THIN-LAYER CHROMATOGRAPHIC METHOD FOR IDENTIFYING VITAMIN C IN FRUITS AND DRUGS

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Abstract

Among the nutrients needed by several essential physiological functions to achieve vital reactions are vitamins. These are a category of substances strictly necessary for superior animals and cannot be synthesized in their body. Of what, vitamins must be procured by food ration.

Separations of the hydrophilic vitamins ascorbic acid (Vitamin C) acid have been compared using 7 mobile phases and commercially available silica gel thin-layer chromatography $_3$

INTRODUCTION

Among the nutrients needed by several essential physiological functions to achieve vital reactions are vitamins. These are a category of substances strictly necessary for superior animals and cannot be synthesized in their body. For this reason, vitamins must be procured by food ration (Gîtea et al, 2012, Gîtea, et al., 2017, 2018).

In organisms vitamins are not structural materials, of the kind of immediate principles (proteins, carbohydrates, lipides) and have no energy value but fulfill important functional scams. The majority of vitamins, or their derivatives, are coenzyatic constituents, Ascorbic acid ($C_6H_8O_6$) is an organic acid, with antioxidant properties, has molecular weight 176.12 g/mol, and the melting Point 190 ÷ 192 ° C. Vitamin C is a 5-carbon lactone and the enolic hydroxyl groups from C_3 and C_2 carbon atoms can disassociate to form a dibasic acid. The remainder of 2.3-endiol enters into conjugation with the lactonic carbonyl group, resulting in the proton of the hydroxyl C_3 group to be Strong acid (pK_1 = 4.25) compared to the proton group protone from C_2 (pK_2 = 11.79). The 2.3-endiol grouping of L-ascorbic acid can donate one or more electrons and thus form the established intermediary (semidehydro-L-ascorbic acid and finally the oxidation product of dehydro-L-ascorbic acid. These reactions accompanied by the transfer of electron are responsible for many but not all chemical and biological reactions body needs very small amounts of these compounds every day. The food ration must ensure the necessary daily (Combs, 2008).

The term "vitamins" was introduced by the Funk in 1911 (Kucharsk, et. al, 2009). Seeking the causes of the disease called beriberi, a disease of the nervous system often encountered in East Asia. This disease is caused by the rice cooker and it was found that it could be cured by the consumption of rice bran. In 1911, Funk isolated from rice bran a substance in the form of water-soluble crystals. This substance was responsible for the healing of Beriberi and was called "Vitamin Beriberi".

The name of vitamins was due to the presence of the functional amino group and the fact that they are vital for life. The name of vitamin was accepted for tpte the essential nutritional factors discovered in the years following Funk's first studies. According to a proposal made by Drummond, in 1920 vitamin Beriberi was called Vitamin B.

From 1928 to 1933, the Hungarian researcher' team composed of Joseph L. Svirbely and Albert Szent-Györgyi and, independently, American Charles Glen King, first isolated vitamin C and showed that it was ascorbic acid. This antiscorbut compound was called vitamin C.

Ascorbic acid, is a hydrosoluble vitamin and one of the most well-known and studied vitamins, being biosynthesized by microbial, vegetable and animal organisms, with the exception of primates and guinea pig.

Due to the presence in the molecule of two asymmetric carbon atoms C_4 and C_5, ascorbic acid is active optic. L-ascorbic acid rotates the plane of positive polarized light. Optical rotation is not significantly affected by incresing the acidity of the solution, but in contrast varies greatly with the increase in the alkalinity of the solution (Seib, 1985).

Ascorbic acid is presented in the form of a crystalline white powder, whose melting point (with decomposition) is 192 ° C. Crystallised from aqueous solutions oversaturated in the form of colourless monocyclic crystals. It is an active optical substance $[\alpha]^{20}_{D} = +32.5°$ (H_2O) and 48 ° (methanol) and has a characteristic absorption spectrum at $\lambda = 265nm$. Ascorbic acid is easily soluble in water (22.4% at 20 ° C) and in methyl alcohol, hard soluble in ethyl alcohol, acetone and glycerin and insoluble in ether, aliphatic and aromatic hydrocarbons. The basic ph-salt of ascorbic acid is insoluble in water and ethyl alcohol, properties on which the methods of isolation of ascorbic acid from vegetable extracts are based. In solutions, ascorbic acid is easily oxidized, especially in the presence of copper and silver ions, the cold reduces the solution Fehling, $AgNO_3$, $KmnO_4$, 2.6-dichlorfenolindophenol, and by heating in the medium of HCl passes in Furfurol at 100 ° C (Bungău, 2014).

Vitamin C is one of the most known antioxidants. It is effective in treating but especially in the prevention of many health problems, being renowned for its action of regulating cholesterol, eliminating free radicals that destroy (Ponder, 2004).

Methods for the determination of ascorbic acid are numerous. Chemical methods are based on the reducer character or on the derivatization of ascorbic acid. These methods allow for the rapid determination of content but do not allow differentiating stereotypes and may inferce other substances that exhibit reducing properties (Parviainen, 1995)

Thin layer chromatography (TLC) is generally regarded as a common analytical method for screening, separation, and preliminary identification of compounds. TLC plays an important role in the quality control of food and drugs in order to investigate the ingredients and to detect the impurities and also for checking the purity and the stability of preparations.

In this paper we present the results obtained from the research effctuated for the qualitative analysis of ascorbic acid by thin layer chromatography of ascorbic acid.

MATERIAL AND METHOD

All reagent al chemical pure from Merck and standard ascorbic acid from Sigma Co.

Preparation of solution

Standard solution Ascorbic acid: weigh 0.1 g ascorbic acid and dissolve in methanol.

