

# Amplify Your Findings with fully integrated CE/MS

## Agilent 7100 Capillary Electrophoresis System



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Product Manager

Low Flow CE & LC Solutions  
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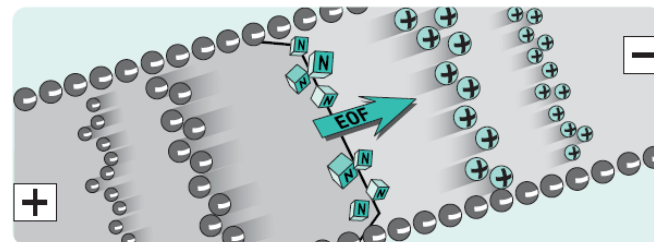
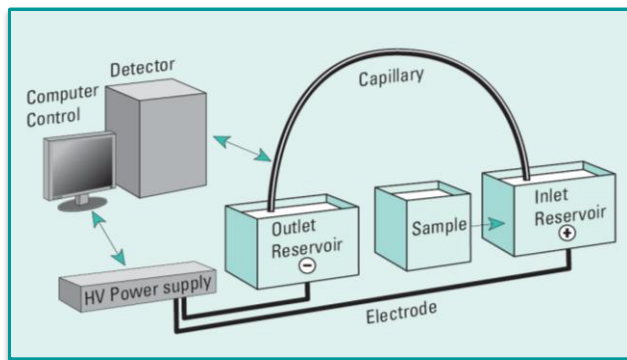
# Overview

- Why Capillary Electrophoresis complements HPLC
- Setup of Agilent CE/MS system
- News and updates on Agilent CE/MS
  - *New Agilent JetStream compatible ESI-sprayer*
  - *Access to iFunnel MS*
  - *New integration of CE into Agilent MassHunter software*
- Application examples CE/MS

# Principles of Capillary Electrophoresis

For charged substances like ions, small basic or acidic drugs and typical biomolecules, CE is an ideal tool for analysis

- Separation based on compound mobility (mass/charge) in an electrical field
- High resolution separations (often > 100,000 plates)
- Fast separation (few minutes)
- Smallest sample volumes (nL Injections)
- Less sample prep required (no stationary phase, just an open glass tube)
- Orthogonal technique complementing HPLC
- Low consumption of sample and aqueous buffer (green method)



# Comparison of Techniques

$$v = \mu_e E$$

where  $v$  = ion velocity  
 $\mu_e$  = electrophoretic mobility  
 $E$  = applied electric field

## HPLC

### Separation principle

Adsorption / desorption between a stationary and a liquid mobile phase

- ▶ Advantage: Chromatography as wide spread method, many publications
- ▶ Disadvantage: Expensive solvents and columns (cost of ownership)

### Typical samples (for reversed phase RP-HPLC)

Neutral or slight polar compounds, small to medium size range

- ▶ Advantage: Fast UHPLC gradients, good reproducibility
- ▶ Disadvantage: Ions / polar compounds often unresolved, intact proteins difficult

### Detection

Various detectors ranging from UV, Fluorescence, Light scattering, Conductivity or a whole suite of MS instruments and methods

- ▶ Advantage: Choice of principle to use, covering a wide range in sensitivity
- ▶ Disadvantage: Not generally compatible with each method/technique

### Flow rate

Pump driven constant flow in typically  $\mu\text{L}/\text{min}$  -  $\text{ml}/\text{min}$  range

- ▶ Advantage: Stable flow, identical speed of compounds at detection point
- ▶ Disadvantage: High solvent consumption, technology needs maintenance

## Electrophoresis

### Separation principle

Compound specific mobility in an electrical field depending on charge state,  $m/z$ , size and shape of the molecule

- ▶ Advantage: High resolution, e.g. isomers can be separated effectively
- ▶ Disadvantage: Solvent and compound chemistry must be aligned

### Typical samples

Ions or charged compounds, from inorganic ions to large polymers

- ▶ Advantage: widest range of molecules, physiological conditions
- ▶ Disadvantage: neutral compounds require often MS-incompatible additives

### Detection

Main technique is UV-DAD with medium sensitivity but broad application range. More sensitive are LIF and Mass Spectrometry

- ▶ Advantage: UV very simple to use for ppm range. MS down to ppt range
- ▶ Disadvantage: Small sample volumes limits choice of detectors

### Flow rate

pH dependent: capillary flow rate plus compound specific migration  
 Typical flow rates are in the range of  $\times 0$  –  $\times 00$   $\text{nL}/\text{min}$

