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scion INSTRUMENTS

LC6000 Application Compendium

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Analysis of Fat Soluble Vitamins by HPLC-DAD

Introduction

Vitamins are critical compounds which are essential for normal metabolism function. They are naturally found in many foods but are also often added to processed food products. Additionally, vitamin supplements are a growing trend among people whose diet is restricted. Vitamins are categorised into two groups; water soluble and fat soluble. The most common fat-soluble vitamin supplements are Vitamin A (retinol), Vitamin A Acetate (Retinol Acetate), Vitamin D_2 (Ergocalciferol), Vitamin D_3 (Cholecalciferol), Vitamin E (dl-α-tocopherol), Vitamin E Acetate (dl-α- tocopherol acetate) and Vitamin K_1 (Phylloquinone).

SCION Instruments developed a qualitative method for the simultaneous identification of seven fat-soluble vitamins. As individual vitamins are unstable, it is recommended for quantitative analysis that each vitamin components is prepared and analysed with individual analytical methods.

Experimental

A SCION 6000 HPLC with DAD was used with a C18 reverse phase column for the simultaneous identification of seven target compounds. Utilising the capability of the DAD to select multiple wavelengths it was possible to identify all seven target analytes over three different wavelengths.

Analytical standards were prepared with a range from 0.01mg/L to 10mg/L for Vitamin A, 0.1mg/L to 100mg/L for Vitamin A acetate, Vitamin D_2, Vitamin D_3, and Vitamin K_1 whilst the calibration range of Vitamin E and Vitamin E acetate was 1mg/L to L. Standard dilutions were made from stocks using methanol. Samples included a Vitamin E enriched supplement and medicated eye drops. All samples were prepared with a 1:10 methanol dilution before being passed through a 0.2µm filter. Analytical conditions for the HPLC-DAD can be found in Table 1.

Table 1. Method Parameters

Parameter	Setting
Column	C18 250mm x 4.6mm x 5µm
Column Temp	40°C
Mobile Phase	Acetonitrile: Methanol (60:40v/v)
Flow Rate	1mL/min
Injection Vol	10µm
DAD	265nm, 280nm and 325nm
Software	CompassCDS

RESULTS

Excellent linearity was observed throughout the analysis with each individual calibration curve exhibiting an R^2 of 0.9999 or greater. Figures 1 and 2 highlight the calibration curves for Vitamin A and Vitamin E.

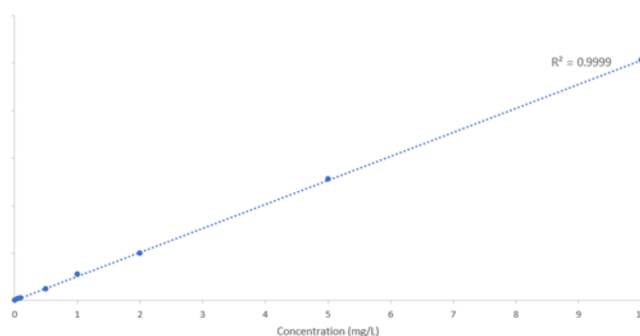

Figure 1. Calibration curve of Vitamin A

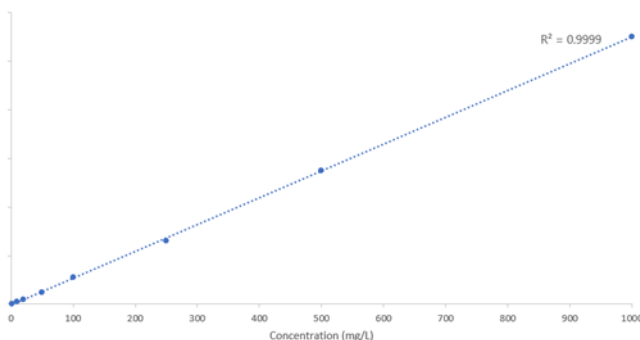

Figure 2. Calibration curve of Vitamin E

As shown in the above figures, it is possible to analyse a wide range of concentrations on the SCION HPLC-DAD without compromising on linearity. This eliminates the need for difficult sample preparation or method adjustments.

Table 2 details the peak identifiers of the seven fat-soluble vitamins analysed and the associated peaks observed in the chromatogram (Figure 3). Additionally, the varying wavelengths used for detection is also listed.

Analysis of Fat Soluble Vitamins by HPLC-DAD

RESULTS

Table 2. Vitamin, detector wavelength and peak number

Peak	Vitamin	Wavelength (nm)
1	D_2	265
2	D_3	265
3	K_1	265
4	E	280
5	E Acetate	280
6	A	325
7	A Acetate	325

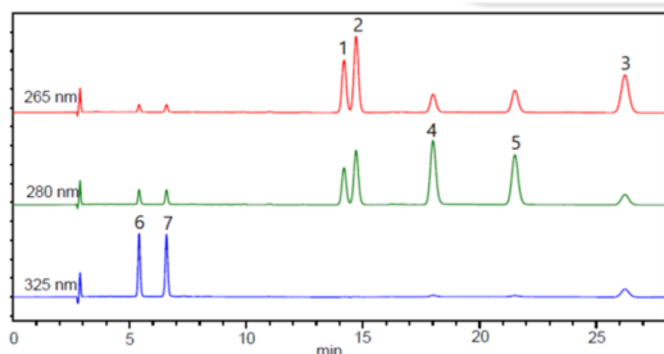

Figure 3. Chromatogram of analytical standard

Both the eye drop sample and supplement sample were analysed with peak identification further confirmed via absorbance spectrum comparisons of both sample and standard. The ability for spectrum comparisons is a vital part of the Compass CDS software offering confidence in results. Figures 4-8 detail the chromatograms of both the eye drop and supplement samples as well as example absorbance spectrum comparison for Vitamin A, Vitamin E Acetate and Vitamin E.

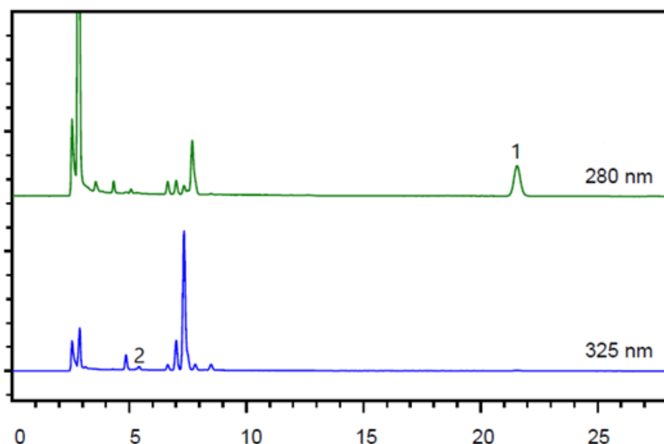

Figure 4. Chromatogram of eye drop sample

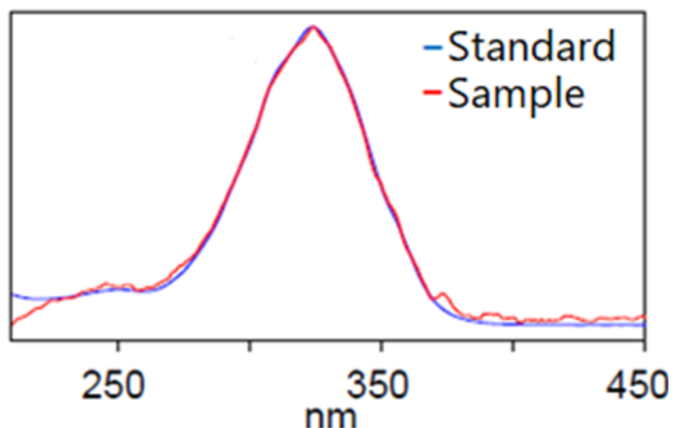

Figure 5. Absorbance spectrum comparison; Vitamin A (eye drop)

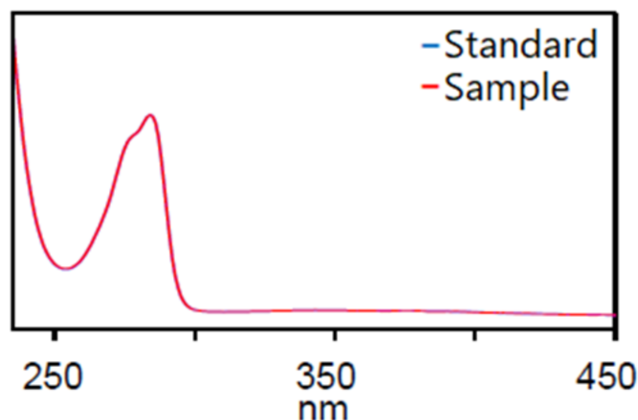

Figure 6. Absorbance spectrum comparison; Vitamin E Acetate (eye drop)

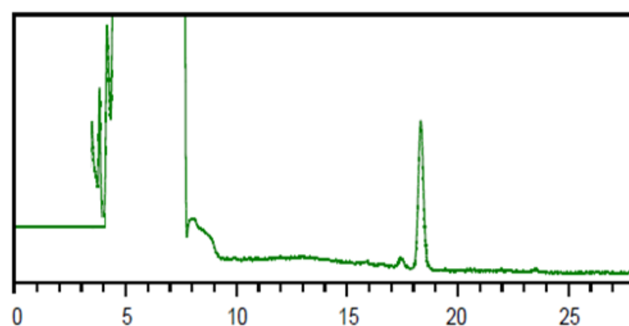

Figure 7. Chromatogram of supplement sample

Analysis of Fat Soluble Vitamins by HPLC-DAD

RESULTS

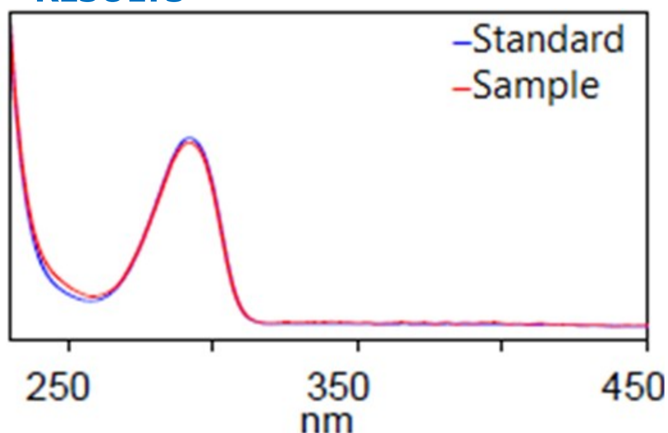

Figure 8. Absorbance spectrum comparison; Vitamin E (supplement sample)

As shown by the above figures, Vitamin A and Vitamin E acetate were detection in the eye drop sample whereas only Vitamin E was detected in the supplement sample. Each identified sample peak gave the same absorbance spectrum as that in the analytical standard, giving confidence in the confirmation of analytes.

Conclusion

Simultaneous identification of seven fat-soluble vitamins was easily achieved using the SCION HPLC with Diode Array Detection and a reverse phase C18 column. A single method utilising multiple wavelengths allowed simultaneous identification of a wide concentration range of vitamins. Compass CDS software allows easy comparison of absorbance spectra for confirmation in identification.

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Analysis of Water Soluble Vitamins by HPLC-DAD

Introduction

Vitamins are critical compounds which are essential for normal metabolism function. They are naturally found in many foods but are also often added to processed food products. Additionally, vitamin supplements are a growing trend among people whose diet is restricted. Vitamins are separated into two groups; water soluble and fat soluble. The most common water-soluble vitamin supplements are Thiamine (B_1), Pyridoxine (B_6), Cyanocobalamin (B_{12}), Riboflavin (B_2), Niacin (B_3) Nicotinamide, Ascorbic Glucoside, Vitamin C and Erythorbic Acid.

Routine analysis of water-soluble vitamins can be challenging due to the unstable nature of the target analytes. Many factors can affect vitamin stability such as exposure to heat, light, air as well as interactions with other food components. By using reverse phase high pressure liquid chromatography (HPLC) with Diode Array Detector (DAD) a qualitative method for the detection of water-soluble vitamins was easily developed. For quantitative analysis, separate HPLC methods are recommended due to Vitamin C and Erythorbic Acid instability in which decomposition regularly occurs during sample preparation.

Experimental

A SCION 6000 HPLC with DAD was used with a C18 (5µm) reverse phase column for the simultaneous identification of nine target compounds.

Analytical standards were prepared with a range from 0.1mg/L to 50mg/L in tetrabutylammonium. For the analysis of vitamins B1 and B6, hydrochloride salt was used. Samples included a vitamin enriched health drink and a nutritional supplement. Samples were diluted 1:10 before being filtered through a 0.45µm filter. Analytical conditions for the HPLC-DAD can be found in Table 1.

Table 1. Method Parameters

Parameter	Setting
Column	C18 250mm x 4.6mm x 5µm
Column Temp	40°C
Mobile Phase	Phosphate Buffer (pH5.2) : Acetonitrile (90:10v/v)
Phosphate Buffer	10mM tetrabutylammonium hydroxide 10mM monopotassium phosphate
Flow Rate	0.8mL/min
Injection Vol	10µL
DAD	260nm

RESULTS

Excellent linearity was observed with all target compounds; R^2 value was 0.996 or greater. To ensure good linearity Vitamin C, Erythorbic Acid and Vitamin B_{12} must be prepared daily, due to the instability of the vitamins in the eluent. Figure 1 shows the linearity of Vitamin B_6 which is representative of all target compounds with the exception of three above (R^2 was 0.996).

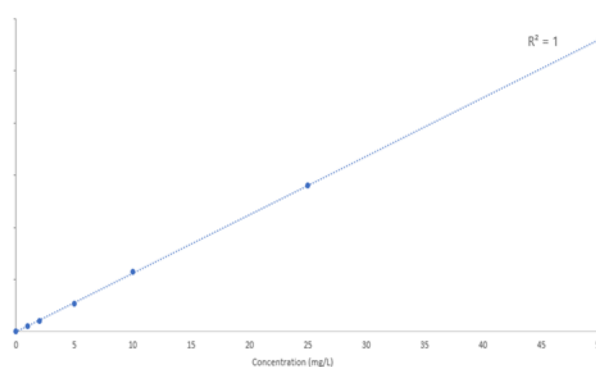

Figure 1. Calibration curve of Vitamin B6

Table 2 lists each target compound and corresponding peak number for Figure 2. Figure 2 shows the chromatogram of the 5mg/L analytical standard.

