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Note

Determination of ophthalmic therapeutic metipranolol and its degradation product by reversed-phase high-performance liquid chromatography

E. VIDAL
Laboratoire de Pharmacognosie–Homéopathie, Faculté de Pharmacie, 27 Boulevard J. Moulin, 13385 Marseille Cedex 5 (France)
M. GUIGUES
Laboratoires Dulcis, "Le Mercator", Rue de l'Industrie, Monaco (Monaco)
and
G. BALANSARD* and R. ELIAS
Laboratoire de Pharmacognosie–Homéopathie, Faculté de Pharmacie, 27 Boulevard J. Moulin, 13385 Marseille Cedex 5 (France)
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Metipranolol, or trimepranol, is a beta-blocker. As with all beta-blockers, it has a hydroxyisopropylamino chain bound to an aromatic ring[1] (Fig. 1a).

Metipranolol can be partially deacetylated, yielding deacetylmetipranolol (Fig. 1b).

Metipranolol is used as a drug for the cardiovascular system[2,3] and also has therapeutic value in the treatment of glaucoma[4,5]. As a result of its use in pharmaceutical preparations, it is necessary to have an analytical method available for testing its purity via the detection of its degradation product.

Reports on beta-blocker analytical methods are numerous[6–14], most of them involving gas–liquid chromatography (GLC). However, this technique requires derivatization prior to injection. High-performance liquid chromatography (HPLC) has also been used to assay beta-blockers in biological fluids[6]. This method is fast and simple and does not need derivatization of the products. An Ion-pair HPLC method has been used with a reversed-phase μBondapak C_{18} column, a water–acetic acid–methanol solvent mixture and 1-heptanesulphonic acid as counter ion.

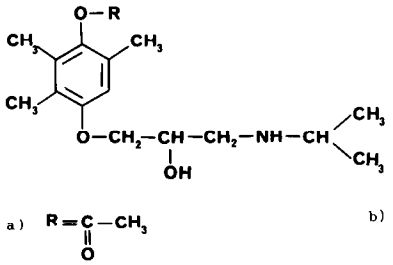

Fig. 1. (a) Structures of (a) metipranolol and (b) deacetylmetipranolol.

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The present work describes a reversed-phase HPLC technique for the separation of metipranolol and deacetylmetipranolol. A reversed-phase μBondapak C_{18} column was used with a mobile phase of acetonitrile, water and 1-hexanesulphonic acid (PIC B_6, Waters Assoc., Paris, France).

EXPERIMENTAL

Equipment

Separations were performed on a Waters liquid chromatograph which was equipped with a Model 6000 A pump, a U6K universal syringe injector and a Model 480 UV detector. The outputs of the detector were connected to a Data Module Model 833 integrator (Merck and Hitachi, France).

