DIONEX

Technical Note 209

Accelerated Solvent Extraction (ASE®) Sample Preparation Techniques for Food and Animal Feed Samples

INTRODUCTION

Sample preparation is an essential part of any solid-liquid extraction. Most food and animal feed samples require some type of treatment either before or after extraction. This can include preparing the sample prior to extraction, adding substances to the ASE cell to retain unwanted extractables, as well as treating the final extract.

This technical note describes different techniques that can be used to prepare various food and animal feed samples, as well as techniques used during and after extraction to ensure the best recoveries of analytes while maintaining a clean extract for analysis.

PRETREATMENT

Wet Food/Animal Feed Samples

Food/Animal Feed samples that contain a high amount of water need to be treated prior to extraction to remove most or all of the water. This can be accomplished several ways:

- Air drying
- Freeze drying (lyophilization)
- Oven drying
- Microwave drying
- Dispersing: mixing sample with ASE Prep DE (P/N 062819, 1 kg) or Ottawa sand (Fisher Scientific S23-3). If Ottawa sand is not available, clean sand* can be used with a mesh size of 30–40.

**Sea sand is not recommended for use in the ASE system.*

Take proper precautions during any thermal drying step. Volatile compounds of interest may be lost during sample pretreatment steps such as drying. If there is concern about the loss of compounds, do not dry the sample using heat. Simply mix the sample with ASE Prep DE until a free-flowing mixture of sample and drying agent is obtained.

Note: Do not use sodium and magnesium sulfate as in-cell drying agents because of potential clogging problems. These compounds can also contribute to the failure of key ASE instrument components such as the static valve.

PLANT MATERIAL

Plant material can contain a varying degree of moisture and often requires treatment prior to extraction. Lyophilization is an excellent pretreatment method for plant materials because this process yields a fine, dry sample that can be easily loaded into an ASE extraction cell. However, if freeze-drying is not a viable option, simply grind (or mix) the plant material with ASE Prep DE using a mortar and pestle. Initially, a ratio of plant material to DE of 1:1 is a good mixture, but the amount of DE may need to be increased if the plant material is very wet. Sufficient DE has been mixed with the sample if the sample can be easily removed from the mortar without adhering to the sides of the mortar. If the plant material is low in moisture, it can be ground in a blender or mill to produce small particles (<2 mm). Cryo-grinders have also been used successfully.

DRY OR LOW MOISTURE FOOD/ANIMAL FEED SAMPLES

Dry food/animal feed samples may not need a drying step, but usually require some pretreatment. Samples such as cereals and grains should be ground and dispersed before extraction. A mortar and pestle sufficient for many samples are excellent tools for this purpose. Other samples may require more extensive grinding to increase the surface area for optimum solvent penetration. For example, some animal feed samples may need to be ground into a course powder for optimal analyte recovery. Usually particles in the range of 1–2 mm (about 10–20 mesh) are sufficiently small.

Dionex has studied the effects of sample surface area on extraction efficiency. Three samples of mozzarella cheese were prepared differently and extracted for fat content using ASE. The ground samples were ground with ASE Prep DE (1:2). Each sample was extracted with a mixture of hexane:2-propanol (3:2) using the following ASE conditions: 1000 psi, 125 °C, 6 min heat-up, 3 x 10 min static cycle, 100% flush, 60 s purge, 11-mL cells containing one cellulose filter. The extracts were analyzed gravimetrically. The ASE results were compared to results obtained from Mojonnier extraction. These results are shown in Figure 1 and demonstrate that increasing the surface area by grinding the sample results in higher extraction efficiencies.

