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Application Note

Antibiotics - Small Molecules, MECC

Analysis of ß-lactam antibiotics in food products, using the deltaDOT Peregrine HPCE

Various penicillin derivatives are widely used to treat infectious diseases in humans and animals, acting to kill or inhibit the growth of the infectious organism. The presence of antibiotics in the food chain is tightly controlled to avoid new strains of antibiotic resistant bacteria developing. EU regulations now determine maximum limits for each of the 8 penicillin derivatives that can be allowed in animal tissue products for the consumer. A mixture of these 8 antibiotic derivatives was analysed using Micellar Electrokinetic Capillary Chromatography producing high quality reproducible data both in terms of peak mobility (<0.5% RSD) and peak area (<3.0% RSD).

INTRODUCTION

A mixture of 8 antibiotic derivatives was analysed using Micellar Electrokinetic Capillary Chromatography (MECC). The Peregrine High Performance Capillary Electrophoresis system was employed, using deltaDOT's proprietary label-free technology. Capillary electrophoresis was applied for the separation of Penicillin V, Penicillin G, Amoxicillin, Ampicillin, Nafcillin, Oxacillin, Cloxacillin and Dicioxacillin, (Figure 1) using a sodium tetraborate buffer supplemented with sodium dodecyl sulphate (SDS).

Sodium dodecyl sulphate (SDS) is the most widely used surfactant in MECC, forming negatively charged droplets. The C_{12}alkylchains of the surfactant penetrate and form the core of the droplet while the negatively charged hydrophilic sulphate groups reside on the surface, in the aqueous phase. Using constant separation conditions, this allows resolution of this set of weakly acidic compounds based upon their hydrophobicity and their variable partitioning into the hydrophobic core of the droplet.

Analysis was performed at pH 8.0, which improves tailing of the peaks and allows the generation of a strong overall electro-osmotic flow towards the anode, in order to allow elution of the hydrophobic compounds while they reside in the aqueous phase.

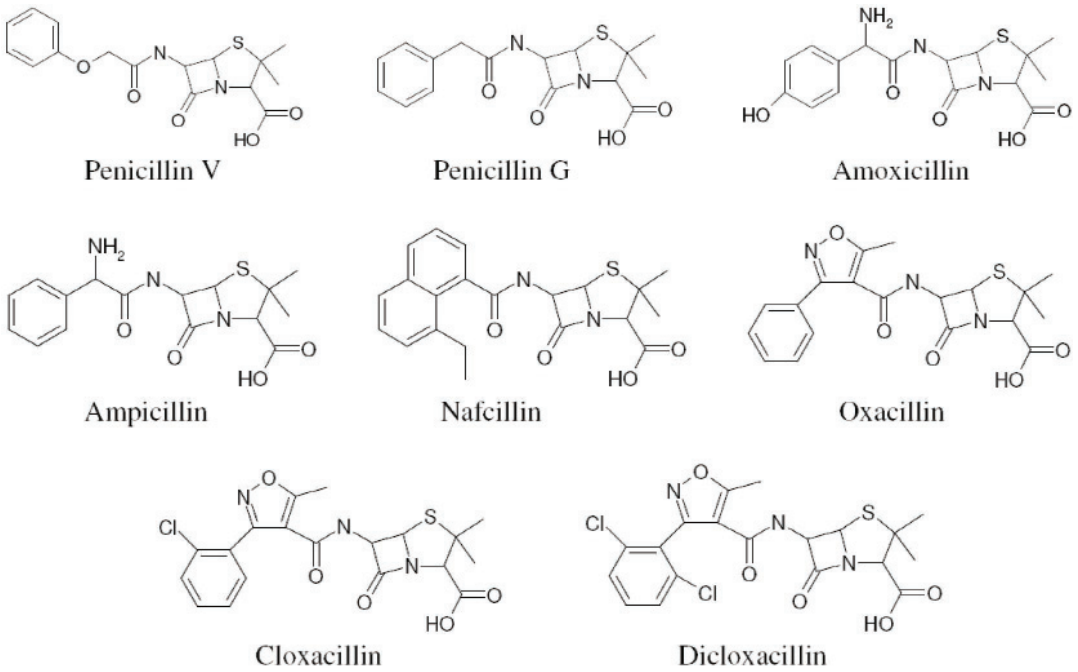

Figure 1: Structures of the eight penicillin derivatives.

