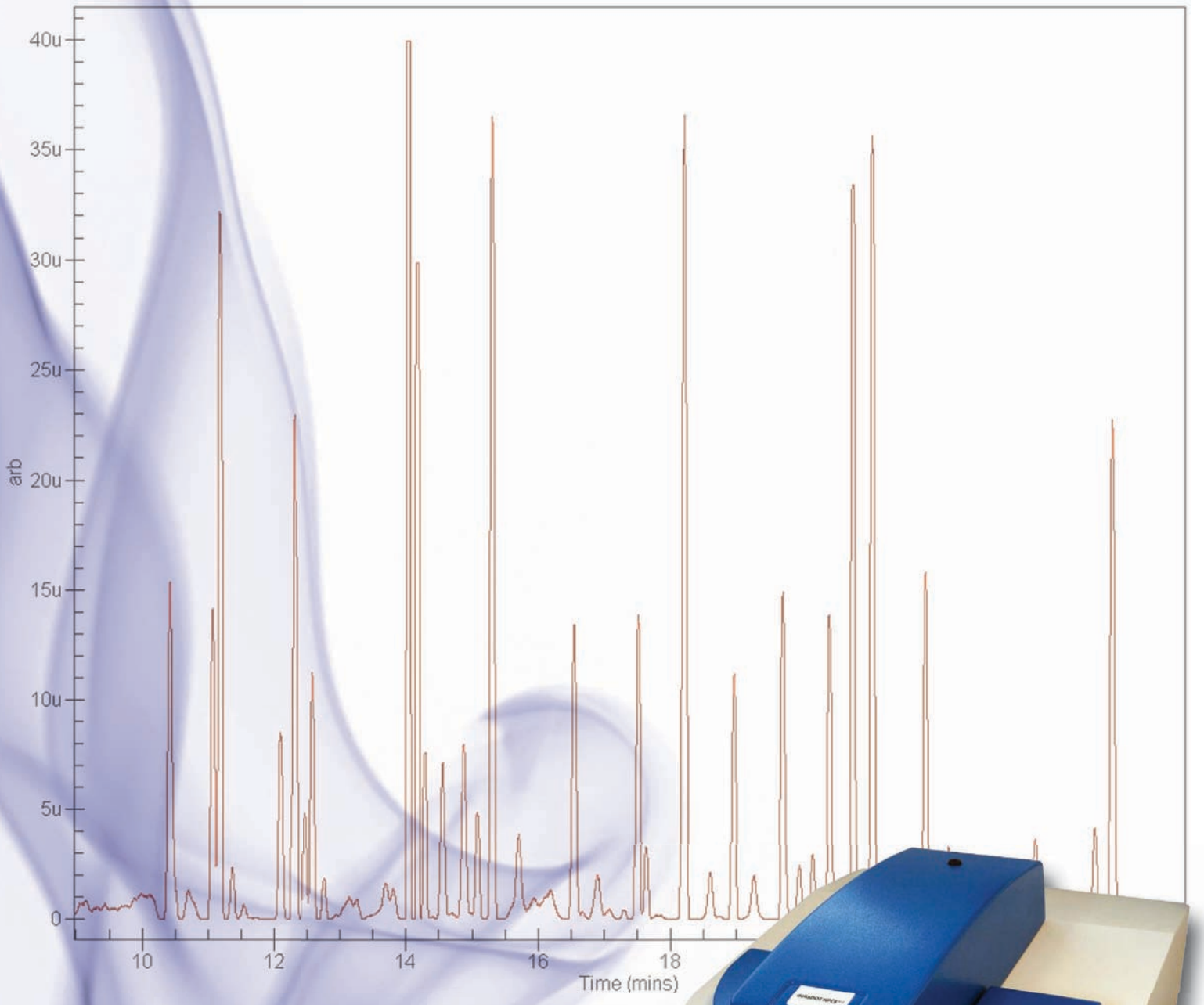
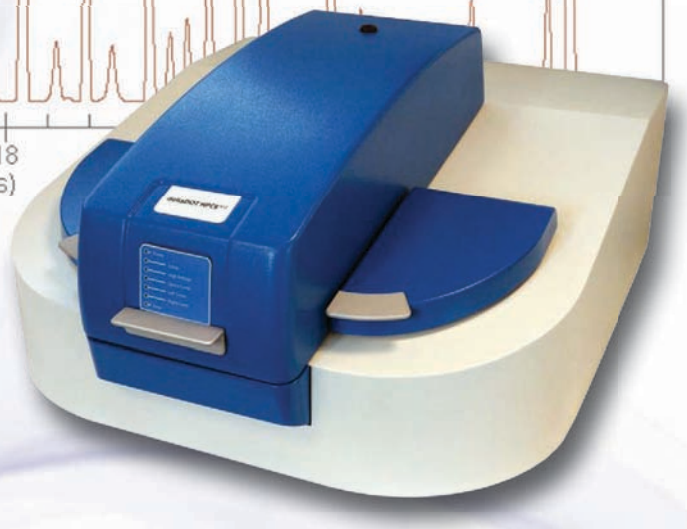
deltaDOT
LONDON

