Supplementary Information

Biocatalytic reduction of α,β-unsaturated carboxylic acids to allylic alcohols

Godwin A. Aleku[1], George W. Roberts[1] & David Leys[1]

[1]*Manchester Institute of Biotechnology, School of Chemistry, University of Manchester, Manchester, UK.*

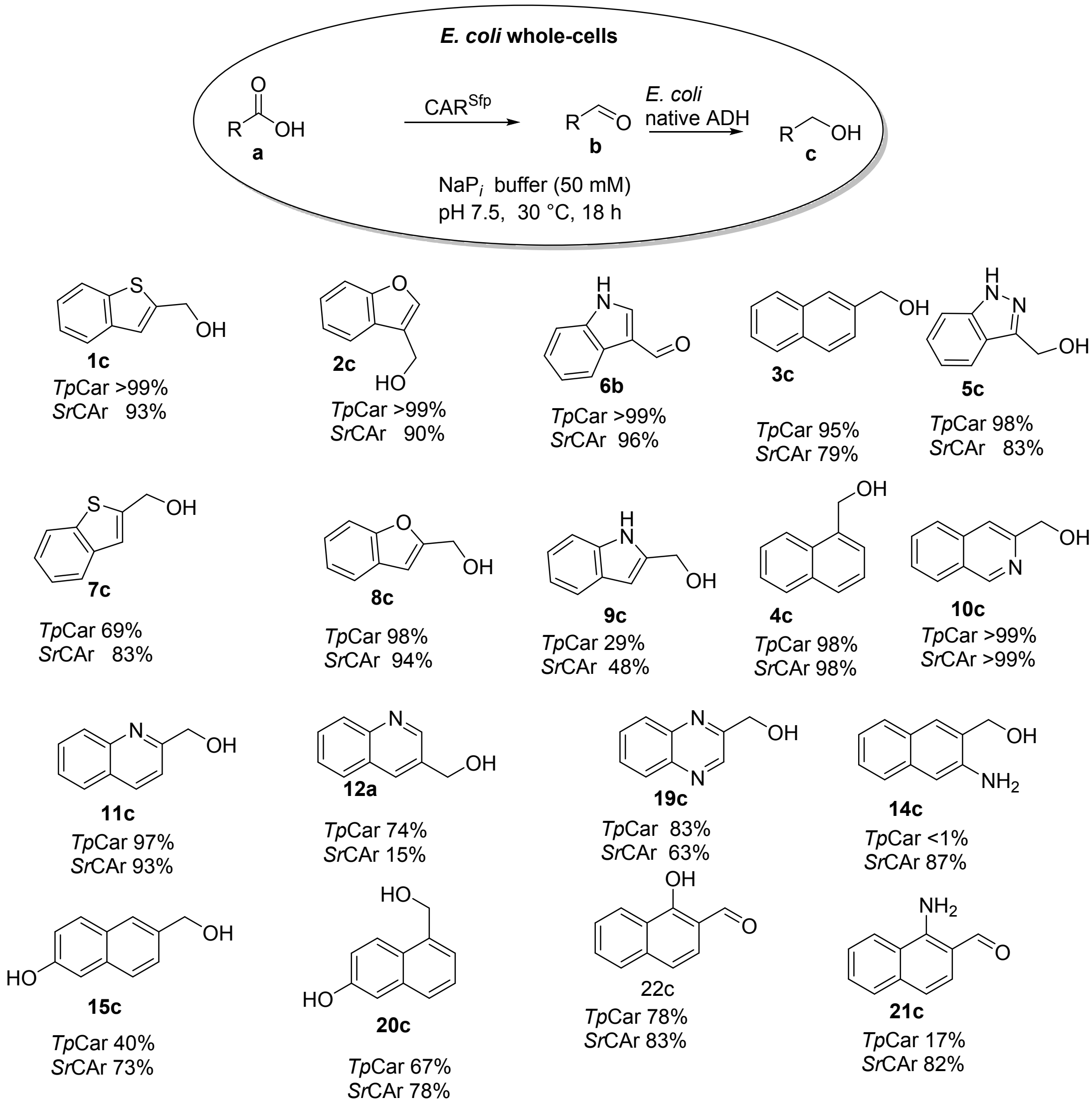

Fig. S1. Screening of carboxylic acids using resting *E. coli* whole cells expressing either *Tp*CAR (co-expressed with Sfp) or *Sr*CAR (co-expressed with Sfp). Reaction contained 5 mM substrate, 20 mM D-glucose, 10 mM $MgCl_2$, 2% v/v DMSO and fresh resting *E. coli* cells containing overexpressed *Sr*CAR at OD600 nm = 30, all in NaP*i* (50 mM, pH7.5), incubated at 30 °C, 250 rpm for 18 h. *Sr*CAR = *Segniliparus rugosus* carboxylic acid reductase. *Tp*CAR= *Tsukamurella paurometabola* carboxylic acid reductase. Sfp= *Bacillus subtilis* phosphopantetheinyl transferase (sfp). Conversion values were determined from HPLC/GC-MS analyses.

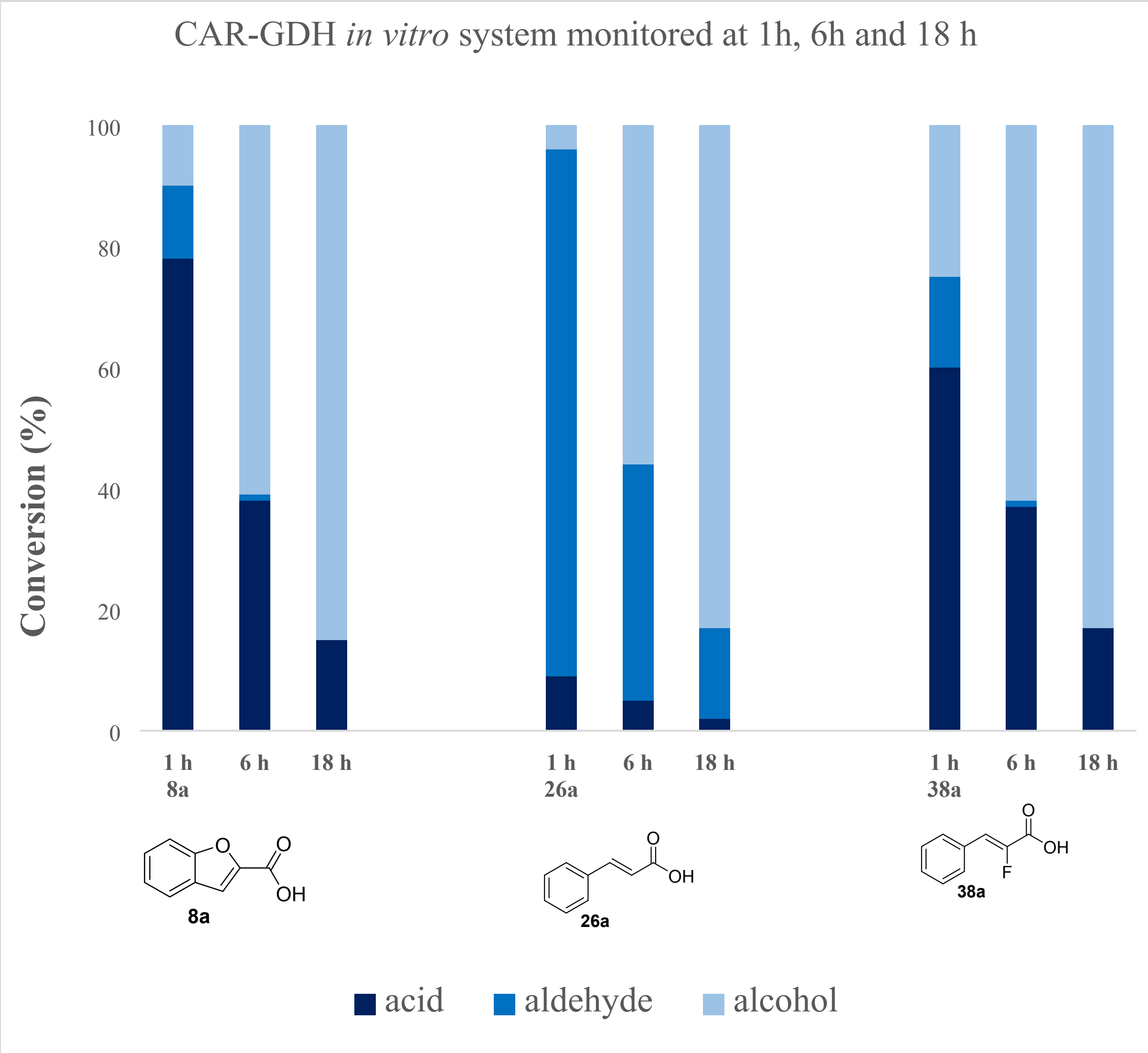

Fig. S2. *In vitro* one-pot CAR-GDH biotransformation for the conversion of carboxylic acids to alcohol *via* intermediate aldehyde, monitored at 1h, 6h, 18h. In this system GDH was used as a bifunctional glucose oxidant (for NADPH recycling) and carbonyl reductase, catalysing reduction of aldehyde to the corresponding alcohol.

