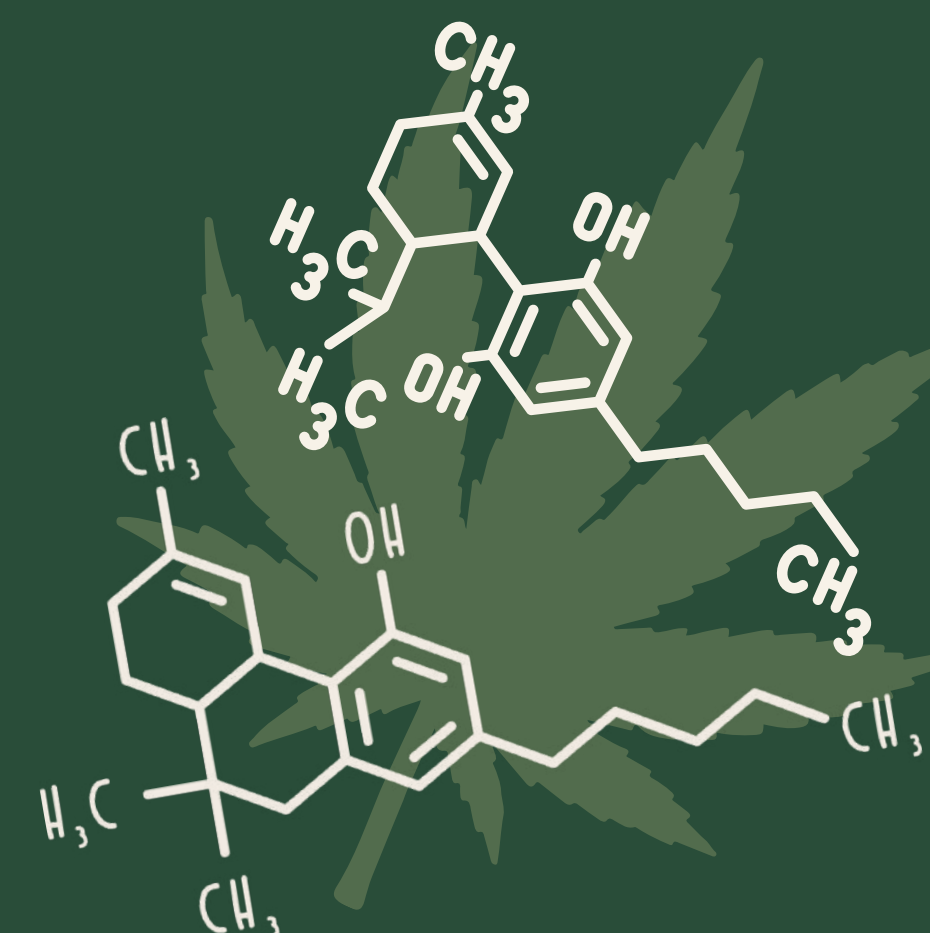


# Assessment of the 4-Aminophenol colorimetric method to differentiate between cannabidiol-rich and $\Delta^9$ -tetrahydrocannabinol-rich cannabis plants

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## Introduction

Portugal is currently the second-largest producer of medical cannabis in the EU, with a growing industrial hemp sector focused on textiles, animal feed, and Cannabidiol (CBD) rich extracts. Legal distinction between hemp and medical cannabis relies on  $\Delta^9$ -Tetrahydrocannabinol ( $\Delta^9$ -THC) content, with a 0.2% (w/w) threshold in dry plant material.<sup>[1]</sup>

Effective differentiation between CBD and  $\Delta^9$ -THC *Cannabis sativa* L. varieties is crucial for regulatory compliance and product control. Among colorimetric methods, only the 4-aminophenol (4-AP) assay distinguishes both cannabinoids through the formation of distinct chromophores—pink for CBD and blue for  $\Delta^9$ -THC.<sup>[2]</sup>

However, oxidation of excess 4-AP over time compromises chromophore stability. This study investigates the influence of 4-AP:cannabinoid stoichiometry on chromophore stability over 24 hours, highlighting the assay's reliability as a rapid, short-term screening tool for cannabinoid profiling.<sup>[3]</sup>

## Aims

Test the effectiveness of the 4-AP assay in distinguishing CBD from  $\Delta^9$ -THC.

