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Detection and Identification of Methylphenidate in Human Urine and Blood Samples

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A method for the detection and identification of methylphenidate (Ritalin ®) in human urine and blood was worked out. The procedure was especially designed for cases in which a high degree of specificity is needed. Extracts from samples are examined by thin-layer and gas chromatography. The procedure also permits the determination of a metabolite of methylphenidate, α-phenyl-2-piperidine acetic acid.

The method has been applied to individuals, who have been driving under the influence of the drug; the results of these analyses are reported. In urine the concentration of the unchanged drug exceeded in most cases that of the metabolite. This is in contrast to the reports on animal experiments by other authors who found that only very small amounts, if any, of the parent drug were excreted.

Methylphenidate, methyl-α-phenyl-2-piperidine acetate (MePPA), was synthetized in 1944 by Panizzon [1] from benzyl cyanide and 2-chloropyridine. It is marketed as the hydrochloride (Ritalin ®, Centedrin ®, Aktilin ®). The pharmacological properties are similar to those of amphetamine and other phenyl-isopropylamines, but methylphenidate differs from these in having only moderate effects on the periferal circulatory system.[2] As seen in Fig. 1, two asymmetric carbon atoms are present. Only one racemate, however, is therapeutically active and has the *threo* configuration, as shown by Weisz and Dudas.[3] In the manufacturing process this racemate is obtained in pure form.[4,5]

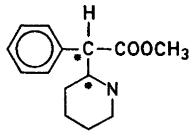

Fig. 1. Methylphenidate, methyl-α-phenyl-piperidine acetate (MePPA), $C_{14}H_{13}NO_2$.*: Asymmetric carbon atoms.
Properties: b.p.$_{0.6}$ = 135–137°, alkaline compound, pK_a = 0.8,[12] crystalline HCl-salt, m.p. = 208–209°.

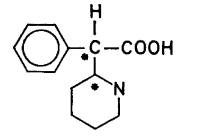

Fig. 2. α-Phenyl-2-piperidine acetic acid (HPPA).*: Asymmetric carbon atoms, Properties: as HCl-salt: m.p. = 234–236° (*threo* racemate), pK values: *threo* form 7.6, *erythro* form 6.6–6.7.

The agent is used in the therapy of narcolepsy and some milder psychiatric disorders, and also in counteracting the sedative action of, *e.g.*, antiallergic drugs. As it has some euphoric properties, especially when taken intravenously, the abuse of the drug is common in many countries, notably in Sweden. Methylphenidate is now listed in this country as a narcotic and is prescribed very sparingly.

Under these circumstances a procedure was needed for detecting and identifying methylphenidate in hospital patients or in car drivers, who are suspected of misusing the drug.

MATERIALS AND METHODS

Apparatus and reagents. The gas chromatographic equipment consisted of a Perkin-Elmer F11 instrument with flame ionization detector and a borosilicate glass column 5′ × 1/4″ O.D. (about 3 mm I.D.) packed with 2 % Hi Eff 3A (neopentyl glycol adipate, from Applied Science Laboratories, State College, Pa., U.S.A.) on Gas Chrom Q (100—120 mesh). The column packing was prepared by dissolving the polyester in chloroform and adding the support material to the solution. The solvent was then evaporated in vacuum. The packed column was conditioned by passing nitrogen through it for 12 h at 245°. During the analysis the column temperature was maintained at 140° and the injection port at about 220°. Nitrogen (carrier gas) and hydrogen were supplied at flow velocities of about 75 ml/min.

Thin-layer chromatography was carried out on glass plates coated with about 0.3 mm aluminium oxide. The plates were developed with propanol – buffer solution pH 4.0 (9+1). The buffer solution was a mixture of 30.7 volumes aqueous citric acid (0.1 M) and 19.3 volumes Na_2HPO_4 (0.2 M). After spraying with iodoplatinate reagent,[6] MePPA gave a dark violet spot with an R_F value of 0.8 at room temperature.

As a reference standard the free base prepared from methylphenidate hydrochloride was used (Ciba Prod. No. 7254, Ord. No. 093355).

Mallinckrodt analytical reagent ether was used throughout, and other reagents were also of analytical grade. Diazomethane was prepared from nitrosomethylurea.[7]

The analytical procedure involves the extraction of MePPA from alkalinized material with ether. HPPA remains in the water layer and is transformed into the methyl ester (= MePPA) with the aid of diazomethane. The MePPA thus formed is extracted with ether, and each of the two extracts is subjected to gas and thin-layer chromatography.

