Journal of Chromatography, 202 (1980) 131–133
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 13,163

Note

Reversed-phase high-performance liquid chromatographic detection of pemoline in doping control

G. P. CARTONI*, M. CIARDI, A. GIARRUSSO and F. ROSATI
Laboratorio Antidoping, F.M.S.I. Roma, Istituto di Chimica Analitica, Università di Roma, Rome (Italy)
(Received July 22nd, 1980)

Pemoline (2-imino-4-oxo-5-phenyloxazolidine) is a psycho-stimulant drug used in many pharmaceutical preparations (Deadyn, Didascon, Stimul, Tradon, etc.), and some cases of drug abuse in athletes have been found.

The determination of pemoline in urine or other biological liquids can be carried out by gas chromatography only after hydrolysis and derivatization. The compound can be detected by a flame ionization detector and identified by mass spectrometry[1], by using a nitrogen-selective detector[2,3] or electron-capture detector[4]. All these procedures are time consuming and not very reliable for routine doping control.

In a previous paper[5] we described a simple method for the determination of pemoline in urine by high-performance liquid chromatography (HPLC) without any chemical transformation. Here we report an improvement that was obtained for this analysis using the recent advances in HPLC, *e.g.*, the use of reversed-phase columns, gradient elution and variable-wavelength detectors, and also a more simple and rapid procedure for sample extraction using XAD-2 macroreticular resin[6].

EXPERIMENTAL

Chromatography

A Hewlett-Packard Model 1084-B liquid chromatograph was used equipped with automatic injection and scanning of the spectra of eluted peaks. The column (25 cm × 4.6 mm I.D.) was packed with 10 μm LiChrosorb RP-18 (or RP-8) and connected to a short (5 cm) pre-column packed with the same phase. In this way the chromatographic column is protected from the many impurities in the biological samples. Other conditions: mobile phase, water–methanol (85:15), flow-rate 2 ml/min; column temperature 40°C; water temperature 70°C; methanol temperature 40°C; detector wavelength, 220 nm.

After the elution of pemoline the solvent composition was raised to 100% methanol with a fast gradient and maintained for 10 min, to clean the column of other retained compounds.

The instrument was programmed to return, after this time, to the initial con-

0021-9673/80/0000–0000/$02.25

ditions and be ready for another analysis. Standard solutions (1 μg/ml) of pemoline in methanol were injected as reference. Pemoline has a retention time of about 8 min.

Sample extraction

Volumes (10 ml) of urine were passed through a column containing 1 g of XAD-2 resin followed by 20 ml of distilled water. The column was allowed to run dry, the effluent discarded and pemoline eluted with 3 ml of methanol. A 40–50 μl volume of this solution was directly injected in the liquid chromatograph. In the case of a suspected peak having a retention time within ± 5% that of pemoline, the sample was injected again, a spectrum recorded at the top of the eluted peak in the wavelength range 190–350 nm and compared with reference spectra.

RESULTS AND DISCUSSION

Pemoline is strongly adsorbed by XAD-2 resin from urine, and a recovery of 80–85% is obtained after elution with methanol. This drug is largely excreted unchanged in urine and a maximum is reached 2–4 h after administration. A urine concentration of 1–2 μg/ml is obtained after administration of a single therapeutical dose (10 mg).

In our method, 10 ng of pemoline injected in the chromatograph show a well detectable peak. This amount corresponds to a minimum detectable concentration in urine of 0.1–0.05 μg/ml.

Fig. 1A shows the chromatogram of a standard solution and Fig. 1B the corresponding UV spectrum of eluted peak. Pemoline has a strong absorption maximum at 220 nm; since this maximum occurs at short wavelengths the best solvents to be used in a mixture with water are methanol or acetonitrile. We prefer the first for routine analysis because it is less expensive and safer.