Define optimal conditions for fast and reliable detection.

Evaluate chromophore stability over 24 hours.

Analyze the impact of 4-AP/cannabinoid ratios on the reaction.



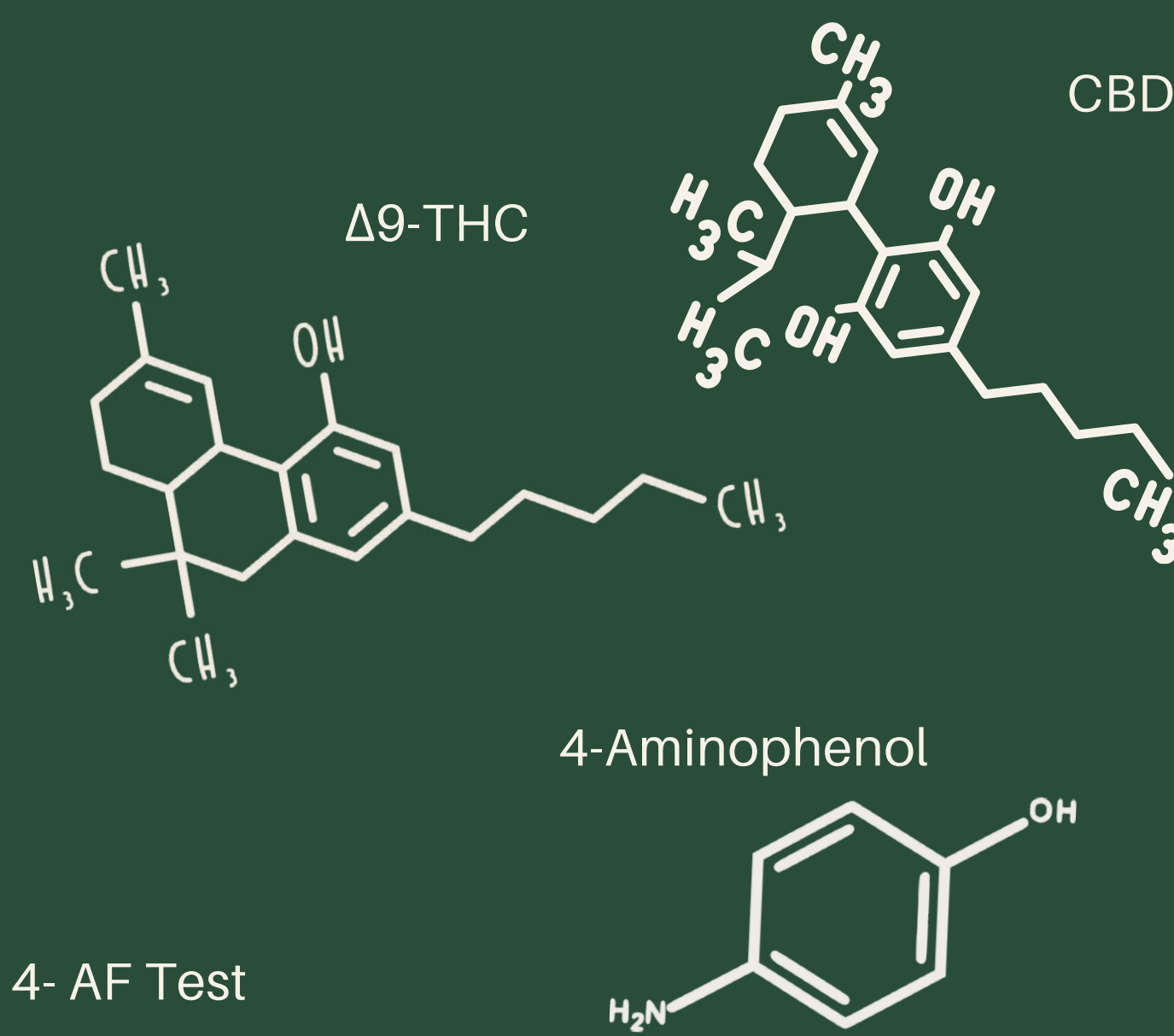
## Materials and Methods

### Reagents

- $\Delta^9$ -THC Solution
- CBD Solution
- 4-AF Reagent (Sigma-Aldrich®)

### Instruments

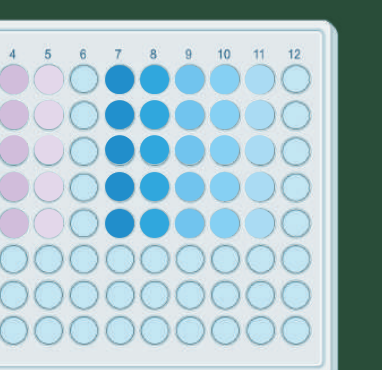
- Tecan Infinity 200 Pro (Tecan®)



