*Journal of Chromatography*, 330 (1985) 315–321
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 17 751

# DETECTION OF PHENOLALKYLAMINES BY CAPILLARY COLUMN GAS CHROMATOGRAPHY–NEGATIVE CHEMICAL IONIZATION MASS SPECTROMETRY

G. P. CARTONI*
*Dipartimento di Chimica, Università di Roma, Rome (Italy)*
and
M. CIARDI, A. GIARRUSSO and F. ROSATI
*Laboratorio Antidoping della FMSI, Rome (Italy)*
(Received March 23rd, 1985)

## SUMMARY

Twelve phenolalkylamines normally used in different pharmaceutical products were determined in urine from volunteers given a therapeutical dose of the drug. The urine extracts were first derivatized with perfluoro propionic or butyric anhydride, then detected by capillary gas chromatography–negative chemical ionization mass spectrometry. The method is highly sensitive and specific in the analysis of biological samples.

## INTRODUCTION

The combination of capillary gas chromatography (GC) with mass spectrometry is a very useful technique for trace analysis of different drugs in biological materials[1,2]. Some difficulties are still encountered in the detection of phenolalkylamines because of their high polarity and low volatility. These compounds can be analysed by gas chromatography only after derivatization, and in electron impact mass spectrometry (EIMS) do not show a specific and sensitive fragmentation. Their detection in biological liquids is difficult.

The perfluoroacyl derivatives of these phenolalkylamines are very stable and volatile, and owing to their high electron affinity a sensitive and specific detection can be obtained by negative chemical ionization mass spectrometry (NCIMS). Many reports have been published on the detection of phenolalkylamine derivatives by GC–MS[3,4]. Octopamine, tyramine and their metabolites have been determined in tissues by NCIMS[6]. Isomeric octopamines and synephrines were measured in urine by capillary GC and NCIMS[7].

In this paper a procedure for detection of twelve phenolalkylamines is reported. The compounds which are present in a pharmaceutical formulation are detected in human urine after drug ingestion. Their derivatives are first formed with pentafluoropropionic or heptafluorobutyric anhydride are then detected by capillary GC and

0021-9673/85/$03.30 

NCIMS. These derivatives give ions which are sufficiently abundant and specific to be very suitable for selected ion monitoring (SIM).

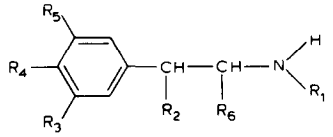
## EXPERIMENTAL

### *Materials*

The compounds reported in Table I were obtained from pharmaceutical products commercially available in Italy. The drugs were dissolved (or suspended) in 2 ml sodium borate solution at pH 9 and extracted with 2 ml of diethyl ether–*tert.*-butanol (5:1), following, the procedure described below for the urine samples. All other reagents were of analytical grade (RPE; Carlo Erba, Italy).

TABLE I

PHENOLALKYLAMINES INVESTIGATED




| *Drug* | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ | $R_6$ | *MW* | *Dose (mg)* |
|---|---|---|---|---|---|---|---|---|
| Bamethan | $C_4H_9$ | OH | H | OH | H | H | 209 | 10 |
| Dimethophrine | $CH_3$ | OH | $OCH_3$ | OH | $OCH_3$ | H | 227 | 60 |
| Ethylephrine | $C_2H_5$ | OH | OH | H | H | H | 181 | 10 |
| Hydroxyamphetamine | H | H | H | OH | H | $CH_3$ | 151 | 20 |
| Isophenephrine | $C_3H_7$ | OH | OH | H | H | H | 195 | 20 |
| Isoproterenol | $C_3H_7$ | OH | OH | OH | H | H | 211 | 10 |
| Metaproterenol | $C_3H_7$ | OH | OH | H | OH | H | 211 | 20 |
| Metaraminol | H | OH | OH | H | H | $CH_3$ | 167 | 10 |
| Octopamine | H | OH | H | OH | H | H | 153 | 50 |
| Phenylephrine | $CH_3$ | OH | OH | H | H | H | 167 | ND* |
| Pholedrine | $CH_3$ | H | H | OH | H | $CH_3$ | 165 | 20 |
| Synephrine | $CH_3$ | OH | H | OH | H | H | 167 | 60 |

* Not determined.

### *Procedure*

A therapeutic dose (10–60 mg) of each drug (Table I) was given orally to volunteers and urine collected before (blank), 4 and 24 h after the drug ingestion. A 5-ml volume of urine was passed through a Sep-Pak-$C_{18}$ cartridge (Waters-Millipore, U.S.A.) which had previously been washed with methanol and water. The Sep-Pak was then washed with 3 ml of water and the compounds were eluted with 2 ml of methanol.

