PO-CON1805E

SHIMADZU
Excellence in Science

High-Throughput Simultaneous Measurement of Vitamins A, D and E in Human Plasma by LDTD-MS/MS

ASMS 2018 WP-128

Tsuyoshi NAKANISHI[1], Mikaël LEVI[1], Takeshi ASHIDA[1],
Hiroyoki YASUDA[1], Ichiro HIRANO[1]
1 SHIMADZU Corporation, MS Business Unit, Kyoto, Japan

High-Throughput Simultaneous Measurement of Vitamins A, D and E in Human Plasma by LDTD-MS/MS

Introduction

Lipohilic vitamins have important roles in physiological functions to maintain good health. Deficiencies in vitamin A, D and E circulating levels are associated with increased risks of degenerative diseases. Such deficiencies, and especially for Vitamin D, have been found to be wide spread within world population. Therefore, as awareness and the need for large epidemiologic studies is increasing, the measurement of vitamins A, D and E status has greatly increased too. Then laboratories need to face this demand with high-throughput, accurate and multiplexed methods. Here we present a method to simultaneously quantify these vitamins by Laser Diode Thermal Desoprtion-tandem mass spectrometry (LDTD™-MS/MS) in human plasma.

Methods and Materials

Reagents

All-trans retinol (Vitamin A), a-tocopherol (Vitamin E), 25-OH vitamin D2 (ergocalciferol) and 25-OH vitamin D3 (cholecalciferol) were selected as target compounds. $^{2}H_{6}$-tocopherol and $^{2}H_{6}$-cholecalciferol were used as internal standards. Certified standard solutions of each compound were purchased from Sigma-Aldrich.
As double-charcoal-stripped plasma was not free of the target components, the calibration standards and quality controls were prepared in a plasma surrogate (Bovine serum albumin at 50 mg/mL in aqueous NaCl at 0.9% (w/v). The calibration range was from 1 to 250 ng/mL for 25-OH vitamins D2/D3, 10 to 4500 ng/mL for Vitamin A and 0.1 to 40 µg/mL for Vitamin E. Seven calibration levels, regularly dispatched and four quality controls levels were independently prepared (LLOQ, 3x LLOQ, 50% of the range and 90% of the range) . Internal standard solution contained $^{2}H_{6}$-tocopherol and $^{2}H_{6}$-cholecalciferol at a concentration of 20 and 2.5 µg/mL in ethanol, respectively. BHT (butylhydroxytoluene) was added at a concentration of 1% (w/v) in all solvents used to prevent Vitamin A degradation.
Solvents used were of LC-MS or Pesticide analysis grade from Wako chemicals.

Sample Preparation

Calibration standards, QC or samples were assayed the same way. One-hundred microliters of sample were mixed with 10 µL of ISTD solution and 100 µL of water/isopropanol (1/1 v/v). After vortexing, the samples were incubated at room temperature for 15 minutes. Then 200 µL were loaded on SLE sorbent (SLE+ 200, Biotage, Sweden) by gravity. After 5 minutes, compounds were eluted with 2x300 µL of hexane. Extracts were then directly deposited in LazWell 96 plate.

Analytical Conditions

Analysis was performed using a LDTD-SH-960 system (Phytronix, Quebec, Canada) coupled with LCMS-8060 triple quad mass spectrometer (Shimadzu Corp. Kyoto, Japan). Parameters are described in Table 1 and 2.

High-Throughput Simultaneous Measurement of Vitamins A, D and E in Human Plasma by LDTD-MS/MS

Figure 1: Overview of the Analytical System

Table 1 LDTD conditions

System	: SH-960
Laser pattern	: 45 % in 3 seconds
Carrier gas flow	: 3 L/min (Air)
Injection Volume	: 2 µL
Total Run Time	: 12 seconds

Table 2 MS/MS conditions

System	: LCMS-8060
Ionization	: APCI
Probe Voltage	: +5 kV (positive ionization)
Temperature	: Desolvation Line: 150°C Heater Block: 200°C
Dwell Time / Pause time	: 24 ms / 1 ms
MRM	:

Compound	MRM Quant	MRM Qual
25-OH D2	395.35 > 269.20	295.35 > 251.20
25-OH D3	383.35 > 257.25	401.35 > 257.25
$^{2}H_{6}$-25-OH D3	389.35 > 263.25	
Vitamin A	269.20 > 93.20	269.20 > 81.05
Vitamin E	431.40 > 137.10	431.40 > 55.15
$^{2}H_{6}$-Vitamin E	437.40 > 143.10	

High-Throughput Simultaneous Measurement of Vitamins A, D and E in Human Plasma by LDTD-MS/MS

Results

Calibration

Calibration curves were calculated by internal standardization using a linear regression model with 1/x weighting. Acceptance criteria was an accuracy comprised between 85 to 115%.
Some typical calibration curves are presented in Figure 2 and mass chromatograms at the LLOQ in Figure 3.