4- AF Test

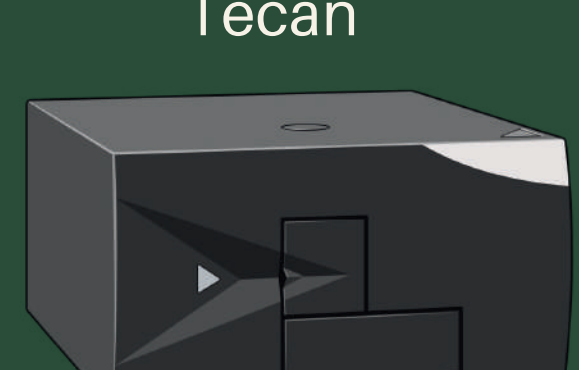


70  $\mu$ l



24 h

Tecan



## Results

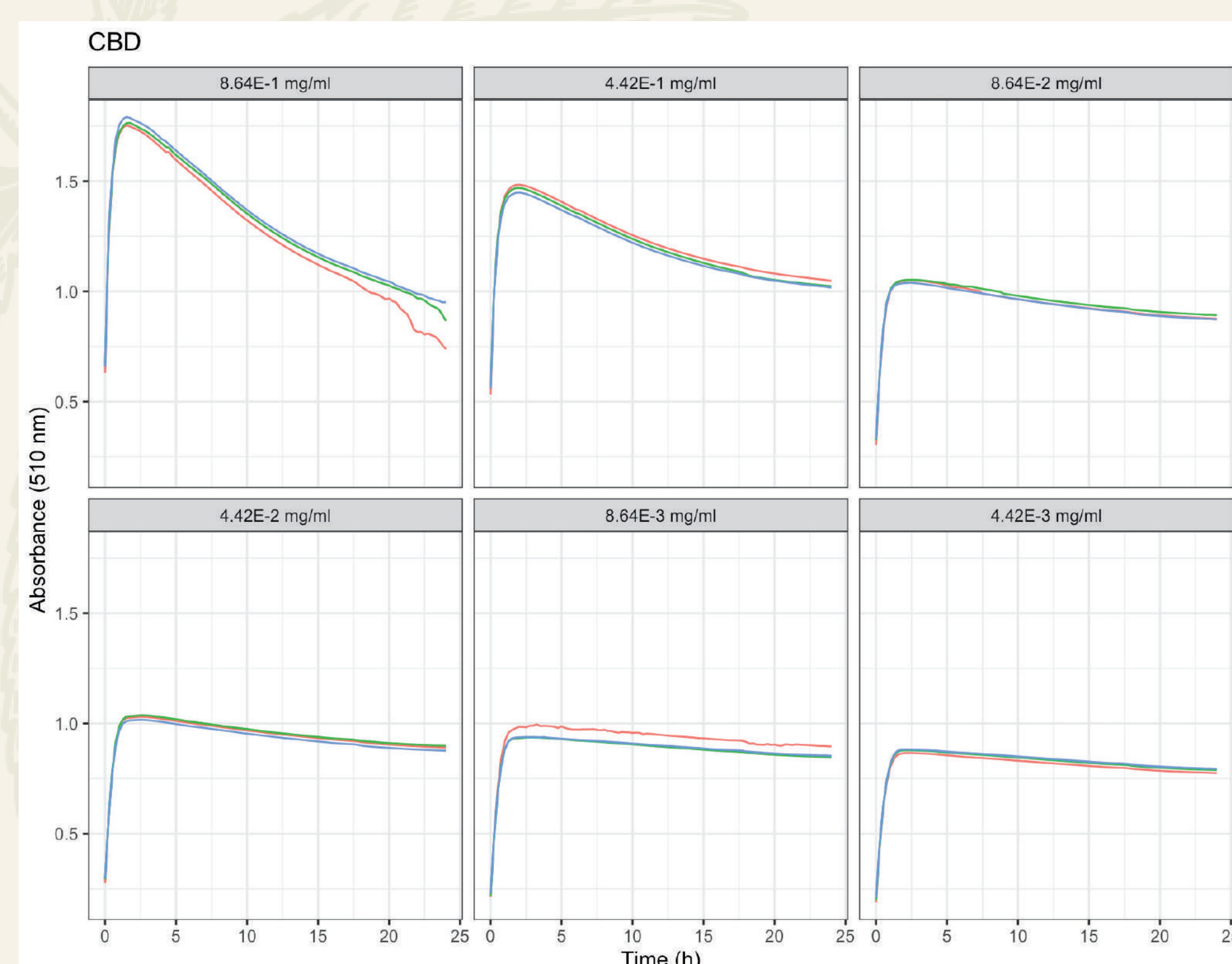


Fig. 2- Kinetics of the reaction between CBD and 4-AP at 510 nm over 24 hours.

