Vitamin A & E in serum/plasma

1080 M VAE

Instruction manual for LC-MS/MS assay
for in vitro diagnostic use

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CE 98/79/EC - IVD Medical Devices

Contents

1. Introduction

1.1 Intended Use

This LC-MS/MS kit is intended for the determination of Vitamin A & E in Serum / Plasma.

The components in this kit must be used as stated in the user manual.

1.2 Intended User

This kit is designed for (healthcare) laboratory professional use. Diagnotix recommends that users adhere to ISO 15189 Medical Laboratories.

1.3 Notice Regarding Serious Incidents

Following (EU) 2017/746 Annex I, Chapter III, 20.4.1 af), any serious incident that has occurred in relation to this device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

1.4 IVD symbols

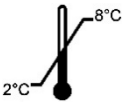
Order Number

Lot Number

For in vitro diagnostic use

See instructions for use

Manufacturer



Temperature limits

Contains sufficient for < n > tests

Expiry date

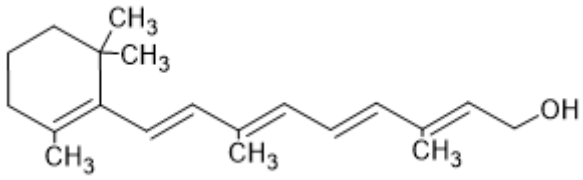
1.2 Clinical background

Vitamins A and E are fat-soluble vitamins, which perform important functions in the body. Vitamin A plays an important role in vision, bone metabolism, and the synthesis of steroid hormones. Deficiency of vitamin A can lead to night blindness, skin dehydration, and hair loss. Vitamin E protects, among others, unsaturated fatty acids in the cell membranes and the LDL cholesterol from attacks by reactive radicals, which can occur as a result of increased oxidative stress in the body. Deficiency of vitamin E can lead to permanent joint pain, making the determination of vitamin E levels essential in this case.
However, overdoses of these vitamins may cause hypervitaminosis with intoxication symptoms. In addition, chemotherapies should not be "accompanied" by extra vitamin supplements – especially high doses of vitamins A and E may limit the success of treatment.

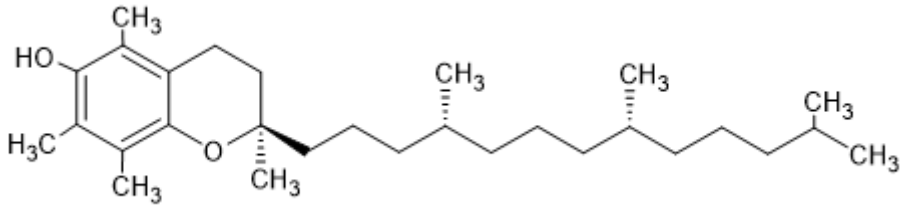
Mass spectrometry based methods have been tested for the determination of Vitamin A & E. Furthermore chromatographic separation for both of the vitamins is critical and not easy. This method can be used for the routine analysis of Vitamin A & E in serum / plasma. Sample preparation is simple and rapid and analogous for the different biological matrices. A six-point lyophilized serum calibrator at clinically relevant levels has been added to the kit. Lyophilized serum controls are also available for quality assurance.

Two isotope-labelled internal standards, Vitamin A-D6 and Vitamin E-D3, are added to compensate for matrix effects and measurement variations. Samples are analyzed using positive ion electrospray in (Multiple Reaction Monitoring) MRM mode for maximum sensitivity and selectivity.

Vitamin A (retinol)



Vitamin E (a-tocopherol)



1.3 Description of the analytical procedure

Vitamin A & E are determined from human serum / plasma by UHPLC with positive ion electrospray LC-MS/MS.

Prior to the LC-MS/MS analysis a sample clean-up is performed to remove the sample matrix and to spike with the internal standard.

After separation by chromatography on an analytical C-18 column, Vitamin A and Vitamin E are ionized by electrospray ionization (ESI) and detected by LC-MS/MS.

Electrospray ionization is a soft ionization technique where a strong electric field is applied to the liquid passing through the ESI-capillary of the MS-source. The ions are mostly performed in solution before desorption and then transferred into the ion path of the tandem mass spectrometer which consists of three quadrupoles (two mass selectors connected by a collision cell).

Measurement of the analytes is carried out in MRM mode. In this mode only selected ions (precursor ions) with a defined mass/charge (M/z) ratio are isolated in the first quadrupole and subsequently transferred into the collision cell, where they are fragmented by impact with an inert gas (argon or nitrogen) at defined voltage settings. Among the fragments generated (known as product ions) only those with a defined M/z ratio can pass the third quadrupole for final detection. In this way the MRM mode ensures a selective identification and quantification of the target analytes.

