

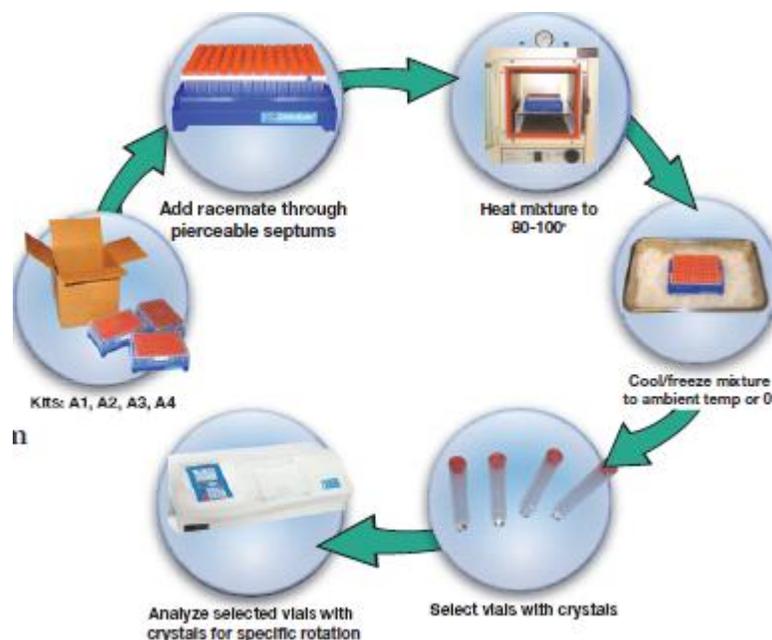
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Chiral Enhancement and resolution process optimization using Diastereomeric crystallization method

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Separation of chiral molecules (enantiomers) from racemic mixture using Diastereomeric Crystallization Technique can be achieved and enhanced by the addition of chiral acids or bases. The resulting formation of salts can be separated on the basis of their differing solubility. Using this principle, ChiroSolve Inc. has developed Chiral Resolution kits that allow scientists to quickly identify the optimum resolution process for a given racemate by providing 576 combinations of resolving agents and solvents to screen against.

Some of the advantages of using ChiroSolv[®] kits:

- **Faster research time:** Racemate can be screened against 288 combinations of resolving agents and solvents in parallel, offering results within 24 hours, a process that would otherwise take over 2 months of scientist's time
- **Accurate and consistent results:** Having the same experimental environment eliminates problems that can lead to inconsistent results
- **Optimized use of skilled staff time:** Kits are designed by an expert in Chiral Resolution; amount and types of chemicals are used with full understanding of solubility diagram. This allows delegation of this work to a Jr. staff
- **Better chance of project success:** Being able to screen a racemate against 100s of combinations of resolving agents and solvents at once and getting quick results increases the probability of project success by multi-fold

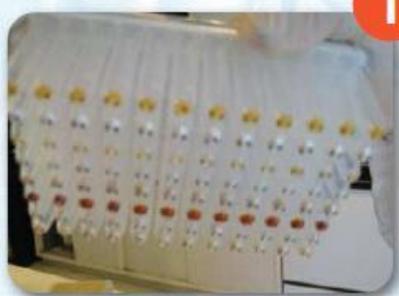
- **Very little amount of racemate is required:** During research time, scientists typically have very little amount of racemate available. Needing less than 3mmol of racemate per kit relieves this concern
- **Tremendous time and money savings**

Typical Resolution procedure

1. Choose the right type of kits depending on whether the unknown racemate is a base or acid respectively
2. If the racemate is of type alcohol, amino acid, aldehyde or ketone, do the pre-processing as described under
3. Add 0.03 mmol of your racemate to each of 96 vials. Depending on the availability of the dispensing auto station and the racemate type (liquid or powder), you may need to remove the thermal seal or cap septums. If so, use the additional septums provided to reseal the vials after adding the racemate. Note that the seal/caps are pierceable to accommodate direct injection of racemate by an autostation
4. Heat the rack along with its vials to 80° C (the optimum temperature for most of these experiments) or until the mixture becomes homogeneous
5. Allow the kit to cool to ambient temperature. Then, if required, further cool it to 4° C and finally to 0° C and observe any crystallization. Vials with crystals are considered positive tests and need further investigation
6. Using crystal initiation techniques see if you can get more vials with crystals. Vials with no crystals even after this effort are considered negative tests
7. Separate out the vials with crystals (positive tests) note down their barcode identification
8. Analyze each of the crystals separately after liberating enantiomers from it's' diastereomeric salts for specific rotation

Reaction Flow of Solid Racemate Kit

ChiroSolve, Inc.