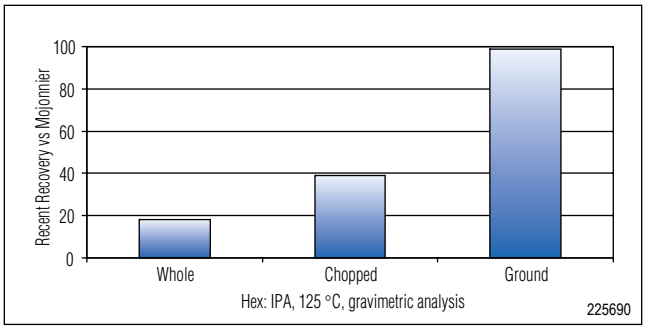

Figure 1. *ASE Sample Preparation Effects vs Mojonnier Extraction.*

MIXING AND DISPERSING

Regardless of whether or not the food/animal feed sample has been pretreated to remove moisture, most samples should be mixed with a dispersing agent. The dispersing agent can be ASE Prep DE, as mentioned above, or dry samples can be dispersed with sand (20–30 mesh particle size or Ottawa sand if available. Sea sand is not recommended for use as a dispersing agent and may cause premature failure of ASE instrument components). Dispersing the sample prevents sample compaction in the extraction cell, increases the surface area, and allows the solvent to better penetrate the sample matrix. As a result, extraction efficiency and precision improve. For dry samples, this pretreatment step should take just a few minutes, but is very important.

CHOOSING THE CORRECT CELL SIZE

As a first approximation choose a cell size that is at least double the mass of the sample. For example, if the sample size is 10 g, consider using a 22- or 33-mL cell. If extracting a 30-g sample consider a 66- or 100-mL cell. This guideline applies to samples that require pretreatment such as dispersion with ASE Prep DE or another drying agent. However, if the sample for extraction is in the form of a finely dispersed, dry powder, 10 g of such a sample will most likely fit into an 11-mL extraction cell. Additional factors to consider when choosing a cell size include sample swelling and the ratio of drying agent to sample used. If the sample tends to swell excessively during extraction, consider using a larger cell and leave some void volume in the extraction cell to accommodate swelling. If the sample is very wet and a large amount of ASE Prep DE is used, a larger cell may be required.

CHOOSING A FILTER

It is very important to use a filter in the bottom of the extraction cell. This will keep fine sample particulates from getting trapped in the frit or from leaving the cell and possibly causing damage to the static valve. Cellulose filters (for ASE 200, Dionex P/N 049458, pkg. of 100 and for ASE 300 and 100, Dionex P/N 056780, pkg. of 100) are the most common filters used, but if the extraction solvent chosen is an aqueous solvent and the temperature is above 100 °C, then the cellulose filter should not be used. Glass-fiber filters (for ASE 200, Dionex P/N 047017, pkg. of 100, and for ASE 300 and 100, Dionex P/N 056781, pkg. of 100) are available for these applications. The glass-fiber filters are available in the same diameter sizes as the cellulose filters. If the sample contains very fine particulates and there is evidence of these particulates passing through into the extraction vial, more than one filter (glass fiber or cellulose) can be used in the extraction cell to help eliminate this problem. In most cases, 2–3 filters will be sufficient.

IN-CELL CLEAN UP

In an effort to eliminate post-extraction clean-up steps, Dionex has studied the addition of various sorbents to the extraction cell. For many sample types, this approach has proven successful in producing clean extracts that are ready for direct analysis. For example, lipids are often co-extracted with fish tissue. Adding alumina (aluminum oxide, Al_2O_3, acidic, activated by oven drying at 350 °C for 15 min) to the extraction cell before adding the sample or sample mixture prevents the extraction of unwanted lipids. Mixing the sample with C18 resin (1:2) retains organic contaminants (C18 bonded silica, 35–70 µm diameter and porosity of 60 Å from Alltech has been used, but similar material from any vendor can be used).

When performing in-cell clean up, solvent choice can impact the retention of unwanted components. For example, a mixture of hexane/acetone (1:1) is a common solvent choice for extracting organochlorine pesticides from animal tissues. However, after extraction, a clean-up step is usually required to remove co-extracted lipids. If alumina is placed in the outlet (or exit) end of the extraction cell (the tissue sample that has been thoroughly mixed with ASE Prep DE is placed on top of the alumina), and hexane is used as the extraction solvent, the lipids will be retained on the alumina and remain in the extraction cell. On the other hand, if hexane/acetone (1:1) is used as the extraction solvent, almost no lipid material will be retained on the alumina. Generally, 60–70 mg of lipid material can be retained per gram of alumina.

