Microscale Recovery of Total Lipids from Fish Tissue by Accelerated Solvent Extraction

Eric D. Dodds[a], Mark R. McCoy[a,e], Adeline Geldenhuys[a,b], Lorrie D. Rea[d,e], and John M. Kennish[a,c,*]

University of Alaska Anchorage: [a]Applied Science, Engineering, and Technology Laboratory; Departments of [b]Biological Sciences and [c]Chemistry; and the [d]Environment and Natural Resources Institute, Anchorage, Alaska 99508, and [e]Physiological Ecology Laboratory, Alaska Department of Fish and Game, Anchorage, Alaska 99518

ABSTRACT: A number of techniques are available for the extraction of lipids from a variety of tissues; however, conventional methods are characteristically labor intensive, typically involve large volumes of toxic solvents, and usually require at least 1 g of tissue. With the availability of accelerated solvent extraction (ASE) technology, the opportunity exists to modify classical lipid extraction techniques such that automated high-pressure, high-temperature extractions may be performed with the use of far smaller volumes of costly and harmful solvents. Moreover, the high extraction efficiency attainable by ASE suggests that significantly less tissue would be required than is routinely used. This paper describes the adaptation of previously developed lipid extraction solvent systems for use with ASE toward the purpose of extracting total lipids from 100 mg of fish tissue. The efficacy of three solvent systems for lipid extraction from representative fish tissues, including a standard reference material, was explored using gravimetry and FA analysis by GC. A TG was used as a surrogate to monitor overall method performance. The findings herein demonstrate that microscale ASE represents an effective and efficient alternative to traditional lipid extraction techniques based on quantity and composition of extracted lipid, surrogate recovery, and precision.

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KEY WORDS: Accelerated solvent extraction (ASE), fatty acid analysis, lipid extraction.

The analysis of lipid composition in a broad range of tissue types is facilitated by an equally varied collection of lipid extraction methodologies. Several classical lipid recovery techniques and numerous variations thereof have been successfully developed, often for a specific sample type. Many of the best studied and widely used methods for extraction of lipids from fish and other marine tissues involve various mixtures of chloroform and methanol as described by Folch *et al.* (1) and Bligh and Dyer (2), and alkane/alcohol mixtures such as that described by Hara and Radin (3) and Radin (4). Methylene chloride has also been advanced as a solvent for lipid extraction owing in part to its already common use in the extraction of organic contaminants from environmental samples, including animal tissues (5). This solvent offers the benefit of allowing lipid analysis and determination of environmental pollutants using the same extract. Although the high volatility of methylene chloride may introduce difficulties related to rapid solvent evaporation, such as erroneously inflated lipid concentrations (6), this very property allows convenient elimination of excess extraction solvent during sample preparation. Moreover, loss of solvent during volume-sensitive preparative steps can often be mitigated by conscientious sample handling. While these serve as examples of suitable extraction methods that are readily available to lipid analysts, there are a number of significant shortcomings of conventional procedures.

First, many lipid extractions are carried out on a somewhat large scale by modern standards; as a result, these protocols require the use of large quantities of harmful and expensive organic solvents. Another consequence of classical-scale lipid extraction is the consumption of substantial amounts of sample tissue. Most procedures recommend 1 to 10 g of tissue, with some suggesting up to 100 g (2,5,7). In cases where the percent lipid is especially low, extraction of tissue on the gram scale may be necessary to obtain sufficient lipid for reliable gravimetric measurement; however, contemporary GC methods allow lipid characterization by hydrolysis and derivatization to FAME with 1 mg lipid or less. Second, despite many reported modifications intended to simplify and expedite traditional lipid extraction methods, the majority of these processes remain cumbersome and time-consuming. Extensive sample handling is required, providing many opportunities for sample contamination and loss or oxidation of lipids.

To address these issues, some alternatives to classical methods of lipid extraction have been explored. Most notably, microwave extraction and supercritical fluid extraction have been thoroughly studied for lipid isolation and have been successfully applied to a number of sample types, as well reviewed by Carrapiso and Garcia (8). Another available technology for lipid extraction is accelerated solvent extraction (ASE), an automated procedure that makes use of pressurized solvents at high temperatures (9). Since the introduction of ASE, a number of extraction techniques have been modified to take advantage of the automation and efficiency of high-pressure, high-temperature ASE.