- ▶ Advantage: Ideal for sensitive low-flow Electro-spray-MS
- ▶ Disadvantage: Flow rates change with chemistry (buffer capacity, wall effects)

# Comparison of Techniques

## HPLC

### Separation Columns

packed with adsorption material, typically RP-modified particles

- ▶ Advantage: many different phases, easy switch, analytical and preparative
- ▶ Disadvantage: risk to damage packing by solvents or by matrix adsorption

### Injections

µL-volumes (typically 1-100) liquid aliquots loaded onto the column

- ▶ Advantage: increased sensitivity through high loading capacity
- ▶ Disadvantage: requires large sample volumes

### UV-Detection

Light path length is an important parameter for UV-sensitivity, in HPLC typical values are 10-60 mm in UV flow cells

- ▶ Advantage: High UV sensitivity through a large light path
- ▶ Disadvantage: Extra detection cell required, balance on volumes & flow rates

## Electrophoresis

### Separation Columns

None, open tube fused silica capillaries usually 25 -100 µm ID

- ▶ Advantage: Low cost, low volumes of buffers (mL) reduced sample prep
- ▶ Disadvantage: Capillary coating might be required, non-preparative technique

### Injections

nL-Aliquots loaded by pressure, alternative: electrokinetic injection

- ▶ Advantage: Stacking procedures (ITP) to concentrate compounds
- ▶ Disadvantage: reduced loading capacity > reduced sensitivity

### UV-Detection

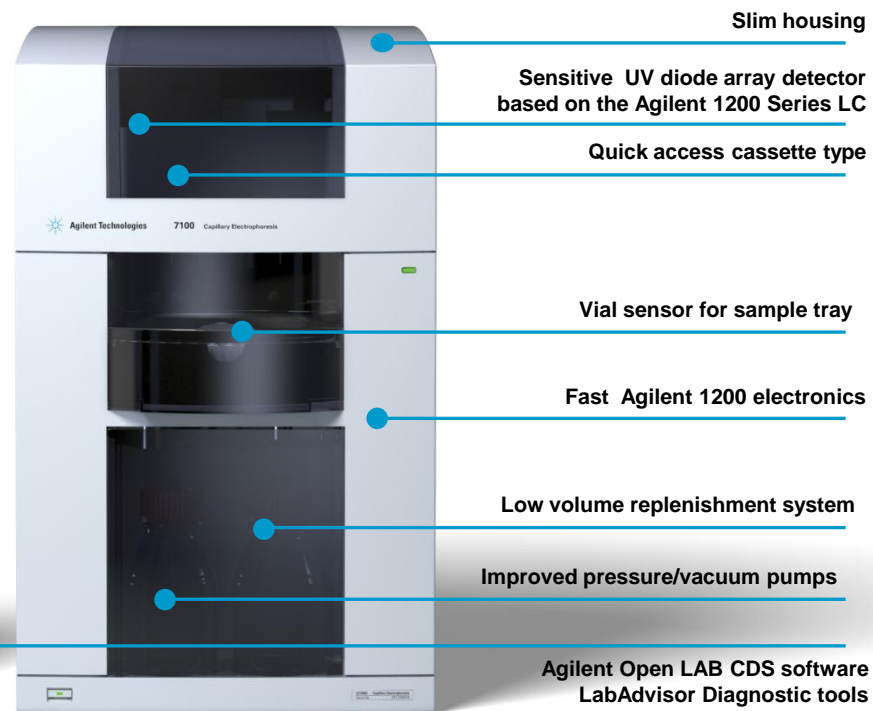
Light path is directly through the capillary without a flow cell, this reduces path length to the ID (25-100 µm)

- ▶ Advantage: Agilent bubble-cell increases ID without a loss in resolution
- ▶ Disadvantage: reduced light-path > reduced sensitivity

	HPLC	CE	
Orthogonal ▲	Familiarity with technique	■■■■	■■■■
	Analyte neutral - polar	■■■■	■■■■
	Range of detectors	■■■■	■■■■
	Sensitivity	■■■■	■■■■
	Reproducibility (qual/quant)	■■■■	■■■■
	Capital cost instruments	■■■■	■■■■
	Sample prep effort	■■■■	■■■■
	Resolution performance	■■■■	■■■■
	Sample volume required	■■■■	■■■■
	Analyte size	■■■■	■■■■
	Cost of ownership	■■■■	■■■■
	Biocompatibility (native Prot.)	■■■■	■■■■
	Analyte polar - fully charged	■■■■	■■■■
	Charged polymers and Proteins	■■■■	■■■■
Complementary ▼			

