White Paper 70632

Use of Accelerated Solvent Extraction with In-Cell Cleanup to Eliminate Sample Cleanup During Sample Preparation

Aaron Kettle
Thermo Fisher Scientific, Sunnyvale, CA, USA

Executive Summary

Accelerated solvent extraction is a sample preparation technique that greatly reduces the amount of time and solvent required to achieve analyte extraction. Use of accelerated solvent extraction with in-cell cleanup can eliminate the need to use gel permeation chromatography (GPC) cleanup procedures and further decrease the amount of time and labor required for sample preparation.

Keywords

Accelerated solvent extraction, ASE, sample preparation, liquid-solid extraction, gel permeation chromatography, GPC, in-cell cleanup, Soxhlet, microwave-assisted extraction, chromatographic interferences

Introduction

Analytical techniques in the environmental and food safety industries are often times preceded by cumbersome and labor-intensive sample preparation procedures. The typical sample preparation workflow is comprised of extraction using a device such as a Soxhlet apparatus, cleanup using GPC, and evaporation to concentrate the sample. Each step involves a manual transfer of sample and increases the likelihood that laboratory error will affect the analysis. These sample preparation techniques have demonstrated to account for greater than 60% of all laboratory error. [25,26]

In 1996, the Thermo Scientific™ Dionex™ ASE™ Accelerated Solvent Extractor system was introduced to the market and revolutionized extraction by using elevated temperature and pressure in an automated system. The rate of extraction was greatly enhanced and the % recovery of analytes consistently increased over traditional techniques such as Soxhlet. Recently, advances in using the Dionex ASE systems have demonstrated the selective removal of interferences during the sample extraction and have thus combined the extraction and cleanup into a single step. Through use of both accelerated solvent extraction and in-cell cleanup, analytical laboratories can further boost sample preparation productivity and further minimize laboratory error.

Thermo
SCIENTIFIC

Accelerated Solvent Extraction Overview

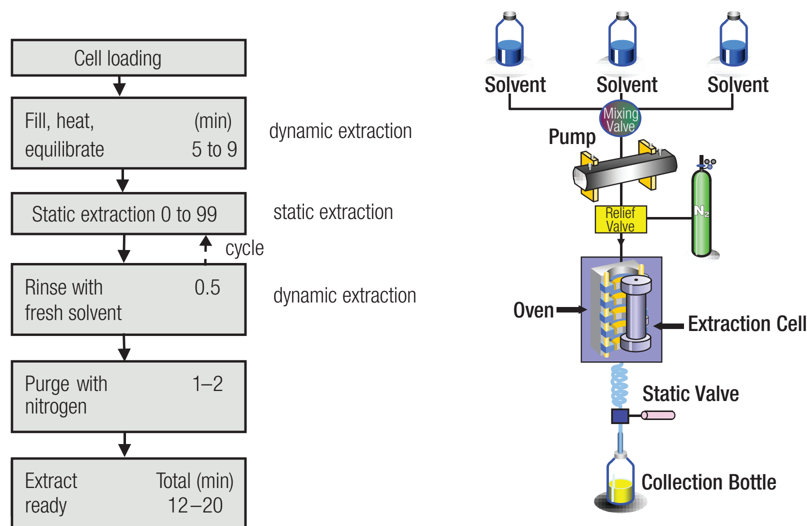

Figure 1. Accelerated solvent extraction process.

Accelerated solvent extraction is an automated extraction technique that uses elevated temperature and pressure to achieve extraction from solid and semi-solid matrices in very short periods. For example, 10 g of sample can be extracted in less than 15 minutes using less than 15 mL of solvent. To perform an extraction, the solid sample is loaded into a sample cell (1 to 100 mL) and the end caps are tightened by hand onto the cells. The filled sample cells are loaded onto a cell tray and collection vessels are loaded onto a collection tray. A robotic arm transfers each cell separately into the oven for extraction. The oven is maintained at the selected operating temperature throughout the extractions. The extraction cell design allows operation of the extractions at elevated pressures (1500 psi) to maintain the solvents as liquids at temperatures above their boiling points. The temperature and pressure are controlled independently for each cell regardless of the solvent used, the moisture or mineral content of the sample, or any characteristic of the matrix that might affect the actual extraction temperature. This is an advantage when compared to microwave extraction, in which the actual pressure and temperature of the extraction are influenced strongly by the above-mentioned sample parameters. Once the cell is placed in the oven, the pump immediately begins to deliver the solvent of choice to the sample cell. Single solvents or premixed solvents can be used from a single collection vessel, or any combination of up to three different solvents can be programmed.