Compared with other methodologies, there have to date been relatively few accounts of lipid recovery by ASE. The presently available reports describe the application of ASE

Present address of first and second authors: University of California Davis, Department of Chemistry, One Shields Ave., Davis, CA 95616.

*To whom correspondence should be addressed at University of Alaska Anchorage, Department of Chemistry, 3211 Providence Dr., Anchorage, AK 99508. E-mail: kennish@uaa.alaska.edu

for lipid isolation from plant and animal tissue (10–13), egg-containing foods (14), and dairy products (15). Although these studies have established ASE as a viable lipid extraction method, several avenues of further development remain to be pursued.

For example, assessment of ASE as a lipid extraction technique using various solvent systems with specific application to fish tissues containing variable lipid quantities has not been described previously. These are key considerations, since it has been well established that the appropriate matching of solvent and tissue type is of utmost importance in any lipid extraction procedure.

Additionally, the application of ASE to lipid extractions at smaller scales has not been well developed; essentially all such methods have been described for extraction of 1–15 g of starting material. Even at a low lipid content, extraction of these sample sizes recovers far more lipid than is required for FA characterization by GC analysis of FAME. Thus, large sample dilutions are necessary for GC analysis, resulting in additional preparation time as well as further waste of solvent. For these reasons, the use of smaller sample sizes is desirable. Furthermore, the use of smaller samples increases the ratio of solvent volume to sample mass, which may be particularly important for recovery of total lipids from tissues of higher lipid content.

Finally, ASE of lipids has typically been assessed by comparison to conventional extractions of the same tissue. Such comparative arguments have been effective in demonstrating the usefulness of ASE for lipid extraction; unfortunately, this approach has not included the design and application of quality control measures for the extraction process and subsequent preparative and analytical steps.

The study presented here was conducted with the dual objective of determining the usefulness of ASE with various solvent systems for the extraction of total lipids from fish tissues on the 100-mg scale, while concurrently establishing rudimentary quality control measures for such a procedure. Salmon muscle, halibut muscle, and NIST SRM (standard reference material) 1946, a standard reference fish tissue homogenate (16), were selected as representative samples encompassing a range of approximately 2 to 20% lipid content. Each sample type was extracted by ASE using chloroform/methanol, hexane/isopropanol, and methylene chloride. The performance of each solvent system for lipid extraction was evaluated for each tissue type using gravimetry and GC analysis of FAME prepared from the extracts. The overall method performance was assessed and monitored through the use of a TG surrogate introduced to the samples before extraction, as well as through comparison of total lipid content and NIST.

EXPERIMENTAL PROCEDURES

Solvents. All solvents used in this study were of residue analysis grade. Methylene chloride (CH_2Cl_2) was purchased from J.T.Baker (Phillipsburg, NJ); methanol (MeOH) was obtained from Fisher Scientific (Fair Lawn, NJ); and chloroform ($CHCl_3$), isopropanol (iPrOH), and hexane were acquired from EM Science (Gibbstown, NJ).

Tissue samples. Three fish sample types were used in this study: king salmon (*Oncorhynchus tshawytscha*) and Pacific halibut (*Hippoglossus stenolepis*) obtained from Cook Inlet, Alaska, during May of 2002; and NIST SRM 1946, Lake Superior fish tissue homogenate (Gaithersburg, MD). Fillets of salmon and halibut muscle were promptly vacuum-packaged and stored at −20°C until the time of extraction. The NIST SRM was stored at −80°C until the time of extraction.