Table 2. Peak Identifiers

Peak	Vitamin
1	B_1
2	B_6
3	Nicotinamide
4	B_{12}
5	Ascorbic Glucoside
6	C
7	Erythorbic Acid
8	B_2
9	Niacin

Analysis of Water Soluble Vitamins by HPLC-DAD

RESULTS

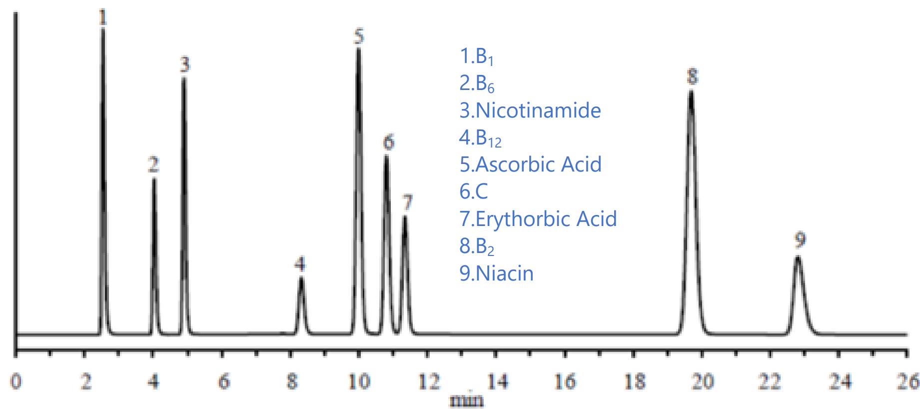

Figure 2. Chromatogram of 5mg/L analytical standard

Figure 2 highlights the excellent separation and peak shape of each target Vitamin.

Figure 3 shows the chromatogram obtained when the vitamin enriched drink sample was analysed. Additionally, Figure 4 highlights the capability of the Compass CDS software to compare the absorption spectrum of the target analyte with the spectrum of the analytical standard, providing extra confidence of the results.

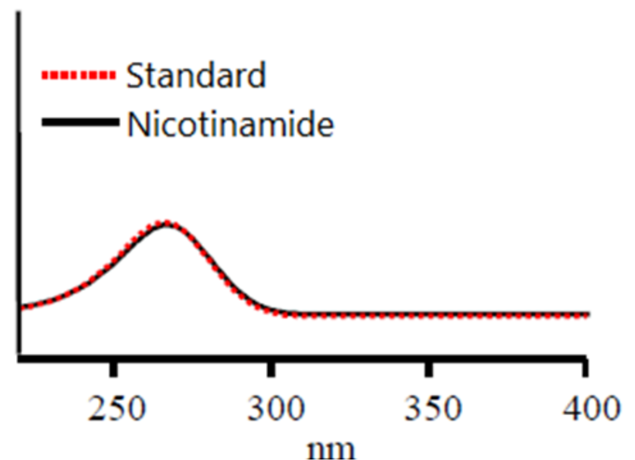

Figure 4. Absorption spectrum comparison of standard and health drink sample

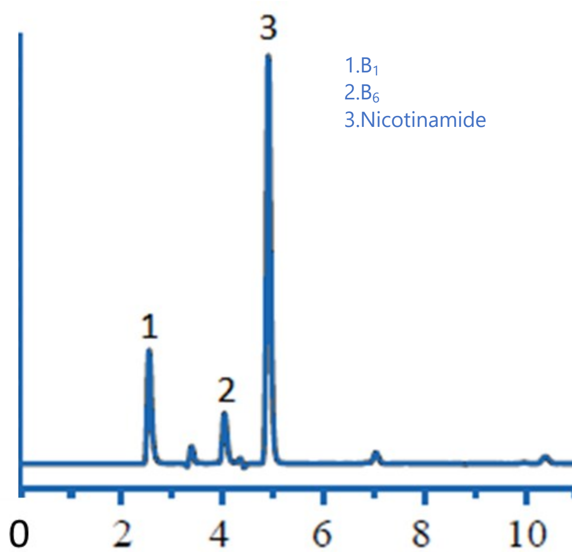

Figure 3. Chromatogram of vitamin enriched health drink

Figure 5 shows the chromatogram of the nutritional supplement sample whilst Figure 6 details the absorption spectrum comparison of Vitamin C to the analytical standard.

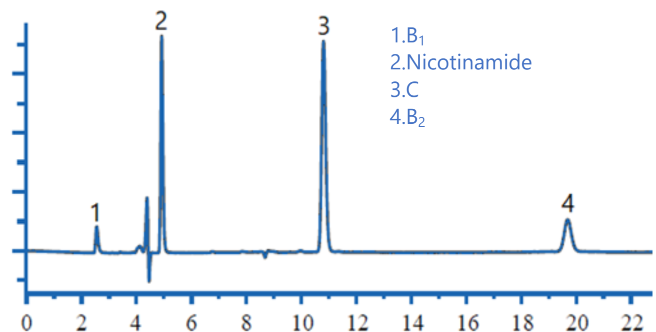

Figure 5. Chromatogram of nutritional supplement sample

Analysis of Water Soluble Vitamins by HPLC-DAD

RESULTS

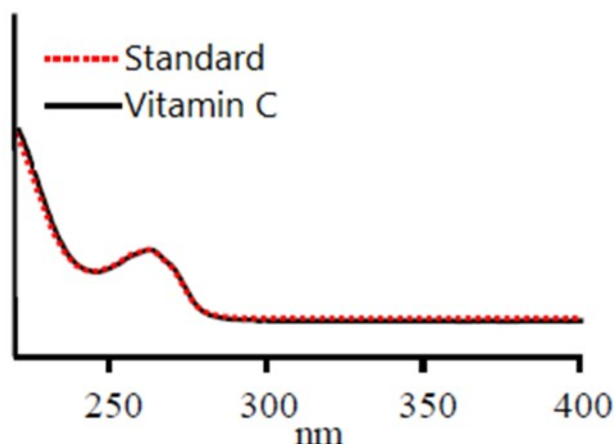

Figure 6. Absorption spectrum comparison of standard and nutritional supplement sample

The nutritional supplement sample contained one more vitamin, Vitamin C, than the vitamin enriched health drink. The only vitamins present in both samples were Vitamin B_1 and Nicotinamide. The absorbance spectrum comparisons confirm that the identified target compounds have the exact same absorbance pattern as the corresponding standard for that compound, adding an extra level of confidence in the results.

CONCLUSION

Simultaneous identification of nine water-soluble vitamins was easily achieved using the SCION HPLC with Diode Array Detection and a reverse phase C18 column. Sample preparation is a key factor that must be considered during this application due to the unstable properties of Vitamin C and Erythorbic Acid. Excellent linearity was observed, even for the unstable vitamins. Compass CDS software allows easy comparison of absorbance spectra for confirmation in identification.

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Analysis of DEHP in Drinking Water by HPLC-DAD

Introduction

DEHP, bis-(2-ethylhexyl)phthalate, is one of the most prominent phthalate contaminants in drinking water. DEHP is also used as a plasticiser during plastic manufacturing. When ingested, it is a cancer-causing hazard and presents a high risk of liver function disorders. For this reason DEHP is a banned substance during food production, under the Restriction of Hazardous Substances (RoHS) Directive by the EU[1], and levels must be monitored. Although a banned substance, commercial sport drink manufactures have been known to substitute palm oil, a common emulsifier, for the more cost effective DEHP.

SCION Instruments developed a method for the identification of DEHP using HPLC and a Diode Array Detector (DAD).

Experimental

A SCION 6000 HPLC with DAD was used with a C18 reverse phase column for the detection of DEHP in mineral water and sports drink samples. A DEHP analytical standard was prepared at a concentration range of 0.1mg/L to 100mg/L. Standard addition was also performed on two negative water and sports drink samples to demonstrate the capability of the instrument. Samples were spiked with 1ppm and 10ppm of DEHP prior to analysis. Table 1 details the analytical conditions of the HPLC-DAD system.

Table 1. Method Parameters

Parameter	Setting
Column	C18 250mm x 4.6mm x 5µm
Column Temp	30°C
Mobile Phase	Water: Acetonitrile (2:98v/v)
Flow Rate	1mL/min
Injection Vol	10µL
DAD	224nm

RESULTS

The chromatogram of a 10mg/L DEHP standard can be observed in Figure 1 whilst Figure 2 shows the calibration curve of DEHP, over a range of 0.1mg/L to 100mg/L.

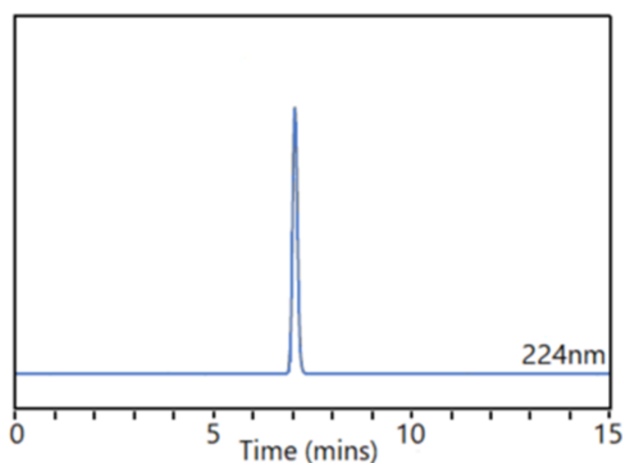

Figure 1. Identification of DEHP at 224nm (10mg/L)

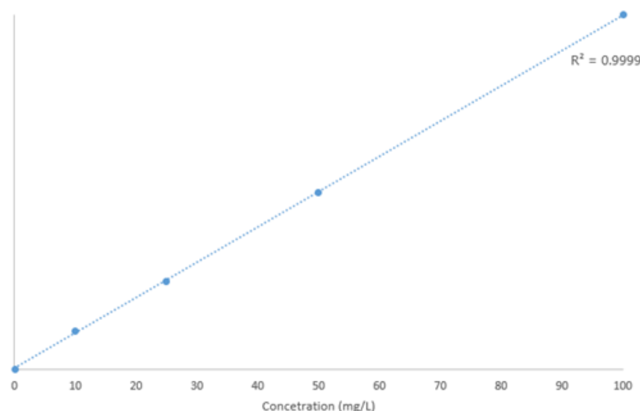

Figure 2. Calibration curve of DEHP; 0.1mg/L to 100mg/L

DEHP exhibits excellent linearity over a wide concentration range, as demonstrated in Figure 2. Retention time and peak are repeatability of the system was tested with six consecutive injections of a 10mg/L standard, the values of which can be found in Table 2.

Analysis of DEHP in Drinking Water by HPLC-DAD

RESULTS

Table 2. Repeatability values of 10mg/L DEHP (n=6)

Run	RT (min)	Peak Area
1	7.058	131486
2	7.057	131742
3	7.056	131456
4	7.057	131769
5	7.056	131069
6	7.057	131743
Mean	7.057	131634
RSD %	0.011	0.11

Excellent repeatability of both retention time and peak value was observed, with RSD% values at 0.011 and 0.11, respectively; highlighting the robustness of the SCION HPLC-DAD system.

Mineral water and a sports drink were both analysed for the presence of DEPH. However, both samples were negative. Standard addition was performed on two separate samples of each sample type with an addition of 1ppm and 10ppm DEHP.

Figures 3 and 4 show the overlay chromatograms of both samples, including the initial blank results and both DEPH additions.

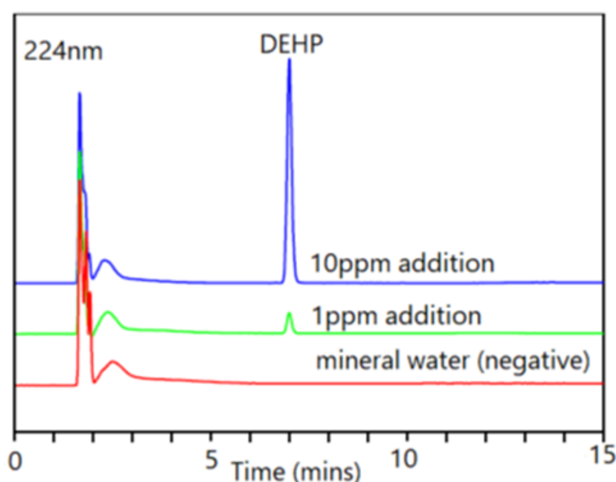

Figure 3. Chromatogram overlay of DEHP standard addition (mineral water)

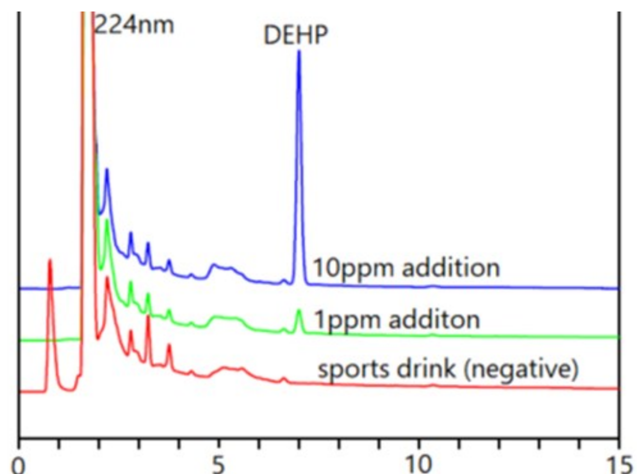

Figure 4. Chromatogram overlay of DEHP standard addition (sports drink)

The above figures demonstrate the excellent sensitivity of the HPLC-DAD system even at low concentrations such as 1ppm.

CONCLUSION

SCION Instruments offers the ideal solution for the identification of DEHP, a restricted contamination compound by HPLC-DAD. Excellent separation, linearity over a wide concentration range and system repeatability was observed for DEHP using a C18 column at 280nm wavelength.

REFERENCES

[1] European Commission (2011). *Directive 2011/65/EU of the European Parliament and of the Council as Regards the List of Restricted Substances*. European Parliament.

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Simultaneous Analysis of Food Dyes by HPLC-DAD

Introduction

Food colourant additives are common dyes used to enhance the colour and palatability of food and drink products. Dyes used during food manufacturing are divided into natural and synthetic dyes. Synthetic dyes are often added to compensate for the loss of natural colour that occurs during processing and storage of food products. Additionally, synthetic dyes offer better stability, brightness and lower cost compared to natural dyes. Due to concerns about the potential health risks from the consumption of artificial food dyes, synthetic colourants are subject to regulation.

