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# Determination of Total Fat in Powdered Infant Formula Using Accelerated Solvent Extraction (ASE)

## **INTRODUCTION**

New government regulations, such as the U.S. Nutritional Labeling and Education Act of 1990, require food products to carry labels that list the content of saturated and unsaturated fats. Accurate determination of fat in certain foods is difficult due to the binding or entrapment of the fat by the matrix. Most methods used to determine fat in these difficult matrices include a pretreatment step to denature or destroy the physical structure of the matrix and allow greater accessibility to the fat (e.g., AOAC Intl. Method 933.05 (Fat in Cheese) calls for sample treatment with ammonium hydroxide followed by hydrochloric acid). Ammonium hydroxide is a common reagent used to pretreat dairy-based products before extraction because it dissolves casein. Common fat determination methods used by the dairy industry are the Roese-Gottlieb and Modified Mojonnier methods (AOAC Intl. Methods 905.02 and 989.05, respectively). These methods specify the use of ammonium hydroxide to dissolve casein and liberate the fat.

Accelerated Solvent Extraction (ASE<sup>®</sup>) is a new automated extraction technique that can significantly reduce extraction time and solvent consumption and yields equivalent results, without requiring a pretreatment step. Samples are loaded onto the ASE system and solvent is pumped into an extraction cell, which is then pressurized and heated for several minutes. The increased temperature allows extractions to be done in a fraction of the time required for traditional extractions performed at room temperature or with warm solvents. Extraction under pressure allows the solvents to be heated while maintaining their liquid state.

Following the extraction step, the ASE system transfers the solvent, along with the dissolved components of interest, into a collection vial (within the instrument) for further cleanup or analysis. The extraction process is automated and up to 24 samples can be extracted sequentially.

ASE has shown significant advantages over competing techniques with regard to time savings, solvent use, automation, and efficiency. In this application note, fat is extracted from infant formulas by ASE without aggressive pretreatments. The results are equivalent to traditional pretreatment/extraction methods. The procedures described in this application note apply to powdered infant formulas and may be suitable for similar matrices. The fat content in these examples is determined by collecting the extract in preweighed vials, evaporating the solvent and any extracted water and reweighing the vials. The extraction efficiency was verified by fatty acid methyl ester (FAME) analysis of the dried extract after the gravimetric recovery had been determined.

## **EQUIPMENT**

ASE 200 Accelerated Solvent Extractor\* equipped with  
11 mL stainless steel extraction cells  
Cellulose filter disks (P/N 49458)  
Analytical balance (0.001 g or better)  
Mortar and pestle  
Solvent evaporator (TurboVap<sup>®</sup>, Caliper Life Sciences)  
Forced air oven  
Sand (Ottawa Standard, Fisher Scientific, Cat. No. S23-3)

\*ASE 150 and 350 can be used for equivalent results.

## REAGENTS

ASE Prep DE (diatomaceous earth) (P/N 062819)

## SOLVENTS

Acetone

Hexane

Water

All solvents are pesticide-grade or equivalent and available from Fisher Scientific.

## EXTRACTION CONDITIONS

Solvent: Hexane acetone, 4:1 volume

Temperature: 125 °C

Pressure: 1500 psi\*

Cell Heatup: 6 min

Static Time: 5 min

Flush Volume: 100%

Purge Time: 60 s

Cycles: 3

Total Time: 24 min

Total Solvent: 20–25 mL

\*Pressure studies show that 1500 psi is the optimum extraction pressure for all ASE applications.

## SAMPLE PREPARATION

### Without Water (Similac®, Enfamil®, SRM 1846)

Place a cellulose filter in each extraction cell before loading the sample. Samples are mixed with ASE Prep DE to prevent sample compaction and ensure efficient solvent contact. Weigh approximately 1 g of sample, to the nearest 0.1 mg. Grind with 3 g of ASE Prep in a mortar and pestle and place it in an 11 mL cell. After the sample is loaded, fill any void volume in the extraction cell with sand.

### With Water (Isomil®, Alsoy®, Good Start®)

Place a cellulose filter in each extraction cell before loading the sample. Weigh 3 g of ASE Prep DE into a suitable weighing dish. Add, by pipette, 0.4 g water to the ASE Prep DE and mix to disperse the water. Weigh approximately 1 g of sample, to the nearest 0.1 mg. Grind the sample with the prepared wet ASE Prep DE in a mortar and pestle and place in an 11 mL cell. After the sample is loaded, fill any void volume in the extraction cell with sand.

