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Review

The market of chiral drugs: Chiral switches versus *de novo* enantiomerically pure compounds

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ABSTRACT

This review article is aimed at providing an overview of the current market of chiral drugs by exploring which is the nowadays tendency, for the pharmaceutical industry, either to exploit the chiral switching practice from already marketed racemates or to develop *de novo* enantiomerically pure compounds. A concise illustration of the main techniques developed to assess the absolute configuration (AC) and enantiomeric purity of chiral drugs has been given, where greater emphasis was placed on the contribution of enantioselective chromatography (HPLC, SFC and UHPC).

Afterwards, we focused our study on the cohort of 45 new drugs that have been approved by the US Food and Drug Administration (FDA) in 2015. We extracted the chemical structure of the new drugs from the FDA approval chemistry reviews available on the database of the agency's Center for Drug Evaluation and Research (CDER), and we selected a subgroup (i.e., 44% of the cohort) of small-molecule active pharmaceutical ingredients (APIs) containing one or more chirality centers.

On the basis of the FDA dossiers examined, it emerged that all the chiral drugs approved by the FDA in 2015 are enantiomerically pure compounds with a well-defined AC, with the exception of one, namely lesinurad, which has been licensed as the racemate of two enantiomeric atropoisomers, arising because of the hindered rotation around the single C–N bond in the naphthalene ring. Finally, none of the previously developed racemates has been switched to the single-enantiomer version in 2015.

Contents

1. Introducing chirality in a drug candidate

It was 1997 (namely, twenty years ago) when, at the Conference of Pharmaceutical Ingredients held in London, the field of chiral drugs proved to have undergone a fundamental change, namely that it has passed through infancy and adolescence to an early adult form [1]. The chiral-switching concept goes back exactly to those

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years: the definition of the term "chiral switch" was introduced by Agranat and Caner in 1999 [2] and later refined [3] in reference to the development of a single enantiomer from a chiral drug that has been developed (and often approved and marketed) previously as a racemate, or as a mixture of diastereoisomers. Notably, a chiral switch for a given drug does not necessarily imply that the racemate has been marketed previously [3]; the essential criterion of a chiral switch is a change in the status of *chirality* [4].

The non-steroidal anti-inflammatory drug (NSAID) ibuprofen (see Table 1) was the first chiral drug of the NSAIDs class to be switched to the single-enantiomer version in 1994 [4]. The reason for such a switch came from the evidence that the (*S*)-enantiomer was over 100-fold more potent as an inhibitor of cyclooxygenase 1 (COX-1) enzyme than (*R*)-ibuprofen [5]. Moreover, ibuprofen, when administered as the racemate, proved to undergo unidirectional chiral inversion from the (*R*)-enantiomer to the (*S*)-enantiomer, the former behaving as a pro-drug for the latter [6,7]. Therefore, the use of the single (*S*)-ibuprofen was thought that it would give faster onset of action at a lower dosage and would reduce the source of configurational individual variability.

After ibuprofen, another "profen" drug (namely, ketoprofen, see Table 1) was submitted to chiral switching and marketed as the (*S*)-(+)-enantiomer in 1998 [4], and this time the switch has been more straightforward, since the metabolic (*R*) ⇋ (*S*) chiral inversion for ketoprofen was shown to be negligible in humans [8].

By merging the data taken from a paper reviewing the chiral switches launched from 1994 to 2002 [4] with those from a more recent review focusing on the period from 2001 to 2011 [9], it emerges that the US Food and Drug Administration (FDA) approved 15 single-enantiomer versions of racemic drugs, *i.e.*, 15 chiral switches have been launched in the period from 1994 to 2011 (see Table 1). The potential advantages of chiral switching include: (1) an improved therapeutic index through increased potency and selectivity and decreased side-effects; (2) a faster onset of action; (3) a reduced propensity for drug-drug interactions, and (4) the exposition of the patient to a lower dosage. It should be noted, however, that the chiral switching practice proved to be sometime controversial [10]. In some cases, in fact, single-enantiomer drugs have offered benefit to patients, particularly when the pharmacological activity resides mainly in one of the two enantiomers: this is the case of ofloxacin, for example, a fluoroquinolone antibacterial agent whose (*S*)-enantiomer (namely, levofloxacin, see Table 1) proved to be more soluble in water than the racemate [11], and thus exhibited an increased antibacterial activity of about two times with respect to the racemate against both gram-positive and gram-negative bacteria, the (*R*)-form being pharmacologically inert [12].

Even more illuminating is the case of the local anesthetic bupivacaine, where the (*S*)-(−)-enantiomer proved to be significantly less cardiotoxic than the (*R*)-enantiomer and the racemate [13,14]. Thus, the chiral switching to the levorotatory enantiomer (namely, levobupivacaine, see Table 1), launched in 2000 in the United States, resulted in the development of a local anesthetic drug with a clinical profile similar to that of the previously marketed racemate, but with a decrease in cardiovascular toxicity.

There have been, however, some cases where single-enantiomer drugs developed from blockbuster racemates offered little clinical advantage over the racemate, and their introduction into the market was exploited by the pharmaceutical companies as a patent protection strategy against generic competitors. The case of omeprazole, a gastric anti-secretory proton pump inhibitor (PPI) which reached the blockbuster status in the United States, is recognized as an example of a commercial strategy aimed at protecting a portion of market against other PPIs (namely, lansoprazole, pantoprazole, and rabeprazole) [10].

Esomeprazole (see Table 1) is the single-enantiomer version launched in 2000 in Europe by the same owner of the racemate [15]. Indeed, a given dose of esomeprazole resulted in an approximately two-fold higher area under curve (AUC) than that achieved after the same dose of omeprazole (the racemate) [16], but the single-enantiomer version was found not statistically superior to omeprazole or, at least, there is a mixed evidence at non-comparable doses [9]. Moreover, no trials have demonstrated an intrinsic therapeutic advantage of esomeprazole over the other PPIs at equivalent doses [17]. After omeprazole, another PPI, namely, lansoprazole (see Table 1) underwent the "chiral switch" as well: in 2009, Takeda launched on the US market the single destrorotatory enantiomer, namely (*R*)-(+)-lansoprazole or dexlansoprazole, which, as in the previous case, did not prove to be superior to the racemate in terms of efficacy in the pre-approval studies [9].

It is the purpose of this review article to analyze which is the tendency of the current market of chiral drugs, either to exploit the chiral switching practice from already marketed racemates or to develop *de novo* enantiomerically pure compounds. In both cases, stringent analytical characterization of drug substances, including a full documentation of the separated pharmacological and pharmacokinetic profiles of the individual enantiomers, as well as their combination, are required by regulatory agencies. As a result, special emphasis is given on the fundamental role played by enantioselective chromatography in the isolation of enantiomerically pure compounds and in assessing the enantiomeric purity of chiral drugs.

2. Methods for assessing the absolute configuration (AC) and enantiomeric purity of chiral drugs

In the year 1992, the FDA issued its long awaited policy statement concerning the development of stereoisomeric drugs [18]. It was the first time that the *chirality* of the active ingredient was put into the foreground of the whole process leading to a new drug, namely, the testing of the bulk drug, the manufacturing of the finished product, the design of stability testing protocols, and the labelling of the drug [19]. This statement had significant implications for all the scientific community working on the development and validation of analytical methods for chiral drug substances and products. In fact, even though the FDA did not mandate development of single enantiomers (racemates may be appropriate in certain cases), single-enantiomer drugs have become the standard in pharmaceutical companies when working with compounds featuring stereogenic centers in their structure. Therefore, the necessity for pure enantiomers required a critical assessment of the most cost-effective way to accomplish their analysis and preparation [20].

Shortening timelines for chiral drug discovery and development usually depends on the efficiency of the determination of the absolute configuration (AC) and enantiomeric purity of the drug to be discovered. To this purpose, based on the experience of one pharmaceutical company (namely, Wyeth), it was proposed in 2007 the building of a "Chiral Technology" toolbox, intended – in contrast to the previous usage – as the ensemble of techniques or tools for (1) the determination of absolute stereochemistry, (2) the enantioseparation of small molecules (both at analytical and preparative level), and (3) the facilitation of asymmetric transformations [21]. The 30 authors of the paper (which contains 400 references to the literature) have strongly encouraged both academic researchers and pharmaceutical companies for the refinement of such just established toolbox, with the design, development and implementation of new tools. The "Chiral Technology" approach was applied by some of the authors of the above-mentioned review article to the enantiomeric separation and spectroscopic evaluation of phenylglycidols [22]. A recent address to the proposal of contributing to the refinement of the "Chiral Technology" toolbox has been given

Table 1
Racemate drugs that have been switched to the single-enantiomer version in the years 1994–2011 (in order of launch on the market).