Global regulations can vary as to which dyes are allowed, specific foods they can be used in and regulatory limits. For example, the Food and Drug Administration (US) allows the use of Sunset Yellow, Brilliant Blue, Indigo Carmine and Erythrosine[1].

SCION Instruments developed a HPLC method for the simultaneous identification of six synthetic dyes at varying wavelengths. Utilising the Diode Array Detector, it was possible to extract the optimal wavelength for each target compound.

Experimental

A SCION 6000 HPLC with DAD was used with a C18 reverse phase column for the simultaneous detection of six synthetic food dyes. The identification of each target compound. Abbreviation used throughout this application note and the extracted wavelength of each compound can be found in Table 1.

Table 1. Target compounds, abbreviations and extracted wavelength (nm)

Compound	Abbreviation	Wavelength (nm)
Sunset Yellow	SY	480
Amaranth	AM	530
Erythrosine	ER	530
Acid Red 52	AR	530
Indigo Carmine	IC	620
Brilliant Blue	BB	620

The DAD was additionally set to 254nm, the primary wavelength. Calibration standards were prepared at a range of 0.5mg/L to 50mg/L for each target compound.

Table 2 details the analytical conditions for this analysis.

Table 2. Analytical Conditions of HPLC-DAD

Parameter	Setting
Column	C18 150mm x 4.6mm x 3µm
Column Temp	10°C
Mobile Phase	A.10mmol/L ammonium acetate: acetontrile(95:5) B.10mmol/L ammonium acetate: acetontrile(50:50)
Flow Rate	1mL/min
Injection Vol	10µL
DAD	224nm

Calibration standards were analysed over a concentration range of 0.5mg/L to 50mg/L.

RESULTS

Figures 1 and 2 show the calibration curve of two target compounds, Brilliant Blue (BB) and Acid Red 52 (AR), which are representative of all target compounds analysed.

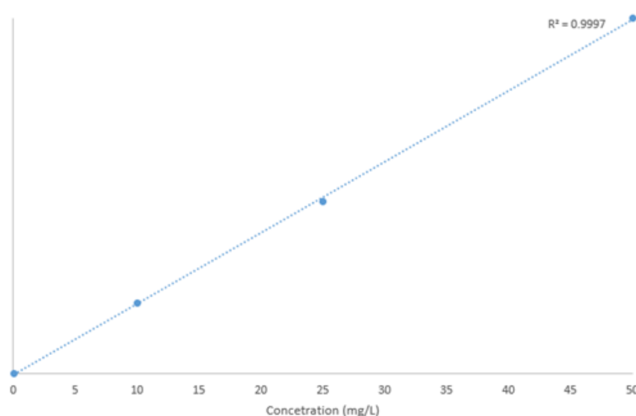

Figure 1. Calibration curve of BB; 0.5mg/L to 50mg/L

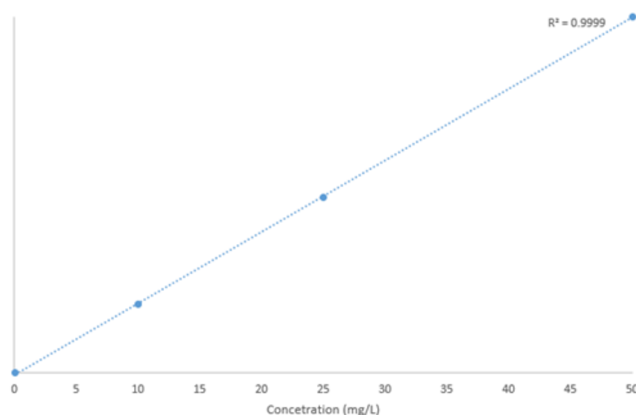

Figure 2. Calibration curve of AR; 0.5mg/L to 50mg/L

Simultaneous Analysis of Food Dyes by HPLC-DAD

RESULTS

All target compounds exhibited high linearity with an R^2 value >0.999, over the wide linear range.

Along with the set wavelength of 254nm, varying wavelengths were extracted as the optimal wavelength for each food dye analysed. Figure 3 highlights the overlay chromatogram of all wavelengths used throughout this application.

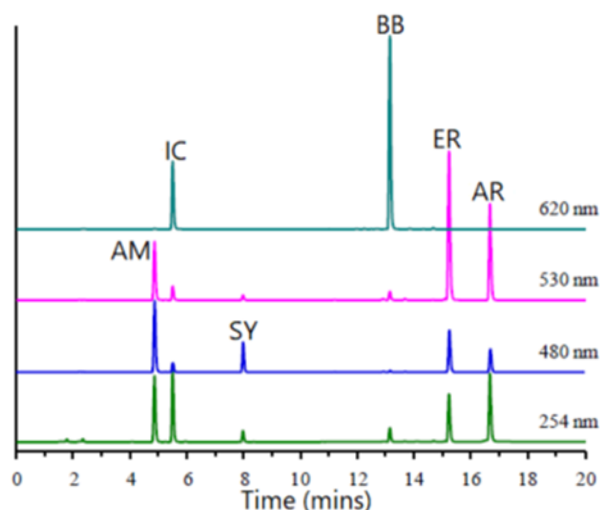

Figure 3. Overlay chromatogram of target analytes (extracted wavelengths)

When analysed at 254nm, all target compounds can be identified. However, all target compounds have different maximum UV absorption wavelengths which were extracted for peak identification. Utilising the extracted wavelength feature of the DAD allows for a simultaneous analysis rather than individual methods for different compounds.

CONCLUSION

SCION Instruments offers the ideal solution for the identification of six simultaneous food dyes by HPLC-DAD. By utilising the extracted wavelength mechanism of the DAD, it is possible to use the optimal absorption wavelengths for each individual compound, eliminating the need for individual methods.

REFERENCES

[1] US Food and Drug Administration (2018). *Federal Food, Drug and Cosmetic Act.* Section 379e.

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Analysis of 2,4-DNPH Derivatised Aldehydes by HPLC-DAD

Introduction

Aldehydes are important compounds regularly use in the chemical industry. Sick house syndrome is a medical condition caused by poor air quality in enclosed indoor spaces and the presence of specific volatile organic compounds (VOCs) such as formaldehyde. It is vital that the level of formaldehyde and associated compounds are regularly measured and controlled especially in working environments in which formaldehyde is handled.

SCION Instruments developed a method for the simultaneous analysis of seven DNPH (2,4-Dinitrophenylhydrazine) derivatised aldehydes plus one derivatised ketone.

Experimental

A SCION 6000 HPLC with DAD was used with a C18 reverse phase column for the simultaneous identification of eight target compounds. An analytical standard containing all target analytes was analysed to demonstrate identification and separation of all compounds.

An ambient air sample was collected in a trap tube filled with silica gel which contained 2,4-DNPH. The aldehydes and ketone present in the ambient air were collected on the trap and derivatised before being eluted with acetonitrile and analysed.

Table 1 details the analytical conditions of the HPLC-DAD.

Table 1. Method Parameters

Parameter	Setting
Column	C18 150mm x 4.6mm x 5µm
Column Temp	40°C
Mobile Phase	Acetonitrile: Methanol (60:40v/v)
Flow Rate	1mL/min
Injection Vol	10µL
DAD	360nm

RESULTS

Figure 1 shows the chromatogram of all target compounds analysed at 0.5mg/L, along with peak identifiers.

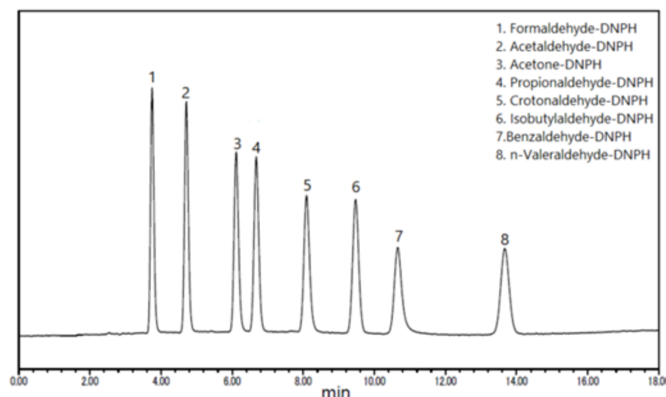

Figure 1. 7 DNPH aldehydes and 1 DNPH ketone (0.5mg/L)

As shown in Figure 1, all target analytes were completely resolved from each other in under 15 minutes.

Limit of detection studies were completed for Formaldehyde and Acetaldehyde. The limited of detection for Formaldehyde in ambient air was 0.02µg/m^3 and 0.1µg/m^3 for Acetaldehyde, respectively.

CONCLUSION

SCION Instruments offers an easy solution for the simultaneous identification of seven DNPH derivitised acetaldehydes and one DNPH derivitised ketone using the SCION 6000 HPLC-DAD system. Complete separation and low detection limits were observed when ambient air samples were analysed.

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Analysis of Glycosides in Medicines by HPLC-DAD

Introduction

Glycosides are molecules in which a sugar is bound to another functional group via a glycosidic bond. Glycosides are mainly O-glycosides, sugar derivatives with various physiological activities that are widely distributed in plants. Additionally, glycosides are widely used as components of unrefined/ herbal medications, such as ginseng or Senna.

SCION Instruments developed a qualitative and quantitative method for the analysis of glycosides in medicines by HPLC-Diode Array Detection (DAD). Confirmation is further certified through the comparison of the absorbance spectrum of both analytical standard and sample analysed.

Experimental

A SCION 6000 HPLC with DAD was used with a C18 reverse phase column for the identification of six common glycosides. Samples included powdered Senna Leaf and a gastrointestinal medicine. Samples were prepared in 50% methanol before being filtered and analysed. Table 1 details the analytical conditions of the HPLC-DAD system.

Figure 1 shows the chromatogram of all target compounds analysed at 0.5mg/L, along with peak identifiers.

Table 1. Method Parameters

Parameter	Setting
Column	C18 150mm x 4.6mm x 3µm
Column Temp	40°C
Mobile Phase	A.10mmol/L monopotassium hydroxide(pH3.0) B.Acetonitrile
Gradient	0 min B 10%, 20 mins B 30%, 25-35 mins B 70%, 35.1mins B 10%
Flow Rate	1mL/min
Injection Vol	50µL
DAD	245nm

Analytical standards were prepared in a concentration range of 0.1mg/L to 100mg/L apart from Puerarin which had a concentration range of 0.1mg/L to 50mg/L.

RESULTS

Figure 1 shows the chromatogram of the 50mg/L calibration mixture, containing six target compounds. Compound identification is also detailed.

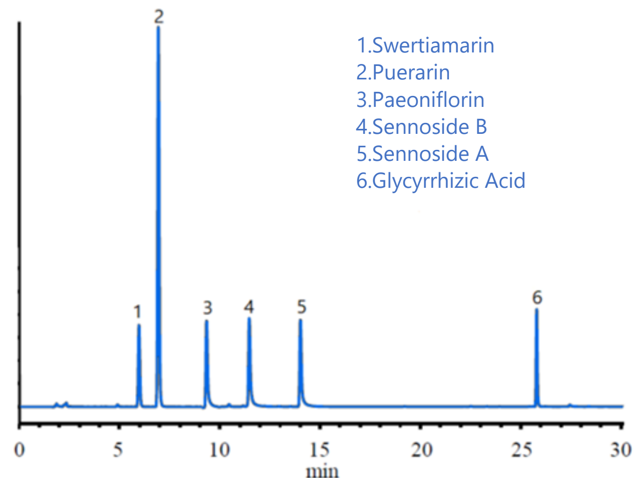

Figure 1. Analytical standard chromatogram (50mg/L)

All target analytes were completely resolved from each other. Figure 2 shows the calibration curve for Sennoside A, which is representative of all target glycosides analysed.

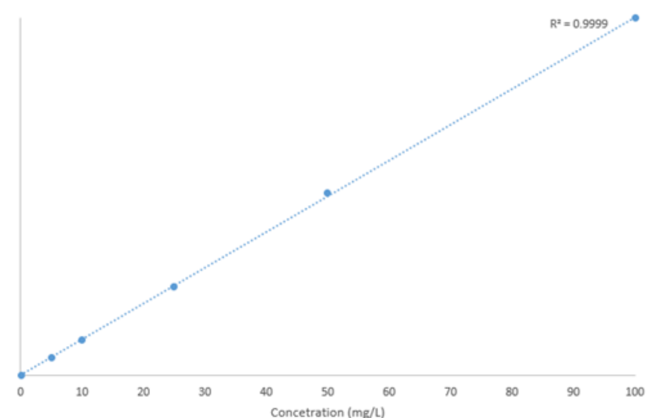

Figure 2. Calibration curve of Sennoside A (0.1mg/L to 100mg/L)

The linearity of all six target glycosides was excellent with an average RSD% of 0.9999, demonstrating the robustness of the LC system. Both samples; the powdered Senna Leaf and the gastrointestinal medicine, were analysed with sample identification confirmed by both retention time matching and comparison of the absorbance spectra of the analytical standards.

Analysis of Glycosides in Medicines by HPLC-DAD

RESULTS

Figures 3-8 show the sample chromatograms as well as the absorbance spectra comparisons, completed in CompassCDS.

