ELSEVIER

Journal of Chromatography B, 708 (1998) 325–329

JOURNAL OF CHROMATOGRAPHY B

Short communication

High-performance liquid chromatographic determination of propiverine and its *N*-oxide in human serum

Klaus Richter[a,*], Steffen Scheithauer[b], Daniela Thümmler[b]

[a]*Institut für Klinische Pharmakologie, Medizinische Fakultät Carl Gustav Carus, Technische Universität Dresden, Fiedlerstrasse 27, D-01307 Dresden, Germany*

[b]*APOGEHA Arzneimittel GmbH, Kyffhäuserstrasse 27, D-01309 Dresden, Germany*

Received 31 July 1997; received in revised form 8 December 1997; accepted 8 December 1997

Abstract

A high-performance liquid chromatographic method has been developed for the determination of propiverine hydrochloride (P4) and its main metabolite, propiverine *N*-oxide (P4NO) in human serum. P4 has been shown to be efficacious in those patients who have either idiopathic bladder instability, or neurogenic bladder (detrusor hyperflexia) resulting from spinal cord injuries. In the present method, the analytes were extracted from serum (1 ml, pH 8) into methyl *tert*.-butyl ether. The separation was performed on a reversed-phase C_8 (RP-select B) column using phosphate buffer–acetonitrile (30:70, v/v). UV absorption was used for measuring the analytes, with a limit of quantitation of about 10 ng/ml, which is appropriate for pharmacokinetic studies. © 1998 Elsevier Science B.V.

Keywords: Propiverine; Propiverine *N*-oxide

1. Introduction

Propiverine hydrochloride (1-methylpiperid-4-yl-2,2-diphenyl-2-(1-propoxy)acetate hydrochloride, P4) is used in the treatment of urinary bladder dysfunction. A gas chromatography–mass spectrometry (GC–MS) method for the determination of P4 and its metabolites [1] is very time-consuming, due to different derivatisation steps. Therefore, a practicable high-performance liquid chromatographic (HPLC) method has been established for determination of P4 and its main metabolite, propiverine *N*-oxide, (P4NO) with a limit of quantitation of about 10 ng/ml, which is appropriate for pharmacokinetic studies in humans. The pK_a of the *tert*. amine propiverine (Fig. 1A) was found to be 4.53. The compound P4NO (Fig. 1B) also has a pK_a value as a base, but this is not precise, being in the range of 3.5 to 4.

2. Experimental

2.1. Chemicals

P4, P4NO (a mixture of two stereoisomers) and 1-methylpiperid-4-yl-2,2-diphenyl-2-(1-butoxy)acetate hydrochloride (butoxy, internal standard, I.S.; Fig. 1C) were provided by APOGEPHA Arzneimittel GmbH (Dresden, Germany).

Acetonitrile LiChrosolv (for chromatography), methanol LiChrosolv (for chromatography), methyl

*Corresponding author.

0378-4347/98/$19.00
PII S0378-4347(97)00630-0

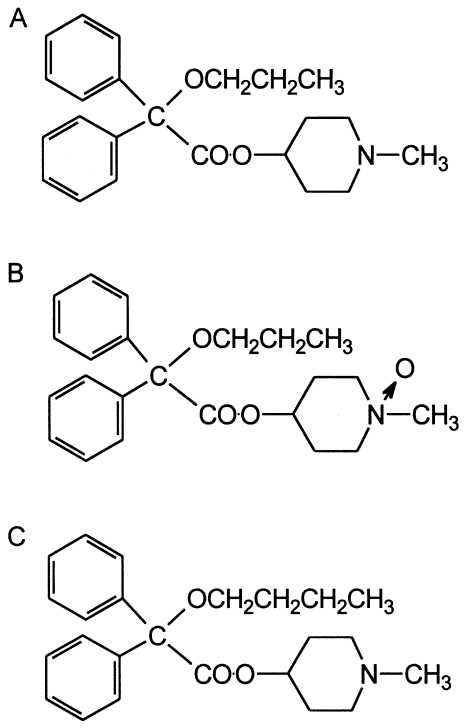

Fig. 1. Structural formulae for propiverine (A), propiverine *N*-oxide (B) and the I.S. (C).

tert.-butyl ether LiChrosolv (for chromatography), sodium acetate trihydrate GR, disodium hydrogen phosphate dihydrate (for chromatography) and potassium dihydrogen phosphate (for molecular biology) were purchased from Merck (Darmstadt, Germany) and acetic acid (HPLC reagent) was from J.T. Baker. Pure water (20 MΩ) was obtained using the ion-exchange system RS 40E, SG Ionenaustauscher (Barsbüttel, Germany).

