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Note

Sequential thin-layer chromatography of propranolol

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Propranolol [1-(isopropylamino)-3-(1-naphthoxy)-2-propanol] is a beta-adrenergic receptor blocking agent which has a variety of pharmacologic actions[1], *i.e.*, antianginal, antiarrhythmic, anticonvulsant, antianxiety, and antihypertensive. It has been used in treatment of pheochromocytoma, throtoxicosis, hypertrophic subaortic stenosis, Parkinson's disease, acne vulgaris, anxiety, tremor, alcoholism, heroin addiction, mania and psychosis. Propranolol has been reported to cause toxic effects which include[2]: hypoglycemia, bronchoconstriction, thrombocytopenic and non-thrombocytopenic purpura, rash, visual and other hallucinations, drowsiness and paresthesias.

Several analytical procedures have been used to determine propranolol: spectrophotofluorometry[3–7], gas–liquid chromatography (GLC)[8–11], mass fragmentography[12], high-pressure liquid chromatography (HPLC)[13] and radioimmunoassay[14]. All of these procedures have primarily been used for determination of propranolol and in some instances of a few metabolites. Furthermore, spectrophotofluorometric methods lack sensitivity. GLC and HPLC procedures, although specific, are laborious and suffer from interferences from substances present in serum. The radioimmunoassay method is limited because of the unavailability of suitable antisera. Recently, sequential thin-layer chromatographic (STLC) methods have been employed to analyze some pesticides[15–17]. This paper describes a rapid STLC method for the sensitive and specific analyses of propranolol and seven of its possible metabolites.

EXPERIMENTAL

Chemicals

DL-Propranolol hydrochloride, 4-hydroxypropranolol hydrochloride and [^{14}C]propranolol were provided by Ayerst Research Laboratories (Montreal, Quebec, Canada). Propranolol glycol [3-(α-naphthoxy)-1,2-propanediol] and α-naphthoxylactic acid [3-(α-naphthoxy)-2-hydroxy-1-propionic acid] were supplied by Dr. T. Walle (Medical University of South Carolina, Charleston, S.C., U.S.A.). Other reference compounds used were α-naphthoxyacetic acid (Pfaltz and Bauer, Stamford,

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Conn., U.S.A.), α-naphthol (Fischer, Pittsburgh, Pa., U.S.A.), 1.4-naphthalendiol and 1,5-naphthalendiol (Eastman-Kodak, Rochester, N.Y., U.S.A.). Two radioactive chemicals were used: DL-[^{14}C]propranolol hydrochloride 1-(isopropylamino)-3-([1-^{14}C]naphthoxy)-2-propanol (specific activity 10.65 mCi/mmole). DL-[^{3}H]propranolol hydrochloride (specific activity 5–10 Ci/mmole) was randomly labeled (New England Nuclear, Boston, Mass., U.S.A.).

Thin-layer plates

Gelman type SG, ITLC, silica gel-impregnated glass fiber sheets (Gelman, Ann Arbor, Mich., U.S.A.), were used.

Solvents

The solvents used were: primary solvent, acetonitrile–benzene–*n*-hexane–ammonia (80:40:40:1) and secondary solvent, *n*-hexane–diethyl ether (9:1).

Procedure

Aliquots (10 μl) of ethanol solution (1 mg/kg) of propranolol hydrochloride and related compounds were spotted on the ITLC sheets. Chromatograms were developed in a lined, pre-equilibrated tank up to 10 cm with the primary solvent, then to 16 cm with the secondary solvent (23 min). The respective regions were identified by visualization in an iodine chamber.

The radiochemical purities of [^{14}C]- and [^{3}H]propranolol were evaluated using the same STLC system. For the characterization of impurities, a mixture of propranolol and structurally related compounds was added to each of the [^{14}C]- and [^{3}H]propranolol solutions, and the chromatography of each solution was subsequently determined. The standards were detected by their color in iodine vapor. The ITLC sheets were cut into 5-mm strips and placed in a scintillation medium. The scintillation solvent was composed of one volume of water, three volumes of Triton X-100, and six volumes of toluene containing 5 g of 2,5-diphenyloxazole (PPO) and 200 mg of 1,4-bis-(5-phenyloxazolyl-2)-benzene (POPOP) per litre. Radioactivity was determined using a Beckman Model LS-100C liquid scintillation spectrometer.

