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Sensitive determination of phenothiazines in body fluids by gas chromatography with surface ionization detection

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ABSTRACT

Fourteen phenothiazine derivatives were tested for their detection by gas chromatography (GC) with surface ionization detection (SID). The sensitivity of GC–SID was highest with trimeprazine and levomepromazine, which contain aliphatic tertiary amino side chains, and lowest with thiethylperazine and thioproperazine, which contain sulphur residues. Chlorpromazine, trimeprazine and promazine showed excellent linearity between the SID response and the drug amount in the range 0.25–3.0 pmol on-column. Their detection limits were as low as *ca.* 5–10 pg (15–30 fmol) on-column (250–500 pg per ml of body fluid). A detailed procedure for isolation of phenothiazines from human whole blood and urine using Sep-Pak C_{18} cartridges, before the GC with SID, is also presented. The recoveries of the drugs (100 pmol), which were added to 1 ml of whole blood or urine, were more than 79%. The baselines remained steady as the column temperature was increased.

INTRODUCTION

Chlorpromazine was developed many years ago, but is still one of the most widely used drugs for treatment of schizophrenia. Until recently, many phenothiazine derivatives have been synthesized and used as antipsychotics, anti-parkinsonism drugs and also antihistaminics. These drugs are frequently encountered in forensic chemistry and clinical toxicology.

In 1985, surface ionization detection (SID) for gas chromatography (GC) was first introduced by Fujii and Arimoto [1]. It was suggested to be very specific and sensitive, especially for compounds having tertiary amino groups in their structures. The present paper describes, for the first time, how phenothiazines can be detected by GC–SID with extremely high sensitivity; a detailed procedure for the isolation of phenothiazines in human whole blood and urine is also presented.

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EXPERIMENTAL

Materials

The chemical structures of fourteen phenothiazine derivatives tested in the present study are listed in Table I, where they are grouped according to their side chain (R_1) structures. IV, VI and VIII–XI were obtained from Yoshitomi Pharmaceutical (Osaka, Japan); XII and XIV from Sandoz (Basle, Switzerland); I from Banyu Pharma-

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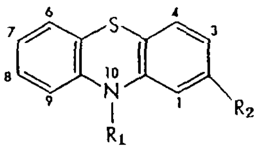
TABLE I

CHEMICAL STRUCTURES OF PHENOTHIAZINE DERIVATIVES USED IN THE PRESENT STUDY



Compound		Molecular mass	R_1	R_2	Miscellaneous
I	Promazine	284	$-CH_2CH_2CH_2N(CH_3)_2$	H	–
II	Chlorpromazine	318	$-CH_2CH_2CH_2N(CH_3)_2$	Cl	–
III	Triflupromazine	352	$-CH_2CH_2CH_2N(CH_3)_2$	CF_3	–
IV	Promethazine	284	$-CH_2CHN(CH_3)_2$ | CH_3	H	–
V	Isothipendyl	285	$-CH_2CHN(CH_3)_2$ | CH_3	H	1:N
VI	Ethopropazine	312	$-CH_2CHN(C_2H_5)_2$ | CH_3	H	–
VII	Trimeprazine	298	$-CH_2CHCH_2N(CH_3)_2$ | CH_3	H	–
VIII	Levomepromazine	328	$-CH_2CHCH_2N(CH_3)_2$ | CH_3	OCH_3	–
IX	Perazine	339	$-CH_2CH_2CH_2-N\;\;N-CH_3$	H	–
X	Prochlorperazine	373	$-CH_2CH_2CH_2-N\;\;N-CH_3$	Cl	–
XI	Trifluoperazine	407	$-CH_2CH_2CH_2-N\;\;N-CH_3$	CF_3	–
XII	Thiethylperazine	399	$-CH_2CH_2CH_2-N\;\;N-CH_3$	SCH_2CH_3	–
XIII	Thioproperazine	446	$-CH_2CH_2CH_2-N\;\;N-CH_3$	$SO_2N(CH_3)_2$	–
XIV	Thioridazine	370	$-CH_2CH_2-$ (N) CH_3	SCH_3	–