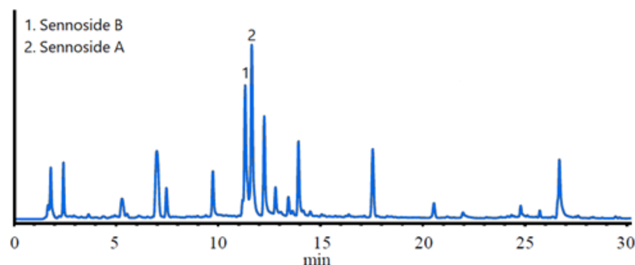

Figure 3. Chromatogram and peak identification of Senna Leaf sample

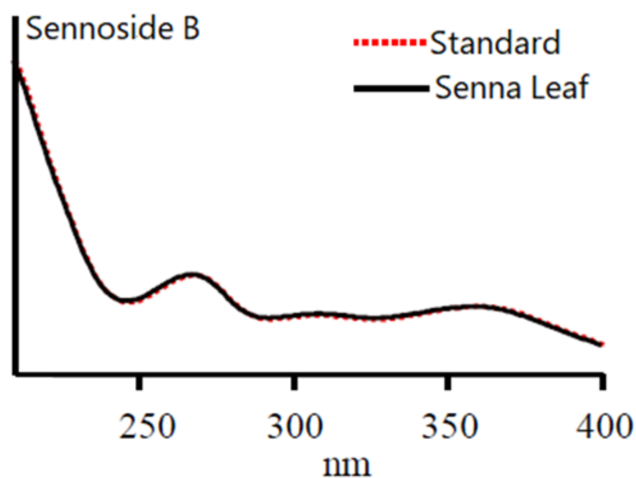

Figure 4. Absorbance spectrum comparison Sennoside B (Senna Leaf)

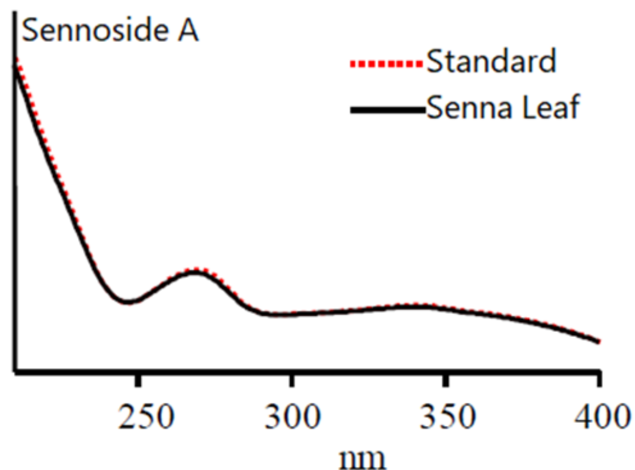

Figure 5. Absorbance spectrum comparison Sennoside A (Senna Leaf)

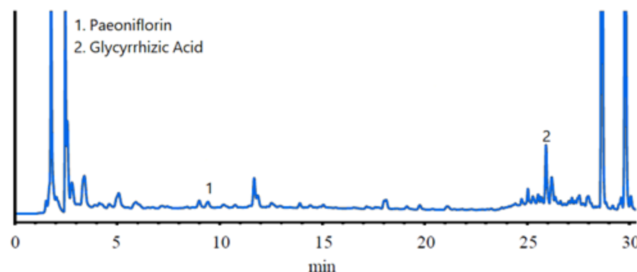

Figure 6. Chromatogram of gastrointestinal medicine

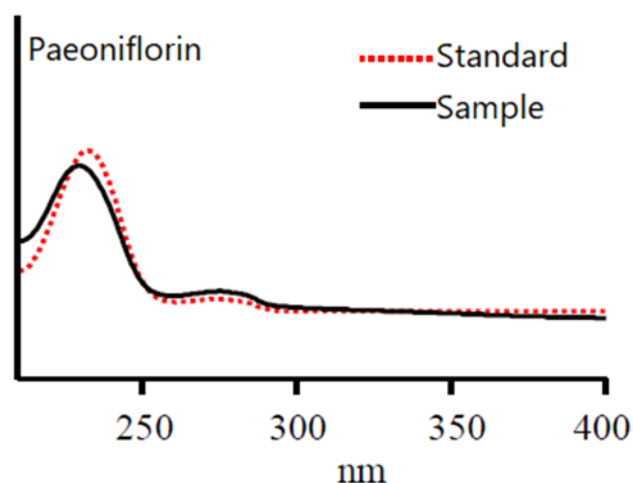

Figure 7. Absorbance spectrum comparison Paeoniflorin (medicine)

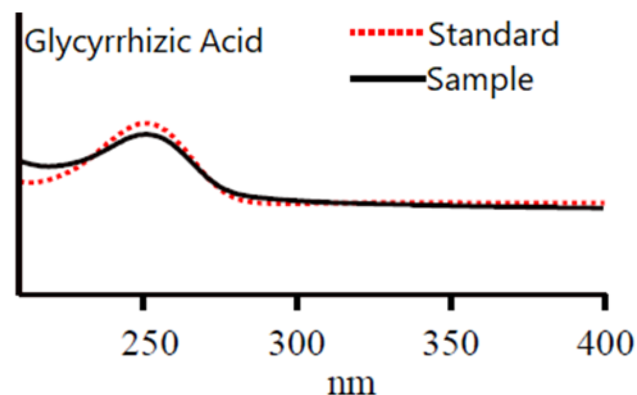

Figure 8. Absorbance spectrum comparison Glycyrrhizic Acid (medicine)

The two samples analysed contained four of the six glycosides commonly found in herbal medicines. Using the absorbance spectrum function of the CompassCDS software, an additional form of peak identification/ confirmation was provided.

Analysis of Glycosides in Medicines by HPLC-DAD

CONCLUSION

SCION Instruments developed a method for the identification of six glycosides by HPLC-DAD. Excellent linearity was observed for all target compounds with additional confidence in peak identification through absorbance spectrum comparison in CompassCDS.

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Analysis of Catechins by HPLC-UV

INTRODUCTION

Hot beverages are one of the most widely consumed drinks worldwide, with tea becoming increasingly more popular. Camellia Sinensis, the leaves used during the production of tea. Studies have shown that tea provides several health benefits such as protecting against cardiovascular disease and the management of cholesterol and obesity. The main antioxidants found in tea are catechins.

The composition of catechins in commercial teas vary due to the species of Camellia Sinensis used, horticultural conditions but most importantly, the degree of oxidation during the manufacturing process. Natural processes such as sun drying or steaming the leaves, therefore preventing oxidation, not only protects the tea flavour but also results in high catechin concentrations with lower caffeine amounts whereas harsh leaf processing results in lower catechins and higher caffeine concentrations. Due to the variability in the composition of catechins in tea, it is vital that catechins can be easily identified in a variety of tea products.

SCION Instruments developed a method for the identification of eight catechins commonly found in tea products as well as caffeine, by HPLC-UV.

EXPERIEMENTAL

A SCION 6000 HPLC with UV was used with a C18 reverse phase column for the simultaneous identification of nine target compounds. An analytical standard containing all target analytes was analysed to demonstrate identification and separation of all compounds. Samples included commercially available bottled green tea and a green tea catechin supplement. Table 1 details the analytical conditions of the HPLC-DAD.

Table 1. Method Parameters

Parameter	Setting
Column	C18 150mm x 4.6mm x 5µm
Column Temp	40°C
Mobile Phase	A.0.05% Phosphoric Acid pH 2.4 B.Methanol: Acetonitrile (3:2)
Gradient	0 min B 10%, 15 mins B25%, 25 mins B60%
Flow Rate	1mL/min
Injection Vol	10µL
UV	280nm

RESULTS

Table 2 identifies all target compounds and associated abbreviations used throughout this application note.

Table 2. Target analytes and abbreviations

Compound	Abbreviation
Gallocatechin	GC
Gallocatechin Gallate	GCG
Epigallocatechin	EGC
Epigallocatechin Gallate	EGCG
Epicatechin	EC
Epicatechin Gallate	ECG
Catechin	C
Catechin Gallate	CG
Caffeine	CA

All target compounds were analysed in a concentration range of 1-50mg/L with the exception of caffeine (1-200mg/L). All target compounds gave a correlation coefficient of >0.999. Figures 1 and 2 show the calibration curve of EGC and CA, the two compounds with the lowest and highest R^2 value.

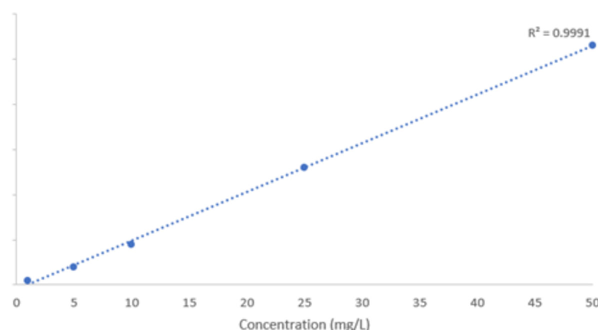

Figure 1. Calibration curve of epigallocatechin (EGC) 1-50mg/L

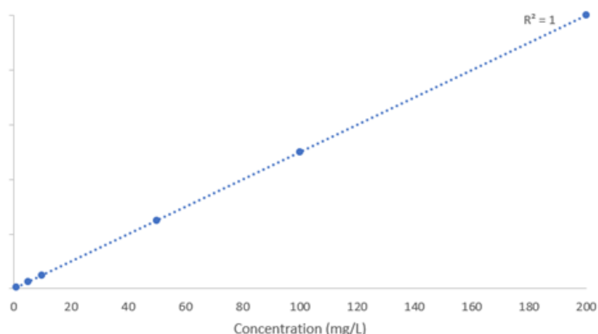

Figure 2. Calibration curve of caffeine (CA) 1-200mg/L

Analysis of Catechins by HPLC-UV

RESULTS

Following the excellent linearity observed of all target compounds, both the green tea and green tea supplement were analysed. Figures 3 and 4 show the obtained chromatograms including peak identification.

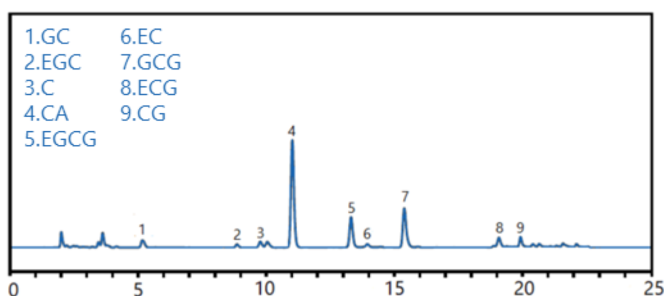

Figure 3. Chromatogram and compound identification of green tea sample

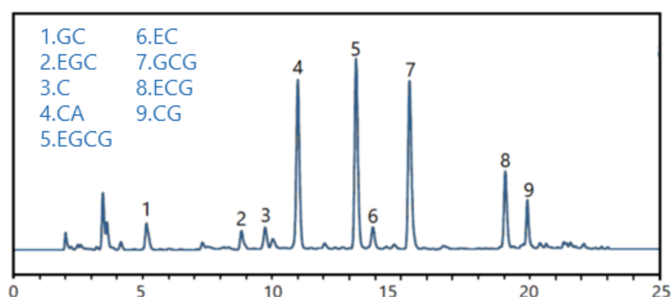

Figure 4. Chromatogram and compound identification of the supplement

As shown in the above figures, the green tea and the green tea supplement exhibit the same catechin profile but at varying concentrations. The naturally derived green tea drink gave considerably lower catechin and caffeine concentrations compared to the supplement sample, which exhibited high levels of CA, EGCG and GCG.

CONCLUSION

SCION Instruments offers an easy solution for the simultaneous identification of eight target catechins plus caffeine using the SCION 6000 HPLC-UV system. Excellent separation and linearity was observed for all target compounds using a C18 column at 280nm wavelength.

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Highly Sensitive Analysis of Organic Acids by HPLC-UV

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 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 they 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 for 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 range

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

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

Parameter	Setting
Column	C18-AQ 250mm x 4.6mm x 5µm
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.

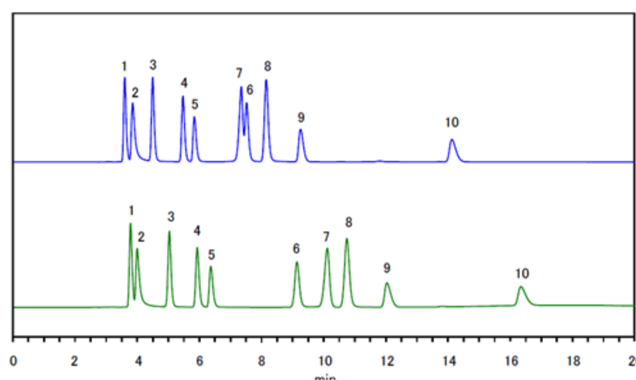

Figure 1. Comparison of separations patters; two column temperatures

RESULTS

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.

Highly Sensitive Analysis of Organic Acids by HPLC-UV

RESULTS

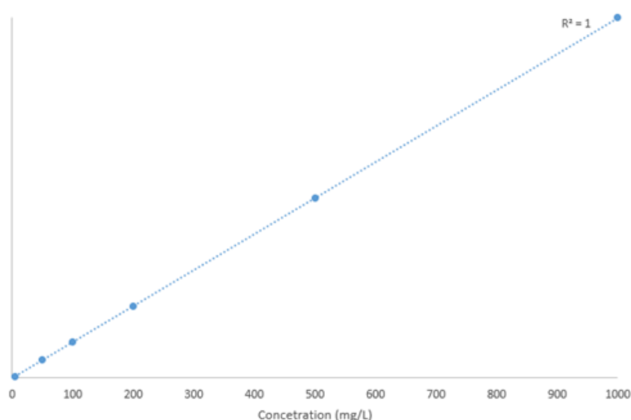

Figure 2. Calibration curve of Acetic Acid; 5mg/L to 1000mg/L

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.

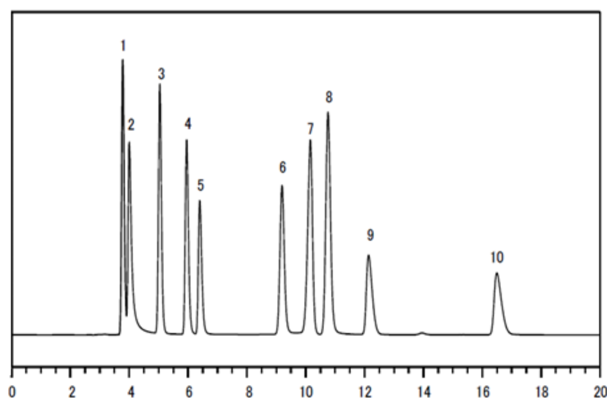

Figure 3. Chromatogram of analytical standard

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.