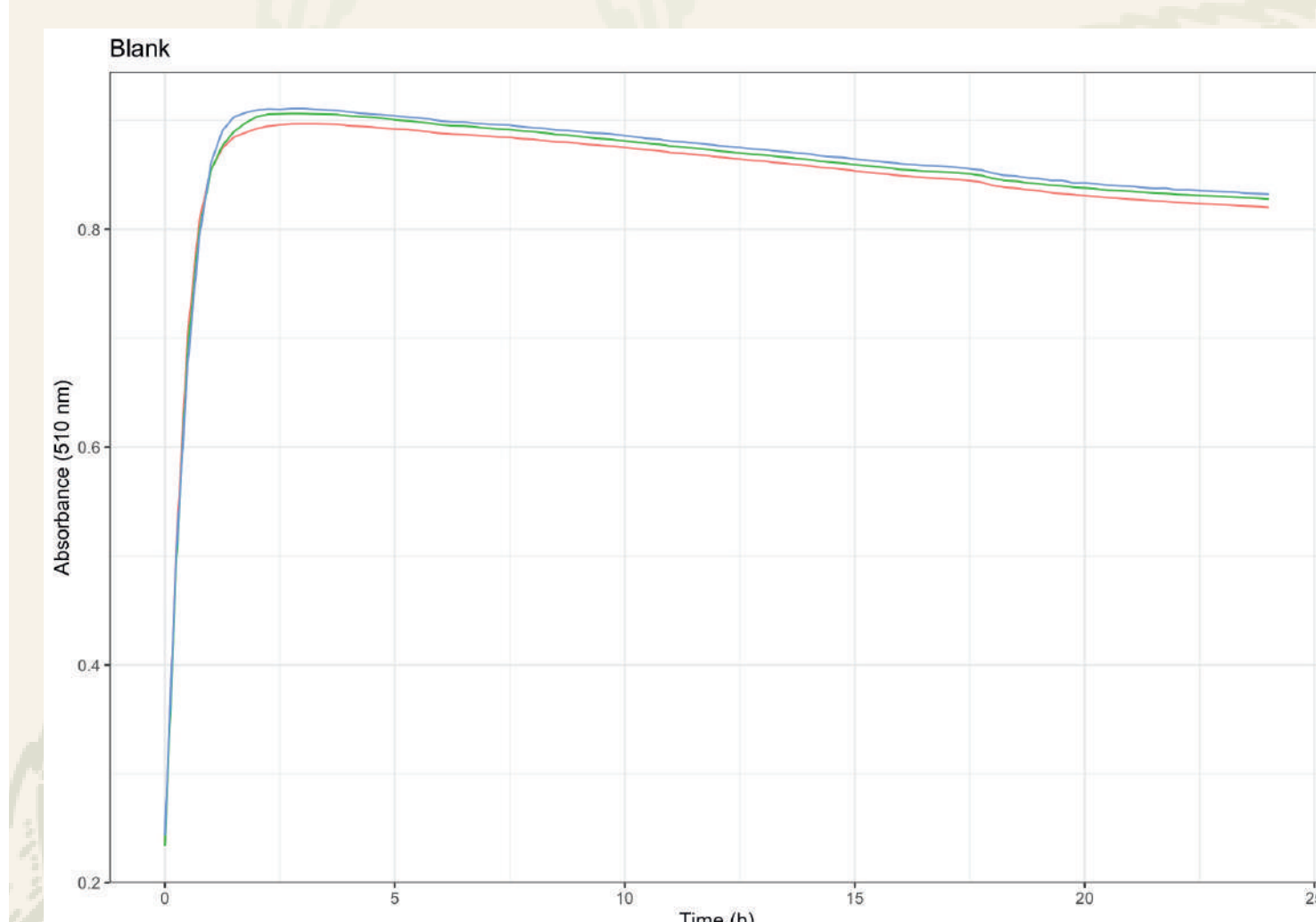


Fig. 3- Kinetics of 4-AP Oxidation at 510 nm over 24 hours.