Chromatography

A reversed-phase μBondapak C_{18} column, 30 cm × 3.9 mm (waters) was used

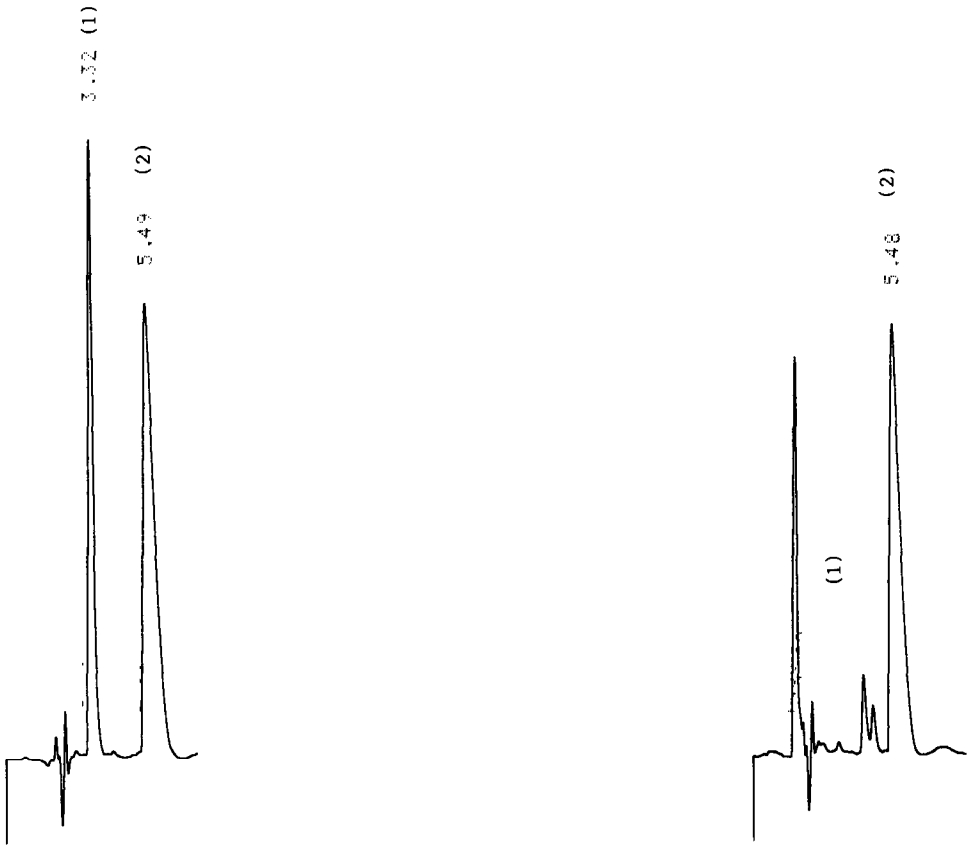

Fig. 2. Representative chromatogram of metipranolol (2, retention time 5.49 min, concentration 1.2 mg/ml) and deacetylmetipranolol (1, 3.32 min, 0.6 mg/ml) in double-distilled water. Attenuation at the time of injection was 0.1.

Fig. 3. Typical chromatogram of a diluted eye-drop solution dosed at 0.3% metipranolol. Attenuation was 0.1. 1 = Deacetylmetipranolol; 2 = metipranolol (retention time 5.48 min).

to separate metipranolol and deacetylmetipranolol. The solvent system was acetonitrile–water–PIC B_6 (175:315:10). The flow-rate was 1.5 ml/min. The column effluent was monitored at 254 nm. Volumes of 20 μl were injected. Sensitivity was 0.1 for the assay of metipranolol and 0.05 for the assay of deacetylmetipranolol.

Samples

The following samples were used: (i) reference metipranolol at 1.2 mg/ml in distilled water; (ii) reference deacetylmetipranolol at 0.6 mg/ml in distilled water; (iii)

