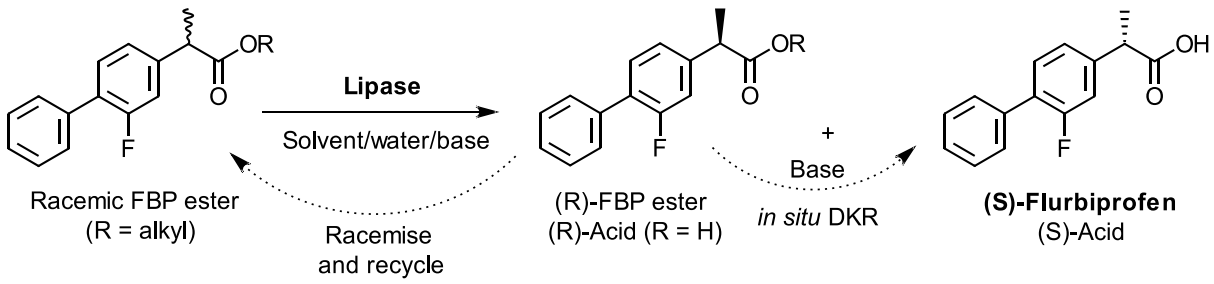
CatSci
BEYOND PROCESS CHEMISTRY

Bio-catalysis Case Study
LIPASES
Development of a DKR for a Generic API

INTRODUCTION
Aesica, a UK contract development and manufacturing organisation (CDMO), wished to develop a **novel bio-catalysed manufacturing route** for one of their products, **(S)-Flurbiprofen** (FBP), a generic active pharmaceutical ingredient (API) similar to ibuprofen. The existing process used a classical but inefficient resolution requiring repetitive crystallisations, and faced imminent patent expiry.

AIM
The aims of the project were to develop **a cheaper manufacturing route** and generate **novel IP.** These would help to maintain an **advantage over other manufacturers**, particularly those in developing economies.



A **bio-catalysed route** would achieve both aims, particularly if coupled with a **dynamic kinetic resolution** (DKR), since this would greatly **increase the efficiency** of the manufacture. A bio-catalysed route would also be more **sustainable** than one based on petrochemical sources

PROPOSAL
A **lipase-catalysed hydrolysis** of the racemic FBP ester to deliver the desired enantiomerically pure (S)-acid was proposed (**Scheme**, above). Ideally a **dynamic kinetic resolution** would be found to allow the unwanted (R)-enantiomer of the ester to be racemised *in situ* and thus also converted to the desired (S)-enantiomer of the acid. However, even a simple resolution to the desired (S)-acid would be **more efficient** than the current process.

A successful application to **Innovate UK**'s High-Value Chemical Manufacturing 2 competition and was funded to 70% for 9 months.

PROJECT PLAN
The plan to test the proposed approach was composed of several stages:
- develop the **analytical methods** (LC)
- **prepare** racemic and enantio-pure ester(s)
- **screen** a range of bio-catalysts (lipases)
- **test** potential racemisation conditions
- develop **preparative processes** on the preferred lipase/ester combination

ANALYSIS
The project required development of three LC methods:
- an **achiral LC** method to monitor reaction progress from ester to acid
- a **chiral LC** method to determine the enantiomeric excess (e.e.) of the acid produced
- a **chiral LC** method to monitor racemisation of the chiral esters for DKR studies

SYNTHESIS
A **range of racemic esters** was readily prepared by simple acid-catalysed esterification from the racemic acid available on scale from the CDMO. Preparation of **several chiral esters** (from chiral acid) required the use of CDI as a coupling agent to avoid any racemisation. In total, 10 esters were prepared in racemic form and three more in enantiomerically pure form. Six were screened against commercial lipases.

BIO-CATALYSIS SCREENING
Bio-catalysis screening was conducted primarily against the preferred ethyl ester in mixed aqueous/organic solvents. Nearly **70 lipases were screened**, the majority being **commercially available**, to reduce future scale-up costs.

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Other racemic esters were synthesised and screened, for comparison. Screening was conducted in **CatSci's standard screening equipment** (**photo**) and multiple sample points were taken by automation to monitor reaction progress.

Those reactions showing good progress by achiral LC were also assessed by chiral LC for their enantioselectivity. Although initial results favoured the unwanted chiral acid, later bio-catalysts were found which favoured the desired (S)-acid in enantiomeric excesses of **>90%.**

RACEMISATION SCREENING

Two enantiomerically pure esters were screened against a range of 30 bases (organic, inorganic and solid-supported bases) in eight solvent mixtures.

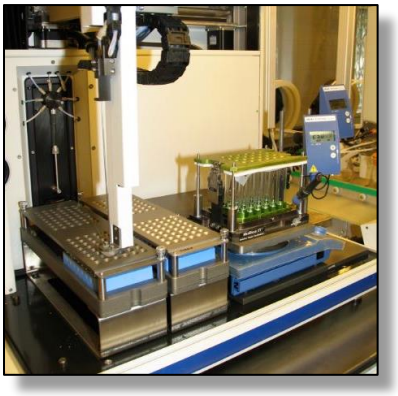
The **preferred ester** was only racemised by two bases, and not without some chemical hydrolysis to racemic acid. The **alternative ester** was racemised quickly with six bases and no hydrolysis to racemic acid, but unfortunately was unsuitable for use on manufacturing scale.

In neither case could a one-pot DKR be established in principle – the bases appeared to poison the lipases used. Despite this, an **off-line process** to racemase the preferred ester using an acceptable base and non-aqueous solvent was established which **completely avoided ester hydrolysis**. It would be possible to incorporate this method into a manufacturing process by engineering and plant changes.

DEVELOPMENT STUDIES

A handful of the best lipases were **screened in more detail**, varying factors such as solvent, concentration, pH and temperature. The two best lipase-catalysed processes were then **scaled to multi-gram level** and the processes further developed to provide an initial process for tech transfer to the CDMO.

The high e.e.s (~90%) achieved from the screening results **were retained** through-out this process and, indeed, improved as development continued. Work-up and isolation processes were developed during this phase. Lastly, a polymorph check confirmed that no changes had occurred in the physical form of the desired enantiopure acid which had been prepared from this **new bio-catalytic process**.



SUMMARY

CatSci conducted all the bio-catalysis screening and initial development for this project as well as much of the early scale-up and development study needed.

Specifically, CatSci achieved the following:

- developed three analytical LC methods, including two chiral ones
- prepared over a dozen racemic and enantiopure FBP esters
- screened several esters against nearly 70 commercially available lipases
- achieved lipase-catalysed hydrolysis to the preferred (S)-acid in >90% e.e.
- conducted extensive studies to establish if a DKR was possible
- developed an off-line recycle of the unreacted (R)-FBP ester as a back-up process
- performed lab-scale development of the process with two preferred lipases
- additionally, identified lipases which provided the alternative (R)-acid in high e.e.

ACKNOWLEDGEMENT

CatSci is grateful to Innovate UK (formerly the TSB) for generous funding and support to this project (project no. TP 131518).

CONCLUSIONS

A **novel bio-catalysed route to the generic API (S)-Flurbiprofen** was identified and developed to demonstrate proof of concept. The formative process was transferred to the CDMO for further scale-up and optimisation studies. The success of the project was validated by a joint patent (WO 2016110708, 2016) to protect this novel bio-catalytic route to (S)-Flurbiprofen and its enantiomer.

Want to find out more?
Contact us at ***technical_enquiries@catsci.com*** *or visit our website* ***catsci.com***

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