E.1 Introduction

Scientists are continually striving to develop a means to accomplish desired results faster, better, cheaper, and more efficiently. However, the application of new technologies to sample preparation has often lagged behind advances in the determinative steps of the overall analytical scheme. Consider the major technological changes in chromatography that have occurred in the last few years. What a contrast exists between the first commercial gas chromatographs (GC) introduced in the late 1950s and the modern GCs we have today! On the other hand, analytical chemists can still be seen preparing samples for chromatographic analysis using the same type of Soxhlet apparatus that was used to prepare samples for the first GC instruments. In fact, Soxhlet apparatus has been in use, with few modifications, for over 100 years. Traditional extraction techniques suffer from several shortcomings. They often take long periods of time (several hours), use large quantities of solvent (hundreds of milliliters per sample), and require a great deal of labor and user intervention.

In addition to the costs associated with sample preparation, studies have shown that sample preparation is the primary source of errors in any analytical process. If we want to have the biggest impact on improving the performance of any analytical method, we would be well-advised to attack the sample preparation portion of the method. That is one reason that Dionex has been involved in research dealing with sample preparation for many years. Accelerated solvent extraction (ASE) was developed by Dionex to address the problems inherent with traditional extraction techniques such as Soxhlet and sonication.

ASE is an automated extraction technique that uses elevated temperatures and pressures to achieve extractions in very short periods of time (for example, 10 g of sample can be extracted in less than 15 minutes, using less than 15 mL of solvent). Why would we do extractions at elevated temperatures and pressures? There are several physicochemical reasons for this. First, let's consider temperature.

It is a well-known fact that the use of higher temperatures increases the capacity of solvents to solubilize analytes. For example, the solubility of anthracene increases nearly 13-fold as we increase temperature from 50 to 150 °C. Increasing the temperature also leads to faster diffusion rates. This means that analytes move

more quickly from the boundary layer near the surface of the matrix from which they are extracted to the bulk solvent at higher temperatures. Higher temperatures also mean lower solvent viscosities, meaning that the solvent can penetrate the pores of the matrix more easily. Finally, increased temperature makes it easier to disrupt solute-matrix interactions (dipole attractions, van der Waals forces, hydrogen bonding, etc.) and remove analytes from the matrix. Taken all together, the net effect is that doing extractions at elevated temperatures means that the extractions happen much faster and use less solvent. However, temperature alone is not enough because many of the organic solvents used in extractions boil at relatively low temperatures. This is one limitation of techniques such as Soxhlet or automated Soxhlet; the highest temperature at which extractions take place in these techniques is the boiling point of the solvent.

If sufficient pressure is exerted on the solvent during the extractions, temperatures above the boiling point can be used. This means that all of the advantages of working at elevated temperature can be realized even with solvents with relatively low boiling points. Operating at elevated pressures also helps the overall extraction process to happen more quickly. Pumping solvent through a packed bed is easier at elevated pressures. Pressurized solvent is forced into the pores of the sample matrix, coming into more intimate contact with analytes in those areas. Hence, the combination of elevated temperatures and pressures allows extractions to occur rapidly and completely. For a more detailed discussion of the theoretical consideration of ASE, consult Richter et al., *Anal. Chem.* **1996**, *68*, 1033-1039.

E.2 Operation

ASE was first described in 1995 and 1996 (1–9). As a technique, it has grown steadily in use since that time. Figure E-1 shows a schematic of the ASE process.

To perform an extraction, the solid sample is loaded into a sample cell (1 to 100 mL) and the end caps (each containing a frit) are tightened by hand onto the cells. The filled sample cells are loaded onto a cell tray and collection vessels (bottles or vials) 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 (room temperature to 200 °C). The cell design and associated fluid apparatus allow operation of the extractions at elevated pressures (500 to 3000 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.