The solvent was evaporated and the residue hydrolysed for 1 h at 90°C with 1 ml of 1 *M* hydrochloric acid containing 100 mg of cysteine. The sample was cooled, extracted with diethyl ether and the solvent discarded. A 1.1-ml volume of a solution containing 166.7 g of boric acid in 1 l of 10 *M* sodium hydroxide was added (the pH of the resulting solution should be about 9), and the solution extracted with 5 ml of diethyl ether–*tert.*-butanol (5:1). The solvent was evaporated to dryness and the residue dissolved in 0.5 ml of cyclohexane.

*Derivative formation*

The cyclohexane solution containing the phenolalkylamine was added to 50 μl of pentafluoropropionic (PFP) or heptafluorobutyric (HFB) anhydride and 25 μl of pyridine. This solution was heated for 15 min at 70°C, cooled then and washed with 1 ml of 1 *M* sodium borate to eliminate the unreacted reagent. A 1–2 μl volume of this solution was injected in the GC–MS system.

*Apparatus*

A Hewlett-Packard gas chromatograph (Model 5846 A) connected to a mass spectrometer (Model 5985) and equipped with an Hewlett-Packard data system was used. A fused-silica capillary column (25 m × 0.31 mm I.D.) coated with bonded SE-54 (film thickness 0.52 μm) was connected directly to the ion source of the mass spectrometer and the sample was introduced with a splitter in the ratio 1:5. Helium at a flow-rate of 2 ml/min was used as carrier gas.

The injector and transfer-line temperatures were 250°C. The column temperature was programmed from 140 to 220°C at a rate of 10°C/min. Negative chemical ionization was carried out with methane as reagent gas; source temperature, 100°C and pressure, 1 Torr; electron multiplier, *ca.* 2000 V.

## RESULTS AND DISCUSSION

The derivatives are readily obtained in high yield according to the described procedure. In the present case the compounds are fully derivatized at the amino and hydroxyl groups. For the HFB derivatives the retention times are *ca.* 30–40% higher than for the corresponding PFP derivatives, and in both cases the derivatives are easily eluted from the capillary column (Figs. 1 and 2).

Comparing the retention times in Tables II and III, it appears that by using HFB a better separation is achieved between octopamine and ethylephrine, and between iso- and metaproterenol. On the other hand with HFB, synephrine is more strongly retained than isosynephrine and pholedrine, and better separated. In Tables II and III are also reported the six most intense peaks and the relative intensities

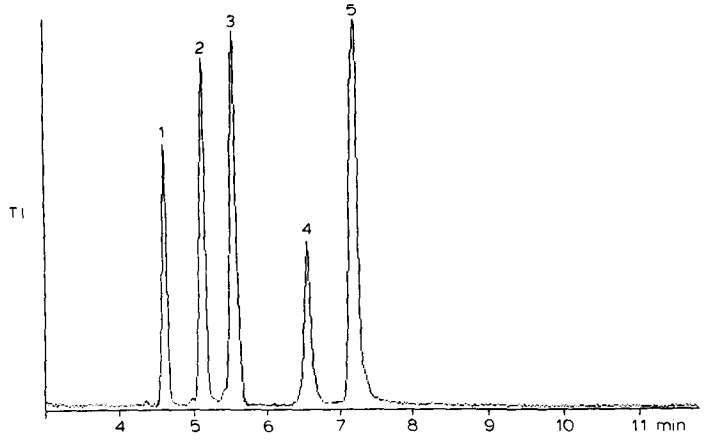



Fig. 1. Gas chromatogram of a standard mixture of PFP derivatives of phenolalkylamines by NCIMS total ion monitor. Peaks: 1 = hydroxyamphetamine; 2 = phenylephrine; 3 = ethylephrine; 4 = synephrine; 5 = pholedrine.

