

Determination of Vitamin E and Vitamin E Acetate in E-liquids Using LC-MS/MS

Method Description

BfR-VitEAc-Liquids-1.0/2020

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1. Application area

Vitamin E and vitamin E acetate are potential additives in e-liquids for electronic cigarettes. In Germany, the addition of vitamins, including vitamin E and vitamin E acetate, to e-liquids is prohibited by the Tobacco Product Ordinance (TabakerzV, Appendix 2). Compliance with this prohibition is particularly relevant for vitamin E acetate. Vitamin E acetate is associated with the outbreak of partially fatal lung diseases (e-cigarette, or vaping, product use-associated lung injury, EVALI) in the USA in summer 2019. Vitamin E acetate was found in the lung fluids of patients and vaporiser fluid in most of the vaporiser products investigated, usually as a diluent in illegal THC oils (CDC, 2020).

The method describes the determination of vitamin E and vitamin E acetate from commercially available e-liquid samples within the trace range. The detection limits are 0.8 pg per mg e-liquid for vitamin E and 0.3 pg per mg liquid for vitamin E acetate.

2. Short description

500 mg of the e-liquid are weighed into a volumetric flask and, after the internal standards have been added, made up to 5 mL with methanol. After the sample has completely dissolved in the methanol, the solution is ready for measurement.

An RP-LC column with a binary gradient is used for the chromatographic separation. The analytes are detected using triple stage quadrupole mass spectrometry. The concentration of vitamin E and vitamin E acetate is determined using a matrix standard series.

3. Chemicals and solutions

3.1 General

Note: The work with harmful chemicals provided for in this method must be carried out under suitable precautionary and protective measures, such as avoiding skin contact and using a fume cupboard.
Unless otherwise specified, analytically pure chemicals and solvents suitable for LC-MS/MS must be used. The water used should be of the highest quality.

3.2 Chemicals

- 3.2.1 Vitamin E (VitE), e.g. 1 mg/ml, purity 96.9 % from Supelco (Bellafonte, PA, USA)
- 3.2.2 Vitamin E acetate (VitEAc), e.g. 1 mg/mL \pm 5 μ g/ml in methanol from Cerillian (Round Rock, TX, USA)
- 3.2.3 Vitamin E d₆ (VitE-d₆), e.g. 500 μ g/mL \pm 2.8 μ g/ml in methanol from Cerilliant (Round Rock, TX, USA)
- 3.2.4 Vitamin E Acetate d₉ (VitEAc-d₉), e.g. 1mg/mL, purity \geq 99 % in methanol from Iris Biotech (Marktredwitz, Germany)
- 3.2.5 Liquid matrix (50:50 PG/VG), e.g. from E-cigarette specialist retailer
- 3.2.6 Formic acid 98 – 100%, e.g. Sigma-Aldrich (Taufkirchen, Germany)
- 3.2.7 Methanol (MeOH), LC-MS quality, e.g. Merck LiChrosolv® (Darmstadt, Germany)
- 3.2.8 Ammonium acetate, LC-MS quality, e.g. Fluka (Morris Plains, NJ, USA)

3.3 Solutions

3.3.1 Eluents for chromatography:

- Eluent A:

385 mg ammonium acetate (3.2.8) is dissolved in 1 L of water with addition of 1 ml formic acid (3.2.5).

- Eluent B:

385 mg ammonium acetate (3.2.8) is dissolved in methanol (3.2.7) with addition of 1 ml formic acid (3.2.5).

3.3.2 Internal standard solution

- Stock solution (10 µg/ml):

Approx. 4.5 ml of methanol (3.2.7) are placed in a 10 ml volumetric flask. 50 µl of the vitamin E acetate d₉ standard solution (3.2.4) are added to this volumetric flask using a piston pipette (100 µl). It is then filled up to the calibration mark using a Pasteur pipette. This results in a vitamin E acetate d₉ stock solution of 10 µg/ml.