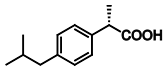
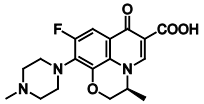
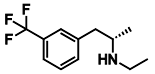
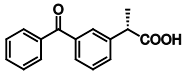
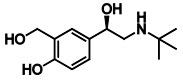
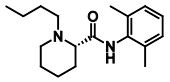
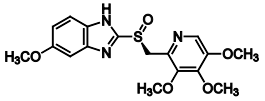
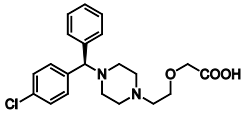
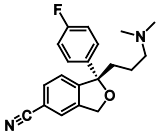
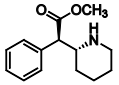
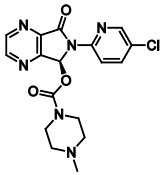
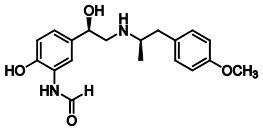
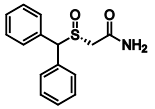
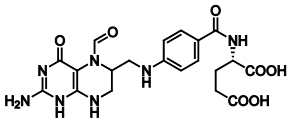
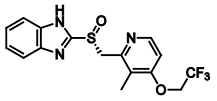
Entry	API	Chemical structure	Pharmacological activity or indications	Single enantiomer	Year of launch (country)	Company
1	Ibuprofen		Anti-inflammatory	(*S*)-(+)-ibuprofen (dexibuprofene)	1994 (Austria)	Spirig AG
2	Ofloxacin		Antibacterial	(*S*)-(−)-ofloxacin (levofloxacin)	1995 (Japan)	Aventis
3	Fenfluramine*		Antiobesity	(*S*)-(+)-fenfluramine (dexfenfluramine)	1996 (USA)	Interneuron Pharmaceuticals
4	Ketoprofen		Anti-inflammatory	(*S*)-(+)-ketoprofen (dexketoprofen)	1998 (Europe)	Menarini
5	Salbutamol (Albuterol)		Antiasthmatic	(*R*)-(−)-albuterol (levalbuterol)	1999 (USA)	Sepracor
6	Bupivacaine		Local anesthetic	(*S*)-(−)-bupivacaine (levobupivacaine)	2000 (USA)	Purdue Pharma
7	Omeprazole		Acid reducer, proton pump inhibitor (PPI)	(*S*)-(−)-omeprazole (esomeprazole)	2000 (Europe) 2001 (USA)	AstraZeneca
8	Cetirizine		Antihistaminic	(*R*)-(−)-cetirizine (levocetirizine)	2001 (Europe) 2007 (USA)	Sepracor/Sanofi-Aventis
9	Citalopram		Antidepressant	(*S*)-(+)-citalopram (escitalopram)	2001 (Europe) 2002 (USA)	Forest

Table 1 (*Continued*)

Entry	API	Chemical structure	Pharmacological activity or indications	Single enantiomer	Year of launch (country)	Company
10	Methylphenidate		Attention deficit, hyperactivity disorder	(*R*,*R*)-(+)-methylphenidate (dexmethylphenidate)	2001 (Europe)	Novartis/ Celgene
11	Zopiclone**		Anxiety and insomnia	(*S*)-(+)-zopiclone (eszopiclone)	2004 (Europe)	Sunovion/ Sepracor
12	Formoterol		Chronic obstructive pulmonary disease	(*R*,*R*)-(−)-formoterol (arformoterol)	2006 (USA)	Sunovion/ Sepracor
13	Modanafil		Narcolepsy	(*R*)-(−)-modanafil (armodanafil)	2007 (USA)	Cephalon
14	Leucovorin (folinic acid)		Rescue after high-dose methotrexate therapy; treatment of colorectal carcinoma in combination with 5-FU; treatment of folate deficiency	(*S*)-(−)-leucovorin (levoleucovorin)	2008 (USA)	Spectrum
15	Lansoprazole		Acid reducer, proton pump inhibitor (PPI)	(*R*)-(+)-lansoprazole (dexlansoprazole)	2009 (USA)	Takeda

*Withdrawn from the market in 1997 for evidence of valvular heart disease.
**Not commercially available in the United States (only in Europe).

by an article published in 2016 and entitled – maybe with a purpose of continuity – "Expanding the chiral toolbox" [23].

Techniques that are validated in both academia and the pharmaceutical industry for the determination of the AC of chiral molecules include X-ray crystallography (see Section 2.1), NMR techniques (see Section 2.2), vibrational circular dichroism (VCD, see Section 2.3), and enantioselective chromatography (see Section 2.4). The fundamental contribution given by the latter both combined with other physicochemical methods and as unique tool (by comparison of the retention data) has been covered in a comprehensive review article [24].

2.1. X-ray crystallography

X-ray crystallography is still considered the most reliable technique for the determination of the AC, but it requires a large single crystal of the pure enantiomer [25] and typically at least one relatively heavy atom (namely, sulfur, selenium, tellurium and heavy halogens), according to the resonant scattering protocol developed by Bijvoet [26,27] and to the so-called Flack parameter, introduced by Flack [25]. The concept is to react an enantiomerically pure compound with a chiral derivatizing agent (CDA) whose AC is known from alternative methods to get a crystalline diastereoisomer suitable for X-ray analysis. The chiral agent thus acts as an internal reference. Notably, the correctness of the AC determination using an internal chiral reference strongly depends on the knowledge of the enantiomeric purity of the reference material, which means that a purification step by enantioselective HPLC is needed. For example, the AC of the enantiomers of indole-3-succinic acid (a synthetic auxin capable of promoting the growth of some seedlings) was determined by isolation of the two enantiomers through reversed-phase HPLC on a Cyclobond I-RSP column, followed by recrystallization of the pure enantiomers as (–)-cinchonidine salts [28]. This produced crystalline products that were suitable for X-ray diffraction analysis.

An elegant protocol was developed by Harada in 1997, where a chiral molecular tool, namely camphorsultam dichlorophthalic (CSDP) acid obtained by connecting (1*S*,2*R*,4*R*)-2,10-camphorsultam with 4,5-dichlorophthalic acid (Fig. 1) was proposed for simultaneously prepare enantiopure alcohols by normal-phase HPLC separation and determine their AC [29,30]. Typically, racemic alcohols are esterified with (–)-CSDP acid yielding covalently bonded diastereoisomeric CSDP esters, which are well separated by HPLC on silica gel. Afterwards, the second-eluted CSDP ester is obtained as colorless prisms by recrystallizing from ethyl acetate, and hence the AC of the CSDP ester can be determined by X-ray crystallography using the heavy atom effects of S and the two Cl atoms [31].

With compounds containing only light atoms, a significant difference between the two crystal-structure models of opposite chirality is not always guaranteed [25]. In this case, the Flack parameter can refine to a physically unrealistic value (less than 0 or greater than 1) and has no meaning.

2.2. Nuclear magnetic resonance (NMR) techniques

NMR spectroscopy has an enormous potential in the field of structure elucidation, but, for the study of enantiomers, a diastereoisomeric environment is needed, to make diastereotopic their nuclei, and hence distinguishable by NMR [32]. The benefits of the technique, however, in the determination of AC are many, including the small amount of sample needed (which can also be recovered) and the applicability to both solid and liquid samples [33,34].

Two general approaches are known for the determination of the AC by NMR, namely (1) the addition of a chiral solvating agent (CSA) to a non-chiral standard NMR solvent [35], and (2) the reaction of the pure enantiomer whose AC is unknown with an enantiopure CDA, producing two diasteroisomeric derivatives [36]. In the first approach, non-covalent interactions between the substrate and the chiral auxiliary are established, and thus the chiral environment produces very small differences in the chemical shifts for the two enantiomers; for this reason, such approach is practically restricted to the determination of the enantiomeric purity, rather than that of the AC [37–39].

In the second case, the substrate and the auxiliary reagent are covalently assembled, and this leads to much greater differences in the chemical shifts than those obtained by the first approach, thus making such approach the method of choice for the assignment of AC by NMR (see Fig. 2).

