

# Extending the Reach of Mass Spectrometry

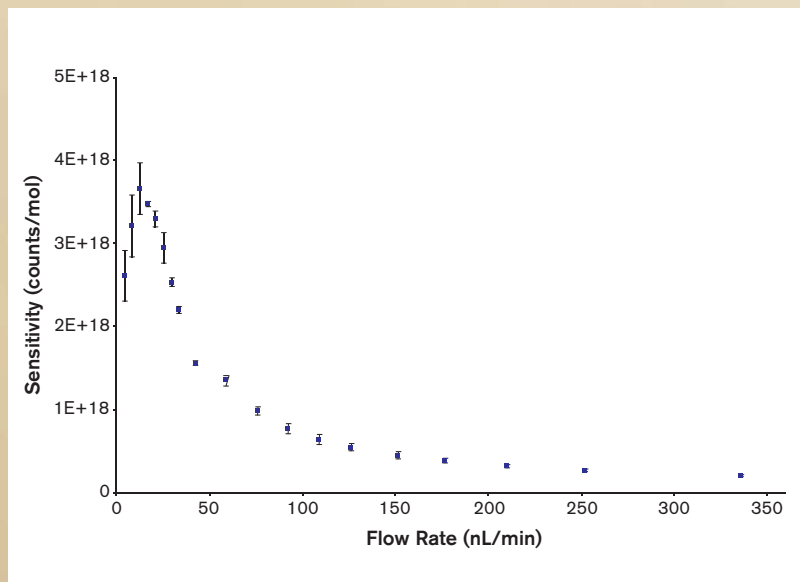
CESI 8000 High Performance Separation-ESI Module  
with OptiMS technology

Genomics  
Cell Analysis  
Particle Characterization  
**Capillary Electrophoresis**  
Lab Automation  
Centrifugation  
Lab Tools

See what  
you've been  
missing.

## Extending the Reach of Mass Spectrometry

Mass spectrometry (MS) has become an indispensable technology for the analysis of compounds of biological interest. Whether you seek to characterize a therapeutic protein; identify the proteins that make up a specific proteome; characterize post-translational modifications; study a metabolomic fingerprint related to a particular condition; or quantify drugs and their metabolites in a minute or complex sample matrix, improvements in assay sensitivity and reductions in ion suppression serve to



The infusion experiments highlighted in the figure above, illustrate the relationship of flow to ionization efficiency. As we decrease flow of the bulk electrolyte we correspondingly increase the efficiency of ionization and reduce ion suppression.

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

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reveal new information. To achieve this, we developed a new front-end separation-and ionization technology called CESI, which combines the high efficiency and ultra-low flow characteristics of capillary electrophoresis with an integrated electrospray ionization source. Reduction of flow with electrospray ionization (ESI) into the nanoflow region has been reported to improve assay sensitivity (1) and reduce ion suppression (2) with ESI-MS. Of course, capillary electrophoresis is, by its very nature, an ultra-low flow separation technology.

## CESI 8000 with OptiMS Technology provides major advantages over other front-end separations to ESI-MS:

- **Increased Sensitivity** – In order of magnitudes depending on analyte and MS
- **High Efficiency Separation** – Peak capacity >300
- **Decreased Cycle Time** – Short analysis and regeneration times
- **Reduced Ion Suppression** – Nanoflow leads to higher sensitivity
- **Minute Sampling** – From nano-litre to micro-litre sample introduction
- **Broader Analyte Coverage** – Ideal for highly charged and polar analytes

