CE/MS/MS as an Orthogonal Technique for Sensitive and Easy Quantification of Peptides in Complex Matrixes

Application Note

Proteomics

Authors

Suresh Babu CV and Srividya Kailasam
Agilent Technologies, Inc.
Bangalore, India

Abstract

This Application Note describes an orthogonal approach to liquid chromatography/mass spectrometry (LC/MS) using an Agilent capillary electrophoresis (CE) system coupled to an Agilent triple quadrupole mass spectrometer for peptide quantification. The newly introduced Agilent Jet Stream (AJS) compatible CE/MS triple tube sprayer and the latest version of MassHunter software (B.05.01), which integrates the control of both CE and MS into a single software platform, have been showcased in this study. Two human serum albumin (HSA) peptides spiked into a tryptic *E. coli* digest have been used as model compounds to demonstrate the CE/MS/MS multiple reaction monitoring (MRM) approach for quantification. With the CE/MS/MS method, peptides of closely related charge states were separated and attomole detection level was achieved.

Agilent Technologies

Introduction

Electrophoretic separation by CE provides an orthogonal tool complimenting liquid chromatographic separation and enhancing coverage and confidence in the results. As CE is an efficient and rapid separation technique, it is increasingly being adopted for the analysis of biological samples such as peptides. Growing interest in this technique has led to a number of developments in the instrument hardware and software to enhance user-friendliness. When coupled with tandem mass spectrometry (MS/MS), it becomes an ideal tool for characterization of biomolecules. Increasingly, CE/MS/MS is also being investigated for quantification of these molecules such as peptides[1]. The recently introduced CE/MS triple tube sprayer is compatible with the AJS source and therefore enhances the sensitivity of analysis of proteins and peptides in complex matrix.

MassHunter acquisition software (B.05.01), is compatible with any of the Agilent TOF, Q-TOF, or Triple Quadrupole CE/MS platforms and integrates control of both CE and MS in a single software. In addition, MassHunter acquisition software for CE/MS/MS incorporates all features of the ChemStation software previously used for CE control. With this new development, it is now easy to switch between an LC/MS or CE/MS setup.

This Application Note demonstrates the performance of CE/MS/MS with the new CE/MS sprayer for the quantification of two human serum albumin (HSA) peptides. In addition, we have shown monitoring 60 MRM transitions for bovine serum albumin (BSA) tryptic peptides within a run time of 35 minutes.

Experimental

Materials

E. coli total protein lysate was purchased from Bio-Rad. Human serum albumin (HSA) Peptides Standard Mix (G245585001), CE grade water (5062-8578), fused silica capillary (G1600-67311), and sample vials (5183-4623) were obtained from Agilent Technologies, Inc. Bovine serum albumin (BSA) and all other chemicals were purchased from Sigma-Aldrich.

Instrumentation and conditions

All analyses were performed using the Agilent 7100 CE system coupled to an Agilent 6490 Triple Quadrupole Mass Spectrometer through a triple tube sheath-liquid interface (G1607B). The MS was equipped with an Agilent Jet Stream source. The CE/MS system was controlled by Agilent MassHunter Acquisition Software (B.05.01). The sheath liquid was delivered by an Agilent 1200 series Isocratic Pump equipped with a 1:100 flow splitter. Agilent MassHunter Peptide Optimizer Software was used to optimize the qualifier and quantifier MRM transitions for HSA peptides. The quantifier was used to generate calibration curves using Agilent MassHunter Quantitative Software. Tables 1 and 2 show the CE/MS parameters and MRM conditions respectively. The approximate amount of sample injected into the separation capillary was calculated using the standard equation for CE.

Table 1. CE/MS/MS parameters.

Agilent Capillary Electrophoresis (CE)	
CE	Agilent 7100 Capillary Electrophoresis System
Sample	HSA peptide mix
Injection	10 s at 50 mbar
Capillary	Fused silica capillary, total length 60 cm, 50 µm id
Buffer	1 M acetic acid, pH 2.3
Voltage	30 kV
Temperature	20 °C
Agilent Triple Quadrupole Mass Spectrometry (MS/MS)	
MS	Agilent 6490 Triple Quadrupole MS
Ionization mode	AJS (positive mode)
Acquisition mode	MRM
Sheath liquid	1:1 methanol:water + 0.5 % acetic acid, 8 µL/min
Drying gas flow	11 L/min
Drying gas temperature	200 °C
Nebulizer	30 psi
Sheath gas temperature	170 °C
Sheath gas flow	7 L/min
iFunnel RF voltage	High pressure 150 V Low pressure 60 V
Vcap	3,500 V
Nozzle voltage	300 V

Table 2. CE/MS/MS MRM parameters.

