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Separation of Vitamin B2 and B12 by Impregnate HPTLC Plates with Boric Acid

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***Abstract*—**A high performance thin layer chromatography system (HPTLC) for the separation of vitamin B2 and B12 has been developed. The separation was successfully using a solvent system of methanol, water, ammonia 7.3.1 (V/V) as mobile phase on HPTLC plates impregnated with boric acid. The effect of other mobile phases on the separation of vitamins was also examined. The method is based on different behavior of investigated compounds in impregnated TLC plates with different amount of boric acid. The Rf values of vitamin B2 and B12 are considered on non impregnated and impregnated silica gel HPTLC plate with boric acid. The effect of boric acid in the mobile phase and on HPTLC plates on the RF values of the vitamins has also been studied.

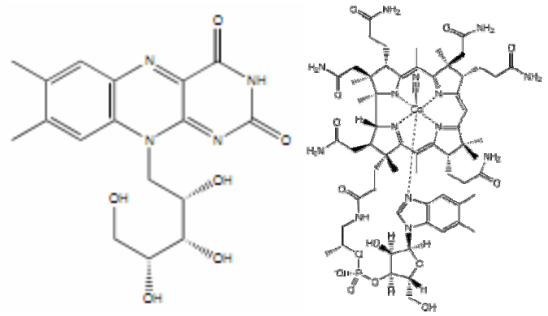
***Keywords*—**High performance thin layer chromatography, HPTLC, Vitamin B2, Vitamin B12, Separation.

I. Introduction

HIGH performance thin layer chromatography (HPTLC) is a sophisticated instrumental technique which utilizes full potential of thin layer chromatographic method. Technically, it is simple to learn and operate. It facilitates automated application and scanning in situ. It offers extreme flexibility for various steps: stationary phase, mobile phase, developing technique, detection (pre and post chromatographic determination). Unlike HPLC, consumption of mobile phase per sample basis is quite low. This saves cost per analysis and analysis time as well. HPTLC facilitates repeated detection (scanning) of the chromatogram with the same or different parameters. HPTLC technique is most suited for impurity profile of drug substances and content uniformity test as per compendia specifications. Simultaneous assay of several components in a multicomponent formulation is possible [1]. Numerous applications of TLC have been reported in the areas of food composition, intentional additives, adulterants, contaminants, and decomposition involving determinations of compound classes such as amino acids (protein quality), lipids and fatty acids (quality and adulteration of fat), sugars (beverage quality), biogenic amines (storage stability), vitamins (added as nutrients, colorants, and antioxidants), and organic acids (preservatives)[2].

The water-soluble B group vitamins include many compounds of differing chemical structure and biological roles, which are essential for the health of children Compared with the requirements of adults [3]. Dietary deficiencies of the B vitamins are associated with several diseases, including degeneration of peripheral nerves and cerebral ataxia in humans [4, 5]. The association of hydrophilic vitamin levels with important biological functions and diseases has led to numerous attempts to quantify these vitamins in foods, including milk, enriched pasta, shellfish, clams, oysters, and muscles [6–8]. Niacin, vitamin B12, and riboflavin, B2, (Fig. 1) are members of the B-group of vitamins that until recently were only of interest in the context of overt deficiency diseases such as anemia and pellagra, or in generalized deficiency conditions where tissues that have a high cell turnover and increased energy demand (i.e. hematopoietic cells, skin, gut, nerve) show a distinct pathology.

The objective of present work is to develop a simple, accurate and precise HPTLC method for separation of diol compounds from others by high resolution using the effect of boric acid which immobilize on HPTLC plate.



(a) (b)

Fig. 1 Molecular structure of a) vitamin B2 and b) vitamin B12

II. Materials and Method

Standards of vitamin B2 and B12 were purchased from sigma. All chemicals and reagents used were of analytical grade and were purchased from Merck (Darmstadt, Germany).

A. Preparation of Standard

Standard of vitamin B2 and vitamin B12 were prepared at a concentration of 0.1 µg μL^{-1} in methanol and water (1:3). All standards and the vitamin mixture were prepared in subdued light and containers were wrapped in aluminum foil, because these vitamins are light-sensitive [9].

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B. Impregnation HPTLC Plates with Boric Acid

Chromatography was performed on 10×10 cm silica gel 60 F 254 HPTLC plates. Before use, the plates were developed with methanol: chloroform (1:1) to remove elutable components, thus producing a clean layer back ground for UV evaluation and a straighter base line and higher signal to noise ratio when scanning [10]. Impregnation was carried out by dipping the silica gel plates in a solution of boric acid 2% in ethanol. The solvent were dried and activated in an air oven at 110°C for 1h [11].

