Scientific paper

Novel HPLC-UV Method for Simultaneous Determination of Fat-soluble Vitamins and Coenzyme Q10 in Medicines and Supplements

Žane Temova Rakuša, Eva Srečnik and Robert Roškar*

University of Ljubljana, Faculty of Pharmacy, Aškerčeva cesta 7, 1000 Ljubljana, Slovenia

* Corresponding author: E-mail: robert.roskar@ffa.uni-lj.si;
Tel: +386 1 4769 655, Fax: +386 1 4258 031

Abstract

A precise, accurate and rapid HPLC-UV method for simultaneous determination of fat-soluble vitamins (vitamin D3, E-acetate, K1, β-carotene, A-palmitate) and coenzyme Q10 was developed and validated according to ICH guidelines. Optimal chromatographic separation of the analytes in minimal analysis time (8 min) was achieved on a Luna C18 150 × 4.6 mm column using a mixture of acetonitrile, tetrahydrofuran and water (50:45:5, *v/v/v*). The described reversed phase HPLC method is the first published for quantification of these five fat-soluble vitamins and coenzyme Q10 within a single chromatographic run. The method was further applied for quantification of the analytes in selected liquid and solid dosage forms, registered as nutritional supplements and prescription medicines, which confirmed its suitability for routine analysis.

Keywords: Fat-soluble vitamins, coenzyme Q10, HPLC, nutrition supplements, pharmaceuticals

1. Introduction

Fat-soluble vitamins (FSVs) are essential for a variety of biochemical and physiological functions in human body, such as epithelial cell differentiation and vision (vitamin A), calcium and phosphate homeostasis (vitamin D), antioxidative protection in cell membranes (vitamin E) and blood coagulation (vitamin K).[1,2] As FSV, with the exception of vitamin D3, cannot be obtained by endogenous synthesis, food or vitamin preparations are their main sources. Due to the large number of commercially available vitamin preparations and their widespread use, the quality control of these preparations is extremely important, especially because of the risk of toxicity from excessive intake of vitamins A and D.[3]

HPLC methods, coupled with different detection techniques: UV,[4–12] FLD,[6] ECD,[13] and MS[5,14] offer the best approach to accurate content determination of the main FSVs in foods, pharmaceuticals and nutritional supplements. The published HPLC-UV methods for quality control of preparations containing FSVs are quite limited in terms of separation and simultaneous determination of FSVs,[4,5,7,8,11] often have run time longer than 15 min[4–6,9,12]

and generally require complicated and time-consuming sample preparation.[11,12,15,16] Further on, to our knowledge none of the published HPLC methods offer simultaneous determination of FSVs and also β-carotene and coenzyme Q10, which are often found along in medicines and nutritional supplements.

We aimed to develop rapid, accurate, precise and selective HPLC-UV method for simultaneous quantification of FSVs (in their most commonly used forms: A-palmitate, D3, E-acetate, K1 and A provitamin – β-carotene) and coenzyme Q10, using simple and fast extraction procedures (without alkaline saponification or solid phase extraction). The optimised HPLC method was successfully validated according to the ICH guidelines. In addition, medicines and nutritional supplements in liquid and solid dosage forms were analysed to confirm the adequacy of the method.

2. Experimental

2. 1. Chemicals and Reagents

All FSVs (vitamin A-palmitate, D3, E-acetate, K1 and β-carotene), butylated hydroxytoluene (BHT), anhy-

drous ferric chloride, *n*-hexane, 85% orthophosphoric acid (H$_3$PO$_4$) and HPLC grade: acetonitrile, methanol and tetrahydrofuran (THF) were purchased from Sigma-Aldrich (Steinheim, Germany). Coenzyme Q10 (CoQ10) was obtained from Kaneka Corporation (Osaka, Japan). Ultra-pure water was obtained through a Milli-Q water purification system A10 Advantage (Millipore Corporation, Bedford, MA, USA).

