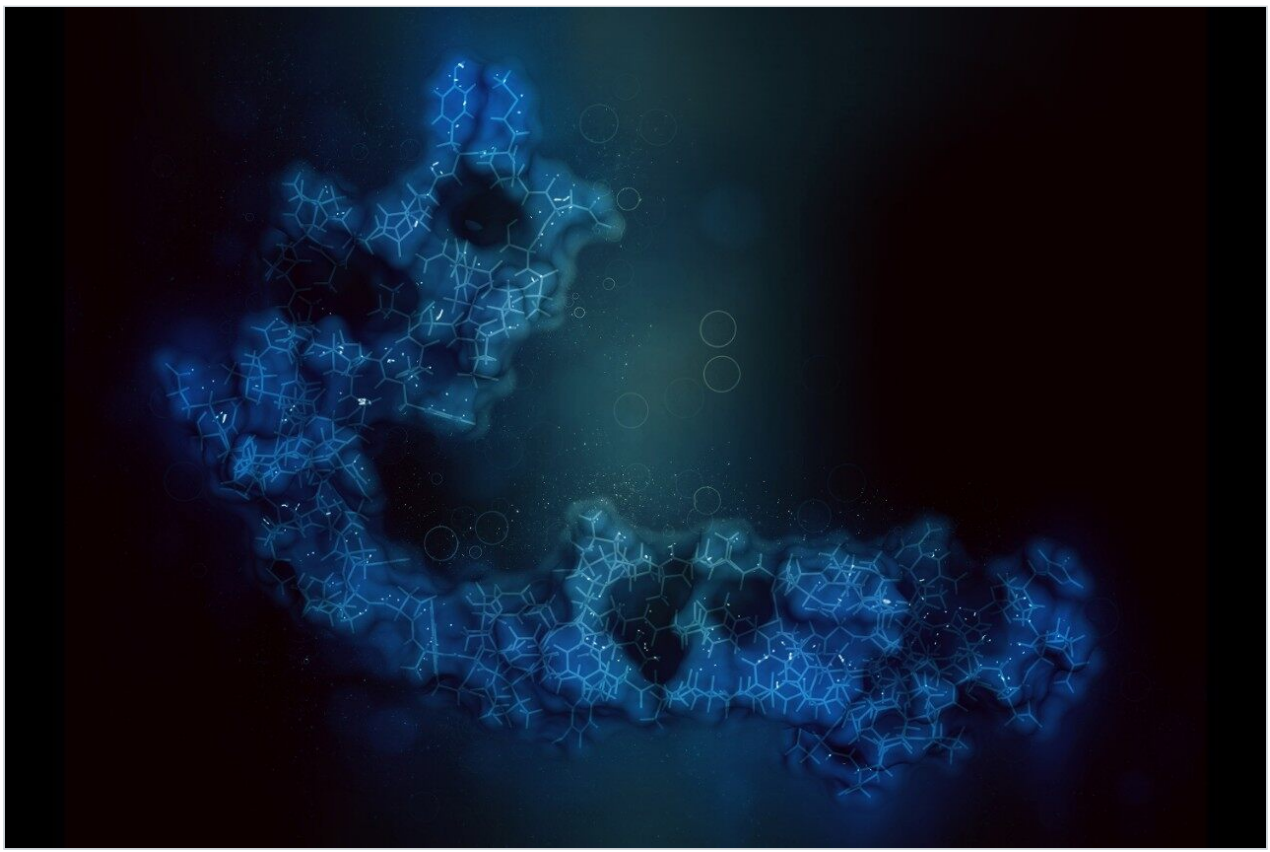
Waters™

□□□□

# Successful Transfer of a 95-minute Nanoflow LC-MS/MS Analysis of Serum Proteins to a 5-minute UPLC-MS/MS Method

Richard G Kay, Philip B Grace, Philip Teale, John Hicks

Quotient Bioresearch, Waters Corporation



*For research use only. Not for use in diagnostic procedures.*

## Abstract

This application note demonstrates the successful transfer of a 95-minute nanoflow HPLC-MS/MS analysis of serum proteins to a 5-minute UPLC-MS/MS method, utilizing the Waters ACQUITY UPLC System.


