydrocarbons from Huadian oil shale in China (10), and more polar PAH metabolites and pesticides (Table 1). Other environmental studies have included the determination of parabens from house dust (14) and personal care products in marine sediments. In a related study, superheated water has been used to release DDE and related compounds, which had been trapped and concentrated on a nanospun fibre (17).

This ability to extract non-polar analytes has also led to the use of superheated water in soil remediation as it does not markedly disrupt the soil structure unlike some alternative techniques. For example, in recent studies Islam and co-workers removed PAHs (18) and lubricating oils (19) from soils; and superheated water has been evaluated for the remediation of pesticide-contaminated soil (20).

The main growth in recent years in superheated water extraction has been for the extraction of plant materials to yield natural products, such as essential oils or nutraceuticals (Table 2). Although similar to other methods that can be slower, such as steam distillation or solvent extraction, the proportions of polar and non-polar compounds can differ, altering the quality of the extracts. In a recent study by two-dimensional gas chromatography (2D–GC), the essential oil from *Rosa damascena Mill* obtained by superheated water extraction was compared with steam distillation and direct thermal desorption (27). With the growth of interest in the identification of traditional Chinese medicines and related products, it has become

Table 2: Recent examples of the application of superheated water to the extraction of plant materials.

Analyte	Matrix	Conditions	Reference
Terpenoids	*Zingiber cassumunar*	140 °C	21
Bioactive species	Chrysanthemum	140 °C	22
Essential oil	*Origanum micranthum*	100–175 °C	23
Essential oils	Camomile *Matricaria Chamomilla* L.	100, 125, 150, and 175 °C	24
Essential oils	*Thymus vulgaris* L.	125 °C	25
Essential oils	Cinnamon bark and leaves	200 °C	26
Essential oil	*Rosa damascena* Mill	150 °C	27
Phenolic and flavouring compounds	Cinnamon bark	150, 200 °C	28
Phenolic compounds	Pomegranate	100–220 °C	29
Curcumin	Turmeric	197 °C	30
Alkaloids	Goldenseal	140 °C	31
Alkaloids	*Sophora flavescens* Ait	79–190 °C	32
Anthraquinones	Roots of *Morinda citrifolia*	150–200 °C	33
Bioactive compounds	*Gastrodia elata* Blume	60–120 °C	34
Flavanoids	Various plants	150–170 °C	35

increasingly important to identify the composition and sources of the constituent plant materials. Superheated water extraction was found by Ong and co-workers (36) to give comparable characteristic biomarkers to conventional Soxhlet extraction (Figure 2).

As well as analytical sample preparation for chromatographic analysis, many of these applications have been used in large-scale extraction processes of materials, such as onion skins (37), to provide potential commercial products. These applications often include HPLC or GC assays and have been reviewed by Mustafa and Turner (38), Chemet and co-workers (39), Mukhopadhyay and Panja (40), and Shi and co-workers (41). One advantage is that the absence of an organic solvent means that potential issues associated with solvent residues are avoided, particularly important if the product is intended for human consumption.

Because superheated water extraction is generally unsuitable for clinical tissues or aqueous samples, it has not been widely applied in the pharmaceutical area. However, Wang and co-workers found that water at 100 °C could be used to extract oxytetracycline, tetracycline, and chloramphenicol antibiotics from animal feeds for HPLC analysis (42). In another study Murakami and co-workers used superheated water up to 225 °C to rapidly extract fluoxetine-hydrochloride from capsules for HPLC analysis (43). Superheated water can also be used for inorganic analysis and Morado Piñeiro and co-workers determined arsenic and arsenic species in scalp hair by using superheated water extraction followed by liquid chromatography coupled to inductively coupled plasma mass spectrometry (LC–ICP–MS) (44).