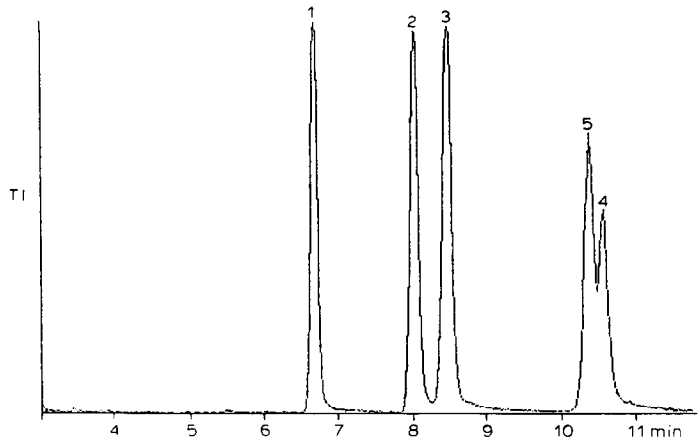



Fig. 2. Gas chromatogram of HFB derivatives of the mixture in Fig. 1 under the same conditions.

obtained from the NCI spectra. Among these peaks, always present is the molecular ion and also the ions $M-C_2F_5COOH$ (or $M-C_3F_7COOH$) and $M-HF$. Also present with a high intensity are the ions $C_2F_5COO^-$ ($m/e$ = 163) (or $C_3F_7COO^-$, $m/e$ = 213) and $C_2F_5CO^-$ ($m/e$ = 147) (or $C_3F_7CO^-$, $m/e$ = 197).

TABLE II

PFP DERIVATIVES OF PHENOALKYLAMINES

| *Drug* | $t_R$ *(min)* | *MW* | *Six most intense peaks and relative intensities* | | | | | |
|---|---|---|---|---|---|---|---|---|
| Metaraminol | 3.70 | 605 | 585 | 163 | 441 | 605 | 457 | 147 |
| | | | 100 | 97 | 78 | 57 | 32 | 32 |
| Hydroxyamphetamine | 4.62 | 443 | 295 | 423 | 128 | 189 | 147 | 443 |
| | | | 100 | 11 | 8 | 2 | 2 | 0.5 |
| Phenylephrine | 5.12 | 605 | 163 | 419 | 605 | 585 | 147 | 176 |
| | | | 100 | 22 | 15 | 13 | 12 | 9 |
| Octopamine | 5.28 | 591 | 427 | 591 | 407 | 279 | 281 | 571 |
| | | | 100 | 8 | 6 | 4 | 3 | 2 |
| Ethylephrine | 5.55 | 619 | 163 | 433 | 599 | 619 | 553 | 190 |
| | | | 100 | 24 | 19 | 15 | 12 | 11 |
| Metaproterenol | 5.88 | 795 | 795 | 649 | 629 | 755 | 163 | 445 |
| | | | 100 | 27 | 26 | 23 | 23 | 14 |
| Isoproterenol | 5.95 | 795 | 795 | 629 | 163 | 649 | 445 | 755 |
| | | | 100 | 65 | 40 | 32 | 26 | 22 |
| Synephrine | 6.58 | 605 | 163 | 585 | 147 | 144 | 176 | 605 |
| | | | 100 | 12 | 12 | 11 | 11 | 8 |
| Isophenephrine | 6.60 | 633 | 163 | 447 | 613 | 633 | 450 | 467 |
| | | | 100 | 46 | 25 | 22 | 15 | 13 |
| Pholedrine | 7.20 | 457 | 147 | 457 | 128 | 290 | 310 | 417 |
| | | | 100 | 26 | 8 | 6 | 2 | 1 |
| Bamethan | 10.52 | 647 | 163 | 218 | 147 | 647 | 500 | 627 |
| | | | 100 | 11 | 10 | 7 | 7 | 4 |
| Dimethophrine | 12.30 | 665 | 163 | 665 | 645 | 144 | 147 | 315 |
| | | | 100 | 34 | 33 | 9 | 8 | 6 |