DNA sequences of carboxylic acid reductases (CAR) and Sfp

>His-*Sr*CAR_gene sequence

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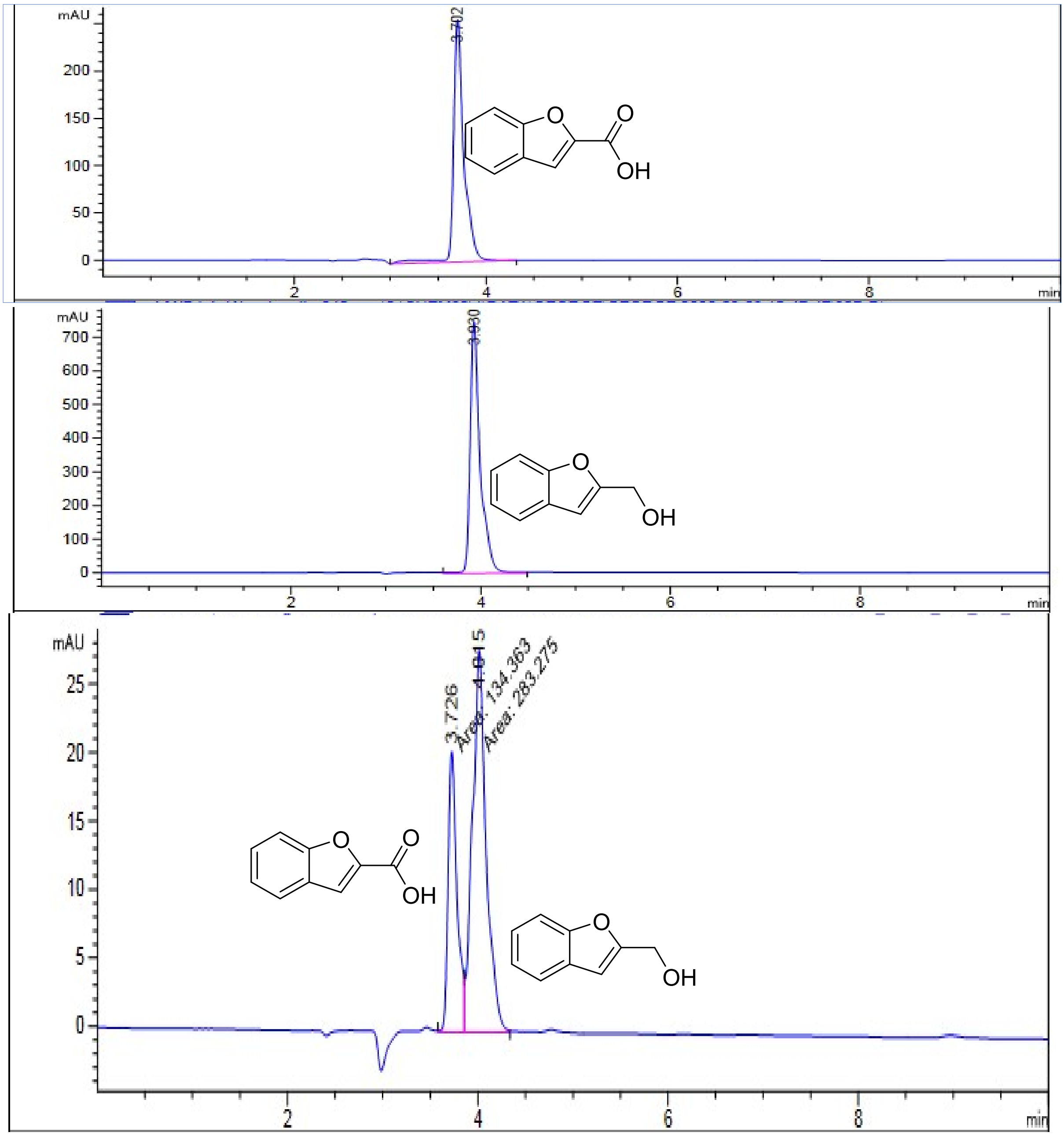

Fig. S3. *In vitro* one-pot CAR_GDH cascade for reduction of benzofuran-2-carboxylic acid to benzofuran-2-methanol: HPLC traces from top to bottom: benzofuran-2-carboxylic acid standard, benzofuran-2-methanol standard, biotransformation samples taken at 6h using purified *Sr*CAR+GDH. Retention times: starting material, benzofuran-2-carboxylic acid (3.70 min), benzofuran-2-methanol (4.02min).

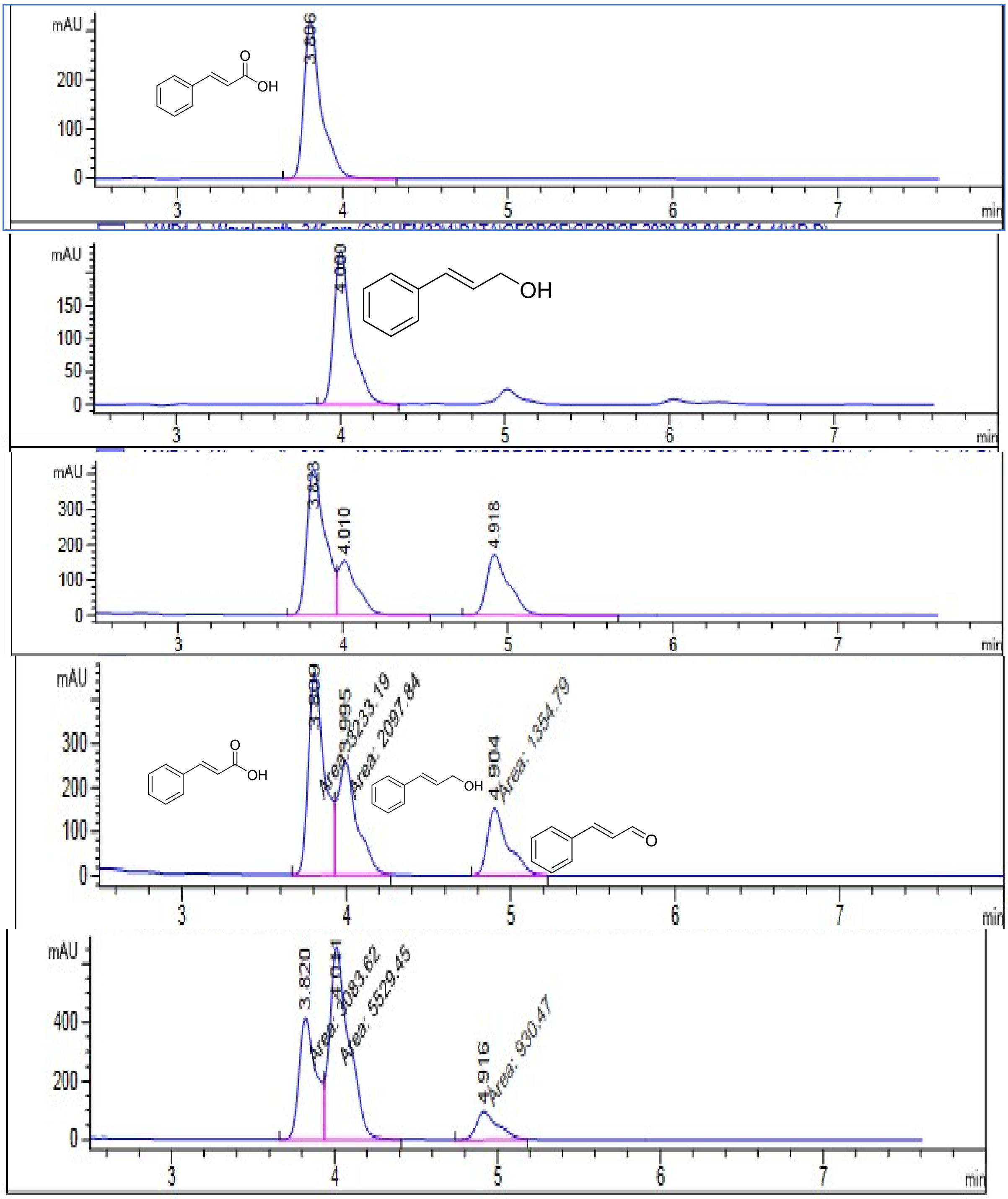

Fig. S4. *In vitro* one-pot CAR-GDH cascade for reduction of cinnamic acid to cinnamyl alcohol: HPLC traces of biotransformation reaction, samples taken at different time points (top to bottom: cinnamic acid standard, cinnamyl alcohol standard, biotransformation samples taken at 2 h, 4 h and 18 h). Retention times: starting material, cinnamic acid (3.80 min), cinnamaldehyde (4.91 min), cinnamyl alcohol (4.30min).