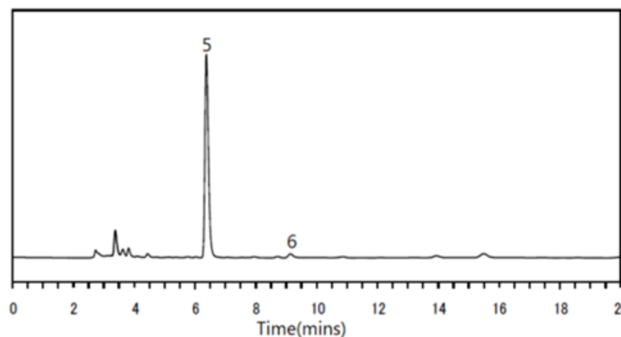

Figure 4. Chromatogram of grain vinegar sample

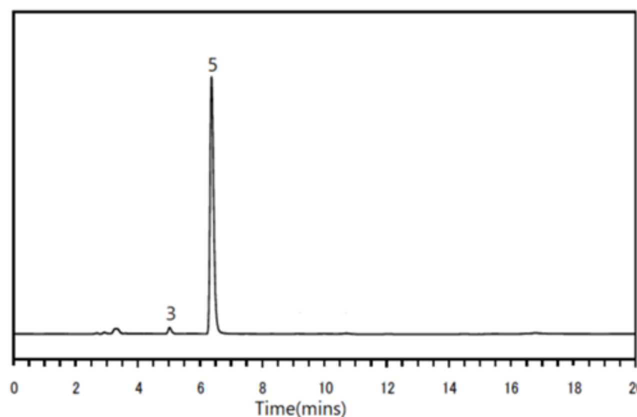

Figure 5. Chromatogram of apple cider vinegar sample

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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Simultaneous Analysis of Four Agriculture Chemical Compounds

INTRODUCTION

The use of herbicides and fungicides are becoming increasingly challenging to both the environment and drinking water supplies. The highly toxic chemicals are easily leached from plants/soils into drinking water systems. Due to the health implications of such chemicals, it is vital that the amount used and exposure to humans are regulated. In particular, Iprodione, Asulam, Thiophanate-methyl and Siduron are four toxic agriculture chemicals that are targeted in water quality management programs globally.

Typically requiring the use of LC-MS for low level detection, Iprodione, Asulam, Thiophanate-methyl and Siduron are challenging to analyse. SCION Instruments developed a method for the simultaneous identification and quantification of all four target compounds using their LC6000, High Performance Liquid Chromatograph (HPLC) with Diode Array Detector (DAD).

EXPERIMENTAL

HPLC Grade water was spiked with the four target compounds. Solid Phase Extraction (SPE) was then used to extract Iprodine, Asulam, Thiophanate-methyl and Siduron from the water before being analysed on the LC6000.

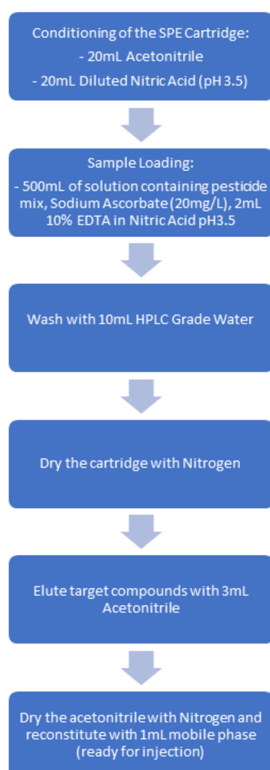

Figure 1. SPE sample preparation procedure

Calibration standards, of each target analyte, were prepared at a range of 0.2mg/L to 20mg/L in acetonitrile. The analytical conditions of the analysis can be found in Table 1.

Table 1. Analytical Conditions of HPLC-DAD

Parameter	Setting
Column	C18 250mm x 4.6mm x 5µm
Column Temp	40°C
Mobile Phase	50mM potassium dihydrogen phosphate pH3.0 Acetonitrile (45:55)
Flow Rate	1mL/min
Injection Vol	10µL
DAD	230nm, 270nm

RESULTS

The calibration curve of Asulam can be found in Figure 2 and is representative of all target compounds. The R^2 value of Asulam was 0.9998 whilst the remaining three target analytes exhibited linearity of 0.9999.

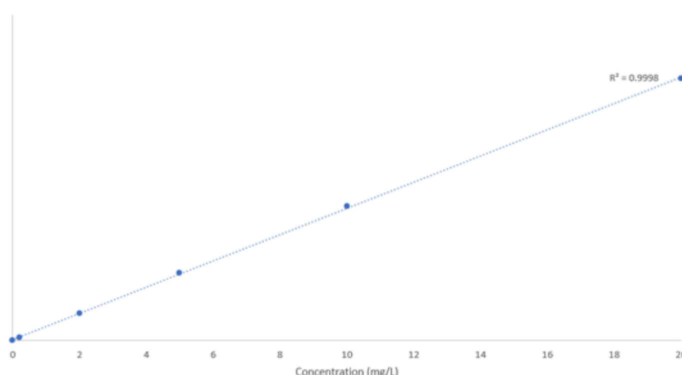

Figure 2. Calibration curve of Asulam

During the analysis, the DAD was set to monitor at two different wavelengths; 230nm and 270nm. 230nm is the optimum absorbance wavelength for Iprodione, Siduron and Thiophanate-methyl whereas the optimum absorbance wavelength for Asulam is 270nm. The multi-wavelength monitoring of the SCION 6430 DAD enables simultaneous detection of all four target compounds, in a single injection.

Figure 3 shows the combined chromatograms of the separation and identification of all four target compounds, at both wavelengths.

Simultaneous Analysis of Four Agriculture Chemical Compounds

RESULTS

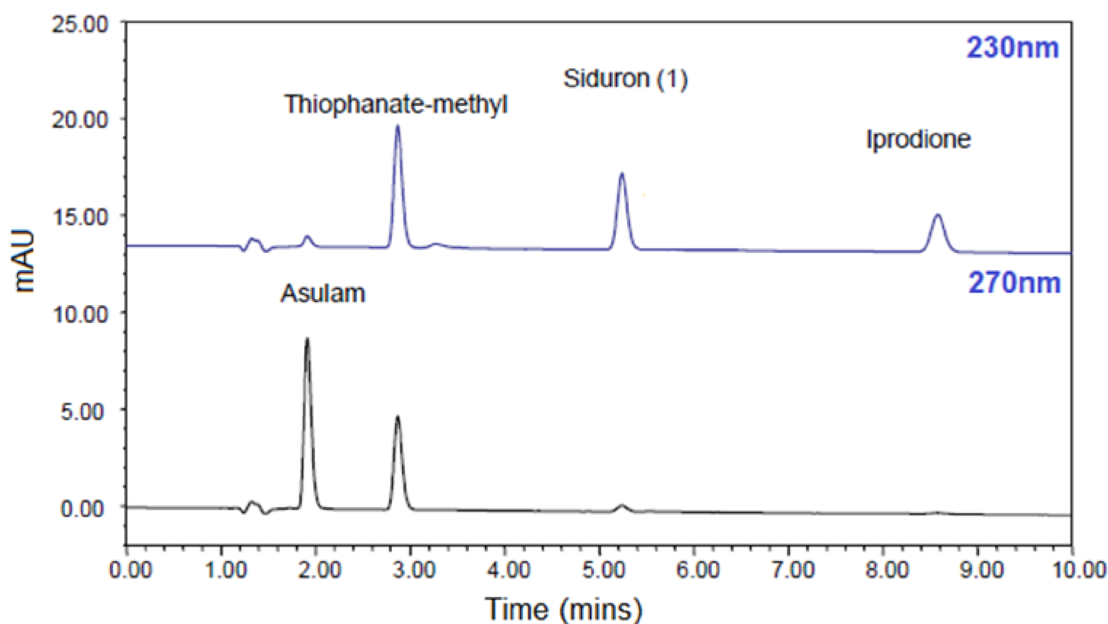

Figure 3. Combined chromatogram of all target compounds at 230nm and 270nm

CONCLUSION

SCION Instruments successfully developed a method for the determination of four herbicides/ fungicides that typically require LC-MS for analysis. Utilising the multiple wavelength monitoring of the highly sensitive Diode Array Detector, it was possible to identify and quantify Iprodine, Asulam, Thiophanate-methyl and Siduron in a single injection. With excellent linearity over a range from 0.2mg/L to 20mg/L, the LC6000 with Diode Array Detector, is the ideal solution for agricultural analysis.

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Analysis of Rare Sugars using HILIC mode HPLC

INTRODUCTION

Naturally occurring in low quantities, Rare Sugars are produced using enzymatic processes. Rare Sugars are often used in low fat and low calorie food products as an alternative to high calorie sugars. Additionally, rare sugars are known to slow down the blood sugar level elevation after digestion which inhibit the accumulation of visceral fat. The use of rare sugars, in this instance, are increasingly popular in commercial food products.

SCION Instruments developed a method for the detection and complete separation of L-Ribose, D-Psicose, Xylitol, D-Tagatose, D-Allose and L-Glucose, using Hydrophilic Interaction Liquid Chromatography (HILIC). HILIC is a type of chromatography that utilises a polar stationary phase in conjunction with a mobile phase containing both water and a higher proportion of a less polar solvent, such as acetonitrile.

A specialist HILICpak VG-50 analytical column was used, alongside method optimisation for the complete separation of the rare sugars. The VG-50 series of columns have a polymer based amino stationary phase specifically designed for saccharide separation. The packing material is a polyvinyl alcohol base, with a hydrophilic functional group; modified tertiary amine. Method optimisation showcasing how eluent composition affects separation of the sugars is also demonstrated.

EXPERIMENTAL

A SCION LC6000 equipped with 6450 RI Detector was used to analyse six Rare Sugars. Calibration standards were prepared in water: acetonitrile (1:1) at a concentration range of 0.01% to 0.5%.

Three different eluent compositions were tested with eluent optimisation critical for successful separation. Table 1 details the method parameters, including the different eluent compositions used for each analysis.

Table 1. Analytical Conditions of HPLC-RI

Parameter	Setting
Column	HILICpak VG-50 250mm x 4.6mm x 5µm
Eluent 1	Water/Acetonitrile 20:80
Eluent 2	Water/Acetonitrile 15:85
Eluent 3	Water/Acetonitrile/ Methanol 5:75:20
Detector	RI

RESULTS

Figure 1 shows the chromatogram obtained when the 0.5% rare sugar calibration standard was analysed using eluent 1; water/acetonitrile at 20:80 composition.

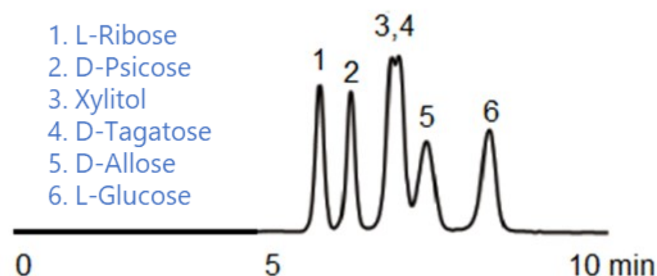

Figure 1. Rare sugar analysis; eluent 20:80 H_2O: ACN

As shown in Figure 1, when the eluent is set at 20:80 water/acetonitrile, the Xylitol and D-Tagatose are not separated, as highlighted by split peaks 3 and 4.

The same calibration standard (0.5%) was then analysed with eluent 2; water/acetonitrile at a composition of 15:85. The concentration of acetonitrile was increased to separate the Xylitol from the D-Tagatose. The chromatogram obtained from this analysis can be found in Figure 2.

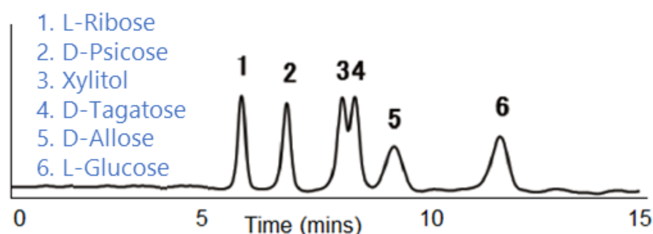

Figure 2. Rare sugar analysis; eluent 15:85 H_2O: ACN

Analysis of Rare Sugars using HILIC mode HPLC

RESULTS

By increasing the ratio of acetonitrile to water, the separation of Xylitol and D-Tagatose only slightly improved. Full separation of all six rare sugars was not achieved.

Further adjustment of the mobile phase was made, to include methanol, a stronger solvent. The final composition was water/acetonitrile and methanol at concentrations of 5:75:20%. Figure 3 shows the chromatogram obtained using this composition.

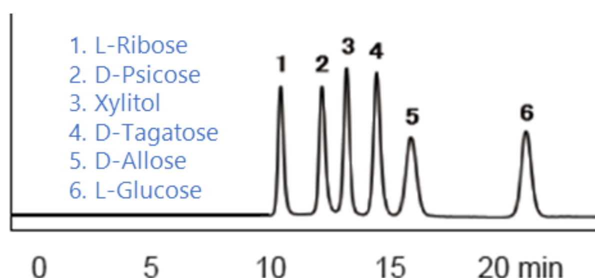

Figure 3. Rare sugar analysis; eluent 5:75:20

The additional of methanol to the eluent resulted in successful separation of all six target compounds. As excellent separation was achieved, calibration curves for each target analyte were generated. Figure 4 shows the calibration curve of Ribose.

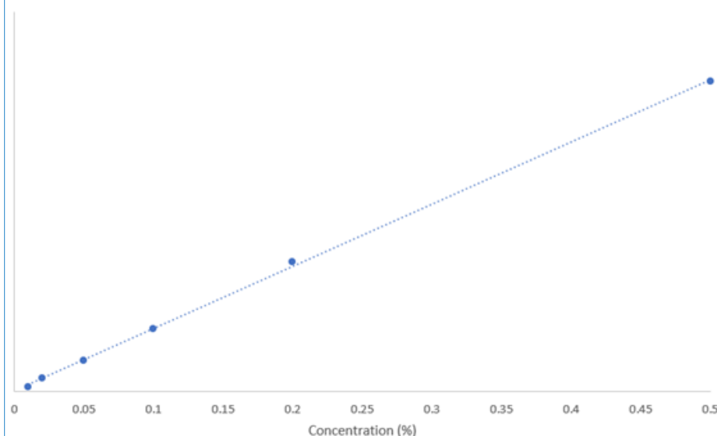

Figure 4. Calibration curve of Ribose

The R^2 value of the Ribose calibration curve was 0.9995. All remaining target compounds had a linearity of 0.9999 with the exception of Psicose which had an R^2 value of 0.9998.

To examine the feasibility of the method with true samples, the same method (with eluent 3 composition) was use to analyse a commercially available syrup containing rare sugars. Reference standards also included Fructose and Galactose.

0.1g of syrup was added to a test tube containing 2.5mL ultra pure water. Once the syrup was completely dissolved, 2.5mL of HPLC grade acetonitrile was added. After gentle shaking, the mixture was filtered through a 0.45µm membrane. A 5µL aliquot was then injected into the LC6000. Figure 5 shows the chromatogram obtained from this analysis.