Although many efforts have been described to develop CDAs that could be useful to assign the AC of different chiral molecules

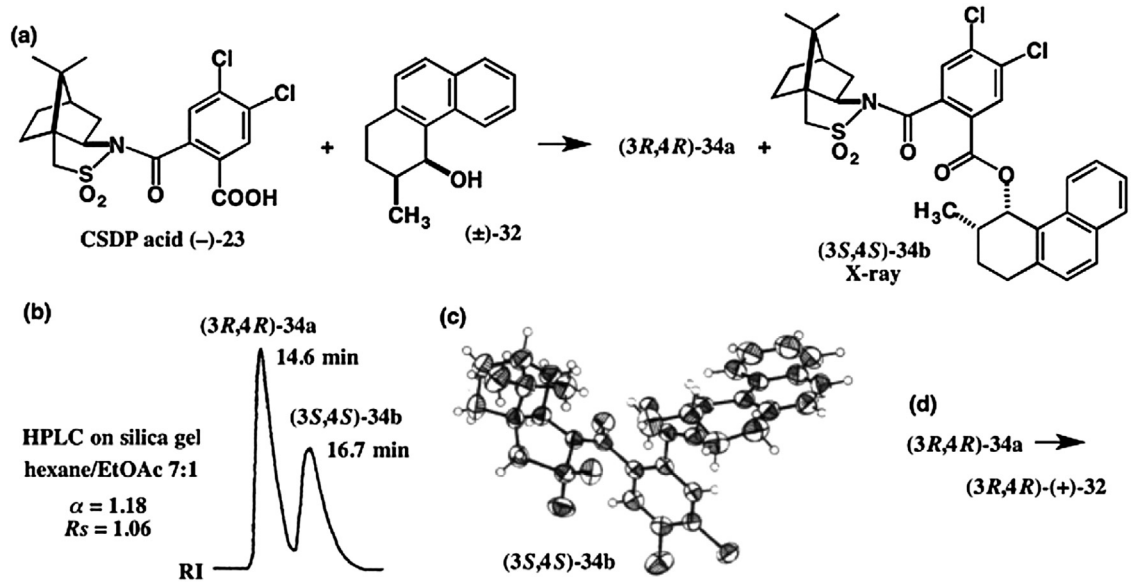


Fig. 1. Preparation (a) and HPLC separation (b) of the CSDP diastereoisomeric esters from a racemic alcohol, and AC determination (c) by X-ray analysis. Reprinted from N. Harada, Molecules 21, 1328 (©2016 by MDPI AG, Basel, Switzerland).

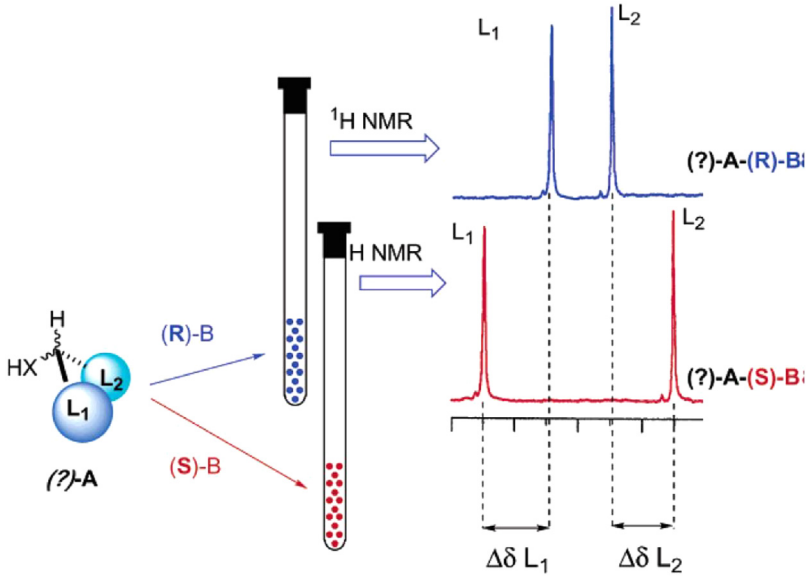


Fig. 2. NMR approach for the AC determination of a chiral sample (A) by derivatization with the two enantiomers of a chiral auxiliary agent (B). Reprinted with permission from J.M. Seco, E. Quiñoá, R. Riguera, Chem. Rev. 104, 17 (©2004 by the American Chemical Society).

[40], the so-named "Mosher's method" (see Section 2.2.1), which uses methoxytrifluoromethylphenylacetic acid (MTPA) as the reagent, has been the most successful, since its implementation in 1973 [41,42], opening the way to many new and more-efficient reagents that are useful for different substrates and are based on the same rationale. A more recent technique which proceeds through the use of chiral liquid crystals as the NMR solvent (see Section 2.2.2) has been described and proposed as a tool for enantiomeric analysis, albeit not completely free of ambiguity [43].

2.2.1. The Mosher's method

α-Methoxy-α-trifluoromethylphenylacetic acid (MTPA; see Fig. 3) has been the most commonly used derivatizing reagent for the determination of the AC of secondary alcohols by NMR since its first report by Mosher and coworkers [41,42] in 1973. Indeed, the paper by Dale and Mosher has received 2411 citations so far (source: Scopus), and the method they developed has come to be known as the "Mosher ester analysis", since the conjugation of the carbinols with MTPA yields the corresponding esters.

Although Mosher originally used ^{19}F NMR spectroscopy, in particular the chemical shifts of the CF_3 groups (see Fig. 3), for the assignment of the AC, in later studies ^{1}H and ^{13}C NMR were utilized, and the chemical shifts of the protons and carbon atoms of the chiral alcohol under examination were used, respectively.

The Mosher's method relies on the fact that the protons in diastereoisomeric MTPA esters display different arrays of chemical shifts (δs) in their ^{1}H NMR spectra. A typical Mosher ester analysis protocol consists of the following [44]: (i) preparation of each of the diastereoisomeric (*R*)- and (*S*)-MTPA esters and (ii) comparative ($\Delta\delta^{SR}$) analysis of the ^{1}H NMR spectral data of these two esters. By analyzing the sign of the difference in chemical shifts for a number of analogous pairs of protons (the set of $\Delta\delta^{SR}$ values) in the diastereoisomeric esters, the AC of the original carbinol stereocenter can be reliably deduced. A typical Mosher analysis requires approximately 4–6 h of active effort over a 1- to 2-days period.

2.2.2. Chiral liquid crystals

Configurational and conformational analysis of organic molecules in liquid crystals was initiated by Emsley and Lindon [45] who introduced an NMR method based on the use of residual dipolar couplings (RDCs) [46] measured in such solvents. More recently, a chiral liquid crystal solvent type (namely, synthetic polypeptides dissolved in deuterated chloroform) was proposed as an NMR tool for distinguishing enantiomers and analyzing enantiomeric excess (e.e.) in chiral compounds [47–49]. The distinction originates from the fact that, in such an anisotropic chiral medium, the averaged molecular ordering parameters are different for each enantiomer (the so-named "differential ordering effect of enantiomers") [47].

A major advantage of using chiral polypeptide liquid crystal solvents as an alignment media is that both the organic molecule and the polypeptide are readily soluble in common low viscosity organic solvents. Thus, also small molecules which are poorly soluble in aqueous based alignment systems can be analyzed with regard to their specific orientation. Second, the chiral differentiation power of these media is very high [43]. In fact, liquid crystal

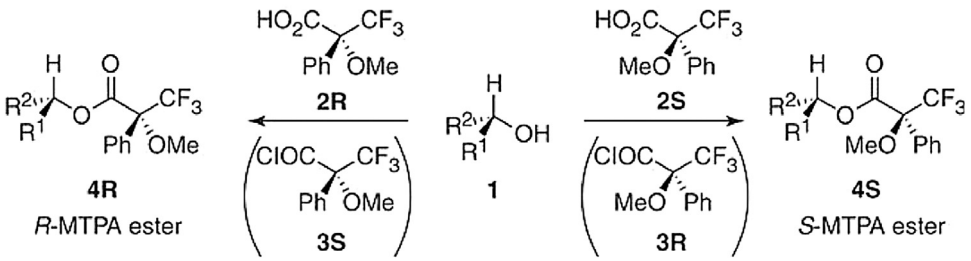


Fig. 3. Scheme for the synthesis of (*R*)- and (*S*)-Mosher esters **4** from the generic carbinol **1**. Both the free Mosher's acids **2** (CO_2H) and the acid chlorides **3** (COCl) can be used as acylating agents. Reprinted with permission from T.R. Hoye, C.S. Jeffrey, F. Shao, Nat. Protoc. 2, 2451 (©2007 by the Nature Publishing Group).

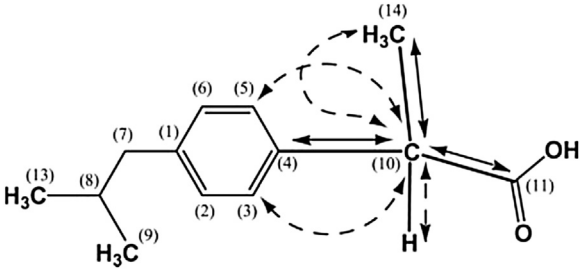


Fig. 4. Discrimination of the enantiomers of ibuprofen by an NMR-based method that utilizes poly-γ-benzyl-L-glutamate (PBLG) dissolved in $CDCl_3$ as chiral liquid crystal. A minimum of five independent RDCs have been measured from the stereocenter of ibuprofen (that is C10). Potential carbon-carbon and carbon-proton RDCs are highlighted by solid and dashed arrows, respectively. Reprinted with permission from V.M. Marathias, G.J. Tawa, I. Goljer, A.C. Bach II, Chirality 19, 741 (©2007 by the Wiley-Liss, Inc.).

solvents work with a wide range of compounds, i.e., alcohols, amines, ethers, ketones, acids [48], amino acids [47], organometallic complexes, alkynes, alkenes and even with alkanes [50].

