Improved CESI-MS sensitivity and repeatability in Glycopeptide Analysis using a Dopant Enriched Nitrogen Gas

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Introduction

Electrospray ionization (ESI)-mass spectrometry (MS) is used routinely in proteomics research. Often it is combined with nano-liquid chromatography (LC)–ESI-MS, whose flow rates are typically kept in a range of 100–1000 nL/min, due to the improved ionization efficiency at these lower flow rates. There is a growing interest in proteomics research for the characterization of glycans, as glycans can interfere with the protein structure and function in multiple manners

Capillary Electrospray Ionization (CESI) is the integration of capillary electrophoresis (CE) and electrospray ionization (ESI) into a single process in a single device (Figure 1)[1]. CESI-MS operates at lower flow rates than nano-LC-ESI-MS (10 – 25 nL/min) and offers several advantages which include increased ionization efficiency and a reduction in ion suppression at the lower flow rate. CESI-MS separates analytes by their charge and size and is, therefore, a complementary separation mechanism to more traditional techniques, such as reverse phase nano-LC-ESI-MS.

This document summarizes the work recently published by the research group at Leiden University Medical Center[2]. In this application note we will show how CESI-MS can be used to characterize glycopeptides from model glycoproteins including a polyclonal immunoglobulin G subclass 1 (IgG1). We will show how a dopant enriched nitrogen (DEN) gas supply, previously shown to improve peptide sensitivity by a factor of ~2.6-fold[3] in combination with an optimized injection volume can be used to improve CESI-MS sensitivity. In this work results from CESI-MS will be compared with a conventional nano-LC-ESI-MS approach.

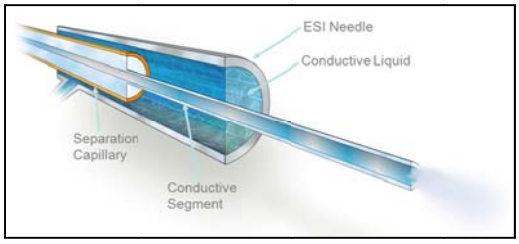

Figure 1: OptiMS® - Ultra low flow ESI Interface.

Materials and Methods

CESI-MS method: Tryptic digest samples[2] (dissolved in 250 mM ammonium acetate at pH 4.0 (3:2, v/v)) were analyzed using a bare fused silica OptiMS CESI cartridge (30 µm ID x 91 cm, polymer coated). Samples were injected hydrodynamically (5 psi, 60 s equivalent to 44 nL or 6.9% capillary volume) and then separated using conditions shown in Table 1.

Action	Time (min)	Pressure (psi)	Direction	Voltage (kV)	Solution
Rinse	2.5	100	Forward	0	0.1 M NaOH
Rinse	2.5	100	Forward	0	0.1 M HCl
Rinse	4	100	Forward	0	Water
Rinse	4	100	Forward	0	10% Acetic acid
Rinse	3	75	Reverse	0	10% Acetic acid
Rinse	60s	5	Forward	0	Sample Vial
Injection	25s	0.5	Forward	0	10% Acetic acid
Separation	35	0	Forward	20	10% Acetic acid
Voltage	5	0	Forward	1	10% Acetic acid

Table 1: CESI separation conditions used for the analysis of tryptic digests.

For MS analysis a UHR-QqTOF maXis Impact HD mass spectrometer (Bruker Daltonics) was coupled to the CESI system using a source adapter available from Sciex. All nano- LC-ESI-MS and CESI-MS experiments were carried out in positive mode using a capillary voltage of 1200 V, end plate offset voltage 0 V, ion energy of 3.0 eV and collision cell energy of 7.0 eV. MS source parameters were set at 1.2 L/min for the drying gas and 150°C for the source temperature. MS spectra were acquired between m/z 200 – 2000 at an acquisition rate of 1 Hz. For the DEN-gas experiments, an in-house made polymer cone was slid onto the capillary housing[2] which allowed a coaxial sheath flow of the DEN-gas around the CESI capillary tip [the concentration of Acetonitrile (MeCN) in the DEN-gas was experimentally determined to be ~4% (mole percentage)]. Nano-LC separations were carried out on an UltiMate 3000 System from Dionex using a core-shell Ascentis Express C18 nano-LC column preceded by a Dionex Acclaim PepMap100 C18 trap column with a gradient elution from 0.1% trifluoroacetic acid to 96% acetonitrile[2].