Sample preparation. Tissue samples were removed from frozen storage and allowed to thaw. Several grams each of salmon and halibut muscle were homogenized with a stainless steel kitchen knife to the finest possible consistency. This processing step was not necessary for the NIST SRM, as the material was provided as a frozen homogenate. For each tissue type, twelve 100-mg portions of homogenate for extraction were weighed on a Mettler AT201 analytical balance (Greifensee, Switzerland), and their exact masses were recorded to the nearest 0.01 mg. Approximately 1 g hydromatrix drying agent (Varian, Walnut Creek, CA) was combined with each portion of tissue. The tissue and hydromatrix mixtures were transferred to 11-mL stainless steel extraction cells, each fitted with three cellulose filters. Each sample was then spiked with 50 μL 10 mg/mL trinonadecanoin (Matreya, State College, PA) in methylene chloride. Trinonadecanoin, the TG of C19:0, was added as a surrogate to assess the performance of all subsequent sample preparation steps. The TG of C19:0 was chosen as the surrogate since the tissues under study were known to lack the corresponding FA. Additional hydromatrix was added to fill the cells. All samples were extracted using a Dionex ASE 200 accelerated solvent extractor (Sunnyvale, CA) operated at 100°C and 13.8 MPa. Each sample was subjected to two static extraction cycles of 5 min each. Nitrogen was used to purge and pressurize the extraction cells. Four replicates of each tissue type were extracted by each of the three solvent systems under study: 60% chloroform/40% methanol; 60% hexane/40% isopropanol; and 100% methylene chloride. All extraction solvents were treated with BHT at a concentration of 100 mg/L (Sigma Chemical, St. Louis, MO) to prevent oxidation of the analytes. Blank preparations containing no tissue were also extracted by each solvent system.

Each extract was poured into an evaporation vessel through approximately 4 g of residue grade anhydrous sodium sulfate (J.T.Baker) using a sintered glass funnel. To recover all lipid quantitatively from the funnel and sodium sulfate, the funnel was thoroughly rinsed with approximately 10 mL of chloroform, hexane, or methylene chloride, depending on the solvent composition of the extract. Each extract was concentrated under nitrogen to roughly 1 mL using a TurboVap solvent evaporator (Zymark, Hopkinton, MA) at a bath temperature of 50°C for $CHCl_3$/MeOH and hexane/iPrOH extracts or 40°C for the CH_2Cl_2 extracts. The concentrates were transferred to clean, tared, round-bottomed reaction tubes, and all remaining solvent was removed by impinging with a stream of nitrogen.

The dry mass of recovered material was measured to the nearest 0.01 mg using the balance described previously, then each sample was treated with 1 mL 0.5 M KOH (VWR, West Chester, PA) in methanol. Hydrolysis was carried out at 80°C for 30 min, and the samples were removed from the heat and allowed to cool. For transesterification, the hydrolyzed samples were treated with 1 mL of newly opened 10% BF_3 in methanol (Supelco, Bellefonte, PA), then heated to 100°C for 10 min. Once cooled to room temperature, 1 mL of distilled water and 2 mL of hexane were added to each sample with vortexing. Following phase separation, the organic phase was collected and transferred to a new vessel. The solvent exchange was repeated with a fresh 2-mL portion of hexane, and the recovered organic phases were pooled. For internal standardization, these preparations were spiked with the methyl ester of C21:0 to result in a final concentration of 50.0 μg/mL after dilution to a total final volume of 10 mL with hexane. The methyl ester of C21:0 was chosen as the internal standard since the tissues under study were known to lack the corresponding FA.

Calibration standards. A series of calibration standards containing the FAME listed in Table 1 were prepared in hexane. These FAME were selected for calibration due to their prevalence in fish tissues and the tissues of other marine animals. FAME of the highest available purity were obtained from Sigma, Supelco, Matreya, and Nu-Chek-Prep (Elysian, MN). Each FAME ranged in concentration from 0.10 to 100.0 μg/mL across the series. The 21:0 FAME internal standard was added to each standard mixture at a concentration of 50.0 μg/mL. All calibration levels were analyzed in quadruplicate by GC, and the mean area ratio for each analyte with respect to the internal standard at each calibration level was used to construct composite calibrations.

Analysis by GC. Analysis of the extracted and derivatized FAME was performed with an HP 5890 Series II Plus GC system (Palo Alto, CA) equipped with a FID. The FID was operated at 300°C, and was supplied with hydrogen and air at flows of 30 and 300 mL/min, respectively. Helium was used as the FID auxiliary gas at a flow of 30 mL/min. Separation of FAME was accomplished using a DB-23 capillary column (60 m × 0.25 mm i.d., film thickness 0.25 μm; Agilent Technologies, Wilmington, DE). Constant flow compensation was used to maintain the flow of helium carrier gas at a rate of 1.0 mL/min. The GC inlet was held at a temperature of 300°C. Sample injections of 1 μL were performed without split for 30 s, followed by a 10:1 split for the remainder of the analysis. Oven temperature was programmed from 125 to 240°C at a rate of 3°C/min. A final hold of 1.67 min was used, for a total run time of 40.00 min.

