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REVIEW- ALTERNATIVES FOR THE SEPARATION OF DRUG ENANTIOMERS: IBUPROFEN AS A MODEL COMPOUND

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Abstract - Given the inherent dangers associated with racemic pharmaceuticals, exhaustive studies of techniques designed to separate enantiomers have been conducted. This paper reports a brief review of the different physical, chemical, and enzymatic methods used for the enantiomeric separation of *rac*-ibuprofen. The possible contribution of each technique to the preparation of enantiomerically pure drugs is reviewed in the context of competitive approaches that depend on the scale of application, with special emphasis on the recent progress achieved in this particular area.
Keywords: Separation of drug enantiomers; Enzimatic methods.

INTRODUCTION

There are two possible approaches to the preparation of optically active compounds by chemical transformation of optically inactive starting materials: kinetic resolution and asymmetric synthesis. Kinetic resolution depends on the fact that the two enantiomers of a racemate react at different rates with a chiral reagent or catalyst, such as an enzyme. Asymmetric synthesis, on the other hand, involves the creation of an asymmetric (stereogenic) center, which, in this case, is by chiral discrimination of equivalent groups in an achiral starting material (prochiral).

The cost of these processes is affected by the price of substrate, and this can have a bearing on the choice of method. Similarly, the price and ease of recycling of the resolving agent or biocatalyst and the reduction of waste streams are obviously important. Another factor that is often overlooked in small-scale experiments is the volume yield or productivity (kilograms of product per unit reactor volume per unit time). For an economically viable process, it is generally essential to obtain high chemical and optical yields at high substrate concentrations and it is sometimes necessary to give percentage points on the former to the benefit of the latter.

Although a number of stereoselective syntheses have been described and applied to the production of single-enantiomer substances, relatively few are selected for large-scale preparation, particularly at the early stages of development of new drugs. At these stages, the use of an asymmetric synthesis would be both expensive and time-consuming; thus preparative techniques for the separation of enantiomers have an interesting potential (Maier et al., 2001).

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CHIRALITY

By definition, a chiral material is one which lacks reflectional symmetry, i.e. it has a nonsuperimposable mirror image structure and is referred to as being "handed". The most common type of chiral compounds are enantiomers. These materials are typically characterized by an asymmetric, tetrahedral carbon atom located at the center of the molecule. These molecules can be as stable, observable stereoisomers if their energy barrier of conversion exceeds 80 KJ/mole. In addition, compounds that are enantiomers have nearly identical physical and chemical properties in an achiral (symmetrical) environment. The specific distinguishing physical property of enantiomers is the rotation of plane-polarized light. If the rotation is to the right (clockwise) the substance is dextrorotatory ((+) or (*d*)); if the rotation is to the left (counterclockwise) it is levorotatory ((-) or (*l*)). When these enantiomers are present in equimolar amounts within a mixture, the resultant mixture is termed racemic. These preparations are optically inactive, because the net rotation of plane-polarized light is counterbalanced by equal concentrations of each enantiomer. Diastereoisomers are nonmirror image stereoisomers that have more than one asymmetric center. Unlike enantiomers, diastereoisomers may be individually isolated because they have different as physical and chemical properties, such solubility and melting point (Sheldon, 1993). Enantiomers may be transformed into diastereoisomers by either covalent or noncovalent coupling of the enantiomers of a racemic mixture to another chiral molecule having at least one asymmetric center. This methodology defines a separation route by which two previously inseparable material may be isolated by conventional techniques.

The interest in chirality and its consequences is not a new phenomenon. However, during the past decade it has raised increasing expectations for scientific and economic reasons, with the pharmaceutical industry being the main contributor and driving force.

