Journal of Chromatography, 581 (1992) 213–218
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 6493

Determination of diphenylmethane antihistaminic drugs and their analogues in body fluids by gas chromatography with surface ionization detection

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(First received January 23rd, 1992; revised manuscript received June 23rd, 1992)

ABSTRACT

Eleven diphenylmethane antihistaminic drugs and their analogues were tested for their detection by capillary gas chromatography (GC) with surface ionization detection (SID). The GC–SID response was highest for doxylamine, diphenhydramine and orphenadrine and lowest for terodiline, clemastine and pipethanate. The detection limits for drugs with the highest response were 2–5 pg (*ca.* 6–20 fmol) on-column (100–250 pg/ml of body fluid). The detection limits with GC–SID were 10–100 times higher than those with GC with nitrogen–phosphorus detection. A detailed procedure for the isolation of the antihistaminics from human whole blood and urine by the use of Sep-Pak C_{18} cartridges, prior to GC–SID, is also presented. The recoveries of the drugs (50 or 500 pmol), which had been added to 1 ml of body fluids, were >60%. The baselines remained steady as the column temperature was increased and the background was clean, especially for whole blood extracts.

INTRODUCTION

Surface ionization detection (SID) for gas chromatography (GC) was developed by Fujii and Arimoto [1] in 1985. SID was reported to provide extremely sensitive and specific responses to compounds containing tertiary amino groups. GC–SID has recently been applied in drug analyses and a few studies have been reported on fentanyl [2], aprindine [3], tricyclic antidepressants [4] and local anaesthetics [5].

This paper reports the detection of diphenylmethane antihistaminics by GC–SID. A detailed procedure for the isolation of the antihistaminics in human whole blood and urine is also presented.

EXPERIMENTAL

Materials

The structures of the eleven diphenylmethane antihistaminics and their analogues tested are shown in Fig. 1. Compounds I–VI and VIII–X were obtained from Sigma (St. Louis, MO, USA), VII from Kissei Pharmaceutical (Nagoya, Japan) and XI from Nippon Shinyaku (Kyoto, Japan). Sep-Pak C_{18} cartridges were purchased

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0378-4347/92/$05.00

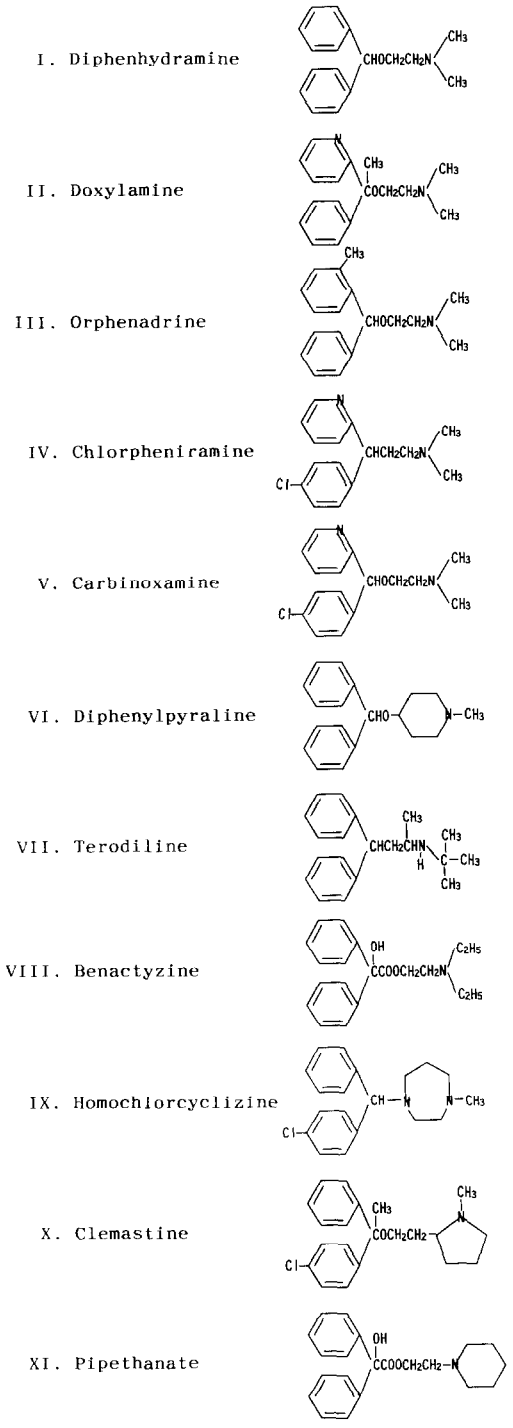

Fig. 1. Structures of the diphenylmethane antihistaminics and their analogues studied.

from Waters (Milford, MA, USA). Other common chemicals used were of the highest purity commercially available. Whole blood and urine were obtained from healthy subjects.