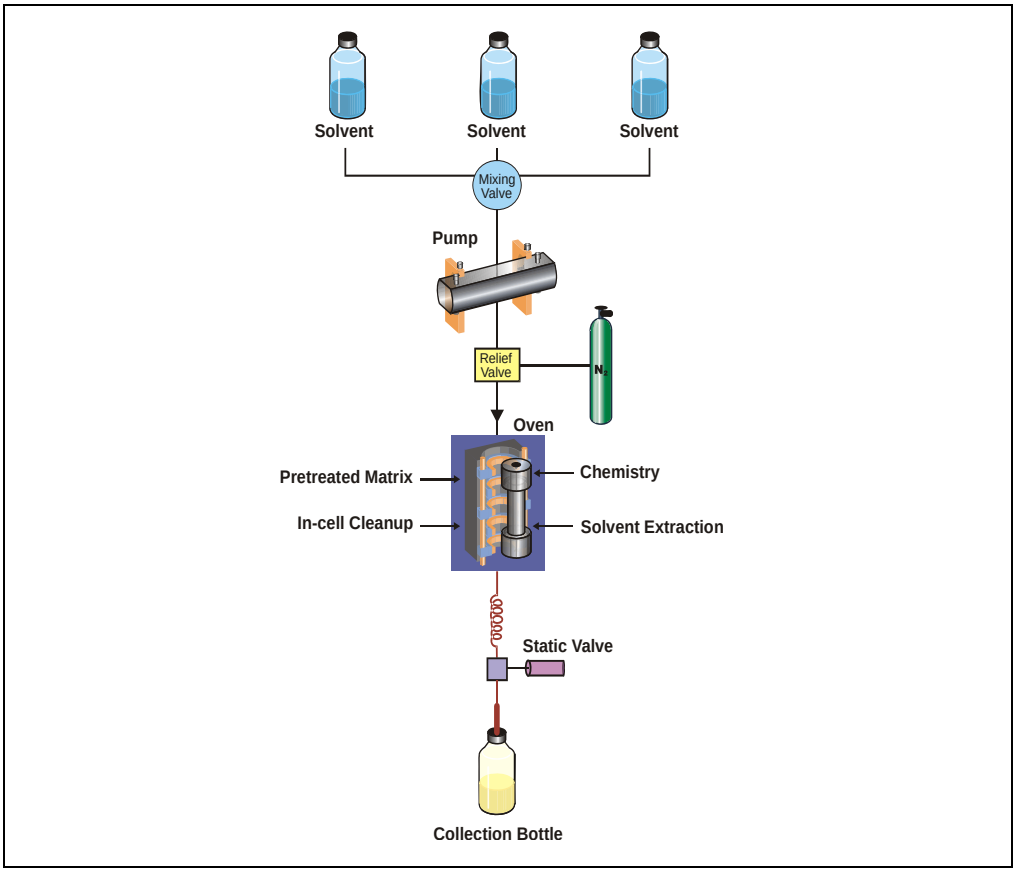

Figure E-1. Schematic of the ASE Process

Once the cell is in place 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 ASE is analogous to fresh solvent dripping down from the condenser onto the extraction thimble during Soxhlet extraction.

During the first phase of a run, called the heat-up time, the cell contents are heated by the oven to the selected operating temperature. Heat-up times vary between 5 minutes for 100 °C and 9 minutes for 200 °C. After the heat-up time, the extraction enters a static period with a duration selected by the user. Typical static times are 5 minutes, but can vary from 1 to 99 minutes. After the static time, fresh solvent is pumped through the cell to remove extracted analytes while the sample and solvent are still hot. The amount used for the rinse can vary from 5% to 150% of the volume of the cell used for the extraction (40% to 60% is most common).

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. The rinse volume is divided by the number of static cycles, so that fresh solvent is present at the beginning of each static cycle. In other words, if one static cycle is selects, the entire rinse volume will be pumped through the cell at the end of the static time. If three static cycles are selected, one-third of the total rinse volume will be pumped through the cell at the end of each static cycle. From one to five static cycles can be programmed; one cycle is the most common.