PHARMACEUTICAL TRENDS

Strong emphasis has been placed on the search for therapeutic benefits with the goal of developing safer and more effective drugs. The importance of determining the pharmacological activity of each component in a drug has gained full acceptance, as shown by the substantial number of single-isomer pharmaceuticals entering the commercial market. The motivation for this single-isomer trend has been provided in part by the FDA (De Castro, 1997) and in part by the production of a host of pharmaceuticals previously protected by 17-20 year patent production laws. Those particular chiral drugs, whose patents are attracting a multitude of overseas producers, would provide price competition and increase the "generic brand" availability from producers with large-scale capacities. Chirally active drugs also represent a large share of the pharmaceutical market. Single-isomer drug sales reached $ 115 billion dollars worldwide in 1999, up 16% from $ 99 billion dollars in 1998. It was estimated that this class of drugs would grow at an average annual rate of 8% up to $ 146 billion in 2003 (Stinson, 2000).

PHARMACOLOGICAL IMPLICATIONS

Drug action is the result of pharmacological and pharmacokinetic processes, by which the drug enters, interacts with and leaves the body. There is a broad range of examples where the stereoisomers of drugs are different in terms of their bioavailability, and distribution, metabolic and excretion behavior and where stereochemical parameters have a fundamental significance in their action and disposition in biological systems (Norbert et al., 2001).

The high degree of stereoselectivity of many biological processes implies that when a given racemic mixture is administered as a drug, the two enantiomers should not have to be equally potent. In fact, very often one of them is the more active isomer for a given action (eutomer), while the other one (distomer), might even be active in a different way, contributing to side effects, displaying toxicity, or acting as an antagonist (Wainer, 1993). For example, it has been established that the *S*-(+) enantiomer of ibuprofen is almost entirely responsible for the anti-inflammatory effects of *rac*-ibuprofen (Mayer and Testa, 1997). It has been reported that the S-(+) enantiomer is 160 times more active than its antipode in the in vitro synthesis of prostaglandins (Adams et al., 1976). To avoid the unwanted effects of the *R*-enantiomer, the use of pure *S*-enantiomer of the profens is desirable. Furthermore, some of the profens generally undergo a unidirectional in vivo chiral inversion from the inactive *R*-enantiomer to the active *S*-form (Caldwell et al., 1988). This peculiar stereoselectivity, which can occur in many of the metabolic routes of a given drug, has to be carefully considered when its development is envisaged or its safety is evaluated.

IBUPROFEN AS A MODEL COMPOUND

In the anti-inflammatory class of pharmaceuticals, *rac*-ibuprofen ((*R,S*)-2-(isobutylphenyl)- propionic acid) is an interesting example of a drug that is still sold as a racemic mixture (Figure 1). The single-isomer product has a higher commercial value than the racemic mixture, but production has been limited by legal and process difficulties. In addition, ibuprofen is the one member of the nonsteroidal anti-inflammatory drug (NSAID) family that has proven to be the most challenging to enantiomerically purify. A large investment has been made in order to research and develop specific techniques for ibuprofen.

Rac-ibuprofen was introduced in the late sixties as a safe NSAID for the treatment of a wide range of complaints, including pain, inflammation, arthritis, fever and dysmenorrhea. Interestingly, the inactive *R* (-) enantiomer undergoes a unidirectional metabolic inversion of configuration to form the active *S* (-) enantiomer (Mills et al., 1973; Adams et al., 1976). In other words, when the drug is administered as a racemate the distomer is converted in vivo into the eutomer, while the latter is unaffected. However, the situation is more complex, as becomes evident upon examining the mechanism of metabolic inversion (Figure 2).

The enantiospecificity of the inversion is controlled by the enzyme acylcoenzime-A synthetase that converts (*R*)-ibuprofen to the corresponding coenzyme A thioester. Racemization of the latter and subsequent hydrolysis yields (*S*)-ibuprofen. In competition with hydrolysis, the thioester intermediate can also undergo acyl exchange with the endogenous triacylglycerols. This results in accumulation of ibuprofen residues in fatty tissue. Since the (*S*)-enantiomer does not form the coenzyme A thioester, it cannot be incorporated into fatty tissue. The long-term effects of the accumulation of ibuprofen residues in fatty tissue are unknown, but toxic side effects cannot be completely ruled out. The risk of side effects can, in any case, be avoided by administering the pure (*S*)-enantiomer (Sheldon, 1993).