As an example of application of the technique to chiral drugs, it is noteworthy the discrimination of the (*R*)- and (*S*)-enantiomers of ibuprofen (see Fig. 4) obtained by using the orientation system composed of poly-γ-benzyl-L-glutamate (PBLG) dissolved in $CDCl_3$ [51]. The method has proved to be suitable for a variety of small molecules with a maximum of one or two stereocenters, which cannot be derivatized with a Mosher's reagent (see Section 2.2.1) and/or for which no large single crystals are easily formed (see Section 2.1). Furthermore, a limited sample amount (namely, 15 milligrams for each enantiomer) is necessary in preparing the NMR samples.

2.3. Vibrational circular dichroism (VCD)

Vibrational circular dichroism (VCD) spectroscopy can be used for many types of analysis related to the structure and conformations of chiral molecules of biological interest, i.e., for determining (1) the absolute stereochemistry [52]; (2) the enantiomeric purity of a sample relative to a known standard [53,54]; (3) the conformation in solution of large and small biological molecules [55].

The AC determination of a chiral molecule is made by comparing the experimental infrared (IR) and VCD spectra of the unknown sample with those of the corresponding DFT calculated VCD spectra of the molecule using a chosen configuration (Fig. 5) [56]. The programs for calculation typically employ the magnetic field perturbation theory of VCD intensities conceived and implemented by Stephens [57,58]. If the sign and relative intensity of the observed bands in the VCD spectrum of the sample are the same as that of the calculated spectrum, the AC of the sample is the same as the AC chosen as the reference. If the bands of the observed VCD spectra feature the opposite sign of those calculated, then the sample has the opposite AC of that used for the calculation. Moreover, when DFT calculations (at a relatively high-level) are applied to all the possible conformers of the enantiomer molecule under study, the resulting relative energies of these conformers will give the relative populations, which will then be used for obtaining the VCD intensities [59].

The basic principles of the application of VCD to the determination of AC of chiral molecules have been nicely described in a review article [52], where a set of applications to organic, pharmaceutical and natural products have also been reviewed.

Furthermore, in 2012 a dedicated book for organic chemists has been published [60], where the authors, which are responsible for much of the existing literature in this field, discuss the applications of VCD spectroscopy to the structural characterization of chiral organic molecules and evaluate the advantages and limitations of the technique in determining molecular structure.

Notably, a huge number of ACs has been determined by VCD in the past twenty years covering a wide variety of compounds [60], but a detailed description of them is beyond the scope of this paper. Anyway, the number of applications is increasing rapidly every year, also thanks to the advances made in state-of-the-art instrumentations. Moreover, the continuous development and upgrade of the computing power and software, together with the availability of higher level functional and basis sets, make the calculation

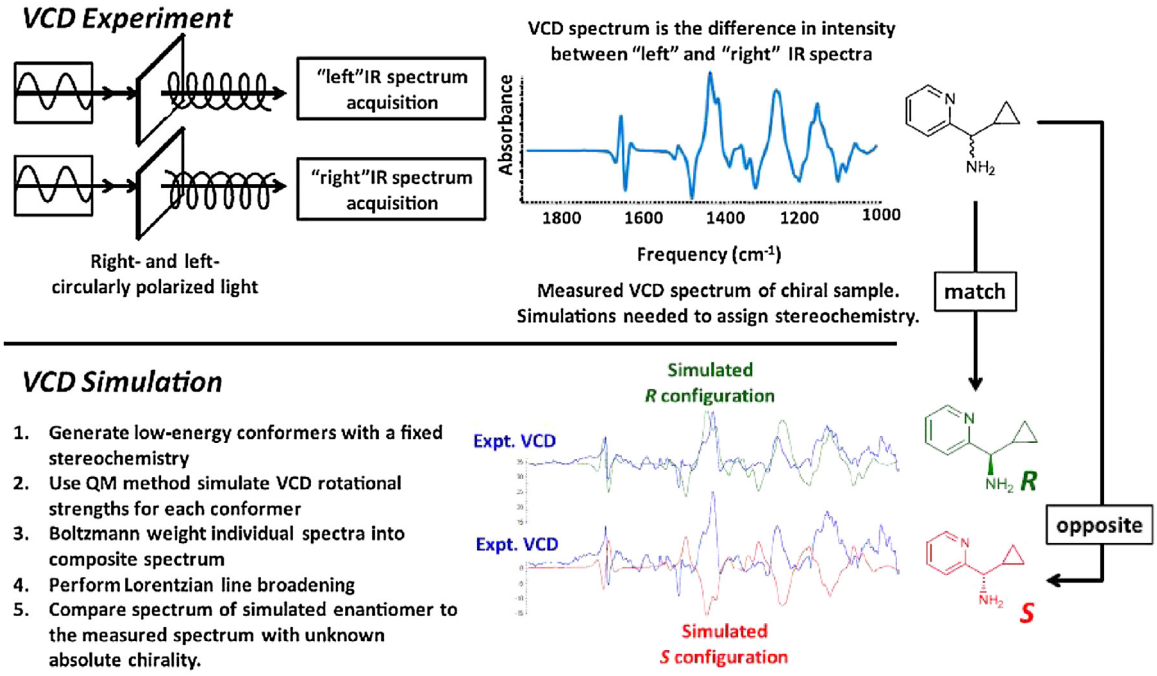


Fig. 5. The essential steps in the VCD assignment of a single enantiomer sample of unknown absolute stereochemistry. Reprinted from S.S. Wesolowski, D.E. Pivonka, Bioorg. Med. Chem. Lett. 23, 4019 (©2013 by Elsevier Ltd.).

of VCD spectra even more accurate and reliable for unambiguously determine the AC of chiral compounds.

In 2013, VCD has been proposed in the Pharmacopeial Forum journal as an emerging technology for determining not only the AC, but also the enantiomeric purity of chiral pharmaceutical ingredients at all phases of the discovery process [61]. More important, this *stimuli* article provided a basis for the development of a general chapter to be included in the US Pharmacopeia for the spectroscopic measurement of AC by VCD in drug substances and products.

As a result, in December 2016, the US Pharmacopeia released a supplement containing two chapters (namely, 782 and 1782) on VCD. The first chapter deals with various features of the VCD practice, such as qualification of VCD spectrometers, sample measurements, validation and verification of the measured spectra. The second one further explains the instrumentation used, provides details on qualitative and quantitative analyses, comparison between measured and calculated spectra, determination of e.e., and concurrent use of VCD for AC and e.e. determination [62].

The impressive growth of applications of VCD spectroscopy for the assignment of the AC has made the practice a rapid alternative to X-ray crystallography (it does not require growing single crystals) in advanced drug discovery projects by AstraZeneca Pharmaceuticals [56]. In the article, selected case studies are illustrated (namely, neurokinin-3 antagonists, re-assignment of the AC of cipralisant, and *N*-Methyl-D-aspartic acid antagonists) with an emphasis on providing utility and impact to pharmaceutical discovery programs.

Finally, VCD proved to be the most discriminatory method also for molecules with multiple stereogenic elements (i.e., diastereoisomers), particularly when it is used in combination with NMR spectroscopy [63]. This is the case of tadalafil, a phosphodiesterase type 5 inhibitor which was approved in 2013 by the FDA for the treatment of male erectile dysfunction, pulmonary arterial hypertension, and benign prostatic hyperplasia. The protocol developed, made by a combination of VCD, electronic circular dichroism (ECD), and optical rotatory dispersion (ORD), proved capable of assigning the stereochemistry of all the four stereoisomers of tadalafil.

The strength and reliability of vibrational optical spectroscopy in the field of drug discovery is furthermore certified by the fact that the FDA has approved vibrational optical activity as a technique for determining the AC of chiral compounds [56].

2.4. Enantioselective chromatography (HPLC, SFC and UHPC)

Enantioselective chromatography has become in the years an essential tool in the area of drug discovery [24,64–69], both by the indirect and direct separation modes. Indirect separation methods based on the use of enantiomerically pure CDAs are frequently used, especially at the large-scale level, although the nature, enantiomeric purity, availability, costs and ease of cleavage of the CDAs are sometimes limiting issues for this strategy [70]. Alternatively, direct methodologies based on the use of chiral stationary phases (CSPs) are most conveniently employed [67,71], where both synthetic building blocks [72,73] and naturally occurring molecules (namely, polysaccharides, proteins, and macrocyclic antibiotics) [74–76] are covalently bonded to the silica surface as chiral selectors. A detailed description of the huge number of CSPs for HPLC reported in the literature and/or those that have been commercialized by the different manufacturers of chromatographic instruments is beyond the scope of the present review article; the reader can refer to [77,78] for a description of the most important contributions published in the field, with an emphasis to emerging CSPs that seem to possess all the requisites necessary to yield even superior performances with respect to the CSPs nowadays available.