Following the final solvent rinse, solvent is purged out of the cell (using nitrogen at 150 psi) for a predetermined period of time. 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 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. Current ASE instrumentation allows for unattended overnight operation of the extraction system without user intervention.

Under method control, each sample is extracted using the same conditions. When using sequence control, each cell can be extracted using different conditions, including different solvents (if multiple solvent reservoirs are installed).

ASE instrumentation includes many features designed to minimize safety issues with the use of solvents at elevated temperatures and pressures. Among the safety measures in place are: flammable vapor sensors, liquid leak detectors, checks for collection vessel overfill conditions, three levels of overpressurization prevention (electronic and mechanical), solvent flow monitoring, and pneumatic source pressure monitoring.

E.3 Method Optimization

Method optimization consists of two main elements: sample preparation and extraction parameters.

E.3.1 Sample Preparation

Sample preparation is an essential part of every solvent-based extraction procedure. While many sample types can be efficiently extracted without any pretreatment, other samples require some manipulation for an efficient extraction to occur. In general, the same sample preparation that is done prior to Soxhlet or sonication extraction should be done prior to extraction by ASE. This section discusses three sample preparation techniques: grinding, dispersing, and drying.

Grinding

For an efficient extraction to occur, the solvent must make contact with the target analytes. The more surface area that can be exposed in a sample, the faster an extraction will occur. Samples with large particle sizes should be ground prior to extraction. Efficient extraction requires a minimum particle size, generally smaller than 1 mm. Grinding can be accomplished with a conventional mortar and pestle or with electric grinders and mills. Often a large, representative sample can be ground and weighed portions of the ground sample can be used for extraction. Soil

and sediment samples generally do not need to be ground, although it may be necessary to remove stones or sticks from the samples prior to extraction. Polymer samples must be in a ground state for an efficient extraction of additive compounds. Materials such as polymers and rubbers are best ground at reduced temperatures (for example, liquid nitrogen). Animal or plant tissue samples can be homogenized using any procedure, including a blender or tissue homogenizer.

The effects of sample surface area on extraction efficiency have been studied. Three samples of mozzarella cheese were prepared differently and extracted for fat content using ASE. The chopped samples were cut into pieces ranging in size between 2 and 5 mm. The ground samples were ground with diatomaceous earth (1:2). Each sample was extracted with a mixture of hexane:2-propanol (3:2) using these ASE conditions: 1000 psi, 125 °C, 6-minute heat-up, three 10-minute static cycles, 100% rinse, 60-second purge, and one 11-mL cell containing one cellulose filter. The extracts were analyzed gravimetrically. The ASE results were compared to results obtained from Mojonnier extraction (see Figure E-2). These results demonstrate that increasing the surface area by grinding the sample results in higher extraction efficiencies.

