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Note

High-performance liquid chromatographic determination of propranolol and its metabolites in rat serum

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Propranolol (PPL), a β-adrenergic receptor blocking agent, is used for treating hypertension, ventricular tachycardia and supraventricular arrhythmias as well as for the prophylaxis of angina pectoris and migraine headache. PPL is a potent inhibitor of sperm motility [1,2], and on account of this property it has been tried clinically as a vaginal chemical contraceptive in women [3].

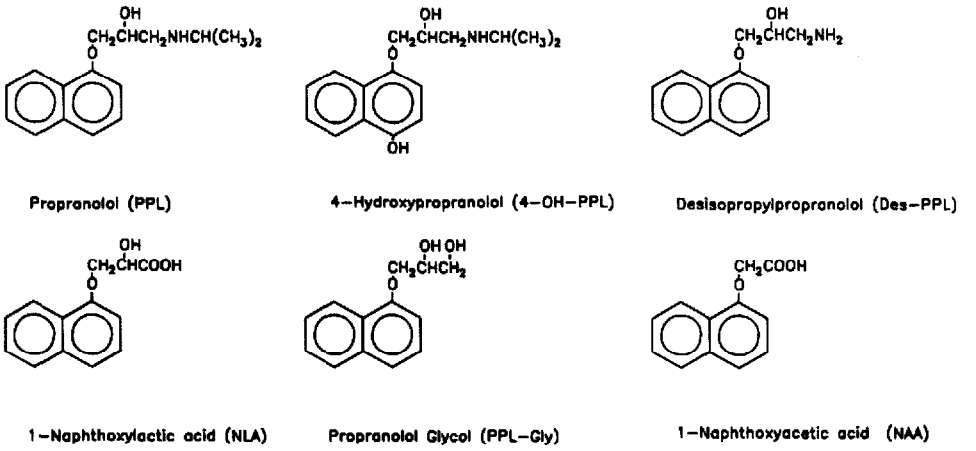

Fig. 1. Chemical structures of PPL and its metabolites.

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PPL is known to undergo extensive first-pass metabolism in dog [4], rat [5] and man [6], and has a large inter-patient variation in bioavailability when administered orally. The chemical structures of PPL and its major metabolites found in plasma of animals and humans are shown in Fig. 1.

A number of high-performance liquid chromatographic (HPLC) methods for the determination of PPL and its metabolites have been reported [7–18]. However, most of these methods are suitable for the determination of only PPL and one or two of its metabolites, and therefore cannot be employed for metabolic profile studies. Presently, to our knowledge, three methods [16–18] are available which can separate PPL and most of its major metabolites. However, these methods have some drawbacks. For example, the method of Kwong and Shen [16] does not include an important PPL metabolite, 1-naphthoxyacetic acid, although it is capable of separating various regioisomeric monohydroxyl metabolites, along with other common metabolites. In addition this method requires a rather complex extraction procedure and has a long chromatographic separation time. The methods of Smith et al. [17] and Pritchard et al. [18] use two different HPLC conditions to determine the acidic and basic metabolites, consequently reducing the efficiency of the analytical system.

In this communication, we describe a simple and rapid (10–15 min) HPLC method capable of separating unchanged PPL and its five metabolites in serum.

EXPERIMENTAL

Materials

Propranolol and 2-naphthoxyacetic acid (internal standard, I.S.) were purchased from Sigma (St. Louis, MO, U.S.A.). The other metabolites, 4-hydroxypropranolol (4-OH-PPL), desisopropylpropranolol (Des-PPL), propranolol glycol (PPL-Gly), 1-naphthoxylactic acid (NLA), 1-naphthoxyacetic acid (NAA) and 4-methylpropranolol (I.S.) were kindly supplied by ICI (Macclesfield, U.K.).

All other chemicals and solvents, such as ascorbic acid, triethylamine (TEA), phosphoric acid, carbonate buffer, diethyl ether, methanol and acetonitrile, were of analytical-reagent grade. All aqueous solutions were prepared using double-distilled water.

Chromatographic conditions

The HPLC system consisted of a Beckman 110 HPLC pump (Fullerton, CA, U.S.A.) connected to a Rheodyne injector with 20-μl sample loop. A Perkin Elmer 650-10S fluorescence detector (Norwalk, CA, U.S.A.) connected to a linear Instrument chart recorder (Irvine, CA, U.S.A.) and a HP 3390A integrator (Palo Alto, CA, U.S.A.) were used for the peak-height measurements. The fluorometer was set at an excitation wavelength of 300 nm and emission wavelength of 375 nm. Both slit openings were set at 20 nm.

