

Supporting information.

A Fast and Sensitive Assay for Measuring the Activity and Enantioselectivity of Transaminases.

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Experimental data.

Commercially available reagents and chemicals were either purchased from Sigma-Aldrich Company Ltd or Fisher Scientific UK Ltd. The three enzymes peroxidase, type VI-A from horseradish (P6782, HRP), D-amino acid oxidase from porcine kidney (A5222, D-AAO) and L-amino acid oxidase from *Crotalus adamanteus* (A9378, L-AAO) were purchased from Sigma-Aldrich Company Ltd. The transaminase enzymes (ATA), glucose dehydrogenase (GDH) and lactate dehydrogenase (LDH) were supplied by Codexis.

1. Sample preparation for Gas chromatography.

An LDH/GDH system was employed to run a transamination in the forward direction. 10 g/L of glucose, 45 g/L of D-Ala, 1 g/L of NAD and 0.5 g/L of PLP were added to 20 mL of 100 mM phosphate buffer and the pH adjusted to 8 using sodium hydroxide (NaOH). 1 g/L of LDH, 1 g/L of GDH and 5 g/L of ATA-117 was then added to the stock solution. 1 mL of the stock solution was added to five different eppendorf tubes and separately 6 mM of acetophenone, 4-chloro-acetophenone, 4-aceylpyridine, 3-acteylpyridine and 4-bromo-acetophenone was added. The solutions were heated to 30 °C and agitated at 900 rpm and samples taken after 5 hours. 40 µL of 5M NaOH was added to stop the reaction and the pH adjusted to 7 followed by extraction (3 ×) with 1 mL of chloroform. The remaining solution was concentrated to 1 mL and the amine derivatised and analysed *via* GC

2. Derivatisation of amines

1 mL of sample was dried in an eppendorf containing MgSO₄. The resulting solution was decanted into a clean GC vial to which 25µL of a 2:1 solution of Et₃N:Ac₂O was added.

3. GC method

Column: Chirasil Dex CB

FID at 200°C

Inlet temperature 200°C

Carrier: He

140°C hold 11 min

Ramp 190°C (5°C/min)
Total time 23min

4. Example of GC data

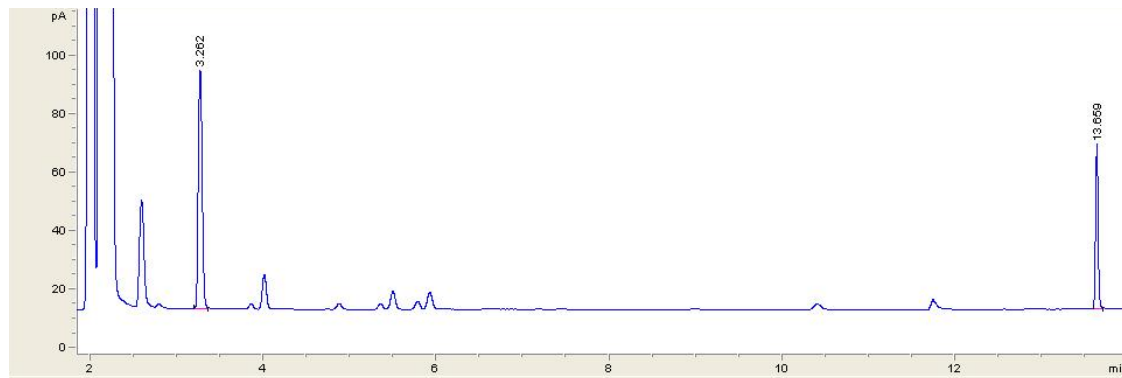


Figure 1: Example of GC chromatogram obtained for compound **7**. RT (*R*)-amine 13.7 min, ketone 3.3 min.

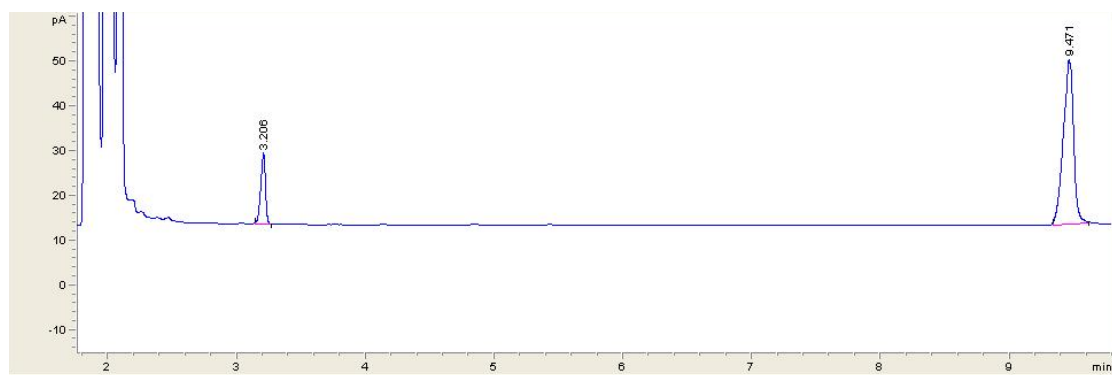


Figure 2: Example of GC chromatogram obtained for compound **6**. RT (*R*)-amine 9.5 min, ketone 3.2 min.