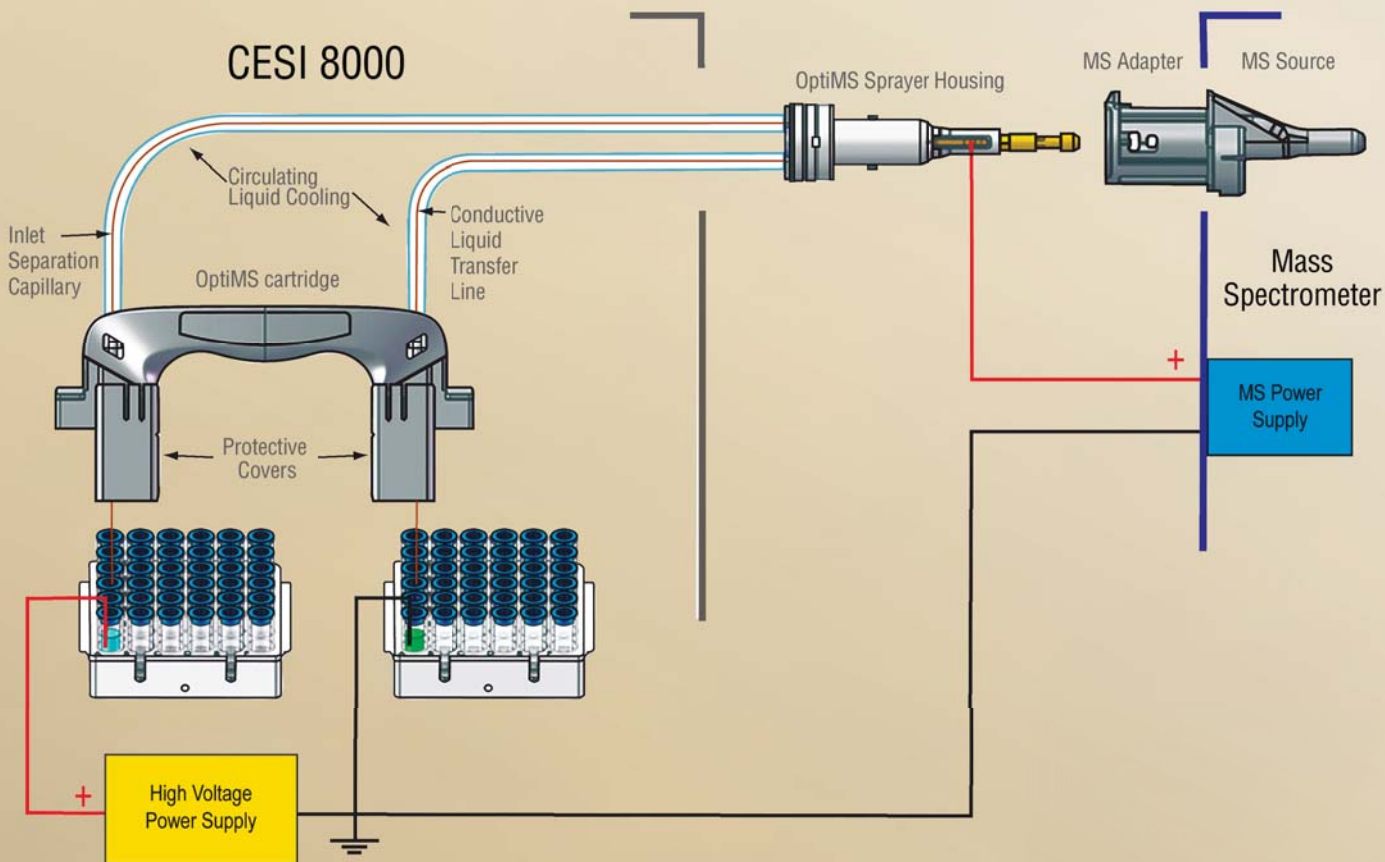
## High Performance Separation and ESI Module Upstream from MS