- Internal standard mix (ISTD mix)

To prepare the internal standard mix, place 9 ml of methanol (3.2.7) in a 10 ml volumetric flask. 500 µl of the vitamin E acetate d₉ stock solution are added using a piston-operated pipette. Then 15 µl of the vitamin E d₆ standard solution (3.2.3) are added using a piston-operated pipette. The volumetric flask is filled up to the calibration mark with methanol (3.2.7) using a Pasteur pipette. This results in the internal standard mix, which is added to all solutions to be analysed.

3.3.3 Standard solution for calibration

- Stock solutions (10 µg/ml):

Approx. 4.5 ml of methanol (3.2.7) are placed in a 5 ml volumetric flask. 50 µl of the vitamin E acetate standard solution (3.2.2) are added to this volumetric flask using a piston pipette. Then, using a Pasteur pipette, it is filled up to the calibration mark with methanol (3.2.7). This results in a vitamin E acetate stock solution of 10 µg/ml.

Approx. 4.5 ml of methanol (3.2.7) are placed in another 5 ml volumetric flask. 50 µl of the vitamin E standard solution (3.2.1) are added to this volumetric flask using a piston pipette (100 µl). Then, using a Pasteur pipette, it is filled up to the calibration mark with methanol (3.2.7). This results in a vitamin E stock solution of 10 µg/ml.

- Standard mix 500 ng/ml

For the standard mix, 250 µl of the vitamin E stock solution and 250 µl of the vitamin E acetate stock solution are pipetted into a 5 mL volumetric flask, which is filled up to the measuring mark with methanol (3.2.7) to obtain a concentration of 500 ng/ml of both analytes.

- Preparation of the calibration standards with matrix (Cal)

To compensate for matrix effects, calibration standards are prepared by adding the amount of analyte-free liquid matrix (3.2.5) equivalent to the sample. 500 mg liquid matrix (3.2.5) are weighed into a 5 mL volumetric flask using a glass Pasteur pipette. Before filling up to the measuring mark with methanol (3.2.7), 100 µl ISTD mix and standard mix are added as described in Table 1.

Table 1: Table for manufacture of the calibration standard

	Concentration of the calibration standard [ng/ml]	Volume of the standard mix [µl]
Kal_1	1.0	10
Kal_2	2.0	20
Kal_3	3.0	30
Kal_4	4.0	40
Kal_5	5.0	50
Kal_6	10.0	100
Kal_7	15.0	150
Kal_8	20.0	200
Kal_9	30.0	300
Kal_10	50.0	500

4. Instruments

4.1 General

The following devices are used, in addition to the normal laboratory equipment:

- 4.1.1 **various piston-operated pipettes**, e.g. from Eppendorf (Wessling-Berzdorf, Germany)
- 4.1.2 **Analytical balance**, accuracy: ± 0.1 mg
- 4.1.3 **Test tube shakers**, vortex mixers, e.g. From Neolab Migge GmbH (Heidelberg, Germany)
- 4.1.4 **Glass Pasteur pipettes**
- 4.1.5 **Glass volumetric flask**, 5 ml
- 4.1.6 **LC sample vials** made of amber glass, 2 ml
- 4.1.7 **LC column**, e.g. from Thermo, Hypersil C18, 50 x 2.1 mm, 2.4 µm (Dreieich, Germany)
- 4.1.8 **LC-MS/MS system**, e.g. by Shimadzu (Duisburg, Germany) and AB Sciex (Darmstadt, Germany)

5. Implementation

5.1 Sample preparation

500 mg samples are weighed in a 5 mL volumetric flask. After adding 100 µL ISTD mix, this is filled up to the measuring mark with methanol (3.2.7). When the sample has mixed completely, the solutions are transferred to LC sample vials and placed in the LC autosampler.

6. LC-MS/MS

6.1 Chromatographic separation

In principle, the analyses can be carried out with various liquid chromatography systems (LC) and separation columns. The chromatographic conditions can be chosen freely. At least double the retention time for the dead volume of the column is suggested as an acceptable criterion for the retention time. The conditions listed in the appendix (8.1) using a C18 column (4.1.7) and the solvents specified under (3.3.1) have proven to be suitable during the in-house validation, but are only to be understood as examples.