HPCE-512

Realising the potential of **Capillary Electrophoresis**



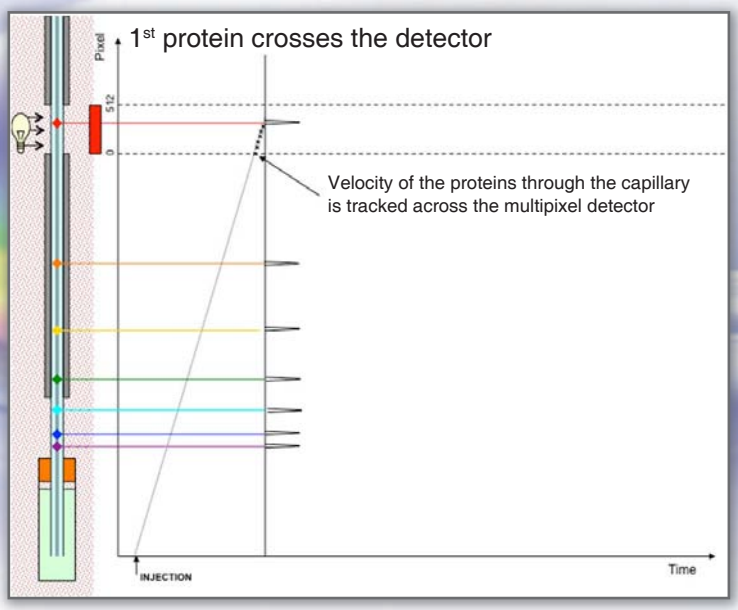


- A Major Advance in High Performance Capillary Electrophoresis
- Label-free Separation and Analysis
- Cost savings and higher quality data

A Major Advance in **Capillary Electrophoresis**

The HPCE-512 system based on multi-point detection overcomes the problems traditionally associated with conventional Capillary Electrophoresis (CE). Data of much higher quality is produced by the instrument compared to other CE systems in terms of quantification, resolution, sensitivity and repeatability. This significant advance in CE technology has been achieved by gathering much more data on the sample than competing systems, and then using proprietary signal processing algorithms to distinguish between signals relating to the analyte and those from background noise.

With conventional CE systems data quality is often poor. Acceptable repeatability of analyses, even those of the same sample, can be difficult to achieve. Sensitivity is deemed inadequate without recourse to expensive, time-consuming labeling techniques that can adversely influence the nature of the analyte. The HPCE-512, however, is a label free system that both reduces the cost of analysis and solves the problems associated with conventional CE systems.



The HPCE-512 images each analyte band across a 512 pixel photo diode array at ten to fifteen times per second so the transit of each band of molecules (in this case protein) is tracked in both time and distance.

