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Application Note

# **Enantiomers** - Small Molecule, CZE

## Enantiomer Analysis using the Peregrine High Performance Capillary Electrophoresis (HPCE) System with Label Free Intrinsic Imaging (LFII®)

Four pairs of enantiomers from a range of basic, neutral and acidic drugs were successfully analysed and resolved using the Peregrine High Performance Capillary Electrophoresis system and deltaDOT's proprietary LFII® technology, demonstrating the system's ability to produce high quality reproducible data in terms of peak mobility (<0.1 % RSD) and peak area (<2.5 % RSD).

In addition the ability to detect 0.1% impurity R or S Atenolol in the presence of 99.9% of the other enantiomer is demonstrated.

## INTRODUCTION

The analysis of four different sets of enantiomers was performed on a Peregrine CE system. Samples were investigated using deltaDOT's proprietary label free intrinsic imaging (LFII®) technology, and analysed by adding highly sulfated cyclodextrins (HSCD) to the run buffer. This allows resolution of a pair of enantiomers under constant separation conditions. Analysis was performed under low pH conditions, allowing the electro-osmotic flow (EOF) to be suppressed, and the negatively charged CDs to migrate towards the anode. The enantiomers therefore migrate through the system by their interaction with the hydrophobic cavity of the HSCDs. Basic compounds will be strong cations at low pH, and therefore interact both hydrophobically and ionically with the negatively charged CDs; while acidic compounds will be protonated at low pH and therefore only interact as a neutral species with the hydrophobic cavity.

Due to the high conductivity of HSCDs, high currents are generated, and therefore separations were carried out in bare fused silica capillaries of 25 $\mu$m internal diameter. Effective capillary thermoregulation is also greatly important to prevent joule heating effects being observed. The Peltier cooling system employed within the Peregrine allows for accurate thermo setting.

Samples of Ketoprofen (acidic), Warfarin (neutral), Propanolol and Atenolol (basic) were analysed demonstrating the capability of deltaDOT's Peregrine Capillary Electrophoresis platform, using Capillary Zone Electrophoresis (CZE).

## MATERIALS AND METHODS

Enantiomer separations were carried out in bare fused silica capillaries of 25 $\mu$m internal diameter, with an effective separation length of 22 cm. Total capillary length was 32 cm. The Peltier cooling system within the Peregrine was set to 22 °C. All samples were made up in run buffer and injected for 2 seconds, using a pressure of 2 psi. A run voltage of 15 kV was employed in reversed polarity mode. Electrophoresis was performed in a proprietary buffer containing an appropriate HSCD. The run buffer was degassed before use. Samples were diluted to the appropriate concentration in 25 mM phosphate buffer pH 2.5.

Between runs the capillary was flushed with a proprietary methodology, followed by sterile water for 1 minute. Subsequently the capillary was flushed for 2 minutes, and then the run buffer (containing the appropriate CD to a concentration of 5%) was injected for 2 minutes, after which the continuity was checked. All data was collected at 214 nm.

## RESULTS

The rapid and efficient separation of each enantiomeric pair was performed using a deltaDOT Peregrine system and data was analysed using both deltaDOT's Equiphase Vertexing Algorithm (EVA) and General 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. We typically observe a 10-fold increase in signal-to-noise using GST as compared to single electropherograms.

EVA is an advanced pattern-recognition tool which maximizes the system resolution, and converts quantitative information to a peak height rather than area. 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.

The single pixel RAW electropherograms, GST- and EVA- processed data for each pair of enantiomers is thus shown, left to right. Ketoprofen (acidic), Propanolol (basic), Warfarin (neutral) and Atenolol (basic) are demonstrated in Figure 1, Figure 2, Figure 3 and Figure 4 respectively.