RESULTS AND DISCUSSION

Gravimetric analysis. The average masses of material extracted from each sample type by each solvent system are given in Table 2. These values are corrected to exclude the mass of the internal standard (trinonadecanoin) and BHT recovered from extraction solvents. With the exception of BHT and trinonadecanoin, essentially no material was recovered from the blank extracts. In the case of salmon tissue, each solvent system recovered a roughly equivalent mass of material (within experimental error); however, in the case of halibut tissue and the NIST SRM, $CHCl_3$/MeOH extracted significantly greater quantities of material than did hexane/iPrOH (by comparison of experimentally determined means and SE). Extracts with CH_2Cl_2 did not recover significantly different amounts of material from either of the other two solvent systems. The mass

TABLE 1
Analyte FAME Present in the Calibration Standards[a]

FAME carbon number	Systematic name	Common name
C14:0	Tetradecanoic acid, methyl ester	Methyl myristate
C16:0	Hexadecanoic acid, methyl ester	Methyl palmitate
C16:1n-7	*cis*-9-Hexadecenoic acid, methyl ester	Methyl palmitoleate
C17:0	Heptadecanoic acid, methyl ester	Methyl margarate
C18:0	Octadecanoic acid, methyl ester	Methyl stearate
C18:1n-9	*cis*-9-Octadecenoic acid, methyl ester	Methyl oleate
C18:1n-7	*cis*-11-Octadecenoic acid, methyl ester	Methyl vaccenate
C18:2n-6	*cis,cis*-9,12-Octadecadienoic acid, methyl ester	Methyl linoleate
C18:3n-3	*cis,cis,cis*-9,12,15-Octadecatrienoic acid, methyl ester	Methyl linolenate
C19:0	Nonadecanoic acid, methyl ester	None
C20:0	Eicosanoic acid, methyl ester	Methyl arachidate
C20:1n-9	*cis*-11-Eicosenoic acid, methyl ester	None
C20:2n-6	*cis,cis*-11,14-Eicosadienoic acid, methyl ester	None
C20:4n-6	*cis,cis,cis,cis*-5,8,11,14-Eicosatetraenoic acid, methyl ester	Methyl arachidonate
C20:5n-3	*cis,cis,cis,cis,cis*-5,8,11,14,17-Eicosapentaenoic acid, methyl ester	None
C22:0	Docosanoic acid, methyl ester	Methyl behenate
C22:1n-9	*cis*-13-Docosenoic acid, methyl ester	Methyl erucate
C22:4n-6	*cis,cis,cis,cis*-7,10,13,16-Docosatetraenoic acid, methyl ester	None
C22:5n-3	*cis,cis,cis,cis,cis*-7,10,13,16,19-Docosapentenoic acid, methyl ester	None
C22:6n-3	*cis,cis,cis,cis,cis,cis*-4,7,10,13,16,19-Docosahexaenoic acid, methyl ester	None

[a]C19:0, known to be absent in the tissues under study, was included as a calibration to assess the surrogate recovery.

TABLE 2
Mass Percent Lipid in Each Tissue as Determined by Gravimetry (mean ± SE)

	Mean percent lipid recovered (n = 4)		
	Salmon	Halibut	NIST SRM[a]
$CHCl_3$/MeOH	23.2 ± 1.9	5.0 ± 0.8	13.5 ± 0.4
Hexane/iPrOH	21.5 ± 3.2	2.5 ± 0.4	10.3 ± 0.7
CH_2Cl_2	24.9 ± 2.3	2.6 ± 0.7	11.6 ± 2.9

[a]The certified value is 10.17 ± 0.48%.

percentage of material recovered from halibut tissue was proportionately more sensitive to extraction by hexane/iPrOH vs. $CHCl_3$/MeOH.

The certified extractable fat content of the NIST SRM 1946 is 10.17 ± 0.48% by mass; thus, the present result in best agreement with the accepted value was obtained by extraction with hexane/iPrOH (10.3 ± 0.7% by mass). Taken alone, this result suggests that a significant quantity of nonlipid content may have been extracted by the $CHCl_3$/MeOH solvent system in particular.

