



## Agilent capillary electrophoresis system



**Agilent Technologies**

# The Agilent CE system - new dimensions in capillary electrophoresis

Capillary electrophoresis (CE), with its high efficiency and resolution, rapid analysis time, plus minimal sample and solvent requirements, is an established technique in many laboratories. Its flexibility covers a broad range of applications in a wide variety of industries, from drug discovery and development through quality control and ion analysis.

Agilent Technologies is the leading global partner for chromatography and capillary separations. When you select Agilent as your partner of choice you can be assured of quality instrumentation, solutions and service.

Agilent's CE system is the first choice for capillary electrophoresis offering benefits such as:

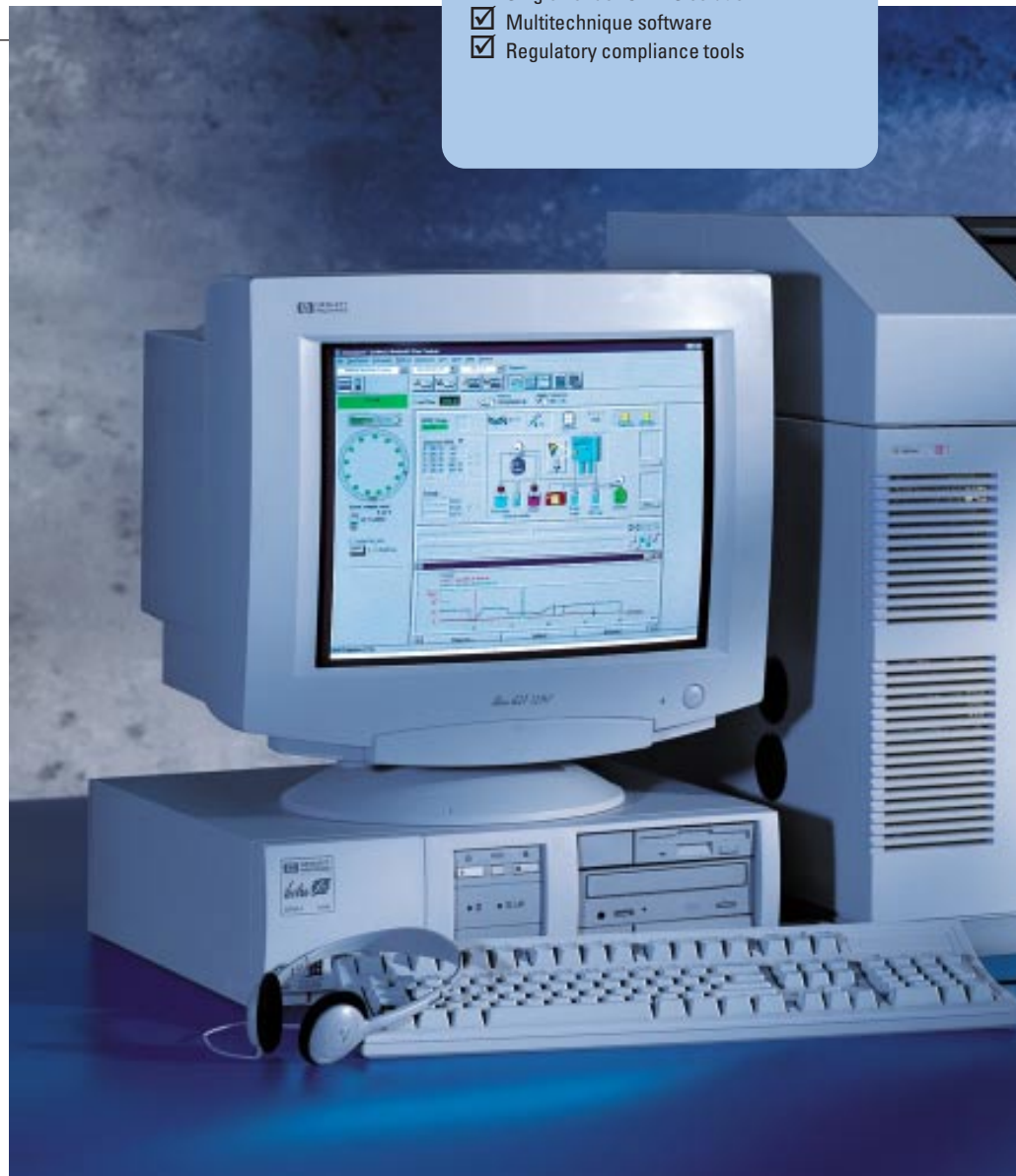
- HPLC-like reproducibility, sensitivity and linearity
- integrated software with graphical user interface
- pioneering new separation techniques, including capillary electrochromatography (CEC) and capillary electrophoresis mass spectrometry (CE-MS)
- extensive regulatory compliance tools
- world wide service and support