2. 2. Instrumentation and Chromatographic Conditions

Chromatographic analysis was performed on an Agilent 1100/1200 series HPLC system (Agilent Technologies, Waldbronn, Germany) equipped with UV-VIS detector and ChemStation data acquisition system. Chromatographic separation was performed on a reversed-phase Luna C18 (2) 150 × 4.6 mm, 5 μm particle size column (Phenomenex, Torrance, USA) at 25 °C using acetonitrile-tetrahydrofuran-water (50:45:5, *v/v/v*) as a mobile phase at flow-rate of 1 mL/min. Injection volume (Vinj) was 20 μL for all tested samples, except for samples from Preparation 2 and 3, where it was adjusted to 3 and 5 μL, respectively. Detection was carried out at 270 nm.

2. 3. Preparation of Standard Solutions

Standard solutions of FSVs and CoQ10 were prepared fresh daily by dissolving appropriate amounts in 0.05% (*m/v*) solution of BHT in *n*-hexane. Nine calibration standards were prepared as mixtures of all tested compounds in 0.05% (*m/v*) solution of BHT in *n*-hexane in different concentration ranges for different analytes, selected considering the concentrations and proportions of the analytes in the investigated pharmaceutical formulations (Table 1). Quality control (QC) samples at three concentration levels were prepared by diluting separately prepared stock solutions with 0.05% (*m/v*) solution of BHT

in *n*-hexane (Table 1). Prepared calibration standards and QC samples were evaporated to dryness under a stream of nitrogen at 40 °C (TurboVap LV, Caliper, Hopkinton-MA, USA). Dry residues were reconstituted in 1.0 mL of 0.01% (*m/v*) solution of BHT in mobile phase.

2. 4. Method Validation

The method was validated according to ICH guidelines Q2(R1)[17] in terms of selectivity, linearity, limit of quantification, precision, accuracy, recovery, sample stability and robustness.

Method selectivity was assessed by comparing chromatograms of individual standard solutions, mixture containing all standards, all used solvents and several common excipients found in commercial preparations (citric acid, sodium hydrogen phosphate, glycerol, propylene glycol, sodium benzoate, methyl- and propylparaben).

Linearity was evaluated based on nine calibration standards (Table 1). The procedure was repeated three times, using different stock solution, for three consecutive days of the validation. Injection volume during validation was 20 μL. Linearity was determined based on least-square linear regression. The acceptance criterion for determination coefficient was R^2 > 0.999.

Limit of quantification (LOQ) was calculated from the regression lines, using the following equations: LOQ = (10 × σ) / S, where σ is the standard deviation of the intercept, S is the average slope of the calibration curve.

Accuracy and precision were examined in terms of repeatability, precision and accuracy (intra- and interday) during three consecutive validation days, based on three QC samples covering low (QC$_L$), medium (QC$_M$) and high ranges (QC$_H$) of the calibration standards (Table 1). QC samples were prepared separately, in triplicate, each day of the validation. Injection repeatability was evaluated on three concentration levels by re-injecting the same QC sample six times. Intra- and inter-day pre-

Table 1. Concentrations of calibration standards and QC samples

		Concentration [mg/L]					
		β-carotene	A-palmitate	D3	CoQ10	E-acetate	K1
Calibration		60	600	50	400	2600	150
standards		36	360	30	240	1560	90
		12	120	10	80	520	30
		6.0	60	5.0	40	260	15
		1.2	12	1.0	8.0	52	3.0
		0.60	6.0	0.50	4.0	26	1.5
		0.30	3.0	0.25	2.0	13	0.75
		0.12	1.2	0.10	0.80	5.2	0.30
		0.06	0.60	0.05	0.40	2.6	0.15
QC samples*	QC$_H$	48	480	40	320	2080	120
	QC$_M$	24	240	20	160	1040	60
	QC$_L$	3.0	30	2.5	20	130	7.5

* QC$_H$, QC$_M$ and QC$_L$ – quality control samples with high, medium and low concentrations

cision (relative standard deviation – RSD) was expected to be not more that 5% (2% for injection repeatability) and intra- and inter-day accuracy (ratio (%) between calculated concentration for the obtained QC response based on the line equation and nominal concentration, which was calculated considering the sample preparation) within ± 5%. Accuracy of the injection volume variations was evaluated by re-injecting different volumes (3, 5, 15, 25 and 30 μL) of the same QC sample three times and comparing it proportionally to the nominal value (injection volume 20 μL) and was expected to be within ± 5%.