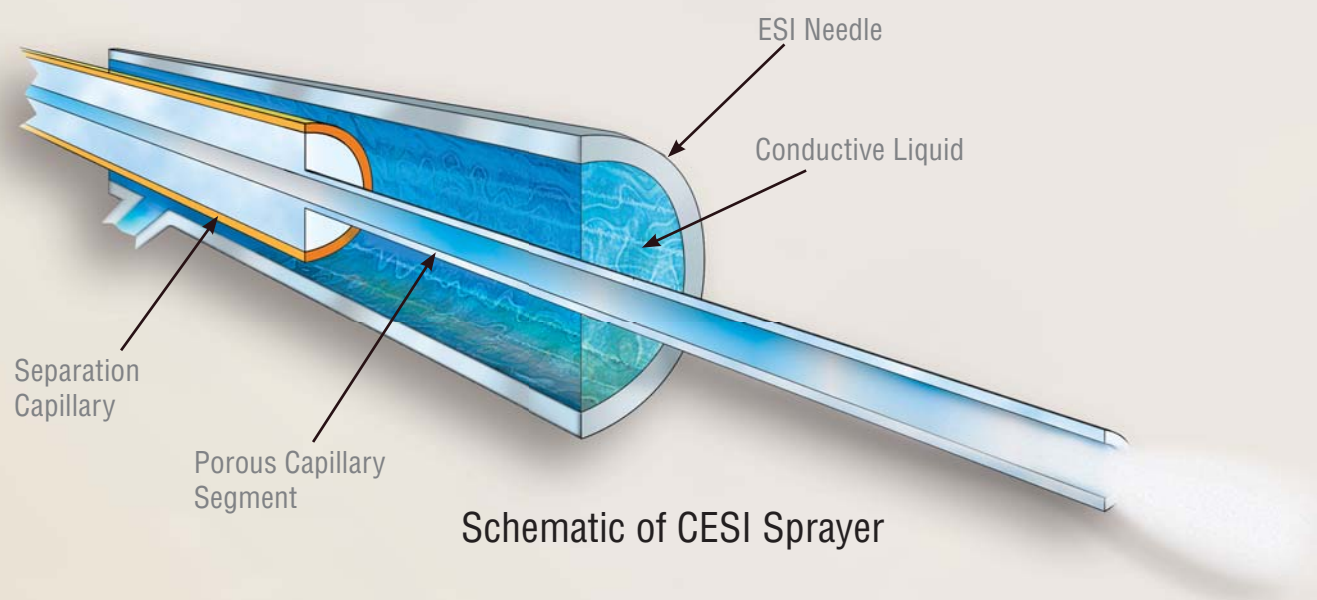
Capillary Electrophoresis (CE) is intrinsically a low flow technology that is known for its unmatched resolving power. The CESI 8000 High Performance Separation-ESI Module is designed for mass spectrometry applications that analyze charged and polar molecules. Thanks to its robust OptiMS technology, the advantages of CE are now delivered to the mass spectrometer without dilution or disturbance. Stable ESI is generated at an ultra low flow in the low nL/min. range, resulting in ultra high sensitivity. These flow rates serve to reduce ion suppression effects.



**The CESI 8000** 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 goal, a low flow pre-MS separation technology was integrated with a novel ESI coupling technology.







The sprayer is located within its protective housing. It combines an intrinsically low flow CE separation with electrospray ionization (ESI) within a single simple device that has no liquid junction or dead volume. The distal end of the capillary is made porous to ion flow. The electrical contact for the CE is achieved through the ESI needle, which is filled with conductive liquid and for the ESI by the porous capillary protruding from the needle allowing electrospray formation at the capillary tip. When ESI voltage is applied, the low flow at the tip terminus instantly vaporizes into a spray. In this manner, all electrochemistry associated with the electrolysis of water are decoupled from the spray. Moreover, the inlet of the capillary and the exit are of the same dimension—reducing the issue of clogging often associated with nanoscale techniques. Using this configuration, ESI is generated using much lower voltage, reducing the current and risks of oxidation artifacts.

