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Review

Gas chromatography with surface ionization detection in forensic analysis

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Abstract

Surface ionization detection (SID) for gas chromatography (GC) is a recent technique that is very sensitive and specific to tertiary amino compounds. The instrumentation for this detector and the application of this method to analyses of drugs, such as tricyclic antidepressants, phenothiazines, butyrophenones, local anaesthetics, narcotic analgesics and diphenylmethane antihistaminics, are reviewed. Specific structures that affect the SID response are discussed and SID is compared with flame ionization, nitrogen–phosphorus and electron-capture detection. Many drugs of medico-legal interest can be determined by GC–SID with extremely high sensitivity and specificity. The high sensitivity may allow trace determintions of drugs present in small samples, such as blood stains and hair, extending its applicability in forensic toxicology.

Contents

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1. Introduction

In 1923, Kingdom and Langmuir [1] first observed the formation of positive caesium ions on an incandescent tungsten surface. When atoms or molecules impinge on an incandescent metal surface, they may evaporate partly as neutral particles and partly as positive or negative ions, as shown in Fig. 1. In 1985, Fujii and Arimoto [2] developed a surface ionization detection (SID) method for gas chromatography (GC), which detected positive ions formed on a hot platinum surface, and showed that it provided extremely high and specific responses to compounds containing tertiary amino groups.

This review demonstrates that many medico-legally important drugs can be determined by GC–SID with extremely high sensitivity and specificity. Recently, Fujii and Arimoto [3] have also published a review mainly on basic aspects of SID, such as its principles, theory and instrumentation.

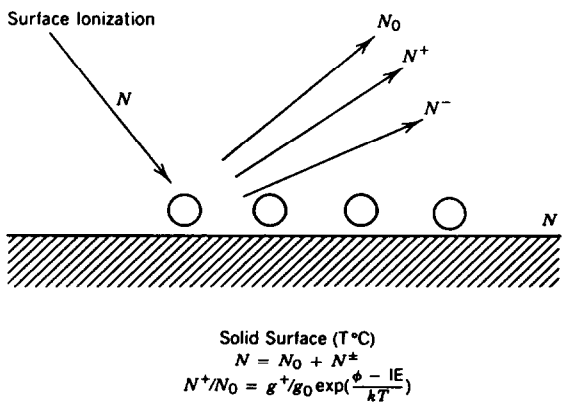

Fig. 1. Formation of neutral atoms and positive and negative ions on a hot metal surface. The surface with a layer of adsorbed particles of concentration N emits fluxes N_0 of neutral particles, N^+ of positive ions and N^- of negative ions. From ref. 3 (© 1992 Wiley).

2. Instrumentation for surface ionization detection

SID is a structural modification of standard nitrogen–phosphorus detection (NPD) for GC [2]; it consists of almost the same components as in NPD except that the alkali-salt bead emitter in NPD is replaced with a platinum emitter and the applied potential is reversed. A conventional flame ionization detection (FID) system might also be modified to a SID system if the platinum emitter is mounted in the gas flow path through the hole of detector envelope that is used for igniting the flame.

Fig. 2 shows the SID system develop by Fujii and Arimoto [2]. The platinum emitter is positioned between the quartz nozzle and the collector electrode. The ring electrode around the quartz nozzle is held at a positive potential of +200 V; the emitter and the ion collector are always at a negative potential of −200 V *versus* the ring electrode. Positive ion current directed to the collector is measured with an electrome-

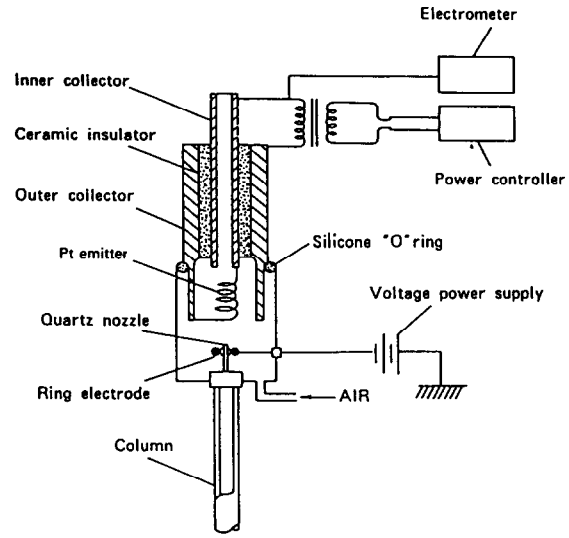

Fig. 2. SID system with a platinum emitter. From ref. 2 (© 1985 American Chemical Society).

