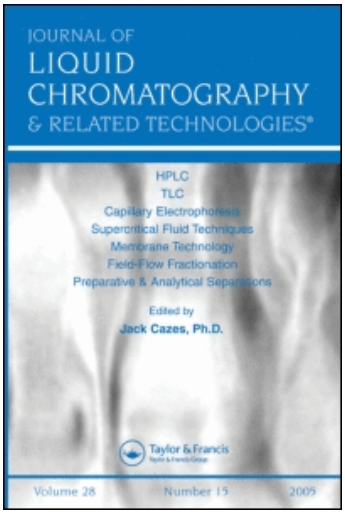

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Pyka, Alina and Gurak, Danuta(2009) 'Use of RP-TLC and Densitometry to Analytical Characteristic of Vitamin K_1', Journal of Liquid Chromatography & Related Technologies, 32: 14, 2097 — 2104

To link to this Article: DOI: 10.1080/10826070903126971

URL: http://dx.doi.org/10.1080/10826070903126971

Journal of Liquid Chromatography & Related Technologies®, 32: 2097–2104, 2009

ISSN: 1082-6076 print/1520-572X online
DOI: 10.1080/10826070903126971

Use of RP-TLC and Densitometry to Analytical Characteristic of Vitamin K_1

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Abstract: Vitamin K_1 (phylloquinone) was investigated with the use of reversed phase thin layer chromatography (RP-TLC) on $RP8F_{254s}$, and $RP18F_{254s}$ (E. Merck) plates, using methanol as a mobile phase. It was stated that a larger amount of the bands on a chromatogram were obtained on $RP8F_{254s}$ plates than on $RP18F_{254s}$ plates. Six densitometric bands on $RP8F_{254s}$ plates were observed, where vitamin K_1 had $R_F = 0.45$ and $\lambda_{max} = 254$ nm; however, the impurities had the R_F values equal to 0.27, 0.54, 0.59, 0.62, and 0.75, respectively. It was stated that RP-TLC with densitometry and spectrodensitometry is a good method for the analysis of the qualitative composition of vitamin K_1.

Keywords: Densitometry, Phylloquinone, RP-TLC, Spectrodensitometry, Vitamin K_1

INTRODUCTION

Vitamins are organic compounds, that have biochemical and physiological proprieties. Because of these qualities, they have been the subject of numerous scientific investigations. Chromatography is useful in the identification and determination of vitamins in pharmaceutical preparations, the

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identification and determination of vitamins and related substances in natural materials and foodstuffs, and the chemical and biochemical determination of vitamins and their metabolites in fats and tissues.[1–5]

Vitamins are classified according to their solubility in water and in fats. Fat soluble vitamins are vitamins A, D, E, and K. Physiological forms of vitamins K are vitamin K_1 (phylloquinone, phytonadione), and vitamin K_2 (farnoquinone). Active analogs and related compounds, known as vitamin K, are menadiol diphosphate, menadione (vitamin K_3), menadione bisulfite, phthiocol, synkayvite, menadiol (vitamin K_4), menaquinone-n (MK-n), ubiquinone (Q-n), and plastoquinone (PQ-n). Phylloquinone (K_1), and menaquinone-4 (MK-4) are natural K vitamins and are often used as medicines to prevent intracranial hemorrhage in the newborn. Vitamin K contributes to the formation and regulation of numerous proteins in the body, but most significantly to prothrombin, a protein essential for blood clotting. Vitamin K is also necessary for converting prothrombin to thrombin, which is also required for blood clotting. Vitamin K is a key factor in the creation of many important nutrients and proteins necessary for essential body functions.[3,6]

Good sources of vitamin K_1 are spinach, collards, broccoli, iceberg lettuce, french salad dressing, green beans, brussels sprouts, green peas, margarine, tuna canned in oil, asparagus, mayonnaise, carrots, eggs, apple pie, taco, and mashed potatoes.[3,7] The scientific literature does not publish the experimental *n*-octanol-water partition coefficient for vitamin K_1. However, the theoretical partition coefficients calculated using different commercial computer programs for vitamin K_1 are equal to ALogPs = 8.48, AClogP = 10.89, AB/logP = 10.00; COSMOFrag = 11.24, miLogP = 8.80, AlogP = 10.25, mLogP = 6.67, KOWWIN = 11.71, xlogP20 = 9.31, and xlogP3 = 10.91.[8,9]

Vitamin K_1 is the component of many pharmaceutical preparations.

The subject of this work was vitamin K_1 (Figure 1) produced by BioChemika. This work concerns the analysis of the qualitative composition of vitamin K_1 by the use of RP-TLC technique with densitometry and spectrodensitometry.

