Journal of Chromatography, 310 (1984) 307—317
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 2198

GAS CHROMATOGRAPHIC QUANTITATION OF METHOXYPHENAMINE AND THREE OF ITS METABOLITES IN PLASMA

S.D. ROY, G. McKAY, E.M. HAWES and K.K. MIDHA*

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

(First received January 24th, 1984; revised manuscript received May 24th, 1984)

SUMMARY

Sensitive gas chromatographic procedures for the determination of methoxyphenamine and three of its metabolites in plasma have been developed. The metabolites were measured using an electron-capture detector. This simple procedure is based on the precipitation of protein from a 1-ml plasma sample with 10% trichloroacetic acid, followed by aqueous derivatization with pentafluorobenzoyl chloride at pH 9.2 and a single-step cyclohexane extraction. The lower limit of detection for the N-desmethyl, O-desmethyl and aromatic 5-hydroxy metabolites of methoxyphenamine were 1.6, 3.1 and 2.2 ng ml^{-1}, respectively, with coefficients of variation less than 10%. The poor electron-capture response of fluorinated derivatives of methoxyphenamine necessitated the use of nitrogen—phosphorus detection. Extractive derivatization with pentafluorobenzoyl chloride, without the need for protein precipitation, enabled quantitation of methoxyphenamine down to 3.8 ng ml^{-1} from a 2-ml aliquot of plasma. In a pilot study involving healthy volunteers who received a single oral dose of methoxyphenamine hydrochloride plasma concentration could be followed in all three subjects for at least 24, 32, 12 and 4 h for methoxyphenamine and the O-desmethyl, 5-hydroxy and N-desmethyl metabolites, respectively.

INTRODUCTION

Methoxyphenamine [MP, 1-(2-methoxyphenyl)-2-methylaminopropane] is a β_2 stimulant used clinically in the treatment of asthma and other allergic conditions. It is metabolized in man and animals by at least three distinctly different metabolic pathways [1—4], which include N-desmethylation, O-desmethylation and hydroxylation at the 5-position of the benzene ring [4]. Inter-individual and species differences in the excretion of metabolites resulting from O-desmethylation and/or N-desmethylation have been observed in a preliminary report [3]. These differences were based on urine analysis by gas

0378-4347/84/$03.00

chromatography (GC) with a flame-ionization detector [5]. In this method, MP and N-desmethylmethoxyphenamine (NDMP), O-desmethylmethoxyphenamine (ODMP) and N,O-didesmethylmethoxyphenamine were analyzed as their trifluoroacetyl derivatives following extraction of the urine samples. However, 5-hydroxymethoxyphenamine (5HMP) has not been quantitated in any of the studies reported so far.

In order to investigate the pharmacokinetics of MP, GC assays for the quantitative determination of MP and its three primary metabolites in plasma were developed. These methods, which are described here, require a 2-ml plasma sample for MP analysis and a 1-ml plasma sample for the simultaneous determination of the three metabolites. The methods are rapid and have adequate sensitivity to determine MP, ODMP, NDMP and 5HMP at concentrations of 3.8, 3.1, 1.6 and 2.2 ng ml^{-1} in plasma, respectively, with coefficients of variation less than 10%.

EXPERIMENTAL

Materials

Pentafluorobenzoyl chloride (PFBCl), distilled cyclohexane, triethanolamine and ethyl acetate were analytical grade. MP hydrochloride was generously donated by Upjohn Canada (Don Mills, Canada).

ODMP, NDMP and 5HMP were synthesized in these laboratories [4]. Phenethylamine (PA) and 4-methoxyphenethylamine (4MPA) (Aldrich, Montreal, Canada) were used as the internal standards for GC using nitrogen—phosphorus detection (NPD) and GC using electron-capture detection (ECD), respectively. All other solvents and chemicals were analytical grade and used without further purification. All glassware used for sample preparation were silanized. The cyclohexane—triethanolamine (CH—TEA) extraction solvent was prepared by refluxing cyclohexane with small amounts of triethanolamine for 2 h, cooling and separating the two phases [6].

The ammonium buffer was a saturated ammonium chloride solution adjusted to pH 9.4 with undiluted ammonia.