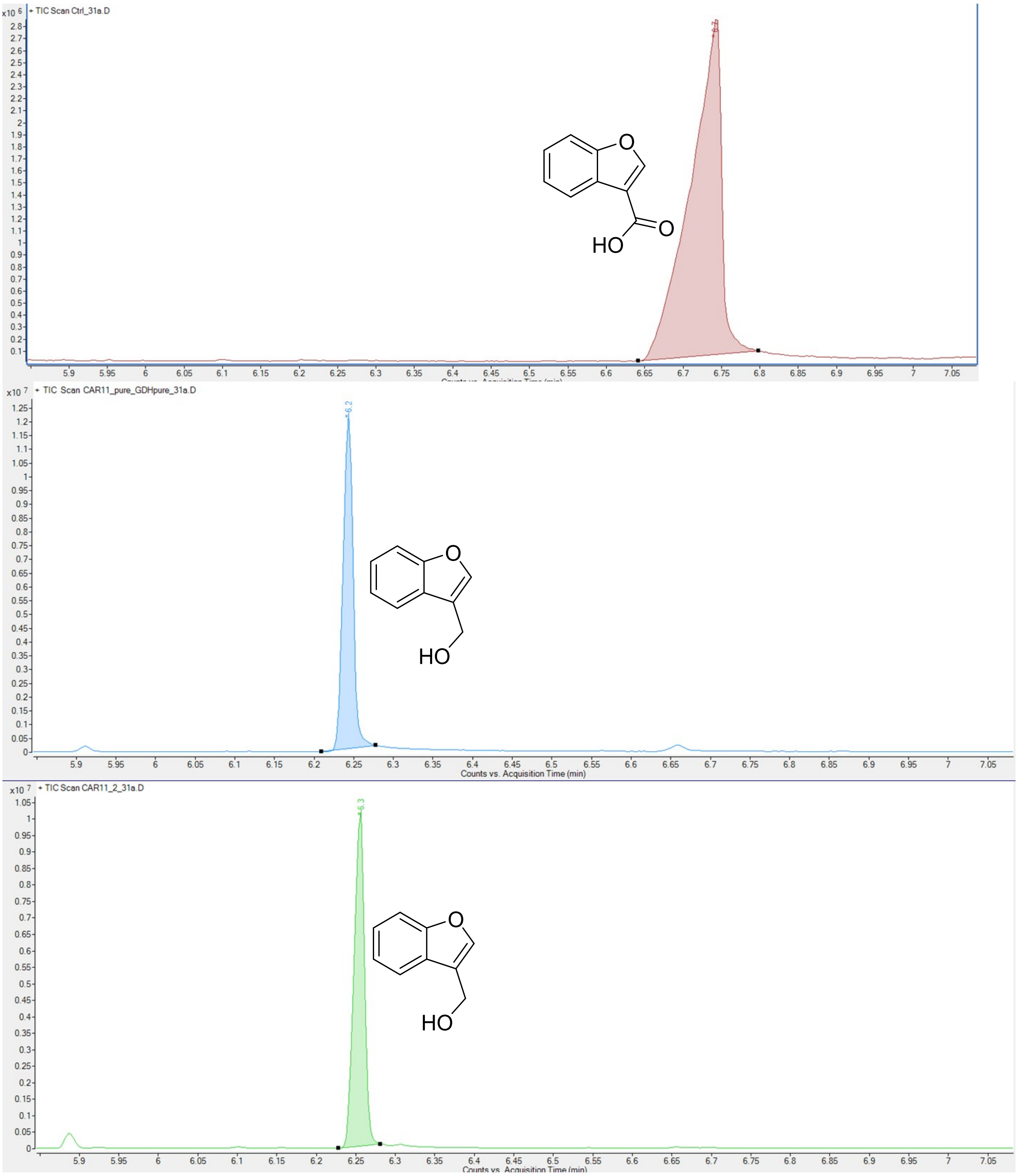

Fig. S5. Biotransformation for conversion of benzofuran-3-carboxylic acid to the corresponding alcohol: GC traces of biotransformation reactions, showing: Row 1 = control reaction performed *E.coli* whole cells lacking the CAR^{sfp} system. Row 2= whole-cell biotransformation reaction catalysed by recombinant *E. coli* whole cells expressing *Sr*CAR. Row 3 = *in vitro In vitro* one-pot CAR-GDH biotansformation.

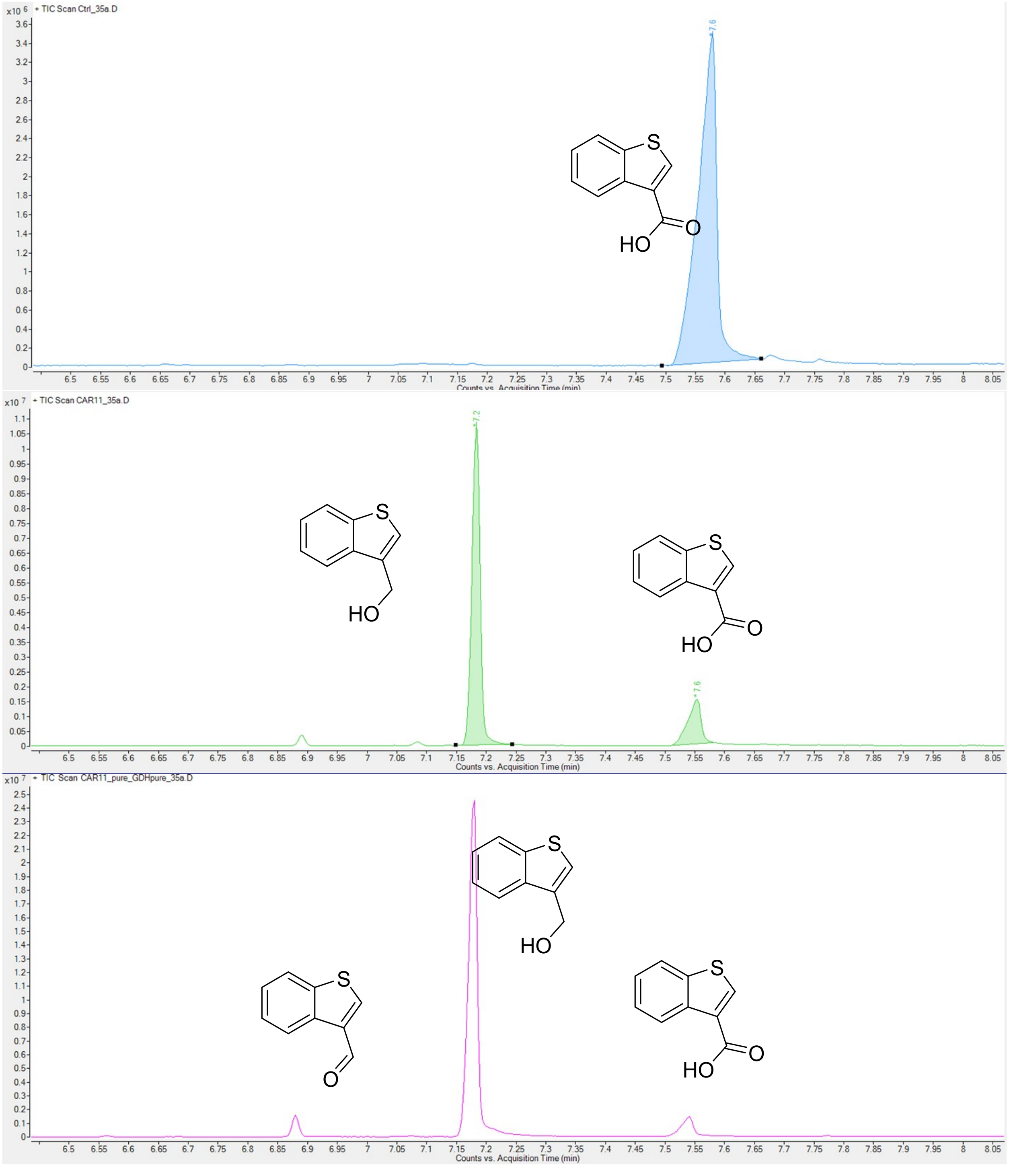

Fig. S6. Biotransformation for conversion of benzothiophene-3-carboxylic acid to corresponding *alcohol*: GC traces of biotransformation reactions, showing: Row 1 = control reaction performed using *E.coli* whole cells lacking the CAR^{sfp} system. Row 2= whole-cell biotransformation reaction catalysed by recombinant *E. coli* whole cells expressing *Sr*CAR. Row 3 = *in vitro In vitro* one-pot CAR-GDH biotansformation.