*Note: With the addition of water to the samples, the optimum extraction temperature is changed to 100 °C.*

## ASE 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 methods 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 MOJONNIER PROCEDURE

To compare the values of fat obtained from an ASE extraction with a traditional extraction, the infant formulas were extracted by the AOAC Intl. Method 932.06, Fat in Dried Milk. This method requires alkaline pretreatment with ammonium hydroxide, heating, and liquid–liquid extraction with a mixture of petroleum ether, diethyl ether, and ethanol. Following the extraction, the combined organic solvent (~125 mL) is evaporated and the residue is dried to a constant weight.

## FAME ANALYSIS

Fatty acid methyl ester analysis was performed on the extracts to verify the extraction efficiency. The method followed was AOAC Intl. Method 991.39, modified by substituting nonadecanoic acid methyl ester for tricosanoic acid methyl ester as the internal standard. Nonadecanoic acid methyl ester was found to be more suitable because it is more representative of the fatty acids in the samples studied here. The gas chromatographic conditions were: split injection, injector temperature 225 °C, oven program 70 °C to 240 °C at 8 °C min<sup>-1</sup> hold for 4 min at 240 °C, FID detector temperature 280 °C. The column used was 30 m × 0.32 mm × 0.25 μm SUPELCOWAX 10™.

## DISCUSSION AND RESULTS

Many laboratories use standard reference materials to verify their procedures and methods. The U.S. National Institute of Standards and Technology (NIST) has made available an SRM with a reported value of fat content. SRM 1846 is a milk-based powdered infant formula. The fat content as listed in the certificate of analysis was determined by nine collaborating labs using three different methods: alkaline pretreatment followed by Roese-Gottlieb extraction, alkaline pretreatment followed by Mojonnier extraction, and acid digestion followed by ether extraction. Alkaline pretreatment followed by Mojonnier extraction (AOAC Intl. Method 932.06, Fat in Dried Milk) was used to confirm accurate results from SRM 1846. The same method was used to extract the other powdered infant formulas and the results are shown in Table 1.

ASE is capable of extracting fat from powdered infant formulas without the use of aggressive pretreatments. Using the extraction conditions previously listed, most of the samples were extracted easily. Nevertheless, a few samples did not give the desired results. It was found that for these samples the addition of water to the ASE Prep DE before the samples were dispersed greatly assisted the extraction of fat. This sequence of adding the water to the dispersant worked better than adding the water directly to the sample or adding water to the extraction solvent. The samples that benefited from the addition of water were the soy protein-based formulas (Alsoy and Isomil) and the hydrolyzed milk protein-based formula (Good Start). The results of the ASE extractions are also shown in Table 1. For each sample, there is close agreement between the traditional Mojonnier method and the ASE methods.

**Table 1. Percent Fat in Powdered Infant Formulas Gravimetric Results from ASE and Mojonnier Extractions**

Sample	ASE Extraction (n = 3)			Mojonnier Extraction (n = 3)		
	Method	% Fat	% RSD	Method	% Fat	% RSD
Isomil	With water	27.86	0.53	932.06	28.71	0.62
Alsoy	With water	25.35	1.10	932.06	25.58	0.82
Good Start	With water	26.21	1.67	932.06	26.72	0.06
Similac	Without water	29.06	0.94	932.06	28.84	0.07
Enfamil	Without water	28.89	0.72	932.06	28.83	0.24
SRM 1846	Without water	27.51	0.70	932.06	26.68	0.41

A potential concern, when using gravimetric analysis with any extraction method, is skewing of the results due to coextraction of nonfat materials. One procedure to ascertain the validity of the values is to determine the sample's fat content by some other technique. Analysis of the fatty acids by gas chromatography is an accepted technique to determine the amount of fat in a sample. The fatty acids in the sample are converted to their methyl esters (FAMES) and are then quantified by gas chromatography. Following each extraction and gravimetric analysis of the commercial infant formulas, FAMES were generated from the residue and analyzed by gas chromatography. The results of the FAMES analyses are shown in Table 2. Again, there is close agreement between the samples extracted by the traditional Mojonnier method and the ASE methods. The composition of the fatty acids can be determined from the GC chromatograms. Examples of chromatograms from both extraction techniques are shown in Figures 1 and 2. The comparison of the fatty acid profile is shown in Table 3. No significant differences are observed in the fatty acid profile obtained from either the ASE or Mojonnier extractions.

