Supplementary Material (ESI) for Chemical Communications

Highly Efficient Asymmetric Reduction of Arylpropionic-aldehydes by Horse Liver Alcohol-Dehydrogenase Through Dynamic Kinetic Resolution.†

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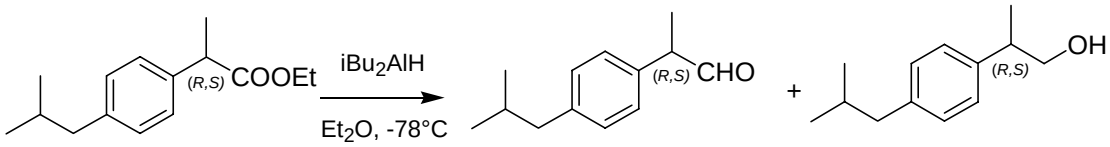
Supporting Information

1. Materials

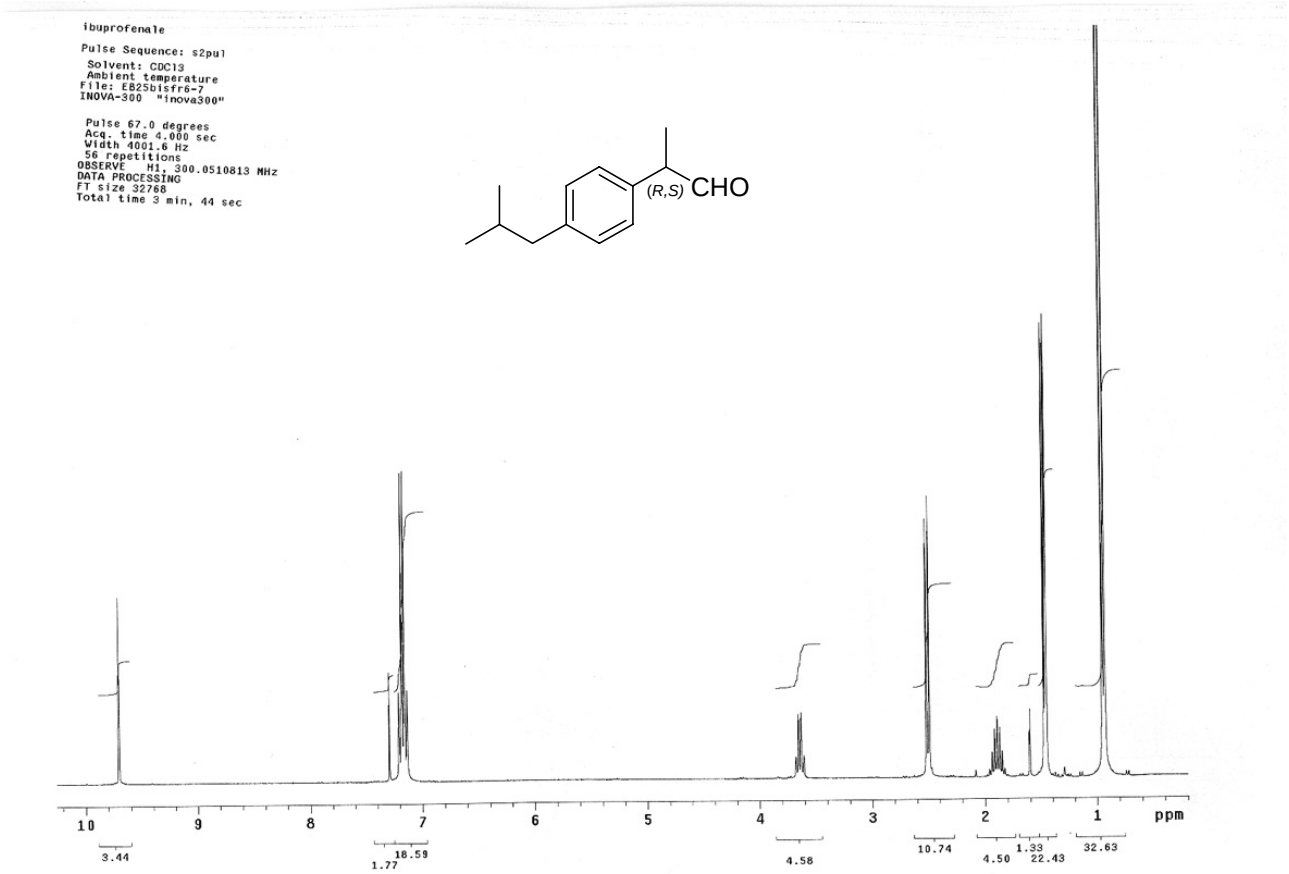
Racemic 2-phenylpropanal, 2-phenylpropanol (racemic mixture and pure *R* or *S* enantiomer), racemic Ibuprofen sodium salt and (*S*) 2-(4-isobutylphenyl)-propanoic acid (*S*-Ibuprofen) are commercially available and were obtained from Sigma-Aldrich. Horse Liver Alcohol Dehydrogenase (A9589) and NADH (N8129) were purchaised from Sigma. All other chemicals were analytical grade and used without further purification.