Sample stability was evaluated at three concentration levels (QC) and additionally in samples from Preparation 6 (tablets) and Preparation 7 (capsules) at 25 °C for two days (at 0 and 24 h) and was expressed as ratio between the concentration at 24 h and the concentration at time 0. The acceptance criterion was 100 ± 5%.

Method recovery was determined by spiking preparation solutions with approximately the same amount of the analytes, as contained in the preparations, in triplicate. Non-spiked preparations and standard solutions containing the added amounts were separately analysed. Average recoveries were calculated by the formula: recovery (%) = 100 × (concentration found in spiked sample – concentration found in unspiked sample) / added concentration.

The robustness of the method was investigated by deliberate modifications made to chromatographic conditions and sample preparation procedure. In sample preparation procedure for preparations in solid dosage form, different vortexing times (10 ± 5 min), shaking times (30 ± 10 min), sonication times (10 ± 5 min) and different drying temperature (40 ± 5 °C) were tested. To evaluate robustness in terms of chromatographic conditions, different injection volumes (20 ± 5 μL), column temperatures (25 ± 2 °C), flow rates (1.00 ± 0.05 mL/min), detection wavelengths (270 ± 5 nm) and mobile phase compositions acetonitrile-tetrahydrofuran-water (50.50:44.55:4.95 and 50:45:5 *v/v/v*) were tested.

2. 5. Sample Preparation of Nutritional Supplements and Medicines

The validated method was further applied to assay vitamins and CoQ10 content in commercially available pharmaceutical preparations: three solutions, three tablets and two soft-shelled capsules, registered as medicines or nutritional supplements. Each preparation was prepared and analysed in triplicate.

Liquid preparations were either directly analysed (Preparation 2 and 3) or diluted 100-fold with 0.01% (*m/v*) solution of BHT in mobile phase prior to analysis (Preparation 1). The extraction procedure for tablets was as follows: one tablet (whole or powdered depending on the preparation) was added to 2.0 mL of 0.1% H_3PO_4 into

a centrifuge tube and vortexed for 10 min. Organic solvent (8.0 mL of 0.05% (*m/v*) solution of BHT in *n*-hexane) was added to the samples, before their further sonication (10 min) and vortexing (2 min). Samples were then centrifuged for 10 min at 25 °C and 5000 rpm. Fraction of the clear supernatant (1–3 mL depending on the preparation) was evaporated to dryness under a stream of nitrogen at 40 °C and reconstituted in 1.0 mL of 0.01% (*m/v*) solution of BHT in mobile phase. The content of vitamins and Co-Q10 in soft-shelled capsules was evaluated by cutting one capsule in half and adding organic solvent (*n*-hexane with 0.05% (*m/v*) BHT : THF = 95:5 (*v/v*)). The samples were sonicated (10 min), shaken (30 min) and centrifuged (10 min, 25 °C, 5000 rpm). The clear supernatant (0.5 mL) was evaporated to dryness under a stream of nitrogen at 40 °C and reconstituted in 1.0 mL of 0.01% (*m/v*) solution of BHT in mobile phase. Samples from preparations containing CoQ10 (Preparation 6 and 7) were additionally reconstituted in 1.0 mL of 0.1% (*m/v*) solution of Fe^{3+} in mobile phase, which acts as an oxidant and enables evaluation of total CoQ10.