Instrumentation and chromatographic conditions

A Hewlett-Packard Model 5840A gas chromatograph equipped with a nitrogen—phosphorus detector was used for the analysis of MP. The silanized glass column, 122 × 0.2 cm I.D., was packed with 3% OV-225 on Gas-Chrom W (100—120 mesh, DMCS-washed). The column was conditioned for 24 h at 285°C at a low helium flow-rate. Temperature was programmed from 185°C, 3.5 min hold-time, to 235°C at a rate of 15°C min^{-1}. The injection port and the detector temperature were maintained at 280°C and 300°C, respectively. The helium gas flow-rate was 30 ml min^{-1}.

For the analysis of metabolites, a Hewlett-Packard Model 5790A gas chromatograph equipped with a ^{63}Ni detector was used. The chromatographic conditions using the above 3% OV-225 column were: injection port temperature 300°C; detector temperature 300°C; column oven programmed from 210°C, 2.1 min hold-time, to a final temperature of 285°C for 2.65 min at 20°C min^{-1}. The carrier gas (argon—methane, 95:5, v/v) flow-rate was 35 ml min^{-1}.

The gas chromatographic—mass spectrometric (GC—MS) analysis was carried out on a V.G. Micromass 7070E mass spectrometer interfaced via a single-stage glass-jet separator to a Hewlett-Packard 5790A gas chromatograph operated under the same conditions as described above. The instrument was operated in the electron-impact (EI) mode at 70 eV and emission current of 200 μA. The GC—MS interface and ion source were held constant at 280°C and 220°C, respectively. Data was collected using a V.G. 2025 data system.

Internal standards

Stock solutions of PA hydrochloride (50 μg ml^{-1} as the free base) and 4MPA hydrochloride (50 μg ml^{-1} as the free base) were prepared every six months in distilled deionized water and stored at 4°C.

Preparation of standard curves

Stock solutions [MP (16 μg ml^{-1}), ODMP (8 μg ml^{-1}), NDMP (8 μg ml^{-1}) and 5HMP (5.8 μg ml^{-1})] were made every six months in distilled deionized water and stored at 4°C. For the preparation of standard curves, appropriate dilutions of these solutions were mixed in distilled deionized water and the appropriate volumes added to 1 or 2 ml of fresh blank plasma for metabolites and MP, respectively. These standard plasma samples for MP and metabolite determinations were then treated in an identical fashion as unknown samples.

Plasma level study

Three healthy male volunteers weighing 65, 70 and 78 kg were fasted overnight and then orally administered 60.3 mg of MP hydrochloride (extemporaneously prepared capsule) with 250 ml of water. Blood samples were drawn at 0, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 12, 24, 28 and 32 h in heparinized evacuated tubes (Vacutainers, Becton and Dickinson, Mississauga, Canada), centrifuged (TJ6 centrifuge, Beckman Instruments, Toronto, Canada) and the separated plasma was stored at −20°C until analysis. During collection of the venous samples, care was taken to avoid contact of the blood with the rubber stoppers of the evacuated tubes.

Extraction and derivative formation

Methoxyphenamine determination in plasma: extractive pentafluorobenzoylation. To a 10-ml PTFE-lined screw-capped test tube were added in turn a 2-ml plasma sample, either spiked or from a MP-dosed volunteer, 20 μl of internal standard solution (6.25 μg ml^{-1}) containing 125 ng of PA, 200 μl of NH_4^+/NH_4OH buffer, 5 ml of CH—TEA and 10 μl of PFBCl (0.5% in cyclohexane). The tube was tightly capped and mixed (Evapomix Fisher Scientific, Edmonton, Canada) for 10 min and centrifuged (Fisher Safety Centrifuge, Fisher Scientific) at 400 *g* for 3 min. The organic phase was transferred to another tube and evaporated under nitrogen at 65°C in a dry bath. The residue was reconstituted in 50 μl of ethyl acetate. Aliquots of 1 μl were analyzed by GC—NPD.

