Phytronix Breakaway Technologies

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Clinical

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Analysis of Vitamin K1 in Serum:

Quantification in Serum at 12 Seconds per Sample Using Luxon Ion Source®

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Introduction

Vitamin K1 (Phylloquinone) is an important compound that plays a key role in the synthesis of several blood coagulation factors, in bone metabolism and in vascular calcifications.

Our goal for this application note is to use a combination of efficient sample preparation and ion mobility technique for the quantification of vitamin K1 in serum using the Luxon Ion Source®, based on the LDTD® technology.

LDTD-MS/MS offers specificity combined with an ultra-fast analysis for an unrivaled quantification method. To develop this application, we focused on performing an efficient sample preparation. Vitamin K1 is analyzed, and results are obtained in less than 12 seconds per sample.

Luxon Ionization Source

The Luxon Ion Source (**Figure 1**) is the second-generation sample introduction and ionization source based on the LDTD technology for mass spectrometry. Luxon Ion Source uses Fiber-Coupled Laser Diode (**Figure 2**) to obtain unmatchable thermal uniformity providing more precision, accuracy, and speed. The process begins with dry samples which are rapidly evaporated using indirect heat. The thermally desorbed neutral molecules are carried into a corona discharge region. High efficiency protonation and strong resistance to ionic saturation characterize this type of ionization and is the result of the absence of solvent and mobile phase. This thermal desorption process yields high-intensity molecular ion signal in less than 1 second sample-to-sample and allows working with very small volumes.

Figure 1 - Luxon Ion Source®

Figure 2 - Schematic of the Luxon Ionization Source

Sample Preparation Method

Due to the instability of vitamin K1, stock solutions were prepared in ethanol (0.01% BHT). Pooled serum was exposed to UV light for at least 24h due to photodegradation of endogenous vitamin K. The pooled exposed serum was then spiked to generate a calibration curve and QC.

- 200 µL of serum sample were transferred to a tube.
- 10 µL of Internal standard (Phylloquinone-D_7, 100 ng/mL in EtOH (0.01% BHT) were added to each sample.
  - Mix
- 200 µL of EtOH (0.01%BHT) were added.
  - Mix
- 1000 µL of Hexane were added.
  - Mix
  - Centrifuge 3 minutes/5000 rpm
- 600 µL of upper layer phase were transferred into a new tube and evaporated to dryness.
- Tubes were reconstituted with 1000 µL of Hexane.
- SPE (Silica: 100 mg/1cc)
  - Activation:
  - 1 mL Hexane: Diethyl ether (1:1)
  - 3 X 1 mL Hexane
- Load:
  - 1 mL reconstituted sample
- Wash:
  - 3 X 1 mL Hexane
- Elution:
  - 1 mL Hexane: Diethyl ether (97:3)
- Spot 6 µL of the elution phase onto a LazWell™ 96 plate
  - Dry 3 minutes at room temperature

LDTD®-MS/MS Parameters

LDTD
Model: Luxon S-960, Phytronix
Carrier gas: 7.5 L/min (air)
Laser pattern:

- 9-second ramp to 75% power

Ion mobility
SelexIon QTrap® System 5500, Sciex
DMS temperature (DT): Low
No modifier
Separation voltage (SV): 4200
Compensation voltage (COV): 18
DMS offset (DMO): -3

MS/MS
MS model: QTrap® System 5500, Sciex
Curtain: 30
CAD: 8
IonSpray Voltage: 4800
Temperature, GS1 and GS2 set to zero.
Scan Time: 100 msec
Total run time: 12 seconds per sample
Analysis Method: Positive MRM mode

Table 1 - MRM transitions for LDTD-MS/MS

	Transition	CE
Vitamin K1	451.2 → 187.1	35
Phylloquinone-d7	458.3 → 194.1	35

Results and Discussion

Validation Test

Calibration curves ranging from 200 to 5200 pg/mL were prepared in a photodegraded serum. Replicate extractions were deposited onto a LazWell™ plate and dried before analysis. The peak area against the internal standard (IS) ratio was used to normalize the signal.

Linearity

The calibration curves were plotted using the peak area ratio and the nominal concentration of standards. For the linearity test, the following acceptance criteria was used:

- Linear regression (r) must be ≥ 0.995

Table 2 shows the inter-day correlation coefficients for Vitamin K1. Values greater than 0.995 are obtained. **Figure 3** shows a typical calibration curve result for Vitamin K.

