Methods Optimization in Accelerated Solvent Extraction

Introduction

Accelerated solvent extraction is an innovative sample preparation technique that combines elevated temperature and pressures with liquid solvents to achieve fast and efficient removal of analytes from various matrices. Accelerated solvent extraction has been demonstrated to be equivalent to existing extraction methodologies such as Soxhlet and automated Soxhlet for most RCRA (Resource Conservation and Recovery Act) analytes from solid and semisolid matrices. It meets the requirements of U.S. EPA Method 3545, Pressurized Fluid Extraction.

Accelerated solvent extraction is an efficient form of liquid solvent extraction, so all of the principles inherent to that technique apply. To achieve efficient extraction, proper sample preparation techniques and operational parameters must be selected. It is normally very easy to transfer an existing solvent-based extraction method to accelerated solvent extraction technology. This Technical Note is a guide to optimize accelerated solvent extraction methods.

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 will require some manipulation for an efficient extraction to occur. As with Soxhlet, the ideal sample for extraction is a dry, finely divided solid. Unfortunately, many samples do not fit this description. Whatever can be done to make the sample similar will have a positive impact on the extraction. In general, the same sample preparation that is done prior to Soxhlet or sonication extraction should be done prior to accelerated solvent extraction.

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 this will occur. Samples with large particle sizes should be ground prior to extraction. Efficient extraction requires a minimum particle size, generally smaller than 0.5 mm. Grinding can be accomplished with a conventional mortar and pestle or with electric grinders and mills. Because quantitative transfer of ground material can be difficult, it is recommended that a large, representative sample be ground, and weighed portions of the ground sample be used for extraction. Polymer samples must be in a ground state for an efficient extraction of additive compounds. Compliant materials such as polymers and rubbers are best ground at reduced temperatures (e.g., liquid nitrogen).

Dispersing

The aggregation of sample particles may prevent efficient extraction. In these cases, dispersing the sample with an inert material such as sand (Ottawa Sand, Fisher Scientific, Cat. No. S23-3) or Thermo Scientific™ Dionex™ ASE™ Prep DE (diatomaceous earth) (P/N 062819) will assist in the extraction process. Dispersing is also recommended with samples that tend to compact in the extraction cell outlet. These samples normally contain very small particles that can adhere tightly to each other when under pressure. Sea sand is not recommended as a dispersing agent, because it contains very small particles that can block system tubing. It is important to periodically run blank extractions of the dispersing agent to verify its cleanliness.

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Drying

Many environmental samples contain water that can prevent nonpolar organic solvents from reaching the target analytes. The use of more polar solvents (e.g., acetone, methanol) or solvent mixtures (e.g., hexane/acetone, methylene chloride/acetone) can assist in the extraction of wet samples. Sample drying prior to extraction is the most efficient way to handle these sample types. Drying is normally accomplished by direct addition of a drying agent such as Dionex ASE Prep DE. The choice of drying agent depends on the sample type. Cellulose may be used for very wet, soft matrices such as fruits and vegetables. The use of magnesium sulfate is not recommended with accelerated solvent extraction due to the potential for melting at higher temperatures. Sodium sulfate should not be used because it could solubilize 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, the recovery of volatile compounds may be compromised by these procedures.

The following ratios have been applied to various sample types for accelerated solvent extraction:

Samples that appear dry:
4 grams of sample to 1 gram of Dionex ASE Prep DE

Samples that appear wet:
4 grams of sample to 2 grams of Dionex ASE Prep DE

The sample and the drying or dispersing agent should be mixed thoroughly in a small vial, beaker, or mortar, and then added to the extraction cell. For quantitative transfer, the mixing vessel can be rinsed with 1–2 mL of the extraction solvent using a Pasteur pipette, and this volume added directly to the extraction cell.

Table 1. Examples of sample preparation.