Problem Areas

One concern that is always present when high temperatures are used is the potential degradation of analytes, but there are few reported examples. Although some degradation of hydrastine and berberine was observed by Mokgadi and coworkers (31) during the extraction of Goldenseal at 160 °C and 180 °C, this was not an issue at 140 °C. Leppänen and co-workers reported that at 240 °C, cellulose in Norway spruce samples could break down and be extracted (45) but not at lower temperatures.

Conclusion

Superheated water extraction between 100 °C and 250 °C can provide an environmentally friendly method of extraction for both polar and non-polar analytes from a wide range of matrices, more rapidly than Soxhlet methods, and without the problems of flammable and often toxic organic solvents.

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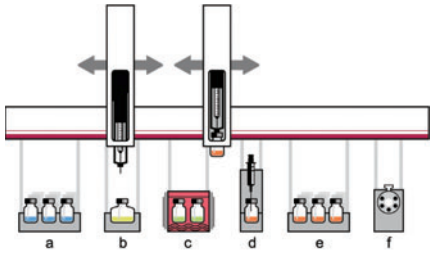


0Sample Preparation for Chromatography: How Much Can Be Automated?

Edward Pfannkoch, Gerstel Inc., Maryland, USA.

Modern autosamplers and workstations possess a range of capabilities, in addition to simple liquid handling, that allow automation of sample preparation steps traditionally performed manually. Scientists in many laboratories are considering automating labour-intensive sample processing steps — such as weighing, filtration, dilution, solid-phase extraction, evaporation, and reconstitution. Most quantitative methods require preparation of calibration standards that can be as time-consuming as preparing the samples. This article reviews the automation capabilities available and some practical considerations to take into account when choosing to automate some or all of the sample preparation and handling steps that must be done before analysis.

Those of us who experienced the early days of high performance liquid chromatography (HPLC) may remember manual injections and strip-chart recorders on chromatographic instruments. In the mid-1980s rapid development of autosamplers, integrators, and personal computers (data systems) greatly simplified sample preparation and contributed to the development of modern automated chromatographic instrumentation.

Fifteen years later, in 1995, an article published in *LCGC North America* (1) provided insights from experts regarding sample preparation for chemical analysis, and predictions regarding where developments would head over the next decade. Increasing sample loads, the need for higher productivity in the face of decreasing analyst skill levels, and increasing issues with worker safety, regulatory constraints, and information management were all listed. Sound familiar?

Noteworthy were consensus opinions on the need for rugged and reliable automation systems that could be automated. It was agreed that future trends would include sample miniaturization and reduced solvent usage. Also, experts agreed that pressure from industry programmes and government agencies could play a significant role in stimulating development. Another major theme was the need for better, more universal communication between instruments to allow automation devices to communicate with any vendor's chromatography platforms.

Opinions differed regarding the importance of integrated (serial) sample processing versus batch processing, and the need for flexible (user-defined) robotic systems that provide a set of "tools" that could be customized versus specific systems designed to provide automated defined solutions. It was stated that developments in detection technology, particularly in the area of liquid chromatography–mass spectrometry (LC–MS), might reduce the need for extensive sample preparation.

A later article (1998) (2) on sample preparation for LC–MS–MS emphasized automation of three common procedures — dilute-and-shoot, solid-phase extraction (SPE), and liquid–liquid extraction — as well as post-injection techniques — column switching and on-line SPE— to manage the sample matrix. The authors also recognized the emerging need for "massively parallel" sample preparation and analysis driven by modern drug discovery practices such as combinatorial chemistry.

