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THIN LAYER CHROMATOGRAPHY SEPARATION OF SOME CAROTENOIDS, RETINOIDS AND TOCOPHEROLS

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ABSTRACT. A simple and reliable thin layer chromatography (TLC) method for simultaneous separation of some carotenoids (β-carotene, lycopene, lutein, astaxanthin and zeaxanthin), retinoids (retinol, retinoic acid, 9-*cis*-retinal and all-*trans*-retinal) and tocopherols (α-tocopherol, γ-tocopherol and δ-tocopherol) was described. For a proper separation of all compounds three different mobile phases (Benzene: Petroleum Ether, 10:90, v/v; Methanol: Benzene: Ethyl acetate, 5:75:20, v/v/v; Benzene: Petroleum Ether, 90:10, v/v) were tested, using silica gel F_{254} as stationary phase. The visualization was performed under UV light (254 and 365 nm). The presented method is simple and adequate for the separation and identification of these compounds from different matrices, such as pharmaceuticals and foods.

Keywords: *carotenoids, retinoids, tocopherols, thin layer chromatography*

INTRODUCTION

The retinoids and tocopherols are known as vitamin A, and E respectively, while carotenoids are vitamin A precursors. The carotenoids are one of the most important groups of natural pigments, because of their wide distribution, structural diversity and numerous functions. Although the classical sources of carotenoids are plants, they are also found in animals and microorganisms [1, 2]. They are classified in two classes, carotenes (i.e. β-carotene and lycopene, Figure 1, 1-2), and xanthophylls (i.e. lutein, astaxanthin and zeaxanthin, Figure 1, 3-5). Nowadays, they are widely associated to retinoids, which are more polar and smaller molecule compounds. Some natural occurring vitamins A are presented in Figures 1, 6-9. Both classes, carotenoids and retinoids, present similar biological activity [3]. On the other part, tocopherols (Figure 1, 10-12) are a series of organic compounds consisting of various methylated phenols. They are biosynthesized by plants. Since animals and humans are unable to biosynthesize

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vitamin E, they have to rely on an external supply of it through their diet. It is well known that some of the tocopherols, the carotenoids and the retinoids are very effective antioxidants and are suspected to reduce the risk of cancer known to be initiated by the production of free radicals [4, 5].

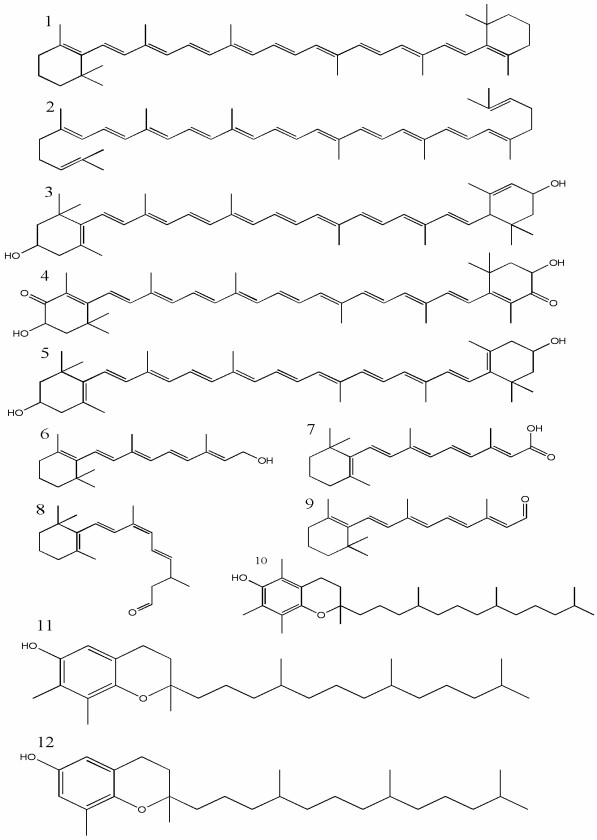

Figure 1. Chemical structure of some carotenoids (1: β-carotene, 2: lycopene, 3: lutein, 4: astaxanthin, 5: zeaxanthin), retinoids (6: retinol, 7: retinoic acid, 8: 9-*cis*-retinal, 9: all-*trans*-retinal), and tocopherols (10: α-tocopherol, 11: γ-tocopherol, 12: δ-tocopherol).

