

APPENDIX 4

Electronic Supplementary Material to the paper “Evaluation between ultrahigh pressure liquid chromatography and high-performance liquid chromatography analytical methods for characterizing natural dyestuffs”

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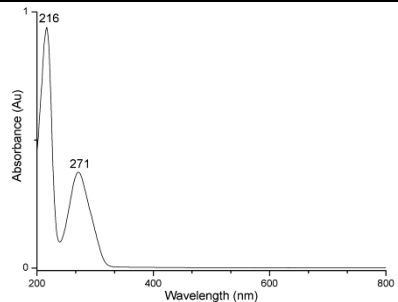
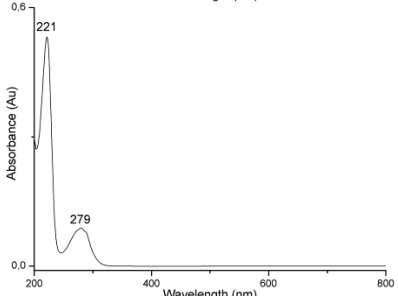
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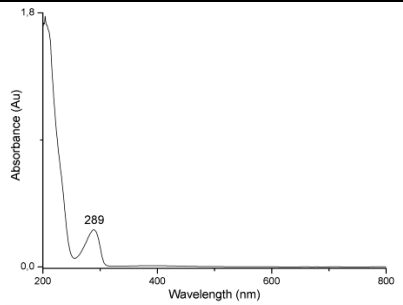
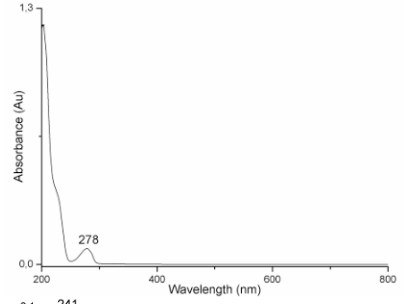
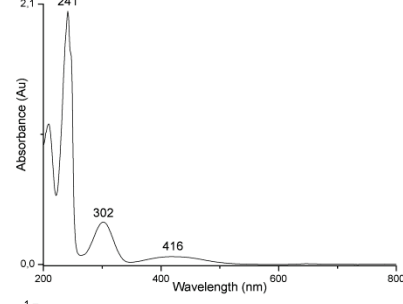
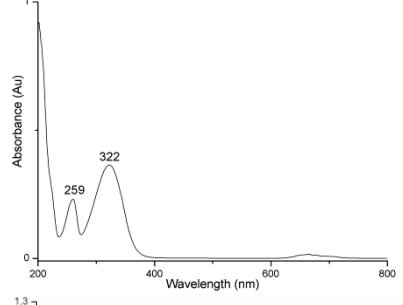
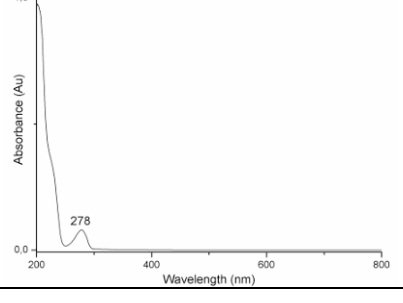
Ana Serrano^{a,b}, Maarten van Bommel^a, Jessica Hallett^b

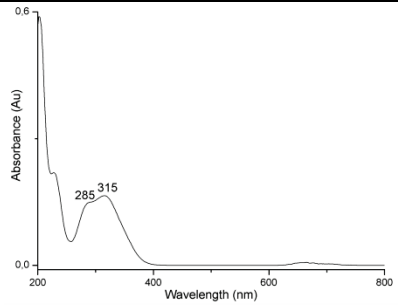
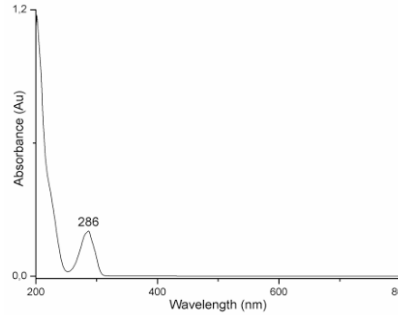
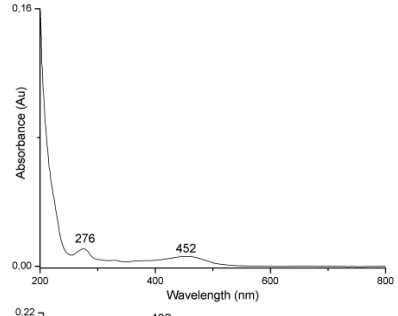
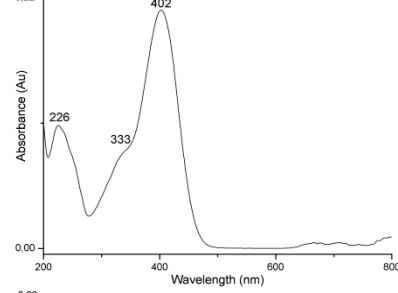
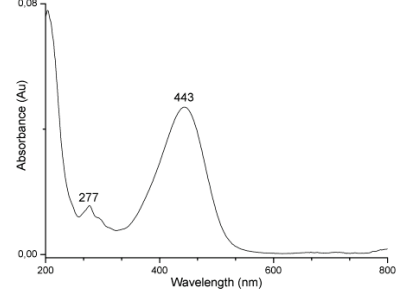
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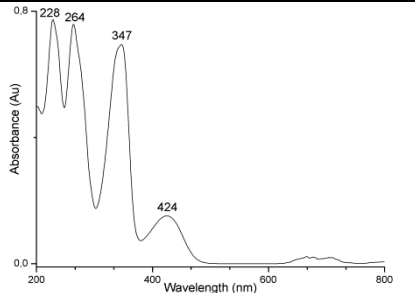
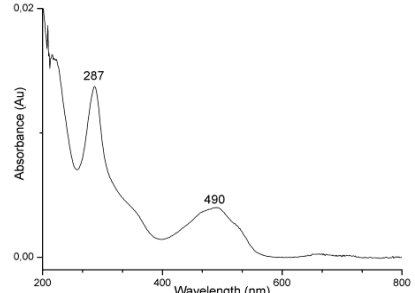
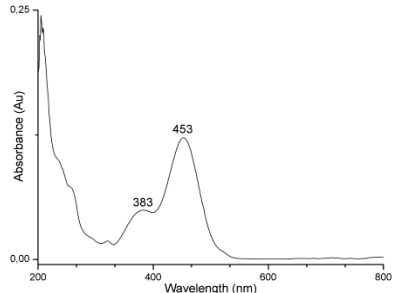
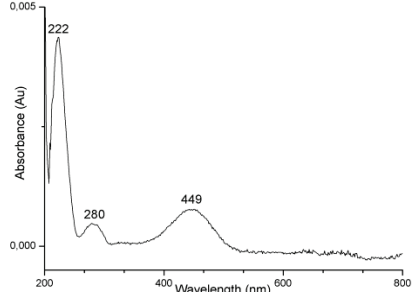
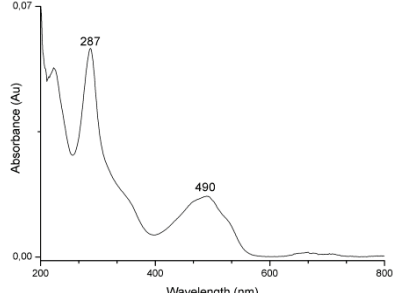
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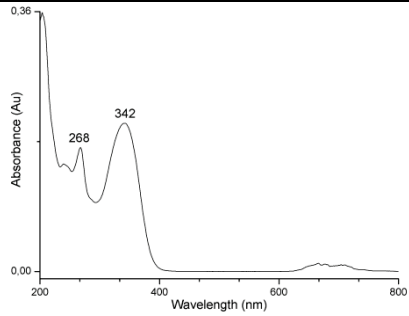
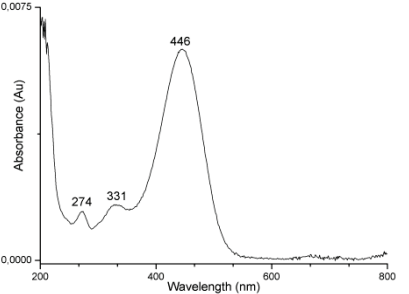
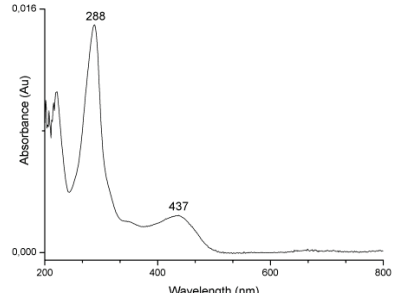
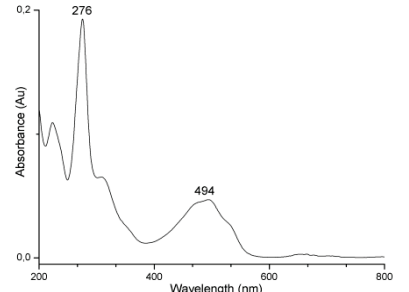
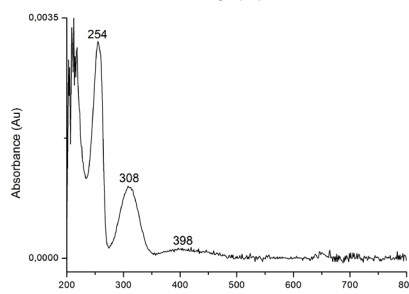
Table 1. Characterization of the retention time and the PDA spectra of the main compounds detected with the analysis of the 59 natural reference materials, belonging to the reference collection of the Cultural Heritage Agency of the Netherlands.