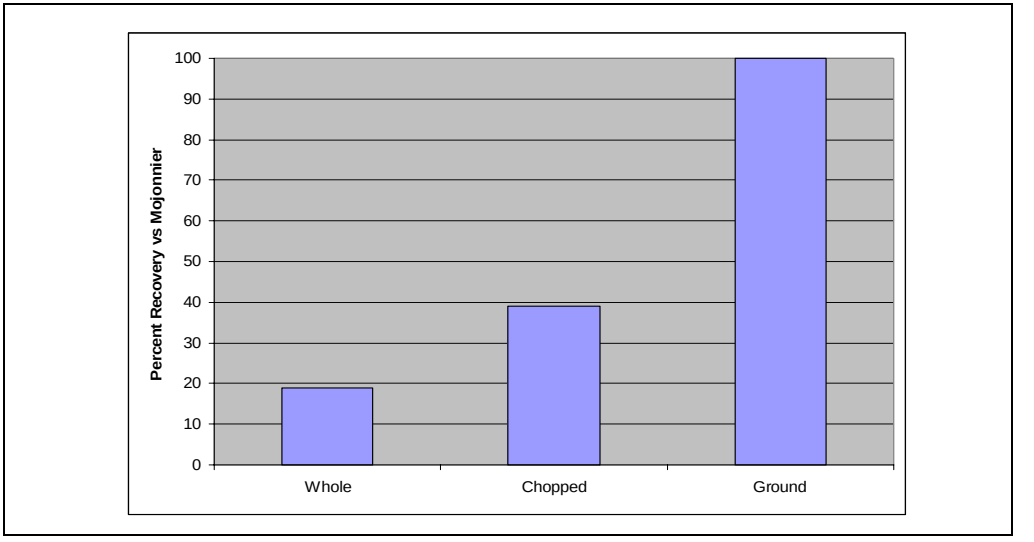

Figure E-2. Effect of Sample Particle Size for ASE vs. Mojonnier Extraction. Whole cheese (0.5 to 1.5 cm size); chopped (2 to 5 mm); ground (mixed thoroughly with diatomaceous earth)

Dispersing

The aggregation of sample particles may prevent efficient extraction. In these cases, dispersing the sample with an inert material such as sand or diatomaceous earth will assist in the extraction process. Dispersing is also recommended with samples that tend to compact in the sample cell outlet. These samples normally contain very small particles that can adhere tightly to each other when under pressure. Some samples may compact in the sample cell due to the high pressures used in ASE. Mixing the sample with sand or diatomaceous earth will prevent this compacting of the sample that can lead to occlusion of the sample cell.

When extracting soil or sediment samples, it is important to mix the samples with a dispersing agent unless the samples are completely dry. If the soil or sediment samples are wet or high in clay, the samples can be mixed with either sand or diatomaceous earth, but the latter is preferred because it will adsorb some of the water and make the sample easier to handle.

It is important to periodically run blank extractions of the dispersing agent to verify its cleanliness.

Drying

Samples do not have to be completely dry to achieve efficient extractions with ASE. This depends on the analytes and the solvent used for extraction. High levels of water can prevent nonpolar organic solvents from reaching the target analytes. The use of more polar solvents (for example, acetone and methanol) or solvent mixtures (for example, hexane/acetone and methylene chloride/acetone) can assist in the extraction of wet samples. Elevated temperatures can also improve the extraction efficiency of wet samples (10). In addition, the solvent being used will impact the type of drying that needs to be done to a sample and whether it need to be done at all. With nonpolar solvents, the need for drying is more important. One can also use a bridge solvent such as acetone (for example, hexane/acetone, 1:1) mixed with a nonpolar solvent to improve the extraction efficiency of wet samples.

Sample drying prior to extraction is an efficient way to handle wet samples. Drying can be accomplished by direct addition of a drying agent such as diatomaceous earth. The use of magnesium sulfate is not recommended with ASE due to the extremely hard, concrete-like material

that can be produced. Sodium sulfate should be used **only** with nonpolar solvents (hexane, heptane, toluene, etc.). Sodium sulfate can become soluble in the extraction process and then be deposited in the exit lines. Oven drying and freeze drying are other viable alternatives for sample drying prior to extraction; however, these procedures may compromise the recovery of volatile compounds.

The sample drying or dispersing agent should be mixed thoroughly in a small vial, beaker, or mortar, and then added to the sample cell. For quantitative transfer, the mixing vessel can be rinsed with 1 to 2 mL of the extraction solvent using a Pasteur pipette, and this volume added directly to the sample cell. The main purpose of mixing with a drying agent like diatomaceous earth is not to remove all water from the sample but to make it easy to transfer the sample into the sample cell. If elevated temperatures are used, then some water will be removed from the sample during the extraction process. In this case, sodium sulfate can be added to the extract after collection to remove water.

