

- Increased coverage from limited samples
- High-resolution separation of polar analytes and critical PTMs
- In-depth characterization of proteoforms and protein complexes

# See What You've Been Missing

CESI 8000 PLUS HIGH PERFORMANCE SEPARATION – ESI MODULE



# Expand the Reach of Your Mass Spectrometer

## The CESI 8000 Plus High Performance Separations-ESI Module

The CESI 8000 Plus is a valuable accessory for your mass spectrometer when analyzing charged and polar compounds in every analytical laboratory:

- Consumes minute samples with extraordinary sensitivity
- Excels at charged and polar metabolites, peptides, and more
- Enables high-resolution separation of proteins and protein-complexes
- Eliminates ion suppression bias so you can see what you've been missing
- Robust and easy-to-use

CESI-MS "showed better separation efficiency and resolution with 100-fold less sample consumption compared to an LC-based intact protein separation."

John Yates III  
The Scripps Research Institute, La Jolla, CA, USA

CESI-MS "shows a separation efficiency for high-molecular-mass compounds like larger peptides and proteins that is remarkably higher than that seen with any LC method"

Herbert Lindner  
Innsbruck Medical University, Austria

"CESI-MS is a high-end tool for Metabolomics work in my lab"

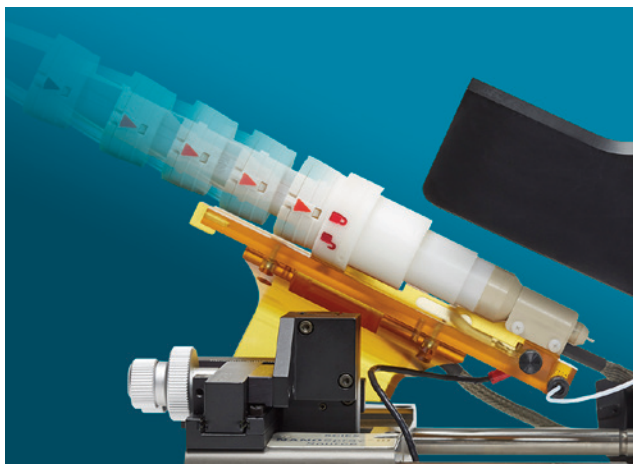
Dr. Rawi Ramautar  
Leiden Academic Center for Drug Research, The Netherlands

"...high resolution CZE separation of intact glycoprotein species coupled to MS has significant potential for the in-depth characterization and quantitative analysis of biopharmaceutical proteoforms."

Barry Karger  
Barnett Institute, Northeastern University, Boston, MA, USA

"With **only** a 200 fmol injection, CESI-MS/MS enables high quality mass spectra while completely characterizing the primary structure of biotherapeutic proteins in terms of amino acid sequence, glycosylation and post-translational modifications. This allows you to see more with less, and strengthens the power of the methodology in the context of biosimilarity assessment."

Yannis-Nicolas Francois  
University of Strasbourg, France



Connecting the CESI 8000 Plus to your MS is as easy as **plug and spray**. Simply click the OptiMS cartridge tip into the source adapter.



"With monoclonal antibodies having become such a significant class of drugs, not just in the BioPharma space but the entire Pharma, capillary electrophoresis has become an indispensable tool for monitoring product related impurities and product variants of these blockbuster drugs. Advances like the CESI 8000 now allow for the coupling of CE and MS and provide a solution for the desperately needed identification of peaks separated by capillary electrophoresis."

