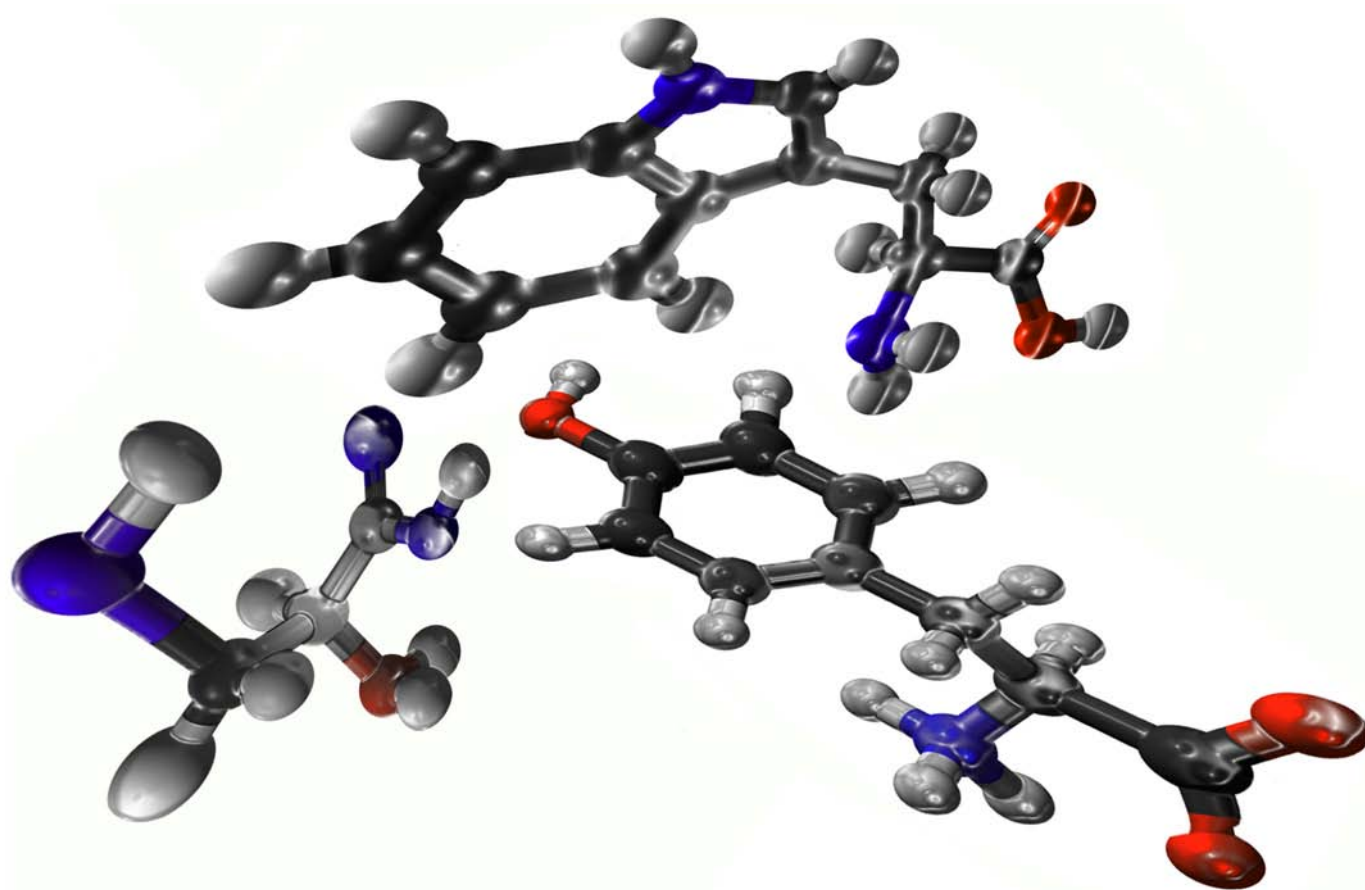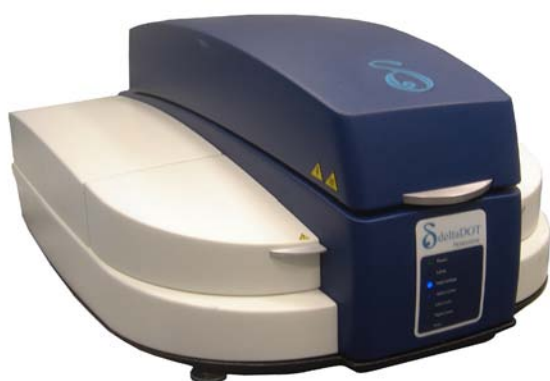# AMINO ACID ANALYSIS