MATERIALS AND METHODS

The analysis of a mixture of 8 ß-Lactam Antibiotics was performed in bare fused silica capillaries of 75 μm internal diameter, with an effective separation length of 40 cm. Total capillary length was 52 cm. The Peltier cooling system was set to 15 °C. Each of the 9 samples was dissolved in dH_2O to generate a serial dilution from 0.5 mg/mL down to 0.05 mg/mL, including a blank. Samples were injected for 5 seconds, using a pressure of 0.8 psi (equivalent 55 mbar). A run voltage of 13 kV was used in standard polarity mode (250 V/cm). Electrophoresis was performed in a proprietary buffer supplemented with SDS, and all data was collected at 214 nm.

RESULTS

The Peregrine HPCE system with LFII® detection was applied to a mixture of 8 ß-lactam antibiotics. Quantifiable detection was measured using the injection parameters given above. Each concentration was run across 4 repeats. Single pixel data, GST, and EVA ("signal") data are all given for concentrations at 5 µg/mL and 0.5 µg/mL as demonstrated in Figures 3 and 4 respectively. For each concentration the Equiphase map is also shown.

The rapid and efficient separation of these 8 penicillin derivatives 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.

At low concentration injections of the penicillin derivatives, no peak was observable (by eye) with respect to the noise in the single pixel data. However when the data is summed across all 512 pixels as demonstrated in the GST electropherograms (maximising the signal-to-noise ratio), and also analysed using deltaDOT's Equiphase Vertexing Algorithm (EVA), a detectable peak can be observed as low as 0.5 µg/mL. The signal-to-noise increase experienced here demonstrates the significance that can be generated from GST and EVA processed data with up to a 20-fold increase in signal-to-noise observed over the single pixel data for the 0.5 mg/mL injection concentration. It can therefore be seen from the GST and EVA data that by applying vertexing, an increased signal output of the peak arising from the injected sample can be produced, leading to greater sensitivity, resolution and reduced noise.

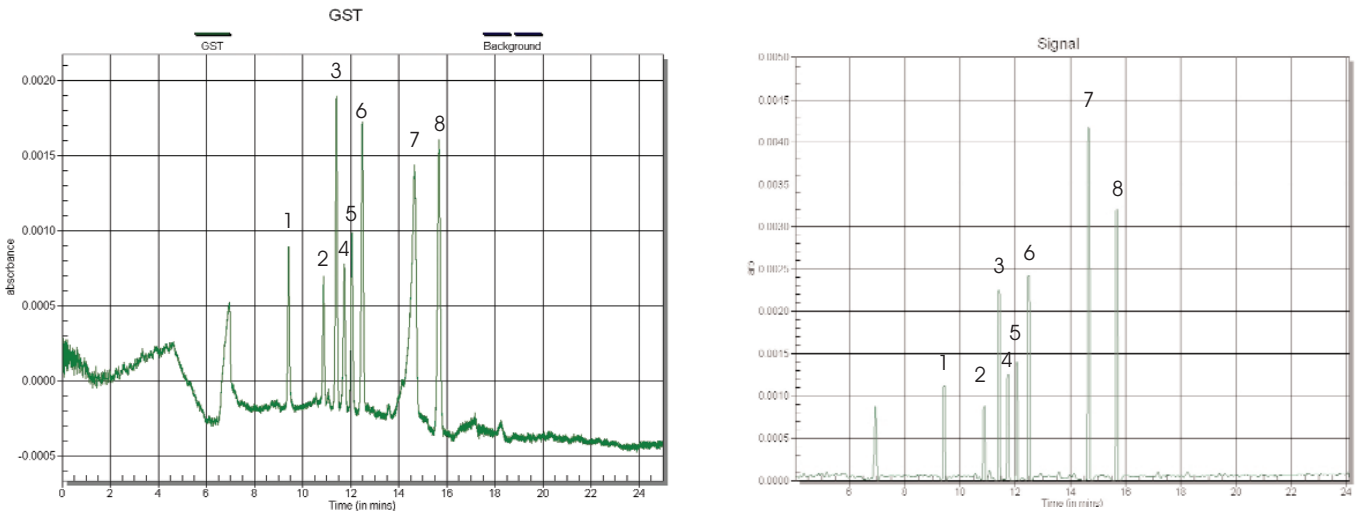

Figure 2: Standard GST electropherogram and EVA trace for the separation of 8 penicillin derivatives. The analytes are (1) amoxicillin, (2) ampicillin, (3) penicillin G, (4) oxacillin, (5) penicillin V, (6) cloxacillin, (7) nafcillin, (8) dicloxacillin.

