

# Coloring the future with sustainable dye extraction from biomass

**Authors:** Chenwah Chreitah  
**Lectorate:** Biobased Building Blocks& products **Research Group/ Project:** Smart Fermentation - TUFUCOL  
**Contact information:** j.meijer8@avans.nl – esgm.mattheussens@avans.nl, Jasper Meijer & Eric Mattheussens  
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## Objective

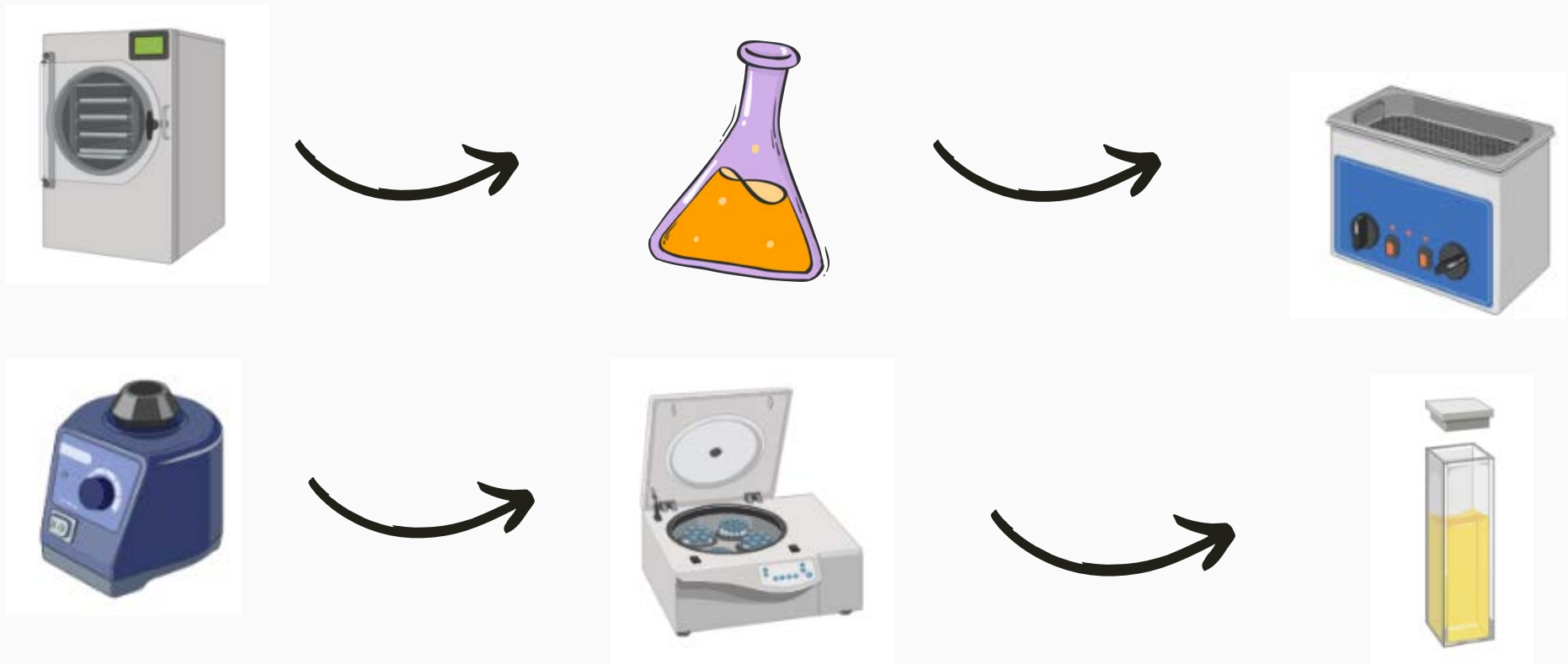
Investigating more sustainable methods for the extraction of natural dyes from biomass, utilizing a short-duration extraction process and environmentally friendly solvents, with the aim of reducing the environmental impact of de applied solvents and optimizing the extraction process. This more sustainable method can contribute to fungal colourants become an alternative to fossil based dyes.