3. Results and Discussion

3. 1. Optimization of Chromatographic Conditions

The main objective of the study was to obtain optimal analytical method in terms of sample preparation and chromatographic conditions for simultaneous quantification of FSVs and CoQ10 in medicines and nutritional supplements. Chromatographic conditions were optimized in order to achieve baseline separated, symmetric peaks of the target analytes in minimal analysis time. Selection of stationary phase (various C18 reversed phase analytical columns) was based on our experience and previous research. Several mixtures of acetonitrile and methanol in isocratic and gradient modes were tested and found suitable for chromatographic separation of all tested analytes except CoQ10, which eluted only after THF was added to the mobile phase. Various mixtures of acetonitrile, THF and water at flow rates 0.5–2.0 mL/min and column temperatures 25–40 °C and detection wavelengths 210–325 nm were tested to optimize the chromatographic separation of all tested vitamins as well as both reduced and oxidized form of CoQ10. Detection wavelength 270 nm was selected as optimal compromise between absorbance of the individual analytes and their concentration in the tested preparations, with particular reference to vitamins D3 and K1, which have the lowest contents in the tested multivitamin preparations. Optimized chromatographic conditions resulted in symmetric and separated peaks with a short run time (Figure 1). The obtained run time is considerably shorter in comparison to other similar published HPLC-UV methods.[4–6,9,12]
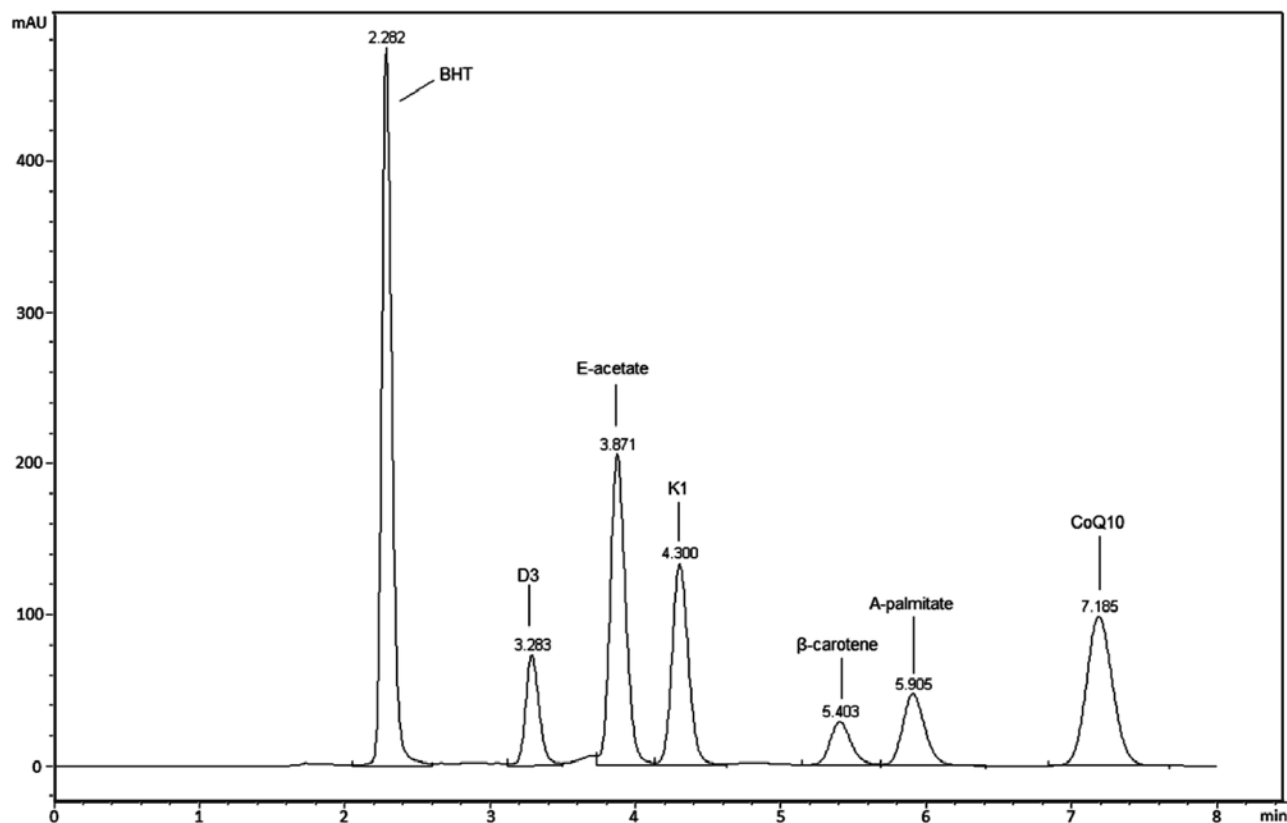
Figure 1: A chromatogram of standard mixture of fat-soluble vitamins and coenzyme Q10.

3. 2. Optimization of Sample Preparation

The optimised HPLC method was further applied to evaluate the content of FSVs and CoQ10 in commercial pharmaceuticals with particular attention to sample preparation, as a crucial step in the analysis of medicines and nutritional supplements. We aimed to develop simple and fast extraction procedure with a minimal number of steps and solvent consumption.