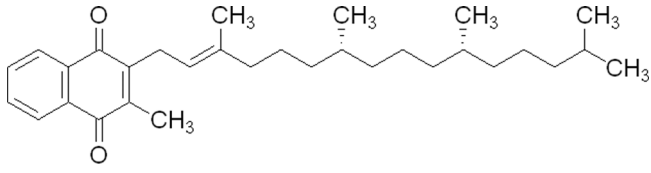

Figure 1. Structural formula of vitamin K_1 (phylloquinone).

EXPERIMENTAL

Chemicals

Methanol (E. Merck, Darmstad, Germany) and ethanol (96%, POCh, Gliwice, Poland) were analytical grade. A pure sample of phylloquinone (vitamin K_1) (BioChemika, pure $\geq$99% HPLC; lot & filing code: 1315018 54606265) was used as test solution. A standard solution of vitamin K_1 (5 mg mL^{-1}) was prepared in ethanol.

Reversed Phase Thin Layer Chromatography

Thin layer chromatography was done on 10 cm $\times$ 20 cm aluminium foil backed TLC plates coated with $RP18F_{254s}$ and on 10 cm $\times$ 20 cm glass backed TLC plates coated with $RP8F_{254s}$ (Merck, Darmstadt, #1.05559, lot: OB687316, and #1.15424; lot: OB549661, respectively). The plates were prewashed with methanol and dried for 24 h at room temperature (22 ± 1°C). Ethanolic solution of vitamin K_1 (5 µL) was spotted manually on the plates. Methanol was used as a mobile phase. The mobile phase (50 mL) was placed in a classical chromatographic chamber (Camag, Switzerland) and the chamber was saturated with methanol vapor for 20 min. The plates were developed vertically, to a distance of 8.5 cm at room temperature (22 ± 1°C). The plates were then dried at room temperature. A Camag densitometer was used for the obtainment of R_F values. The chromatograms were done in a five fold manner and each track was scanned three times; the mean retardation factor R_F values were calculated.

Densitometric Analysis

Densitometric scanning was then performed at 254 nm. The radiation source was a deuterium lamp emitting a continuous spectrum between 190 and 450 nm. The slit dimensions were 8.00 $\times$ 0.40 mm, Macro; the optimized optical system was light; the scanning speed was 20 mm s^{-1}; the data resolution was 100 µm step^{-1}; the measurement type was remission; and the measurement mode was absorption; the optical filter was second order. Each track was scanned three times and baseline correction (lowest slope) was used.

Spectrodensitometric Analysis

A spectrum scan was recorded using a Camag Scanner TLC 3 operated in absorbance mode and controlled by WinCATS 1.4.2 software. The

radiation sources were a deuterium lamp emitting a continuous UV spectrum between 190 and 450 nm. Start wavelength was 200 nm and ending wavelength was 450 nm. The slit dimensions were 8.00×0.40 mm, mm, Macro; the optimized optical system was resolution; the scanning speed was $20\,\text{nm}\,\text{s}^{-1}$; the data resolution was $1\,\text{nm}\,\text{step}^{-1}$; the

Table 1. R_F values and spectrodensitogram characteristics of vitamin K_1 and its impurities after their separation on $RP18F_{254s}$ and $RP8F_{254s}$ plates using methanol as mobile phase

		Spectrodensitogram characteristics		
		Fundamental absorption band	Remaining absorption bands	
Compounds	R_F value	λ_{max} (nm)[a]	λ (nm)	Intensity (AU)
$RP18F_{254s}$ plate				
Impurity 1	0.08	337	217	39.2
			252	73.7
			273	73.3
Vitamin K_1	0.19	270	256	92.7
			309	76.1
			343	75.6
Impurity 2	0.36	271	223	52.2
			310	67.1
Impurity 3	0.40	333	217	47.9
			272	78.0
Impurity 4	0.72	270	218	40.6
			263	92.6
			309	79.3
$RP8F_{254s}$ plate				
Impurity 1	0.27	335	219	37.7
			253	68.6
			274	70.4
Vitamin K_1	0.45	254	222	60.2
			270	94.2
			308	64.6
			341	70.6
Impurity 2	0.54	330	271	70.1
Impurity 3	0.59	262	222	55.9
			307	68.3
Impurity 4	0.62	270	222	51.3
			317	69.6
Impurity 5	0.75	270	219	50.3
			313	53.3

[a]Intensity of all fundamental absorption band is equal to 95 AU.

measurement type was remission; and the measurement mode was absorption; the optical filter was second order.

