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Expanding phosphorylation and post translational site mapping in proteomics using CESI-MS

Sensitive workflow for the detection of PTMs in biological samples from small sample volumes

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Overview

Who Should Read This: Senior Scientists, Lab Directors and Proteomics researchers.

Focus: Expanding Post Translational Modification (PTMs) identification using capillary electrospray ionization (CESI) in combination with accurate MS detection in Histone analysis.

Goals: Show how CESI-MS can detect additional PTMs compared to nano LC-MS and how it is a complementary and an orthogonal technique which can expand your PTM coverage when used together with nano LC-MS analysis.

Problem: Traditional nano LC-MS methods involve the injection of µLs of sample to obtain peptide mapping of biological digests. Repeating injections for additional analysis is not always possible if sample volume is limited and additional PTM coverage from repeat analysis is therefore not available. Nano LC-MS/MS separates peptides based on hydrophobic interactions with the LC columns stationary phase and sometimes polar PTMs and very hydrophobic peptides can be missed depending on the gradient used.

Results: The Sciex CESI 8000 system in combination with accurate MS detection provides a complementary solution to nano LC-MS for the characterization of post translational modifications sites in H1 histone samples. A total of 70 different modified peptides, including 50 phospho-peptides, were identified in rat histones and out of the 70 modified H1 histone peptides, 27 peptides could be identified solely by CESI-MS with11 solely by LC-MS. CESI-MS was able to detect 157 histone H1 peptides compared to 154 from LC-MS but only consumed 300 fmol of sample compared with 30 pmol by LC-MS.

Key Challenges:

• Separation and detection of modifications sites for H1 Histones.

• Expanding PTM coverage with limited sample volume.

Key Features:

• Sample consumption by CESI-MS was 1% of that by LC-MS allowing the analysis of limited samples firstly by CESI-MS and then by LC-MS increasing peptide coverage.

• CESI-MS was able to detect unique PTM sites which were missed when only LC-MS was used.

Experimental Design

Sample Preparation: Nuclei from rat testes (Sprague-Dawley) were extracted with perchloric acid (5%, v/v) for H1 histone preparation.[1] Once extracted the Histones were digested using endoproteinase Arg-C (Sigma-Aldrich) in 5 mM ammonium bicarbonate buffer (pH 8.0, 1 h at 37° C).

CESI-MS methods. For CESI-MS analysis a CESI 8000 equipped with positively coated or neutrally coated fused-silica capillaries was coupled to a Thermo Scientific LTQ Orbitrap XL mass spectrometer.[2] Before each analysis the separation capillary and the conductive liquid capillary were rinsed with BGE to refresh the buffer. Samples were injected for 10 s at 5 psi (7.5 nl) followed by an injection plug of BGE (5 psi for 5 s). For the separation the conditions depended on the capillary coating but for the neutrally coated capillaries separations were performed at 30 kV (normal polarity mode), with a pressure

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gradient of 0 - 43 min, 0.5 psi; 43 - 51 min, 2 psi; and 51 - 60 min, 5 psi. Acetic acid 10% (v/v) was used as the BGE for neutral coated capillaries.[2]

Nano LC methods. Nano-HPLC—Protein digests were analyzed using an UltiMate 3000 nano-HPLC system coupled to an LTQ Orbitrap XL mass spectrometer. A homemade frit less fused silica micro-capillary column (75 µm inner diameter x 280 µm outer diameter) packed with 10 cm of 3 µm RP C18 material was used with a gradient elution at a flowrate of 250 nl/min. The gradient (solvent A: 0.1% formic acid; solvent B: 0.1% formic acid in 85% acetonitrile) started at 4% B with B increased linearly from 4% to 50% over 50 min and then from 50% to 100% over 5 min.