## Method

### Extraction method

The wet biomass was first freeze-dried and then finely ground into a homogeneous powder. Approximately 1.0 g of biomass was accurately weighed and extracted using ethanol (70% or 96%) at various biomass-to-solvent ratios (1:5 to 1:50 g/ml). Samples were treated in an ultrasonic bath for 15 minutes, followed by shaking for 15, 30, or 45 minutes. After centrifugation (7000–10000 rcf, 15 minutes), the supernatant was carefully collected and analyzed using UV-Vis spectrophotometry. Both the remaining residue and extracts were stored under refrigerated conditions for further analysis.



### Analytical method

The analysis was performed using an isocratic HPLC system with a mobile phase consisting of acetonitrile and 2% acetic acid (75:25, v/v) at a flow rate of 1.0 mL/min. The column temperature was maintained at 24°C, and detection was carried out at 250 and 450 nm. Samples were filtered through a 0.45 µm nylon membrane prior to injection, with an injection volume of 20 µL. The total analysis time per sample was 12 minutes.



## Current Results



Figure 1: Dye extract in 70% ethanol (light yellow) and 96% ethanol (dark orange).

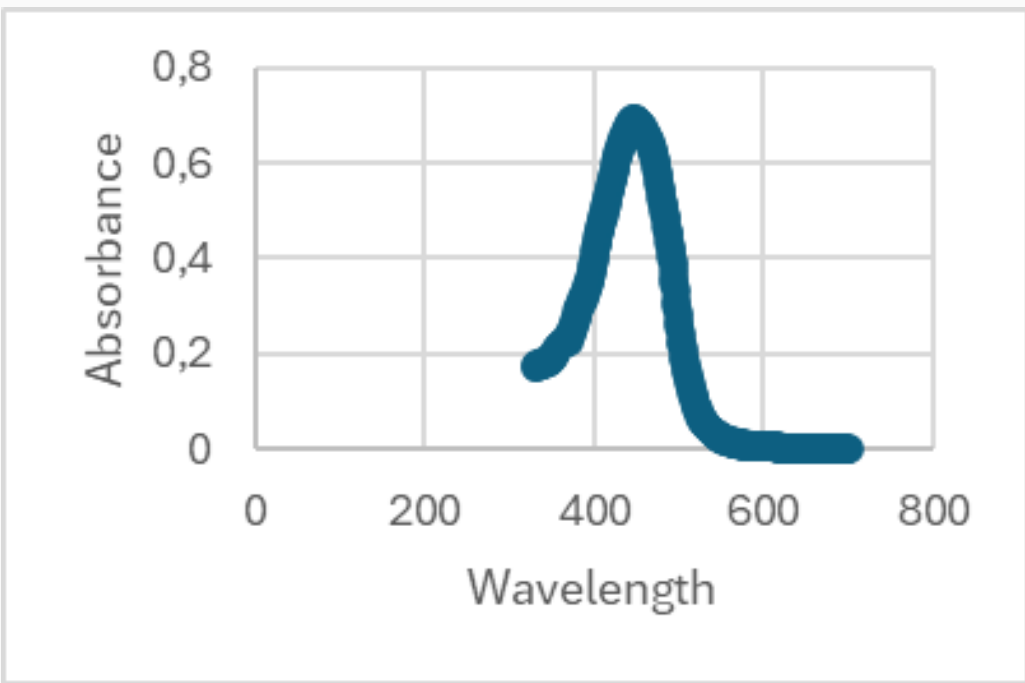


Figure 2: Spectrophotometric measurement of the sample extracted with ethanol in a 1:5 ratio.