Clean-up procedure

Drugs were extracted with Sep-Pak C_{18} cartridges according to Suzuki *et al.* [6] with a minor modification. For pretreatment of a cartridge, 10 ml of methanol and 10 ml of distilled water were passed through it.

A 1-ml volume of whole blood or urine, with or without addition of drugs, was mixed with 7 ml of distilled water with shaking, followed by addition of 3 ml of 1 *M* $NaHCO_3$ solution. The mixture was centrifuged at 800 *g* for 10 min (the centrifugation is not necessary for urine) and the supernatant fraction was loaded on a Sep-Pak cartridge at a flow-rate not greater than 5 ml/min. The cartridge was washed twice with 10 ml of water each and finally 3 ml of chloroform–methanol (8:2) were passed through to elute the antihistaminics, which were then collected in a vial. The eluate consisted of a major amount of an organic layer (lower phase) and a minor amount of an aqueous layer (upper phase); the latter was removed by aspiration with a Pasteur pipette and discarded. The organic layer was evaporated to dryness under a stream of nitrogen and the residue was dissolved in 100 μl of methanol. A 2-μl aliquot was subjected to GC analysis.

GC conditions

GC analyses were carried out on a Shimadzu GC-15A instrument equipped with a SID system and on a Hewlett-Packard Model 5890 gas chromatograph with nitrogen–phosphorus detection (NPD). A DB-1 non-polar fused-silica capillary column (30 m × 0.32 mm I.D., film thickness 0.25 μm) (J&W Scientific, Folsom, CA, USA) and split–splitless injectors were used for both GC instruments. The GC conditions for both instruments were as follows: column temperature, programmed from 100 to 280°C at 8°C/min; injection temperature, 280°C; detector temperature for both SID and NPD, 280°C; and helium flow-rate, 22 cm/s. The SID conditions were as follows:

heating current through the platinum emitter, 2.2 A; emitter temperature. *ca.* 600°C; and ring electrode bias voltage, +200 V with respect to the collector electrode. The samples were injected in the splitless mode at a column temperature of 100°C and the splitter was opened after 1 min.

RESULTS

Table I shows the retention times and relative peak-height intensities of the eleven diphenylmethane antihistaminics and their analogues (2 pmol each for injection), measured by GC–SID. The response was highest with doxylamine, diphenhydramine, orphenadrine, chlorpheniramine and diphenylpyraline and lowest with terodiline, clemastine and pipethanate.

Calibration graphs were constructed by plotting five points with different amounts of non-extracted drugs (200, 400, 600, 800 and 1000 fmol on-column, which are equivalent to 10, 20, 30, 40 and 50 pmol/ml, respectively, for I–VI; 2, 4, 6, 8 and 10 pmol on-column, which are equivalent to 100, 200, 300, 400 and 500 pmol/ml, respectively, for VII–XI). All drugs showed excellent linearity between peak height and amount, with r values of 0.9957–0.9999. The equations, for example, were $y = 1.05x + 2.71$ for diphenhydramine, $y = 1.07x + 12.5$ for doxylamine, $y = 1.01x + 11.8$ for orphenadrine, $y = 0.639x + 8.28$ for chlorpheniramine and $y = 0.574x + 2.91$ for diphenylpyraline (y = peak height; x = amount of drug). The detection limits of these five drugs were 2–5 pg (*ca.* 6–20 fmol) on-column (100–250 pg/ml body fluid). Compounds VII–XI gave a sensitivity about ten times lower than I–VI.

Fig. 2 shows gas chromatograms for whole blood and urine, with and without addition of 50 pmol each of I–VI to 1-ml samples. The separation of the test peaks from each other and from impurities was satisfactory. The recovery of I–VI added to whole blood and urine was >60% (Table II). Fig. 3 shows the same addition test for VII–XI, where 500 pmol of each compound was added to 1-ml samples because of the lower response of SID to these compounds. The separation and recovery (Table II) are also satisfactory for VII–XI. In all chromatograms (Figs. 2 and 3), the baselines were steady at low levels and did not rise with increase in column temperature.