<b>Table 2. FAME Analysis of ASE and Mojonnier Extractions</b>			
<b>ASE FAME results (n = 1)</b>		<b>Mojonnier FAME results (n = 1)</b>	
<b>Sample</b>	<b>% Fat</b>	<b>Sample</b>	<b>% Fat</b>
Isomil	27.12	Isomil	27.13
Alsoy	25.13	Alsoy	24.03
Good Start	25.27	Good Start	25.38
Similac	27.52	Similac	27.57
Enfamil	26.52	Enfamil	26.06
SRM 1846	27.24	SRM 1846	26.73

<b>Table 3. Fatty Acid Comparison (Sample: Isomil) Normalized Weight Percent</b>			
<b>ASE Extraction</b>		<b>Mojonnier Extraction</b>	
<b>Fatty Acid</b>	<b>% Fat</b>	<b>Fatty Acid</b>	<b>% Fat</b>
Saturated fatty acids	34.9	Saturated fatty acids	34.5
Monounsaturated	37.2	Monounsaturated	37.5
Polyunsaturated	27.8	Polyunsaturated	28.0

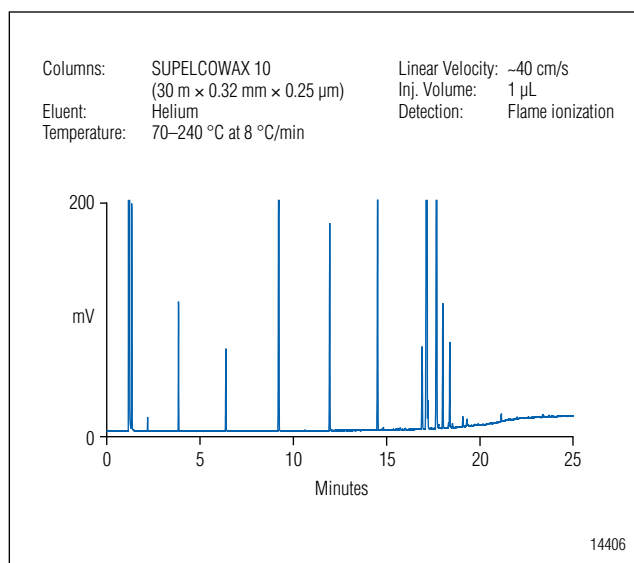


Figure 1. Chromatogram of FAMES from ASE extraction of Isomil.

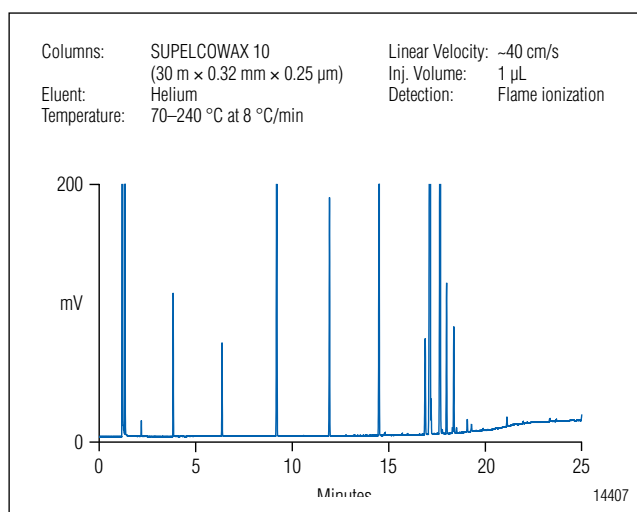


Figure 2. Chromatogram of FAMES from Mojonnier extraction of Isomil.

## CONCLUSION

ASE can extract total fat from infant formulas with recoveries comparable to traditional hydrolysis/extraction methods. The amount of fat obtained from infant formula using ASE represents the total fat in the sample as confirmed by good agreement between the gravimetric and FAME analysis methods. Furthermore, ASE provides a method that is faster and requires far less solvent and labor than traditional methods. The automation features of ASE allow up to 24 samples to be run sequentially and unattended, unlike the labor-intensive Mojonnier method. In addition, ASE requires only 20 mL of solvent compared to 125 mL for the Mojonnier method.

## SUPPLIERS

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Caliper Life Sciences, 68 Elm Street, Hopkinton, MA 01748 USA, Tel: 508-435-9500, [www.caliperls.com](http://www.caliperls.com).

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