CE/MS and LC/MS Synergy

Complementary Solutions for Peptide Mapping

Application Note

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Introduction

Peptide mapping is commonly used to identify or characterize the protein of interest. Monitoring peptide mapping is a critical part of regulatory guidelines for the characterization of biopharmaceuticals. Peptide mapping serves as a primary QC step in pharmaceutical development. It involves chemical or enzymatic treatment of protein to obtain peptide fragments, followed by separation and detection using mass spectrometry (MS). Both chromatographic and electrophoretic-separation techniques have to be employed to study peptide mapping in coupling to MS. Even though LC/MS is the most commonly used method for peptide mapping, CE/MS is an alternate method that offers additional information on peptide separation and different selectivity. This Application Note used both CE/MS and LC/MS methods to monitor peptide separation and provide the orthogonal solutions.

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Experimental

CE/MS

The CE/MS analysis was performed using the Agilent 7100 CE system coupled to the Agilent 6520 Accurate-Mass Q-TOF LC/MS equipped with an electrospray source and an orthogonal coaxial sheath liquid interface (G1607B). Separations and spray stability were optimized using the blank buffers and a standard. A sheath-liquid CE/MS interface with a low flow rate (4 µL/min) was maintained to preserve the high efficiency separation of CE and to provide the stable flow and spray conditions essential for electrospray ionization. Q-TOF parameters were optimized automatically through MS tuning programs, and the MS system was calibrated using an ESI tuning mixture.

LC/MS

The Agilent 1260 Infinity HPLC-Chip/MS system was coupled with the Agilent 6520 Accurate-Mass Q-TOF LC/MS platform for LC/MS analyses. The HPLC-Chip/MS interface enables automatic chip loading, sample and solvent delivery to the chip, high pressure switching of flows, and automated and reproducible chip positioning to the MS source for accurate mass measurement.

Table 1. CE/MS and LC/MS parameters.

CE conditions	
CE	Agilent 7100 CE
Sample	BSA digest
Injection	Different injection times at 50 mbar
Capillary	Bare fused silica, total length 60 cm, 65 cm, 85 cm, 50 µm id
Buffer	10 mM acetic acid
Voltage	27 kV (0.3 minutes ramp)
Temperature	20 °C
Preconditioning	15 minutes flush with buffer at 1 bar
MS conditions	
MS	Agilent 6520 Accurate-Mass Q-TOF LC/MS
Ionization mode	ESI
Acquisition mode	MS (mass range 100–3,200 *m/z*)
Sheath liquid	0.5 % acetic acid in 50 % methanol, 4 µL/minute
Drying gas flow	5 L/min
Nebulizer	10 psi
Drying gas temperature	175 °C
Fragmentor	175 V
Vcap	3,500 V
LC conditions	
LC	Agilent 1260 Infinity Nanoflow LC System and an Agilent 1260 Infinity Capillary LC System
Sample	BSA digest
Injection	2 µL
HPLC-Chip	G4240-62005 , 5 µm, Agilent ZORBAX 300SB-C18, 40 nL enrichment column, a 75 µm × 43 mm analytical column
Flow rate	3 µL/min (Cap pump), 0.6 µL/min (Nano pump)
Solvents	A) 0.1 % formic acid (FA) in water; B) 90 % ACN in water with 0.1 % FA
Gradient	Time (min) B (%) Initial 3 30 50 32 95 34 95 34.10 3
MS conditions	
MS	Agilent 6540 Accurate-Mass Q-TOF LC/MS
Ionization mode	ESI
Acquisition mode	MS (mass range 100–3,200 *m/z*)
Drying gas flow	5 L/min
Nebulizer	10 psi
Drying gas temperature	325 °C
Fragmentor	175 V
Vcap	3,500 V

Results and Discussion

Capillary electrophoresis (CE) is an electrically-driven liquid-based separation in which analytes are separated according to their difference in migration velocity in an electrical field. The individual compound velocity depends on the charge and size of the analyte. Conversely, Liquid Chromatography is a pressure-driven liquid-based separation where analytes are separated based on adsorption/desorption kinetics. This Application Note shows a BSA peptide mapping study as an example to understand the orthogonality of CE/MS and LC/MS techniques. Furthermore, HPLC-Chip/MS setup was chosen as LC/MS because of the nL flow rate delivered into the electrospray source by LC/MS, which is similar to the flow rate generated by CE/MS. Nano-LC/MS is one of the most sensitive LC techniques and, therefore, provides a more valid comparison.

