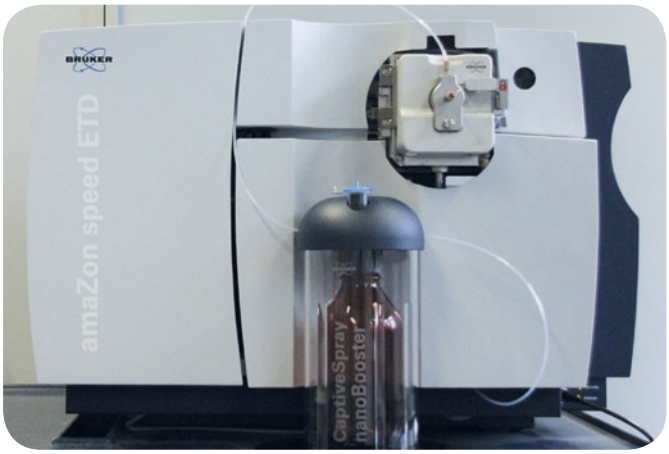

Technical Note # TN-44

Increasing Peptide Identification Rates for Proteomics Samples by Controlling Peptide Charge States Using CaptiveSpray nanoBooster

Introduction

Reliable and reproducible mass spectrometric results from nanoflow rate separations of complex peptide mixtures are strongly reliant on the ESI process. Whereas standard nanospray sources are prone to spray-instabilities and clogging, the CaptiveSpray ionization source provides a operationally robust and sensitive ESI source at nanoflow rates. This source utilizes a tapered etched capillary emitter tip attached to the inlet of the MS, which draws in gas around the emitter, desolvating and focusing the ions into the MS without the need for complicated and time-consuming xyz positioning of the source.

The CaptiveSpray nanoBooster ionization source allows the modification and vapor enrichment of the gas that flows around the emitter (Figure 1). Dependent on the dopant used, either charge stripping or charge enhancement of peptides and proteins can be achieved during the ionization process. Charge enhancement is advantageous for the analysis of large biomolecules due to the reduction of the mass-to-charge (m/z) ratio. Furthermore higher charge stages increase efficiency of electron transfer dissociation (ETD) MS/MS.

Authors

Stephanie Kaspar, Thorsten Ledertheil, Ralf Hartmer, Thomas Hagedorn, Carsten Baessmann
Bruker Daltonics GmbH, Bremen, Germany

Kerry Nugent
Bruker Daltonics Inc., Auburn, USA

Keywords	Instrumentation and Software
impact	impact
Proteomics	CaptiveSpray nanoBooster
CaptiveSpray nanoBooster	DataAnalysis
Charge States	ProteinScape

This TN describes the effect of the nanoBooster on the analysis of complex tryptic digests with regard to the average charge state and signal intensities. Furthermore, the optimization of peptide and protein identification rates using acetonitrile-enriched gas streams guided into the CaptiveSpray source is shown.

Experimental

Tryptic peptides were separated on a nanoLC system. For MS-detection at nano flow rates of 300 nL/min the CaptiveSpray nanoBooster ionization source was coupled to an Ultrahigh Resolution (UHR) Q-TOF system (impact). This second-generation CaptiveSpray source incorporates the nanoBooster, which allows the addition of vapor from organic solvents to the inlet gas of the CaptiveSpray source (Figure 1).

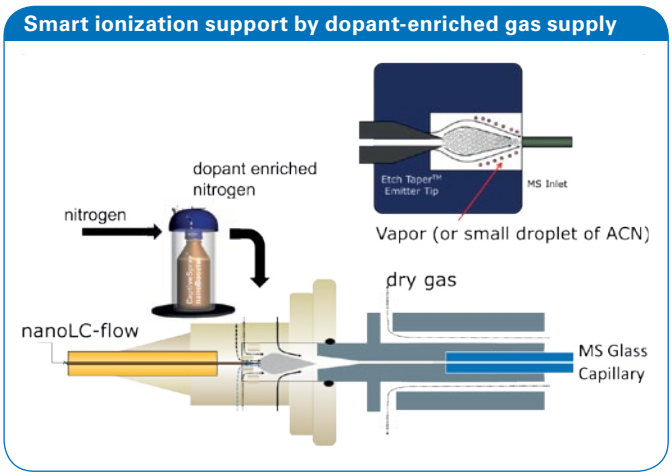

Figure 1: Schematics of the CaptiveSpray ionization source equipped with the nanoBooster for charge state controlling. Solvent-vapor-enriched nitrogen is introduced into the source.

The use of acetonitrile as dopant shows the effect of the enrichment of the inlet gas with organic solvents on peptide charge distribution, signal intensities, signal-to-noise behaviour and overall identification rates.