The extraction procedure for tablets was adjusted from Temova and Roškar's method for vitamin D3 extraction,[18] which comprised of addition of 0.1% H_3PO_4 to two tablets, vortexing for 2 min, subsequent addition of methanol (8.0 mL), sonication (10 min), vortexing (2 min) and centrifugation of the samples. In order to determine the optimal method, studies were done with (2.0 mL) and without the addition of 0.1% H_3PO_4 to one powdered tablet. Hereafter, different shaking times (0, 2, 4, 5 and 15 min with vortex mixer and 30 min with rotary mixer), organic solvents (methanol, *n*-hexane and mobile phase), sonication times (0, 5, 10, 15 min), further shaking times (0, 2 and 4 min with vortex mixer and 30 min with rotary mixer) and centrifugation times (0, 10 and 15 min) at 25 °C and 5000 rpm were tested. The best extraction in terms of method recoveries was achieved with the addition of 2.0 mL of 0.1% H_3PO_4 into a centrifuge tube with one tablet, vortexing for 10 min and addition of 0.05% (*m/v*) solution of BHT in *n*-hexane (8.0 mL), before their further sonication (10 min) and vortexing (2 min). Samples were then centrifuged for 10 min at 25 °C and 5000 rpm.

The optimization of the extraction procedure for soft-shelled capsules started with investigation of extraction organic solvents (anhydrous ethanol, methanol, *n*-hexane, different mixtures of *n*-hexane and methanol, mobile phase, *n*-hexane:THF = 95:5 (*v/v*)), to determine the optimal solvent, which was found to be *n*-hexane with 0.05% (*m/v*) BHT : THF = 95:5 (*v/v*). THF was added to solve the issue of poor solubility of β-carotene. Further on, sonication time (0, 5, 10, 15 min), shaking time (15, 30, 45, 60 min) and reconstitution solvent (methanol, mobile phase and 0.01% (*m/v*) solution of BHT in mobile phase) were tested in order to obtain optimal recoveries of the extraction procedure. The final extraction parameters (10 min sonication, 30 min shaking and 0.01% (*m/v*) solution of BHT in mobile phase as reconstitution solvent) provided suitable recoveries and sample stability of all analytes (Table 3).

3. 3. Method Validation

Selectivity of the method was confirmed as no interfering peaks were found at retention times of the selected

analytes (Figure 1). The standard calibration curves were linear over concentration ranges as presented in Table 2. Appropriate linearity was achieved with the addition of lipophilic antioxidant – BHT, especially for vitamin A-palmitate (R^2 = 0.9945 without BHT) and β-carotene (R^2 = 0.9935 without BHT). As shown in Table 2 on the example of QC medium sample, intra-day, inter-day and accuracy of the injection volume variations was better than ± 5% at all times, which is within the defined acceptance criteria. Similarly, intra and inter-day precision were below 3% in all cases and injection repeatability was less than 2% (Table 2). Sample stability after 24 h was assessed in order to ensure that the obtained concentration results adequately reflect those directly after sampling. Stability of all tested analytes in QC samples and samples from Preparation 6 and 7 after 24 h in the autosampler at 25 °C was within the defined acceptance criteria 100 ± 5%. Such sample stability in QC samples was obtained after the addition of BHT, especially for vitamin A-palmitate and β-carotene, which were found subject to extensive degradation without appropriate stabilization with BHT (22 and 70% after 24 h for vitamin A-palmitate and β-carotene, respectively). Method was found robust to slight changes in both chromatographic and sample preparation

Table 2. Validation data

| Analyte | Range [mg/L] | LOQ [mg/L] | R^2 | Accuracy | | | Precision | | |
				Intra-day	Inter-day	Variation of Vinj*	Intra-day	Inter-day	Injection repeatability
A-palmitate	1.2 – 600	1.2	0.9996	100.90	100.96	99.7–101.4	0.27	0.20	0.19
CoQ10	0.80 – 400	0.80	0.9997	100.89	100.94	100.0–101.5	0.53	0.98	0.10
D3	0.25 – 50	0.15	0.9998	100.99	101.06	99.9–100.9	0.55	2.69	0.24
E-acetate	2.6 – 2600	1.5	0.9996	99.93	99.90	99.1–103.2	0.56	2.32	0.14
K1	0.30 – 150	0.20	0.9997	101.18	101.24	100.0–101.7	0.40	0.87	0.13
β-carotene	0.12 – 60	0.12	0.9997	101.24	101.30	100.0–100.4	0.52	1.28	1.83

