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Note

Separation of optical isomers of methoxyphenamine and its metabolites as N-heptafluorobutyryl-L-prolyl derivatives by fused-silica capillary gas chromatography

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Methoxyphenamine is a β_2-adrenergic agonist which is used clinically in the treatment of asthma and other allergic conditions. It is metabolized in man and animals via O-dealkylation, N-dealkylation and aromatic hydroxylation [1]. Genetic polymorphism in the metabolism of methoxyphenamine is well documented [2,3]. It has been reported that O-demethylation and aromatic hydroxylation of methoxyphenamine are deficient in those subjects who have been phenotyped as poor metabolizers of debrisoquine [2]. Therefore, the relatively innocuous methoxyphenamine appears to be suitable as a multi-metabolic pathway genetic probe for assessing polymorphism in biotransformation of xenobiotics [4].

Methoxyphenamine is a chiral drug. Interestingly, the major metabolites of this drug, namely 2-hydroxymethamphetamine, 2-methoxyamphetamine and 2-methoxy-5-hydroxymethamphetamine are also optically active since the chiral center of the parent compound was retained in each case. Racemic methoxyphenamine and its metabolites were previously quantitated in biological fluids by gas chromatography (GC) using either electron-capture or nitrogen-selective detection [5]. However, to the best of our knowledge, no literature reports are available on the enantioselective assay of these compounds in biological fluids.

In this paper, we applied the previously reported aqueous derivatization technique with N-heptafluorobutyryl-L-prolyl chloride (HPC) [6] to the analysis of methoxyphenamine and its three metabolites. The diastereomers are then resolved using fused-silica capillary GC with nitrogen-selective detection.

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EXPERIMENTAL

Materials

Methoxyphenamine hydrochloride was generously donated by Upjohn Canada (Don Mills, Canada). 2-Hydroxymethamphetamine, 2-methoxyamphetamine and 2-methoxy-5-hydroxymethamphetamine were synthesized according to previously reported procedure [1]. HPC was prepared according to the procedure of Lim et al. [6]. All glassware used for sample preparation was silanized with 5% dichlorodimethylisilazane in toluene.

Apparatus

A Hewlett-Packard Model 5790A gas chromatograph equipped with a nitrogen–phosphorus detector was used. The GC separation was achieved using a 15 m × 0.25 mm I.D., 0.1-μm cross-linked dimethyl silicone fused-silica capillary column (Hewlett-Packard, NJ, U.S.A.).

Chromatographic conditions

Flow-rates of carrier gas and make-up gas (helium) were 1.5 and 60 ml/min, respectively. Oven temperature was held at 150°C for 1 min following injection and then programmed to 260°C at a rate of 10°C/min and held for 20 min at the final temperature. Split vent and septum vent flow-rates were 10 and 2 ml/min, respectively.

Sample preparation

The parent drug and its three metabolites were extracted from urine following adjustment to pH 9.2 using ethyl acetate. Following centrifugation the organic phase was transferred to another screw-cap test tube. Methanol saturated with hydrochloric acid (10 μl) was added and evaporation was carried out in a dry bath at 60°C under a gentle stream of nitrogen. Then 1 ml of $NaHCO_3$–Na_2CO_3 (7:3, 5%, w/v) buffer was added to the dried residue and allowed to stand in an ice-bath for another 15 min. HPC (10 μl) was added to the sample and vortexing was carried out at 1700 rpm for 10 min. The sample was then allowed to stand in ice-bath for 20 min. Cyclohexane (5 ml) was added and extraction of diastereomers was carried out by vortexing at 1600 rpm for 10 min. After centrifugation, the organic layer was transferred to another test tube. Evaporation of solvent was carried out under nitrogen at 75°C. The dried residue was reconstituted in 100 μl of ethyl acetate and an aliquot of 2 μl was injected into the capillary gas chromatograph.