2. Components of the Vitamin A & E Kit

2.1 Ordering information

1080 KIT M VAE - Complete Kit for Vitamin A & E in serum/plasma

Contents (for 300 assays):

Vitamin A & E Calibrator Set (Calibrator 1 – 6)	2001 CAL HM VAE	6 x 2 x 500 µl
Vitamin A & E Deproteinization Solution with Internal Standard	1090 M VAE	3 x 100 ml
Vitamin A & E Mobile Phase I	1091 M VAE	1 x 250 ml
Vitamin A & E Mobile Phase II	1092 M VAE	1 x 500 ml
Vitamin A & E Autosampler Washing Solution	1096 M VAE	1 x 1000 ml
Vitamin A & E Manual		

Separately available components:

Vitamin A & E Calibrator Set (Calibrator 1 – 6)	2001 CAL HM VAE	6 x 2 x 500 µl
Vitamin A & E Deproteinization Solution With Internal Standard	1090 M VAE	1 x 100 ml
Vitamin A & E Mobile Phase I	1091 M VAE	1 x 250 ml
Vitamin A & E Mobile Phase II	1092 M VAE	1 x 500 ml
Vitamin A & E Autosampler Washing Solution	1096 M VAE	1 x 1000 ml

Analytical column Vitamin A & E BEH C18 1.7 µm 2.1 x 50mm	186002350	1 pc
Vitamin A & E Control I	2012 HM VAE	10 x 500 µl
Vitamin A & E Control II	2013 HM VAE	10 x 500 µl
Vitamin A & E Control III	2014 HM VAE	10 x 500 µl
Vitamin A & E Control Set	2002 CON M VAE	3 x 3 x 500 µl

2.2 Safety information

Several components are chemical preparations and may contain hazardous substances. For safety information, please consult the Material Safety Data Sheet (MSDS) of each component.

The human serum used for manufacturing calibrators and controls was tested for HIV1/2-, HBV- and HCV-antibodies, Hepatitis B-surface antigen, HIV1- and HCV-RNA, HBV-DNA (NAT). However, because no test method can offer complete assurance that products derived from human sources will not transmit infectious agents, it is recommended that this product be handled with the same precautions as patient samples.

2.3 Storage conditions and lifetime of kit components

Please unpack the kit components from the transport packaging *immediately upon receipt* and follow the instructions for storage conditions indicated on the product labels.

2.3.1 Calibrators and controls

2001 CAL HM VAE | Vitamin A & E Calibrator Set
2002 CON HM VAE | Vitamin A & E Control Set
2012 HM VAE | Vitamin A & E Control I
2013 HM VAE | Vitamin A & E Control II
2014 HM VAE | Vitamin A & E Control III

2.3.1.1 Handling

Reconstitute the calibrators and controls as follows:

1. Carefully remove the cap and rubber plug avoiding any loss of contents.
2. Reconstitute Vitamin A & E Calibrator Set and Controls with exactly 500 µl distilled or deionised water using a volumetric pipette.
3. Replace the plug and let stand during 15 minutes.
4. Swirl the vial carefully and mix thoroughly making sure that all traces of dry material have dissolved, do not shake. Avoid foaming.
5. Let stand for 15 minutes at room temperature.
6. Swirl the vial carefully, do not shake. Avoid foaming.
7. Use the preparation as a patient sample.

2.3.1.2 Stability and storage

The stability of the calibrators and controls are:

Before reconstitution:	2 - 8 °C	Until expiry date printed on the product label
After reconstitution:	2 - 8 °C	48 hours
After reconstitution:	- 20 °C	1 week

The declared stated stabilities are only valid in case of no bacterial contamination.

2.3.3 Deproteinization Solution with Internal Standard

1090 M VAE | Vitamin A & E Deproteinization Solution with Internal Standard

2.3.3.1 Handling

The Reagent is liquid and ready for use.

2.3.3.2 Stability and storage

The stability of the Deproteinization with Internal Standard is:

Before opening: 2 - 8 °C	Until expiry date printed on the product label
After opening: 2 - 8 °C	2 weeks
After opening: -20 °C	1 month

2.3.4 Mobile Phases

1091 M VAE | Vitamin A & E Mobile Phase I
1092 M VAE | Vitamin A & E Mobile Phase II

2.3.4.1 Handling

The Reagents are liquid and ready for use.

2.3.4.2 Stability and storage

Store at 2 - 8 °C	After first opening the Reagent can be used for 6 weeks if closed and stored at 2 - 8 °C or 2 weeks on the UHPLC

2.3.5 Autosampler washing solution

1096 M VAE | Vitamin A & E Autosampler Washing Solution

2.3.5.1 Handling

The Reagent is liquid and ready for use.

2.3.5.2 Stability and storage

Store at 2 - 8 °C	After first opening the Reagent can be used for 6 weeks if closed and stored at 2 - 8 °C or 2 weeks on the UHPLC

3. Required instruments

Using this test kit requires a UHPLC system with tandem mass spectrometer (LC-MS/MS).