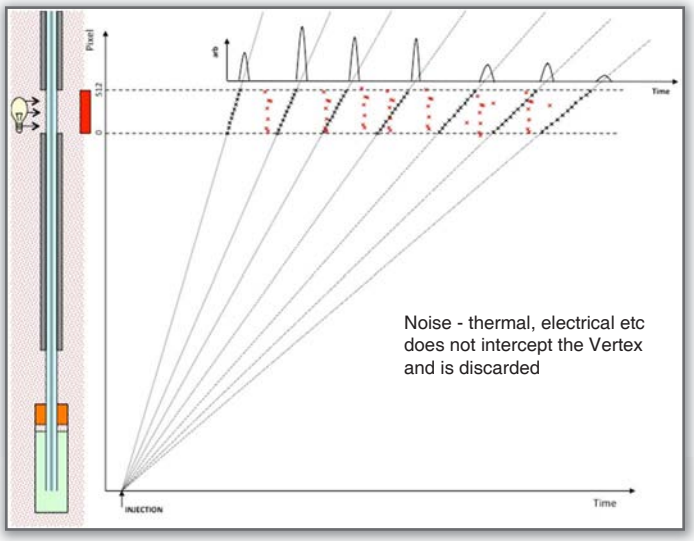
The resultant slope of the line tracking the analyte's progress is dependent on characteristics of the molecules (e.g. molecular weight, charge / mass depending on the chosen mode of operation).

The straightness of the line indicates the fidelity of the separation conditions. The tracks of all the various proteins lead back to a common injection point or vertex.

Signals which are detected but which do not coincide with any of the vertex tracks (shown in red) may be identified and discarded as background noise.

The result is an analysis of clearly defined peaks with very low background interference.

This, in turn, leads to the realisation of high-resolution, quantifiable and repeatable analyses without the need to use labeling chemicals.