A tryptic digest of the human HeLa cell line is used as a model system. Different sample amounts (100 ng and 1 µg) and different gradient lengths (60, 240 min) were used to investigate the effect of the nanoBooster.

The database search was performed with Mascot 2.4 using SwissProt entries for human. The result was limited to proteins identified with a false discovery rate < 1%.

Results and Discussion

This TN describes the effects of the nanoBooster using acetonitrile as dopant compared to the standard lab air setup. The nanoBooster is directly coupled to the air inlet of the CaptiveSpray ionization source. Nitrogen is provided from the mass spectrometer's gas supply and is enriched with organic solvent before entering the CaptiveSpray ionization source.

The most prominent effect of the nanoBooster using acetonitrile-enriched nitrogen is an increase in the average charge state. Initial experiments analyzing tryptic peptides of BSA revealed an increase of the average charge state from 2.32+ to 2.66+ when using acetonitrile-enriched gas supply. For a HeLa digest, which represents a very complex peptide mixture, the average charge state increased from 2.16+ to 2.34+. Peptides, which were detected as singly charged species using purified ambient air, were predominantly detected as doubly charged ions using acetonitrile-enriched nitrogen (Figure 2 a). Besides in-spray charge state enhancement of peptides and proteins, the nanoBooster also enables charge stripping using organic solvents like methanol or isopropanol.

Another effect of the nanoBooster is an increase in signal intensity (Figure 2 b). The observable effect is strongly dependent on sample amounts. Base peak signal intensities increase up to five-fold for 100 ng of HeLa digest (Figure 3) due to charge-state enhancement. The effect on signal intensity using higher sample amounts (e.g. > 1 µg) was less distinct but still detectable.

The resulting increase in signal intensity and the charge state enhancement directly results in increased protein identification rates on the Q-TOF system (Figure 4). Because singly charged peptides are typically not considered for the fragmentation process and thus do not contribute to identification, an increase in identification rate occurs using the charge enhancement caused by the addition of acetonitrile vapor to the inlet gas. Using the nanoBooster is especially effective when limited amounts of sample are available. Compared to the standard CaptiveSpray lab air setup, using nanoBooster provided a 25% increase in the number of proteins identified in a 100 ng HeLa cell lysate sample.

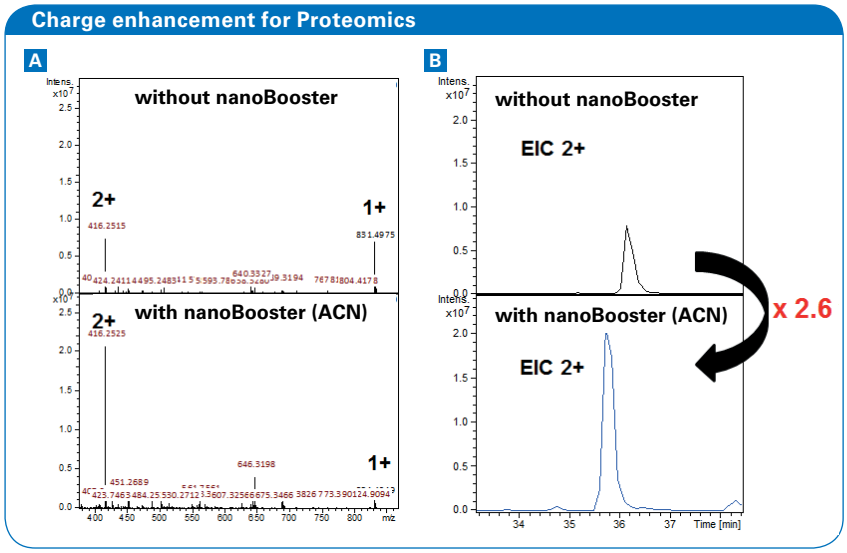

Figure 2: Effect of the nanoBooster on average charge. (a) Using acetonitrile as a dopant shifts charge states, leading to a reduction of the 1+ signal and an increase in intensity of doubly charged ions for tryptic HeLa peptides. (b) Increase in signal intensity of m/z 416.25 when nanoBooster (using acetonitrile as dopant) is used instead of the standard lab air setup of the CaptiveSpray ionization source.