For example, suppose a 10-g sample of fish tissue that is approximately 10% lipid by weight (1000 mg of total lipids) needs to be extracted. The amount of alumina necessary to retain all of the lipids from this sample is 1000 mg lipid/60 mg alumina (mg lipid)$^{-1}$ or approximately 17 g of alumina (16.67 g). In this case a 33-mL ASE cell or larger would be required to contain the fish tissue, the ASE Prep DE, and the necessary volume of alumina.

Another procedure that has been used to eliminate lipids is to put the collected extract in a refrigerator or freezer. Any collected lipids will turn solid and the liquid extract can be carefully decanted or the vial can be centrifuged.

CHOOSING A SOLVENT

When transferring extraction methods from traditional or manual techniques to ASE, the question, "What solvent should I use?" often arises. In most cases, the same solvent that was used in the previous technique can be used in the ASE system with excellent results. Remember that the ASE system is compatible with almost all liquid solvents with the exception of mineral acids (HCl, H_2SO_4, and HNO_3) and strong caustic solutions (KOH and NaOH). The use of mineral acids in the ASE system will etch the stainless steel cells and tubing, which leads to premature failure of key instrument components. Strong bases destroy the ASE pump components, leading to pump failure. As stated in the ASE User's Manual, buffers, weak acids, and weak bases have all been successfully employed as ASE solvents. If acidic extraction conditions are required, weaker acids, such as acetic or phosphoric should be used, usually in the 1–10% (v/v) range mixed with aqueous or polar solvents.

If there is uncertainty about which solvent to use, note that the polarity of the extraction solvent should be similar to the polarity of the target analytes. The solvent should not only be able to extract the analytes but leave the sample matrix generally intact. For example, when the goal is to extract an analyte that is moderately polar using an extremely polar solvent, the polar solvent may extract not only the target analytes, but much of the sample matrix as well. This scenario could create an extract that contains co-extractable compounds that may require extensive clean-up procedures prior to analy-

sis—or cause damage to an expensive piece of analytical instrumentation. To eliminate this concern, a less polar extraction solvent or a mixture of solvents may work better. Generally, if a particular solvent has worked well for a traditional extraction procedure, it should also work well for ASE. Mixing solvents of different polarities can be used to extract a broad range of target compounds present in the same sample. Solvent choice may also depend on which solvent is compatible with the chosen analytical technique. Most liquid solvents, including water and buffered aqueous mixtures, can be used in the ASE instrument.

Table 1 lists some suggested ASE solvents for various food matrices and analytes. Please note that these are only suggestions and other solvents or solvent mixtures may work better for particular samples. In ASE method development, the extraction temperature and choice of solvent are the most important parameters to consider. As mentioned previously, if an extraction procedure for a particular sample already exists, then begin by using the same solvent in the ASE instrument. In some cases, the choice of solvent will be determined by the analytical procedure. For example, when extracting polycyclic aromatic hydrocarbons (PAHs) for GC or GC-MS analysis, dichloromethane (DCM)/acetone (1:1) is the recommended ASE extraction solvent. However, if HPLC is the preferred analytical technique, acetonitrile is the recommended ASE solvent.

