Enabling enantiopurity: Combining racemization and dual drug co-crystal resolution

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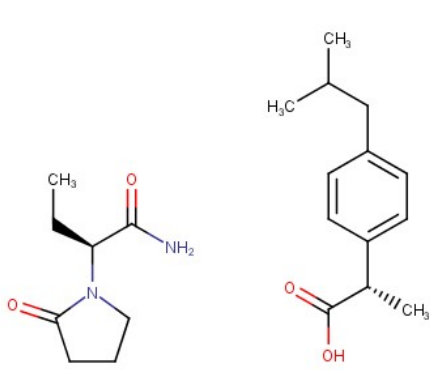
Abstract
A new tool to obtain enantiopure (*S*)-Ibuprofen has been designed. Starting from racemic Ibuprofen, co-crystallization with Levetiracetam is used as a resolution tool to obtain only the target enantiomer of (*S*)-Ibuprofen in the solid state. The resulting mother liquor, enriched in (*R*)-Ibuprofen, is recovered and submitted to a racemization cycle, after which another co-crystallization step is introduced. Levetiracem can be recovered from the co-crystal phase and reused, resulting in an economic use of the resolution agent. Overall, a novel approach to transform a racemic compound into enantiopure material has been developed.

Introduction
In the pharmaceutical industry, chirality is of key importance. Where one enantiomer has the desired effect, the other can be inactive or in other scenarios even have detrimental effects.[1–3] Some well-described examples are Thalidomide[4–6] ((*R*) works as a sedative, whereas the (*S*) has teratogenic effects), Ethambutol[4,7] (The (*S,S*)-enantiomer is a tuberculostatic, whereas the (*R,R*) counterpart causes blindness) and Dopa[4,8] (L-enantiomer treats Parkinson's, D-enantiomer causes granulocytopenia). To prevent issues like this, regulatory instances prefer drugs to be marketed in the enantiopure form[9], which circumvents the potential adverse effects.[10,11] To obtain enantiopure drugs, the drug is either synthesized in an enantiopure manner or obtained from a racemic mixture applying a post-synthetic physical chiral separation process. Common synthetic approaches are the chiral pool approach (using enantiopure starting materials) or utilizing an asymmetric approach (reaction preferentially leads to one enantiomer).[12–17] Alternatively, the separation is performed using a physical separation step after the synthesis. The most common physical separation processes are crystallization based (mainly diastereomeric salt formation) or chromatography based. [18–23] The downsides of these methods are apparent: chiral chromatography is expensive and diastereomeric salt formation is only feasible when the compound is salsifiable. More importantly, in both cases the enantiomers are separated and thus a 50% loss (unwanted enantiomer) occurs. To prevent such a loss one would have to transform the unwanted enantiomer into the desired one, which can be achieved through racemization. Racemization for organic molecules typically occurs via two pathways or combinations thereof.[24–26] A basic pathway (proton abstraction), where the intermediate structure is a carbanion in which the lone-pair can exhibit inversion or an acidic pathway involving protonation followed by displacement of the now protonated group, resulting in stereoinversion.



Scheme 1: Chemical structures of Levetiracetam and (*S*)-Ibuprofen

Over the recent decade, racemization has been combined with crystallization processes to lead to one pot deracemization processes, which ultimately lead to enantiopure material with a theoretical 100% yield. Viedma ripening[27,28], preferential dynamic crystallization[29,30] and crystallization induced diastereomeric transformations[31] have been described for the transformation of a racemic mixture into enantiopure material.

As each of these methods remains subject to limitations (e.g. requirement to form a conglomerate, salsification ability, etc), such a one pot deracemization procedure is not always feasible. In this case, an alternative approach is to introduce a step-wise deracemization procedure, where the separation and racemization steps are performed seperately.