RESULTS AND DISCUSSION

Vitamin K_1 is a basic lipophilic vitamin. Therefore, the investigations of vitamin K_1 were performed by RP-TLC technique, densitometry, and spectrodensitometry. The investigations were performed on $RP18F_{254s}$ and $RP8F_{254s}$ plates using methanol as a mobile phase. Obtained densitogram on $RP18F_{254s}$ plate shows five chromatographic spots; where the general component, namely vitamin K_1 had $R_F = 0.19$ and $\lambda_{max} = 270$ nm. However, remaining densitometric bands of existing compounds besides vitamin K_1 had the R_F values equal to 0.08, 0.36, 0.40, and 0.72, respectively (Table 1). However, six densitometric bands on $RP8F_{254s}$ plates were observed where vitamin K_1 had $R_F = 0.45$ and $\lambda_{max} = 254$ nm; however the impurities had the R_F values equal to 0.27, 0.54, 0.59, 0.62, and 0.75, respectively (Figure 2). Detailed densitomeric characteristic

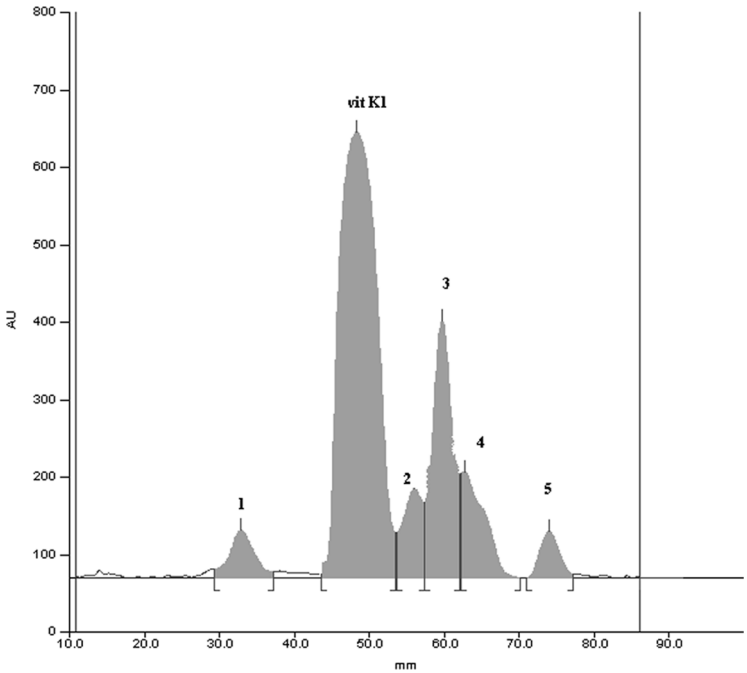

Figure 2. Densitogram of 25.00 μg of vitamin K_1 (phylloquinone) separated on $RP8F_{254s}$ plate. Where: vit.K1 – vitamin K_1 (phylloquinone); 1, 2, 3, 4, and 5 – the impurities.

data of the obtained densitomeric bands are presented in Table 1. The obtained spectrodensitograms of vitamin K_1 indicate that applied sorbents influences the wavelength of the obtained fundamental absorption band (λ_{max}) and the additional absorption bands, as well as on their intensity values [AU]. This fact indicates the necessary standaridization of the spectrodensitometric investigations regarding the applied chromatographic conditions. Therefore, the spectrodensitograms of vitamin K_1 can be correctly compared only on the same chromatographic sorbent. This fact was also observed for other drugs.[10,11]

It was stated that a larger amount of the bands on a chromatogram were obtained on $RP8F_{254s}$ plates than on $RP18F_{254s}$ plates. Obtained data indicate that vitamin K_1 had quite a few of the impurities of an unidentified structure. A spectrodensitogram of vitamin K_1 on a $RP8F_{254s}$ plate is presented in Figure 3. The spectrodensitograms of vitamin K_1 impurities are presented in Figures 4 and 5.

Obtained results indicate that the investigated chromatographic and spectrodensitometric conditions are a proper method for the evaluation of the degree of purity and analytical characteristic of vitamin K_1. It was stated that RP-TLC with densitometry and spectrodensitometry is a good method for the analysis of the qualitative composition of vitamin K_1.

