deltaDOT
See the molecule

Application Note

Artificial Sweeteners - Small Molecule, CZE

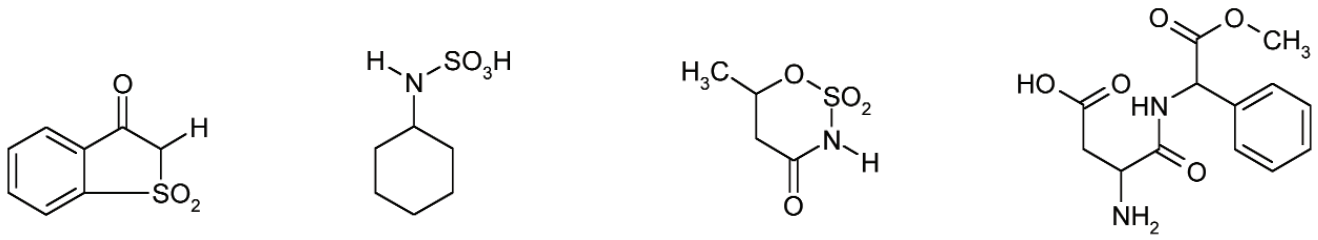
Analysis of Artificial Sweeteners using the Peregrine High Performance Capillary Electrophoresis (HPCE) System with Label Free Intrinsic Imaging (LFII®)

Rapid capillary electrophoresis separation of a mixture of the most common artificial sweeteners has been demonstrated using the Peregrine HPCE system and deltaDOT's proprietary LFII® technology. These sweetener compounds have been separated with migration reproducibilities of <0.3% RSD and peak area reproducibilities of <3% RSD. This establishes deltaDOT's LFII® technology as the best alternative to the more complex HPLC methods currently employed to monitor these compounds in the food industry.

INTRODUCTION

Artificial sweeteners have been used as sugar substitutes in beverages and food for over 30 years. The last 15 years have seen a variety of health scares and rising obesity levels. This has led to the efficient analysis of these food additives to become increasingly significant. Four commonly used synthetic sweeteners were analysed in this study; Saccharine, Cyclamate, Acesulfame K and Aspartame (Figure 1). Combinations of these sweetener compounds are employed to help simulate the natural sugar taste in various diet beverages.

Using deltaDOT's propriety LFII® technology, a mixture of these sweetener compounds and some of their derivatives were separated using capillary zone electrophoresis (CZE). A sodium tetraborate buffer (pH 9.4) was used to generate a strong Electro-osmotic Flow (EOF). This method was shown to be highly applicable to beverage analysis, providing a rapid and simple tool for quality control and analysis due to the excellent reproducibility achieved using deltaDOT's Peregrine HPCE system.



Saccharine | Cyclamate | Acesulfame K | Aspartame

Figure 1: Structure of sweeteners used in this study.

MATERIALS AND METHODS

Sweetener separations were carried out using bare fused silica capillaries with a 50 μm internal diameter. All analyses were carried out with a 50 cm effective separation length and a total capillary length of 62 cm. All samples were prepared in deionised water and hydrodynamically injected for 1 second at a pressure of 1 psi. A run voltage of 25 kV was employed, producing an electric field of 400 V/cm. All separations were carried out at 25 °C, using a proprietary electrolyte.

New capillaries were flushed for 15 minutes with a proprietary buffer sequence; subsequent runs underwent a similar proprietary flush methodology. All data was collected at 200 nm.

RESULTS AND DISCUSSION

The Peregrine HPCE system with LFII® detection was applied to a mixture of 7 artificial sweeteners and sweetener derivatives. All compounds were successfully separated with a lower limit of quantifiable detection of 0.25 μg/mL. Figure 2 shows the GST electropherogram and EVA signal plots of a typical separation with the corresponding single pixel electropherogram and equiphase map shown in Figure 3.

