

Chromatography

Simultaneous Analysis of Combination Drugs by HPLC-DAD

Ashleigh Mellor, Applications Specialist, SCION Instruments

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 vital for any toxicology laboratory, especially when the target analytes are common in overdose cases.

SCION Instruments developed an HPLC-DAD method for the simultaneous determination of two common over the counter medications; Ibuprofen and Paracetamol. Both Ibuprofen 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.



Figure 1. SCION HPLC-DAD.

Experimental

A SCION HPLC with Diode Array Detector was used for the analysis of Ibuprofen and Paracetamol, as shown in Figure 1.

CompassCDS software was used for the whole analysis including method acquisition, data interpretation and reporting of results. Calibration standards containing both Ibuprofen 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 an 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.

Conditions

Table 1. Analytical conditions of HPLC-DAD.

Column	C18 5µm x 4.6mm x 150mm
Column Temperature	25°C
Mobile Phase	A:Phosphoric Acid Buffer B: Methanol
Gradient	0-6 minutes: A30:B70%
	6.1-15 minutes: A10:B90%
Flow Rate	1mL/min
Detection Wavelength	220-400nm (Ibup 237nm, Para 254nm)
Injection Volume	20µL

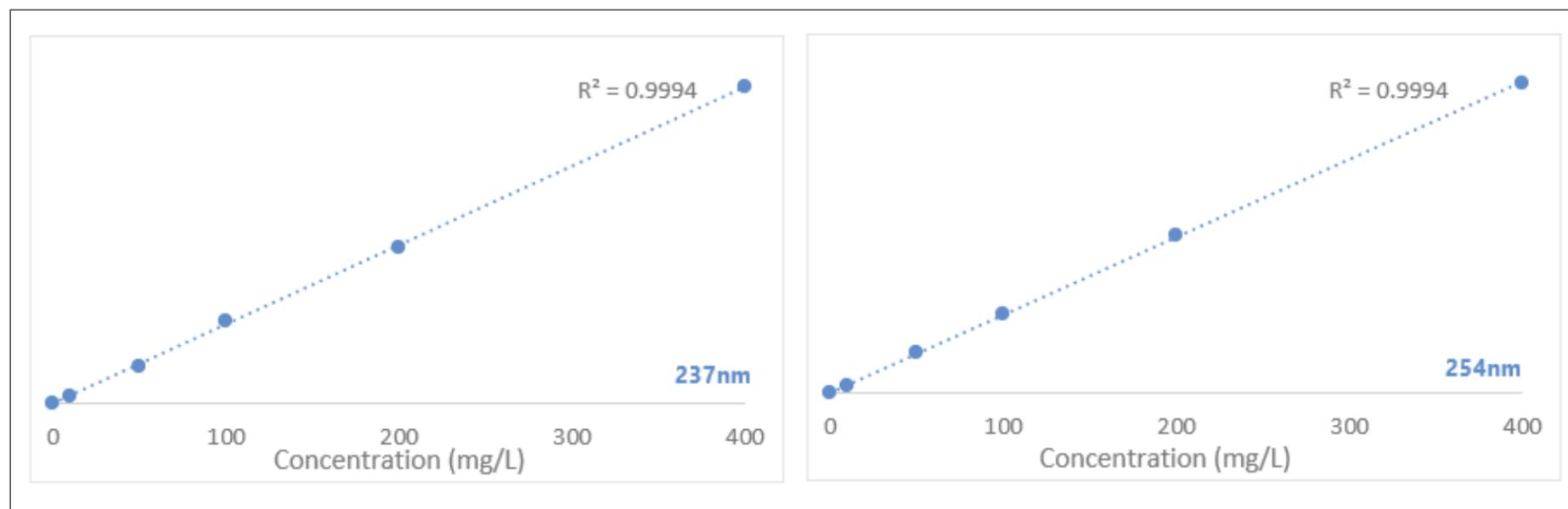


Figure 2. Calibration Curve of Ibuprofen at 237nm and Paracetamol at 254nm. HPLC-DAD.

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 2* show the calibration curves of both target analytes, at the maximum absorbance wavelengths.

The SCION HPLC exhibited excellent linearity over the concentration range of 10-400mg/L for both target compounds, each with an R2 value of 0.9994. Following the calibration standards, the sample containing the Ibuprofen and Paracetamol tablet extracts was analysed. *Figure 3* shows the stacked chromatograms of both target compounds at their specific wavelengths.