## Introduction

Serum and plasma are good sources of protein biomarkers to disease, as they perfuse all the tissues in the body. Therefore, the detection and measurement of these proteins could enable the early diagnosis of the onset of a specific disease.

The application of LC-MS/MS to biomarker identification requires the proteins to be tryptically digested and traditionally involves nanoflow chromatographic analysis. Although this approach gives high sensitivity and specificity, it involves long analytical run times. If LC-MS/MS is to be used for serum protein quantitation in a clinical environment, the throughput and reproducibility of analysis needs to be significantly increased to handle the large number of samples that would be involved for disease screening purposes.

In this work, we demonstrate the successful transfer of a 95-minute nanoflow HPLC-MS/MS analysis of serum proteins to a 5-minute UPLC-MS/MS method, utilizing the Waters ACQUITY UPLC System.

## Experimental

### Sample Preparation and HPLC-MS/MS Conditions

A human serum sample was depleted of high abundance proteins using an acetonitrile-based extraction method.[1]

The sample was then tryptically digested and analysed by nanoflow liquid chromatography tandem mass spectrometry using an UltiMate 3000 (Dionex) and a 4000 Q Trap (Applied Biosystems/SCIEX) using a 95-minute method (Figure 1, Table 1). The column used for this analysis was a PepMap C18 (Dionex) fused silica nanobore column (0.075 x 150 mm, 3.5 µm), with a constant flow rate of 300 nL/min.

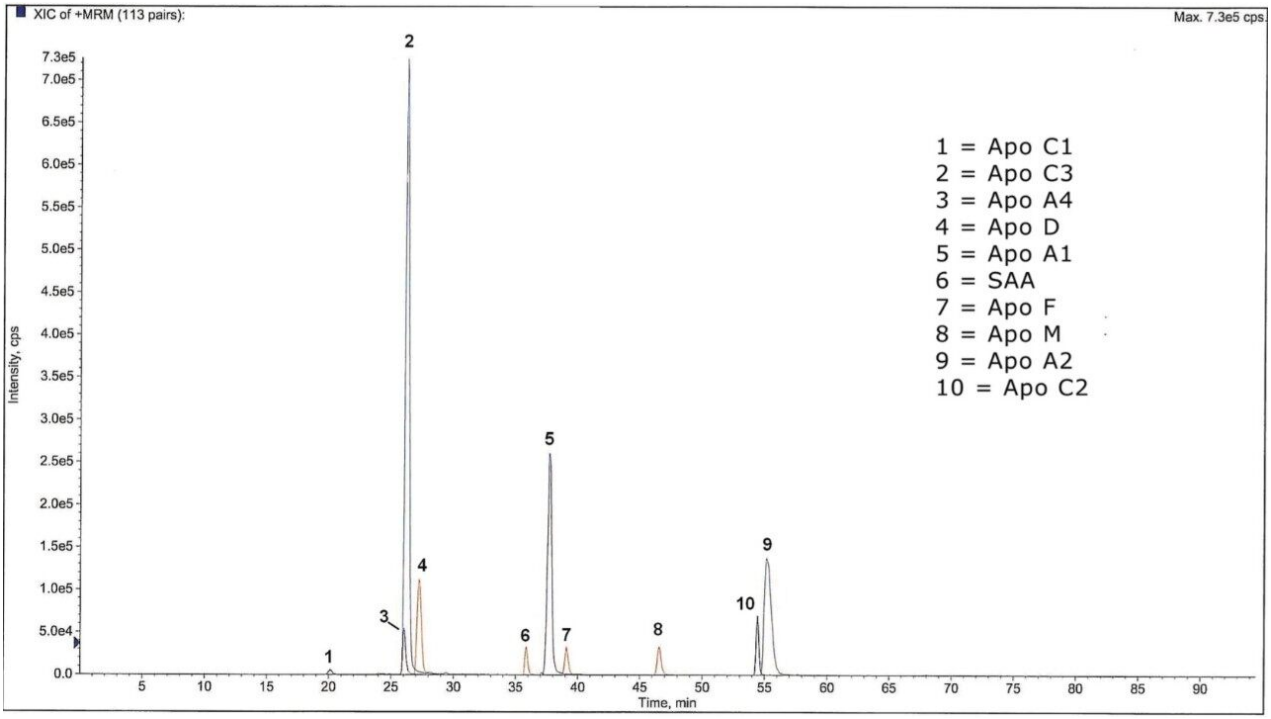



*Figure 1. Nano LC-MS/MS analysis.*

| Time (min) | %A |
|---|---|
| 0 | 100 |
| 3 | 100 |
| 5 | 95 |
| 60 | 40 |
| 70 | 10 |
| 82.5 | 10 |
| 82.6 | 100 |
| 95 | 100 |