RESULTS AND DISCUSSION

The chiral reagent, HPC, has been used for resolution of racemic amines [6–8] by formation of diastereomers. These diastereomers have different physical and chemical properties and, therefore, they can then be resolved using an appropriate achiral GC stationary phase [8]. The optical purity of HPC was determined by GC separation of the diastereomers formed by reaction of HPC with

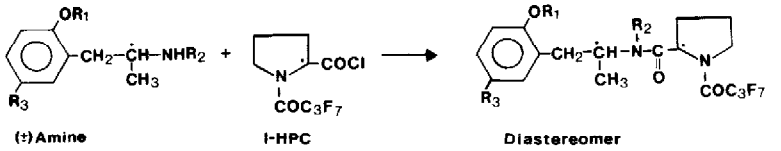

Fig. 1. Reaction of methoxyphenamine and its three metabolites with HPC to form diastereomeric pairs.

Compound	R_1	R_2	R_3
Methoxyphenamine	CH_3	CH_3	H
2-Hydroxymethamphetamine	H	CH_3	H
2-Methoxyamphetamine	CH_3	H	H
2-Methoxy-5-hydroxymethamphetamine	CH_3	CH_3	OH

optically pure enantiomers and racemic α-methylbenzylamine [6]. The optical purity of HPC (99%) was similar to that published previously [6].

The reaction of racemic methoxyphenamine, 2-hydroxymethamphetamine, 2-methoxyamphetamine and 2-methoxy-5-hydroxymethamphetamine with HPC to form the corresponding diastereomeric pairs are shown in Fig. 1. N-Monosubstituted derivatives were obtained with methoxyphenamine and 2-methoxyamphetamine. Formation of N-monosubstituted derivatives was established by reacting the individual racemic compound with HPC and the final product was analyzed by GC. As expected racemic methoxyphenamine and 2-methoxyamphetamine were resolved into their enantiomeric pair. However, derivatization of individual synthetic phenolic amine metabolites of methoxyphenamine with HPC resulted in mono- and diderivatives. Formation of mono- and diderivatives has also been observed following derivatization of these phenolic amine metabolites with pentafluorobenzoyl chloride [5]. The structure of the mono- and diderivatives of N- and N,O-pentafluorobenzamide of these phenolic amines were confirmed by GC–mass spectroscopy [5]. For these pentafluorobenzamides, the monoderivatives eluted earlier than the diderivatives. Although no mass spectral data are available for these HPC derivatives of the phenolic amines, similar elution order is expected (Fig. 2B). The mono- and diderivatives were then resolved into their enantiomeric pair (Fig. 2B).

The resolution of the diastereomeric pairs of methoxyphenamine and its metabolites by GC are summarized in Table I. The separation of two chromatographic peaks can be inferred from the resolution factor (R) which is defined as follows [9]:

$$R = 2\frac{t_{R(1)} - t_{R(2)}}{W_{B(1)} - W_{B(2)}}$$

where $t_{R(1)}$ and $t_{R(2)}$ are the retention times and $W_{B(1)}$ and $W_{B(2)}$ are the peak widths of each isomer. Accordingly, baseline resolution (100%) of a peak is achieved when R is 1.5 as compared to only 98% resolution of the peaks when R

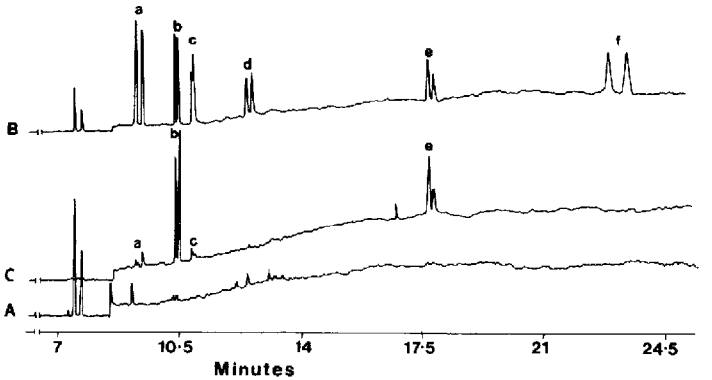

Fig. 2. Enantiomeric resolution of methoxyphenamine and its three metabolites after derivatization with HPC. (A) Extract from blank urine; (B) extract from urine spiked with racemic methoxyphenamine and its three metabolites; (C) extract from 12-h unhydrolyzed urine from a volunteer dosed with 60.3 mg of methoxyphenamine hydrochloride. Peaks are HPC derivatives of (±) 2-methoxyamphetamine (a), (±) methoxyphenamine (b), (±) 2-hydroxymethamphetamine monoderivative (c), (±) 2-hydroxymethamphetamine diderivative (e), (±) 2-methoxy-5-hydroxymethamphetamine monoderivative (d) and 2-methoxy-5-hydroxymethamphetamine diderivative (f).