Data is also given for the separations carried out at an effective concentration of 5 μg/mL (Figure 4), at this point the signal-to-noise ratio is too low for single pixel detection. However as a consequence of deltaDOT's superior detection system and signal processing algorithms, signal can be easily observed in GST and EVA signal plots.

deltaDOT's Peregrine system employs sophisticated algorithms and detection technology to improve signal-to-noise. The sweeteners were analysed with both deltaDOT's Equiphase Vertexing Algorithm (EVA) and General Separation Transform (GST) algorithm. GST is a method of combining the data from all 512 pixels in a natural way which preserves the peak shape information of individual electropherograms, while at the same time maximising the signal-to-noise ratio. A 10-fold increase in signal-to-noise using GST as compared to single electropherograms is typically observed.

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.

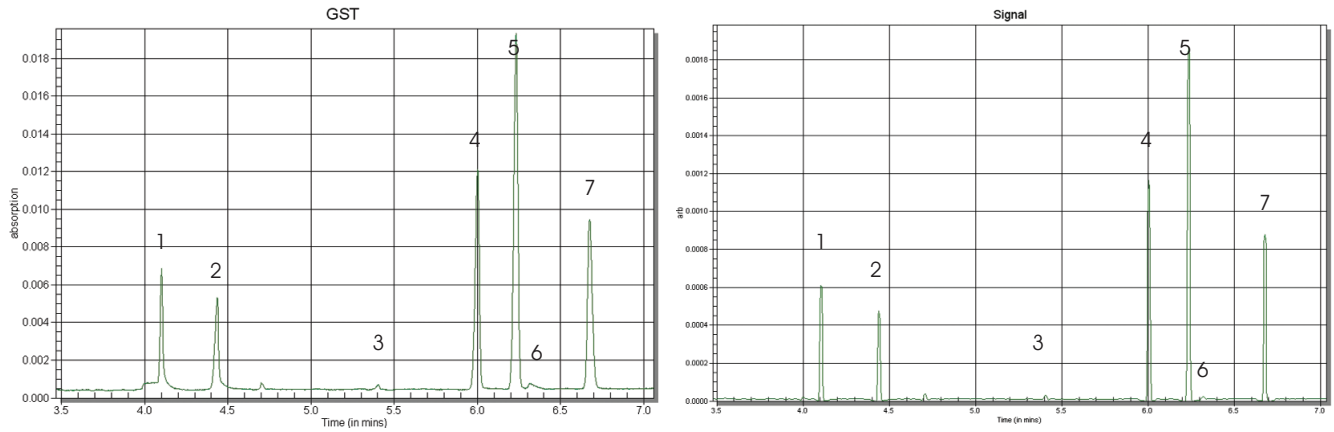


Figure 2: Standard GST electropherogram and EVA trace for the separation of 7 sweeteners and sweetener derivatives, (100mg/ml). The analytes are (1) Phenylalanine, (2) Aspartame, (3) Cyclamate, (4) Saccharine, (5) Benzoic acid, (6) Aspartic Acid, (7) Acesulfame K (8).