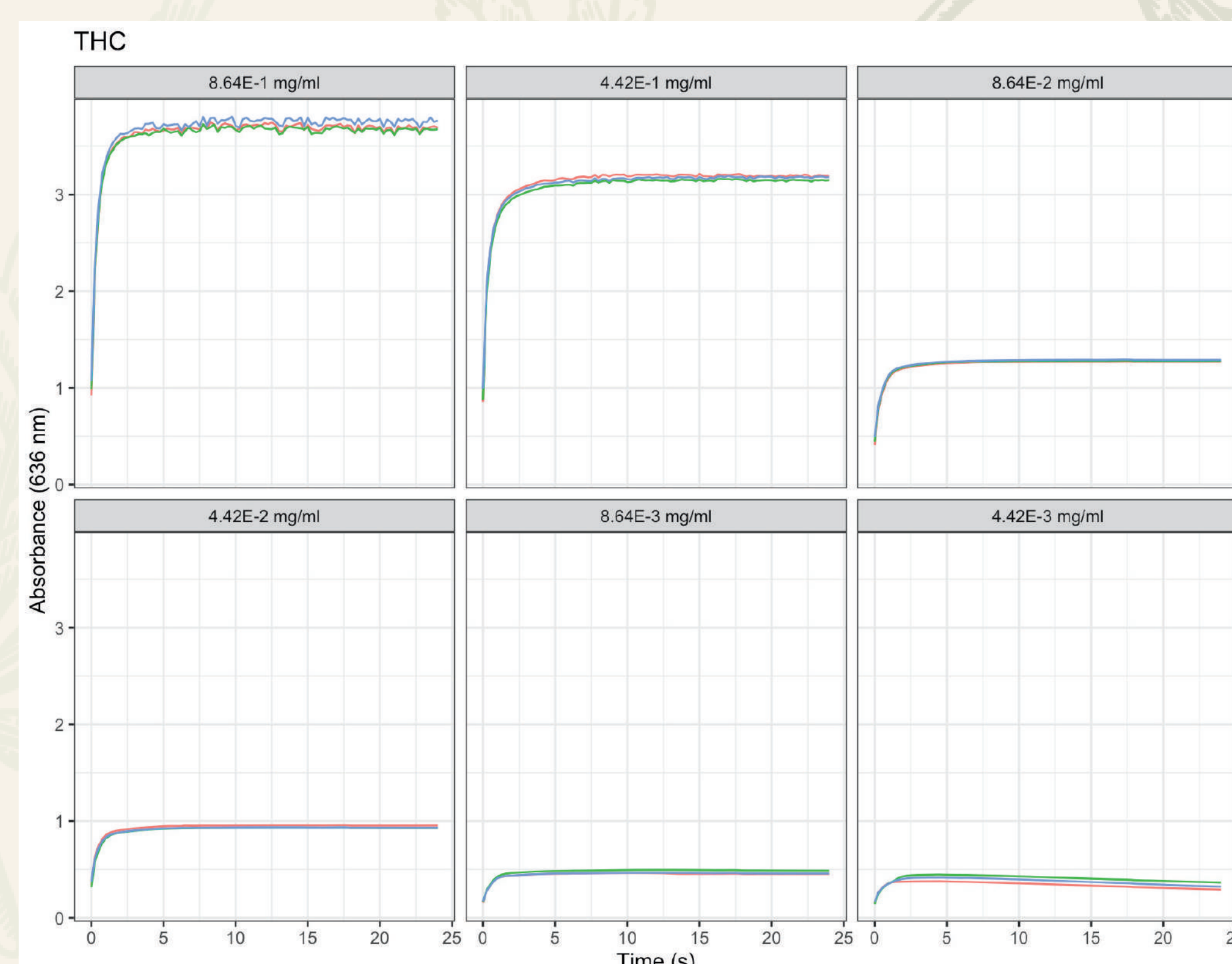


Fig. 4- Kinetics of the reaction between  $\Delta^9$ -THC and 4-AP at 636 nm over 24 hours

