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Note

Use of sulfo-phospho-vanillin to quantitate unsaturated neutral lipids in thin-layer chromatography

EDWARD B. TAPSCOTT and G. LYNIS DOHM

Department of Biochemistry, School of Medicine, East Carolina University, Greenville, N.C. 27834 (U.S.A.)

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The sulfo-phospho-vanillin reaction has been established as a routine procedure for quantitation of total serum lipids[1,2]. This technique has been extended to analysis of cholesterol and unsaturated fatty acids separated by thin-layer chromatography (TLC). Although there are limitations in the method which result from specificity of phospho-vanillin for double bonds, this may be used to advantage in determining the percentage of neutral lipids that are unsaturated.

MATERIALS AND METHODS

Lipid standards obtained from Sigma (St. Louis, Mo., U.S.A.) were spotted on 20 × 20 cm plates prepared by spreading Adsorbosil I ($CaSO_4$ binder, Applied Science Labs., State College, Pa., U.S.A.) to 0.25-mm thickness. The prepared plates were air dried and activated at 100° for 1.5 h. Samples were spotted alternately on 1.0-cm lanes leaving 1.5 cm between lanes. Plates were first developed in diethyl ether–benzene–acetic acid (40:50:1) to 7 cm above the origin, air dried and redeveloped to the top in hexane–diethyl ether (96:4). These solvent systems afforded good separation of cholesterol, oleic acid, triolein, methyl oleate, and cholesteryl oleate in that order (Fig. 1). Spots were visualized by spraying with Rhodamine 6G (0.001%, w/v) in methanol.

Each spot and its corresponding blank were scraped from the plate into test tubes and 1.0 ml concentrated sulfuric acid was added. The mixture was heated for 10 min in a boiling water-bath and cooled for 5 min in cold water. To the sample were added 2.0 ml of phospho-vanillin reagent prepared as previously described[1]. The mixture was then incubated for 15 min at 37° and centrifuged for 5 min at 800 *g* to separate the gel. The absorbance was read at 540 nm.

RESULTS AND DISCUSSION

With this procedure, standard curves for fatty acids and cholesterol are linear from 15 through 100 μg for both pure lipid standards and for standards scraped from plates (Figs. 2 and 3). We have also found that standard curves for cholesterol oleate, methyloleate and triolein are linear. The assay provides good sensitivity and reproducibility despite a relatively high blank resulting from the gel and Rhodamine 6G.

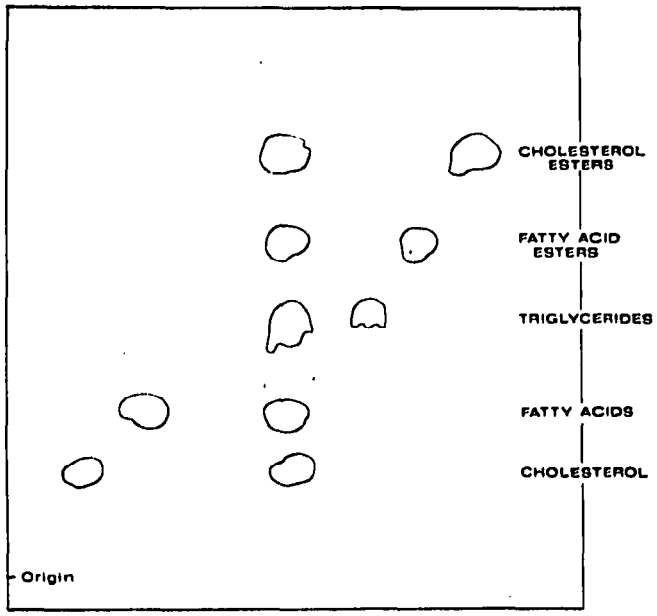

Fig. 1. Representative separation pattern obtained when plates were spotted and developed as described in Materials and methods.