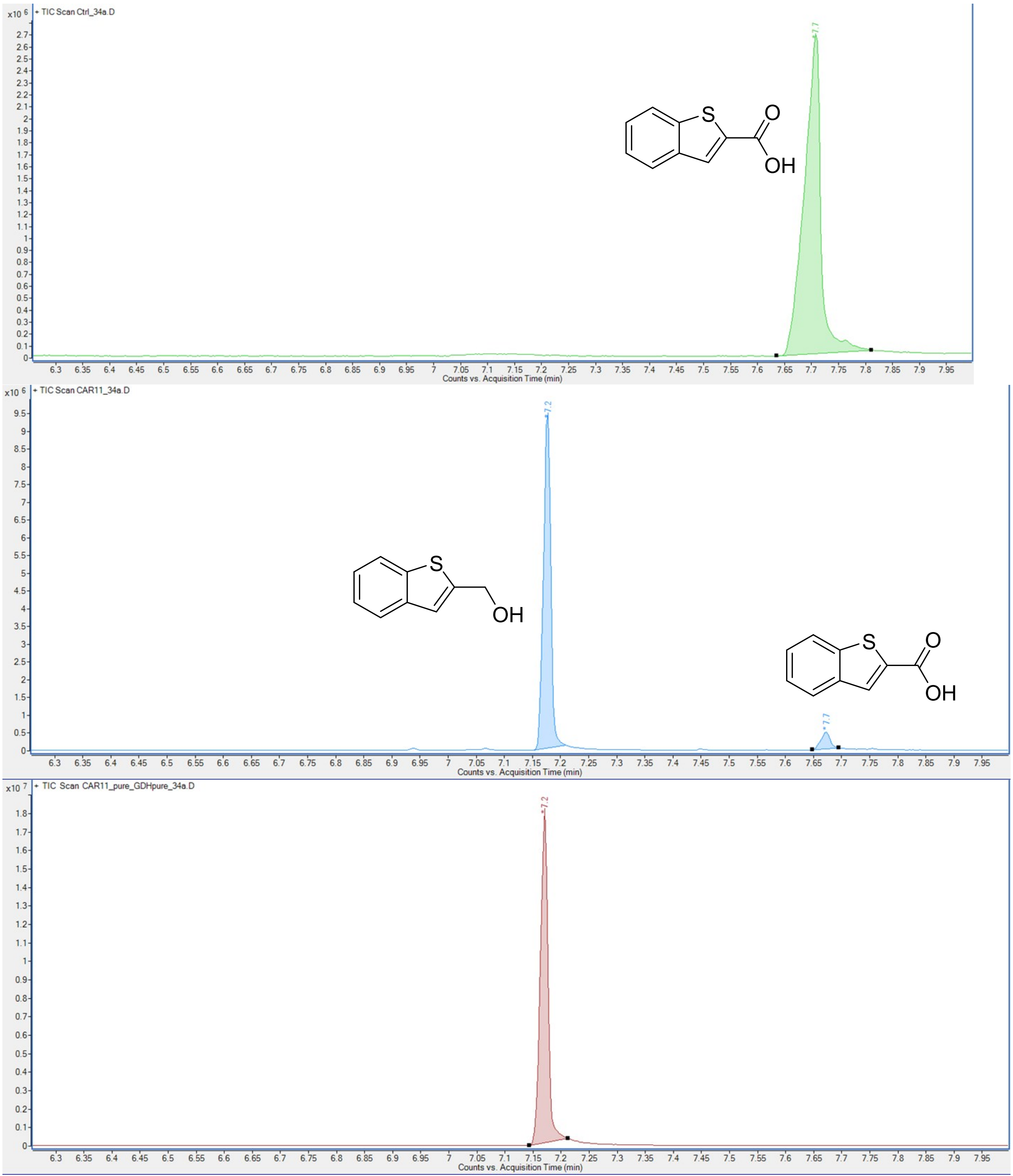

Fig. S7. *Biotransformation for conversion of benzothiophe-2-carboxylic acid to corresponding alcohol*: GC traces of biotransformation reactions, showing: Row 1 = control reaction performed using *E.coli* whole-cells lacking the CAR^{sfp} system. Row 2= whole-cell biotransformation reaction catalysed by recombinant *E. coli* whole cells expressing *Sr*CAR. Row 3 = *in vitro In vitro* one-pot CAR-GDH biotansformation.

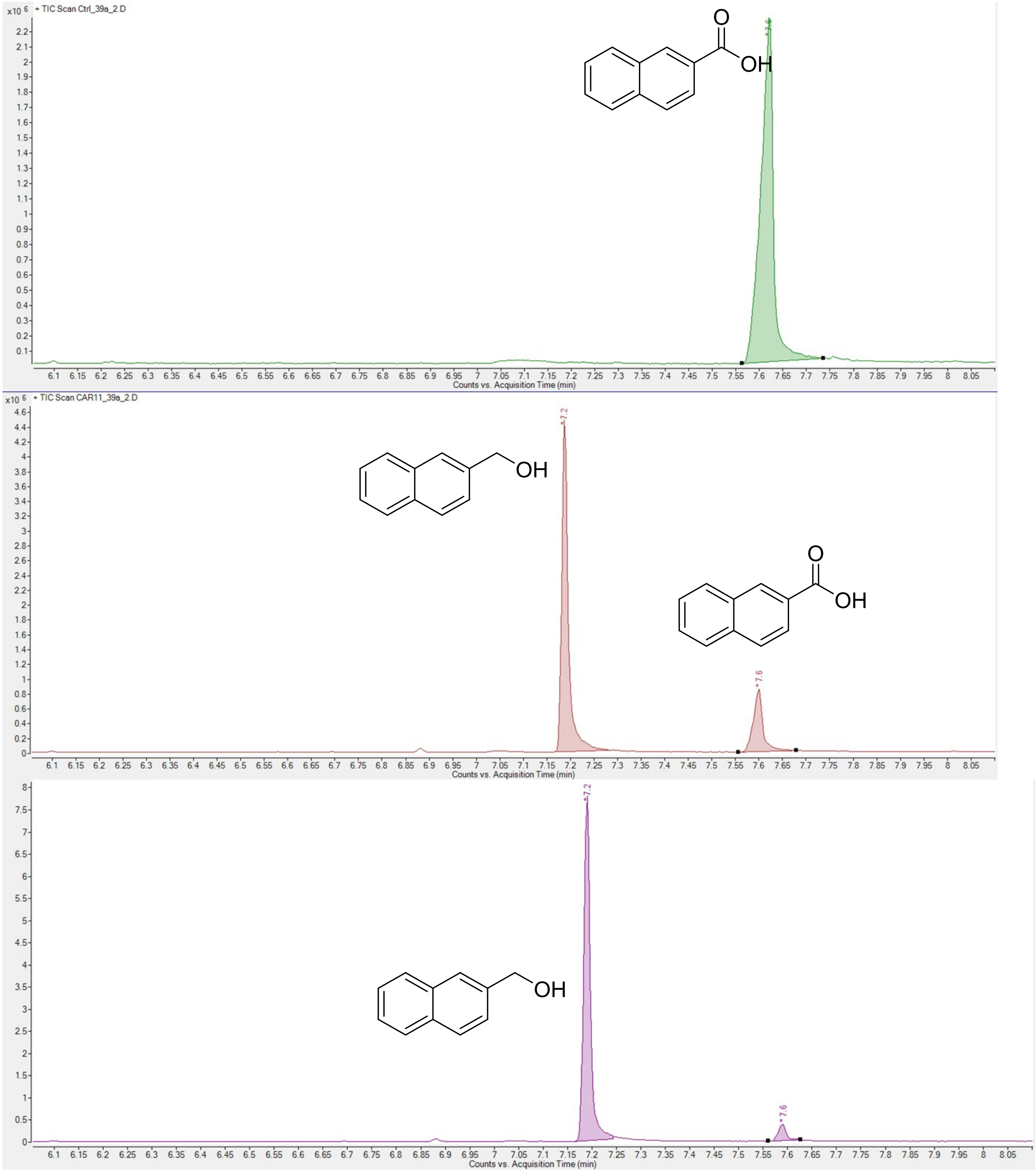

Fig. S8. Biotransformation for conversion of 2-naphthoic acid to the corresponding alcohol: GC traces of biotransformation reactions, showing: Row1 = control reaction performed using *E.coli* whole cells lacking the CAR^{sfp} system. Row2= whole-cell biotransformation reaction catalysed by recombinant *E. coli* whole cells expressing *Sr*CAR. Row = *in vitro In vitro* one-pot CAR-GDH biotansformation.