ter. Electronics for variable heating of the emitter filament are required. The emitter is ten-turn coiled platinum (99.9%, 0.25 mm diameter), which is capable of withstanding temperatures around 1200°C for much longer than 1 month in the environment of a fast flow of a helium–air mixture. Platinum was chosen as an emitter material primarily because it has a higher work function (5.65 eV) than other typical refractory metals such as tungsten and rhenium. This property easily allows positive thermionic emission from the surface. The SID system is now commercially available from Shimadzu (Kyoto, Japan).

The SID conditions for capillary GC routinely used in our laboratories are as follows: heating current through the platinum emitter, 2.2 A; emitter temperature, *ca.* 600°C; and ring electrode bias voltage, +200 V *versus* the collector electrode.

3. Application to drug analyses

3.1. Tricyclic antidepressants

As a first study of the application of GC–SID, we tested the tricyclic antidepressants imipramine, amitriptyrine, trimipramine, chlorimipramine, desipramine, carpipramine, clocapramine and lofepramine [4]. Of these eight compounds, lofepramine, carpipramine and clocapramine, which had cyclic or aromatic side-chain structures, were found to give multiple peaks owing to heat decomposition in their underivatized form under medium-bore capillary GC conditions. Desipramine, with a secondary amino side-chain, showed much lower sensitivity. Therefore, the main experiments were performed on imipramine, amitriptyrine, trimipramine and chlorimipramine, which have tertiary amino groups with straight aliphatic side-chain structures, because they were relatively stable to heat.

Imipramine, amitriptyline, trimipramine and chlorimipramine (5 ng of each) were added to 1 ml of urine, plasma and whole blood, and extracted with Sep-Pak C_{18} cartridges. The four drugs were separated well from biological impurities on the gas chromatograms, but imipramine and trimipramine appeared overlapped with the use of an SPB-1 fused-silica capillary column (30 m × 0.32 mm I.D., film thickness 0.25 μm) (Fig. 3). The recovery of these drugs added to each body fluid was more than 60%.

The backgrounds obtained from plasma and whole blood were fairly clean, but that for urine showed many impurity peaks, which may be due to the excretion of many methylated metabolites of endogenous and exogenous amines in the urine; however, the drug peaks did not overlap any impurity peak in the urine extract. The baselines remained steady as the column temperature was increased (Fig. 3).

The four drugs showed satisfactory linearity in the range 10–80 pg in the injected volume. The detection limit (signal-to-noise ratio = 3) was 5–10 pg on-column (0.5–1.0 ng/ml in a sample).

Fujii *et al.* [5] also mentioned briefly that imipramine and chlorimipuramine can be determined with high sensitivity by GC–SID.

3.2. Phenothiazines

Phenothiazines are used widely as antipsychotics (major tranquillizers), anti-parkinsonism drugs and antihistaminics. They are frequently encountered in forensic chemistry and clinical toxicology because of their relatively small safety dose ranges.

We tested fourteen phenothiazines as shown in Table 1 [6], which gives their relative peak-area intensities measured 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 graphs of peak area *versus* drug amount were drawn for chlorpromazine, trimeprazine and promazine. The three drugs showed excellent linearity with r values of 0.9984–0.9999 in the range of 0.25–3 pmol on-column. The detection limits of these phenothiazines were *ca.* 5–10 pg on-column, which is equivalent to 250–500 pg/ml in a sample.

