Paper chromatography of lipids: use of cupric hydroxide impregnated paper

A method of paper chromatography was developed using cupric hydroxide impregnated paper for the analysis of lipids.

Whatman 3 MM paper was impregnated by immersion, first in 5 % cupric sulphate solution and then, after drying, in a 5 % sodium hydroxide solution. The paper was then washed with tap water until no basic reaction was observed anymore, and finally with distilled water. After drying (at a temperature not exceeding 80°), the prepared paper may be stored for an indefinitive time, but away from acid vapours.

The following ^{14}C-labelled fatty acids and glycerides were examined with this paper: oleic, linoleic, linolenic, palmitic, stearic, myristic and lauric acids, diolein and triolein.

A sample (25–50 μg), dissolved in chloroform, was spotted on the paper and developed by ascending chromatography using the solvents shown in Table I. After development, the chromatograms were scanned with a thin-window Geiger-Müller counter to determine the corresponding peaks of radioactivity (see Table I).

TABLE I

R_F VALUES

	*Solvents**			
	1	*2*	*3*	*4*
Oleic acid	0.68	0.99	0.72	0.02
Linoleic acid	0.54	0.98	0.74	0.04
Linolenic acid	0.80	0.96	0.80	0.09
Triolein	0.00	0.99	0.00	0.98
Diolein	0.00	0.96	0.02	0.94
Stearic acid	—	0.00	0.72	0.04
Palmitic acid	—	0.00	0.68	0.50
Myristic acid	Do not form copper soaps			
Lauric acid	Do not form copper soaps			

* Solvent 1: 10% cyclohexylamine in water.
Solvent 2: Chloroform–ethanol (1:1).
Solvent 3: Methanol–water–cyclohexylamine (120:60:10).
Solvent 4: Benzene–chloroform (1:2).

The spots were also detected, after a short treatment with 0.1 % acetic acid to eliminate the excess of cupric hydroxide from the paper, by staining according to KAUFMANN AND NITSCH[1] with potassium ferrocyanide.

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1 H. P. KAUFMAN AND W. H. NITSCH, *Fette und Seifen*, 56 (1954) 154.

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