Rapid supercritical fluid chromatography tandem mass spectrometry (SFC-MS/MS) for the routine quantification of retinol and alpha-tocopherol in human serum/plasma

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Introduction

Supercritical fluid chromatography (SFC) employs carbon dioxide as the mobile phase when near its critical temperature and pressure. These conditions allow for low viscosity and high diffusivity during separation, resulting in faster and improved separation. We used Ultra Performance Convergence Chromatography™ (UPC^2), a new generation of SFC, coupled to an Xevo TQSmicro mass spectrometer (MS) to determine endogenous levels of retinol and alpha-tocopherol in human serum and plasma.

Analytical Method

Samples were prepared for analysis using an automated MultiPurpose Sampler (MPS) in which a protein crash using 1% acetic acid in acetonitrile solution was followed by extraction of the lipid phase into heptane. Each sample is subsequently vortexed and centrifuged for 30 seconds to assist protein precipitation and separation. Samples were transferred to the UPC^2 sample manager and 1 µl of extracts were further separated in an ACQUITY UPC2 HSS C18 column maintained at 35°C. A flow rate of 1.5ml/min was applied to the column, using a gradient elution of supercritical carbon dioxide and methanol (2%-10% methanol), and 1% formic acid in methanol solution for the make-up pump. Detection and quantification was carried out by operating the MS in positive electrospray ionisation (ESI) multiple reaction monitoring (MRM) mode. Chromatographic baseline separation of the vitamins was achieved in 2.5 min.