Addition tests were made with a non-polar

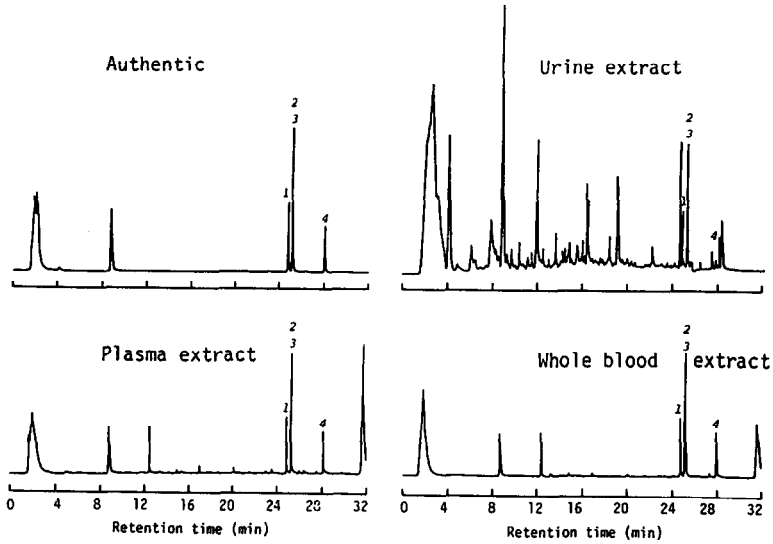

Fig. 3. Capillary GC–SID for tricyclic antidepressants extracted from urine, plasma and whole blood with the use of Sep-Pak C_{18} cartridges. Peaks 1 = amitriptyline; 2 = imipramine; 3 = trimipramine; 4 = chlorimipramine. GC was carried out with an SPB-1 fused-silica capillary column (30 m × 0.32 mm I.D., film thickness 0.25 μm). GC conditions: column temperature, increased from 100 to 280°C at 6°C/min; injection temperature, 200°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 2 min. A mixture of the four tricyclic antidepressants (5 ng of each) was added to 1 ml of urine, plasma or whole blood.

Table 1
Retention times and relative peak-area intensities of phenothiazines measured by GC–SID

Compound[a]	Retention time (min)	Relative peak-area intensity[b]
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.
[b] Chlorpromazine = 1.00.

capillary column for whole blood and urine, with and without the addition of 100 pmol each of ten phenothiazines to 1-ml whole blood and urine samples. Prochlorperazine, thiethylperazine, thioproperazine and thioridazine were omitted because of their low responses or long retention times. All drug peaks were well separated from impurity peaks except that of promethazine, which partially overlapped an impurity peak for the whole blood extract. The recoveries of the ten phenothiazines were more than 79% for both whole blood and urine samples. The baselines remained steady during the increase in column temperature.

3.3. Butyrophenones

Butyrophenones are classified as major tranquillizers and are widely used for the treatment of schizophrenia. They are also frequently encountered in forensic science practice. We found

that some drugs of this group showed a high response to SID [7]. The drugs tested were haloperidol, moperone, bromperidol and pipamperone. They were added (50 pmol of each) to 1 ml of whole blood or urine and extracted with Sep-Pak C_{18} cartridges. The four drugs were well separated from each other and from impurities with a DB-1 fused-silica capillary column (15 m × 0.32 mm I.D., film thickness 0.25 μm).

Excellent linearity was observed in the range of 0.2–4 pmol on-column for the four compounds. The detection limit of the drugs was about 40 pg (0.1 pmol) on-column.

Haloperidol was determined with this method in whole blood and urine samples obtained from a 76-year-old male schizophenic patient who had received 3 mg of haloperidol daily; the levels found were 7.18 and 43.2 pmol/ml for blood and urine, respectively.

3.4. Local anaesthetics

Local anaesthetics are occasionally encountered in forensic science practice, especially in medical accidents. Fujii *et al.* [5] reported that lidocaine, a typical local anaesthetic, could be determined with high sensitivity by GC–SID. We also tested lidocaine, procaine, dibucaine, ethyl *p*-aminobenzoate (benzocaine), tetracaine, mepivacaine, bupivacaine, propitocaine, oxybuprocaine and *p*-(butylamino)benzoic acid 2-(diethylamino)ethyl ester by this method [8,9]. All drugs were relatively stable to heat and did not decompose during capillary GC analysis. They can be separated from each other and from impurities with use of either a non-polar or intermediate-polarity capillary column [10].

The sensitivity of the local anaesthetics to SID varied considerably. The highest sensitivity was apparent with lidocaine, mepivacaine and bupivacaine. These three drugs showed excellent linearity of the calibration graphs in the range 10–100 pg on-column. The detection limits of the drugs were 5–10 pg on-column (0.5–1.0 ng/ml in a sample). The three drugs (5 ng of each) added to 1 ml of whole blood and cerebrospinal fluid (CSF) could be measured with excellent recoveries.

The sensitivity for procaine, dibucaine, tetracaine, oxybuprocaine and *p*-(butylamino)benzoic acid 2-(diethylamino)ethyl ester was *ca.* 20 times lower and that for ethyl *p*-aminobenzoate and propitocaine was *ca.* 100 times lower.