### **The simple and modular “plug-and-spray” design simplifies operational efficiency:**

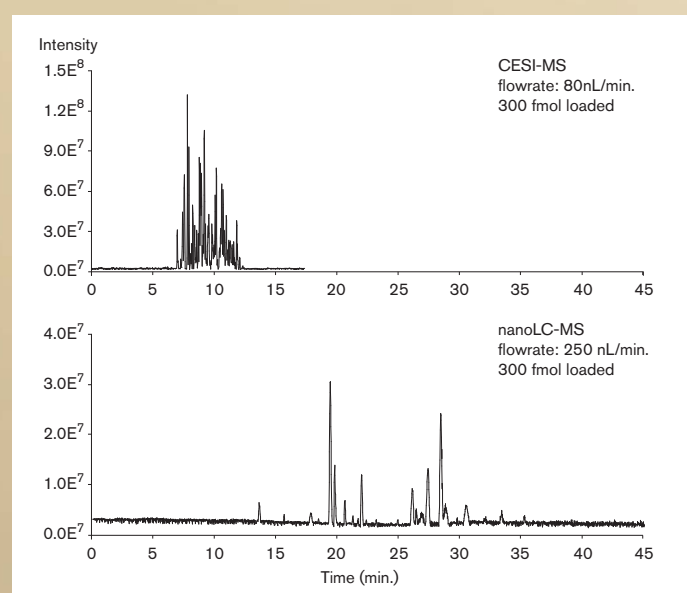
- Automated sample handling from microvials
- Simple interfacing, with automatic triggering of MS data collection
- Easy switching between liquid chromatography (LC) and CESI-MS
- Software as easy to use as 1 – 2 – 3

## Complex Peptide Mixtures

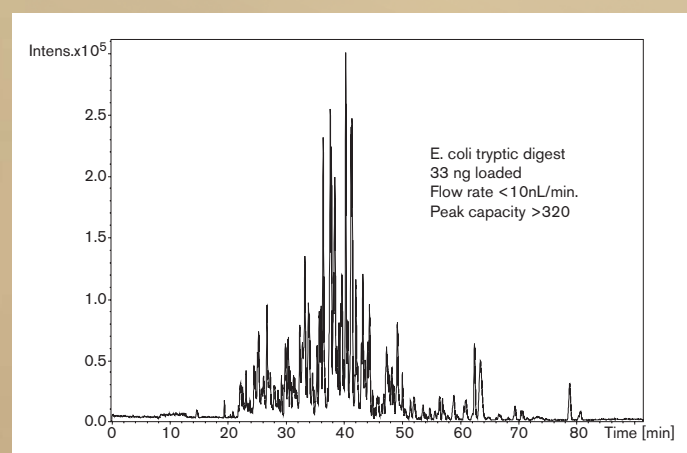
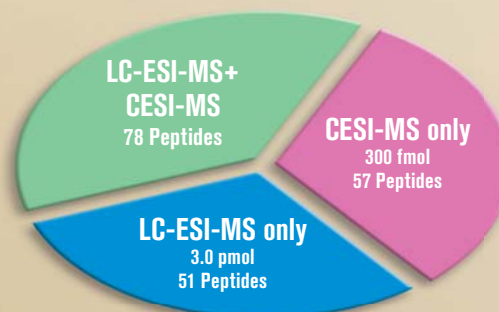
The CESI 8000 High Performance Separation - ESI Module with OptiMS technology significantly enhances the sensitivity of peptide analysis while simultaneously expanding the coverage of the smaller, highly polar peptides often missed by other technologies. The ultra-low flow rates achievable during electrospray ionization reduce ion suppression and make it possible to detect those post-translational modifications (PTMs) like phosphorylation and sialylation that typically hamper efficient ionization. The high separation efficiency and high sensitivity of the technology permit detection of peptides present in low abundance and/or of low molecular weight; even microsequence variants differing only slightly in mass or charge.

### Advantages of the CESI 8000 with OptiMS technology for proteomic studies include:

- Orthogonal approach to nanoLC-ESI-MS or UPLC-ESI-MS, yielding complementary results
- Expanded coverage of phosphorylated, low molecular weight, and hydrophilic peptides
- High resolution separations with sensitivity in the low picomolar range
- Faster cycle times



Analysis of ~300 fmol of Arg- digested Rat Testis Linker Histone H1 protein loaded onto both the CESI 8000 with OptiMS technology and nanoLC-MS. Note the higher signal and resolution of the CESI-MS separation despite its shorter analysis time and lower flow rate.