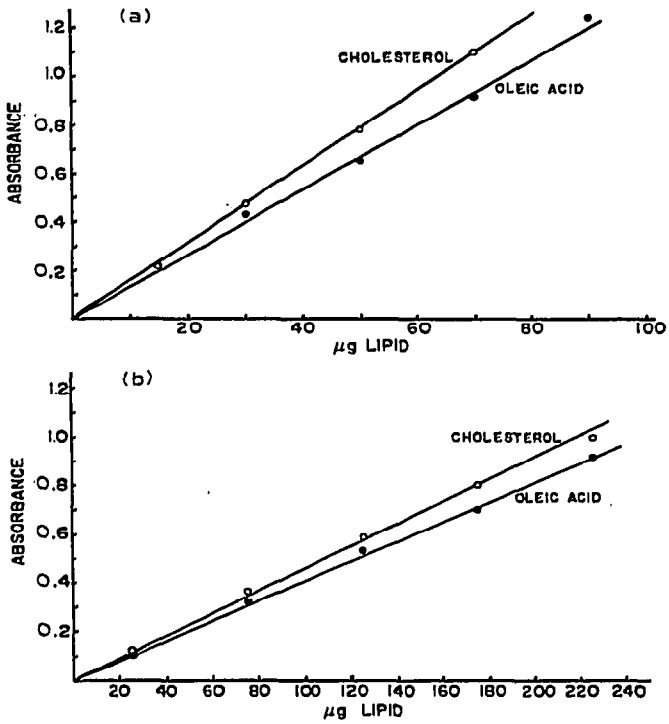

Fig. 2. Examples of lipid standards taken from TLC plates and assayed by the phospho-vanillin technique. (a) Reagents as described in methods. (b) Expanded range with reagent volumes doubled.

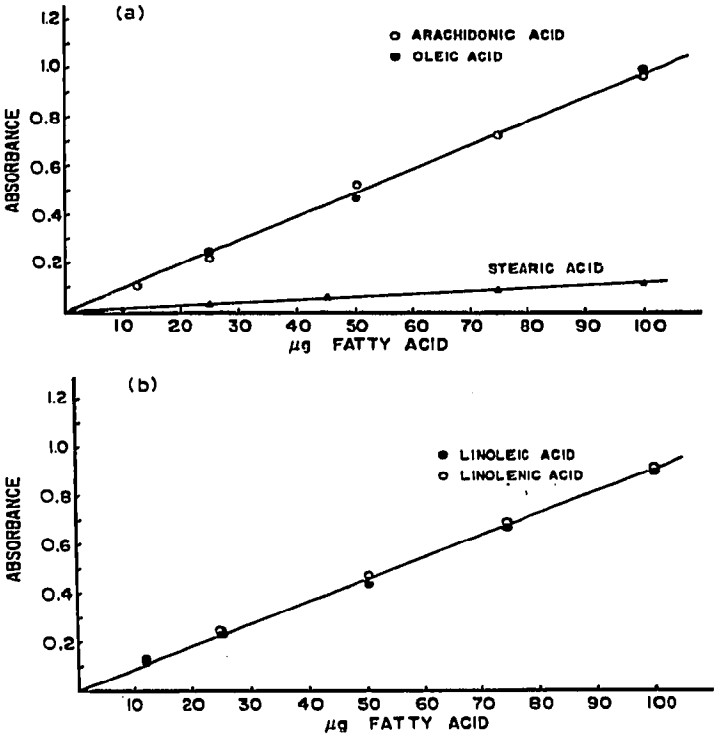

Fig. 3. Lipid standards analyzed with phospho-vanillin indicating the specificity for unsaturation and the lack of color enhancement with polyunsaturation. (a), Arachidonic, oleic and stearic acids; (b), linoleic and linolenic acids.

Doubling the volumes of sulfuric acid and phospho-vanillin (Fig. 2b) decreases the blank absorbance and improves reproducibility at the expense of sensitivity.

Mlekusch *et al.*[3] described the use of the sulfo-phospho-vanillin reaction for quantitation of neutral lipids separated by TLC. Their technique employs visualization of the lipid spots by spraying with sulfuric acid and charring at 150°. The method we present is preferred over the previously reported method because it eliminates variations which might result from variation in spraying acid, charring temperature and time.

It was been shown that a carbon–carbon double bond is necessary for the reaction[1,2]. As indicated in Fig. 3, stearic acid fails to react whereas oleic, linoleic, linolenic and arachidonic acids react with little variation in the degree of color formation. Hence, the quantity of unsaturated fatty acids in a sample can be determined by this method. By using this method in conjunction with a method for the determination of total lipids (such as used by Amenta[4]) the percentage of fatty acids which are unsaturated may be determined.

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