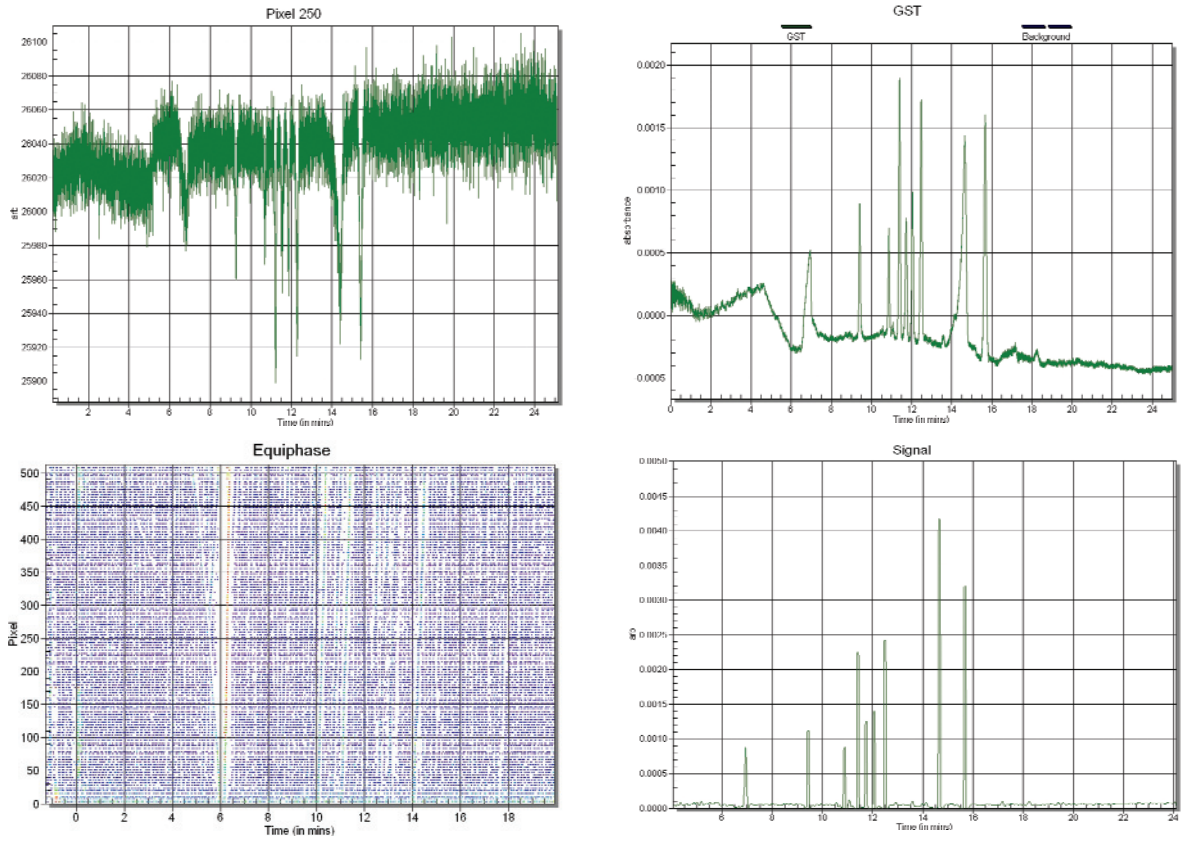

Figure 3: Comparison of single pixel, GST and EVA data for 5 mg/mL sample concentration.