TABLE I

RESOLUTION FACTORS FOR DIASTEREOMERS OBTAINED ON A 15 m×0.25 mm I.D. DIMETHYL SILICONE CAPILLARY COLUMN

Compound*	Retention times (min)		Resolution factor
HPC(±)NDMP	9.17	9.37	1.62
HPC(±)MP	10.32	10.42	1.16
HPC(±)ODMP (monoderivative)	10.83	10.86	0.35
HPC(±)ODMP (diderivative)	17.63	17.80	1.41
HPC(±)5HMP (monoderivative)	12.37	12.54	1.14
HPC(±)5HMP (diderivative)	22.86	23.41	2.10

*MP = methoxyphenamine; NDMP = 2-methoxyamphetamine; ODMP = 2-hydroxymethamphetamine; 5HMP = 2-methoxy-5-hydroxymethamphetamine.

equals 1.0. Except for the monoderivative of 2-hydroxymethamphetamine, almost baseline resolution was obtained for the rest of the compounds.

Fig. 2A is a chromatogram of an extract of blank urine. No extraneous peaks with retention times similar to the peaks of interest were observed. Fig. 2C shows a typical chromatogram of extract of 12-h unhydrolyzed urine obtained from a volunteer following oral administration of 60.3 mg of methoxyphenamine hydrochloride. Present in the extract of unhydrolyzed urine are methoxyphenamine, 2-methoxyamphetamine and 2-hydroxymethamphetamine. The absence of 2-methoxy-5-hydroxymethamphetamine in the urine extract clearly suggests that this metabolite is predominantly excreted as conjugates (glucuronide and/or sulfate) which is in agreement with earlier reports [2,3].

TABLE II

ENANTIOMERIC RATIOS OF METHOXYPHENAMINE AND ITS THREE METABOLITES IN STANDARD AND DOSED URINE SAMPLES

Peak 1 is earlier eluting peak, peak 2 is later eluting peak, ratio is peak 1/peak 2. N.D. = not detected.

Compound*	Standard sample			Dosed urine sample		
	Peak 1 (cm^2)	Peak 2 (cm^2)	Ratio	Peak 1 (cm^2)	Peak 2 (cm^2)	Ratio
MP	1.48	1.45	1.02	2.40	2.99	0.80
NDMP	2.86	2.63	1.09	0.23	0.37	0.61
ODMP (monoderivative)	1.03	1.42	0.72	-	-	-
ODMP (diderivative)	0.84	0.65	1.29	1.43	0.55	2.59
5HMP (monoderivative)	0.85	0.65	0.92	N.D.	N.D.	N.D.
5HMP (diderivative)	2.50	2.25	1.11	N.D.	N.D.	N.D.

*For abbreviations, see Table I.

The peak-area ratios of each enantiomeric pairs of methoxyphenamine and its three metabolites in spiked and dosed urine are summarized in Table II. Enantiomeric peak-area ratios corresponding to methoxyphenamine, 2-methoxyamphetamine and 2-methoxy-5-hydroxymethamphetamine are close to unity, thereby suggesting that kinetic racemic resolution for these compounds is minimum. However, extensive kinetic racemic resolution was observed for 2-hydroxymethamphetamine derivatives (Table II). Deviation of the enantiomeric ratios from unity for methoxyphenamine and metabolites in dosed urine suggests that methoxyphenamine undergoes stereoselective disposition in man. In view of the unavailability of pure enantiomers of methoxyphenamine and its metabolites, the elution order of these enantiomeric pairs could perhaps be predicted on the basis of correlation between absolute configuration and elution order of amphetamine and analogues possessing only an α asymmetric carbon [10–13], that is, the *l*-antipode elutes earlier than the *d*-enantiomer.

CONCLUSION

The reported aqueous derivatization procedure for chiral amine with HPC by Lim et al. [6] was found to be suitable for derivatization of chiral phenolic amines. Methoxyphenamine undergoes stereoselective disposition in man. A possible application of such an enantioselective assay is in the investigation of the phenotype-dependent stereoselective disposition of methoxyphenamine in extensive and poor metabilizers of debrisoquine.

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