In this work, we develop such a step-wise transformation process for (*RS*)-Ibuprofen, for which no one-step single pot deracemization has been described so far. To do so, we start by using enantiospecific co-crystallization from solution, a recently introduced resolution technique for compounds that do not form salts. [32,33] Enantiospecific co-crystallization occurs when only one enantiomer of the target API is able to co-crystallize with the resolving agent. Recent findings have shown such behavior to be more commonplace for co-crystals compared to diastereomeric co-crystal formation.[32,34–36] In a recent contribution, we showed how resolution through co-crystallization can be used in a dual-drug approach, separating (*R*)- and (*S*)-Ibuprofen using Levetiracetam.[33] In that contribution, we showed how enantiopure (*S*)-Ibuprofen can be obtained upon enantiospecific co-crystallization followed by dissociation of the co-crystal to yield the pure (*S*)-Ibuprofen. The maximum theoretical yield of such a process would be 50%. In this contribution, we go beyond by combining this resolution process with a racemization process for the opposite enantiomer. After racemization of the undesired enantiomer and recovery of the resolution agent, a second co-crystallization resolution can be performed by feeding the starting materials back into the system. By looping this system, ultimately a zero waste process in terms of APIs is achieved.

Materials and Methods

Starting Materials

S-2-(2-oxopyrrolidin-1-yl)butanamide ((*S*)-Etiracetam or Leviracetam) was purchased from Xiamen Top Health Biochem. Tech. Co., Ltd. (*RS*)-Ibuprofen was purchased from HoaHua Industry Co., Ltd. (*S*)-Ibuprofen was purchased from Thermofischer Acros Organics. Acetonitrile was purchased from VWR International S.A.S. Octane (98%) was obtained from Sigma Aldrich Co. LLC. Triethylamine (>99.5%) was obtained from Honeywell Fluka – Fischer Scientific. All materials were used without further purification.

Enantiospecific crystallization conditions

Typically co-crystal seeds were created via ball milling by adding (*S*)-Ibuprofen and Levetiracetam together in a 1:1 molar ratio, followed by milling at 30Hz for 60 minutes(See supporting information, section 1). A typical co-crystallization process is as follows: 10394.09mg (RS)-Ibuprofen (50.4mmol) and 8575.22mg Levetiracetam (50.4mmol) were added to 100mL of acetonitrile and heated until dissolution was complete. The solution was left to cool to -10°C and subsequently seeded with 1 wt% of the solids in the system. A second seeding (also 1 wt%) is done 24h after the initial seeding event. Deviations from this starting ratio and other process changes are listed in supplementary information section 2. Standard conditions are used in the text, unless mentioned otherwise. The yields after each step were calculated by comparing the total input of solid material to the output after crystallization.

HPLC Analysis

Chiral High Performance Liquid Chromatography (Chiral HPLC) measurements were performed on a reverse phase Waters Alliance 2695 system, with a Photo Array Detector (Waters 2998) at 210nm. Samples were measured using a 1mL flow of 50/50 H_2O/MeCN with 0.1% formic acid added, on a Lux 5µm Amylose-1 250 x 4.6mm column from Phenomenex Inc. The samples were dissolved in 50/50 H_2O/MeCN.

NMR Analysis

^{1}H-Nuclear Magnetic Resonance Spectroscopy (^{1}HNMR) measurements were performed on a 300MHz Bruker Avance. DMSO-d6 was used as a solvent.