# Agilent 7100 CE System

<b>Performance</b>	Highest sensitivity for UV
<b>Handling</b>	Quick, direct and easy
<b>Automation</b>	Agilent replenishment system
<b>Flexibility</b>	All modes, open to external detectors
<b>CE/MS</b>	Complete single vendor solution
<b>Economic</b>	Reducing cost of ownership



**Slim housing**

**Sensitive UV diode array detector based on the Agilent 1200 Series LC**

**Quick access cassette type**

**Vial sensor for sample tray**

**Fast Agilent 1200 electronics**

**Low volume replenishment system**

**Improved pressure/vacuum pumps**

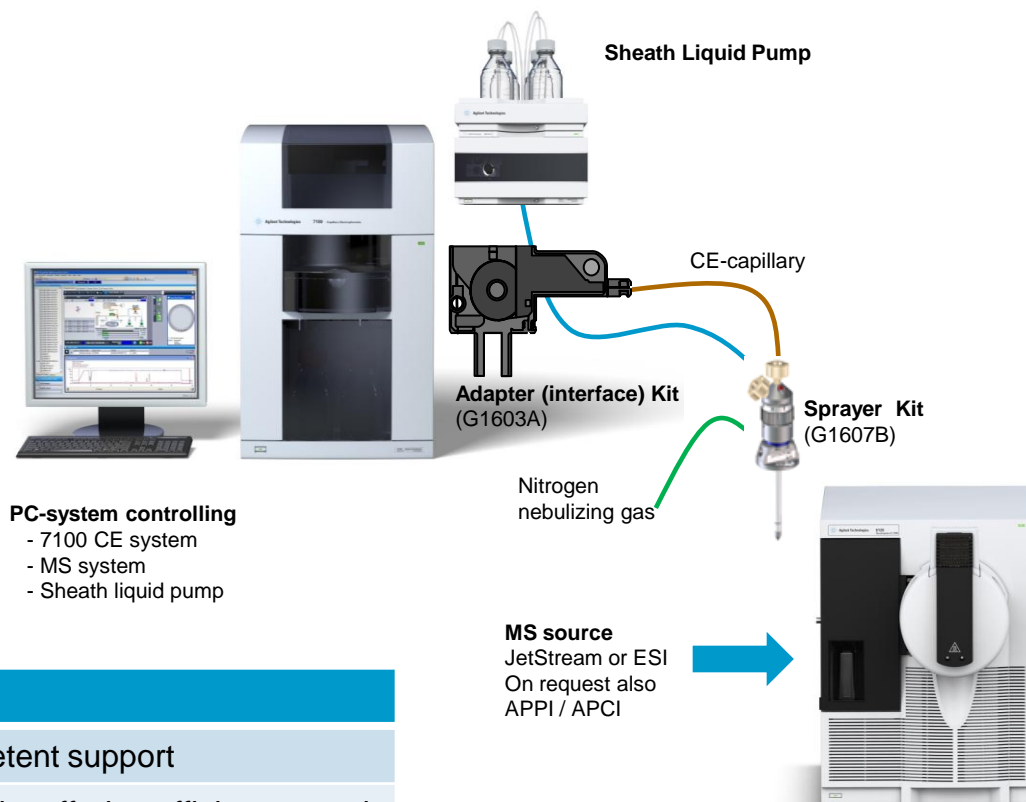
**Agilent Open LAB CDS software  
LabAdvisor Diagnostic tools**