2. Synthesis of 2-(4-isobutylphenyl)-propanal (2-Ibuprofenal) and 2-(4-isobutylphenyl)-propanol (2-Ibuprofenol).

Ibuprofenal, 2-(4-isobutyl-phenyl)-propanal, was prepared by reducing the ethyl ester of commercially available racemic Ibuprofen (ref. 1): under inert atmosphere, ethyl 2-(4-isobutylphenyl)-propanoate (30 mmol, 7.03 g) was dissolved in Et_2O (100 mL) at -78°C, then iBu_2AlH (1M solution in hexane, 36 mL, 36 mmol) was added dropwise. Conversion was followed by GC, at completion the reaction mixture was poured into a 2M aqueous solution of potassium sodium tartrate, diluted with Et_2O (100 mL), vigorously shaken until phase separation and re-extracted with Et_2O (3× 50 mL). Ibuprofenol, 2-(4-isobutyl-phenyl)-propanol, is obtained as over-reduction-by-product in the same reaction. The alcohol and aldehyde can be easily separated by flash-chromatography (cyclohexane/ethylacetate) and were obtained in 65% and 15% yield respectively. For an efficient purification of the aldehyde, the starting ester must be completed reacted.



Structure of 2-(4-isobutylphenyl)-propanal and 2-(4-isobutylphenyl)-propanol were consistent with already reported spectra. (ref. 2 and 3)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Solvent: CDCl3
Ambient temperature
File: EB25bisfr6-7
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Pulse 67.0 degrees
Acq. time 4.000 sec
