Thin-layer chromatography of hydroxamic acids

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SUMMARY The separation of very short-chain from long-chain fatty acyl hydroxamates by thin-layer chromatography is described. The detection limit was 2 μg.

KEY WORDS fatty acyl hydroxamates · thin-layer chromatography

THE PREPARATION OF a hydroxamic acid derivative is often a convenient method to establish a bond configuration or a reaction process. Previously, fatty acyl hydroxamates have been identified by paper chromatography (1, 2). The method described below utilizes the more convenient and less time-consuming thin-layer chromatography. Hydroxamic acid derivatives of saturated straight-chain fatty acids were prepared from their chlorides or anhydrides (3). Derivatives with chain-lengths C_2 and C_4 were recrystallized from ether or acetone; those with a chain length above C_{14}, from chloroform–methanol mixtures. 2–40 μg of hydroxamic acid was applied per spot to Silica Gel G thin-layer plates (Merck, precoated TLC plates F_{254}). The plates were developed in toluene–methanol (8:2 or 6:4) at room temperature. The air-dried plates were sprayed with Hill's reagent (4) that had been diluted with an equal volume of methanol. The hydroxamates appeared as purple or brown spots; the minimum detectable amount of palmitoyl hydroxamic acid was 2 μg. The R_f values were independent of the applied amount in the range studied here.

TABLE 1 R_f VALUES FOR HYDROXAMIC ACIDS ON SILICA GEL G (MERCK F_{254})

	Toluene–Methanol 8:2	Toluene–Methanol 6:4
C_2	0.20	0.43
C_4	0.31	0.59
C_{14}	0.42	0.77
C_{16}	0.43	0.77
C_{18}	0.43	0.79
C_{24}	0.43	0.80

Abbreviation: TLC, thin-layer chromatography.

As shown in Table 1, resolution of derivatives according to chain length was not obtained above C_{14} but the method allows for a convenient separation of short-chain from long-chain products and would be useful in the study of the interconversion of acetyl or malonyl CoA derivatives to higher homologues. The TLC plates used can be easily scored and broken into strips. These strips can be assayed for radioactivity on a scanner with good resolution since the long-chain derivatives chromatograph as very narrow bands.

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