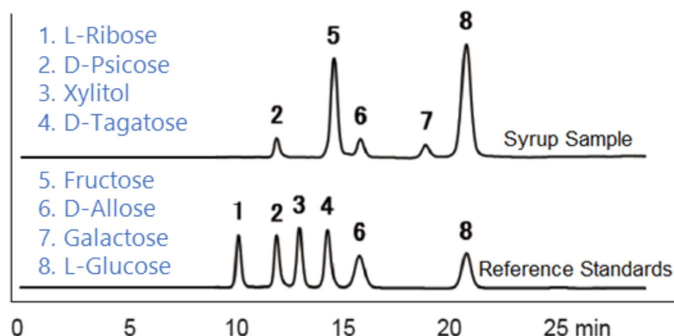

Figure 5. Chromatogram of sugar syrup

By comparing the two above chromatograms, successful separation and identification of rare sugars was possible, using the eluent 3 LC method.

CONCLUSION

Method development, specifically mobile phase optimisation is critical to any HPLC application. By adjusting not only the ratio of solvents but the strength of the solvents used in mobile phase choice, SCION Instruments demonstrated how small changes of mobile phase composition can result in significant chromatography improvements.

SCION Instruments developed a method for the analysis of rare sugars using Hydrophilic Interaction Liquid Chromatography, on the LC6000 equipped with RI detector. Excellent linearity and separation was observed for all target compounds.

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SCION INSTRUMENTS

Analysis of Functional Sugars using HILIC mode HPLC

INTRODUCTION

There has been an increasing demand for control on the amount and quality of functional sugars in dairy products world wide. Lactulose is a functional sugar found in dairy products and is found to regulate intestinal health when consumed in small amounts.

The chemical structure of Lactulose, Lactose and Epilactose are very similar, resulting in chromatographic separation difficult.

SCION Instruments developed a method for the detection and complete separation of Lactulose, Lactose and Epilactose using Hydrophilic Interaction Liquid Chromatography (HILIC). HILIC is a type of chromatography that utilises a polar stationary phase in conjunction with a mobile phase containing both water and a higher proportion of a less polar solvent, such as acetonitrile.

A specialist HILICpak VG-50 analytical column was used, alongside method optimisation for the complete separation of the functional sugars. The VG-50 series of columns have a polymer based amino stationary phase specifically designed for saccharide separation. The packing material is a polyvinyl alcohol base, with a hydrophilic functional group; modified tertiary amine.

EXPERIMENTAL

A SCION Instruments LC6000 with RI detector was used for the simultaneous determination of Lactulose, Lactose and Epilactose.

Calibration standards of containing each target analyte were prepared from a range of 0.01% to 0.5%, in water/acetonitrile (1:1). Table 1 details the method parameters of the LC6000.

Table 1. Analytical Conditions of HPLC-RI

Parameter	Setting
Column	HILICpak VG-50 250mm x 4.6mm x 5µm
Temperature	40°C
Eluent	Water/Acetonitrile/Methanol 5:75:20
Flow Rate	1mL/min
Detector	RI

RESULTS

Figures 1a, 1b and 1c show the calibration curve of all target compounds over a range of 0.01% to 0.05%. All target compounds exhibited excellent linearity.

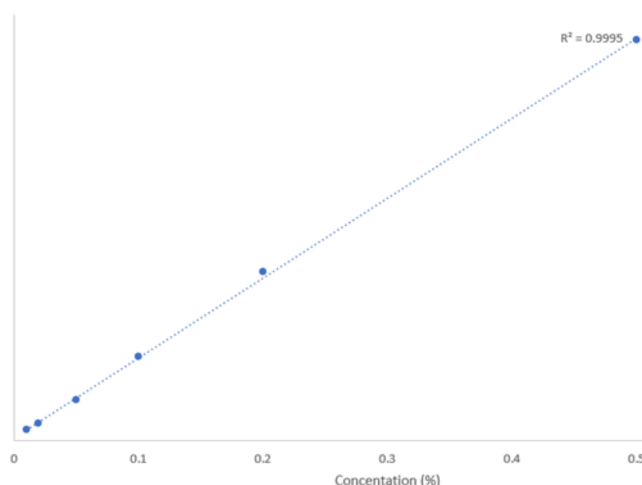

Figure 1a. Calibration Curve of Lactulose

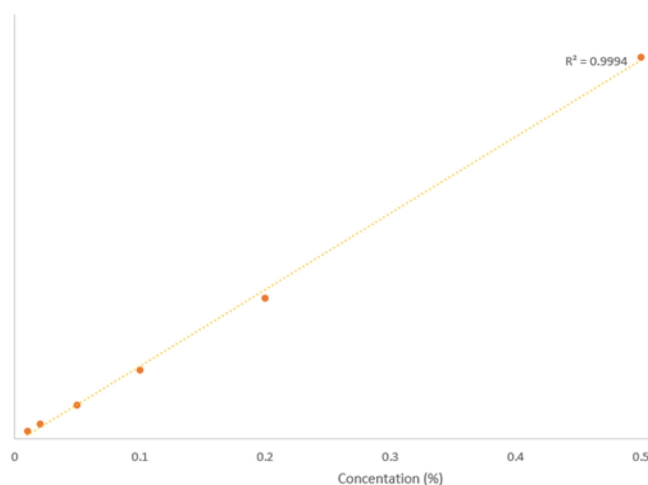

Figure 1b. Calibration Curve of Epilactose

Analysis of Functional Sugars using HILIC mode HPLC

RESULTS

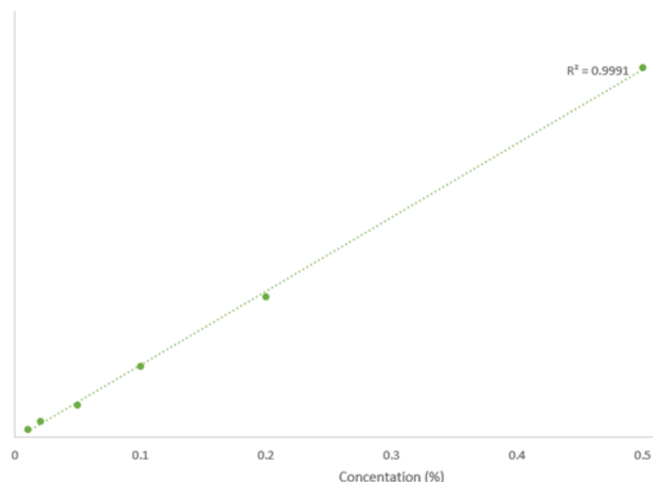

Figure 1c. Calibration Curve of Lactose

When analysing functional sugars the most crucial outcome for a successful analysis is the complete resolution and separation of each target compound. Figure 2 highlights the chromatography achieved using the HILICpak VG-50 column.

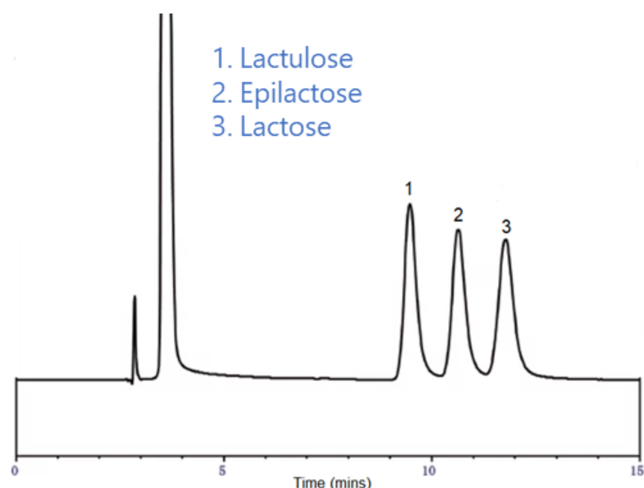

Figure 2. Chromatogram of Lactulose, Epilactose and Lactose

Many USP Pharmacopeia methods describe separation specifications which must be met during the analysis of functional sugars, using an RI detector with HILIC mode chromatography.

The USP Monograph (USP 35) states that '*The Resolution between Lactulose and Epilactose is not less than 1.5 and that between Epilactose and Lactose is not less than 0.9*'.

Using the HILICpak VG-50 column in combination with the SCION Instruments RI detector, the resolution obtained from the analysis exceeded these specifications, highlighting the excellence of chromatographic separations using the LC6000 in HILIC Mode.

Table 2 details the resolution of Lactulose and Lactose plus Lactulose and Epilactose as well as the specifications set out by USP 35.

Table 2. Resolution of all target compounds

Compound	Resolution	USP 35 Specification
Lactulose/ Epilactose	2.29	>1.5
Epilactose/ Lactose	1.98	>0.9

CONCLUSION

When operated in HILIC mode and combined with the specialist HILICpak VG-50 sugar column, the LC6000 with 6450 RI Detector is the ideal solution for challenging sugar analyses. With complete separation, optimum resolution and excellent linearity, the analysis of Lactulose, Epilactose and Lactose can be successfully achieved in under fifteen minutes.

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Simultaneous Analysis of Combination Drugs by HPLC-DAD

INTRODUCTION

Both prescription drugs and over the counter medications contain a single active pharmaceutical ingredient (API); the biologically active substance that treats illnesses. Often, more than one API is used in combination to treat symptoms. The demand for accurate qualitative and quantitative methods of combination drugs is increasing, in a variety of industries.

During manufacturing of pharmaceuticals, the level of active ingredient must be highly regulated. Long term users of combination drugs must also be monitored to ensure the individual is not at risk of overdose and/or long-term side effects associated with chronic exposure to the API's. Most importantly, the analysis of combination drugs is a vital for any toxicology laboratory, especially when the target analytes are common in overdose cases.

SCION Instruments developed a HPLC-DAD method for the simultaneous determination of two common over the counter medications; Ibuprofen and Paracetamol. Both Ibruprofen and Paracetamol are commonly used to treat the common cold or minor aches and pains. The Diode Array Detector enables a single injection for the identification of the two API's by utilising specific wavelengths of the two target compounds as well as using the absorbance spectra for further confirmation. Targeting the compounds at the specific wavelengths ensures maximum absorbance of the compound, at the set wavelength, enhancing identification.

EXPERIMENTAL

A SCION Instruments LC6000 with Diode Array Detector (DAD) was used for the analysis of Ibuprofen and Paracetamol.

CompassCDS software was used for the whole analysis including method acquisition, data interpretation and reporting of results. Calibration standards containing both Ibruprofen and Paracetamol were prepared at the following concentrations: 10,50,100,200 and 400mg/L in methanol. Over the counter Ibuprofen and Paracetamol were purchased and prepared. One of each tablet was dissolved in 100mL of the mobile phase (phosphoric acid buffer/ methanol (30:70) before being sonicated and diluted with another 100mL of mobile phase.

Samples were then centrifuged for 10 minutes at 3000rpm before being filtered through a 0.2µm filter. The sample was then transferred to a HPLC autosampler vial. If blood is the sample for analysis, liquid-liquid extraction must be performed. Analytical conditions for this analysis can be found in Table 1.

Table 1. Analytical Conditions of HPLC-DAD

Parameter	Setting
Column	C18 250mm x 4.6mm x 5µm
Temperature	25°C
Eluent	A: Phosphoric Acid Buffer B: Methanol
Gradient	0-6mins: B70% 6.1-15mins B90%
Flow Rate	1mL/min
DAD	220-400nm Ibu 237nm, Para 254nm
Injection	20µL

RESULTS

The linearity of the HPLC system was tested using analytical standards of Ibuprofen and Paracetamol at a range of 10-400mg/L. The specific wavelengths of both Ibuprofen and Paracetamol were utilised by the Diode Array Detector. Figures 1a shows the calibration curve of Ibuprofen at wavelength 237nm whereas Figure 1b shows the calibration curve of Paracetamol, at wavelength 254nm.

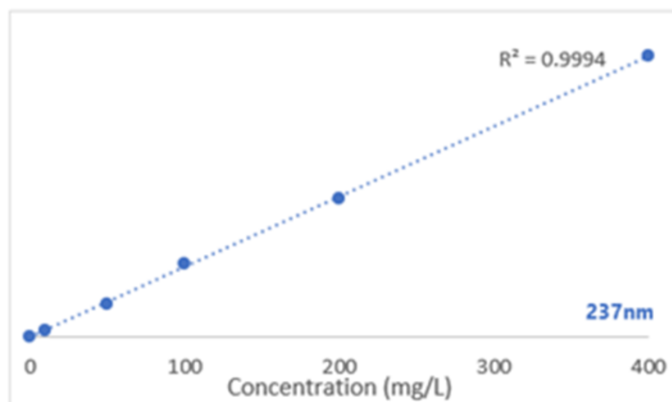

Figure 1a. Calibration Curve of Ibuprofen at 237nm

Simultaneous Analysis of Combination Drugs by HPLC-DAD

RESULTS

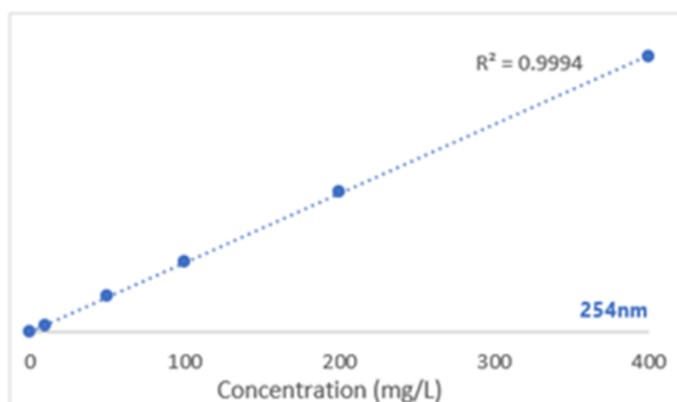

Figure 1b. Calibration Curve of Paracetamol at 254nm

The SCION HPLC exhibited excellent linearity over the concentration range of 10-400mg/L for both target compounds, each with an R^2 value of 0.9994. Following the calibration standards, the sample containing the Ibuprofen and Paracetamol tablet extracts was analysed. Figure 2 shows the stacked chromatograms of both target compounds at their specific wavelengths.

Ibuprofen has a specific absorbance wavelength of 237nm whereas Paracetamol has a maximum absorbance at 254nm. Utilising the multi-wavelength capability of the Diode Array Detector it was possible to detect both target compounds in a single injection, in under 12 minutes. The concentration of both target compounds was determined using the respective calibration curves. The quantitative values for Ibuprofen was 34.11mg/L whilst the Paracetamol was calculated at 39.24mg/L.