Fruit Extract Solution: (fruits: apple, kiwi and lemon juice). To obtain the extract containing ascorbic acid, weigh 10 g fruit, grinds with 5 g sand and add 100 ml of distilled water, homogenise and filter it. The extract is subject to determinations.

Chromatografic Analysis

In a first step of the study, was determined the optimum composition of the mobile phase used for proper separation of ascorbic acid.

Separations of vitamin standard and extract solution were compared on 5 cm x 10 cm silica gel 60 GF 254 TLC plates.

In view of this study, the compositions of the mobile phase evaluated are summarized in Table 1. Plates were developed 8 cm beyond the origin.

Table 1

The compositions of the mobile phase

No	Component	Volume ratio	Rf
1	n-Butanol:Chloroform:Acetic Acid:Ammonia:Water	7:4:5:1:1	7
2	Ethanol:Chloform:Acetic Acid:Amoniac:Apa	7:4:5:1:1	7
3	Acetonă:Methanol:Benzen	1:2:8	8
4	Acetonă:Methanol:Toluene	1:2:8	This study
5	Toluene:Metanol:Acetone:Acetic Acid	14:4:1:1	This study
6	Chloroform:Ethanol:Acetone:Ammonia	2:2:2:1	9
7	Metanol:Water	7:3	10
8	Etanol:Water	2:1	11

On the chromatographic plates are spotted with samples and standards. The chromatographic plates are dried and were developed with mobile phases1 to 7 presented in table no.1. Follow the development that takes place up to the front of the solvent reaches about 1 cm from the top of the plate. The plate are removed dry out.

System provinding clear separations of the standards were used to separate the vitamin mixture and samples from fruits.

Vitamin were detected by fluorescence quenching under 254 nm ultraviolet light.

RESULTS AND DISCUSSION

R_f values of vitamin standards are summarized în table 2.

Table 2

R_f values of vitamin C from separation with a variety of combination of mabile phase

No	Component	Volume ratio	R_f
1	n-Butanol:Chloroform:Acetic Acid:Ammonia:Water	7:4:5:1:1	0.46; 0,81
2	Ethanol:Chloform:Acetic Acid:Amoniac:Apa	7:4:5:1:1	0,75
3	Acetonă:Methanol:Benzen	1:2:8	0,73
4	Acetonă:Methanol:Toluene	1:2:8	0,50
5	Toluene:Metanol:Acetone:Acetic Acid	14:4:1:1	0,90
6	Chloroform:Ethanol:Acetone:Ammonia	2:2:2:1	0,15
7	Metanol:Water	7:3	0,84
8	Etanol:Water	2:1	-

In case of sample 8 the separation was not satisfactorily. A more poor separation was also achieved when using the mobil phase 6 (Table 2), the value of the Rf parameter was only 0.15. In the rest of the determinations the separations are satisfactory, the shape of the corresponding feet. A very careful attention represents the molil phase system 1. The ascorbic acid standard was partly impurified with

dehydroascorbic acid. With this eluent system, separation and highlighting of dehydroascorbic acid has been managed.

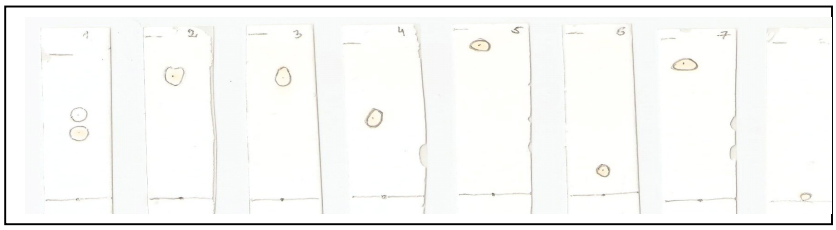

Fig. 1. Chromatograme plate from separation vitamin C with a variety of combination of mabile phase

The good results obtained in these determinations by TLC of vitamin C permit to identify the presence of ascorbic acid in fruit extracts to be carried out under these conditions. In table 3 are presented the results obtained in TLC analysis of extract of apple kiwi and lemon juice. Mobil phase used in this determination was selected as the mixture with which was obtained good separation (n-Butanol : Chloroform : Acetic Acid : Ammonia: Water 7:4:5:1:1)

Table 3

R_f values of vitamin C from separation fruit extract

No	Fruct	R_f
1	Kiwi	0,85
2	Apple	0,83
3	Lemon juice	0,81

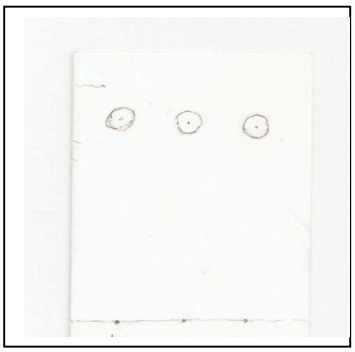

Fig. 2. Chromatogram of fruit extract

For each sample of fruit extract was determined R_f parameter. The experimental values obtained demonstrated the presence of ascorbic acid in each extract of these fruits, the separation being good, the Rf value falling

within the range 0.81 – 0.85, comparable to Those obtained in the case of Rf standard = 0.81 (table 3 and Figure 2)

Separation, detection, and quantification of vitamin C acid using the mobile phases n-butanol–chloroform–acetic acid–ammonia–water, 7:4:5:1:1, in combination with detection reagents and natural fluorescent colors is an alternative to using individual chemical assays. The methods developed in this research, which have the potential for qualitative analysis of vitamin C in fruit and drugs.

CONCLUSIONS

Separation, detection, and quantification of vitamin C acid using the mobile phases n-butanol – chloroform – acetic acid – ammonia – water, 7:4:5:1:1, in combination with detection reagents and natural fluorescent colorsis an alternative tousing individual chemical assays. The methods developed in this research, which have the potential for qualitative analysis of vitamin C in fruit and drugs.

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