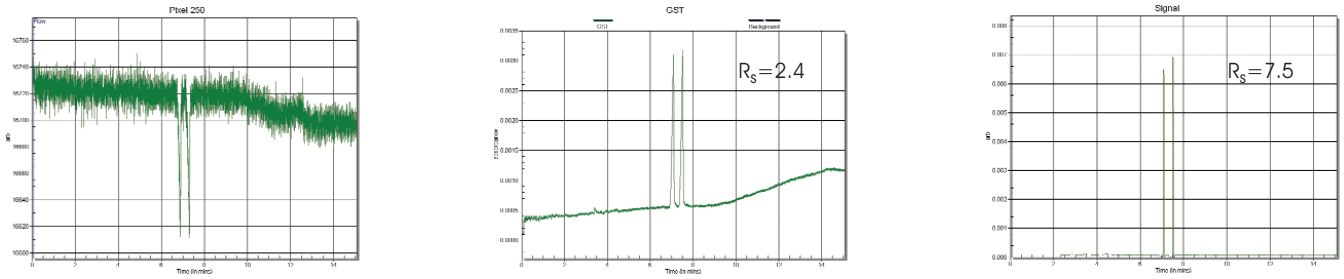


Figure 1: Comparison of single pixel, GST and EVA data for Ketoprofen.

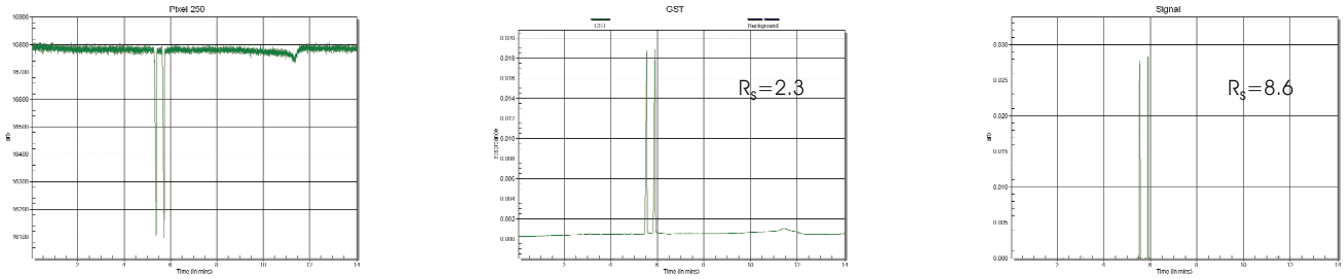


Figure 2: Comparison of single pixel, GST and EVA data for Propanolol.

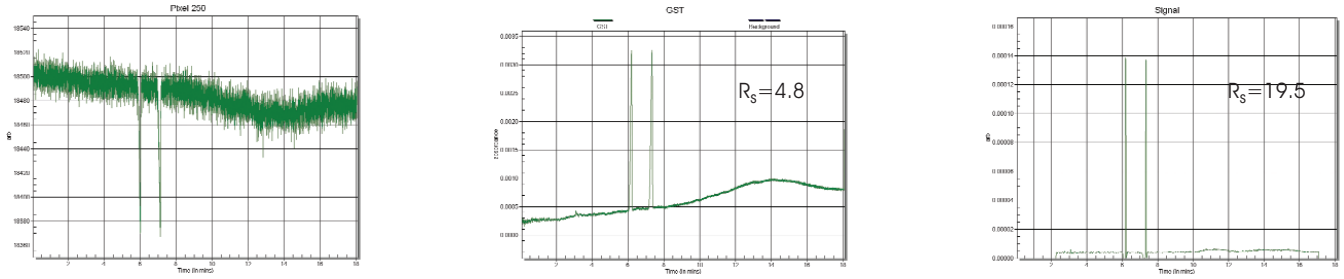


Figure 3: Comparison of single pixel, GST and EVA data for Warfarin.