ceutical (Tokyo, Japan); II from Sigma (St. Louis, MO, USA); III from E. R. Squibb & Sons (New Brunswick, NJ, USA); V from Sumitomo Pharmaceutical (Osaka, Japan); VII from Daiichi Seiyaku (Tokyo, Japan) and XIII from Shionogi (Osaka, Japan). Sep-Pak C_{18} cartridges were purchased 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 from biological fluids with Sep-Pak C_{18} cartridges according to Suzuki *et al.* [2] 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 (100 pmol each), was mixed with 16 ml of distilled water with shaking, followed by the addition of 3 ml of 1 *M* sodium hydrogencarbonate solution. The mix-

ture was centrifuged at 800 *g* for 10 min (the centrifugation step is not necessary for urine), and the supernatant fraction was loaded on the Sep-Pak cartridge at a flow-rate not greater than 5 ml/min. The cartridge was washed twice with 10 ml of water and finally 3 ml of chloroform–acetonitrile (8:2) were passed through it to elute the phenothiazines, which were 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 discarded by aspiration with a Pasteur pipette. 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 made on a Shimadzu GC-15A instrument equipped with an SID system. A non-polar fused-silica DB-1 capillary column (30 m × 0.32 mm I.D., film thickness 0.25 μm, J&W Scientific, Folsom, CA, USA) and a split–splitless injector were used. The GC conditions were: column temperature, 120–280°C (6°C/min); injection temperature, 280°C; and helium flow-rate, 22 cm/s. The SID conditions were: detector temperature, 280°C; 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 120°C, and the splitter was opened after 1 min.

RESULTS

Table II shows retention times and relative peak area intensities of fourteen phenothiazines detected by GC–SID, when 2 pmol of each were injected into the GC port. The sensitivity was highest for trimeprazine, levomepromazine and promazine, and lowest for thioproperazine and thiethylperazine.

Calibration curves were drawn by plotting five points with different amounts of non-extracted drugs (0.25, 0.50, 1.0, 2.0 and 3.0 pmol on-column, which are equivalent to 12.5, 25, 50, 100 and 150 pmol/ml, respectively) for chlorpromazine, trimeprazine and promazine. All drugs tested showed excellent linearity between peak areas and the drug amounts with *r* values of 0.9984–

TABLE II

RETENTION TIMES AND RELATIVE INTENSITIES OF PHENOTHIAZINES DETECTED BY GC–SID

Compound[a]	Retention time (min)	Relative peak area intensity (chlorpromazine = 1.00)
Trifluprom azine	19.3	0.67
Isothipendyl	19.8	0.80
Promethazine	19.9	1.36
Trimeprazine	20.3	2.30
Promazine	20.6	1.95
Ethopropazine	21.4	1.33
Chlorpromazine	23.0	1.00
Levomepromazine	23.6	2.01
Trifluoperazine	25.6	0.41
Perazine	27.1	0.64
Prochlorperazine	28.7	0.20
Thioridazine	31.1	0.34
Thiethylperazine	32.7	0.09
Thioproperazine	46.9	0.04

[a] Aliquots of 2 pmol of each drug were injected into the GC port.