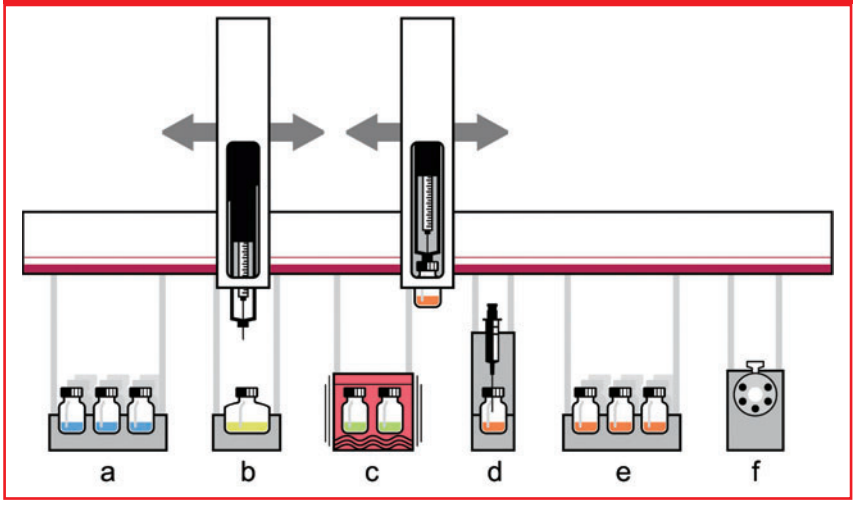
This review addresses the following questions: What driving forces are stimulating the continued development of the automation of sample preparation for gas chromatography (GC) and HPLC today? Where do we stand regarding current automation capabilities? What are the next developments on the horizon?

Defining Sample Preparation

One of the most formidable challenges to standardization and automation in the analytical laboratory is the broad range of sample types. Samples can be gaseous, liquid, solid, or a mix of phases (for example, biological tissues). Some sample types lend themselves to relatively straightforward collection of homogeneous samples — for example water, some liquids, and air samples. Other solid sample types, such as whole fruits and vegetables or production batches of pharmaceuticals, require physical homogenation of larger representative samples before reducing the sample size to a more easily manipulated volume. This article will address only the automation of processing steps for small, homogeneous, or homogenized samples.

Samples are often intact prior to sample preparation, but there are a few examples of sample collection devices that perform partial sample preparation as part of the process of collecting the sample. Air sampling onto adsorbent tubes concentrates analytes and simplifies transport. Similarly, solid-phase microextraction (SPME) (3) and stir-bar sorptive extraction (SBSE) (4) concentrate analytes onto extraction phases on fibres or stir bars, respectively, that can stabilize

Figure 1: xyz-robotic autosampler configured for automated solid-phase extraction (SPE) before high performance liquid chromatography (HPLC) injection: a) sample vial storage, b) standards, c) incubation and agitation, d) SPE, e) eluate storage, and f) liquid chromatography (LC) instrument valve.



the analytes and facilitate transport. Although typically performed manually, these methods simplify downstream sampling and analysis, and can be automated with relative ease.

Automating Sample Introduction

Virtually all manufacturers of chromatographic instrumentation provide some type of automation for sample introduction into their systems and users expect this capability as a standard offering. Numerous companies also manufacture devices for sample introduction that can be interfaced with a variety of chromatographic platforms. Basic engineering decisions made about the core design of these systems often defines whether they are dedicated or flexible; compact or expandable; push-button-intuitive; or user-programmable. It is not possible to review all available instrumentation for sample preparation here. Instead, features of representative commercially available instruments are described. Given that upgrades and new designs are constantly released, this review will provide a snapshot in time of this continuously evolving field.

Modern autosamplers used for sample injection already include some sample preparation. Liquid injections, used almost universally for injections into LC and GC instruments, may not normally be considered sample preparation. However, when coupled with a programmed temperature vaporizing (PTV) inlet in a GC system, an autosampler can perform large volume injection (LVI) eliminating manual sample concentration or evaporation (5).

Likewise, autosamplers for headspace injection techniques — whether dedicated units (the Agilent 7694E or the PerkinElmer TurboMatrix) or flexible syringe-based instruments such as those based on the CTC PAL platform (CTC Analytics) — already transport, thermostat, and reproducibly transfer samples from the vial headspace into the GC inlet eliminating manual handling steps and interference from nonvolatile sample matrices. More specialized samplers and accessories from suppliers such as Dani, Tekmar, EST Analytical, OI Analytical, Gerstel, and others can automate dynamic headspace sampling techniques.