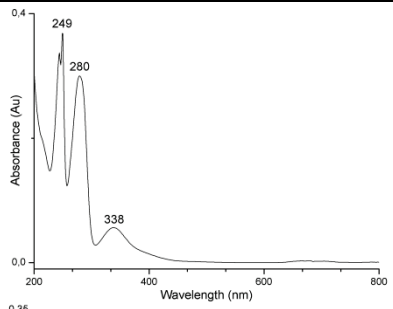
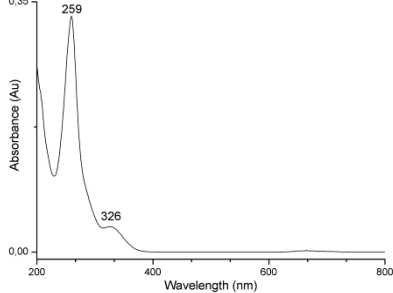
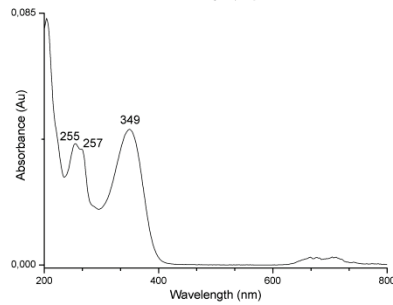
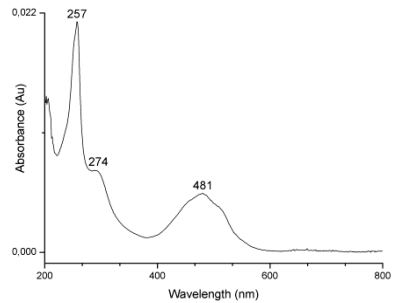
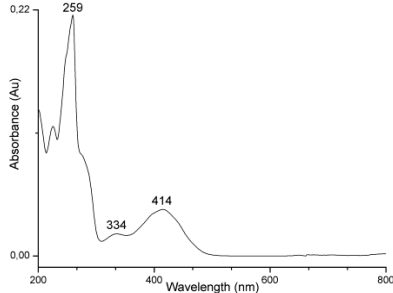
Reference samples	Colour Index number	Main compound detected	Retention time (min)	PDA spectra
Gallic acid		Gallic acid	2.98	
Indoxyl		Indoxyl	4.51	

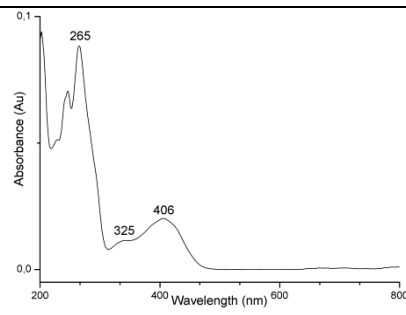
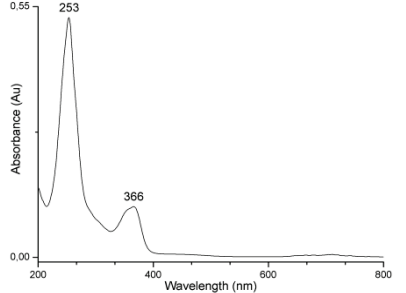
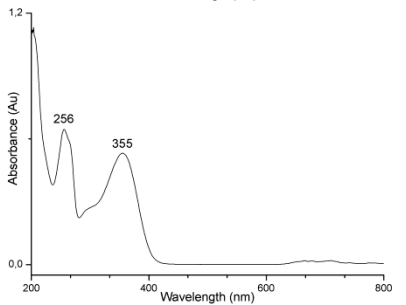
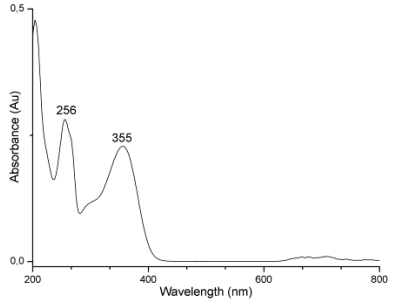
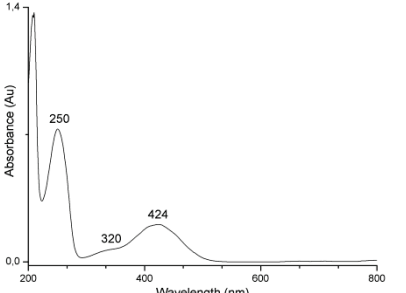
Reference samples	Colour Index number	Main compound detected	Retention time (min)	PDA spectra
Haematoxylin	75290	Haematoxylin	4.57	
Catechin hydrated	75250	Catechin	5.99	
Isatin	730000	Isatin	6	
Daphnetin		Daphnetin	6.88	
Epicatechin		Epicatechin	7.17	

