BEYOND MEASURE

SCION INSTRUMENTS

Analysis of Glycosides in Medicines

Application Note

AN0067

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.

Table 1. Analytical conditions of the HPLC-DAD

Conditions	
Column	C18 3µm x 4.6mm ID x 150mm
Column Temp	40°C
Mobile Phase	(a) 10mmol/L monopotassium hydroxide (pH3.0) (b) Acetonitrile
Gradient	0 min B10%: A90%, 20 min B30%: A70%, 25-35mins B70%:A30%, 35.1-50mins B10%:A90%
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.

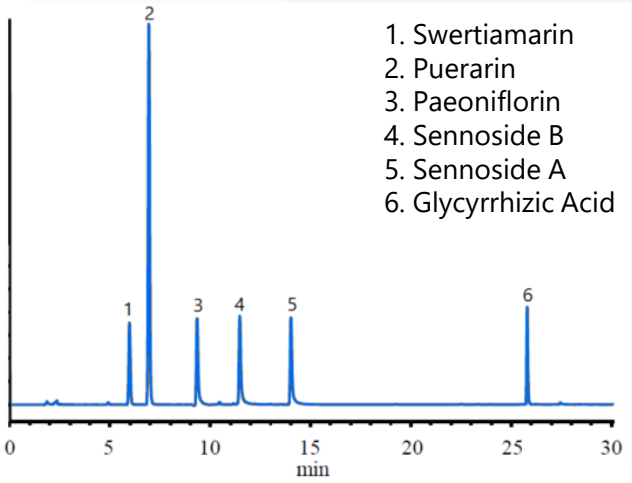

Figure 1. Analytical standard chromatogram (50mg/L)

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.

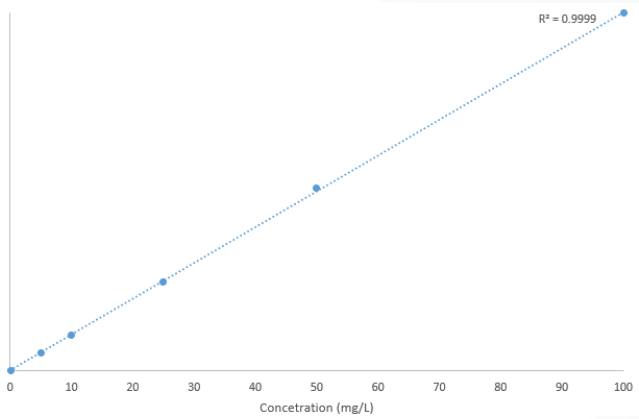

Figure 2. Calibration curve of Sennoside A (0.1mg/L to 100mg/L)

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

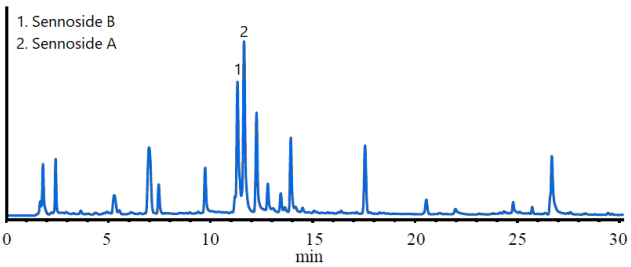

Figure 3. Chromatogram and peak identification of Senna Leaf sample

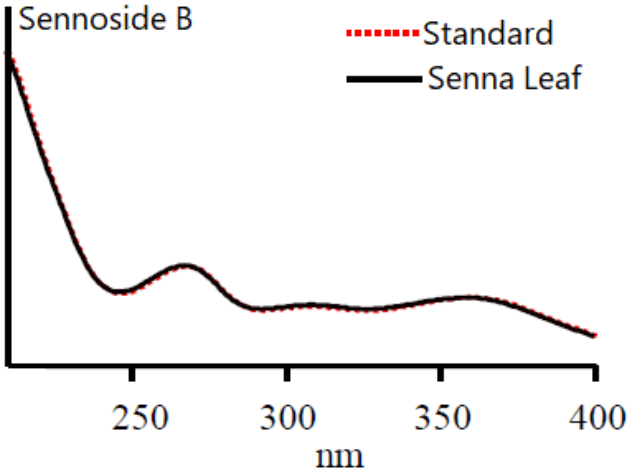

Figure 4. Absorbance spectrum comparison Sennoside B (Senna Leaf)

