ChiroSolve

Enantiomer preparation for discovery, development and manufacturing

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Background on Chiral Chemistry

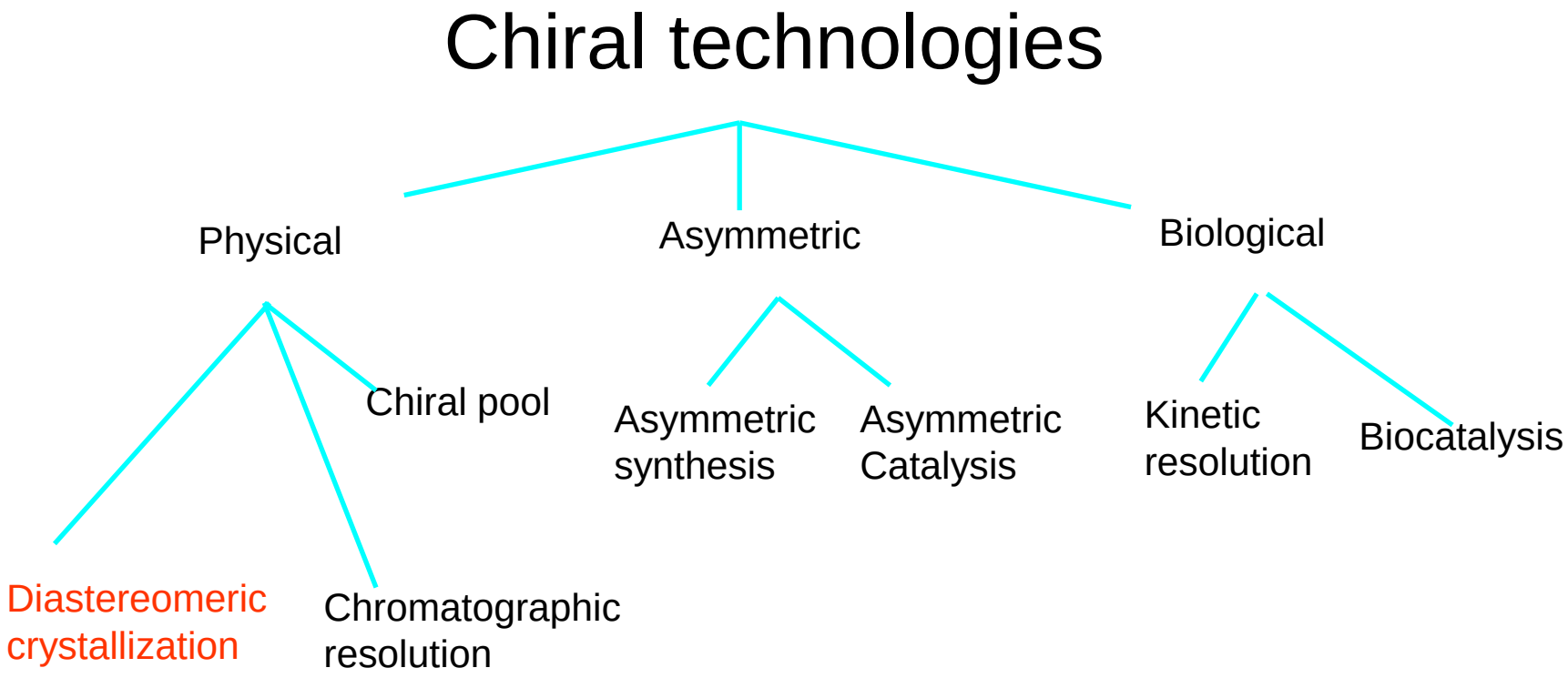
Chirality in pharmaceutical market

- Chiral molecules are closely related, mirror-image molecules with +ve or –ve optical rotation; very often with different bioactivity
- Worldwide single enantiomer drugs market is over $200 billion; 40% of total drug market
- 9 out of 10 top selling drugs are chiral in nature
- Number of chiral molecules as new drug candidates entering pre-clinical stage per year: 400
- Number of chiral drug molecules being considered as potential candidates: 40,000

Why chirally pure drugs?