TABLE III

HFB DERIVATIVES OF PHENOLALKYLAMINES

| *Drug* | $t_R$ *(min)* | *MW* | *Six most intense peaks and relative intensities* | | | | | |
|---|---|---|---|---|---|---|---|---|
| Metaraminol | 5.77 | 755 | 213 | 735 | 541 | 755 | 197 | 557 |
| | | | 100 | 98 | 92 | 87 | 64 | 56 |
| Hydroxyamphetamine | 6.68 | 543 | 345 | 365 | 523 | 178 | 479 | 543 |
| | | | 100 | 16 | 12 | 4 | 1 | 0.5 |
| Phenylephrine | 8.02 | 755 | 213 | 577 | 519 | 755 | 735 | 539 |
| | | | 100 | 35 | 31 | 29 | 25 | 19 |
| Ethylephrine | 8.47 | 769 | 213 | 533 | 591 | 769 | 553 | 749 |
| | | | 100 | 36 | 36 | 28 | 27 | 27 |
| Octopamine | 8.50 | 741 | 527 | 741 | 329 | 213 | 507 | 721 |
| | | | 100 | 35 | 11 | 10 | 10 | 4 |
| Isoproterenol | 9.97 | 995 | 779 | 995 | 213 | 798 | 601 | 545 |
| | | | 100 | 90 | 84 | 76 | 65 | 52 |
| Metaproterenol | 9.98 | 995 | 995 | 779 | 798 | 213 | 601 | 955 |
| | | | 100 | 65 | 53 | 42 | 40 | 35 |
| Isophenephrine | 10.00 | 783 | 213 | 547 | 783 | 763 | 567 | 605 |
| | | | 100 | 71 | 43 | 35 | 33 | 15 |
| Pholedrine | 10.37 | 557 | 197 | 557 | 537 | 340 | 360 | 519 |
| | | | 100 | 9 | 6 | 5 | 2 | 1 |
| Synephrine | 10.55 | 755 | 213 | 197 | 735 | 558 | 755 | 178 |
| | | | 100 | 25 | 16 | 10 | 9 | 8 |
| Bamethan | 14.22 | 797 | 213 | 797 | 777 | 600 | 268 | 347 |
| | | | 100 | 41 | 26 | 24 | 8 | 2 |
| Dimethophrine | 15.95 | 815 | 795 | 815 | 213 | 583 | 617 | 775 |
| | | | 100 | 90 | 76 | 7 | 6 | 6 |

The advantage of NCI compared with EI mass spectrometry is that with these highly electronegative derivatives there is a large production of negative ions and consequently a high current intensity. As is seen from the values in Tables II and III for the two classes of derivatives, with NCI there is always the possibility to select

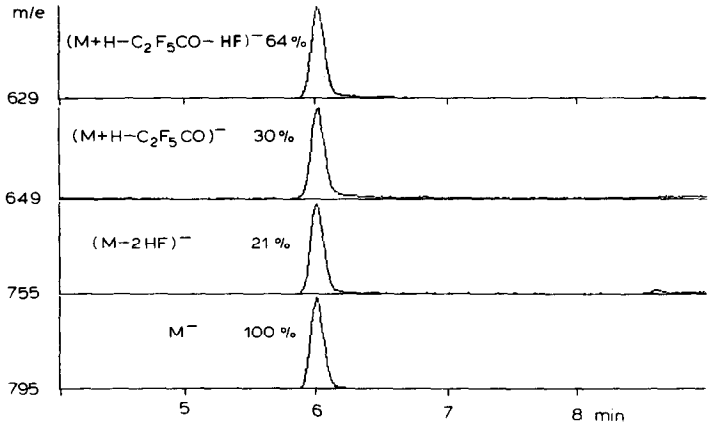



Fig. 3. SIM gas chromatogram of an urine sample taken 4 h after ingestion of 10 mg of isoproterenol, with PFP derivatization.

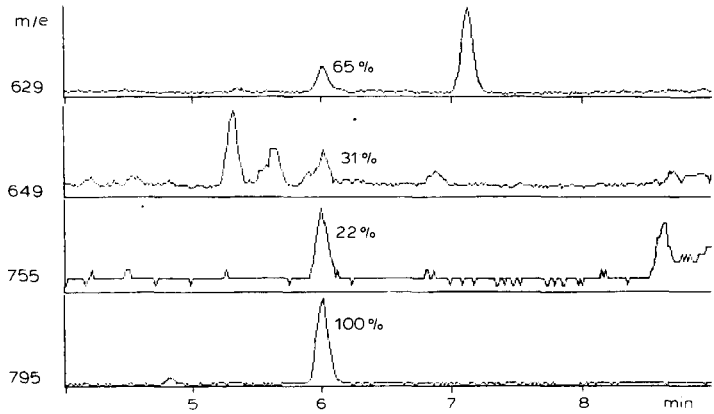



Fig. 4. SIM gas chromatogram of the urine sample taken the same subject as in Fig. 3, 24 h after drug ingestion.

