

arsenite in 100 ml of acetone previously acidified with 0.66 ml of 10 *N* HCl and filtering the solution. The filtrate is diluted to 500 ml.

Reagent C. Ethanol saturated with thiobarbituric acid.

The paper is dipped successively through reagents A, B and C, with drying after each dip. It is then heated as above with identical results.

Solutions of 3-deoxyxylose, 3-deoxyxylitol, 2-deoxyribose and 2-deoxyglucose (0.2 *M*–0.003 *M*) were applied as 4 μ l spots (about 1 cm diam.) to strips of Whatman No. 1 chromatography paper. 3-Deoxyxylitol, 2-deoxyribose and 2-deoxyglucose could be readily detected down to 1 μ g/sq.cm. At the highest concentration used, 3-deoxyxylitol showed a red "halo" because of incomplete cleavage in the centre of the spot. The spot from 0.1 *M* 3-deoxyxylose was faint and at lower concentrations undetectable.

None of the following sugars gave reactions: glucose, fructose, sorbose, xylose, xylulose and 3-deoxyxylulose.

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Thin-layer chromatography of vitamin B₁₂ and its analogues

Vitamin B₁₂, isolated from microbial fermentation, is often accompanied by its analogues, some of which have no biological activity for either man or animals. The separation of these compounds is difficult because they are chemically closely related to cyanocobalamin.

The known methods for the separation of vitamin B₁₂ from its analogues include the use of column chromatography on cellulose¹ and ion exchangers^{2–8}, electrophoresis^{9,10}, and paper chromatography^{6,11–17}.

Recently, there have been some reports on the use of thin-layer chromatography on silica gel for the separation of these compounds^{19,20}.

This report relates to a new method for separation of some vitamin B₁₂ compounds on thin layers of alumina.

Experimental and results

The experiments were performed with the following cobalamins: cyanocobalamin (B₁₂), factor B_{12III}, pseudovitamin B₁₂, factor A, factor B, factor V(nB), and a mixture of them in water with an addition of NaCN (5 % aqueous solution) to obtain the dicyano complexes. During chromatography dicyano complexes decomposed into the monocyano forms. Different mixtures of the following solvents were used:

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isobutanol, *n*-butanol, isopropanol and water, with and without addition of NaCN, NH₄OH, and CH₃COOH. The best separation was obtained with the mixture isobutanol-isopropanol-water (1:1:1) on basic alumina (second degree of activity according to Brockmann). The use of neutral and acid alumina under the same conditions did not give a good separation of the individual compounds.

The following procedure was used. For the preparation of the chromatographic plate, covered by a thin layer (about 1 mm thick) of dry alumina, frosted glass plates of 30 × 15 cm size, were used. The plates were smoothed with a roller according to the usual method¹⁸. The solutions of the compounds and the mixture of them were applied as spots at a distance of about 3 cm from the edge of the plate. After the spots had been dried (in air) the plate was placed in an inclined position (about 20°) in a chromatographic chamber, dipping it 2–3 cm in the solvent system which had been previously added. The chromatogram was developed by the ascending technique until the solvent penetrated about 24–26 cm. The time of development did not exceed

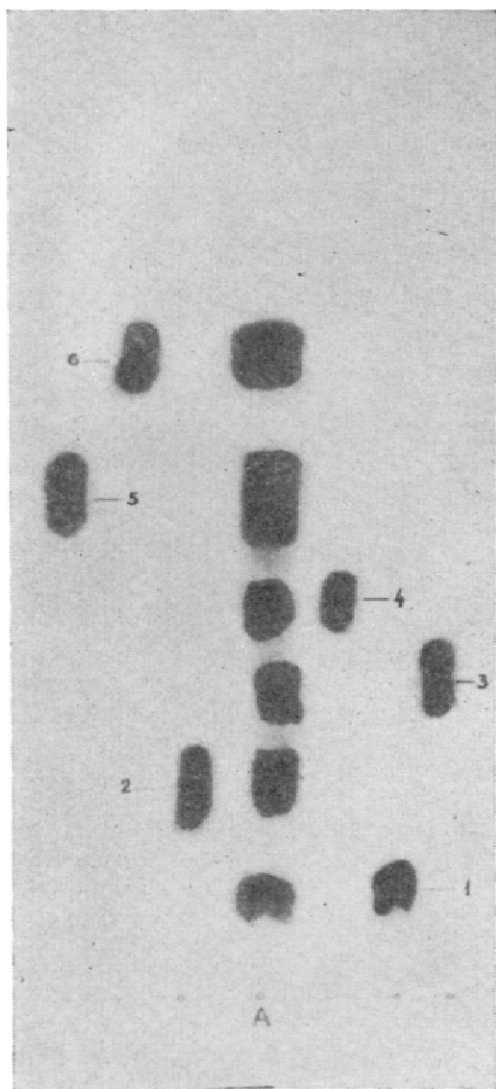


Fig. 1. The separation of vitamin B₁₂ analogues on alumina. 1 = Factor V(nB); 2 = pseudo-vitamin B₁₂; 3 = factor A; 4 = factor B₁₂III; 5 = cyanocobalamin (B₁₂); 6 = factor B; A = mixture of compounds 1, 2, 3, 4, 5 and 6.

4-5 h at $20^{\circ} \pm 0.5^{\circ}$. Under the conditions described one can obtain a clear separation of the individual analogues in the mixture. The spots of the factors B and V(nB) had a good shape and compactness. The spots of the other compounds were also well defined, but they were longer and there were weak tails between them. The minimum amount detectable of each compound was 0.5 γ .

TABLE I
 R_F VALUES OF CYANOCOBALAMIN AND ITS ANALOGUES

Compounds	R_F values
Cyanocobalamin	0.62
Factor B	0.74
Pseudovitamin B ₁₂	0.25
Factor B ₁₂	0.46
Factor V(nB)	0.12
Factor A	0.37

The R_F values of the individual substances are shown in Table I and the developed chromatographic plate is shown in Fig. 1.

The method described is simple and can be easily carried out. It is applicable to the control of the purity of concentrates and commercial preparations of vitamin B₁₂.

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