Width 4001.6 Hz
56 repetitions
OBSERVE H1, 300.0510813 MHz
DATA PROCESSING
FT size 32768
Total time 3 min, 44 sec
(R,S) CHO
10
9
8
7
6
5
4
3
2
1
ppm
3.44
1.77
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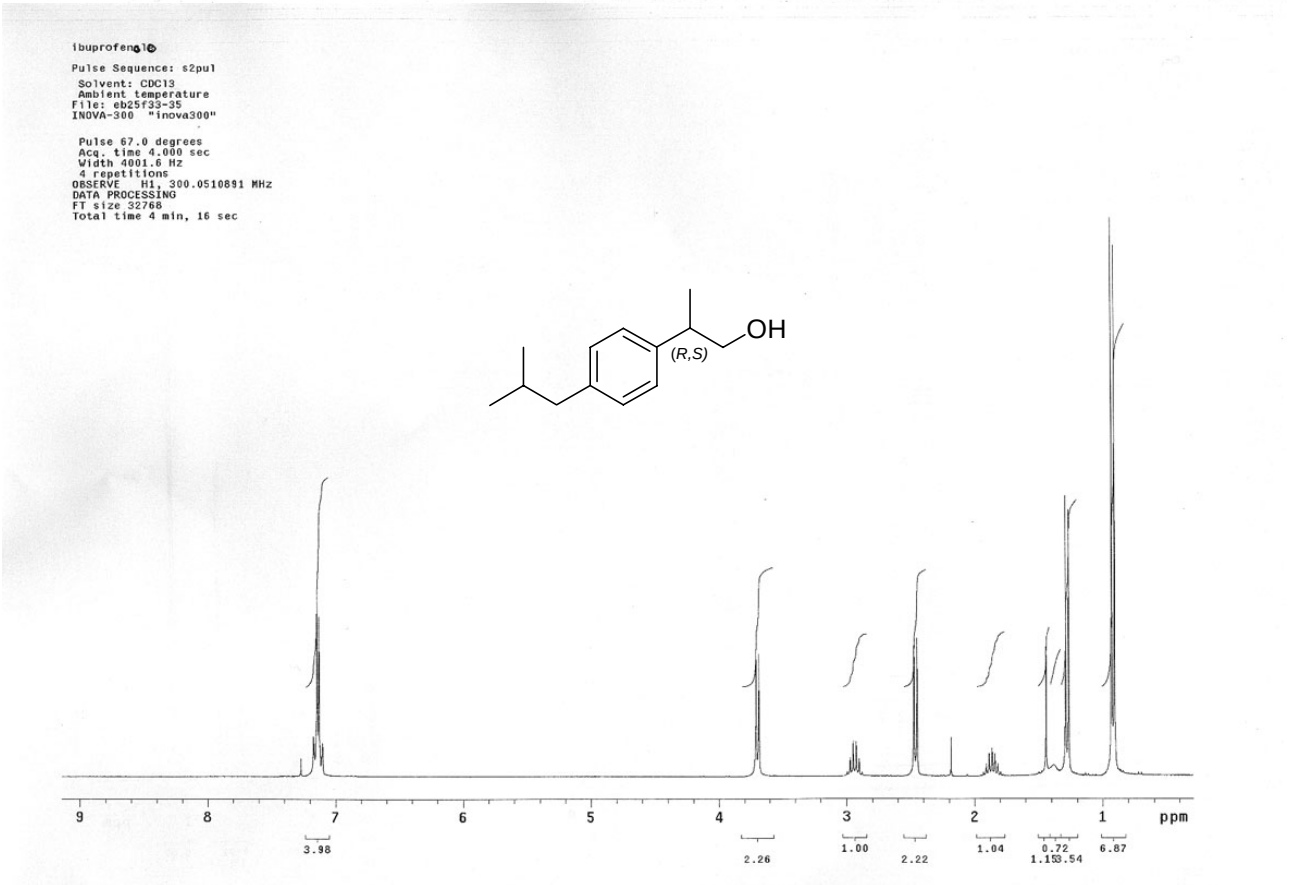
4.58
10.74
4.50
1.33
22.43
32.63


ibuprofenolo
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
File: eb25f33-35
INOVA-300 "inova300"
Pulse 67.0 degrees
Acq. time 4.000 sec
Width 4001.6 Hz
4 repetitions
OBSERVE H1, 300.0510891 MHz
DATA PROCESSING
FT size 32768
Total time 4 min, 16 sec
OH
(R,S)
9
8
7
6
5
4
3
2
1
ppm
3.98
2.26
1.00
2.22
1.04
0.72
1.15
3.54
6.87

3. Procedure of the enzymatic reduction

3.1 Preparative enzymatic reduction using CH_3CN as a co-solvent, mmol scale.

2-Phenylpropanal enzymatic reduction has been conducted on a mmol scale, using CH_3CN as a co-solvent. Reagents have been added in the following order: 2-phenylpropanal (1 mmol, 134 mg), EtOH (0.3 mL), CH_3CN (4 mL), phosphate buffer (20 mL), NADH (1 μmol, 7 mg), the mixture has been homogenized (gentle mixing) and the enzyme (2 mg) added last. Conversion has been followed by HPLC and after aldehyde total consumption, the enzyme has been filtered off, the CH_3CN concentrated and the crude extracted with Et_2O. The organic phase has been anhydrified, concentrated and the product purified by flash-chromatography (cyclohexane/EtOAc).

