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Note

Detection of low nanogram quantities of phencyclidine extracted from human urine

Preparation of an acetylated column packing material for use in gas chromatography with nitrogen–phosphorus detection

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The detection of low nanogram levels of phencyclidine [PCP, 1-(1-phenylcyclohexyl)piperidine] extracted from urine has been difficult to achieve with conventional gas chromatography (GC). In order to detect this drug below 100 ng/ml, it has been necessary to use high-cost mass spectrometry in conjunction with GC [1,2]. GC with non-heat-treated columns and flame ionization detection have been shown to have detection limits above 100 ng/ml [3–7], while this paper describes a technique that enables PCP detection at 15 ng/ml.

In order to increase sensitivity of GC systems to alkaloids, it is imperative to reduce the adsorptive properties of the column packing support material [8–10]. Street et al. [11] demonstrated that it was possible to detect 50 ng of morphine in a 1-μl extract of putrefied post-mortem urine, but a heat-treated acylated support and nitrogen–phosphorus detection (NPD) was required.

The purpose of this paper is to show that PCP may be detected at 15 ng/ml using GC–NPD and acetylated Chromosorb W AW-DMCS (80–100 mesh) packing material. A nitrogen–phosphorus detector was chosen for its selectivity toward nitrogen-containing organic compounds. This detector has been shown to be ten to fifty times more sensitive to alkaloids than flame ionization

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detectors [12]. The heat-treated acylated support remained sensitive to PCP for approximately one year at column temperatures of 230°C. The column packing material was prepared by a modification of the method of Street et al. [11].

EXPERIMENTAL

Chromosorb W AW-DMCS, 80–100 mesh, was purchased from Varian Assoc. (Dallas, TX, U.S.A.). Potassium hydroxide, hydrochloric acid, sulfuric acid, and benzene (thiophene-free) were purchased from J.T. Baker (Phillipsburg, PA, U.S.A.). Pyridine, sodium hydroxide, and diethyl ether were purchased from Fisher Scientific (Chicago, IL, U.S.A.). Acetic anhydride and acetone were purchased from Malinckrodt (St. Louis, MO, U.S.A.). Ethanol was purchased from U.S. Industrial (San Antonio, TX, U.S.A.). Ketamine·HCl was purchased from Parke-Davis (Morris Plains, NJ, U.S.A.). Phencyclidine·HCl was purchased from Applied Science Lab. (State College, PA, U.S.A.). The GC system was purchased from Perkin-Elmer (Houston, TX, U.S.A.).

Gas chromatographic system

All analyses were performed on a Perkin-Elmer Sigma 1 GC system. The glass column was 91.4 cm in length, with inner and outer diameters of 2 mm and 6.25 mm, respectively. The injector port and nitrogen–phosphorus detector were each set at 250°C. The column temperature was set at 230°C. The bead potentiometer was set at 500. The air pressure was set at 1.79 bar, while the hydrogen gas pressure was set at 0.69 bar. The flow-rate of the carrier gas (nitrogen) was kept constant at 30 ml/min. The attenuation and the chart speed were set at 1 and 10 mm/min, respectively.

Treatment of support for column

Chromosorb W AW-DMCS (40 g) was washed in 0.35 mol/l potassium hydroxide and allowed to stand for 1 h (20 g support per 80 ml potassium hydroxide). This mixture was boiled for 10 min and rinsed with distilled water. The distilled water was decanted from the support material and the support air-dried. A pyridine–acetic anhydride mixture (3:2, v/v) (100 ml) was added to the dried support. This mixture was allowed to stand at room temperature for 48 h. The support was rinsed with acetone until the pyridine odor was removed. The acetylated support was allowed to air-dry. 10% SE-30 in benzene (3:2, v/v) was added to the dried support. This mixture was allowed to stand for 48 h at room temperature. The SE-30-coated support was air-dried at room temperature. Post-drying, the coated support was transferred to a 20 cm × 4 cm I.D. glass column. The glass tube was wrapped in aluminum foil and heated