MS methods. Both separations used the same Thermo Scientific LTQ Orbitrap XL mass spectrometer to detect the peptides. The MS was operated in data-dependent mode, switching between MS, MS2, and MS3 acquisition. Survey full-scan MS spectra (m/z 250–1800) were acquired in the Orbitrap with a resolution of 15,000 and the 3 most intense ions detected in the survey scan (above a threshold of 1000 counts) were fragmented in the linear ion trap (LTQ) using collision-induced dissociation. All the data acquired was searched against a rat database using Sequest, Proteome Discoverer search engine.[2]

Analysis of Histone H1 digests by CESI-MS

When positively coated capillaries are used for the separation of protein digests the electroosmotic flow (EOF), which is dependent on the level of charge on the capillary surface, speeds up separations reducing the separation length which can adversely affect peptide identification. Neutral coated capillaries can reduce EOF lengthening separation windows and improving peptide detection. When 300-fmol of histone H1 digest was

analyzed by CESI-MS 157 H1 peptides were identified within a 38.5 minute separation window (Figure 1). Of these peptides 105 were unmodified and 52 were modified this was an increase when compared to when a positively charged (polyethyleneimine) coated capillary was used where only 83 peptides were detected due to its shorter separation window.[2]
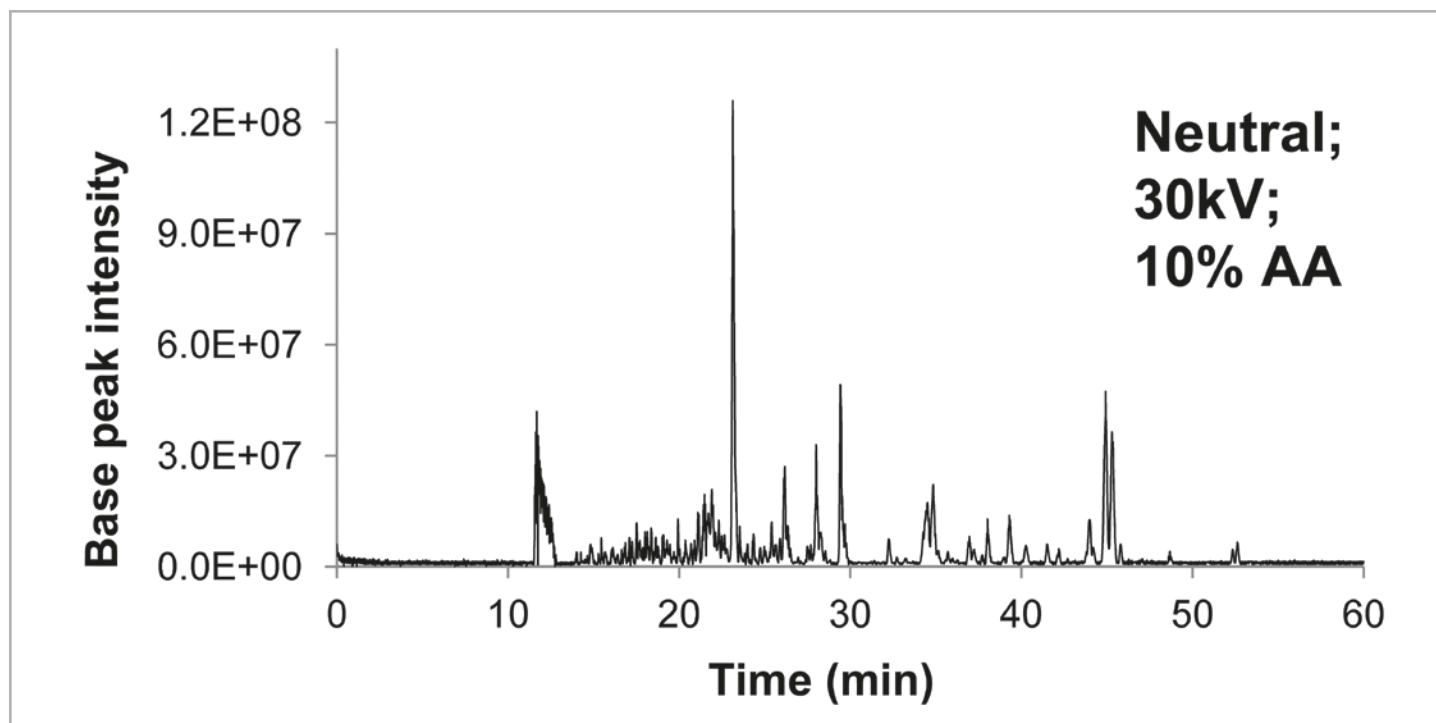
Figure 1. Base peak electropherogram of rat testis H1 histones digested with endoproteinase Arg-C analysed by CESI-MS using a neutrally coated capillary, a separation voltage of 30 kV, and a BGE of 10% (v/v) acetic acid. Sample amount consumed was 6.15 ng (300 fmol).

