



SiliaPrep™

## Analysis of Vitamin K1 in Serum

Analysis of Vitamin K1 (*phylloquinone*) in human serum requires low limit of quantitation (LOQ), therefore efficient sample preparation to eliminate all potential interferants prior to analysis. Phytronix Technologies demonstrated that combining liquid-liquid extraction (LLE) to a solid-phase extraction (SPE) using SiliaPrep SPE cartridges allowed to get enough sensitivity. The samples were then successfully analyzed with a runtime of 12 seconds using LDTD-MS/MS technique.

---

**LEARN MORE**

about SiliaPrep SPE cartridges in our brochure "*Solutions For Sample Preparation*".

Sufficient level of Vitamin K1 is necessary to stay healthy as it plays a key role in the formation of blood coagulation factors. An efficient and sensitive method of analysis is therefore required to check on a patient's condition if a health problem is suspected.

## EXTRACTION

### Step 1 – LLE

- Transfer 200  $\mu$ L of serum to a tube.
- Add 10  $\mu$ L of internal standard (*Phylloquinone-D7*, 100 ng/mL in EtOH (0.01 % BHT)) to each sample.
- Add 200  $\mu$ L of EtOH (0.01 % BHT).
- Add 1,000  $\mu$ L of hexane.
- Centrifuge 3 minutes at 5,000 rpm.
- Transfer 600  $\mu$ L of the upper layer phase into a new tube and evaporate to dryness.
- Reconstitute the tubes with 1,000  $\mu$ L of hexane.

### Step 2 – SPE

**Table 1:** Human serum extraction using SiliaPrep SPE cartridges

Human Serum Extraction Using SiliaPrep SPE Cartridges	
Parameter	Value
CARTRIDGE	SiliaPrep SPE cartridge, Silica, 100 mg, 1 mL, 40 - 63 $\mu$ m, 60 Å
PART NUMBER	SPE-R10030B-01C
CONDITIONING / EQUILIBRATION STEPS	1 mL of hexane:diethyl ether (1:1) 3 x 1 mL of hexane
LOADING STEP	1 mL of the reconstituted sample
WASHING STEP	3 x 1 mL of hexane
ELUTION STEP	1 mL of hexane:diethyl ether (97:3)
FURTHER ANALYSIS	Spot 6 $\mu$ L of the elution fraction onto a LazWell™ 96 plate Dry 2 minutes at room temperature

## RESULTS

Six serum samples from real patients were extracted following this protocol then analysed by LDTD-MS/MS. Vitamin K1 concentrations obtained are presented in Table 2.

**Table 2:** Vitamin K1 concentration from 6 real patients

Vitamin K1 Concentration from 6 Real Patients	
Patient Number	Vitamin K1 Concentration (pg/mL)
1	615.9
2	604.8
3	362.1
4	975.6
5	628.6
6	788.9

## CONCLUSION

The extraction method allowed efficient and selective sample preparation to extract vitamin K1. Samples were efficiently analyzed by LDTD-MS/MS in 12 seconds with an excellent linearity, accuracy, and precision.

Thanks to Phytronix Technologies for sharing their results.