Chromatographic separations were achieved on a 5-μm C_{18} reversed-phase column (15 cm $\times$ 4.6 mm) obtained from Supelco Canada. The mobile phase consisted of a mixture of methanol–acetonitrile–0.1% triethylamine in water (25:25:50), the pH adjusted to 2.5 with 1 *M* orthophosphoric acid. Flow-rate was

set at 1 ml/min at ambient temperature, yielding a column back-pressure of 80 bars.

Standard solutions

Stock solutions of 1.0 mg/ml of all the compounds were prepared in methanol, whereas the standard solutions were in a 10% methanol–water mixture having the following final concentrations: solution A: 500 ng/ml 4-OH-PPL, 100 ng/ml PPL, 100 ng/ml Des-PPL; solution B: 100 ng/ml NLA, 500 ng/ml NAA; solution C: 50 ng/ml PPL-Gly.

Another standard solution containing all of the six standards at identical concentrations was prepared for spiking purposes.

Internal standards

Methanolic solutions (1.0 mg/ml) of 2-naphthoxyacetic acid and 4-methylpropranolol were prepared separately, and further mixed and diluted to give working solution of 0.05 and 0.5 μg/ml for 4-methylpropranolol and 2-naphthoxyacetic acid, respectively. An aliquot of 100 μl of this internal standard mixture was used for each 0.5 ml of serum sample.

Extraction procedure

To a 0.5-ml rat serum sample, placed in a 15-ml centrifuge tube fitted with PTFE-lined screw cap, were added 100 μl of 0.5% ascorbic acid solution, 1.0 ml of 1.0 *M* carbonate buffer (pH 10) and 100 μl of internal standard solution. The samples were vortexed for 1.0 min with 3.0 ml of diethyl ether for extraction. After centrifugation at 1500 *g* at 4 °C for 10 min, the upper (organic) layer was back-extracted into 0.5 ml of 0.1 *M* orthophosphoric acid and separated by centrifugation. The aqueous phase containing basic metabolites was assayed by HPLC, while the ether phase containing PPL-Gly was evaporated to dryness under nitrogen, and the residue was reconstituted in 0.5 ml of the HPLC mobile phase prior to analysis.

The lower aqueous phase of the basic extract was acidified with 1.0 ml of 6 *M* hydrochloric acid and extracted with 3.0 ml of diethyl ether. After centrifugation the ether phase was separated, evaporated to dryness and reconstituted with 0.5 ml of HPLC mobile phase. Fig. 2 illustrates the scheme of this extraction procedure.

Calibration

Linear calibration curves were prepared by least-squares regression analysis of peak-height ratios of analyte to I.S. against standard concentrations of PPL or metabolites in spiked serum samples. In the case of PPL-Gly the calibration curve was generated by regression of peak heights against concentrations of standard in spiked serum samples. The concentrations of PPL and its metabolites in serum for calibration curves ranged from 1.25 to 10 ng/ml for PPL-Gly, 2.5 to 20 ng/ml for PPL, Des-PPL and NLA and 12.5 to 100 ng/ml for 4-OH-PPL and NAA. These concentration levels were attained by mixing 100 μl of the spiking standard

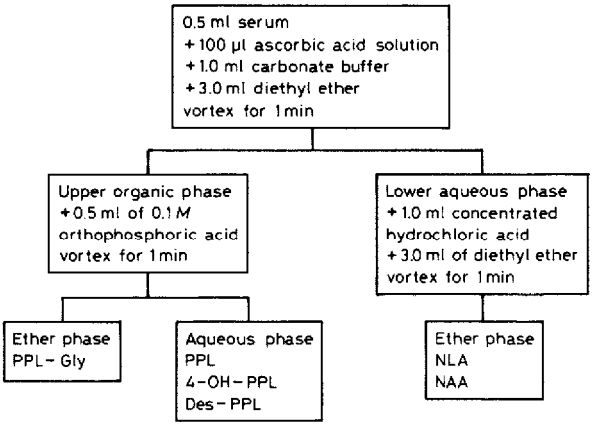

Fig. 2. Flow diagram of extraction procedure.

solution with the 0.5-ml serum sample for the highest concentration. For the lower concentrations, the standard solution was serially diluted to the required concentrations prior to spiking with 100 µl of these diluted solutions.