using LABEL FREE INTRINSIC IMAGING

Application Note: Amino Acids

ANALYSIS OF AMINO ACID STANDARDS USING THE DELTADOT HPCE 512 PIXEL LABEL FREE CAPILLARY ELECTROPHORESIS INSTRUMENT

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ABSTRACT

Capillary electrophoresis using indirect UV detection was applied for the analysis of 9 amino acids. This is a fast and convenient method for the analysis of amino acids, compared to HPLC. A reversed polarity mode was employed, with the sample being injected at the cathode and electrophoresed towards the detector at the anode.

INTRODUCTION

deltaDOT's core technology, Label Free Intrinsic Imaging, (LFII®) was applied to indirect UV measurements, using a UV-absorbing buffer. This allows the transmission of light as the non-UV-absorbing analytes pass the detector window. Indirect UV can be used in the analysis of substances inaccessible to the normal LFII® methodology. These include carbohydrates, lipids and sugars, which typically absorb little in the 200-300 nm spectral range favoured by deltaDOT's LFII® technology.

In this instance a mixture of amino acids was investigated using deltaDOT's proprietary LFII® technology and analysed using a high pH run buffer in order to fully ionize the amino acid residues. A quaternary ammonium salt was added to the electrolyte in order to reverse the direction of the EOF, allowing anion migration in the same direction, towards the anode.

