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High-Throughput Vitamin K Profiling in Human Plasma by LDTD-MS/MS

ASMS 2018 TP-128

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Introduction

Vitamin K has an important role in blood coagulation and bone mineralization. However, current research is still trying to unravel the functions of vitamin K. For better understanding and large epidemiologic studies, performant analytical methods are necessary. Vitamin K is a combination of several lipophilic vitamers including phylloquinone (K1), menaquinones (K2) and menadiones (K3). Usually, circulating levels of vitamin K are lower than other fat-soluble vitamins, making the assay more challenging. As not all vitamers and their metabolites possess the same potency or toxicity, it is interesting to assay as much as possible of these compounds.
Here we present a method to simultaneously quantify vitamers and metabolites by Laser Diode Thermal Desoprtion-tandem mass spectrometry (LDTD™-MS/MS) in human plasma.

Methods and Materials

Reagents

Phylloquinone (Vitamin K1), Menaquinone-4 (MK-4), Menaquinone-7 (MK-7) and 2,3-epoxy-menaquinone-4 (Epoxy-MK-4) were selected as target compounds and provided by IsoSciences. $^{2}H_{6}$-tocopherol was used as internal standard (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 0.1 to 100 ng/mL for all compounds. 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 at a concentration of 1 µg/mL in ethanol.
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.

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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	: 15 ms / 1 ms

MRM :

Compound	MRM Quant	MRM Qual
Vitamin K1	451.40 > 186.95	451.40 > 199.10
MK-4	445.30 > 81.00	445.30 > 187.05
MK-7	649.50 > 95.30	649.50 > 187.00
Epoxy-MK-4	461.30 > 81.15	461.30 > 107.25
$^{2}H_{6}$-Tocopherol	437.40 > 143.10	

Results

Calibration

Calibration curves were calculated by internal standardization using a quadratic 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.