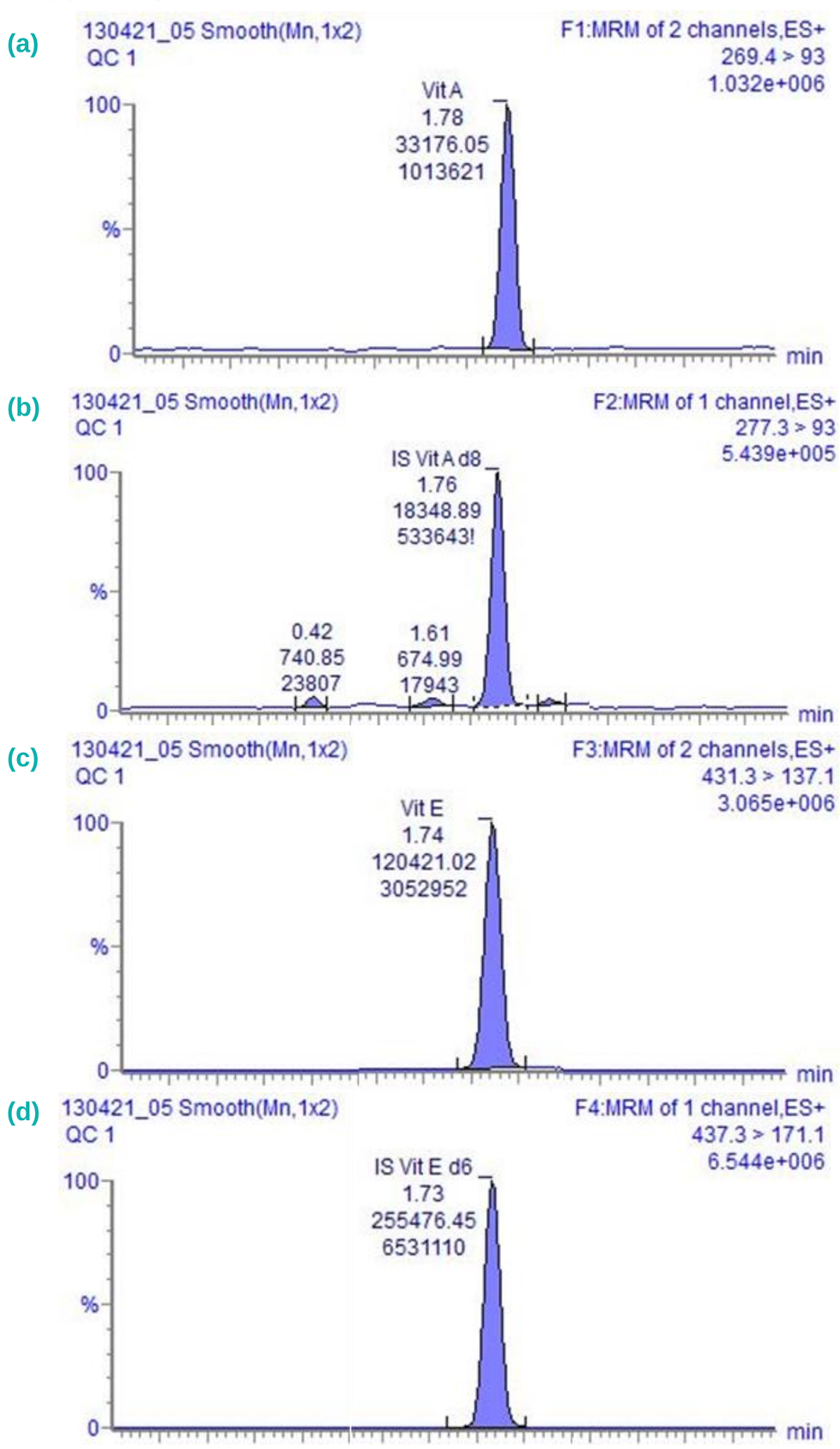

Figure 1 – Typical chromatograms: (a) Retinol quantifier ion, (b) Retinol-d8 IS, (c) alpha-tocopherol quantifier ion, (d) Alpha-tocopherol- d6 IS

Method Summary

Instrumentation:	ACQUITY UPC2 with Xevo TQSmicro MS
Column:	ACQUITY UPC2 HSS C18 column, 1.8µm, 2.1 mm X 100 mm
Analyte:	Retinol and alpha-tocopherol
Internal Standard:	Retinol-d8 and alpha-tocopherol-d6
Mass Transitions:	Retinol: 269.4 > 93 Alpha-tocopherol: 431.3 > 137.1
Mobile phase:	Solvent A: CO2 Solvent B: Methanol
ABPR:	2200 psi
Make-up pump (ISM)	Solvent: 1% Formic Acid in methanol
Flow rate:	1.5ml/min
LLOQ:	Retinol: 0.09 µmol/L Alpha-tocopherol: 0.78 µmol/L
Sample extraction volume:	1µl
Injection time:	4.0 minutes

Results

Representative chromatograms are shown in Figure 1. The method proved to be linear in the calibration range for retinol up to 14 µmol/L and for alpha-tocopherol up to 111 µmol/L, with lower limits of quantification of 0.09 µmol/L and 0.78 µmol/L respectively. Inter- and intra-assay CVs were ≤ 8% for both vitamins, mean recoveries were 83-116%. Performance of the method has been assessed by UK NEQAS for the past two years. Reports show superior accuracy (A) scores of 65 and 80 for retinol and alpha-tocopherol respectively when compared to the median accuracy scores of all labs and method specific labs, illustrated in Figure 2.

	Method Principle	Your Method	A score with trend arrow	Method median A score	All lab median A score
Vitamin A	Mass spectrometry	Recipe calibrant [2RP]	65 ● ↗	148	86
Vitamin E	Mass spectrometry	Recipe calibrant [2RP]	80 ● ↔	125	99

Figure 2 - UK NEQAS EQA performance, January 2021: accuracy (A) scores of UPC^2–MS/MS method for retinol (vitamin A) and alpha-tocopherol (vitamin E) analysis. A satisfactory A score is defined as below 100. Both scores are superior to all lab median A score and method specific median A score.

Conclusion

SFC-MS/MS provides improved specificity and significantly faster analysis compared with HPLC-based methodologies. We have used this methodology for the analysis of >10,000 samples over a period of two years. EQA assessment reports the analysis to be performing consistently well. The MPS reduces sample preparation time and complexity, limiting the potential for human errors and freeing up time of laboratory staff. Other benefits of this method include reduced use of solvents; approximately 85% less.

References used during method validation:

Hinchliffe E, Rudge J, Reed P. A novel high-throughput method for supported liquid extraction of retinol and alpha-tocopherol from human serum and simultaneous quantitation by liquid chromatography tandem mass spectrometry. Ann Clin Biochem. 2016;53(4):434-445.

Greaves R, Jolly L, Woollard G, Hoad K. Serum vitamin A and E analysis: comparison of methods between laboratories enrolled in an external quality assurance programme. Ann Clin Biochem. 2010;47:78-80

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