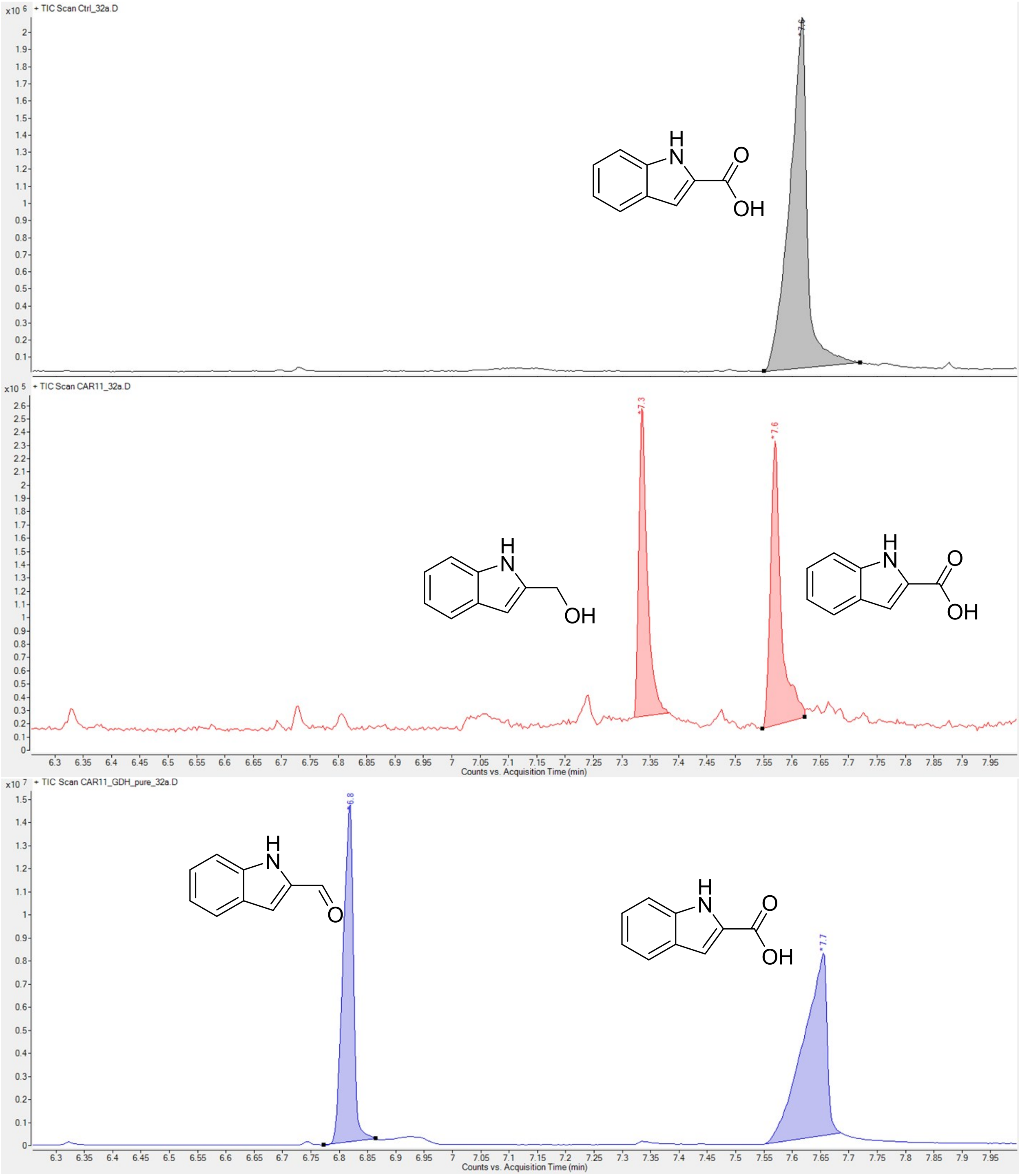

Fig. S9. Biotransformation for conversion of indole-2-carboxylic acid to the corresponding aldehyde/alcohol: GC traces of biotransformation reactions, showing: Row 1 = control reaction performed using *E.coli* whole cells lacking the CAR^{sfp} system. Row 2= whole-cell biotransformation reaction catalysed by recombinant *E. coli* whole cells expressing *Sr*CAR. Row 3 = *in vitro In vitro* one-pot CAR-GDH biotansformation.

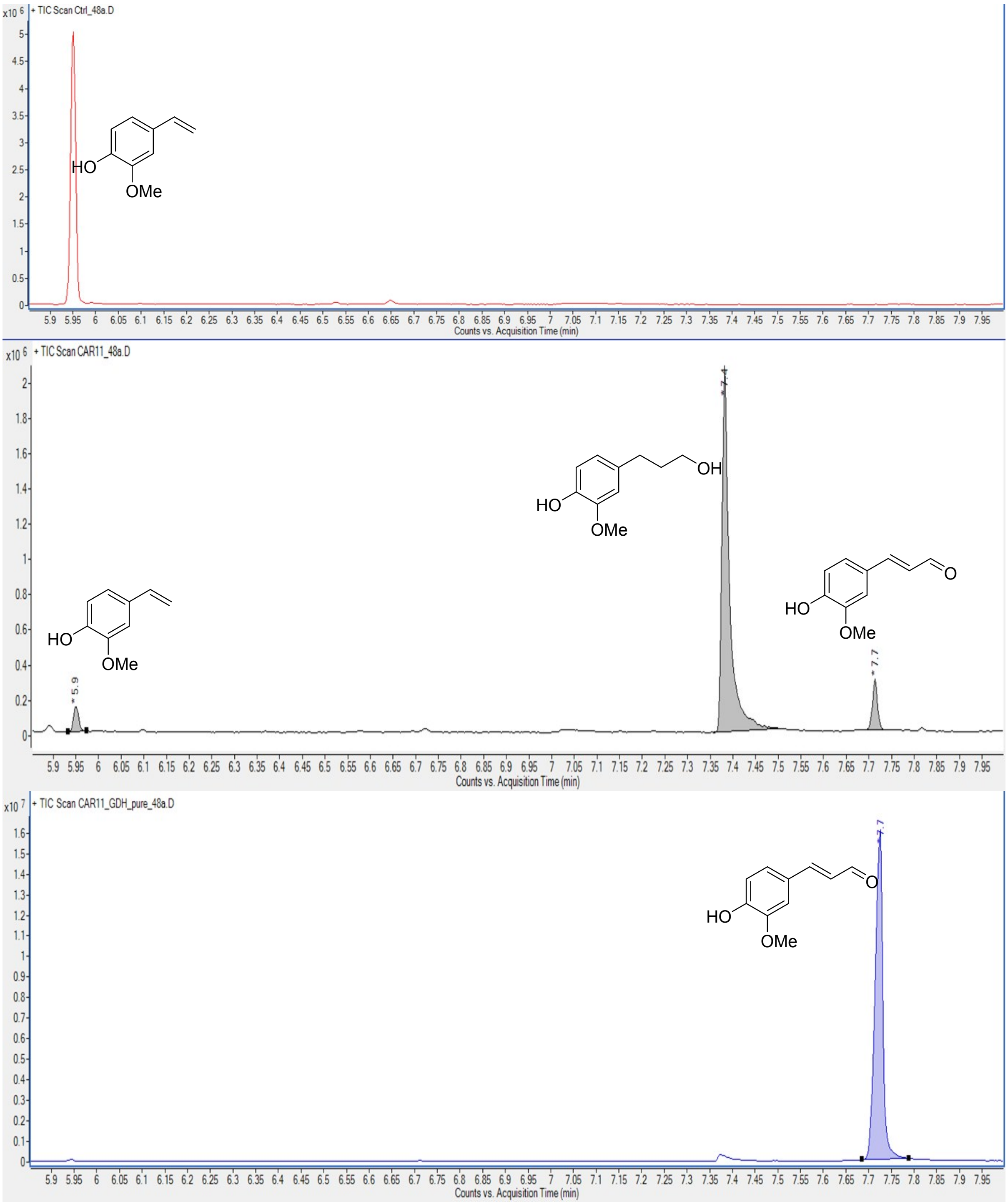

Fig. S10. Biotransformation for conversion of ferulic acid to the corresponding aldehyde/alcohol: GC traces of biotransformation reactions, showing: Row 1 = control reaction performed using *E.coli* whole cells lacking the CAR^{sfp} system. Row 2= whole-cell biotransformation reaction catalysed by recombinant *E. coli* whole cells expressing *Sr*CAR. Row 3 = *in vitro In vitro* one-pot CAR-GDH biotansformation. NB: E. coli whole cell lacking CAR^{sfp} system fully converted the acid to the decarboxylated product.