# Agilent CE/MS Setup

## OpenLAB CDS (ChemStation edition)

### Single Point Software Control

- 7100 CE Instrument
- MS Single Quadropole
- LC Make-Up Flow



### Agilent CE/MS Advantages

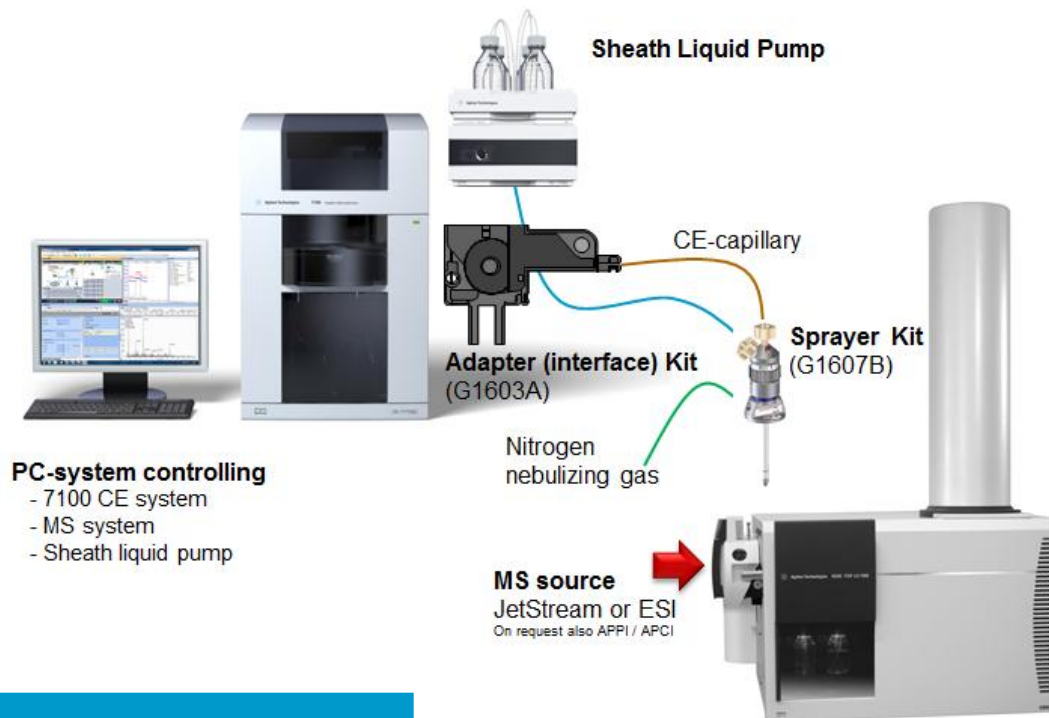
Single vendor solution:	• direct and competent support
Sheath Liquid Interface:	• robust and reliable, offering efficient control on chemistry
Capillary outlet on ground:	• no compromises on voltages for CE or ESI-MS

# Agilent CE/MS Setup

## New: MassHunter Software Control

### Single Point Software Control

- 7100 CE Instrument
- OF, QTOF, QQQ
- LC Make-Up Flow



### Agilent CE/MS Advantages

Single vendor solution:	• direct and competent support
Sheath Liquid Interface:	• robust and reliable, offering efficient control on chemistry
Capillary outlet on ground:	• no compromises on voltages for CE or ESI-MS



# Agilent CE/MS Solutions

## Components

### Agilent's complete CE/MS systems consists of

- Agilent Agilent 7100 CE instrument
- Agilent CE/MS adapter kit (G1603A)
- Agilent CE/MS sprayer Kit (G1607B)
- Agilent 6000 series MS instrument portfolio (Q, QQQ, TOF, Q-TOF)
- Agilent MS source (JetStream, Standard ESI, APCI, APPI)
- Agilent MassHunter software B.05.01 (or higher)
- Agilent HPLC pump with degasser (any 12xx-type)
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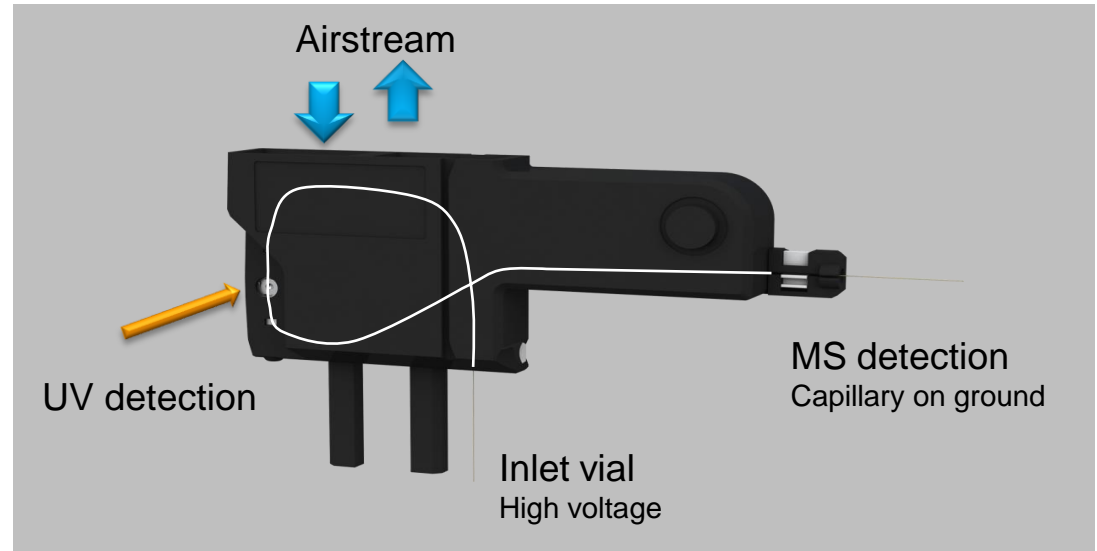
### Other detectors can be run in parallel to the MS-detection:

