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Note

Determination of pemoline by high-pressure liquid chromatography

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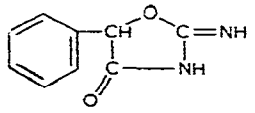
Pemoline (2-imino-4-oxo-5-phenyloxazolidine), having the structural formula I, is a psycho-stimulant drug used in many pharmaceutical preparations (Deadyn, Didascon, etc.).



I

At the present, no satisfactory methods exist for the quantitative determination of this drug in pharmaceutical products or in biological liquids. Gas chromatography (GC) cannot be applied because pemoline is not directly eluted, and when derivatized pemoline forms various compounds in different yields. However, GC has been applied for the determination of benzaldehyde which is formed after oxidative degradation of pemoline[1]. Thin-layer chromatography (TLC) has been applied for the analysis of this drug in urine, but only for a qualitative detection[2].

High-pressure liquid chromatography (HPLC) has been employed successfully for the determination of many other common drugs in pharmaceutical compositions[3] and for drugs of abuse[4]. In this work we report the application of HPLC to the determination of pemoline in pharmaceutical products, or in biological liquids. This technique has many advantages over previous methods, including high sensitivity and selectivity, ease of quantitation and the possibility of application to non-volatile samples without their chemical transformation.

EXPERIMENTAL

Apparatus

A DuPont Model 840 liquid chromatograph was used with a UV detector at 254 nm. The column (25 cm × 2.1 mm I.D.) was packed with Zorbax (DuPont, Frieberg/Hessen, G.F.R.) at room temperature. The liquid phase for routine analysis was *n*-hexane–isopropanol–concentrated ammonia (37:12:1). Pressure, 120 atm; flow-rate, 0.5 ml/min.

Extraction of the drug from pharmaceutical products

The product, containing 1–10 mg of pemoline, was suspended in 100 ml of

water. The suspension was filtered, and to 10 ml of this solution were added 0.5 g of NaCl and few drops of NH_3 (to pH 8–9). The resulting solution was extracted three times with methyl acetate (1 × 10 ml, 2 × 5 ml). The organic extracts were collected, dried over sodium sulphate, an appropriate amount of an internal standard was added for quantitative determination and the solution was concentrated to 0.1 ml. 1–2 μl of this concentrated solution were injected into the chromatograph. Linear calibration graphs were constructed from the peak area ratios at several sample concentrations.

Extraction of the drug from urine

To 10 ml of urine were added 0.5 g of NaCl. The solution was adjusted to pH 8–9 with a few drops of dilute NH_3 and extracted three times with methyl acetate (1 × 10 ml, 2 × 5 ml). The collected organic extracts were extracted with 5 ml of 5 *N* HCl and 0.5 g of NaCl. The organic phase was discarded and the aqueous solution was extracted with methyl acetate (3 × 10 ml). The organic phase was again discarded. The aqueous solution was adjusted to *ca.* pH 10 with concentrated NH_3 and again extracted with methyl acetate (1 × 10 ml, 2 × 5 ml). The collected organic extracts were concentrated as above, dried and examined with the chromatograph.

RESULTS AND DISCUSSION

Solvent extraction

Pemoline is soluble in water and in other polar solvents such as methanol and methyl acetate; it is almost insoluble in non-polar solvents. For this reason, methyl acetate was used for the extraction of the drug from aqueous solutions, and NaCl was added in order to decrease the solubility of the drug in water.