3.1 Required LC Modules

- Auto sampler
- UHPLC gradient pump
- Column heater
- Degasser

4. The analytical system

4.1 Preparation of the analytical system

- Flush the LC system excluding the column.
- Set the UHPLC pump at a flow rate of 1 ml/min and flush the system for 10 minutes with Mobile Phase I and II (50 : 50).
- Connect the column with the column heater. (see arrow marking on the column)

After flushing the system, the equilibration is performed as follows:

- Set the UHPLC pump to a flow rate of 0.55 ml/min.
- Set the column heater to 30°C.
- Equilibrate the column for 15 minutes with Mobile Phase II.
- Start the program for the gradient and equilibrate for another 10 minutes.

4.2 Starting the analytical system

- Equilibrate the system.
- Check the temperature of the column.
- Initialize the injector.
- Start the programme on the LCMSMS system.

4.3 LC-MS/MS Parameters and Conditions

4.3.1 LC Parameters

UHPLC pump	Flow rate 0.55 ml/min
Mobile Phases I and II	Close the bottles to avoid alteration of RT's through evaporation of the mobile phases
Column	The column is installed in the column heater 30°C For the complete UHPLC system the backpressure should not exceed 800 bar. 1 bar = 14.5 PSI

4.3.2 Autosampler Conditions

Injection volume:	2 µL
Sample temperature:	10 °C
Runtime:	4 min
Column temperature:	45 °C ± 2 °C alarm
Needle wash:	wash twice for 6 seconds
Seal Wash:	10:90 ACN:H2O
Wash Solvent:	Autosampler Washing Solution; 1096 M VAE

4.3.3 Gradient

Time (min)	**Flow Rate (mL/min)**	**%A**	**%B**	**Curve**
0.00	0.55	35	65	Initial
2.00	0.55	5	95	6
3.00	0.55	5	95	6
3.10	0.55	35	65	6
4.00	0.55	35	65	6

Please note that the gradient is dependent on the analyser used. End users will need to define the optimal gradient for the analyser in use.

4.3.4 MS Conditions (e.g. Waters Xevo TQS)

MS System:	(Waters Xevo TQS)
Ion mode:	Electrospray
Capillary voltage:	1.0 kV
Polarity:	positive
Source temperature:	150 °C
Desolvation temperature:	650 °C
Desolvation gas flow:	1000 L/hr
Detection mode:	MRM
Dwell time:	0.019 sec
Collision gas:	Argon / Nitrogen

Substance	**Precursor**	**Product**
Vitamin A	269.30	81.00
Vitamin A	269.30	93.00
Vitamin A D6	275.30	81.00
Vitamin A D6	275.30	93.00

Substance	**Precursor**	**Product**
Vitamin E	431.40	83.00
Vitamin E	431.40	165.10
Vitamin E D6	437.40	83.00
Vitamin E D6	437.40	171.10

These conditions are an indication, the optima can differ slightly between different LC-MS/MS systems.

5. Sample

5.1 Sample material

Use serum /plasma (EDTA- and Heparin-tubes)

5.2 Sample preparation

5.2.1 Reconstitution of the lyophilised Calibrators / Controls.

See 2.3.1.1 and the product data sheets.

5.2.2 Sample preparation (patient sample, calibrator or control)

1. 25 µl sample, calibrator or control.
2. Add 975 µl Deproteinization solution with Internal Standard deuterated.
3. Mix immediately using a vortex mixer for 30 seconds.
4. Centrifuge (5 min, 10000 x g or more).
5. Transfer 400 µl centrifuged supernatant to a vial or 96 well plate, which is suitable for the auto sampler in use and Inject 2 µl in the LC-MS/MS.

