

## ANALYTICAL HPLC TEST METHOD

### Analysis Purpose

In-process monitoring of Stage 1 and Stage 2a

### Conditions

Column: Waters Xbridge C18 (50 mm x 4.6 mm x 5 microns)

Flow Rate: 1.0 mL/min Oven Temp: 40 °C

Inj. volume: 5 µL Detection: 260 nm 100-500MS POS

Mobile Phase: Acetonitrile/methanol vs. 10 mM phosphate buffer, pH 5.5

Sample Prep: Dissolve/dilute samples in acetonitrile

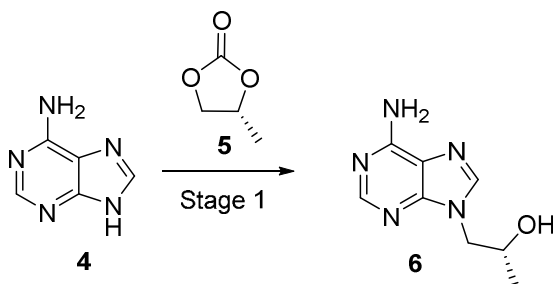
Prepare 10 mM phosphate buffer by dissolving 1.28 g dibasic sodium phosphate in 900 mL water and adjust pH to 5.5. Add 50 mL each of acetonitrile and methanol and mix. This is premixed 'aqueous', which should be resistant to microbial growth. The 'organic' portion of the mobile phase is a 1:1 mix of acetonitrile:methanol. Inclusion of alcohol prevents on-column equilibration problems for several intermediates.

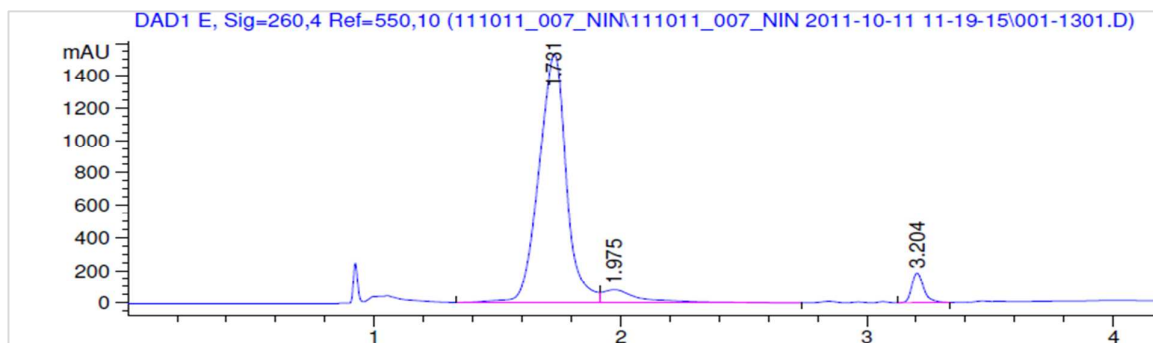
### Retention Times (chromatograms attached) S

Stage 1 Adenine (**4**) 1.00 min, HPA (**6**) 1.73 min, HPA-NH-substituted regioisomer 3.20 min.

Stage 2 HPA (**6**) 1.69 min, PPA-HH (**9**) 3.18 min and PPA (**8**) 3.52 min.

### Stage 1





### Stage 2a