Indirect UV detection using LFII® was employed in order to visualise the 9 amino acids (Figure 1). The UV absorbing amino acids of Tyrosine and Tryptophan were analysed within the mixture demonstrating that both chromophore-containing and non-chromophore-containing amino acids can be detected simultaneously.
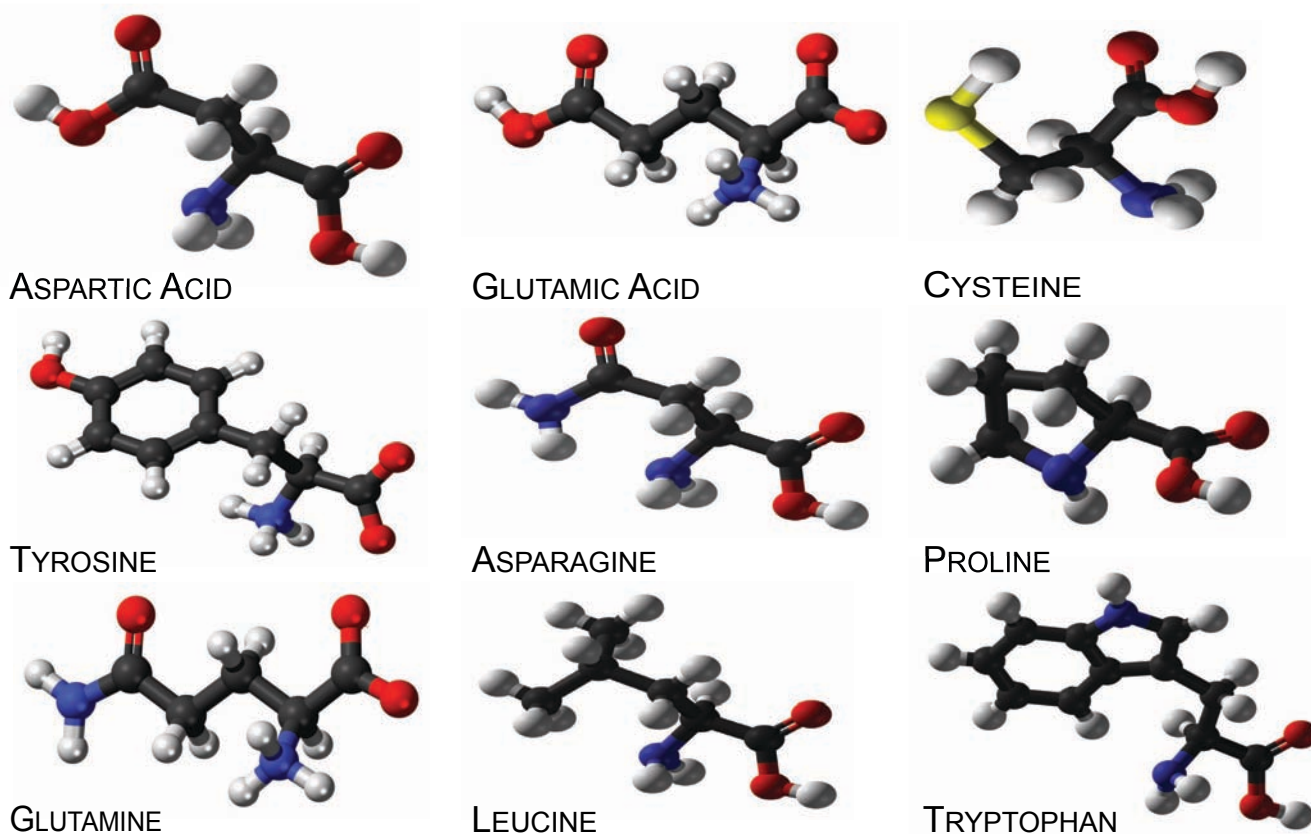

ASPARTIC ACID   GLUTAMIC ACID   CYSTEINE

TYROSINE   ASPARAGINE   PROLINE

GLUTAMINE   LEUCINE   TRYPTOPHAN

Figure 1. *Structures for the given 9 amino acids.*

When applying indirect UV detection, the background electrolyte (BGE) or probe added for UV absorbance is critical. Not only is the pH and wavelength of the chromophore present important, but also the mobility of the probe relative to that of the analytes of interest, as well as the concentration that is added to the run buffer.

Due to the addition of a UV absorbing probe to the running buffer, high currents can be generated, and therefore 50 µM i.d. capillary was employed. Full preconditioning of the capillary was essential to ensure a stable coating and generation of a reversed EOF.

MATERIALS AND METHODS

The data presented in this report were collected on a PEREGRINE I HPCE. All detection was performed at 254nm. Separation of the 9 amino acid residues was carried out in bare fused silica capillaries of 50μm internal diameter, with an effective separation length of 69.9 cm. The total capillary length was 82.1cm total length. The Peltier cooling system within the PEREGRINE I was set to 18°C. Amino acids were prepared by dilution in distilled water from their powder forms, to a concentration of 150 μg/ml, for method development and reproducibility studies.

All samples were injected hydrodynamically at 1.0 psi for 5 seconds, and run in reversed polarity mode. Electrophoresis was performed in a proprietary buffer system (pH 12.0). New capillaries were conditioned by flushing with run buffer for 15 minutes at a pressure of 30 psi.

