Journal of Chromatography, 226 (1981) 488—491
Biomedical Applications
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROMBIO. 1020

Note

Determination of biperiden in human serum by glass capillary gas chromatography with isothermal splitless injection and nitrogen-sensitive detection

PEKKA OTTOILA and JYRKI TASKINEN*

Orion-Yhtymä Oy, Orion Pharmaceutical Co., Research Laboratories, P.O. Box 19, SF-00101 Helsinki 10 (Finland)

(First received April 21st, 1981; revised manuscript received June 24th, 1981)

Biperiden, 1-bicyclo[2.2.1]hept-5-en-2-yl-1-phenyl-3-piperidinopropan-1-ol hydrochloride (Fig. 1A), is an anticholinergic drug which is used in the treatment of Parkinson's disease [1]. So far only one method based on gas chromatography—mass spectrometry has been reported for the determination of biperiden in serum [2]. Although the method involves a complex extraction and derivatization procedure, the sub-nanogram sensitivity required for bioavailability and pharmacokinetic studies is not obtained. In this report we describe a simpler and more sensitive method, which involves extraction of biperiden from serum with hexane and quantitation by glass capillary gas chromatography with nitrogen-sensitive detection.

EXPERIMENTAL

Materials

Biperiden hydrochloride was supplied by Orion Pharmaceutical Co. (Helsinki, Finland). Internal standard, 1-bicyclo[2.2.1]hept-2-yl-1-phenyl-3-piperidinopropan-1-ol (Fig. 1B), was obtained by catalytic hydrogenation (Pd-C) of

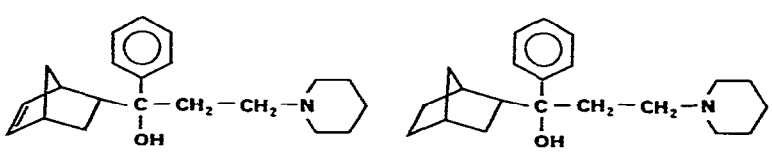

Fig. 1. The structures of biperiden (A) and internal standard (B).

0378-4347/81/0000—0000/$02.50

biperiden. Hexane (p.a., E. Merck, Darmstadt, G.F.R.) and tridecane (puriss., Koch-Light, Colnbrook, Great Britain) were used without further purification.

Procedure

After adding internal standard (2.5 ng/ml), 2 ml of serum were alkalinized (pH 9.5) with 1 *M* NH_4OH and extracted twice with 5 ml of hexane. The combined hexane layers were evaporated to almost dryness with a stream of nitrogen at 40°C and the residue was dissolved in 25 μl of tridecane. The extracts were protected from light and stored in the refrigerator.

Analyses were performed using a Hewlett-Packard 5730A gas chromatograph with an HP 18704B capillary system inlet and an HP 18789A N—P flame ionization detector. The OV-101 glass capillary column, 25 m × 0.32 mm I.D., was prepared by Dr. S. Räisänen (Helsinki University, Helsinki, Finland). The column was operated isothermally at 215°C. Aliquots of 1.5 μl of extracts in tridecane were injected using splitless injection technique with a 15-sec splitless period. The injector temperature was 250°C and the detector temperature 300°C. Gas flow-rates were: carrier (helium) 2 ml/min, hydrogen 3 ml/min, air 40 ml/min and auxiliary (helium) 30 ml/min. Biperiden was quantitated by comparing peak height ratios of biperiden and internal standard to a calibration curve obtained by analyzing spiked serum samples over the range 0.25—10 ng/ml.

RESULTS AND DISCUSSION

Biperiden chromatographed well on the OV-101 glass capillary column with a detection limit of about 20 pg injected onto the column. Derivatization was not necessary to prevent adsorption as was reported to be the case with packed columns [2]. For the sensitivity required splitless injection was a prerequisite. In this case the solvent effect [3] can be utilized to prevent band broadening. This can be achieved in a case of lower boiling solvents by injecting at low temperature and by subsequent temperature programming of the column. The use of long-chain alkanes as solvents to obtain the solvent effect at higher injection temperatures, in order to make faster and simpler isothermal operation feasible, is well known although not commonly adopted for quantitative drug analysis [4—7]. At higher temperatures the injection conditions, especially the choice of solvent, are critical to obtain optimum effect and maximum peak height. In this case best results were obtained using isothermal conditions at 215°C with tridecane as the solvent. Biperiden and internal standard eluted fully separated within 5 min and samples could be analyzed with an interval of 6 min.

After injecting about 200 samples the contamination of the beginning of the column started to cause some broadening of peaks. The performance of the column could be restored by washing the first 20—30 cm of the column with hexane and then treating with 0.5% OV-101 in methylene chloride.