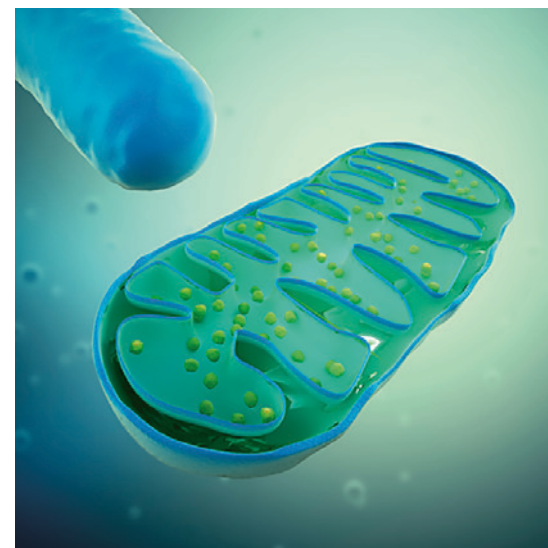
Timothy Blanc  
Eli Lilly



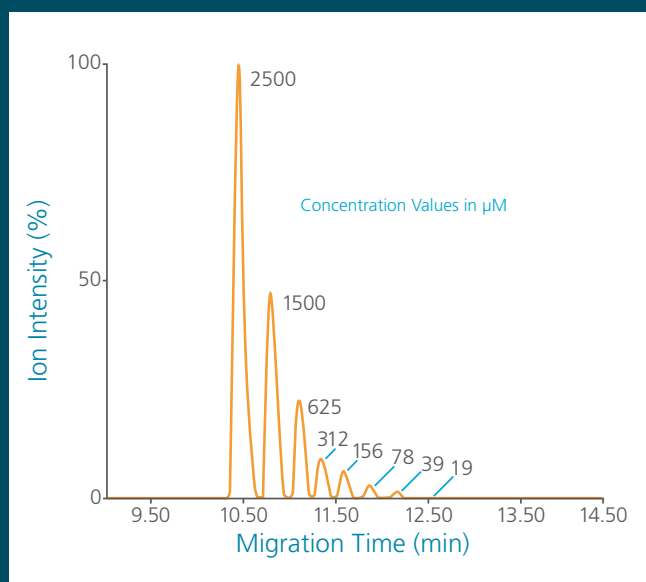
# More Data from Less Sample

CESI-MS is particularly valuable for precious samples such as: subcellular fractions, microdialysates, CSF, murine samples, CTCs, needle biopsies, FFPE archives, or highly toxic samples such as ADCs and venoms.

- Unique multi-segment injection increases throughput 10X
- Extraordinary sensitivity and high resolution enable maximum information with minimal sample consumption (~50 nL per injection)



**Multiple experiments  
in the time of one, with  
<1  $\mu$ L sample injected.**



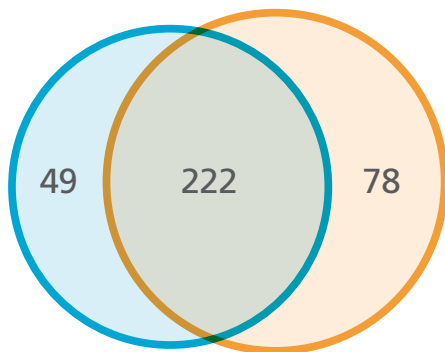
Data from multiple samples in single MS detection run.

The unique multi-segment injection (MSI) feature significantly increases throughput. Multiple injections are separated by 60 seconds of background electrolyte injections for sequential MS analysis. This technique is commonly used with MRM targeted analyte detection.

## Example of high sensitivity from a limited sample

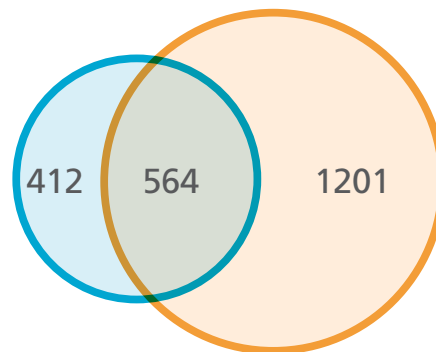
Using only 100 ng of yeast mitochondrial extract, CESI-MS significantly increased peptide identification, resulting in a substantial increase in the number and variety of proteins identified.