*Table 1. Nano LC Conditions.*

Peptides specific for 9 Apolipoproteins (Apo) including A1, A2, A4, C1, C2, C3, D, F, and M, and also a peptide from serum amyloid A (SAA), were among a number detected using a multiple reaction monitoring (MRM) based MS approach.

The solvents used in the analysis were A = 0.1% formic acid in water (v/v) with 2% ACN, and B = 0.1% formic acid in water (v/v) with 90% ACN.

## Sample Preparation and UPLC-MS/MS Conditions

The same sample was subsequently analysed on a system consisting of a Waters ACQUITY UPLC System coupled to an API 5000 (Applied Biosystems/SCIEX) Mass Spectrometer. The column used for the UPLC analysis was an ACQUITY UPLC BEH $C_{18}$ Column (2.1 x 100 mm, 1.7 µm), with a flow rate at a constant 700 µ L/min.

The same solvents were used as in the nanoflow analysis, however, possible particulates were removed from the solvents using a 0.2 micron filter prior to purging the solutions through the ACQUITY UPLC System. Only 10 transitions were used – those that were specific for the apolipoproteins detected in the nanoflow analysis.

Only four injections were performed for method transfer, where the LC Conditions were modified following results from the previous analysis. The first injection involved a 14-minute method (Figure 2), the second an 8-minute method (Figure 3) and the final two were 5-minute methods (Figures 4 and 5). LC Conditions for the four UPLC runs are displayed in Tables 2 to 5.

