WEB302 - SOMT Activity Factorial

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August 4, 2014, Halle

1 Deduced from WEB299

SOMT seems to be active:

- in reducing conditions (1 mM DTT)
- in the presence of divalent cations (2 mM Mg^{2+})
- at a pH of 8.5

SOMT activity:

- is somewhat peculiar (at least over long incubation times)
- peaks appear in the chromatograms, that are not supposed to be there (e.g. very early retention time [Peak 1], early shoulder of Naringenin [Peak 2] Fig.1)

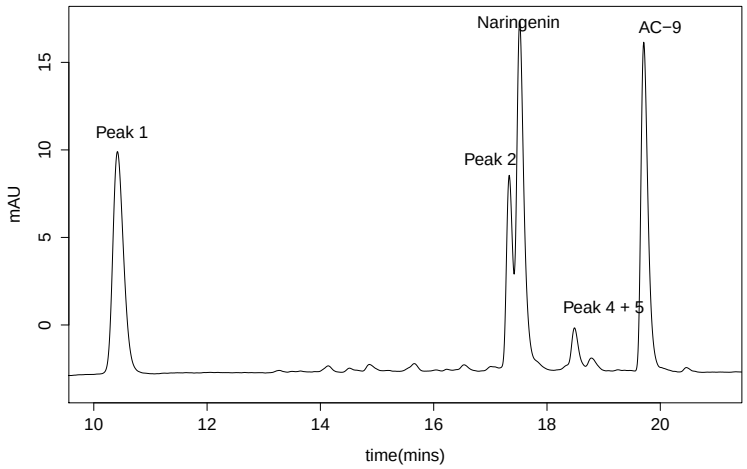

Figure 1: HPLC Trace at 325 nm of WEB299.12 activity test.

2 (Fractional) Factorial to optimize assay conditions

- optimize asssay conditions with refolded protein from WEB301.12 (Tab. 1)

***variable* Factors:**

- temperature	- pH	- *reaction time*
- *divalent cations*	- *redox conditions*	- *DES*
- SAM concentration	- concentration of substrate	- substrate
- enzyme concentration		

	Mg^{2+} [mM]	Ca^{2+} [mM]	DTT [mM]	DES [% (V/V)]	e1	e2	e3
1	0.00	5.00	2.00	25.00	-1.00	1.00	-1.00
2	0.00	0.00	0.00	0.00	-1.00	-1.00	-1.00
3	5.00	5.00	0.00	25.00	-1.00	-1.00	1.00
4	0.00	5.00	0.00	0.00	1.00	1.00	1.00
5	5.00	5.00	2.00	0.00	1.00	-1.00	-1.00
6	5.00	0.00	0.00	25.00	1.00	1.00	-1.00
7	0.00	0.00	2.00	25.00	1.00	-1.00	1.00
8	5.00	0.00	2.00	0.00	-1.00	1.00	1.00
9	2.50	2.50	1.00	12.50	0.00	0.00	0.00

Table 1: 4 Factor fractional factorial design with centerpoint for assessing SOMT activity influences. The variable factors are divalent cations (Mg^{2+} and Ca^{2+}), DTT and 1,2-propanediol:cholin chloride:water (PCH) DES.e1, e2, e3 are dummy variables important for the factorial design process.