2.2. HPLC method

2.2.1. Apparatus and chromatographic conditions

The HPLC system (Shimadzu, Kyoto, Japan) was set up with two LC-10AS pumps, a SIL-10A auto-injector equipped with a 50-μl loop, an SCL-10A controller, a column oven (CTO-10A), a diode array spectrophotometric detector (UVD 340 S, Gynkotek, Germeringen, Germany) and the chromatography data system GynkoSoft (V 5.50, Softron) for controlling the diode array detector, evaluation of the chromatograms and for data processing. A reversed-phase column (RP-select B, 125×3 mm I.D., Superspher 60, 5 μm, LiChroCART 125-3 HPLC cartridge) with a 4-mm guard column (RP-select B, LiChrospher 60, 5 μm, LiChroCART 4-4, Merck) was used for the analyses and the column was maintained at a temperature of 35°C and equilibrated with the mobile phase. The mobile phase was phosphate buffer (pH 7.3, 0.007 mol/l)–acetonitrile (30:70, v/v). The solvent flow-rate was 0.7 ml/min.

2.3. Sample preparation

2.3.1. Extraction procedure

Serum samples of 1.0 ml, spiked with 600 ng of the I.S. dissolved in 15 μl of water, were extracted twice with 4.5 ml of methyl *tert*.-butyl ether. This mixture was shaken for 20 s (REAX 1 R, Heidolph, Kelkheim, Germany). After centrifugation for 10 min at 2500 *g*, the organic phases were separated and evaporated to dryness at 80°C in a vacuum centrifuge (Jouan Evaporator centrifuge 10.22). The residue was redissolved in 100 μl of the mobile phase and a 50-μl aliquot was then injected onto the column.

2.3.2. Standard solutions

Stock solutions of P4, P4NO and butoxy were prepared by dissolving each of the substances in water to a final concentration of 1 mg/ml. Working solutions were obtained by further dilution of the stock solutions with water.

3. Results

3.1. Chromatography

Symmetrical peaks were observed. The retention times of P4, P4NO and butoxy were about 9.5, 12.8 and 15.5 min, respectively. The overall chromatographic run time was 17 min. Typical chromatograms are shown in Fig. 2.

3.1.1. Composition of the mixture of P4NO isomers

A chromatogram of the reference substance, P4NO, shown in Fig. 3, demonstrates three peaks; two of the isomers and one of P4, the parent

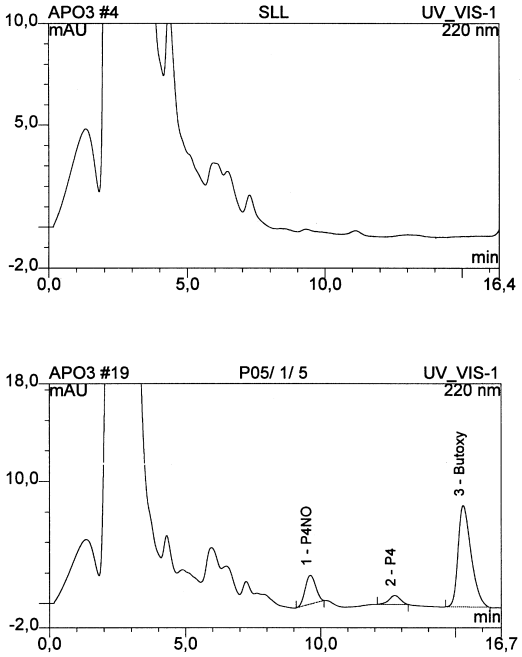

Fig. 2. Chromatograms of plasma samples. SLL=blank; P05/1/5=plasma sample of a subject 5 h after an oral dose of 15 mg of propiverine hydrochloride (P4=28 ng/ml, P4NO=249 ng/ml).

compound in the synthesis. P4NO contains 7% of P4 and 66% of the second peak isomer. In human serum, only the second peak isomer of P4NO could be detected. An assignment to the absolute configuration of the *N*-oxide is not yet possible. The chromatography of the P4NO reference substance was performed with an eluent containing 75% acetonitrile and, therefore, the retention times are shorter than in chromatograms of serum samples.

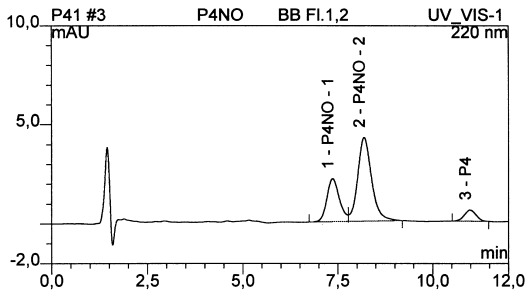

Fig. 3. Chromatogram of the reference substance of P4NO, with the two peaks (P4NO-1 and P4NO-2; 66%) of the isomer mixture, and 7% of the parent compound, P4.