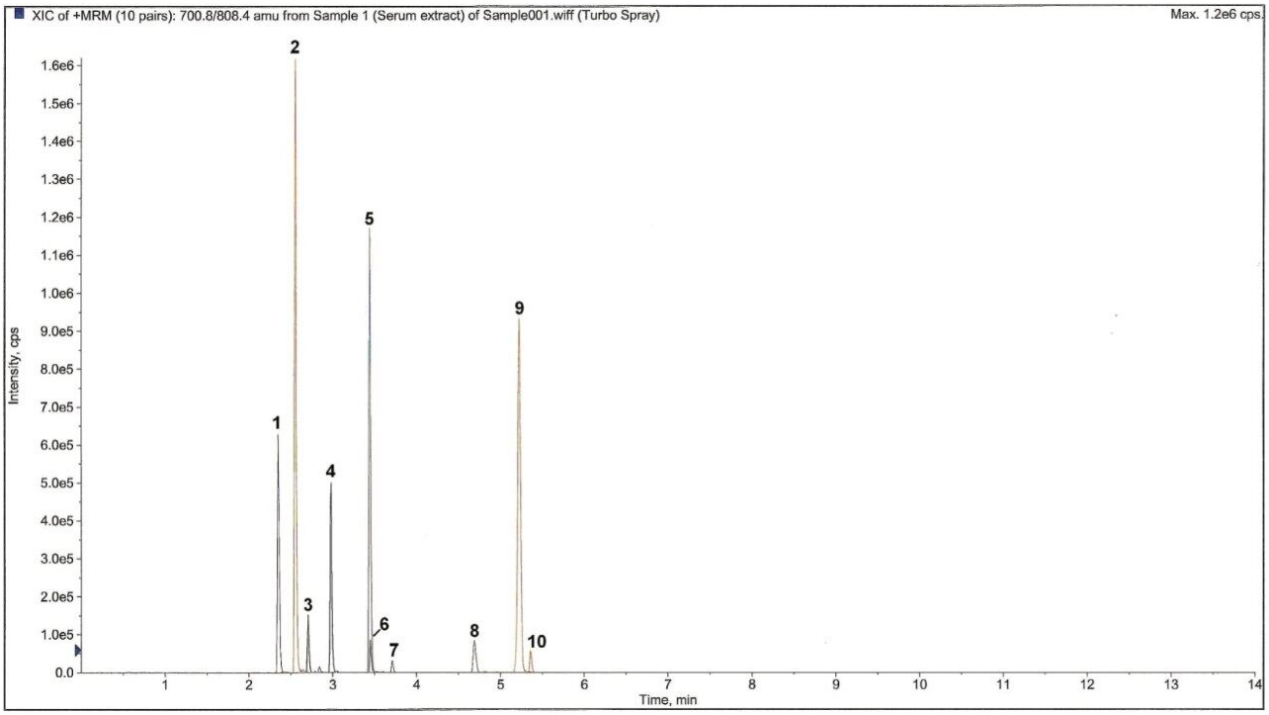



*Figure 2. UPLC-MS/MS analysis, a 14-min method.*

| Time (min) | %A |
|---|---|
| 0 | 0 |
| 8 | 60 |
| 10 | 90 |
| 12 | 90 |
| 12.1 | 0 |
| 14 | 0 |

*Table 2. 14-min method conditions.*

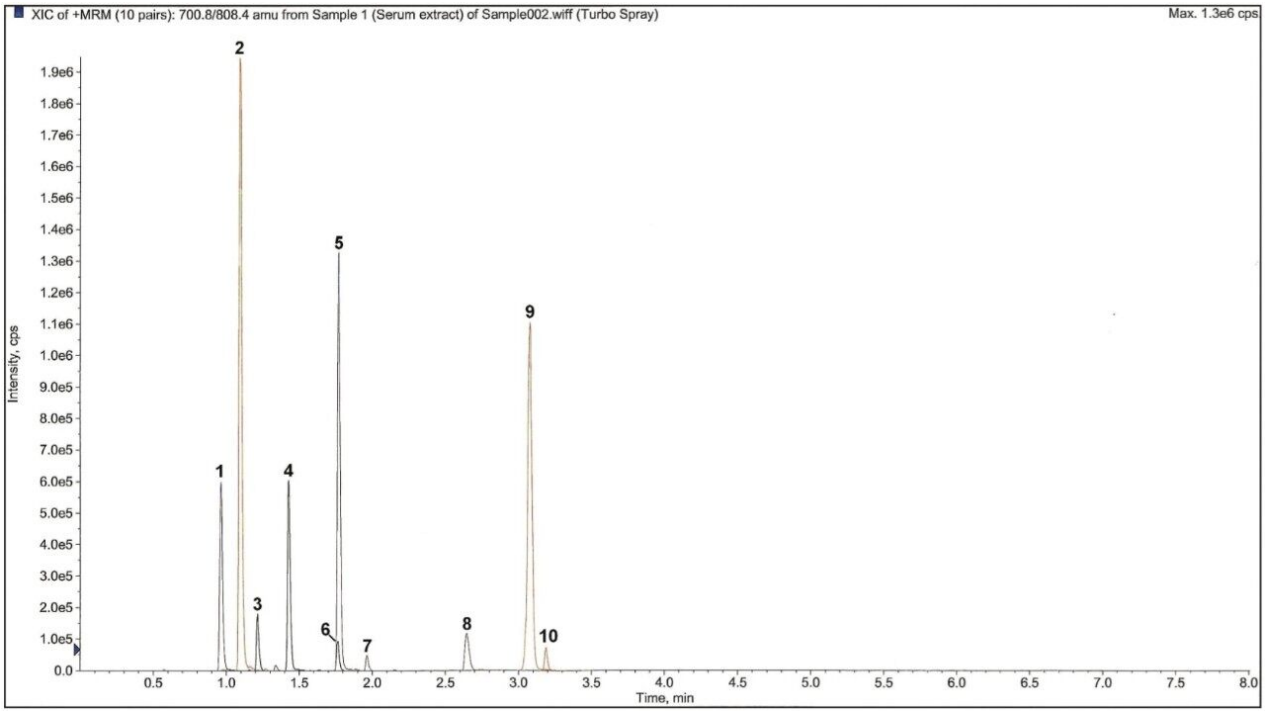



*Figure 3. UPLC-MS/MS analysis, an 8-min method.*

| Time (min) | %A |
|---|---|
| 0 | 0 |
| 4 | 60 |
| 5 | 90 |
| 6 | 90 |
| 6.1 | 0 |
| 8 | 0 |