They are found together, often in different pharmaceutical formulations or as food additives, because of their synergic activity [6]. The chromatographic determinations of mixtures of these compounds were intensely studied. The high performance liquid chromatography (HPLC) is, unequivocally the most used chromatographic technique in their determination, because of its sensibility and variability [7-13]. Furthermore, gas chromatography (GC) has extensive applications for tocopherols [14-16] and retinoids [17, 18], while the carotenoid

determinations are more difficult because of compounds physicochemical properties. Other chromatographic techniques, such as sub- and supercritical chromatography are rarely used [19].

Thin layer chromatography methods have been widely employed for the analysis of lipophilic vitamins and their precursors [20, 21]. Normal phases systems based on silica gel and magnesium oxide sorbents and chemically bonded sorbents with largely non-aqueous mobile phases have been most often used for TLC determinations of carotens, while for xanthophylls and retinoids more polar mobile phases are required [22-26]. The tocopherols TLC analysis demands, as well, low polarity mobile phases when silica gel plates are used [27-29]. Moreover, some reverse phase TLC separations were reported [30].

The objective of this work was to develop a proper separation procedure for some carotenoids, retinoids and tocopherols in normal phase chromatography, able to be used for TLC separation from pharmaceutical preparative and foods.

RESULTS AND DISCUSSION

Because of the high variance of analytes polarities the direct separation of compounds by one development was impossible. The polarities of β-carotene and lycopene are lower than of all other compounds. These aspects lead to the using of three different mobile phases. The MP1 had as target the separation of β-carotene and lycopene. A proper separation of these compounds can be observed in Figure 2.

A second elution of the same plate with MP2 can solve the separation of xanthophylls and retinoids. Moreover, the method allows a sufficient separation of all xanthophylls, including the lutein and zeaxanthin isomers. The retinoids are well separated by xanthophylls (Figure 3). The isomers 9-*cis*-retinal and all-*trans*-retinal could not be separated by each other. However, the tocopherols remained at start by using the MP1, while the MP2 moves them in the front. The MP1 and MP2 were not able to offer a proper separation of tocopherols, but the problem was solved by the MP3, which offered a high resolution separation. As can be seen the selectivity of MP1 was modified by changing the solvents ratio, obtaining MP3. The MP3 strength is intermediary between MP1 and MP2. In Figure 4 the sensitive separation of tocopherols can be observed. To create a better estimation of the separation, the R_F values were calculated as a proportion between the migration distance and solvent front of analites. The R_F values were presented in Table 1.