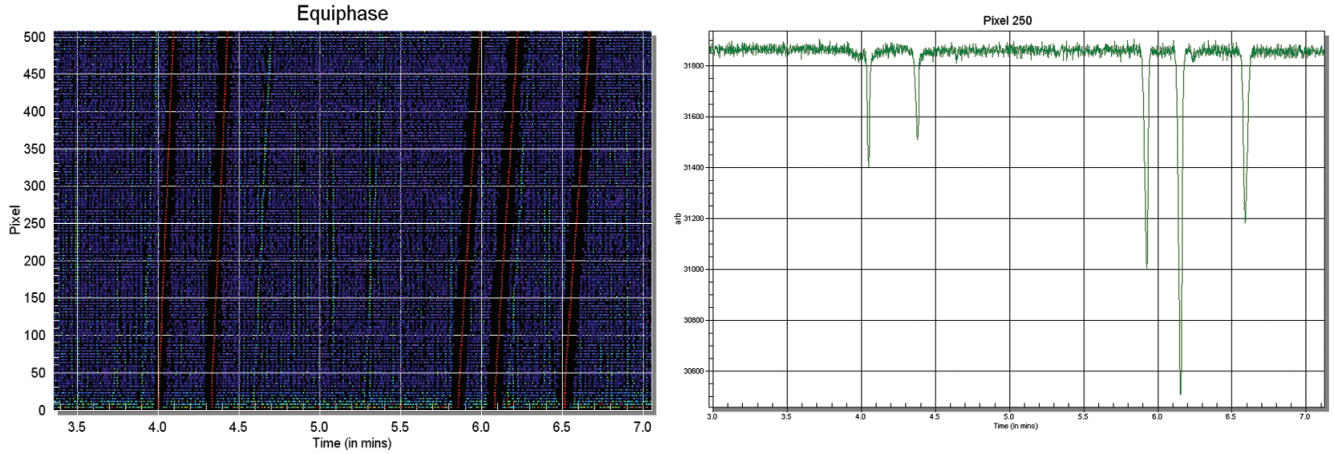


Figure 3: Corresponding single pixel and equiphase map data for the GST and EVA signal data from figure 2.