*Table 3. 8-min method conditions.*

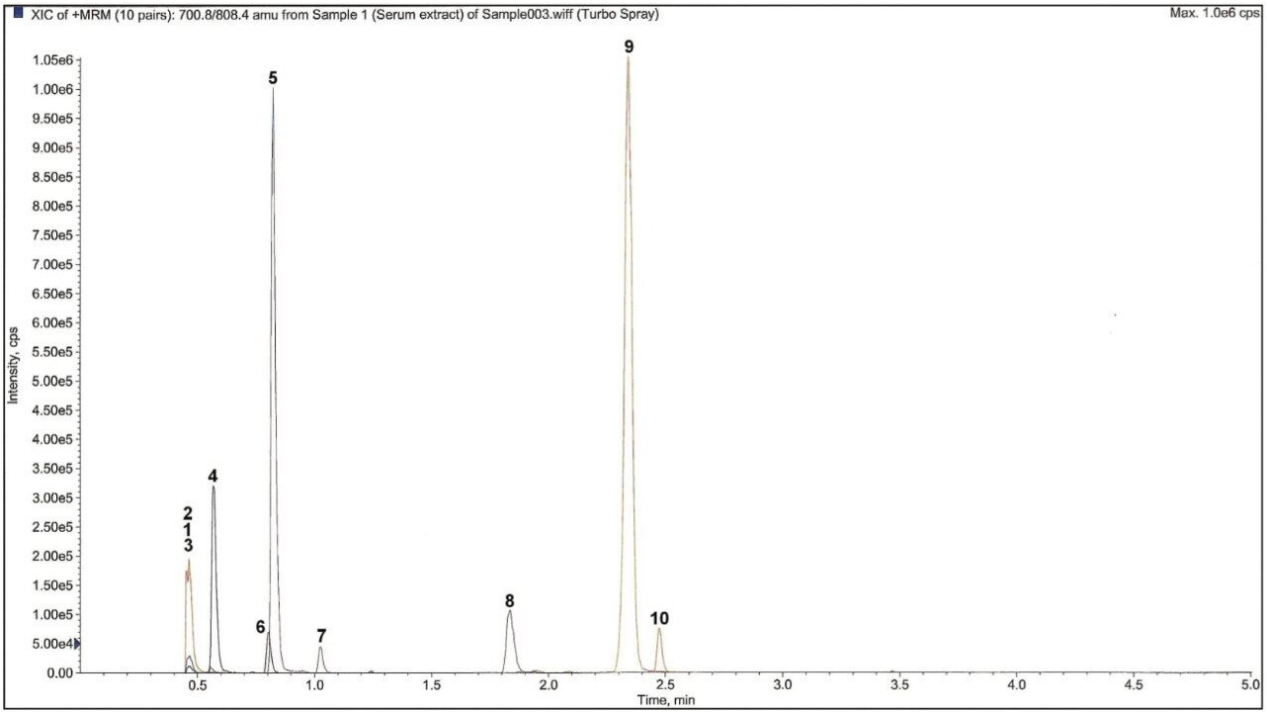



*Figure 4. UPLC-MS/MS analysis, a 5-min method.*

| Time (min) | %A |
|---|---|
| 0 | 20 |
| 3 | 45 |
| 3.1 | 90 |
| 4 | 90 |
| 4.1 | 20 |
| 5 | 20 |

