BEYOND MEASURE

SCION INSTRUMENTS

Highly Sensitive Analysis of Organic Acids by HPLC-UV

Application Note AN0066

INTRODUCTION

Organic acids such as malic, ascorbic and citric acid, are commonly found in food and beverage products. Derived by both natural biochemical processes and added as preservatives/stabilisers, organic acids contribute to the sensory properties of food and drink, including both aroma and taste. The monitoring of these organic acids is essential for both quality control during production of said products but also for evaluating food authenticity and purity. Although regulations vary widely, regulations are in place to prevent the bulk use of these ingredients.

SCION Instruments developed a HPLC method for the simultaneous identification of ten organic acids, using a single wavelength by UV detection. UV detection is possible by the detection of absorption via the carboxyl groups of the organic acids. Additionally, the use of a low carbon octadecylsilyl (ODS) column reduced the hydrophobicity of the silica surface, providing a stable analysis for the separation of high polarity compounds, such as organic acids, in a 100% aqueous solution.

EXPERIMENTAL

A SCION 6000 HPLC with UV was used with a C18-AQ (ODS) reverse phase column for the simultaneous detection of ten organic acids in food products. Table 1 details the ten target compounds analysed, associated peak number for identification in the chromatogram as well as calibration range.

Table 1. Target compounds and concentration of calibration standards

Peak Number	Compound	Calibration Concentration
1	Tartaric Acid	2.5-500mg/L
2	Formic Acid	5-1000mg/L
3	Malic Acid	5-1000mg/L
4	Lactic Acid	5-1000mg/L
5	Acetic Acid	5-1000mg/L
6	Pyroglutamic Acid	0.5-100mg/L
7	Citric Acid	5-1000mg/L
8	Fumaric Acid	0.05-10mg/L
9	Succinic Acid	5-1000mg/L
10	Propionic Acid	5-1000mg/L

Commercial grain vinegar and apple cider vinegar were diluted 1:50 with pure water before being filtered and analysed. Analytical conditions can be found in Table 2.

Table 2. Analytical Conditions of HPLC-UV

Conditions	
Column	C18-AQ 5µm x 4.6mm ID x 250mm
Column Temp	25°C
Mobile Phase	1mmol/L Sulphuric Acid + 8mmol/L Sodium Sulphate (pH 2.8)
Flow Rate	1mL/min
Injection Vol	10µL
UV	210nm

During development of this analytical method, varying column temperatures were measured to determine their effect on peak separation. Figure 1 shows the comparison of separation patterns of two different column temperatures; 40°C, the typical LC column operating temperature, and 25°C.

RESULTS

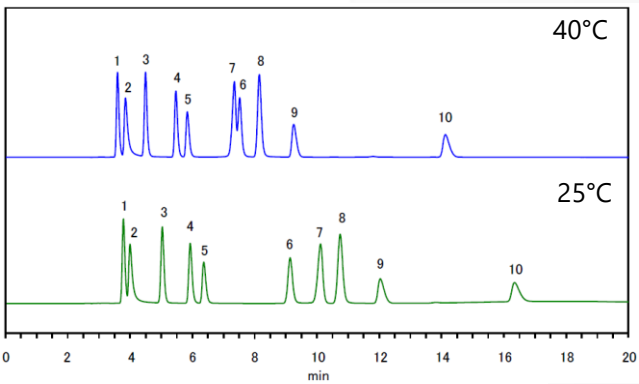

Figure 1. Comparison of separations patters; two column temperatures

As observed in Figure 1, conducting the analysis with a column temperature of 40°C provides poor separation of Citric Acid and Pyroglutamic Acid. By reducing the column temperature to 25°C, separation of all target compounds is achieved, with only an additional two minutes to the analysis time. The column temperature of 25°C was used for this application.

Calibration standards were analysed over a variety of concentration ranges, depending upon the target compound. Figure 2 shows the calibration curve for Acetic Acid and is representative of all organic acids analysed.

