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Determination of vitamins A, D and E in a small volume of human plasma by a high-throughput method based on liquid chromatography/tandem mass spectrometry

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We have developed an automated high-throughput assay for the determination of vitamin A (retinol), ergocalciferol (25-OH D2), cholecalciferol (25-OH D3) and vitamin E (α-tocopherol) in a small volume of human plasma. Sample preparation involved mixing 50 μL of plasma with 100 μL of ethanol containing isotope-labelled internal standards, followed by mixing with isooctane/chloroform (3:1, 300 μL). The organic phase was evaporated, and the sample reconstituted in 50 μL methanol. The analysis was performed using reversed-phase liquid chromatography with a gradient mobile phase containing water, methanol and ammonium formate. Chromatographic run-time was 5 min, and positive mode electrospray tandem mass spectrometry (MS/MS) was used for detection. The limits of detection were 0.10 μM for all-trans retinol and 3.3 nM for 25-OH D2 and 25-OH D3. Recoveries were 91.9–105.0%, and within- and between-day coefficients of variance (CVs) 2.4–5.3 and 3.1–8.2, respectively. The assay is presently being used in large-scale studies.

Vitamin A is required for normal vision, growth, reproduction and immune system function.[1] The main function of vitamin D is to enhance the absorption of calcium and phosphorous in the small intestine, and this vitamin is thus central to bone formation.[2] Vitamin E is an important biological antioxidant.[3] Assessment of nutritional status requires measurements of multiple vitamins.[4,5]

In epidemiologic studies of associations of various health outcomes with multiple biomarkers in serum/plasma, a limited sample volume is often available. High-throughput methods that are capable of combined measurement of several vitamins in a small sample volume are therefore useful in studies of nutrition and health.

Few assays for combined measurement of plasma or serum concentrations of several fat-soluble vitamins have been published.[6–12] Such method development is a challenge because of the diverse chemical structures of these vitamins and their large span of plasma concentrations, which include the nanomolar to the micromolar range. Published assays have the drawback of relatively long chromatographic run-times (15–35 min)[6–9,12] and/or consumption of large sample volumes (300–2000 μL).[6,8,10–12]

The present assay includes established plasma markers of the vitamins A (retinol),[1,13,14] D (ergocalciferol and cholecalciferol)[13–18] and E (α-tocopherol).[13,14,19,20] Low sample volume requirement, simple and automated sample preparation, short chromatographic run-time as well as adequate method performance makes the assay suitable for use in large-scale studies.

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EXPERIMENTAL

Materials

All-trans retinol (95%), 25-OH D3 (98%), butylated hydroxytoluene (BHT, 99%), isooctane (>99.5%) and bovine serum albumin (99%) were from Sigma-Aldrich. 25-OH D2 (>98%) and ammonium formate (99%) were from Fluka and α-tocopherol (99.9%) from Supelco. $^{2}H_{6}$-all-trans retinol (99.2%) was purchased from Buchem (Apeldoorn, The Netherlands), $^{2}H_{6}$-25-OH D3 (95%) from Synthetica (Oslo, Norway), $^{2}H_{9}$-α-tocopherol (99%) from Chemaphor (Toronto, Canada), chloroform (>99%) from Merck, methanol (99.9%) from Lab-scan and ethanol (96%) from Arcus.[21] Doubly deionized water was used.

Individual stock solutions were prepared on ice in methanol with BHT (1 g/L) and stored at −80°C until use. The concentrations of the stock solutions were 356 μM (all-trans retinol), 532 μM (25-OH D2), 498 μM (25-OH D3), 2320 μM (α-tocopherol), 3420 μM ($^{6}H_{2}$-all-trans retinol), 245.8 μM ($^{6}H_{2}$-25-OH D3) and 2300 μM ($^{6}H_{2}$-α-tocopherol). The stock solutions of all-trans retinol, α-tocopherol, $^{2}H_{6}$-all-trans retinol and $^{2}H_{9}$-α-tocopherol were prepared in red light under nitrogen atmosphere. No unlabelled compounds were detected in any of the isotope-labelled internal standards.