Once solvent has made its way through the sample cell and reaches the collection vessel, the static valve closes to allow pressurization of the cell. Since the solvent expands as it heats, the pressure in the cell will increase when the static valve closes. When the pressure reaches 200 psi above the set point, the static valve rapidly opens to relieve the pressure and then closes again. The pump also delivers fresh solvent to the cell in an effort to return the pressure to the set point value. This addition of fresh solvent during accelerated solvent extraction is analogous to fresh solvent dripping down from the condenser onto the extraction thimble during Soxhlet extraction. After the static time, fresh solvent is pumped through the cell to remove extracted analytes while the sample and solvent are still hot. The user can select the number of times the sample will be in the static mode, and enter this as the number of static cycles.

Following the final solvent rinse, solvent is purged out of the cell. The total time for the extraction is usually less than 15 minutes and the amount of solvent used is approximately 1.5 times the volume of the sample cell (for example, about 15 mL for a 10 mL cell). The extracts are delivered to the collection vessels through a filter, and in many cases do not need any additional preparation prior to analysis. Since the extract is diluted by the total volume of extraction solvent plus the rinse solvent, a further concentration step (evaporation, solid-phase concentration step (evaporation, solid-phase extraction, etc.) may be required when performing trace analysis. Upon completion of the purge step, the cell is returned to the tray and the next sample is taken to the oven to begin the extraction process again.

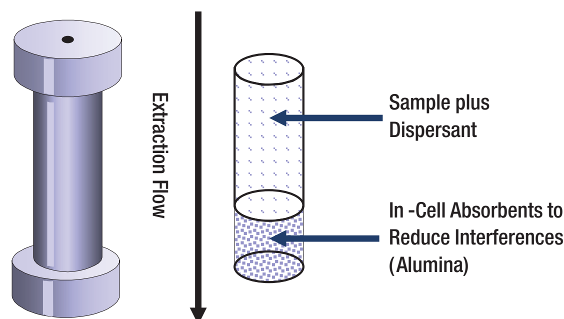

Figure 2. Schematic of selective extraction using accelerated solvent extraction.

The extraction cells used for the Dionex ASE systems are available in stainless steel or zirconium. Stainless steel is the standard for most extractions however; the zirconium cells will accommodate samples pretreated with acids or bases. Each cell type offers a flow through pathway that accommodates the addition of filters for in-line filtration and dispersants for samples with particulate matter. Additionally, various resins can be layered in the extraction cell to retain interferences, providing a clean extract that is ready for analysis and requires no post-extraction cleanup. The process of adding these resins is referred to as in-cell cleanup and is used to increase the selectivity of the accelerated solvent extraction.

Selective Extraction Using Accelerated Solvent Extraction

Selectivity in extraction is defined as the ability to extract compounds of interest with little or no interfering coextracted compounds. Accelerated solvent extraction is generally considered to be an exhaustive extraction technique, and often the extracts obtained from complex samples contain compounds that can interfere with the determination of the desired analytes. Selectivity in accelerated solvent extraction could come from the manipulation of the extraction conditions to minimize coextractables while maximizing analyte recovery. There are three basic procedures to obtain selective extraction in accelerated solvent extraction or, in other words, to generate extracts that contain the compounds of interest and few, if any, interfering compounds. The three techniques are choice of temperature, choice of solvent, and use of adsorbents in the sample cell. Of course, the most powerful method is to use variations of all three to fine-tune the selectivity during the accelerated solvent extraction process.