Number of Proteins Identified



● nanoLC-MS/MS ● CESI-MS/MS

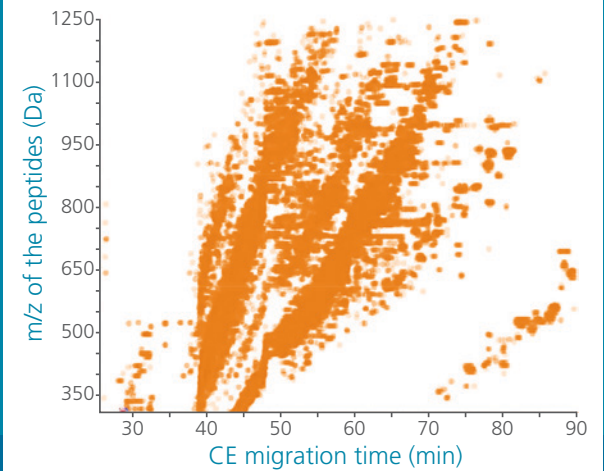
Number of Peptides Identified



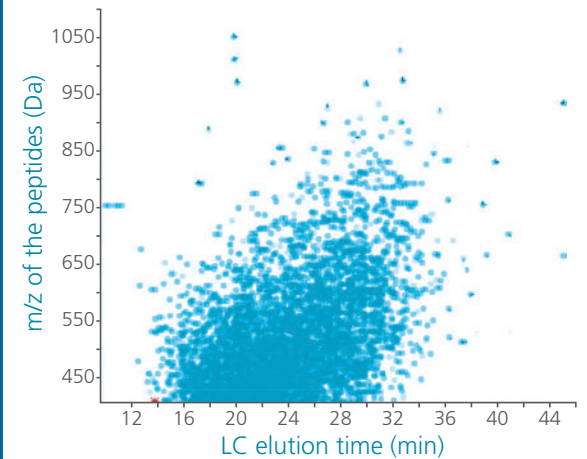
● nanoLC-MS/MS ● CESI-MS/MS

Each dot is related to a single MS/MS fragmentation spectrum.

CESI-MS/MS



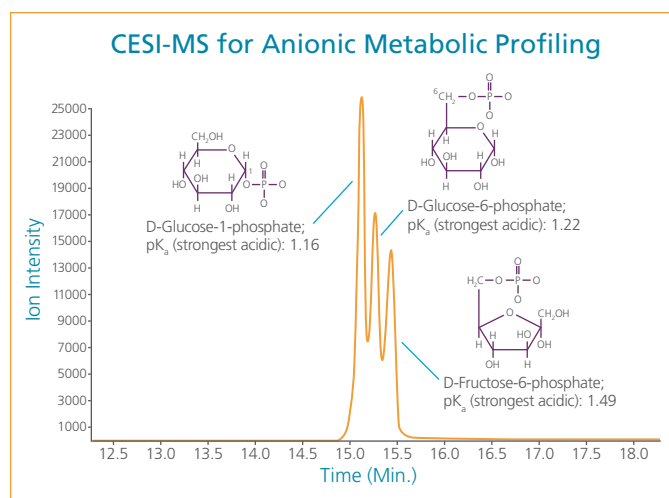
NanoLC-MS/MS





Measurements of highly charged analytes pose considerable challenges to current technologies. CESI is the method of choice for high-resolution separation of polar metabolites, peptides and proteins rich in particular post-translational modifications (such as glycosylation, citrullination, methylation, and phosphorylation).