*Table 4. 5-min method conditions.*

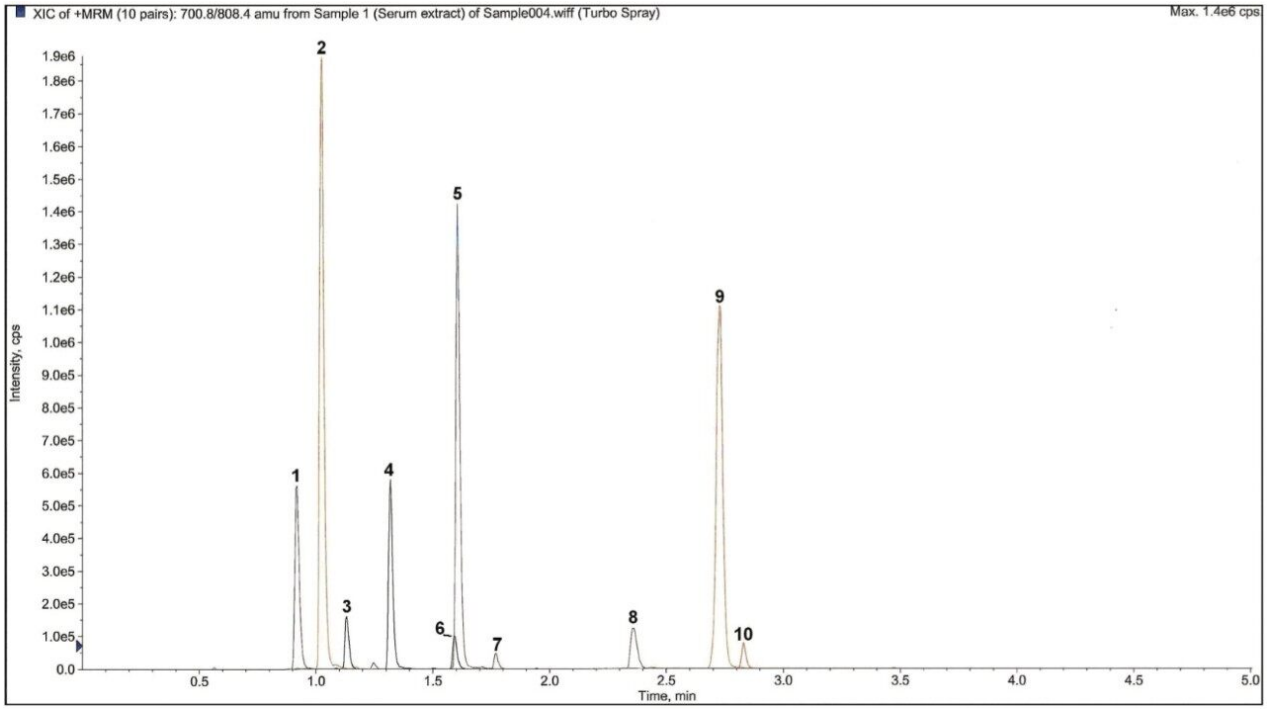



*Figure 5. UPLC-MS/MS analysis, also a 5-min method.*

| Time (min) | %A |
|---|---|
| 0 | 10 |
| 3 | 45 |
| 3.1 | 90 |
| 4 | 90 |
| 4.1 | 10 |
| 5 | 10 |

*Table 5. Final method conditions.*

## Results and Discussion

In transferring the assay from a 90-minute nanoflow method to a UPLC method, four injections were made, resulting in the reduction of the run time from 14 minutes in the first run, to a final 5-minute run time (Figures 2 to 5).

The final analysis method demonstrates that the retention order of the peptides is very similar to the nanoflow analysis method, and that eight of the 10 peaks are baseline-separated.

UPLC method development, including all four injections, was performed within a 45-minute period, indicating the versatility of the system for quick method transfer experiments.

## Conclusion

This UPLC method was subsequently used to generate a quantitative UPLC-MS/MS method for Apo A1 in human serum,[2] where we demonstrated that the accuracy and reproducibility of the UPLC-MS/MS approach was very similar to the immunochemistry-based clinical analysers that currently perform the quantitation of Apo A1 in clinical laboratories.

## References


1. Enrichment of Low Molecular Weight Serum Proteins using Acetonitrile Precipitation for Mass Spectrometry Based Proteomic Analysis. Kay R, Barton C, Ratcliffe L, Matharoo-Ball B, Brown P, Roberts J, Teale P, Creaser C. *Rapid Commun. Mass Spectrom*. 2008; 22: 3255–3260.
2. The Application of Ultra-Performance Liquid Chromatography/Tandem Mass Spectrometry to the Detection and Quantitation of Apolipoproteins in Human Serum. Kay R, Gregory B, Grace P, Pleasance S. *Rapid Commun. Mass Spectrom*. 2007; 21: 2585–93.


## Featured Products

ACQUITY UPLC System <https://www.waters.com/514207>

720002801, September 2008