Table 1
Precision of the analytical method for P4 and P4NO from six sets of samples

Concentration added (ng/ml)	Concentration found (mean±S.D.) (ng/ml)	Coefficient of variation (%)
P4		
10.7	10.60±0.33	3.1
21.4	21.62±1.16	5.4
42.8	43.62±1.23	2.8
85.6	84.64±3.28	3.9
128.4	128.79±4.14	3.2
171.2	170.66±2.53	1.5
214.0	212.91±2.87	1.3
428.0	428.90±6.24	1.5
P4NO		
26.4	25.07±2.81	11.2
52.8	54.30±7.07	13.0
79.2	77.02±6.60	8.6
105.6	102.79±5.99	5.8
132.0	129.16±7.46	5.8
264.0	268.26±6.10	2.3

3.2. Precision and linearity

The precision and accuracy of the method were assessed by the determination of eight concentrations in six independently prepared series of serum samples from different subjects, as shown in Table 1. In Table 2, the day-to-day precision and accuracy on twelve different days are given. The lower limit of

Table 2
Precision of the analytical method for P4 from twelve sets of samples on different days

Concentration added (ng/ml)	Concentration found (mean±S.D.) (ng/ml)	Coefficient of variation (%)
P4		
10.7	10.7±0.1	9.9
21.4	20.6±1.8	8.9
42.8	42.9±2.9	6.7
85.6	85.8±3.3	3.9
171.2	166.0±8.0	4.8
214.0	211.9±6.5	3.1
428.0	423.4±8.1	1.9
P4NO		
26.4	23.7±4.6	19.4
52.8	52.1±5.6	10.7
105.6	109.2±8.1	7.4
132.0	141.5±8.2	5.8
264.0	287.7±16.9	5.9

quantitation, i.e., with a coefficient of variation <10% for six repeated measurements, is about 10 ng/ml for P4 and about 25 ng/ml for P4NO. The linearity of the method was confirmed in the range of at least 10–430 ng/ml for P4 and 25-260 ng/ml for P4NO.

3.3. Extraction recovery

The recovery was evaluated from the peak heights of standard serum samples used in different series on different days and from those measured by direct injection of the analytes. The recovery from serum was found to be 83% for P4, 44% for P4NO and 84% for the I.S., as shown in Table 3. The one-step extraction of the relatively polar P4NO and the less polar P4 and I.S. delivered the best results with regards to recovery and purity with methyl *tert*.-butyl ether. The recovery is not dependent on the pH at values higher than five.

3.4. Stability

The stability of P4, P4NO and butoxy was investigated in water, serum and plasma. A degradation or N-oxidation of P4 and butoxy did not occur in frozen samples during a two-month or even longer storage period, nor after three freeze–thaw cycles. The analytes were stable in samples stored at 5°C for at least two weeks and at room temperature in the autosampler for at least one day. Degradation of the extracted analytes could not be observed during evaporation of the methyl *tert*.-butyl ether, but the residue of the extract should be redissolved immediately with the HPLC eluent. This solution remains stable for a few days.

Table 3
Recovery of P4, P4NO and butoxy, calculated from the reduced peak heights (=peak height/amount) of standard serum samples and the reduced peak heights of directly injected amounts

	Amount added (ng/ml)	Mean height (mAU)	Mean reduced height (mAU/ng)	Mean S.D. (mAU/ng)	C.V. (%)	*n*
P4						
	21.4	1.229	0.0574	0.0064	11.2	6
	42.8	2.384	0.0557	0.0036	6.4	6
	64.2	3.761	0.0586	0.0016	2.8	6
	85.6	5.155	0.0595	0.0028	4.7	6
	107.0	6.534	0.0611	0.0019	3.1	6
	214.0	13.189	0.0616	0.0037	6.1	6
Mean extracted			0.0590			
Mean direct	107.0	7.606	0.0711	0.0019	2.7	12
Recovery (%)			83			
P4NO						
	26.4	0.634	0.0240	0.0066	27.7	6
	39.6	1.022	0.0258	0.0029	11.3	6
	52.8	1.452	0.0269	0.0018	6.8	6
	66.0	1.837	0.0278	0.0027	9.9	6
	132.0	3.712	0.0281	0.0011	3.8	6
Mean extracted			0.0265			
Mean direct	66.0	4.015	0.0608	0.0032	5.2	30
Recovery (%)			44			
Butoxy						
Mean extracted	300	15.086	0.0503	0.008	15.7	36
Mean direct	300	17.863	0.0595	0.002	3.5	36
Recovery (%)			84			

4. Conclusions

The HPLC method presented proved to be selective, sensitive and robust for the measurement of P4 and P4NO in serum. We also assayed plasma because it might happen that only plasma is available from some of the studies in humans; we could not find any differences when compared with the results for serum samples. The assay is fast and requires relatively simple sample preparation. From our recent experience, we can confirm that a large number of samples can be processed daily.

Acknowledgements

This work was supported by APOGEHA Arzneimittel GmbH (Dresden, Germany). The authors acknowledge the technical assistance of Ms. M. Pescheck.

References

[1] T. Marunaka, Y. Umeno, Y. Minami, E. Matsushima, M. Maniwa, K. Yoshida, M. Nagamachi, J. Chromatogr. 420(1) (1987) 43.