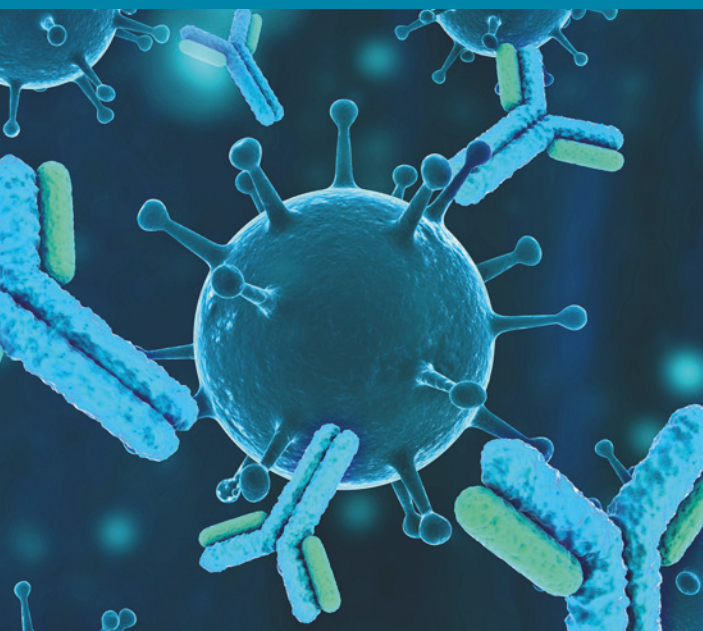
## Separate Charged / Polar Molecules and Proteins in High Resolution



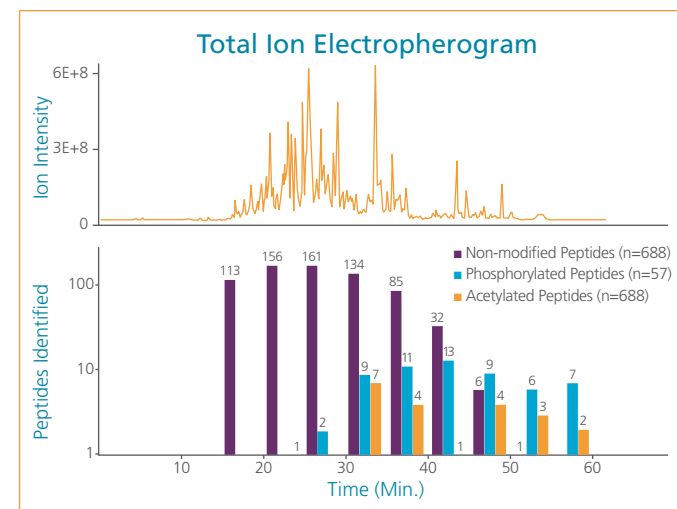
Kindly provided by Dr. Rawi Ramautar, Leiden Academic Center for Drug Research, The Netherlands.

Electrophoretic separation combined with highly efficient ESI supports a variety of applications, from investigating the anionic and cationic metabolome, to the study of proteoforms, glycoforms, and multimeric protein complexes.

The separation of sugar phosphates in central metabolism and baseline separation of isobaric positional isomers such as citrate and isocitrate demonstrates CESI-MS' high resolving power. CESI is also the preferred technology for chiral compound separations in a cost effective manner compared to chiral LC columns.

