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Chiral interconversion monitoring of a drug candidate by supercritical fluid chromatography (SFC)

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ABSTRACT

Stereoisomer interconversion of chiral drug substances is of significant importance if it occurs within pharmacological and pharmaceutical time scales and under physiological and shelf life conditions. Several analytical techniques exist for the determination of first order rate constants and enantiomerization energy barriers by dynamic and stopped flow chromatography, mathematical models and functions, and computer programs. The focus of this work is to utilize a simple supercritical fluid chromatography (SFC) chiral assay to determine the possibility of interconversion of the desired R and less active S isomers of a drug candidate. The rate constants of racemization and enantiomerization, the half life of racemization, and enantiomerization energy barriers were determined for the R → S (or, forward) and S → R (or, reverse) conversions. The method was selective and sensitive enough to detect less than 1% interconversion occurring under the conditions studied. The method also demonstrated that R ⇌ S racemization was possible only under extreme conditions of prolonged heating (80 °C) and highly basic pH (9.5).

1. Introduction

Chiral separation has gained significant attention in pharmaceutical analysis over the past few years with an increasing trend in synthesis of chiral compounds and exploration of chiral drug switches. Configurational stability of chiral drug substances is of pharmaceutical and pharmacological concern, as most biochemical processes are stereochemically regulated and enantiomers can have different interactions in terms of pharmacokinetics, pharmacodynamics, and toxicological properties. The monitoring of configurational and conformational lability in terms of chiral interconversion is particularly important in the following cases:

- The distomer has pharmacological activity markedly different from the intended isomer (eutomer). Associated with this phenomenon, an inverse relationship exists between the effective dose of chiral drugs and their enantiomeric potency ratios, also known as Pfeiffer's rule [1–3].
- The distomer is pharmacologically toxic or causes detrimental side effects. A well-known pharmaceutical example of this is the case of thalidomide.

Since the late 1980s, the U.S. Food and Drug Administration (FDA) has recognized the importance of drug stereomers and has set forth guidelines requiring configurational characterization of chiral drugs during pharmaceutical development [4,5]. These dictate that drug manufacturers assess the stereochemical integrity of enantiomers as well as examine the potential of interconversion of individual isomers. Further impetus for characterizing chiral interconversion is supported by observations described by Testa et al. [6] and Reist et al. [7] that no stereoisomer is configurationally stable under all combinations of temperature and pH. Also, two time scales and related conditions are relevant to stability of drugs: the pharmaceutical time scale, in the range of days to years to include manufacturing and shelf life, and the pharmacological time scale, in the range of minutes to days to cover physiological conditions (37 °C, pH 7.4) during exposure. Enzymatic inversion of chiral molecules is also possible and has been reported [6]. Physiologic enantiomeric ratio can also change as a result of metabolism and excretion rate differences. These, however, are considered to be in vivo enrichment of a particular enantiomer and not a stereochemical interconversion [6].

It is important to point out the differences between enantiomerization and racemization, which are both associated with compounds possessing one chiral center. Enantiomerization refers to the reversible first order interconversion of one enantiomer to the other [7]. Typically this passes through a transition state (e.g., Ar–CO bond rotation) [8–11], or an intermediate (e.g., base catalyzed pyramidal carbanion or acid catalyzed acyl carbonium ion [12–14], a fast keto ⇌ enol tautomerism [15,16], etc.).

In contrast, racemization refers to the irreversible conversion of an enantiomer (be it optically pure or impure) into a racemic

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mixture (50:50 molar ratio of original and the converted enantiomer) [7,17,18]. This is also associated with a loss in optical activity of the chiral substance over time. A racemic mixture is, therefore, the endpoint of the interconversion of two enantiomers. The rate constant of racemization k_{rac} refers to this first order kinetic process. In an achiral environment, the two antipodal enantiomers have identical free energies, and their k_{rac} should be the same. The relationship between the rate constant for enantiomerization (k_{enant}) and the rate constant of racemization (k_{rac}) is: $k_{rac} = 2 \times k_{enant}$, under a given set of conditions [7,19].

The half life of racemization ($t_{1/2rac}$) is the time during which the enantiomeric purity of a chiral compound reduces to 50% of its original enantiomeric excess (e.e). The e.e is calculated as the % difference in the mole fractions (or, peak area% in chromatography) of the two enantiomers. Thus, a racemate has an e.e = 0.

An experimental approach to k_{enant} determination is to measure the rate during very early phase of conversion, when essentially most of the enantiorich form is still unchanged. During this period, the other isomer has not yet accumulated to a significant amount and the reverse conversion rate is negligible. The overall observed (or, apparent) rate constant of the kinetics during this period is a fairly accurate estimate of the true k_{enant} for the relevant process.