TABLE 1. SUGGESTED ASE SOLVENTS FOR VARIOUS FOOD MATRICES AND ANALYTES

Matrix	Analytes Group	Suggested Solvents
Fruits and vegetables	Environmental toxins (organic)[a]	DCM/acetone (1:1, v:v), hexane/acetone (1:1, v:v), toluene, ethyl acetate
Fruits and vegetables	Environmental toxins (inorganic)[b]	Water
Fruits and vegetables	Antioxidants	7% acetic acid in methanol, 0.1% TFA in methanol
Grains and seeds	Oils	Hexane, hexane/isopropanol (3:2, v:v), petroleum ether
Grains and seeds	Pesticides	DCM/acetone (1:1, v:v), hexane/acetone (1:1, v:v), toluene, ethyl acetate
Grains and seeds	Mycotoxins	Methanol/water (1:1, v:v), acetonitrile/water (1:1, v:v), methanol/water/ H_3PO_4
Meats (beef, pork and poultry)	Pesticides	Acetone/hexane (1:1, v:v), DCM/acetone (1:1, v:v)
Meats (beef, pork and poultry)	Sterols	Chloroform/methanol (2:1, v:v), hexane /isopropanol (3:2, v:v)
Meats (beef, pork and poultry)	Ions: sulfates and nitrates	Water
Meats (beef, pork and poultry)	Antibiotics	Water
Fish and shellfish	Environmental toxins (organic)	DCM/acetone (1:1, v:v), hexane/acetone (1:1, v:v), toluene
Fish and shellfish	Environmental toxins (inorganic)	Methanol, methanol with 1% acetic acid
Dairy	Environmental toxins (organic)	DCM/acetone (1:1, v:v), hexane/acetone (1:1, v:v)
Dairy	Environmental toxins (inorganic)	Water
Dairy	Fats and lipids	Hexane, hexane/isopropanol (3:2, v:v), petroleum ether
Snack foods	Fats and lipids	Hexane, hexane/isopropanol (3:2, v:v), petroleum ether
Snack foods and potatoes	Acrylamide	Water with 10 mM formic acid or acetonitrile (100%)

[a] PAH, PCB, OCP, OPP, Dioxins
[b] Anions and cations

CHOOSING THE ASE METHOD PARAMETERS

Dionex has published several application notes describing extraction of various food and animal feed samples. These application notes provide recommended methods and ASE parameters for these particular applications. But, because every sample matrix may be different and some scenarios may not fit exactly with each application note, a new method may need to be developed for some samples.

Extraction parameters used in Standard ASE Conditions (SAC) are a good starting point for method development. To load the SAC method parameters, select Method "0" in the METHOD EDITOR screen. SAC conditions are as follows:

Oven Temperature:	100 °C
Static Time:	5 min
Flush Volume:	60 % of cell volume[a]
Purge Time:	60 s on ASE 200, 100 s on ASE 300[b]
Static Cycles:	1
Pressure:	1500 psi (only applies to ASE 200 instruments)

[a]Decrease flush volume to 50% if instrument was manufactured in 1998 or later.
[b]Remember the purge time depends on the ASE cell size: 60 s for 11-mL cells and 100 s for 34-mL cells. Purge time may need to be increased depending on the size of the ASE extraction cell.

Save the SAC conditions as a method number between 1 and 24 (the instrument will not allow Method 0 to be loaded from the LOAD METHOD/SCHEDULE Screen). Once the results from the initial ASE method have been determined, adjust the ASE method parameters for optimum extraction recovery for a particular sample. Remember that the temperature, solvent, and time are the three most important ASE method parameters influencing extraction efficiency. Changing the extraction temperature will, in most cases, give the most dramatic results. Once the extraction temperature has been established, "fine tune" the ASE method by adjusting the static time, the static cycles, and the flush volume. A finely tuned ASE method will result in a fast, efficient extraction that consumes a minimum volume of solvent. For more information on ASE method development, see Dionex Technical Note 208.

