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A simple and efficient one-step method for the extraction of lipid-soluble compounds from tissue and food

Adam Metherel

There are many reasons for measuring lipids in a wide array of sample matrices. Lipids such as omega-3 fatty acids, *trans* fatty acids, and cholesterol play significant roles in human health and are commonly determined in blood and tissue for biomarker assessments. Lipid analysis is further required for government-mandated food labeling and has the potential to support efforts in food traceability. Other important applications of lipid analyses include biodiesel production for greener fuels and basic biomedical and biomolecular research. This broad spectrum of applications is accompanied by an equally broad number of tissue and food samples on which to perform fatty acid and lipid analysis. Regardless of whether one is performing lipid assessments in humans, rats, mice, eggs, or milk, or whether that assessment is for purposes of omega-3 profiling, cell function quantification, or food labeling, the single most common theme among them all is the complex analytical process.

Traditionally, lipid analysis is a tedious process requiring (i) sample collection, (ii) sample storage, (iii) sample preparation, (iv) measurement of analytes, and (v) data handling (Stark, 2008). Sample preparation can then be broken down further into (i) homogenization, (ii) extraction, and (iii) derivatization steps. Occasionally, and depending on the final goal of analysis, one or more of these steps can be combined into a single step, but these steps are rarely completely removed. Adding to the significant requirements of fatty acid analysis is the potential for additional analytical steps such as thin-layer chromatography for the separation of more specific fatty acid fractions. Although the analytical process for lipids is a challenge, significant advances have been made over the past 10–15 years in collection (e.g., fingertip prick blood collection) (Marangoni *et al.*, 2004), extraction (e.g., ultrasonic energy) (Luque-Garcia and Luque de Castro, 2007), derivatization (e.g., microwave energy) (Armstrong *et al.*, 2008), and fast gas chromatographic steps (Masood *et al.*, 2008). Despite these advances, more traditional methods remain entrenched as standard procedure in many research institutions.

LIMITATIONS OF CURRENT METHODS

A comparison of the three most commonly used fatty acid extraction methods from food was carried out in flaxseed (Taha *et al.*, 2012). These three methods are (i) the International Organization for Standardization (ISO) method, (ii) the AOAC International method, used most commonly in industry for nutritional labeling purposes, and (iii) the Folch method, used most commonly in academic settings for agricultural, nutritional, and biochemical research. These methods commonly require between 4 and 24 hours to complete and can require up to 10 grams (g) of

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- **Traditional methods of analyzing lipids in tissue and food can take between 4 and 24 hours to complete and may require up to 10 grams of sample and as much as 500 milliliters of solvents for the extraction process.**

- **The Folch method—commonly used in agricultural, nutritional, and biochemical research—requires manual pipetting, for which yields may vary from day to day and from individual to individual, thus making comparative analysis difficult.**

- **The following article describes a simple and efficient one-step method for extracting cholesterol and fatty acids that requires no pipetting, no centrifugation, and no mixing of standards and solvents. The method increases analytical precision while reducing the time required to perform the separation technique.**

FIG. 1.
Image of extraction filter equipped with syringe.

sample and as much as 500 milliliters (mL) of solvents for the extraction process. Specifically, the Folch method uses a 2:1:0.8 chloroform/methanol/aqueous buffer solvent mixture. Extraction of the lipids is performed with 2:1 chloroform/methanol, and the aqueous buffer is added to facilitate the separation of polar (methanol) and nonpolar (chloroform) layers. This separation step requires centrifugation of the mixture followed by the manual pipetting of the bottom lipid-containing chloroform layer for the isolation of extracted lipids.

Manual laboratory techniques can yield significant variability from day to day and sample to sample within an individual. This variability is even larger between individuals within the same lab or between different labs and makes comparative analyses difficult. Methods and techniques designed to automate and simplify these procedures can reduce variability thereby improving analysis between multiple datasets.

A SIMPLE AND ACCURATE ONE-STEP EXTRACTION METHOD

Certo Labs has recently developed both a fatty acid and a cholesterol extraction kit to expedite the process of extracting lipids from various tissue and food samples. These extraction kits are based on the Folch extraction method, which requires significant experience and skill to achieve accuracy and precision. The aims are to simplify the method without compromising this accuracy and precision and to allow non-experts to perform the lipid extractions with ease and confidence, and in significantly less time than would traditionally be required.

Using the Certo kit requires no pipetting, no centrifugation, and no mixing of standards and solvents. The pre-mixed extraction solvents are added to the tissue or food sample, and subsequently homogenized and extracted as normal. Following this, the aqueous buffer is added and the sample/solvent, inverted twice, added to the extraction syringe and passed through the treated filter (Fig. 1) by the gentle plunging of the syringe. The lipid-containing chloroform mixture is collected, and the non-lipid-containing water/methanol is selectively trapped and left behind in the filter. The Certo kit uses a 2:1:0.5 chloroform/methanol/aqueous buffer solvent mixture that is slightly different from the Folch method (2:1:0.8). The Folch solvent ratios are intended for optimal separation of polar and nonpolar phases by centrifugation. Conversely, the modification of this ratio in the Certo kit is intended for optimal separation of polar and nonpolar phases by the aqueous filter.