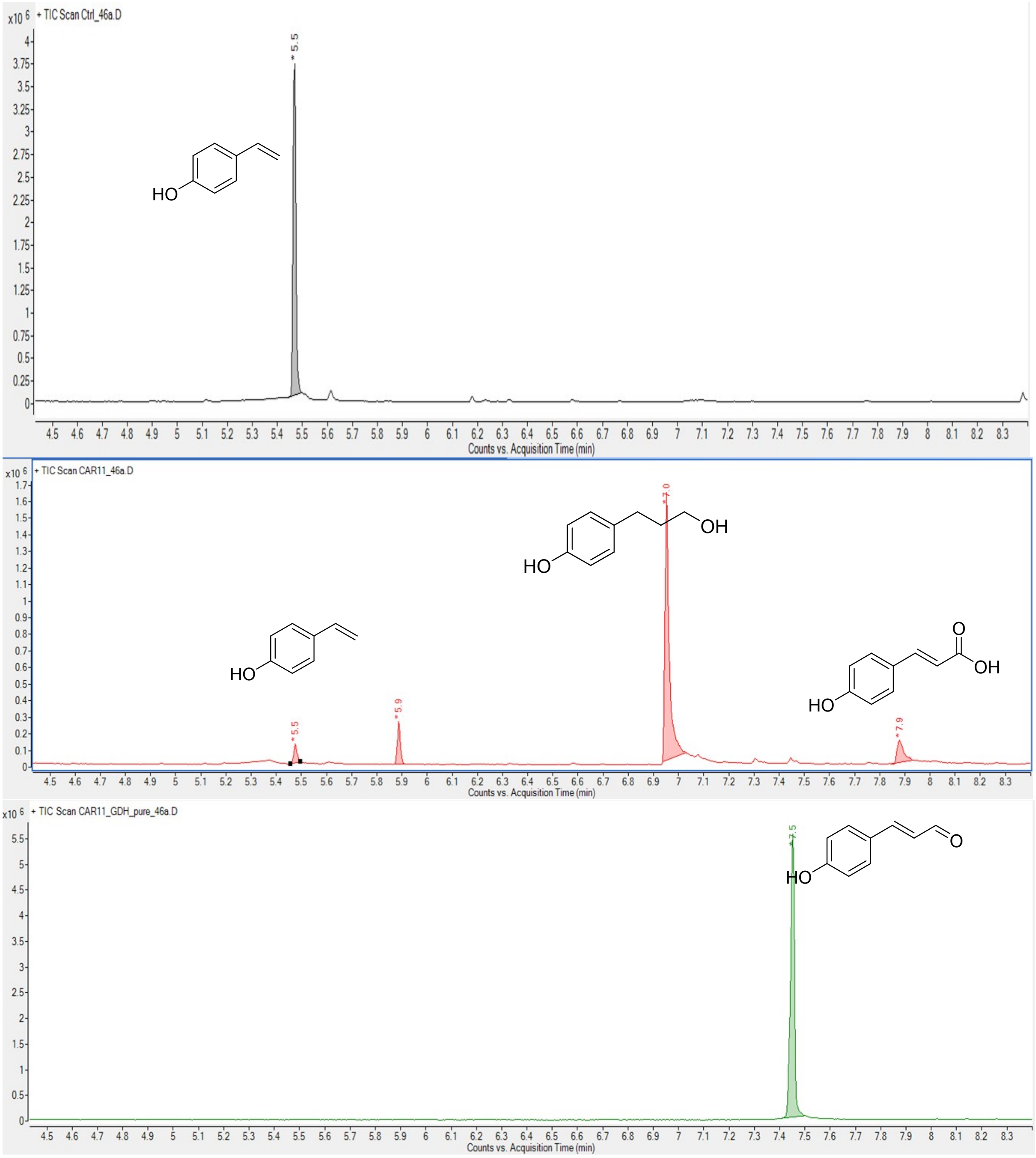

Fig. S11. Biotransformation for conversion of *para*-coumaric acid to the corresponding aldehyde/alcohol: GC traces of biotransformation reactions, showing: Row 1 = control reaction performed using *E.coli* whole cells lacking the CAR^{sfp} system. Row 2= whole-cell biotransformation reaction catalysed by recombinant *E. coli* whole cells expressing *Sr*CAR. Row 3 = *in vitro In vitro* one-pot CAR-GDH biotansformation. NB: E. coli whole cell lacking CAR^{sfp} system fully converted the acid to the decarboxylated product.

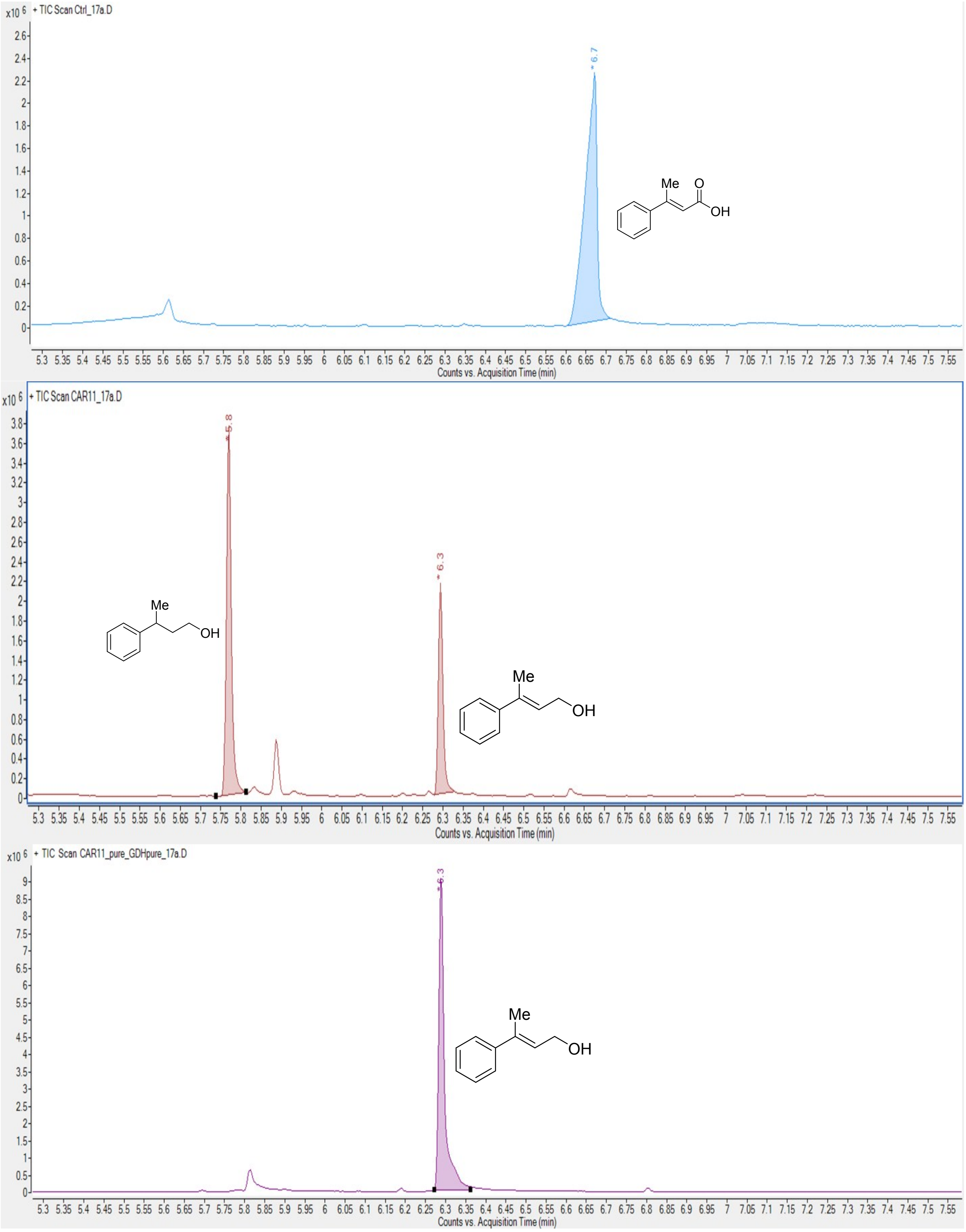

Fig. S12. Biotransformation for conversion of beta-methylcinnamic acid to the corresponding alcohol: GC traces of biotransformation reactions, showing: Row 1 = control reaction performed using *E.coli* whole cells lacking the CAR^{sfp} system. Row 2= whole-cell biotransformation reaction catalysed by recombinant *E. coli* whole cells expressing *Sr*CAR. Row 3 = *in vitro In vitro* one-pot CAR-GDH biotansformation.