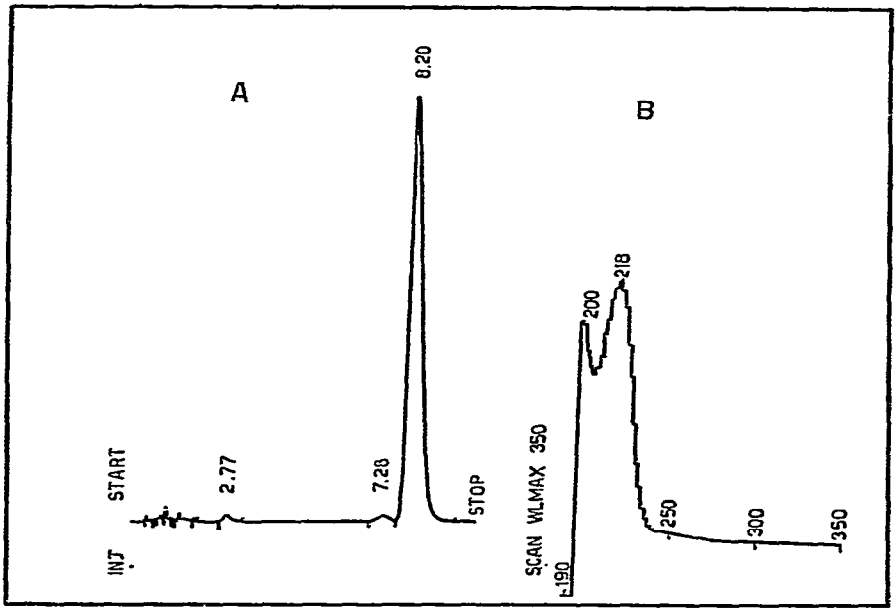

Fig. 1. A, Chromatogram of a standard solution of pemoline. Column: LiChrosorb RP-8 (25 cm × 4.6 mm I.D.). Mobile phase: water–methanol (85:15); flow-rate 2 ml/min. Retention times in minutes. B, UV spectrum recorded on the eluted peak of pemoline.

Fig. 2A shows the chromatogram of a urine blank and Fig. 2B that of the urine of the same subject 4 h after ingestion of a single therapeutical dose (10 mg) of pemoline. As can be observed, in reversed-phase HPLC, many compounds are eluted well before pemoline as non-interfering peaks. At the end of each analysis it is important to clean the column by increasing the concentration of methanol to 100% to eliminate interferences of late eluted peaks from the previous injections.

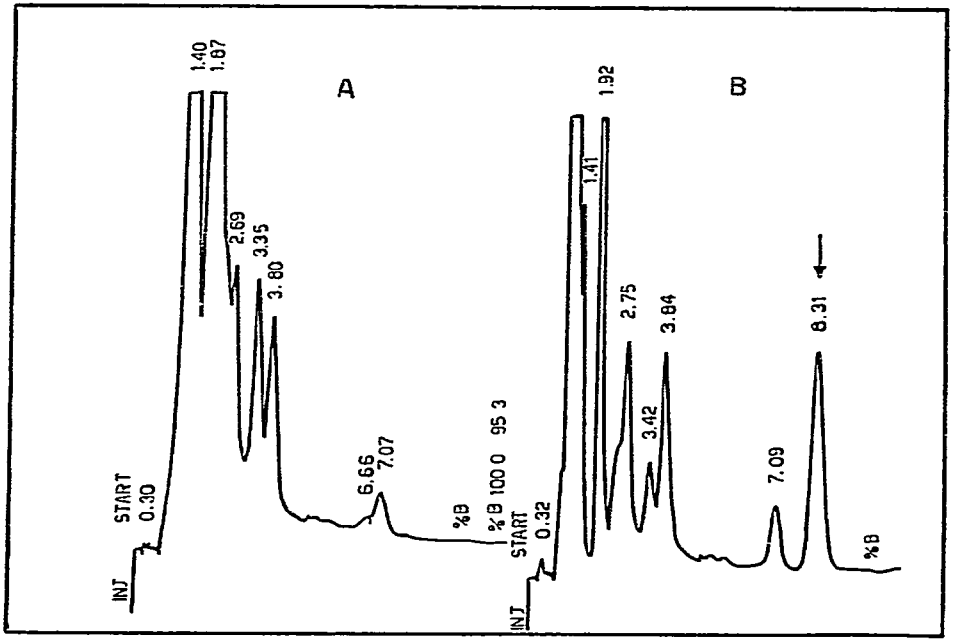

Fig. 2. Chromatogram of a urine blank (A) and of urine 4 h after the administration of 10 mg of pemoline. Conditions as in Fig. 1.

The remaining part of the sample extracts can be utilized for other confirmatory tests; the suspected peak can be easily collected and examined by HPLC on a different column, by gas–liquid chromatography after hydrolysis and esterification and by mass spectrometry either by direct injection of the collected peak or in combination with gas chromatography.

Following this procedure we have carried out numerous analyses for the detection of pemoline in doping control with a good selectivity and sensitivity, in a short time and without any complicated sample treatment.

ACKNOWLEDGEMENT

The work was supported by the Consiglio Nazionale delle Ricerche, Italy.

REFERENCES

1 M. Van Boven and P. Daenneus, *J. Chromatogr.*, 134 (1977) 415.
2 N. P. E. Vermeulen, D. De Roode and D. D. Breimer, *J. Chromatogr.*, 137 (1977) 333.
3 N. P. E. Vermeulen, M. W. E. Teunissen and D. D. Breimer, *J. Chromatogr.*, 157 (1978) 133.
4 Sou-Yie Chu and L. T. Sennello, *J. Chromatogr.*, 137 (1977) 343.
5 G. P. Cartoni and F. Natalizia, *J. Chromatogr.*, 123 (1976) 474.
6 F. T. Delbeke and M. Debackere, *J. Chromatogr.*, 133 (1977) 214.