Metabolite determination in plasma: pentafluorobenzoylation in aqueous media. To a 1-ml plasma sample either spiked or from a dosed volunteer were added 20 μl of internal standard solution (2.5 μg ml^{-1}) containing 50 ng of

4MPA, 1 ml water and 1 ml of 10% trichloroacetic acid to precipitate the plasma proteins. The 10-ml tube was tightly capped and centrifuged (TJ6 centrifuge) at 1720 *g* for 10 min. The supernatant was transferred to another 10-ml tube and added sequentially were 300 μl of saturated sodium carbonate solution to neutralize the trichloroacetic acid, 1 ml of 10% sodium bicarbonate solution and 1 μl of PFBCl. The tube was shaken vigorously for 5 min and allowed to stand for 30 min at room temperature. The aqueous layer was extracted with 5 ml of cyclohexane by mixing on an Evapomix for 10 min followed by centrifugation at 400 *g* for 3 min. The organic phase was transferred to a clean tube and evaporated in a dry bath under nitrogen. The dried residue was reconstituted with 50 μl of anhydrous ethyl acetate by mixing on a Vortex mixer for 5 sec. Aliquots (1 μl) of the resulting solution were analyzed by GC—ECD.

Recovery study

For the determination of recovery, four replicate samples at levels of 120, 100, 50, 5, 50 and 125 ng ml^{-1} for MP, ODMP, 5HMP, NDMP, 4MPA and PA, respectively, were spiked in fresh blank plasma and run through the appropriate procedure as described above. The external standard method was employed and the peak height ratios obtained for the extracted samples were compared with those of fresh standards prepared in organic solvents.

Temperature dependence study

MP (400 ng) and each of its metabolites (80 ng) were derivatized with PFBCl as described above. These benzoyl derivatives were analyzed with the column oven temperature programmed from 200°C to 270°C at a rate of 12°C min^{-1}. The detector temperature was varied at 25°C installments from 150°C to 300°C. The effect of detector temperature on the ECD response of each compound at a fixed concentration was examined by plotting the peak area against detector temperature.

RESULTS AND DISCUSSION

N-Pentafluorobenzoyl derivatives of primary and secondary alkylamine drugs and doping agents have been prepared for their sensitive analysis by GC—ECD [6—11]. Pentafluorobenzoylation not only facilitates gas chromatography, it also provides electron-capturing ability to these amines, which is generally much greater to that obtained with derivatizing agents such as trifluoroacetic anhydride, pentafluoropropionic anhydride and heptafluorobutyric anhydride [7—9]. Therefore, for the sensitive analysis of MP and three of its metabolites, N- and N,O-pentafluorobenzoyl derivatives were prepared. Generally, amine compounds are extracted from biological fluids with organic solvents before acylation. More recently extractive acylation, especially benzoylation with PFBCl, has been found to be a very efficient method for the analysis of doping agents [6], because it combines extraction and derivatization in one step. This extractive pentafluorobenzoylation, when applied to the GC—ECD or GC—NPD analysis of MP, gave recoveries of 99.9% from plasma (Table I) with no interferences from endogenous plasma constituents. The metabolites

TABLE I

RECOVERY OF METHOXYPHENAMINE, METHOXYPHENAMINE METABOLITES, AND THE INTERNAL STANDARDS FROM PLASMA

Compound added	Amount added to 1 ml plasma (ng)	Amount recovered (ng)	*n*	Percentage recovery (mean ± S.D.)
Methoxyphenamine	120	119.9	4	99.9 ± 2.6
Phenethylamine	125	89.87	4	71.9 ± 1.2
O-Desmethylmethoxyphenamine	100	65.80	4	65.8 ± 3.6
5-Hydroxymethoxyphenamine	50	29.65	4	59.3 ± 1.9
N-Desmethylmethoxyphenamine	5	2.53	4	50.7 ± 3.4
4-Methoxyphenethylamine	50	45.50	5	91.0 ± 3.0

of MP were analyzed by adapting an aqueous derivatization procedure [12] in which the pentafluorobenzoyl derivatives of phenols are formed in aqueous medium by the addition of sodium bicarbonate and PFBCl. These derivatives can then be conveniently extracted in high yields with organic solvents. This overcomes the problems associated with the poor recoveries of phenolic amines from biological medium. However, in the analysis of MP metabolites in plasma, the aqueous O- and N-derivatization was performed after the precipitation of plasma proteins with 10% trichloroacetic acid. In the presence of plasma proteins, the phenolic amines did not react efficiently with PFBCl. This protein precipitation improved the recoveries about four-fold as compared to when protein precipitation was not carried out. Aqueous derivatization was found to be highly pH-dependent. Therefore, the control of pH was important in order to have reproducible recoveries of the derivatized phenolic amines. Pentafluorobenzoylation of phenols is known to be facilitated above pH 9, therefore, in the present study, after protein precipitation, derivatization in an aqueous medium was carried out at around pH 9.4 in the presence of sodium bicarbonate.