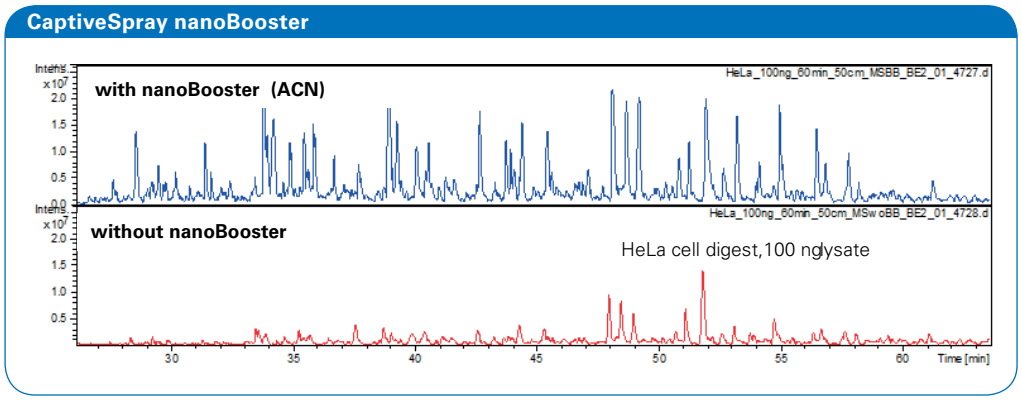

Figure 3: Comparison of base peak chromatograms of the analysis of 100 ng HeLa separated on a 60 min gradient using CaptiveSpray ionization source and CaptiveSpray nanoBooster. A significant increase of signal intensity can be observed.

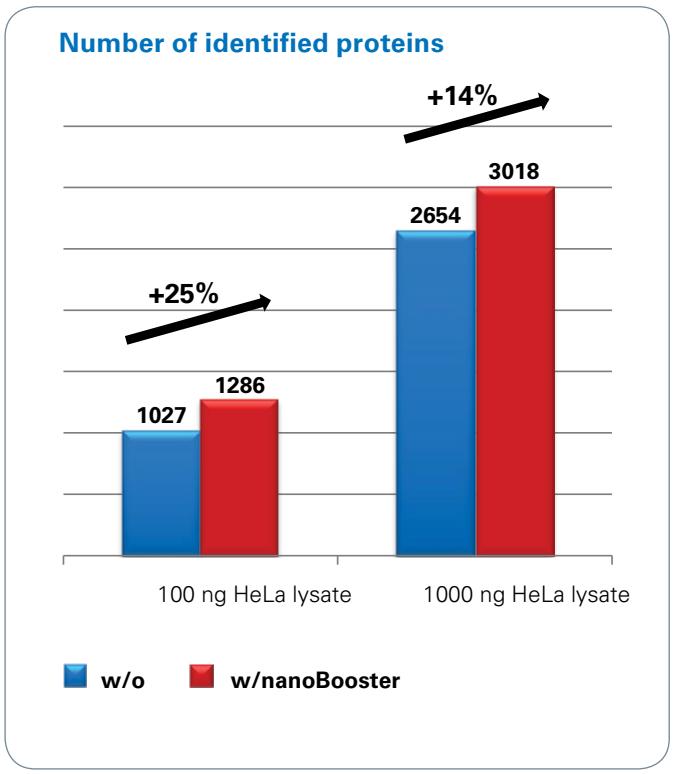

Figure 4: Comparison of the number of proteins identified from HeLa digest samples using either the standard lab air setup of the CaptiveSpray source or the nanoBooster with acetonitrile. The largest impact on identification rates was observed for the lower sample amount (100 ng lysate).

Conclusion

Equipping the CaptiveSpray ionization source with the new nanoBooster allows addition of vapor from organic solvents to the gas inlet. Depending on the solvent, in-spray charging enables charge-enrichment or charge-stripping for peptides and proteins.

The increase in the charge state combines with an increase in signal intensity, resulting in increased protein identification rates on the Q-TOF system (impact). The effect on protein identification rates depends on the sample amount loaded onto the column and is more significant for lower sample amounts.

The data presented here clearly demonstrate efficient in-spray charging of peptides without the need for addition of supercharging agents to LC solvents that may influence chromatographic performance. The CaptiveSpray ionization source equipped with the nanoBooster is not only advantageous for peptide identification experiments on Q-TOF systems, but also for ETD measurements requiring higher charge states (>+3). Further application areas are intact protein analysis – where shifting of charge states enables analyses of larger proteins – and glycoproteomics, where identification of glycan structures and localization of glycosylation sites can be improved (see LCMS 76).

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

Bruker Daltonik GmbH

Bremen · Germany
Phone +49 (0)421-2205-0
Fax +49 (0)421-2205-103
sales@bdal.de

www.bruker.com

Bruker Daltonics Inc.

Billerica, MA · USA
Phone +1 (978) 663-3660
Fax +1 (978) 667-5993
ms-sales@bdal.com

Bruker Daltonics Inc.

Fremont, CA · USA
Phone +1 (510) 683-4300
Fax +1 (510) 687-1217
cam-sales@bruker.com