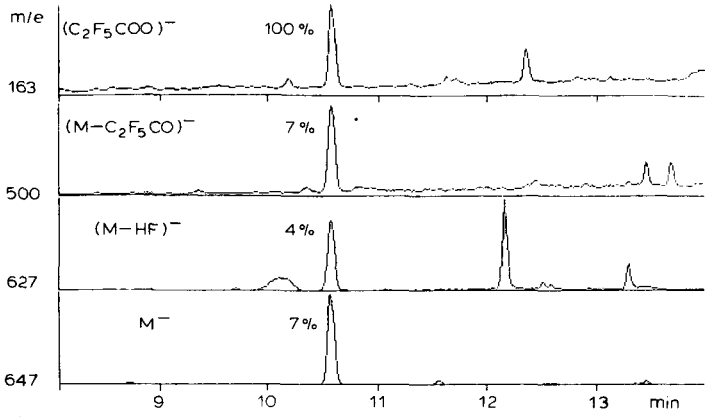



Fig. 5. SIM gas chromatogram of a urine sample taken 24 h after ingestion of 10 mg of bamethan.

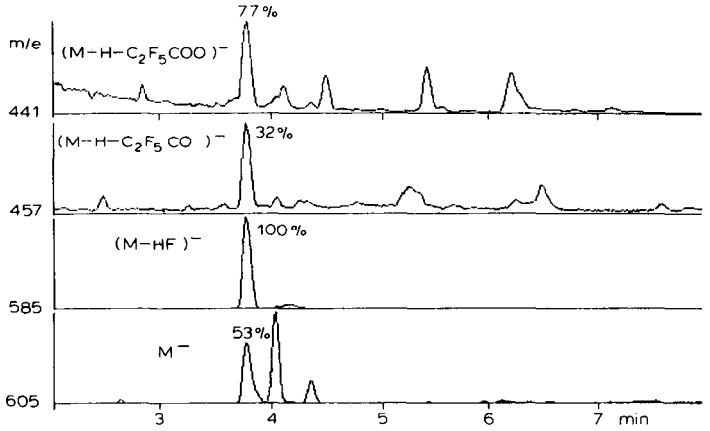



Fig. 6. SIM gas chromatogram of a urine sample taken 24 h after ingestion of 10 mg of metaraminol.

at least three or four ions, having a high intensity, which are specific for these phenolalkylamines and can be used in a screening procedure by selected ion monitoring (SIM). By using a quadrupole mass spectrometer with a data system as described, it is possible to carry out a routine analysis for these drugs in urine samples, by changing the selected $m/e$ values at prefixed time intervals in such a way as to monitor the appropriate ions during the retention time range of the products under analysis.

This procedure is chiefly used in our laboratory for a routine analysis of phenolalkylamines in urine samples for antidoping control. NCI mass spectrometry is especially suitable for trace analysis of these derivatized compounds in biological samples; there is less interference compared with EI. The signal to noise ratio is higher and consequently detection limits lower than 1 ppb are easily obtained.

In Fig. 3 is shown the SIM analysis of a urine sample extracted 4 h after the ingestion of isoproterenol (a single dose of 10 mg). The recorded peaks correspond to the ions $M^-$ ($m/e$ = 795), M − 2HF ($m/e$ = 755), (M + H) $-COC_2F_5$ ($m/e$ = 649) and 649 − HF ($m/e$ = 629) with the expected intensity ratios (Table II). In Fig. 4 is shown the same urine analysis for this subject but 24 h later. In Figs. 5 and 6 are reported the SIM analyses of urine samples taken 24 h after the ingestion of a single therapeutic dose of 10 mg of bamethan and metaraminol.

The analyses are carried out by monitoring with NCI four selected ions among the ones reported in Table II. In this way many urine samples can be analyzed with a high sensitivity and selectivity. When such an analysis indicated the presence of a drug, a full NCI spectra was recorded by use of a new injection of the sample.

## REFERENCES

1 G. P. Cartoni, M. Ciardi, A. Giarrusso and F. Rosati, *J. Chromatogr.*, 279 (1983) 515.
2 G. P. Cartoni and F. Coccioli,, *J. Chromatogr.*, 278 (1983) 144.
3 M. Donike, *J. Chromatogr.*, 115 (1975) 591.
4 M. Donike, *J. Chromatogr.*, 103 (1975) 91.
5 P. H. Duffield, D. F. H. Dougan, D. N. Wade, G. K. C. Low and A. M. Duffield, *Spectrosc. Int. J.*, 2 (4-5) (1983) 311.
6 J. T. Martin, J. D. Barchas and K. F. Faull, *Anal. Chem.*, 54 (1982) 1806.
7 K. E. Ibrahim, M. W. Couch, C. M. Williams, M. B. Budd, R. A. Yost and J. M. Midgley, *Anal. Chem.*, 56 (1984) 1695.