Formation of N- and O-pentafluorobenzoyl derivatives was checked by GC—MS (Table II) under the conditions described in Experimental; MP and its N-desmethyl metabolite were both converted into their N-monoacyl deivatives, while ODMP and 5HMP yielded N,O-dipentafluorobenzoyl derivatives. Each of these derivatives gave a mass spectrum with the appropriate molecular ions and diagnostic ion. Rationalization of these diagnostic ions in terms of the structures of the N- and N,O-pentafluorobenzoyl derivatives of MP, NDMP, ODMP and 5HMP can be readily elucidated from literature reports [4, 6, 10, 11].

Detector temperature was observed to have significant effect on the electron-capturing abilities of the PFB derivatives of MP and the three metabolites. The PFB derivative of MP showed maximum electron-capturing response at a temperature of 150°C (Fig. 1) and this response decreased considerably as the detector temperature was raised, especially at temperatures of 200°C and above. However, the PFB derivatives of NDMP, ODMP and 5HMP each showed one maximum in the range 175—200°C and, after a minimum in the range 225—250°C, a subsequent rise in their ECD response up to the maximum

TABLE II

TABULATION OF PRINCIPAL FRAGMENTATION PATHWAYS OF PFB DERIVATIVES OF METHOXYPHENAMINE AND ITS THREE METABOLITES

Methoxyphenamine	$M^{+\cdot}$ 373 (0.2)	M−121$^+$ 252 (53)	M−178$^+$ 195 (100)	M−206$^+$ 167 (9)	M−225$^+$ 148 (47)	M−282$^+$ 91 (19)	
N-Desmethylmethoxyphenamine	$M^{+\cdot}$ 359 (0.7)	M−121$^+$ 238 (21)	M−164$^+$ 195 (100)	M−192$^+$ 167 (12)	M−211$^+$ 148 (97)	M−268$^+$ 91 (42)	
O-Desmethylmethoxyphenamine	$M^{+\cdot}$ 553 (0.1)	M−225$^+$ 328 (0.8)	M−301$^+$ 252 (100)	M−358$^+$ 195 (100)	M−386$^+$ 167 (15)	M−436$^+$ 117 (4)	M−462$^+$ 91 (1)
5-Hydroxymethoxyphenamine	$M^{+\cdot}$ 583 (1)	M−225$^+$ 358 (10)	M−331$^+$ 252 (68)	M−388$^+$ 195 (100)	M−416$^+$ 167 (15)	M−466$^+$ 117 (3)	M−492$^+$ 91 (2)