The specificity achieved with glass capillary gas chromatography and nitrogen-sensitive detection allowed the complex extraction procedure suggested by previous workers [2] to be replaced by a simple extraction with hexane. Gas chromatograms obtained by analyzing serum extracts are shown in Fig. 2. The

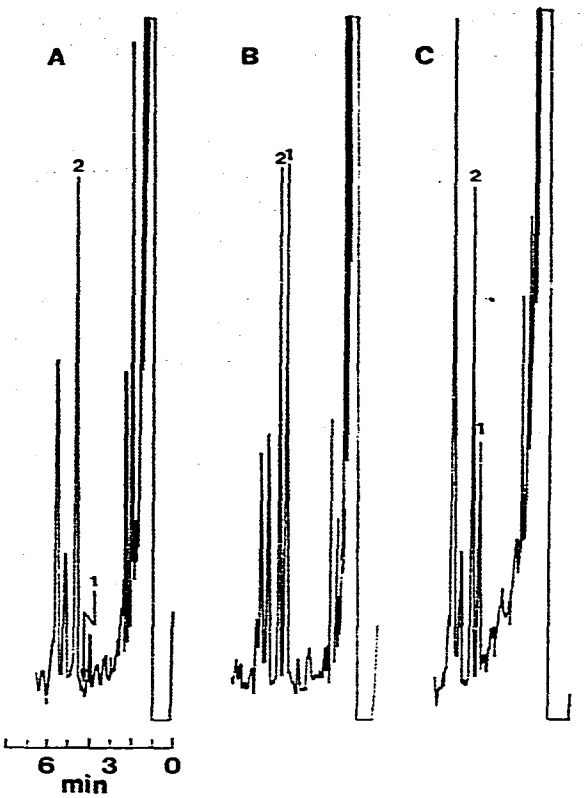

Fig. 2. Gas chromatograms obtained by analyzing serum extracts with a 25-m OV-101 glass capillary column isothermally at 215°C. (A) Blank serum with internal standard. (B) 2.5 ng/ml biperiden and internal standard added to blank serum. (C) Serum sample of a volunteer 2 h after an oral dose of 4 mg. Peaks: 1 = biperiden, 2 = internal standard.

recovery test was carried out using standard serum samples spiked with 2.5 ng/ml biperiden. Internal standard was added in tridecane at the end of the evaporation step. In reference samples both biperiden and internal standard were added to blank serum extracts. Recovery of biperiden was found to be 60 ± 6% (S.D., n = 6). Despite the relatively low recovery the precision was reasonable, even near the lower limit of quantitation. This is due to the suitable internal standard, which differed from biperiden only by the absence of the double bond in the bicycloheptane ring. Precision was studied by analyzing replicate spiked samples at the concentrations of 2.5 ng/ml and 0.5 ng/ml, and was 3.3% and 6.8% (C.V., n = 6), respectively.

Biperiden was quantitated by comparing peak height ratios with a calibration curve, which was constructed daily by linear regression after analyzing spiked serum samples in the concentration range 0.25—10 ng/ml. In this range linearity was good (typically r = 0.999). The lower limit of quantitation, 250 pg/ml, was adequate for analyzing samples up to 8 h after a single dose of 4 mg, as is demonstrated in Fig. 3. The assay method has been used successfully for moni-

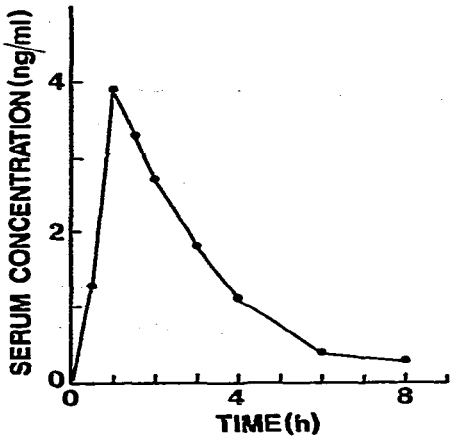

Fig. 3. Serum concentration curve from a healthy volunteer after a 4-mg dose of biperiden.

toring serum concentrations in a steady-state situation. After a dose of 4 mg twice a day of long-acting formulations, serum concentrations of 0.5—4 ng/ml were measured.

REFERENCES

1 N.S. Kline, B.T. Mason and L. Winick, Curr. Ther. Res., 16 (1974) 838.
2 R.K. Harle, B.J. Millard and P.R. Wood, in E. Reid (Editor), Methodological Surveys in Biochemistry, Vol. 7, Ellis Horwood, Chichester, 1978, p. 167.
3 K. Grob and K. Grob, Jr., J. High Resolut. Chromatogr. Chromatogr. Commun., 1 (1978) 57.
4 K. Grob and K. Grob, Jr., J. Chromatogr., 94 (1974) 53.
5 H. Buser, Anal. Chem., 48 (1976) 1553.
6 H. Buser, Anal. Chem., 49 (1977) 918.
7 P. Ottoila, U. Lamminsivu and J. Taskinen, Kemia-Kemi, 7 (1980) 756.