A NEW METHOD FOR THE ASSAY OF VITAMIN B12 BY TLC

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Abstract

A new simple, easy and inexpensive method for the determination of cyano-cobalamine (B_{12}) from compound preparations, has been developed. The method hold good for the seperation of trace amounts of B_{12} in compound preparations. It may prove interesting for adoptation by pharmacists and clinical laboratories desiring to save time, labor and money.

Introduction

Cyanocobalamine, the most common form of B_{12} is odourless, tasteless, and occurs as red needle-like crystals or as red powder. It is insoluble in ether, acetone and chloroform, hygroscopic but soluble in water upto 1.25%. The crystals began to darken at 210-2015°C, and melt above 300°C. Its empirical formulae C_{63} H_{88} Co N_4 O_{14} P and molecular weight 1355 4.

There are number of chemical and physico-chemical methods available for the determination of vitamin B_{12} (The National Formulary, 1975; Cords and Ratycz, 1959; Bruening and Kline, 1961; Pharmacopoeia of the United States, 1970; Mitra et al., 1962; Monnier et al., 1963, 1963a; Beige et al., 1968; Bacher et al., 1964; Konecny et al., 1967; British Pharmacopoeia, 1973); and for the purification of B_{12} from other constituents of Multivitamin, liver extract, blood and compound preparations.

Usually ion exchange resins of the amberlite type have been employed for the seperation purpose. Cellulose ion exchangers have also been used. The ion exchangers generally employed are aluminium oxide (Lens et al., 1952), Amber-lite XE-97 (Van Melle, 1956) and IRA-400 (Marsh and Kuzel, 1951). In the method presented here cyanocobal-amin is seperated from non-cyanocobalamins by thin layer chromatography using n-butanol, potassium dihydrogen phosphate (KH_2PO_4) acetic acid and methanol (4:4:2:1) as eluting solvent. The spots are extracted with the developing solvent and determined spectrophotometri-cally.

Material and Method

Glass plates 10 x 20 x 0.2 cm are coated with silica Gel G(PF 254) to a thickness of 0.25 mm by a suitable TLC spreader. They are dried overnight at room temperature and then heated in an oven at 110°C for 30 minutes, cooled and stored in a clean, covered container.

A measured volume of assay solution containing 25-100 meg of vitamin B_{12} is applied 2 cm from the edge of the chromatogram. The spots are dried at 24°C. A seperate spot of pure cyano-cobalmain is also applied on the same plate. The solvent system consists of n-butanol, potassium dihydrogen phosphate (KH_2PO_4) (0.066M), acetic acid and methanol (4:4:2:1). The plate eluted in a TLC tank is kept in dark at room temperature. The solvent system is allowed to ascend 12-14 cm from the starting line. After elution the plate is dried at 110°C for 30 minutes, the spots are developed and removed with a sharp spatulla under UV(254 nm) light and transferred to two test tubes. These are extracted with the developing solvent and the extract collected in a volumetric flasks.

Developing Solvents:
Either of the following developing solvents may be used.
1. Potassium cyanide 0.1% aqueous solution adjusted to pH6 with 2N acetic acid, or NaOH. The solvent must be handled carefully because of HCN vapours, and is freshly prepared twice a week.
2. Polysorbate: For extraction purpose 7-10 ml of polvsorbate in each test tube is added and heated to 40-45°C.
The amount of cyanocobalamin is determined by measuring the absorbance at 361 mu, l%E1cm =207 or at 550 mu, $l\%E1_{cm}$=63 of solution containing not more than 25 meg of cyanocobalamin per ml, using eluting solvent as blank.

Results and Discussion

A measure of specificity is introduced by determining the ratios of the extinctions at 278, 361 and 550 mu or at 341 and 376 mu as proposed by Bruening and Kline (1961). The authors determined the relative purity of cyano-cobalamine in pharmaceutical products by measuring the ratios of extintions at 341 and 376 rou.; the average ratio is 0.99 with none exceeding 0.998. Ratios above 1.02 would indicate that product in question contained cyanocobalamine of less than USP purity.
As vitamin B12 preparations contain preservatives, water cannot be used as a blank, because preservatives may also absorb in the same region. In a quality control laboratory, it is not usually possible to prepare a blank except manufacturer; as he has the advantage to prepare the blank from the same solvents without vitamin addition.
Formation of aquocobalamin from cyano-cobalamin due to exposure to light has been avoided in the above method, as the assay has been carried out in dark. Aquocobalamin has a maximum absorption of 351 mu which differ from the maximum absorption of cyanocobalamin at 361 mu only by 10 mu. It is a very short shift in wave length and upto about 10% of aquocobalamin present in solution may not show any shift in absorption.

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