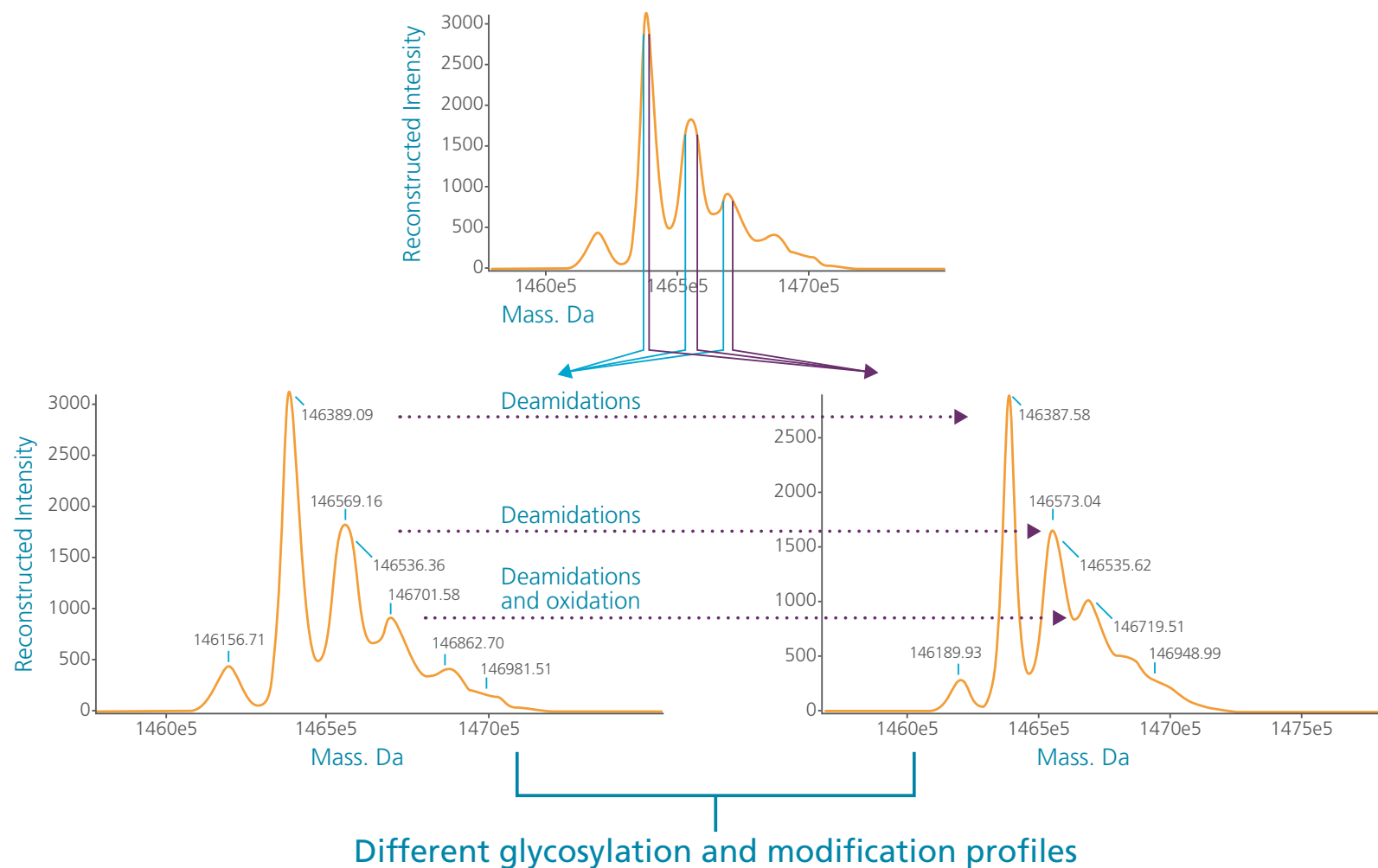


For complex peptide mixture analysis, CESI excels at various post-translational modifications (PTMs) revealing important biological functional variations.



Kindly provided by Herbert Lindner, Innsbruck Medical University, Austria.

## Intact MAb Separation of IgG1 by CESI-MS



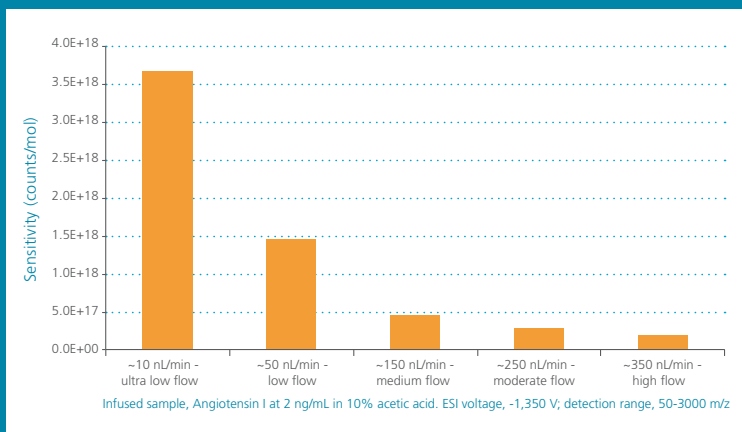
For deep characterization of proteoforms, glycoforms, charge states, and PTMs, CESI is the only front end technology that enables liquid phase high resolution separation of proteins, metal binding, and protein complexes. CESI-MS is an invaluable tool to drive biopharmaceutical development and biochemical mechanistic studies.