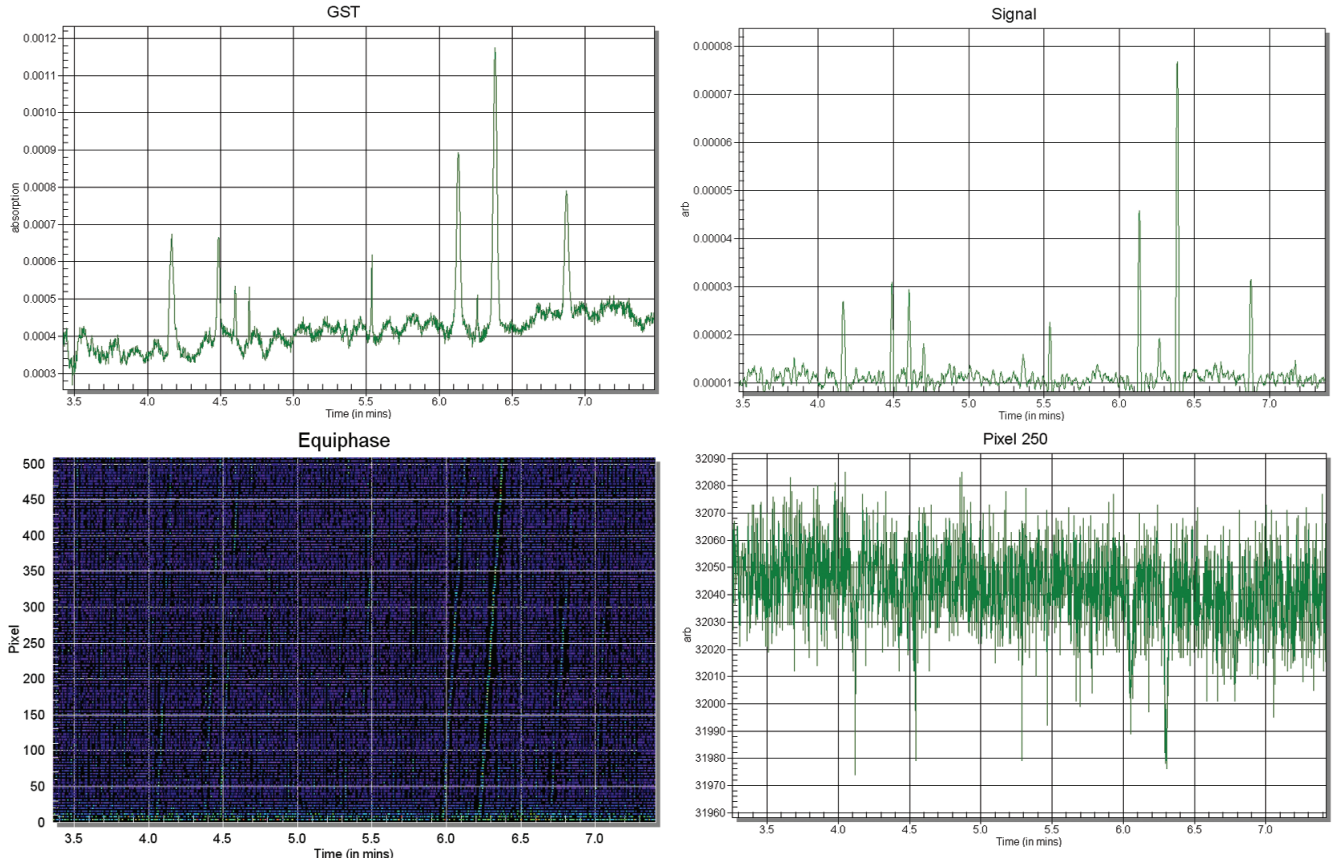


Figure 4: Comparison of GST, EVA signal, equiphase and single pixel data, for 5 μg/mL effective sample concentration.

As well as excellent sensitivity, the Peregrine's Peltier temperature control system provides superb thermal control. A set of 8 repeat runs (Figure 5) generated peak migration times of <0.3% RSD (Table 2) and peak area reproducibility of <3.0% RSD (Table 4). An overlay of these 8 repeats is shown in Figure 5.

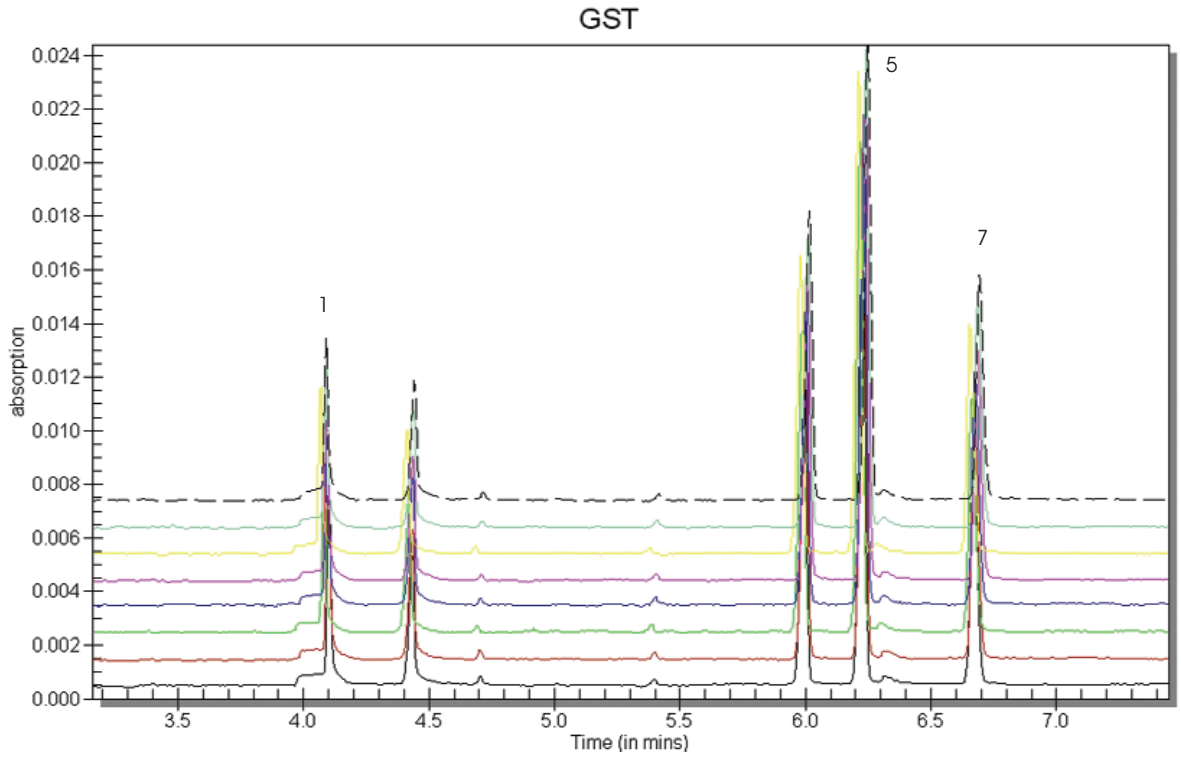


Figure 5: Overlay of 8 repeat runs.