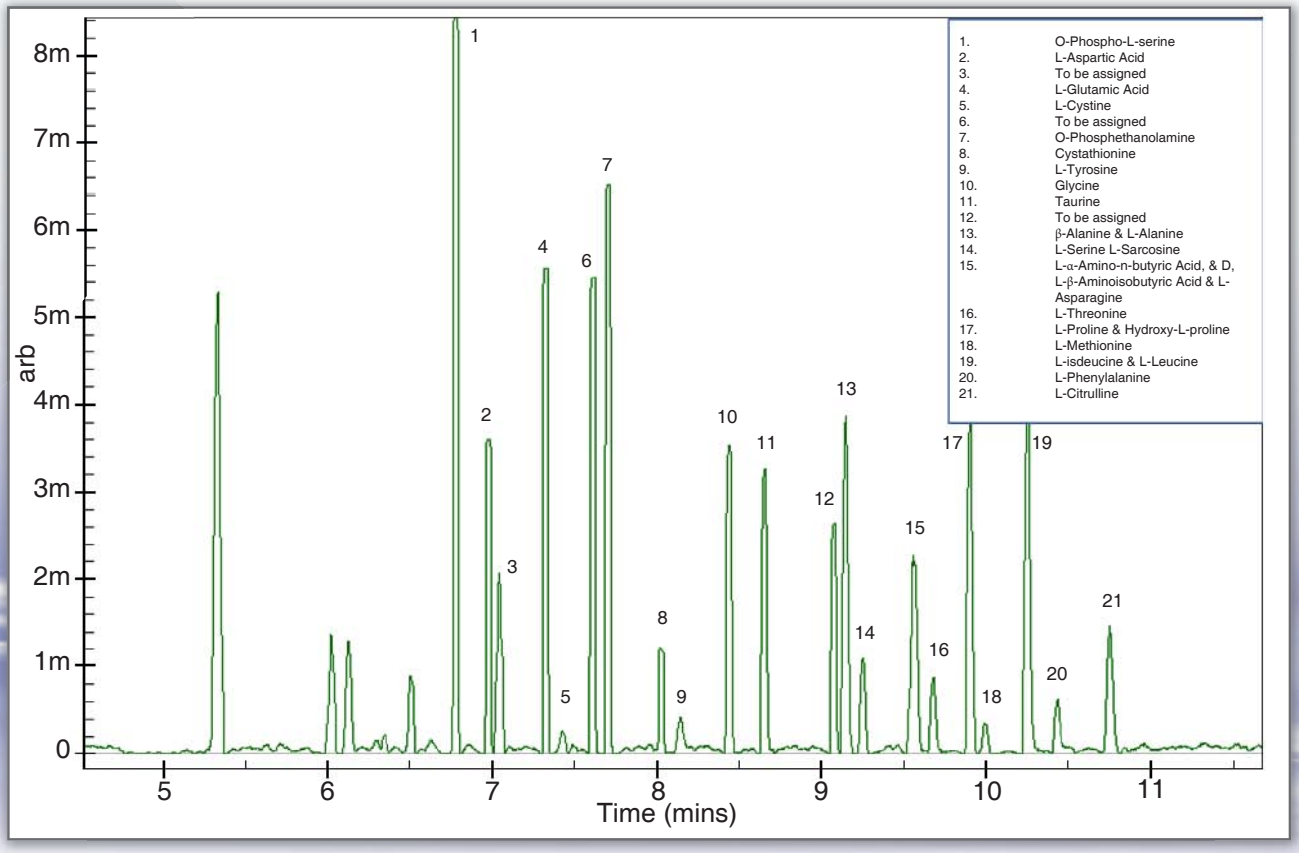


Better Data Quality

Resolution, Repeatability & Quantification Accuracy

The 512-pixel photo diode array detector used in the HPCE-512 system, allows very large datasets to be captured through each analysis.

Powerful signal processing algorithms enable the background noise to be eliminated and individual components to be separated and quantified. Identification of each analyte peak can often be achieved from the molecular weight.

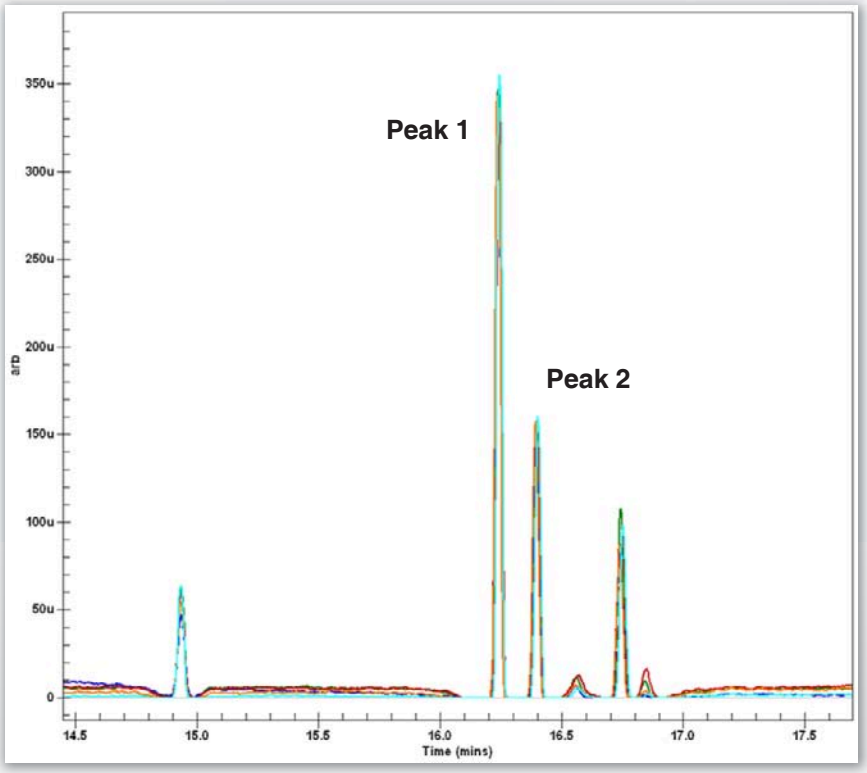


EVA processed data of amino acid standard solution (Sigma A6407)