Figure 1 shows the CE/MS and LC/MS peptide map of BSA. The theoretical digestion list of peptide masses matched 80 % with experimental masses, with an 81 % sequence coverage (showing a 5 ppm mass error) for CE/MS and LC/MS runs, respectively. Among the total number of peptides identified (114), 37 peptides are unique to CE/MS and 33 peptides are unique to LC/MS. Each of the techniques contained 44 common peptides, showing affinity towards both modes of separation. The detection of distinct tryptic peptides revealed the complementary values of CE/MS and LC/MS techniques.

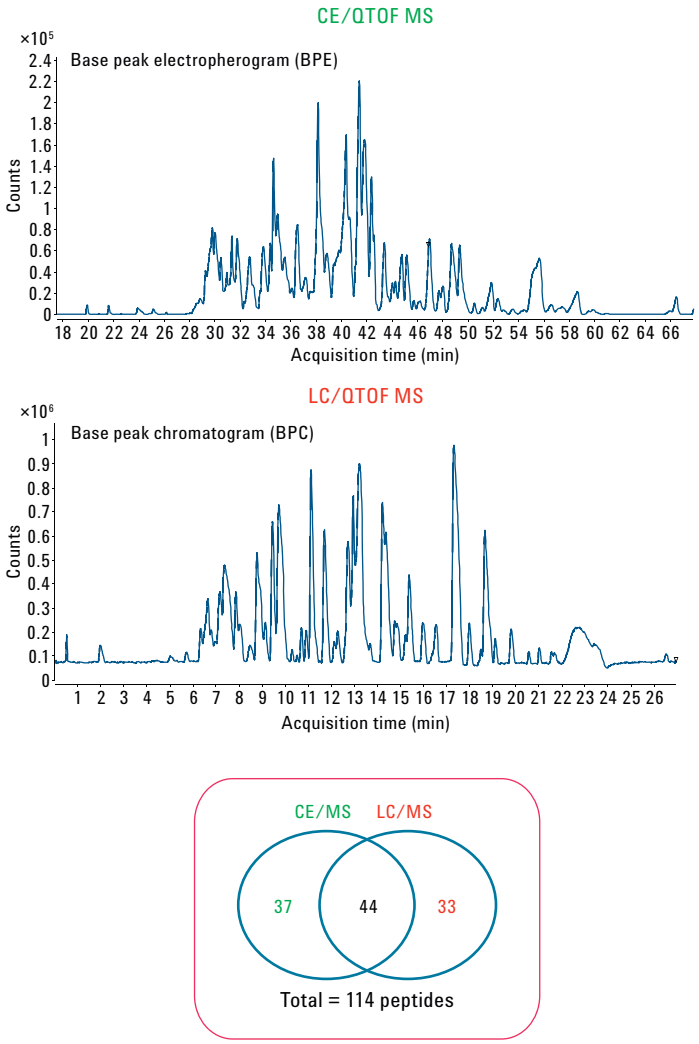

Figure 1. CE/MS and LC/MS of peptide mapping.

Given the different separation mechanisms of both techniques, the analytes elution order of the CE/MS profile will be different than the LC/MS profile. Elution orders are dictated by hydrophobicity and mobility. Figure 2 depicts the change in elution order for the same set of BSA peptides. Different selectivity is the reason for the altered elution order for both techniques, which is caused by their different physical separation principles. In CE, the change in selectivity can be obtained by simply switching to a different pH of the background electrolyte (run buffer). However, different column chemistry and solvent set are required in LC to make major selectivity changes.

Usually, hydrophilic compounds do not retain well, and elute quickly, on reverse-phase materials such as a C18 column. Figure 3 shows the CE/MS and LC/MS results for two hydrophilic peptides. The ATEEQLK peptide was hardly detected in LC/MS, whereas CE/MS produced better signals for all the hydrophilic peptides shown. CE/MS also produced a better peak shape for TCVADESHAGCEK peptide.