- UV-DAD (included in 7100 CE)
- LIF (Laser induced Fluorescence – e.g. Picometrics)
- CCD (contactless conductivity – e.g. ISTech or eDAQ)

# Agilent interface for CE/MS

## Cassette type

- **Cassette without liquid cooling**, temperature control by fast airstream providing efficient cooling and heating using a Peltier element
- Quick change of capillaries, no sealings, no liquids no leaks.
- Access to 7100 built in UV-DAD providing UV monitoring (traces and full spectra)
- CCD Sensors can be placed inside the temperature controlled cassette for conductivity measurements



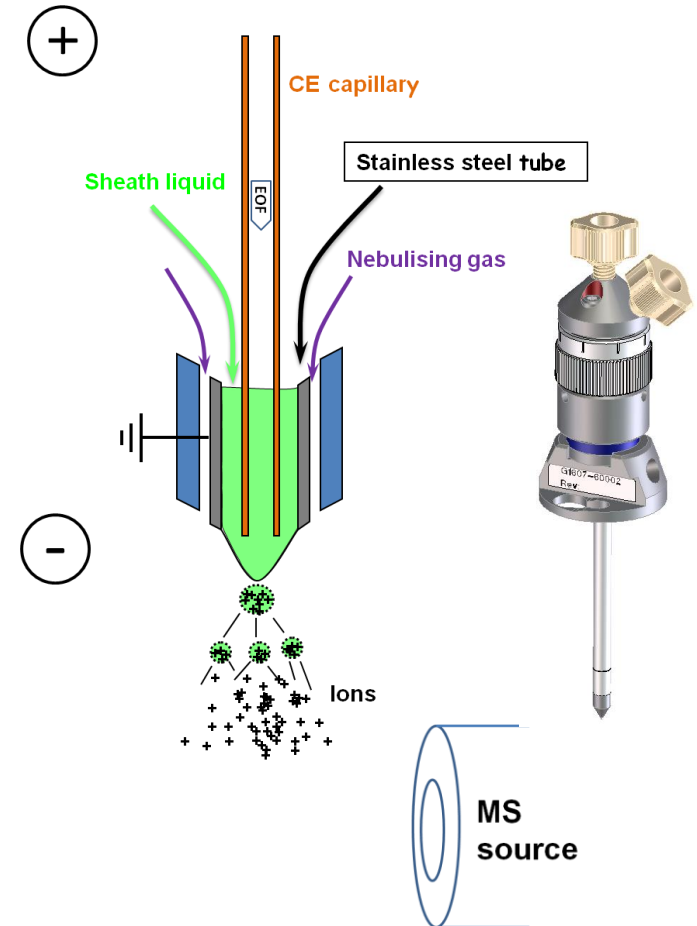
# Agilent interface for CE/MS

## Sheath-liquid type

- **Sheath liquid** is added to the CE eluent by a software controlled LC pump at a rate of typically 1 - 5  $\mu\text{L}/\text{min}$ .
- It often consists of a mix of water, methanol or isopropanol, adjusted for desired pH range (by volatile acids or bases)
- Besides controlling flow rate and chemical conditions for ESI ionization of molecules it allows grounding of the non-conductive fused silica capillary to the metal tube of the spray needle

### Advantages

- High stability & reproducibility for routine analysis
- Decoupling chemistry (CE separation / MS ionization)
- Constant flow rates during runs and sequences
- No modification of capillary / columns required



# Capillary Electrophoresis

## The Advantage of Agilent JetStream source compatibility

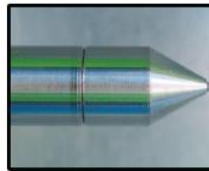
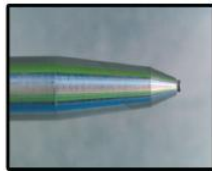
- Access to latest and most sensitive MS
- Saving money, no need for extra ESI sources



