ANALYTICAL BIOCHEMISTRY 110, 240–241 (1981)

# Radiochemical Assay of Long-Chain Fatty Acids Using $^{63}Ni$

HUBERT F. DE BRABANDER AND ROGER VERBEKE

*Laboratory of Chemical Analysis of Food of Animal Origin, Veterinary Faculty of the University of Ghent, Casinoplein 24, B-9000 Ghent, Belgium*

Received August 25, 1980.

The reproducibility and sensitivity of the radiochemical assay of fatty acids, described by R. J. Ho, can be enhanced by replacing the centrifugation step with absorption of water by anhydrous sodium sulfate.

During studies of the effect of chilling temperatures on the quality of bovine meat, small amounts of free fatty acids had to be monitored.

Using a radiochemical assay of long-chain fatty acids, with $^{63}Ni$ as a tracer (1), we found that this procedure was improved considerably by replacing the centrifugation step with absorption of water using anhydrous sodium sulfate. It was found that this modification of the method of Ho (1) resulted in an increased reproducibility and a higher recovery of the complexed Ni.

## MATERIALS AND METHODS

### *A. Reagents*

A1. Nickelous nitrate, 2 mmol, and 0.8 ml of glacial acetic acid were dissolved in saturated sodium sulfate solution and taken to 100 ml with the same solution.

A2. $^{63}Ni$ chloride, 1 mCi (The Radiochemical Centre, Amersham; dissolved in 1 ml 0.1 M HCl), was mixed with 4 ml of A1.

A3. Standard solutions of stearic acid (0.08–0.8 mM) in heptane.

A4. Aqualuma (a ready-to-use microcolloidal scintillator) was obtained from Lumac.

### *B. Working Solution*

One milliliter of A1, 0.85 ml saturated potassium sulfate solution, 10 $\mu$l $^{63}Ni$ solution (A2), and 0.15 ml of triethanolamine were mixed (100 $\mu$l of this solution contains 1 $\mu$mol $Ni^{2+}$ and 0.0995 $\mu$Ci $^{63}Ni$).

### *C. Centrifugation Procedure*

To a silanized 10-ml glass tube (10 cm length, 1 cm i.d., round bottomed) are added 0.5 fatty acid solution (A3 or sample), 1 ml of chloroform–heptane (4:1, v/v), and 100 $\mu$l of working solution B. The tube is shaken vigorously and centrifuged at 500*g* for 10 min. Two hundred microliters of the organic layer is transferred into a liquid scintillation counting vial containing 5 ml Aqualuma. The radioactivity is measured in a scintillation counter and corrected for reagent blank.

### *D. Sodium Sulfate Procedure*

The partitioning step is the same as in the other procedure but after mixing, anhydrous sodium sulfate ($\simeq$1*g*) is added, and the tube is shaken until the solution is clear. Two hundred microliters of the organic solvent is transferred into liquid scintillation counting vials containing 5 ml Aqualuma.

0003-2697/81/010240-02$02.00/0

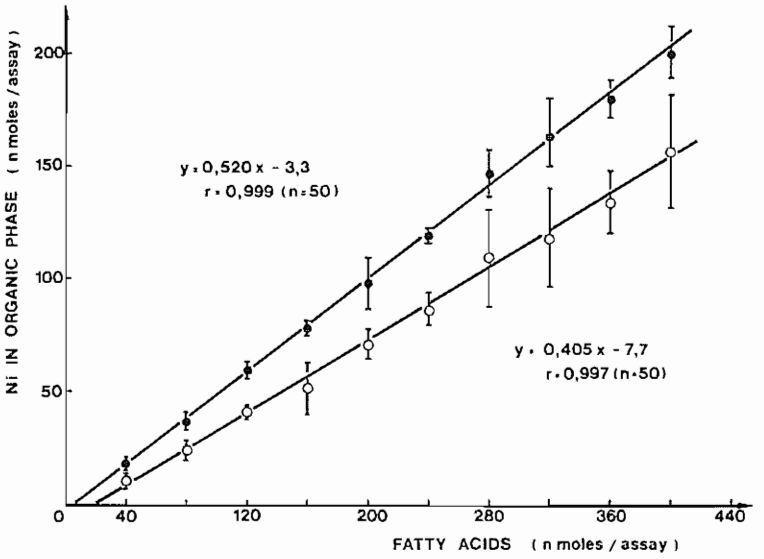


FIG. 1. Stearic acid standard curves obtained by $^{63}Ni$ radiochemical assay using centrifugation (○) or addition of sodium sulfate (●). Each point was a mean of five values (2 SE).

## RESULTS AND DISCUSSION

The reagents used in the present study were similar to those described by Ho (1) except for scaling up of the volumes by a factor of 10 and decreasing the ratio of $^{63}Ni$ to $Ni(NO_3)_2$ by 100. The amount of lipid-soluble $^{63}Ni$ could be accurately estimated using liquid scintillation counting.

Standard curves of 0.08 to 0.8 mM stearic acid (40 to 400 nmol/assay) were prepared with both methods. The results are shown in Fig. 1.

In contrast to the results obtained with sodium sulfate, the intercept of the regression line using centrifugation was significantly different from zero ($P < 0.001$). The slope of the standard curve obtained with sodium sulfate is significantly higher ($P < 0.001$) than that obtained by centrifugation. The slope of the sodium sulfate standard curve corresponds to a ligand to metal ratio of $1.92 \pm 0.02$. This ratio agrees with the formation of a $Ni(\text{free fatty acids})_2$ complex. With centrifugation the ligand to metal ratio was calculated as $2.47 \pm 0.05$. The differences between the ligand to metal ratios cannot be explained by volume changes of the two phases during extraction (measured to be less than 1%). Concentration of the complex at the water–heptane interphase may explain the negative intercept and the lower slope of the centrifugation standard curve. The reproducibility of the sodium sulfate procedure (coefficient of variation 3.2%) was significantly better than the centrifugation method (coefficient of variation 6.7%).

These results show that the centrifugation step in the procedure of R. J. Ho (1) can advantageously be replaced by absorption of the water through addition of solid sodium sulfate. Moreover, omitting the centrifugation step avoids breaking of glassware and spreading of radioactive material.

## ACKNOWLEDGMENT

The authors thank Mrs. A. M. Van Meir-Grégoire for valuable technical assistance.

## REFERENCE

1. Ho, R. J. (1970) *Anal. Biochem.* **36**, 105–113.