Sample Type	Sample Preparation
Wet Soil/Sediment	10 g of sample with 5 g of Dionex ASE Prep DE
Fish Tissue (80% Moisture)	3 g of sample with 2 g of Dionex ASE Prep DE
Creams/Lotions	2 g of sample with 3 g of Dionex ASE Prep DE
Fruits/Vegetables	10 g of sample with 5 g of Dionex ASE Prep DE or 2 g cellulose
Ground Polymer	1–3 g of sample with 1–3 g of sand

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. 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 accelerated solvent extraction. Compatibility with the postextraction analytical technique, the need for extract concentration (solvent volatility), and the cost of the solvent should all be considered. While many accelerated solvent extraction methods recommend solvents or solvent mixtures for specific analyte classes, there may be alternatives that better fit the needs of a particular laboratory. Solvents that exhibit marginal results at ambient conditions may perform adequately under accelerated solvent extraction conditions. Most liquid solvents, including water and buffered aqueous mixtures, can be used in accelerated solvent extraction. Strong acids (HCl, HNO_3, H_2SO_4) are not recommended, due to their ability to react with the stainless steel in the system. For extraction of polymer samples, select a solvent that will extract the additives but not the matrix itself. A 1:1 mixture of acetonitrile and ethyl acetate has been shown to work well. 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.

Temperature

Temperature is the most important parameter used in accelerated solvent 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 to 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 accelerated solvent extraction applications operate in the 75 to 125 °C range, with 100 °C the standard temperature for all environmental applications except dioxins. If the sample has a tendency to melt in the extraction cell, a cellulose Soxhlet thimble can be used to facilitate extraction and sample removal. An example of the effect of temperature is shown below for the extraction of total petroleum hydrocarbons (TPH) from soil. Note that not only does the analyte recovery increase, but the reproducibility improves as a function of temperature.

Table 2. Effect of temperature on TPH extraction from soil.

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.

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 accelerated solvent extraction 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 accelerated solvent extractions are performed between 1000 psi (7 MPa) and 2000 psi (14 MPa), with 1500 psi (10 MPa) the standard operating pressure.

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 flush volume is divided by that number. When the first static time is complete, the divided portion of the flush volume is delivered to the cell, with the "used" solvent directed to the collection vial. 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 flush 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-min static cycles can be used in place of one 10-min static step. When low temperature extractions are desired (< 75 °C), multiple static cycles should be used to compensate for the lack of fresh solvent normally introduced during the heatup 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.

Method Development and Validation

A representative sample should be prepared as outlined above. Select an extraction cell size that most closely matches the sample size. The extraction 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. Select the extraction solvent using the considerations listed above and rinse the system. Program the method starting with the default method values on the Method Edit screen (the default method can be accessed by selecting "0" as the method).

Table 3. Default method condtions (Method 0).

Pressure	1500 psi (10 MPa)
Temperature	100 °C
Static Time	5 min
Flush Volume	50%
Purge Time	60 s
Cycles	1

Use the default method as the starting point, or adjust the temperature as required. The automation capabilities of the system can now be used to assess the efficiency of the extraction. Create a schedule that will extract the test sample two or three times into separate vials. Extract the sample and analyze all of the vials for target analytes. If there is significant analyte present in the second or third vials, adjust the following parameters (one at a time) and repeat the validation process:

1. Increase the temperature (use 20 °C steps).
2. Add a second or third static cycle.
3. Increase the static time (use 5-min increments).

If these steps do not result in a complete extraction, reexamine the sample preparation steps and the choice of extraction solvent.

Conclusion

With proper sample preparation and optimization of extraction parameters, nearly any sample currently extracted with a liquid solvent can be performed in less time and with smaller quantities of solvent using accelerated solvent extraction. Combining the efficiency with the automation of the Thermo Scientific™ Dionex™ ASE™ 350 Accelerated Solvent Extractor system can dramatically improve the throughput of the analytical laboratory.

Suppliers

Sigma-Aldrich®, P.O. Box 14508, St. Louis, MO 63178 USA, Tel: 800-325-3010, www.sigmaaldrich.com

Fisher Scientific, 2000 Park Lane, Pittsburgh, PA 15275-1126 USA, Tel: 800-766-7000, www.fishersci.com.

Whatman®, Inc., 9 Bridewell Place, Clifton, NJ 070014 USA, Tel: 800-631-7290, www.whatman.com.

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