3.2 General procedure for enzymatic reduction

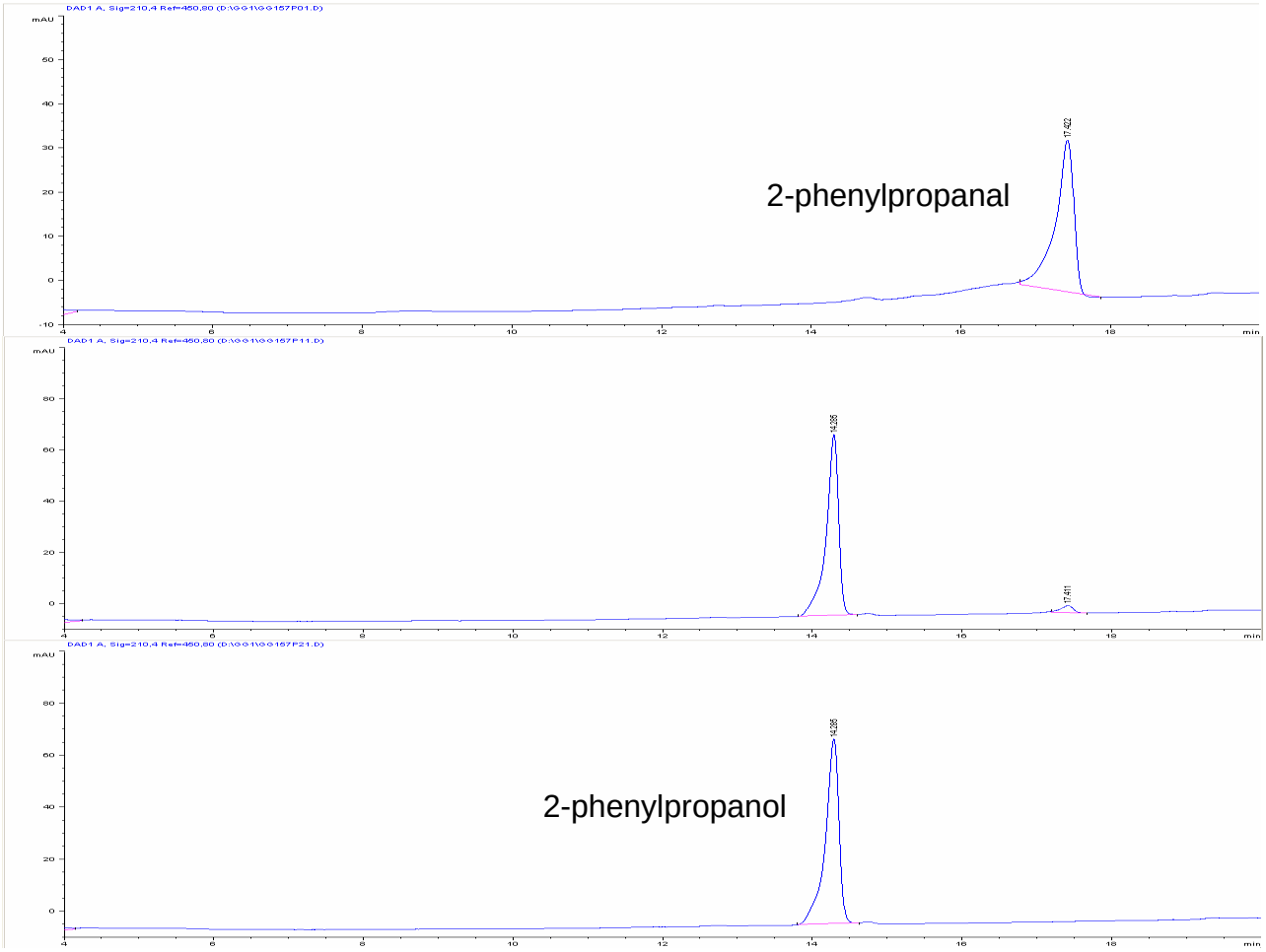
NADH recycling: into a vial equipped with a magnetic stirrer (double spinfin magnetic stirring bar) all reagents were added in the following order: 0.5 mL of a 5mM solution of the starting aldehyde in CH_3CN or THF, 0.15 mL of EtOH, 0.5 mL of a 0.1 mM solution of NADH freshly prepared in phosphate buffer (pH 7.5), phosphate buffer (pH 7.5) to reach a total final volume of 5 mL and the chosen amount of enzyme from a freshly prepared solution in phosphate buffer. In the case of co-solvent absence, 2-phenylpropanal was added starting from a 1mM solution in phosphate buffer whereas ibuprofenal, which is insoluble in buffer, was diluted in EtOH.

