Letter to the Editor

Selection of internal standard for quantitative analysis of enantiomers following precolumn chiral derivatization

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The employment of techniques like high-performance liquid chromatography (HPLC) and gas chromatography (GC) with associated chromatographic conditions and detection systems has enabled the enantioselective quantitative analysis of the parent entity and metabolite(s) of several racemic drug substrates. Application of enantioselective analysis has permitted delineation of pharmacokinetic/disposition characteristics of enantiomers belonging to important classes such as β-blockers, nonsteroidal antiinflammatory agents, amphetamine congeners and antiarrhythmic agents.

Similar to quantitative analysis of nonchiral drugs, the use of internal standard has been practiced for enantioselective quantitation; where quantitation is performed by regression (linear, quadratic or curve fitting) using either peak-height or peak-area detector response ratios of the enantiomer to the internal standard versus the enantiomer concentration. However, unlike a nonchiral drug, two computations of the regression equation are performed, one for each enantiomer.

Review of the literature regarding quantitative analysis of enantiomers involving precolumn chiral derivatization indicates very different approaches in the selection of the internal standard. As depicted in Table 1, the internal standards that are used for enantioselective quantitation can be classified as: (1) nonchiral internal standard; (2) single enantiomer internal standard; (3) nonreactive racemic internal standard; and (4) racemic internal standard.

The use of racemic internal standard presents a substrate stereochemically comparable to the racemic drug and thus, provides an ideal environment for the chiral derivatization reaction. Furthermore, generation of two peaks of internal standard diastereomers, as a result of chiral derivatization, provides the chromatographer with a choice for the selection of the internal standard peak for the purpose of quantitation; this may also prove advantageous because in the event of any endogenous interference with one peak of internal standard, it permits switching to the other peak.

However, the use of a racemic internal standard may also present problems. Firstly, due to differential reaction rates of internal standard enantiomers with the chiral derivatization reagent, artifactually greater formation of one diastereomer over the other may occur. Secondly, the chromatographer is faced with an additional challenge to resolve satisfactorily, the two diastereomeric peaks of the internal standard generated by the chiral derivatization reaction.

On the other hand, the use of either a nonchiral or single enantiomer internal standard does not present

Table 1
Classification of internal standards used for quantitative analysis of racemic substrates

Type	Internal standard	Racemic substrate	Matrix	Chiral derivatization	Detection	Reference
Nonchiral internal standard	Benzyl cinnamate	Flunoxaprofen	Serum urine	*S*-1-phenylethylamine	HPLC–ultraviolet	[7]
	Chlorophenetermine	Fenfluramine	plasma	*S*-heptafluorobutyrylprolyl chloride	GC–ECD	[12]
		Methylphenidate	plasma	*S*-heptafluorobutyrylprolyl chloride	GC–ECD	[11]
	Amantadine	Methoxyphenamine *N*-desmethyl metabolite	urine	*S*-heptafluorobutyrylporlyl chloride	GC–ECD	[13]
	1-Nitronapthalene	Pindolol	plasma	2,3,4,6-tetra-*O*-acetyl-β-glucopyranosyl isothio-cyanate	HPLC–ultraviolet	[5]
	4-Methoxyphenyl acetic acid	Ibuprofen	plasma	*R*-(−)-2,2,3-trifluoro-1-(9-anthryl) ethanol	GC–MS	[15]
	Aniline sulfate	Methamphetamine Amphetamine	serum	(−) fluoroenylethyl chloroformate	HPLC–fluorescence	[6]
Single enantiomer internal standard	D-Ephedrine	*O*-Desmethyl methoxy-phenamine; 5-hydroxy methoxyphenamine	urine	*S*-heptafluorobutyrylprolyl chloride	GC–ECD	[13]
	L-Tryptophan	Ritalinic acid	plasma	*S*-heptafluorobutyrylprolyl chloride	GC–ECD	[14]
	(+) Flecainide	Propranolol	plasma	(−) mentyl chloroformate	HPLC–fluorescence	[8]
	S-Cicloprolol	Betaxolol	blood	*R*-1-(1-napthyl)ethyl isocyanate	HPLC–fluorescence	[3]
Nonreactive racemic internal standard	(±) *N,N*,Bis-carvedilol	Carvedilol *O*-desmethyl carvedilol	plasma	2,3,4,6-tetra-*O*-acetyl-β-glucopyranosyl isothio cyanate	HPLC–fluorescence	[4]
Racemic internal standard	(±) Pindolol	Verapamil Norverapamil	plasma	(−) menthyl chloroformate	HPLC–fluorescence	[1]
	(±) Atenolol	Sotalol	plasma	*S*-1-(1-naphthyl)ethyl isocyanate	HPLC–fluorescence	[2]
	(±) Pronethalol	Propranolol	plasma	*S*-flunoxaprofen chloride	HPLC–fluorescence	[10]
	(±) Practolol	Atenolol	plasma	(+)-1-(9-fluoroenyl)ethyl chloroformate	HPLC–fluorescence	[9]

problems associated with different enantiomeric re-action kinetics with the chiral derivatization reagent. However, it is very important that the single enantio-mer be of the highest optical purity to avoid any degree of racemization during the chiral derivatiza-tion process. Because the reaction of either nonchiral or single enantiomer internal standard with the chiral derivatization reagent will produce a single dia-stereomer peak, the chromatography is relatively easy and the resolution may not pose a problem for the chromatographer.

Although it has equal proportions of the two enantiomers, because the nonreactive racemic inter-nal standard does not have a free functional group to

react with the chiral derivatization reagent, it fails to serve as a marker for the chiral derivatization reaction employed in the analysis.

Overall, there are many approaches for the selection of internal standard for use in quantitative analysis of drug enantiomers following chiral derivatization as enumerated by examples in the literature. Selection of any of these approaches is influenced by the nature of the racemic drug, and the complexity of biological extraction and chiral derivatization processes. However, it should be noted that the chosen internal standard should be a reliable indicator of both extraction and derivatization processes involved in the enantioselective analysis.

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