TABLE I

RETENTION TIMES AND RELATIVE PEAK-HEIGHT INTENSITIES OF DIPHENYLMETHANE ANTIHISTAMINICS AND THEIR ANALOGUES MEASURED BY GC–SID

A 2-pmol amount of each drug was injected into the GC port.

No.	Compound	Retention time (min)	Relative intensity[a]
I	Diphenhydramine	13.7	1.00
II	Doxylamine	14.3	1.19
III	Orphenadrine	14.7	0.84
IV	Chlorpheniramine	15.5	0.66
V	Carbinoxamine	16.4	0.64
VI	Diphenylpyraline	16.7	0.54
VII	Terodiline	14.9	0.04
VIII	Benactyzine	18.7	0.21
IX	Homochlorcyclizine	19.7	0.19
X	Clemastine	20.5	0.09
XI	Pipethanate	21.0	0.13

[a] Diphenhydramine = 1.00.

TABLE II

RECOVERIES OF DIPHENYLMETHANE ANTIHISTAMINICS AND THEIR ANALOGUES ADDED TO WHOLE BLOOD AND URINE

The amount of each drug added to 1 ml of whole blood or urine was 50 pmol (1 pmol on-column in case of 100% recovery) for I–VI and 500 pmol (10 pmol on-column in case of 100% recovery) for VII–XI.

No.	Compound	Recovery (%)	
		Whole blood	Urine
I	Diphenhydramine	103	117
II	Doxylamine	89.3	79.0
III	Orphenadrine	90.6	97.5
IV	Chlorpheniramine	78.4	88.7
V	Carbinoxamine	86.6	92.0
VI	Diphenylpyraline	65.0	90.4
VII	Terodiline	94.5	89.1
VIII	Benactyzine	98.6	106
IX	Homochlorcyclizine	74.0	99.2
X	Clemastine	60.9	127
XI	Pipethanate	103	130