The choice of temperature alone can affect selectivity. The higher the temperature for the extraction, the less selective the results. Lowering the temperature will make accelerated solvent extraction more selective, but the recovery of analytes can diminish unless the time is increased. Similarly, selectivity is decreased when using more polar solvents. However, an analyst can use a series of solvents of increasing polarity to obtain selective extractions or "fractionation". For example, dark-colored fruits such as blueberries are being studied for their antioxidant content. These compounds are polyphenolic in nature and require polar solvents for extraction. However, if the fruit sample is extracted with a polar solvent like methanol, acetonitrile, ethanol, or water, the resulting extract contains many compounds that make the analysis for antioxidant compounds more challenging. By using accelerated solvent extraction, the samples can first be extracted with nonpolar solvents likehexane or DCM to remove unwanted wax compounds. Then, by extracting the same sample with solvents of increasing polarity and collecting the fractions in separate vessels, extracts are obtained that are easier to analyze. Figure 3 is a photo of extracts obtained from a single sample of wild blueberries extracted with hexane, followed by DCM, ethyl acetate, acetonitrile, and then ethanol. Clearly, using a fractionation procedure like this can offer advantages when analyzing extracts of plant materials that can contain several hundred compounds of interest.

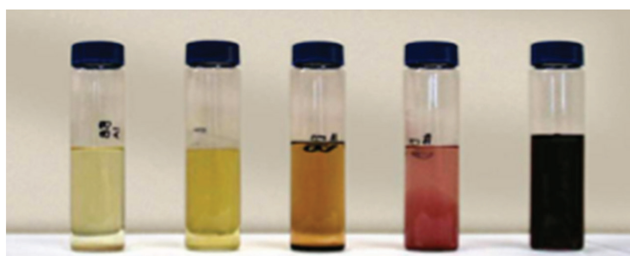

Figure 3. Selectivity in accelerated solvent extraction. The same sample of blueberries was extracted with (from left to right) hexane, DCM, ethyl acetate, acetonitrile, and ethanol.

The use of sorbents in the sample cell along with the sample has offered some of the highest level of selectivity in accelerated solvent extraction. Typically, the adsorbent is loaded into the sample cell first (outlet end) and the sample is loaded on top of the adsorbent. This way, the flow of solvent during the extraction is such that unwanted compounds are retained in the cell by the adsorbent. Francis et al.[1] first reported the use of alumina in sample cells to retain lipids when extracting PCBs from fish tissue using accelerated solvent extraction. They reported that, as the temperature of the extraction goes up or as the polarity of the solvent increases, the capacity of the alumina to retain lipids decreases. For example, if hexane or heptane is the extraction solvent, heat activated alumina will retain about 70 mg of lipid per gram of alumina. If DCM is used, then the capacity is only about 35 mg lipid/g of alumina. If the correct amount of sorbent is used, then extracts can be produced using accelerated solvent extraction that requires no additional post-extraction treatment other than volume adjustment. The most common use of adsorbents in accelerated solvent extraction has been for the extraction and determination of nonpolar compounds such as PCBs, OCPs, PCDDs, and PCDFs in high lipid content matrices such as food or animal tissue. Other adsorbents that have been used include copper, C18 resin, Florisil, silica gel, acid-impregnated silica gel, and ion-exchange resins. Table 1 shows a summary, with related references.

Table 1. List of adsorbents used in accelerated solvent extraction.

Adsorbent	Uses	References
Silica	Removes nonpolar lipids	2
Florisil	Removes nonpolar lipids	3,4
Alumina	Removes nonpolar lipids and colored compounds	1,5,6
C18 resin	Removes nonpolar lipids	7,8
Ion-exchange resins	Removes ionic interferences	9
Copper powder	Removes sulfur	10,11
Carbon	Assist with purification of PCDDs, PCDFs, and coplanar PCBs	12,13

There are a few interesting things to note from this table. Acid-impregnated silica gel has the highest capacity for lipid retention of all the adsorbents that were reported: It has roughly double the capacity of alumina. The sulfuric acid is typically present on the silica at 40% (wt/wt). Copper powder (cleaned with HCl first) can be used to retain sulfur when extracting sediment samples. Gentili and colleagues[14] reported an interesting use of a C18 adsorbent. In this case, they were extracting polar antibiotics (sulfonamides) from animal tissues. They put C18 material in the outlet of the cell to retain some of the lipids that extracted with the analytes. Then, the samples were placed in a freezer to allow separating and hardening. The samples were centrifuged and aliquots were removed and analyzed. This is a case in which the analytes were polar, the solvent was polar (water), and the matrices (baby food and meat) were polar. This is in contrast to several publications in which the analytes were relatively nonpolar (PCBs, PCDDs, OCPs, etc.) and the solvents were nonpolar (like hexane). In this case, the adsorbents have higher capacity than when the extraction solvent is polar.