Depending on the stage in chiral drug discovery, goals and needs are different. At the initial stages of the process, a quick screening of large libraries of chiral molecules is necessary [79]. High-throughput methods need enantioselective systems capable of analyzing the largest number of molecules with minimum changes of the experimental conditions. On the other hand, once a racemic candidate has been selected for the following steps of the drug development, the optimization of the separation is strongly facilitated by the chance of transferring the information gathered in the screening phase with great practical and economic advantages.

Supercritical fluid chromatography (SFC) has recently witnessed a remarkable breakthrough in the enantioseparation of pharmaceuticals [80,81] particularly in the initial screening stage of the drug discovery process [82]. In fact, supercritical or subcritical CO_2-based mobile phases posses a reduced viscosity and hence allow for faster mass transfer with respect to typical LC eluents. As a result, high flow-rates can be employed with a large gain in the analysis times, concomitant with chromatographic efficiencies as high as those achieved by gas chromatography. Moreover, preparative systems for bulk-scale SFC purification allow purifying a large amount of drug substance [83,84]. Therefore, SFC, in its new metamorphosed meaning [85], may no longer be considered as a technique with some special application scope, but rather a complementary tool to the widely accepted analytical techniques [86,87].

Almost all of the chiral selectors used in HPLC have been successfully applied to sub-/supercritical chromatography [88], and they are characterized by a high variety. Among them, polysaccharide derivatives have been used to the greatest extent [89–91], because of their easy accessibility and broad enantioselectivity, followed by Pirkle-type selectors [92,93], cyclodextrins [94,95], and macrocyclic antibiotics [89,96,97]. In a recent review it is reported a list of enantioselective separations achieved by using supercritical fluids for a range of drugs and drug-like compounds [98].

The revival of interest in SFC in recent years has been predominantly the result of the introduction of new state-of-the art instrumentation [99,100]. Second, the development of CSPs in sub-2.0 μm format has opened new frontiers in the field of enantioselective "e" Ultra-High Performance SFC (eUHPSFC) [101,102], allowing to perform very fast separations (in the order of seconds, or something like that) and simultaneously keep very high the efficiency of the system. It should be noted, however, that the extraordinary advancement in developing sub-2-μm particles for ultra-high performance applications (Fig. 6) has been limited so far to brush-type CSPs (namely, DACH-DNB-CSP, Whelk O1, and macrocyclic antibiotics-based CSPs) [103–105], whereas, from a practical point of view, there would be a pressing need for other kinds of sub-2-μm CSPs, in addition to brush-type [106,107].

With regard to the AC determination by enantioselective chromatography, it must be said that it was typically achieved by comparison of the HPLC elution order of the unknown sample with that of enantiomeric standards of closely related analogs, which means that the elution order of the analyte is assumed to be correlated with that of a known enantiomer of the analog. Obviously, this approach is limited to analytes for which enantiomeric standards of structurally-related analogs are available [108]. The introduction of suitable chiro-optical detection systems (namely, optical rotation (OR)- and circular dichroism (CD)-based) in the field of enantioselective HPLC separations has indeed represented a large analytical innovation, because of the selective monitoring of optically active molecules in complex mixtures of chemically diverse organic compounds [109]. However, chiro-optical detectors do not directly allow getting information on the AC of a given chiral molecule, unless they are coupled with quantum chemical CD calculations for both the enantiomers under investigation [110]. In any case, the stereochemical information contained in the bisignate response at a suitable wavelength could be exploited to establish the elution

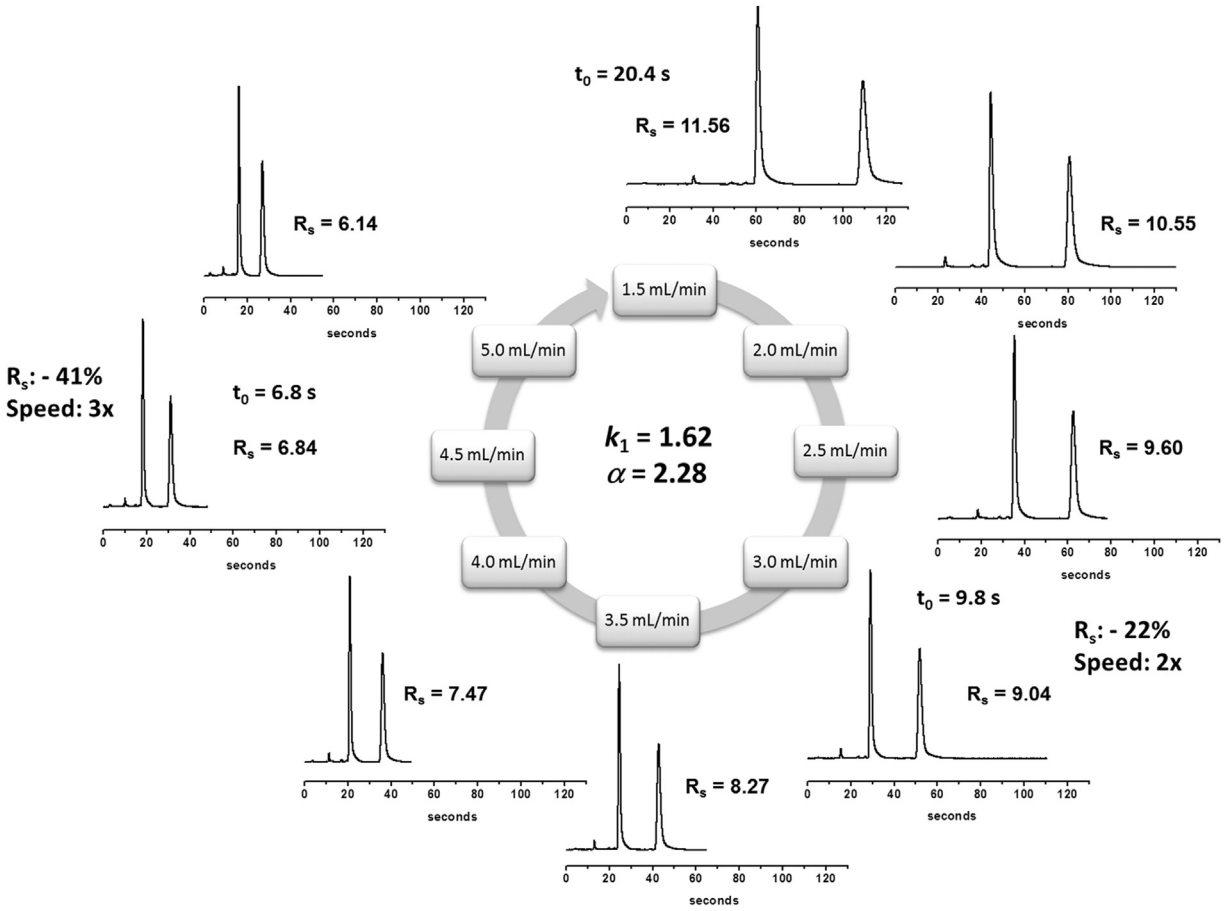


Fig. 6. Ultrafast separation of naproxen and its enantiomer on the UHPLC 1.7 μm (*R,R*)-Whelk-O1 (5 cm × 4.6 mm I.D.) column at different flow-rates. Reprinted with permission from D. Kotoni, A. Ciogli, C. Molinaro, I. D'Acquarica, J. Kocergin, T. Szczerba, H. Ritchie, C. Villani, F. Gasparrini, Anal. Chem. 84 (©2012 by the American Chemical Society).

order for those compounds not available as single enantiomers of known configuration [76].

The combination of enantioselective HPLC with the chemical correlation method proved to supply the AC for a broad variety of chiral compounds, and the results obtained have been collected in a dedicated review article [24]. Briefly, the technique is based on the comparison of the retention time of the isolated enantiomer resulting from a series of non-racemizing chemical transformations with the retention times of the enantiomers of the corresponding racemate, whose AC is known [111].

Enantiomeric purity of chiral compounds (typically expressed by the enantiomeric excess) is conveniently achieved by enantioselective chromatography provided that or the racemate or the two enantiomers are available as the reference. Whilst such standards can be easily obtained in the case of molecules derived from a synthetic route, the world of naturally occurring products is full of examples of single-enantiomer formats, and a large portion of drugs draws fully from the natural products. To overtake such limitation it was developed, ten years ago, a new approach named "Inverted Chirality Columns Approach (ICCA)", which has proved to be very useful in the enantiomeric trace analysis, when the minor enantiomer follows the major one and is partially hidden by the tailing of the leading enantiomer: on the CSP with opposite configuration, the trace enantiomer is eluted first, thus enabling a more precise and accurate quantitation by peak area integration [112,113].