### New setup offers:

- Access to 6490 QQQ and 6550 QTOF iFunnel technology
- No need to purchase separate ESI source (G1948B) for CE/MS
- Full backwards compatibility of G1607B sprayer to existing ESI sources
- Very robust sprayer

Old G1607A  
Sprayer tip



New G1607B  
Sprayer tip

# Agilent interface for CE/MS

## Agilent MassHunter software for LC-MS & CE/MS

MassHunter versions B.05.01 and higher are integrating and controlling Capillary Electrophoresis for CE/MS analysis as a single software package under Windows 7 (64 bit)

The screenshot displays the 'Agilent MassHunter Workstation Data Acquisition' window. The 'Instrument Status' panel shows a schematic of the CE/MS system with various components like the Inlet, Electrolyte, Waste, and Q-TOF. The 'Method Limits' table is visible, listing parameters such as Voltage, Current, Power, and Time. The 'Chromatogram Plot' shows a TIC with several peaks, and the 'Actuals' panel on the right provides a summary of current instrument parameters.

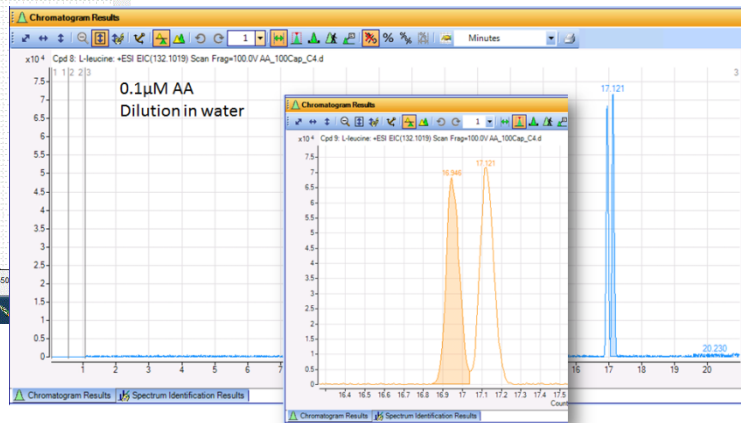
Parameter	Value
CE: Current Actual	34.76 $\mu$ A
CE: Cassette Temperature	25.02 $^{\circ}$ C
CE: Run State	Run
DAD: UV/LampState	Off
Iso. Pump: Flow	0.8 mL/min
Q-TOF: Gas Temp	300 $^{\circ}$ C
Q-TOF: Vaporizer/Sheath Gas T.	195 $^{\circ}$ C
Q-TOF: Nebulizer	10 psig
Q-TOF: Rough Vac	2.14E+00 Torr
Q-TOF: TOF Vac	2.22E-07 Torr
Q-TOF: Not Ready Text Long	
Q-TOF: Error State	

Graphical UI

This section shows the 'Method Editor' for 'AminoAcids\_2\_01082012.m'. The 'CE (G7150A)' properties are configured, including inlet/outlet home positions, cassette temperature (25.0  $^{\circ}$ C), and high voltage system settings. The 'MS TOF (Expt)' parameters are also visible, such as Gas Temp (300  $^{\circ}$ C), Drying Gas flow (8.0 L/min), and Nebulizer pressure (2 psig). The 'MS online spectra' plot shows abundance versus m/z, with a prominent peak at 176.1195.

MS TIC and EIC

MS online spectra



MS parameters

# Agilent 7100 CE/MS software options

Software type	OpenLAB CDS	MassHunter	ChemStation (+MassHunter)
Supported CE instrument	Agilent 7100 CE	Agilent 7100 CE	old Agilent 1600 CE
Supported MS instrument	Agilent 61xx MSD	Agilent TOF,QTOF,QQQ Incl. iFunnel Systems	MSD only (ChemStation) TOF,QTOF,QQQ (CS+MH)
Workstation	Single PC	Single PC	1 PC co-excecution CS + MH 1 PC-CS + 1 PC- MH
SW Version	C.01.03 and higher	B.05.01. and higher	Last CS: B.04.03 MH: B.04.xx
Additional requirements			Macro (sequence > worklists)

# CE/MS Applications

## CE/MS analysis provides specific advantages

- Orthogonal separation method vs. chromatographic separations
- Very high resolution by combining CE and MS resolution
- Improved MS sensitivity vs. standard UV analysis
- Fast analysis especially with TOF/QTOF instrumentation
- CE separation with reduced sample preparation
- Non-denaturing separations of bio molecules (e.g. Proteins)