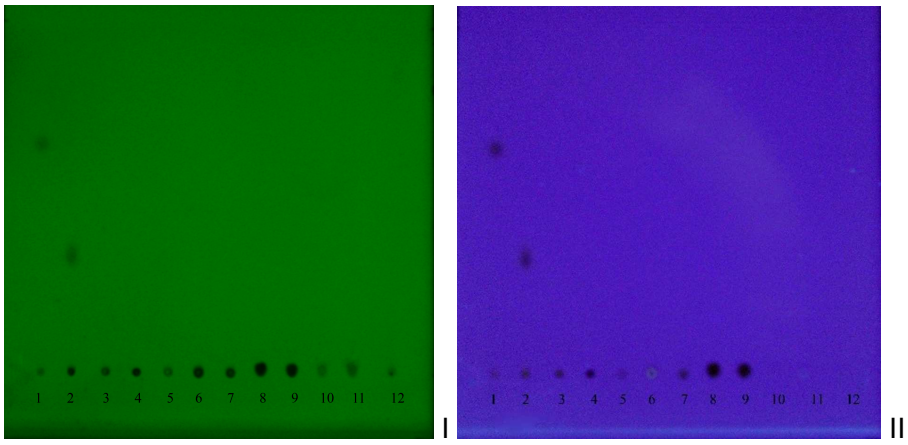

Figure 2. The TLC separation of β-carotene (1) and lycopene (2) by the other compounds (3- lutein, 4- astaxanthin, 5- zeaxanthin, 6- retinol, 7- retinoic acid, 8- 9-*cis*-retinal, 9- all-*trans*-retinal, 10- α-tocopherol, 11- γ-tocopherol, 12- δ-tocopherol) by using the MP1 mobile phase (Benzene: Petroleum Ether; 10:90; v/v) and visualized under UV light at 254 nm (I) and 365 nm (II).

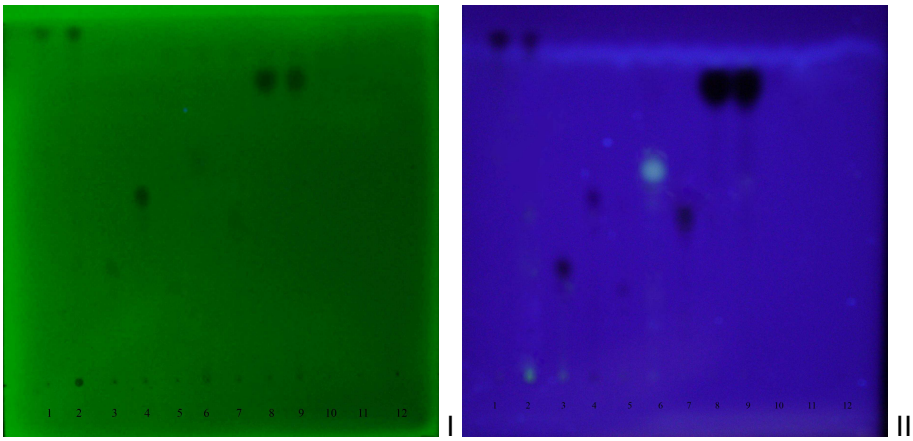

Figure 3. The TLC separation of xanthophylls (3- lutein, 4- astaxanthin, 5- zeaxanthin) and retinoids (6- retinol, 7- retinoic acid, 8- 9-*cis*-retinal, 9- all-*trans*-retinal) by the other compounds (1- β-carotene, 2- lycopene, 10- α-tocopherol, 11- γ-tocopherol, 12- δ-tocopherol) by using the MP2 mobile phase (Methanol: Benzene: Ethyl acetate; 5:75:20; v/v/v) and visualized under UV light at 254 nm (I) and 365 nm (II).

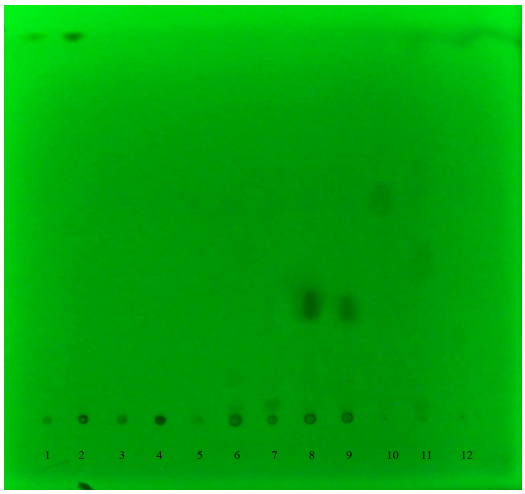

Figure 4. The TLC separation of tocopherols (10- α-tocopherol, 11-γ-tocopherol, 12- δ-tocopherol) by the other compounds (1- β-carotene, 2- lycopene, 3- lutein, 4- astaxanthin, 5- zeaxanthin, 6- retinol, 7- retinoic acid, 8- 9-cis-retinal, 9- all-trans-retinal) by using the MP3 mobile phase (Benzene: Petroleum Ether; 90:10; v/v) and visualized under UV light at 254 nm (A).

Table 1. Rf values of carotenoids, retinoids and tocopherols.