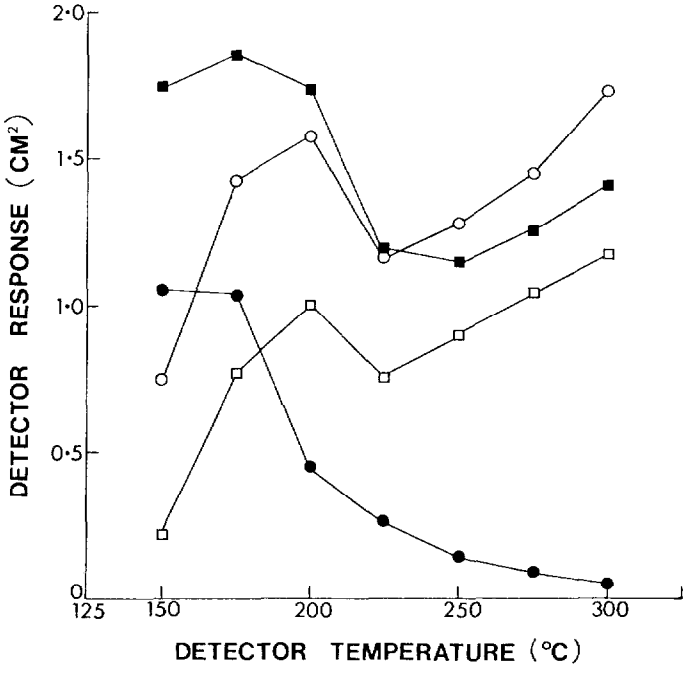

Fig. 1. Relationship between ECD response and detector temperature for methoxyphenamine PFB (•), N-desmethylmethoxyphenamine PFB (▪), O-desmethylmethoxyphenamine di-PFB (○) and 5-hydroxymethoxyphenamine di-PFB (□).

examined temperature of 300°C. In each case both the maximum and the subsequent rise occurred at temperatures where the MP derivative had negligible ECD response. Due to the significant reduction in ECD response of the MP derivative at detector temperatures above 150°C, it became necessary to analyze MP separately from its metabolites, and hence, a GC—NPD assay was developed for the analysis of MP from plasma. The phenomenon of a lower ECD response at higher detector temperatures for secondary amine compounds lacking other derivatizable functional groups as compared to their analogous primary amine derivatives, has also been observed for other secondary amines such as ethylamphetamine [11] and fenfluramine [9].

N- and N,O-pentafluorobenzoyl derivatives of MP, NDMP, ODMP, 5HMP and the internal standards phenethylamine and 4-methoxyphenethylamine gave sharp symmetrical peaks. Fig. 2A shows a typical chromatogram obtained by processing control blank plasma, without the internal standard, through the GC—ECD assay. The extraneous peak observed at a retention time of 7.8 min did not interfere with the assay as it elutes after the peaks due to the derivatives of the metabolites and the internal standard. A chromatogram obtained when the GC—ECD method was applied to spiked human plasma (1 ml) containing 25 ng ml^{-1} each of NDMP, ODMP, 5HMP and 50 ng ml^{-1} internal