Screening	Target Analysis
MS choice: TOF/QTOF	MS choice: MSD/QQQ
	<ul style="list-style-type: none"><li>• Protein ID</li><li>• Peptide mapping</li><li>• Metabolomics</li><li>• Small Molecule ID</li><li>• Impurity analysis</li><li>• ....</li></ul>

# Applications: Small anionic molecules

## Metabolomics by CE-TOF/MS

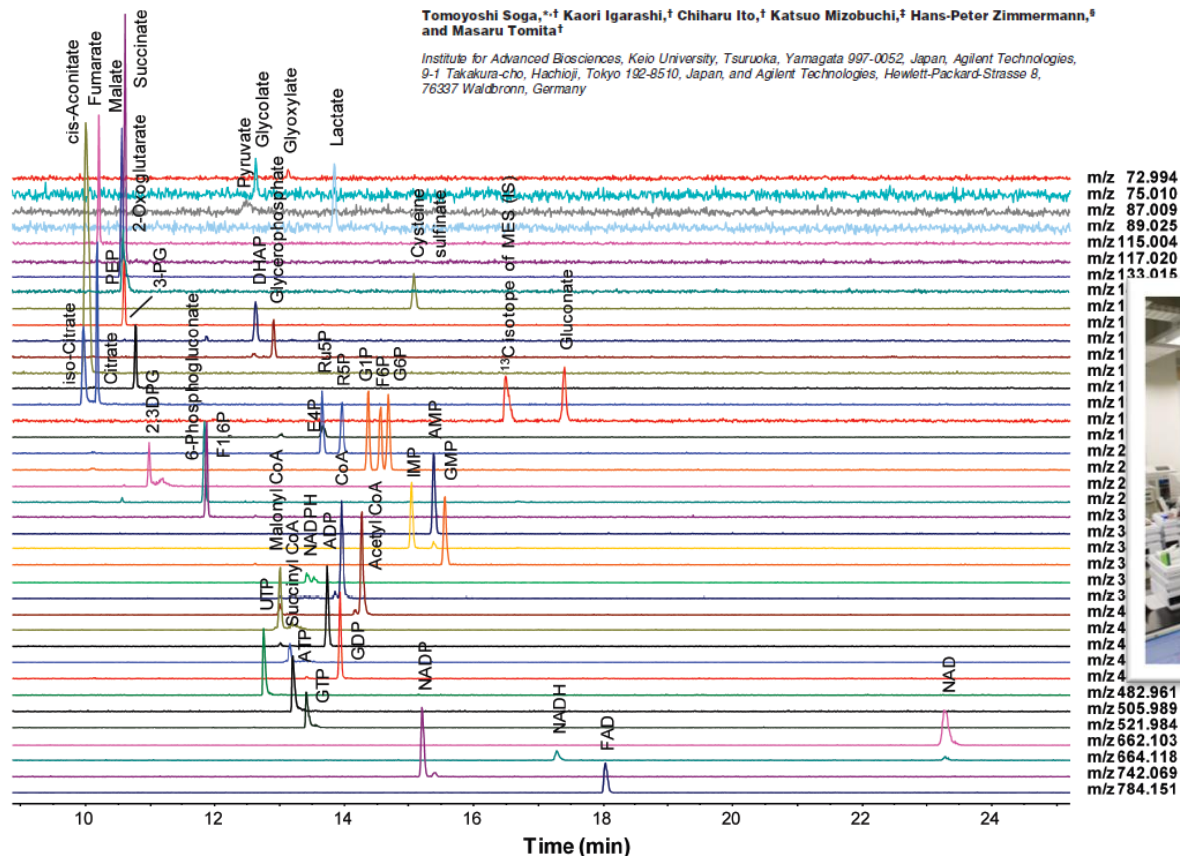
Instrument type: Agilent TOF

Journal: Analytical Chemistry

### Metabolomic Profiling of Anionic Metabolites by Capillary Electrophoresis Mass Spectrometry

Tomoyoshi Soga,<sup>\*,†</sup> Kaori Igarashi,<sup>†</sup> Chiharu Ito,<sup>†</sup> Katsuo Mizobuchi,<sup>‡</sup> Hans-Peter Zimmermann,<sup>§</sup> and Masaru Tomita<sup>†</sup>

