# Quantitative Extraction of Hamster Liver Lipid and Cholesterol with Supercritical Carbon Dioxide

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**ABSTRACT:** A quantitative method for liver lipid and cholesterol extraction with supercritical $CO_2$ and ethanol entrainer (SCE) is reported and compared with the Folch (chloroform/methanol) procedure. Mean values for lipid and cholesterol in hamster livers ($n$ = 48) were similar between the SCE and Folch methods. Correlation coefficients between the two methods were 0.9866 for total lipid and 0.9546 for cholesterol. Similar mean values and high correlations between the two methods validate the SCE procedure as a precise alternative method for quantitative liver lipid extractions. The SCE method also reduces the use of hazardous solvents.
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**KEY WORDS:** Cholesterol, hamster, lipid, liver, supercritical $CO_2$.

Lipid metabolism studies in experimental animals require quantitative measurements of lipid components in relatively limited amounts of tissue. The Folch method (1) for lipid extraction and another similar procedure (2) in which chloroform and methanol are used is the principal method currently available. These procedures are laborious and time-consuming and use toxic, hazardous hydrocarbon solvents, which have high disposal costs. Supercritical $CO_2$ (SC) is a nontoxic, nonflammable economical alternative for lipid extraction and has no disposal costs. The solvating properties of SC are similar to those of a liquid, but it has the viscosity and diffusivity of a gas, permitting rapid mass transfer of solute to solvent. When combined with an entrainer, such as ethanol (E), sample solubility and selectivity can be enhanced (3). SC extraction methods have been developed largely for applications in the food industry, such as seed oil extractions (4,5) or reduction of fat and/or cholesterol content of ground beef (6), dehydrated beef (7) lard, ham and luncheon meats (8), fish muscle (9), and egg yolk (10). Analytical applications of SC have been reported for pesticide quantitation from a variety of food matrices (11) and for quantitative oil determination in oilseeds (12). To our knowledge, there are no published reports on the use of SC for quantitative determination of lipids and cholesterol in liver tissue. The high moisture content of liver interferes with SC extraction by acting as a barrier to SC penetration (8,12) and must be reduced or eliminated for complete extraction. In this report, a procedure with SC and absolute ethanol entrainer (SCE) is presented for quantitative extraction of lipid and cholesterol in hamster liver and compared with the Folch method.

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## EXPERIMENTAL PROCEDURES

*Sample preparation.* Hamster livers ($n$ = 48), stored at –70°C, collected from animals fed various levels of cholesterol (from 0–3% of the diet) up to 12 wk in nutritional studies were extracted for lipid by the Folch method with chloroform and methanol (1) and with SCE. Before extraction, each liver was individually thawed and minced homogeneously. To eliminate sample moisture as a source of interference with SC extraction, pelletized diatomaceous earth was used to absorb water and enhance sample dispersion (11). Liver aliquots, weighing from 0.25 to 0.30 g, were mixed with approximately 1.2 g of pelletized diatomaceous earth (Hydromatrix; Varian, Harbor City, CA), previously sieved over 30 mesh (600 μm) to remove fines, and transferred quantitatively to 10-mL crystalline polymer extraction cartridges (Isco, Inc., Lincoln, NE) for supercritical $CO_2$ extraction.

*Extraction conditions and procedures.* Because preliminary extractions with SC alone gave modest (63–75%) recoveries, absolute ethanol was selected as an environmentally acceptable entrainer to enhance extraction of lipid and cholesterol (9). The supercritical fluid extraction system consisted of the following components: a 260-mL syringe pump (Model 260D; Isco, Inc.) for liquid $CO_2$, chilled to 5°C with a circulating water jacket; a 100-mL syringe pump (Isco Model 100 DX) for entrainer (100% ethanol) delivery at ambient temperature; a temperature-controlled dual-chamber extractor (Isco Model SFX 220) wherein cartridges, containing sample, were maintained at 80°C for optimum solubilization of lipids in SC (8); a fixed volume (1.5 mL) insulated coaxially heated capillary restrictor (Isco, Inc), heated to 100°C to control the flow of $CO_2$ as it emerges from the extractor and undergoes depressurization, and to prevent clogging due to ice or lipid buildup; and vented pre-weighed screw-capped 20 × 150 mm sample collection tubes that contained ethanol. Coleman-grade liquid $CO_2$ was pressurized to 7500 psi (517 bar)

in the 260-mL pump, while ethanol was pressurized to 10,000 psi (690 bar) in the 100-mL pump. Samples were extracted with a mixture of 75% SC and 25% (by volume) entrainer for 20 min, followed by 20 min with 65% SC and 35% entrainer, to maximize extraction with the least amount of entrainer in a time-efficient manner. The combined flow rate of SC and ethanol averaged approximately 5 mL/min for two samples extracted simultaneously, with an estimated total volume of 72 mL (64 g) of SC and 28 mL of ethanol for each sample. Extracted total lipid was determined after evaporating ethanol under $N_2$ and drying in a 90°C oven for 45 min. Lipid extracts were stored at −17°C until dissolved in 10 mL of chloroform/methanol, (86:14), for cholesterol analysis. Homogeneous 1-g samples from the same livers were also extracted by the Folch procedure (1). Aliquots of lipid extract from both methods were evaporated under nitrogen, solubilized with Triton X-100 (13), and analyzed for total cholesterol using a procedure described previously (14).

*Statistical analyses*. Each of the 48 livers was extracted by both SCE and Folch procedures. Liver cholesterol analyses of each extract were conducted in triplicate. Mean values resulting from the two extraction procedures for lipid and cholesterol were compared by *t*-test, and efficiency of the SCE procedure relative to the Folch method was measured by determining correlation coefficients (15).

## RESULTS AND DISCUSSION

Lipid extraction with the SCE procedure was completed in 40 min on liver samples that weighed 0.25–0.3 g. Total liver lipid values as percentage of liver (fresh weight basis) by the SCE method ranged from 8.1 to 26.7%, and by the Folch method from 7.8 to 28.2%. Cholesterol values by these two methods were 16.9–120.9 and 18.9–129.7 mg/g liver, respectively. The means ($n = 48$) and standard error of the means by the SCE and Folch methods for lipid were 16.7 ± 0.9 and 17.0 ± 1.0%, respectively, and for cholesterol 62.2 ± 4.7 and 65.5 ± 5.0 mg/g, respectively. There were no significant differences in the mean lipid and cholesterol values by the SCE and Folch procedures. Correlation coefficients between the two methods were 0.9866 for lipid and 0.9546 for cholesterol and showed good agreement between the SCE and Folch methods for both liver lipid and cholesterol analytical determinations over a wide range of concentrations. The SCE method is environmentally safer, less laborious, faster, and more economical than hydrocarbon solvent methods for lipid extraction. Similar mean values and high correlations with the Folch procedure indicate that the SCE method is suitable for quantitative lipid extraction of small amounts of experimental animal liver tissue.

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