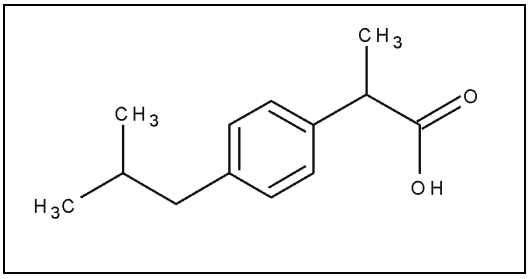

Figure 1: Chemical structure of ibuprofen

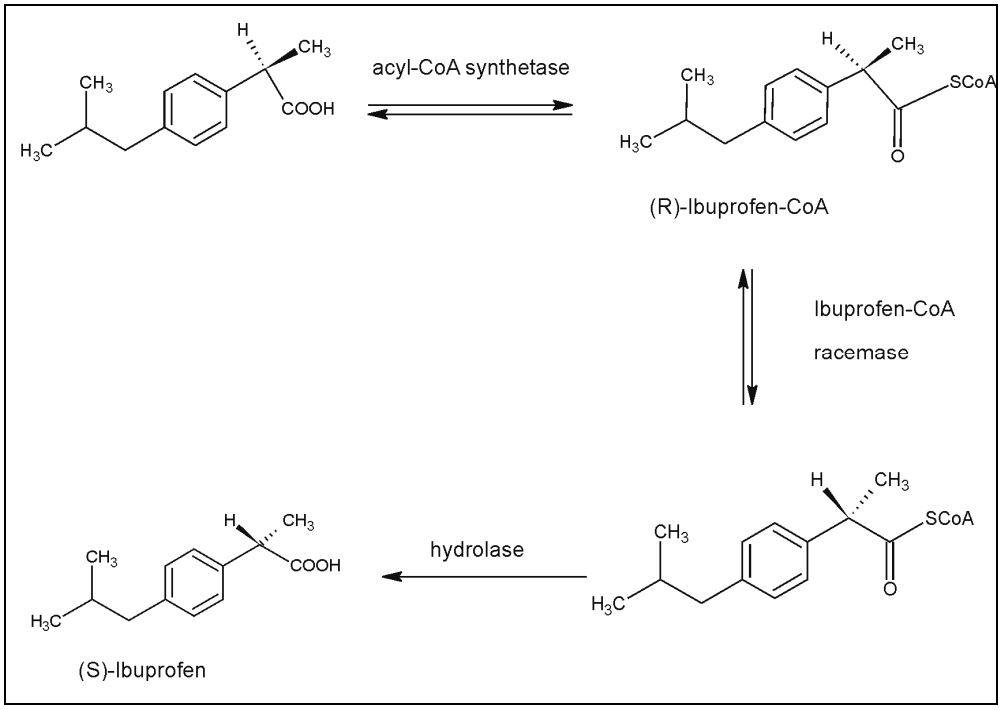

Figure 2: Mechanism of metabolic inversion of ibuprofen

METHODS OF SEPARATION OF ENANTIOMERS

The first successful attempt to resolve enantiomers from their racemic mixture was performed by Louis Pasteur, in which he manually resolved a racemic mixture of sodium ammonium tartrate into its individual enantiomers (Pasteur, 1848). A multitude of methods and techniques for the separation of enantiomers exists, though not all methods are equally applicable for every racemic mixture. The main groups of techniques for the separation of enantiomers are schematized in Figure 3.

The text below describes the main techniques applied to the enantioselective separation of *rac*-ibuprofen. For each method, several advantages and disadvantages prevail, depending upon factors such as time, purity, chemical processing, and inherent side reactions. A large investment has been made in research to develop specific techniques for ibuprofen.