SPME is another automatable GC injection technique that includes sample preparation. A number of SPME innovations have been automated including:

- Pre- or post-extraction derivatization: The autosampler inserts the SPME fibre into a vial containing reagent either before or after exposure to the sample.
- Hot injection and trapping (HIT): The SPME fibre is inserted into a thermal desorption unit mounted above a cold inlet that allows refocusing of the analytes improving peak shapes for early eluting compounds.

New multi-fibre exchange modules for the CTC-type sampler use Supelco's new Fast Fit Fibre Assemblies (developed jointly with Chromline srl Prato) designed for easier field sampling and offer barcode labelling and stable transport after sampling. Modules are available that offer three preconditioned SPME fibres, or alternatively a fibre can be picked from a tray (preloaded in the field) and automatically desorbed into the GC inlet.

Thermal introduction techniques for GC (thermal desorption and thermal extraction) also provide some automated sample preparation capabilities. Thermal desorption is the process of heating a sampling device containing a sorbent medium, such as an air sampling adsorbent tube or phase-coated stir bar, on which analytes have been trapped and concentrated. Thermal extraction involves directly heating a solid sample in a tube under a gas stream to extract and concentrate the volatiles on an integral secondary trap (available from Markes International, Frontier, Perkin Elmer, or CDS Analytical) or in a cryogenically cooled inlet (Gerstel) from which they are transferred directly to the GC column. Direct thermal extraction requires small sample sizes (5–100 mg) of ideally finely divided powder or thin films, but can eliminate sample preparation steps such as liquid extraction and filtration. Accessories are available (from Gerstel and CDS Analytical) to automate gas switching during thermal extraction or pyrolysis (to allow heating in the presence of air for oxidation studies or addition of reagents to perform thermochemolysis (Gerstel, Frontier), for example to study lignin structure (6).

Dedicated accessory instruments are available for automating some types of sample cleanup and extraction as part of HPLC injection. Figure 1 shows a diagram of an autosampler platform with accessories for automated SPE using a disposable cartridge format. On-line SPE is available in a variety of formats and is used for rapid preconcentration and cleanup of samples. Valco Instruments lists a number of valving configurations, with formats ranging from regenerated and reused SPE cartridges configured as part of the sample loop to multivalve column switching systems such as those offered by Cohesive Technologies (Thermo Fisher Scientific). Alternatively, systems are available from Spark Holland and Gerstel that automatically exchange disposable

Table 1: Typical features of software available for sample preparation.

General Features
Controls multiple instruments from one computer
Interfaces with multiple chromatography data systems
Compatible with LIMS
Logfiles for traceability
21 CFR Part 11 capable
User-friendly, Microsoft Windows or icon based programming
Simplified command lists based on hardware configuration
On-line help menus and prompts
Integrated testing and debugging of program steps
Automated error notification
Graphical display of sample processing schedule
Running methods and sequences that can be edited
Multiple programmable inputs and outputs available
High-throughput capabilities (prepare ahead, parallel preparation)

SPE cartridges placed in-line between the injection valve and the analytical column.

The need for rapid and widespread screening of blood samples has stimulated the development of methods for collecting dried blood spots (DBS) on filter paper cards. This technology was reviewed recently (7) and offers less invasive, cost-effective sample collection, and small sampling volumes. Also, when dry, samples are more stable and pose less risk to analysts handling the samples compared to liquid samples. Currently, the technique is mostly manual or semi-automated by punching out blood spots from the DBS cards. However, new instrumentation that extracts the spots on the card for further workup and analysis has been introduced. It will be of interest to see if major regulatory bodies will endorse this new form of blood sample handing.