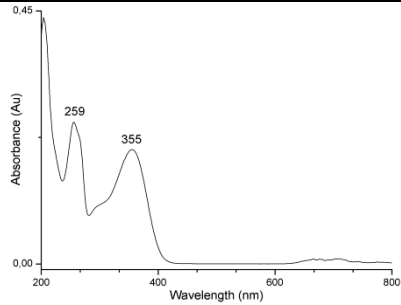
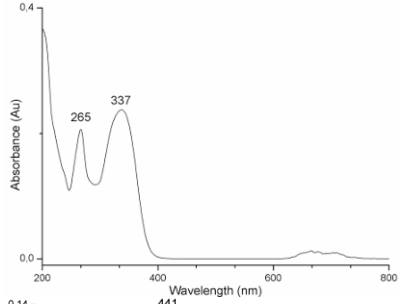
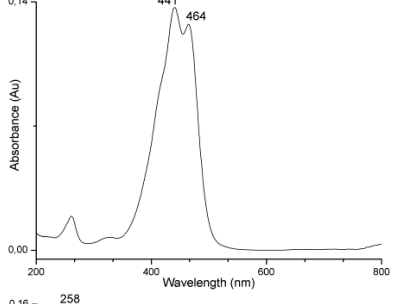
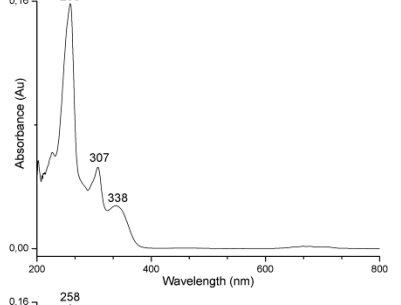
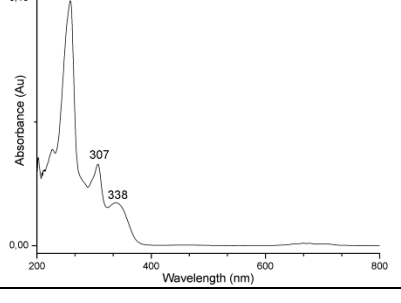
Reference samples	Colour Index number	Main compound detected	Retention time (min)	PDA spectra
Maclurin	75240	Maclurin	7.49	 The PDA spectrum for Maclurin shows a sharp initial peak at 200 nm, followed by a shoulder at 285 nm and a main peak at 315 nm. The absorbance scale ranges from 0.0 to 0.6, and the wavelength scale ranges from 200 to 800 nm.
Brazilin		Brazilin	7.5	 The PDA spectrum for Brazilin shows a sharp initial peak at 200 nm, followed by a shoulder at 286 nm. The absorbance scale ranges from 0.0 to 1.2, and the wavelength scale ranges from 200 to 800 nm.
Logwood	75290	Haematein prime	7.8	 The PDA spectrum for Logwood shows a sharp initial peak at 200 nm, followed by a shoulder at 276 nm and a main peak at 452 nm. The absorbance scale ranges from 0.00 to 0.16, and the wavelength scale ranges from 200 to 800 nm.
Carthamin yellow		Carthamin yellow	7.89	 The PDA spectrum for Carthamin yellow shows a sharp initial peak at 200 nm, followed by a shoulder at 226 nm, a main peak at 333 nm, and a secondary peak at 402 nm. The absorbance scale ranges from 0.00 to 0.22, and the wavelength scale ranges from 200 to 800 nm.
Haematein	75290	Haematein	7.93	 The PDA spectrum for Haematein shows a sharp initial peak at 200 nm, followed by a shoulder at 277 nm and a main peak at 443 nm. The absorbance scale ranges from 0.00 to 0.08, and the wavelength scale ranges from 200 to 800 nm.

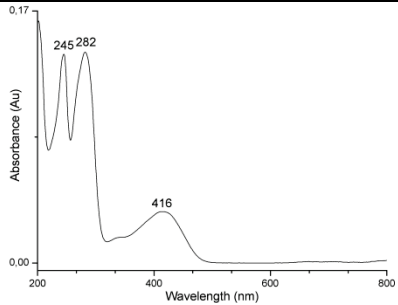
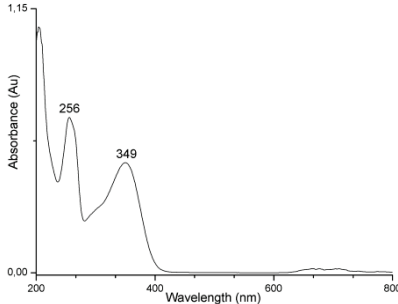
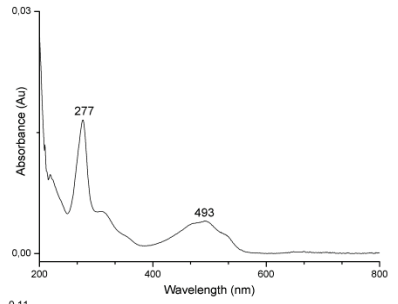
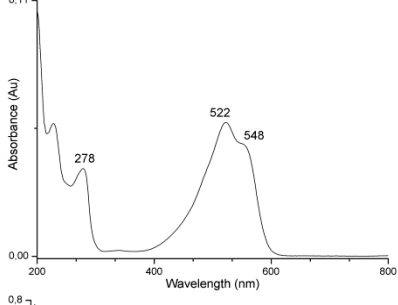
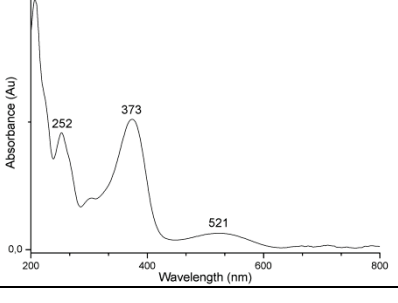
Reference samples	Colour Index number	Main compound detected	Retention time (min)	PDA spectra
Barberry (Berberis vulgaris)	75160	Berberine	7.96	 The PDA spectrum for Berberine shows absorbance (Au) on the y-axis (0.0 to 0.8) and wavelength (nm) on the x-axis (200 to 800). The spectrum features four distinct peaks labeled with their wavelengths: 228 nm, 264 nm, 347 nm, and 424 nm. The peak at 228 nm is the highest, followed by 264 nm, 347 nm, and 424 nm.
Lac dye	75450	Laccaic acid C	8.18	 The PDA spectrum for Laccaic acid C shows absorbance (Au) on the y-axis (0.00 to 0.02) and wavelength (nm) on the x-axis (200 to 800). The spectrum features two distinct peaks labeled with their wavelengths: 287 nm and 490 nm. The peak at 287 nm is significantly higher than the peak at 490 nm.
Redwood		Anhydro brasilein (brasilein prime)	8.32	 The PDA spectrum for Anhydro brasilein shows absorbance (Au) on the y-axis (0.00 to 0.25) and wavelength (nm) on the x-axis (200 to 800). The spectrum features two distinct peaks labeled with their wavelengths: 383 nm and 453 nm. The peak at 453 nm is higher than the peak at 383 nm.
Brasilein		Brasilein	8.42	 The PDA spectrum for Brasilein shows absorbance (Au) on the y-axis (0.000 to 0.005) and wavelength (nm) on the x-axis (200 to 800). The spectrum features three distinct peaks labeled with their wavelengths: 222 nm, 280 nm, and 449 nm. The peak at 222 nm is the highest, followed by 449 nm, and 280 nm.
Lac dye	75450	Laccaic acid E	8.46	 The PDA spectrum for Laccaic acid E shows absorbance (Au) on the y-axis (0.00 to 0.07) and wavelength (nm) on the x-axis (200 to 800). The spectrum features two distinct peaks labeled with their wavelengths: 287 nm and 490 nm. The peak at 287 nm is significantly higher than the peak at 490 nm.