The Certo kit has been tested for both cholesterol and fatty acids in a variety of foods and tissues and compared with the classic Folch method. Accuracy of lipid concentrations (mg/g) in egg and brain samples was similar (Fig. 2). The Certo kit also provided greater precision as the relative standard deviations averaged 4% in egg and 10% in brain, whereas Folch results averaged 6% in egg and 17% in brain. We hypothesize this is due to the removal of human error associated with manual pipetting, and it should be noted the Folch results were generated from a highly trained technician with 5+ years of experience in fatty acid determinations.

In addition to the increased analytical precision, using the Certo kit appreciably reduces the time required to perform the separation technique. The extraction time for the egg and brain data presented herein (6 samples each) required only 3 minutes using the Certo kit, while it took 12 minutes using the conventional Folch method. This correlates to an average of 30 seconds per extraction when using the Certo kit and 2 minutes per extraction for the Folch method.

Currently, the Certo kit has been assessed and developed for use in cholesterol and fatty acid extractions from approximately 100 mg of tissue or food samples; however, the kit has the potential for application to the preparation of any lipid-soluble compound including vitamins A, D and E; pesticides; and antibiotics. In addition, a larger-volume extraction kit that will allow for the analysis of up to 1 g of a sample and uses 20 mL of 2:1 chloroform/methanol for extraction is in development. These simple, easy, and high-throughput extraction kits promise to save academic and industry researchers' additional time and costs without compromising the accuracy and precision of the determination of lipid compounds.

Adam Metherel is the Mitacs Elevate Industrial Post-Doctoral Fellow at Certo Labs, Inc., and the University of Waterloo in Ontario, Canada. He can be reached at ahmether@uwaterloo.ca or adam@certolabs.com.

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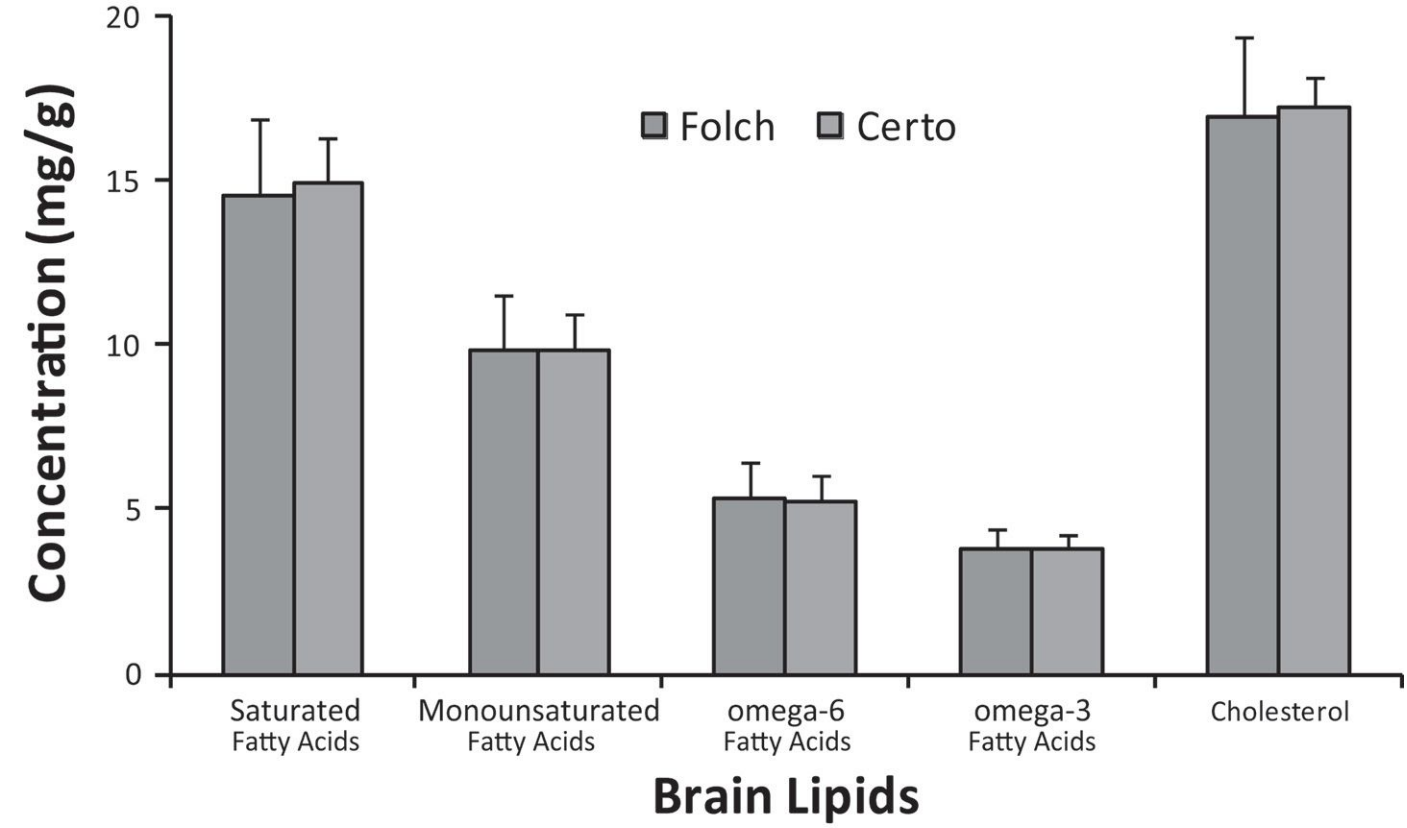

