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Letter to the Editor

Isothermal capillary gas chromatography with electron-capture detection of heptafluorobutyryl-L-prolyl derivatives of chiral amphetamines

Sir,

From the beginning of this decade reports on the enantiomeric separation of drug racemates have increased in number, perhaps due to the emphasis that racemates need to be looked at as a mixture of two independent drugs with unique pharmacokinetic, pharmacodynamic and metabolic profiles [1–4]. The two well established analytical tools for chiral separations are high-performance liquid chromatography and gas chromatography (GC) [5–7]. The most commonly used GC procedure for enantiomeric separations is based on 'indirect method', where an optically pure chiral reagent is employed to convert drug enantiomers into their corresponding diastereomeric derivatives, which are then separated on an achiral stationary phase using appropriate GC conditions. Several factors need to be evaluated in the selection of a chiral reagent for the successful development of the indirect method. (i) The availability of a chiral reagent with highest optical purity from a commercial outlet must be established or, if the chiral reagent is synthesized in the laboratory, the availability of starting materials of the highest optical purity and maintenance of optical purity throughout the synthetic sequence to yield the chiral reagent; (ii) it is necessary to optimize the amount of chiral reagent added and the time for completion of the reaction; (iii) it is important to confirm that there is no racemisation during derivatisation procedure or during the chromatography; (iv) it is essential to check for the stability of the chiral reagent upon storage; and (v) to check for the reproducibility of the results from time to time.

A very useful chiral derivatising agent is heptafluoroburyryl-L-prolyl chloride (L-HPC), which must be synthesized in the laboratory [8], but which satisfies all the essential requirements of a chiral reagent listed above. Enantioselective assays of L-HPC-derivatised methylphenidate [9], fenfluramine

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TABLE I

RETENTION TIMES AND COLUMN OVEN TEMPERATURES FOR THE SEPARATION OF HEPTAFLUOROBUTYRYL-L-PROLYL DERIVATIVES OF CHIRAL AMPHETAMINES ON CAPILLARY OV-225 COLUMN

Other GC conditions were: injection port temperature, 280°C; detector temperature, 300°C, carrier gas, argon–methane; column flow-rate, 1 ml/min; auxillary flow-rate, 60 ml/min; head pressure on column, 15 p.s.i.; split vent flow-rate, 28 ml/min; septum purge flow-rate, 2 ml/min; chart speed, 0.5 cm/min. P_1 = first eluting peak; P_2 = second eluting peak.

Compound	Column oven temperature (°C)	Retention time (min)	
		P_1	P_2
dl-Norfenfluramine	190	10.77 (*l*)	11.54 (*d*)
dl-Fenfluramine	190	11.85 (*l*)	13.55 (*d*)
dl-Amphetamine	200	6.54 (*l*)	7.24 (*d*)
dl-Methamphetamine	200	8.67 (*l*)	9.21 (*d*)
dl-N-Desmethylmethoxyphenamine	220	6.99	7.63
dl-Methoxyphenamine	220	9.10	9.94
dl-*p*-Methoxyamphetamine	230	4.81	5.32
dl-3,4-Dimethoxyamphetamine	230	7.92	8.88
dl-Methoxamine	240	6.93	7.64
dl-Methylphenidate	250	4.53 (*d*)	6.17 (*l*)
dl-Norephedrine	250	6.60	7.47
dl-Ephedrine	250	7.57 (*d*)	8.24 (*l*)

[10], norfenfluramine [10] and methoxyphenamine [11] performed previously in our laboratories have indicated both satisfactory chromatography and excellent electron-capture detection (ECD) response of the diastereomeric amide derivatives of the various analytes. It has been shown that chiral derivatisation was complete after reaction times of 30–60 min for various analytes. Since chiral derivatisation with L-HPC is carried out in an aqueous–alkaline condition (pH 9.5), any excess of the chiral reagent is destroyed and thus will not interfere in the chromatography.

To confirm the general applicability of L-HPC as a chiral reagent, several racemic amphetamines were derivatised with L-HPC in aqueous–alkaline condition (pH 9.5). After a reaction time of 45 min, the diastereomers of the various amphetamines were extracted with *n*-pentane. The residue obtained after the evaporation (65°C) of *n*-pentane was taken up in ethyl acetate. Suitable aliquots were injected into the GC–ECD system using an appropriate isothermal column oven temperature. The chromatography of the diastereomeric pairs of peaks of the various analytes showed satisfactory resolution. The retention time for each diastereomeric pair of peaks and details of GC conditions are provided in Table I.

In conclusion, the results of our study confirm the versatility and reliability

of L-HPC in its use as a chiral reagent. Thus L-HPC can be readily employed as a chiral reagent for enantiomeric disposition studies of chiral amphetamines in humans and animals.

College of Pharmacy,
University of Saskatchewan,
Saskatoon,
Saskatchewan S7N 0W0 (Canada)

NUGGEHALLY R. SRINIVAS
JOHN K. COOPER
JOHN W. HUBBARD*
KAMAL K. MIDHA

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