APPENDIX 1. PDA HPLC METHOD

A.1.1 pda HPLC method

All solvents were HPLC grade and water of deionised, distilled quality. Glassware was cleaned with detergent, rinsed thoroughly with purified water then methanol, and dried.

A.1.2 Sample preparation

The sample was weighed to the nearest 0.1mg on an electronic microbalance, then hydrolysed with 400µl of 1:1:2 (v/v/v) 37% hydrochloric acid:methanol:water in 2ml glass conical test tubes for precisely 10 mins at 100°C. After rapid cooling, the extract was filtered through 5mm Analytichem polypropylene frits under positive pressure, the test tube rinsed with 2 × 200ml 1: 1 (v/v) methanol:water and the combined filtrates dried by rotary vacuum evaporation at 40°C. The dry residue was then reconstituted with 25ml of methanol followed by 25ml water immediately before analysis.

A reagent blank was also prepared with the sample to identify contamination arising through sample preparation.

A.1.3 Instrumental method

A Waters 660 gradient pump and a Waters 996 PDA detector were controlled by Waters Millennium version 2.1 software, which also collected and manipulated the data.

The reversed-phase Spherisorb ODS2 5mm particle size analytical column, 150 × 4.6mm (length × i.d.), was enclosed in a heat-controlled chamber and maintained at 25±1°C. Sample extracts were injected onto the column via a Rheodyne injector with a 20ml sample loop.

A tertiary solvent gradient eluent system of A: pure methanol, B: 25mM aqueous sodium orthophosphate buffer and C: 5% (w/v) aqueous phosphoric acid was used at a flowrate of 1.2ml min^{-1}. The elution programmes followed:

0–2 min isocratic elution 34A:66B:10C

2–30 min linear gradient elution to 90A:0B:10C 30–33 min isocratic elution 90A:0B:10C

Systems were re-equilibrated at the starting composition for 5 mins before next injection. Solvents were sparged on-line with helium at 30ml min^{-1}.

Chromatographed peaks were monitored at 254nm although the PDA detector measured all spectral information between 250 and 750nm. The bandwidth (resolution) was 2.4nm, and response time set to 1s.

Major and minor colouring components were identified by matching their retention times and spectra with reference solutions and extracts of reference dyed fibres.

Full results of the High Performance Liquid Chromatography with Photodiode Array Detection can be consulted. They will be held with the original data, currently at the National Museums of Scotland Collections Centre.