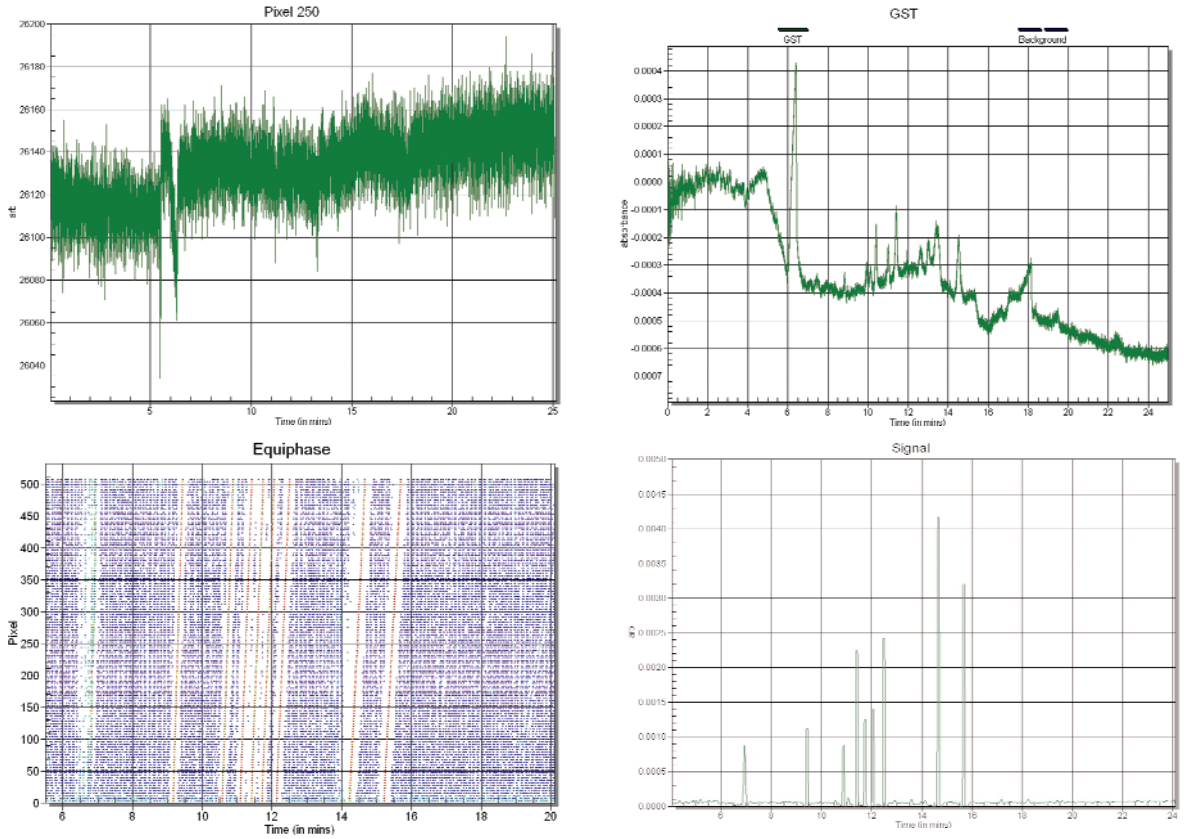

Figure 4: Comparison of single pixel, GST and EVA data for 0.5 mg/mL sample concentration.