Excess NADH: into a vial equipped with a magnetic stirrer (double spinfin magnetic stirring bar) all reagents were added in the following order: 0.5 mL of a 5mM solution of the starting aldehyde in CH_3CN or THF, 0.5 mL of a 10 mM solution of NADH freshly prepared in phosphate buffer (pH 7.5), phosphate buffer (pH 7.5) to reach a total final volume of 5 mL and the chosen amount of enzyme from a freshly prepared solution in phosphate buffer. In the case of co-solvent absence the aldehyde was directly added to the reaction mixture.

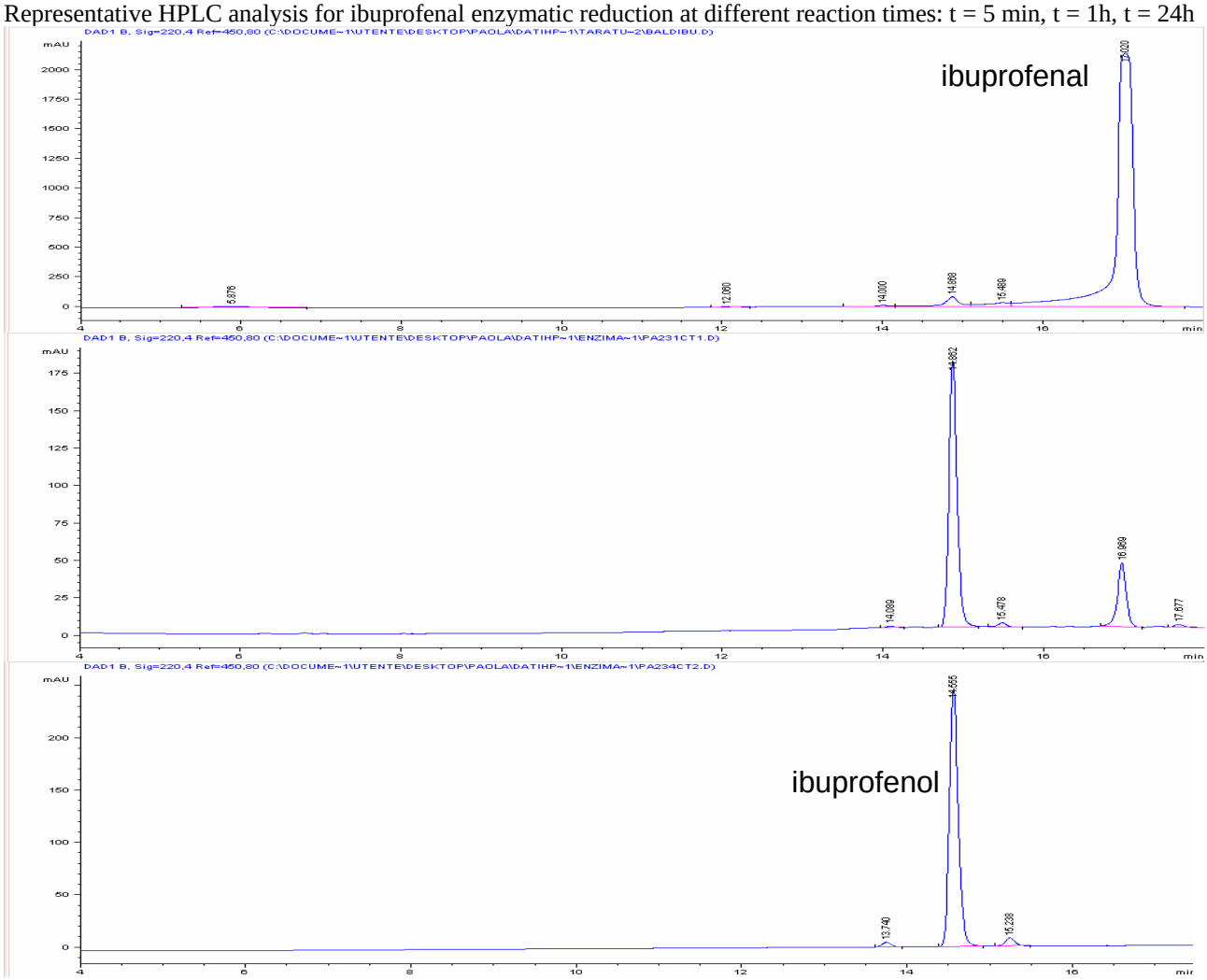
In the case of enzymatic reductions in hexane, data were obtained by adding lyophilized cofactor together with lyophilized enzyme into the reaction vessel before adding the solvent mixture and the substrate.