Analytical procedure. 1—3 ml of urine or about 10 ml of blood is acidified with a few drops of concentrated hydrochloric acid to a pH of less than 4 and extracted with 2 × 3 volumes of ether. The ether layers are discarded. The pH of the aqueous phase is adjusted to about 11 with solid sodium carbonate, and the solution is shaken with 3 × 3 volumes of ether. The organic layers are pooled, dried with anhydrous sodium sulfate and evaporated under an air-stream at slightly elevated temperature. The residue is dissolved in ethanol to a known volume (0.5—1.0 ml).

The aqueous solution is acidified with N hydrochloric acid to pH about 3 and evaporated to dryness *in vacuo*. The residue is suspended in about 2 ml of ethanol and cooled. Diazomethane in ether is added. After 5 min a drop of glacial acetic acid is added. Diazomethane must be present in excess (bubbles of nitrogen gas are formed). The mixture is again evaporated, the residue dissolved in about 2 ml of water and ether extraction is again carried out at acidic and alkaline pH as already described above.

Aliquots of 1—5 μl from the two extracts as well as standard solutions of MePPA (0.050—0.010 mg/ml) are injected into the gas chromatograph. The peak heights are used for *quantitative* determination. For *identification* the retention times, both of the compound itself and of its *N*-acetyl derivative, are employed. This derivative is formed inside the gas chromatograph by first injecting the sample and then after 5 sec or less, 2 μl of acetic anhydride.[8]

Aliquots containing about 10 μg of MePPA are chromatographed on thin layer plates under the condition mentioned above.

RESULTS AND DISCUSSION

The analytical procedure. The purpose of this investigation was to develop a method of analysis which is rapid and simple rather than highly accurate. However, a considerable degree of specificity was considered necessary and was achieved by a combination of thin layer and gas chromatography together with the formation of the *N*-acetyl derivative. In many cases extraction of the unchanged MePPA only, with subsequent gas chromatography is sufficient.

In a few experiments 1—10 μg MePPA was added to one milliliter of urine and run through the procedure; the recovery was almost quantitative.

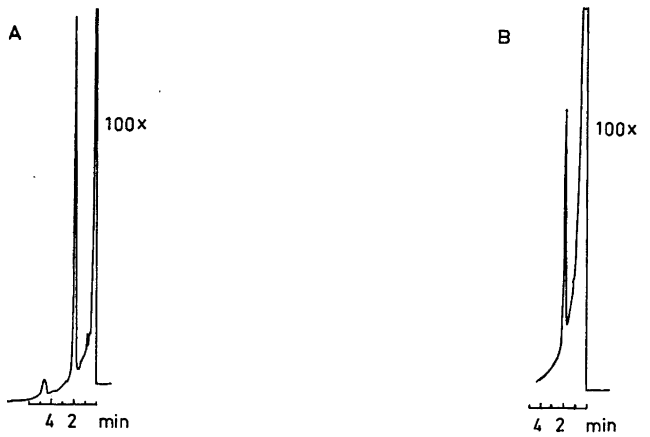

Fig. 3. Gas chromatogram of an extract from the urine of a person, who had been driving a car under the influence of the drug (A), as well as of pure methylphenidate (solution of the base in cyclohexane, 0.05 mg/ml; B).

Fig. 3A shows the actual chart from a gas chromatographic analysis of an extract from urine, originating from a case of driving under the influence of the drug. Fig. 3B shows the analysis of pure MePPA, and Figs. 4A and 4B show the corresponding results after acetylation.

Excretion of methylphenidate. The author could not find any reports about the excretion of methylphenidate or its metabolites in humans. Bernhard *et al.*[9] could not find any unchanged MePPA in the urine of rats, which had received about 50 mg MePPA per kg body weight. In a corresponding experiment with guinea pigs only small amounts of unchanged drug were found in the urine.[10] Both groups of authors used labelled MePPA (carboxyl-^{14}C); during 24 h the rats had excreted 90 % and the guinea pigs about 70 % of the original radioactivity. About one half of this radioactivity was found to represent the hydrolysis product of MePPA, namely α-phenyl-2-piperidine acetic acid (HPPA; see Fig. 2). The remaining part of the radioactivity represented more water soluble metabolites, some of them probably glucuronide conjugates, but their structures were not established.