# Principles and Design

## CESI

The integration of capillary electrophoresis (CE) with electrospray ionization (ESI) into a single dynamic process within the same device.

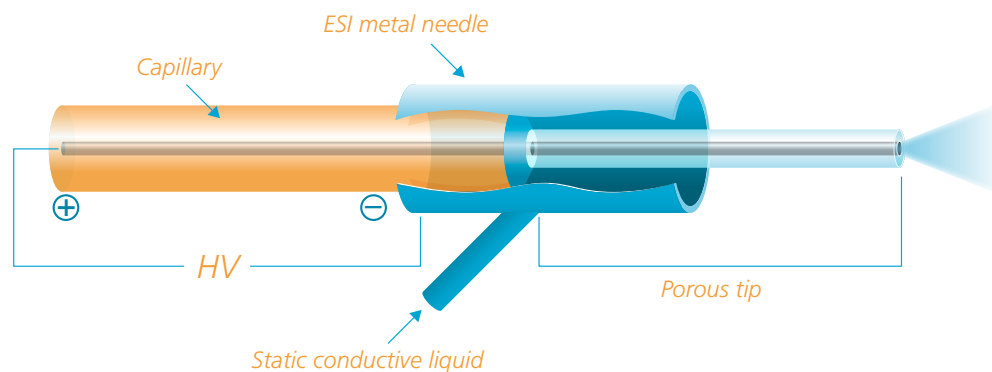
CESI was developed as a new front-end separation and ionization technique, combining the high efficiency and ultra-low flow of CE with ESI. This ESI optimization has shown to greatly improve assay sensitivity while reducing ion suppression bias.<sup>1, 2</sup>



1. Kelly RT, Page JS, Zhao R, Qian W, Mottaz HM, Tang K, Smith RD, Anal.Chem. 2008, 80, 143-149

2. Schmidt A, Karas M, Dulks T, J., Am. Soc. Mass Spectrom. 2003, 14, 492-500

Schematic of CESI sprayer

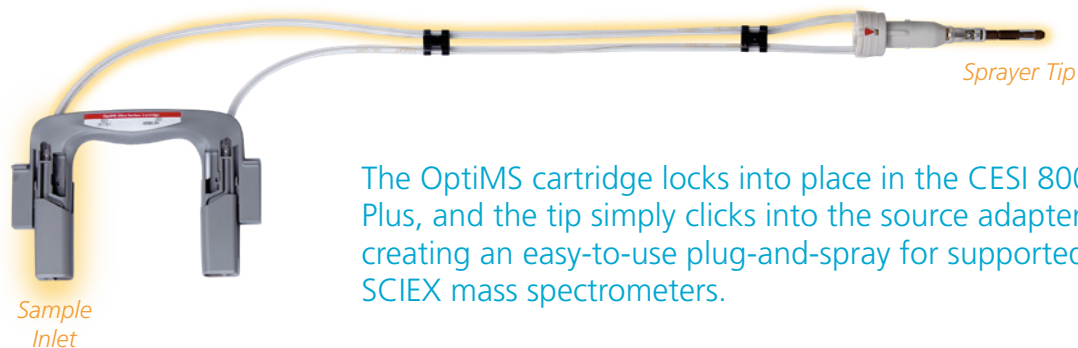


The CESI 8000 Plus was developed in collaboration with mass spectrometry researchers covering a number of applications. They sought to expand their range of detection and increase sensitivity. In order to achieve this, ultra-low flow separation was integrated with mass spectrometry using proprietary CESI-MS technology.

The sprayer, located within a protective housing of the OptiMS cartridge, combines an intrinsically low-flow CE separation with electrospray ionization (ESI) within a single device.