III. Result and Discussion

Compounds that contain hydroxylic group can react with boronat. The interaction that may occur is the formation of a cyclic ester complex in alkaline pH (Fig.2). These complexes can form with 1, 2 or 1, 3-diol- containing compounds when the hydroxyl groups are oriented in the proper geometry like carbohydrates and glycoproteins. It was found that the some vitamins like vitamin B2 could be effectively retarded by boric acid on plates in TLC. This result indicated binding of this vitamin to boric acid on the TLC plates. The basis of impregnation in silica-gel plates was developed by Mezzetti et al [12], when they observed that addition of boric acid to a solvent system, led to the preferential movement of certain sugars over the others. This led to the trying of various impregnates [11].

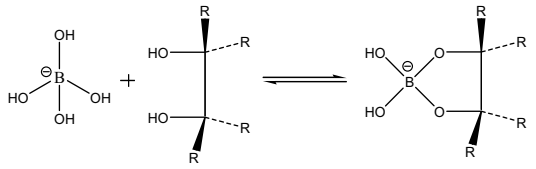

Fig. 2 Cyclic ester formed from the reaction of 1, 2-diols with borate

Separation of vitamins B2 and B12 have been compared on impregnated and non impregnated HPTLC plates and different solvent systems. Plates were spotted with 5µL of each vitamin in the form of bands of width 2mm at a distance of 10mm from the edge of plate using a Camag linomate IV sample applicator (Switzerland). The plates were run in different solvent systems in Camag TLC chamber to a distance of 80mm, the developed HPTLC plates were dried, and then scanned at 254 nm with a Camag scanner II having a deuterium lamp in conjunction with Cats 3 version software. The R_f values for vitamins were observed on each kinds of plates and solvent systems. The results are listed in Table I.

TABLE I
R_F Values of Vitamins on Impregnated and Non Impregnated HPTLC Plates using Different Solvent Systems

solvent system	Compound	R_f value on non impregnated plate	R_f value on impregnated plate
Methanol, water ,ammonia 7:3:1	vitamin B2	0.91	0.71
	vitamin B12	0.97	0.97
Ethanol, chloroform, acetone, ammonia 2:2:2:1	vitamin B2	0.50	0.17
	vitamin B12	0.31	0.30
Water, pyridine,1-butanol 15:35:50	vitamin B2	0.57	0.20
	vitamin B12	0.72	0.66
Ethanol, chloroform, acetic acid, water ,ammonia 5:4:2.5:1:2	vitamin B2	0.93	0.86
	vitamin B12	0.90	0.91
Ethanol, chloroform, acetonitril, toluene, water ,ammonia 7:4:4.5:0.5:1:1	vitamin B2	0.6	0.27
	vitamin B12	0.46	0.41
Ethanol(with boric acid 2%), chloroform, acetonitril, toluene, water ,ammonia 7:4:4.5:0.5:1:1	vitamin B2	0.72	0.27
	vitamin B12	0.57	0.40

Among the various solvent systems studied for an effective separation of the mentioned compounds (unpublished data), just solvent systems have been listed in the Table that could separate the mentioned vitamins.

Because of strong interaction of boric acid with vitamin B2, having diol group in its structure, Rf values of this vitamin decrease in impregnated plates with boric acid (see Table I). This Table also introduces some mobile phase system which can separate two vitamins from each other in both non impregnated and impregnated silica gel HPTLC plates with boric acid. But in case of plates with non impregnation that can not separate the two mentioned vitamins and Rf values of respective vitamins were close together and an unsatisfactory resolution was observed (case of 2 and 4 in the Table), using of impregnated plates with boric acid is effective. In other words the methods developed in this research, which have no potential for qualitative analysis, can be worked by using the impregnated plates with boric acid. It was found that solvent system namely methanol, water, ammonia 7.3.1 (V/V) could impart the better separation of examined solvent systems and

mentioned mobile phase gave a comparatively satisfactory separation with all impregnates studied.

Effect of boric acid contents of TLC plates on Rf of mentioned compounds was also considered. There is no significant different among the plates which impregnated with 2, 3 and 4% boric acid in ethanol.

IV. Conclusion

A new simple high performance thin-layer chromatography (HPTLC) method has been established for separation of two important water-soluble vitamins (B2 and B12), using silica gel impregnated plates with boric acid. The effect of impregnation was observed by the ability of boric acid to bind to diol compounds. Complex formation of boric acid with diol functional groups is well known. This paper reports the retention behavior of two water soluble B group vitamins on thin layers of the inorganic silica gel impregnated with boric acid. So many methods have been developed for separation of these vitamins, but our method is fast, selective and can be done in a single-step process.

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