POST-EXTRACTION TREATMENT

Sometimes, in spite of pretreatment efforts, some type of post-extraction treatment is necessary to produce high quality analytical data. For example, at ASE extraction temperatures above 100 °C, water from wet samples usually appears in the final extract, even after pretreatment with ASE Prep DE. If acetone is used as the extraction solvent, the extracted water will be miscible with the acetone and may not be visible until the evaporation step (in most cases, the extract will appear cloudy if water is mixed with acetone). On the other hand, if hexane is used as the extraction solvent, the extract will contain two distinct phases: (1) a visible water layer, and (2) a hexane layer. To easily remove unwanted water from extracts, add sodium sulfate directly to the collection vial containing the extract (remember it is important not to use sodium sulfate in the extraction cell). Shake the collection vial and decant the solvent into a clean collection vial or other suitable container. Rinse the sodium sulfate with fresh solvent to ensure that analytes of interest are not trapped in the sodium sulfate layer.

EXAMPLE OF METHOD DEVELOPMENT OF AN ANIMAL FEED SAMPLE

The following is an example of developing an ASE method for an animal feed sample:

Sample: Pelletized rodent food (dry)

Analyte: Anti-schizophrenic drugs

Step One: Pretreatment of sample

- Pellets were ground into a course powder using a hand grinder.
- 10 g of ground sample was mixed with 5 g of Ottawa sand (20–30 mesh particle size).

Step Two: Preparation of the extraction cell

- A 33-mL ASE extraction cell was selected.
- A cellulose filter was placed into the extraction cell outlet.
- The sample/sand mixture was added to the cell.

Step Three: Selection of ASE extraction solvent

- Because methanol was used for the traditional extraction of this sample (wrist shaker) methanol was also chosen as the extraction solvent for the ASE.

Step Four: Selection of first ASE method parameters

- The temperature of 75 °C was chosen (because of the high carbohydrate content of the rodent food, a temperature that was 25 °C below the SAC of 100 °C was chosen as the preliminary temperature).
- A flush volume of 30% was chosen. (ASE instruments manufactured after 1998 contain large-bore tubing. To adjust for this, the original 60% flush volume was decreased by half.)
- One static cycle was chosen (SAC).
- A 3-min static time was chosen (3 min was chosen instead of the SAC of 5 min to cut down on the extraction time if possible).
- The purge time was chosen to be 100 s (SAC)
- This method was saved as Method 1.

RESULTS OF ASE METHOD 1

Analysis of the samples extracted using the conditions of ASE Method 1 showed the presence of the target analyte, but at a lower concentration than desired. From the initial results of ASE Method 1, methanol appears to be a good choice for the extraction solvent because the target analyte was detected. However, the concentration of the target compound was lower than expected. After an examination of the extraction parameters of ASE Method 1, it was decided to increase the extraction temperature. This decision was based on the fact that solvent, temperature, and extraction time are the three most important ASE method parameters governing extraction efficiency. A fresh portion of the sample was extracted using a revised method shown below:

Step Five: Revision of ASE Method 1

- Temperature increased to 150 °C (the temperature was increased due to the low recoveries).
- All other parameters stayed the same.
- Saved new ASE parameters as Method 2.

RESULTS OF ASE METHOD 2

Analysis of the samples extracted using the conditions of ASE Method 2 resulted in better recovery of the target analyte, but the extract was deeply colored and contained some co-extracted material. It appeared that the ASE extraction temperature was a key parameter for the extraction of this particular analyte, however, because some unwanted matrix material was co-extracted with the target compound, it was thought that perhaps the extraction temperature in ASE Method 2 was too high.

Step Six: Revision of ASE Method 2

- Temperature decreased to 100 °C (temperature was decreased in an effort to eliminate the co-extractables and to eliminate the dark color of the extract).
- Static time increased to 5 min (static time was increased to 5 min to ensure good analyte recovery with a lower temperature).
- All other parameters stayed the same.
- Saved new ASE parameters as Method 3.

RESULTS OF ASE METHOD 3

Analysis of the samples extracted using the conditions of ASE Method 3 resulted in excellent recovery of the target analyte. In addition, the ASE extract was clear and contained no co-extracted matrix material.

