

# Rapid Determination of Fat in Meat Using Accelerated Solvent Extraction

## Introduction

Soxhlet extraction is an accepted technique for extracting fat from meat samples. Though it is simple and robust, there are drawbacks to Soxhlet extraction, such as long drying and extraction times, lack of automation, and the amount of solvent used per sample. Accelerated solvent extraction is a technique that was developed to replace Soxhlet and other extraction techniques for many samples. The automation and rapid extraction time of accelerated solvent extraction overcome the shortcomings of Soxhlet extraction.

Accelerated solvent extraction uses common liquid solvents at increased temperatures and pressures to accelerate the extraction process. Analytes are more soluble and thus dissolve more quickly in hot solvents, whereas, in the Soxhlet method, the upper temperature is limited by the boiling point of the solvent. Pressurized solvent can be heated to well above its boiling point. By using hot, pressurized liquid solvents, accelerated solvent extraction decreases the amount of solvent and the time needed to complete an extraction. Most extractions can be completed in less than 20 min, using less than 20 mL of solvent.

This application note describes an accelerated solvent extraction method for determining the amount of fat in meat samples. The method provides a rapid means of extracting fat from all of the types of meat investigated. Fresh or processed meat, as well as high- and low-fat meat products can be extracted with accelerated solvent extraction very easily. The Thermo Scientific Dionex ASE 200 accelerated solvent extraction system allows automated, uninterrupted extractions of up to 24 samples and is available with computer control of all the extraction parameters.

The details of the accelerated solvent meat extraction method and how it compares with the traditional Soxhlet method are presented in this application note.

## Equipment

Dionex ASE™ 200 accelerated solvent extraction system, with 11- or 22-mL stainless steel extraction cells (P/Ns 048765 and 048764)

Cellulose Filters (P/N 049458)

Collection Vials, 40 mL (P/N 048783)

Fisher Scientific Ottawa sand standard

Thermo Scientific Dionex ASE Prep DE (diatomaceous earth) (P/N 062819)

Analytical balance (to read to nearest 0.0001 g or better)

Fisher Scientific mortar and pestle, or equivalent

Solvent evaporator (N-EVAP®, Organomation Associates, Inc., Berlin, USA, or equivalent)

Forced air oven

Microwave oven (800 W) with carousel\*

\*Dionex ASE 150 and 350 accelerated solvent extraction systems can be used for equivalent results

## Solvents

Fisher Scientific petroleum ether or hexane, pesticide-grade, or equivalent

## Extraction Conditions

Solvent:	Petroleum ether or hexane*	Extraction solvent
Temperature:	125 °C	The temperature during extraction
Pressure:	1500 psi†	The pressure during extraction
Heat:	6 min	Cell temperature equilibration time
Static:	1 or 2 min**	Extraction time
Flush:	60%	Fresh solvent added after the static time as a percent of cell volume

## Extraction Conditions Continued

Purge:	60 s	Nitrogen purge of cell after extraction
Cycles:	2	Number of times static period is repeated during the extraction
Total Time:	12 min	Extraction time per sample
Total Solvent:	20 mL	Solvent use per sample

\*Petroleum ether and hexane were found to be equivalent as extraction solvents for fat in meat.

\*\*When extracting more than 1 g of a high-fat sample, a 2-min static time may be beneficial.

† Pressure studies show that 1500 psi is the optimum extraction pressure for all accelerated solvent extraction applications.

## Sample Preparation

Place approximately 6 g of Dionex ASE™ Prep DE in a mortar and then weigh and record to the nearest 0.1 mg, 3–4 g of homogenous sample on top of the Dionex ASE Prep DE. Using a pestle, grind the sample with the Dionex ASE Prep DE and then transfer the mortar with its contents to a microwave oven for drying. Up to five 3–4 g samples can be dried at once in an 800-W oven at full power for 3 min (actual drying time may vary).

Place a cellulose filter in each extraction cell before loading the sample. Remove the sample from the oven and place it in a 22-mL cell. If desired, the mortar and pestle may be rinsed with a few mL of petroleum ether and the rinsings added to the extraction cell. After the sample is loaded, fill any void volume in the extraction cell with sand.

The sample weight and cell size cited above are optimized for low-fat samples. For high-fat samples, a 1-g sample is sufficient and an 11-mL cell will minimize the solvent volume used.

## Extraction Procedure

Place the loaded cells into the upper carousel and the appropriate number of clean, preweighed collection vials in the lower carousel. Set up the method to be used and start the extraction. After the extractions are complete, remove the collection vials from the lower carousel. Evaporate the extraction solvent with a nitrogen stream and then dry each extract in an oven at 100 °C, until a constant weight is achieved. The extract weight is obtained by subtracting the vial tare weight from the total weight.

## Traditional Soxhlet Procedure

To compare the values of fat obtained from an accelerated solvent extraction with a traditional extraction, the meat samples were extracted using Association of Analytical Chemists (AOAC) Intl. Official Method 960.39 (Fat or Ether Extract in Meat). A brief description of the method follows. Weigh a 3–4 g sample by difference into a thimble containing a small amount of sand. Mix with a glass rod, place a thimble and rod in a 50 mL beaker, and dry in an oven for 1.5 h at 125 °C. Extract the sample with petroleum ether for 4 h at a condensation rate of 5–6 drops/s. Dry the extract to a constant weight at 100 °C. Allow the sample to cool, and then determine the weight.

