## Thin-layer chromatography of 2,4-dinitrophenylhydrazones of α-alkyl substituted carbonyl compounds

The carbonyls isolated from edible products are generally of a very complicated mixture. On converting them into their 2,4-dinitrophenylhydrazones (DNPHs) almost complete separation of such mixtures is possible by a combination of partition and adsorption thin-layer chromatography:

(i) according to chain length, *e.g.* with Kieselguhr G plates impregnated with Carbowax[1],

(ii) according to number and position of double bonds, *e.g.* with silica gel or alumina plates impregnated with silver nitrate[2–4].

On using TLC plates impregnated with silver nitrate we have now found that DNPHs of some branched, saturated carbonyls can be separated from other DNPHs having the same chain length.

The plates (20 × 20 cm) were impregnated with 44 wt. % $AgNO_3$ (layer thickness: 0.25 mm), as described earlier[2,5,6].

An amount of 2–4 μg of the DNPHs, dissolved in a minimum quantity of chloroform, was spotted on to the plates, which were then developed in a mixture of light petroleum (b.p. 40–60°) and diethyl ether (85:15, v/v), until the liquid front had travelled 13 cm.

The separation of a number of DNPHs of straight-chain, as well as branched, saturated aldehydes and ketones on an alumina plate impregnated with silver nitrate is shown in Fig. 1.

On both silica gel and alumina plates, impregnated with silver nitrate, the

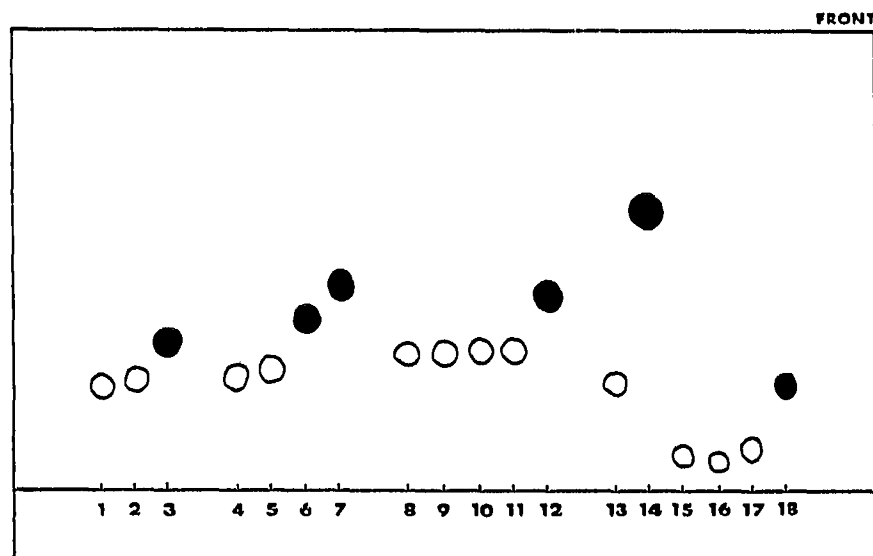


Fig. 1. Separation of carbonyl-DNPHs by TLC on alumina impregnated with silver nitrate. Mobile phase: light petroleum (b.p. 40–60°)–ether (85:15, v/v). Plate: 20 × 20 cm. The DNPHs of α-alkyl substituted carbonyls are shown as black spots. The $R_F$ values of the compounds are given in brackets. 1 = pentanal (0.22); 2 = 3-methyl butanal (0.23); 3 = 2-methyl butanal (0.32); 4 = heptanal (0.24); 5 = 3-ethyl pentanal (0.26); 6 = 2-ethyl butanal (0.37); 7 = 2-ethyl hexanal (0.45); 8 = dodecanal (0.29); 9 = 10-methyl dodecanal (0.30); 10 = tetradecanal (0.29); 11 = 12-methyl tridecanal (0.30); 12 = 2-methyl undecanal (0.42); 13 = methyl butyl ketone (0.23); 14 = 3,3-dimethyl-2-butanone (0.61); 15 = cyclopentanone (0.08); 16 = cyclohexanone (0.06); 17 = 3-methyl cyclopentanone (0.09); 18 = 2-methyl cyclopentanone (0.23).

DNPHs of α-alkyl substituted saturated carbonyls showed higher $R_F$ values than other DNPHs having the same chain lengths. The results obtained with the two types of plate did not show any marked differences. Separation was also possible on non-impregnated plates, but here the $R_F$ value differences were smaller than with the impregnated plates.

It is immaterial whether the α-alkyl substituent is a methyl or an ethyl group. At the time, corresponding aldehyde-DNPHs with an even longer side chain at the α-position, and comparable ketone-DNPHs, were not available. A ketone-DNPH having two substituents at the α-position (3,3-dimethyl 2-butanone) had an $R_F$ value which differed appreciably from that of the normal ketone-DNPH. DNPHs of saturated ketones with one substituent at the α-position can probably also be separated on silver nitrate plates from other ketone-DNPHs having the same chain length.

We have also compared 2-methyl cyclopentanone-DNPH with the DNPHs of 3-methyl cyclopentanone, cyclopentanone, and cyclohexanone. Surprisingly, the DNPHs of cyclic ketones were absorbed much more strongly on silver nitrate plates than DNPHs of aliphatic ketones. 2-Methyl cyclopentanone-DNPH, however, migrated much faster than the other cyclic ketone-DNPHs. This difference was less pronounced on plates not impregnated with silver nitrate.

We suppose that the bond between the C=N bond and the silver nitrate is weakened by the α-alkyl substituent, as a result of which separation becomes possible.

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## Thin-layer chromatography of cyanocobalamin, hydroxocobalamin and $B_{12}$ coenzymes

Vitamin $B_{12}$ vitamers and their coenzyme forms can be separated by paper partition chromatography and paper electrophoresis. Paper partition chromatography takes a long time for the development and a shortcoming is that spots tail. On the other hand, paper electrophoresis is performed within a comparatively short time and has a good resolution. However, with the latter method not many samples can be examined at the same time a factor which is very desirable during the preparation and purification of such coenzyme forms of the vitamin.

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