Plasma was collected from 8 healthy persons, pooled, and a fraction of this pool was spiked with 1 μM all-trans retinol, 220 nM 25-OH D2, 200 nM 25-OH D3 and 10 μM α-tocopherol. The unspiked and spiked plasma were

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aliquoted and stored at −80°C, and the spiked plasma was used for calibration of the assay. Analyte concentrations of the unspiked plasma were calculated by using the standard addition method and analysing 24 replicates of the unspiked and spiked plasma. The vitamer concentrations of the unspiked plasma were 1.89 μM for retinol, 50.1 nM for 25-OH D3 and 25.4 μM for α-tocopherol; 25-OH D2 was below LOD.

Method development is of the Quality Control Category, which under the Norwegian regulations in force is exempt from full review by the Institutional Review Board.

Sample collection and processing

EDTA plasma was obtained by collecting blood into Vacutainer tubes (Becton Dickinson), giving a final EDTA concentration of 4 mM. The samples were immediately put on ice and centrifuged (at 2000 g/4°C for 10 min) within 60 min. The plasma was then processed further or stored at −80°C until use.

Sample processing was performed in 1 mL Costar polypropylene plates and liquid handling by a robotic workstation (MicrolabAT Plus, Reno, NV, USA) fitted with Hamilton disposable tips with liquid detection. A plasma volume of 50 μL was deproteinized by adding 100 μL of ethanol containing BHT (1 g/L) and internal standards (1000 nM of $^{2}H_{6}$-all-trans retinol and $^{2}H_{9}$-α-tocopherol, 100 nM of $^{2}H_{6}$-25-hydroxyvitamin D3), followed by rigorous mixing for 15 s and then gentle mixing for 5 min. Then 300 μL of isooctane/chloroform (3:1, v/v) were added, gentle mixing repeated for 5 min, followed by 3 min of centrifugation (4000 g/4°C). After washing the pipette tips 10 times with the organic solvent, 200 μL of the organic layer were transferred to a new plate, and the solvent evaporated at 23°C under a flow of nitrogen gas, using a TurboVap96 (Caliper Lifescience, Hopkinton, MA, USA). The resulting material was then dissolved in methanol (50 μL) containing BHT (1 g/L) and vigorously mixed for 20 s. The samples were protected from light at all stages.

Instruments

We used an Agilent series 1100 high-performance liquid chromatography (HPLC) system equipped with a thermostated autosampler and degasser, and an API 4000 triple-quadrupole tandem mass spectrometer from Applied Biosystems/MSD SCIEX equipped with an electrospray ionization source (ESI). A column switcher from Valco (type EMHA) was used to divert the flow to waste during the first minute of each run and between each injection. Analyst v.1.4.2 from Applied Biosystems/MDS SCIEX was used for data acquisition and analysis.

HPLC/MS/MS

The processed samples were kept in a autosampler at 8°C in subdued light, and volumes of 20 μL were injected into an Ascentis Express C18 column (50 × 4.6 mm, particle size 2.7 μm) fitted with a solvent filter and kept in a thermostatted column compartment at 55°C. The mobile phase consisted of two components and was delivered at a flow rate of 1100 μL/min according to the following time table: 0–4.0 min, 2.5 mM ammonium formate in MeOH; 4.1–5.0 min, 80% MeOH, 20% H_2O. Samples were injected every 6 min, and delivered to the mass spectrometer with no split.

Mass spectrometric parameters were optimized by infusing 10 μM solutions of each analyte at a rate of 100 μL/min. This solution was mixed with 2.5 mM ammonium formate in MeOH delivered at a flow rate of 1000 μL/min, using the T-junction located at the front of the mass spectrometer. In order to increase the number of data points for each chromatographic peak, the ms-scan was divided into two segments (segment 1: 0–2.3 min; segment 2: 2.3–5.0 min). The following parameters were set for all analytes and internal standards within each scan segment (segment1, segment 2): collision-activated dissociation gas (3 psig, 2 psig), ion source temperature (500°C, 600°C), interface heater off, ionspray (4500 V, 5000 V). Curtain gas (20 psig), ion source gas 1 (90 psig) and 2 (70 psig), and entrance potential (10 V) were identical for both scan segments.