Results & Discussion

Discussion on the system used

(*S*)-Ibuprofen (active enantiomer),[37,38] forms an enantiospecific co-crystal with Levetiracetam (active ingredient of Keppra, an anti-convulsant)[39,40]. The chemical structures of both compounds are shown in scheme 1. The co-crystal does not form between Levetiracetam and (*R*)-Ibuprofen, which means that Levetiracetam serves as a chiral discriminator[33]. This also implies that Levetiracetam can be used as a chiral resolving agent for chiral resolution from solution. We designed such a dual drug resolution process in our earlier work. Identifying appropriate conditions, the addition of Levetiracetam to a solution of racemic Ibuprofen can lead to co-crystal formation, and consequently to opposing enantioenrichment in the solid and liquid phases. To identify the appropriate process conditions, ideally phase diagrams are constructed. In our earlier work[33] we constructed such a diagram (shown in figure 1), for the Ibuprofen Levetiracetam co-crystal system. As temperature, pressure and amounts of (*R*)- and (*S*)-Ibuprofen, Levetiracetam and solvent can be varied, these diagrams can become very complex. Even when fixing temperature and pressure, one technically ends up with a quaternary phase diagram (amount of solvent, amount of (*R*)- and (*S*)-Ibuprofen and amount of Levetiracetam) which is hard to represent graphically. To reduce the complexity further, the total solvent amount was fixed (95 mol% in our case), as described in our earlier work. This results in a ternary diagram as shown in figure 1. The edges correspond to (*R*)-Ibuprofen, (*S*)-Ibuprofen and Levetiracetam, respectively, in a suspension composed of 95 mol% solvent and 5 mol% overall in other compounds (solids). The vertical line in the middle (left-most line) corresponds to the racemic line, represented by a 50:50 ratio of (*S*)- and (*R*)-Ibuprofen. Going upwards along this line from the base of the triangle to the top, the only variable is the amount of Levetiracetam with respect to the amount of racemic compound. Along this line, the nature of the solid state which is stable in suspension alters according to the ratio Ibuprofen to Levetiracetam. When no Levetiracetam is present, (*RS*)-Ibuprofen is stable in suspension. For small amounts of Levetiracetam (up to at least 20% of overall solid composition) (*RS*)-Ibuprofen is the only stable phase in suspension. Increasing the amount of Levetiracetam even further (40-60% of overall solid composition), one enters a zone where three solid phases are stable in suspension: (*RS*)-Ibuprofen, Levetiracetam and the co-crystal of (*S*)-Ibuprofen with Levetiracetam. For even higher amounts of Levetiracetam (80% of overall solid composition), Levetiracetam is the only solid phase stable in suspension. Following a non-racemic mixture for Ibuprofen, e.g. an (*S*):(*R*) ratio of 60:40 or 70:30, new zones emerge. Along the 60:40 line, increasing amounts of Levetiracetam successively result in the following zones: (*RS*)-Ibuprofen; (*RS*)-Ibuprofen and Co-crystal; Co-crystal and Levetiracetam. Along the 70:30 line, increasing amounts of Levetiracetam successively lead to zones with: (*RS*)-Ibuprofen; Co-crystal; Co-crystal and Levetiracetam. The width of the zone where the Co-crystal is the only solid form emerging increases as the starting Ibuprofen composition moves towards the (*S*)-enriched direction as could be expected.