Compound	Qual/Quant	Precursor ion	MS1 Res	Product ion	MS2 Res	Dwell	CE	Cell accelerator voltage
YLYEIAR	Quantifier	464.3	Wide	651.0	Unit	50	10	5
	Qualifier	464.3	Wide	136.0	Unit	50	10	5
LVNEVTEFAK	Quantifier	575.3	Wide	213.0	Unit	50	15	5
	Qualifier	575.3	Wide	937.0	Unit	50	15	5

Sample preparation

For the calibration curve experiments, stock solutions of HSA peptide standards were made in water at 20 pmol/µL, then serially diluted to the respective concentrations. In the spiking experiments, different concentrations of HSA peptides were spiked into a tryptic digest[2] of *E. coli*. A tryptic digest of BSA was generated as described[2] and MRM transitions for the tryptic peptides were generated using Skyline software.

Results and Discussion

The CE/MS AJS sprayer was developed specifically for CE flow rates and has a modified sprayer tip. The performance of this AJS compatible CE/MS sprayer has been shown to provide enhanced sensitivity as compared to the standard CE/MS sprayer[3].

To demonstrate the CE/MS/MS MRM approach for quantification, two synthetic peptides derived from HSA were chosen as model compounds. Two MRM transitions were monitored for each of the two peptides. Figure 1 shows the overlay of CE/MS/MS MRM quantifier transitions of the two HSA peptides. Peptide 1 (YLYEIAR) migrates at 3.8 minutes and peptide 2 (LVNEVTEFAK) migrates at 3.9 minutes. The close migration times of the two peptides results from a minimal mobility difference between the two under the acidic CE electrolyte conditions (pH 2.3) employed in the experiment. The use of unique MRM quantifier transitions for each of the two peptides enables their simultaneous quantification under the present conditions. The qualifier transitions helped confirm the identities of the two peptides. The MRM transitions selected for peptide 1 (464.3 → 136) and peptide 2 (575.3 → 213) were used in the present study as they provided superior sensitivity under the CE/MS/MS conditions employed. However, these low *m/z* transitions lack specificity and might not be the best transitions in a complex mixture. Although only one MRM transition per peptide was monitored in the present proof-of-concept study, typically three to five MRM transitions per peptide are monitored to ensure specificity.

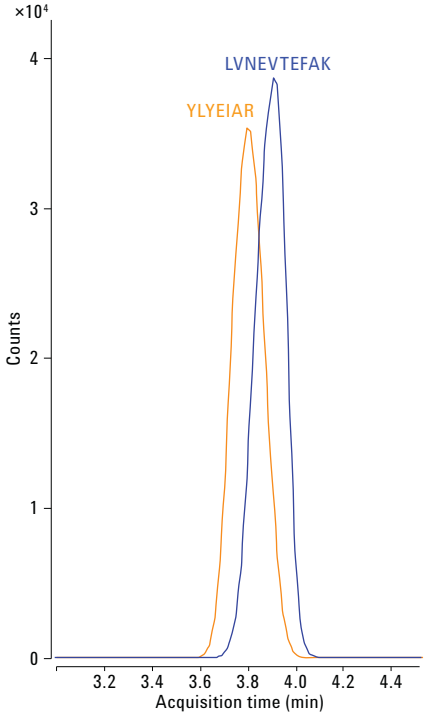

Figure 1. Overlaid CE/MS/MS MRM electropherograms for HSA peptides: YLYEIAR and LVNEVTEFAK.

Calibration curves for the two peptides in aqueous buffer were generated over the concentration range of 0.002 pmol/µL–20 pmol/µL. Both peptides showed excellent linearity, with $R^2 > 0.999$, as seen in the log-log plots (Figures 2A, 2B). The data demonstrates a wide linear dynamic range of up to four orders of magnitude. Under the experimental conditions described, for both peptides, the calculated limits of detection (LOD) were found to be 2.3 amol and the limits of quantitation (LOQ) were determined to be 23.5 amol. Figure 3 shows the peaks obtained at the LOD level for the two peptides, as well as overlays of the quantifier and qualifier transitions. The LOD of the method was also determined in *E. coli* digest to ascertain the method performance in a complex matrix. Different concentrations of the HSA peptides were spiked into this matrix and the quantifier transitions shown in Figure 4 were monitored. The two peptides could be detected at the 2.3 amol level even in this complex background.