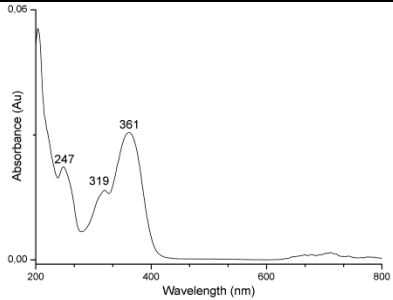
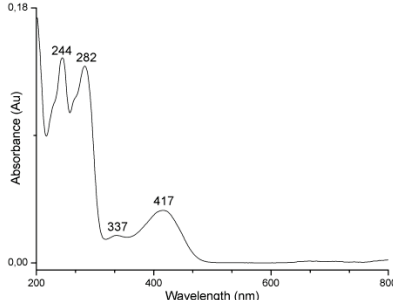
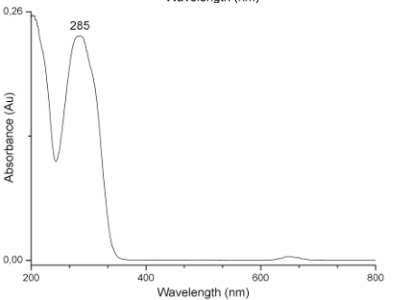
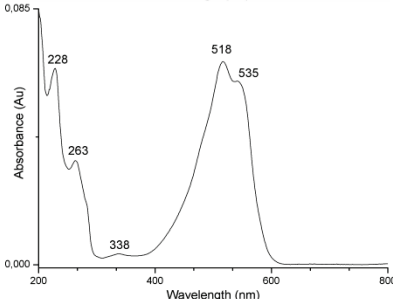
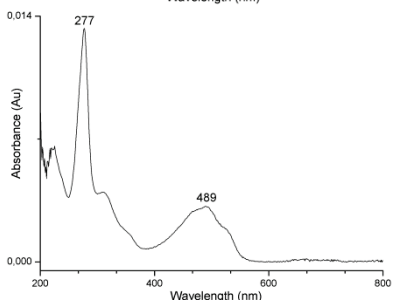
Reference samples	Colour Index number	Main compound detected	Retention time (min)	PDA spectra
Luteolin-3', 7-diglycoside		Luteolin-3', 7-diglycoside	9.74	 Absorbance (Au) vs Wavelength (nm) for Luteolin-3', 7-diglycoside. The spectrum shows a sharp peak at 268 nm and a broader peak at 342 nm. The y-axis ranges from 0.00 to 0.36, and the x-axis ranges from 200 to 800 nm.
Brasilein		Anhydro brasilein equivalent	10.16	 Absorbance (Au) vs Wavelength (nm) for Anhydro brasilein equivalent. The spectrum shows a small peak at 274 nm and a large, broad peak at 446 nm. The y-axis ranges from 0.0000 to 0.0075, and the x-axis ranges from 200 to 800 nm.
American cochineal (<i>D. coccus</i>)	75470	dcII (flavokermesic acid glycoside)	10.43	 Absorbance (Au) vs Wavelength (nm) for dcII (flavokermesic acid glycoside). The spectrum shows a peak at 288 nm and a smaller peak at 437 nm. The y-axis ranges from 0.000 to 0.016, and the x-axis ranges from 200 to 800 nm.
American cochineal (<i>D. coccus</i>)	75470	Carminic acid	10.94	 Absorbance (Au) vs Wavelength (nm) for Carminic acid. The spectrum shows a peak at 276 nm and a broad peak at 494 nm. The y-axis ranges from 0.0 to 0.2, and the x-axis ranges from 200 to 800 nm.
Purpur		Mono bromo isatin	11.26	 Absorbance (Au) vs Wavelength (nm) for Mono bromo isatin. The spectrum shows three distinct peaks at 254 nm, 308 nm, and 398 nm. The y-axis ranges from 0.0000 to 0.0035, and the x-axis ranges from 200 to 800 nm.

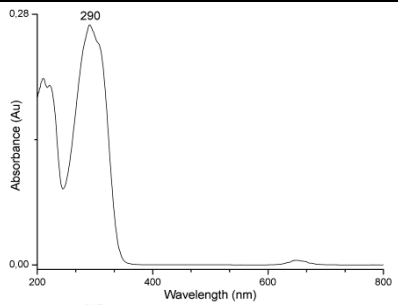
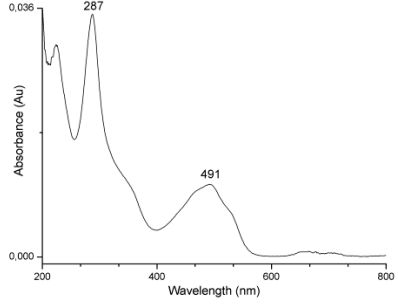
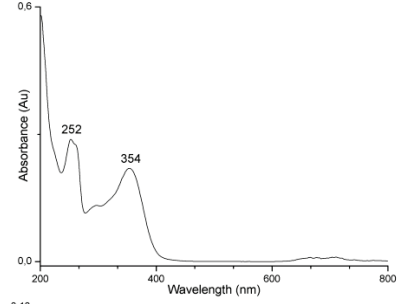
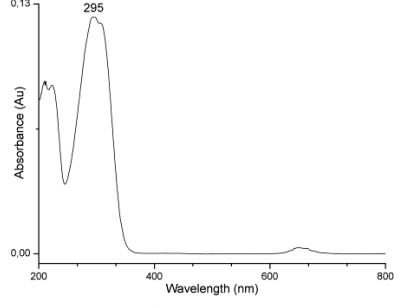
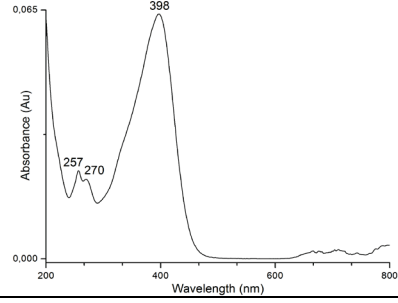
Reference samples	Colour Index number	Main compound detected	Retention time (min)	PDA spectra
Lawson	75480	Lawson	11.47	 The PDA spectrum for Lawson shows absorbance (Au) on the y-axis (0.0 to 0.4) and wavelength (nm) on the x-axis (200 to 800). The spectrum features three distinct peaks labeled with their wavelengths: 249 nm (highest absorbance), 280 nm, and 338 nm. The absorbance decreases significantly after 400 nm.
Genistin		Genistin	11.75	 The PDA spectrum for Genistin shows absorbance (Au) on the y-axis (0.00 to 0.35) and wavelength (nm) on the x-axis (200 to 800). The spectrum features two main peaks labeled with their wavelengths: 259 nm and 326 nm. The absorbance is highest at 259 nm and decreases towards longer wavelengths.
Luteolin-7-O- β -D-glucoside		Luteolin-7-O- β -D-glucoside	12.37	 The PDA spectrum for Luteolin-7-O-β-D-glucoside shows absorbance (Au) on the y-axis (0.000 to 0.085) and wavelength (nm) on the x-axis (200 to 800). The spectrum features three peaks labeled with their wavelengths: 255 nm, 257 nm, and 349 nm. The absorbance is highest at 255 nm and decreases after 400 nm.
Purpurin	75410	Purpurin equivalent	13.12	 The PDA spectrum for Purpurin shows absorbance (Au) on the y-axis (0.000 to 0.022) and wavelength (nm) on the x-axis (200 to 800). The spectrum features three peaks labeled with their wavelengths: 257 nm, 274 nm, and 481 nm. The absorbance is highest at 257 nm and shows a broad shoulder around 481 nm.
Madder		Ruberythric acid	13.258	 The PDA spectrum for Madder shows absorbance (Au) on the y-axis (0.00 to 0.22) and wavelength (nm) on the x-axis (200 to 800). The spectrum features three peaks labeled with their wavelengths: 259 nm, 334 nm, and 414 nm. The absorbance is highest at 259 nm and decreases after 400 nm.

