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Hydrophilic Vitamins

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I. INTRODUCTION

The benefit of using TLC for identification of unknown vitamins and related compounds by comparing R_f values of the unknown compounds with those of authentic vitamins is beyond doubt. The quantification of the separated vitamins can be performed by the use of modern densitometry. TLC as a powerful separation and analytic tool is used particularly for pharmaceutical preparations and food products. Because amounts of most hydrophilic vitamins are low or very low in tissue or body fluid, bioautography or derivatization is used before densitometry. Various high-quality precoated plates with small, uniform particle diameters are available for TLC or high-performance TLC (HPTLC). Stationary phases of silica gel, cellulose, or various reversed phases are available. TLC has great advantages (simplicity, flexibility, speed, and relatively low cost) for the separation and analysis of hydrophilic vitamins.

II. THIAMINE (VITAMIN B_1)

The chemical structure of thiamine is shown in Fig. 1. A pyrimidine moiety (2-methyl-4-amino-5-hydroxymethypyrimidine) and a thiazole moiety (4-methyl-5-hydroxyethylthiazole) are connected by a methylene group. The double salt form of thiamine with hydrochloric acid is readily soluble in water. Thiamine in water is most stable between pH 2 and 4 and unstable at alkaline pH; it is heat-labile, with its decomposition dependent on pH and length of exposure to heat.

The structures of the phosphate esters of thiamine are also shown in Fig. 1. Thiamine monophosphate (TMP), thiamine pyrophosphate (TPP), and thiamine triphosphate (TTP) are commonly found in organisms. About 80–90% of the total thiamine content in cells is TTP, the coenzyme form of thiamine.

To investigate thiamine metabolism in mammals, thiamine (R_f values 0.16, 0.04, and 0.03), thiamine metabolites excreted in urine [thiochrome (R_f values 0.31, 0.28, and 0.33), thiazole (R_f values 0.85, 0.79, and 0.81), 2-methyl-4-amino-pyrimidinecarboxylic acid (R_f values 0.42, 0.21, and 0.26)], and the related compounds pyrimidinesulfonic acid (R_f values 0.48, 0.39, and 0.46), α-hydroxyethylthiamine (R_f values 0.23, 0.09, and 0.06), and N'-methylnicotinamide (R_f values 0.31, 0.06, and 0.05) were analyzed and identified by TLC on silica gel with acetonitrile–water (40:10 v/v) adjusted to pH values of 2.54, 4.03, and 7.85, respectively, with formic acid as solvent (1). Although N'-methylnicotinamide and thiochrome could not be separated in single-phase chromatography at pH 2.54, a second phase at right angles to the first in pH 4.03 solvent separated these quite clearly without affecting the resolution of the other compounds (1).

The quantitative analysis of thiamine hydrochloride (vitamin B_1) using HPTLC on silica gel plates with two different mobile phases was elaborated (2). After the TLC separation, vitamin B_1 was derivatized by the use of *tert*-butyl hypochlorite or potassium hexocyanoferrate(III)–sodium hydroxide as reagent. The *tert*-butyl hypochlorite reagent formed yellow fluorescing derivatives