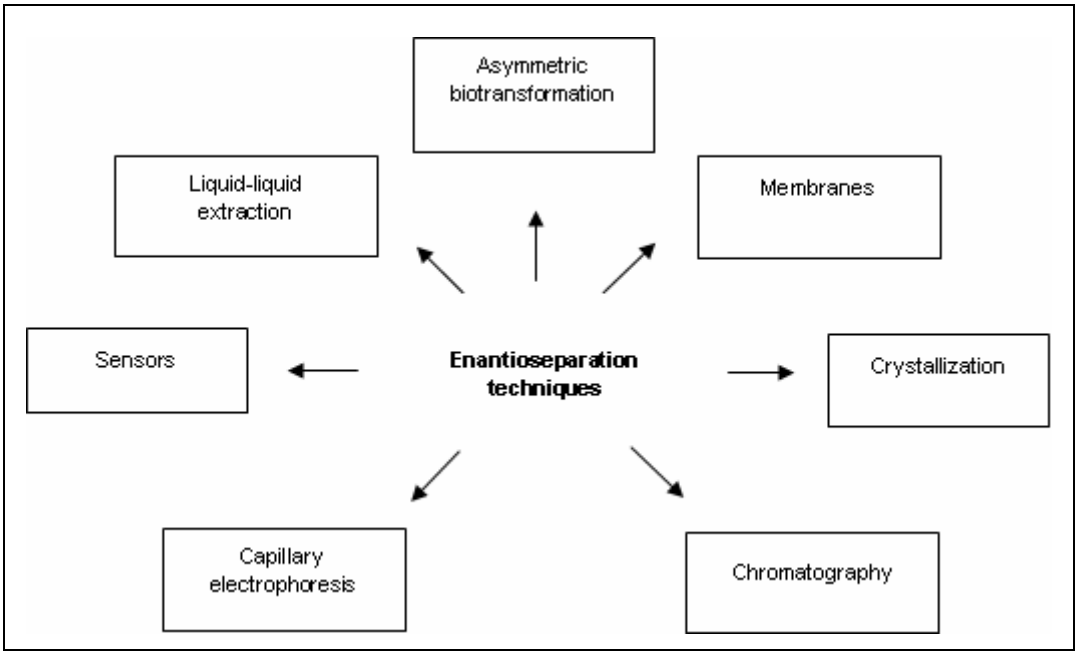

Figure 3: Techniques used for the separation of enantiomers

Chromatographic Separation Methods

The most common method to date for the enantiomeric resolution of ibuprofen enantiomers and chiral materials in general is high performance liquid chromatography. For the direct separation of enantiomers by chromatography on chiral stationary phases (CSP), two strategies are essentially applicable. The first strategy consists of selecting the best available CSP for the racemic compound of interest, while the second option consists of modifying (derivatizing) the racemic solute to accommodate it to a defined CSP until it separates on this particular CSP. Construction of a chiral stationary phase or prederivatization of the individual enantiomers to produce the diastereisomeric pair are the two important techniques employed. Though these techniques vary from normal to reverse phase and utilize a variety of detector systems, the separations achieved thus far are similar (Ahn et al., 1994).

There are extensive reports in the literature concerning prederivatization techniques (Khoa et al., 1994; Griffith et al., 1993; Lemko et al., 1993; Wright et al., 1992; Toyo'oka et al., 1992). A chiral derivatization reagent that has an appropriate chromophore or fluorophore is a useful tool for analysis of ibuprofen with respect to selectivity, sensitivity, and versatility (Kondo et al., 1993; Toyo'oka et al., 1992). In most instances ethyl chloroformate is used to couple a chiral ligand to the racemic enantiomers in order to produce the diastereoisomeric pair. Though the enantiomers of ibuprofen were resolved in less than 20 minutes, there are inherent drawbacks to this method. These drawbacks include the following (Griffith et al., 1993): the rates of formation for the diastereoisomeric pair may be different, resulting in

an incorrect ratio of the two enantiomers; impurities within the reaction mixture could result in a multiple number of diastereisomeric pairs; racemization may occur and its extent is dependent upon the coupling agent and reaction solvent; and a chemical, covalent bond is formed between the coupling agent and ibuprofen, which is difficult to disassociate in order to recover the free acid of ibuprofen.