Industry need to	How chirally pure product solves this need
Ensure product* safety	Unwanted enantiomer can be toxic, may cause side-effects
Ensure product* efficacy	Enantiomers have different (sometimes opposite) bioactivity
Minimize dosing	Enantiomerically pure products cut dosage by up to 50%, increase patient tolerance
Save time and money	Pure enantiomer saves clinical testing time, reduces bring-to-market cost – no requirement to test racemate or the other enantiomer
Comply with regulations	FDA and EU require fully study and disclosure of bio-activities of each enantiomer in drug; proof of enantiomeric purity using prescribed methods

Pathways to pure enantiomers



65% of chirally pure products are manufactured using diastereomeric crystallization. Chirosolve products are based on Diastereomeric crystallization technique

Diastereomeric crystallization: example separation

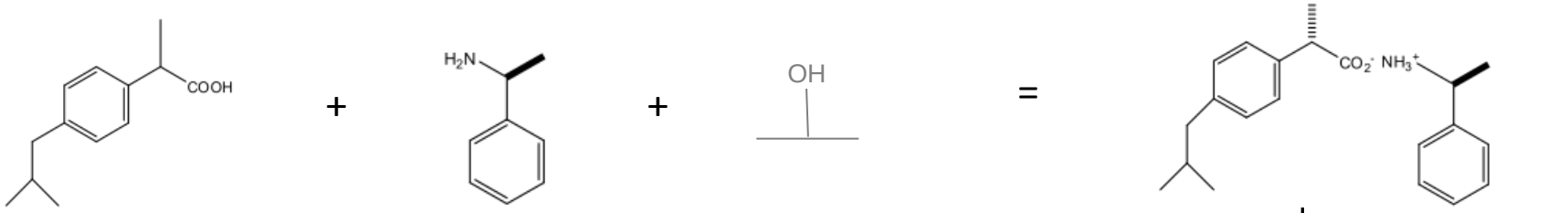
(+/-)Ibuprofen

(-) phenethyl amine

2-propanol

Diastereomeric salt

(-) crystalline and (+) filtrate



Treat with strong acid and separate out

(-)isomer (crystalline)

(+)isomer (filtrate)

Scale-up to identify
# of re-crystallizations
needed to get target purity

Diastereomeric crystallization challenges

- Identifying the optimal separation using diastereomeric crystallization technique involves choosing the right reagent and solvent out of 100s of combinations.
- High cost of initial material during discovery stage limits the ability to explore chiral separation routes effectively
- Due to time and resource constraints, pre-selecting small set of reagents to screen the racemate leads to sub-optimized method development
- Manual approach to both initial separation and optimization is monotonous, time consuming, and error-prone

ChiroSolve Products

Solution: ChiroSolve Products

- **Screen**
  - Exhaustive screening of racemate against 384 combinations provides comprehensive separation conditions for IP coverage
  - Short-lists the ideal separation conditions to be explored during method development
- **EnantioPrep (Screen + Purification kits)**
  - Isolates pure enantiomer from racemate for discovery and preclinical
  - Produces small quantity (up to 20 gm) of enantiomer within matter of days
- **ScalePrep (screen + Purification + Optimization kits)**
  - Explores short-listed separation conditions to identify shorted route to produce pure enantiomer
  - Further optimizes selected condition to identify ideal ratio between racemate and reagent; ideal concentration of solvent(s)
  - Optimized method is scalable to kilo quantities

ChiroSolv® advantage

- Short Term
  - Exhaustive and parallel screening provides several suitable options
  - Consistent results; robust method
  - Considerable time saving, resource cost saving
  - High versatility increases project success
  - Well documented step-by-step procedure eliminates human errors

- Long Term
  - Minimize research and manufacturing costs by finding the optimum strategy to employ when dealing with chiral separation
  - Reduce time-to-market by streamlining your research processes
  - Low cost, robust method to follow
  - Expand research options
  - Unbiased results ensure full exploration of separation methods
  - Establish IP foundation

ChiroSolv® Screen kits

- **384 combinations of resolving agents/solvents to screen against in parallel**
  - 4 kits per racemate; each with 96 vials (4 acid kits, 4 base kits)
  - Consistent results; identifies comprehensive set of conditions
  - Exhaustive screening offers unbiased results