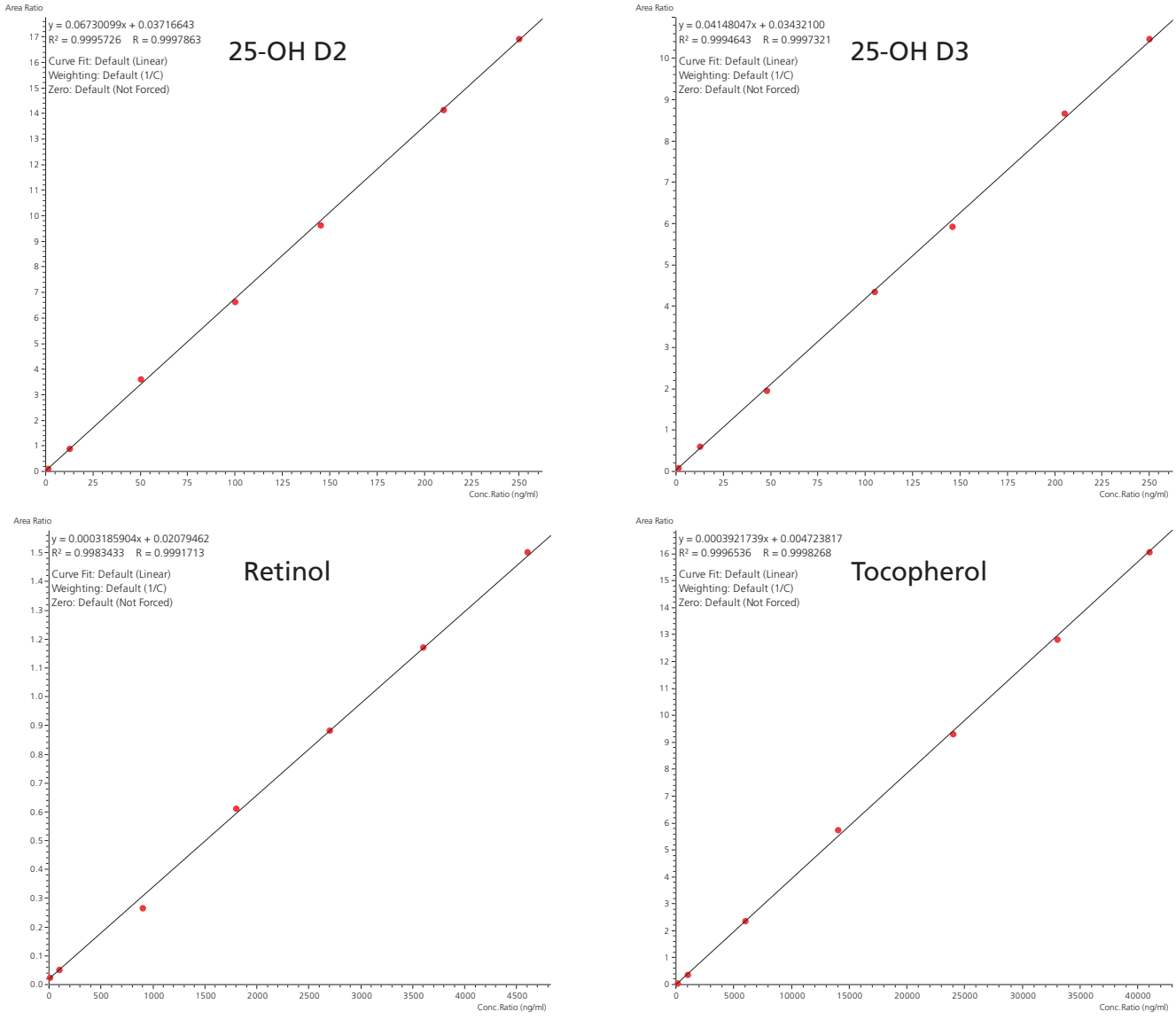

Figure 2 Typical Calibration Curves

High-Throughput Simultaneous Measurement of Vitamins A, D and E in Human Plasma by LDTD-MS/MS