The deficiencies in the prederivatization method prompted researchers to develop a technique by which ibuprofen would remain chemically unmodified. Instead, a column which had a chiral packing material was employed (Oi et al., 1995; Haginaka et al., 1993; Haikala et al., 1991; Farkas et al., 1993; Castellani et al., 1994). Based on the applications published over the last ten years, it appears that the most successful chiral packing materials comprise the derivatives of cellulose, amylose, cyclodextrins, proteins, and amino acids (Oi et al., 1995). Baseline resolution of enantiomeric ibuprofen in less than 30 minutes has been successful. The disadvantage of this method is that there appears to be no universal chiral column and analysis conditions. In addition, large volumes of organic-based mobile phase are required for component elution, and this volume of solvent must be evaporated in order to extract the free acid of ibuprofen. Obtaining ibuprofen with the minimal solvent contamination required by the FDA becomes more costly as the amount of ibuprofen processed increases. This method, however, is still preferred to prederivatization, since the number and availability of different chirals available commercially has increased.

Physical Separation Methods

Crystallization has been the predominant separation technique to resolve an enantiomeric mixture into its individual isomers on the industrial scale. There are three primary methods of crystallization for enantiomeric resolution (Tung et al., 1995): 1. Preferential crystallization or resolution by entrainment – stereospecific growth of each individual isomer in two different crystallizers from solution. This process requires no resolving agent. 2. Diastereoisomeric crystallization – the resolving agent binds to enantiomers to a diastereoisomeric salt pair. These salts are separated as a function of their phase behavior. 3. Catalytic kinetic resolution – the resolving agent reacts at a different rate with each enantiomer.

The most attractive method involves the formation of diastereisomeric salts between acids and bases and, in this case, the resolving agent can be readily removed and recycled. Diastereoisomeric crystallization has been the dominant technique among industrial pharmaceutical companies for the resolution of ibuprofen enantiomers (Tung et al., 1995; Merk, 1990). This method is often referred to as classical resolution.

Considerable research efforts have been invested to create rational concepts for diastereoisomeric salt crystallization, but the possibilities of predicting ideal combinations of resolution agents for a given racemic mixture are still very limited. Therefore, identifying appropriate resolution agents and conditions often requires substantial “trial-and-error” experimentation and might be expensive in time, labor and material (Norbert et al., 2001).

A common resolving agent used for the enantiomeric separation of ibuprofen is L-lysine. It has been found that upon salt formation, the *D*-ibuprofen-*L*-lysinate salt has about two-thirds the solubility of the *L*-ibuprofen-*L*-lysinate salt (Tung et al., 1995). This indicates that a decent separation of the *L*-ibuprofen-*L*-lysinate from the *D*-ibuprofen-*L*-lysinate salt may be readily achieved. The formation of an optically active salt is also desirable, since salt is more soluble in water than the corresponding free acid of ibuprofen. Pharmacological studies have proven that the amino acid salt of ibuprofen is better adsorbed in the blood stream than the free acid (Jeon et al., 1994). Thus, an effective, inexpensive, and pharmacologically valuable product is produced by this technique.

Enzymatic Kinetic Resolution

Two types of enzyme that have received increasing attention in the context of organic synthesis are esterases and lipases. Lipases (E.C. 3.1.1.3) are a special example of carboxyl esterases. The main sources of commercial lipases are porcine pancreas and a variety of bacteria and yeast (Table 1). The extracellular microbial lipases are particularly suited to synthetic applications because they have broad substrate specificities and no coenzyme requirement for catalysis. Although the natural substrates of lipases are acylglycerols, they can also catalyze the hydrolysis of a wide range of artificial water-insoluble esters with a high degree of enantiospecificity. In low-water media, lipases catalyze esterification, transesterification, and interesterification. These transformations can be employed for the kinetic resolution of different chiral compounds (Sih, et al., 1988; Margolin, 1993; Carvalho et al., 2005b).