## Multitechnique software with graphical user interface

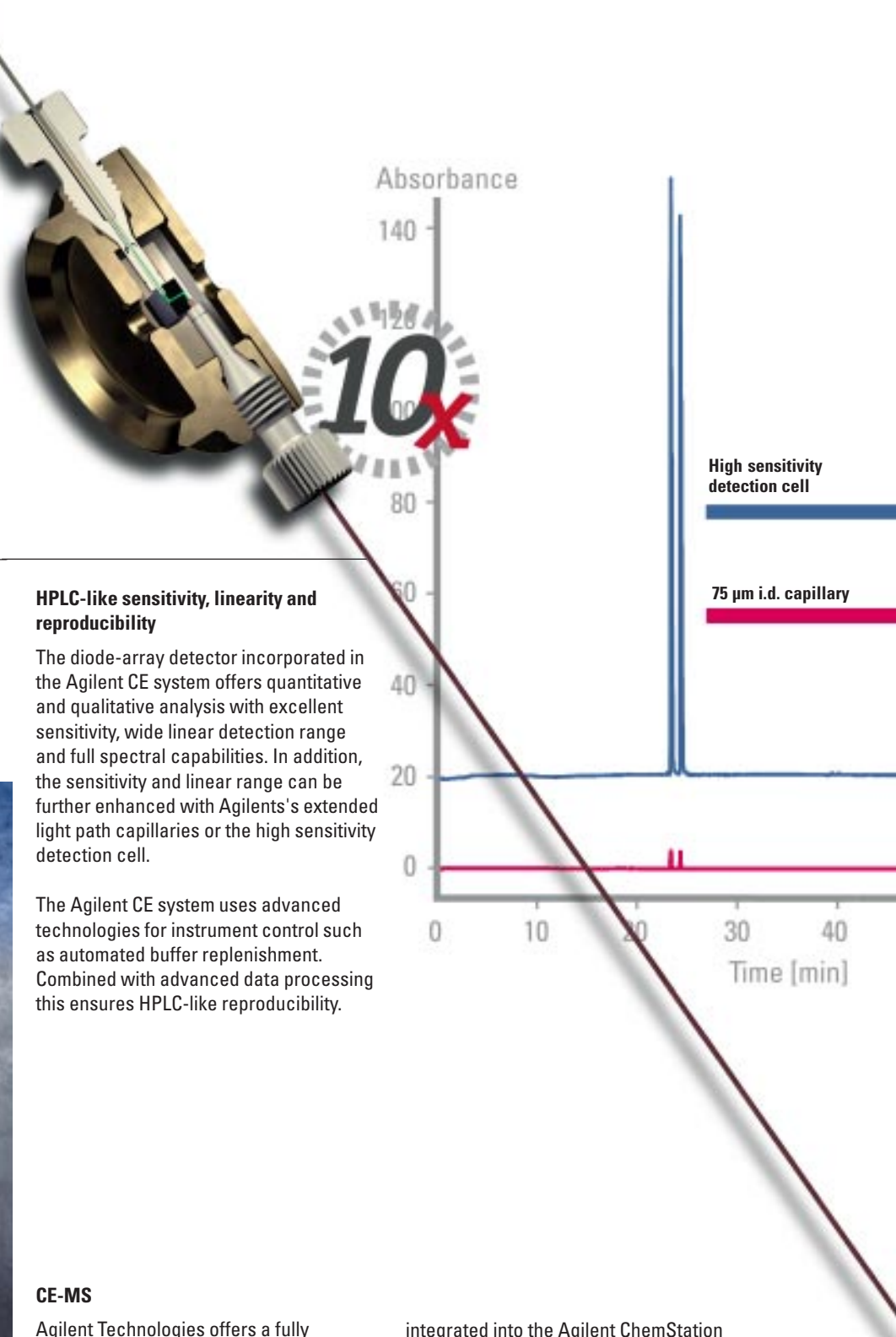
Many users will already be familiar with the Agilent ChemStation software, and new users will learn quickly with the simple graphical user interface. This results in increased efficiency and a reduction in training costs.

## Checklist

- HPLC-like sensitivity and linearity
- HPLC-like reproducibility
- Self-correcting injection system
- Enhanced integrator
- Replenishment system
- CEC
- Single vendor CE-MS solution
- Multitechnique software
- Regulatory compliance tools



The Agilent high sensitivity detection cell provides a 10-fold increase in sensitivity, unsurpassed spectral fidelity and an extended linear range. All with a unique decoupled cell design allowing simple capillary replacement.