*Institute for Advanced Biosciences, Keio University, Tsuruoka, Yamagata 997-0052, Japan, Agilent Technologies, 9-1 Takakura-cho, Hachioji, Tokyo 192-8510, Japan, and Agilent Technologies, Hewlett-Packard-Strasse 8, 76337 Waldbronn, Germany*

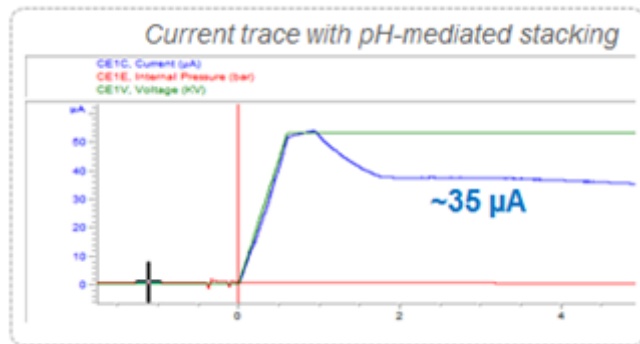






# Quantification of Drugs of abuse

Instrument type: Agilent QQQ



Combination of effective sample stacking method with a highly sensitive iFunnel QQQ instrument



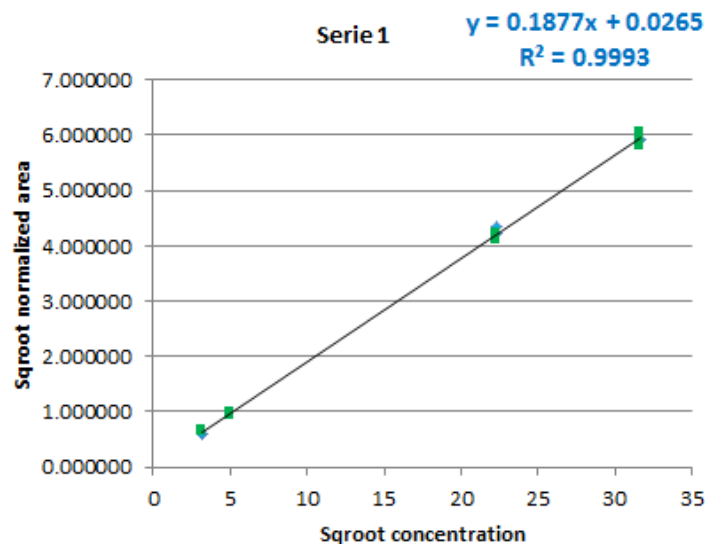
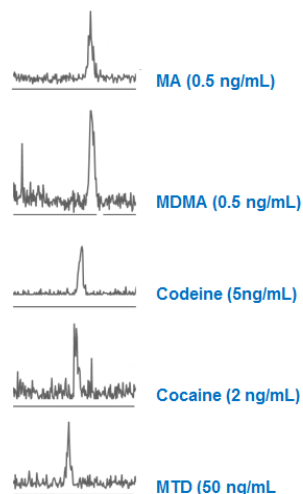
## Cocaine

### LIMITS OF DETECTION

Compound	LOD [ng/mL]
MA	0.5
d <sub>5</sub> -MA	0.2
MDMA	0.5
d <sub>3</sub> -MDMA	1
Codeine	5
d <sub>3</sub> -codeine	5
Cocaine	2
d <sub>3</sub> -cocaine	1
MTD	50
d <sub>3</sub> -MTD	50



Electropherograms obtained at each LOD  
(main compounds shown as example)



Data: Isabelle Kohler, University of Geneva, School of Pharmaceutical Sciences, Geneva, Switzerland

# Summary

Agilent is the only sole vendor to provide a completely integrated robust and sensitive CE/MS solution for research and for routine analysis

- Full Agilent series 6000 MS portfolio available – single quad, QQQ, TOF, and QTOF
- Triple-tube interface to optimize individually separation and MS ionization – no compromises
- Range of ion sources available - standard ESI and Agilent JetStream (APPI and APCI on demand)
- Flexibility on additional detectors – UV-DAD, LIF, and CCD in parallel to MS
- iFunnel-Sensitivity for small molecules down to the ppt range
- Agilent MassHunter software control – one software, one workstation
- Single-vendor solution – integrated system and single-source support

## More information

Integrated CE/MS for orthogonal analysis is one of a wave of innovative Agilent technologies for ensuring your analytical lab stays at the forefront of separation potential.

[www.agilent.com/chem/cems](http://www.agilent.com/chem/cems)