- **Very little racemate needed** per experiment (0.001 to 0.03 mmol per vial)
  - Researchers do not have lot of sample to work with during early research

- **High-throughput standard kits**, compatible with most auto-stations
  - Vials and rack are chemically inert and withstand extreme temperature
  - Whole experiment can be done inside the rack without taking out vials
  - No additional setup required; kits are ready to use out of the box

ChiroSolv® Screen kits (cont')

- **Designed to screen any racemate, giving results within 24 hours**
  - Racemate can be acid, base, alcohol, aldehyde, ketone or amino acid and can have single or multiple chiral centres

- **Resolving Agents and Solvents chosen on the following criteria**:
  - Literature precedent for optimum crystallisation
  - Compliance/traceability
  - Availability and cost
  - Environmental/FDA regulations (i.e. no acetone due to shipping issues)

- **Ideal for easy robotic manipulations**
  - Kits come with peelable seal or pierceable cap-mat to accommodate direct injection of racemate by liquid dispensers

Types of Screen kits

Primary Screening

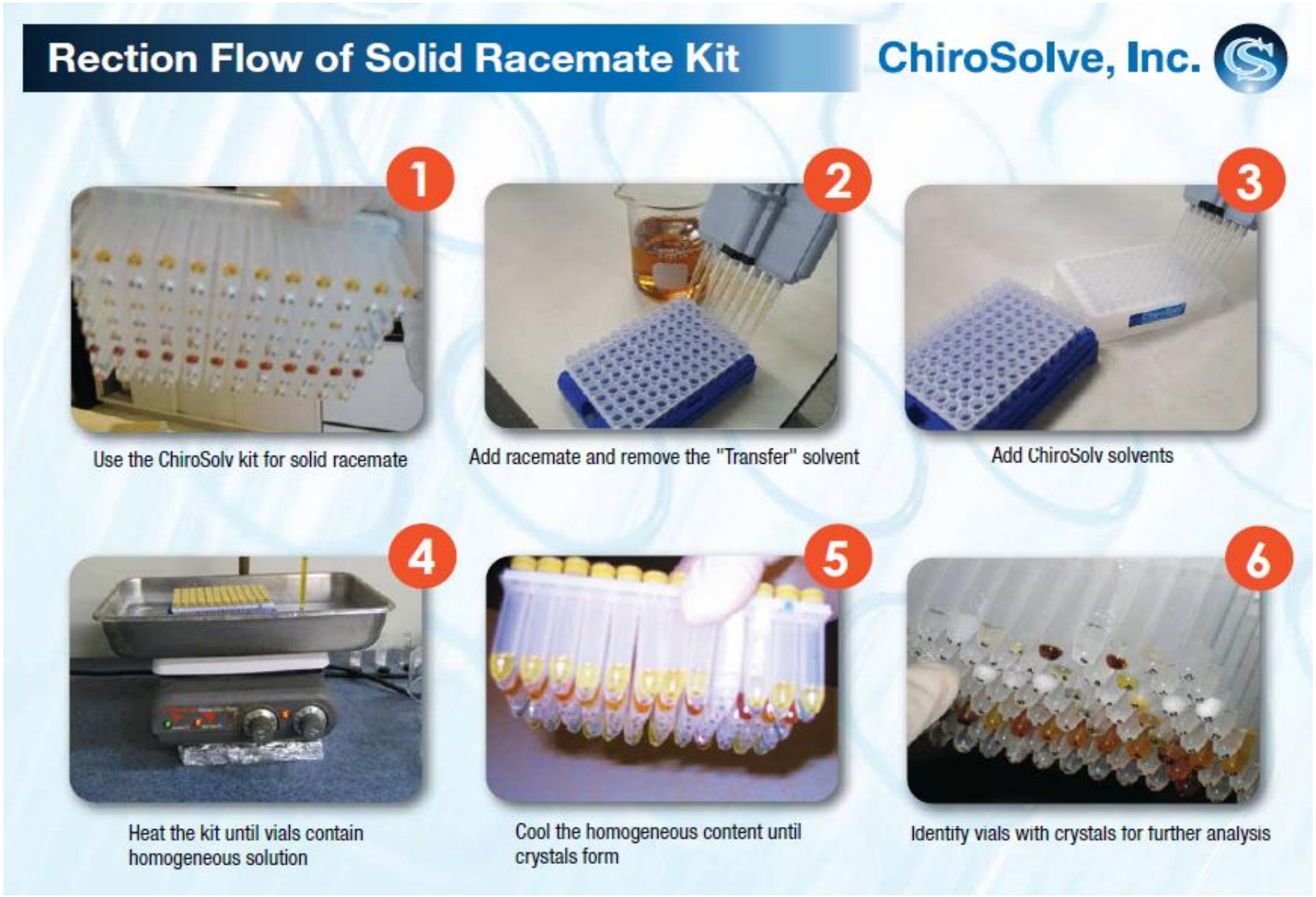
- Set of four (4) kits per racemate
  - Comprehensively covers 384 conditions known to offer best separation
  - Racemate/reagent ratio is 1:1
  - Requires totally up to 12 mmol or avg. 3.5 gm racemate (so that diastereomeric salt crystals can be identified with naked eyes; no optical detection needed)
- Kits have 2 components:
  - 96-vial plate has 8 resolving agents (unique reagents in each row); and each vial contains 0.03 mmol of reagent
  - 96-well plate has 12 solvents (unique solvent in each column) which have to be added to the reagent-racemate mixture during reaction