* different injection volumes (3, 5, 15, 25 and 30 µL), calculated to nominal injection volume 20 µL
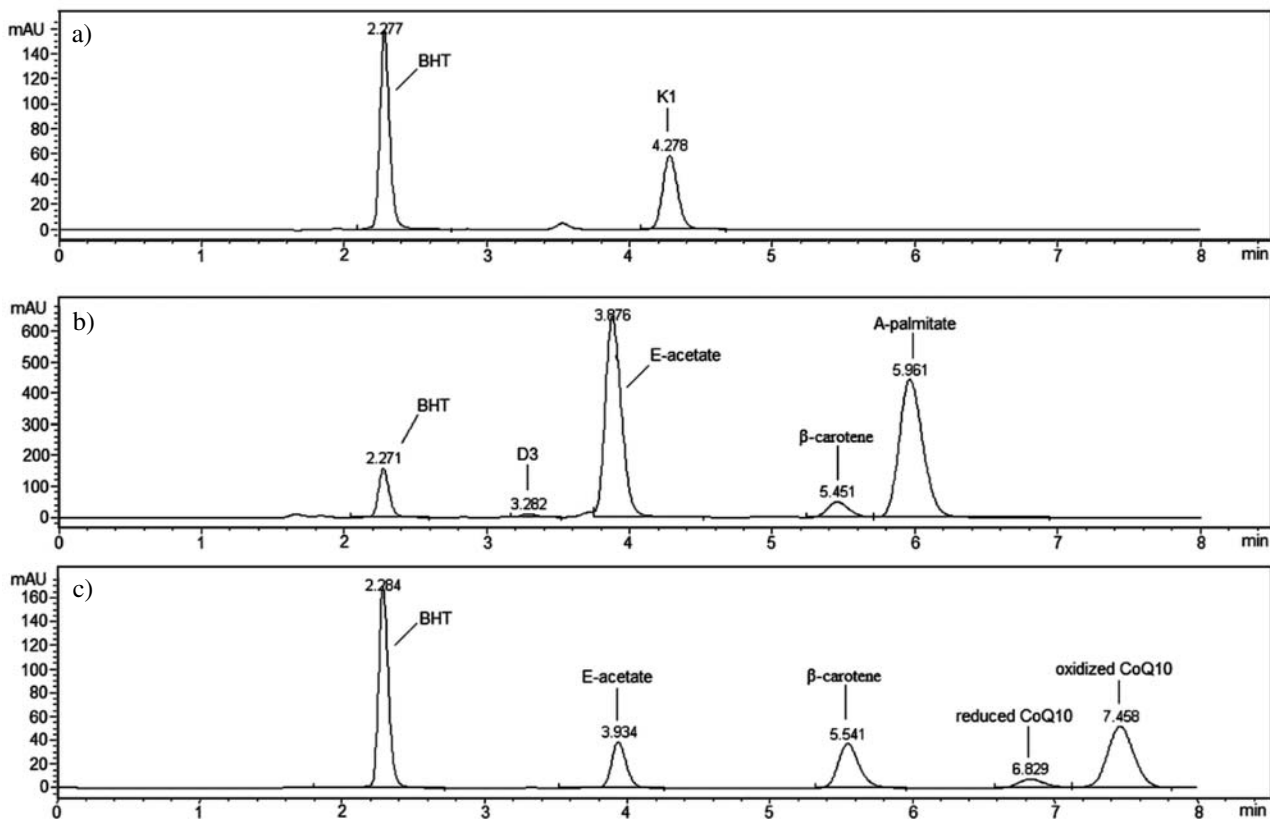

Figure 2: Representative chromatograms of a): Preparation 1 – solution, b): Preparation 5 – tablets and c): Preparation 7 – capsules, which also contains a reduced form of CoQ10 that is chromatographically separated from its general form (oxidized CoQ10).

parameters. The obtained results from samples prepared with deviations in the sample preparation procedure, were accurate (100 ± 5%) and precise (RSD < 5%). In all varied chromatographic conditions the resolution between the studied compounds was not altered, demonstrating the robustness of the method.

