A high throughput LCMS/MS-method for Vitamin A and Vitamin E

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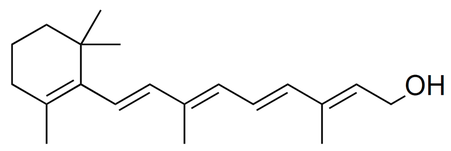
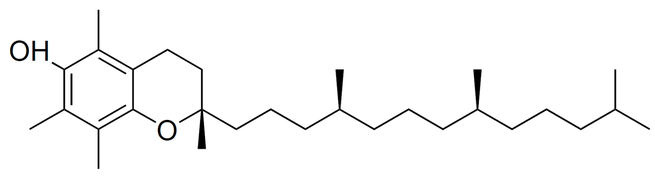
1. Aim

To develop a robust high-throughput LCMS/MS method for all-*trans*-retinol (Vitamin A) and α-tocopherol (Vitamin E) in serum.



All-*trans*-retinol



α-Tocopherol

2. Introduction

Assessment of vitamin status is of clinical as well as nutritional importance. Clinical indications include:

- Conditions causing malabsorption of fat
- Liver diseases
- Poisoning due to kidney failure or overconsumption (vitamin A)

3. Method

Sample preparation (LLE):

- Pipetting robot: Tecan Freedom Evoware 150
- Sample: **25 µL patient serum** (fasting)
- Internal standard: ^{13}C-labelled analytes in 100 µl ethanol
- Sample extraction with 650 µL hexane
- Evaporation (N_2) in 96-well sample plate
- Reconstitution in 100 µL 75 % methanol containing 0.2 g/L butylated hydroxytoluene (BHT) for stability
- Time: 80 minutes for 96 samples (81 patient samples)

UPLC chromatography:

- Instrument: Nexera X2 UPLC-system with an Agilent ZORBAX Eclipse Plus C18 column
- Mobile phases: Water (A) and methanol (B) containing 0.1 % formic acid in a stepwise gradient (% B) from 71-92 % over 10 min
- Flow: 600 µL/min
- Injection volume: **1 µL**
- Time: 18 hours for 96 samples

Mass spectrometry:

- Instrument: Sciex QTrap 4500
- MRM parameters: see Table 1.

Table 1: Parameter list for the MRM transition detection of vitamin A and vitamin E, including their ^{13}C-labelled internal standard analytes (IS).

ID	RT	Q1	Q3	DP	EP	CE	CXP
Vitamin A 1	5.8	269.1	95.1	26	10	13	10
Vitamin A 2	5.8	269.1	93.1	26	10	22	8
Vitamin A IS 1	5.8	290.2	84.1	6	10	23	7
Vitamin A IS 2	5.8	290.2	98.1	6	10	26	15
Vitamin E 1	9.1	431.4	137.1	60	10	59	12
Vitamin E 2	9.1	429.4	165.1	60	10	37	9
Vitamin E IS 1	9.1	437.4	142.1	60	10	65	10
Vitamin E IS 2	9.1	435.4	171.1	60	10	34	16

4. Method validation

Calibrators (made in-house):

- Matrix: UV-radiated pooled human serum
- Six levels + blank

Measurement range:

- Vitamin A: 0.16-12.4 µmol/L
- Vitamin E: 1.7-113 µmol/L

Linearity (extended calibrator curve):

Vitamin A: 0.05-60 µmol/L

Vitamin E: 1.0-125 µmol/L

Traceability:

- NIST Standard Reference Material 968f.

Selectivity and interference:

- No observed interfering peaks
- No effect of added Intralipid up to a lipemic index of 1967 mg/dL

Precision:

- Analytical precision: standard deviation of 20 parallels within one day
- Within-series precision: standard deviation of differences between two parallels for each of 20 series over 18 days
- Total precision: standard deviation of one parallel from each of 20-21 series over 18 days

The results are presented in Table 2.

Table 2: Precision results. In-house made quality control (QC) samples. Matrix: low; UV-radiated pooled human serum (spiked), medium; pooled human serum, high; pooled human serum (spiked)

		Vitamin A		Vitamin E	
		Mean conc. [µmol/L]	CV %	Mean conc. [µmol/L]	CV %
Analytical	Low	0.65	3.6	12.5	4.3
	Medium	1.97	4.8	29.0	4.3
	High	2.96	5.3	39.2	4.8
Within-series	Low	-	10.2	-	4.5
	Medium	-	8.5	-	5.6
	High	-	7.3	-	5.2
Total	Low	0.66	9.4	12.1	5.1
	Medium	1.95	6.6	29.1	4.4
	High	3.08	7.5	39.5	4.6

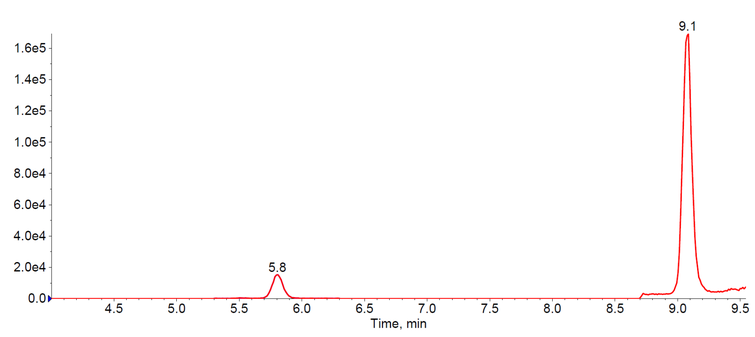


Figure 1: Chromatogram of vitamin A and vitamin E (TIC from all MRM transitions).

Reference ranges:

- Adapted from Mayo Clinic, USA
- Validated using samples from 20 fasting laboratory employees (Fig 2)

Note the relatively wide reference ranges compared to the results.

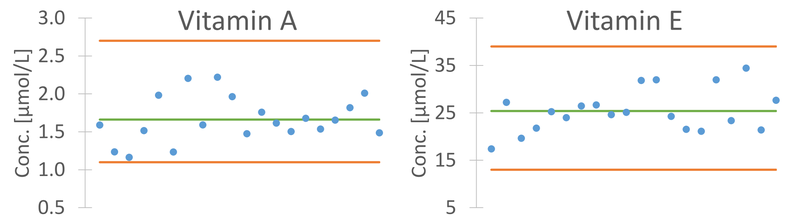


Figure 2: 20 samples used for validation of the reference ranges. Vitamin A: 1.1-2.7 µmol/L, vitamin E: 13-39 µmol/L. The average is marked with a green line.

5. Troubleshooting

Drift in concentration within series:

Observations: A consistent positive bias (average +11 %) between the first and the last QC samples in a series (vitamin A)

Finding:

- Temperature of IS-solution affects results
- IS solution was originally pipetted from a cold solution. The first samples of the series received colder IS solution than the last

Possible explanation:

- Solubility of IS is affected by temperature
- The pipetted volume may change with temperature

Solution: Equilibrate the IS solution to room temperature before pipetting.

6. Implementation

This method was implemented for routine use in august 2019, and is accredited according to ISO15189. The method replaced a discontinued LCMS/MS-method for vitamin A and E.

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