Formation of arylpropanols was monitored by HPLC analysis on a Agilent Eclipse XDB-C8 column (5μm, 4.6 × 150 mm), flow = 0.5 mL/min, eluent .H_20/CH_3CN 80/20 till 100% of CH_3CN in 20 min. At different reaction times, aliquot samples were filtered on a PTFE filter (0.45 μm), diluted and directly injected. Calibration curves obtained with pure arylpropanols were used for quantitative analysis.

Representative HPLC analysis for 2-phenylpropanal enzymatic reduction at different reaction times: t = 5 min, t = 1h, t = 24h



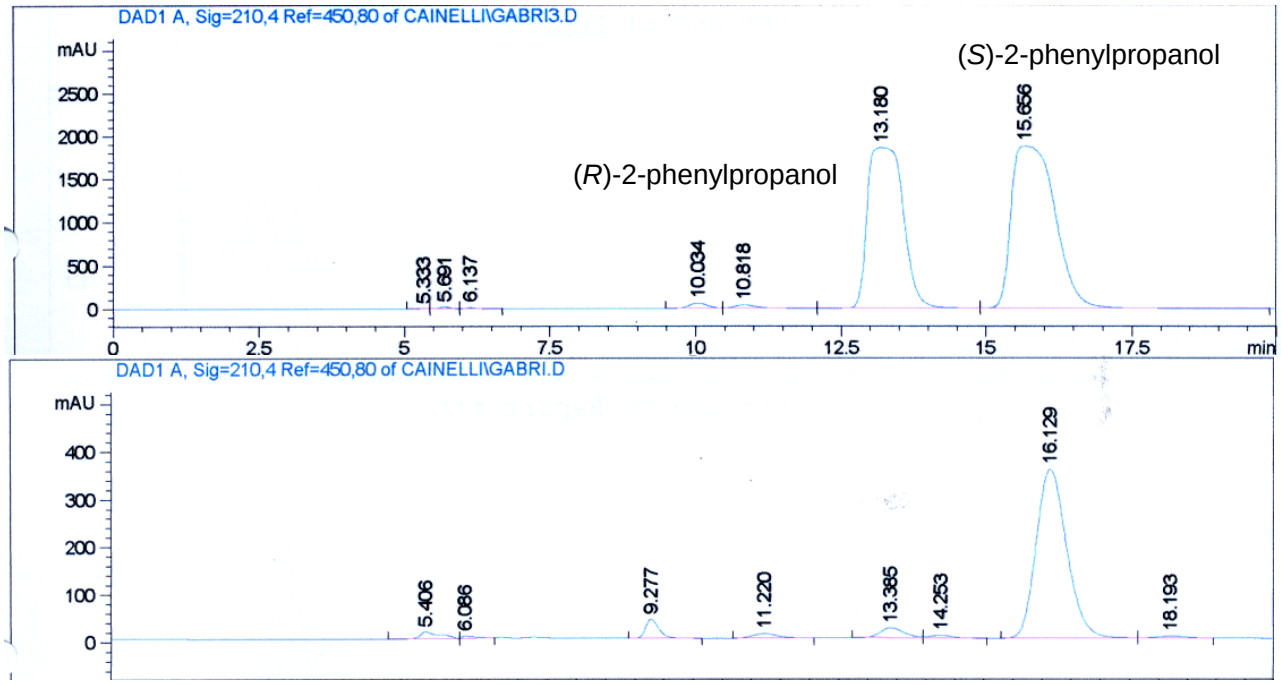
Representative HPLC analysis for ibuprofenal enzymatic reduction at different reaction times: t = 5 min, t = 1h, t = 24h