Name	Rf		
	MP1	**MP2**	**MP3**
β-carotene	0.83	1.00	1.00
Lycopene	0.37	1.00	1.00
Lutein	0.00	0.48	0.00
Astaxanthin	0.00	0.71	0.00
Zeaxanthin	0.00	0.45	0.00
Retinol	0.00	0.74	0.00
Retinoic Acid	0.00	0.61	0.00
9-*cis*-retinal	0.00	0.94	0.36
All-*trans*- retinal	0.00	0.94	0.37
α-tocopherol	0.00	1.00	0.44
γ-tocopherol	0.00	1.00	0.31
δ-tocopherol	0.00	1.00	0.24

The method described above was tested on two types of samples, vegetables and pharmaceutical products. Three extracts of carrots, tomatoes and tomato sauce were spotted near by standard solutions of carotenoids and tocopherols. After developing using MP1 a good identification of β-carotene and lycopene was obtained (Figure 5). The rest of carotenoids and tocopherols were not presented in the analyzed extracts.

The second test for method ability was performed on an antioxidant pharmaceutical product containing β-carotene, α-tocopherol acetate and ascorbic acid. The β-carotene, tocopherols and ascorbic acid were spotted near by the product solution. The mobile phase chosen for developing was the MP3. A certain identification of β-carotene could be observed (Figure 6). However, the method is not proper for the identification of ascorbic acid, because it was not moved from the start position. The R_F values obtained for standard and identified compounds in both, vegetables and pharmaceutical product, presented in table 2, confirm the identity of extracted compounds.

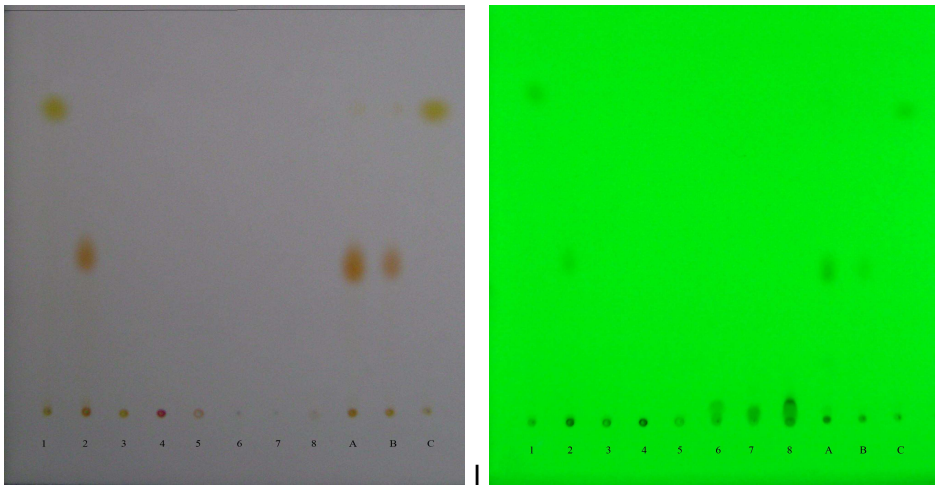

Figure 5. The identification of β-catotene (1) and lycopene (2) in the extracts of tomatoes (A), tomato souce (B) and carrots (C) by using MP1 mobile phase (Benzene: Petroleum Ether; 10:90; v/v) and visualized under visible (I) and UV light (II). The other spotted compounds were lutein (3), astaxanthin (4), zeaxanthin (5), α-tocopherol (6), γ-tocopherol (7), δ-tocopherol (8).

Table 2. The Rf values of identified compounds into different vegetable and pharmaceutical products using the proper mobile phases.