SUMMARY

When developing a method for the ASE system, there are many parameters to consider. First pretreat the sample, if required, to ensure proper contact with the extraction solvent. Sample pretreatment can be accomplished in many ways, such as removing excess water and properly dispersing the sample. Second, determine the best ASE solvent to extract the target analytes. The solvent that was used for the traditional or manual extraction method is usually a good starting point. If needed, adjust or change the ASE solvent based on results from the instrumental or determinative procedure used to analyze the extract. Third, optimize the ASE method parameters. Standard ASE Conditions (SAC) are typically a good starting point when developing a new ASE method. Finally, determine if any post-extraction treatment is required, such as removing any unwanted water from the extract or filtering the extract prior to analysis.

SUPPLIERS

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

Alltech Associates, Inc., 2051 Waukegan Road, Deerfield, IL 60015 USA, Tel: 847-948-8600, www.alltechweb.com.

REFERENCES

1. *Extraction of Drugs from Animal Feeds Using Accelerated Solvent Extraction (ASE)*. Application Note 326; Dionex Corporation, Sunnyvale, CA.
2. *Rapid Determination of Organochlorine Pesticides in Animal Feed Using Accelerated Solvent Extraction (ASE).* Application Note 349; Dionex Corporation, Sunnyvale, CA.
3. *Rapid Determination of Sulfonamide Residues in Animal Tissue and Infant Food Containing Animal Products Using Accelerated Solvent Extraction (ASE).* Application Note 353; Dionex Corporation, Sunnyvale, CA.
4. *Selective Extraction of PCBs from Fish Tissue Using Accelerated Solvent Extraction (ASE).* Application Note 322; Dionex Corporation, Sunnyvale, CA.
5. Determination of Unbound Fat in Various Food Matrices Using Accelerated Solvent Extraction (ASE). Application Note 321; Dionex Corporation, Sunnyvale, CA.
6. *Extraction of Oil from Oilseeds by Accelerated Solvent Extraction (ASE).* Application Note 325; Dionex Corporation, Sunnyvale, CA.
7. *Determination of Total Fat in Powdered Infant Formula Using Accelerated Solvent Extraction (ASE).* Application Note 329; Dionex Corporation, Sunnyvale, CA.
8. *Accelerated Solvent Extraction (ASE) of Pesticide Residues in Food Products.* Application Note 332; Dionex Corporation, Sunnyvale, CA.
9. *Rapid Determination of Fat in Meat Using Accelerated Solvent Extraction (ASE).* Application Note 334; Dionex Corporation, Sunnyvale, CA.
10. *Accelerated Solvent Extraction (ASE) of Active Ingredients from Natural Products.* Application Note 335; Dionex Corporation, Sunnyvale, CA.
11. *Determination of Fat in Dried Milk Products Using Accelerated Solvent Extraction (ASE).* Application Note 340; Dionex Corporation, Sunnyvale, CA.
12. *Determination of PCBs in Large Volume Fish Tissue Samples Using Accelerated Solvent Extraction (ASE).* Application Note 342; Dionex Corporation, Sunnyvale, CA.
13. *Determination of Pesticides in Large Volume Food Samples Using Accelerated Solvent Extraction (ASE).* Application Note 343; Dionex Corporation, Sunnyvale, CA.
14. *Extraction of Fat from Chocolate Using Accelerated Solvent Extraction (ASE).* Application Note 344; Dionex Corporation, Sunnyvale, CA.
15. *Extraction of Fat from Dairy Products (Cheese, Butter, and Liquid Milks) Using Accelerated Solvent Extraction (ASE).* Application Note 345; Dionex Corporation, Sunnyvale, CA.
16. *Extraction of Zearalenone from Wheat and Corn by Accelerated Solvent Extraction (ASE).* Application Note 350; Dionex Corporation, Sunnyvale, CA.
17. *Fast Determination of Acrylamide in Food Using ASE/ICUV or ASE/ICMS.* Application Note 409; Dionex Corporation, Sunnyvale, CA.
18. Gentili, A., Perret, D., Marchese, S., Sergi, M., Olmi, C., Curini, R. 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.* **2004**, *52*, 4614–4624.