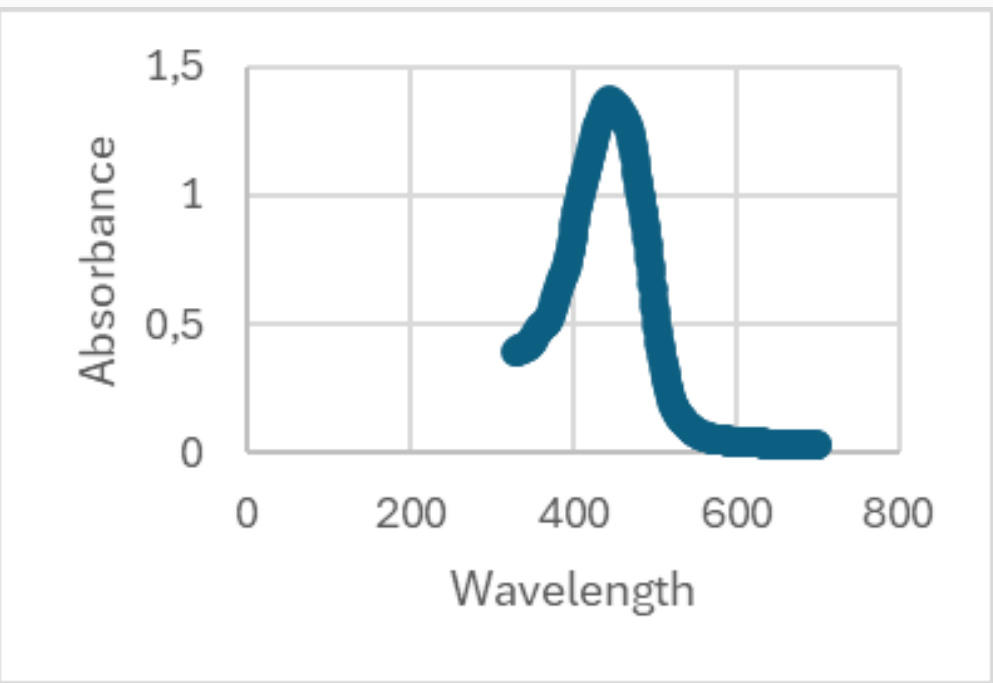


Figure 3: Spectrophotometric measurement of the sample extracted with ethanol in a 1:50 ratio.