The ions were detected in positive mode by multiple reaction monitoring (MRM) using unit resolution at both quadrupoles. The retention times, protonated precursor ions and product ions, as well as ion-pair specific parameters for the analytes and internal standards, are summarized in Table 1.

Plasma concentrations were calculated by dividing the analyte peak area by the area of the corresponding deuterated internal standard. $^{2}H_{6}$-25-OH D3 was also used as internal standard for 25-OH D2.

Quantitation was evaluated using additional ion-pair combinations (Table 1) for 25-OH D3, α-tocopherol and the corresponding internal standards. This procedure was used for detection of possible interference with vitamin D forms from coeluting isobaric compounds resulting from loss of water. Since we used the third most abundant isotope of α-tocopherol to avoid signal saturation, such validation was performed to investigate possible isotope effects compromising quantitation of α-tocopherol. A total of 137 plasma samples were used for these experiments. The resulting concentrations were compared by Passing-Bablok regression[22,23] using the Excel tool provided by ACOMED statistic.[24]

Matrix effects

We mapped matrix effects[25] in 15 different plasma specimens spiked after sample treatment with 1.67 μM all-trans retinol, 73.3 nM 25-OH D2, 66.6 nM 25-OH D3 and 11.1 μM α-tocopherol. By comparing the peak areas to the corresponding peak areas obtained by identical spiking of methanol, the matrix effects were calculated as:

$$\text{Matrix effect} = \frac{\left[\begin{matrix}\text{Analyte}\\ \text{peak area}\end{matrix}\right]_{\text{spiked}} - \left[\begin{matrix}\text{Analyte}\\ \text{peak area}\end{matrix}\right]_{\text{endogenous}}}{\left[\text{Analyte peak area}\right]_{\text{methanol}}} * 100\%$$

and also by replacing the analyte peak area by the peak area ratio (peak area analyte)/(peak area ISTD) in the formula.

Linearity and limit of detection (LOD)

Linearity and LOD were determined by preparing 24 different solutions of 4% albumin in phosphate-buffered saline

Table 1. Retention times and instrument settings

Analyte	t_r (min)	Transitions, *m/z*		DT (ms)	DP (V)	CE (V)	CXP (V)
		Precursor ion	Product ion				
Scan segment 1, 0.0–2.3 min							
$^{6}H_2$-all-trans retinol[a]	1.69	275.5	96.2	30	85	30	8
retinol[a]	1.73	269.1	93.2	30	60	30	5
$^{6}H_2$-25-OH D3[a]	1.58	407.2	389.2	30	95	17	15
$^{6}H_2$-25-OH D3[b]	1.58	407.2	263.0	30	90	21	15
25-OH D3[a]	1.59	401.4	383.2	30	80	10	10
25-OH D3[b]	1.59	401.4	257.0	30	90	21	15
25-OH D2[a]	1.61	413.5	395.4	50	70	14	10
Scan segment 2, 2.3–5.0 min							
$^{9}H_2$-α-tocopherol[a]	2.99	440.4	174.4	30	65	32	15
$^{9}H_2$-α-tocopherol[b]	2.99	440.4	146.4	30	65	60	15
α-tocopherol[a]	3.01	433.4	167.4	30	65	30	15
α-tocopherol[b]	3.01	431.4	137.4	30	65	50	15

Abbreviations: t_r, retention time; DT, dwell time; IS, ion spray; DP, declustering potential; CE, collision energy; CXP, collision cell exit potential.
[a]Ion-pairs used for routine quantitation.
[b]Ion-pairs used for validation of quantitation.