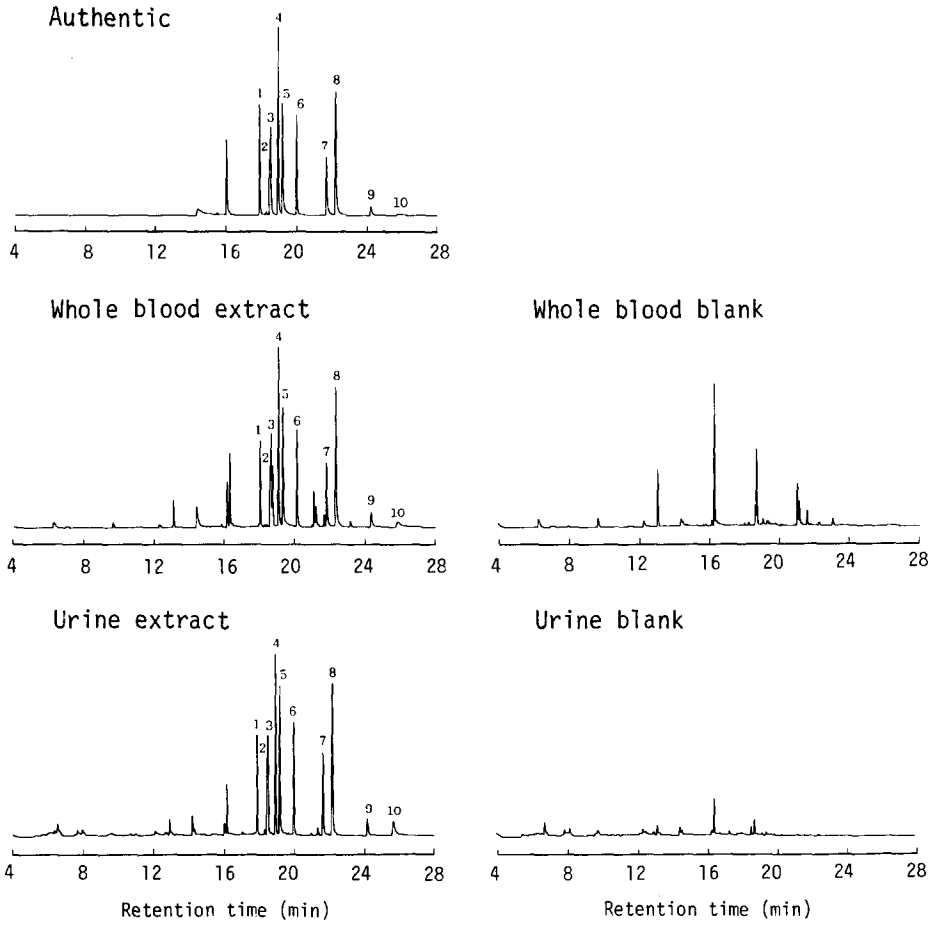


Fig. 1. Capillary GC–SID for human whole-blood and urine extracts in the presence and absence of phenothiazines using Sep-Pak C_{18} cartridges for isolation. Peaks: 1 = trifluprazine; 2 = isothipendyl; 3 = promethazine; 4 = trimeprazine; 5 = promazine; 6 = ethopropazine; 7 = chlorpromazine; 8 = levomepromazine; 9 = trifluoperazine; 10 = perazine. GC was carried out with a DB-1 fused-silica capillary column (30 m × 0.32 mm I.D., film thickness 0.25 μm). GC conditions: column temperature, 120–280°C (6°C/min); injection temperature, 280°C; detector temperature, 280°C; helium flow-rate, 22 cm/s. The samples were injected in the splitless mode at a column temperature of 120°C and the splitter was opened after 1 min. The mixture of ten phenothiazines (100 pmol each) was added to 1 ml of whole blood or urine.

0.9999. The equations were: $y = 41.8x - 4.75$ for chlorpromazine, $y = 97.1x + 50.2$ for trimeprazine, and $y = 69.2x - 6.24$ for promazine. The detection limits (signal-to-noise ratio = *ca.* 5) of these phenothiazines were *ca.* 5–10 pg (15–30 fmol) on-column, which is equivalent to 250–500 pg (0.75–1.5 pmol)/ml.

Fig. 1 shows gas chromatograms for whole blood and urine, with and without addition of 100 pmol each of ten phenothiazines to 1-ml whole-blood and urine samples. Prochlorperazine, thiethylperazine, thioproperazine and thioridazine were omitted from these addition experiments because of their low response and long retention times. All drug peaks were well separated from impurity peaks except that of promethazine (peak 3), which partially overlapped an impurity peak for the whole-blood extract. The baselines remained steady during the increase in column temperature. The recoveries of ten phenotiazines were generally excellent (more than 79%) for both whole-blood and urine samples (Table III).