- The CESI OptiMS capillary inlet and outlet are of the same inner diameter
- The electrical contact for CE separation as well as for ESI generation is achieved through the ESI metal needle filled with conductive fluid
- Ultra low flow at the porous capillary tip instantly generates a fine ESI spray when a low voltage ~ 1.25 KV is applied
- The low voltage of CESI-MS reduces oxidation artifacts





### Zero dead-volume design

A single continuous separation capillary is contained within the OptiMS cartridge, with the same internal diameter from sample to spray. This design:

- Does not require lengthy column equilibration
- Eliminates sample carry-over
- Reduces sample-to-sample injection time
- Increases robustness

### Multiple experiments / sample analyses from as little as 5 $\mu$ L

The nanoVials have been validated for use with 5  $\mu$ L of sample. With careful pipetting, injections from as little as 1  $\mu$ L are possible. CESI-MS has ultra-low sample consumption, leaving most of the material available for analysis by orthogonal techniques.



### CESI 8000 Plus mobile design

The CESI 8000 Plus comes equipped with an electric height adjustable mobile lab bench.

### Service, support, and consumables

Our customer support organization has access to the latest product updates, software revisions, methods and repair procedures. The following is available to further optimize your use of CESI-MS:

- Application Training
- Operation Qualification (OQ)
- Chemistries for MS as well as optical detection

### Multi-MS connectivity and multi-detection capability

In CESI-MS mode, the CESI 8000 Plus can be connected to industry-leading SCIEX mass spectrometers. In stand-alone CE mode, UV/VIS, PDA, or LIF detection can be selected. This allows for the analysis of biologics using our CE-SDS, cIEF, and glycan chemistries.

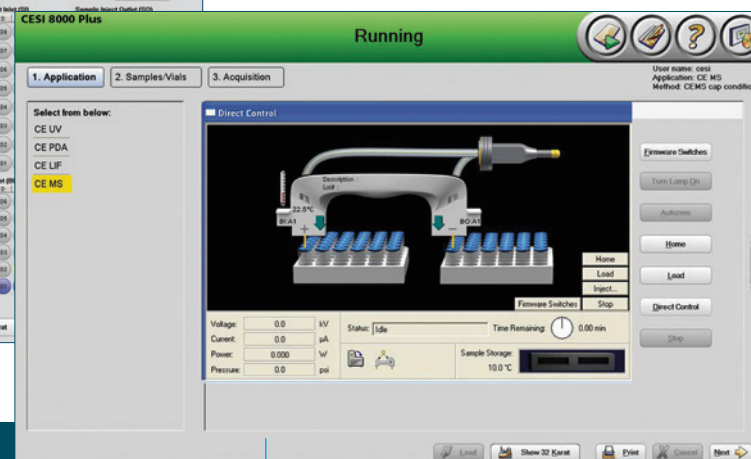
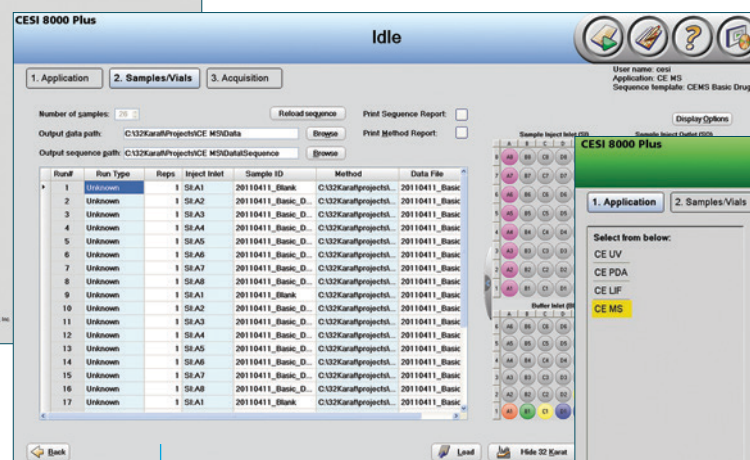
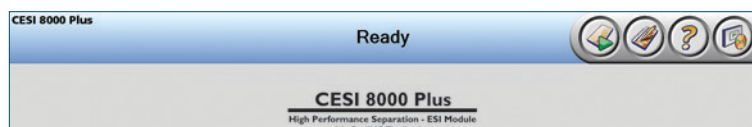
### CE Assays and Method Development



### MS Analysis



# CESI 8000 Plus Easy-to-Use Software



## Ready for Use Within Minutes

Simply select one of the pre-loaded methods, follow visuals to place samples and reagents, and hit START.