E.3.2 Extraction Parameters

Solvent

For an efficient extraction, the solvent must be able to solubilize the target analytes while leaving the sample matrix intact. The polarity of the extraction solvent should closely match that of the target compounds. The common adage of "like dissolves like" is very applicable in ASE. Mixing solvents of differing polarities can be used to extract a broad range of compound classes. Generally, if a particular solvent has been shown to work well in a conventional procedure, it will also work well in ASE. Compatibility with the post-extraction analytical technique, the need for extract concentration (solvent volatility), and the cost of the solvent should all be considered. While many ASE methods recommend solvents or solvent mixtures for specific analyte classes, there may be alternatives that better fit the needs of a particular laboratory. For example, Schantz (11) showed that dichloromethane/acetone or acetonitrile can be used to get complete extraction of polycyclic aromatic compounds. If GC or GC-MS is the analytical method, then dichloromethane/acetone would be the solvent of choice. If HPLC is the determinative step, then acetonitrile would be the solvent of choice. Solvent choice will also determine the

level of coextractables along with the analytes. Generally, the more polar the solvent or solvent mixture, the less selective it will be.

Solvents that exhibit marginal results at ambient conditions may perform adequately under ASE conditions. Most liquid solvents, including water and buffered aqueous mixtures, can be used in ASE. Strong acids (HCl, HNO_3, H_2SO_4) are not recommended for use with stainless steel cells because they may damage the cells. However, small amounts of these acids can be used with zirconium cells. When required, weak acids such as acetic or phosphoric can be used. These should be added to aqueous or polar solvents in the 1-10% (v/v) range.

For extraction of polymer samples, select a solvent that will extract the additives but not the matrix itself. For example, isopropanol with small levels of cyclohexane has been shown to work well for the extraction of phenolic antioxidant additives in polymers.

Temperature

Temperature is the most important parameter used in ASE extraction. As the temperature is increased, the viscosity of the solvent is reduced, thereby increasing its ability to wet the matrix and solubilize the target analytes. The added thermal energy also assists in breaking analyte-matrix bonds and encourages analyte diffusion from the matrix surface.

When developing a new method, start at 100 °C or, if the target analytes have a known thermal degradation point, start at 20 °C below this level. Most ASE applications operate in the 75 to 125 °C range, with 100 °C the most common temperature for environmental applications. If the sample has a tendency to melt in the sample cell, a cellulose thimble can be used to facilitate extraction and sample removal.

Table E-1 shows an example of the effect of temperature for the extraction of petroleum hydrocarbons (TPH) from soil. Note that not only does the analyte recovery increase, but the reproducibility improves as a function of temperature.

Temperature	Recovery	RSD (%)
27 °C	81.2	6.0
50 °C	93.2	5.0
75 °C	99.2	2.0
100 °C	102.7	1.0

Samples were analyzed by IR, with n = 5.

Table E-1. Effect of Temperature on TPH; Extraction from Soil

There appears to be confusion about the purpose of the preheat function available on ASE systems. When the preheat function is used, a cell is placed in the oven and heated without any solvent being pumped into the cell. This function was originally developed to allow pre-extraction derivatization of analytes or the drying of samples by blowing nitrogen through the cell while it is heated. The preheat function should **not** be used in normal operation of ASE. In general, this should be set to zero minutes. If used, recoveries of more volatile compounds (for example, organochlorine pesticides) will be greatly reduced.

Pressure

The effect of pressure is to maintain the solvents as liquids while above their atmospheric boiling points, and to rapidly move the fluids through the system. The pressures used in ASE are well above the thresholds required to maintain the solvents in their liquid states, so pressure adjustments for changing solvents are not required. Changing the pressure will have very little impact on analyte recovery, and it is not considered a critical experimental parameter. Most ASE 350 extractions are performed at 1500 psi.

Static Cycles

The use of static cycles was developed to introduce fresh solvent during the extraction process, which helps to maintain a favorable extraction equilibrium. This effectively approximates dynamic extraction conditions without the need for troublesome flow restrictors to maintain pressure. When more than one cycle is used in a method, the rinse volume is divided by that number. When the first static time is complete, the divided portion of the rinse volume is delivered to the cell, with the "used"

solvent directed to the collection vessel. The system then holds the sample and solvent for a second static period. The nitrogen purge step is initiated only after the final static cycle. Because the original rinse volume has only been divided, no additional solvent is used for the extraction.