FAME analysis: FA composition and sum of FA. The content of representative FA recovered from the three tissues by each solvent system is illustrated in Figures 1–3. The most abundant FA are shown for salmon and halibut tissues, and a suite of FA with NIST-certified values is shown for the SRM. In Figure 3, the certified value for each individual FA involved is shown beside the present findings. Furthermore, Table 3 provides the sum of all quantified FA (those listed in Table 1), expressed as tissue mass percentage, for each combination of sample type and extraction solvent. Owing largely to the fact that not all FA present in each sample were quantified, the sums of FA (Table 3) are not expected to equate to the gravimetric recoveries listed in Table 2; nonetheless, a comparison of the values shown in Table 3 with those in Table 2 can be informative.

In extracts of salmon muscle, the FA compositions of the

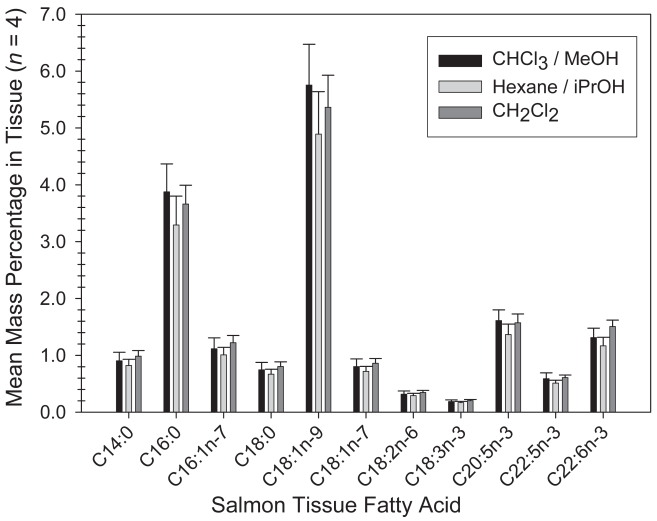

FIG. 1. Mean mass percentage of representative FA as the TG in salmon muscle. Error bars represent the SD.

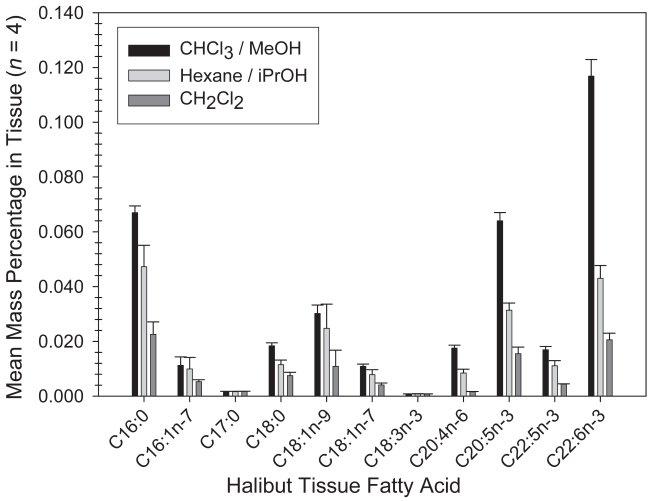

FIG. 2. Mean mass percentage of representative FA as the TG in halibut muscle. Error bars represent the SD.

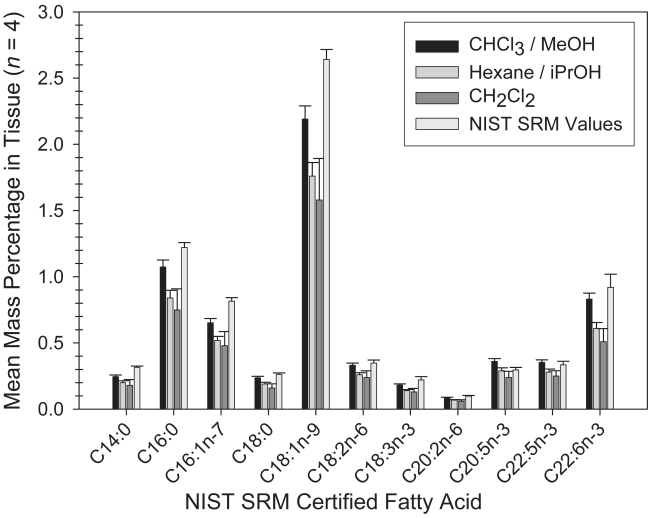

FIG. 3. Mean mass percentage of representative FA as the TG in NIST SRM 1946. The certified reference values reported by NIST are also shown. Error bars represent the SD, except for the NIST SRM; in this case, the error bars represent the uncertainties reported by NIST.