## Visual and Intuitive

The layout of the user interface mimics the instrument to enable direct tracking of sample contents.

## Flexible yet Expandable

Select programmed control or direct control to maximize throughput and optimize separation performance.

## Module Specifications

### Dimensions:

Height: 29.2 in (74.2 cm)  
Door Open: 38.8 in (98.6 cm)  
Width: 25 in (63.5 cm)  
Depth: 28.4 in (72.1 cm)

### Height Adjustable Portable Lab Bench with Memory Settings:

36 in x 30 in (91.4 cm x 76.2 cm)  
Height adjustable from 27 in to 44 in (69 - 112 cm)

### Weight (uncrated):

188 lbs (85.3 kg)

### Electrical Requirements:

Voltage: 100 - 240 V; 50/60 Hz

### Voltage Range:

1 to 30 kV programmable  
at 0.1 kV increments

### Current Range:

3 to 300  $\mu$ A programmable  
at 0.1  $\mu$ A increments

### Operating Environment Range:

15 - 30° C  
20 - 60% relative humidity  
(non-condensing)

### Sample Trays:

2 x 48 CESI vials / 0.3 mL microvials

### Buffer Tray:

2 x 36 CESI vials

### Sample Temperature

#### Adjustment Range:

4 - 60° C

### Capillary Temperature

#### Adjustment Range:

15 - 30° C

### Stand-alone Capillary Temperature

#### Adjustment Range:

15 - 60° C

### Pressure Delivery Range:

-5 to +100 psi

### Minimum Required Sample

#### Volume:

50  $\mu$ L when using microvials  
5  $\mu$ L when using nanoVials



## Ordering Information

### A98089: MS CESI-MS base unit

Includes CE separation module, OptiMS Sprayer technology, and UV detector ready for stand-alone or CESI-MS analysis. Also includes computer, monitor, system controller pre-loaded with the CESI 8000 Plus software, electric height adjustable mobile lab bench making switching between CESI-MS and other front end separation instrument is swift and simple.

### A98089 MD 2: CESI-MS base unit with UV and Diode Array Detectors (for standalone CE applications)

### A98089 MD 3: CESI-MS base unit with UV and LIF Detectors (for standalone CE applications)

### A98089 MD 4: CESI-MS base unit with UV, Diode Array and LIF Detectors (for standalone CE applications)

### B07367: Silica Surface OptiMS Cartridge

30  $\mu$ m ID, 150  $\mu$ m OD x 90 cm

Contains bare fused-silica capillary for high-resolution separation of peptides, glycans, amino acids and nucleotides/nucleosides

### B07368: Neutral OptiMS Cartridge

30  $\mu$ m ID, 150  $\mu$ m OD x 90 cm

Contains covalently attached neutral hydrophilic capillary surface enabling high resolution separation of intact proteins and protein/peptide/small molecule/metal complexes.

### 5043467: nanoVial

5  $\mu$ L starting volume validated, 1  $\mu$ L starting volume demonstrated

### Biologic Characterization Kits

390953: SDS-MW Assay Kit/Purity/Sizing

477600: Carbohydrate Labeling and Analysis Assay

A58481: cIEF Peptide Marker Kit (pI Marker Kit)

A80976: Advanced cIEF Starter Kit

### CESI OptiMS Adapter Kits

B07363: OptiMS Adapter for SCIEX Nanospray III source

For more product information on our capillary electrophoresis systems visit [sciex.com/ceproducts](http://sciex.com/ceproducts).

For information on CESI 8000 Plus and supported MS models/sources, contact your SCIEX representative.

# Your Success is Our Success

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