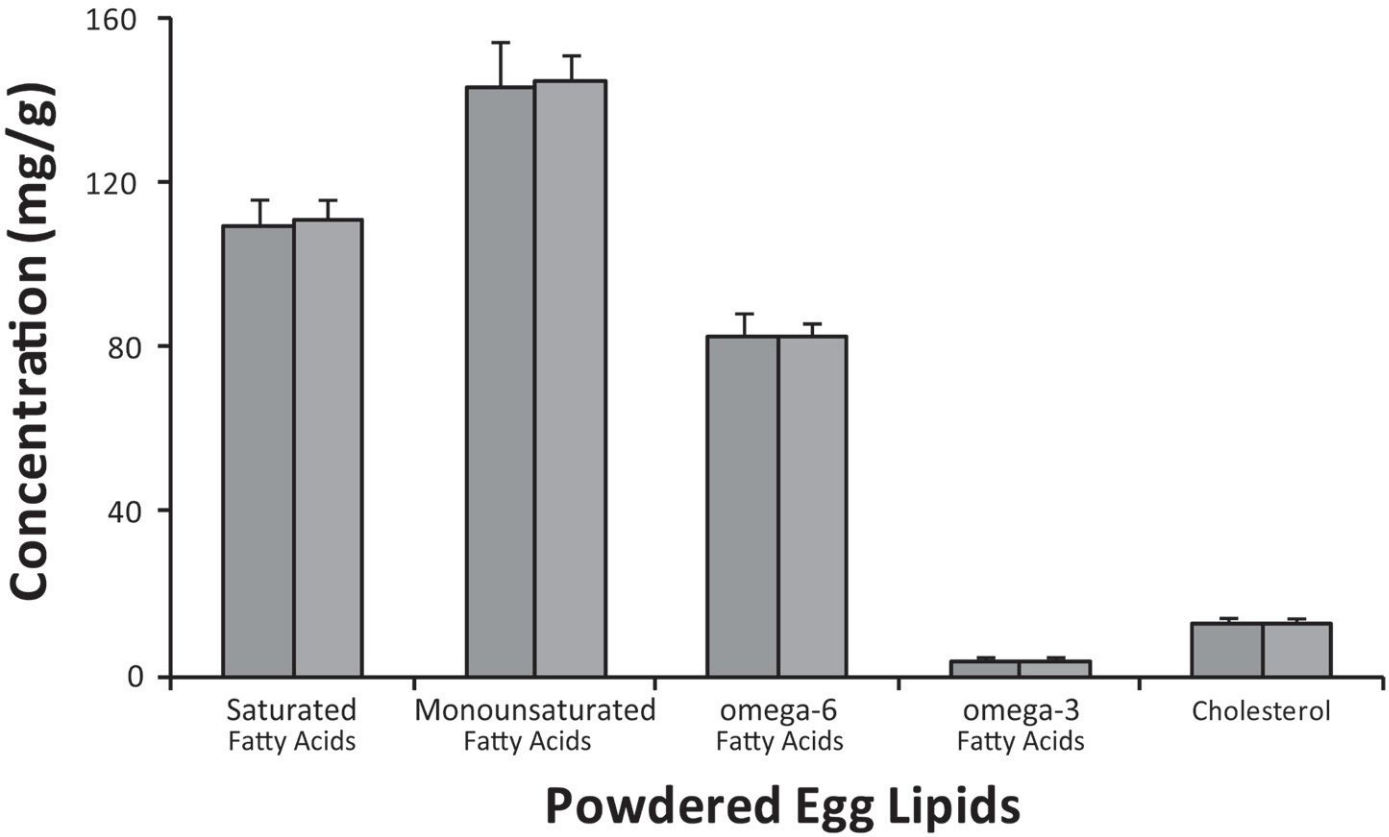

***FIG. 2**. Lipid determinations by Folch method vs. Certo kit in brain and egg.*