Ion-exchange resins can be used to remove unwanted ionic species.[9] For example, Thermo Fisher Scientific has been involved in projects to determine perchlorate in soils and vegetation samples. Water at 80 °C was used as the extraction solvent. Alumina was used to remove colored compounds such as chlorophyll. Ion-exchange resins were placed in the ASE cells to remove chloride and sulfate. This allowed the determination of perchlorate at the sub-ppb levels. Björklund and his coworkers have published many articles on the use of adsorbents in-line in ASE cells to improve selectivity,[13, 15–20] especially for PCB and PCDD analysis. One article discusses the results of the development of a scheme that allows the separation of non-ortho PCBs and PCDD/Fs from the bulk of the PCBs, gravimetric fat determinations, and minimum post-extraction cleanup prior to analysis.[13] This offers an obvious savings in time, labor, and solvent costs over traditional extraction procedures followed by cleanup methods such as GPC.

Accelerated solvent extraction has been shown to work well for the extraction of acrylamide from many food matrices.[21] The use of adsorbents in the ASE cell has now been extended to this application.[22] Florisil was added to the cell when extracting coffee or chocolate samples. The authors report that 6 g of Florisil in the cell, along with 2 g of sample, produced clear extracts without any interference. Figure 4 shows the effect of varying the level of the Florisil.

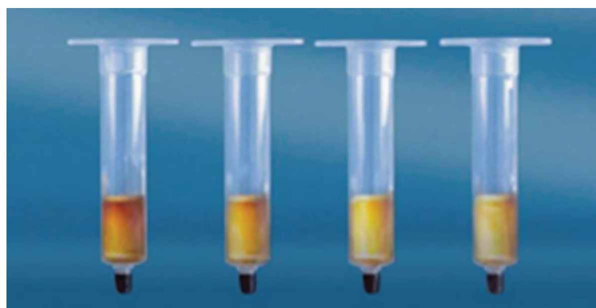

Figure 4. Residual co-extractives after the accelerated solvent extraction of a coffee sample trapped on a SPE cartridge, the ASE cells containing varying from 0 to 6 g Florisil. From left to right: 1) No Florisil in the ASE cell, 2) 2 g Florisil in the ASE cell, 3) 4 g Florisil in the ASE cell, and 4) 6 g Florisil in the Dionex ASE cell.

One of the most fascinating uses of selective extraction with the Dionex ASE system was reported by Poerschmann et al.[23, 24] In this work, a combination of adsorbents in the cells, varying temperature, and solvent allowed fractionation to be achieved that was superior to conventional solid-phase extraction (SPE) after exhaustive extraction. This work demonstrated the fractionation of lipid classes using sequential extractions. Unmodified silica and cyanopropyl silica were used as the adsorbents in the outlet of the cells. The biological samples were extracted with hexane/acetone (9:1, v/v) at 50°C to remove the neutral lipids. Then, the same samples were extracted with chloroform/methanol (1:4, v/v) at 110 °C to remove polar lipids such as phospholipids and hydroxy-containing fatty acids. What is intriguing about this work is that this fractionation scheme could be used to screen for diagnostic central nervous system (CNS) lipid markers in meat products. This is of particular interest for risk assessment studies for bovine spongiform encephalopathy (BSE) and food labeling legislation. The accelerated solvent extraction fractionation scheme worked better than the widely used exhaustive lipid extraction procedure followed by SPE with regard to lipid recoveries and clean fractionation of the lipid classes. Clearly, the combination of temperature, solvent, and adsorbent materials in the ASE cell can provide unique selectivity and capability and provide additional advantages over other sample preparation techniques.

Conclusion

When the Dionex ASE system was introduced to the market in 1996, the amount of time and solvent used for extraction was reduced remarkably compared to traditional sample preparation techniques. However, accelerated solvent extraction originally did not address the need for sample cleanup with GPC following extraction. Now, the Dionex ASE system is able to automate the sample preparation a step further by eliminating the need to perform post-extraction cleanup. The combination of temperature, solvent, and adsorbent materials in the ASE cell can provide unique selectitivity and provide additional advantages over other sample preparation and GPC techniques. Eliminating post-extraction cleanup steps reduces solvent usage and waste, and saves the analyst time and labor. This makes the Dionex ASE system a powerful tool for sample preparation and greatly improves laboratory productivity.