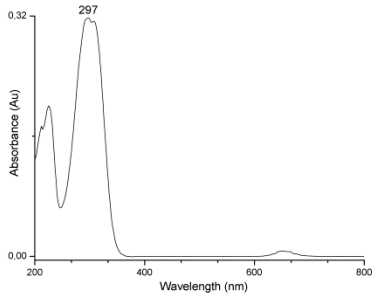
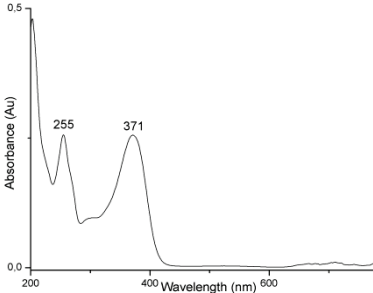
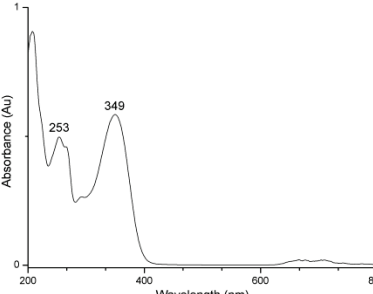
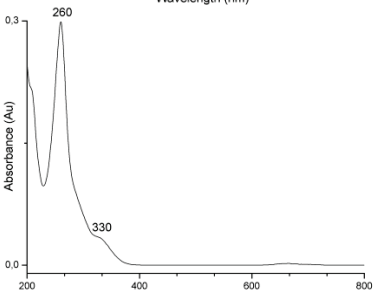
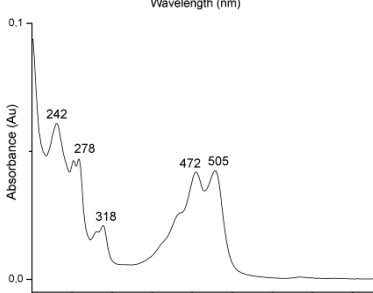
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Madder		Lucidin-3-O-primeveroside	13.483	 0.1 Absorbance (Au) 0.0 200 400 600 800 Wavelength (nm)
Ellagic acid		Ellagic acid	13.62	 0.55 Absorbance (Au) 0.00 200 400 600 800 Wavelength (nm)
Rutin	75730	Rutin	13.67	 1.2 Absorbance (Au) 0.0 200 400 600 800 Wavelength (nm)
Hyperoside		Hyperoside	14.02	 0.5 Absorbance (Au) 0.0 200 400 600 800 Wavelength (nm)
Juglon	755500	Juglon	14.03	 1.4 Absorbance (Au) 0.0 200 400 600 800 Wavelength (nm)

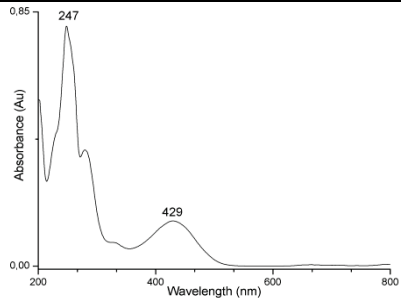
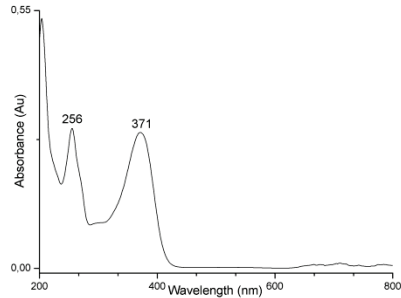
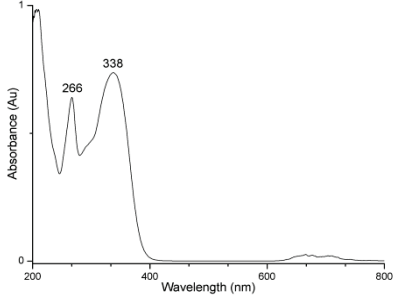
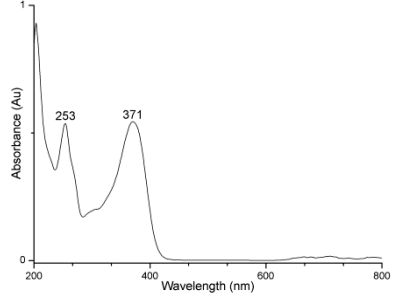
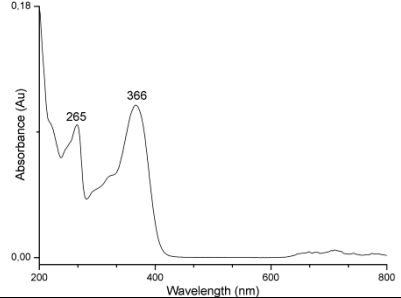
Reference samples	Colour Index number	Main compound detected	Retention time (min)	PDA spectra
Isoquercetin		Isoquercetin	14.14	
Apigenin-7-glycoside		Apigenin-7-glucoside	14.41	
Crocin		Crocin	14.51	
Redwood		Type C compound	14.51	
Redwood		Type C compound equivalent	14.62	