Static cycles have proven to be useful for sample types with a very high concentration of analyte, or samples with difficult to penetrate matrices. The static time can be adjusted to minimize the total extraction time. For example, three 3-minute static cycles can be used in place of one 10-minute static step. When low temperature extractions are desired (< 75 °C), multiple static cycles should be used to compensate for the smaller amount of solvent introduced during the heat-up step, as the static valve pulses to regulate the pressure.

Time

Certain sample matrices can retain analytes within pores or other structures. Increasing the static time at elevated temperatures can allow these compounds to diffuse into the extraction solvent. The effect of static time should always be explored in conjunction with static cycles, in order to produce a complete extraction in the most efficient way possible. Generally, ASE extractions are completed in less than 20 minutes; if times longer than this are necessary to get complete extraction, then a higher temperature, different solvent, or multiple static cycles should be explored to reduce the overall extraction time.

E.4 Method Development

A representative sample should be prepared as outlined in the discussion on sample preparation (see Section E.3.1). Select a sample cell size that most closely matches the sample size. The sample cells do not need to be filled completely; however, a full cell will use less solvent in the extraction process than a partially filled one. A recommended set of conditions to begin method development are as follows: 1500 psi, 100 °C (or 20 °C below analyte decomposition temperature), 5-minute static time, 40% rinse volume, 60-second purge (up to 300 seconds for a 100-mL cell), and one static cycle. If desired, a sequence can be set up to extract each of a series of samples under a different set of conditions and collect them in separate collection vessels. Samples can also be extracted multiple times to

determine extraction completeness. If analyte is present in the second or third vessel, then the initial conditions need to be changed.

The order of change would be (1) change the solvent, (2) increase the temperature, (3) add a second or third cycle, and (4) increase the time. In this way, one can quickly determine the optimized extraction conditions for a particular analyte and matrix.

E.5 Selectivity in ASE

Selectivity in extraction is defined here as being able to extract compounds of interest with little or no interfering coextracted compounds. ASE 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 ASE 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 ASE 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 ASE 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 ASE more selective, but the recovery of analytes can diminish unless the time is increased. Similarly, selectivity is decreased when using more polar solvents. However, one can use a series of solvents of increasing polarity to obtain selective extractions or "fractionation."

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. We have found that the samples can first be extracted with nonpolar solvents like hexane 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, one can obtain extracts that are easier to analyze.

Figure E-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.

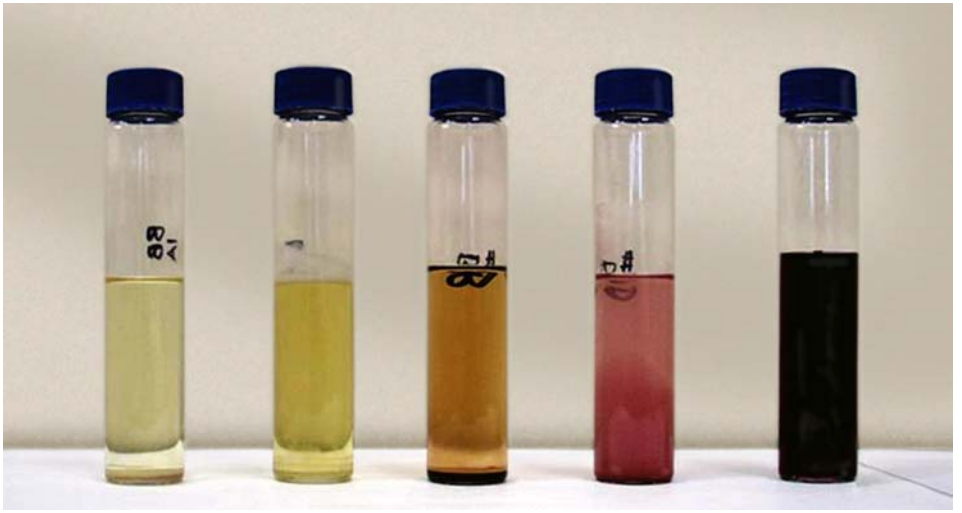

Figure E-3. Selectivity in ASE. The same sample of blueberries was extracted with (from left to right) hexane, DCM, ethyl acetate, acetonitrile, and ethanol