References

1. J. Ezzell, B. Richter, and E. Francis, "Selective Extraction of Polychlorinated Biphenyls from Fish Tissue Using Accelerated Solvent Extraction," *American Environmental Laboratory*, **8(12)**, 12-13 (1996).
2. J. L. Ezzell, B. E. Richter, W. D. Felix, S. R. Black, and J. E. Meikle, "A Comparison of Accelerated Solvent Extraction with Conventional Solvent Extraction for Organophosphorus Pesticides and Herbicides," *LC/GC* **13**, 390-398 (1995).
3. J. L. Gomez-Ariza, M. Bujalance, I. Giraldez, A. Velasco, and E. Morales, "Determination of Polychlorinated Biphenyls in Biota Samples Using Simultaneous Pressurized Liquid Extraction and Purification," *J. Chromatogr., A.*, **946**, 209-219 (2002).
4. U.S. EPA Method 3620C, Florisil Cleanup. U.S. Environmental Protection Agency, Cincinnati, OH, 2000.
5. Thermo Fisher Scientific Inc., "Selective Extraction of PCBs from Fish Tissue Using Accelerated Solvent Extraction (ASE)," Application Note 322, LPN 0764, Sunnyvale, CA, 1996.
6. Thermo Fisher Scientific Inc., "Determination of PCBs in Large-Volume Fish Tissue Samples Using Accelerated Solvent Extraction (ASE)," Application Note 342, LPN 1204, Sunnyvale, CA, 2000.
7. E. Björklund, A. Muller, and C. von Holst, "Comparison of Fat Retainers in Accelerated Solvent Extraction for the Selective Extraction of PCBs from Fat-Containing Samples," *Anal. Chem.*, **73**, 4050-4053 (2001).
8. S. Sporring and E. Björklund, "Selective Pressurized Liquid Extraction of Polychlorinated Biphenyls from Fat-Containing Food and Feed Samples: Influence of Cell Dimensions, Solvent Type, Temperature and Flush Volume," *J. Chromatogr., A.*, **1040**, 155-161 (2004).
9. Thermo Fisher Scientific Inc., "Determination of Perchlorate in Vegetation Samples Using Accelerated Solvent Extraction (ASE) and Ion Chromatography," Application Note 356, LPN 1830, Sunnyvale, CA, 2006.
10. M. M. Schantz, J. J. Nichols, and S. A. Wise, "Evaluation of Pressurized Fluid Extraction for the Extraction of Environmental Matrix Reference Materials," *Anal. Chem.* **69**, 4210-4219 (1997).
11. U.S. EPA Method 3660B, Sulfur Cleanup. U.S. Environmental Protection Agency, Cincinnati, OH, 1996.
12. M. Nording, S. Sporring, K. Wiberg, E. Björklund, and P. Haglund, "Monitoring Dioxins in Food and Feedstuffs Using Accelerated Solvent Extraction with a Novel Integrated Carbon Fractionation Cell in Combination with CAFLUX Bioassay," *Anal. Bioanal. Chem.*, **381**, 1472-1475 (2005).

13. P. Haglund, S. Sporring, K. Wiberg, and E. Björklund, "Shape-Selective Extraction of PCBs and Dioxins from Fish and Fish Oil Using In-Cell Carbon Fractionation Pressurized Liquid Extraction," *Anal. Chem.*, **79**, 2945-2951 (2007).

14. A. Gentili, D. Perret, S. Marchese, M. Sergi, C. Olmi, and R. Curini, "Accelerated Solvent Extraction and Confirmatory Analysis of Sulfonamide Residues in Raw Meat and Infant Foods by Liquid Chromatography Electrospray Tandem Mass Spectrometry," *J. Agric. Food Chem.*, **52**, 4614-4624 (2004).

15. E. Björklund, S. Sporring, K. Wiberg, P. Haglund, and C. von Holst, "New Strategies for Extraction and Clean-up of Persistent Organic Pollutants from Food and Feed Samples Using Selective Pressurized Liquid Extraction," *Trends in Anal. Chem.*, **25**(**4**), 318-325 (2006).