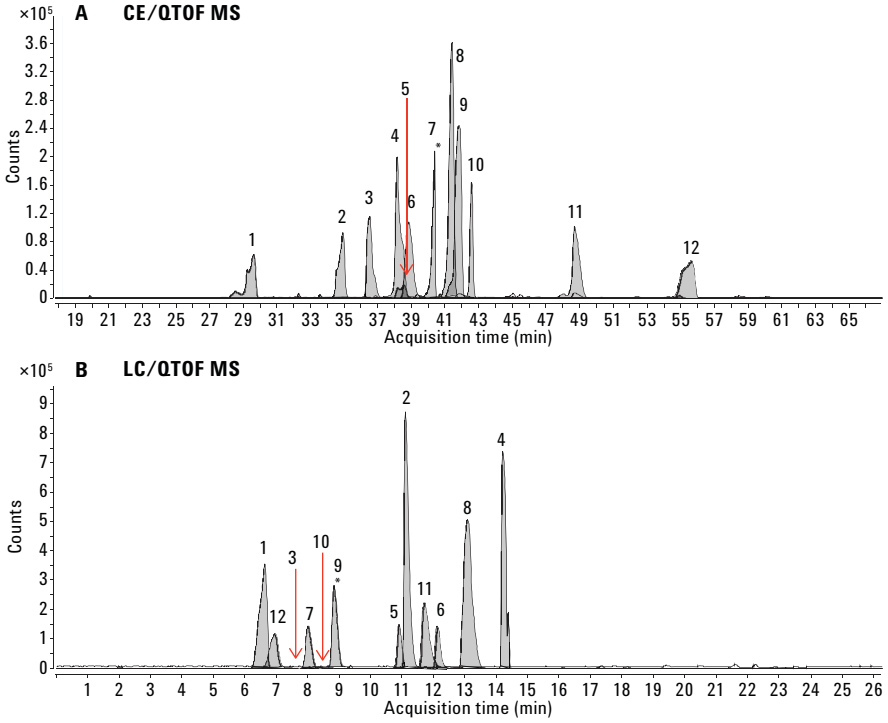

Figure 2. (A) CE/QTOF MS and (B) LC/QTOF MS separation comparison of same set of BSA tryptic peptides. Peptides: 1. LCVLHEK, 2. HLVDEPQNLIK, 3. NYQEAK, 4. QTALVELLK, 5. YLYEIAR, 6. ECCHGDLLECADDR, 7. LVTDLTK, 8. LVNELTEFAK, 9. AEFVEVTK, 10. LCVLHEKTPVSEK, 11. LVVSTQTA, 12. DDSPDLPK.

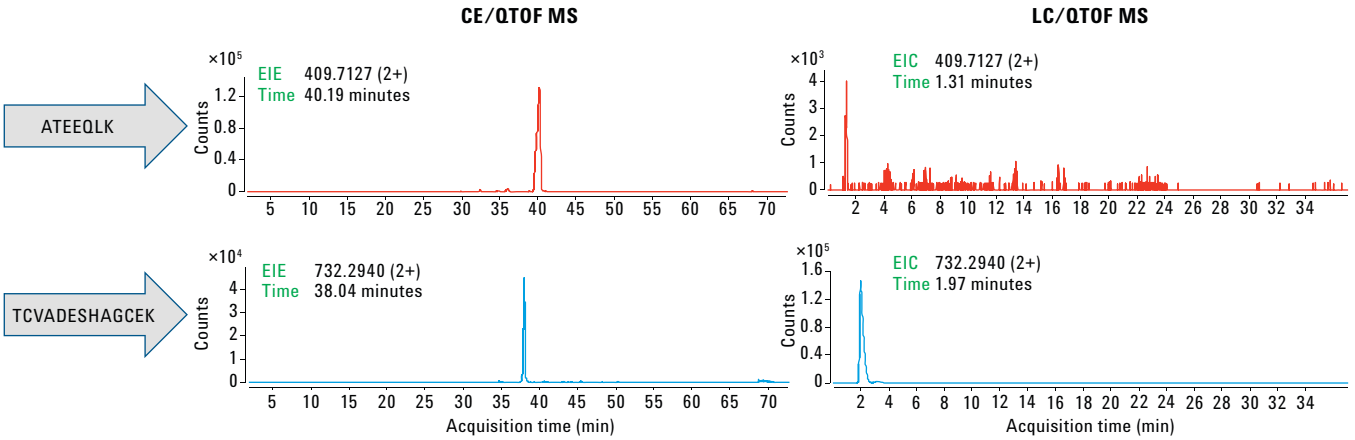

Figure 3. CE/MS and LC/MS comparison of hydrophilic peptide behavior.

