Poster

[P27-8] P27-8: Assay and monitoring
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[P27-8-7] A new LC-MS/MS method for determination of vitamin K1 and vitamin K2 (MK-4, MK-7) in serum

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Background
Vitamins K1 and K2 are the cofactors for the enzyme gamma-glutamylcarboxylase, which is involved in carboxylation of the vitamin K-dependent proteins. The role of vitamin K extends well beyond the regulation of blood clotting to impact bone formation, and development of heart disease, and possibly cancer. Objective of this study was to evaluate a new LC-MS/MS method for determination of vitamin K1 and two forms of vitamin K2 (MK-4, MK-7) in patient serum.

Methods
A UPLC-tandem mass spectrometry method for the determination of vitamin K1 and vitamin K2 (MK-4 and MK-7) in human serum was developed. Mass spectrometric detection was performed on a triple-quadrupole in the possitive elecrospray ionization mode by multiple reaction monitoring (Agilent 1290 with Triple Quad 6470, Agilent Technologies). K1-d7 and MK-4-d7 were used as internal standards. 20 μL of IS mixture were added to 500 μL of serum and 2 mL of ethanol were added to precipitate the proteins. The mixture was extracted with 4 mL of hexane for 10 min and then centrifuged. Solid phase extraction was then used. Chromatographic separation was curried out on SB-C8 column (2.1x100 mm, 1.8 m, Agilent Technologies) using a binary gradient of mobile phases (A - 0.1% ammonium fluoride in 50% methanol, B –0.1% ammonium fluoride in methanol). The separation was accomplished at 40 ˚C. The analysis time was 9 minutes.

Results
The LC-MS/MS method has been successfully validated. The linearity was excellent up to 10 ng/mL for all three forms of vitamin K. Lower limits of quantitation were 0.03 ng/mL for K1 and MK-4, and 0.05 ng/mL for MK-7. The intra and interday accuracy and precision were evaluated on two QC samples by multiple analysis and coefficients of variation were 2.3-7.0% for intra-assay, 7.2-11.3% for inter-assay, and 0.5-6.6% for precision.

Conclusions
We developed and fully validated a new LC-MS/MS method for determination of vitamin K1, MK-4 and MK-7, which could be implemented into the practise, because new studies have reported that daily vitamin K intake improves bone metabolism, vascular calcification, and cardiovascular risk, and this method allows the determination of the optimal dose.