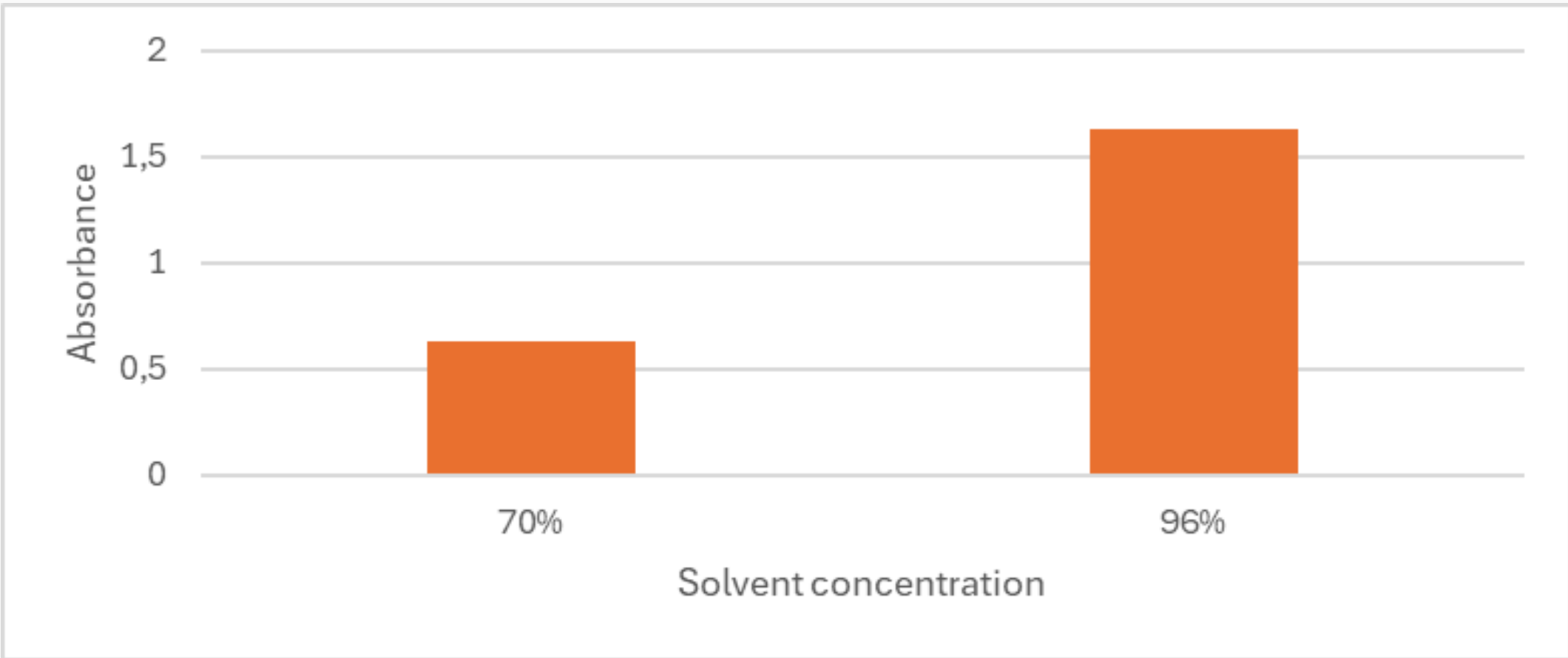


Figure 4: Graph showing absorption during extraction with 70% and 96% ethanol.