RESULTS AND DISCUSSION

The reported inconsistency in the development of pharmacologic and adverse effects of propranolol might be attributed to the variability of its pharmacokinetics and metabolism in man[18]. Furthermore, since more than 95% of propranolol is metabolized in man[4], some of the drug's effects may be caused by one or more of its metabolic products. One metabolite of propranolol, 4-hydroxypropranolol, showed beta-blocking activity[19]. Another metabolite, propranolol glycol, caused central nervous system effects[2]. It is therefore important to develop a single technique, for accurate separation, isolation, and identification of propranolol and its possible metabolites in man and animals.

The R_F values (average of three developments) of propranolol and related compounds in two single and one sequential system are listed in Table I. In a preliminary study, no single system was found capable of separating all these compounds. These chemicals could be classified into two groups according to their separation on

TABLE I

R_F VALUES FOR PROPRANOLOL AND RELATED COMPOUNDS ON ITLC SHEETS USING SINGLE AND SEQUENTIAL SOLVENT SYSTEMS

Solvents: primary, acetonitrile–benzene–*n*-hexane–ammonia (80:40:40:1); secondary, *n*-hexane–diethyl ether (9:1); sequential solvent system, primary solvent for 10 cm and secondary solvent 16 cm.

Compound	*Primary solvent*	*Secondary solvent*	*Primary solvent followed by secondary solvent*
α-Naphthoxylactic acid	0.03	0.00	0.02
α-Naphthoxyacetic acid	0.15	0.05	0.10
4-Hydroxypropranolol	0.62	0.00	0.38
Propranolol	0.85	0.00	0.51
1,5-Naphthalenediol	0.93	0.50	0.56
1,4-Naphthalenediol	1.00	0.85	0.62
Propranolol glycol	0.98	0.10	0.66
α-Naphthol	1.00	0.88	0.95

ITLC: group A consisted of propranolol, 4-hydroxypropranolol, α-naphthoxylactic acid and α-naphthoxyacetic acid and group B consisted of α-naphthol, 1,4-naphthalenediol and 1,5-naphthalenediol. Propranolol glycol behaved as a group A compound in the secondary solvent and as a group B compound in the primary solvent.

The best solvent system for the separation of a group A compounds from each other, as well as from compounds which belong to group B, was the primary solvent. In this system all group B compounds and propranol glycol moved together and too quickly with the solvent front (R_F = 0.93–1.0) to interfere with the group A compounds. The latter substances moved at good separable distances from each other (see Table I). On the other hand, when the secondary solvent was used, compounds in group B moved and separated from the stationary group A compounds (Table I).

By employing a two-solvent sequential TLC system, which developed the ITLC sheets first with the primary solvent for 10 cm followed by the secondary solvent for 16 cm, a good resolution of all compounds tested was obtained (Table I).

It is of interest to report that the width of the ITLC sheet used is of critical importance in determining the R_F values of the compound analyzed. Table II shows that when propranolol was run on sheets with different widths using this STLC

TABLE II

EFFECTS OF THE WIDTH OF ITLC SHEETS ON THE DEVELOPING TIMES OF THE PRIMARY AND SECONDARY SOLVENT FRONTS AND R_F VALUE FOR PROPRANOLOL

Solvents: primary, acetonitrile–benzene–*n*-hexane–ammonia (80:40:40:1); secondary, *n*-hexane–diethyl ether (9:1); sequential solvent system, primary solvent for 10 cm and secondary solvent 16 cm.

ITLC width (cm)	*Developing time (min)*		*Propranolol* R_F
	Primary solvent	*Secondary solvent*	
2.0	14	31	0.11
5.0	13	32	0.12
10.0	13	32	0.13
20.0	7	16	0.53

system, both solvents moved at slower rate and the R_F value of propranolol decreased by the decrease in the sheet width. This effect seems to be the result of inefficient equilibration of the small-width thin-layer sheets with the solvent vapors, despite pre-equilibrating and lining the tank with filter paper. However, the movement of the solvent front and the R_F value of propranolol were reproducible when a 20-cm-wide ITLC sheet was used, and they were not affected by the distance of the spot from the edge of the ITLC sheet.