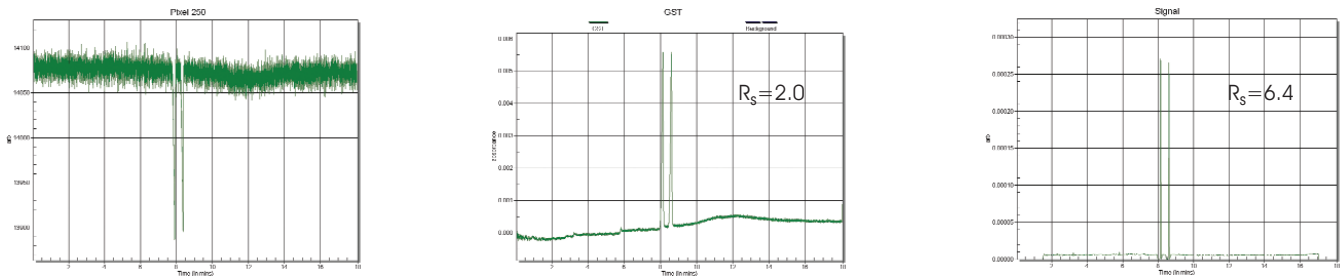


Figure 4: Comparison of single pixel, GST and EVA data for Atenolol.

Reproducibility of the Peregrine HPCE with LFII® system was demonstrated with each of the enantiomeric pairs. Reproducibility was calculated from GST data, based upon peak migration times and peak areas, as shown in Table 1 and Table 2 respectively.

| | Mean | std dev | % RSD |
|---|---|---|---|
| Ketoprofen | 6.9988 | 0.0067 | 0.0957 |
| Propanolol | 5.5046 | 0.0055 | 0.1002 |
| Warfarin | 6.1581 | 0.0067 | 0.1901 |
| Atenolol | 8.1285 | 0.0148 | 0.1818 |

Table 1: GST Reproducibility of Peak Migration Times for each enantiomeric pair (based on peak 1).

| | Mean | std dev | % RSD |
|---|---|---|---|
| Ketoprofen | 0.3293 | 0.0082 | 2.4900 |
| Propanolol | 1.3808 | 0.0341 | 2,4697 |
| Warfarin | 0.2655 | 0.0033 | 1.2346 |
| Atenolol | 0.7162 | 0.0143 | 1.9929 |

Table 2: GST Reproducibility of Peak Area for each enantiomeric pair (based on peak 1).

Reproducibility was also calculated from EVA processed data, for peak migration time and peak area, as shown in Table 3 and Table 4 respectively.

| | Mean | std dev | % RSD |
|---|---|---|---|
| Ketoprofen | 7.0283 | 0.0058 | 0.0323 |
| Propanolol | 5.5202 | 0.0051 | 0.0916 |
| Warfarin | 6.1835 | 0.0069 | 0.1008 |
| Atenolol | 8.1551 | 0.0150 | 0.1843 |

Table 3: EVA Reproducibility of Peak Migration Times for each enantiomeric pair (based on peak 1).

| | Mean | std dev | % RSD |
|---|---|---|---|
| Ketoprofen | 0.0058 | 7.9E-05 | 1.3521 |
| Propanolol | 1.0411 | 0.0006 | 1.3846 |
| Warfarin | 0.0058 | 6.92E-05 | 1.1927 |
| Atenolol | 0.0127 | 7.03E-05 | 0.5522 |

Table 4: EVA Reproducibility of Peak Area for each enantiomeric pair (based on peak 1).

Figure 5 shows the analysis of the R-enantiomer of Atenolol in the presence of trace amounts of the S-enantiomer. The S-form of the basic drug is present at a level of 0.1% of the R-enantiomer, while Figure 6 shows a plot of concentration of the R-Form (as a percent of the S-Form) versus the percentage peak area observed.