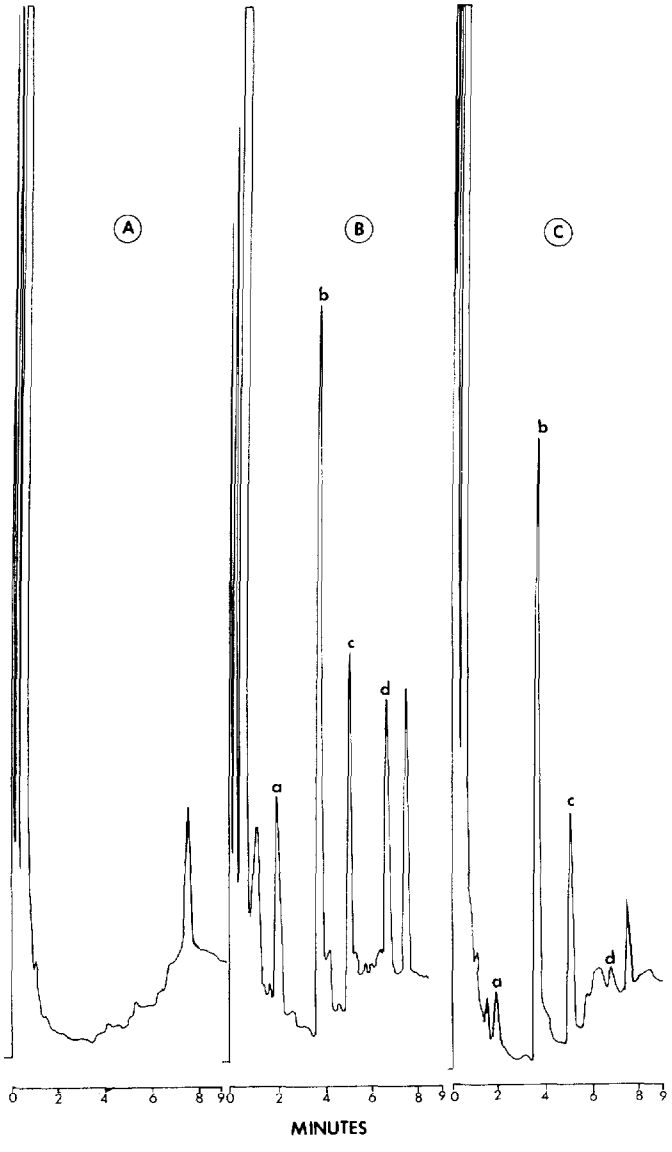

Fig. 2. Electron-capture chromatograms of extracts from 1 ml of plasma. A, Blank plasma; B, plasma spiked with N-desmethylmethoxyphenamine (a), O-desmethylmethoxyphenamine (c), 5-hydroxymethoxyphenamine (d) and internal standard (b); C, plasma sample from a volunteer 4 h after oral administration of methoxyphenamine hydrochloride.

standard (4 MPA) is shown in Fig. 2B. The chromatogram of the extract from a plasma sample (1 ml) from blood withdrawn from a male volunteer (78 kg) at 4 h after oral administration of 60.3 mg of MP · HCl is shown in Fig. 2C. GC analysis time was 9.0 min and 2.4, 22.3 and 2.6 ng ml^{-1} NDMP, ODMP and 5HMP, respectively, were estimated in this sample.

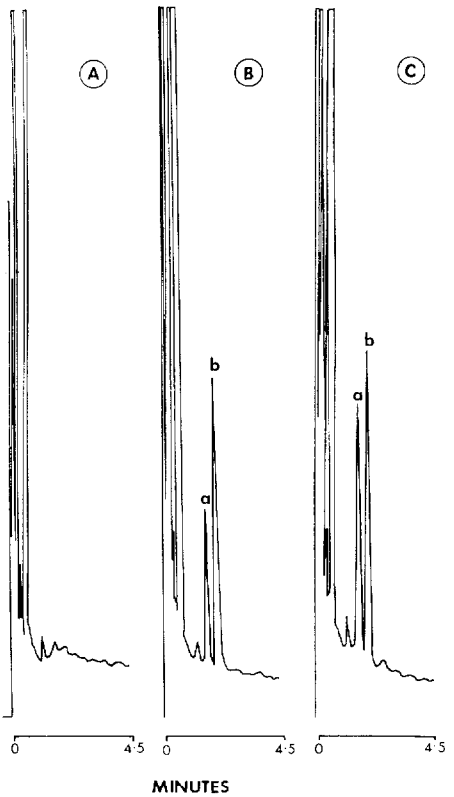

Fig. 3. Chromatograms with nitrogen-selective detection of extracts from 2 ml of plasma. A, Blank plasma; B, plasma spiked with methoxyphenamine (a) and internal standard (b); C, plasma sample from a volunteer 4 h after oral administration of methoxyphenamine hydrochloride.

Fig. 3A shows a typical chromatogram obtained by processing control blank plasma, without the internal standard, through the GC—NPD assay of unmetabolized MP. There were no endogenous peaks which interfered with the peaks due to the N-pentafluorobenzoyl derivatives of MP (t_R 2.4 min) and the internal standard PA (t_R 2.8 min). Also shown (Fig. 3B) is a chromatogram obtained when the GC—NPD method was applied to spiked human plasma (2 ml) containing 60 ng ml^{-1} MP and 125 ng ml^{-1} PA. Fig. 3C shows a GC—NPD chromatogram of a plasma sample (2 ml) from blood withdrawn from a healthy male volunteer (70 kg) 1 h after oral administration of 60.3 mg of MP · HCl. GC analysis time was less than 4.0 min and 89 ng ml^{-1} MP was found in this sample.

Column selection was found to be critical for separation of the N-pentafluorobenzamides of MP and NDMP. Initial attempts to resolve these

derivatives on non-polar to polar columns such as OV-1, OV-17, OV-25 and OV-210 were unsuccessful. These two derivatives could only be separated on the polar stationary phase OV-225, which was thermally stable with minimal drift [13] in the GC—ECD analysis of the metabolites at column oven temperatures of 210—285°C. However, this drift of baseline became more significant in the GC—NPD analysis of MP, although the column oven temperatures were lower than those used in the metabolite assay by GC—ECD. This problem of baseline drift, which may be due to bleed from a cyano column contaminating the nitrogen—phosphorus detector, was overcome by preconditioning the column just before analysis at 285°C for 6 h with a low flow of helium.