TABLE 3
Mass Percent FA in Each Tissue as Determined by GC Analysis of FAME (mean ± SE)

	Mean percent FA (n = 4)		
	Salmon	Halibut	NIST SRM[a]
$CHCl_3$/MeOH	18.4 ± 2.3	0.37 ± 0.01	7.4 ± 0.4
Hexane/iPrOH	16.2 ± 2.2	0.21 ± 0.03	5.8 ± 0.4
CH_2Cl_2	18.4 ± 1.4	0.10 ± 0.02	5.2 ± 1.0

[a]The certified value is 8.76 ± 0.17%.

extracts were found to be very similar, regardless of the solvent extraction system used (Fig. 1). Indeed, the extracted quantity of individual FA closely resembled the gravimetric results for salmon tissue, with the different solvents extracting essentially equivalent (that is, within the experimentally

determined SE) FA contents in most cases, regardless of whether gravimetric recoveries or individual FA quantities were compared. This is good evidence that, for this sample type, negligible quantities of nonlipid material were extracted, irrespective of the extraction solvent employed.

Conversely, the FA contents determined in the various extracts of halibut muscle were found to be in relatively poor agreement (Fig. 2). In general, $CHCl_3$/MeOH recovered the most FA, with hexane/iPrOH recovering significantly less (that is, not within the experimentally determined SE) in almost all cases. The CH_2Cl_2 extracts were consistently lowest in FA content by a considerable margin. It should also be noted that, whereas the results obtained by gravimetry suggest a similar amount of lipid recovered by hexane/iPrOH and CH_2Cl_2, the GC analysis clearly demonstrates far lower FA content in the CH_2Cl_2 extracts. This may be indicative of the recovery of relatively large amounts of nonlipid material by CH_2Cl_2.

Extractions of the NIST SRM performed using $CHCl_3$/MeOH were found to most closely approximate the certified FA values provided by NIST. Indeed, these results yield a FA composition reasonably similar to the NIST values, with most results falling within experimental error when $CHCl_3$/MeOH is used (Fig. 3). The most important exception is the result for 18:1n-9, the most abundant of the FA in the tissue, which fell marginally short of the NIST SRM value. The extracts using hexane/iPrOH and CH_2Cl_2 recovered somewhat less of the higher-abundance FA, although for the less-abundant FA these solvent systems exhibited reasonable agreement with $CHCl_3$/MeOH and NIST values. In general, the NIST sample was less sensitive to solvent composition than the halibut tissue, but still more sensitive than the salmon tissue. Although the $CHCl_3$/MeOH extracts produced a FA composition that had the best agreement with the NIST values, the gravimetric results implied that a greater-than-expected amount of lipid was recovered (13.4 ± 0.6% by mass, as compared with the NIST value of 10.17 ± 0.48% by mass), further implicating a tendency of this solvent system to extract nontrivial quantities of nonlipid materials. Also, whereas hexane/iPrOH produced extracts in best gravimetric agreement with the NIST extractable fat value, that extract fell significantly short of the reference values for a number of individual FA.

The capability of ASE to provide extracts that reproduce the FA content values reported by NIST is especially notable when the differences in sample size and time investment between the present methodology and that employed by NIST are considered. The reference values for the FA composition of the SRM were produced by extracting 2.5-g samples for 18–22 h using a Soxhlet apparatus and 1:1 hexane/acetone. The present results were obtained using 100-mg samples requiring a total of less than 35 mL of solvent per sample (including rinsings and final dilution), and with an extraction time of less than 20 min per sample.

Another important result illustrated by Figure 3 is that comparison of the ASE extraction results with the NIST SRM reference values furnishes no evidence of method bias against PUFA. It therefore appears that oxidation of PUFA is not occurring, despite the high temperatures at which ASE is performed. Some protection against analyte oxidation is afforded by the use of nitrogen for purging and pressurizing ASE cells, providing inert extraction conditions. This, in tandem with treatment of the extraction solvents with BHT, is clearly effective in preserving the PUFA content.

