Assay of O-Acetylhomoserine sulfhydrylase (*Ca*Met15p) activity.

Objectives: Optimisation of method and conditions suitable for activity assessment of O-acetyl-L-homoserine sulfhydrylase from *C. albicans* (*Ca*Met15p) via HPLC detection of L-homocysteine (HCT) formed by the enzyme.

Methods: The reaction buffer consists of substrates; O-acetyl-L-homoserine, sodium sulfate, and pyridoxal-5-phosphate (PLP) as well as an appropriate amount of enzyme (*Ca*Met15p). Enzyme was added to start the reaction, the final volume of the reaction mixture was 50 µL. The reaction was stopped by the addition of 100 µL of stopping buffer (3.2 mM guanidinium-HCl in 100 mM sodium phosphate buffer pH 5.8). Negative controls were prepared with addition of an enzyme after termination of the reaction with the stopping buffer. 20 µL of 3 mM 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) dissolved in 100 mM sodium phosphate buffer pH 6.8 containing 10 mM EDTA, was added to derivatise HCT. The samples were injected into Zorbax Eclipse C18 column 5um, 250 mmx4,6 mm (Agilent) equilibrated by 0.45 % HCOOH (A) and acetonitrile (B). Formed HCT-TNB complex was detected by the DAD detector at λ=330nm, retention time ~ 7.8 min. The presence of the formed HCT-TNB complex was confirmed by mass spectrometry.

Results: The best activity of Met15p was detected in 100 mM Tris-HCl buffer of pH 8.0. Optimal reaction conditions were found to be 10 min at 37 °C. For best results, the reaction buffer consisted of 10 mM O-acetyl-L-homoserine, 0.1 mM sodium sulfate nonahydrate, 0.1 mM PLP, and 10 nM of *Ca*Met15p. The optimal elution profile was found to be: 1.2 ml min^{-1}, 0 - 5 min. – 20% B, 5 - 6 min. 20– 80% B, 6 - 7 min. – 80% B, 7.1-15 min. – 80–20% B.