High Resolution Data

Example - Ribonuclease B and its 5 glycoforms

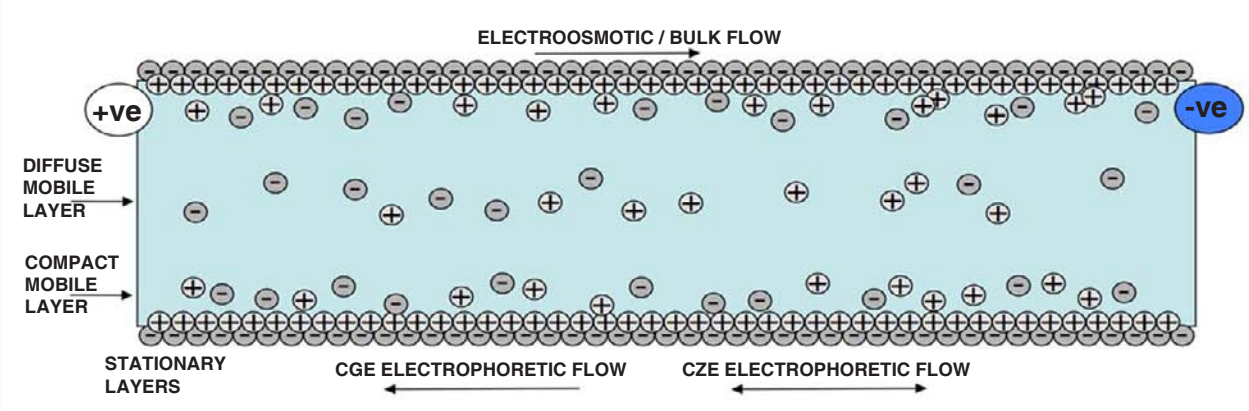
The image shows the Capillary Gel Electrophoresis of this glycoprotein with resolutions of <200Da (1 Mannose unit = 162Da), data quality that is comparable to some forms of Mass Spectroscopy.



The powerful data processing algorithm resolves glycoproteins at resolutions under 200 Daltons

Self Diagnostics

Continuous Monitoring of Capillary and Buffer Conditions



The **Electroosmotic Flow** issue

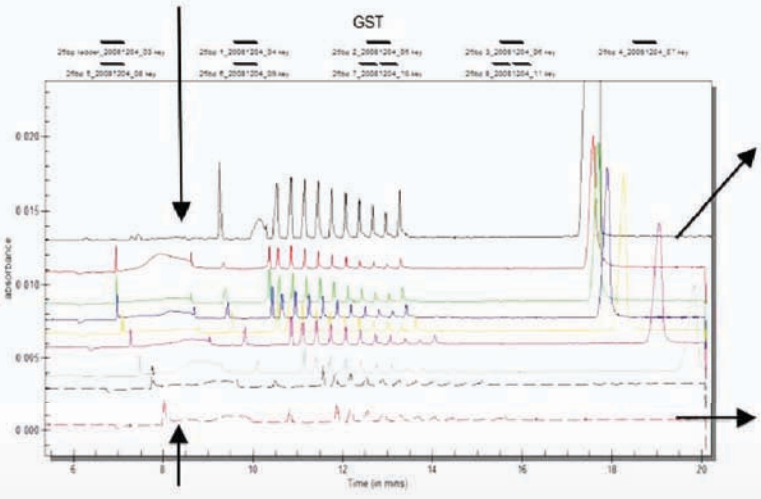
The fused silica capillary contains surface silanol groups that become ionized in the presence of common electrophoretic media so it is negatively charged under most common pH conditions. This results in the formation of an electric double-layer which is immobile even under the application of an electric field and a mobile region with substantial cationic character. Beyond this compact layer is a diffuse layer that is a neutral bulk electrolyte solution.

When a potential difference is applied to the capillary, the cations in the mobile layer migrate in the direction of the cathode. The ions are solvated in the electrolyte, thus the bulk electrolyte buffer is also mobilised in the direction of the cathode. This fluid flow is called electroosmotic flow (EOF) and if not monitored by multi-point detection and controlled by capillary conditioning will lead to poor data, especially with respect to repeatability.

The **HPCE** approach

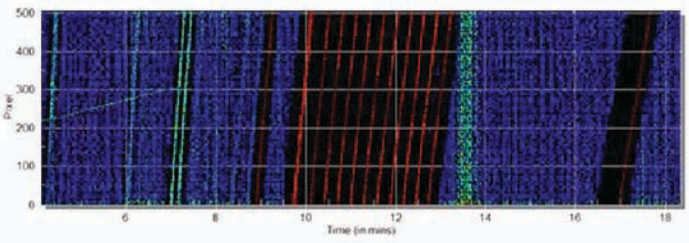
The multi-point detection allows space and distance based data sets to be generated in their signal processing algorithms as an Equiphase map. This can then be used to check each run to see if conditions in the capillary have affected the data. The EOF can be monitored for any specific separation by observing the behaviour of analyte bands or buffer components in the Equiphase map. This allows capillary conditioning and other parameters (such as pH, temperature, buffers etc.) to be optimised to generate significantly superior data in terms of repeatability, as well as resolution and quantification.