The extraction curve of pemoline with these solvents at various pH values is shown in Fig. 1. The extraction can be carried out at any pH between 0.5 and 11. At

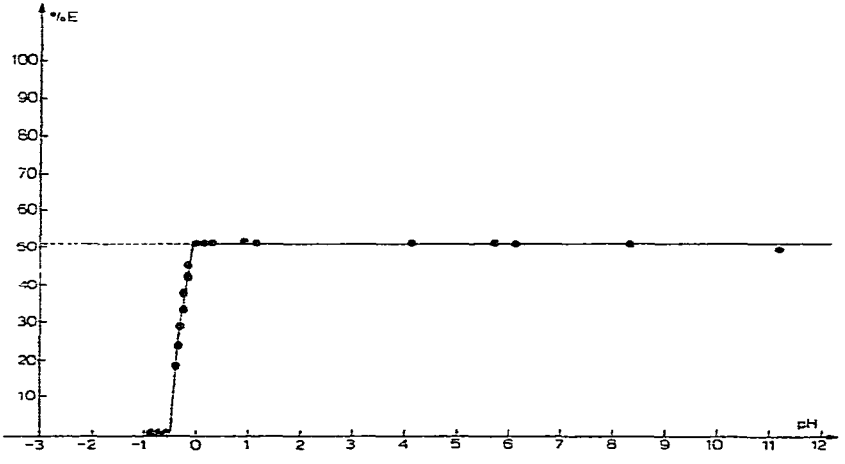


Fig. 1. Percentage of extraction of pemoline in methyl acetate from water (1:1) containing 0.5 g of NaCl and buffered at various pH values.

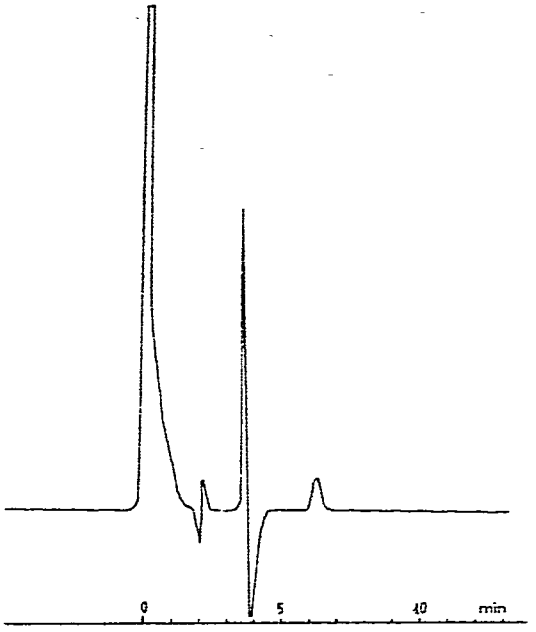


Fig. 2. Chromatogram of 0.1 μg of pemoline from a pharmaceutical product. Column (25 cm × 2.1 mm I.D.) packed with Zorbax. Solvent, *n*-hexane–isopropanol–concentrated ammonia (37:12:1). Pressure, 120 atm; flow-rate, 0.5 ml/min. Chart speed, 1.25 cm/min.

higher pH values a decrease in the percentage of drug extracted was observed, due probably to decomposition of the product. Pemoline shows very weak basic properties and only in strong acid solution did the percentage of drug extracted decrease rapidly; all of the drug remained in the aqueous phase in the presence of 5 *N* HCl. The procedure was thus established for cleaning the extract from biological samples, *i.e.*, a double extraction was carried out at different pH values as described above.

Chromatographic behaviour

Two types of column packings, both containing silica particles for adsorption chromatography, were tested, Zipak and Zorbax (DuPont). Zorbax, having a smaller particle diameter (10 μm), gives a column of higher efficiency. Under the conditions described above, 2000 theoretical plates were obtained for the pemoline peak. Liquid phases of different compositions were also tested. It was found that, for a good peak shape without tailing, 2–3% of concentrated ammonia should be added to the liquid phase. The ratio of *n*-hexane to isopropanol largely influences the retention time, which decreased on increasing the percentage of the alcohol in the mixture. 24% of isopropanol was used which gives a capacity ratio of $k' = 2.3$, a reasonable value for analytical determinations. Fig. 2 shows a chromatogram of pemoline extracted from a pharmaceutical product; a well defined peak was obtained when 0.1 μg of sample was injected.