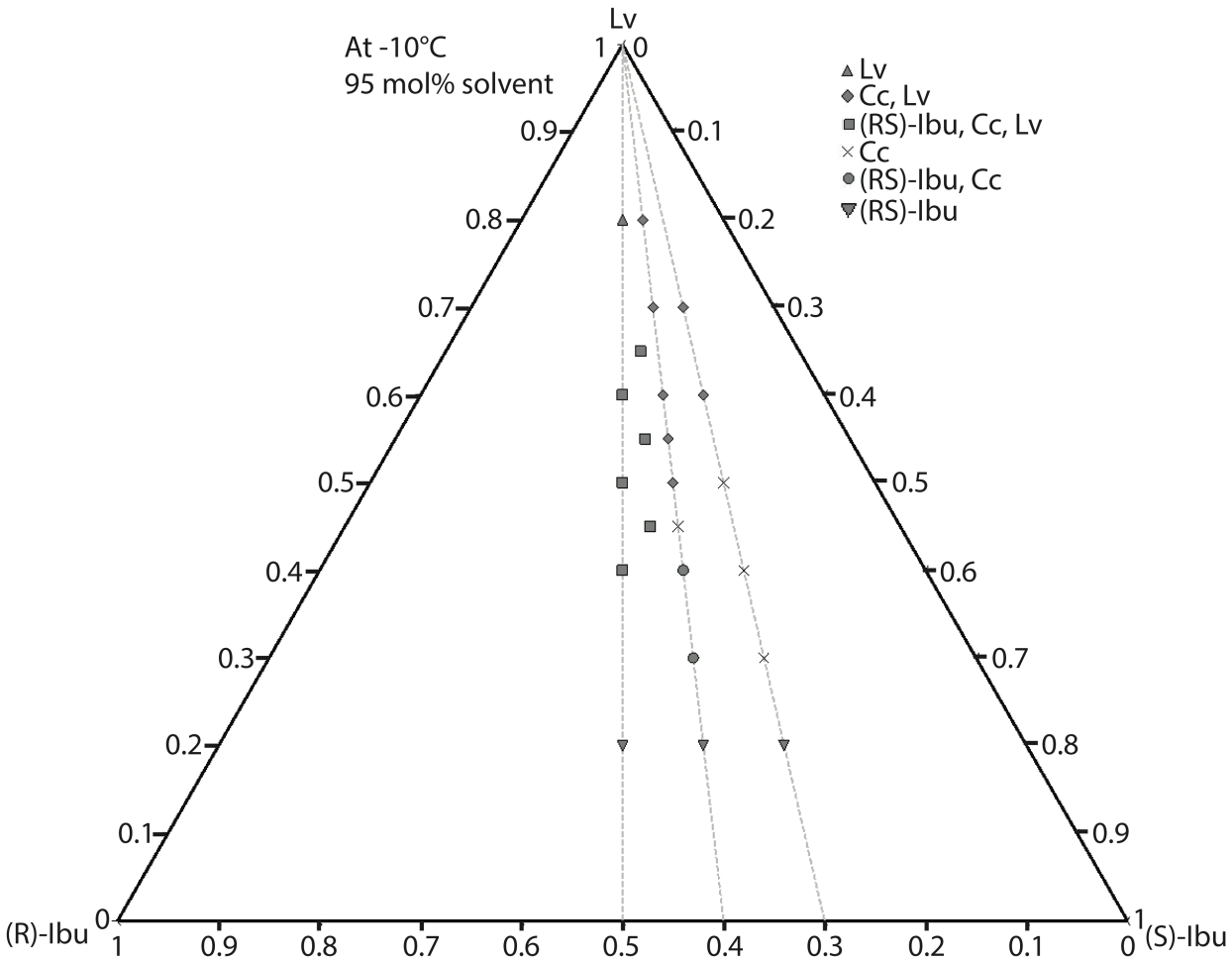


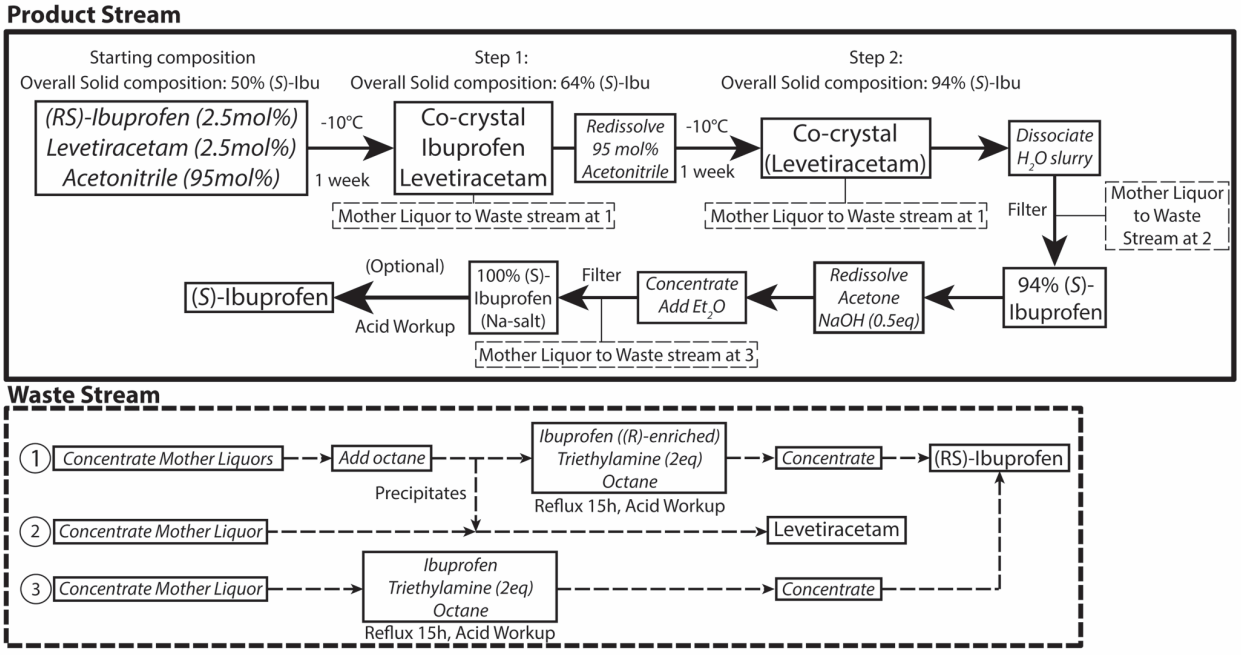
Figure 1: Ternary phase diagram for (RS)-Ibuprofen and Levetiracetam, with 95 mol% acetonitrile as solvent at -10°C. Image taken and adapted from our previous communication[33].

To obtain an imbalance of enantiomeric excess between the solid and liquid phases, one requires the crystallization of the co-crystal phase (as it only contains (*S*)-Ibuprofen). Starting from racemic Ibuprofen, the only relevant zone where this would occur is given by the area where three solid forms present as shown by the filled squares in figure 1. Along the 60:40 and 70:30 lines, this also occurs for the zones where the co-crystal is among the phases stable in suspension, as denoted by crosses (only co-crystal stable phase), filled dots (Co-crystal and (*RS*)-Ibuprofen stable phases) and filled diamonds (Co-crystal and Levetiracetam are stable phases in suspension).

<u>Towards enantiopure (S)-Ibuprofen</u>

Using the phase diagram of figure 1, a scaled-up process has been designed as shown in scheme 2 which ultimately enables enantiopure (*S*)-Ibuprofen combining racemization and co-crystallization. In the product stream, the starting point is the exact center of figure 1 (racemic Ibuprofen with Levetiracetam in a 1:1 ratio, with 95 mol% acetonitrile, solvent). The solids were dissolved by heating to 40°C after which the solution was cooled to -10°C, and seeded with co-crystal (1 wt%), which triggered the crystallization of all forms. 24 hours after the initial seeding event the suspension was seeded again. After a one week isothermal hold, the crystals were harvested, washed with 2 times 20 mL of cold (-10°C) acetonitrile, filtered and the enrichment of the solid phase was determined via chiral HPLC. Simultaneously, the overall composition of Ibuprofen to Levetiracetam was determined via ^{1}H-NMR.