3. 4. Assay in Nutritional Supplements and Medicines

In order to test the applicability of the developed and validated HPLC method to commercial multi-vitamin preparations, six pharmaceutical preparations, registered as medicines and two nutritional supplements in various dosage forms (solutions, tablets, soft shelled capsules) were tested to evaluate the content of the analytes in relation to the value claimed on the label. Liquid preparations were either directly analysed (Preparation 2 and 3) or diluted with 0.01% (*m/v*) solution of BHT in mobile phase prior to analysis (Preparation 1). Samples from tablets and capsules were prepared according to the optimised extraction procedure (Section 3.2) Representative chromatograms of samples from all three dosage forms are presented in Fi-

gure 2. The results for average contents and calculated method recoveries are presented in Table 3. High recoveries and low standard error values (Table 3) confirmed the suitability of the established HPLC method for quantification of the target analytes in liquid and solid preparation without any pre-treatment (Preparation 2 and 3) or after a simple and rapid pre-treatment (other preparations).

4. Conclusions

The main novelty in our approach is the developed HPLC method, which to our knowledge, is the first published method for simultaneous determination of five fat-soluble vitamins (A-palmitate, D3, E-acetate, K1 and A provitamin – β-carotene) and coenzyme Q10. Moreover, analysis time of only 8 min is favourable in comparison to other published HPLC-UV methods for determination of fewer fat-soluble vitamins. The obtained results from the assay in commercial nutrition supplements and medicines confirmed that the method is appropriate for routine analysis and quality control of multi-vitamin products in pharmaceutical and health food industries. Considering

Table 3. Average content in medicines and nutritional supplements along with method recoveries (n = 3)

		Analyte	Declared content	Determined content (average ± standard error [%])	Method recovery[#] [%]
Solutions	Medicines			**PREPARATION 1**	
		K1	10 mg/mL	9.97 ± 0.02 mg/mL	103.8
				PREPARATION 2	
		A-palmitate	3300 μg/mL	4594 ± 3 μg/mL	
		D3	50 μg/mL	62.4 ± 0.2 μg/mL	100.1
				PREPARATION 3	
		D3	100 μg/mL	114.7 ± 0.3 μg/mL	98.4
Tablets	Medicines			**PREPARATION 4**	
		A-palmitate	330 μg/tbl	394 ± 1 μg/tbl	99.3
		D3	2 μg/tbl	2.43 ± 0.04 μg/tbl	99.3
				PREPARATION 5	
		A-palmitate	2750 μg/tbl	3336 ± 55 μg/tbl	94.2
		D3	10 μg/tbl	12.2 ± 0.1 μg/tbl	95.8
		E-acetate	15 mg/tbl	17.8 ± 0.3 mg/tbl	96.9
		β-carotene*	/	0.120 ± 0.003 mg/tbl	104.2
	Nutritional supplement			**PREPARATION 6**	
		A-palmitate	800 μg/tbl	863 ± 16 μg/tbl	99.6
		CoQ10	4.5 mg/tbl	3.92 ± 0.17 mg/tbl	99.2
		D3	5 μg/tbl	7.32 ± 0.07 μg/tbl	104.8
		E-acetate	12 mg/tbl	12.2 ± 0.8 mg/tbl	97.2
		K1	25 μg/tbl	18.1 ± 0.7 μg/tbl	97.4
Capsules	Medicine			**PREPARATION 7**	
		CoQ10	30 mg/caps	28.6 ± 0.1 mg/caps	102.3
		E-acetate	24 mg/caps	34.3 ± 0.2 mg/caps	98.1
		β-carotene*	/	3.36 ± 0.02 mg/caps	108.0
	Nutritional supplement			**PREPARATION 8**	
		E-acetate	30 mg/caps	45.5 ± 0.8 mg/caps	99.8
		β-carotene	7 mg/caps	8.03 ± 0.10 mg/caps	108.2

* content not defined; # method recovery calculated according to the formula in section 2.4.

that most of the tested preparations had considerably higher contents than labelled (up to 150%), quality control of these preparations is of extreme importance.