**CESI-MS is capable of generating separations of peptides with a high peak capacity (>320) as illustrated by the E.coli tryptic digest highlighted above. This allows for the analysis of very complex samples at a proteome level.**

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Further analysis of the peptides and identified proteins showed the advantages of the CESI with OptiMS technology in breadth of coverage. Merging the results of the 300 fmol CESI-MS and 3.0 pmol LC-ESI-MS analyses, a total number of 186 different histone H1 peptides could be identified. 42% (78 peptides) of these peptides were identified by both methods, 30.6% (57 peptides) could be identified solely by CESI-MS and 27.4% (51 peptides) by LC-ESI-MS. This result clearly demonstrates that CESI-MS complements LC-ESI-MS in an ideal manner since the number of peptides identified was increased by 44% from 129 (LC-ESI-MS only) to 186 (LC-ESI-MS + CESI-MS)"

\* Data courtesy of Prof. H. Lindner, Innsbruck Medical University.

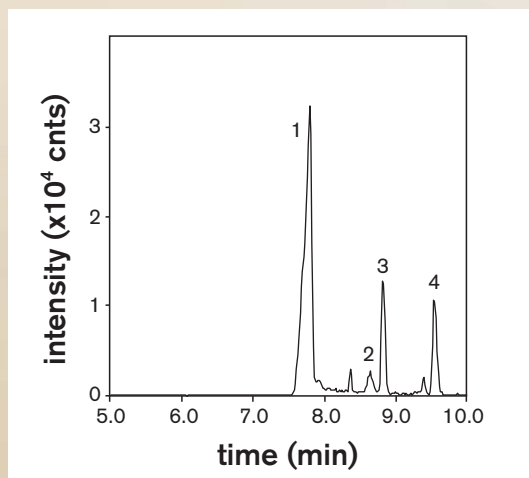
## Intact Protein Analysis

Whether your research focus is with proteomics or the characterization of biopharmaceuticals, the rigorous analysis of intact proteins contains many analytical challenges. The use of mass spectrometry (MS) has emerged as the leading means for achieving such protein structure determination, for which the proteins of interest need to be ionized, such as by electrospray ionization (ESI). The efficacy of ESI-MS depends in part on the efficiency and flow rate of the separation technology upstream from MS.

Capillary electrophoresis (CE) is an established technology for the separation of intact proteins, determination of molecular weight, characterization of glycans and the detection of other PTMs. Through the use of OptiMS technology, the CESI 8000 High Performance Separation – ESI Module delivers ionized proteins to the MS source, while preserving intact protein structure and the high resolution CE separation. High sensitivity is ensured by the ultra-low flow rate of the technology in the mass sensitive range of MS.

### Advantages of the CESI with OptiMS technology for intact protein MS analysis:

- High resolution and significantly improved sensitivity enable the separation and detection of intact proteins, their isoforms, subunits and cleaved fragments
- Use of native electrolytes preserve structure allowing analysis of protein complexes



BPE obtained with sheathless CESI-MS of a mixture of insulin (1), carbonic anhydrase II (2), ribonuclease A (3) and lysozyme (4) (5 µg/mL each) using the nanoESI source.

### Linearity ( $R^2$ )<sup>a</sup> and LODs (nM)<sup>b</sup> for the four model proteins obtained with sheath liquid CE-MS and CESI-MS

Protein	Sheath liquid CE-MS <sup>c</sup>		CESI-MS <sup>d</sup>	
	$R^2$	LOD	$R^2$	LOD
Insulin	0.992	106	0.999	1.28
Carbonic anhydrase II	0.981	79	0.989	0.58
Ribonuclease A	0.989	33	0.992	0.62
Lysozyme	0.990	41	0.997	0.50

a Concentration range, 1-100 µg/mL (sheath liquid) and 0.05-25 µg/mL (CESI-MS).

b Concentration to yield S/N ratio of 3 as calculated by extrapolation from 1-µg/mL injection. For carbonic anhydrase analyzed with sheath-liquid interfacing, a 5-µg/mL injection was used.

c BGE, 100 mM ammonium acetate (pH 3.1).

d BGE, 100 mM ammonium acetate (pH 3.1) containing 5%(v/v) isopropanol.