Name	**R_F**			
	Vegetable Products Extracts (MP1)			**Triovit (MP3)**
	Tomato souce	**Tomatoes**	**Carrots**	
β-carotene	0.84	0.83	0.83	0.84
Lycopene	0.37	0.36	nd	-
Lutein	nd	nd	nd	-
Astaxanthin	nd	nd	nd	-
Zeaxanthin	nd	nd	nd	-
α-tocopherol	nd	nd	nd	nd
γ-tocopherol	nd	nd	nd	nd
δ-tocopherol	nd	nd	nd	nd
Ascorbic Acid	-	-	-	0.00

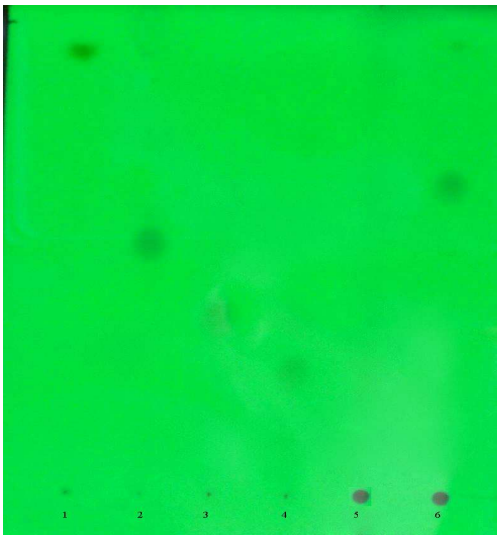

Figure 6. Separation and identification of some vitamins in Triovit capsule unde UV light (254 nm) with MP3 mobile phase (Benzene: Petroleum Ether; 90:10; v/v). Spots: 1- β-carotene, 2- α-tocopherol, 3- γ-tocopherol, 4- δ-tocopherol, 5- ascorbic acid, and 6- Triovit capsule.

CONCLUSIONS

The chromatographic separation of some complex mixtures of vitamins and precursors is difficult because of the variability of compound nature. The thin layer chromatography is an effective method for the separation of large groups of substances, especially if more chromatographic systems with different selectivities are used. The separation of carotenoids, retinoids and tocopherols on silica gel F_{254} plates with three different mobile phases could be obtained. Also, the separation of carotenes and tocopherols using benzene: petroleum ether (10:90 and 90:10 v/v) indicates that, by changing the solvents ratio were provided different selectivity mobile phase. The obtained results indicate that the method is suitable to be used for qualitative determination of investigated compounds into real samples of vegetable and pharmaceutical products.

EXPERIMENTAL SECTION

Materials. The β-carotene, lycopene, lutein, astaxanthin, 9-*cis*-reinal, all-*trans*-retinal, δ-tocopherol and ascorbic acid standards were obtained from Sigma, while zeaxanthin, retinol and retinoic acid were Fluka products. α and γ-tocopherols were purchased from Acros Organics. The solvents used (petroleum ether, methanol, benzene and ethyl acetate) were obtained from

Chimopar. All the chemicals were analytical degree purity. As stationary phases were used TLC silica gel F_{254} plates 10 x 10 cm from Merck. The spotting was performed using a Hamilton microsyringe of 10 µL.

Samples of carrots, tomatoes, tomato sauce, and pharmaceutical product (Triovit capsule) were obtained commercially. The Triovit is a diet supplement containing β-carotene (10 mg), α-tocopherol acetate (40 mg), ascorbic acid (100 mg), selenium (50 µg) and excipients (magnesium stearate and silicon dioxide anhydrous).

Extraction Procedure. 10 g of each vegetable product were extracted according to Cimpan *et al.* [31]. The pharmaceutical product (1 capsule of Triovit) was dissolved in 5 mL of chloroform followed by a filtration step performed in order to remote the excipients. The obtained extracts could be directly applied on the chromatographic plate.

Chromatography. 2 µL of standard solutions (1 mg mL^{-1}) and extracts were spotted on the TLC plates at 1.5 cm from the bottom edge and at 1 cm from lateral edges. The distance between spots were 0.7 cm. Plates were developed on 8 cm using three different mobile phases:

MP1. Benzene: Petroleum Ether (10:90; v/v);

MP2. Methanol: Benzene: Ethyl acetate (5:75:20; v/v/v);

MP3. Benzene: Petroleum Ether (90:10; v/v).

The visualization was performed under UV light at 254 and 365 nm.

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