3.5. Narcotic analgesics and their analogues

Meperidine, a narcotic analgesic drug with a piperidine ring structure, has been reported to be determined with high sensitivity by GC–SID [11]. Meperidine, together with the internal standard (diphenylpyraline), was added to whole blood and urine and extracted with a Sep-Pak C_{18} cartridge. The compounds were determined by GC–SID was a DB-17 fused-silica capillary column (15 m × 0.32 mm I.D., film thickness 0.25 μm). The recovery of meperidine was close to 100% and the detection limit was 10 pg on-column.

Pentazocine has also been determined by GC–SID [12], although its sensitivity was lower than that of meperidine. Pentazocine, which had been added to whole blood and urine samples and extracted with a Sep-Pak C_{18} cartridge, could be detected with a good recovery of more than 90%. The detection limit of this drug was about 50 pg on-column. Extracts of whole blood and urine samples obtained 3 h after intramuscular injection of 15 mg of pentazocine showed intense peaks of the drug.

Dextromethorphan and dimemorphan, morphine analogue antitussives, could be determined with relatively high sensitivity by this method [13]. The detection limit of these drugs was about 20 pg on-column.

Suzuki *et al.* [14] found that fentanyl and its derivatives could be determined with high sensitivity by GC–SID, although their experiments were carried out only with authentic standards.

3.6. Diphenylmethane antihistaminics and their analogues

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 often abused;

fatal cases involving their ingestion are frequently encountered.

Most of the drugs contain tertiary amino groups and are very suitable for determination by GC–SID. We tested eleven drugs of this group [15] and obtained extremely high sensitivities for some of them. Table 2 gives the retention times and relative peak-height intensities of eleven diphenylmethane antihistaminics and their analogues (2 pmol of each injected). The response was highest with doxylamine, diphenhydramine, orphenadrine, chlorpheniramine, carbinoxamine and diphenylpyraline; their detection limits were 2–5 pg (*ca.* 6–20 fmol) on-column. The other drugs in Table 2 had sensitivities about ten times lower than those for the above six drugs.

The gas chromatograms for the extracts of whole blood and urine, with and without addition of 50 pmol each of the six drugs to 1-ml samples, with the use of a non-polar DB-1 fused-silica capillary column, showed satisfactory separation of the test peaks from each other and from impurities. The recovery of the drugs added to whole blood and urine was more than 60% with extraction with Sep-Pak C_{18} cartridges.

Table 2
Retention times and relative peak-height intensities of diphenylmethane antihistaminics and their analogues measured by GC–SID

Compound[a]	Retention time (min)[b]	Relative intensity[c]
Diphenhydramine	13.7	1.00
Doxylamine	14.3	1.19
Orphenadrine	14.7	0.84
Chlorpheniramine	15.5	0.66
Carbinoxamine	16.4	0.64
Diphenylpyraline	16.7	0.54
Terodiline	14.9	0.04
Benactyzine	18.7	0.21
Homochlorcyclizine	19.7	0.19
Clemastine	20.5	0.09
Pipethanate	21.0	0.13

[a] A 2-pmol amount of each drug was injected into the GC port.
[b] GC conditions as in Fig. 4.
[c] Diphenydramine = 1.00.

In all the chromatograms, baselines were also steady and did not rise with increase in column temperature.

3.7. Miscellaneous

Kawano *et al.* [16] reported the sensitive determination of aprindine, an antiarrhythmic drug, in serum; its detection limit was 16 pg.

We have recently obtained good results by GC–SID for cocaine, benzoylecgonine, atropine, antitussive drugs such as clobutinol, carbetapentane and oxeladin and paraquat after reduction.

4. Specific structures that affect the response of surface ionization detection

4.1. Tertiary amino groups

SID gives a high sensitivity to compounds with tertiary amino groups [2]. It seems that tertiary amino groups with straight side-chain structures give a slightly higher SID response than cyclic tertiary amino groups when the sensitivities for various types of phenothiazines (Table 1) and for diphenylmethane antihistaminics are compared (Table 2). It should be mentioned that compounds with N-alkylpiperidine rings, such as mepivacaine, bupivacaine [8], meperidine [11], butyrophenones [7] and fentanyl [14], give relatively high sensitivities.

Secondary amino compounds, such as terodiline [15] and propitocaine [8], gave sensitivities 25–100 times lower than tertiary amino compounds.