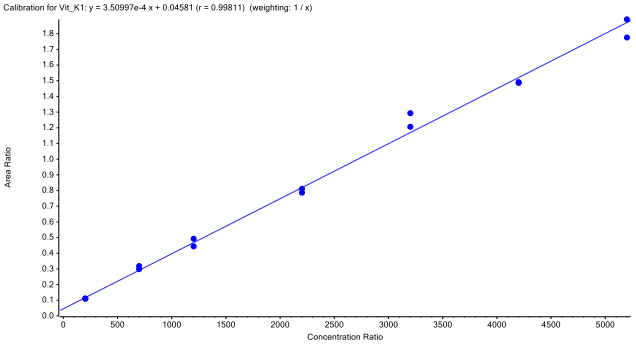

Figure 3 – Vitamin K1 calibration curve

Table 2 – Inter-day calibration curve correlation coefficients

	Vitamin K1
Curve 1	0.99811
Curve 2	0.99718
Curve 3	0.99714
Curve 4	0.99796
Curve 5	0.99835

Precision and Accuracy

For the accuracy and precision evaluation, the following acceptance criteria were used:

- Each concentration must not exceed 15% CV
- Each concentration must be within 100 ± 15% of the nominal concentration

For the intra and inter-run precision and accuracy experiment, each QC was analyzed in sextuplicate, on five different days. **Table 3 and 4** shows the intra and inter-run precision and accuracy results for Vitamin K1. The obtained %CV was below 15% and the accuracy was within 15% of the nominal value.

Table 3 - Intra-Run Precision and Accuracy of Vitamin K1

Vitamin K1	QC-L	QC-M	QC-H
Conc (pg/mL)	700	2200	4200
N	6	6	6
Mean (pg/mL)	679.9	2186.1	4228.5
%CV	10.9	5.5	3.9
%Nom	97.1	99.4	100.7

Table 4 - Inter-Run Precision and Accuracy of Vitamin K1

Vitamin K1	QC-L	QC-M	QC-H
Conc (pg/mL)	700	2200	4200
N	30	30	30
Mean (pg/mL)	674.7	2156.7	4280.8
%CV	12.5	5.2	4.3
%Nom	96.4	98.0	101.9

Wet Stability of Sample Extracts

Following the extraction, sample extracts are kept at 4°C in closed containers protected from light. After 4 days, sample extracts are spotted on a LazWell™ plate, dried and analyzed. Precision and accuracy of QC samples are reported in **Table 5**. All the results are within the acceptable criteria range for 4 days at 4°C.

Dry Stability of Samples Spotted in LazWell™

Extracted samples are spotted onto a LazWell™ plate, dried and kept at room temperature for 1 hour before analysis. The precision and accuracy results of QC samples are reported in **Table 5**. All the results are within the acceptable criteria range for 1 hour at room temperature.

Table 5 - Wet and Dry Stability of Vitamin K1

Parameters	Dry stability (1 hour / RT)			Wet stability (4 days / 4°C)		
QC	QC-L	QC-M	QC-H	QC-L	QC-M	QC-H
Conc. (pg/mL)	700	2200	4200	700	2200	4200
N	6	6	6	6	6	6
Mean (pg/mL)	682.5	2086.8	4181.2	649.6	2206.7	4191.0
%CV	5.3	7.4	6.3	4.0	3.7	1.9
%Nom	97.5	94.9	99.6	92.8	100.3	99.8

Cross validation study

Real patients' serum samples (N=6) have been tested with this method to correlate with results obtained by traditional LC-MS/MS. The percentage of difference between the values are evaluated. A difference below 15% is obtained. Results are reported in Table 6.

Table 6 - Comparison between Vitamin K1 concentration values

Vitamin K1	LC (pg/mL)	Luxon (pg/mL)	%Diff (%)
M1	528.2	615.9	7.7
M2	641.2	604.8	-2.9
M3	324.3	362.1	5.5
M4	984.6	975.6	-0.5
M5	484.4	628.6	13.0
M6	810.8	788.9	-1.4

Conclusion

The Luxon Ion Source® combined with Sciex QTrap® 5500 mass spectrometer system allows the ultra-fast (**12 seconds per sample**) analysis of Vitamin K1 in serum.