FAME analysis: surrogate recovery. The mean percentages of surrogates recovered from each set of samples as determined by GC analysis are summarized in Table 4. Complete recovery of the surrogate would correspond to an analytical determination of 50.0 µg/mL C19:0 FAME in the extract; recovery of the surrogate is expressed in terms of a percentage of that value. Among all surrogate recoveries measured, 75% fell within 80–120% of the ideal value, with 56% falling within 90–110% of the ideal value. During the study, a low surrogate recovery (in this example, <60%) was indicative of an unsatisfactory sample, even though a typical gravimetric recovery was obtained. It is therefore likely that the sample was mishandled or otherwise corrupted postextraction; not surprisingly, the analyte FA were also much lower in concentration compared with the other replicates of the sample whose surrogate recoveries were >90%. It can also be seen that the set of extracts with the consistently poorest surrogate recovery (namely, halibut tissue extracted by CH_2Cl_2) were also found to consistently contain the lowest analyte FA content, further corroborating the inadequate performance of this solvent system in this particular matrix. Although surrogate recovery is useful for monitoring the quality of sample handling and analytical procedures, high recovery of the surrogate does not necessarily imply quantitative extraction of lipids from the tissue of interest. Therefore, the surrogate recoveries alone cannot be used to draw inferences with respect to the effectiveness of a particular solvent system for lipid extraction from a given sample. The choice of a solvent system appropriate to the tissue of interest is a separate issue and should be addressed as such.

Based on the results presented here, it may be concluded that microscale ASE of lipids for FA analysis of fish tissues can be accomplished with quantitative accuracy and a level of precision that is satisfactory for many applications. Provided that the appropriate solvent system is chosen, the FA composition can be quantitatively characterized with 100 mg of tissue for samples ranging from roughly 2 to about 20% lipid by mass, with the possibility of extending this to tissues of other lipid contents with only the need for minor changes in scale or dilution scheme.

In addition, the technique can be easily augmented by the application of elementary quality control in the form of a surrogate, allowing the method performance to be monitored.

TABLE 4
Mean Surrogate Recoveries for Replicate Extractions (mean ± SE)

	Mean percent surrogate recovery (n = 4)		
	Salmon	Halibut	NIST SRM
$CHCl_3$/MeOH	102.3 ± 0.7	93.8 ± 3.1	107.1 ± 1.7
Hexane/iPrOH	79.8 ± 9.7	87.6 ± 6.2	90.0 ± 6.4
CH_2Cl_2	108.5 ± 1.6	58.3 ± 4.3	83.1 ± 5.3

Although the surrogate will allow the detection of certain chemical errors in preparation and instrumental errors in analysis, it is important to acknowledge that good surrogate recovery cannot be interpreted as evidence of appropriate matching of extraction solvent system and sample type; this determination must be made independently. The use of a standard reference material for performance monitoring is therefore recommended.

Carrying out lipid extraction on a 100-mg scale offers the advantage of requiring the collection and/or consumption of smaller tissue samples and provides the capability to study localized lipid composition from small samples. However, when overall lipid content is of interest, the analyst should be aware that such small portions may not be representative of the whole, unless the sample is taken from a larger composite of well-homogenized material.

In general, extraction by $CHCl_3$/MeOH consistently gave the highest recovery of FA and yielded the best results for the NIST SRM FA composition; however, based on comparison of gravimetric measurements and FAME analysis, this solvent system would also seem prone to extraction of significant amounts of nonlipid material. Hexane/iPrOH and CH_2Cl_2 also appear effective for relatively high lipid samples, but both are increasingly unsuitable as the lipid content of the sample is reduced. This is not unexpected, since the ratio of polar to nonpolar lipid is typically highest in low lipid tissues, and $CHCl_3$/MeOH is well known to be a most effective solvent system for the extraction of polar lipids.

The large degree to which the FA recovery of hexane/iPrOH and CH_2Cl_2 is reduced with lessened lipid content is not necessarily reflected by gravimetric measurements of extracted material; indeed, these results have illustrated multiple occasions on which gravimetric lipid recovery results were not consistent with the FA composition of the sample. This is an important consideration, as a number of examples of lipid extraction method comparisons in which gravimetry alone was used to assess the relative effectiveness of each method have been published. This approach appears particularly prevalent among studies of lipid extraction from fish and marine tissues (5–7,11). Such studies fail to provide information on the composition of the recovered material, whereas the findings presented here clearly advocate the analysis of FA composition in addition to gravimetry for the comparison of lipid extraction procedures. Thus, gravimetrically derived lipid quantities alone are tenuous and should be regarded as preliminary.

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