RESULTS AND DISCUSSION

A PEREGRINE I HPCE system with LFII® detection was used for the separation of 9 amino acids residues. Analysis of the separations was performed using deltaDOT's Equiphase Vertexing Algorithm (EVA) and Generalised Separation Transform (GST) algorithm. GST is a method of combining the data from the 512 pixels in a natural way which preserves the peak shape information of the electropherograms while at the same time maximising the signal-to-noise ratio. Typically a 10-fold increase in signal-to-noise using GST is observed compared to single electropherograms. EVA is an advanced pattern-recognition tool which maximizes the system resolution. In EVA the electropherograms are first analyzed to find local peaks. These are used first to perform vertexing (determine the point of origin of the bands) and then to produce a signal output.

EVA is an advanced pattern-recognition tool which maximizes the system resolution, and converts quantitative information to a peak height rather than area using vertexing to determine the point of origin of the bands and produce a signal output. The results quoted in this report are based upon the GST and EVA analyzed traces.

All 9 amino acid residues were resolved and identified with baseline resolution using GST processed data. **Figures 2** and **3** demonstrate representative GST and EVA traces respectively. The EVA processing software was modified to depict negative inflection peaks within the GST processed traces as positive peaks.
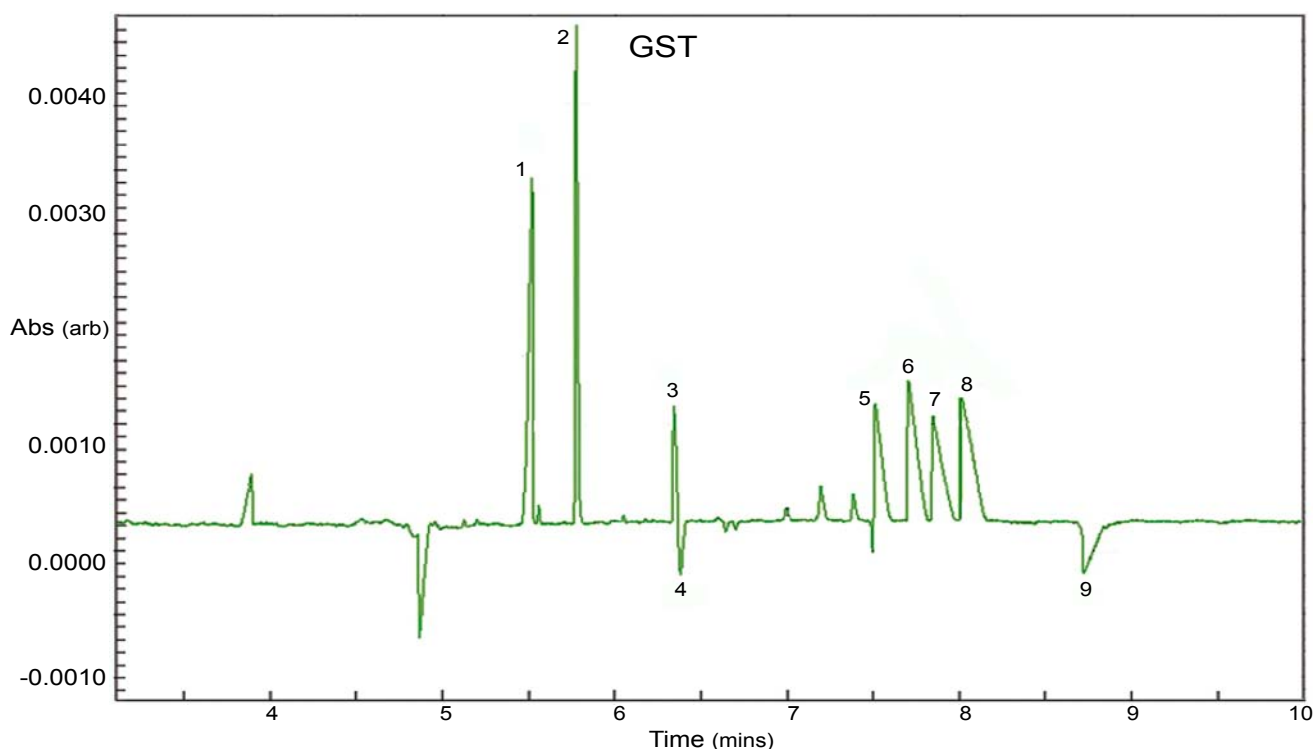

