An Improved Extraction Procedure for the Determination of Triglycerides and Cholesterol in Plasma or Serum

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A simple method of preparing extracts of plasma or serum suitable for determination of both triglycerides and cholesterol is described. Its chief advantage over present procedures is in a considerable saving of time and labor involved in the preparation of large numbers of samples. The method appears to give a more complete extraction of glycerides and compares favorably with existing methods of extracting cholesterol.

The recent interest in the level of plasma triglycerides, as well as cholesterol, in the investigation of hyperlipemic and atherosclerotic disease states has made desirable rapid and simple procedures for their determination. This need has been met in large part by the development of the Van Handel and Zilversmit procedure for the determination of triglycerides (1, 2) and the Zlatkis and Zak procedure for the determination of cholesterol (3, 4). Unfortunately, the extraction procedures used for these methods have not lent themselves well to the determination of both on a single extract of plasma—the requirement for a phospholipid-free extract in the determination of triglycerides has precluded the use of the more polar solvents necessary for the extraction of cholesterol. Thus, solvents used in the determination of triglycerides are inadequate for the extraction of cholesterol. Consequently, either separate extracts must be prepared or, following the extraction of total plasma lipids, phospholipids must be laboriously removed. Both these alternatives are cumbersome and time consuming when a large number of samples are involved.

While some investigators have stated that single extracts of plasma are suitable for the determination of both triglycerides and cholesterol, these have proved unsatisfactory in our experience.

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After numerous empirical trials of solvent combinations we have found a method which yields a complete extraction of glycerides which is free of phospholipids and, at the same time, provides a complete extraction of cholesterol.

The present report describes the procedure in detail and gives results determined on extracts of plasma by this method, as compared with those previously used.

Materials and Methods

Diethyl ether (Fisher or Merck), isopropanol (Fisher or Merck), chloroform (Fisher or Merck), and isopropyl ether (Fisher or Merck) were reagent grade, but were not redistilled. Where indicated, isopropyl ether was freed of peroxides by passage through an alumina column. Florisil 60/100 mesh (Fisher) was used as obtained from the supplier. Silicic acid (Malinkrodt or Baker) and Zeolite (W. A. Taylor Co.) were sized and activated as recommended by the various authors whose methods were compared with the present one. Triglyceride content of the extracts was determined by the method of Van Handel and Zilversmit (1, 2), cholesterol content was determined by a modification of the method of Zlatkis and Zak (3, 4), and phospholipid phosphorous was determined by the method of Fiske and SubbaRow (5).

Extraction Procedure

About 2 gm. of Florisil (60/100 mesh) are placed in a 50-ml. screw-cap (Teflon-lined) tube. Serum or plasma (0.5 ml.) is pipeted onto the Florisil. Added is 10 ml. of diethyl ether and the tube is capped tightly. The tube is then mixed vigorously (vortex mixer) for about 1 min. (× 3), and then let stand for about 30 min. Then, 5 ml. of isopropanol is added and the tube is again capped tightly, mixed for about 3 min., and let stand again for 30 min. During this period the Florisil settles, allowing aliquots of the extract to be taken without filtration. Duplicate 0.5-ml. aliquots of supernatant solution are evaporated rapidly at 80° in a vacuum oven and then treated as in the procedure of Van Handel and Zilversmit (1) for triglycerides, or as in the procedure of Zlatkis and Zak (3) for cholesterol.

Results

Twenty separate aliquots of pooled serum were extracted simultaneously from Florisil using chloroform and ether-isopropanol (Table 1). The mean triglyceride concentration using chloroform was 100.6 mg./100 ml. (± 1.8 S.E., range 88–115). With ether-isopropanol extracts the mean result was 134.0 mg./100 ml. (± 0.8 S.E., range 129–139). The
blank values obtained with the ether-isopropanol extract were no higher than those obtained with chloroform. Random serum samples extracted in a comparable manner showed consistently higher results with ether-isopropanol extraction (mean 12%, range 3.4–41.3%).

Corn oil and tripalmitin standards (5 mg./100 ml.) were applied to Florisil and then extracted with chloroform or ether-isopropanol. The absorbance, after color development of those extracted with chloroform, was 20% lower than the standard not treated with Florisil, whereas those extracted with ether-isopropanol were not significantly different from the untreated standard. Standards made up in chloroform or ether-isopropanol had identical absorbances.

Radioactive tripalmitin-14C (Applied Science Laboratories) was purified by thin layer chromatography on silica gel. This was then applied to Florisil and extracted with ether-isopropanol. The recovery was 97%.

Ten chloroform and 10 ether-isopropanol extracts were then tested for phospholipid content (Table 1). The phospholipid content of the chloroform extract was small (mean 4.7 mg./100 ml., range 1–9), but there was no detectable phospholipid phosphorus in the ether-isopropanol extract.

A comparison of effectiveness of cholesterol extraction from serum was then made between ether-isopropanol and isopropanol alone and can be seen in Table 1 (6). Twenty separate extractions of pooled serum applied to Florisil were made with each solvent. The mean result with isopropanol was 230.4 mg./100 ml. (±S.E., range 224–238) and with ether-isopropanol, 229.2 mg./100 ml. (± 1.1 S.E., range 221–234). Cholesterol content was determined on chloroform extracts of Florisil. The mean result was 197.0 mg./100 ml. (± 2.5 S.E.), indicating incomplete extraction.

Other procedures reported to be suitable for the combined extraction of triglycerides and cholesterol were then compared with the present procedure. The procedure recommended by Azarnoff (7) employing Baker's silicic acid and isopropyl ether, and that of Mendelsohn and Antonis (8) for both triglycerides and cholesterol, gave much lower values than those obtained with the present extraction procedure.

The procedure described by Lofland (9) employing isopropanol and

<table>
<thead>
<tr>
<th>Extract</th>
<th>Triglycerides*</th>
<th>Cholesterol*</th>
<th>Phospholipids*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ether-isopropanol-florisil</td>
<td>134.0 ± 0.8</td>
<td>229.2 ± 1.1</td>
<td>0</td>
</tr>
<tr>
<td>Chloroform-florisil</td>
<td>100.6 ± 1.8</td>
<td>197.0 ± 2.5</td>
<td>4.7 ± 0.8</td>
</tr>
<tr>
<td>Isopropanol</td>
<td>—</td>
<td>230.4 ± 1.0</td>
<td>—</td>
</tr>
</tbody>
</table>

* Expressed as mg./100 ml. serum ± S.E. (n = 20).
zeolite was also tried. With this procedure we were unable to obtain extracts of sufficiently low phospholipid content for the determination of glycerides.

**Discussion**

The extraction procedure described here is simple, rapid, and requires reagents that can be obtained readily and used without further treatment. It apparently gives a more complete extraction of glycerides from plasma than other methods currently in use and, at the same time, provides a suitable extract for determination of cholesterol. The treatment with diethyl ether of the plasma mixed with Florisil fixes the phospholipids so tightly that we have been unable to devise a method for their recovery.

Florisil, in our hands, has given more consistent results regardless of the extraction procedure used. We found that Zeolite varied considerably in its consistency and, frequently, fine particles had to be washed out. Van Handel noted that Zeolite should be ground and sized to 80–100 mesh, and then activated (2). The Florisil we have used has not required any further treatment. Blankenhorn et al. found that recovery of monoglycerides from Zeolite was poor (10).

The use of diethyl ether has not posed problems. Evaporation is negligible so long as the sample tubes are tightly capped; we have not encountered the difficulties with subsequent determination of glycerides described by Jagannathan (11).

**References**