TABLE III

CALIBRATION CURVE DATA FOR METHOXYPHENAMINE GC—NPD

Concentration (ng ml^{-1})	n	Mean peak height ratio	S.D.	C.V. (%)
3.75	3	0.036	0.002	5.7
7.5	7	0.073	0.005	6.4
15	7	0.145	0.007	5.3
30	7	0.292	0.014	5.3
60	7	0.584	0.027	4.9
120	7	1.122	0.053	4.7

$Y = 0.0093X - 0.006$ ($r^2 = 0.99$)

TABLE IV

CALIBRATION CURVE FOR O-DESMETHYLMETHOXYPHENAMINE GC—ECD

Concentration (ng ml^{-1})	n	Mean peak height ratio	S.D.	C.V. (%)
3.125	5	0.056	0.005	8.9
6.25	5	0.107	0.003	2.8
12.5	5	0.198	0.019	9.7
25	5	0.364	0.024	6.6
50	5	0.683	0.036	5.3
100	5	1.350	0.076	6.1

$Y = 0.0132X + 0.024$ ($r^2 = 0.99$)

TABLE V

CALIBRATION CURVE DATA FOR N-DESMETHYLMETHOXYPHENAMINE GC—ECD

Concentration (ng ml^{-1})	n	Mean peak height ratio	S.D.	C.V. (%)
1.563	4	0.0289	0.008	2.7
3.125	5	0.0433	0.003	6.7
6.25	5	0.0830	0.002	3.3
12.5	5	0.1644	0.010	6.1
25	5	0.3364	0.020	6.0

$Y = 0.0132X + 0.003$ ($r^2 = 0.99$)

TABLE VI

CALIBRATION CURVE DATA FOR 5-HYDROXYMETHOXYPHENAMINE GC—ECD

Concentration (ng ml^{-1})	n	Mean peak height ratio	S.D.	C.V. (%)
2.22	4	0.0550	0.002	3.3
4.44	4	0.1096	0.008	7.0
8.88	4	0.2000	0.004	2.2
17.76	4	0.3775	0.010	2.6

$Y = 0.0205X + 0.019$ ($r^2 = 0.99$)

The accuracy and preccision of the GC—NPD assay of MP are demonstrated in Table III. Tables IV—VI show the accuracy and precision for the GC—ECD assays of ODMP, NDMP and 5HMP, respectively. The calibration curves were linear in the concentration ranges 3.8—120 ng ml^{-1} for MP, 3.1—100 ng ml^{-1} for ODMP, 1.6—25 ng ml^{-1} for NDMP and 2.2—17.8 ng ml^{-1} for 5HMP, while their respective overall coefficients of variation in these ranges were 5.4%; 6.6%; 5.0% and 3.8%.

The overall recoveries of MP, ODMP, 5HMP, NDMP and the internal standards are given in Table I. The mean recoveries of MP, NDMP, ODMP and 5HMP were found to be 99.9 ± 2.6%, 50.7 ± 3.4%, 65.8 ± 3.6% and 59.3 ± 1.9%, respectively. The internal standards 4MPA and PA gave overall recoveries of 91.0 ± 3.0% and 71.9 ± 1.2%, respectively.

