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Note

Detection of tricyclic antidepressants in body fluids by gas chromatography with a surface ionization detector

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Surface ionization detection (SID) for gas chromatography (GC) was first introduced by Fujii and Arimoto in 1985 [1]. It is extremely specific and sensitive to organic compounds, such as secondary and tertiary amines, that form their dissociative species at a low ionization potential. Very recently, Suzuki et al. [2] have tried detection of the authentic fentanyl and its methylated derivatives by GC–SID. However, to our knowledge, this method has never been applied to biological samples. In this brief report, we present data on GC–SID for some tricyclic antidepressants extracted from human urine, plasma and whole blood.

EXPERIMENTAL

Materials

Imipramine·HCl, chlorimipramine·HCl and desipramine·HCl were obtained from Ciba-Geigy (Basel, Switzerland), carpipramine·HCl and clocapramine·HCl from Yoshitomi Pharmaceutical (Osaka, Japan), amitriptyline·HCl from Yamanouchi Pharmaceutical (Tokyo, Japan), trimipramine

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maleate from Shionogi (Osaka, Japan) and lofepramine·HCl from Daiichi Seiyaku (Tokyo, Japan). Sep-Pak C_{18} cartridges were purchased from Waters Assoc. (Milford, MA, U.S.A.). Other common chemicals used were of the highest purity commercially available. Urine, plasma and whole blood were obtained from healthy subjects.

Isolation with Sep-Pak C_{18} cartridges

Drugs were extracted on Sep-Pak C_{18} cartridges according to Suzuki et al. [3]. For pretreatment of a cartridge, 10 ml of chloroform–2-propanol (9:1), 10 ml of acetonitrile and 10 ml of distilled water were passed through it.

A 1-ml volume of urine, plasma or whole blood containing tricyclic antidepressants was mixed with 1 ml of 1 *M* sodium bicarbonate and 2 ml of water, and loaded on a Sep-Pak cartridge; in the case of whole blood, the mixture was centrifuged at 500 *g* for 5 min and the supernatant was used for the next procedure. The sample solution was poured into the pretreated cartridges at a flow-rate not greater than 5 ml/min. It was washed with 10 ml of water, and finally 3 ml of chloroform–2-propanol (9:1) were passed through it to elute the antidepressants, 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 1-μl aliquot was subjected to GC analysis.

GC conditions

A Shimadzu GC-15A instrument equipped with a SID system with a fused-silica SPB-1 capillary column (30 m×0.32 mm I.D., film thickness 0.25 μm, Supelco, Bellefonte, PA, U.S.A.) and a split–splitless injector was used. The GC conditions were: column temperature, 100–280°C (6°C/min); injection temperature, 200°C; helium flow-rate, 22.0 cm/s. The SID conditions were: platinum emitter current, 2.2 A; emitter temperature, ca. 600°C; ring electrode bias voltage, +200 V with respect to the collector electrode. The samples were injected in the splitless mode at 100°C of the column temperature and splitter was opened after 2 min.

RESULTS AND DISCUSSION

Although eight tricyclic antidepressants were tested for GC–SID, desipramine, lofepramine, carpipramine and clocapramine were found to give multiple peaks owing to heat decomposition in their underivatized forms under the present conditions. Thus the main experiments were performed on imipramine, amitriptyline, trimipramine and chlorimipramine, which were relatively heat-stable. Fig. 1 shows gas chromatograms with SID for 5 ng each of these

four compounds, which had been added to 1 ml of urine, plasma and whole blood and extracted with Sep-Pak C_{18} cartridges. The four drugs were separated from biological impurities on the gas chromatograms, but imipramine and trimipramine appeared overlapped under the present conditions. The recoveries of the tricyclic antidepressants added to each body fluid were more than 60%.

To check the backgrounds, the three body fluids, 1 ml of each, were treated as above in the absence of drugs and subjected to GC–SID (Fig. 2). The backgrounds obtained from plasma and whole blood were fairly clean, but that for urine showed many impurity peaks; fortunately the drug peaks did not overlap any impurity peak in the urine extract (Fig. 1). The baselines remained steady as the column temperature was increased (Figs. 1 and 2).

Fig. 3 shows calibration curves for the four drugs. They showed satisfactory linearity in the range 10–80 pg in an injected volume. The detection limit of each drug was 0.5–1.0 ng per ml of sample (5–10 pg in an injected volume). The detection limits for desipramine, lofepramine, carpipramine and clocapramine were also low and not more than 1 ng in an injected volume, though they decomposed and gave multiple peaks.