## Method Comparison (Accelerated Solvent Extraction and Soxhlet)

To compare the accelerated solvent extraction and Soxhlet methods, the main procedures are summarized in Table 1. Each method uses the same amount of sample. Each involves sample drying, extraction with petroleum ether, and solvent evaporation for a gravimetric determination. Samples are treated in a similar manner to ensure that the fat residue will be similar in composition to that obtained from a traditional Soxhlet extraction. Notable differences in sample treatment include the microwave drying and the heated and pressurized extraction conditions for the accelerated solvent extraction method.

## Time Comparison (Accelerated Solvent Extraction and Soxhlet)

Accelerated solvent extraction requires much less time than the Soxhlet method. The microwave-drying step reduces the drying time from 90 min to as little as 3 min. Even with large numbers of samples (>20) the drying time is considerably reduced. The difference in the extraction step also reduces the total method time. Because accelerated solvent extraction uses heated solvents, the extraction occurs much faster than Soxhlet. The heated solvents hold more lipid material and thus a smaller total amount of solvent is needed for complete extractions. For a single sample, accelerated solvent extraction can deliver results in about an hour when time for sample preparation and final solvent removal is included. When large numbers of samples need to be processed, the total method time for accelerated solvent extraction becomes comparable to multiple Soxhlets running simultaneously. It would take

Table 1. Comparison of the Accelerated Solvent Extraction and Soxhlet Methods

Accelerated Solvent Extraction Method	Soxhlet Method
Weigh 3–4 g of sample by difference into a mortar containing 6 g of Dionex ASE Prep DE.	Weigh 3–4 g of sample by difference into thimble containing a small amount of sand.
Grind with mortar and pestle until well dispersed.	Mix with glass rod and place thimble and rod in 50-mL beaker.
Dry in microwave for 3 min. Place dried sample in 22-mL accelerated solvent extraction cell.	Dry in oven for 1.5 h at 125 °C.
Extract with petroleum ether at 125 °C. Extraction period is 12 min per sample.	Extract sample with petroleum ether. Use a thimble porosity permitting rapid passage of ether. Extraction period is 4 h at condensation rate of 5–6 drops/s.
Dry extract to constant weight at 100 °C, cool, and weigh.	Dry extract to constant weight at 100 °C, cool, and weigh.

Table 2. Time Comparison of the Accelerated Solvent Extraction and Soxhlet Methods

Method Steps	Accelerated Solvent Extraction 2 Samples	Soxhlet 2 Samples (in parallel)	Accelerated Solvent Extraction 20 Samples	Soxhlet 20 Samples (in parallel)
Weigh Sample	1 min	1 min	10 min	10 min
Prepare Sample	9 min	4 min	90 min	40 min
Dry Sample	3 min	90 min	12 min*	90 min
Extract Sample	24 min	240 min	240 min	240 min
Evaporate Solvent	15 min	15 min	15 min	15 min
Dry Extract	30 min	30 min	30 min	30 min
Weigh Extract	1 min	1 min	10 min	10 min
Total Method Time	83 min	381 min	407 min	435 min

\* Up to five 4-g samples can be dried at once. See *Sample Preparation* for details.

more than 20 Soxhlet extractors working in parallel to compete with accelerated solvent extraction in terms of total time expended. The time comparisons for two samples and 20 samples are shown in Table 2.

### Results and Discussion

Several low-fat and high-fat meat samples were obtained from a lab that routinely provides nutritional analyses for meat. The issuing lab had determined the percent fat by weight of the samples using Soxhlet extraction (AOAC Intl. Official Method 960.39). After all samples arrived in our lab, they were extracted with the Dionex ASE 200 accelerated solvent extraction system using the methods described and the results were returned to the lab that provided the samples. Only then were the accelerated

solvent extraction and Soxhlet values compared. The samples ranged from “nonfat” meats with a percent fat by weight of less than 1% to high-fat samples where the percent fat by weight was greater than 50%.

#### Extraction of Low-Fat Meats

With the health consciousness of consumers increasing, more food companies are providing meat products with very low fat content. Accelerated solvent extraction shows good accuracy and precision when extracting the fat from these low-fat matrices as shown in Table 3 and Table 4. Hexane and petroleum ether were found to be equivalent as extraction solvents. Petroleum ether has a lower boiling point and may be preferred during postextraction solvent evaporation.