## Metabolomics

Development of new drugs and research on biomarkers for toxicity depend on the analysis and understanding of the composition, concentration, and dynamics of the entire compilation of small compounds present in biological samples. The large chemical variety and dynamic range of these metabolites present a major analytical challenge for any single separation technology to completely address. This creates a special demand on analytical methods for achieving a satisfactory coverage of metabolites.

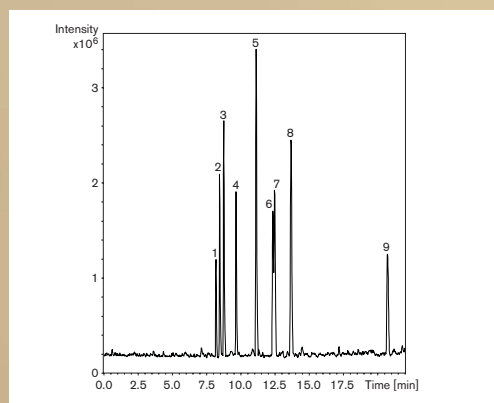
A large fraction of the endogenous metabolites present in biological samples is highly polar and ionic and, therefore, capillary electrophoresis (CE) is a very attractive separation technique for the analysis of such samples, as compounds are separated on the basis of their charge-to-size ratio. The CESI 8000 High Performance Separation - ESI Module with OptiMS technology provides fast and efficient separation, and significantly increases MS sensitivity while simultaneously expanding coverage of organic acids, amino acids, low molecular weight amines, peptides, nucleic acids, nucleosides, and related metabolites. Molecules often missed or altogether undetectable by technologies like Reversed Phase LC-MS now become visible.

### Advantages the CESI 8000 with OptiMS technology brings to metabolomics analysis:

- In-depth profiling with expanded coverage of charged and polar compounds
- Orthogonal approach to RPLC-MS, yielding complementary results
- High sensitivity for low abundant metabolite identification in the sub-nM range
- High resolution separation upstream from MS
- Minimal loss of metabolites: simple sample preparation, no derivatization required
- Good tolerance to sample matrix components
- Short cycle times and high reproducibility using simple rinses between separations
- Cationic and anionic compounds can be analyzed in sequence, separated only by a simple rinse

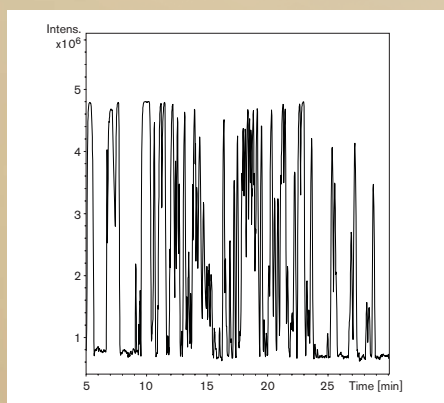
### LODs (S/N=3) for test compounds

Compound	nM
Lysine	0.8
Arginine	0.7
Isoleucine	1.9
Leucine	1.8
Phenylalanine	0.6
Tyrosine	0.7
Aspartate	5.2
Histidine	0.8
Methionine	2.2
Glutamate	4.1



### Multiple extracted ion electropherogram for metabolite mixture of:

1. Lysine
2. Arginine
3. Histidine
4. Dopamine
5. Phenylalanylglycine
6. Isoleucine
7. Leucine
8. Tyrosine
9. Hippuric acid



1:1 diluted with  
run buffer.  
Run buffer: 10%  
acetic acid  
(pH 2.2)  
MS scan range:  
50-450 m/z  
Data courtesy of  
Dr. R. Ramautar,  
Leiden University  
Medical Center

Analysis of a metabolite mixture comprised of compounds from various chemical families, by CESI-MS yielded a good separation and excellent analyte intensities