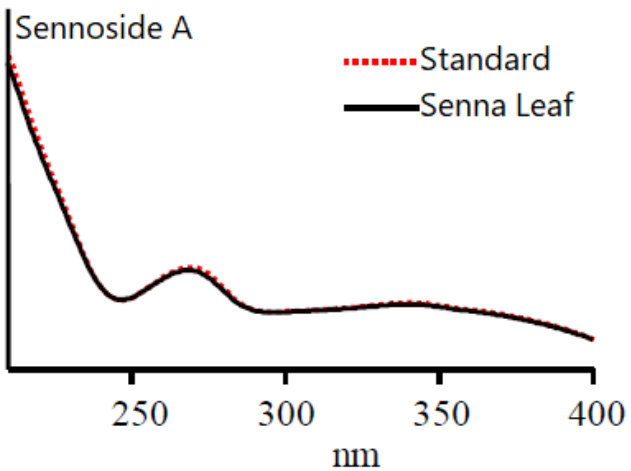

Figure 5. Absorbance spectrum comparison Sennoside A (Senna Leaf)

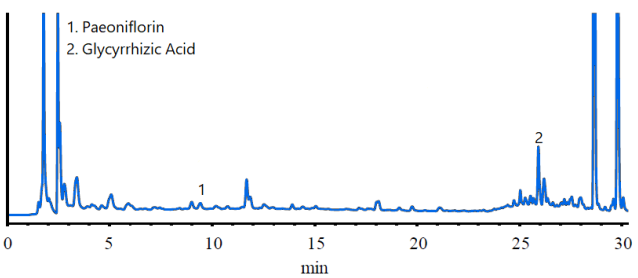

Figure 6. Chromatogram of gastrointestinal medicine

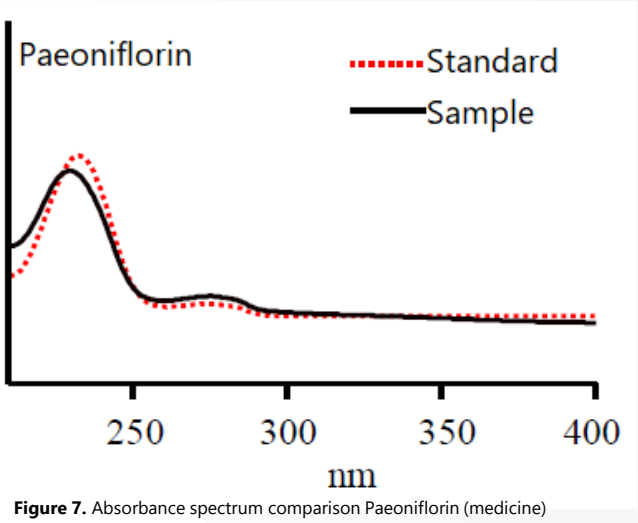

Figure 7. Absorbance spectrum comparison Paeoniflorin (medicine)

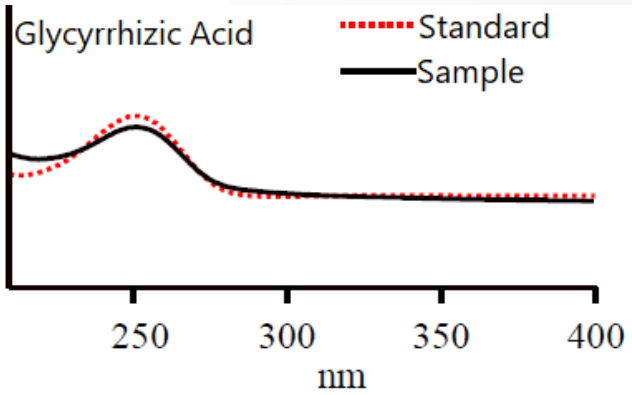

Figure 8. Absorbance spectrum comparison Glycyrrhizic Acid (medicine)

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.

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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