Table 3. Percent Fat in Low-Fat Fresh Turkey Breast (Accelerated Solvent Extraction vs Soxhlet)

Sample	Accelerated Solvent Extraction Run #1*	Accelerated Solvent Extraction Run #2	Accelerated Solvent Extraction Run #3	Accelerated Solvent Extraction Run #4	Accelerated Solvent Extraction Run #5	Accelerated Solvent Extraction Average	Standard Deviation	Soxhlet
Turkey #1	0.48	0.38	0.44	0.38	0.33	0.40	0.058	0.37
Turkey #2	0.32	0.46	0.28	0.33	0.24	0.33	0.084	0.32
Turkey #3	0.39	0.35	0.40	0.52	0.52	0.40	0.068	0.36
Turkey #4	0.72	0.66	0.62	0.68	0.68	0.65	0.047	0.67
Turkey #5	0.48	0.40	0.42	0.48	0.48	0.42	0.064	0.47

Conditions: 4 g sample, 6 g Dionex ASE Prep DE, samples dried for 3 min in 800-W microwave oven, 22-mL cells, petroleum ether solvent (\*run #1, hexane solvent), 125 °C, 1500 psi, 2 × 1 min cycles, 60% flush, 60 s purge.

Table 4. Percent Fat in Low-Fat Processed Meat Samples (Accelerated Solvent Extraction vs Soxhlet)

Sample	Accelerated Solvent Extraction Run #1*	Accelerated Solvent Extraction Run #2	Accelerated Solvent Extraction Run #3	Accelerated Solvent Extraction Average	Standard Deviation	Soxhlet
Beef	2.82	2.90	2.83	2.85	0.046	2.81
Chicken	0.84	0.84	0.79	0.82	0.025	0.75
Ham	1.85	1.74	1.87	1.82	0.069	1.72
Franks	1.90	1.97	1.97	1.94	0.041	1.54
Turkey	1.04	1.00	1.04	1.02	0.026	0.94

Conditions: 4 g sample, 6 g Dionex ASE Prep DE, samples dried for 3 min in 800-W microwave, 22-mL cells, petroleum ether solvent, 125 °C, 1500 psi, 2 × 1 min cycles, 60% flush, 60 s purge.

## Extraction of High-Fat Meats

The high-fat meat samples are also separated into two categories: fresh and processed meat. The fresh meat samples were pork meat trimmings from a production line. To illustrate the importance of good sample preparation, pork sample #2 was prepared twice in triplicate. It was prepared as received and also after being reground with a small lab grinder. When sample #2 was received, it was evident that the sample contained a lot of nonfatty connective tissue. However, the sample was prepared and run “as is” with the rest of the samples.

The results for sample #2 were: 52.77%, 54.16%, and 47.67% fat, with an average of 51.53% fat and a standard deviation of 3.42% fat. The relatively high standard deviation was due to the inhomogeneous nature of the sample and not a reflection of the instrument performance. The data presented in Table 5 show the results of sample #2 after more thorough grinding. These data show that a homogeneous sample is necessary for precise results. Table 6 shows the results of the extraction of fat from high-fat processed meat. Both sets of data show that Accelerated Solvent Extraction provides good accuracy and precision for the extraction of fat from meat samples.

Table 5. Percent Fat in High-Fat Fresh Pork Samples (Accelerated Solvent Extraction vs Soxhlet)

Sample	Accelerated Solvent Extraction Run #1	Accelerated Solvent Extraction Run #2	Accelerated Solvent Extraction Run #3	Accelerated Solvent Extraction Average	Standard Deviation	Soxhlet
Pork #1	32.60	32.95	32.72	32.76	0.18	35.19
Pork #2	52.62	52.20	52.97	52.60	0.38	52.66
Pork #3	24.46	24.29	24.31	24.35	0.09	24.57
Pork #4	28.96	28.53	27.57	28.35	0.71	27.39
Pork #5	27.91	28.33	29.30	28.51	0.71	28.85

Conditions: 1 g sample, 3 g Dionex ASE Prep DE, samples dried for 3 min in 800-W microwave, 11-mL cells, hexane solvent, 125 °C, 1500 psi, 2 × 1 min cycles, 60% flush, 60 s purge.

Table 6. Percent Fat in High-Fat Processed Meat Samples (Accelerated Solvent Extraction vs Soxhlet)

Sample	Accelerated Solvent Extraction Run #1	Accelerated Solvent Extraction Run #2	Accelerated Solvent Extraction Run #3	Accelerated Solvent Extraction Average	Standard Deviation	Soxhlet
Beef	41.39	41.37	39.44	40.74	1.12	40.85
Pepperoni	42.97	42.56	42.43	42.66	0.28	43.15
Chorizo	28.03	28.17	27.73	27.98	0.22	27.84
Bacon	47.08	45.71	47.19	46.66	0.82	46.83
Sausage	33.67	34.11	33.61	33.80	0.28	33.54

Conditions: 1 g sample, 3 g Dionex ASE Prep DE, samples dried for 3 min in 800-W microwave oven, 11-mL cells, hexane solvent, 125 °C, 1500 psi, 2 × 1 min cycles, 60% flush, 60 s purge.

## Conclusion

Extraction of fat from meat with accelerated solvent extraction is rapid and delivers accurate and precise results. Hexane and petroleum ether and were found to be equivalent extraction solvents. This accelerated solvent extraction method is competitive with multiple Soxhlet extractors, and the automation of accelerated solvent extraction allows unattended overnight runs of up to 24 samples. The results obtained from the accelerated solvent extraction method match the values obtained using the Soxhlet method.

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## Suppliers

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