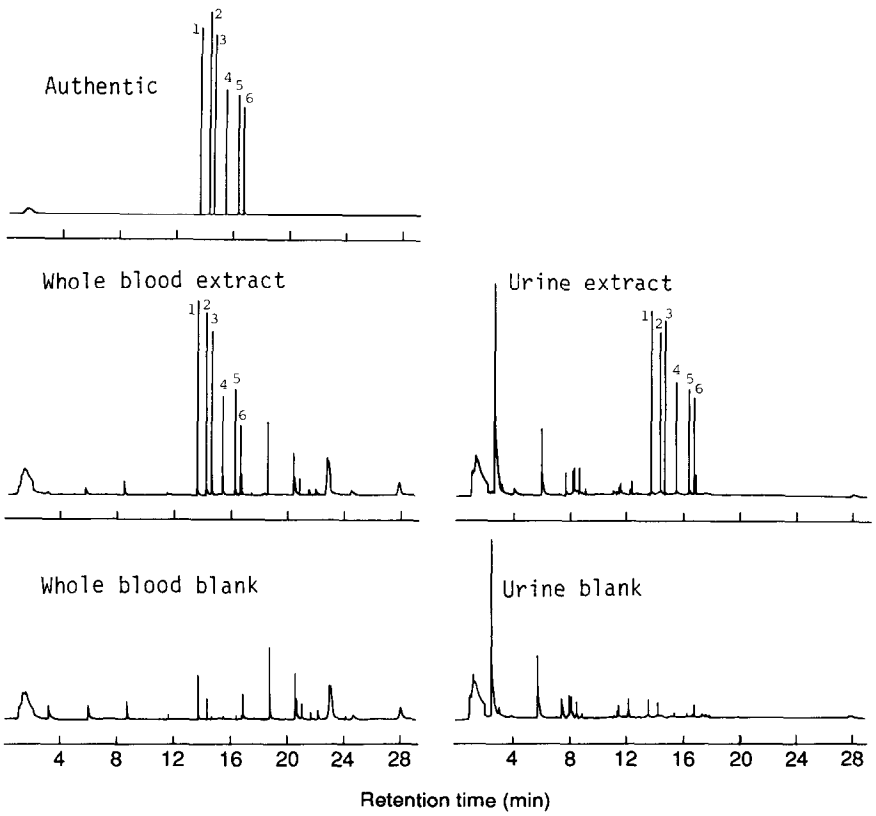

Fig. 2. Capillary GC–SID of human whole blood and urine extracts in the presence and absence of diphenylmethane antihistaminics (I–VI) with the use of Sep-Pak C_{18} cartridges for isolation. Peaks: 1 = diphenhydramine; 2 = doxylamine; 3 = orphenadrine; 4 = chlorpheniramine; 5 = carbinoxamine; 6 = diphenylpyraline. GC was carried out with a DB-1 fused-silica capillary column (30 m × 0.32 mm I.D., film thickness 0.25 μm). The GC conditions were as follows: column temperature, programmed from 100 to 280°C at 8°C/min; injection temperature, 280°C; detector temperature, 280°C; and helium flow-rate, 22 cm/s. The samples were injected in the splitless mode at a column temperature of 100°C and the splitter was opened after 1 min. A mixture of I–VI (50 pmol each) was added to 1 ml of whole blood or urine.

The same whole blood extract sample that had been used for GC–SID of I–VI (Fig. 2, middle left panel) was subjected to GC–NPD under similar conditions for comparison, as shown in Fig. 4. Many large impurity peaks appeared at various stages of the GC measurements with NPD. The baseline fluctuated and rose slightly with increase in the column temperature. It is obvious that the sensitivity with SID is much higher (10–100 times) than that with NPD.

DISCUSSION

Diphenylmethane antihistaminics and their analogues are one of the most commonly used drug groups for the treatment of colds, asthma and other allergic diseases, and are easily obtainable at drug stores for their abuse. Fatal cases involving their ingestion have been repeatedly reported [7,8]. In this paper, we have shown that antihistaminics such as doxylamine, diphenhydramine and orphenadrine can be detected by GC–SID with extremely high sensitivity; the detection limits of I–VI are as low as 2–5 pg on-column (100–250 pg/ml of blood or urine). The detection limits obtained with GC–SID are comparable to or even higher than those given by GC with electron-capture detection (ECD), which