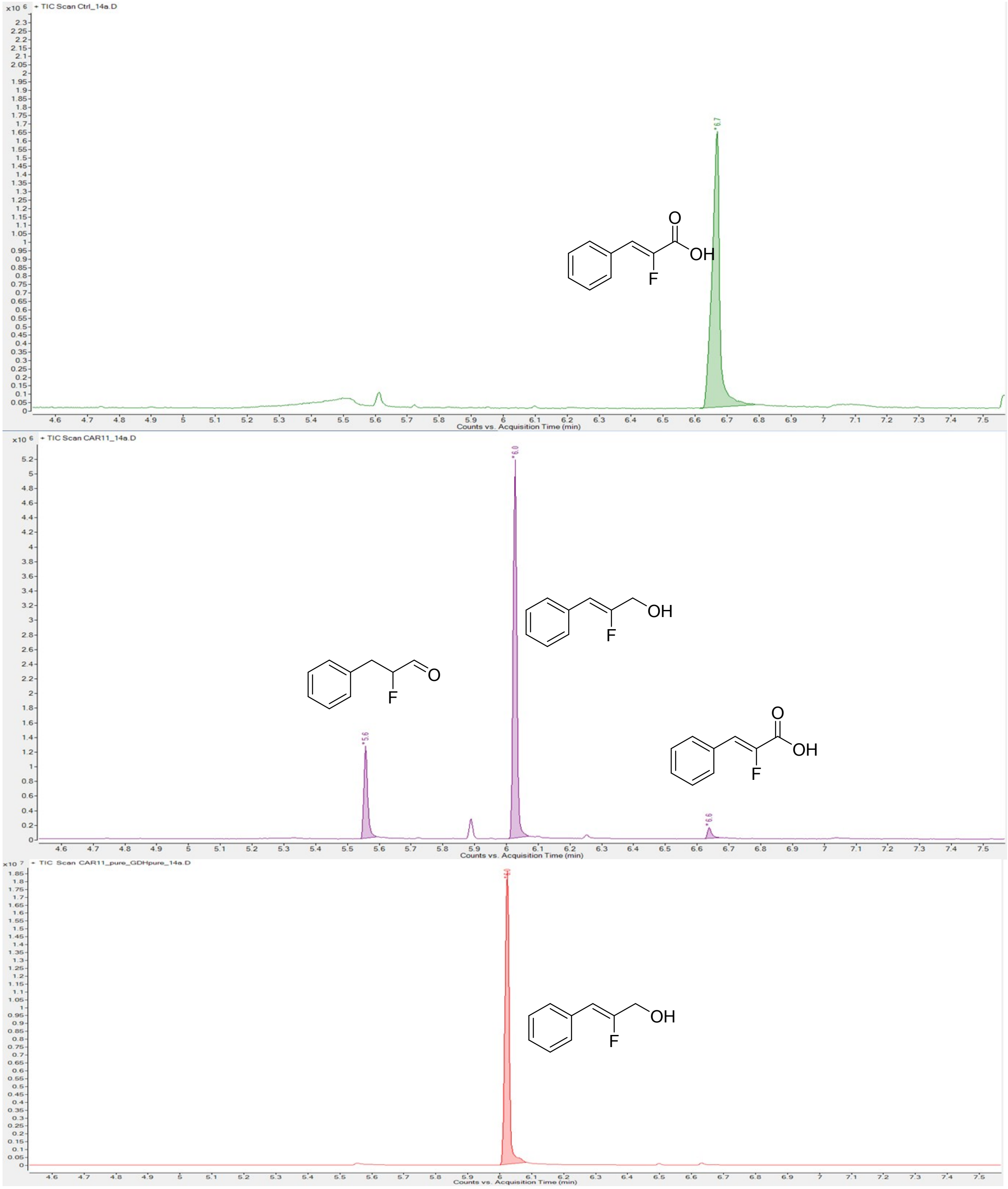

Fig. S13. Biotransformation for conversion of alpha-fluorocinnamic acid to the corresponding alcohol: GC traces of biotransformation reactions, showing: Row 1 = control reaction performed using *E.coli* whole cells lacking the CAR^{sfp} system. Row 2= whole-cell biotransformation reaction catalysed by recombinant *E. coli* whole cells expressing *Sr*CAR. Row 3 = *in vitro In vitro* one-pot CAR-GDH biotansformation.

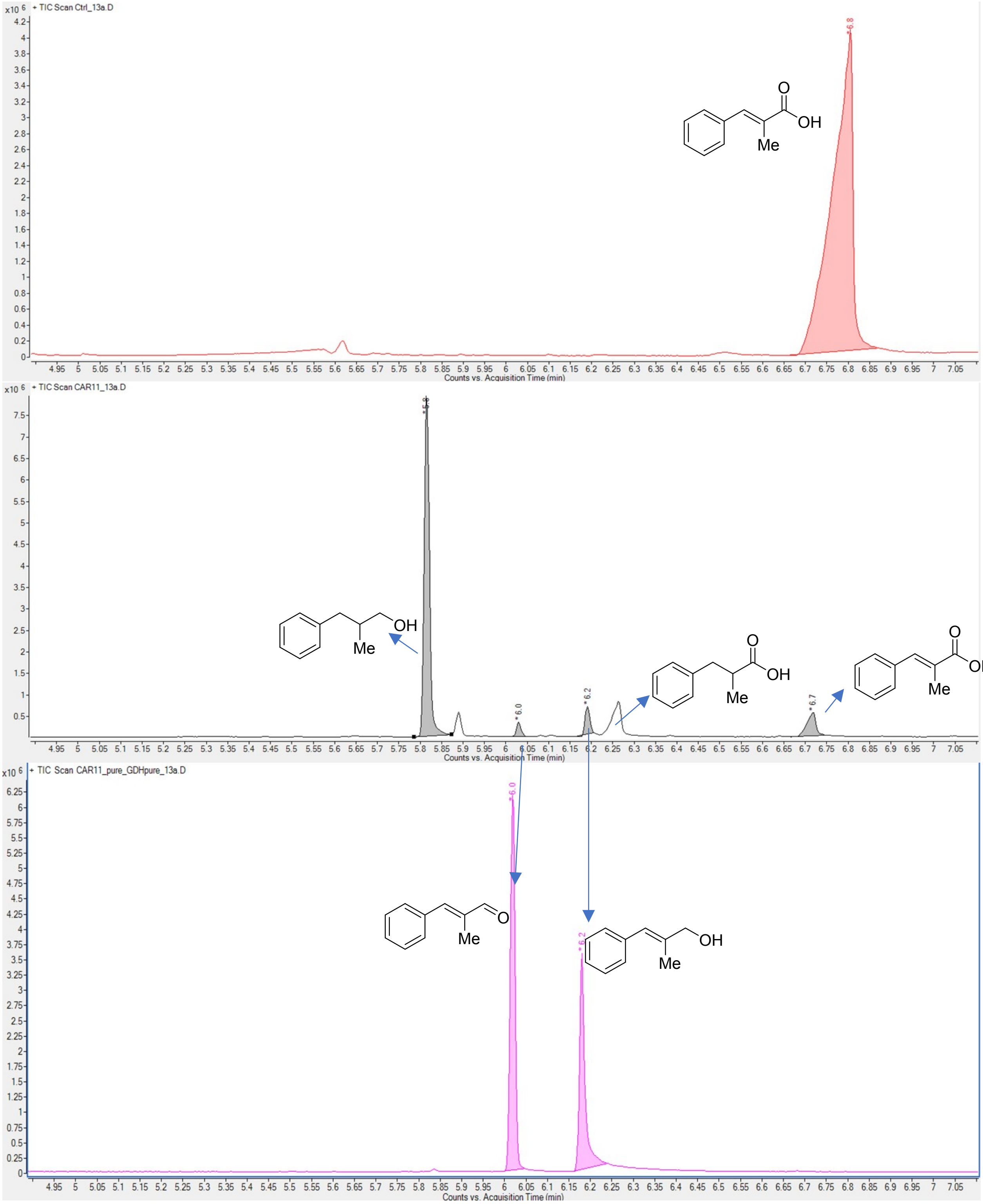

Fig. S14. Biotransformation for conversion of alpha-methylcinnamic acid to the corresponding alcohol: GC traces of biotransformation reactions, showing: Row 1 = control reaction performed using *E.coli* whole cells lacking the CAR^{sfp} system. Row 2= whole-cell biotransformation reaction catalysed by recombinant *E. coli* whole cells expressing *Sr*CAR. Row 3 = *in vitro In vitro* one-pot CAR-GDH biotansformation.