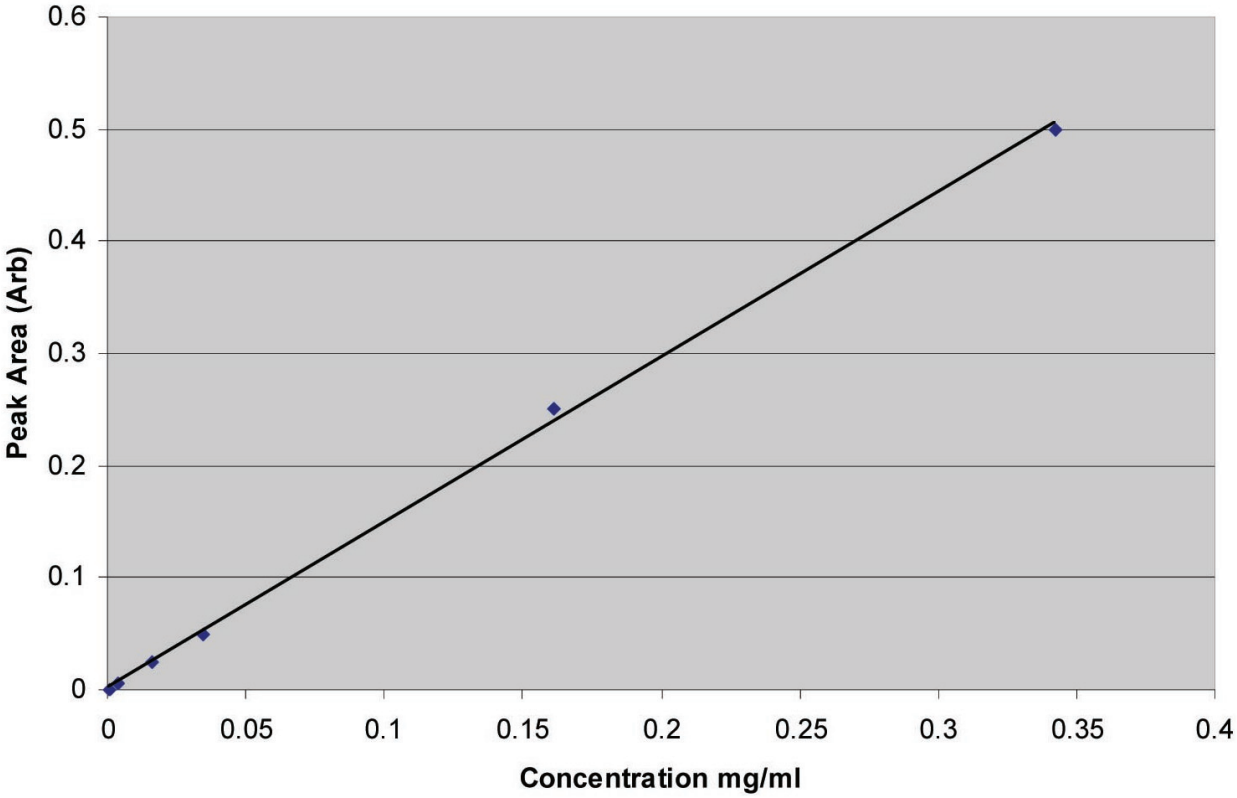

Figure 5: Linearity of sample concentration versus peak area.

	mean	std dev	% RSD
Peak migration time	11.38507	0.034436	0.302465
Peak area	0.23407	0.004955	2.116688

Table 1: GST Reproducibility of Peak Migration Times (based on peak 1).

	mean	std dev	% RSD
Peak migration time	11.37822	0.02802	0.246261
Peak area	0.003197	5.69E-05	1.781004

Table 2: EVA Reproducibility of Peak Migration Times (based on peak 1).

CONCLUSION

The Peregrine HPCE system with LFII® detection has been used to analyse 8 penicillin derivatives, demonstrating both rapid and efficient resolution of each of the analytes throughout the concentration range. The GST data shows an impressive signal-to-noise increase, and that by applying vertexing in GST and EVA data, an increased signal output from the injected sample can be produced, leading to greater sensitivity, resolution and reduced noise.

The relative standard deviations (RSD) of peak migration and peak elution times have demonstrated the excellent reproducibility between runs, for both GST and EVA processed data (table 1 and 2). HPLC is commonly used for the quantification of ß-lactam antibiotics; however high purity organic solvents, special sample preparation and long HPLC stabilisation times are required. deltaDOT's Peregrine capillary electrophoresis system with LFII® provides a simpler, cheaper and faster analysis and has routinely been applied to solve many other environmental and food analysis problems.

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deltaDOT (http://www.deltadot.com) is developing and commercialising innovative enabling technologies and products in the bioscience arena. The company is focused on applying its Label Free Intrinsic Imaging technology to drug discovery.

deltaDOT was founded in 2000 and is a spin out from the Imperial College London, UK. It is focused on the harnessing of cutting-edge particle physics technology and its application to the needs of biomolecular separation, including proteins, DNA and RNA analysis. The company has a strong proprietary position and extensive expertise in instrumentation, microfluidics, automation, computing and analysis which will contribute to improvements in knowledge, profitability and process time throughout drug discovery and general life sciences research.