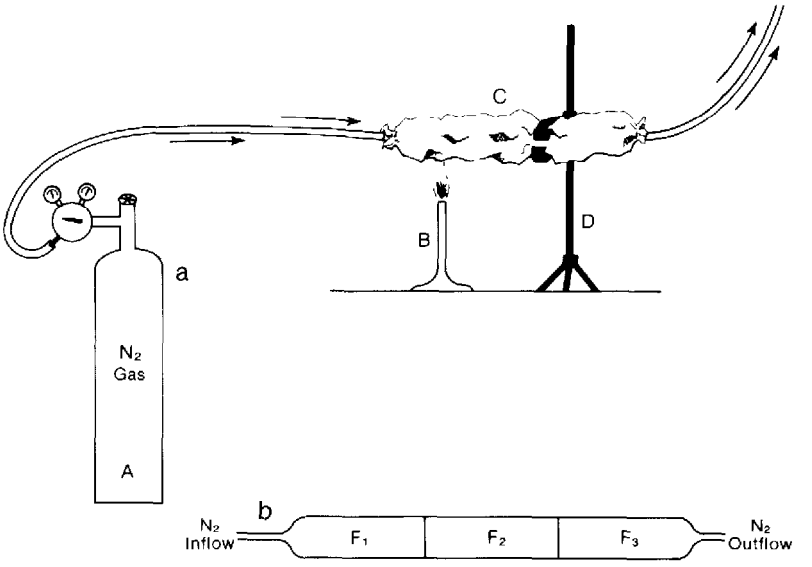

Fig. 1.(a) Method used to heat-treat the acetylated support under nitrogen gas flow. The arrows indicate the direction of the gas flow. A = Nitrogen gas cylinder; B = bunsen burner; C = glass tube filled with acetylated support; D = ring stand and clamp. (b) Method by which the heat-treated acetylated support was divided into three fractions. The fraction nearest to the nitrogen gas inflow was assigned 1 The middle one third was assigned 2, while the latter one third was assigned 3.

under a flame for 1 h as nitrogen flowed through the column (see Fig. 1a). The heat-treated SE-30 acetylated support was divided into three fractions as described by Street [8]. The fractions were labeled in one thirds as shown in Fig. 1b. Fraction 1 was closest to the nitrogen gas inflow, while fraction 3 was farthest from this inflow.

Treatment of the glass column

The glass column was treated in a similar manner as the support material. Initially, the glass column was cleansed in concentrated hydrochloric acid, washed with distilled water, rinsed with acetone, and air-dried. The column was filled with a 3:2 (v/v) mixture of pyridine and acetic anhydride. Air bubbles were removed and the column stoppered for three days at room temperature. The column was emptied, rinsed with benzene, and air-dried in an oven at 100°C for 1 h.

Consolidation of acetylated support and glass column

The dry prepared column and the fractionated support material were combined and treated as described by Street [8]. Briefly, the column was filled with fraction 1 and heat-treated for 1 h at 400°C in the GC system. The column was emptied and refilled with fraction 3. This fraction 3 column was attached to the Sigma 1 system and heated to 350°C, while nitrogen gas flowed through

the column. When a stable baseline was obtained, the column temperature was reset at 230°C and the column was ready for use.

PCP extraction procedure from human urine

PCP was added to drug-free urine, obtained from human volunteers. The final PCP concentration in the urine prior to the extraction was 50, 25, and 15 ng/ml. The extraction of PCP from the urine was as described briefly in the next paragraph.

The pH of 10 ml of the PCP urine was adjusted between 6.5 and 7.0. Ketamine·HCl (0.3 ml of 100 μg/ml) was added to the pH-adjusted urine. Ketamine served as the internal standard for this procedure. Diethyl ether (20 ml, an-