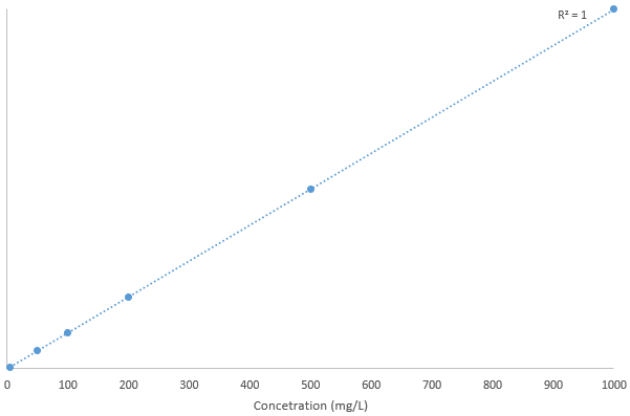

Figure 2. Calibration curve of Acetic Acid; 5mg/L to 1000mg/L

The SCION HPLC-UV system demonstrates excellent linearity for all target compounds, even over a wide concentration range. A calibration standard chromatogram can be found in Figure 3.

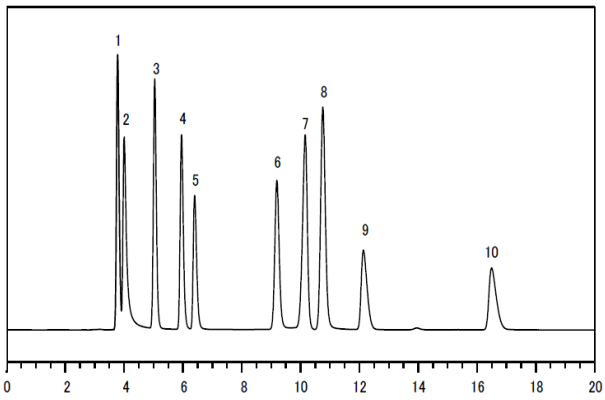

Figure 3. Chromatogram of analytical standard

Two commercially available vinegars, grain vinegar and apple cider vinegar, were analysed under the same conditions. Their respective chromatograms can be found in Figures 4 and 5.

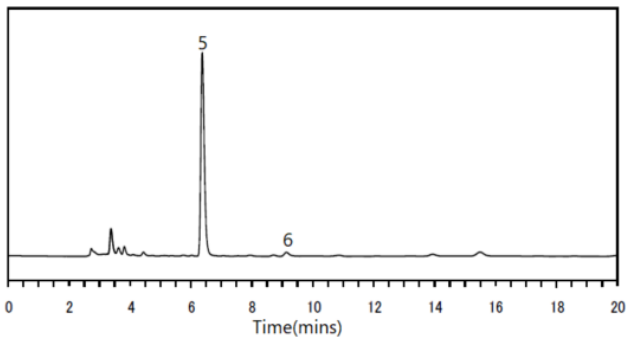

Figure 4. Chromatogram of grain vinegar sample

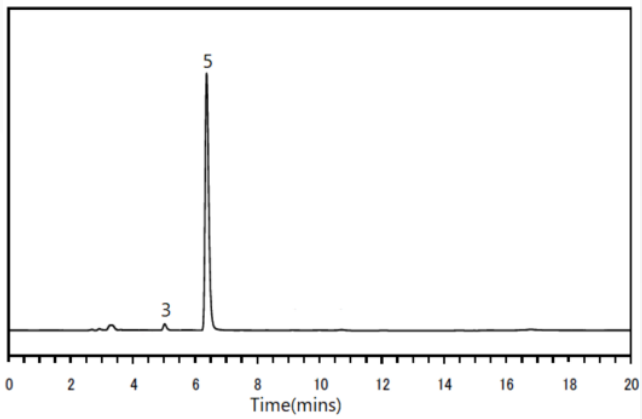

Figure 5. Chromatogram of apple cider vinegar sample

Both vinegar samples analysed only contained two organic acids. More importantly, the main ingredient found in both samples is Acetic Acid (peak 5). This identification was expected as Acetic Acid is the main ingredient in vinegar, apart from water. Malic Acid, identified in the apple cider vinegar sample, is a common food additive responsible for a sour fruity taste.

CONCLUSION

SCION Instruments developed a method for the simultaneous determination of ten organic acids by HPLC-UV. Separation of the target compounds was improved by simply lowering the column temperature. Excellent linearity was observed for all target compounds.

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