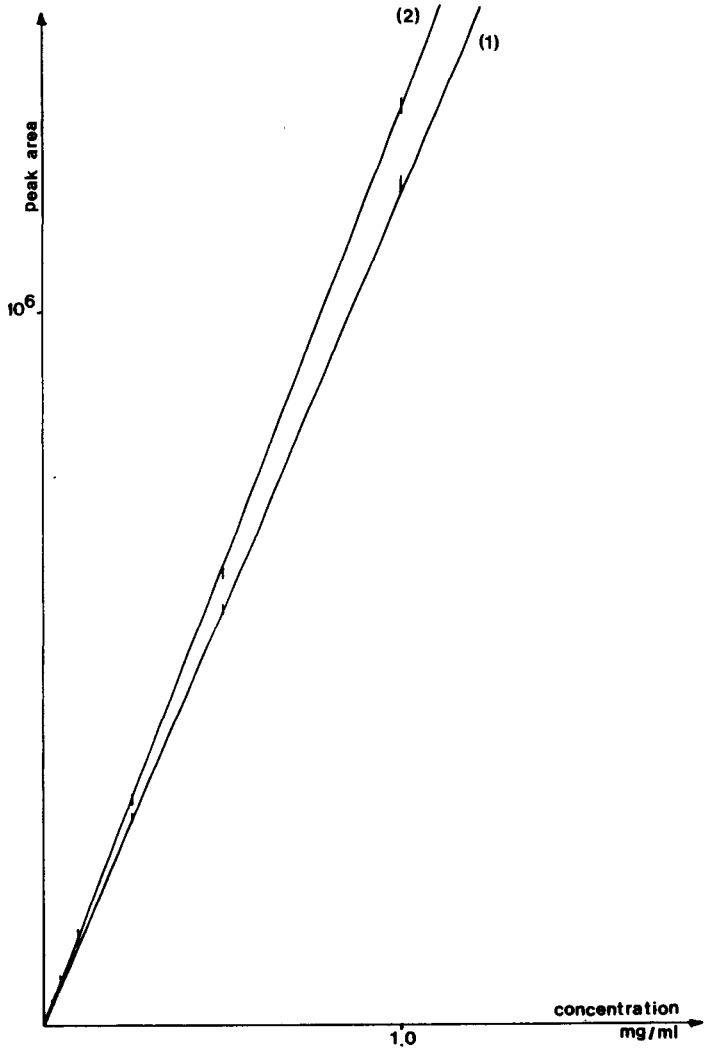

Fig. 4. Standard curves of metipranolol (1) and deacetylmetipranolol (2) *versus* peak area. Data represent mean ± S.D. of replicate samples (n = 4) run on the same day. Equations for the lines were calculated by linear regression analysis. Peak areas were expressed in integrator units.

TABLE I

COEFFICIENTS OF VARIATION FOR REPLICATE SAMPLES (n = 4) OBTAINED ON THE SAME DAY

Compound	*Concentration (mg/ml)*	*Mean peak area**	*S.D.*	*Coefficients of variation (%)*
Metipranolol	0.025	30 976	630	2.0
	0.050	59 229	1667	2.8
	0.100	121 117	869	0.7
	0.250	294 940	730	0.2
	0.500	592 163	9122	1.5
	1.000	1 182 212	25 558	2.1
	2.000	2 337 410	42 141	1.8
Deacetylmetipranolol	0.005	6634	241	3.6
	0.010	12 694	691	5.4
	0.025	31 898	221	0.7
	0.050	63 137	1028	1.6
	0.100	126 725	845	0.7
	0.250	314 774	4834	1.5
	0.500	632 336	13 405	2.1
	1.000	1 294 765	25 130	1.9

* Peak areas are expressed as arbitrary electric impulsion units of the integrator.

eye drops dosed at 0.3% metipranolol and diluted (4 ml/10 ml distilled water); and (iv) eye drops dosed at 0.6% metipranolol and diluted (2 ml/10 ml distilled water).

RESULTS AND DISCUSSION

Resolution

Fig. 2 shows a chromatogram of metipranolol and deacetylmetipranolol standards. The retention times of metipranolol and deacetylmetipranolol standards. The retention times of metipranolol and deacetylmetipranolol were 5.49 and 3.32 min, respectively. A typical chromatogram from one diluted eye-drop solution is shown in Fig. 3. Each product was well resolved (resolution = 2.84) and has symmetrical peaks. Interfering peaks from eye-drop solutions were not observed at 254 nm.

Standard curves

Linear standard curves were obtained for concentrations of metipranolol from 0.025 to 2 mg/ml and for concentrations of deacetylmetipranolol from 0.005 to 1

TABLE II

METIPRANOLOL AND DEACETYLMETIPRANOLOL CONTENT OF EYE-DROP SOLUTIONS (g/100 ml)

Sample	*Eye drops dosed at 0.3% metipranolol*	*Eye drops dosed at 0.6% metipranolol*
Metipranolol	0.295	0.610
Deacetylmetipranolol	0.0046	0.0095

mg/ml (Fig. 4). The coefficients of variation for replicate samples obtained on the same day are shown in Table I. The practical limits of detection for metipranolol and deacetylmetipranolol are 0.02 and 0.005 mg/ml, respectively.

Assays of metipranolol and deacetylmetipranolol in eye-drop solutions were performed by using an external standard method of calculation and assays of two eye-drop solutions were realized (Table II). Each result is the mean of three assays.

A rapid, reliable and sensitive procedure was developed that allows the separation of metipranolol and its degradation product, even at low concentrations ($\leqslant$ 5 μg/ml). This method should be well adapted for routine control of eye-drop solutions.

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