For further identification and confidence in results, the absorbance spectra of the target compounds were also compared with a spectral library. Figure 3a shows the absorbance spectra comparison of the Ibuprofen sample against the spectra of a Ibuprofen standard whereas Figure 3b shows the comparison absorbance spectra's of the Paracetamol sample and Paracetamol standard.

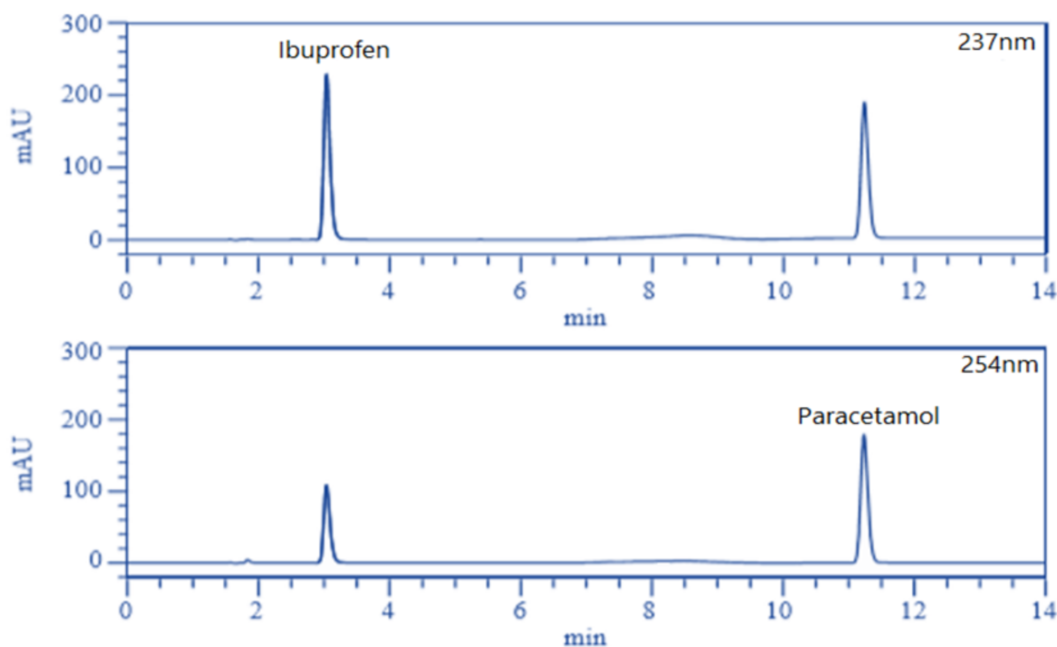

Figure 2. Stacked chromatogram of target compounds

Simultaneous Analysis of Combination Drugs by HPLC-DAD

RESULTS

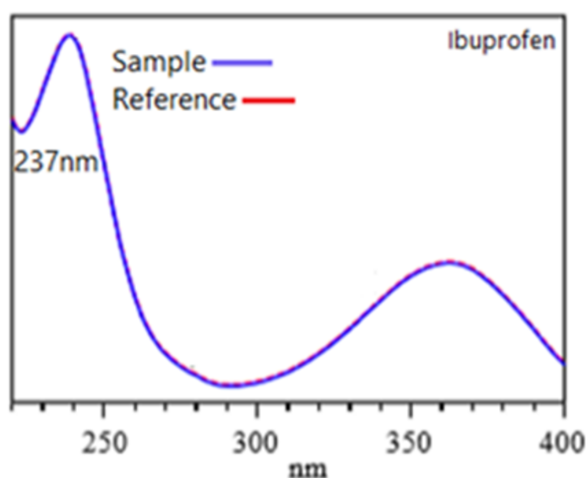

Figure 3a. Absorbance spectra comparison of Ibuprofen

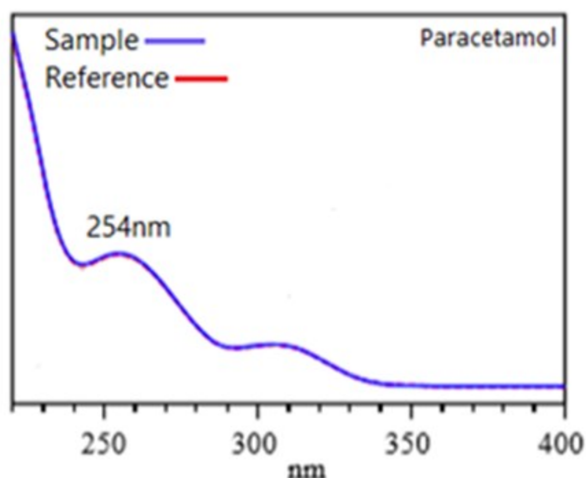

Figure 3b. Absorbance spectra comparison of Paracetamol

CompassCDS software was used for the spectral library comparisons, in which an in house reference library was used. Figure 3a/b shows the overlay of the absorbance spectra generated during both the sample analysis and the reference standard, in which both Ibuprofen and Paracetamol exhibited a 100% match, thus confirming the positive identification of both target compounds (as the spectra of the sample is overlaid with the reference and the match is 100%; the reference spectra is not visible). Repeatability of the system was performed with six consecutive injections of the 10mg/L calibration standard. Figure 4 shows the overlay chromatograms for both Ibuprofen and Paracetamol.

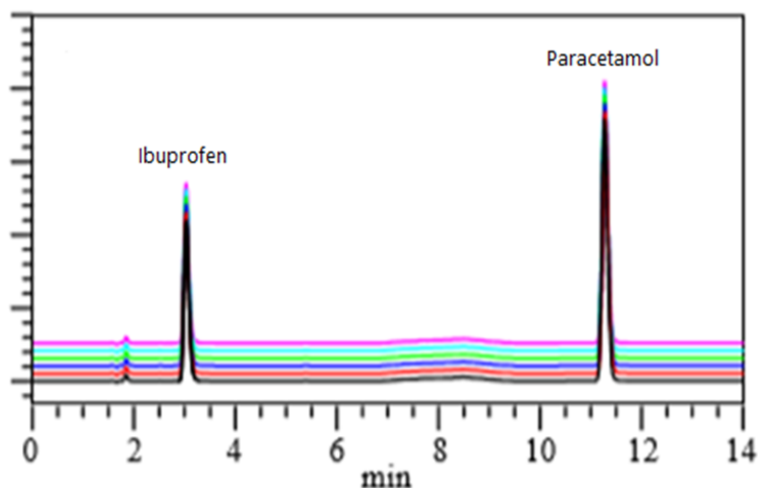

Figure 4. Overlay of 10mg/L analytical standard chromatograms

Excellent repeatability of the 10mg/L analytical standard was observed, over six consecutive injections; with an RSD of 0.27% for Ibuprofen at 237nm and 0.18% for Paracetamol at 254nm. Retention times were also stable and reproducible with RSD values of 0.06% and 0.04%, respectively. The excellent repeatability and linearity observed demonstrates the robustness of the SCION HPLC system.

CONCLUSION

SCION Instruments developed a fast, accurate method for the simultaneous identification and quantification of combination drugs by HPLC-DAD. This method is critical to numerous industries including pharmaceutical and toxicology. With a single injection and utilising the multi-wavelength capabilities of the Diode Array Detector it was possible to clearly separate Ibuprofen and Paracetamol, two commonly used over the counter medications, in under 12 minutes. Compound identification was further confirmed through absorbance spectral comparisons using CompassCDS. Excellent linearity and repeatability of the SCION HPLC-DAD was demonstrated with low RSD values and high R^2 values.

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SCION INSTRUMENTS

Simultaneous Analysis of Phenoxyethanol and Parabens by HPLC-DAD

INTRODUCTION

Parabens are a class of widely used preservatives in cosmetic and pharmaceutical products, primarily due to their bactericidal and fungicidal properties. Parabens are a series of parahydroxybenzoates which are currently under scrutiny due to the possibility that long-term use of products containing parabens can cause hormone disruption and fertility issues, although this has not been confirmed[1]. Methyl *p*-hydroxybenzoate, ethyl *p*-hydroxybenzoate, propyl *p*-hydroxybenzoate and butyl *p*-hydroxybenzoate are the most common used parabens in the cosmetic and pharmaceutical industry. Phenoxyethanol is an additional component used as a germicide and antiseptic ingredient in cosmetics. Naturally occurring in plant material such as green tea, phenoxyethanol is often used in tandem with parabens to reduce the number of parabens used during the manufacturing of such products. Monitoring paraben and phenoxyethanol concentrations are a vital part of any cosmetic of pharmaceutical manufacturer.

SCION Instruments developed a method for the simultaneous analysis of parabens and phenoxyethanol by HPLC-Diode Array Detection (DAD). Utilising the flexibility of the DAD, it was possible to extract the optimal wavelength for each target compound. Confirmation of peak identity is further certified through the comparison of the absorbance spectrum of both analytical standard and sample analysed.

EXPERIMENTAL

A SCION 6000 HPLC with DAD was used with a C18 reverse phase column for the identification of six parabens and phenoxyethanol. Samples included commercially available hand lotion and face cream. Samples were diluted in ethanol before being filtered and analysed. Table 1 details the analytical conditions of the HPLC-DAD system. The optimum wavelengths for the phenoxyethanol and parabens were 220nm with 254nm, respectively.

Calibration curves were generated for all parabens plus phenoxyethanol at a concentration range of 0.1-50mg/L.

Table 1. Analytical Conditions of HPLC-DAD

Parameter	Setting
Column	C18 150mm x 4.6mm x 5µm
Temperature	40°C
Eluent	Acetonitrile: 0.01% Phosphoric Acid* 35:65 v/v
Flow Rate	1mL/min
DAD	220nm + 254nm
Injection	10µL

RESULTS

Figure 1 shows the calibration curve of ethyl *p*-hydroxybenzoate and is representative of all target compounds in this analysis.

All parabens and phenoxyethanol had excellent linearity over a range of 0.1mg/L to 50mg/L with R^2 values of no less than 0.9999. Table 2 details the target compounds and the associated peak number on the chromatograms.

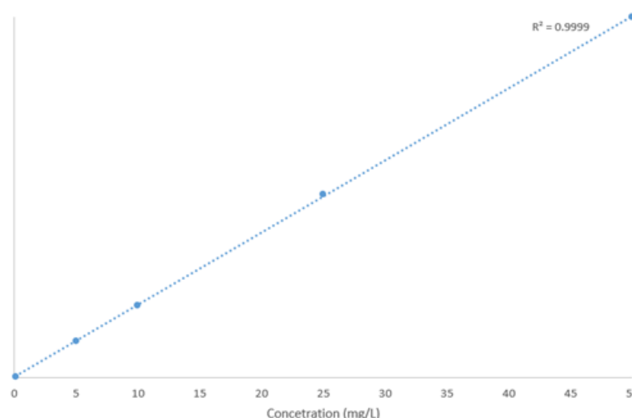

Figure 1. Calibration curve of ethyl p-hydroxybenzoate

Table 2. Peak number and compound identifier

Peak	Compound
1	2-phenoxyethanol
2	Methyl p-hydroxybenzoate
3	Ethyl p-hydroxybenzoate
4	Isopropyl p-hydroxybenzoate
5	Propyl p-hydroxybenzoate
6	Isobutyl p-hydroxybenzoate
7	Butyl p-hydroxybenzoate

Figure 2 shows the two chromatograms obtained when a 10mg/L standard was analysed, over both wavelengths.

Simultaneous Analysis of Phenoxyethanol and Parabens by HPLC-DAD

RESULTS

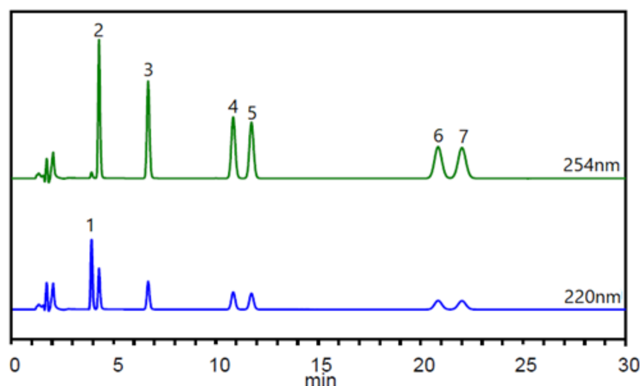

Figure 2. Standard chromatogram over two wavelengths 10mg/L)

As shown in Figure 2, all target compounds are detected over both wavelengths, although response and peak shape varies. By utilising the dual wavelengths, chromatography and selectivity increases as a result of the optimum wavelength for the parabens and phenoxyethanol being selected.

Along with retention time comparison to standards, absorbance spectra comparisons were also made in CompassCDS, which provided an additional level of confirmation for peak identification. Figures 3-8 show the chromatogram of the hand lotion and face cream samples along with the comparison of absorbance spectra for a selection of identified compounds.