4.2. Amide groups

A nitrogen atom directly bound to a carbonyl group does not give a high SID response; we failed to detect barbiturates, benzodiazepines, pyrazolones and hydantoins, all of which contain amide groups, by GC–SID with high sensitivity (unpublished observations). However, a carbonyl group present in the β-position to the nitrogen of a tertiary amino group showed no negative effects for SID; this conclusion is based on the

very high sensitivity observed for lidocaine, bupivacaine and mepivacaine, which contain amide structures in their side-chains together with tertiary amino groups [8].

4.3. Halogen groups

Chlorpromazine and triflupromazine, which contain halogen groups on the phenothiazine nucleus, showed lower responses than promazine (Table 1). The same is true when the data for prochlorperazine and trifluoroperazine are compared with those for perazine. Chlorpheniramine and carbinoxamine gave lower responses than diphenhydramine and doxylamine (Table 2). Hence, the presence of a halogen group may lower the SID response. This is not surprising because of the electrophilic properties of halogen groups.

4.4. Sulphur groups

Thiethylperazine and thioproperazine, which contain sulphur groups on the phenothiazine nucleus, showed the lowest sensitivity, suggesting negative effects of sulphur groups for SID (Table 1).

5. Comparison with other detectors for gas chromatography

5.1. Flame ionization detection

Compounds with tertiary amino groups could be determined by GC–SID with a sensitivity 100–1000 times higher than that of conventional FID for tricyclic antidepressants [4], local anaesthetics [8], dextromethorphan, dimemorphan [13] and diphenylmethane antihistaminics [15].

5.2. Nitrogen–phosphorus detection

In each study of the GC–SID of drugs with tertiary amino groups [4,6–9,11–13,15], the sensitivity of SID was carefully compared with that of NPD either on the basis of our own experiments or literature data. In view of the signal-to-

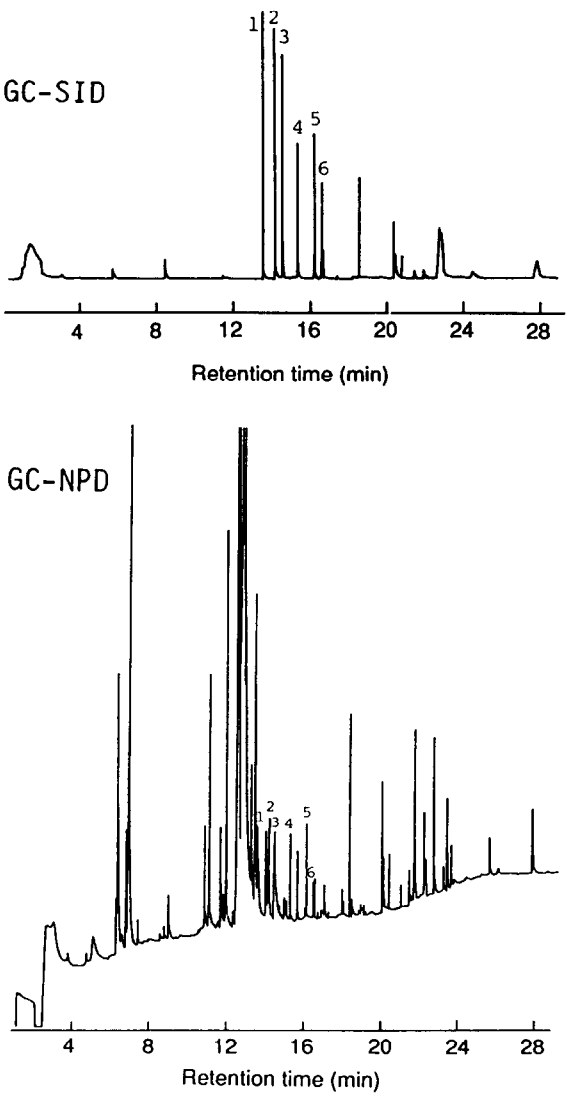

Fig. 4. Comparison of GC–SID with GC–NPD for a human whole blood extract in the presence of six diphenylmethane antihistaminics (50 pmol of each for 1-ml samples) with the use of Sep-Pak C_{18} cartridges for extraction. Peaks: 1 = diphenhydramine; 2 = doxylamine; 3 = orphenadrine; 4 = chlorpheniramine; 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). GC conditions: column temperature, increased 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 drug mixture of 50 pmol of each was added to 1 ml of whole blood. Both chromatograms were obtained with samples from the same extract vial.