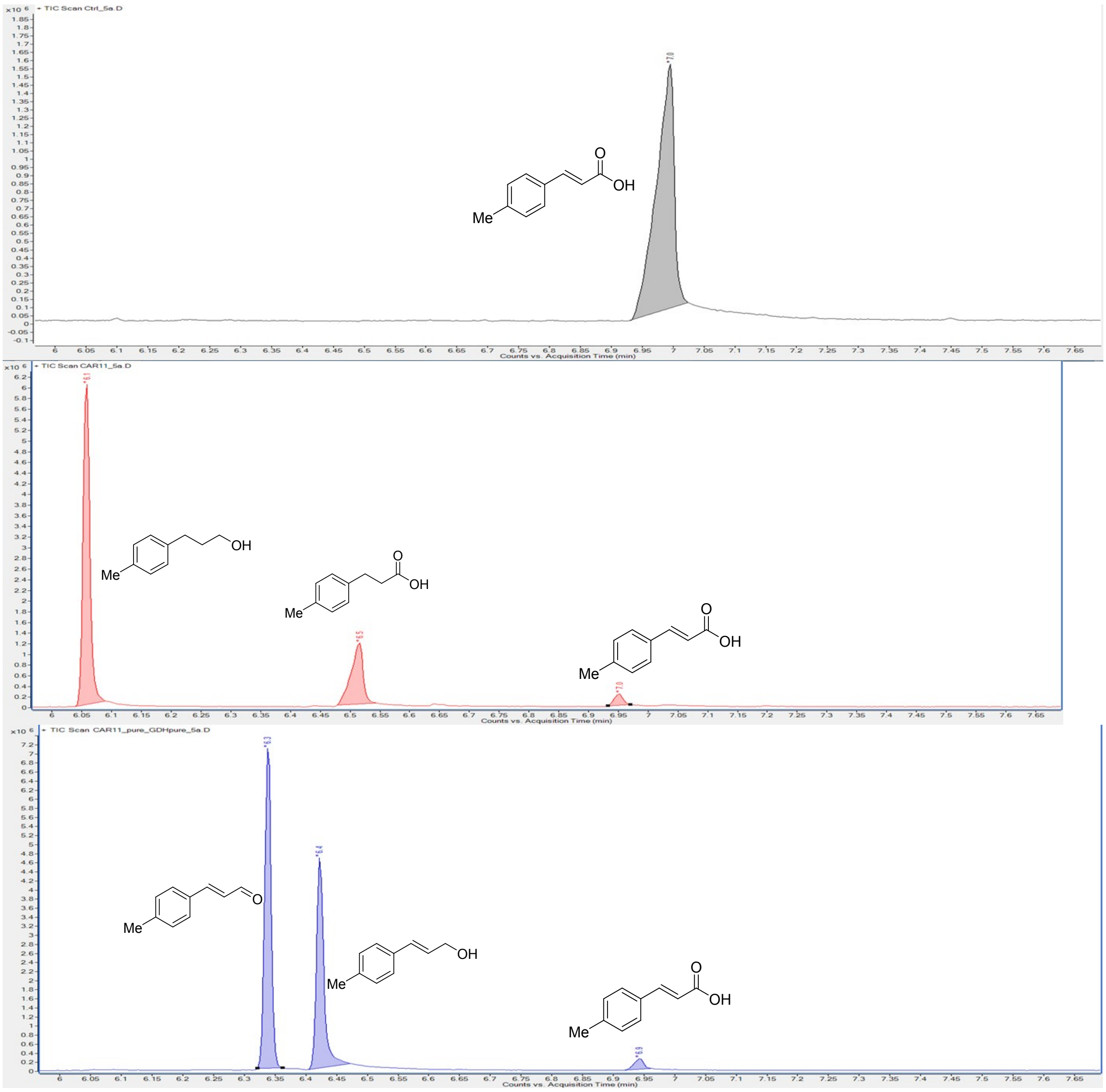

Fig. S15. Biotransformation for conversion of *p*-methylcinnamic acid to the corresponding alcohol: GC traces of biotransformation reactions, showing: Row 1 = control reaction performed using *E.coli* whole cells lacking the CAR^{sfp} system. Row 2= whole-cell biotransformation reaction catalysed by recombinant *E. coli* whole cells expressing *Sr*CAR. Row 3 = *in vitro In vitro* one-pot CAR-GDH biotansformation.

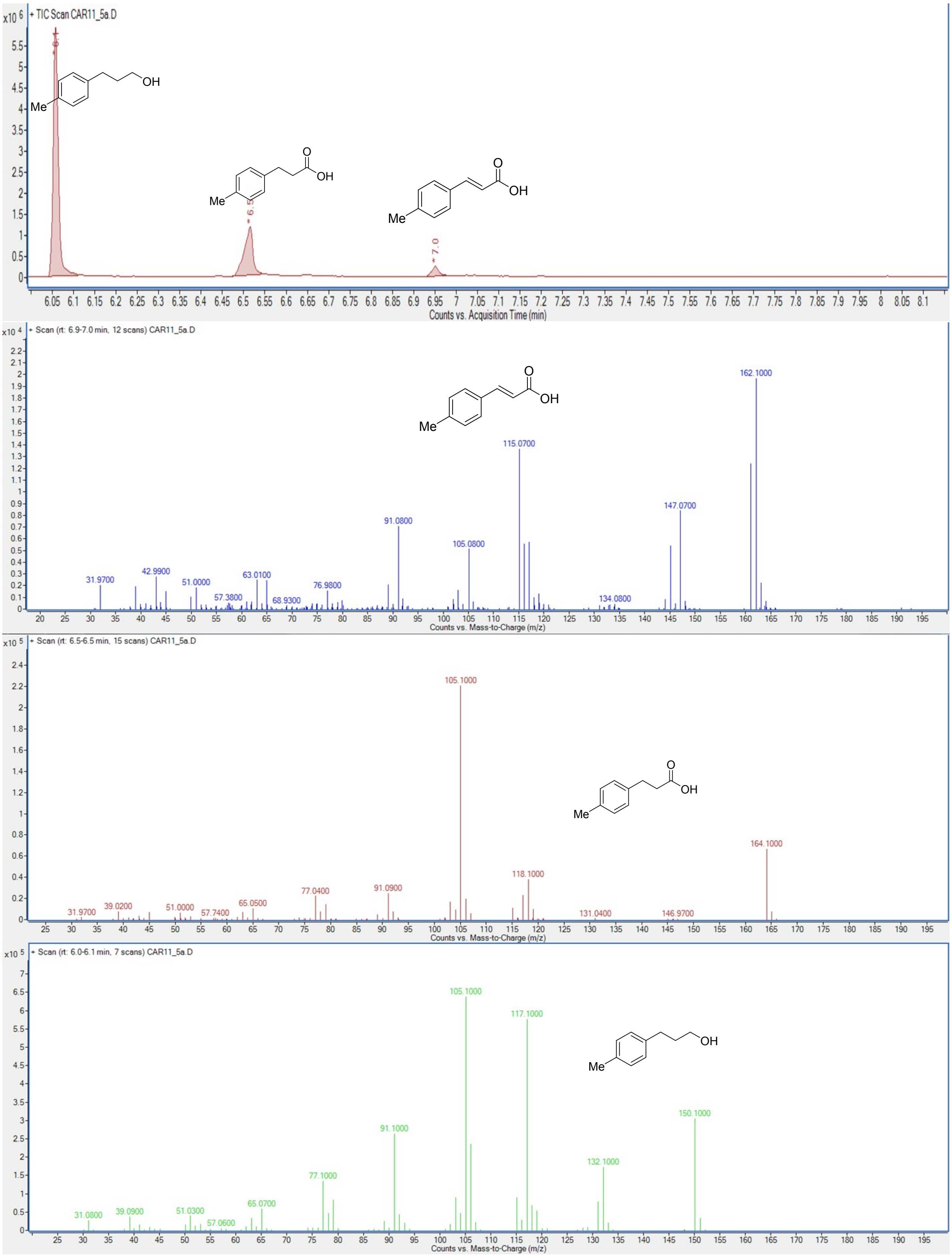

Fig. S16. Biotransformation for conversion of 4-methylcinnamic acid to the corresponding alcohol: GC-MS trace of biotransformation reactions, showing. Row 1= whole-cell biotransformation reaction catalysed by recombinant *E. coli* whole cells expressing *Sr*CAR. Rows 2-4 = mass spectra extracted from biotransformation trace, showing products and intermediates.