



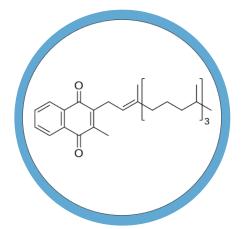
SiliaPrep[™]

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

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	

Table 2: Vitamin K1 concentration from 6 real patients





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.

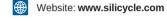
Thanks to Phytronix Technologies for sharing their results.



SiliCycle Inc. 2500, Parc-Technologique Blvd, Quebec City (Quebec) G1P 4S6 CANADA Phone: +1 418.874.0054

Toll Free +1 877.745.4292 (North America only)

Email: info@silicycle.com



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