Science Together

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A D E K - Easy separation of fat-soluble vitamins using GPC/SEC

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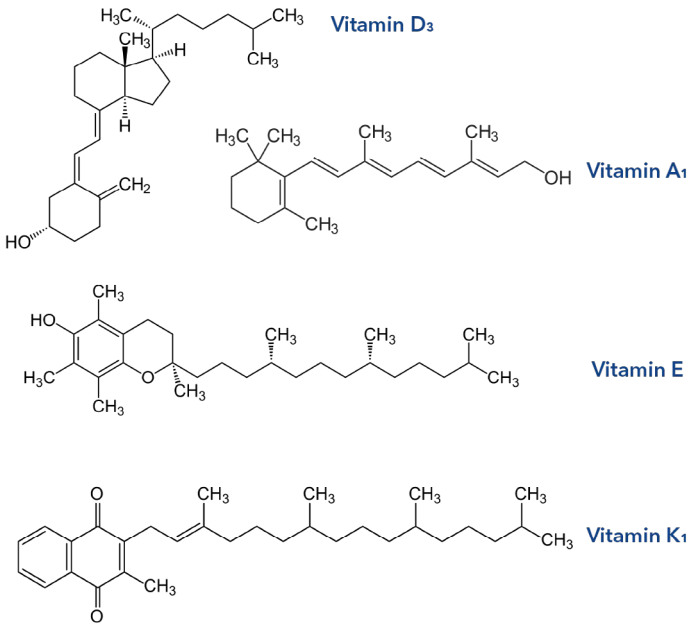
SUMMARY

Vitamins are essential micronutrients that are needed in small amounts for various roles throughout the human body. Vitamins are divided into two groups: water-soluble (B-complex vitamins and C vitamins) and fat-soluble vitamins (A, D, E, and K). The fat-soluble vitamins are stored in the body for long periods of time and generally pose a greater risk for toxicity when consumed in excess than water-soluble vitamins [1]. Here, an analytical HPLC method based on size exclusion chromatography is described.

INTRODUCTION

Fat-soluble vitamins are required for a wide variety of physiological functions. They are absorbed in the intestine in the presence of fat. Classical deficiencies of these vitamins can manifest clinically as night blindness (vitamin A), osteomalacia (vitamin D), increased oxidative cell stress (vitamin E), and haemorrhage (vitamin K) [2]. Since megadoses of vitamins A, D, E , or K can be toxic and may lead to health problems, it is necessary to provide quality control of dietary supplement products to guarantee the right indication of vitamin concentration. Therefore, a HPLC method for the analysis of fat-soluble vitamins was developed based on the separation principle of size exclusion.



Structural formulas of typical fat-soluble vitamins

Additional Information

A D E K - **Separation of fat-soluble vitamins using GPC/SEC**

RESULTS

A mixed standard of the fat-soluble vitamins was prepared and dissolved in tetrahydrofuran. For quantification, calibration curves for the four vitamins in ranges from 0.002 mg/mL to 0.1 mg/mL for vitamins A, D, K and from 0.0045 mg/mL to 0.18 mg/mL for vitamin E were determined. **Fig. 1** shows the mixed vitamin standard at a concentration of 0.05 mg/mL (A, D, K) and 0.09 mg/mL (E). The calibration showed a good linearity and for all compounds $R^2 > 0.999$ was achieved **(Fig. 2)**. **Tab. 1** summarizes the calculated LOD (S/N=3) and LOQ (S/N=10) values for the separation. Vitamin E showed the least sensitivity but nevertheless LOD and LOQ values reside in an approriate range e.g. the analysis of dietary supplement products, where high amounts of vitamins are expected.

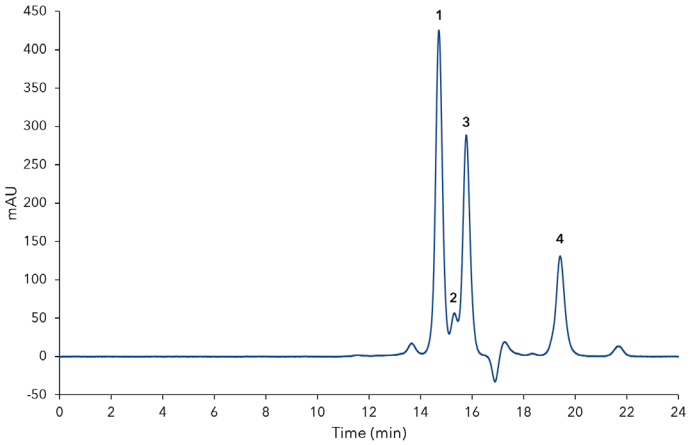


Fig. 1 Chromatogram of a mixed standard of fat-soluble vitamins at 0.05 mg/mL (A, D, K) and 0.09 mg/mL (E), 1) vitamin A palmitate, 2) vitamin E, 3) vitamin D3, 4) vitamin K