Run 1 shows clear, well formed peaks

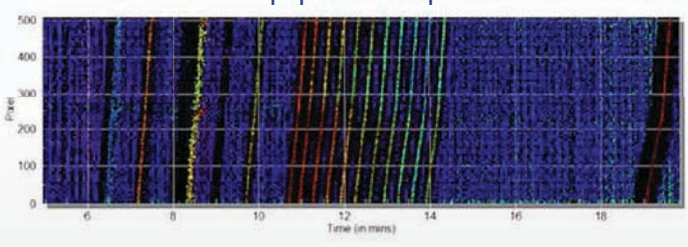


Run 11 shows weak, indeterminate peaks

Equiphase Map 1



Equiphase Map 11



The picture above would indicate that the product had degraded, but the Equiphase maps show that it is the separation conditions that have changed.

Example: *E.coli* Cell Lysate

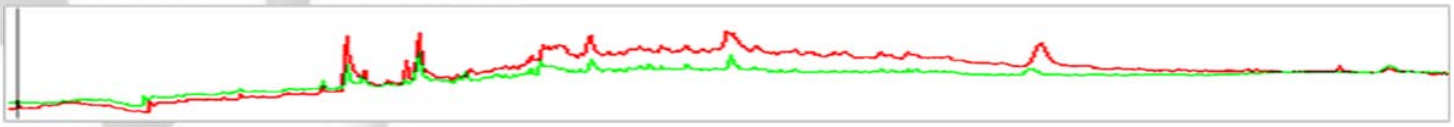
Analysis with **Conventional Capillary Electrophoresis (CE)**

The analysis of cell lysate samples is required in many biopharmaceutical areas:-

- Expression of protein products
- Purification of biological products such as Monoclonal Antibodies
- Analysis of up and down-regulation of proteins in disease states

The analysis of complex protein mixtures, such as cell lysates often requires the use of fluorescent or chemical labels. The analysis of proteins with these expensive labels can cause changes in their characteristics leading to increased errors in the analysis process.

Limitations inherent to single-point detection systems used in conventional CE instruments lead to poor resolution and repeatability (caused by the inability to adequately monitor capillary and buffer conditions).



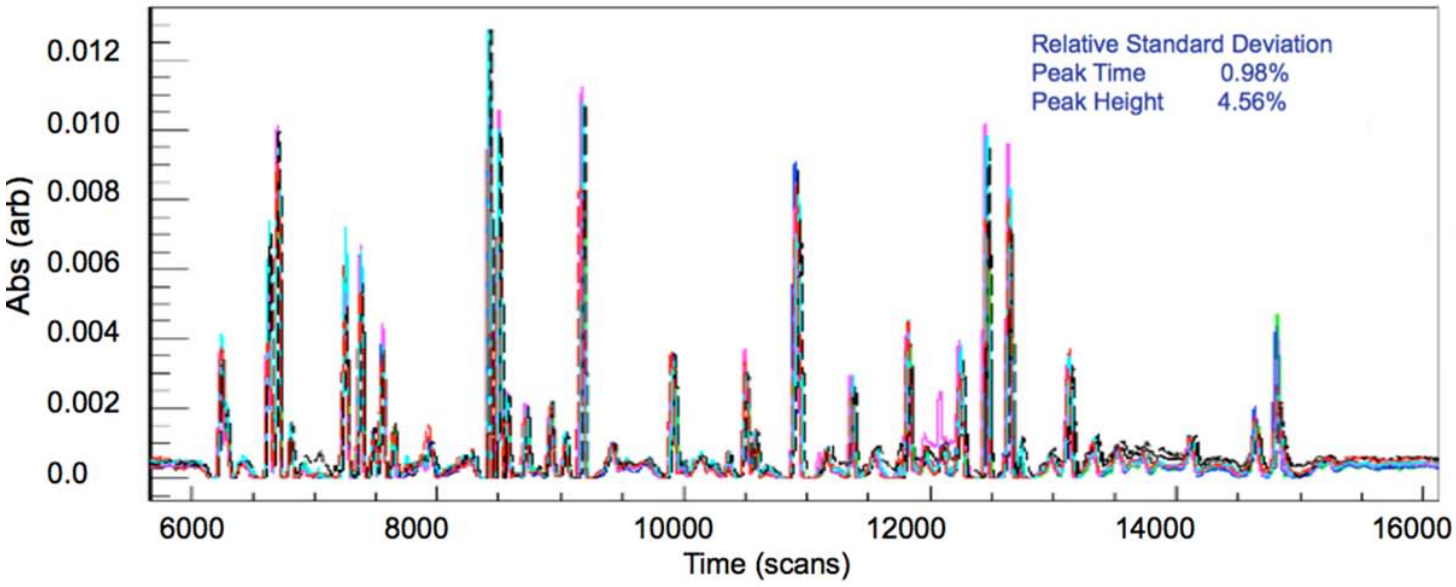
Single point CGE analysis of an E.coli *cell lysate*