Figure 2. GST separation of all 9 amino acids. The analytes are (1) Aspartic acid, (2) Glutamic acid, (3) Cysteine, (4) Tyrosine, (5) Asparagine, (6) Proline, (7) Glutamine, (8) Leucine, (9) Tryptophan (4 and 9 both contain a chromophore).
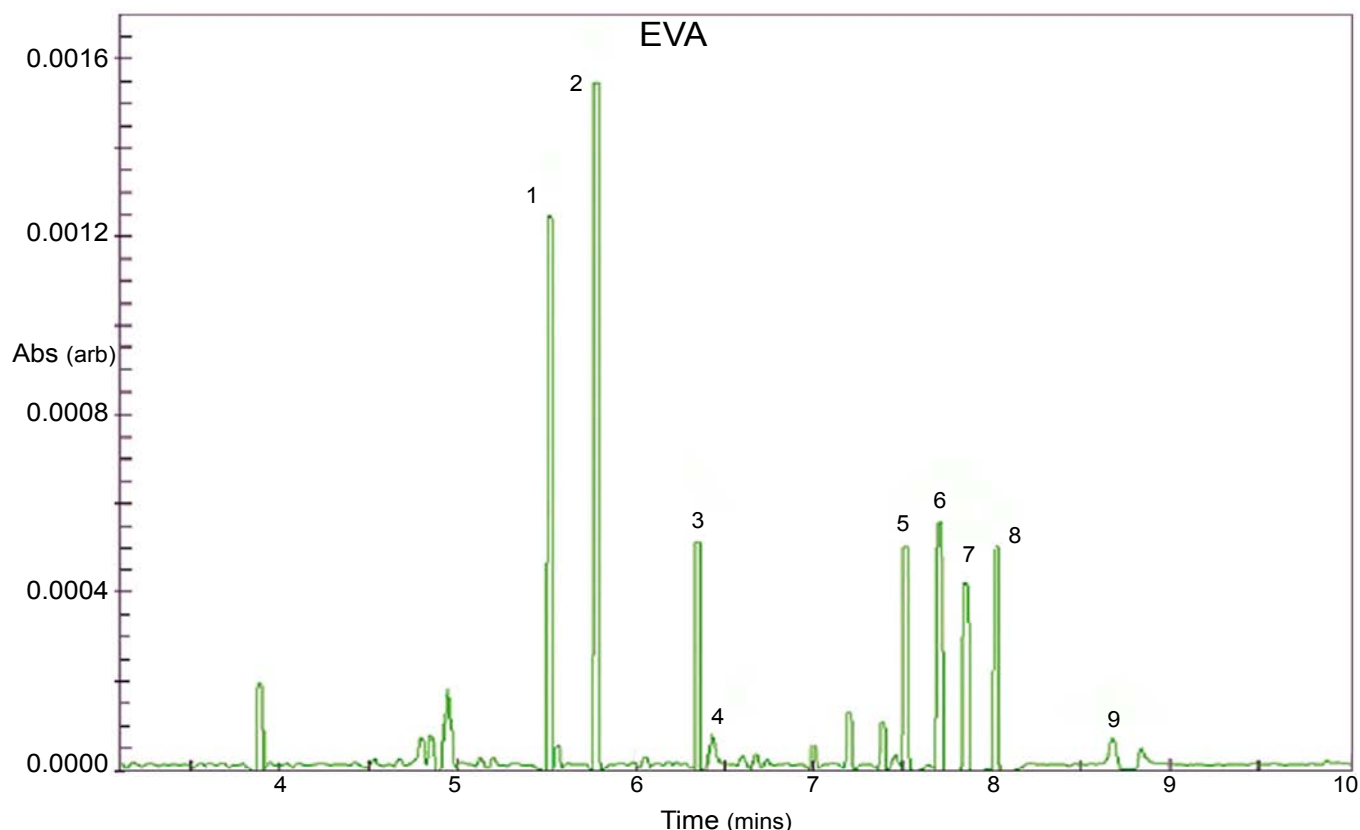
Figure 3. *EVA separation of all 9 amino acids. The analytes are (1) Aspartic acid, (2) Glutamic acid, (3) Cysteine, (4) Tyrosine, (5) Asparagine, (6) Proline, (7) Glutamine, (8) Leucine, (9) Tryptophan.*