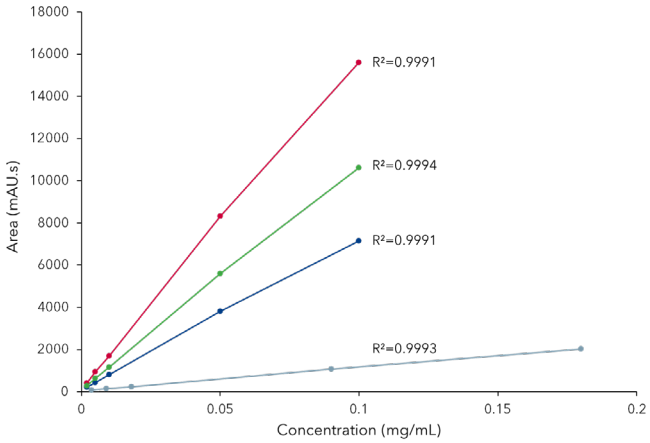


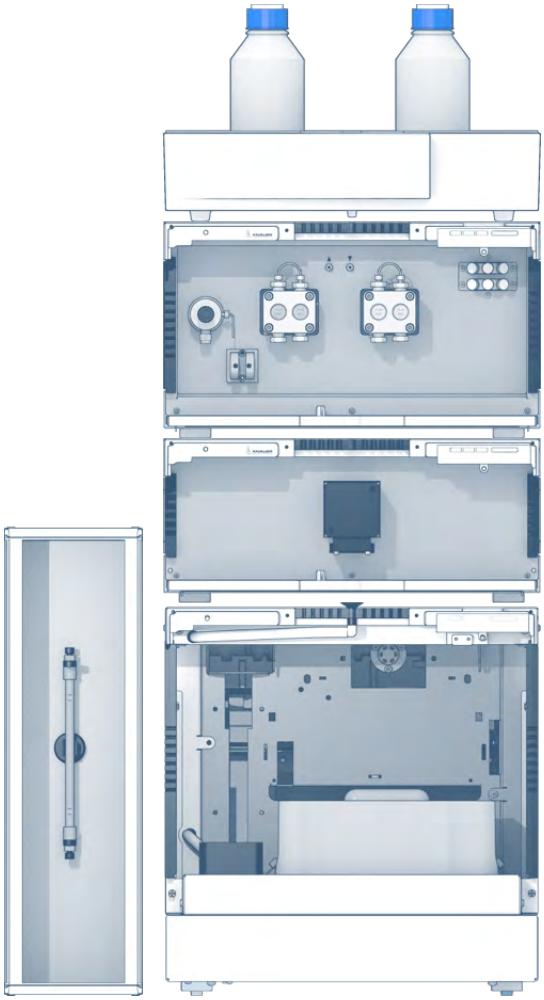
Fig. 2 Calibration curves for vitamin D3 (red), vitamin A (green), vitamin K (blue), and vitamin E (grey); corresponding linearity values are indicated

Tab. 1 Calculated LOD and LOQ values

Substance	LOD (S/N=3) in µg/mL	LOQ (S/N=10) in µg/mL
Vitamin A palmitate	0.10	0.34
Vitamin E	2.00	6.40
Vitamin D3	0.16	0.54
Vitamin K	0.40	1.33

MATRIALS AND METHODS

The used AZURA® analytical system was equipped with an AZURA P 6.1L pump suitable for normal phase applications. Furthermore a 2 channel GPC degasser was used. Aquisition was performed with an AZURA UVD 2.1L and an analytical flow cell. For injection, an AZURA autosampler AS 6.1L was used. The column thermostat CT 2.1 was part of the system. The isocratic method ran at a flow rate of 1 mL/min for 25 minutes. Stabilized tetrahydrofuran was used as eluent. The column temperature was set to 40 °C and vitamins were detected at 280 nm. A column tandem was used of two times AppliChrom ABOA StyDiViBe, with a pore size of 35 Å, covering a molecular weight range from 100 to 2500 Da in a dimension 300 x 8 mm ID.



CONCLUSION

The isocratic method based on size exclusion separation mechanism is an easy possibility for the determination of the four fat-soluble vitamins and a valuable addition to commonly used reversed phase gradient methods. Although, the peaks are not completely baseline separated it is possible to perform quantification. The method can be used for the quality control of dietary supplement products. To obtain a better resolution for vitamin E, the extension of the separation distance would be reasonable. This could be achieved by adding a third column with the same pore size.

REFERENCES

[1] Fat-Soluble Vitamins: A, D, E, and K; Fact Sheet No. 9.315, Food and Nutrition Series/Health, http://extension.colostate.edu/topic-areas/nutrition-food-safety-health/fat-soluble-vitamins-a-d-e-and-k-9-315/

[2] Fat-Soluble Vitamins: Clinical Indications and Current Challenges for Chromatographic Measurement, Ali A. Albahrani and Ronda F. Greaves; https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4810759/

ADDITIONAL MATERIALS AND METHODS

Tab. A1 Method parameters

Column temperature	40°C
Injection volume	100 µL
Injection mode	Full loop
Detection	UV 280 nm
Data rate	20 Hz
Time constant	0.05 s

Tab. A2 Pump parameters

Eluent A	Tetrahydrofuran (stabilized)
Flow rate	1 mL/min
Pump program	isocratic
Run time	25 min

Tab. A3 System configuration

Instrument	Description	Article No.
Pump	AZURA P 6.1L, HPG for normal Phase	APH38ED
Degasser	2 channel GPC degasser	A5335
Autosampler	AZURA AS 6.1L	AAA00AA
Detector	AZURA UVD 2.1L	ADA01XA
Flow cell	Analytical UV Flow Cell	A4061XB
Thermostat	AZURA CT 2.1	A05852
Column	2 x AppliChrom ABOA StyDiViBe, 35 Å (100 – 2500 Da), 5 µm, 300 x 8 mm ID	30GA470ABJ
Software	ClarityChrom 7.4.2 – Workstation, autosampler control included	A1670

RELATED KNAUER APPLICATIONS

VFD0162 – Separation of ascorbic acid and vitamin B complexes - essentially required nutrients