Table 1: Examples of commerciallly available lipases

Source	Suppliers
Alcaligenes sp.	Amano
Aspergillus niger	Amano, Fluka
Bacillus subtilis	Towa Koso
Candida cylindracea	Sigma, Amano, Meito Sangyo, Boehringer-Mannheim, Fluka, Aldrich
Candida lipolytica	Amano, Fluka
Chromobacterium viscosum	Sigma, Toyo Jozo
Geotrichum candidum	Sigma, Amano
Humicola lanuginosa	Amano
Mucor miehei	Amano, Novo
Penicilium camemberti	Rhône-Poulenk
Penicilium roquefort	Fluka
Porcine pancreas	Sigma, Amano, Boehringer- Mannheim, Fluka, Aldrich
Pseudomomas aeruginosa	Amano
Pseudomomas fluorescens	Amano, Fluka
Rhizopus arrhizus	Sigma, Boehringer-Mannheim, Fluka

Considerable efforts have been made to use racemic profens (2-arylpropionic acids) or their ester derivatives as the substrate in enantioselective esterification (De la Casa et al., 2002; Bhandarkar and Neau, 2000; Wu and Liu, 2000; Sánchez et al., 2000; Carvalho et al., 2005a), hydrolysis (Lee et al., 1995; Xin et al., 2000; Steenkamp and Brady, 2003; Long et al., 2005) or transesterification (Chang and Tsai, 1999; Santaniello et al., 1993) in order to produce the desired (*S*)-profens and their (*S*)-esters by employing lipases as the biocatalyst in the presence of organic solvents. Taking advantage of the naturally inherent selectivity of the enzyme's active site, reactive discrimination occurs between the enantiomers in the racemic mixture. Though not exclusively, one enantiomeric in general “fits” better on the active site and is therefore converted to its corresponding ester at a higher rate. (Faber, 1992)

The preparation of (*S*)-ibuprofen and (*S*)-naproxen by enzymatic hydrolysis of these racemic esters has been exhaustively researched using *Candida cylindracea (rugosa)* lipase (Lee et al., 1995; Xin et al., 2000; Lin and Tsai, 2000; Lopez et al., 2004) A continuous process has been described for the enantiospecific hydrolysis of the ethoxyethyl ester of naproxen using a column packed with *Candida cylindracea* immobilized on an ion exchange resin (Battistel et al., 1991).

Another method for producing enantiomerically pure ibuprofen is selective enzymatic esterification using lipases. Sánchez et al. (2000) reported high ester yields (49.9%) and enantiomeric excess (93.8%) of ibuprofen using immobilized lipase from *Rhizomucor miehei* with isooctane as solvent and butanol as etherification agent. The stability of the reaction and the enzyme (up to 100h) as well as the low cost and simple scale-up of the system make this process an alternative option for highly stereoselective esterification in organic media (Sánchez et al., 2000). This process is dependent upon a number of variables including optimum temperature, ibuprofen and alcohol concentration, pressure, water content, and enzymatic lifetime.

The standard kinetic resolution process has the disadvantage of obtaining a maximum 50 % yield of the desired enantiomer. To overcome this limitation, dynamic kinetic resolution has been tested as a potentially efficient process in which the standard kinetic resolution is coupled with continuous *in situ* racemization of the initial substrate (Ebbers et al., 1997; Lin and Tsai, 2000; Lu et al., 2002). In organic media, two new parameters which are not found in aqueous solutions may be of paramount importance namely the nature of the solvent and its water content. The effect of these parameters on the rates of enzymatic reactions as well as on the thermodynamic equilibrium has been studied (Lee et al., 1995; Arroyo et al., 1995 1999; Ducret et al., 1998).

Different strategies have been developed to improve the stereoselectivity of enzyme-catalyzed resolutions. The most popular are recycling of the product, modification of the substrate, improvement of the enzymatic stability, and changing the reaction conditions. Several immobilization methods and supports have been tested for the covalent bonding of lipase from *Candida antarctica* (Arroyo et al., 1995 1999). Coupling lipase reactions with a supported liquid membrane based on ionic liquids showed facilitative and selective permeation of (*S*)-ibuprofen

through the supported liquid membrane, indicating successful optical resolution of a racemic mixture using the enzyme-facilitative supported liquid membrane (Miyako et al., 2003).