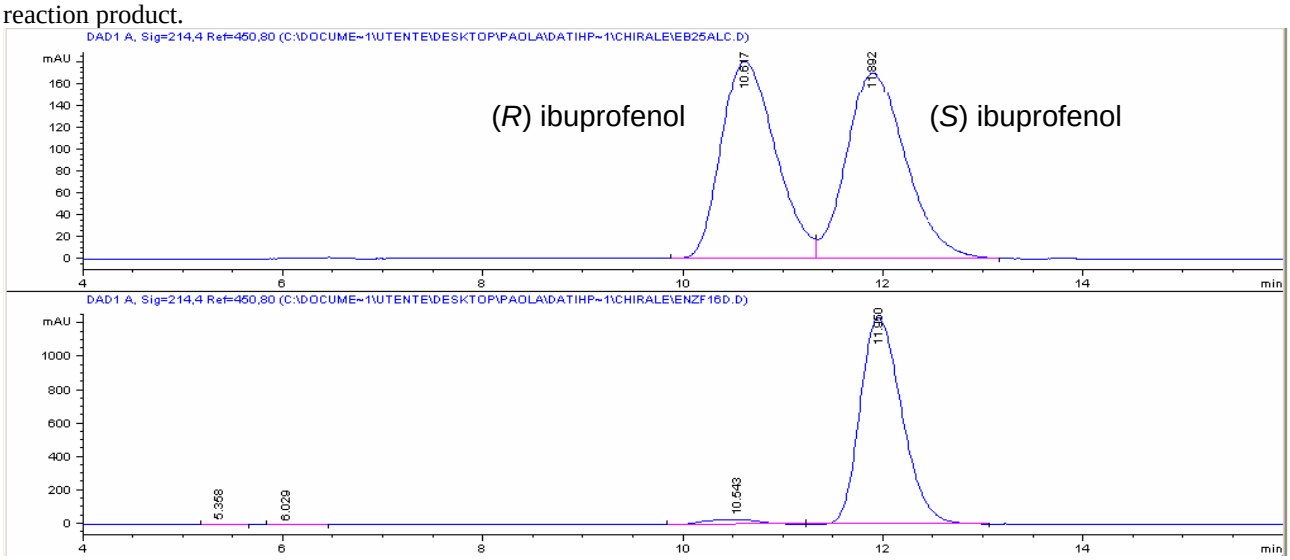
4. Determination of enantiomeric excess

Enantiomeric excesses were determined by HPLC analysis on chiral columns (Chiralcel OF, 0.5 mL/min, hexane/*i*PrOH 93/7)
Absolute configuration was established by comparison with commercial (S) 2-phenylpropanol or (S) ibuprofenol obtained by reduction with $BH_3.Me_2S$ of commercial (S) Ibuprofen following the procedure reported in ref. 4.

Representative chiral HPLC analysis for racemic mixture of 2-phenylpropanol and (S)-2-phenylpropanol obtained as enzymatic reaction product.



Representative chiral HPLC analysis for racemic mixture of Ibuprofenol and (S)-ibuprofenol obtained as enzymatic reaction product.



References

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4) A. Chen, L. ren, C. M. Crudden *J. Org. Chem*. **1999**, *64*, 9704-9710.