Reproducibility studies were performed using pressure injections of the mixture of 9 amino acids, at a concentration of 150 μg/ml. The reproducibility data for the peaks arising from aspartic acid, glutamic acid and leucine are presented for both GST and EVA data in the **Tables 1** and **2.**

GENERALISED SEPARATION TRANSFORM	Peak migration reproducibility			Peak area reproducibility		
	Mean	Std dev	% RSD	Mean	Std dev	% RSD
Aspartic acid	5.514	0.012	0.212	0.043	0.001	1.973
Glutamic acid	5.781	0.014	0.238	0.035	0.001	1.896
Leucine	8.035	0.022	0.271	0.045	0.001	1.372

Table 1*, GST reproducibilities for 3 amino acids.*

EQUIPHASE VERTEXING ALGORITHM	Peak migration reproducibility			Peak area reproducibility		
	Mean	Std dev	% RSD	Mean	Std dev	% RSD
Aspartic acid	5.521	0.012	0.212	0.006	0.0002	4.142
Glutamic acid	5.782	0.012	0.224	0.007	0.0001	1.597
Leucine	8.031	0.019	0.234	0.002	2.47E-05	1.054

Table 2*, EVA reproducibilities for 3 amino acids.*

LIMIT OF DETECTION AND QUANTIFICATION

1. Pressure injection - For pressure injection of 1 psi for 5 seconds, a serial dilution between 10 to 0.039 mM was created for each of the analyte listed in the **Table 3**. LoD and LoQ were calculated on five points ranging from 1.25 mM to 0.039 mM for each of the analyte (with the exception of lysine)
The LoD ranged from 0.037 mM and LoQ ranged from 0.01 to 1.38 mM to 0.45 mM. Lysine was injected at 7 psi for 3 seconds using a different UV absorbing buffer the LoD was calculated to be 0.06 μm and LoQ was calculated to be 0.2 μm.
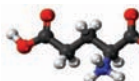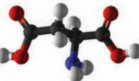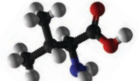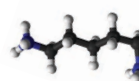
	Cysteine (N=6)	Glutamic Acid (N=6)	Homocysteine (N=4)	Aspartic Acid (N=1)	Valine (N=1)	Lysine (N=1)
Structure						
MW	240.30	147.13	268.35	133.1	117.15	182.65
LoQ	10μM	150μM	1380μM	250μM	400μM	0.2μM
LoD	45μM	37μM	450μM	80μM	130μM	0.06μM
R²	0.9968	0.999	0.996	0.9816	0.9816	0.9995

Table 3.

2. Electrokinetic Stacking injection

-Stacking is a process which is generally used when an analyte present at low concentrations in a long injected sample zone, is concentrated into a short zone (stack). The stacked analytes are then separated and individual zones are detected. Thus stacking can provide better separation efficiency and detection sensitivity.