#### HPLC-like sensitivity, linearity and reproducibility

The diode-array detector incorporated in the Agilent CE system offers quantitative and qualitative analysis with excellent sensitivity, wide linear detection range and full spectral capabilities. In addition, the sensitivity and linear range can be further enhanced with Agilent's extended light path capillaries or the high sensitivity detection cell.

The Agilent CE system uses advanced technologies for instrument control such as automated buffer replenishment. Combined with advanced data processing this ensures HPLC-like reproducibility.

#### CE-MS

Agilent Technologies offers a fully integrated CE-MS solution with all system components and support coming from Agilent. A unique feature of the system is its ease of use—the mass selective detector (MSD) control is smoothly

integrated into the Agilent ChemStation software, the Agilent spraying system needs no positional adjustments, and the unique design of the MS inlet makes the CE separation conditions independent of the MSD operation conditions.



# CE solutions – for discovery, development and quality control of drugs

In today's demanding pharmaceutical industry, fast and robust separation methods are needed in order to reduce the time to market for new drugs. Capillary electrophoresis, the powerful instrumental approach to electrophoresis, responds to these needs in all stages from drug discovery through quality control. CE offers fast separations with orthogonal selectivities to chromatographic techniques. In addition, the integration of CE into the US Pharmacopeia as a standard physical test—initiated in 1996, ensures its acceptance worldwide.

Agilent Technologies has made a long-term commitment to the pharmaceutical industry. Changing regulatory requirements are monitored all over the world to ensure you get the right product features and services—a large number of applications and solution kits have already been developed as part of this process.

Vitamins

Oligonucleotides

## Checklist

### Method:

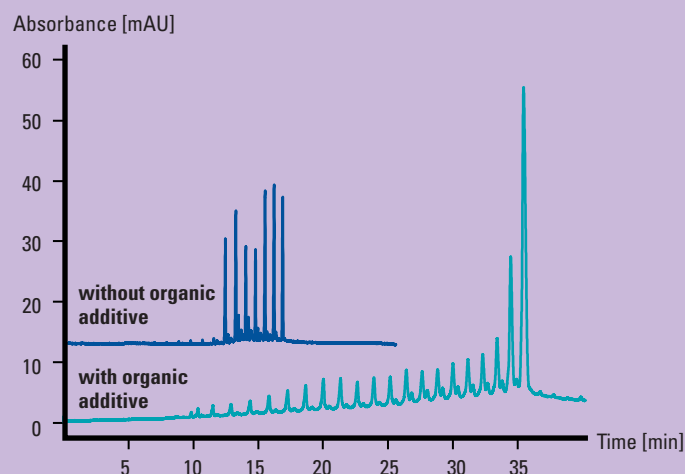
- Fast separation
- Robust
- Low cost per analysis
- Regulatory compliance tools
- Simultaneous quantification of main component and trace contaminants

### Hardware:

- Agilent high sensitivity detection cell
- Replenishment for unattended operation
- CEC capability
- Single vendor CE-MS solution

### Software:

- Multitechnique software
- Self-explanatory user interface
- Direct access to instrument control
- Enhanced integrator for CE peaks
- Mobility calculation for improved reproducibility
- Integrated CE-MS control
- Data accessibility for the next millennium



## Oligonucleotides

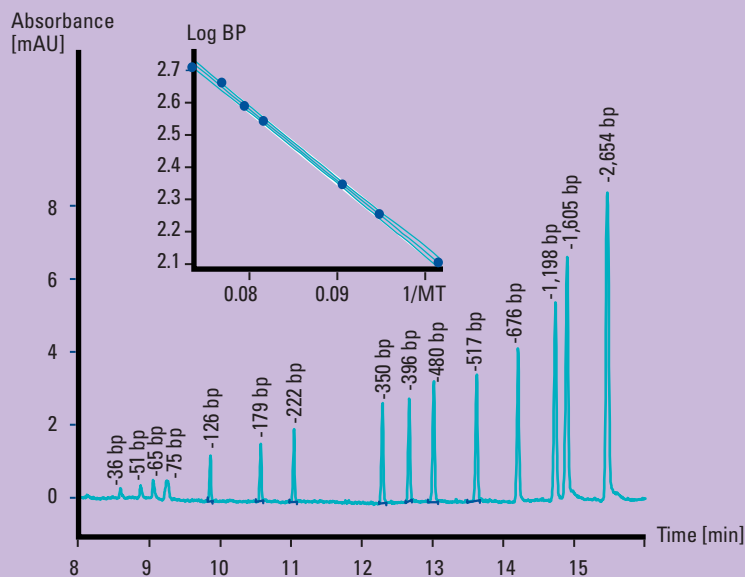
The analysis of oligonucleotides or antisense oligonucleotide therapeutics by capillary electrophoresis is fast, reproducible and highly automated. This makes it ideally suitable for quality control applications in oligonucleotide core facilities, as well as in many phases of bio pharmaceutical drug development.