	Mean	Std Dev	% RSD
1.phenylalanine	4.0936825	0.01174	0.286784
2.aspartame	4.4358275	0.010583	0.238573
3.cyclamate	5.40220125	0.011717	0.216884
4.benzoic acid	6.00340375	0.012364	0.205947
5.saccharine	6.23511	0.012542	0.201152
6.aspartic acid	6.31117875	0.012068	0.19122
7.acesulfame K	6.678345	0.013211	0.197824

Table 1: Reproducibility of sweetener peak migration times for EVA signal

	Mean	Std Dev	% RSD
1.phenylalanine	4.087433	0.011537	0.282257
2.aspartame	4.433851	0.011475	0.258794
3.cyclamate	5.401239	0.011933	0.220916
4.benzoic acid	6.010125	0.012182	0.202675
5.saccharine	6.229058	0.012861	0.206457
6.aspartic acid	6.306759	0.011917	0.188966
7.acesulfame K	6.679628	0.013294	0.199031

Table 2: Reproducibility of sweetener peak migration times for GST

	Mean	Std Dev	% RSD
1.phenylalanine	0.005989376	0.000146	2.441649
2.aspartame	0.004650409	0.000115	2.475944
3.cyclamate	3.22E-04	2.57E-05	7.966744
4.benzoic acid	0.011375013	0.00027	2.376869
5.saccharine	0.018149838	0.000399	2.199434
6.aspartic acid	3.10E-04	0.00013	41.7822
7.acesulfame K	0.008535708	0.000192	2.244734

Table 3: Reproducibility of sweetener peak areas for EVA signal

	Mean	Std Dev	% RSD
1.phenylalanine	0.111588	0.003172	2.843024
2.aspartame	0.093772	0.004200	4.479112
3.cyclamate	0.005623	0.000837	14.892363
4.benzoic acid	0.202170	0.005378	2.660376
5.saccharine	0.320531	0.007756	2.419599
6.aspartic acid	0.011859	0.000987	8.326897
7.acesulfame K	0.179480	0.003689	2.055123