This solid phase obtained from step 1 in scheme 2 has an average (S):(R)-Ibuprofen ratio of 64:36 and a yield of 31% (See table 1 below, weight of solid recovered with respect to weight of solids initially introduced). XRPD data shows that all three forms are present ((*RS*)-Ibuprofen, co-crystal and Levetiracetam), as expected from figure 1. From figure 1, one observes that in principle for a 64:36 ratio of S- vs R-Ibuprofen, one could add an appropriate amount of Levetiracetam, so that that (*RS*)-Ibuprofen no longer crystallizes (diamond squares in Figure 1). The solids of step 1 are redissolved again using 95% of acetonitrile solvent (see supporting information section 3) and if needed Levetiracetam was added to arrive in the area where the co-crystal is the only stable form in suspension.. As enrichment has occurred, the redissolved solid ratio is no longer racemic for Ibuprofen and has moved to the right in figure 1. Furthermore, since the composition obtained in step 1 is known (via Chiral HPLC and ^{1}H-NMR), the expected outcome can directly be read off of figure 1 based on the input composition. A similar heating, cooling and seeding procedure to step 1 is followed in step 2 and after another one week isothermal hold period the solids are again harvested, washed, filtered and analyzed. The resulting enrichment after the second dissolution and crystallization cycle appears to be the limit for our system (94% (*S*)-Ibuprofen as solid solution of the co-crystal). This means that a near enantiopure end-state is achieved in only two cycles. At this point the co-crystal (which forms a partial solid solution of 94:6 (*S*):(*R*)-Ibuprofen) could be used as potential drug form, since Levetiracetam and Ibuprofen exhibit synergistic behavior. When combined, the effect of Ibuprofen improves up to 11-fold.[41]



Scheme 2: Flowchart showing a two step process for enantiopure (*S*)-Ibuprofen, with a racemization and recycling route (Waste Stream).

If the co-crystal is not the wanted phase, the API can be dissociated from the co-crystal by slurrying the entire amount of solid recovered in step 2 in 50mL of water. Ibuprofen is insoluble in water, whereas Levetiracetam dissolves readily, which means the remaining solid phase being filtered off solely consists of Ibuprofen. At this stage a 94% (S)-Ibuprofen solid phase is still recovered. To come to enantiopure Ibuprofen, a literature based approach was used, first transforming Ibuprofen to its sodium salt.[42] Ibuprofen is characterized by a eutectic at around 90:10 (*R*) vs (*S*) (ratio varies slightly based on solvent used), whereas the sodium salt has a eutectic at 62:38. The use of the sodium salt will therefore lead to an increased yield during the enantiopurification. The salt is synthesized by dissolving the Ibuprofen in acetone (10mL/g material) and adding half a molar equivalent of NaOH. Unlike in literature,[42] the

suspension was heated to 50°C to aid dissolution. After dissolution was complete, the solvent was left to evaporate and the residue was triturated with diethyl ether (10mL/g of material) and filtered to yield 99+% pure (*S*)-Ibuprofen Na-salt as determined via HPLC. At this stage the Ibuprofen salt can be recovered for use as final API product, or alternatively an acidic workup (using 1M HCl) followed by filtration gives the liberated final enantiopure (*S*)-Ibuprofen.

In scheme 2 at various stages during the product stream, waste is generated. As we aim for an economically viable process, all the waste was collected at these specific points and processed in a respective waste stream. This not only allows to recover the resolving agent (Levetiracetam) but also to recover (R)-enriched Ibuprofen. Through racemization this latter is transformed into a racemic Ibuprofen mixture, which can in turn be reinjected into the process, thus leading to an overall deracemization process. The waste stream at point 1 uses an orthogonal solvent strategy, where Levetiracetam precipitates from the mother liquor's concentrate when octane is added. The (*R*)-rich Ibuprofen octane solution is racemized with triethylamine in octane under reflux conditions (2mL of octane/mmol of material, 2 equivalents of triethylamine, 15h reflux)[43] followed by an acidic workup. The solvent is evaporated and racemic Ibuprofen is obtained. A similar process is performed with the waste stream at point 3. The only difference here is that there is no removal of Levetiracetam required. The waste stream at point 2 only contains Levetiracetam, which can be recovered by concentration of the solvent before reintroducing it into the product stream.
The advantages of the proposed product and waste streams is that there is almost no waste generated. The racemized Ibuprofen and separated Levetiracetam are recycled and reintroduced in the product stream, reducing the amount of material needed, and achieving the ultimate goal of transforming RS-Ibuprofen into its enantiopure counterpart.