Proteins

# Peptides

## Double stranded DNA and PCR products

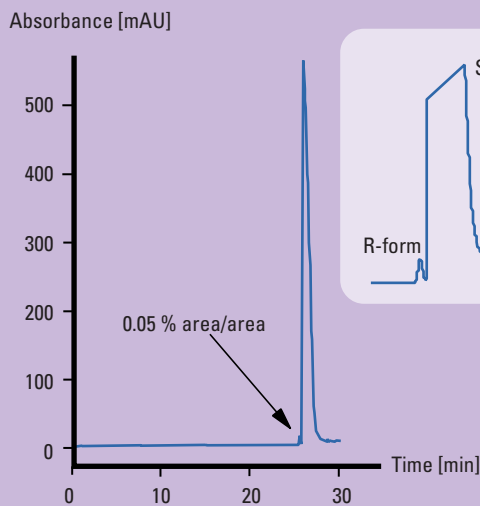
Capillary electrophoresis provides a convenient method to determine the size of double-stranded DNA and PCR products. The Agilent ChemStation automatically generates a Ferguson plot for size determination.



# DNA

*“Regulatory authorities are increasingly requesting that the pharmaceutical industry assess the enantiomeric purity of their chiral building blocks, intermediates and final drug substances. This, coupled with the ever increasing demands imposed on the analyst to reduce method development times, makes chiral CE a very attractive technique. It offers unprecedented chiral discrimination through its high resolving power, chiral selector versatility and rapid automated method development.”*

**Dr. Melvin Euerby**  
(Astra Charnwood, UK)



## Chiral drug compound

When used with the high sensitivity detection cell the simultaneous quantification of the main component and trace impurities below 0.1 % is possible without sample overloading.

# Chirals

# Glycoproteins

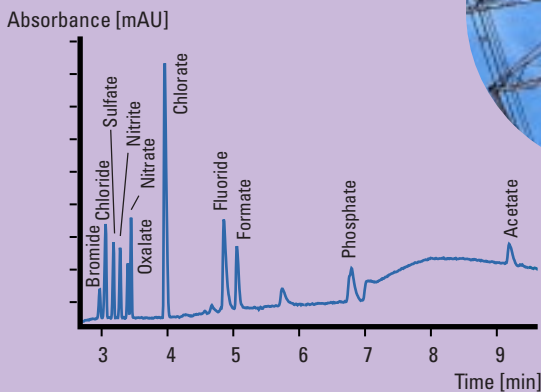
# CE solutions – the alternative for ions

Capillary electrophoresis is a true alternative to ion chromatography.

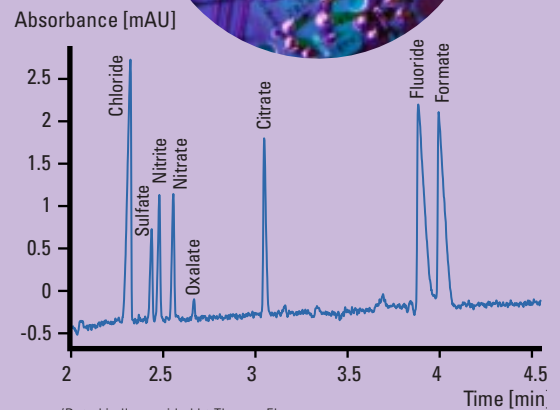
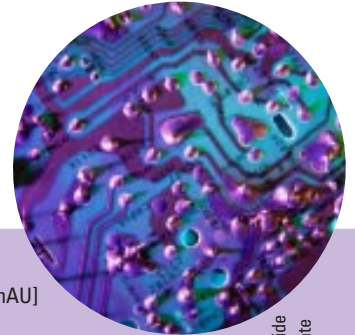
Its main features include:

- unmatched analysis time—enhancing your sample throughput
- high resolving power—allowing analysis of complex samples in one run
- typically < 1 ml of solvent required with only a small amount of waste— saving money and helping to protect the environment
- no need for specialized columns, precolumns and membranes—easy instrument setup and maintenance
- often no laborious sample preparation needed—just sample dilution
- only nanoliter amounts of samples injected—allowing different methods or analytical techniques to be applied to one sample

Ionic contamination in primary and secondary circuit water leading to metal corrosion, is a major problem for the nuclear power generating industry. For this reason low levels of small anions and cations must be monitored. CE provides a rapid and facile method for their determination in the low ppb range.