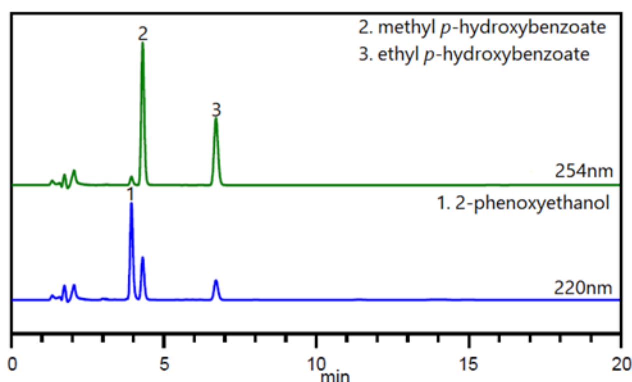

Figure 3. Chromatogram and peak identification of face cream sample

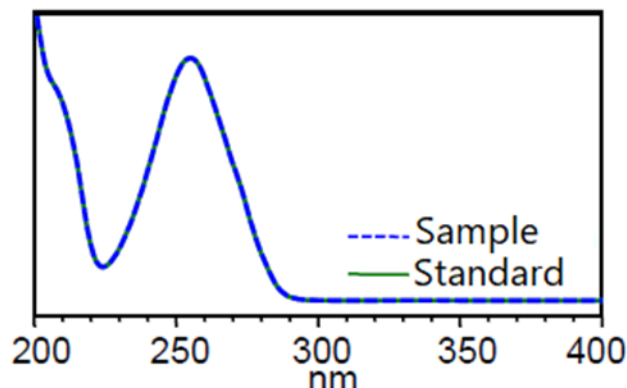

Figure 4. Absorbance spectrum comparison methyl *p*-hydroxybenzoate (face cream)

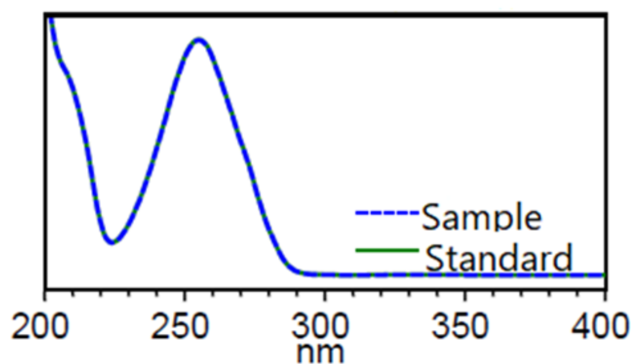

Figure 5. Absorbance spectrum comparison ethyl *p*-hydroxybenzoate (face cream)

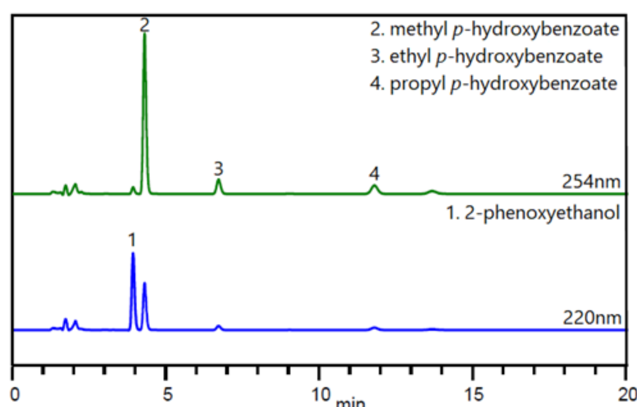

Figure 6. Chromatogram and peak identification of body lotion sample

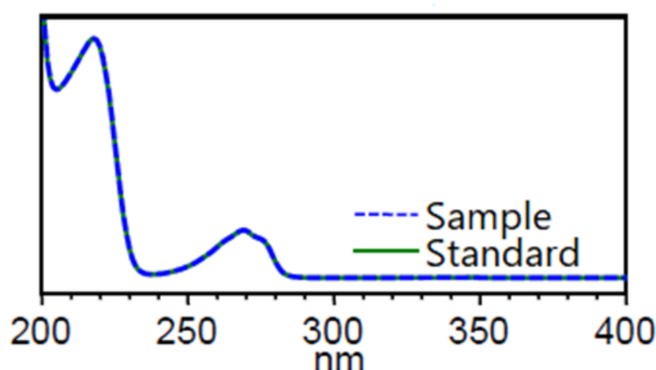

Figure 7. Absorbance spectrum 2-phenoxyethanol (hand lotion)

Simultaneous Analysis of Phenoxyethanol and Parabens by HPLC-DAD

RESULTS

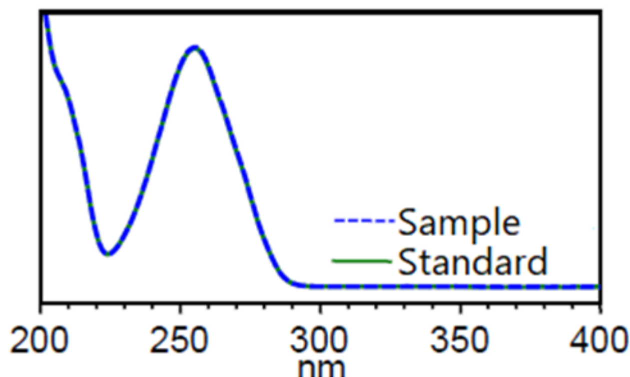

Figure 8. Absorbance spectrum propyl *p*-hydroxybenzoate (hand lotion)

As per the analytical standards, utilising the two separate wavelengths gave better detection of the individual components, compared to using a single wavelength. Additionally, the comparison spectra confirms that the identified target compounds have the exact same absorbance pattern as the corresponding standard for that compound, adding an extra level of confidence in the results.

The hand cream contained more parabens than that of the body lotion. This is typical in cosmetics due to the storage conditions of hand cream; it is more likely to be moved from place to place requiring a higher preservation level than a body lotion or other cosmetics. Both samples contained 2-phenoxyethanol, the common germicide ingredient in cosmetics.

CONCLUSION

SCION Instruments developed a method for the identification of six parabens and 2-phenoxyethanol by HPLC-DAD. By utilising the extracted wavelength mechanism of the DAD, it is possible to use the optimal absorption wavelengths for each individual compound, eliminating the need for individual methods. Excellent linearity was observed for all target compounds with additional confidence in peak identification through absorbance spectrum comparison in CompassCDS.

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Simultaneous Analysis of Triazole Fungicides by HPLC-DAD

INTRODUCTION

Fungicides are chemical compounds commonly used to kill parasitic fungi and their spores. Fungi can have devastating impacts in agriculture resulting in critical losses of yield, quality and profit. Fungicides are common upon crop growers due to the longevity that the fungicides work for in the environment. Specifically, Paclobutrazol and Uniconazole are triazole fungicides which act as a plant growth regulators. Plant growth regulators are commonly used to prevent the growth of weeds in households but to also enhance crop yield in plants such as tomatoes and peppers. When used on food crops, Paclobutrazol results in products with a higher resistance to drought and fungi, enabling a higher profit for the producer. Uniconazole is traditionally used to restrain plant grown and delay flowering, enabling the plant to be more manageable.

Although both Paclobutrazol and Uniconazole are traditional triazole chemicals, care must be taken when using on crops as over application can be devastating to the harvesting of the crop. Additionally, the overuse of Paclobutrazol and Uniconazole can result in elevated levels of residues in the harvested fruit and vegetables, which are a threat to consumers. There are numerous regulations worldwide regarding the maximum allowable residue levels in various fruits so the monitoring of these chemicals is critical during crop harvesting. SCION Instruments developed a method for the simultaneous determination of Paclobutrazol and Uniconazole by High Performance Liquid Chromatography (HPLC) with Diode Array Detection (DAD).

EXPERIMENTAL

A SCION Instruments LC6000 equipped with autosampler, column oven and DAD was used for the simultaneous analysis of Triazole Fungicides. Paclobutrazol and Uniconazole calibration standards were prepared over a concentration range of 0.10mg/L to 1mg/L. Five different samples, each containing the target compounds were quantified using the calibration curves from the calibration standards.

The DAD was operated at a range of 220-400nm with the target absorbance wavelengths at 230nm for both compounds. Table 1 details the analytical conditions of the LC6000.

Table 1. Analytical Conditions of HPLC-DAD

Parameter	Setting
Column	C18 250mm x 4.6mm x 5µm
Temperature	30°C
Eluent	Methanol: Water (0.1% formic acid) 70:30v/v
Flow Rate	1mL/min
DAD	220-400nm, 230nm
Injection	20µL

RESULTS

Calibration standards were analysed at concentrations of 0.1, 0.2, 0.5, 0.8 and 1mg/L for each triazole fungicide. The calibration curves can be found in Figures 1a and 1b.

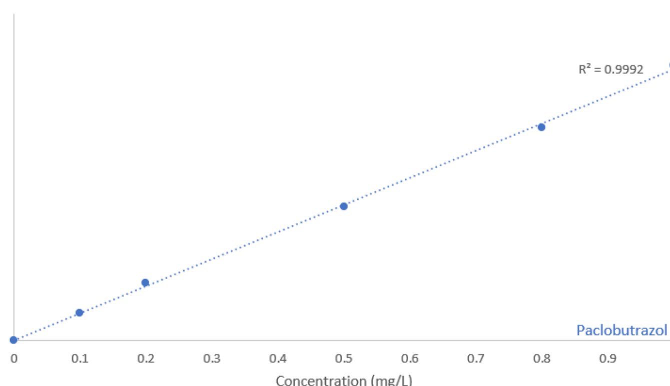

Figure 1a. Calibration Curve of Paclobutrazol

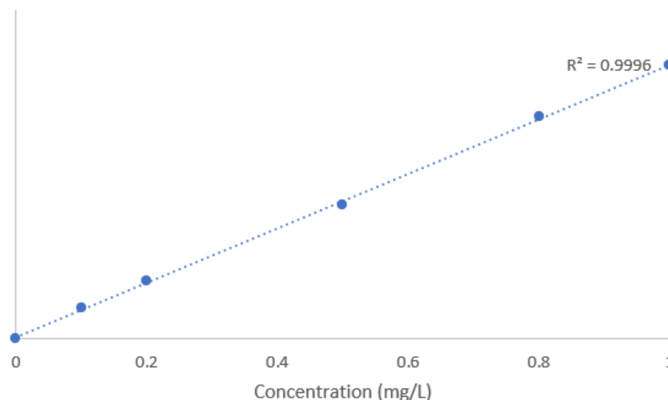

Figure 1b. Calibration Curve of Uniconazole

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SCION INSTRUMENTS

Simultaneous Analysis of Triazole Fungicides by HPLC-DAD

RESULTS

Both Paclobutrazol and Uniconazole exhibited excellent linearity over a concentration range of 0.1-1mg/L. Five samples containing both target compounds were diluted in methanol and analysed under the same conditions as shown in Table 1.

The repeatability of the method was determined using 13 simultaneous injections of the 0.5mg/L standard, for each target compound. Figure 2a shows the overlay chromatogram for Paclobutrazol whilst 2b shows the overlay chromatogram for Uniconazole.

Figure 2a. Repeatability of 0.5mg/L Paclobutrazol

Figure 2b. Repeatability of 0.5mg/L Uniconazole (n=13)

As shown in Figures 2a and 2b, the repeatability of 13 consecutive injections of both 0.5mg/L calibration standards was excellent. The RSD% values for both target compounds, for retention time and peak area can be found in Table 2.

Table 2. Repeatability values (n=13)

Compound	Peak Area (RSD%)	Retention Time (RSD%)
Paclobutrazol	0.74	0.21
Uniconazole	0.33	0.21

The excellent repeatability values highlights the robustness of the method. With minimum variability between both peak area and retention time, the five samples containing Paclobutrazol and Uniconazole were analysed. Quantitation of all samples was performed using the calibration curves (Figures 1a and 1b). Figure 3 shows an example chromatogram of sample 1.

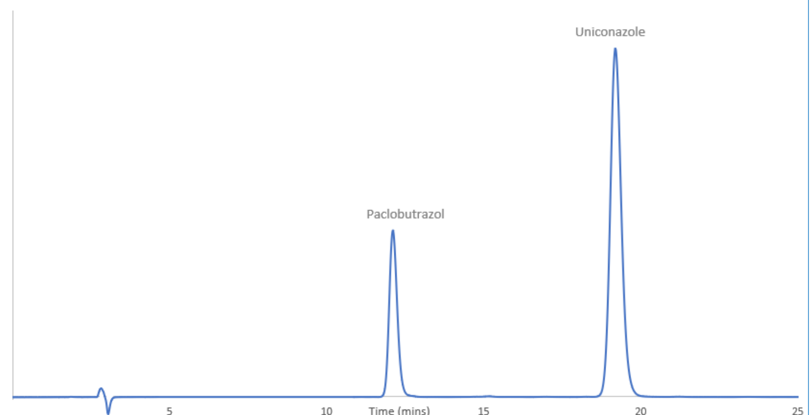

Figure 3. Chromatogram of sample containing target compounds

Figure 3 is an example chromatogram from one of the analysed samples; and is representative of all samples. Retention time and quantification of each triazole fungicide was based off the calibration standards. Table 3 details the results from the analysis of all five samples.

Simultaneous Analysis of Triazole Fungicides by HPLC-DAD

RESULTS

Table 3. Concentration of Triazole Fungicides

Sample	Paclobutrazol Concentration (mg/l)	Uniconazole Concentration (mg/L)
1	0.54	0.51
2	0.81	0.81
3	0.08	0.09
4	0.18	0.19
5	1.0	1.0

Each sample contained varying amounts of Paclobutrazol and Uniconazole. To further confirm peak identification, the three dimensional data from the diode array detector was used; in the form of spectral comparisons.

The spectra from both Paclobutrazol and Uniconazole identified in all samples were compared with a spectral reference library. An example of this comparison can be found in Figure 4.

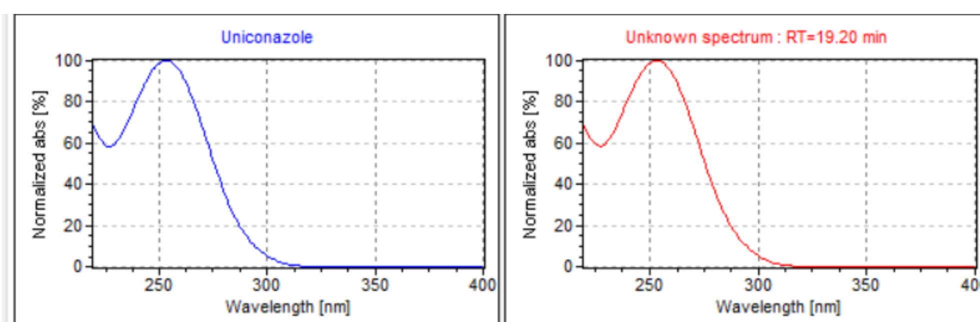

Figure 4. Spectral comparison of Uniconazole sample with stored reference

Figure 4 shows the spectral comparison of the identified Uniconazole in sample one with a stored reference standard. The blue window shows the spectra of the reference standard whilst the red window shows the spectra obtained when the sample was analysed. The match between the two spectra's was 1000; confirming with 100% that the two compounds are the same.

Each sample was compared to the known references for both compounds; each giving a 100% spectral match. This further confirms the presence of both compounds in all samples. Using the diode array detector 3D spectra gives additional certainty and confidence in peak identification.

CONCLUSION

SCION Instruments developed a method for the simultaneous analysis of two triazole fungicides. Triazole fungicides are highly regulated compounds which can be damaging to not only human health but also crop harvesting if they are used in vast amounts. Using the three dimensional data modelling of the diode array detector provided additional confirmation of peak identification of both Paclobutrazol and Uniconazole. With excellent linearity and repeatability, the SCION Instruments method and LC6000 is perfect for any environmental laboratory.

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