For a chiral compound, any possible on-column interconversion can be measured by a variety of dynamic chromatographic methods, as noted by Krupcik et al. [20], if the process occurs within the time frame of separation. These different dynamic approaches are used for the determination of rate constants and interconversion energy barriers of enantiomers. On-column stopped flow techniques [21], computer simulation (e.g., discontinuous plate, stochastic, and continuous flow models) [21,22], programs (e.g., SIMUL, Mimesis, ChromWin) [21,23], approximation functions, and deconvolution methods [24,25] have also been employed for the determination of rate constants and enantiomerization energy barriers.

Combination of chiral separation with classical kinetic methods has also been reported for the determination of interconversion rate constants [20]. In this approach, the interconversion of an isomer (pure or enantiorich) in solution is performed outside the separation system (off-line) by controlling temperature for a required time. Aliquots of converting enantiomer solution are sampled at regular intervals and analyzed by enantioselective chromatographic methods. The chiral column is maintained at conditions (e.g., temperature, mobile phase, etc.) which quench any possible on-column interconversion. Achiral chromatographic detectors are typically used and give equal responses for both enantiomers. The apparent rate constants may be determined directly from peak areas, in this case.

In general, an assay to monitor the chiral interconversion should have the following attributes: (a) be stereospecific for the chiral center of interest, (b) provides baseline or better resolution of the intended and the other isomer, and (c) sensitive enough to detect the interconversion occurring at a low concentration, particularly if the minor isomer elutes immediately following a tailing major isomer peak [26].

SFC has gained particular attention in monitoring both off-column (classic) and on-column (dynamic) interconversion of stereoisomers due to the advantages of faster separation speed, higher efficiency, less mobile phase consumption, and significantly less organic solvent waste generation offered by this technique, as compared to traditional HPLC [27]. In this work, the interconversion possibility of a therapeutically important chiral drug candidate was investigated by SFC. The R isomer of this compound was found to possess different potency compared to the S (minor) isomer. It was, therefore, necessary to investigate if the R isomer would racemize to the less active S isomer during typical pharmacological and pharmaceutical time scales, and thus lose its therapeutic activity. It would potentially also provide the synthetic chemist with useful information to optimize conditions and maximize yield of the desired isomer, if significant interconversion is occurring. A sensitive and selective chiral SFC method was employed to accurately demonstrate any interconversion from R to S and vice versa, and preliminary studies have been conducted to measure the interconversion rates in co-solvent containing media at a range of pH from 3 to 9.5.

2. Experimental

2.1. Materials and reagents

The basic drug candidate (initial optical purity 99%) and its S enantiomer (initial optical purity 99%) consisted of one chiral center (of the configuration of $R_1R_2R_3C$–H adjacent to a carbonyl group), and were obtained from AstraZeneca compound management (Wilmington, DE). This is identical with AZM in a previously reported work [28]. Among the various lots of the compound studied, the S (minor) enantiomer was present at a minimal 1% level. HPLC grade methanol and USP grade 200 proof ethanol were obtained from J.T. Baker (Phillipsburg, NJ) and Pharmco (Brookefield, CT), respectively. Dimethylethylamine (DMEA) was used in the SFC method development as additive, and purchased from Acros Organics (NJ). The drug compound is a diprotic base and it was necessary to include basic additives in the mobile phase to reduce the possibility of peak tailing. Lactic acid (pH adjusted to 3.0) was prepared in-house. Potassium phosphate monobasic buffer and sodium borate buffer solutions were products of Fisher Scientific (Fairlawn, NJ) and LabChem (Pittsburgh, PA), respectively. The pH of the three buffer solutions were checked prior to use by a Beckman pH meter model Φ40. SFC grade carbon dioxide was supplied by MC Industries (Malvern, PA).

2.2. Preparation of method development reference solution

An in-house, fit-for-purpose SFC method optimized for this drug candidate was used during the interconversion evaluation study. The details of the method development and validation have been published earlier [28,29] and therefore beyond the scope of this paper. For feasibility evaluation of interconversion, the following solution was prepared:

Approximately 5 mg of each enantiomer were weighed out in a 5 ml volumetric flask and brought to volume with 0.1 M lactic acid (pH 3.0) as vehicle. The solution was sonicated for approximately 5 min using a sonication bath (Branson model 2200) and also vortexed briefly to ensure complete dissolution, using a Thermolyne Maxi Mix II model 37600 mixer. This produced a stock solution (2 mg/ml) of racemic mixture. This stock solution was further diluted 50:50 with mobile phase modifier and used as the reference, to ensure baseline resolution of the enantiomers by SFC.

2.3. Preparation of solutions of R isomer for interconversion study

For evaluation of the effect of pH on the interconversion of R to S, the following solutions were prepared:

(1) Approximately 1 mg/ml of R isomer in 1 ml of 0.1 M lactic acid (pH 3.0).
(2) Approximately 1 mg/ml of R isomer in 1 ml of 0.05 M potassium phosphate monobasic buffer (pH 7.0).
(3) Approximately 1 mg/ml of R isomer in 1 ml of 40 g/l sodium borate buffer (pH 9.5).

The R isomer was completely soluble in pH 3.0 solution following brief sonication. The compound was only sparingly soluble at pH