Power plants



(Data kindly provided by Thomas Ehmann, Wacker Siltronic AG, Burghausen, Germany)

## Semiconductor industry

*“Capillary electrophoresis, as a microanalytical technique, features merits in the determination of contaminants on wafer surfaces. As the diameter increases, the financial value of the wafers increases exponentially, therefore it is mandatory to apply a great variety of different analysis techniques when working on one single wafer. This is possible with CE due to the low sample volume. Moreover, by changing only the composition of the electrolyte, a broad variety of analytes in different matrices can be determined.*

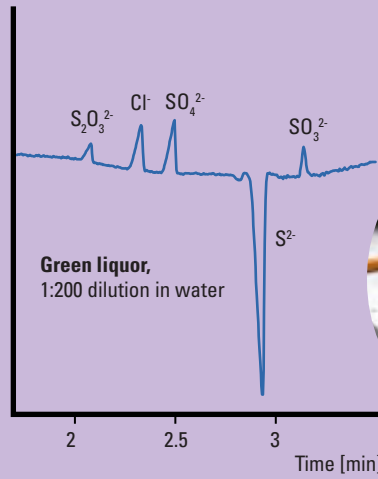
*CE covers a range from ionic to non-ionic analytes that are important in the semiconductor industry.”*

**Dr. Laszlo Fabry**  
(Wacker Siltronic AG, Germany)

## Checklist

- Ease of sample preparation
- Low sample consumption
- Fast separation
- Minimal maintenance
- Low cost of ownership
- Low ppb sensitivity

Recycling for cost reduction and environmental concerns necessitates the monitoring of Kraft liquors for anionic content. The caustic nature of the samples results in labor intensive maintenance procedures. CE analyses are three times faster and require minimal sample preparation compared to ion chromatography.



(Data kindly provided by Dwayne Van, Longview Fibre, Longview WA, USA)

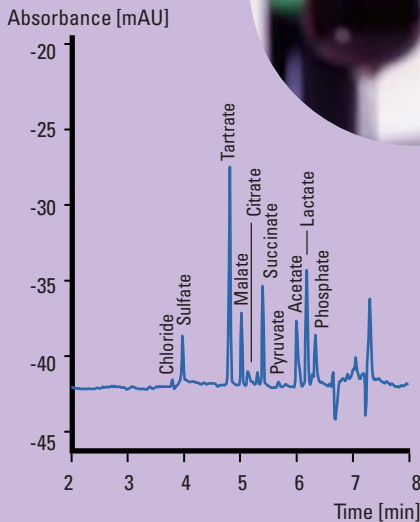
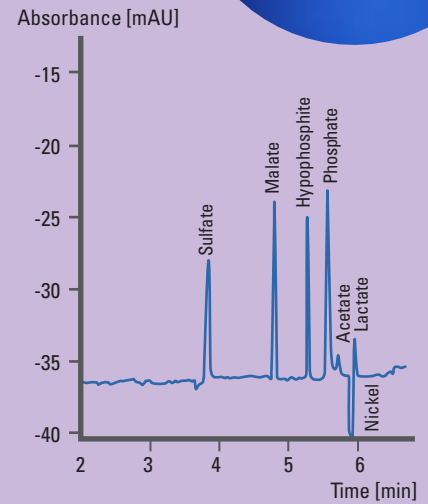


### Pulp and paper industry

### Plating bath industry

*"In the plating bath industry, the monitoring of additives in bath solutions or waste is essential for quality control and cost saving. The analysis by capillary electrophoresis is advantageous over ion chromatography with respect to sample preparation, resolution and simplicity."*

**Nobuo Ebina**  
(Ebina Denka Kogyo Co., Japan)



### Food and beverages industry

*"Validation of CE for organic acid analysis in grape juice and wine has enabled us to replace other methods like ion chromatography (IC), or HPLC. Major advantages are, speed of analysis—six major organic acids in less than 13 minutes; instrumental simplicity; its adaptability in a routine QC lab—currently running 24 hours; no sample cleanup; low cost of supplies and maintenance, and minimal waste disposal."*

**Steve Kupina**  
(Canandaigua Wine Co., USA)

# CEC – for chromatographic selectivity and highest efficiencies

Capillary electrochromatography (CEC) is a fusion of the techniques of capillary electrophoresis and liquid chromatography, resulting in an increase in efficiency by as much as an order of magnitude over conventional HPLC.