Another unique use of solvent selectivity was reported by Draisci et al. (12). The authors reported the use of ASE for the extraction of corticosteroids from beef liver prior to analytical determination by LC-MS. First, the sample was extracted with hexane to remove the majority of lipids that would interfere in the determinative step. Next, the samples were extracted with hexane/ethyl acetate to remove the steroid compounds. Solvent fractionation using ASE is an area that needs to be explored to more fully understand its potential.

The use of sorbents in the sample cell along with the sample has offered some of the highest level of selectivity in ASE. 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. Azalea et al. (13) first reported the use of alumina in sample cells to retain lipids when extracting PCBs from fish tissue

using ASE. 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 ASE that require no additional post-extraction treatment other than volume adjustment.

The most common use of adsorbents in ASE 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 E-2 shows a summary, with related references.

Adsorbent	Uses	References
Silica	Removes nonpolar lipids	4
Florisil	Removes nonpolar lipids	15, 16
Alumina	Removes nonpolar lipids and colored compounds	13, 17, 18
C18 resin	Removes nonpolar lipids	19, 20
Ion-exchange resins	Removes ionic interferences	22
Copper powder	Removes sulfur	11, 23
Carbon	Assists with purification of PCDDs, PCDFs, and coplanar PCBs	24, 25

Table E-2. List of Adsorbents Used in ASE

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 (21) 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

them to separate and harden. 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 (22). For example, Dionex 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 (25–31), 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 clean-up prior to analysis (25). This offers an obvious savings in time, labor, and solvent costs over traditional extraction procedures followed by clean-up methods such as GPC.

ASE has been shown to work well for the extraction of acrylamide from many food matrices (32). The use of adsorbents in the ASE cell has now been extended to this application (33). 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 interferences. Figure E-4 shows the effect of varying the level of the Florisil.

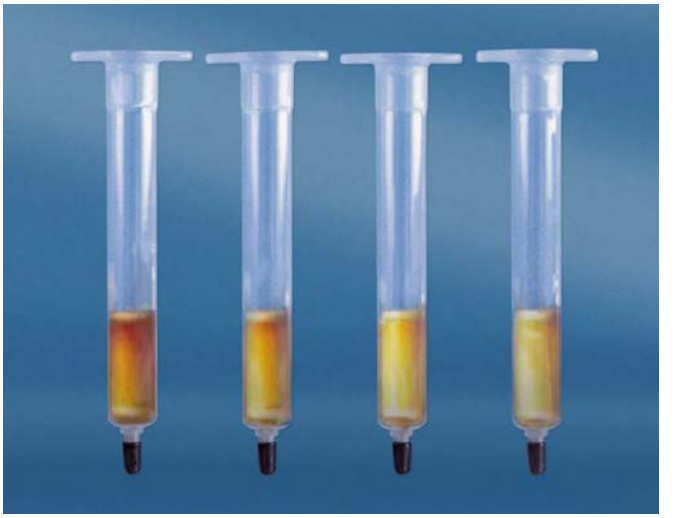

Figure E-4. Residual co-extractives after the ASE extraction of a coffee sample trapped on an SPE cartridge, the ASE cells containing varying from 0 to 6 g Florisil. From left to right: 1) No Florisil in the ASE cell, 2) 2 g Florisil in the ASE cell, 3) 4 g Florisil in the ASE cell, and 4) 6 g Florisil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

One of the most fascinating uses of selective extraction with ASE was reported by Poerschmann et al. (34, 35). In this work, a combination of adsorbents in the cells, varying temperature, and solvent allowed fractionation to be achieved that was superior to conventional 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 was intriguing about this work was that the authors demonstrated 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 assessments studies for bovine spongiform encephalopathy (BSE) and food labeling legislation. The ASE 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.

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