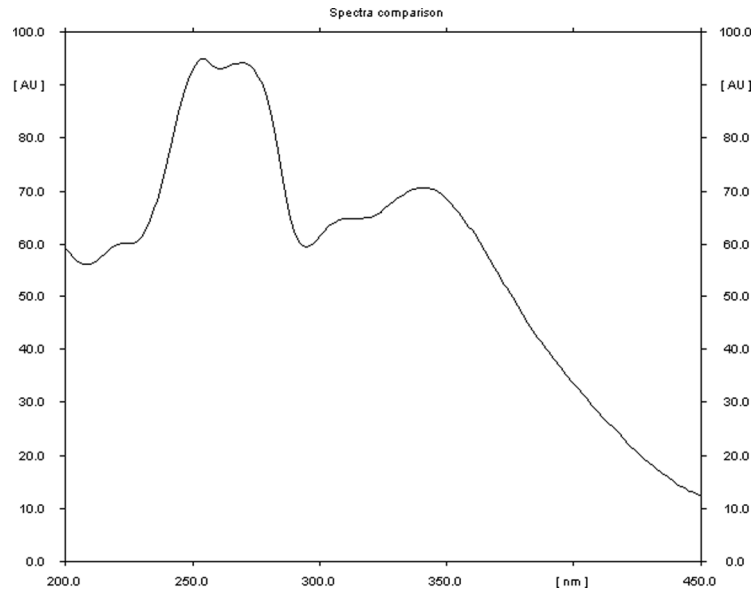

Figure 3. Spectrodensitogram of vitamin K_1 (phylloquinone) on $RP8F_{254s}$ plate.

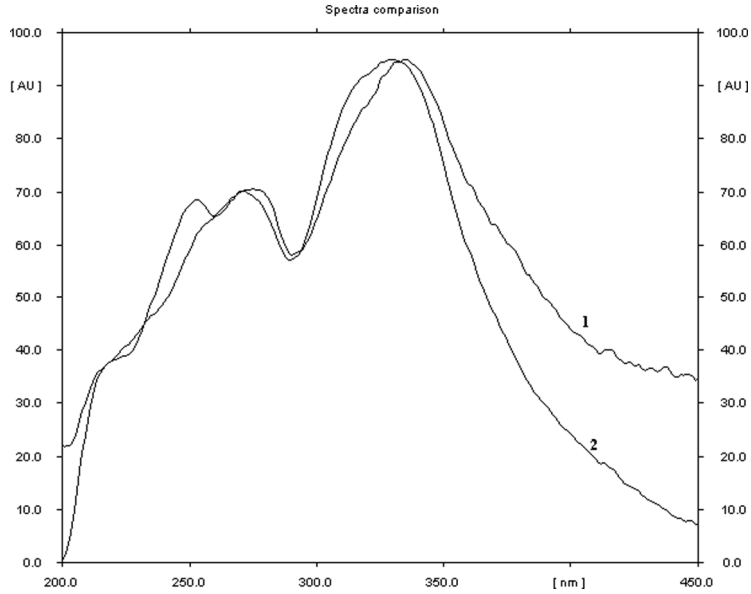

Figure 4. Spectrodensitograms of the impurities 1 and 2 of vitamin K_1 (phylloquinone) separated on $RP8F_{254s}$ plate.

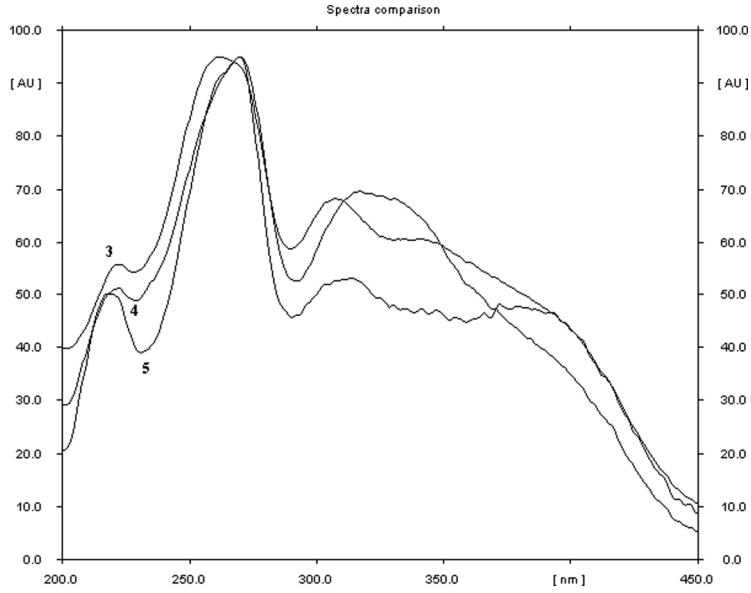

Figure 5. Spectrodensitograms of the impurities 3, 4, and 5 of vitamin K_1 (phylloquinone) separated on $RP8F_{254s}$ plate.

Further investigations will concern the identification of the impurities of vitamin K_1.

ACKNOWLEDGEMENT

This research was financed by the Medical University of Silesia as part of statutory research project KNW-1-012/08.

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Received February 12, 2009
Accepted March 5, 2009
Manuscript 6474