Expanding Autosampler Capabilities

Samplers configured for headspace or SPME injection are typically preprogrammed with standard injection cycle options with few additional automation options. However, the capabilites of modern autosamplers for liquid injection can often be expanded. Samplers designed for GC injection typically use a single small (5–10-µL) syringe limiting the volume range that can be manipulated for other reagents, but they may still be able to automatically add internal standards to sample vials. Samplers injecting into HPLC valves using the loop overfill method, or GC autosamplers performing LVI, may be able to use larger syringes to add relevant volumes of internal standards, derivatizing reagents, or sample modifiers (salts, acids, or bases) as part of the automated sample processing steps.

Versatile Prepstations

In the past 10 years robotic xyz samplers capable of GC or HPLC injection have become the industry standard, although lower-cost dedicated autosamplers are still quite common. The evolution of robotic samplers has been driven by the addition of specialized hardware modules, and the development of versatile, user-friendly software. One of the first steps in this development was to mount two robotic samplers one above the other (referred to as a "twin" or "dual-rail" configuration). CTC Analytics then more recently created a longer autosampler platform (PAL-xt) to accommodate more accessories and, soon after, integrated two injection units into a single rail xyz sampler. Two different syringe types can be mounted simultaneously, allowing a wider range of sample processing options. This configuration has been used to automate a variety of headspace GC methods with liquid internal standard or reagent addition including blood alcohol (8), and cyanide in drinking water (9).

Alternatively, specialized liquid delivery modules are available that can deliver millilitre volumes of a dilution solvent. A configuration using a dilutor module from CTC Analytics was used to automate ASTM standard method D6584-07 for biodiesel (10). The widely practiced dilute-and-shoot technique used in LC–MS–MS to reduce interference and ion suppression can also be automated by using autosamplers capable of handling both larger diluent and smaller microlitre volumes.

Automated manipulation of the sample is not the only benefit provided by these flexible prepstations. The additional liquid-handling capability has been routinely used for serial dilution and automated preparation of calibration standards for liquid, headspace, and SPME injection methods (11,12). This is particularly beneficial to automating matrix-matched standard preparation — blank sample matrix samples are processed at the same time as test samples, before being spiked with standards. For example, automated extraction and analysis of pesticides using the QuEChERS (quick, easy, cheap, effective, rugged, safe) approach using matrix-matched standards improved quantification (13).

The proliferation of flexible xyz robotic autosamplers created the need for software control that was user-programmable so that customized processing steps could be created by the analyst. Firmware and software provides the initial framework and tools for customization of autosampler actions. For example, CycleComposer software (CTC Analytics) provides detailed control features enabling the analyst to create customized actions, but requires an understanding of the underlying macro language. Instrument manufacturers subsequently provided drivers or other integrated control of these samplers within their proprietary chromatographic platforms, but most of the embedded control is for standard injection modes, with relatively little flexibility.

There is clearly a need for user-friendly, flexible software control of customizable autosampler platforms that can communicate effectively with a variety of different chromatographic platforms such as ChemStation (Agilent Technologies), MassHunter (Agilent Technologies), Analyst (AB Sciex), Xcalibur (Thermo Fisher Scientific), Millenium (Waters Corp.), and ChromaTOF (Leco). Third

Table 2: Sample preparation hardware modules available for several platforms.

Mixing (orbital, stirring, high speed vortex)
Headspace (static, dynamic, purge-and-trap, full evaporation)
SPME fibre exchange
Heating (conventional, microwave)
Cooling (Peltier, circulator)
SPE (standard cartridge, ITSP microcartridge, 96-well plate)
dSPE (vial format, disposable pipette extraction)
Thermal desorption or extraction (SBSE, adsorbent tube, solids, pyrolysis)
Evaporation or solvent exchange
Filtration
Barcode reading
Ultrasonication
Weighing
Centrifugation

party instrument distributors focused on front-end sample automation may be able to meet this need. Typical software features currently available on some of these instruments are listed in Table 1. For example, Maestro software (Gerstel) provides a user-friendly Microsoft Windows-based programming interface to control the CTC autosampler platform. Standard injection modes are programmed with on-screen help and prompts for recommended parameter ranges. A list of typical sample preparation modules from a variety of vendors is provided in Table 2.