Results

The best source conditions for the CESI-MS (determined based on signal intensities, background noise, in-source fragmentation and repeatability of the relative abundances of tryptic glycopeptides from polyclonal IgG) indicated that MeCN as dopant gave the best results and similar to what was reported for nano-LC–ESI-MS[2]. Figure 2, compares the relative peak areas observed for glycopeptides with or without DEN-gas and demonstrate a ~2-fold enhancement for all glycopeptides when the DEN-gas was used. Moreover, the addition of DEN-gas led to a lower abundance of noise and interferences over the whole MS detection range (i.e., m/z 200–2000), especially in the region between m/z 500–800 which showed the highest level of background interferences (Figure 3).

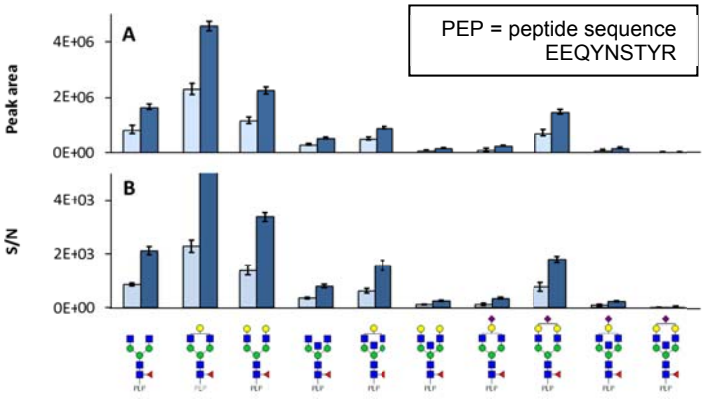

Figure 2 (S-8). Differences in peak areas and signal-to-noise ratio (S/N) observed for the tryptic fucosylated glycopeptides from polyclonal antibody IgG. CE–ESI-MS (light blue) and CE–ESI-MS with DEN-gas setup (dark blue) with (A) Absolute peak areas and (B) S/N ratios.

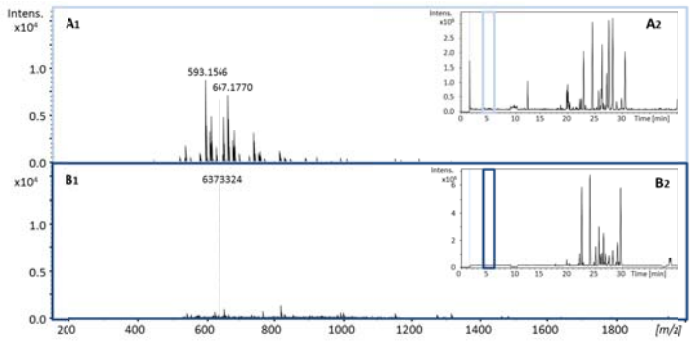

Figure 3 (S-9). Background spectra collected between 4.5 and 5.5 min from the analysis of tryptic glycopeptides. (A1) MS spectrum obtained with conventional CESI-MS setup and (B1) CESI-MS using DEN-gas.

Limits of Detection (LODs) were determined for the G2F glycopeptide of IgG monoclonal antibody (mAb) 1 for both CESI-MS and nano-LC–ESI-MS. Table 2 highlights the lowest concentration that was detected with a S/N ratio ≥3.

	IgG conc (pg/µL)	IgG injected amount (pg)
Nano LC-ESI-MS	250	250
CESI-MS	75	3.3
CESI-MS + DEN gas	3	0.1

Table 2. Limits of Detection observed (lowest concentration where the detected S/N ratio was ≥3) for IgG G2F glycopeptide of IgGmAb1in nano-LC–ESI-MS and CESI-MS with and without DEN-Gas.

