WEB314 - MTAN Activity measurement for DIM

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1 Experimental

- Calibration curve Adenine (HPLC)
- measurements (4 time points, 0, 5, 10, 20, 30 min)

1.1 Assay

Assay buffer: 0.1 M Tris/HCl, 0.2 M KCl, 20 mM $MgCl_2$, pH 7.5

Substrate Solution: 10 mM SAH in 50 mM HCl

Reaction Mix (1000 μl):

2.5 mM SAH	316.2 μl 7.9 mM SAH
2 μM $\sim$ 53 $\frac{\mu g}{mL}$ MTAN	38 μl 1.4 $\frac{mg}{mL}$ MTAN (**omit until start of reaction**)
in assay buffer	100 μl 1 M Tris/HCl
	50 μl 4 M KCl
	50 μl 0.4 M $MgCl_2$
	445.8 μl ddH_2O

Reaction:

1. 192.4 μl Reaction Mix
2. add 7.6 μl 1.4 $\frac{mg}{mL}$ MTAN
3. measure UV absorption at 276 nm (JASCO) in 1 mm cuvette R abline dashedfor 300 seconds at room temp (25°C)

2 Fitting the data

sample	lower fit limit (s)	upper fit limit (s)
WEB314.buffer	0	300
WEB314.denat.1	0	300
WEB314.S.1	0	30
WEB314.S.2	0	30
WEB314.S.3	0	30

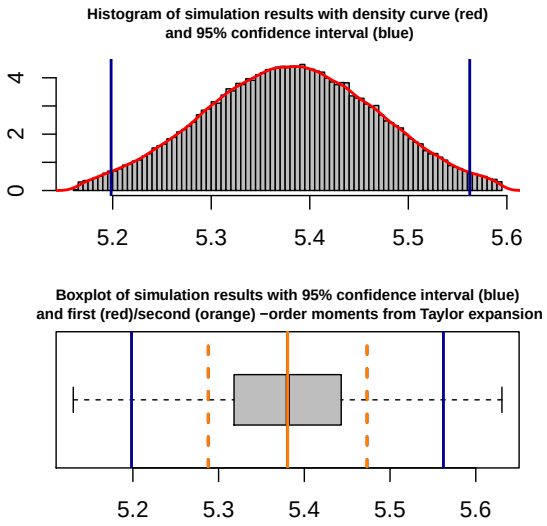

Figure 1: The plot of the object obtained from the error propagation

Table 1: The specific activity of MTAN in $\frac{U}{mg}$ as calculated from the slopes of the progress curves. Calculations from triplicate measurements were made using the *propagate* package for R.

	Mean.1	Mean.2	sd.1	sd.2	2.5%	97.5%
$\frac{U}{mg}$	5.3805248	5.3805248	0.0926499	0.0926499	5.1989500	5.5617620