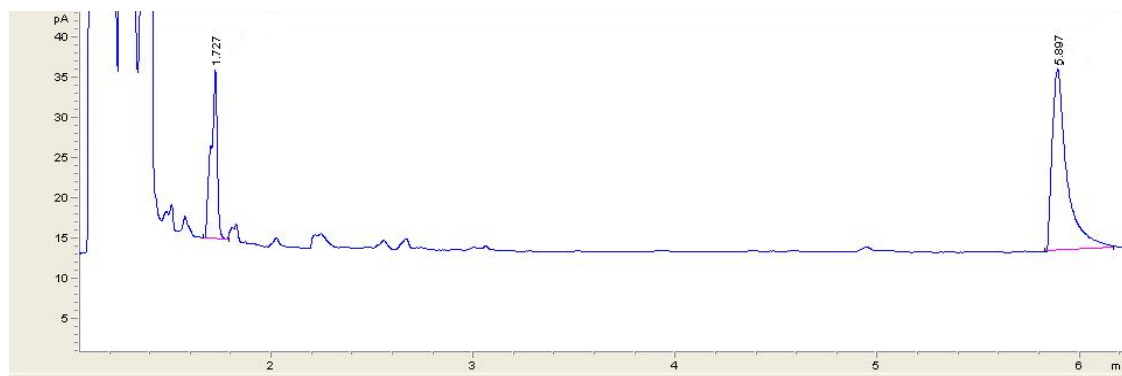


Figure 3: Example of GC chromatogram obtained for compound **13**. RT (*R*)-amine 5.9 min, ketone 1.7 min.

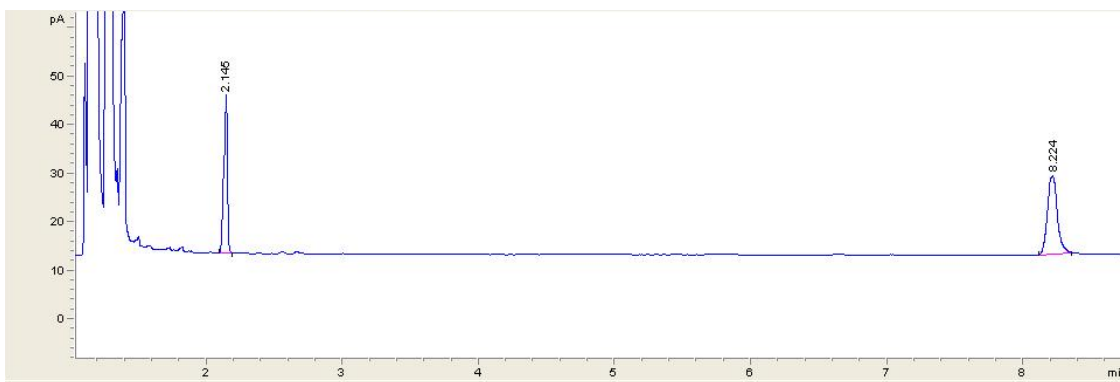


Figure 4: Example of GC chromatogram obtained for compound **8**. RT (*R*)-amine 8.2 min, ketone 2.1 min.

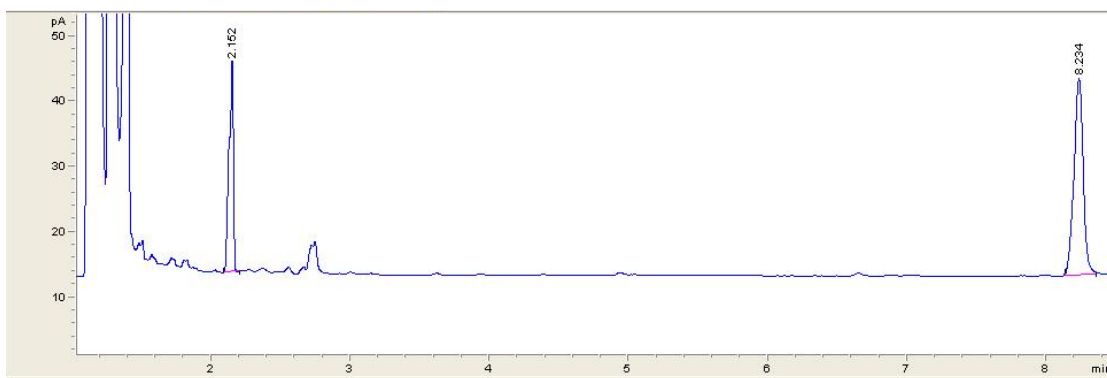


Figure 4: Example of GC chromatogram obtained for compound **12**. RT (*R*)-amine 8.2 min, ketone 2.2 min.