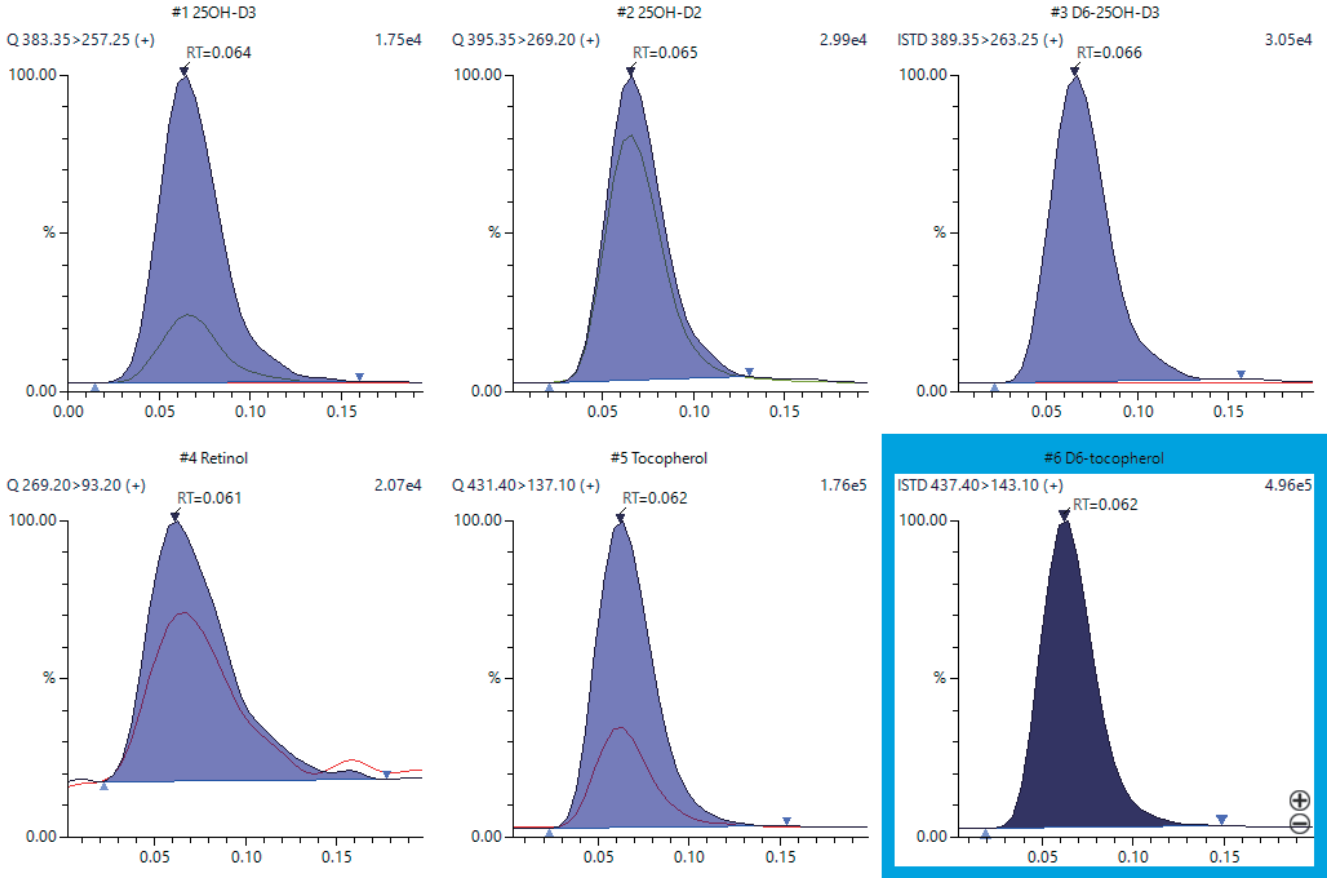

Figure 3 Chromatograms at the LLOQ

Recovery

Total recovery (i.e. combining extraction and matrix effect) was evaluated by comparing ISTD peak areas in real plasma samples to an equivalent prepared in solution. Each type of sample was prepared in triplicate. The mean recoveries were of 95% and 89% for $^{2}H_{6}$-cholecalciferol and $^{2}H_{6}$-tocopherol, respectively, illustrating the good extraction rate and the low matrix effect.

Precision and Accuracy

Precision and accuracy were evaluated by measuring the concentration of QC samples at four levels across 4 independent runs. In each run, 5 replicates of each QC were prepared and analyzed. Acceptance criteria were a relative standard deviation <15% (20% at the LOQ) and accuracy between 80-120%. Results are presented in Table 3.

High-Throughput Simultaneous Measurement of Vitamins A, D and E in Human Plasma by LDTD-MS/MS

Table 3 Precision and Accuracy

	25-OH D2	25-OH D3	Retinol	Tocopherol
Intra-run precision (%)	9.3 / 5.5 / 5.1 / 4.8	7.6 / 6.4 / 3.2 / 4.1	8.2 / 2.3 / 3.1 / 2.5	5.1 / 4.4 / 4.6 / 6.5
Inter-run precision (%)	10.2 / 6.3 / 5.2 / 5.6	8.0 / 6.4 / 5.0 / 4.8	8.5 / 4.2 / 3.0 / 4.8	7.2 / 4.5 / 6. 5 / 7.1
Average accuracy	92.5%	101%	96.2%	105%

Conclusions

A very high-throughput method to simultaneously analyze vitamin D, A and E was set up. The sample preparation remained simple and automatable to face high volumes of sample, and giving very good recoveries.

Next steps will include the addition of an internal standard for retinol (not received at the time of experiments) and evaluation of result correlation between LDTD-MS/MS and UHPLC-APCI-MS/MS methods on real samples.

Disclaimer: The products and applications in this presentation are intended for Research Use Only (RUO). Not for use in diagnostic procedures. Not available in China.

First Edition: June, 2018

SHIMADZU

Shimadzu Corporation

www.shimadzu.com/an/