(PBS) containing 0.00125–10 μM all-trans retinol, 0.275–2200 nM of 25-OH D2 and 0.250–2000 nM 25-OH D3. Because of the high endogenous plasma concentrations of α-tocopherol, LOD was not determined for this analyte, and linearity was investigated by spiking plasma pooled from four individuals, containing 18.9 μM α-tocopherol, with 0.033–33.3 μM α-tocopherol. Signal-to-noise (S/N) ratios were calculated for each ion-pair as the peak height divided by the standard deviation of the baseline, using the script supplied by Applied Biosystems (Analyst v. 1.4.2). LODs were determined as the lowest concentrations that gave S/N values higher than five, lower limit of quantification (LLQ) as two times LOD, and linearity parameters were assessed by linear regression using concentrations above LOD for each analyte.

Recovery and precision

Recovery and precision were studied by dividing plasma from a healthy person into three portions. One portion was left unspiked and contained endogenous levels of each vitamer (2.19 μM retinol, 57.3 nM 25-OH D3 and 38.1 μM α-tocopherol; 25-OH D2 was below LLQ), which is defined as low concentrations. A second portion of the plasma was spiked (with 1.0 μM all-trans retinol, 110 nM 25-OH D2, 100 nM 25-OH D3 and 5 μM α-tocopherol) to medium concentrations and the third portion (with 3.0 μM all-trans retinol, 330 nM 25-OH D2, 300 nM 25-OH D3, 15 μM α-tocopherol) to high concentrations. Twenty-four replicates for each analyte concentration were analyzed on the same day. The recovery was calculated as:

$$\text{Recovery}(\%) = \frac{\text{Measured concentration} - \text{Endogenous concentration}}{\text{Added concentration}} \times 100$$

Within-day precision was calculated from the recovery experiments, while between-day precision was estimated by analyzing the same samples on 18 different days.

Accuracy

Five serum pools were obtained from the Vitamin D External Quality Assessment Scheme (DEQAS)[26] to check the accuracy of 25-OH D3 determination.

Plasma concentrations of A, D and E vitamers

In order to establish the concentration ranges of the analytes, we obtained plasma samples collected in southern Norway in February from 168 anonymous, assumed healthy individuals with mean age 42.8 (range: 21–69) years, 41% female.

RESULTS AND DISCUSSION

This assay use automated sample preparation and fast chromatography to quantitate established markers of the fat-soluble vitamins A, D and E in 50 μL of human plasma. The method is suitable for use in large-scale investigations.

Sample preparation, stability and adsorption of analytes

Procedures for preparation of standard solutions, sample processing and storage were established based on published data on stability and degradation.

Retinol[27] and α-tocopherol[28] are considered susceptible to degradation. Standard solutions of these analytes and the corresponding internal standards were thus prepared with antioxidant (BHT, 1 g/L), on ice, under red light in a nitrogen atmosphere. Retinol,[9,29] vitamin D forms[9,30] and α-tocopherol[9,29] are reported to be stable in plasma.

All standard solutions and plasma samples were stored at −80°C, and no signs of degradation of analytes or internal standards were observed over a period of 3 months, which is consistent with published results.[12] Analytes were stable in

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processed plasma samples for at least 48 h while stored in the autosampler at 8°C in microtiter plates under subdued light.

Sample processing was performed at room temperature and protected from light. Some authors[31–33] specify the use of borosilicate glassware for sample preparation in the analysis of vitamin D forms. We therefore investigated if adsorption of analytes onto the polypropylene plates occurred by comparing the signal intensity of analytes and internal standards in samples processed in borosilicate glass vials versus in polypropylene plates. No differences were found (not shown), suggesting no adsorption of analytes to the plates. However, we observed adsorption of retinol onto the pipette tips when transferring the organic phase to a new plate. Washing the tips ten times with the organic phase before this transfer step prevented such loss of retinol.

Chromatography

The selection of column, mobile phase components and gradient program was optimized to obtain short retention times, which are a prerequisite for high-throughput assays. A chromatogram of the spiked plasma used for calibration is shown in Fig. 1; no interfering peaks were observed for any of the analytes or internal standards. The retention times are listed in Table 1. All analytes eluted within 3 min, and another 2 min were allowed for reequilibration of the column before the next sample injection.