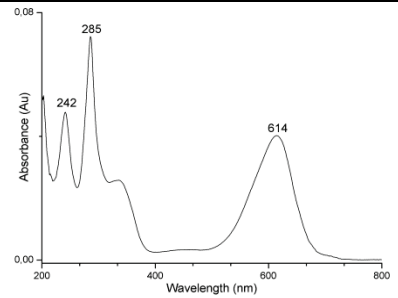
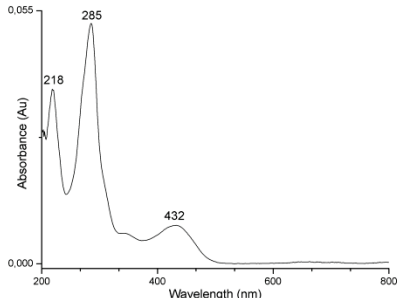
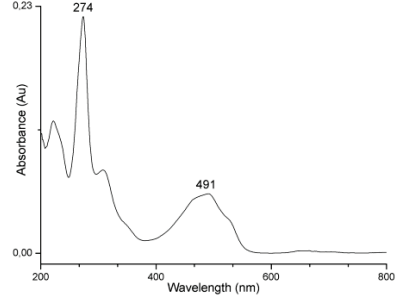
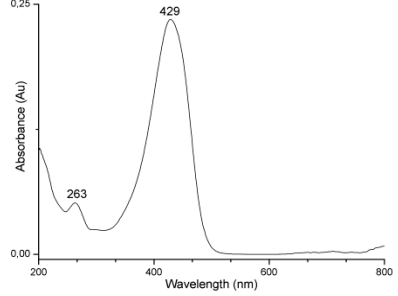
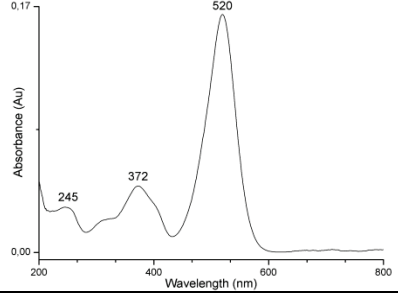
Reference samples	Colour Index number	Main compound detected	Retention time (min)	PDA spectra
Madder		Rubiadin-3-O-primeveroside	15.62	 Absorbance (Au) vs Wavelength (nm) for Rubiadin-3-O-primeveroside. The spectrum shows three distinct peaks: a small peak at 245 nm, a slightly higher peak at 282 nm, and a broad peak at 416 nm. The y-axis ranges from 0.00 to 0.17, and the x-axis ranges from 200 to 800 nm.
Quercitrin		Quercitrin	15.70	 Absorbance (Au) vs Wavelength (nm) for Quercitrin. The spectrum shows two main peaks: a sharp peak at 256 nm and a broader peak at 349 nm. The y-axis ranges from 0.00 to 1.15, and the x-axis ranges from 200 to 800 nm.
American cochineal (<i>D. coccus</i>)	75470	dcVI (carminic acid glycoside)	15.73	 Absorbance (Au) vs Wavelength (nm) for dcVI (carminic acid glycoside). The spectrum shows two peaks: a sharp peak at 277 nm and a broad peak at 493 nm. The y-axis ranges from 0.00 to 0.03, and the x-axis ranges from 200 to 800 nm.
Orcein		α or β hydroxyl orcein	15.99	 Absorbance (Au) vs Wavelength (nm) for α or β hydroxyl orcein. The spectrum shows three peaks: a small peak at 278 nm, a broad peak at 522 nm, and a smaller peak at 548 nm. The y-axis ranges from 0.00 to 0.11, and the x-axis ranges from 200 to 800 nm.
Myricetin		Myricetin	16.06	 Absorbance (Au) vs Wavelength (nm) for Myricetin. The spectrum shows three peaks: a small peak at 252 nm, a broad peak at 373 nm, and a very small peak at 521 nm. The y-axis ranges from 0.0 to 0.8, and the x-axis ranges from 200 to 800 nm.

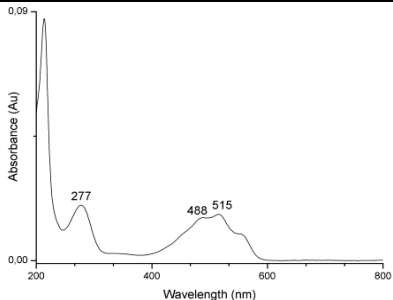
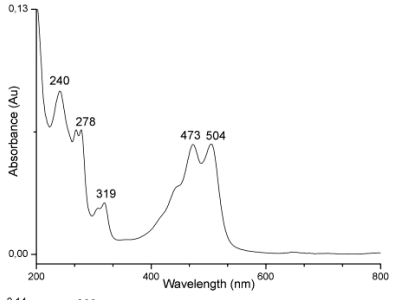
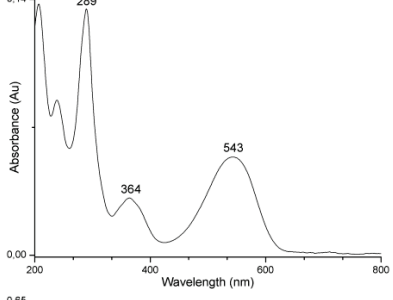
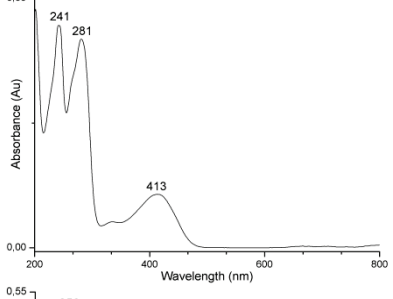
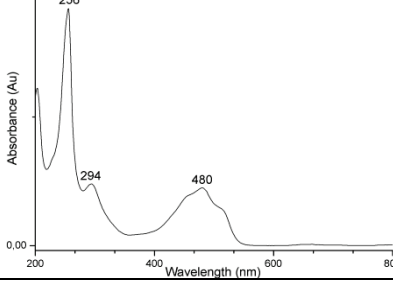
Reference samples	Colour Index number	Main compound detected	Retention time (min)	PDA spectra
Fisetin	75620	Fisetin	16.19	 0.05 Absorbance (Au) 247 319 361 0.00 200 400 600 800 Wavelength (nm)
Munjistin	75370	Munjistin	16.27	 0.18 Absorbance (Au) 244 282 337 417 0.00 200 400 600 800 Wavelength (nm)
Safflower		CTO safflower related	16.22	 0.26 Absorbance (Au) 285 0.00 200 400 600 800 Wavelength (nm)
Orcein		α or β hydroxyl orcein (2nd compound)	16.45	 0.085 Absorbance (Au) 228 263 338 518 535 0.000 200 400 600 800 Wavelength (nm)
American cochineal (<i>D. coccus</i>)	75470	dcVII (carminic acid glycoside)	16.49	 0.014 Absorbance (Au) 277 489 0.000 200 400 600 800 Wavelength (nm)