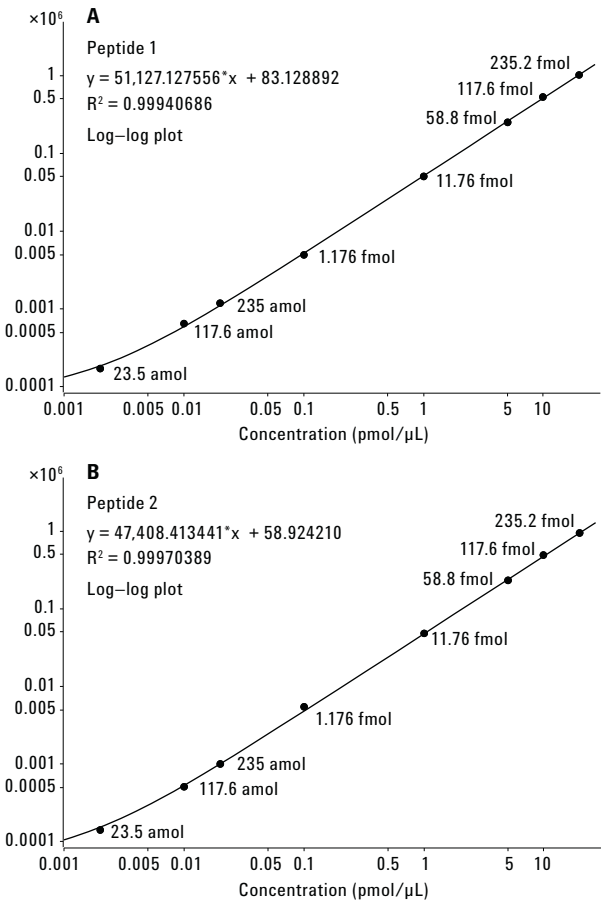

Figure 2. Calibration curves of (A) YLYEIAR and (B) LVNEVTEFAK from 0.002 pmol/µL to 20 pmol/µL. Linear fit with the origin ignored and 1/x weighting.