The graph in **Figure 4** demonstrates a linear relationship between injection voltage and time when the analyte is made in water. The greatest peak area was observed when analyte made in water was injected at 10 kV for 60 seconds. Samples made in 0.1 M and 0.02M HCl had large effect on peak area at the two different injection voltage conditions at the four different time points. To determine LoD and LoQ, a serial dilution of Aspartic acid and Glutamic acid made in water were injected at 5 kV for 15 seconds.
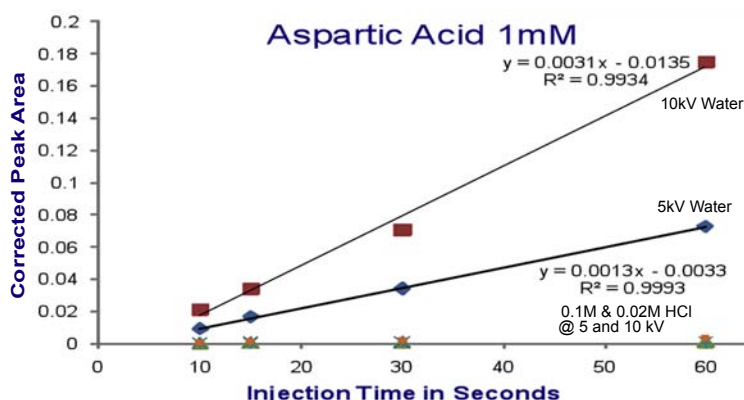

Figure 4. The effect of four injection time in seconds (10, 15, 30 and 60 seconds) using two different injection voltage conditions (5 kV and 10 kV) for aspartic acid made in three different stack buffer

LoD and LoQ for aspartic acid is 0.1 and 0.3 μM respectively and for Glutamic acid, LoD and LoQ is 0.085 and 0.250 μM - **Table 4**).

	Glutamic Acid (N=6)	Aspartic Acid (N=1)
LoQ	0.250μM	0.300μM
LoD	0.085μM	0.100μM
R²	0.9995	0.9952

Table 4

CONCLUSION

The PEREGRINE I HPCE system with LFII® with indirect UV detection was successfully applied for the analysis of 9 amino acid residues, demonstrating both rapid and efficient separation throughout the concentration range.

Excellent resolution has been demonstrated between all 9 amino acid residues, with high reproducibility for migration time (<0.5%) and peak area (<2%). Using the appropriate UV absorbing probe added to the run buffer at high pH, it is possible to simultaneously identify a variety of non-chromophore and chromophore containing containing analytes. It is interesting to note the negative defection of the UV absorbing amino acids. The GST data shows an impressive signal to noise increase, and excellent resolution between amino acid residues, while the EVA processed data shows dramatically increased resolution of all 9 peaks, by applying the vertexing.

A minor modification to the EVA software algorithms allows us to continue to employ this powerful processing technique, allowing the amino acids to be highly resolved and clearly identified.

Analysis of these 9 amino acids demonstrates the powerful capability of the PEREGRINE I indirect UV analysis technique, and using a universal buffer this technique can allow the analysis of many analytes such as carbohydrates, lipids, anions and cations, all within the in the same system.

Amino Acids - Small Molecules

Indirect UV Analysis of Amino Acids using Capillary Zone Electrophoresis with LFII® Detection

A mixture of 9 amino acids was analysed using deltaDOT's PEREGRINE I HPCE at high pH to fully ionize each of the amino acid residues. Indirect UV detection was employed to visualise the 9 amino acids. Excellent resolution was demonstrated between all 9 amino acid residues, with high reproducibility for migration time (<0.5% RSD) and peak area (<2% RSD).

Applications for this type of measurement include IVF embryo selection, neonatal screeninig and media optimisation.

deltaDOT's core technology of Label Free Intrinsic Imaging, (LFII®) may be applied to indirect UV measurements for the analysis of substances including carbohydrates, lipids and sugars, which typically absorb little in the 200-300 nm spectral range.

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deltaDOT has developed and is commercialising innovative capillary electrophoresis technologies and products in the bioscience sector. The company has a strong proprietary position and extensive expertise in instrumentation automation, computing and analysis which will contribute to improvements in knowledge, profitability and process time throughout drug discovery and general life sciences research.