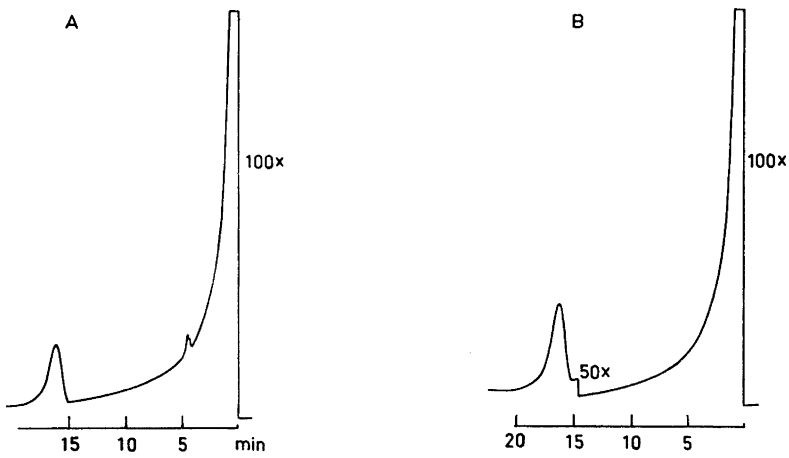

Fig. 4. Gas chromatograms of the same samples as in Fig. 3. Within 5 sec from injecting the sample, 2 μl of acetic anhydride was injected. The peak represents the *N*-acetyl derivative of methylphenidate from the urine extract (A) and the pure standard solution (B), respectively.

Table 1. Methylphenidate (MePPA) and α-phenyl-2-piperidine acetic acid (HPPA) in urine samples from drivers, analyzed in 1968. The concentrations are expressed as μg of methylphenidate per ml of urine.

Analysis No.	Case history; Patient's report	Examinating physiscian's report	Concentration in the urine MePPA	HPPA
1	Took 50 tabl. Ritalina 1 h before sampling	Drowsiness, no orientation in time	14	—[a]
2	Took 25 tablets 9 h before sampling	Hyperactivity	40	—[a]
3	Injected dissolved tablets 24 h before sampling	Hyperactivity	25	35
4	Took tablets about 12 h before sampling	Bad performance of balance tests	11[b]	1.6[b]
5	Injected dissolved tablets (20)	Drowsiness, dilatation of pupils of the eye	0.8	—[a]
6	Injected dissolved tablets	Drowsiness	4.9	0.6

[a] Not determined.
[b] Corresponding values for blood were 0.5 and 2.5, respectively.

In the present study, some analyses were made of blood and urine from humans suspected of having ingested or injected methylphenidate. Positive cases are shown in Table 1. This table shows that the urine contains considerable amounts of unchanged drug. Together with the low percentage of HPPA found, this is in great contrast to the above mentioned reports. As it was not possible in the present investigation to obtain urine, which had been quantitatively collected during a longer period of time, no conclusive data about the excretion of MePPA and HPPA could be obtained. However, it seems likely that an essential difference exists between the fate of the drug in humans on one hand and in rats and guinea pigs on the other. Whether this difference depends upon man's lower ability to metabolize MePPA, or upon the rapid elimination through the kidneys before the drug has been metabolized to any greater extent, is yet an open question. The excretion of amphetamine can be mentioned for a comparison; the bulk of an ingested dose is excreted unchanged by man during 48 h if the urine is acid. The corresponding time interval for rats is considerably lower. An important parameter for the excretion rate of this and many other drug is the pH of urine; Beckett and Rowland [11] found that man excreted about 60 % of an ingested dose of amphetamine during 16 h when the urine was acid (pH about 5), but only 3 % if the urine was alkaline (pH about 7.9). The apparent difference in elimination rates of methylphenidate between different species may have the same cause, since human urine as a rule is more acidic than urine from rats and guinea pigs.

In order to investigate the possibility of HPPA formation from MePPA *in vitro* in the samples, the following experiment was carried out: To each of three centrifuge tubes containing ammonium fluoride (preserving agent), 50 μg of MePPA was added. To each of tubes 1) and 2) 10.0 ml of urine was further added and to 3) 10.0 ml of 0.1 N sulfuric acid. Tube 2) was stored at 4° and the others at 21°. After nine days the tube contents were analyzed for MePPA by extraction and gas chromatography. In these cases *p*-dimethylaminobenzaldehyde was employed as an internal standard. In tube 1) only 52 % of the original MePPA was recovered, in tube 2) 74 % and in tube 3) 88 %.

It is thus possible that at least part of the HPPA found in the samples stems from hydrolysis of methylphenidate *in vitro*. This process can be minimized by storing the sample in the cold and in analyzing it as soon as possible. The pH of the urine is another important factor; MePPA will be rapidly hydrolyzed at alkaline pH, and the lowest rate of hydrolysis occurs at a pH of about 2.9.[12]

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