DETERMINATION OF PROPRANOLOL AND 4-HYDROXYPROPRANOLOL IN TISSUE HOMOGENATES BY DIRECT INJECTION AND COLUMN SWITCHING HPLC

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An improved HPLC method is proposed for simultaneous determination of propranolol (PL) and 4-hydroxypropranolol (4-OH PL) in tissue homogenates.

KEYWORDS propranolol; 4-hydroxypropranolol; tissue homogenates; direct injection; column switching; HPLC

We have already reported on the direct injection and column switching HPLC,[1] and also on the HPLC determination of PL and 4-OH PL in plasma.[2] But in tissue homogenates air oxidation of 4-OH PL proceeded quickly. So an improved procedure was developed for the simultaneous determination in tissue homogenates as follows.

1. Preparation of tissue homogenates: A mixture of 100 mg tissue/ml of 0.25 M saccharose solution containing 30 mM phosphate (pH 3.0) was homogenized in a small Potter-Elvehjem homogenizer dipped in ice water. In this medium 4-OH PL was stable for at least 5 h.

2. HPLC: We used three columns, i.e., a guard column (1 cm x 4 mm ID, pore size of the inlet and outlet endfitting filter 70 μm and 2 μm, respectively) packed with TSK gel HW-65C (particle size 50-100 μm), a precolumn (1 cm x 4 mm ID) packed with TSK SP-SW gel (a cation exchanger) coated with protein[3] in an acidic medium and an analytical column (5 cm x 4 mm ID, maintained at 45°C) packed with TSK gel ODS 80TM (particle size 5 μm).

At first, the guard column and precolumn were equilibrated with 10 mM citrate buffer (CB, pH 2.7, flow rate 1 ml/min). Then, freshly prepared tissue homogenate sample (100 μl) was injected into the guard column and precolumn. Proteins in the sample flowed out into the drain, minute particles and adsorptive endogenous components were trapped in the guard column, and PL and 4-OH PL were adsorbed in the precolumn. At 6 min after injection the precolumn was disconnected from the guard column and connected to the analytical column in a backflushing mode. Then, the first eluent of 0.1 M CB (pH 4.0) containing 15% acetonitrile (MeCN) was allowed to flow at 1 ml/min for 3 min to elute 4-OH PL, then the eluent was switched to 0.1 M CB (pH 4.0) containing 22% MeCN for 7 min to elute PL. The PL and 4-OH PL were detected by two fluorometers connected in series, λ_{ex}= 310 nm, λ_{em}= 350 nm for PL, and λ_{ex}= 320 nm, λ_{em}= 420 nm for 4-OH PL. The guard column was rinsed with 0.5% sodium dodecyl sulfate solution containing 0.1 M NaOH for 5 min and methanol for 5 min by backflushing.

The guard column could be used about 20 times for routine analyses. When the endfitting filter was clogged, it was taken

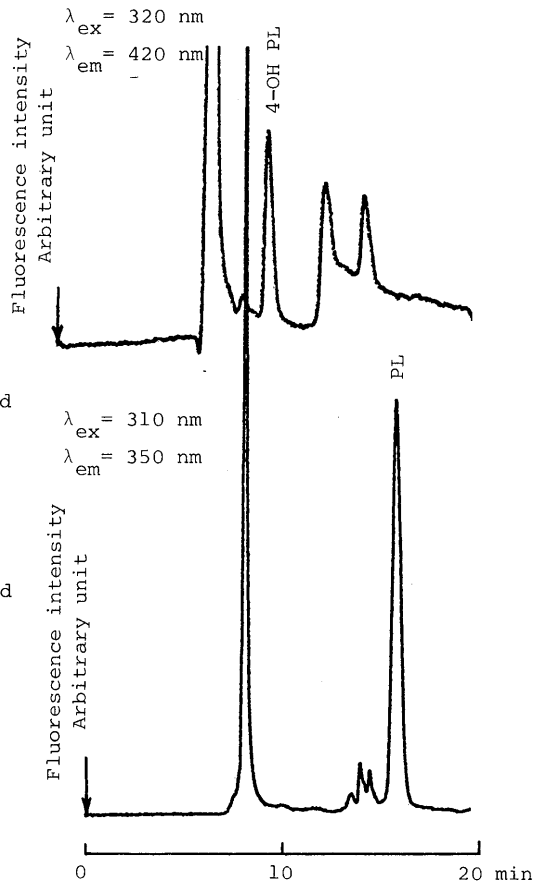

Fig. 1. Elution Profile of PL and 4-OH PL

Sample: Mouse liver homogenate (100 μl) spiked with PL (13 ng) and 4-OH PL (14 ng).

Flow rate: 1 ml/min.

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off and washed with 0.2 M NaOH solution under sonication.

The elution profile is shown in Fig. 1. The detection limits were 0.13 ng (S/N = 5) for PL and 0.28 ng (S/N = 5) for 4-OH PL. The recoveries were 100.4-103.6% with CV value less than 4% (n = 25) from the added 13 ng of PL and 14 ng of 4-OH PL. After denaturating precipitation of proteins by using 0.4 M perchloric acid or 5% trichloroacetic acid, the recoveries from kidney homogenates were only 51.4% and 49.3% from the added 13 ng of PL (n = 5) and 65.4% and 60.3% from 14 ng of 4-OH PL (n = 5), respectively. The loss seems to be caused by the coprecipitation. When 50% MeCN was used for denaturation, the recoveries were 101.6% and 101.5% for PL and 4-OH PL, respectively. But, 100% recovery by the column switching method was feasible without such time-consuming pretreatment.

The result of an application is shown in Table I. PL was administered in mice (3.3 μg/g wt.) by abdominal injection, and liver, kidney and heart samples were collected at intervals of 0.5, 1.0 and 2.0 h. In liver and kidney, the concentration of PL was ten times as high as 4-OH PL. PL in heart occurred at almost the same concentration as liver, but 4-OH PL could hardly be detected in heart.

Table I. PL and 4-OH PL in Mouse Liver, Kidney and Heart after Abdominal Injection

Time after injection, h		Liver	Kidney	Heart
		(μg/g-tissue)		
0.5	PL	1.24	2.60	1.01
	4-OH PL	0.13	0.34	0
1.0	PL	0.52	0.93	0.56
	4-OH PL	0.06	0.17	0
2.0	PL	0.26	0.47	0.18
	4-OH PL	0.05	0.05	0

(Data are the average of three experiments.)
(Dose: 3.3 μg PL/g wt. DDY mouse.)

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