Steps To Automate

Highlighted here are a few simple steps that can be automated and that are likely to become much more commonly integrated into routine automated operations. Coupling sample preparation with GC analysis was recently reviewed in depth (14).

Liquid–liquid extraction is often used before injection, and when large sample volumes are not needed, in-vial liquid–liquid extraction may become more common. Mixing devices are available to achieve efficient extraction that can achieve orbital mixing speeds of 750 to 3000 RPM. When smaller (2-, 4-, or 10-mL) vials are used the extracting organic solvent may be more or less dense than the aqueous phase, and the layer to be injected onto the instrument can be selected by adjusting the penetration depth of the autosampler syringe needle. When larger (20-, 40-, or 100-mL) vessels and dense solvents are required, the sampler must allow liquid to be withdrawn from the lower layer. One alternative approach is membrane-assisted solvent extraction (MASE) that uses a small polypropylene bag filled with the extraction solvent suspended in the vial (15). Dense solvents can be used because the bag holds the liquid in a position easily accessible by the autosampler syringe.

Evaporation and solvent exchange are common procedures to dissolve analytes into a solution compatible with the HPLC eluent. Single- and multi-position evaporation modules are available on some instruments allowing full automation through serial- or semi-batch processing.

Filtration is often the final step when manually processing samples for injection, particularly before HPLC or ultrahigh-pressure liquid chromatography (UHPLC), to prevent blocking the narrow orifices in valves and connection tubing. Particulates may already be present in samples or may form after experimental steps — in this instance, pH adjustment, solvent addition, various digestion procedures, or even the simple dilute-and-shoot approach. In-vial devices are available (Whatman, Thomson) that allow manual filtration of the sample as it is placed into the vial. An accessory is available from Leap Technologies that allows the CTC autosampler to perform the filtration with these devices. Filtration using conventional syringe filters can also be automated, although care must be taken to ensure particulates do not contaminate the autosampler syringe or needle.

The newest autosampler platform from CTC Analytics is known as the PAL RTC, or Robotic Tool Change system. In addition to automating standard sample introduction capabilities — liquid, headspace, and SPME — the RTC can automatically exchange tools (in this instance, syringe types) enabling a single injection unit system to carry out multiple functions without being limited by one single syringe type. As new autosampler platforms enter the market new software control will be needed, but this will take time.

A number of sample preparation steps required immediately before sample injection can now be automated, allowing “just in time” sample preparation. For this to be efficient, sample preparation steps must be completed during the chromatographic separation of the previous sample, to prevent automated sample preparation from becoming a bottleneck and limit throughput.

Some software (Gerstel Maestro) is capable of automatically overlapping or grouping sample preparation steps to maximize throughput without the need for cumbersome analyst programming. For example, automated saponification and esterification for analysis of fatty acid methyl esters (FAMES) and pain management drug screening in urine have been automated (16,17).

Standalone Workstations

A number of high-throughput analysis methods are being developed based on fast GC, HPLC, or UHPLC where the analytical runtime is too short to allow efficient coupling of sample preparation steps. For example, high throughput LC–MS–MS analyses may be completed in a couple of minutes. Automated sample preparation supporting these types of analyses have therefore been taken off-line.

Off-line automation allows interfacing with equipment or processing steps that are currently incompatible with the chromatographic system. Application examples include high-speed centrifugation, or weight verification after liquid transfer using a balance. Weighing solids and powders typically requires more sophisticated robotic modules. Sometimes the sample load for one analysis type may not justify the expenditure for automation, but a single workstation platform automating several sample preparation procedures,

potentially "feeding" several different analytical instruments, may fit into a laboratory workflow.