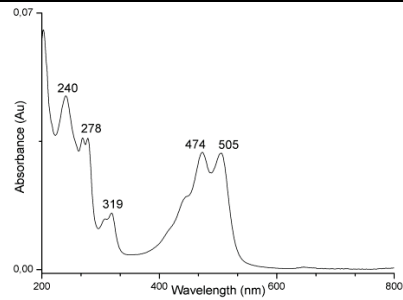
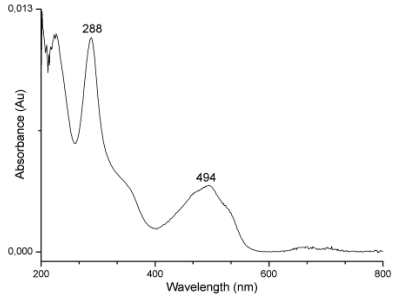
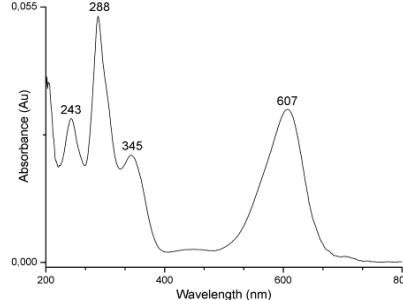
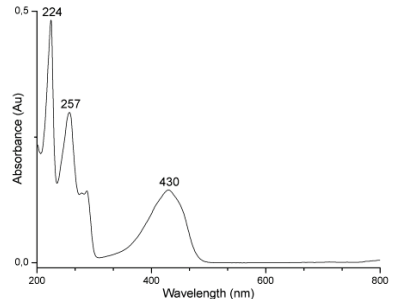
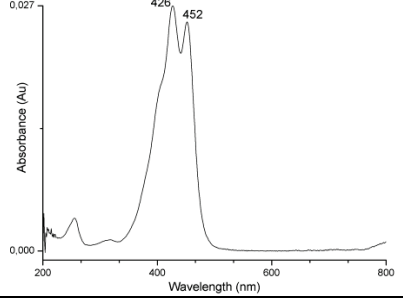
Reference samples	Colour Index number	Main compound detected	Retention time (min)	PDA spectra
Safflower		CT1 safflower related	16.84	
Lac dye	75450	Laccaic acid B	16.97	
Morin	75660	Morin	17.04	
Safflower		CT2 safflower related	16.99	
Fisetin	75620	Sulfuretin	17.31	

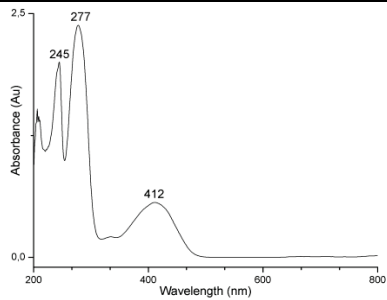
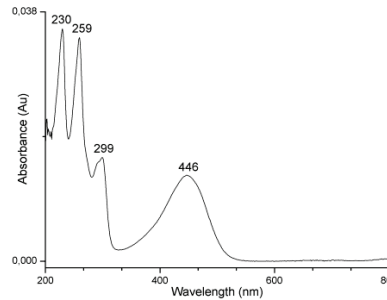
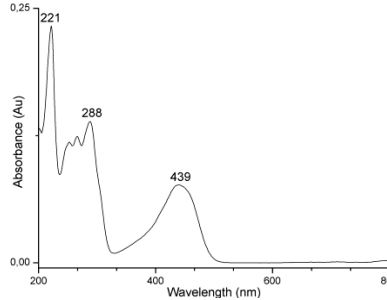
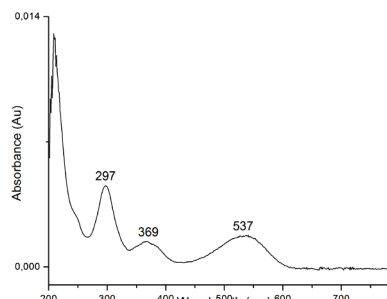
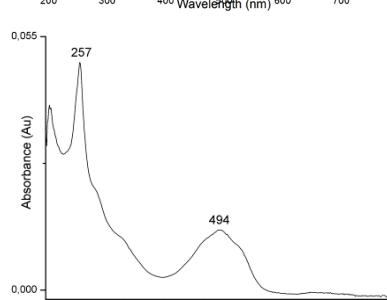
Reference samples	Colour Index number	Main compound detected	Retention time (min)	PDA spectra
Safflower		CT3 safflower related	17.56	 Absorbance (Au) vs Wavelength (nm) for Safflower. The spectrum shows a sharp peak at 297 nm with an absorbance of approximately 0.32. There is a smaller peak around 220 nm and a very low absorbance at higher wavelengths up to 800 nm.
Quercetin	75670	Quercetin	18.56	 Absorbance (Au) vs Wavelength (nm) for Quercetin. The spectrum shows a peak at 255 nm (absorbance ~0.4) and a broader peak at 371 nm (absorbance ~0.3). Absorbance drops significantly after 400 nm.
Luteolin	75660	Luteolin	18.79	 Absorbance (Au) vs Wavelength (nm) for Luteolin. The spectrum shows a peak at 253 nm (absorbance ~0.8) and a broader peak at 349 nm (absorbance ~0.6). Absorbance drops significantly after 400 nm.
Genistein	75610	Genistein	19.07	 Absorbance (Au) vs Wavelength (nm) for Genistein. The spectrum shows a peak at 260 nm (absorbance ~0.3) and a smaller peak at 330 nm (absorbance ~0.1). Absorbance drops significantly after 400 nm.
Santalin		Santalin A	20.32	 Absorbance (Au) vs Wavelength (nm) for Santalin A. The spectrum shows multiple peaks: 242 nm (absorbance ~0.08), 278 nm (absorbance ~0.05), 318 nm (absorbance ~0.02), 472 nm (absorbance ~0.05), and 505 nm (absorbance ~0.05). Absorbance is higher in the 200-400 nm range and lower in the 400-800 nm range.

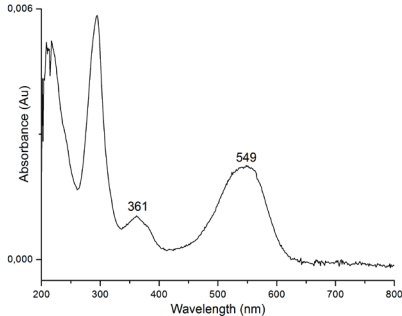
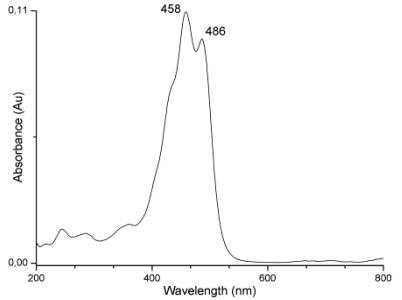
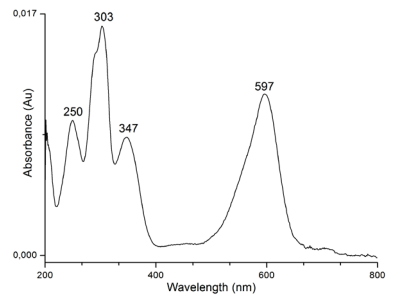
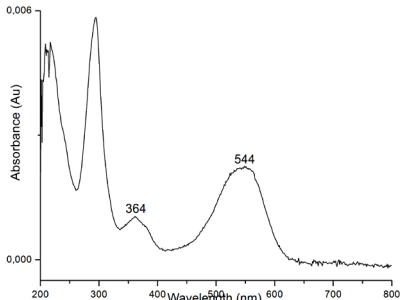
Reference samples	Colour Index number	Main compound detected	Retention time (min)	PDA spectra
Alizarin	75330	Alizarin	20.59	
Rhamnetin	75690	Rhamnetin	20.78	
Apigenin	75580	Apigenin	20.85	
Isorhamnetin		Isorhamnetin	21.1	
Kampferol	75640	Kampferol	21.20	

