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Editorial

Determination of Enantiomeric Excess in Pharmaceutical Active Agents: The Role of High Performance Liquid Chromatography

Chika J Mbah*

Department of Pharmaceutical and Medicinal Chemistry, University of Nigeria, Nsukka, Nigeria

Editorial

Chiral drugs are composed of molecules with the same chemical structure, but different 3-dimensional molecular arrangements. Chirality is defined as non-super imposable mirror images.

A pair of stereoisomer's that is non-super imposable mirror images of one another is an enantiomer and therefore has different 3-dimensional configuration. Several drugs of clinical importance are enantiomers. However, there is an increasing trend for the pharmaceutical industries to develop and market drug products containing only a single enantomer. Some of them can be new chemical entities such as salmeterol, sertraline etc. Others such as esomeprazole from omeprazole (racemate) and escitalopram from citalopram (racemate) are developed from currently marketed drugs.

The development of these single enantiomer from chiral drugs (racemates) is called chiral switching [1,2]. The rationale towards chiral switching include improved therapeutic index, decreased inter-individual variability, decreased potential for drug-drug interactions and more appropriate dosing frequency. Information on the composition of the enantiomers does assist in the development of a single enantiomer and invariably the enantiomeric excess [3]. Enantiomeric excess is used to describe the optical purity of an enantiomer. The optical purity (expressed as percentage) is a comparison of the optical rotation of a pure sample of unknown stereochemistry versus the optical rotation of a sample of pure enantiomer. The value of the enantiomeric excess can range from 0% to 100%. For instance a drug possessing a single chiral center, theoretically will be a racemic mixture consisting of 50% of the dextro-enantiomer (+) and 50% of the levo-enantiomer (-) and have zero as the enantiomeric excess. However, if a drug contains 100% of one enantiomer, then its enantiomeric excess is 100%. Determination of Enantiomeric Excess (EE), is of special importance in the control of the purity of chiral pharmaceutical active agents. A plethora of methods for the determination of Enantiomeric Excess (EE) are available [4]. They include (i) Covalent synthesis and detection of diastereomers using enantiomerically pure derivatizing agents (ii) Nuclear Magnetic Resonance (NMR) - detection of transient diastereomeric interactions using chiral shift reagents or solvating agents [5] (iii) Chromogenic host molecules - cause different UV-Visible spectral changes following interaction between a chiral host molecule and one or the other of the enantiomers of the guest (drug sample) molecule (iv) Mass spectrometry (v) Chromatography (vi) Chiroptical - based on the principle that anisotropy g, is proportional to the enantiomeric excess (vii) Fluorescence (viii) Liquid crystals - based on the principle that the helical pitch p, is inversely proportional to enantiomeric excess. The pitch is obtained when a small amount of an optically active drug molecule is added to a nematic liquid crystal, resulting in the induction of a cholesteric phase (ix) Enzymatic methods and immunoassays (x) Kinetic resolution - based on the use of stereoselectivity factor s, to obtain enantiomeric excess following kinetic resolution of a drug racemic mixture by an enantiopure reagent (xi) Polarimetry- based on the measurement of optical rotation or circular dichroism [6]. The use chromatographic methods such as High Performance Liquid Chromatography (HPLC), Gas Chromatography (GC) and Capillary Electrophoresis (CE) to evaluate enantiomeric excess have taken dominance over the other methods. Although capillary electrophoresis in chiral separation has low consumption of both analyte and chiral selector as well as lack of need for expensive chiral stationary phases (because the chiral selector is added to the buffer) however, when compared to GC and HPLC, its drawbacks such as lack of usefulness as a preparative separation tool and low detection limit restraint the use of the method to determine enantiomeric excess.

High performance liquid chromatographic and gas chromatographic methods constitute the present state of the art in analyzing enantiomers. Gas chromatography despite its high sensitivity and specificity in the determination of enantiomers, the need to have low molecular weight samples, extract the samples into organic solvents and the tendency to have samples destroyed in the detector make HPLC to become the method of choice to determine enantiomers (hence enantiomeric excess) in pharmaceutical active agents. The determination of enantiomeric excess of chiral compounds is generally done by chiral or achiral HPLC [7-9]. Chiral HPLC uses nonspecific detectors such as UV or fluorescence [10,11] while achiral HPLC

***Corresponding author:** Chika J Mbah, Department of Pharmaceutical and Medicinal Chemistry, University of Nigeria, Nsukka, Nigeria, Tel: +234 8036599955; Email: chika.mbah@unn.edu.ng; cjmbah1234@yahoo.com

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is coupled to chiroptical detectors such as Circular Dichroism (CD) or Optical Rotation (OR). Circular dichroism or optical rotation detector [12,13] allows quantification of enantiomeric excess by using anisotropy factor (g) as an analytical signal. Other detectors often coupled to HPLC in the determination of enantiomeric excess include Nuclear Magnetic Resonance (NMR), Mass Spectrometric (MS) or hybrid types such as NMR/CD, MS/CD, NMR/OR, MS/OR [14,15] respectively. Although MS does not provide chiral information, it is the detector of choice because of its high sensitivity, tolerance of impurities and potential for speed. Despite chiral specific character of circular dichroism and optical detectors, their use in HPLC has limitation because of lack of sensitivity compared with UV/fluorescence detectors. HPLC has versatile columns [5,16] consisting of different types of chiral stationary phases. Such stationary phases (chiral selectors) include but not limited to cellulose tris (3,5-dimethylphenylcarbamate), amylose tris (3,5-dimethylcarbamate), phenylcarbamylated β-cyclodextrin, cellulose tris (4-methylbenzoate), 3,4,6-Tribenzoyl-2-benzoylamino-2-deoxy-D- glucose etc. Others such as N-Acetyl-L-cysteine/o-phthalaldehyde, (S)-1-(1-naphthyl) ethyl isocyanate, (–) - Menthyl chloroformate, 2,3,4,6-Tetra-O-acetyl- β -D-glucopyranosyl isothiocyanate, (S)-N-trifluoroacetylprolyl chloridese etc. are used as a chiral derivatizing agents.

Qualitative identification and quantitative determination of enantiomers by HPLC usually require the use of pure enantiomer standards. However, in the absence of reference standard, synthesized purified enantiomers could be used to determine enantiomeric excess by employing chiral HPLC coupled with MS/CD or MS/OR detectors.

Some of the drugs that HPLC analytical technique has been used to determine their enantiomeric excess include but not limited to duloxetine (antidepressant, urinary incontinence), fluoxetine (antidepressant), ifosfamide (antiviral), labetalol (antihypertensive), atenolol (antihypertensive), ketoprofen (anti-inflammatory), fluribiprofen (anti-inflammatory), warfarin (anticoagulant), chlorthialidone (direutic), clarithromycin (macrolide antibiotic), camptothecin (antineoplastic) and rifampicin (antibacterial).

In summary, of all the methods available to determine enantiomeric excess, HPLC is the instrument of choice because of high resolution power of the stationary phase (chiral or achiral) high sensitivity and specificity of the detector (single or tandem), high accuracy and precision of the analytical technique.

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