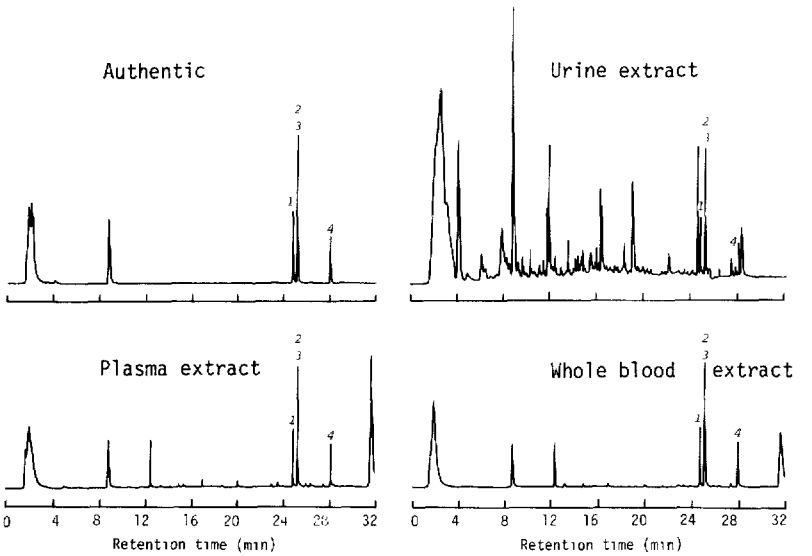

Fig. 1. Capillary GC–SID for tricyclic antidepressants extracted from urine, plasma and whole blood with use of Sep-Pak C_{18} cartridges. Peaks: 1 = amitriptyline; 2 = imipramine; 3 = trimipramine; 4 = chlorimipramine GC was carried out with a SPB-1 fused-silica capillary column (30 m × 0.32 mm I.D., film thickness 0.25 μm). The GC conditions were: column temperature, 100–280°C (6°C/min); injection temperature, 200°C; helium flow-rate, 22 cm/s. The samples were injected in the splitless mode at 100°C column temperature, and splitter was opened after 2 min. The mixture of four tricyclic antidepressants (5 ng each) was added to 1 ml of urine, plasma or whole blood.

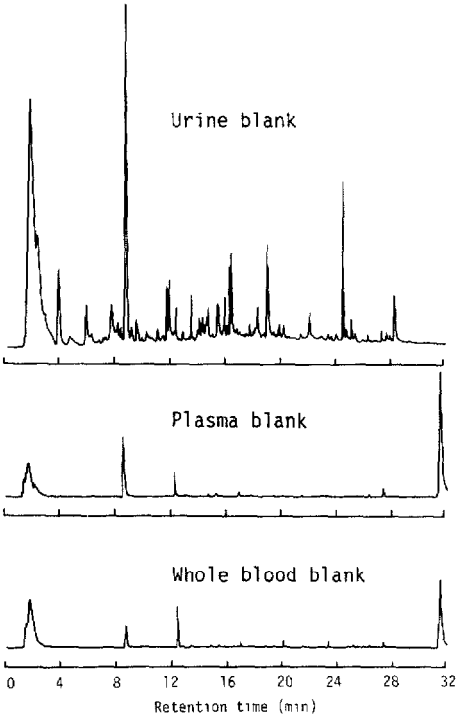

Fig. 2. Capillary GC–SID showing backgrounds obtained from human urine, plasma and whole blood (1 ml of each) in the absence of tricyclic antidepressants. GC conditions were as specified in Fig. 1.

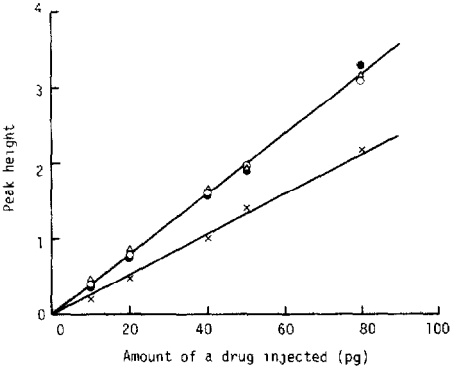

Fig. 3. Calibration curves by GC–SID for tricyclic antidepressants. (△) Amitriptyline; (○) imipramine; (●) trimipramine; (×) chlorimipramine. GC conditions were as specified in Fig. 1. The equations and r values were: $y=0.03842x+0.08033$, $r=0.9992$ for amitriptyline; $y=0.03867x+0.03333$, $r=0.9997$ for imipramine; $y=0.04153x-0.08533$, $r=0.9985$ for trimipramine; $y=0.02887x-0.1127$, $r=0.9983$ for chlorimipramine.

In this study, we have been able to detect some tricyclic antidepressants present in human body fluids with extremely high sensitivity by GC–SID. Data on SID are very scant at present, because it was developed only in 1985 [1] and is not yet in widespread use. However, the necessary equipment is neither complicated nor expensive; it is a modification of the standard thermionic ionization detector (TID) [1]. SID provides extremely sensitive and specific responses to compounds with secondary or tertiary amino groups in their structures. We carefully compared the sensitivity of SID with that of TID for the present tricyclic antidepressants, and found that SID was about ten times as sensitive as TID (unpublished observation) and about 2000 times as sensitive as flame ionization detection with a packed column [4].

The backgrounds for plasma and whole blood were much cleaner than that for urine (Fig. 2). This is probably due to the excretion of many methylated metabolites of amines into urine. In this respect, SID is more suitable for plasma or blood samples than for urine samples.

Probably a number of other drugs, poisons and biogenic amines in biological samples could be analysed by GC–SID with extremely high sensitivity and selectivity. Many studies with this detector should be possible.

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