6.2 MS conditions

The analyses can be carried out with MS/MS instruments from different manufacturers. Instrument-specific measuring system conditions, which have proven their suitability during in-house validation, are listed in the appendix (8.1) as examples.

Note: For qualitative detection and quantification, it is necessary that at least two substance-specific mass transitions are detected per analyte. The ratio of the two mass transitions should remain constant across all measurements.

6.3 Measurement

The calibration solutions should be injected in ascending order at the beginning and at the end of the sequence. After approximately 25 samples, the calibration solutions should be injected again. A blank value should be injected after each sample and the blank value should be injected twice according to the highest calibration standard (50 ng/mL).

7. Calculations

Quantitative determination is carried out using the calibration series with matrix. The calculation is performed by entering the ratios of the peak areas of the analytes to the peak areas of the internal standard in the calibration function. The peak area of vitamin E is related to the peak area of vitamin E d₆ and the peak area of vitamin E acetate to the peak area of vitamin E acetate d₉.

7.1 Calibration function

Equation 1: Calibration function

$$f(x) = y = ax + b$$

Legend:

y	Ratio of peak areas of the target analytes and the respective internal standards
a	Gradient of the calibration function
x	Concentration of the target analytes [ng/ml] = β (mass concentration)
b	Axis intercept of the calibration function

Equation 2: Calculation of the vitamin E / vitamin E acetate concentration of the sample

$$Gehalt = \beta \times DF = \left[(y - b) \times \frac{1}{a} \right] \times \frac{V_{Diluent}}{m_{Einwaage}} = \beta \times 0,01$$

Legend:

β	Mass concentration in the measurement solution [ng/ml]
DF	Conversion factor for ng/ml to ng/mg
y	Peak area of the target analytes
b	Axis intercept of matrix calibration
a	Gradient of matrix calibration
$V_{Diluent}$	Volumes of the diluents (methanol (3.2.7)) [ml]
$m_{Einwaage}$	Mass of the weighed sample [mg]

7.2 Presentation of results

The results are presented in ng/mg to three significant figures. To convert the concentration in the measurement solution in ng/ml to the concentration in the e-liquid in ng/mg, subject to the sample preparation procedure described in Chapter 5, the conversion factor is 0.01.

References

- CDC (2020), US Centers for Disease Control and Prevention, Outbreak of Lung Injury Associated with Use of E-cigarette, or Vaping, Products, https://www.cdc.gov/tobacco/basic_information/e-cigarettes/severe-lung-disease.html [accessed: 16 September 2020]
- DIN ISO 32645. (1994) Chemical Analysis; Decision limit, Detection limit and determination limit, Estimation in case of repeatability, terms, methods, evaluation. Deutsches Institut für Normung DIN.

8. Appendix

8.1 LC-MS/MS measurement

LC-MS/MS system consists of

Control software (e.g. Analyst 1.7, AB Sciex)

Triple quadrupole mass spectrometer (e.g. API 4000 Qtrap, AB Sciex)

LC system (e.g. Prominence series, Shimadzu)

LC pump (e.g. LC-20AD, Shimadzu)

Degasser (e.g. DGU-20As, Shimadzu)

Autosampler (e.g. SIL-20AC HT, Shimadzu)

Column oven (e.g. CTO-20AC, Shimadzu)

Bus interface (e.g. CBM-20A)

LC settings

Eluent A	see 3.3.1
Eluent B	see 3.3.1
Column temperature	40 °C
Flow rate	300 µl/min
Injection volume	10 µl
Autosample temperature	15 °C
Column	e.g. Hypersil C18, 50 x 2.1 mm, 2.4 µm (Thermo Scientific, part no.28102-052130)
Total running time	10 minutes

Gradient

Time [min]	% A	% B
0.0	20	80
1.0	1	99
5.0	1	99
5.5	20	80
10.0	20	80

MS/MS instrument settings

Ionisation	Electro-spray positive mode (ESI +)
Ion spray voltage (IS)	5500 V
Temperature (TEM)	350 °C
Curtain gas (CUR)	Nitrogen, 18 psi
Collision gas (CAD)	Medium
Ion source gas 1 (GS1)	Nitrogen, 55 psi
Ion source gas 2 (GS2)	Nitrogen, 50 psi
Interface heater (ihe)	On
Entrance potential (EP)	10 V

Substance-specific parameters

The analytes were detected via the multiple reaction monitoring (MRM) analysis mode. Three specific transitions to daughter ions were selected for identification. The respective mass

transitions and collisions energy (CE), declustering potential (DP) and collision cell exit potential (CXP) should be taken from Table 2.