Strong Acid Kits: contain strong acids to be used against weakly base racemate

Reduced Volume Glass Vial Kits: Same as primary screening but totally require only 0.4 mmol or avg. 0.12 gm racemate

- Must be used with HPLC or other optical method of analysis
- Ideal for fully automating screening of library of compounds

How to use ChiroSolv® Screen kits



Stage-specific chiral resolution needs

Medicinal Chemistry

- Require pure enantiomer for preclinical assessment to avoid confounding effects of testing with racemate
- Fast access to enantiomer with target purity for lead identification

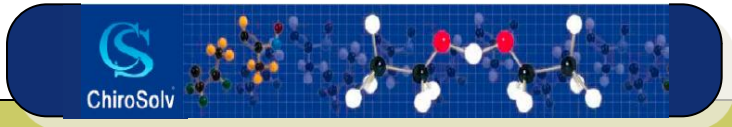
Development Chemistry

- Find efficient, safe, and cost-effective routes for producing enantiomer
- Identify best prospects for scale up and commercial manufacturing
- Adopt ‘Green’ processes

ChiroSolve medicinal chemistry solution

Medicinal Chemistry

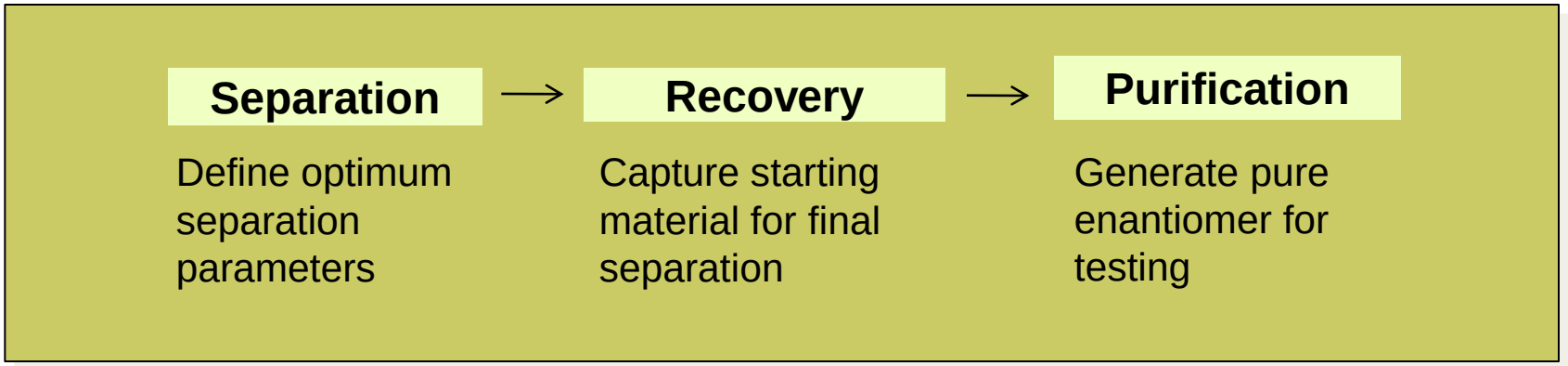
- Require pure enantiomers for preclinical assessment to avoid confounding effects of testing with racemates
- Fast access to enantiomers with target purity for initial testing