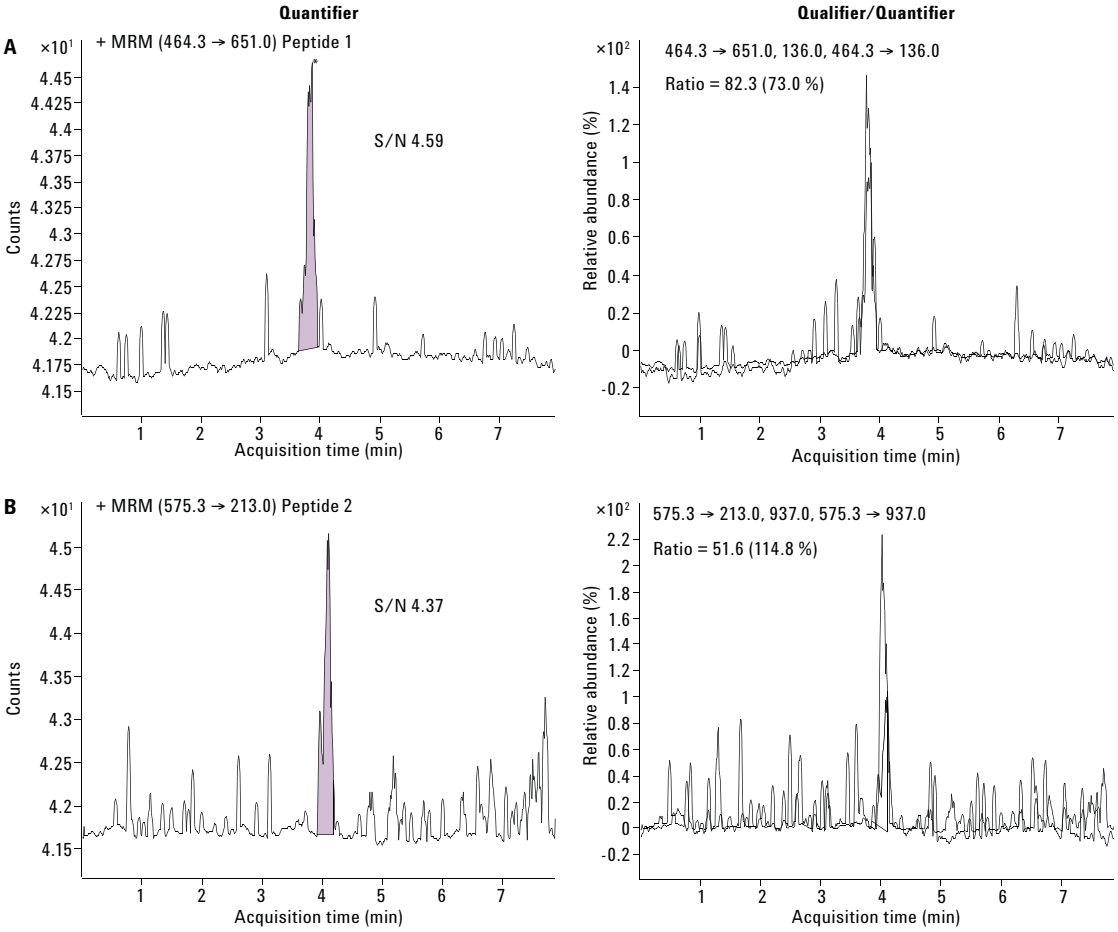

Figure 3. CE/MS/MS MRM electropherograms of (A) YLYEIAR and (B) LVNEVTEFAK at 2.3 amol.