To prepare a pure product, one needs to obtain a very high enzyme enantioselectivity. The product might be obtained in highly pure form at a particular degree of conversion, provided that the reaction equilibrium is favorable (Chen et al.,1982). From the standpoint of high productivity, easy product separation, and few reaction steps, lipase-catalyzed asymmetric hydrolysis is considered to be better for the practical resolution of racemic acid (Cambou and Klibanov, 1984).

The advantage of the enzymatic method is the high enantiomeric excess, resulting from the inherent selectivity of the enzyme. The enzyme is reusable and the products of the reaction are easy to separate. The disadvantages of this method are related to the number of parameters that must be optimized for the enzyme and the selectivity of the system is limited by the extent of conversion (Chen et al., 1982).

OTHER METHODS

Further techniques have been researched as potential methods for the preparation of optically pure compounds. Gas chromatography-mass spectrometry (Jack and Rumble, 1992), electrochromatography (Mayer and Shurig, 1993), capillary electrophoresis (Soini et al., 1994; Bjornsdottir et al., 1998), and nuclear magnetic resonance (Fulwood and Parker, 1992) have also been utilized as potential analytical techniques in enantiomeric separation of ibuprofen. These methods have proven to be less useful due to their inability to achieve baseline resolutions or their inability to removed the free acid of ibuprofen after separation. In addition, the effectiveness of high performance liquid chromatography has masked the advances in the development of these methods.

A recent development in the separation of ibuprofen enantiomers has been in supercritical fluid chromatography (Khundker et al., 1993; Wilson, 1994; Wilkins et al., 1995; Johannsen, 2001). The most commonly used supercritical fluid is carbon dioxide, which has chemical inertness and low toxicity. Ibuprofen has been shown to be soluble in supercritical carbon dioxide at optimum conditions of 70ºC and a supercritical carbon dioxide density of 0.7 g/ml. (Khundker et al., 1993). Supercritical fluid chromatography (SFC) behaves in a way similar to normal phase high pressure liquid chromatography, but can be run three to ten times faster than normal phase HPLC. In addition, SFC does not produce problems of column deactivation, long equilibration times, and leaching of a chiral constituent. (Wilson, 1994). When performed with carbon dioxide, SFC also provides an environmentally sound separation technique. This technique utilizes a chiral column and a mobile phase, which usually consists of a combination of carbon dioxide and a polar modifier such as methanol or tetrahydrofuran. One advantage that traditional HPLC has over SFC is peak shape and resolution. Separation in SFC tends to produce peaks on which tailing is evident. This is thought to be attributed to hydrogen bonding interactions with the chiral packing of the column (Wilkins et al., 1995). Despite this disadvantage, SFC is attractive due to the use of a benign mobile phase and the speed at which separations may be performed. This technique has not been well received as a preparative scale separation method.

CONCLUSION

The impact of chirality on almost any pharmacological or biological process is well recognized and has strong repercussions in many fields of economic interest, such as the development of drugs. Besides the ethical or environmental reasons for developing single enantiomers, they may have a real therapeutic benefit, and in some cases, their development has been used as a strategy for extending patent life. In this context, a large number of methods for the preparation of optically pure compounds have been studied and widely reported. Most methods are intrinsically expensive, consume vast quantities of organic solvent, and involve combinations of time-consuming crystallization and/or chromatographic procedures. In the calculation cost of, many factors are involved including equipment and packing investment, operational costs (solvent, energy), recycling costs, manpower, and environmental impact. There are currently only a very limited number of reported applications of enantioselective separation. On a industrial scale, resolution is still favored over asymmetric synthesis, in particular at the early stages of development of bioactive compounds, where time constraints are a major issue. Further advances in the area of preparation will obviously favor environmentally benign protocols for technical processes, and the reduction in waste streams and in recycling of solvents and materials will be very important.

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