A final mention must be done in this Section on the procedures and acceptance criteria to be followed in applications for approval of new chiral drug substances: where a new drug substance is predominantly made by one enantiomer, the opposite enantiomer is excluded from the qualification and identification thresholds given in the ICH Guidelines on Impurities in New Drug Substances and Impurities in New Drug Products [114,115], because of practical difficulties in quantifying it at those levels (technical limitations may preclude the same limits of quantification or qualification from being applied). However, that impurity in the chiral new drug substance and the resulting new drug product(s) should otherwise be treated according to the principles established in those Guidelines.

3. The chiral drug development process

One of the attractive benefits of introducing chirality in a drug candidate is that it leads to increased complexity to a specific target, i.e., it gives access to a greater diversity of compounds to be explored [116]. There are two principal scenarios, for a pharmaceutical company, in the development process of chiral drugs: (1) the above-mentioned switch from an existing racemic drug to one of the two enantiomers of that drug [2,4], typically the so-called eutomer [117], and (2) the *de novo* development of an enantiomerically pure drug.

For the sake of clarity, when we talk about a novel molecule or a new entity that is approved by the regulatory authorities or it is introduced into the market, we should refer to an unequivocal designation; unfortunately, each country and its drug-regulatory agency have their own definitions. Therefore, terms like new

Fig. 7. Designations of new drugs for all the scientists involved in the field of drug discovery and at regulatory agencies. Reprinted with permission from S.K. Branch, I. Agranat, J. Med. Chem. 57, 8729 (©2014 by the American Chemical Society).

molecular entity (NME), new chemical entity (NCE), new biological entity (NBE) as well as new active substance (NAS) are frequently used with some ambiguity, by both medicinal chemists and the other scientists involved in drug discovery and development. With the aim of shading some light on the ambiguity and inconsistency surrounding the above-mentioned terms, a perspective article was published [118], where the authors also recommended, for the future, to refer the term new therapeutic entity (NTE), which is a general, comprehensive term for a new drug designation, to either an NME or an NBE (Fig. 7).

With regard to the first scenario, it must be noted that the chiral switching practice (see Section 1) has been an important feature of drug development portfolios, particularly in the period from 1994 to 2011 (see Table 1). However, it has been showed that just a few pre-approval randomized controlled trials (RCTs) has been provided that included the racemic precursor as a direct comparator, that is for one third of the chiral switches approved from 2001 to 2011 (namely, for esomeprazole, levocetirizine and dexlansoprazole) [9]. On the other hand, it should be acknowledged that the FDA does not possess the legal authority to require comparative efficacy testing of the single enantiomers versus the previously developed racemate prior to approval [9].

For the *de novo* development of an enantiomerically pure drug, three main pathways are available for the pharmaceutical industry to access the chiral product: (1) to start from a pure enantiomer of a natural product (chiral pool); (2) to employ a stereoselective synthesis (including enzymatic and biological procedures); and (3) to separate a racemate obtained by a non-stereoselective synthetic protocol (chiral resolution). In all the cases, the company must provide detailed specifications for the final product which assure identity, strength, quality, and purity from a stereochemical point of view [18]. At the discovery stage of drug development, when a large number of molecules are required in milligrams amount for initial testing, stereoselective syntheses are not time- or cost-efficient. Moreover, since both the enantiomers of the new drug candidate are needed for biological testing (as required by the FDA's policy statement), the development of a chiral active pharmaceutical ingredient as the racemate can be more suitable, for the pharmaceutical industry, from a commercial and strategic point of view: in fact, the cost for large-scale non-stereoselective reactions is greatly reduced with respect to that affording a single enantiomer; afterwards, the chiral resolution of the racemate can be achieved at any level of the development process (i.e., on starting materials, intermediates or final products), using several methods including crystallization, diastereoisomeric salt or complex formation and enantioselective chromatography (see also Section 2.4) [119]. The latter has become the most time- and cost-effective approach for preparative purposes at the discovery stage in the pharmaceutical industry, where it also proved to accelerate drug development [119,120] and therefore facilitate an earlier regulatory approval [121].

In the later stages of the drug discovery, when the pharmaceutical company decides to focus just on one of the two component of the racemate, the direct production of the desired enantiomer by a stereoselective synthetic route remains a primary target. Several transformations with significant potential for use in API manufacture have been published in 2015, and a few of them have been highlighted in a dedicated article [23].

Finally, it should be noted that racemic NMEs continue to be approved, as a result of two recent surveys [121], particularly when the difference in therapeutic properties between a single enantiomer of a racemic NME and the racemate is sufficiently substantial to justify subsequent development and patenting of the enantiomer.

3.1. A focus on the chiral drugs approved by the FDA in 2015

The group of Agranat from The Hebrew University of Jerusalem (Israel) has been giving a fundamental contribution in the statistical and critical elaboration of the whole data concerning the intellectual property [2] and the trends in the development of chiral drugs [3,4,122] since 1999. It is sufficient to say that the review article entitled "Putting chirality to work: the strategy of chiral switches" [4] has received 291 citations so far (source: Scopus). For their studies, the authors have surveyed both the database *To Market, to Market* in Annual Reports in Medicinal Chemistry, which lists all the new drugs launched each year in the worldwide markets, and the FDA database of drug approvals, to identify and classify all new products (divided into achiral, single-enantiomer and racemate) approved for use in a desired range of years.

A similar statistical survey has been performed in 2007, where the field of chiral drugs, focused on the new drugs launched in the years 1983–2004, has been observed mainly from a synthetic point of view [123].

The picture that emerges from the above-mentioned studies is that single-enantiomer drugs are a rapidly growing proportion of the NME drugs that are introduced into the market each year, and their percentage has ranged, for instance, from about 40% of the new worldwide approved drugs in 1992 to almost 70% in 2010. However, it must be kept in mind that new racemic drugs are not dead [121].

With the aim of getting a rapid picture of the current market of chiral drugs nowadays, we examined the database of the agency's Center for Drug Evaluation and Research (CDER) at the FDA, focusing on the 2015 FDA drug approvals [124]. In 2015, a cohort of 45 new drugs (exactly the double of the average approval number in the years 2006–2009) were approved by the FDA, including 33 new molecular entities (NMEs) and 12 biologics license applications (BLAs).

By therapeutic area, anticancer drugs (31% of the cohort) proved to dominate the CDER approval list, as already happened in 2011, 2012 and 2013 (the exception was 2014, when oncology accounted for only 22% of approvals). Cardiology and infectious disease follow oncology with the same abundance (9%).

We extracted the chemical structure of the 45 new drugs from the FDA approval chemistry reviews at the CDER website and we selected a subgroup (i.e., 44% of the cohort) of small-molecule active pharmaceutical ingredients (APIs) containing one or more chirality centers (see Table 2). The percentage of chiral drugs in the 2015 approval list was calculated by considering as individual drugs

Table 2
Selected chiral small molecule drugs from the 45 new drugs approved by FDA in 2015.