This has three specific benefits:

- separation of closely related compounds
- shorter run times and increased sample throughput, and
- substitution of gradient HPLC methods by isocratic CEC.

CEC uses capillary columns packed with LC stationary phases. As in LC, CEC separations are achieved by the partition of a solute between the mobile and stationary phases. The additional separation by mobilities resolves charged and neutral components.

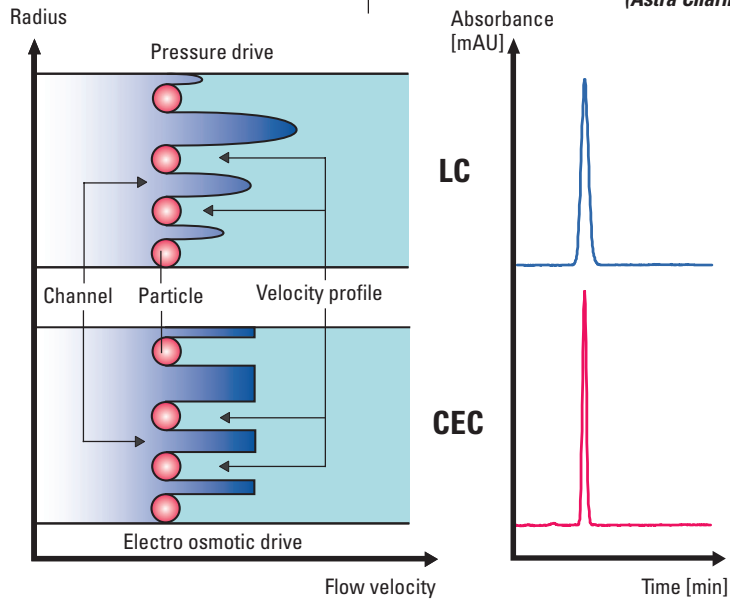
In CEC, the pressure driven flow of LC is removed and replaced by an electrically driven flow—the electro osmotic flow (EOF). This means:

- no pump with moving mechanical parts or seals,
- elimination of eddy diffusion, resulting in increased efficiency, and
- column length and particle size that are not restricted by a pressure limit.

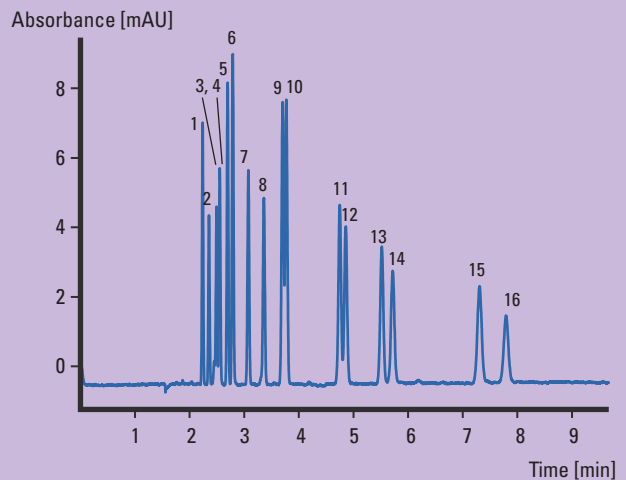
Robust, longterm operation of CEC columns requires pressurization of both capillary ends. This is available as a core function of the Agilent CE system.

*“CEC’s vastly increased resolving power coupled to the high speed analysis which this technique also offers, is essential in a modern pharmaceutical industry which demands the separation of increasingly complex mixtures.”*

**Dr. Melvin Euerby**  
(Astra Charnwood, UK)



Separation of an EPA PAH standard by CEC



## Checklist

- Microanalytical technique
- Separation of closely related compounds without gradient
- High sample throughput
- Chromatographic selectivity plus separation mechanism by charge

# CE-MS – one vendor, one software, one solution

CE-MS combines the short analysis time and high separation efficiency of CE with the molecular weight and structural information from the MS. The technique has been successfully used for the analysis of biopolymers, drugs and drug metabolites, and agrochemicals, among others.

Unlike other systems, Agilent Technologies offers a fully integrated solution—all the system components come from one vendor and are controlled by one software package, combined to give you one solution. In addition, Agilent's unique design features surpass previous limitations of CE-MS.