Analysis with **High Performance Capillary Electrophoresis - HPCE-512**

In the analysis shown below, *E.coli* lysate samples were analysed in standard running buffer containing ß-mercaptoethanol. After de-gassing, they were transferred to vials and loaded on the HPCE-512 carousel.

The data were acquired in Capillary Gel Electrophoresis (CGE) mode. The increase in resolution is due to the multipixel technology used in deltaDOT's Label Free Intrinsic Imaging.

Repeatability data show less than 1% RSD* in peak time (Molecular weight) and <5% RSD in peak area (quantification).



Overlay of the processed data of nine consecutive E.coli *lysate* runs all separated under the same conditions*

* Relative Standard Deviation over 9 runs

Specifications

Physical Characteristics
D <700 mm; W <500 mm, H <500 mm, Weight <50 kg

Sample Handling
The system includes two carousels: - an input carousel and an output carousel. Analyses can be run either way.
The number of tube positions is 24 per carousel.
Sample injection can be electrokinetic or hydrodynamic.
Temperature control is by Peltier cell cooling.

Electrical Requirements

Maximum capillary voltage (bipolar)	28 kV (-14kV to +14kV)
Filter wavelength range	200–300nm
Voltage requirements	85 to 264 VAC 50/60 Hz
Power requirements	<750 Watts total

Operating Requirements

Temperature	14 to 30 °C
Humidity	0 to 90% Non-condensing

Optional Extras

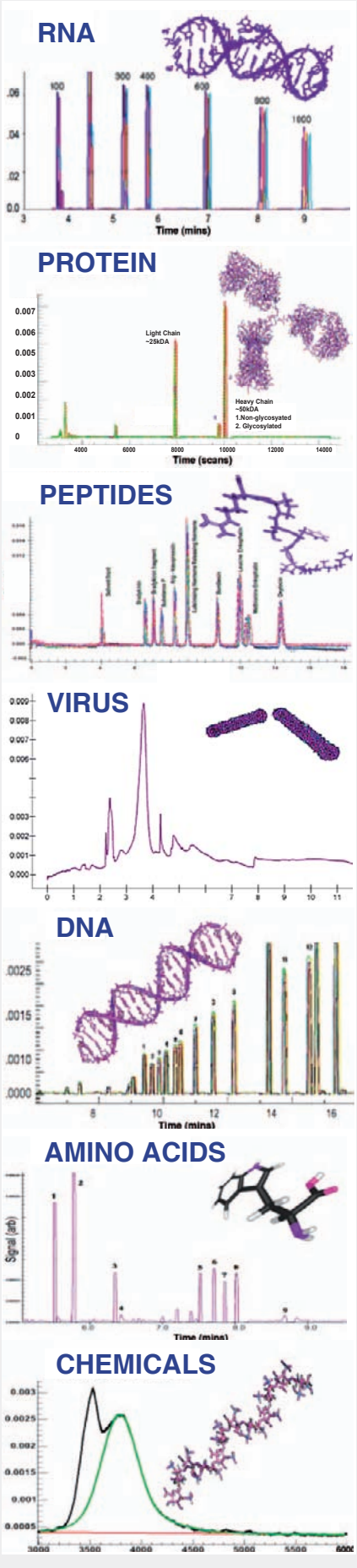
Sample Cooling	Between 10°C and 40°C

Sample Analysis and Application Support

We are always willing to arrange for analysis of your samples to demonstrate the superior data produced by the HPCE-512.

Please contact us for more information.

Application Areas



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