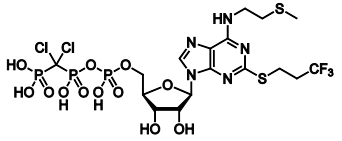
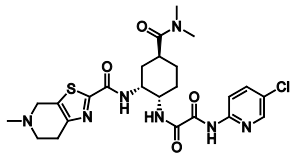
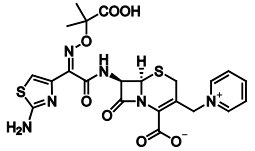
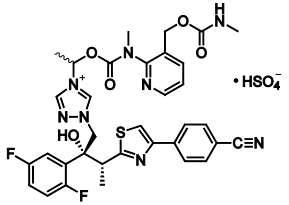
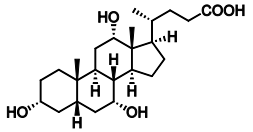
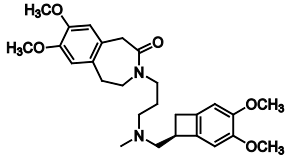
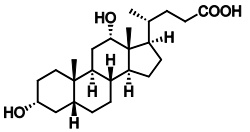
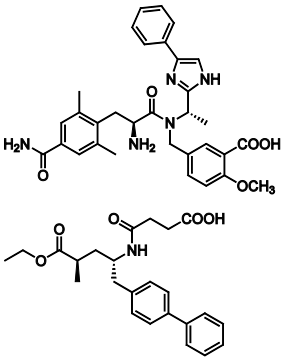
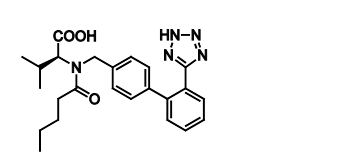
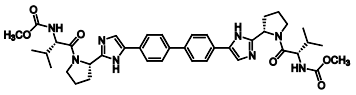
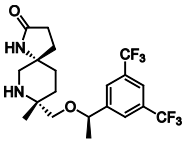
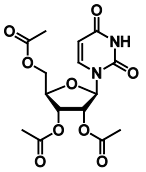
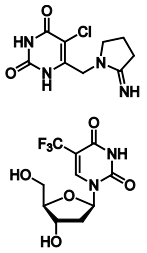
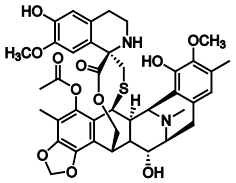
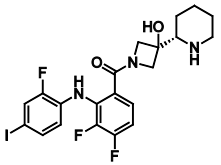
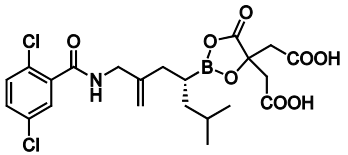
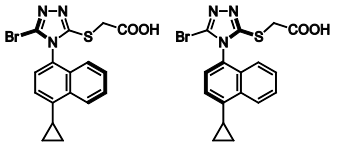
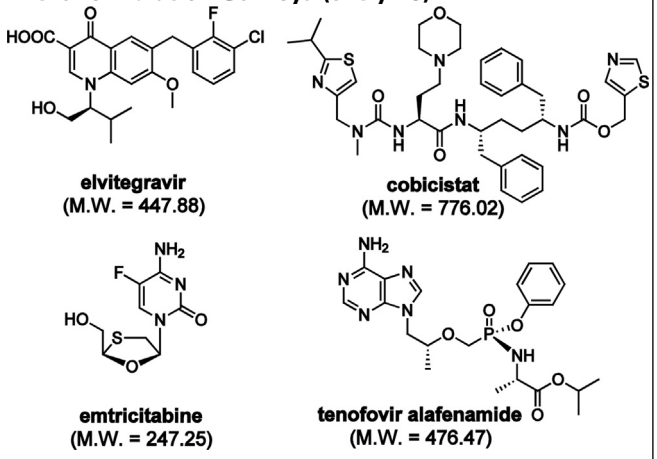
Entry	API	Chemical structure	Molecular weight (Da)	Treatment or indications	Number of stereocenters	e.e. determination	Company
1	Cangrelor (NDA 204958)		776.36	Myocardial infarction	4	Specific optical rotation	Medicines Company
2	Edoxaban (NDA 206316)		548.06	Acute ischemic stroke, systemic embolism and deep vein thrombosis	3	Chiral HPLC	Daiichi Sankyo
3	Avibactam + ceftazidime (NDA 206494)		256.24 546.58	Complicated intra-abdominal and urinary tract infections	3 (avibactam) 2 (ceftazidima)	Chiral HPLC (for avibactam)	Allergan
4	Isavuconazonium sulfate (NDA 207500)		800.81	Antifungal	3	Chiral HPLC	Astellas
5	Cholic acid (NDA 205750)		408.57	Bile acids synthesis and peroxisomal disorders	11	Specific optical rotation	Retrophin
6	Ivabradine (NDA 206143)		468.59	Chronic heart failure	1	Chiral HPLC	Amgen

Table 2 (*Continued*)

Entry	API	Chemical structure	Molecular weight (Da)	Treatment or indications	Number of stereocenters	e.e. determination	Company
7	Deoxycholic acid (NDA 206333)		392.57	Reducing moderate-to-severe fat below the chin	11	not disclosed	Kythera
8	Eluxadoline (NDA 206940)		569.65	Irritable bowel syndrome with diarrhea	2	Chiral HPLC	Allergan
9	Sacubitril + valsartan (NDA 207620)		411.49 435.52	Chronic heart failure	2 (sacubitril) 1 (valsartan)	Chiral HPLC	Novartis
10	Daclatasvir (NDA 206843)		738.88	Chronic HCV infections	4	Chiral HPLC	Bristol-Myers Squibb
11	Rolapitant (NDA 206500)		438.41	Chemotherapy-induced nausea and vomiting	3	Chiral HPLC	Tesaro
12	Uridine triacetate (NDA 208169)		370.31	Hereditary orotic aciduria	2	not disclosed	Wellstat

13	Tipiracil + trifluridine (NDA 207981)		242.66 296.20	Unresectable advanced or recurrent colorectal cancer	3 (trifluridine)	Specific optical rotation (for trifluridine)	Taiho
14	Trabectedin (NDA 207953)		760.85	Unresectable or metastatic liposarcoma and leiomyosarcoma	7	Specific optical rotation	Johnson & Johnson
15	Elvitegravir + cobicistat + emtricitabine + tenofovir alafenamide (NDA 207561)	See Box 1	See Box 1	HIV	1 (elvitegravir) 3 (cobicistat) 2 (emtricitabine) 3 (tenofovir alafenamide)	Chiral HPLC	Gilead
16	Cobimetinib (NDA 206192)		531.31	Melanoma with $BRAF^{V600E/K}$ mutations	1	Chiral HPLC	Genentech
17	Ixazomib (NDA 208462)		514.16	Multiple myeloma	1	Chiral HPLC	Takeda
18	Lesinurad (NDA 207988)		404.28	Hyperuricaemia/Gout	1 chirality axis	Chiral SFC	AstraZeneca

Box 1: Chemical structures of the four APIs combined in the formulation Genvoya (entry 15).



those which are part of a combination (namely, entry 3, entry 9, and entry 15, Genvoya, the latter being indeed an association of four chiral molecules, see Box1).

The number of chirality centers ranged from one (entry 6, entry 9, and entries 15–17) to seven (entry 14), with the exception of cholic (entry 5) and deoxycholic (entry 7) acids, which, being naturally occurring compounds, feature a more complex stereochemical architecture (namely, eleven chirality centers are present).

The molecular weights (MW) of the selected items ranged from approximately 250 to 800 Dalton; notably, more than the half of them does not meet one of the cut-off of the Lipinski rule-of-five [125], namely that regarding the MW which should be less than 500 Da for the "drug-likeness" [126] of a compound.

On the basis of the FDA dossiers examined, it emerged that all the chiral drugs approved by the FDA in 2015 are enantiomerically pure compounds with a well-defined AC, with the exception of one, namely lesinurad (entry 18), which has been licensed as the racemate of two enantiomeric atropoisomers, arising because of the hindered rotation around the single C–N bond in the naphthalene ring. Furthermore, none of the previously developed racemates has been switched to the single-enantiomer version in 2015, even though newly approved single-enantiomer drug developed by application of the chiral switch strategy would not have been included in FDA's approvals (NMEs and BLAs) for 2015.

We became interested in surveying the methods proposed by the drug sponsors for the determination of the e.e. of the above-mentioned new drugs and/or for their enantioseparation. Generally, we failed in finding such information in the FDA dossiers (it was not disclosed because commercially confidential), and therefore we consulted the database of the European Medicines Agency (EMA), which provided us the data we were looking for.

With the exception of cangrelor (entry 1), cholic acid (entry 5), trifluridine (entry 13) and trabectedin (entry 14), for which the enantiomeric purity was routinely checked by specific optical rotation, for almost all the other drugs the e.e. was measured by "chiral HPLC".

In some cases (namely, entries 3, 6, 10 and 11) we found also the information regarding the enantioselective columns used (mainly the polysaccharides-based family), whereas for the other APIs such an information is not yet available in the literature.

Avibactam (entry 3) is a beta-lactamase inhibitor approved in combination with the semisynthetic cephalosporin ceftazidimine for the treatment of complicated intra-abdominal and urinary tract infections. To separate the two enantiomers (namely, 1*R*,2*S*,5*R* and 1*S*,2*R*,5*S*) of an immediate precursor of avibactam, whose preparation is described in a dedicated PCT application [127], the Chiralpak ADH (250 × 4.6 mm I.D.) column was used, the mobile phase consisting of *n*-heptane/ethanol/diethylamine (65:35:0.005, v/v/v).

Ivabradine (entry 6) contains just one chirality centre in the benzocyclobutane ring, the claimed enantiomer being the (*S*). The e.e. was measured on a Chiralpak 1A column, using a mobile phase consisting of *n*-heptane/2-propanol (95:5, v/v) and PDA detection at 220 nm [128].

For daclatasvir (DCV, entry 10), which was approved for the treatment of chronic hepatitis C virus (HCV) genotype 3 infections, an enantioselective HPLC method has just been published [129], where an excellent resolution was achieved on the Chiralpak ID-3 column, using a binary gradient containing acetonitrile/diethylamine and methanol/diethylamine as the mobile phases, and UV detection at 315 nm. The *all*-(*S*) enantiomer (i.e., the eutomer) was separated from its *all*-(*R*) enantiomer and the corresponding diastereoisomeric impurities under the same chromatographic run, with resolution values (Rs) ranging from 5.27 and 6.21 (chosen as the optimal in the above-mentioned conditions), and from 1.79 and 2.56, respectively (Fig. 8). Notably, daclatasvir represents an "arc of triumph" for medicinal chemistry [130], since it is the first direct-acting antiviral agent which demonstrates that a chronic HCV infection could be cured in the absence of interferon therapy.

