A Simultaneous Quantification of Fat Soluble Vitamins Using Liquid Chromatography-Tandem Mass Spectrometry

A Albahrai1, V Rotaru1, P Roche1, R Greaves1,2,3
1School of Medical Sciences, RMIT University, Bundoora, VIC, Australia
2Centre for Hormone Research, Murdoch Children’s Research Institute Parkville, VIC, Australia
3Australasian Association of Clinical Biochemists Vitamins Working Party, Australia

Introduction
Non-classical roles of fat soluble vitamins (FSVs) in many pathologies including cancer have been identified. It is challenging to accurately and simultaneously quantify FSVs (vitamin A, D and E) because of lack of reference methods for vitamin A and E recognised by the Joint Committee for Traceability in Laboratory Medicine (JCTLM). Reference methods are essential to achieve traceable measurement. This study aimed to develop a method for simultaneous quantification of 25-hydroxyvitamin-D2 (25-OH-vit-D2), 25-hydroxyvitamin-D3 (25-OH-vit-D3) and its 3-epi-isomer (3-epi-OH-vit-D3), retinol and α-tocopherol in human serum using liquid chromatography-tandem mass spectrometry (LC-MSMS).

Method
This method was developed and validated using commercial calibrators and controls, and deuterated internal standards. Commercial calibrators for vitamin D (RECIPe) and for vitamin A and E (Bio-Rad Laboratories), commercial controls (UTAK Laboratories) and in-house controls were used. The samples were liquid-liquid extracted and injected into Agilent LC-MSMS 6490 system using Pursuit-PFP column (150 mm x 2 mm) with ESI (positive mode) and multiple reaction monitoring (MRM).

Results
Separation and quantification of 25-OH-vit-D3 from its 3-epi-isomer as well as 25-OH-vit-D2, retinol and α-tocopherol were achieved. The dynamic ranges were 4-160 nmol/L for 25-OH-vit-D2 and 3-epi-25-OH-vit-D3, 4-200 nmol/L for 25-OH-vit-D3, 0.1-4 μmol/L for retinol and 4-70 μmol/L for α-tocopherol with linear regression (r²) 0.957-0.998. Method validation experiments demonstrated < 6% and < 15% for the intra-run and inter-run imprecision (CV%), respectively, and 87-112% for the recoveries of the investigated vitamins.

Conclusion
A simple LC-MSMS method was developed and validated for simultaneous quantification of the three vitamin D compounds as well as vitamin A (retinol) and vitamin E (α-tocopherol) in serum.

Sample Type Source
Blank • Milli-Q water
Calibrators • Vitamin A and E calibrator, multi-level in-house prepared using single concentration level calibrator (Bio-Rad Laboratories)
• Vitamin D (25-OH-vit-D2/D3) calibrator*, multi-level set (RECIPe)
Controls • Vitamin D control, tri-level set (UTAK Laboratories)
• Vitamin A and E control, tri-level set (UTAK Laboratories)
• 3-Epi-25-OH-vit-D3 controls**, tri-level set (in-house prepared)
Human serum • Healthy volunteers

Sample Preparation
• 100 μL of serum + 100 μL of Milli-Q water were vortexed
• 200 μL of methanol containing deuterated internal standards was added and vortexed then equilibrated for 10 minutes
• 1.5 mL of hexane was added and vortexed extensively prior to centrifugation at 3000 rpm for 5 minutes
• Organic layer was transferred into a new glass tube then dried under nitrogen gas at room temperature
• Samples were reconstituted in 250 μL methanol and injected into LC-MSMS system

Acknowledgement:
The work was conducted on an Agilent LC-MSMS 6490 system as part of the RMIT University-Agilent Technologies collaboration Program