TABLE III

RECOVERIES OF PHENOTHIAZINES ADDED TO WHOLE BLOOD OR URINE

Compound[a]	Recovery (%)	
	Whole blood	Urine
Triflupromazine	79.9	91.7
Isothipendyl	113	140
Promethazine	108.5	89.3
Trimeprazine	95.8	96.3
Promazine	107.6	133
Ethopropazine	99.2	114.5
Chlorpromazine	113	145
Levomepromazine	113	123
Trifluoperazine	173	187
Perazine	–[b]	–

[a] A 100-pmol aliquot of each drug had been added to 1 ml of whole blood or urine.

[b] For the authentic sample, the perazine (100 pmol dissolved in 100 μl of methanol) did not show a clear peak (see Fig. 1), which made the recovery calculation impossible.

DISCUSSION

In this paper, we have tested various phenothiazines for their detection by GC–SID; extremely high sensitivity was obtained for trimeprazine, levomepromazine and promazine (Table II). The application of GC–SID to drug analyses has recently begun, and only a few studies have been reported on fentanyl [3], aprindine [4], tricyclic antidepressants [5] and local anaesthetics [6].

Chlorpromazine and triflupromazine, which contain halogen groups on the phenothiazine nucleus (R_2), showed lower responses than promazine (Tables I and II). The same relation is true when the data for prochlorperazine and trifluoperazine are compared with that of perazine (Table II). Thus, it seems to be the case that the presence of a halogen group in the R_2 position lowers the response of SID. Phenothiazines with aliphatic tertiary amino side chains (I–VIII) generally showed higher sensitivity than those with piperazinyl side chains (IX–XIII).

Thiethylperazine and thioproperazine, which contain sulphur groups on the phenothiazine nucleus, showed the lowest sensitivity, suggesting negative effects of sulphur groups for SID.

The recovery of some phenothiazines, especially in urine extracts, exceeded 100% (Table III and Fig. 1). This phenomenon is not due to contamination by impurities: the gas chromatograms for the extracts without addition of the drugs did not show any impurity peak where the drugs would be expected to appear (Fig. 1, right panels). It may be attributable to certain factors contained in the biological extracts, which may prevent drugs from adsorbing to the column and/or from decomposing during exposure to heat; this is not the case for the non-extracted (authentic) phenothiazines.

We have carefully compared the gas chromatograms for phenothiazines obtained by the present SID method with those obtained by nitrogen–phosphorus detection (NPD); large impurity peaks appeared at early stages of GC measurements and the baseline rose during increase in column temperature with NPD. In view of the signal-to-noise ratio and baselines, the sensitivity by the GC–SID was 10–100 times higher than that with GC with NPD (unpublished results). In comparison with other GC methods in the literature, our sensitivity is also 10–100 times higher than that reported for GC of chlorpromazine with NPD [7] and with electron-capture detection [8]; it is similar to or even higher than that by GC–mass spectrometry [9–11].

Phenothiazines are metabolized very quickly via N-demethylation, hydroxylation, N-oxidation and sulphoxidation in the human body, and therapeutic plasma concentrations of unchanged phenothiazines are usually very low (10–26 ng per ml of plasma for chlorpromazine) in contrast to their high daily doses (75–300 mg) [12]. The detection limits of chlorpromazine, trimeprazine and promazine by the present GC–SID were as low as *ca.* 5–10 pg (15–30 fmol) on-column, which is equal to 250–500 pg per ml of body fluid. Thus, the present GC–SID method is sensitive enough for therapeutic monitoring of low blood levels of phenothiazines in clinical pharmacology, in addition to the detection of high levels in forensic and clinical toxicology.

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