Reference samples	Colour Index number	Main compound detected	Retention time (min)	PDA spectra
Indigotin	73000	Indigotin	21.7	 The PDA spectrum for Indigotin shows absorbance (Au) on the y-axis (0.00 to 0.08) and wavelength (nm) on the x-axis (200 to 800). The spectrum features three distinct peaks: a small peak at 242 nm, a larger peak at 285 nm, and a broad peak at 614 nm.
Kermes (<i>K. vermillio</i>)	75460	Flavokermesic acid	21.85	 The PDA spectrum for Flavokermesic acid shows absorbance (Au) on the y-axis (0.000 to 0.055) and wavelength (nm) on the x-axis (200 to 800). The spectrum features three distinct peaks: a small peak at 218 nm, a larger peak at 285 nm, and a broad peak at 432 nm.
Kermes (<i>K. vermillio</i>)	75460	Kermesic acid	22.18	 The PDA spectrum for Kermesic acid shows absorbance (Au) on the y-axis (0.00 to 0.23) and wavelength (nm) on the x-axis (200 to 800). The spectrum features two distinct peaks: a small peak at 274 nm and a broad peak at 491 nm.
Curcuma (turmeric)	75300	Curcumin	22.88	 The PDA spectrum for Curcumin shows absorbance (Au) on the y-axis (0.00 to 0.25) and wavelength (nm) on the x-axis (200 to 800). The spectrum features two distinct peaks: a small peak at 263 nm and a broad peak at 429 nm.
Safflower (<i>Carthamus</i>)	75140	Carthamin	23.24	 The PDA spectrum for Carthamin shows absorbance (Au) on the y-axis (0.00 to 0.17) and wavelength (nm) on the x-axis (200 to 800). The spectrum features three distinct peaks: a small peak at 245 nm, a broad peak at 372 nm, and a large peak at 520 nm.

Reference samples	Colour Index number	Main compound detected	Retention time (min)	PDA spectra
Alkanin	75530	Alkanin	23.26	 Absorbance (Au) Wavelength (nm) 277 488 515
Santalin		Santalin B	23.27	 Absorbance (Au) Wavelength (nm) 240 278 319 473 504
Indigotin	73000	Indirubin	23.93	 Absorbance (Au) Wavelength (nm) 289 364 543
Xanthopurpurin	75340	Xanthopurpurin	24.02	 Absorbance (Au) Wavelength (nm) 241 281 413
Purpurin	58205	Purpurin	24.32	 Absorbance (Au) Wavelength (nm) 256 294 480

Reference samples	Colour Index number	Main compound detected	Retention time (min)	PDA spectra
Santalin		Santalin C	24.8	 Absorbance (Au) vs Wavelength (nm) for Santalin C. The spectrum shows several peaks, with the most prominent ones labeled at 240, 278, 319, 474, and 505 nm. The y-axis ranges from 0.00 to 0.07, and the x-axis ranges from 200 to 800 nm.
Lac dye	75450	Laccaic acid A	25.15	 Absorbance (Au) vs Wavelength (nm) for Laccaic acid A. The spectrum shows two main peaks labeled at 288 and 494 nm. The y-axis ranges from 0.000 to 0.013, and the x-axis ranges from 200 to 800 nm.
Purpur		6-monobromoindigotin	26.27	 Absorbance (Au) vs Wavelength (nm) for 6-monobromoindigotin. The spectrum shows four labeled peaks at 243, 288, 345, and 607 nm. The y-axis ranges from 0.000 to 0.055, and the x-axis ranges from 200 to 800 nm.
Chrysophanic acid		Chrysophanol	26.32	 Absorbance (Au) vs Wavelength (nm) for Chrysophanol. The spectrum shows three labeled peaks at 224, 257, and 430 nm. The y-axis ranges from 0.0 to 0.5, and the x-axis ranges from 200 to 800 nm.
Crocetin		Crocetin	26.47	 Absorbance (Au) vs Wavelength (nm) for Crocetin. The spectrum shows two labeled peaks at 426 and 452 nm. The y-axis ranges from 0.000 to 0.027, and the x-axis ranges from 200 to 800 nm.

Reference samples	Colour Index number	Main compound detected	Retention time (min)	PDA spectra
Rubiadin		Rubiadin	26.72	 The PDA spectrum for Rubiadin shows absorbance (Au) on the y-axis (0.0 to 2.5) and wavelength (nm) on the x-axis (200 to 800). The spectrum features three distinct peaks: a small peak at 245 nm, a major peak at 277 nm, and a smaller peak at 412 nm.
Morinda citrifolia	75380	Morindone	27.54	 The PDA spectrum for Morindone shows absorbance (Au) on the y-axis (0.000 to 0.038) and wavelength (nm) on the x-axis (200 to 800). The spectrum features four peaks: a small peak at 230 nm, a major peak at 259 nm, a smaller peak at 299 nm, and a broad peak at 446 nm.
Emodin	75440	Emodin	27.81	 The PDA spectrum for Emodin shows absorbance (Au) on the y-axis (0.00 to 0.25) and wavelength (nm) on the x-axis (200 to 800). The spectrum features three peaks: a major peak at 221 nm, a smaller peak at 288 nm, and a broad peak at 439 nm.
Purpur		6 - mono bromo indirubin	28.01	 The PDA spectrum for 6-mono bromo indirubin shows absorbance (Au) on the y-axis (0.000 to 0.014) and wavelength (nm) on the x-axis (200 to 800). The spectrum features three peaks: a small peak at 297 nm, a smaller peak at 369 nm, and a broad peak at 537 nm.
Pseudo-purpurin		Pseudo-purpurin	28.68	 The PDA spectrum for Pseudo-purpurin shows absorbance (Au) on the y-axis (0.000 to 0.055) and wavelength (nm) on the x-axis (200 to 800). The spectrum features two peaks: a major peak at 257 nm and a broad peak at 494 nm.

Reference samples	Colour Index number	Main compound detected	Retention time (min)	PDA spectra
Purpur		mono-bromo- indirubin equivalent	28.68	 Absorbance (Au) vs Wavelength (nm) for mono-bromo-indirubin equivalent. The spectrum shows a small peak at 361 nm and a larger peak at 549 nm. The y-axis ranges from 0.000 to 0.006, and the x-axis ranges from 200 to 800 nm.
Anatto		Bixin	29.37	 Absorbance (Au) vs Wavelength (nm) for Bixin. The spectrum shows two distinct peaks at 458 nm and 486 nm. The y-axis ranges from 0.00 to 0.11, and the x-axis ranges from 200 to 800 nm.
Purpur		6,6-di-bromo- indigotin	29.46	 Absorbance (Au) vs Wavelength (nm) for 6,6-di-bromo-indigotin. The spectrum shows four peaks at 250 nm, 303 nm, 347 nm, and 597 nm. The y-axis ranges from 0.000 to 0.017, and the x-axis ranges from 200 to 800 nm.
Purpur		6,6'-di-bromo- indirubin	30.33	 Absorbance (Au) vs Wavelength (nm) for 6,6'-di-bromo-indirubin. The spectrum shows a small peak at 364 nm and a larger peak at 544 nm. The y-axis ranges from 0.000 to 0.006, and the x-axis ranges from 200 to 800 nm.