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Analysis of Histone H1 digests by nano LC-MS

Nano LC-MS was performed using a homemade fritless 10 cm column packed with 3 µm reverse-phase C18 resin. In Figure 2A, the base peak chromatogram of the histone H1 digest for the same amount of sample as in the CESI-MS experiments is displayed. Due to the low signal in the nano LC- MS analysis only 73 peptides were identified (62 unmodified and 11 modified).

Nano LC has a much larger loading capacity than CE 30 pmol of the Arg-C digest was injected (a 100-fold increase relative to the CESI-MS analyses) this resulted in broader peaks but yielded a similar amount of identified H1 peptides, 154 (111 unmodified and 43 modified).
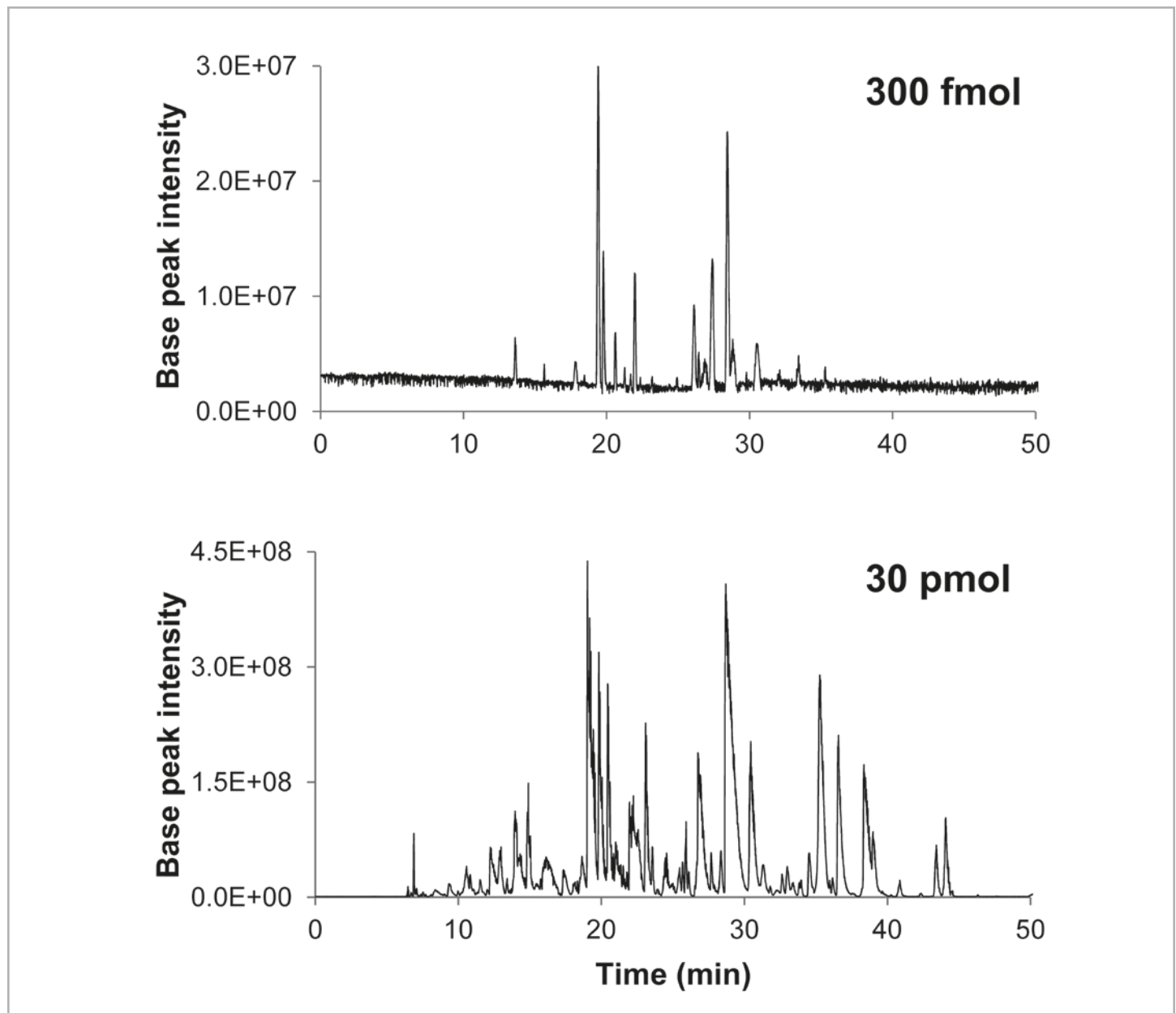