The Agilent 7696A Workbench is an example of a standalone sample preparation platform designed to provide flexible liquid handling and some additional features including heating, cooling, mixing, and weighing. The system is based on handling small volumes in 2-mL vials reducing solvent use and waste generation; but may require modification and revalidation of the analytical method to accommodate the reduced sample size. The Agilent Workbench has been used to miniaturize and automate liquid–liquid extractions of drugs in plasma, perform serial dilutions to prepare standards for calibration, and fatty acid methyl ester (FAME) analysis in biodiesel (18,19).

Gilson offers a number of systems for off-line liquid handling and SPE. The Gilson GX-281, for example, is based on a syringeless pump design and can function as a standalone workstation or can be coupled to an HPLC instrument by incorporating a proprietary injection valve in the configuration. It is designed to handle samples in a variety of test tubes or scintillation vials, offering automated valve control to access up to five solvent reservoirs and six rinse solvents, and all flowpaths are constructed of biocompatible materials. With additional accessories, tubes can be moved to a compatible balance option. Other models such as the GX-274 allow automated positive pressure elution SPE in 1-, 3-, and 6-mL cartridge formats.

Finally, there is a class of dedicated robotic systems designed for either complete automation of a specific procedure or for high-throughput, parallel processing of small sample volumes, often in a 96- or 384-well plate format. Tecan, Tomtec, and Zinsser NA provide a range of robotic systems for liquid handling. Dedicated, robotic workstations are available from PerkinElmer (Janus workstation), Agilent, and others. Teledyne Tekmar has developed the AutoMate-Q40 for complete automation of the QuEChERS extraction for pesticides from fruit, vegetables, and other foodstuffs. With this system, homogenized sample is prepared and appropriate amounts of sample (typically 15 g) in 50-mL conical centrifuge tubes are loaded onto the AutoMate-Q-40. The automated system adds solvents, internal standards, and solid salts, performing all mixing and centrifugation steps. A portion of the upper acetonitrile layer is removed automatically and added to a tube containing dispersive SPE sorbent that is mixed and centrifuged. A portion of the cleaned-up extract is then transferred to a final sample tube from which it can be placed into a sample vial for GC–MS analysis.

Conclusion

The key themes cited by the experts almost two decades ago have largely been realized in modern instrumental sample preparation methods. We now use smaller samples and less organic solvent and handle a wider variety of tasks automatically with instrumentation providing better traceability and communicating more universally with different vendor's platforms. In the areas where the experts envisioned parallel paths, we have seen parallel development, with the growth of both serial "just in time" processing and batch type "workstation" automation systems, as well as the development of both dedicated analyzers and flexible, customizable platforms to meet quickly evolving needs.

One clear challenge is to develop flexible software tools that can keep up with changing computer operating systems and chromatography platforms. There is a need for software to take advantage of the hardware capabilities available, combining them in unique ways to address emerging issues in the analytical laboratory.

Pressure to increase capacity to screen more samples faster and more accurately continues from a number of areas (such as pharmaceutical, food and beverage, medical, and biological). High-throughput and fast analyses are likely to require more development in standalone workstations that can prepare samples off-line. Automation is unlikely to replace all manual sample preparation in the laboratory, but is likely to become an increasingly common part of modern methods.

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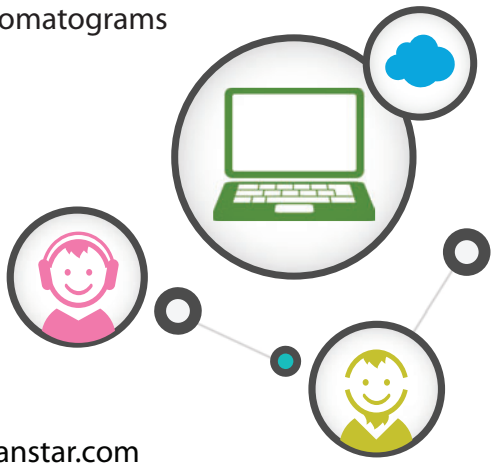

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