Isotopologues may have different retention times,[34–36] but for the vitamers included in the present assay, such differences were minor (0.04 min or less, Table 1).

Mass spectrometry

The precursor ions for retinol[37] and $^{6}H_2$-all-trans retinol were the $[M–H_2O]^+$ ions, while for 25-OH D2, 25-OH D3, $^{6}H_2$-25-OH D3, α-tocopherol and $^{9}H_2$-α-tocopherol precursor ions corresponded to $[M + H]^+$. For retinol, vitamin D forms and all isotope-labelled internal standards we selected the product ions with the highest signal yield, while for α-tocopherol we used the third most abundant isotope in order to prevent saturation of the detector. Acquisition parameters were optimized for each analyte and internal standard, and are summarized in Table 1. In order to increase the number of data points across each chromatographic peak, we divided the analysis into two scan segments (Table 1).

Ion-pairs used for vitamin D and E forms

The combination of chromatographic separation and tandem mass spectrometry is considered to result in highly specific detection, but interferences may occur in analysis of complex biological samples. The magnitude of such interference depends on factors such as concentration and ionization efficiency of analytes and interfering compounds. In addition, different compounds may have different yield of various fragments formed.[38] Thus, the use of more than one ion-pair will greatly enhance the specificity of an assay.[38] Using more than one ion-pair has also been suggested to be the best method to identify the presence of interfering isobaric compounds, but this approach is presently only rarely used in routine application of LC/MS/MS.[39] As the ion-pairs used for detection of vitamin D forms were due to the common fragmentation caused by loss of water, we were concerned that coeluting isobaric substances might interfere with detection of these compounds. We therefore included one additional ion-pair for both 25-OH D3 and $^{6}H_2$-25-OH D3, and used these to verify quantitation of 25-OH D3. Due to the generally low concentration of 25-OH D2 in plasma we did not investigate this aspect for this analyte.

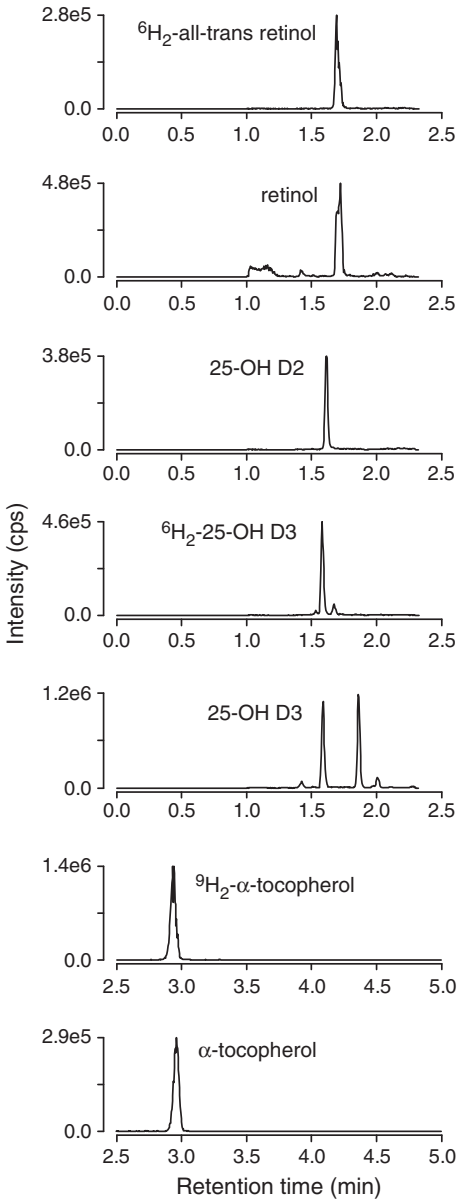

Figure 1. Normalized HPLC/MS/MS chromatogram of the (spiked) calibrator plasma containing $^{6}H_2$-all-trans retinol (1.00 μM), retinol (2.89 μM), 25-OH D2 (220 nM), $^{6}H_2$-25-OH D3 (200 nM), 25-OH D3 (250.1 nM), $^{9}H_2$-α-tocopherol (10.0 μM), α-tocopherol (35.4 μM).