5. Plate reader method

Experiments were run on a spectrophotometer (Molecular devices SpectraMax M₂) at 30 °C with a wavelength of 540 nm and absorbance readings taken every 30 seconds.

6. Example of plate reader data

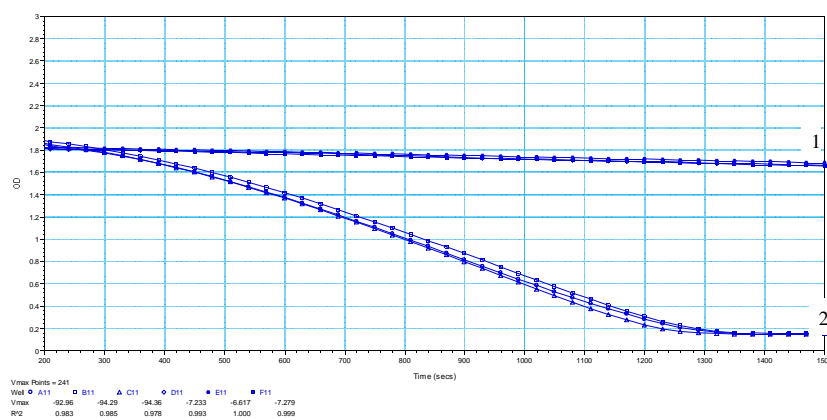


Figure 1: Absorbance data taken at 540nm. 1: Blank 2: Compound **10**.

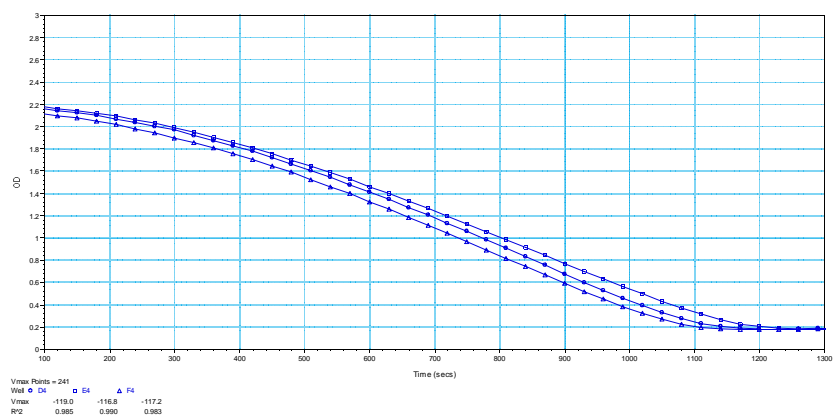


Figure 2: Absorbance data taken at 540nm. Compound 7.

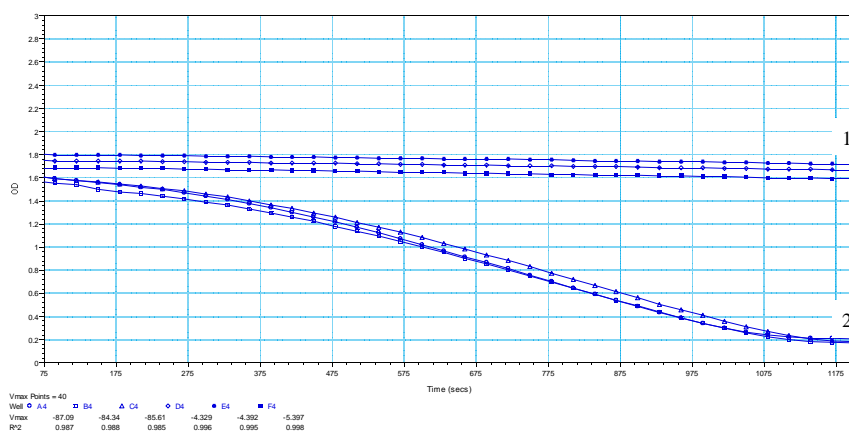


Figure 3: Absorbance data taken at 540nm. 1: Blank 2: Compound 11.