RESULTS AND DISCUSSION

Using this method, PPL and its metabolites were extracted into three fractions: basic, acidic and neutral. Although this extraction procedure is essentially similar to the methods of Smith et al. [17] and Pritchard et al. [18], it has two important modifications. Firstly, the basic metabolites are extracted back into phosphoric acid, which gives a cleaner extract. Secondly, instead of sodium metabisulphite, ascorbic acid is used as an antioxidant which improves reproducibility and recovery of the metabolites, especially that of 4-OH-PPL, which is consistent with the findings of Lo and Riegelman [10]. On the other hand, unlike the method described by Kwong and Shen [16], the present method not only provides a cleaner extract with the extraction solvent system used, but also furnishes nearly threefold reduction in the chromatographic separation time (viz. 10–15 min versus 40 min).

All extracted fractions were injected separately, and each run took about 10–15 min. Fig. 3 shows the chromatographic traces of blank and spiked serum samples. In this study two separate internal standards were used, i.e. 4-methylpropranolol for the analysis of the basic fraction (PPL, 4-OH-PPL, Des-PPL), and 2-naphthoxyacetic acid for the analysis of the acidic fraction (NAA, NLA). The neutral fraction (PPL-Gly) was measured by using the external standard method.

Calibration curves were linear over the concentration ranges mentioned in the text with minimal intercepts and high correlation coefficients (Table I). The recoveries (%) of the metabolites were determined at the standard solutions concentration levels by comparing the peak-height ratios (metabolites to internal

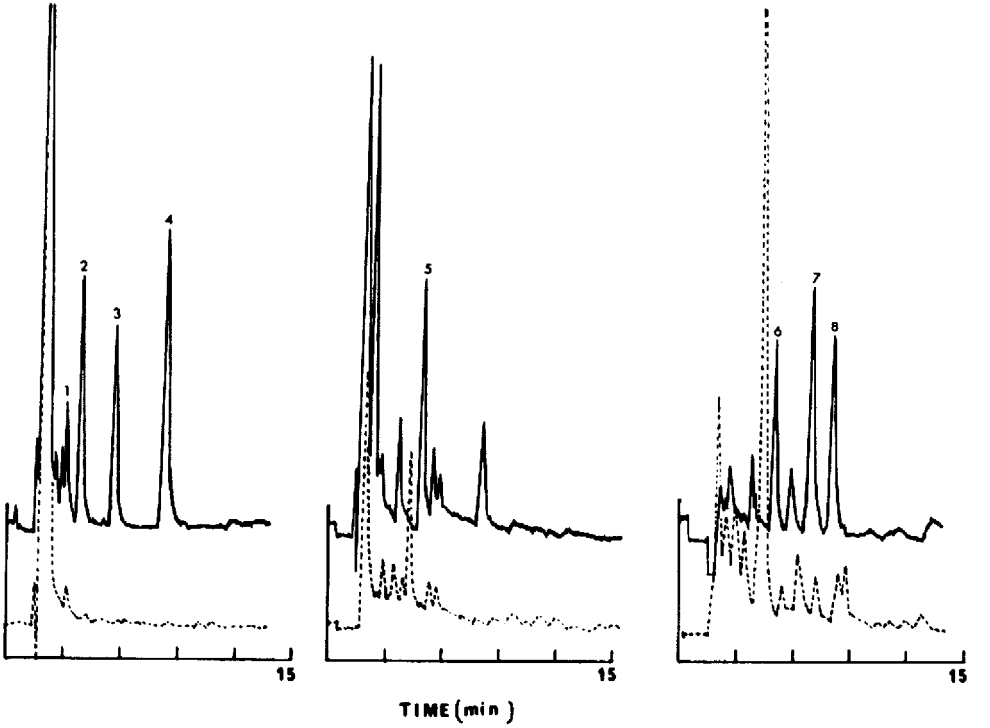

Fig. 3. Chromatographic traces of PPL and metabolites. (-----) Blank sample: (——) spiked rat serum sample. Peaks: 1 = 100 ng/ml 4-OH-PPL; 2 = 20 ng/ml Des-PPL; 3 = 20 ng/ml PPL; 4 = 10 ng/ml 4-methylpropranolol (I.S.); 5 = 10 ng/ml PPL-Gly; 6 = 20 ng/ml NLA; 7 = 100 ng/ml 2-naphthoxyacetic acid (I.S.); 8 = 100 ng/ml NAA. See Fig. 1 for the chemical names of the abbreviations used.

