Meditsiiniline keemia/Medical chemistry   LOKT.00.009

Determination of vitamins using thin layer chromatography.

08.09.15

http://tera.chem.ut.ee/~koit/arstpr/vita_en.pdf

1   Introduction

1.1   Method basics

Planar chromatograpy belongs to the family of chromatographic methods used for separation and determination of substances. It allows to carry out qualitative and sometimes also quantitative analysis of chemical components in complex mixtures. Substances to be determined are transferred onto the layer of sorbent as a spot or band using a capillary, microsyringe or special aplicator. Sorbent may comprise of a layer (0,05...0,2 mm thick) of fine-grain material (silicagel, cellulose, aluminium oxide, ion exchange resin) on a solid support (glass, plastic, metal). Instead of sorbent, porous materials (paper, plastic etc) can be used as well.

Spots of samples are applied on the plate in a straight line called **starting line**. The mobile phase starts moving upwards due to capillary forces when the plate edge is inserted into the eluent in chromatographic chamber. While the eluent is moving the components of sample separate from each other because of the interactions between the molecules of eluent, sorbent and substances to be separated. The elution process is stopped when the eluent has traveled up the plate until 5-10 mm from the upper edge of the chromatographic plate. This eluent front is marked and this line is called **stop line**. The elution distance, h_0, is the distance between the starting line and the stop line.

After the chromatographic separation, all the components of the analysed mixture are located somewhere between the starting line and stop line. In order to visualize the separated substances (if they are not colored) different techniques are used: chemical reactions, adding fluorescence indicators to the sorbent layer during the process of preparation of the plates or spraying the plates with fluorescent solutions and then observing under ultraviolet lamp.

As the distances travelled by the different substances differ, their mobilities can be used for qualitative analysis. Therefor, the distance between the starting line and the center of the spot of substance h_x (mm) characterizes each substance. Retention factor, R_F, provides better way to indentify substances. R_F is calculated from the following formula:

$$R_F = \frac{h_x}{h_0}$$

Provided that exactly the same amount of sample is applied, the intensity of the spot can be used for quantitative determination: the bigger the amount of substance in the mixture, the more intensive is the spot. Also the size of the spot can give quantitative information – the bigger the spot, the bigger the content of this compound in the mixture. Intensity of the spots is evaluated by comparing with the intensities of analyte spots with known amounts visually or using densitometer (device for measuring optical density).

1.2   Water-soluble vitamins

In cure and prophylactical medicine the most useful water-soluble vitamins are vitamin C (ascorbic acid), B_1 (thiamine), B_2 (riboflavin), B_6 (pyridoxine), B_3 (niacin, nicotinic acid) ja B_5 (pantothenic acid).

1.3 Determination of water-soluble vitamins using thin layer chromatography

Separation of the mixture of vitamins is carried out on a silicagel sorbent on aluminium support. For visualization the eluted and dried plate is placed under ultraviolet lamp. Table 1 presents the R_F values of vitamines and colours of spots in ultraviolet light.

Table 1. Retention factors and colors of vitamins.

Vitamin	R_F	Color in UV-light
B$_1$ (thiamine)	0,40	violet
B$_2$ (riboflavin)	0,30	yellow
B$_3$ (niacin, nicotinic acid)	0,36	violet
B$_6$ (pyridoxine)	0,49	dark blue
C (ascorbic acid)	< 0,1	dark blue

2   Aim of the work

Qualitative analysis of vitamins in a polyvitamin tablet.

3   Instruments, chemicals and glassware

1. Elution chamber
2. Plates with silicagel and fluorescence indicator
3. Aplicators of samples (matches)
4. Beaker, 50 ml
5. Funnel
6. Filter paper
7. Mortar and pestle
8. UV lamp
9. Graduated test tube
10. Glass plate for preparing standard solutions
11. Ruler
12. B$_2$ – vitamin powder
13. Nicotinic acid (B$_3$)
14. Ascorbic acid (C)
15. Eluent (chloroform, ethanol, acetone, conc. NH$_4$OH in volume ratio 2:2:2:1)
16. A polyvitamin tablet

4   Analytical procedure

Pour 5 ml of eluent to elution chamber. Close the chamber with a lid and let stand for 10-15 min.

While the elution chamber is saturating with solvent vapours, take the tablet given by the supervisor, crush it in the mortar and add ~5 ml of distilled water. Filtrate the solution into a beaker. The filtrate is used for analysis

Mark the starting line to silicagel plate - 6-8 mm from the edge of the plate - with graphite pencil (be careful, don't scratch the silicagel layer!). Also mark the locations where the samples will be spotted (4, including the analysed sample). The distance between neighboring spots should be about 10 mm and the the spots should be at least 5 mm away from the plate edge. Usually the spot of unknown substance is applied to the center of the starting line.

Few crystals of each standard substance is put on a glass plate (wash and dry the spatula before taking the next substance!). Add a drop of distilled water to each standard substance and mix with a match. Transfer each solution on the chromatographic plate using the sulfur free end of a match. The spots on the sorbent must not be bigger than 3 mm in diameter. Let the spots dry.

The chromatographic plate is placed into the elution chamber and the chamber is covered with a lid. Elution is stopped when the solvent front has traveled up the plate until 5-10 mm from the top (about 20-30 min). The elution front is marked with a graphite pencil. Dry the plate in an oven at 60° C for 10 min. Use UV-lamp to visulize the bands and draw the contours of the bands on the plate.

Presence or absence of the vitamins in the polyvitamin tablet is ascertained by the distances from starting line and color of the bands. Measure the distances between starting and stop lines, h_0, and between the starting line and each band center, h_x. Calculate the values of R_F and compare the results with the data in Table 1.

5   Results

Names of vitamins found in the tablet.