Table 4: Reproducibility of sweetener peak areas for GST

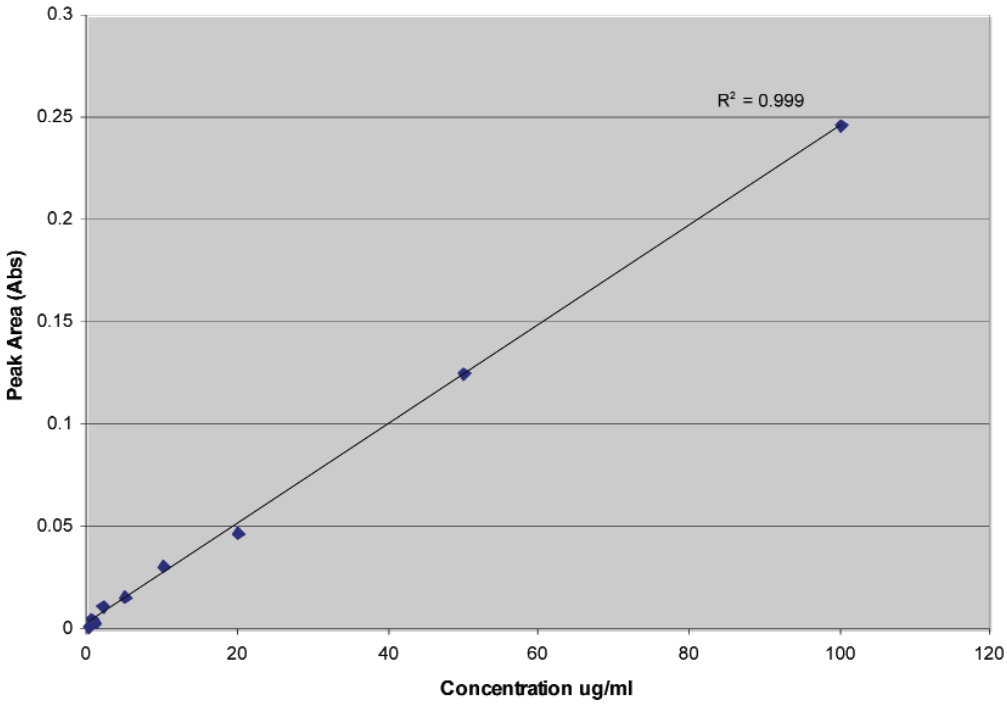


Figure 6: Linearity of sample concentration versus peak area for saccharine peak.

2 Diet Colas were also compared with our standard sweetener separation, and this is represented in Figure 7. Both soft drinks analysed contained Aspartame and Acesulfame K, which are known ingredients, interestingly however, **benzoic acid** did not appear to be present in Diet Cola 2.

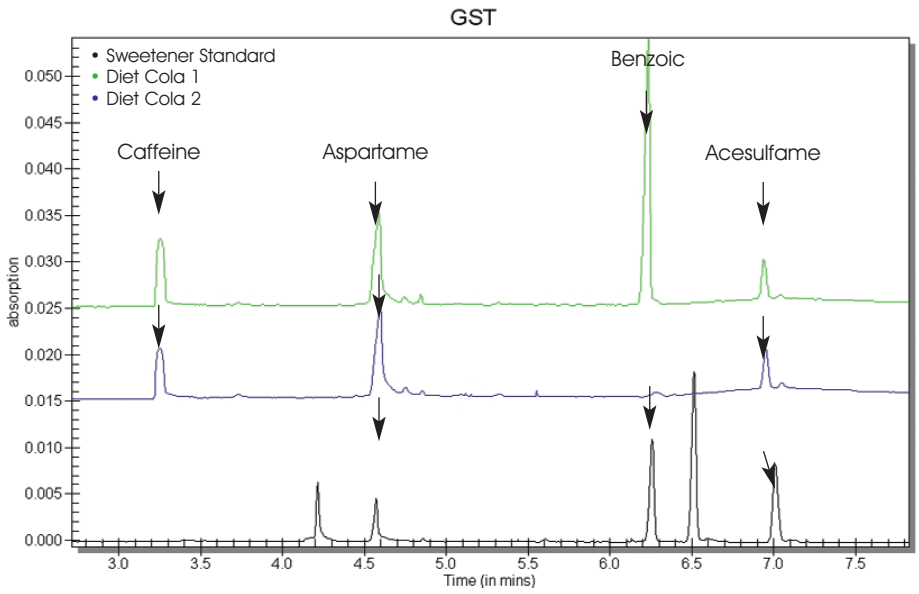


Figure 7: GST electropherograms for standard sweeter and derivatives (black), and Diet Cola 1 (green) and Diet Cola 2 (blue). The caffeine peak at 3.25 minutes was confirmed by separate migration analysis.

CONCLUSION

The Peregrine HPCE system with LFII® detection was successfully applied for the analysis of 7 sweetener compounds and their derivatives. Excellent quantifiable signal detection of better than 0.25 μg/ml at a low detection wavelength is demonstrated. Being able to observe cyclamate in the same separation as the other sweetener compounds demonstrated a method that can be used to analyse all the major sweetener types in one run. Excellent linearity was observed across the concentration range, as demonstrated by the Saccharine data (Figure 6). This demonstrates efficient hydrodynamic pressure control, coupled with excellent signal-to-noise detection, using deltaDOT's LFII® system. Finally, highly reproducible peak migration times, attained with the Peregrine's Peltier temperature control and instrumentation, give confidence for the accurate detection of sweetener compounds in beverages and food derivatives.

deltaDOT Ltd
Bessemer Building (RSM)
Prince Consort Road
London SW7 2BP UK

T +44 (0)20 7594 1001
F +44 (0)20 7594 1006
E info@deltadot.com
W www.deltadot.com