Table 3
Summary of determinations of drugs by GC–SID without derivatization

Drug	Sample[a]	Extraction	Elution solvent	GC column[b]	Detection limit (on-column) (pg)	Ref.
Tricyclic antidepressants						
Amitriptyline, chlorimipramine, imipramine, trimipramine	WB, P, U	Sep-Pak C_{18}	Chloroform–2-propanol (9:1)	SPB-1 (100–280°C)	5–10	4
Phenothiazines						
Chlorpromazine, ethopropazine, isothipendyl, levomepromazine, promazine, promethazine, triflupromazine, trimepromazine	WB, U	Sep-Pak C_{18}	Chloroform–acetonitrile (8:2)	DB-1 (120–280°C)	5–10	6
Perazine, trifluoperazine	WB, U	Sep-Pak C_{18}	Chloroform–acetonitrile (8:2)	DB-1 (120–280°C)	25–50	6
Butyrophenones						
Bromperidol, haloperidol, moperone, pipamperone	WB, U	Sep-Pak C_{18}	Chloroform–ethanol (9:1)	DB-1 (180–300°C)	40	7
Local anaesthetics						
Bupivacaine, lidocaine, mepivacaine	WB, CSF	Sep-Pak C_{18}	Chloroform–methanol (9:1)	Ulbon HR-1 (100–280°C)	5–10	8
Benoxinate, dibucaine, procaine	WB, CSF	Sep-Pak C_{18}	Chloroform–ethanol (4:1)	DB-17 (120–180°C)	100–200	9
Narcotic analgesics and analogues						
Meperidine	WB, U	Sep-Pak C_{18}	Chloroform–ethanol (9:1)	DB-17 (100–220°C)	10	11
Pentazocine	WB, U	Sep-Pak C_{18}	Chloroform–ethanol (9:1)	DB-17 (150–280°C)	50	12
Dextromethorphan, dimemorphan	WB, U	Sep-Pak C_{18}	Chloroform–ethanol (9:1)	DB-17 (100–280°C)	20	13
Fentanyl and derivatives	Authentic standard	–	–	CBP-1 (100–320°C)	5	14
Diphenylmethane antihistaminics and analogues						
Carbinoxamine, chlorpheniramine, diphenhydramine, diphenylpyraline, doxylamine, orphenadrine	WB, U	Sep-Pak C_{18}	Chloroform–methanol (8:2)	DB-1 (100–280°C)	2–5	15
Benactyzine, clemastine, homochlorcyclizine, pipethanate, terodiline	WB, U	Sep-Pak C_{18}	Chloroform–methanol (8:2)	DB-1 (100–280°C)	20–50	15
Other						
Aprindine	Serum	Ethyl acetate–hexane (9:1)	–	DB-17 DB-1 (240°C)	16	16

[a] WB = Whole blood; P = plasma; U = urine.
[b] Fused-silica capillaries.

noise ratio and baselines, the sensitivity of GC–SID is 10–100 times higher than that of GC–NPD. A typical comparison of SID with NPD for diphenylmethane antihistaminics is shown in Fig. 4; the two traces were obtained with the same sample. In the SID chromatogram, the baseline was steady and did not rise with increase in column temperature, whereas in the NPD chromatogram many large impurity peaks appeared at various stages of the GC measurements and the baseline fluctuated and rose slightly with increase in column temperature.

Compounds with secondary or primary amino groups can probably be determined with higher sensitivity by GC–NPD than by GC–SID.

5.3. Electron-capture detection (ECD)

GC–ECD often gives a very high sensitivity comparable to that of GC–mass spectrometry with selected-ion monitoring for compounds with halogen or nitro groups. GC–SID gave a sensitivity comparable to or even higher than that given by GC–ECD for haloperidol [7] and chlorpheniramine [15].

6. Conclusions

GC–SID is a recent method developed in 1985 [2] and its application to drug analyses has begun. A brief summary of the applications is given in Table 3, which covers many drugs important in forensic science practice. GC–SID is especially recommendable for the determination of compounds with tertiary amino groups without halogen or nitro groups. The extremely high sensitivity of this method may also allow trace determinations of drugs present in small samples, such as blood stains and hair, extending its applicability in forensic toxicology.

7. Acknowledgements

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