Further analysis of peptide distribution reveals that low molecular weight peptides and shorter amino acid peptide lengths are well represented by CE/MS, but LC/MS covers a wide range of peptide length identification. In addition, the acidic peptides behave well in the CE/MS conditions.

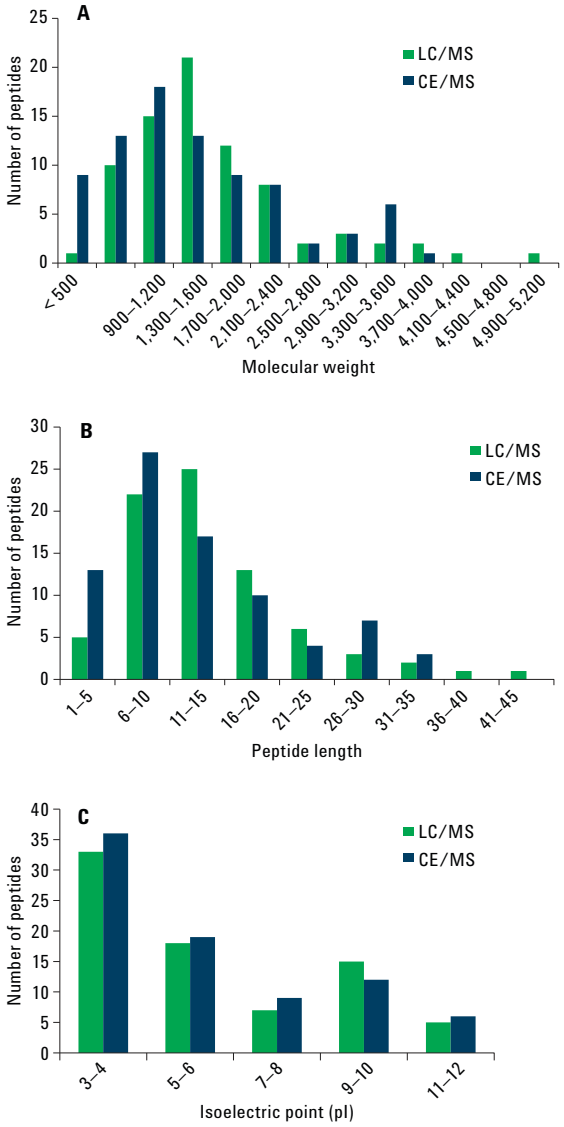

Figure 4. CE/MS and LC/MS comparison of peptide distributions. (A) Molecular weight plot; (B) peptide length plot; (C) Isoelectric point plot.

Conclusion

The comparison study done in this Application Note shows the advantages of both CE/MS and LC/MS techniques (Table 2). It is demonstrated that both CE/MS and LC/MS techniques provide additional information and, thereby, serve as an orthogonal and complementary approach for separating peptides.

Table 2. Advantages of CE/MS and LC/MS techniques.

	CE/QTOF MS (Agilent 6520 Accurate-Mass Q-TOF LC/MS)	LC/QTOF MS (Chipcube-Agilent 6540 Accurate-Mass Q-TOF LC/MS)
Sample injected	44 nL (0.34 pmole)	2 µL (15 pmole)
Peptide elution window	30 minutes	16 minutes
Sequence coverage	80 %	81 %
Total peptides identified	82	78
Distinct peptides identified	37	33
Selectivity and resolution	Change in the elution order of peptide shows the complementary value of the two techniques	
Selectivity	CE/MS shows the best separation/ionization for hydrophilic peptides	
Peptide distribution	Shorter peptides are represented (1–5 amino acid peptide length)	Shorter peptides are less represented (1–5 amino acid peptide length) and also cover a wide range of peptide length
	Identified peptides starting with three amino acid length	Identified peptides starting with four amino acid length
	Low MW peptides are well represented (< 500 Da)	Low MW peptides are less represented (< 500 Da)
	Acidic peptides (pI 3-4) are well represented	

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