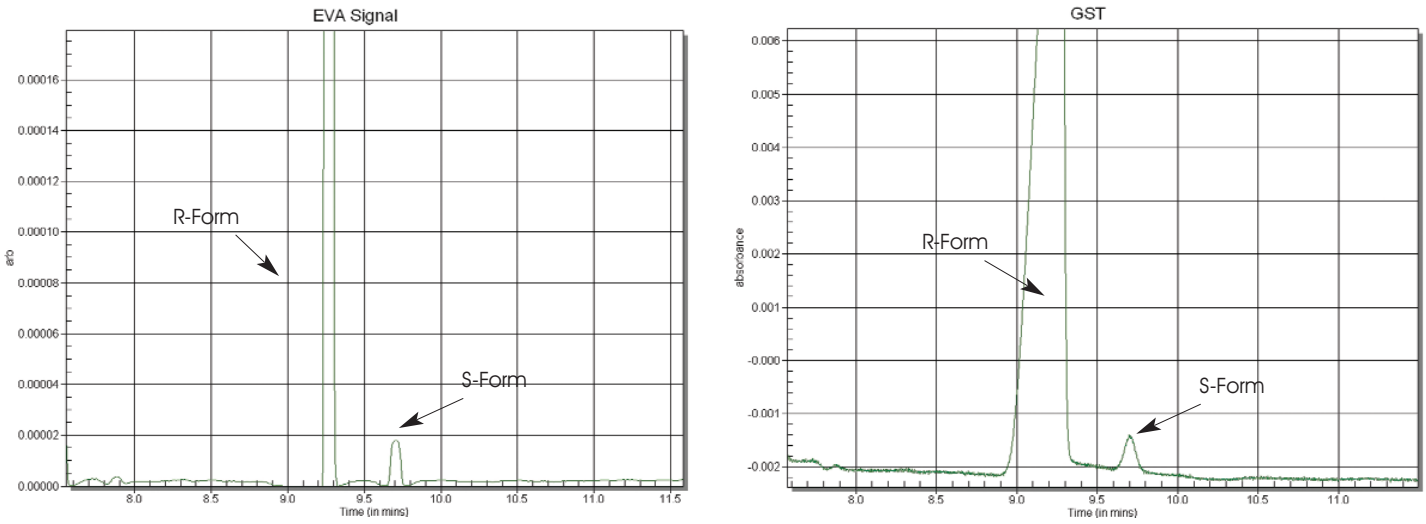


Figure 5: GST and EVA data for 0.1% S-Atenolol in the presence of R-Atenolol.

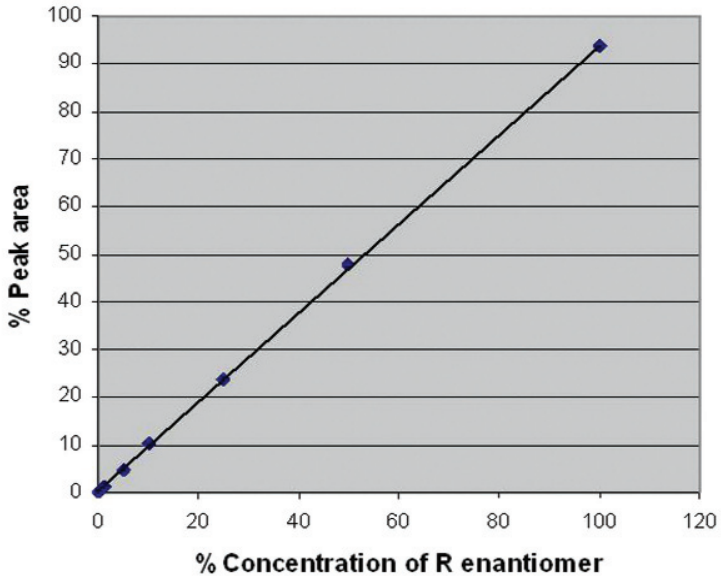


Figure 6: GST and EVA data for 0.1% R-Atenolol in the presence of S-Atenolol.

## CONCLUSION

The Peregrine HPCE system with LFII® detection has been successfully applied to chiral analysis, demonstrating rapid and efficient separation of each enantiomeric pair. Excellent resolution has been demonstrated between all four sets of enantiomers, with high reproducibility. Good linearity was also demonstrated between the peak area with respect to concentration, and quantification of 0.1% of the minor enantiomeric form was demonstrated in the presence of the major form. Using the appropriate highly sulphated cyclodextrin added to the run buffer, it is possible to resolve a variety of enantiomeric mixtures, as demonstrated here with the analysis of acidic, neutral and basic drugs.

The GST data shows an impressive signal-to-noise increase, and excellent resolution between enantiomers, while the EVA processed data shows dramatically increased resolution of the two peaks.

This demonstrates the powerful separation capability of the Peregrine system when employed for enantiomer separations, especially when processed using deltaDOT's EVA signal analysis.

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