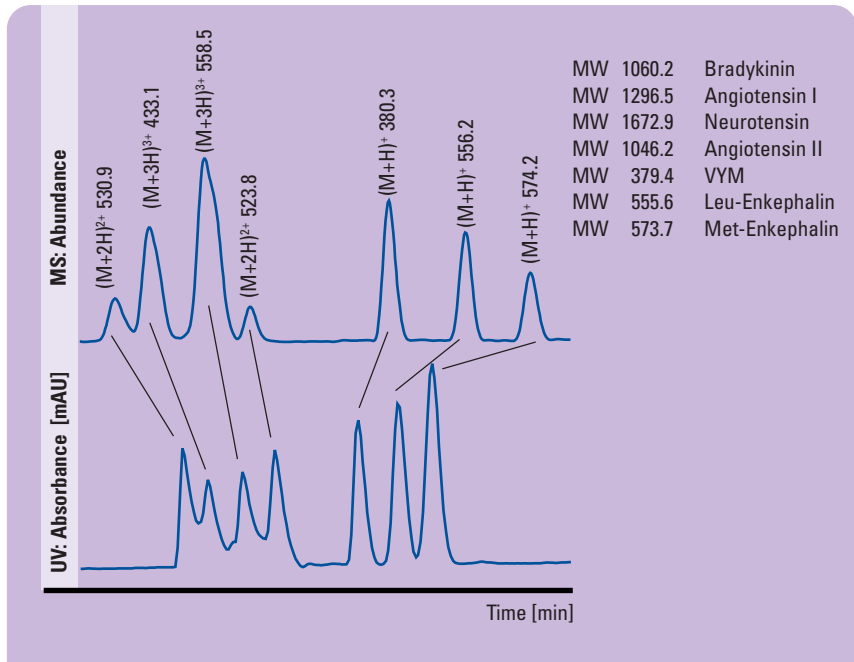


Agilent's spraying system

- The spraying system from Agilent is now arranged at right angles and needs no positional adjustments, enabling very simple and stable operation and allowing the use of moderate amounts of conventional electrophoretic buffers or additives, for example phosphate or cyclodextrins,
- the nebulizer is operated at ground potential increasing the effective field strength over the separation capillary and making the CE separation conditions independent from the MS operation conditions,
- the high-efficiency solvent drying system ensures excellent sensitivity.



## Peptide separation with simultaneous UV and MS detection



Fully integrated and automated, Agilent's CE-MS software guarantees ease of use from method development and data processing

# CE – for highest confidence in your results

Typically, there are five steps required for full instrument, method and data validation. Agilent Technologies offers software tools and kits to facilitate and automate these steps for the Agilent CE system.

*"I have been successful in validating capillary electrophoretic analytical methods employing the same rigorous acceptance criteria for precision, accuracy, ruggedness and linearity as I would use for either HPLC or GC."*

**Todd Wielgos**  
(Baxter Health Care Corporation, USA)

## Step 1: Qualify your vendor and your vendor's design

Ensured with Agilent's reputation and documentation of successful development validation.

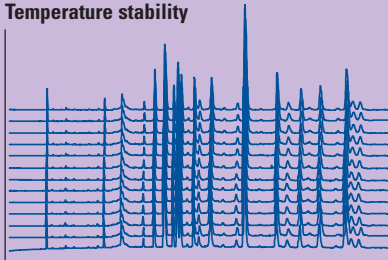
## Step 2: Qualify the instrument in your laboratory before beginning operation

Follow the appropriate test procedures and certification for Installation Qualification (IQ) and Operation Qualification/Performance Verification (OQ/PV). Agilent offers a kit which provides instructions, methods and materials for performing OQ/PV for the Agilent CE system.

## Step 3: Validate your analytical method

The Agilent ChemStation includes system suitability software for automated method validation.

### Temperature stability

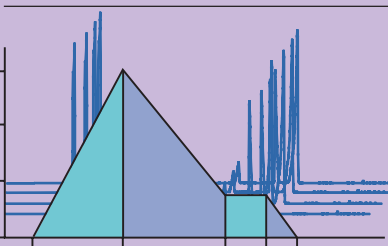


### DAD function

- wavelength accuracy
- linearity
- noise and drift



### Injector reproducibility and linearity



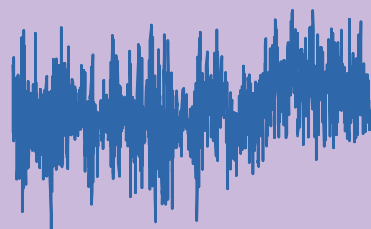
#### Step 4: Qualify system performance during routine operation

Agilent's software features automated daily system suitability testing, with an optional database with online quality control charts for documenting performance parameters.