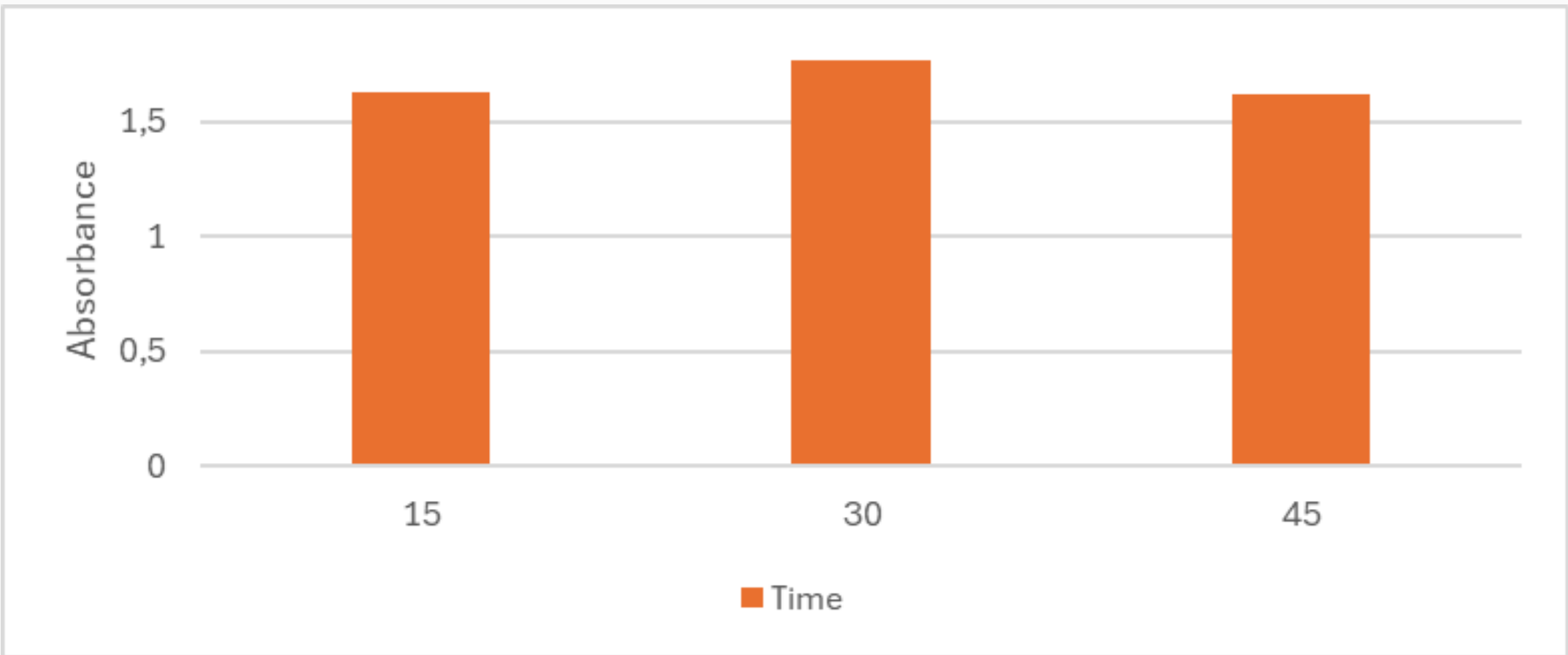


Figure 5: Graph showing the three different extraction times: 15, 30, and 45 minutes.

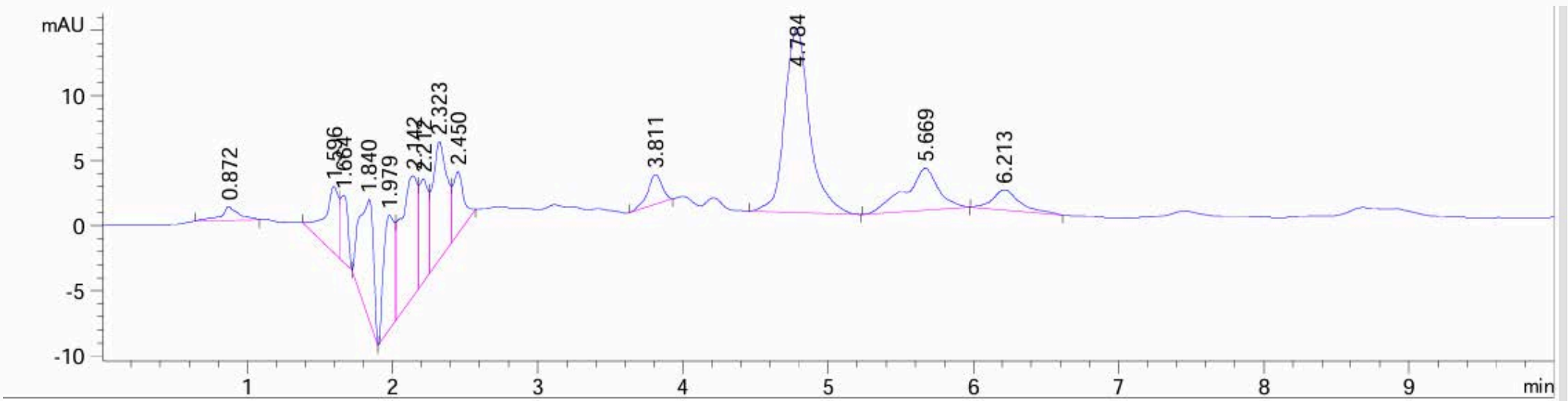


Figure 6: HPLC results showing that the retention time of the extracted sample starts at 3.8 minutes and indicating that the dye consists of four different molecules.



## Conclusion

Spectrophotometer measurements show a clear absorption peak at 445 nm, corresponding to the expected absorption of the target dye. The study demonstrates that a higher solvent-to-sample ratio, in this case 1:50, results in more efficient dye extraction. This effect is further enhanced when using ethanol at a higher concentration of 96% instead of 70%. These results indicate that higher solvent concentration improves the extraction process. Regarding extraction time, the study shows no significant difference between 15, 30, and 45 minutes, suggesting that shorter extraction times are sufficient for optimal yield. HPLC analysis highlights that spectrophotometry alone is insufficient for full dye characterization. The HPLC results reveal that the dye consists of four different molecules, indicating a more complex composition than what was suggested by the spectrophotometer data.

I would like to express my sincere gratitude to my supervisors Jasper Meijer and Eric Mattheussens