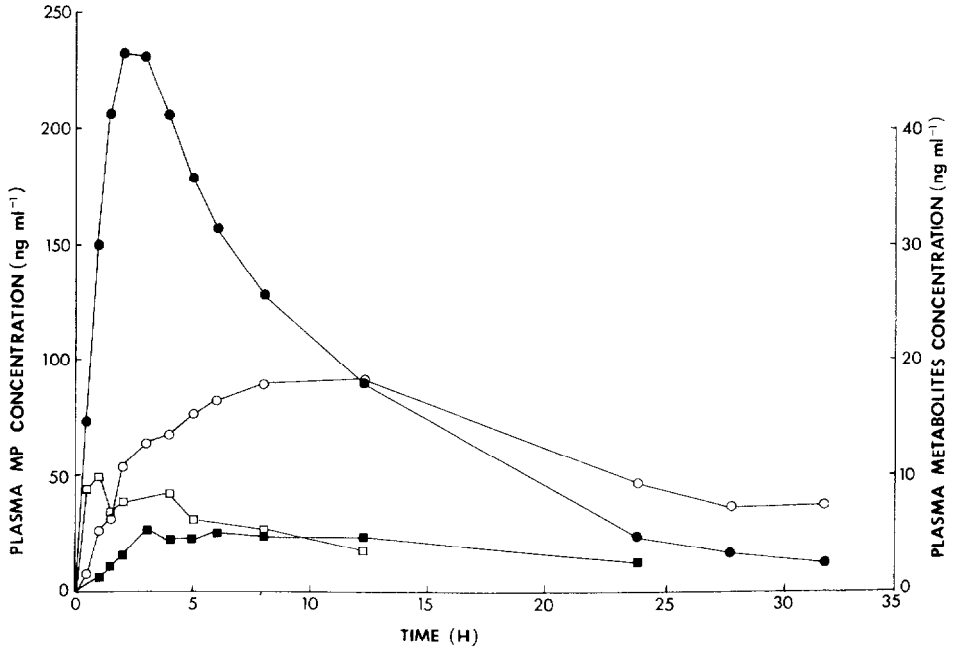

Fig. 4. Plasma concentration—time profiles for methoxyphenamine (•), O-desmethylmethoxyphenamine (○), N-desmethylmethoxyphenamine (▪) and 5-hydroxymethoxyphenamine (□) obtained from a volunteer (65 kg) who received a single oral dose of methoxyphenamine hydrochloride.

The application of the present methods to the determination of MP and its metabolites in plasma is shown in Fig. 4. The sensitivity of the GC—NPD assay of MP was such that plasma concentrations could be followed up to 24 h in all three subjects who were each orally administered 60.3 mg of MP · HCl. Also, the GC—ECD assay allowed the anlaysis of ODMP in plasma samples collected as late as 32 h after MP · HCl administration, whereas due to their lower plasma levels, NDMP and 5HMP could only be quantitated for 4 and 12 h, respectively, in all three subjects following single doses of MP · HCl.

In summary, GC methods are reported for the quantitative analysis of MP and three of its metabolites in plasma. The GC—NPD method for MP and the GC—ECD method for NDMP, ODMP and 5HMP are sensitive and reproducible and will be used to determine the pharmacokinetics of the drug in healthy volunteers following single oral doses.

ACKNOWLEDGEMENT

The support of the Medical Research Council of Canada (Grant No. MA-6950) is gratefully acknowledged.

REFERENCES

1 K.K. Midha, J.K. Cooper, I.J. McGilveray, R.T. Coutts and R. Dawe, Drug Metab. Dispos., 4 (1976) 568.
2 K.K. Midha and R.T. Coutts, J. Chromatogr., 144 (1977) 280.
3 T. Inoue, T. Niwaguchi and M. Emoto, Kagaku Keisatsu Kenkyusho Hokoku, Hokagaku Hen, 33 (1980) 89.
4 G. McKay, J.K. Cooper, E.M. Hawes, S.D. Roy and K.K. Midha, Xenobiotica, 13 (1983) 257.
5 T. Niwaguchi and T. Inoue, J. Chromatogr., 161 (1978) 223.
6 F.T. Delbeke, M. Debackere, J.A.A. Jonckheere and A.P. De Leenheer, J. Chromatogr., 273 (1983) 141.
7 S.B. Matin and M. Rowland, J. Pharm. Sci., 61 (1972) 1235.
8 A.C. Moffat, E.C. Horning, S.B. Matin and M. Rowland, J. Chromatogr., 66 (1972) 255.
9 K.K. Midha, J.K. Cooper and I.J. McGilveray, J. Pharm. Sci., 68 (1979) 557.
10 K.K. Midha, I.J. McGilveray and J.K. Cooper, Can. J. Pharm. Sci., 14 (1979) 18.
11 K.K. Midha, J.K. Cooper, D. Gagné and K. Bailey, J. Anal. Toxicol., 3 (1979) 53.
12 N. Nose, S. Kobayashi, A. Tanaka, A. Hirose and A. Watanabe, J. Chromatogr., 125 (1976) 439.
13 G.L. Dadish, W. Vycudilik and G. Machata, Forensic Sci., 10 (1977) 205.