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Review

Applications of chiral chromatography coupled with mass spectrometry in the analysis of chiral pharmaceuticals in the environment

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ABSTRACT

The aim of this paper is to introduce the subject of chirality within pharmaceuticals and its implications in environmental contamination. The paper describes contemporary techniques and stationary phases in the analysis of chiral pharmaceuticals and illicit drugs, the main focus being on liquid chromatography coupled with mass spectrometry. A critical review of the methods employed including sample collection, preparation and analysis is undertaken. Special attention is paid to the possible analytical pitfalls of chromatographic separations at enantiomeric level, which could potentially lead to erroneous measurements of enantiomers. Several applications of chiral analysis and results gained in the field are also discussed. Among them are: (i) study of fate and effects of chiral pharmaceuticals in the environment and during wastewater treatment, (ii) estimation of community-wide drugs use via newly emerging field of sewage epidemiology and (iii) usage of chiral drugs as chemical markers of water contamination with sewage. The paper also aims to identify gaps within current knowledge.

Contents

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1. Introduction

Pharmacologically active compounds (PACs) are widely regarded as emerging contaminants and many of them possess at least one stereogenic centre. The aim of this paper is to introduce the subject of chirality within PACs and its implications in environmental contamination. The paper describes contemporary techniques utilized in the analysis of chiral contaminants and provides a critical review of the methods employed and results gained in the field.

1.1. Phenomenon of chirality

Enantiomers are molecular entities which are non-superimposable mirror images. The chirality (handedness) of enantiomeric molecules is caused by the presence of one or more chiral elements (chirality axis, chirality plane, or chirality centre, e.g., asymmetric carbon atom) in the structure. The chirality and optical activity of the enantiomers is determined by their absolute configuration, i.e., the spatial arrangement of the atoms in the molecule.

1.2. Nomenclature

IUPAC approved naming system is the Cahn-Ingold and Prelog designation for four- and six-coordinate stereogenic centres, prefixed with R or S, or R_p or S_p when discussing molecules with planar chirality [1]. Dextro and Levo (+/−) may also be used to describe enantiomers where the absolute configuration may not be known or to describe the rotation of light under prescribed conditions, although the prefixes 'd' and 'l' are discouraged [1]. Readers may also encounter *E*1, *E*2, etc. in papers regarding the chromatographic separation of enantiomers. These ad hoc prefixes only indicate the elution order of the enantiomers under the presented chromatographic conditions when the Cahn-Ingold and Prelog and rotatory designations are not known.

The composition of enantiomers can be calculated and expressed in three different ways and thus data conveying this proportion should be carefully interpreted accordingly (see Eqs. (1)–(3)):

$$e.e. = 100(F_{(+)} - F_{(-)}) \quad (1)$$

where *e.e.* represents *Enantiomeric Excess* and *F* the mole fraction, this is an expression of the difference between the total weight, or mole, of each enantiomer [1], often expressed as a percentage. If the elution order is not known, *F*(+) and (−) may be substituted for *E*1 or *E*2 under defined chromatographic conditions:

$$ER = \frac{+}{-} \quad (2)$$

where *ER* represents enantiomeric ratio (the difference in the proportion of the enantiomers expressed as a ratio) [1].

$$EF = \frac{(+)}{(+) + (-)} \quad (3)$$

where *EF* represents enantiomeric fraction, (+) and (−) may be substituted with *E*1 and *E*2 if the elution order is not known.

Thus a racemate can be expressed as *e.e.* = 0%, *EF* = 0.5 or *ER* = 1. Although the enantiomeric ratio is a widely recognized way of presenting enantiomeric signature, the enantiomeric fraction is gaining in popularity due to more meaningful representation of environmental data [2].

Resolution of enantiomers is defined as "the separation of a racemate in the component enantiomers" [1]. In chromatography this is usually calculated with the use of the following equation (Eq. (4)):

$$R_s = \frac{t_{r2} - t_{r1}}{0.5(w1 + w2)} \quad (4)$$

where R_s represents resolution between two symmetrical peaks, t_{r2} the elution time of enantiomer *E*2, t_{r1} the elution time of enantiomer *E*1, *w*1 the base width of peak for *E*1 and *w*2 represents the base width of peak for *E*2. More detailed discussion on R_s calculation can be found elsewhere [3].

Accuracy and precision relies on full separation of enantiomers. Unfortunately in practice, quantification of enantiomers of chiral contaminants is often performed using partially resolved peaks. The two main techniques used to integrate partially resolved peaks are the valley drop and the deconvolution method. The most popular, the valley drop method, simply separates any non-separated areas of the peaks with a vertical line. The deconvolution method uses Gaussian based functions fitted to each peak individually. The valley drop method biases results towards a more racemate fraction, as overlapped regions from larger peaks are disproportionately ascribed to smaller peaks, particularly if tailing occurs. The deconvolution method eliminates this bias between uneven peaks and can even account for tailing if appropriate software is used [4].

1.3. Chirality in pharmaceuticals

Two enantiomers of the same compound, despite having the same physical and chemical properties, show different interactions with other chiral molecules due to differences in spatial arrangement of the atoms and therefore binding affinity. This phenomenon is particularly significant in biological interactions as all proteins, enzymes and carbohydrates are chiral [10]. Thus organisms might respond uniquely to each enantiomer, a phenomenon discovered by Pasteur in 1857 [5]. This is of particular importance in the case of chemicals such as PACs and pesticides, which are designed to illicit biological action.

Many drugs are chiral with well documented stereoselective pharmacodynamic responses in humans such as those summarized in Table 1. A drug's pharmacological action is dependent on its pharmacokinetics, i.e. absorption, distribution, metabolism and excretion. These processes require interaction with many chiral molecules within an organism. For example albumin, the major protein responsible for transporting pharmaceuticals within the blood is chiral therefore stereoselective transportation may occur. In addition metabolism may result in chiral inversion (common among anti-inflammatory drugs), or the addition of a chiral centre into an achiral parent compound (for example metabolism of cimetidine leading to the chiral metabolite, cimetidine S-oxide). Table 2 summarizes some examples of these phenomena in humans.

For a more detailed discussion see a critical review by Kasprzyk-Hordern [6].

1.4. Implications of chirality in pharmaceuticals to risk assessments and drug development

Because of the often wide ranging implications of the enantiomeric composition of a drug each enantiomer is assessed individually during drug design. In addition, the enantiomeric fraction is measured at each step throughout the patient's exposure to ascertain any chiral inversion or stereoselective or stereospecific pharmacokinetics which may take place.

However environmental risk assessments have lagged behind those required in medicine. The European Medicines Agency (EMEA) Guideline On the Environmental Risk Assessment of Medicinal Products for Human Use [19] states that estimation of exposure and the prediction of risk, Phase's 1 and 2A of the EMEA guideline, is only conducted on whole parent compounds i.e. as a racemate or as a single enantiomer if prescribed as such. This is an inappropriate risk assessment as parent compounds do not enter the environment in their original racemic or enantiomerically pure