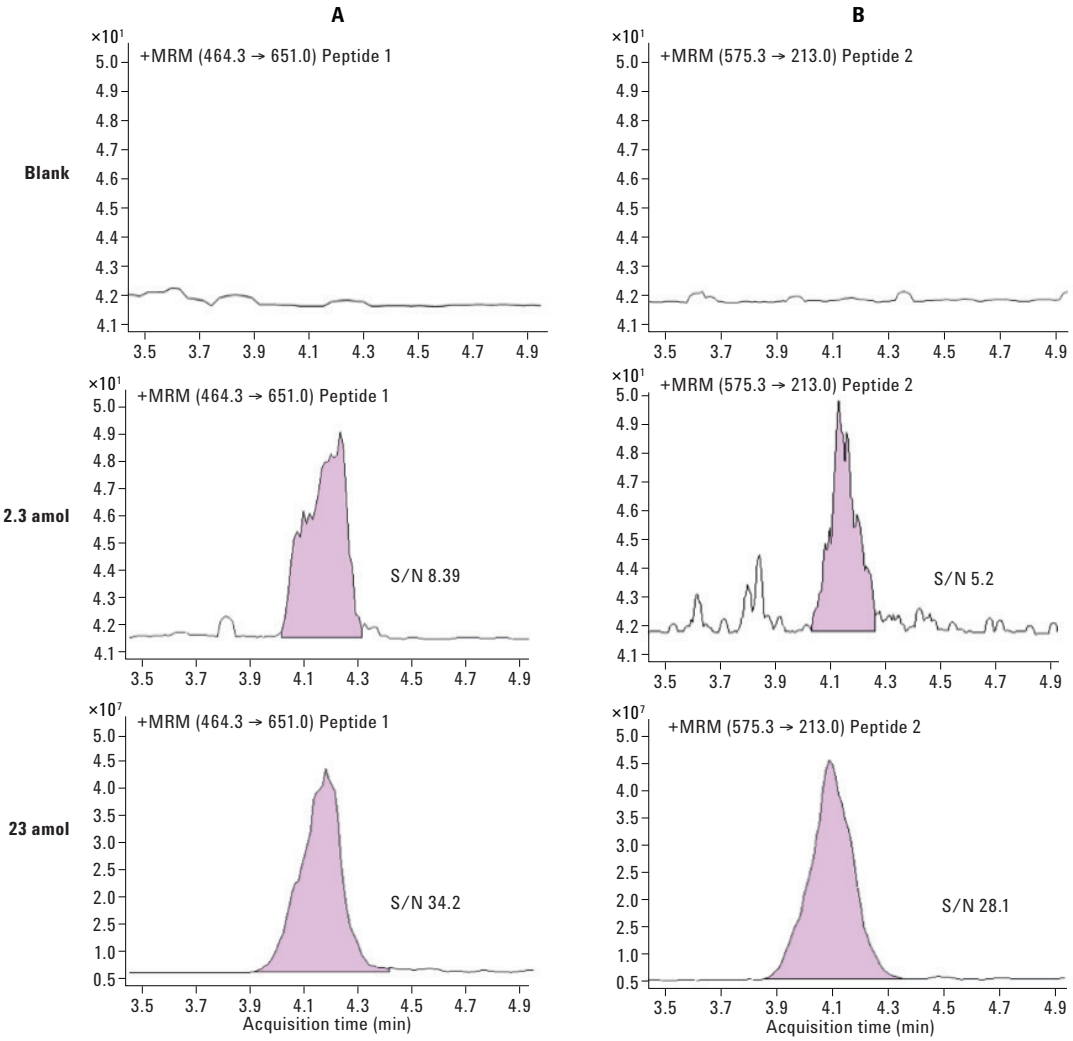

Figure 4. CE/MS/MS MRM electropherograms of different levels of A) YLYEIAR and B) LVNEVTEFAK in *E. coli* tryptic digest.

Further, the capability of the instrument and the software used in the study to handle multiple transitions was shown by analyzing a tryptic digest of BSA. The list of MRM transitions for this experiment was generated using Skyline software[4]. Figure 5 shows the overlay of 60 MRM transitions for BSA tryptic peptides. The result demonstrates the feasibility of monitoring numerous MRMs in a single CE/MS/MS run. The data quality can be further improved by using dynamic MRM (DMRM) acquisition mode where the peptide transitions are monitored in their respective migration time windows.

Conclusions

This Application Note demonstrates CE/MS/MS as a sensitive orthogonal approach for peptide quantification over a wide linear dynamic range, even in complex matrixes. The Agilent MassHunter software allows easy control of the CE/MS system and provides the flexibility to easily switch between LC/MS and CE/MS configurations.

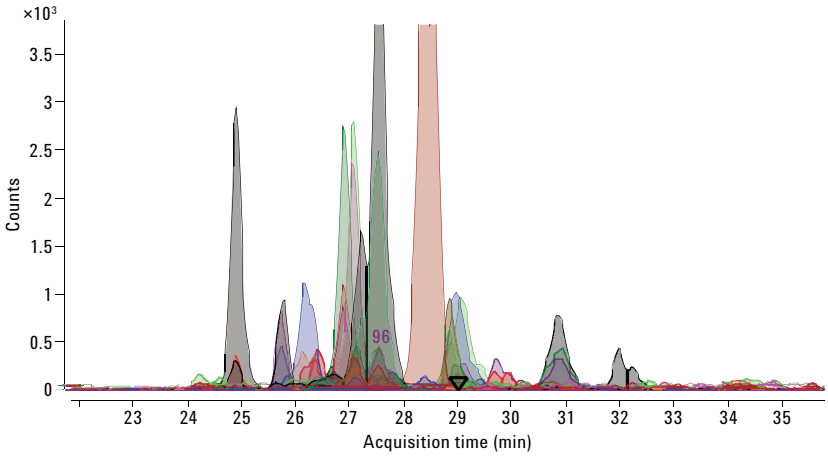

Figure 5. Overlay of CE/MS/MS MRM transitions of BSA tryptic peptides. The 60 peptide MRM transitions were imported to MassHunter Acquisition software from a Skyline document and the MRM transition signals were acquired using CE/MS/MS.

References

1. Sun, L., *et al.*, Capillary zone electrophoresis-multiple reaction monitoring from 100 pg of RAW 264.7 cell lysate digest, *Analyst*, **2013**, 138:3181-3188.

2. Glycopeptide Analysis of Antibodies by Capillary Electrophoresis and Q-TOF Mass Spectrometry, Agilent publication 5990-7138EN.

3. Compatibility of Agilent Jet Stream thermal gradient focusing technology with CE/MS, Agilent publication 5990-9716EN.

4. MacLean, B., *et al.*, Skyline: an open source document editor for creating and analyzing targeted proteomics experiments, *Bioinformatics*, **2010**, 26(7):966-968.

www.agilent.com/chem

Published in the USA, April 1, 2014
5991-2729EN

Agilent Technologies