

# Degradation products and coloring components of shellfish purple identified by UHPLC-MS/MS

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**Abstract:** Silk sample dyed with shellfish purple were left under artificially accelerated aging with UV radiation ( $\lambda=254$  nm) for 25 days. Standard solutions of indigotin (IND) and 6-bromoindigotin (MBI) and shellfish purple dye were also used to detect degradation products in solutions after 30 days under ambient conditions and coloring components. A new UHPLC-MS/MS method was used to identify the degradation products of shellfish purple aged samples ( $t=25$  days) and four coloring components in the samples before aging ( $t=0$ ). Chromatographic separation in UHPLC method was carried out on an ACQUITY UPLC BEH C18 column (1.7  $\mu\text{m}$ , 2.1 x 1.8 mm). MS was performed using a heated electrospray ion source (HESI). In summary, the four coloring components were identified in shellfish purple samples (shellfish purple dye and dyed silk before and after aging) in small quantities. Isatin, isatoic anhydride (DP3) and other degradation products were also detected in IND and MBI standard solutions as well as in shellfish purple dye and dyed silk samples, which left under artificially accelerated aging.

## Experimental Chromatographic conditions

Time (min)	A (%)	B (%)
0	95	5
3	95	5
13	70	30
19	40	60
22	40	60
37	5	95
37.01	95	5
38	95	5

Gradient elution	A: H <sub>2</sub> O + FA 0.1% (v/v) B: CH <sub>3</sub> CN + FA 0.1% (v/v)
Detection	MS/MS
Flow rate	0.3 mL min <sup>-1</sup>
Temperature	40°C
Injection volume	10µL

### MS/MS conditions

Ionization mode	ESI-	ESI+
Spray voltage (V)	2500	3000
Capillary temperature (°C)	380	380
Vaporizer temperature (°C)	340	340
Sheath gas pressure (Arb)	40	40
Aux. gas pressure (Arb)	10	40
Collision gas (Argon) pressure (mTorr)	1.5	1.5

### Sample preparation

#### Solution preparation

- Weighing of 200 µg of standard compounds
- Dilution in 500µL of a **1.18 M NaOH aqueous solution** and ultrasonication at 30°C
- Addition of 4mL 1:1 water-methanol for 10 min
- Addition of 6mL 1:1 water-methanol at 40°C for 20 min; solution pH 8
- Injection of clear supernatant

#### Sample preparation

- Weighing of 1-1.2 mg of dye and dyed silk
- Dilution in 200µL DMSO, heating at 80°C for 15min
- Injection of clear supernatant

## Results and discussion

Compound	IND		MBI		Shellfish purple		Silk dyed with Shellfish purple		
	FRESH	AGED <sup>1</sup>	FRESH	AGED <sup>1</sup>	FRESH	AGED <sup>1</sup>	FRESH	AGED <sup>2</sup>	AGED <sup>1</sup>
IS		+		+		+			+
DP3		+		+		+			+
DP4		+		+	+	+	+	+	+
DP5		+		+					
DP6						+			
DP7		+		+					
DP9		+							+
DP10		+		+	+	+	+	+	+
Co A					+	+	+		+
Co B					+	+	+		+
Co C					+	+	+		+
Co D					+	+	+	+	+
IND	+	+	+	+	+	+	+	+	+
INR					+	+	+	+	+
MBI			+	+	+	+	+	+	+
6'MBIR					+		+		+
6MBIR					+		+		
DBI					+	+	+	+	+
DBIR					+		+		+

**AGED<sup>1</sup>** Left at room temperature without light for 30 days; **AGED<sup>2</sup>** Artificially accelerated aging with UV radiation (homemade chamber: four 25 W UV lamps at 250 nm, 25 days, 30 cm distance between the fabrics and the lamps)

## Acknowledgements

This research has been financially supported by the General Secretariat for Research and Technology (GSRT) and the Hellenic Foundation for Research and Innovation (HFRI), Scholarship Code: 1514 (16<sup>th</sup>).

## Conclusions

- Better ionization of standard solutions of coloring components.
- Degradation products of indigotin were detected in shellfish purple and in standard solutions of indigotin and 6-bromoindigotin.
- The four colourants of shellfish purple which are not often detected, were identified in both shellfish dye and in the silk fabric dyed with shellfish purple.

## References

- [1] Witkos<sup>''</sup> K, Lech K, Jarosz M. Identification of degradation products of indigoids by tandem mass spectrometry. *Journal of Mass Spectrometry* 2015;50:1245–51.
- [2] Surowiec I, Nowik W, Moritz T. Mass spectrometric identification of new minor indigoids in shellfish purple dye from *Hexaplex trunculus*. *Dyes and Pigments* 2012;94:363–9.