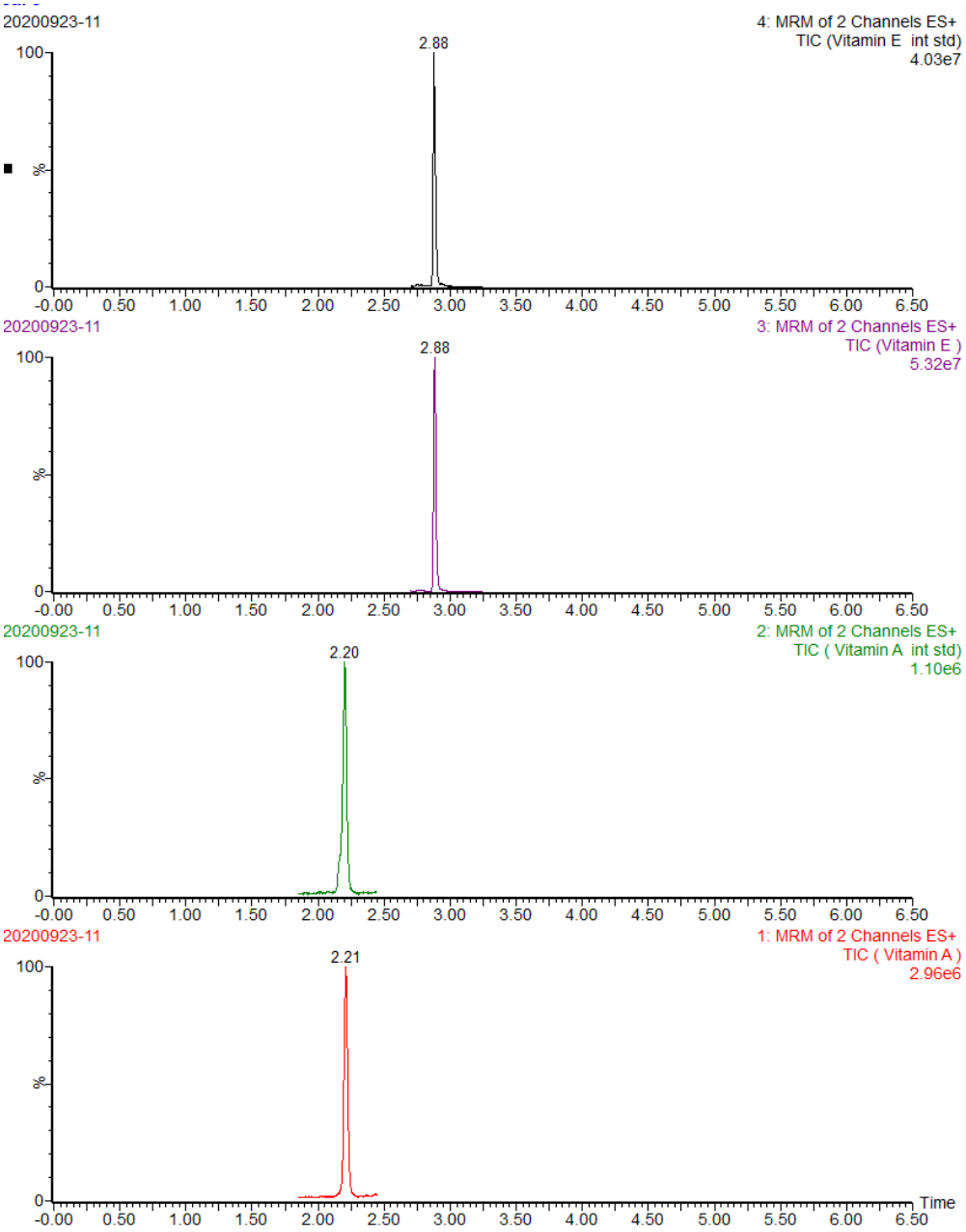
5.2.3 Sample Preparation with pipette robot

Into a 2 ml 96 well plate:

1. 25 µl sample (Calibrator, Control, Patient sample),
2. Whilst mixing the plate, add 975 µl Vitamin A & E deproteinization solution with Internal Standard.
3. Once mixing is complete, centrifuge (5 min , 10,000x g or more).
4. Transfer the samples into a 1 ml 96 well collection plate for injection on the UHPLC/MS/MS system (Needle placement 2 mm) or inject directly off the pellet (Needle placement 10 mm) 2 µl in the LC-MS/MS.

5.3 Examples of chromatograms

Example chromatogram of a Patient sample, recorded with the Waters LC-MS/MS TQS-µ:



6. Test data (Validation report)

6.1 Linearity

	µmol/l
Vitamin A	8.5
Vitamin E	60.0

6.2 Limit of quantification

	µmol/l
Vitamin A	0.25
Vitamin E	3.5

6.3 Repeatability

Vitamin A

Item	**Measured value (µmol/l)**	**Standard Deviation (µmol/l)**	**CV (%)**	**N**
Level I	0.44	0.007	1.6	20
Level III	2.9	0.011	0.4	20
Patient material	2.5	0.087	0.5	20

Vitamin E

Item	**Measured value (µmol/l)**	**Standard Deviation (µmol/l)**	**CV (%)**	**N**
Level I	6.0	0.098	1.6	20
Level III	44.1	0.273	0.6	20
Patient material	27.2	0.199	0.7	20

6.4 Reference Ranges

	µmol/l
Vitamin A	1.2 – 2.7[1]
Vitamin E	15 - 35

The indicated reference ranges are taken from scientific literature. It is recommended that each laboratory establishes its own reference ranges.

[1] https://www.nvkc.nl/algemeen-overzicht-referentiewaarden

7. References

1. *NVKC, Algemeen overzicht referentiewaarden*, accessed at https://www.nvkc.nl/algemeen-overzicht-referentiewaarden on the 24th of September 2020