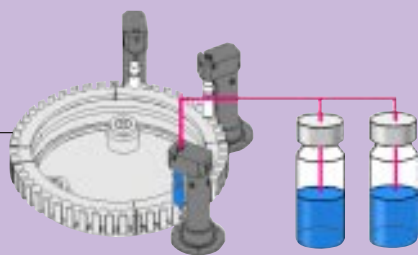
#### Step 5: Ensure data security, integrity and traceability

The software has a password-protected user access. It saves instrument conditions and logbooks together with raw data in checksum-protected binary files.

#### Voltage stability



#### Replenishment functionality



## Specifications

|  |  |   |
|--|--|---|
| <b>Dimensions</b>                          | <b>Width</b>                           | 42.5 cm, (16.8 in)  |
|  | <b>Height</b>                          | 57.5 cm, (22.7 in)  |
|  | <b>Depth</b>                           | 52 cm, (20.5 in)  |
|  | <b>Weight</b>                          | 52 kg, (115 lb)   |
| <b>Environment</b>                         | <b>Temperature:</b>                    | 5–40 °C   |
|  | <b>Humidity:</b>                       | up to 80 %, at 30 °C (non-condensing)   |
| <b>Power requirements</b>                  | <b>Line voltage:</b>                   | 100/120/220/240 VAC ; +5 %, -10 %, 650 VA   |
|  | <b>Line frequency:</b>                 | 50 (48–55) Hz; 60 (57–66 ) Hz   |
| <b>Pressure system</b>                     |  | Programmable with 0–50 mbar bidirectional<br>Flushing with 1 bar or with high pressure 2–12 bar bidirectional<br>Vial pressurization with high pressure 2–12 bar  |
| <b>Safety features</b>                     |  | Current leak detection; Low current limit<br>Safety sensors at door and cover disabling high voltage<br>Diagnostic functions  |
| <b>Electrophoresis power</b>               | <b>Voltage range:</b>                  | setable 0 to $\pm$ 30 kV supply   |
|  | <b>Current:</b>                        | setable 0–300 $\mu$ A   |
|  | <b>Power:</b>                          | setable 0–6 W   |
|  |  | Operation under constant voltage, current or power<br>Programmable polarity switch  |
| <b>Injection modes</b>                     |  | Self correcting injection system with injection from both ends  |
|  | <b>Programmable range:</b>             | up to 10,000 seconds  |
|  | <b>Pressure:</b>                       | 0–50 mbar   |
|  | <b>Electrokinetic:</b>                 | 0–30 kV   |
| <b>Autosampler/<br/>fraction collector</b> |  | 48-position carousel. All vials are randomly accessible from<br>cathode and anode end of capillary. Temperature control with<br>external waterbath with vial temperature from 10–40 °C<br>(non-condensing conditions) |
| <b>Replenishment</b>                       |  | Satellite station for refilling anode and cathode buffer vials with fresh<br>buffer for automatic continuous operation and buffer selectable<br>buffer levelling  |
| <b>Vials</b>                               |  | 100 $\mu$ l sample vials, 1 ml or 2 ml buffer vials (polypropylene or<br>glass) with resealing snap caps  |
| <b>Capillary cassette</b>                  |  | Forced-air temperature-controlled with Peltier element  |
|  | <b>Temperature range:</b>              | 10 °C below ambient to 60 °C<br>( $\pm$ 0.1 °C) with a min. of 4 °C   |
|  | <b>Minimum total capillary length:</b> | 33 cm   |
|  | <b>Capillary compatibility</b>         | 365 $\mu$ m o.d.  |
| <b>Detector</b>                            |  | Real time UV-Visible diode-array detector (190–600 nm)  |
|  | <b>Wavelength accuracy:</b>            | 1 nm  |
|  | <b>Response time:</b>                  | 0.1 to 10 s (8 choices)   |
|  | <b>Light source:</b>                   | prealigned deuterium lamp<br>up to five signals simultaneously,<br>full spectral acquisition with<br>Agilent ChemStation  |
| <b>Raw data channels</b>                   |  | Detector signals, voltage, current, power, capillary temperature<br>and pressure.   |
| <b>System control</b>                      |  | Operating with graphical user interface under Microsoft®<br>Windows 95 and Windows NT®  |
|  | <b>Time programmable parameters:</b>   | voltage, current, power, polarity<br>pressure, inlet and outlet vial<br>capillary temperature,<br>pre and post-run conditioning<br>with pressure and/or voltage,<br>replenishment, fraction collection                |
| <b>CE specific software</b>                |  | Mobility report, time corrected areas, pl calibration and bio polymer<br>size calibration   |

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