WEB305 - Chromabond HR-X Test

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1 Introduction

For the analysis of methyl transferase reaction via the LAAO-assay it is necessary to remove flavonoids and phenyl propanoids. Those substances interfere with the detection of H_2O_2 via HRP, because rather than TMB these substances tend to get oxidized.

Trying to remove the flavonoid substrates and products of a MT-reaction by solid phase extraction columns(SPE).

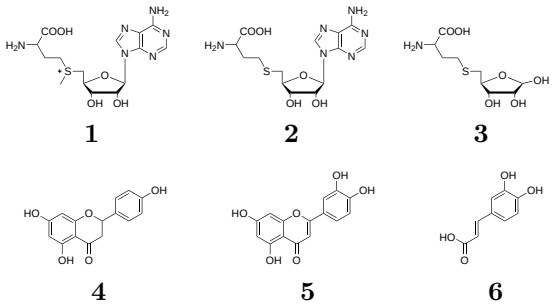

Figure 1: Substances to be separated for analysis by LAAO assay. Polar substances to be eluted for analysis are **1** - SAM, **2** - SAH and **3** - Ribosyl homocysteine. Flavonoids and phenyl propanoids (**4**–**5**) are problematic during detection with LAAO/HRP.

2 Experimental

2.1 Sample preparation

Sample solutions:

1. 100 μM SAH in ddH_2O
2. 100 μM SAH, 0.2 mM Eriodictyol in ddH_2O

Sample treatment:

A1: 100 μM SAH in ddH_2O passed through HR-X column
A2: 100 μM SAH, 0.2 mM Eriodictyol in ddH_2O passed through HR-X column
B1: 100 μM SAH in ddH_2O
B2: 100 μM SAH, 0.2 mM Eriodictyol in ddH_2O

Column preparation:

Conditioning of HR-X column:
1.5 mL MeOH, then 1.5 mL Water

Sample aspiration:
0.5 mL sample passed through column by pressure (syringe)

Washing/Elution:
eluted polar components by washing with 0.5 mL water

Regeneration:
regenerated by passing through 2 mL of MeOH

2.2 LAAO-Reaction

1. add 75 μL LAAO-reagent to 25 μL of sample
2. incubate for 10 min @ RT
3. stop reaction with 50 μL 12 N H_2SO_4
4. measure absorption at 450 nm