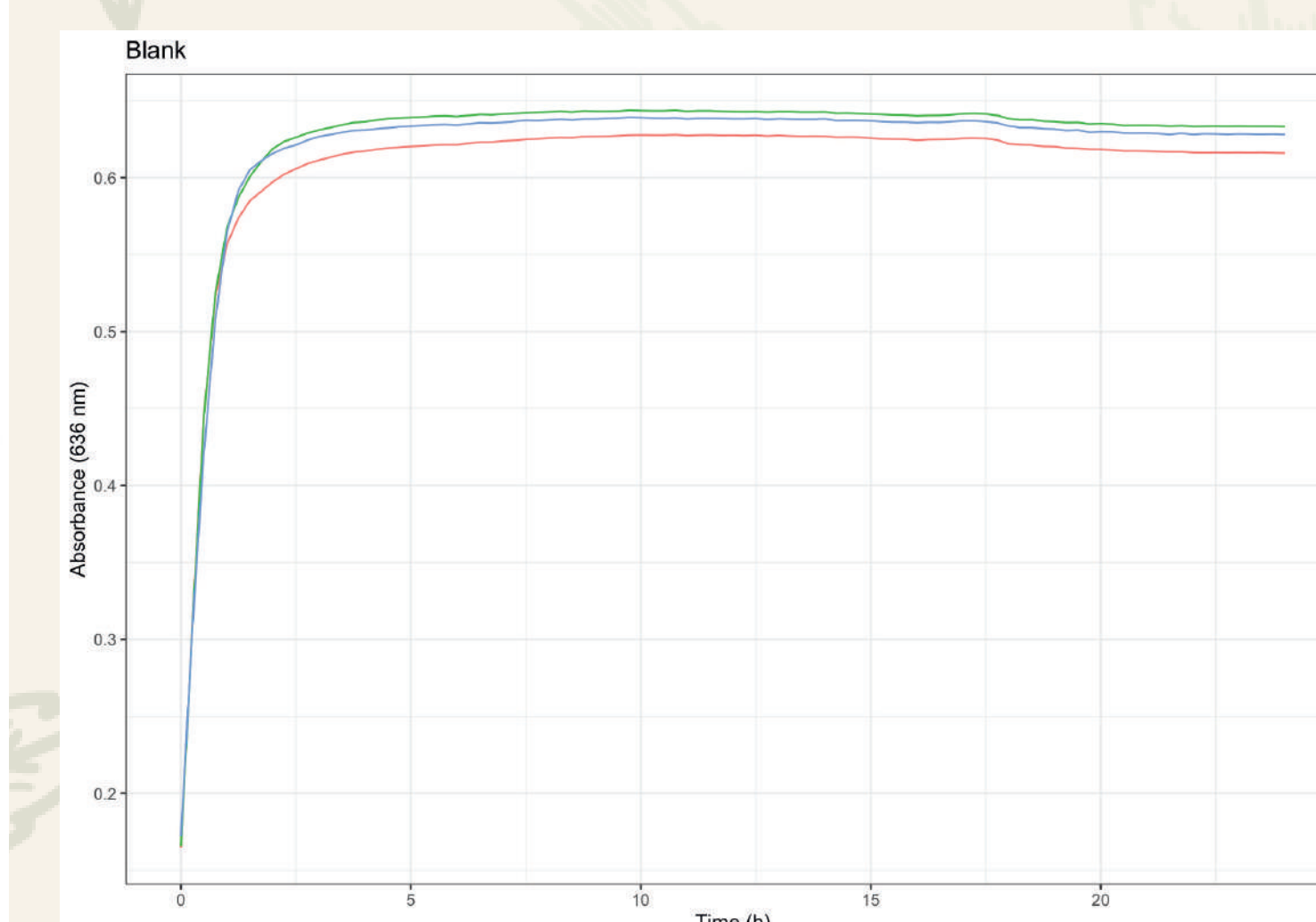


Fig. 5- Kinetics of 4-AP Oxidation at 636 nm over 24 hours.

## Discussion

Results presented in Figures 2 and 4, show the reaction behaviour between the compounds and 4-AP over the 24-hour period, followed by spectrophotometry. These showed an rapid increase in absorbance over the first two hours, both when there is a cannabinoid present and when there is not, which indicates a slow reaction with the cannabinoids, and a rapid oxidation of the 4-AP within this time frame.

## Conclusion

The results indicate that 4-aminophenol (4-AP) exhibits limited temporal stability, with significant oxidation occurring within the first few minutes post-reaction initiation, as evidenced by an increase in absorbance. Nevertheless, the precise time window during which reliable differentiation between CBD and  $\Delta^9$ -THC remains feasible has not yet been fully established. This limitation arises from the progressive formation of dark oxidative by-products, which ultimately mask the characteristic chromogenic responses of the cannabinoid-4-AP complexes.

## References

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3. Lewis K, Wagner R, Rodriguez-Cruz SE, Weaver MJ, Dumke JC. Validation of the 4-aminophenol color test for the differentiation of marijuana-type and hemp-type cannabis. J Forensic Sci. 2021 Jan;66(1):285–94. doi:10.1111/1556-4029.14562

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