- Prepare enantiomer for discovery and preclinical study in matter of days
- Establish early IP foundation by identifying complete *scope of* reagents offering the separation
- Get small quantity of enantiomer for lead identification

Medicinal chemistry: *EnantioPrep*™

- Prepare pure enantiomer for discovery and preclinical study
- Establish IP scope
- Generate 500 mg to 20 grams of desired enantiomer
- Extract Up to 90% enantiomer from racemate, purity over 95%
- Define the ideal resolution pathway



Medicinal chemistry: *EnantioPrep*™

Screen

Matrix of 32 resolving agents and 12 solvents

Typical: 10 ‘hits’
Analyze with HPLC

Use the supplied chemicals and tools to recover racemate from all wells

Purify

Use selected resolving agent and solvent and purification tool for enrichment

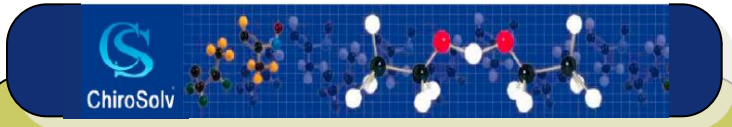
Pure enantiomer

- Prepare pure enantiomer within days
- Maximize use of racemate
- Establish IP scope
- Generate 500 mg to 20 grams of desired enantiomer
- Define the ideal resolution pathway

ChiroSolve development chemistry solution

Development Chemistry

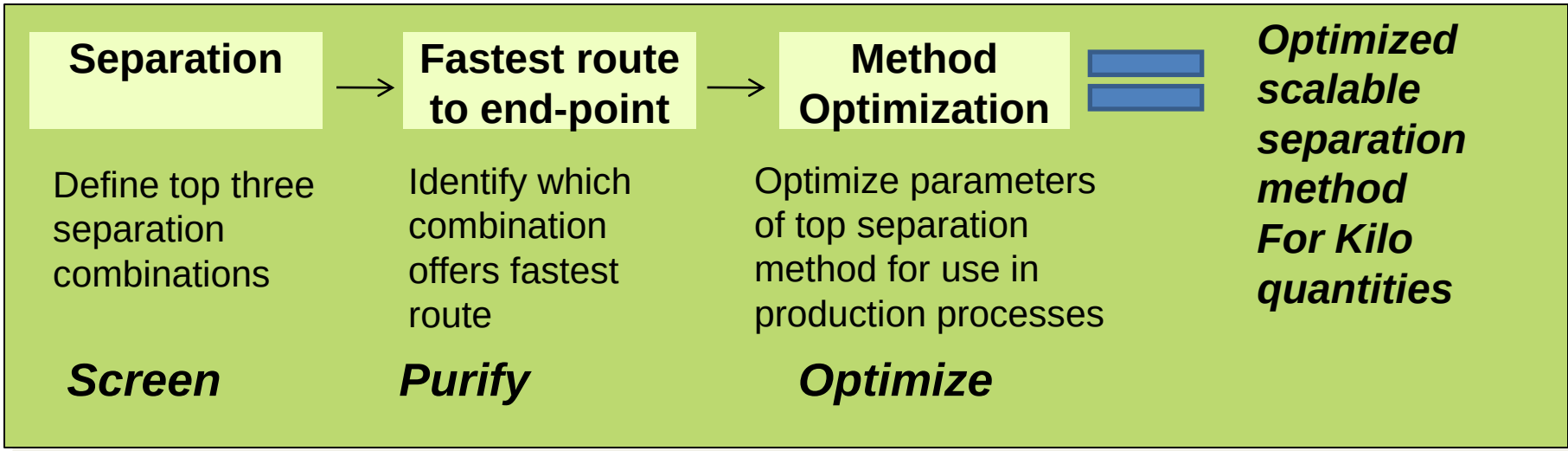
- Find efficient, safe, and cost-effective routes for producing enantiomers
- Identify best prospects for scale up and commercial manufacturing
- Adopt ‘Green’ processes’



