Limits of detection of some lipids in thin-layer chromatography

The use of thin-layer chromatography in lipid biochemistry is now widespread. In our laboratory we have found it convenient to employ thin-layer chromatography to monitor eluants of chromatographic columns or to determine the purity of compounds before use. It is therefore necessary to know the minimum quantities detectable in order to know the degree of contamination of the compounds tested. Accordingly, employing the three detection systems commonly used in our laboratory, we have undertaken experiments to determine the limits of detectability of various lipids.

Experimental

Thin-layer chromatographic plates, 250 μ thick, were made in the usual manner. We customarily dissolve 10 mg of Rhodamine 6G (National Aniline Division, Allied Chemical Corporation*) in the water used to make the slurry. The solution must be filtered before use. Silica Gel G (Brinkmann Instruments, Inc.*) and Silica Gel H (Brinkmann Instruments, Inc.*) work equally well in our laboratory.

The lipid compounds, with the exception of lecithin, were passed through silicic acid columns[1] before use. The compounds were weighed on an analytical balance and serial dilutions in benzene, chloroform or hexane were made, so that the proper quantity of compound (0.01–3 μg) might be applied to the plate in less than 5 μl of solvent.

The thin layers were developed with either a mixture of petroleum ether (b.p. 60–70°), diethyl ether and acetic acid (90:10:1, v/v)[2] or benzene and ethyl acetate (9:1, v/v) (for sterol and alcohol only)[3]. The spots were observed as follows: First, under ultraviolet illumination, which caused the Rhodamine to fluoresce pale green and the lipids to appear pink; secondly, after exposure to iodine vapors for 2–5 min, once again under ultraviolet light, the spots appearing intense blue; and thirdly, after spraying the plate with 50 % sulfuric acid and charring at 110°. In the last procedure the Rhodamine 6G is decolorized and does not mask the charring of the lipid.

Results

The results are summarized in Table I. It can be seen that the iodine in conjunction with the Rhodamine increases the sensitivity several fold. Experiments not recorded here have shown that iodine alone is not as sensitive as iodine–Rhodamine fluorescence. Unsaturation increases the sensitivity with the iodine–Rhodamine and with the charring. Some of the compounds did not char under our conditions (110°), although they do char at higher temperatures (200°); but we have not determined how leuco-Rhodamine reacts at the higher temperature.

Discussion

Most of the lipids have a lower limit of detection of about 0.1 μg. When 20 μg (a commonly used quantity) of compound is chromatographed, 0.1 μg represents 0.5 %. By increasing the load, a greater purity test can be obtained.

Although the Rhodamine–iodine detection is more sensitive than the charring

* Mention of a proprietary name or company does not necessarily imply endorsement by the U.S. Department of Agriculture.

TABLE I

MINIMUM AMOUNT (IN μg) OF COMPOUND DETECTED

Compound	*U.V.*	I_2 + *U.V.*	H_2SO_4
Saturated compounds			
Eicosane	3	0.1	0
Tristearin	1.2	0.7	0
Behenic acid	0.5	0.1	0
Hexadecanol	1	0.5	0.5
Cholestanol	0.2	0.05	0.1
Cholestan-3-one	1	0.1	0.5
Lecithin (dipalmitoyl)	0.5	0.01	0.5
Unsaturated compounds			
Squalene	1.8	0.02	0.2
Cholesteryl oleate	1	0.05	0.1
Cholesteryl stearate	1	0.05	0.2
Triolein	2	0.05	1
Oleic acid	0.5	0.05	0.2
Cholesterol	0.5	0.05	0.1
Δ^4-Cholesten-3-one	1	0.05	0.5

method, we often employ both methods to obtain additional useful information. The Δ^5-sterols and their esters give an intense purple color after 5–10 min of heating at 110° and can be readily distinguished from other lipids which only char. Also lanosterol, which turns a distinctive pink-black color, can be distinguished from some long-chain alcohols which run in almost the same position.

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Received August 27th, 1964

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