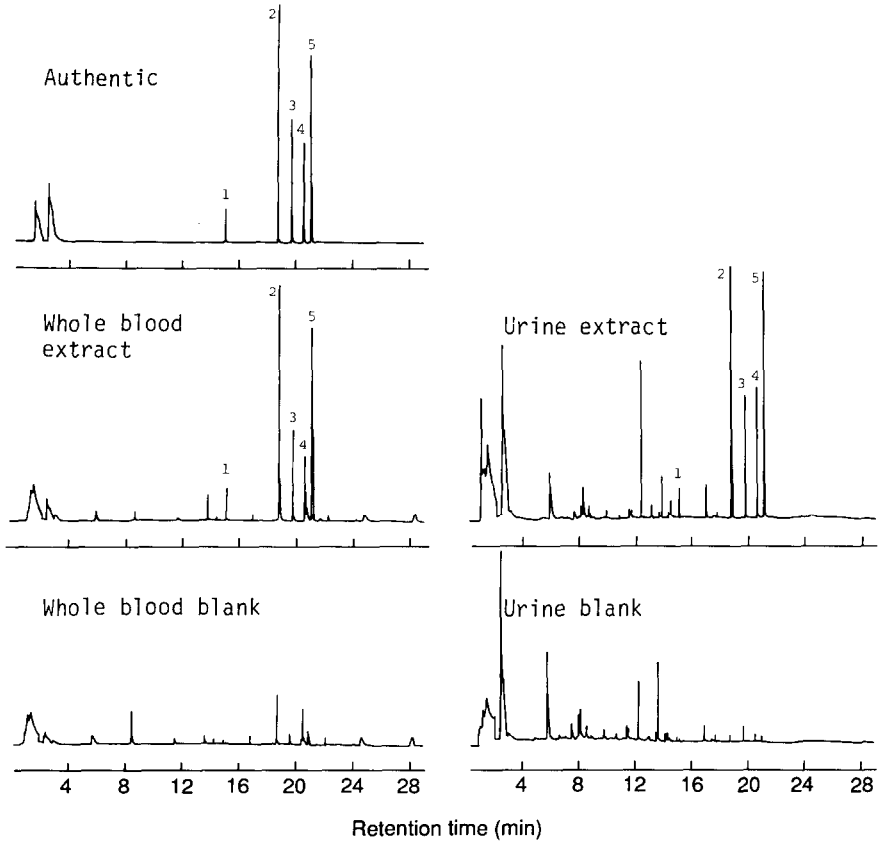

Fig. 3. Capillary GC–SID of human whole blood and urine extracts in the presence and absence of diphenylmethane antihistaminics and their analogues (VII–XI) with the use of Sep-Pak C_{18} cartridges for isolation. Peaks: 1 = terodiline; 2 = benactyzine; 3 = homochlorcyclizine; 4 = clemastine; 5 = pipethanate. A mixture of VII–XI (500 pmol each) was added to 1 ml of whole blood or urine. GC conditions as in Fig. 2.

can be applied to compounds with halogen groups; the detection limit of chlorpheniramine, for example, was reported to be 1 ng/ml of serum by ECD [9] and 0.1–0.5 μg/ml of blood and urine by conventional flame ionization detection (unpublished observation), and those reported for diphenhydramine and chlorphenylamine by GC–NPD were 4–10 ng/ml of plasma or serum and 0.4 ng/ml of plasma, respectively [10–12]. As shown in Fig. 4, NPD was confirmed to give a sensitivity 10–100 times lower than that of the present GC–SID. In addition, the baselines with SID were very stable even with temperature programming (Figs. 2 and 3). This is due to the highly specific response of the detector to tertiary amino compounds. Therefore, GC–SID can be recommended for the sensitive detection of antihistaminics.

It is of interest to define specific molecular structures that cause high and low sensitivity with the GC–SID method. Fujii and Arimoto [1] reported that compounds with an aliphatic tertiary amino group gave the highest sensitivity by GC–SID. Such a trend seems also true for the present diphenylmethane antihistaminics and their analogues when the sensitivity for I–V is compared with that for IX–XI with ring side-chain structures (Table I and Fig. 1). However, diphenylpyraline, with an N-methylpiperidine ring, showed a relatively higher response (0.64) of the detector than expected.

Doxylamine gave a slightly higher detection

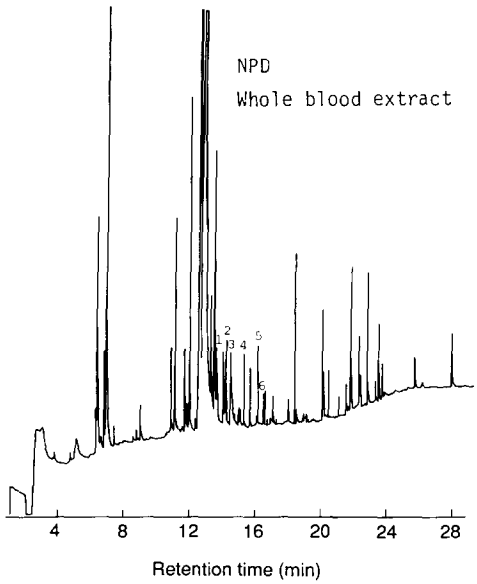

Fig. 4. Capillary GC–NPD of human whole blood extract in the presence of six diphenylmethane antihistaminics (I–VI, 50 pmol of each for 1-ml samples) with the use of Sep-Pak C_{18} cartridges for isolation. Peak numbers and GC conditions as in Fig. 2.

limit than diphenhydramine (Table I), suggesting that neither the pyridinyl group nor the methyl group attached to the central carbon atom has negative effects on the response of SID. Chlorpheniramine and carbinoxamine gave lower responses (0.66 and 0.64, respectively) than diphenhydramine or doxylamine. This may be due to the negative effects of a chlorine group present in the *para* position on a phenyl group in both compounds, if we assume that the pyridinyl group has no negative effect on the SID response as mentioned above.

The therapeutic concentration of diphenhydramine in human plasma was 0.08–0.16 μg/ml 2–4 h after a single oral administration; its toxic concentration was > 1 μg/ml [13]. The detection limit of the present GC–SID method is far below the therapeutic levels. Hence the present method is useful for the therapeutic monitoring of diphenylmethane antihistaminics and their analogues in clinical pharmacology. The high sensitivity of this method may also allow trace determinations of the antihistaminics present in small samples such as bloodstains and hair, extending its applicability in forensic toxicology.

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