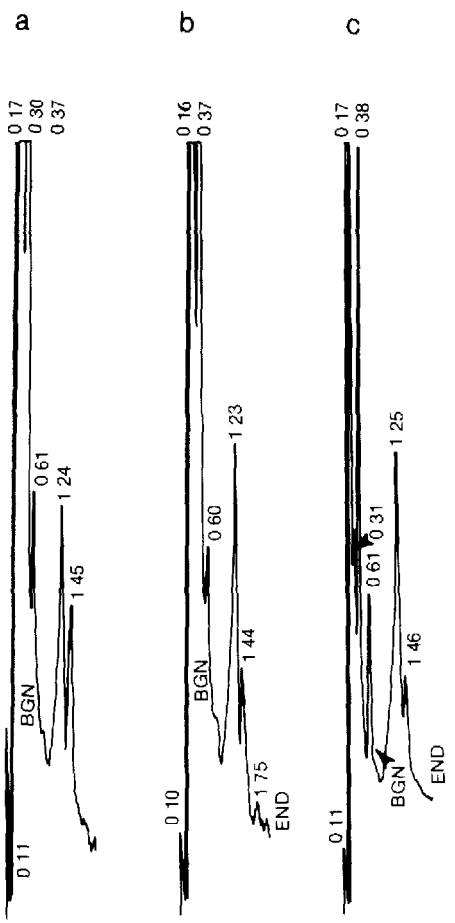

Fig. 2 Chromatograms representing three different extracted concentrations of PCP from urine. The concentrations of PCP are 50 ng/ml (a), 25 ng/ml (b), and 15 ng/ml (c). The internal standard peak (ketamine) was registered between 1.19 and 1.27 min with PCP following 0.2 min later.

hydrous) was added to the above mixture and it was placed on a shaker for 10 min. Sulfuric acid (0.5 mol/l, 5 ml) was added to the ether layer and it was laced on a shaker for 10 min. Sodium hydroxide (5 mol/l, 2 ml) was added to the acid layer, followed by 5 ml of anhydrous diethyl ether. This mixture was shaken for 10 min. The ether layer was placed in a 30–50°C water bath and evaporated to dryness. The residue was reconstituted in 100 μl of absolute ethanol. The samples were at this time ready for analysis by GC. Aliquots of 1 μl of the reconstituted samples were injected into the Sigma 1 system.

RESULTS

The typical chromatographic pattern obtained from PCP analysis using the procedure described in the Experimental section is shown in Fig. 2. All three chromatograms exhibit excellent separation of ketamine and PCP. In addition, the chromatograms are devoid of extraneous peak interference. Thus, the extraction procedure was found to provide excellent isolation of ketamine and PCP, while maintaining an outstanding analysis time of only 2 min per PCP test. The retention times for ketamine were between 1.19 and 1.27 min with PCP following approximately 0.2 min later. The short analysis time was made possible by the 230°C column temperature. Ketamine exhibited no peak interference with PCP when it was analyzed in the absence of PCP. In addition, PCP did not interfere with the ketamine reference peak (data not shown).

DISCUSSION

The method described in this paper demonstrates that PCP may be detected at 15 ng/ml using conventional GC–NPD and a special acetylated column packing material. The acetylated column and packing material aid in the sensitivity of the method toward identifying PCP extracted from urine. The column sensitivity is most likely due to reduced hydrogen bonding between the support and PCP and ketamine. Street et al. [11] indicated that acylation of the diatomaceous earth eliminated one of two types of hydrogen bonding inherent in the support material. The first is the Si–OH terminal hydroxy group, which is capable of acting as a proton donor for hydrogen bonding [11]. The second type of hydrogen bonding occurring within the column is the Si–O–Si functional group, which acts as a proton acceptor. This interaction was deemed not to be possible due to the high molecular weight of the compounds involved in this study (PCP and ketamine). Street et al. [11] had shown previously that high-molecular-weight alkaloids such as morphine do not form hydrogen bonds with the Si–O–Si functional moiety due to steric hindrance.

This PCP procedure should be employed by drug detection laboratories, which are using GC as the confirmatory method of drug identification. There are two major advantages for using the method described in this paper. First,

the analysis time is only 2 min per specimen. Thus, a great deal of time can be saved per analysis, which will free technicians for other projects in the laboratory. Second, the column life is extraordinarily long. Once a stable baseline is achieved, the column will maintain its sensitivity toward PCP for approximately one year. The column was designated as stable when the drift from the chart recorder was eliminated for the Sigma 1 system. Therefore, maintenance is kept to a minimum and productivity is enhanced, since time is not lost in preparing new columns monthly. Although different batches of packing material will vary in the time required to achieve a stable baseline, the overall sensitivity to PCP was found to be similar (data not presented) between batches. The major disadvantage is the time required to prepare the column, but the quick analysis time and long column life more than compensate for this deficit.

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