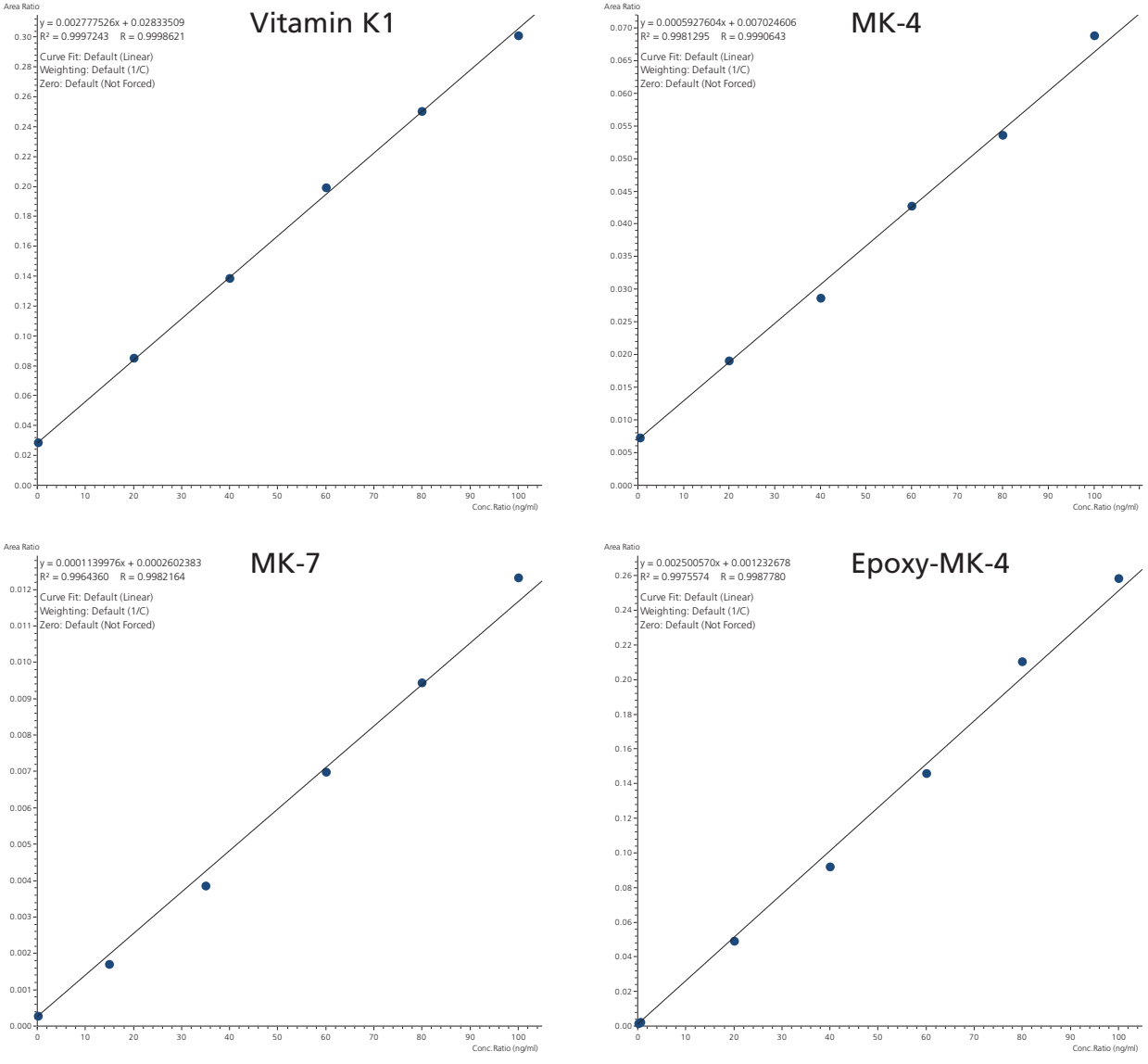

Figure 2 Typical Calibration Curves

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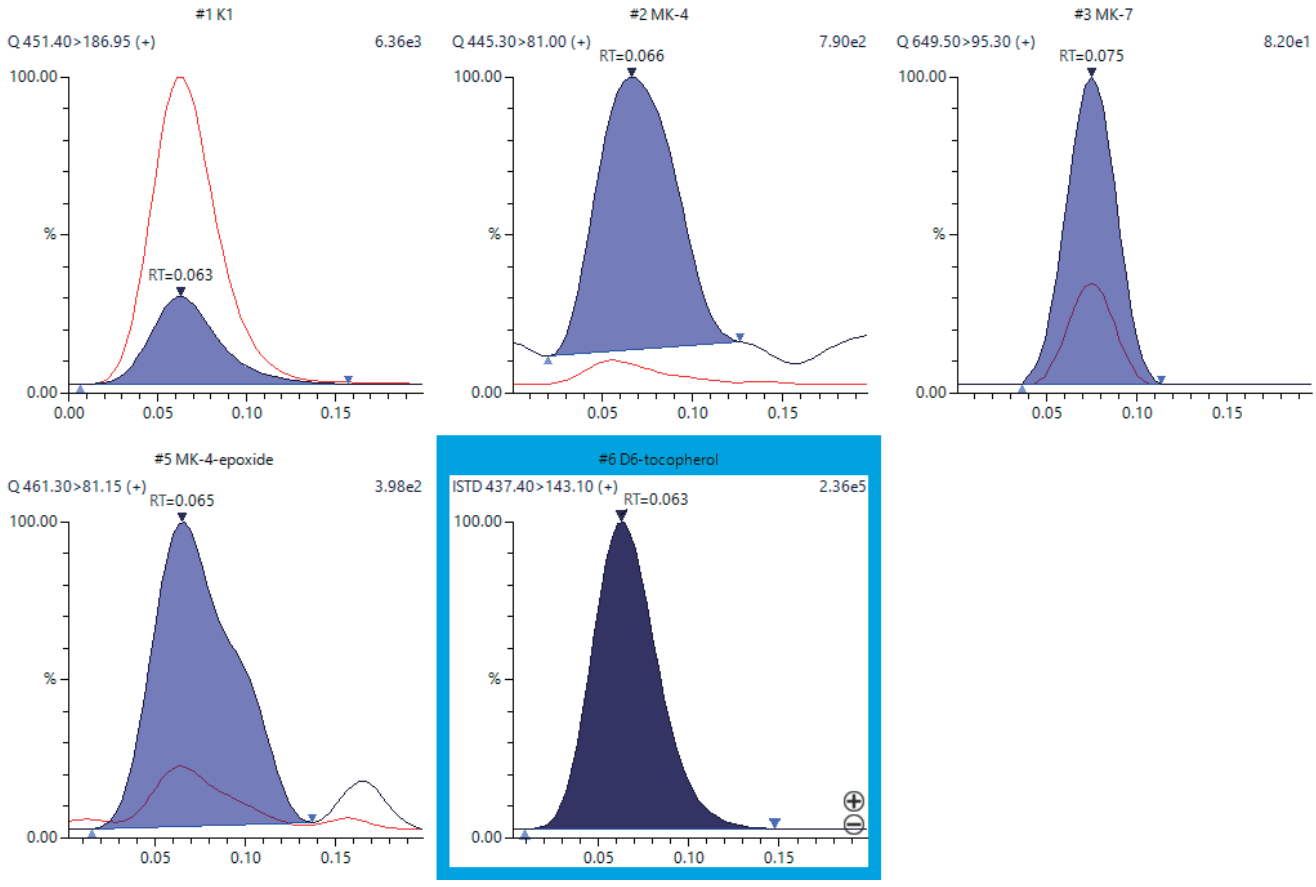

Figure 3 Mass Chromatograms at the Lower Limit of Quantification (equivalent to 33 fg per shot)

Recovery

Extraction recovery was evaluated by comparing peak areas in middle range QC to an equivalent prepared in solution. Each type of sample was prepared in five replicates. The mean recoveries were from 85 to 105% illustrating the good extraction rate.

Precision and Accuracy

Precision and accuracy were evaluated by measuring the concentration of QC samples at four levels across 3 independent runs. In each run, 5 replicates of each QC were prepared and analyzed. All intra-run and inter-run precision were inferior to 10%. Accuracies were comprised between 89.6 to 102%.

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Conclusions

A very high-throughput method to simultaneously analysis of vitamin K vitamers in 12 seconds was set up. The sample preparation remained simple and automatable to face high volumes of sample, and giving very good recoveries. Precision and accuracies are within classical requirements for quantitative analysis in biological matrices.
Next steps will include the addition of more specific internal standards, addition of Vitamin K1 epoxyde and evaluation of result correlation between LDTD-MS/MS and UHPLC-APCI-MS/MS methods on real samples.

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First Edition: June, 2018

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