These results are also depicted in Figure 4 which shows the differences observed in the S/N ratios between the three methods at relatively high, medium, and low concentrations.

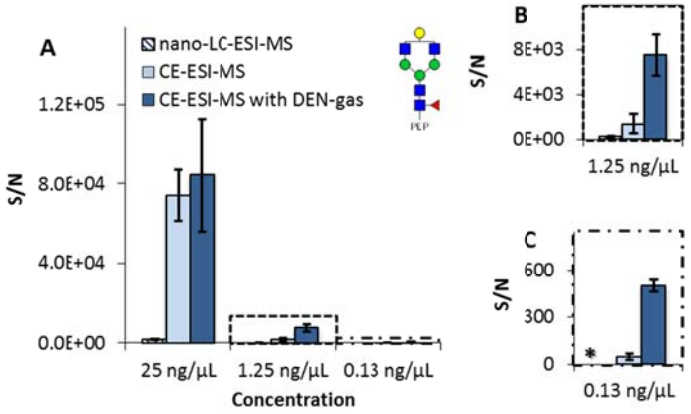

Figure 4. Peak areas of tryptic Fc N-glycopeptide G1F from the IgGmAb1 obtained with nano-LC–ESI-MS, conventional CE–ESI-MS, and CE–ESI-MS with DEN-gas at different concentrations. (A) Peak areas observed at relatively high, medium, and low concentrations. Magnifications of the middle and low concentration is displayed in (B) Peak areas observed at 1.25 ng/µL and (C) Peak areas observed at 0.13 ng/µL. This concentration was below the LOD of nano-LC–ESI-MS and hence G1F was not detected (*). Error bars represent the standard deviation (N = 3). The PEP illustrates the tryptic peptide sequence EEQYNSTYR.

At relatively low concentrations (Figure 4C), no signal was detected with the reference method (nano-LC–ESI-MS) while CE–ESI-MS with DEN-gas led to higher S/N ratios (10-fold) compared to the conventional

setup. CE-MS is usually considered to have lower concentration sensitivity than chromatographic approaches (due to the significantly lower loading levels (nanoliter versus microliter range). However, by combining CESI-MS with a DEN-gas supply and an on-line pre-concentration technique, CESI-MS showed better concentration sensitivity than the state-of-the-art nano-LC–ESI-MS approaches, making it a very competitive and attractive technique for glycopeptide analysis.

Finally repeatability and intermediate precision was investigated at low, medium, and high concentrations for the three most abundant tryptic glycopeptides from IgGmAb1. Tables 3 displays the areas observed for repeatability (intraday variability). Relative standards deviations (RSDs) were lower than 4% for all studied concentrations, which is similar to results reported for nano-LC–ESI-MS[4].

	Relative abundance		
Conc, (ng/µL)	GOF	G1F	G2F
0.03	37 % (3.2%)	51 % (2.6%)	12 % (1.5%)
0.30	37 % (1.1%)	51 % (0.9%)	12 % (2.7%)
3.00	37 % (0.6%)	51 % (0.4%)	12 % (1.0%)

Table 2. Repeatability (N = 3) of Tryptic Fc *N*-Glycopeptides from IgGmAb1 with CE–ESI-MS Using the DEN-Gas.

Conclusions

A CESI-MS method integrating the use of a DEN-gas supply has been investigated for the use in the detection of glycopeptides. CESI-MS with DEN-gas offered several advantages including:-

- Improved sensitivities for glycopeptide analysis when compared to CESI-MS without DEN-gas.
- Lower LODs than observed with state-of-the-art nano-LC–ESI-MS methods.
- Excellent repeatability for glycopeptide detection with RSDs lower than 4%.

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

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3. Meyer, J. and Komives, E. A. “Charge State Coalescence during electrospray ionization improves peptide identification by tandem mass spectrometry”. J. Am. Soc. Mass Spectrom. 2012, 23, 1390–1399.
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