5. References

1. S. Kucukkolbasi, N. Ires, H. Kara. *J. Selcuk Univ. Nat. Appl. Sci.* **2001**, 30–47.

2. O. Heudi, M. Trisconi, C. Blake. *J. Chromatogr. A* **2004**, 1022, 115–123.
   https://doi.org/10.1016/j.chroma.2003.09.062

3. F. Granado-Lorencio, E. Rubio, I. Blanco-Navarro, B. Pérez-Sacristán, R. Rodríguez-Pena, F. J. García López. *Food Chem. Toxicol.* **2012**, 50, 2106–8.
   https://doi.org/10.1016/j.fct.2012.03.001

4. S. N. Anuradha, S. Arunkumar. *Int. J. Pharma. Bio. Sci.* **2012**, 3**,** 322–327.

5. D. E. Breithaupt, S. Kraut. *Eur. Food Res. Technol.* **2006**, 643–649. https://doi.org/10.1007/s00217-005-0195-7

6. S. Kucukkolbasi, O. Bilber, H. Filiz Ayyildiz, H. Kara. *Quim. Nov.* **2013**, 36**,** 1044–1051.

7. G. Kλackow, E. L. Anuszewska. *Acta Pol. Pharm.* **2000**, 57, 167–170.

8. B. Buszewski, W. Zbanyszek. *J. Liq. Chromatogr. Relat. Technol.* **2002**, 25, 1229–1241.

https://doi.org/10.1081/JLC-120004021

9. L. Chen, L. Zhiyong, K. Xuejun, Z. Xiaoling, Z. Shenglan, G. Zhongze. *Procedia Environ. Sci.* **2011**, 8, 588–595.
   https://doi.org/10.1016/j.proenv.2011.10.091

10. S. Wielinski, A. Olszanowski. *J. Liq. Chrom. Rel. Technol.* **2001**, 24, 201–213.
    https://doi.org/10.1081/JLC-100001482

11. P. Moreno, V. Salvadó. *J. Chromatogr. A* **2000**, 870, 207–215. https://doi.org/10.1016/S0021-9673(99)01021-3

12. V. Kienen, W. F. Costa, J. V. Visentainer, N. E. Souza, C. C. Oliveira. *Talanta* **2008**, 75, 141–146.
    https://doi.org/10.1016/j.talanta.2007.10.043

13. I. Acworth, P. Gamache. Thermo Fisher Scientific, Chelmsford, MA, USA.

14. C. Nimalaratne, C. Sun, J. Wu, J. M. Curtis, A. Schieber. *Food Res. Int.* **2014**, 66, 69–77.
    https://doi.org/10.1016/j.foodres.2014.08.034

15. J. L. Luque-García, L. Castro. *J. Chromatogr. A* **2001**, 935, 3–11. https://doi.org/10.1016/S0021-9673(01)01118-9

16. A. Jedlička, J. Klimeš. *Chem. Pap.* **2005**, 59, 202—222.

17. International Conference on Harmonization (ICH) Guidelines, Q2(R1) Validation of analytical procedures, Commission of the European Communities, **2005**.

18. Ž. Temova, R. Roškar. *J. Chromatogr. Sci.* **2016**, 54, 1180–6.
    https://doi.org/10.1093/chromsci/bmw048

Povzetek

Razvili in validirali smo natančno, točno in hitro HPLC-UV metodo za sočasno vrednotenje lipofilnih vitaminov (vitamin D3, E-acetat, K1, β-karoten, A-palmitat) in koencima Q10. Optimalno kromatografsko ločbo analitov v kratkem času analize (8 min) smo dosegli na koloni Luna C18 150 × 4,6 mm z uporabo mobilne faze: acetonitril, tetrahidrofuran in voda (50: 45: 5, v/v/v). Opisana reverznofazna HPLC-UV metoda je prva objavljena analizna metoda za sočasno vrednotenje izbranih lipofilnih vitaminov in koencima Q10. Metodo smo nadalje uporabili za vrednotenje teh analitov v izbranih zdravilih in prehranskih dopolnilih, v tekočih in trdnih farmacevtskih oblikah ter tako potrdili njeno primernost za rutinsko uporabo.