Journal of Chromatography, 374 (1986) 223—225
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 2848

Letter to the Editor

Determination of α-naphthoxylactic acid, a major metabolite of propranolol, in plasma by high-performance liquid chromatography

Sir,

Recently we published a high-performance liquid chromatographic (HPLC) method for the determination of propranolol, its basic and neutral metabolites in plasma [1]. Although the HPLC methodology was not significantly new, a new approach for the extraction procedure based on solid-phase extraction columns (C_{18} Bond-Elut^{TM}) afforded a very simple, rapid and reproducible assay compared to the many previously published methods.

We have now extended the solid-phase extraction procedure to include the assay of naphthoxylactic acid (NLA), the major acidic metabolite of propranolol. Methods presently available for the determination of NLA involve either gas chromatography (GC) [2], HPLC [3, 4] or thin-layer chromatography (TLC) [5]. The GC methods require lengthy derivatization steps and the TLC methods require lengthy and tedious extraction steps. The HPLC methods also involve lengthy procedures and are characterised by poor reproducibility at low concentrations (< 20 ng/ml) [3]. The method we describe here is rapid (total extraction and analysis time of less than 5 min) and is highly reproducible (coefficient of variation of 4.2% at 10 ng/ml in plasma).

NLA was extracted from 0.5 ml of acidified plasma (0.5-ml plasma sample or standard made from drug-free plasma combined with 0.2 ml of 1.0 *M* hydrochloric acid) by passing through a previously activated C_{18} Bond-Elut column [1]. The Bond-Elut column was then washed with 0.5 ml of distilled water twice. The NLA was then eluted from the Bond-Elut into a polypropylene tube with 0.5 ml of acetonitrile—0.01 *M* phosphate buffer, pH 7 (25:75). A 20-μl aliquot of this extract was then injected onto the HPLC column (15 cm × 0.4 cm I.D., 5-μm C_{18} Nova-Pak, Waters Assoc., Sydney, Australia) which was eluted at a flow-rate of 2 ml/min with a mobile phase of acetonitrile—0.1% orthophosphoric acid (30:70). The NLA was detected with a Varian Flurichrom fluorescence detector (Varian Assoc., Palo Alto, CA, U.S.A.) fitted with a 220-nm interference excitation filter and a 7-60 (Varian) cut-off emission filter.

0378-4347/86/$03.50

The recovery of NLA from plasma was estimated by comparison with a non-extracted standard at the same concentration in the acetonitrile–phosphate buffer used to elute the Bond-Elut. The mean recoveries (± S.D.) from eight replicates were 76.6 ± 4.2%, 85.5 ± 1.5% and 81.3 ± 1.6% at 10, 100 and 500 ng/ml NLA, respectively.

A coefficient of variation of 5.5, 1.7 and 2.0% was obtained from eight replicate assays of plasma containing 10, 100 and 500 ng/ml NLA, respectively. The inter-assay variability of the slope of the standard plot was less than 8% over a range of 50—600 ng/ml NLA. The assay was linear over a range of 50—1000 ng/ml NLA ($y = 0.18x$, $r = 0.997$).

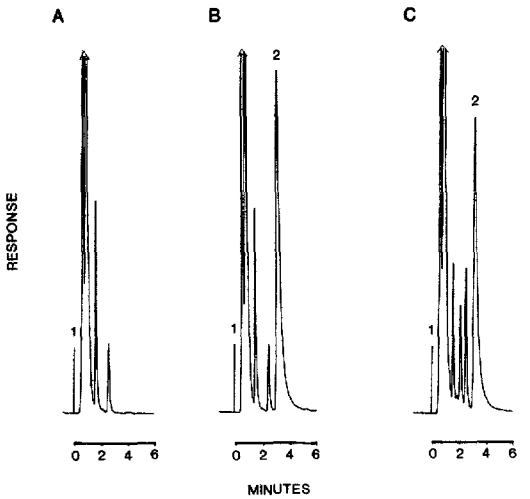

Fig. 1. Chromatograms obtained from (A) drug-free plasma, (B) plasma spiked with 400 ng/ml NLA and (C) plasma from a volunteer 1.25 h after an 80-mg oral dose of propranolol. Peaks: 1 = injection point; 2 = NLA.

Fig. 1 shows the chromatograms obtained from (A) drug-free plasma; (B) plasma spiked with 400 ng/ml NLA and (C) plasma from a volunteer 1.25 h after an 80-mg oral dose of propranolol. The assay was free of interference from any endogenous plasma component or from other drugs commonly co-administered with propranolol (atenolol, metoprolol, hydrallazine, minoxidil, methyldopa, pindolol, frusemide, chlorothiazide, sorbide nitrate, clofibrate, nifedipine, verapamil, dipyridamole, tolbutamide and aspirin).

In summary, the method we describe here offers a rapid and straightforward extraction of NLA from plasma which can be analysed easily by HPLC. The method is reproducible and sensitive and does not suffer any interference from drugs commonly coadministered with propranolol.

Department of Clinical Pharmacology,
Alfred Hospital, Prahran,
3181 Melbourne
(Australia)

P.M. HARRISON*
A.M. TONKIN
S.T. DIXON
A.J. McLEAN

1 P.M. Harrison, A.M. Tonkin, C.M. Cahill and A.J. McLean, J. Chromatogr., 343 (1985) 349.
2 D.E. Easterling, T. Walle, E.C. Conradi and T.E. Gaffney, J. Chromatogr., 162 (1979) 439.
3 M.T. Smith, I. Livingstone, W.D. Hooper, M.J. Eadie and E.J. Triggs, Ther. Drug Monitor., 5 (1983) 87.
4 M. Lo and S. Riegelman, J. Chromatogr., 183 (1980) 213.
5 Y. Garceau, I. Davis and J. Hasegwa, J. Pharm. Sci., 67 (1978) 826.

(First received June 6th, 1985; revised manuscript received August 29th, 1985)