### CESI-MS metabolic profiling of human urine.

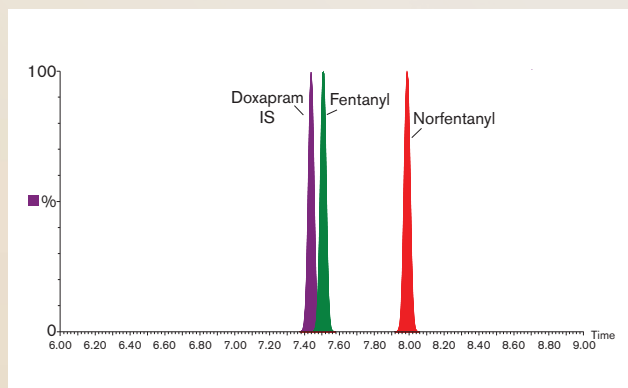
Highly information-rich metabolic profile with high peak responses was obtained over nearly the entire separation window using an injection volume of ~8 nL only.



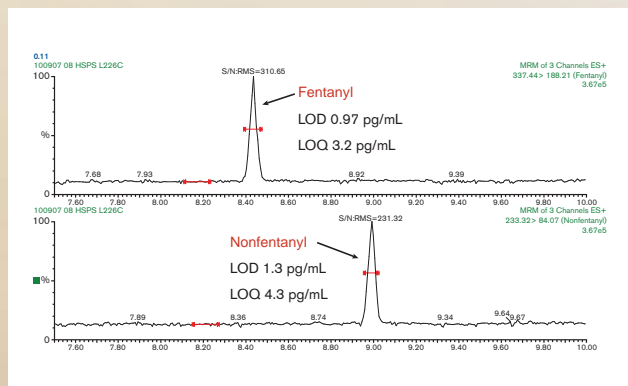
## Drugs and Their Metabolites in Biological Fluids

A significant challenge for analysts is to reliably identify, confirm and quantify drugs, their metabolites and impurities in samples of biofluids such as blood, plasma, serum and urine. Metabolites of drugs of forensic interest are often highly polar and charged. Capillary Electrophoresis has been providing the solution for routine analysis of these compounds for over 15 years. In demanding circumstances, when only minimum specimen is available and drug levels in the biofluid are low, a more sensitive hyphenated approach is required, such as CESI-MS. Effective extraction and separation techniques are currently available with appropriate internal standards and test mixtures, along with methods for the CESI 8000 software.

### Fentanyl & Norfentanyl

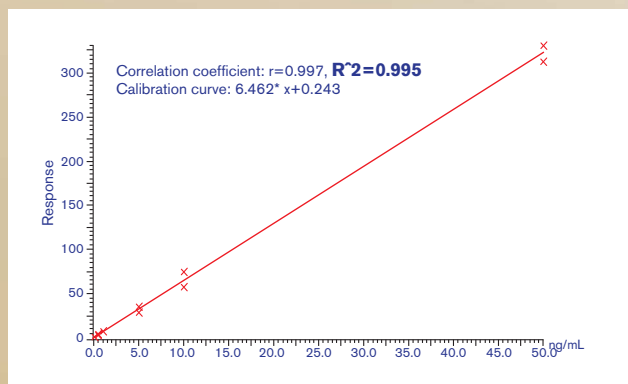


### Calculated LOD and LOQ

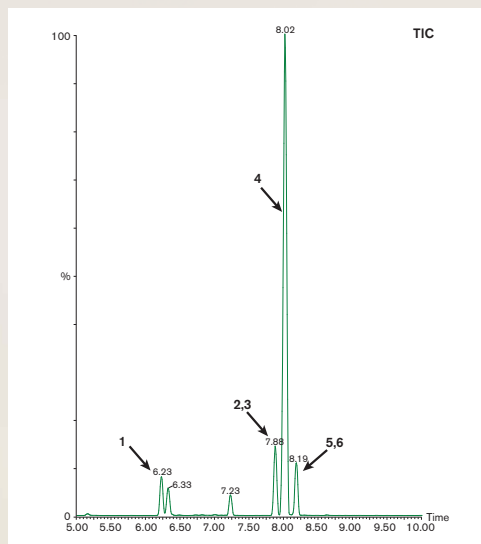


The data were derived using only 6 nL of sample injected.