The radiochemical purities of [^{14}C]- and [^{3}H]propranolol were evaluated using the two solvent sequential TLC system. [^{14}C]propranolol was found to be 97.72% pure (Fig. 1). The impurities were identified by STLC to be α-naphthoxylacetic acid (0.38%), α-naphthoxyacetic acid (0.15%), 4-hydroxypropranolol (1.05%) propranolol glycol (0.23%) and α-naphthol (0.10%). Two other impurities which were not identified, X_1 and X_2, had R_F values of 0.25 and 0.78 and accounted for 0.27% and 0.10% of total radioactivity, respectively. The purity of [^{3}H] propranolol was 84.02% (Fig. 2), with 3.23% α-naphthoxylacetic acid, 0.83% α-naphthoxyacetic acid, 4.39% 4-hydroxypropranolol, 0.80% propranolol glycol, and 0.10% α-naphthol. Two unidentified impurities had the same R_F values as X_1 and X_2 in [^{14}C]propranolol and accounted for 1.58% and 1.72% of total radio-activity, respectively.

This STLC method is very useful for the separation and identification of propranolol and some of its possible degradation products. It is rapid (23 min), convenient in handling the chromatogram, and is highly reproducible. The ITLC strips could be cut for quantitative determination by liquid scintillation counting or extracted for unequivocal identification of each compound using other techniques such as infrared spectroscopy and mass spectrometry. The radioactive purities of [^{14}C]- and [^{3}H]propranolol have been determined using this system.

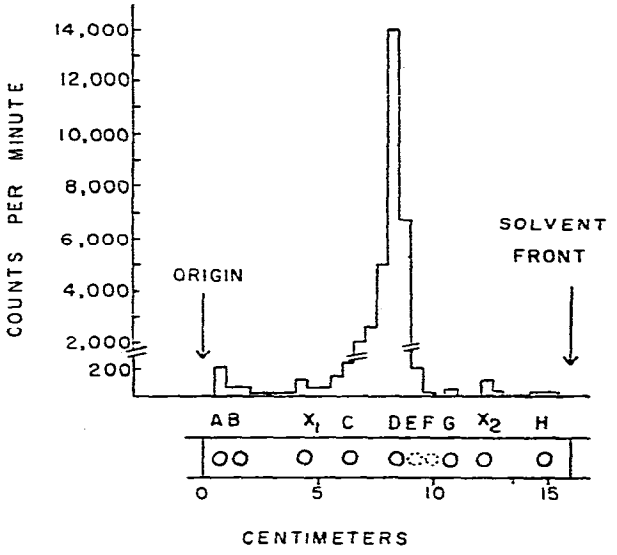

Fig. 1. Chromatogram and scan for [^{14}C]propranolol, using Gelman type SG, ITLC silica gel-impregnated glass fiber sheets, following sequential elution with the primary solvent for 10 cm and the secondary solvent for 16 cm. A = α-Naphthoxylactic acid; B = α-naphthoxyacetic acid; C = 4-hydroxypropranolol; D = propranolol; E = 1,5-naphthalenediol; F = 1,4-naphthalenediol; G = propranolol glycol; H = α-naphthol; X_1 and X_2 = unknowns.

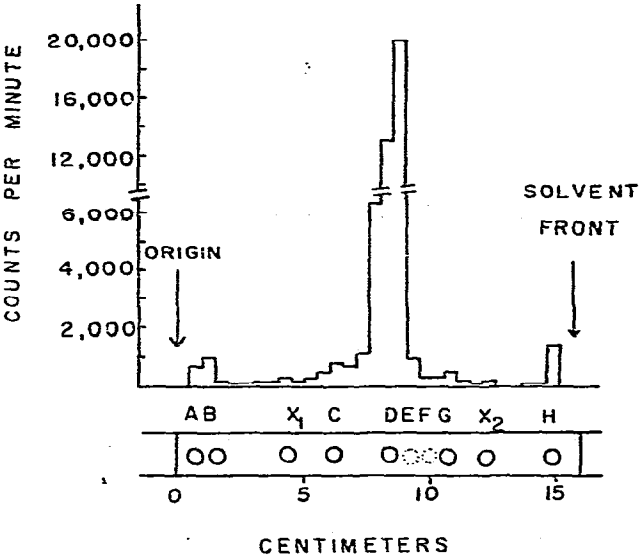

Fig. 2. Chromatogram and scan for [^{3}H]propranolol, using Gelman type SG, ITLC silica gel-impregnated glass fiber sheets as described in legend of Fig. 1.

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