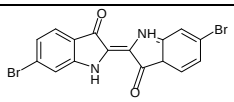
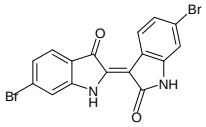
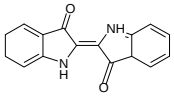
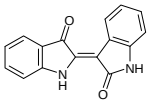
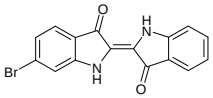
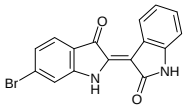
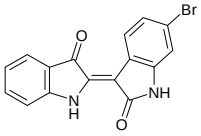
Appendix I

Characteristics of indigoid and indirubin standards and diagnostic parameters obtained by liquid chromatography-mass spectrometry

Compound Number	Standard	Structure	$[M-H]^+$	t_R
5	6,6'-dibromoindigo[1]		417, 419, 421	15.4
7	6,6'-dibromoindirubin		417, 419, 421	16.5
8	Indigo		261	10.7
9	Indirubin		261	10.5
10	6-bromoindigo		339, 341	12.7
11	6-bromoindirubin		339, 341	13.5
12	6'-bromoindirubin		339, 341	13.2

$[M-H]^+$ = the pseuomolecular ion (Br^{79}, Br^{81}) registered as the dominant signal in ESI mass spectrums in the negative ionization mode.

t_R = the retention time in minutes.

[1] Female extracts displayed a shift in retention time compared to the synthetic standard (Table 1, Chapter 2). This was attributed to HPLC column replacement (despite identical specifications). To confirm the identity of dye components, a female hypobranchial extract was spiked with the dibromoindirubin standard, which also contained trace amounts of the dibromoindigo isomer. In comparison to un-spiked extracts, an increase in relative peak intensity in the spiked extract at 14.5min and an additional peak at 16.0min confirmed the dominance of dibromoindigo in female extracts. Subsequent re-analysis of male extracts confirmed that the retention time shift was due to column replacement rather than any specific properties of the female extracts.