Table 2: Substance-specific parameters of the LC-MS/MS methods

Analyte	Q1 mass [Da]	Q3 mass [Da]	Dwell time [ms]	DP [V]	CE [V]	CXP [V]
Vitamin E acetate (NH ₄ adduct) Quantifier	490.4	207.0	70	51	31	0
Vitamin E acetate	473.4	207.3	70	111	25	12
Vitamin E acetate	473.4	164.9	70	111	53	8
Vitamin E acetate Quantifier	431.4	165.2	70	76	27	10
Vitamin E	431.4	69.0	70	76	57	12
Vitamin E	431.4	137.0	70	76	59	24
Vitamin E acetate d ₉ (NH ₄ -adduct) Quantifier	499.5	482.5	70	76	19	14
Vitamin E acetate d ₉ (NH ₄ -adduct)	499.5	216.2	70	76	33	14
Vitamin E acetate d ₉ (NH ₄ -adduct)	499.5	174.2	70	76	61	10
Vitamin E d ₆ Quantifier	437.4	171.2	70	87	29	10
Vitamin E d ₆	437.4	69.1	70	87	57	12
Vitamin E d ₆	437.4	143.2	70	87	61	8

Table 3: Detection (LOD) and quantification limit (LOQ) based on the e-liquid, determined in an in-house validation using the method described in accordance with DIN EN ISO 32645

Analyte	LOD [pg/mg]	LOQ [pg/mg]
Vitamin E	0.8	2.7
Vitamin E acetate	0.3	1.0

8.2 Example chromatogram

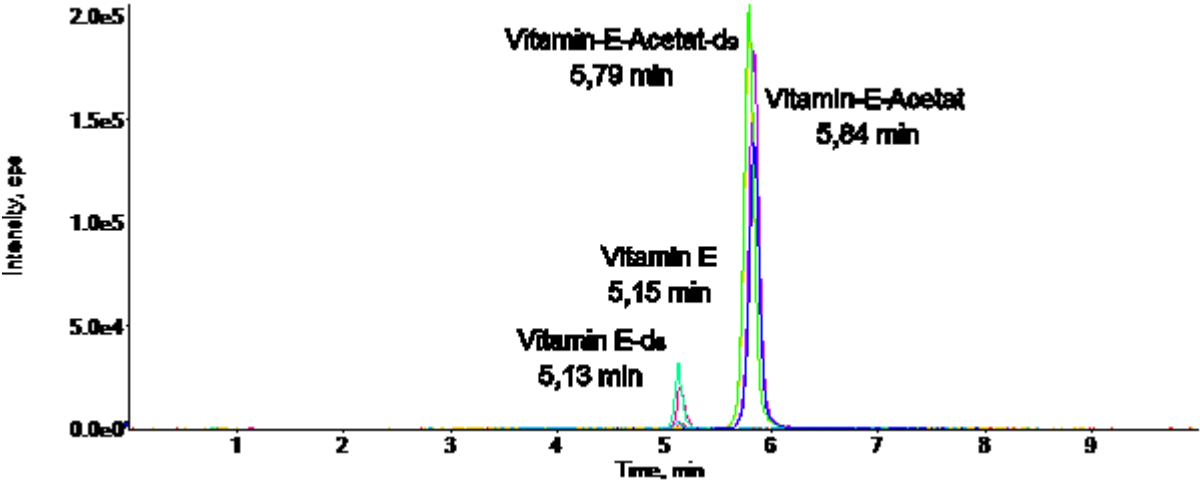


Figure 1: Chromatogram of a matrix standard [c = 10 ng/ml], MRM