TABLE I

STANDARD CURVE PARAMETERS FOR PPL AND ITS METABOLITES

Compound	Slope ± S.E. of estimates	Intercept ± S.E. of estimates	Correlation coefficient (r)
4-OH-PPL	0.511 ± 0.011	0.037 ± 0.007	0.9995
Des-PPL	0.914 ± 0.015	0.027 ± 0.010	0.9997
PPL	0.737 ± 0.010	0.085 ± 0.007	0.9998
PPL-Gly	0.771 ± 0.023	0.027 ± 0.016	0.9991
NLA	0.566 ± 0.015	0.021 ± 0.010	0.9993
NAA	0.636 ± 0.012	0.043 ± 0.008	0.9996

standard) following direct injection of the aqueous standard solutions with those obtained after the whole extraction procedure (Table II).

The present method not only uses one set of conditions for the extraction of the acidic, basic and neutral metabolites but also employs one set of HPLC conditions for the separation and quantitation of these classes. An important characteristic of the present method is its capability of separating PPL and its major

TABLE II

RECOVERIES OF PROPRANOLOL AND ITS METABOLITES FROM SPIKED RAT SERUM

Compound	Recovery (%)		Coefficient of variation ($n=4$) (%)
	Mean	Range	
4-OH-PPL	82.93	71.34–89.63	9.69
Des-PPL	63.95	58.93–76.34	12.94
PPL	80.54	73.89–94.58	9.50
PPL-Gly	94.45	90.37–105.19	7.17
NLA	89.23	70.72–103.31	15.28
NAA	96.53	88.44–106.36	7.39

metabolites without changing any of the extraction or chromatographic conditions. On account of its simplicity and short analysis time, this method may have wide applications in clinical and pharmacokinetic studies, where detailed metabolic profiles of PPL and its metabolites are required.

In summary, the method described herein is relatively simple, sensitive and rapid for the determination of PPL and its five metabolites in serum.

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REFERENCES

1 J. Zipper, M.E. Bruzzone, S. Angelo, V. Munoz and R.G. Wheeler, Int. J. Fertil., 27 (1982) 342.
2 R.M. Pearson, C.Y. Hong, L.G. Patel, P. Turner, D.M. Potts, R. Wheeler and J. Zipper, Contracept. Deliv. Syst., 5 (1984) 303.
3 J. Zipper, R.G. Wheeler, D.M. Potts and M. Rivera, Br. Med. J., 287 (1983) 1245.
4 D.G. Shand, G.H. Evans and A.S. Nies, Life Sci., 10 (1971) 1417.
5 D.G. Shand, R.E. Rangno and G.H. Evans, Pharmacology, 8 (1972) 344.
6 T. Walle, T.C. Fagan, E.C. Conradi, U.K. Walli and T.E. Gaffney, Clin. Pharmacol. Ther., 26 (1979) 167.
7 W.D. Mason, E.N. Amick and O.H. Weddle, Anal. Lett., 10 (1977) 515.
8 R.L. Nation, G.W. Peng and W.L. Chiou, J. Chromatogr., 145 (1978) 429.
9 A. Taburet, A.A. Taylor, J.R. Mitchell, D.E. Rollins and J.L. Pool, Life Sci., 24 (1979) 209.
10 M. Lo and S. Riegelman, J. Chromatogr., 183 (1980) 213.
11 Y. Yamamura, K. Uchino, H. Kotaki, S. Isozaki and Y. Saitoh, J. Chromatogr., 374 (1986) 311.
12 R.R. Koshakji and A.J.J. Wood, J. Pharm. Sci., 75 (1986) 87.
13 K.A. Smith, S. Wood and M. Crous, Analyst, 112 (1987) 407.
14 W. Li and R.C. Lanman, Anal. Lett., 20 (1987) 603.
15 P.M. Harrison, A.M. Tonkin, C.M. Cahill and A.J. McLean, J. Chromatogr., 343 (1985) 349.
16 E.C. Kwong and D.D. Shen, J. Chromatogr., 414 (1987) 365.
17 M.T. Smith, I. Livingstone, W.D. Hooper, M.J. Eadie and E.J. Triggs, Ther. Drug Monit., 5 (1983) 87.
18 J.F. Pritchard, D.W. Schneck and A.H. Hayes, J. Chromatogr., 162 (1979) 47.