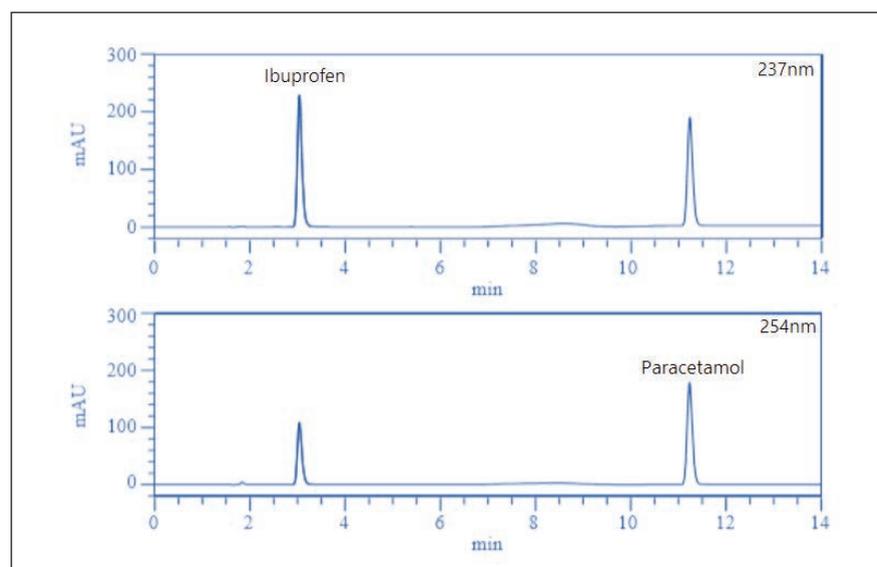


Figure 3. Stacked chromatograms of target compounds at their specific absorbance wavelengths.

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 value 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 4* shows the absorbance spectra of both Ibuprofen and Paracetamol.

CompassCDS software was used for the spectral library comparisons, in which an in house reference library was used. *Figure 4* 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 5* shows the overlay chromatograms for both Ibuprofen and Paracetamol.

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.

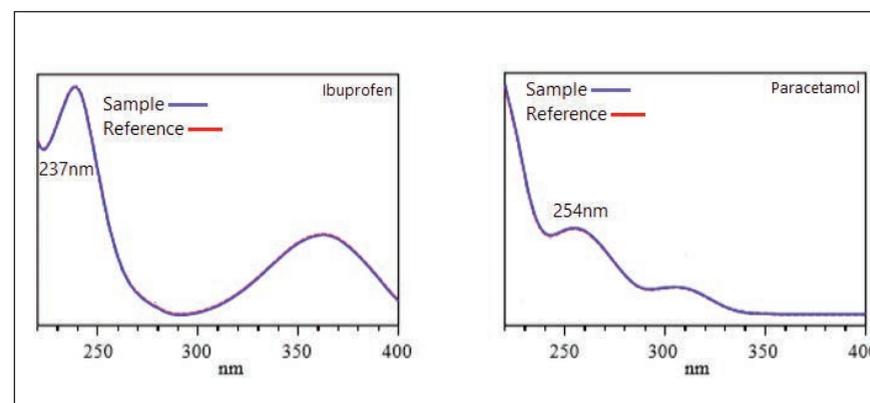


Figure 4. Absorbance spectra comparison of both Ibuprofen and Paracetamol.

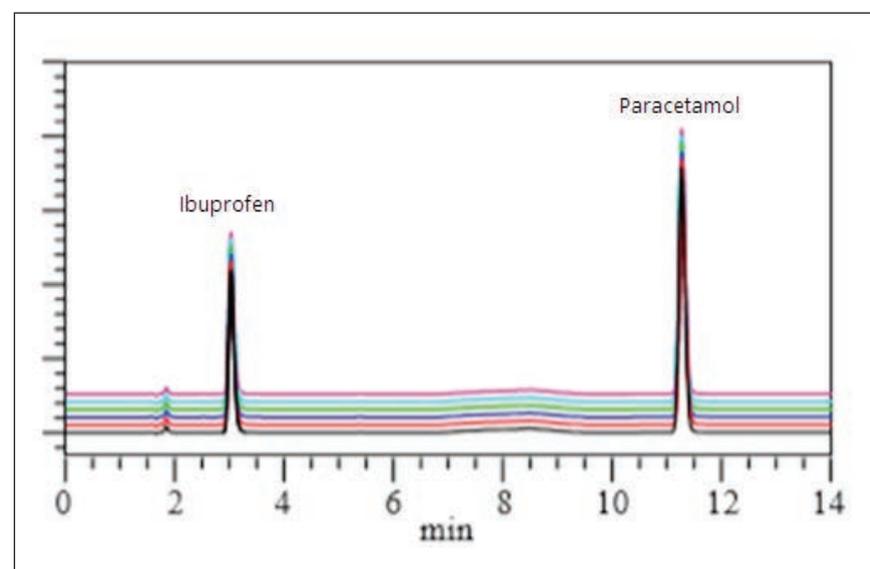


Figure 5. Overlay of 10mg/L analytical standard chromatograms (n=6).

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 R2 values.