16. A. Hussen, R. Westbom, N. Megersa, N. Retta, L. Mathiasson, and E. Björklund, "Optimisation of Pressurized Liquid Extraction for the Determination of p,p'-DDT and p,p'-DDE in Aged Contaminated Ethiopian Soils," *Anal. Bioanal. Chem.*, **386**(**5**), 1525-1533 (2006).

17. A. Hussen, R. Westbom, N. Megersa, L. Mathiasson, and E. Björklund, "Development of a Pressurized Liquid Extraction and Clean-up Procedure for the Determination of Alpha-Endosulfan, Beta-Endosulfan and Endosulfan Sulfate in Aged Contaminated Ethiopian Soils," *J. Chromatogr. A*, **1103**, 202-210 (2006).

18. S. Sporring, C. von Holst and E. Björklund, "Selective Pressurized Liquid Extraction of PCB's from Food and Feed Samples: Effects of High Lipid Amounts and Lipid Type on Fat Retention," *Chromatograpia*, **64**(**9-10**), 553-557 (2006).

19. K. Wiberg, S. Sporring, P. Haglund, and E. Björklund, "Selective Pressurized Liquid Extraction of Polychlorinated Dibenzo-p-dioxins, dibenzofurans and dioxin-like polychlorinated biphenyls from Food and Feed Samples," *J. Chromatogr. A*, **1138**, 55-64 (2007).

20. A. Hussen, R. Westbom, N. Megersa, L. Mathiasson, and E. Björklund, "Selective Pressurized Liquid Extraction for Multi-residue Analysis of Organochlorine Pesticides in Soil," *J. Chromatogr. A*, **1152**, 247-253 (2007).

21. Thermo Fisher Scientific Inc., "Fast Determination of Acrylamide in Food Samples Using Accelerated Solvent Extraction (ASE) Followed by Ion Chromatography with UV or MS Detection," Application Note 409, LPN 1497, Sunnyvale, CA, 2003.

22. Thermo Fisher Scientific Inc., "Extraction and Cleanup of Acrylamide in Complex Matrices Using Accelerated Solvent Extraction (ASE) Followed by Liquid Chromatography, Tandem Mass Spectrometry (LC-MS/MS)," Application Note 358, LPN 1922, Sunnyvale, CA, 2007.

23. J. Poerschmann and R. Carlson, "New Fractionation Scheme for Lipid Classes Based on 'In-Cell Fractionation' Using Sequential Pressurized Liquid Extraction," *J. Chromatogr. A*, **1127**, 18-25 (2006).

24. J. Poerschmann, U. Trommler, W. Biedermann, U. Truyen, and E. Lucker, "Sequential Pressurized Liquid Extraction to Determine Brain-Originating Fatty Acids in Meat Products as Markers in Bovine Spongiform Encephalopathy Risk Assessment Studies," *J. Chromatogr. A*, **1127**, 26-33 (2006).

25. Majors, R.E. LC-GC, 1995, 13, 742-749.

26. Majors, R.E. LC-GC, 1999, 17, S8-S13.

www.thermoscientific.com/ase

©2013 Thermo Fisher Scientific Inc. All rights reserved. ISO is a trademark of the International Standards Organization. All other trademarks are the property of Thermo Fisher Scientific Inc. and its subsidiaries. This information is presented as an example of the capabilities of Thermo Fisher Scientific Inc. products. It is not intended to encourage use of these products in any manners that might infringe the intellectual property rights of others. Specifications, terms and pricing are subject to change. Not all products are available in all countries. Please consult your local sales representative for details.

Thermo Fisher Scientific, Sunnyvale, CA USA is ISO 9001:2008 Certified.

Australia +61 3 9757 4486
Austria +43 1 333 50 34 0
Belgium +32 53 73 42 41
Brazil +55 11 3731 5140
China +852 2428 3282
Denmark +45 70 23 62 60
France +33 1 60 92 48 00
Germany +49 6126 991 0
India +91 22 6742 9494
Italy +39 02 51 62 1267
Japan +81 6 6885 1213
Korea +82 2 3420 8600
Netherlands +31 76 579 55 55
Singapore +65 6289 1190
Sweden +46 8 473 3380
Switzerland +41 62 205 9966
Taiwan +886 2 8751 6655
UK/Ireland +44 1442 233555
USA and Canada +847 295 7500

Thermo SCIENTIFIC
Part of Thermo Fisher Scientific