The process described in scheme 2 was repeated multiple times, and the results are shown in table 1. Table 1 shows the % of (S)-Ibuprofen with respect to the total amount of ibuprofen in the solid phase recovered after each step. Even though there was some variation in the amount of Levetiracetam vs. Ibuprofen in step 2 experiments, in all cases, pure co-crystal was obtained as a 94:6 (*S*):(*R*) solid solution.. Individual experiments are detailed in the supporting information section 2. In step 1 the average of seven experiments was taken, in step two the average of three experiments.

Starting composition	Enrichment after step 1[1]	Enrichment after step 2
2.5mol% (*RS*)-Ibuprofen 2.5mol% Levetiracetam 95mol% Acetonitrile	(*S*)-Ibuprofen: 64%(avg of 7 exp.) Yield: 31%(avg of 7 exp.)	(*S*)-Ibuprofen: 96%(avg of 3 exp.) Yield: 34%(avg of 3 exp.)

Table 1: % of S-Ibuprofen upon completion of the process stepshown in Scheme 2. The yield is given as the total mass of solid phase recovered upon crystallization vs total mass of solid introduced initially in the specific step.

When the starting composition was created using recycled material obtained from the waste stream in scheme 2, the outcome of step 1 did not change and gave enrichment in (*S*)-Ibuprofen as expected. This shows that the compounds from the mother liquor(s) after waste processing can be reused without problems in the product stream. After the second crystallization step in the product stream, the Ibuprofen salt was created as described[43] earlier, but the suspension was heated to 50°C to complete the dissolution of sodium hydroxide (unlike literature). The washed product of the concentrated sodium

[1] Interestingly, stirring (at 200 rpm) seemed to have a strong negative influence on the enrichment of the process. The average enrichment after step 1 without stirring (as listed in table 1) is 64% and the average with stirring was 53% (average of 8 experiments). The reason for this difference has not been investigated as it is outside the scope of this article, but it is likely that the agitation prevents the co-crystal from forming.

salt (concentrate was initially slurried until evaporation was complete, then washed and filtered twice via displacement) was enantiopure as expected, but no yields were recorded. When the sodium salt of Ibuprofen was liberated via an acidic workup, an enantiopurity of 99+% for (*S*)-Ibuprofen was obtained.

The overall average yield over the two crystallization steps is low (11%) and has not been fully optimized, as the goal of this work was to demonstrate a working process not utilizing diastereomeric salts nor separation via chiral column. However, since we showed API and resolving agent are fully recovered via the waste stream (no API material is lost or discarded), this low yield is is not a limiting issue, as one can cycle through the process multiple times. For example, Levetiracetam is used as the chiral resolving agent and does not get consumed at all during the entire process. The undesired (*R*)-Ibuprofen (and in solution remaining (*S*)-Ibuprofen) also gets recovered and racemized, achieving full transformation of (RS)-Ibuprofen into the enantiopure counterpart. The only product leaving the system is the final pure (*S*)-Ibuprofen, all the remaining material being reused.

Conclusion

A novel process to transform racemic material into enantiomerically pure material was developed based on co-crystallization principles, as an alternative for known procedures. In two steps, near enantiopure (*S*)-Ibuprofen is obtained in the form of the co-crystal as a partial solid solution. After further optional purification (*S*)-Ibuprofen is obtained with 99+% enantiopurity. The resolution agent is recovered during the process, and can be reused as such whereas the (*R*)- Ibuprofen enriched waste is recovered and fully racemized, so that it can also be recycled into the stream. This ultimately implies that the entire amount of (RS)-Ibuprofen can be successfully transformed into the desired enantiopure (S)-Ibuprofen end product. Interestingly this process can be interrupted at different stages, as the co-crystal, as well as the sodium salts of Ibuprofen are also viable solid states. The process developed here is thermodynamic and can be extended to all co-crystal forming compounds, with no limitation to conglomerate formation, nor salt formation.

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