Use the ChiroSolv kit for solid racemate



Add racemate and remove the "Transfer" solvent



Add ChiroSolv solvents



Heat the kit until vials contain homogeneous solution



Cool the homogeneous content until crystals form

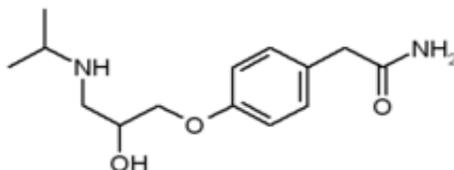


Identify vials with crystals for further analysis

Example Chiral Enhancements done and their results

A. Atenolol

Atenolol belongs to a class of drugs known as beta blockers, which are prescribed for high blood pressure, angina and to prevent repeat heart attacks. It is the most widely prescribed beta-blockers in the world and is sold under the brand name Tenormin by AstraZeneca Inc. since 1976. The drug is a basic compound with a molecular weight of 266.3 gm, a chemical formula of $C_{14}H_{22}N_2O_3$, and a chemical structure of this form



This is a β 1- adrenergic receptor antagonist. This activity is due to the (-) isomer.

In order to identify the optimal resolution condition for this basic compound and to separate out the enantiomers, acidic ChiroSolv[®] kits A-1, A-2 and A-3 were used.

Actual work

1. 9 mmol of atenolol or 2.397 gm was dissolved into 60 mL of methanol. 200 uL was dispensed through the pierceable septums into each of the 288 vials of the kits A-1, A-2 and A-3 using robotic liquid dispenser. This gave each vial approximately 0.03 mmol of atenolol
2. Kits were placed in an 80°C water bath for a few minutes until the racemate was completely dissolved and then cooled to ambient temperature
3. To encourage maximum crystallization, kits were placed in a 4°C refrigerator for 15 minutes. In case of some of the vials, some basic initiation was needed for crystal formation
4. A basic workup of 500 uL 1 N NaOH and 400 uL of ethyl acetate was performed on each of the kits to release the potentially diastereomer drug bound to the chiral resolving acid
5. The basic workup was mixed with the crystals and then the top layer of ethyl acetate containing the released compound was taken out and added to a pre-weighed tube
6. The ethyl acetate was then evaporated and the potentially diastereomeric compound was left and weighed
7. A Rudolph Research Analytical Autopol[®] III – Automatic Polarimeter was used to measure the optical rotation, α , of the crystals dissolved in methanol
8. Based on the α rotation and the crystal yield, specific rotation was calculated $[\alpha]$ and the best results that provided highest yield and rotation were identified (shown below)

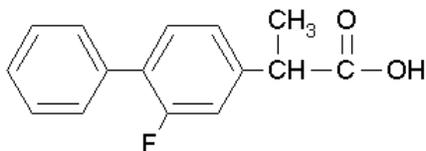
Results

- Maximum positive specific rotation of +0.133 in the A-1 kit's D-6 vial which contains (-) malic acid and 90% isopropanol.
- Maximum negative specific rotation of -0.114 in the A-2 kit's D-7 vial which contains (-) dibenzoyl-l-tartaric acid and 80% methanol.

Exact enantiomeric purity can be further measured using Chiral HPLC that tells the extent of chiral enhancement achieved. We recommend that this procedure should be repeated twice to ensure consistent results. We also recommend that further scale-up of 2 or 3 best results should be performed to identify the best resolution process during manufacturing.

B. Flurbiprofen

Flurbiprofen is a member of the phenylalkanoic acid derivative family of non-steroidal anti-inflammatory drugs (NSAIDs) used to treat the inflammation and pain of arthritis and can be used for the treatment of metastatic prostate cancer, and Alzheimer's disease. It is also known by the trade name **ANSAID** and marketed by Pfizer. The drug is an acidic compound with a molecular weight of 244.27 gm, the chemical formula $C_{15}H_{13}FO_2$, and a chemical structure of this form:



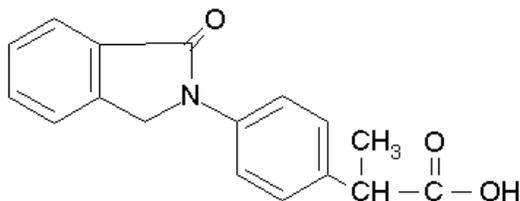
In order to identify the optimal resolution condition for this acidic compound and to separate out the enantiomers, base ChiroSolv[®] kits B-1, B-2 and B-3 were used.

Results

It was found that Quinine in methanol would offer the ideal condition (more data available when paper is published)

C. INDOPROFEN

This is an off the market analgesic and anti-inflammatory drug that may be a starting point for finding a new drug to treat spinal muscular atrophy (SMA), a devastating childhood neurological disorder. The drug is an acidic compound with a molecular weight of 281.31 gm, the chemical formula C₁₇H₁₅NO₃, and a chemical structure of this form:



In order to identify the optimal resolution condition for this acidic compound and to separate out the enantiomers, base ChiroSolv[®] kits B-1, B-2 and B-3 were used.

Results

It was found that L-prolinol in 90% IPA or 95% Ethanol would offer the ideal condition (more data available when paper is published)