Detection in biological samples

Samples of a man's urine were collected before, and 2 and 4 h after adminis-

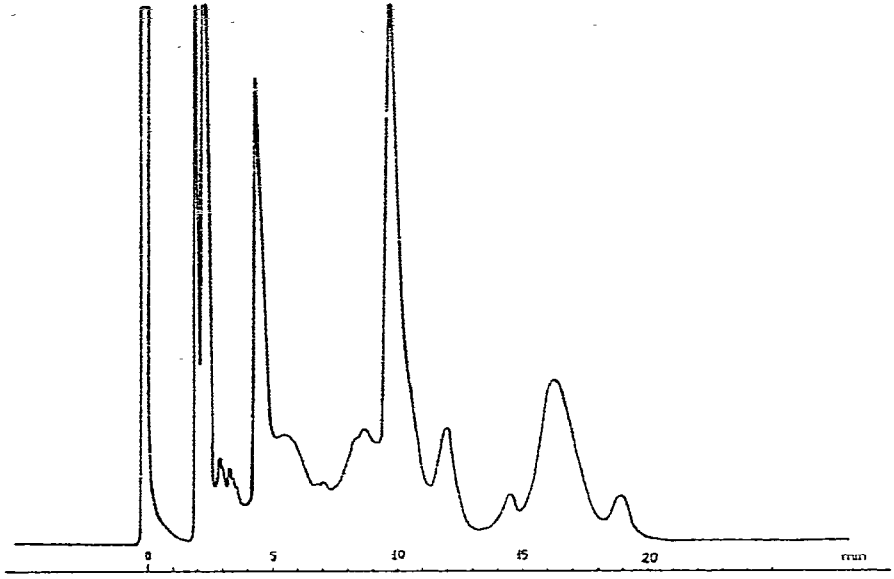


Fig. 3. Chromatogram of an urine extract before administration of the drug. Conditions as in Fig. 2.

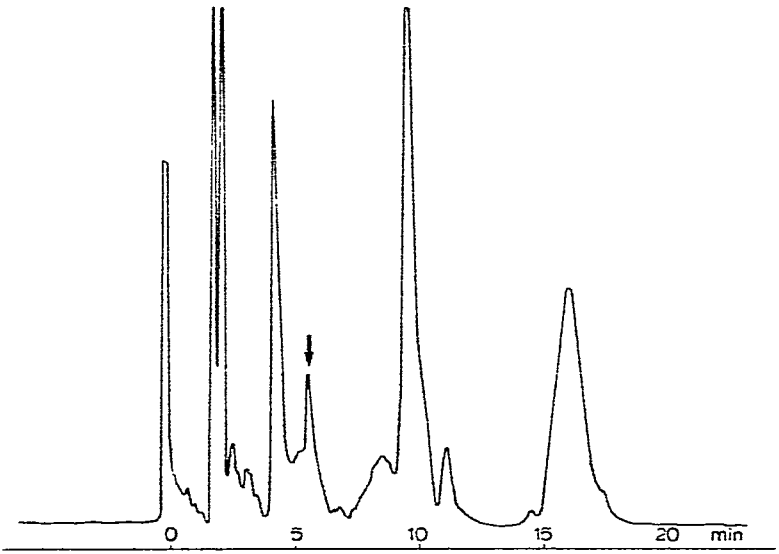


Fig. 4. Chromatogram of an urine extract 4 h after the administration of 10 mg of the drug. Conditions as in Fig. 2.

tration of a single therapeutic dose of 10 mg of the drug. Figs. 3 and 4 show the chromatograms obtained for the samples of urine, extracted as described above, before and 4 h after the administration of the drug. A concentration of 2.2 μg of pemoline per ml of urine was found in the latter sample.

As shown by these chromatograms, it is possible to determine pemoline in biological samples by HPLC with a high sensitivity and selectivity. It is thus possible to follow the rate of drug excretion, to study the therapeutic effects and to detect cases of abuse.

ACKNOWLEDGEMENT

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