Many chemical elements may be present as more than one isotope, giving rise to isotopic molecules. Isotopic ion-pairs may be used for quantitation,[38] and when using isotopic precursor ions, the fragments may be monoisotopic (i.e. not containing isotopes other than those of a monoisotopic precursor ion) or isotopic. The presence of various isotopes may influence the abundance of fragments in mass spectrometry.[40,41] The

Table 2. Matrix effects[a]

Analyte	Based on analyte area	Based on analyte/ISTD area ratio
all-trans retinol	71 (11)	63 (6)
25-OH D2	97 (8)	100 (6)
25-OH D3	96 (11)	95 (8)
α-tocopherol	52 (35)	82 (6)

Abbreviations: ISTD, internal standard.
[a]Based on 15 different plasma samples. All values (%) are given as mean (CV).

abundance of isotopic elements may vary between samples as a result of different sources of the material,[42] and the abundance of isotopic fragments will depend on the molecular weight and structure of the fragment. Such phenomena could compromise quantitation, and if the same concentrations were obtained by monoisotopic and isotopic ion-pairs, this suggests that such effects are small. Unless isotope effects are large, adequate correction should be obtained if the distributions of various isotopes are similar in samples and calibrators. Our results based on isotope-labelled internal standards reduced both the matrix effect and the relative matrix effect for α-tocopherol, suggesting that isotope effects were minor. Since the third most abundant isotope was used for quantitation of tocopherol, we were concerned that the detection of different isotopomers of α-tocopherol might compromise quantitation of this analyte. An ion-pair for α-tocopherol with the $[M+H]^+$ ion as precursor (plus the analogue ion-pair for $^9H_2$-α-tocopherol) was therefore included to verify quantitation of this vitamer.

In 137 plasma samples the median (5–95 percentiles) plasma concentrations were 61.1 (30.1–104.2) nM for 25-OH D3 and 25.8 (18.2–35.5) μM for α-tocopherol. Comparing the plasma concentrations obtained with two ion-pair ratios using Passing-Bablok regression gave 95% confidence intervals for 25-OH D3 of 0.91 to 1.01 (slope) and −3.6 to 1.9 (intercept); for α-tocopherol, these were 0.93 to 1.08 and −1.3 to 2.9. Thus the different ion-pair ratios gave concentrations that did not differ, demonstrating that the chosen ion-pairs were adequate for quantitation.

Matrix effects

Matrix effects can have a negative impact on the performance of HPLC/MS/MS assays by reducing precision and accuracy. For the present assay, matrix effects were most pronounced for α-tocopherol (52%) and all-trans retinol (71%), while for the vitamin D forms the matrix effects were small (96–97%). By dividing the analyte area by the area of the internal standard, we obtained absolute matrix effects which were increased for all-trans retinol, were essentially unchanged for the vitamin D forms, and reduced for α-tocopherol. It is conceivable that retinol forms other than all-trans retinol may influence the matrix effects of this analyte. The relative matrix effects[25] ranged from 8% (25-OH D2) to 35% (α-tocopherol) (Table 2). Notably, the use of isotope-labelled internal standards reduced the relative matrix effects for all analytes to values ranging from 6 to 8%, thereby improving the performance of the assay, demonstrating the importance of using appropriate internal standards. This result also demonstrates that isotope effects were minor.

Performance of the method

Results for LOD and linearity are shown in Table 3. The LODs were 0.10 μM for all-trans retinol and 3.3 nM for 25-OH D2 and 25-OH D3. The upper limits of the linear ranges were 5 μM for all-trans retinol and 500 nM for 25-OH D2 and 25-OH D3. α-Tocopherol is present in very high concentrations in plasma (in the range 20–40 μM),[43] and the third most abundant isotope was used to avoid saturation of the detector at endogenous plasma concentrations. Investigation of LOD was therefore not performed for this analyte. The response for α-tocopherol was linear through the investigated range (18.9–52.2 μM). Thus, the method is suitable to quantify endogenous plasma concentrations of all-trans retinol, 25-OH D3 and α-tocopherol, as well as 25-OH D2 concentrations obtained after supplementation with this vitamer. Recoveries were 91.9–105.0% (Table 4). Within-day coefficients of variance (CVs) were 2.4–5.3% and between-day CVs were 3.9–8.2% (Table 5).