- Efficient routes for producing enantiomer
- Define commercially viable and cost effective processes
- Efficient conversion of existing methods to Green Chemistry processes

Development chemistry - *ScalePrep*™

- Identify cost-effective and shortest route for manufacturing enantiomers
- Predictive for scaling from kilo lab to ton production
- Optimize process using factors: energy efficiency, safe chemical usage, minimization of waste
- Collect data on alternative processes



Development chemistry: *ScalePrep*™

Screen

Matrix of 32 resolving agents and 12 solvents

Typical: 10 ‘hits’;
Analyze with HPLC;
Select best 3 hits

Purify

Using each of 3 combos, purify and see which offers pure enantiomer with minimum steps

Optimize

Order Optimize kits from Chirosolve to evaluate optimization factors (temperature ramps, stoichiometry)

Optimized crystallization method

- Choose reagents that offer shortest route to end-point
- Define the ideal resolution conditions for scale-up in terms of proportions of starting material, energy savings

Recovery and Purification kits

After the screening, these kits recover the racemate used; and then obtain pure enantiomer through series of re-crystallization steps. Each kit includes:

- **Recovery solution and recovery unit**: When the contents of the screening kits (after the expertiment) is collected into the recovery unit containing the recovery solution, 2 liquid layers are formed: bottom layer contains the racemate; while the top layer contains the resolving agent
- **Purification and Recovery Bottles** that allow easy collection of purified enantiomer and filtrate containing the other enantiomer respectively
- **Filtering unit** with vacuum adapter that allows fast crystal separation during re-crystallization steps

Optimization kits

Based on the results of the screening, customer may request for one or more Optimization Kits. These kits include:

- **Rows of a single resolving agent** in difference quantity to match the ratio 1:1, 1:1.5, 1:2, 1:2.5, 1:3, 1:3.5, 1:4, 1:4.5 with the racemate
- Different columns of the kit containing the **target solvent(s) in different concentration**

Using these kits, process optimization can be achieved within matter of days. These optimization parameters can improve the enantiomeric yield; or can reduce the number of re-crystallization steps. One or more optimization kits containing different combination of reagent/solvents identified as best candidates during screening process can be explored in parallel.

Success stories

- **Discovery group** from **big pharma** was unable to separate out enantiomers; Chirosolve Screening services were able to give them 4 combinations of resolving agents and solvents that gave good crystals. As a result, they were able to complete the project successfully.

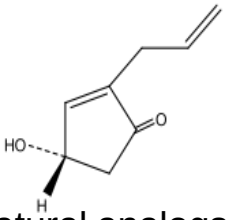
- **Discovery startup** needed a very quick turn-around to explore different separation methods for IP prosecution. Using Chirosolv kits, they were able to quickly explore the methods without having to compromise quality, leading to patentable results.

- **Process chemistry** group from **major Bio-pharma** used ChiroSolv kits to identify higher purity, and in the process, found a better method that reduced re-crystallization steps from 4 to 3.

- **CRO firm** was able to choose the best derivatisation route that would give optimum end results in terms of enantiomeric purity and yield

Example separations (performed in-house)

- **Alcoholic fragment of synthetic pyrethrins**



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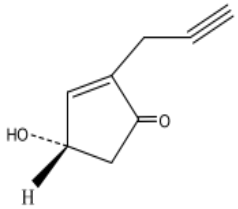
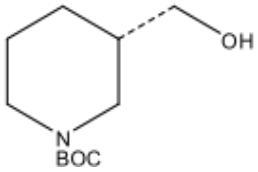


Allethrin is a potent insecticide and is more stable than the natural analogs. In order to identify the separation process using ChiroSolv, we modified this material with phthalic anhydride & succinic anhydride to prepare acidic monophthalates and succinates.

- **n-BOCS-(+)-β-hydroxymethylpiperidine**



This is an intermediate for tryptase inhibitor project.

- **Pyridine-containing β-amino acid**

1 **(**3-pyridyl-β-amino-3-propionic acid) and its ester hydrochloride 2. This combination of acidic and basic functional groups offers a wide choice of resolving agents.