Figure 2. Base peak chromatograms of rat testis H1 histones digested with endoproteinase Arg-C using nano LC –MS. A) represents an injection of 6.15 ng (300 fmol) and C 615 ng (30.0 pmol). Analyses were performed using a homemade fritless 10 cm column packed with 3 µm reversed-phase C18.

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Comparison of nano LC-MS with CESI-MS for Histone 1 digest analysis

When the CESI-MS analysis was compared with LC-MS analysis more modified peptides were detected when only 1% of the sample was injected (300fmol compared to 30 pmol). Figure 3 shows a comparison of both techniques. Each analysis was repeated in triplicate and combining all the data sets a total of 70 different modified peptides were identified[2] the majority of which were phosphorylated. When the individual peptide identifications were compared there was an overlap of 47% of the total peptides detected between the CESI-MS analysis (neutrally coated capillary) and the LC-MS analysis (30 pmol injected). When the modified peptides were compared 10 were only detected by CESI-MS and 11 only by LC-MS highlighting the complementary nature of these techniques.[2] Of this group of modified peptides CESI-MS detected 3 times the amount of the phosphorylated peptides (27 compared to 11) due to polar nature of this modification.
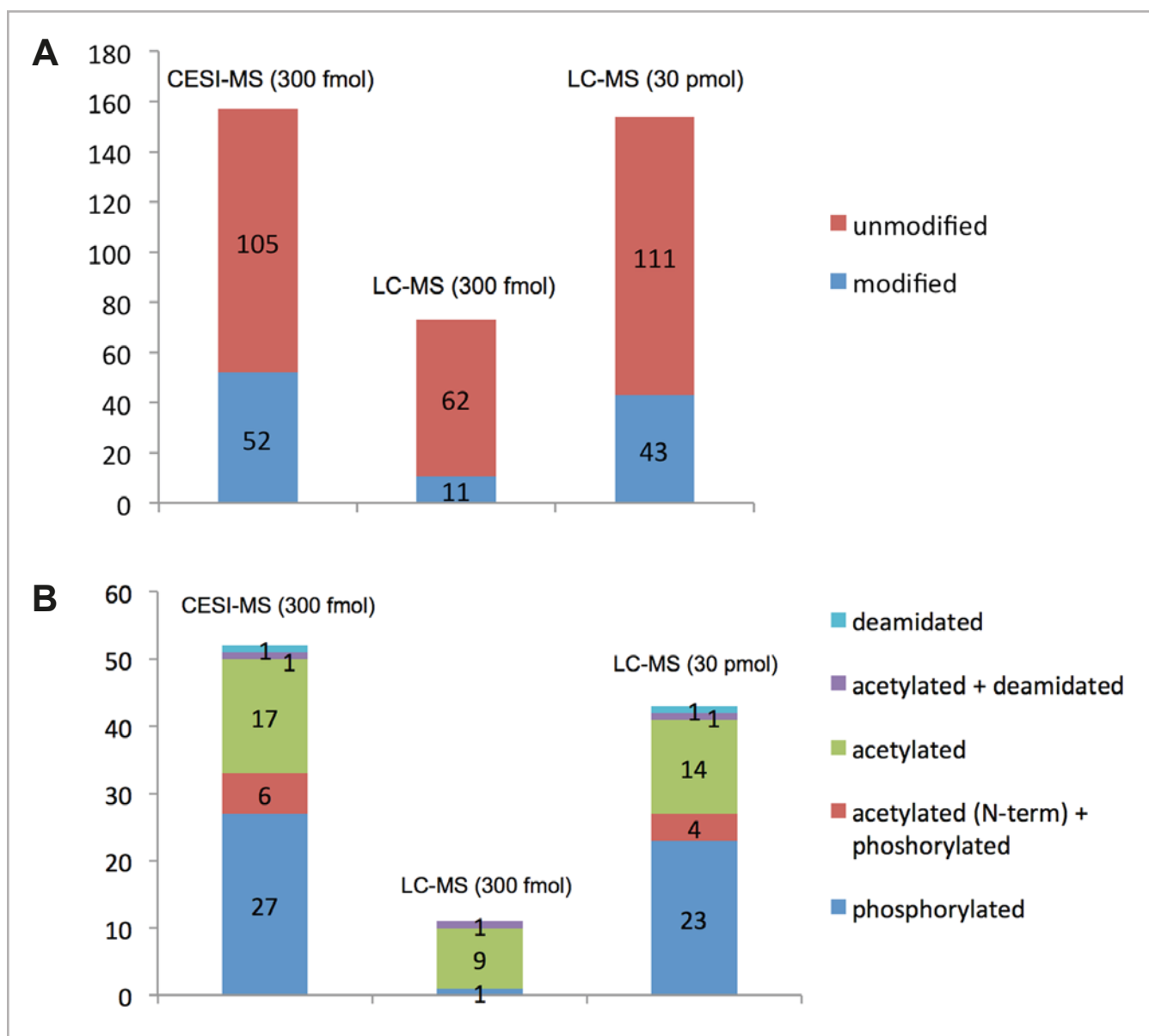

Figure 3. Comparison of CESI-MS using a neutrally coated capillary and LC-MS analysis of Histone H1 digests. A) The total number of histone peptides detected; B) Breakdown of the modified peptides detected by both techniques. For each technique the data from triplicate runs was merged to give the overall number of identifications.

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Further Information

For further information on this topic we would like to refer readers to the full scientific publication on which this application note is based.[2]

References

1. Sarg, B., Koutzamani, E., Helliger, W., Rundquist, I., and Lindner, H. H. (2002) Post synthetic trimethylation of histone H4 at lysine 20 in mammalian tissues is associated with aging. *J. Biol. Chem.* 277, 39195–39201.

2. Sarg, B., Faserl, K., Kremser, L., Halfinger, B., Sebastiano, R., and. H. H. Lindner (2013). Comparing and Combining Capillary Electrophoresis Electrospray Ionization Mass Spectrometry and Nano–Liquid Chromatography Electrospray Ionization Mass Spectrometry for the Characterization of Post-translationally Modified Histones. *Mol. Cell Proteomics*; 12(9): 2640–2656.

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