Rolapitant (entry 11) is a tachykinin neurokinin 1 (NK1) antagonist approved for the prevention of chemotherapy-induced nausea and vomiting. It exhibits stereoisomerism due to the presence of three chirality centres, all of which originate in raw materials. Enantiomeric purity is controlled by using the Chiralcel OD-H (25 cm × 4.6 mm I.D.) column, with a mobile phase made by *n*-hexane/2-propanol (95:5, v/v) + 1% DEA at a flow-rate of 1.3 ml/min and UV detection at 215/240 nm [131].

During the writing of this review article, it was published the first study dealing with the synthesis and the isolation of the two atropoisomers of lesinurad by enantioselective SFC [132]. Briefly, the racemic ethyl ester precursor of the drug (Fig. 9) was submitted to semi-preparative SFC, which afforded the two atropoisomers as ethyl esters with e.e. ranging from 94% (for the second eluting enantiomer) to 100% (for the first eluting). The column used was a Chiralpak AS (250 mm × 30 mm, 5 μm), the eluent being a mixture of supercritical CO_2/ethanol (0.05% DEA) = 70/30, flushed at a flow-rate of 60 ml/min. Afterwards, the two enantiomers were individually hydrolyzed with aqueous LiOH to the enantiomerically pure free carboxylic acids.

Notably, the authors did not observe any enantiomerization upon heating in solution and in pharmacokinetic studies in vivo. Furthermore, considering the significant difference found for the two atropoisomers, it was speculated by them that the (+)-lesinurad might offer a better hyperuricaemia/gout activity than (−)-lesinurad or the racemate. We believe that, most likely, in a very near future, lesinurad will be one of the candidates to a chiral switch that will lead to the release of a patent that claims the single (+)-enantiomer.

For the sake of truth, the National Institute for Health and Care Excellence (NICE) has just published an appraisal consultation document turning down lesinurad's use within its marketing authorization, that is, for treating hyperuricaemia in patients whose blood level of uric acid was not sufficiently controlled with allopurinol, a xanthine oxidase inhibitor. The final decision on the appraisal is expected for September 2017 [133].

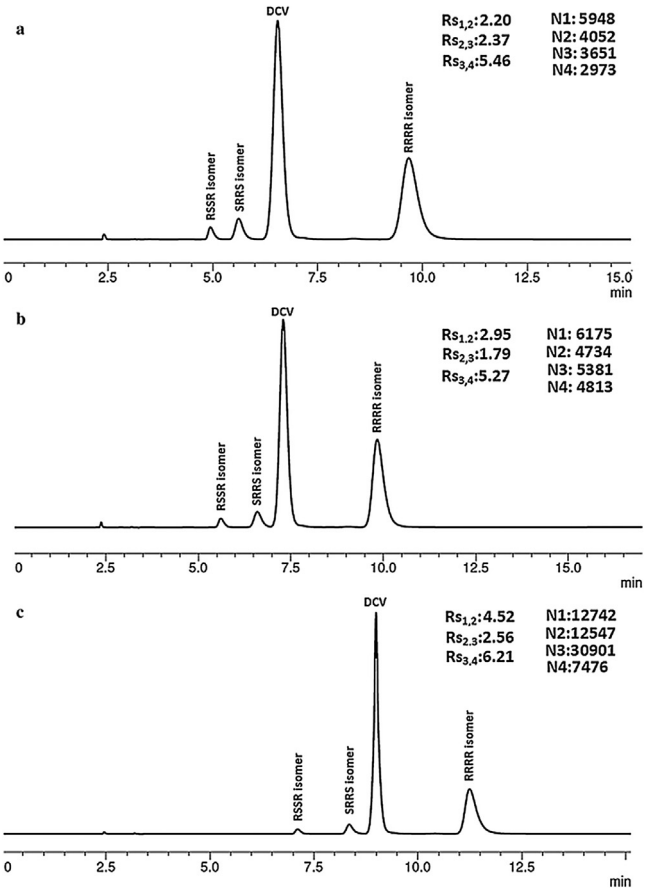


Fig. 8. Typical chromatograms obtained for the separation of daclatasvir (*all-S*-DCV), its enantiomer (*all-R*), and the other diastereoisomeric impurities on the Chiralpak ID-3 column. The results collected in the bottom trace have been taken as the optimal by the authors. Reprinted with permission from G. Srinivasu, K. Nagesh Kumar, Ch. Thirupathi, Ch. Lakshmi Narayana, Ch. Parameswara Murthy, Chromatographia 79, 1457 (©2016 by Springer-Verlag Berlin Heidelberg).

4. Conclusions

We became interested in taking a look – from the point of view of academia – to the current trend of the market of chiral drugs from the evidence that most of the new drugs introduced annually to the market are single enantiomers [121]. The basic query was the way by which the pharmaceutical industry achieves the goal to launch enantiomerically pure compounds, either by resorting to the chiral switching practice (as frequently happened for blockbuster drugs in the period 1994–2011) or by manufacturing *de novo* a single-enantiomer drug.

Starting from the 45 new drugs approved by the FDA in 2015, we extracted a subgroup of small molecules (ranging within an approximate 250–800 Da molecular weight interval) featuring one or more chirality centres (namely, 44% of the cohort), and we made a check about their stereochemical profile. We used the FDA database of drugs approval (namely, the CDER website) for extracting the physico-chemical properties of the drugs, and the database of the European Medicines Agency (EMA) to gather information about the methods proposed by the drug sponsors for the determination of the e.e. and/or their enantioseparation.

We found out that all the NME drugs selected have been approved as single enantiomers with a well-defined absolute stereochemistry, except for one (i.e., lesinurad), which has been licensed as the racemate of two enantiomeric atropoisomers. Furthermore, none of the previously developed racemates has been switched to the single-enantiomer version in 2015.

With regard to the methods routinely employed to check the enantiomeric purity at the different stages of the discovery process, the term "chiral HPLC" proved to dominate the list of the selected items, followed by "specific optical rotation".

Unfortunately, chromatographic details for the enantiomeric purity test of both assay and impurities were not provided in the chemical reviews examined, obviously since they are the subject of commercial confidentiality. Anyway, for avibactam, ivabradine, daclatasvir, rolapitant and lesinurad the analytical methods for the enantioseparation of the diverse stereoisomers were available in the literature, both included in application patents (it is the case of avibactam, ivabradine and rolapitant) and in extremely recent experimental articles (for daclatasvir and lesinurad,). For lesinu-

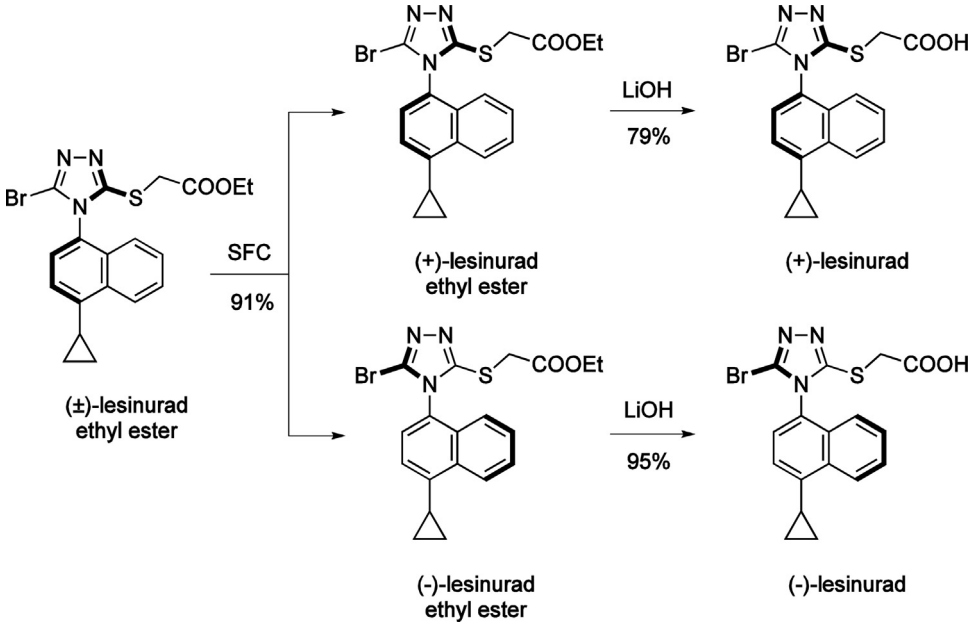


Fig. 9. The strategy for the separation of the two atropoisomers of lesinurad by semi-preparative enantioselective SFC.

rad, considering the significant activity difference found for the two enantiomers (namely, atropoisomers), it can be expected the release of a patent that claims the single (+)-enantiomer.

This review article could represent an invitation, for the researchers working in the academia as well as in the pharmaceutical industry, to fill the gap, i.e., to design and develop enantioselective analytical methods for those compounds in Table 2 for which they have not yet been described.

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