### Linearity of Fentanyl



### Chloroquine (CQ) Metabolite Identification



CQ is a compound with complex metabolism, and its analysis requires good resolution. Its metabolites are easily detected, differentiated, and maintained throughout the CESI 8000 separation and MRMs.

1. Doxapram IS 379.5
2. CQ, N-Desethyl-hydroxy- 308.2
3. CQ, Hydroxy- 336.4
4. CQ 320.3
5. CQ, N-Desethyl- 294.2
6. CQ, N,N-Didesethyl- 264.3

# CESI 8000 Software



The CESI 8000 software simply requires the routine user to select one of the laboratory's pre-loaded methods, set the number of samples, load the reagents, and start running.



CESI 8000 software is graphical and very easy to work with. All vial positions may be annotated, allowing you to easily track the contents of any given vial.



Either programmed control or direct control can be implemented, allowing the user maximum flexibility to optimize the separation.

## System Specifications

### Dimensions:

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

### Height Adjustable Gear Driven Portable Lab Bench:

36" x 30" Height adjustable  
from 27" to 53"

### Weight: (uncrated):

188 lbs (85.3 kg)  
(includes UV detection)

### Electrical Requirements:

Voltage: 100 - 240V 50/60Hz

### 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

### Sample Trays:

2 x 96-well plates  
2 x 48 universal vials  
2 x 48 0.3 ml vials or microfuge tubes

### Buffer Tray:

2 x 36 universal vials

### Detection Capability:

UV/Vis  
200, 214, 254, 280 nm standard filter  
190 - 600 nm (custom filter option)

### Sample Temperature Adjustment Range:

4 - 60°C

### Capillary Temperature Adjustment Range:

15 - 60°C

### Pressure Delivery Range:

-5 to +100 psi



## Ordering Information

### A98089: CESI 8000 High Performance Separation - ESI Module

Includes CE separation module with UV detector (CE methods development mode only), OptiMS Sprayer cartridge, system controller pre-loaded with CESI 8000 software, portable height-adjustable (electric – gear driven) lab bench designed for rapid change between LC and CESI front end, and system startup reagents.

#### Reagents

OptiMS Test Kit	B07663
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#### Supplies

OptiMS - Neutral Surface Cartridge - 30 µm ID x 100 cm total length	B07368
OptiMS - Silica Surface Cartridge - 30 µm ID x 90 cm total length	B07367
Universal Vials (pkg of 100)	A62251
Micro Vials, 200 µL (pkg of 100)	144709
Universal Vial Caps (pkg of 100)	A62250
Electrode Replacement Kit	A47775
Vial Cap Opener Assembly	A95348
Buffer Vial Tray (36 vials)	A58254
Sample Vial Tray (48 vials)	A58255
Coolant, 450 mL, (qty 1)	359976

### A59494: Solid-State LIF and Detector Upgrade

For installations requiring laser induced fluorescence (LIF) detection.  
0 - 1000 RFU, Source Lasers with 3 mW Power Output: Includes  
488 nm solid-state laser and LIF detector module. 635 nm diode laser (optional).

### 149819: Diode Array Detector Upgrade Kit

For installations requiring photodiode array detection. 190 - 600 nm  
(programmable), 0.5 - 32 Hz scan collection frequency (programmable)

OptiMS adapter for AB Sciex MS Nanospray III source	B07363
OptiMS adapter for Bruker MS ESI Nanospray source	B07365
OptiMS adapter for Thermo Nanospray II MS source	B07366
OptiMS adapter for Waters MS Nano source	B07362

Items can be ordered at:

**[www.beckmancoulter.com/CESI](http://www.beckmancoulter.com/CESI)**

For more information on our capillary electrophoresis systems, contact your local  
Beckman Coulter representative, or visit our web site at [www.beckmancoulter.com/CESI](http://www.beckmancoulter.com/CESI)



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