We obtained five serum samples from DEQAS, with mean (CV) concentrations of 25-OH D given as 19.7 (23.2), 38.7 (14.1), 49.7 (13.5), 57.2 (12.9), 75.1(13.0) nmol/L. In these samples 25-OH D2 was below the LLQ of the assay, while there was a strong correlation between 25-OH D3 quantified by this assay (y) and the 25-OH D concentrations given by DEQAS (x); ($y = 1.14x - 3.05$, $r^2 = 0.999$).

The overall performance of the method is comparable to assays including several fat-soluble vitamins,[6–12,44] and also with assays that separately measured vitamin A,[45,46] D[18,31,32] or E.[28,47,48]

Table 3. Linearity and limit of detection[a]

Analyte	Calibration range	LOD[b]	Regression parameters		
			Slope	Intercept	r^2
all-trans retinol (μM)	0.00125–10	0.10	0.886	0.0145	0.9984
25-OH D2 (nM)	0.250–1000	3.3	0.0069	0.0213	0.9925
25-OH D3 (nM)	0.250–1000	3.3	0.0067	0.0864	0.9939
α-tocopherol (μM)	18.93–52.2	n.d.[c]	0.0055	-0.0193	0.9988

[a]Performed in PBS for all-trans retinol and D vitamers, in spiked plasma for α-tocopherol.
[b]LOD, limit of detection, defined as S/N ratio >5.
[c]Not determined due to high plasma concentrations.

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Table 4. Analytical recovery of the assay

	Concentration[a]			Recovery[b] (%)	
	Endogenous	Endogenous + Added		Added	
Analyte	Low	Medium	High	Medium	High
all-trans retinol (μM)	2.19	3.20	5.05	100.6	95.2
25-OH D2 (nM)	4.9	113.8	318.0	99.0	94.9
25-OH D3 (nM)	57.3	160.5	348.9	103.2	97.2
α-tocopherol (μM)	38.1	43.3	51.8	105.0	91.9

[a]n = 24 for all concentrations.
[b]Data are given as mean values for 24 observations.

Table 5. Imprecision of the assay

	Within-day CV (n = 24)[a] (%)			Between-day CV (n = 19) (%)		
Analyte	Low	Medium	High	Low	Medium	High
(all-trans) retinol[b]	3.8	3.9	4.1	6.2	8.0	5.9
25-OH D2	n.d.[c]	4.3	4.5	n.d.[c]	4.6	7.7
25-OH D3	4.6	4.4	5.3	8.2	7.3	7.7
α-tocopherol	2.4	3.6	3.6	5.3	4.7	3.9

[a]Data from the recovery experiments.
[b]These values refer to retinol for endogenous concentrations (Low) and all-trans retinol for spiked concentrations (Medium and High).
[c]Not determined as the concentration was below LLQ (6.6 nM).

Plasma concentrations of the analytes

The median (5–95 percentiles) plasma concentrations found in a group of 168 presumed healthy individuals were 2.08 (1.46–2.84) μM for retinol, <LLQ (<LLQ – 9.7) nM for 25-OH D2, 60.2 (32.4–98.5) nM for 25-OH D3 and 26.5 (18.9–38.8) μM for α-tocopherol. These concentrations are in line with those reported for healthy subjects by others for retinol,[43] vitamin D[49] and α-tocopherol.[43]

CONCLUSIONS

An automated HPLC/MS/MS assay suitable for analysis of established markers of vitamins A, D and E in 50 μL of human plasma in 5 min has been developed. The present method has been implemented in our laboratory for determination of endogenous concentrations of these vitamers in large-scale studies with limited sample volume available from serum/plasma repositories.

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