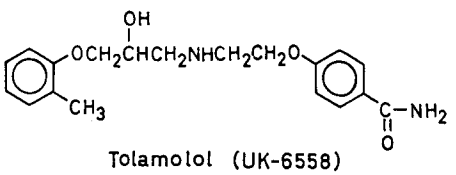
Thin layer chromatography — fluorimetric estimation of tolamolol in human plasma

Tolamolol is a new β-adrenoceptor blocking agent, (Augstein, Cox & others, 1973) which has been shown to be cardio-selective in man (Briant, Dollery & others, 1973). A quantitative thin layer chromatographic method has been developed for the estimation of unchanged drug concentration in human plasma or serum.



Tolamolol (UK-6558)

4-[2-(2-Hydroxy-3-*o*-tolyloxypropylamine)ethoxy]benzamide

A Vitatron TLD 100 densitometer (Fisons Scientific Apparatus) was used to measure the fluorescence of the t.l.c. spots. Peak areas were measured with a Hewlett Packard integrator (Model 3370 B). The t.l.c. plates were Merck precoated silica gel 60 without fluorescent indicator.

Ethyl acetate, methanol and isopropanol were Analar grade. The ethyl acetate was dried over molecular sieve 4A and then distilled. Hexane, heptane and liquid paraffin were fractions from petroleum (BDH). The fluorescent reagent, 1-ethoxy-4-(dichloro-s-triazinyl)naphthalene (EDTN) was synthesized by the method of Shaw & Ward (1968) and used as a solution in dry ethyl acetate, 0·2 mg ml^{-1}. The internal standard, 1-[2-(2-carbamoyl-4-methylphenoxy)-ethylamino]-3-(2-methylphenoxy)-propan-2-ol, (UK-6633) was synthesized according to Augstein, Ham & others (1971). Tolamolol hydrochloride was used to prepare standard solutions. The internal standard was dissolved in methanol (1·0 mg ml^{-1}) and this stock solution was diluted with distilled water on the day of use to give a solution of 100 ng ml^{-1}.

1·0 ml of water containing 100 ng of internal standard was added to 1·0 ml of human heparinized plasma or serum in a 10 ml screw-capped centrifuge tube. Ethyl acetate (4 ml) was added and the solutions were mixed mechanically for 10 min and the phases separated by centrifugation. The ethyl acetate was transferred to a clean centrifuge tube, care being taken not to carry over any of the aqueous layer or material at the interface. The extract was heated to 40° and a stream of nitrogen passed over the surface to evaporate the solvent. To the residue in 0·5 ml methanol, heptane (1·0 ml) was added and the tubes shaken for 20 s. After centrifugation for 2 min, the heptane (upper layer) was discarded and the methanol was evaporated in a stream of nitrogen at 40°. The residue was dissolved in 50 μl of a solution of EDTN in dry ethyl acetate and the reaction allowed to proceed for 10 min at 40°. The reaction mixture was then transferred to a t.l.c. plate taking care to keep spot size less than 4 mm by directing a stream of unheated air over the plate. The plates were placed in filter paper lined tanks containing the solvent system hexane–isopropanol (75 : 25) and the solvent was allowed to ascend 15 cm above the origin. After the plates had been dried in a stream of unheated air (2 min) the fluorescent spots were stabilized by spraying with liquid paraffin–diethyl ether (1 : 2).

The fluorescence of the spots due to the derivatives of tolamolol and the internal standard was measured using the densitometer in the fluorescence mode with UVB and 430 nm filters. The integrator was used to determine the peak areas.

Measured quantities of tolamolol were added to 1·0 ml aliquots of human plasma

over the range 20–200 ng free base ml^{-1} plasma. These samples were assayed according to the above procedure and the results used to prepare a standard curve. The ratio of the areas of the peaks due to the derivatives of tolamolol and the internal standard were plotted against the concentration of tolamolol in plasma. The relation is linear up to 200 ng.

The R_F value for the derivatives of tolamolol and the internal standard were 0·23 and 0·35 respectively.

A hydroxylated metabolite of tolamolol is extracted from the plasma with ethyl acetate and the fluorescent derivatives of this metabolite and tolamolol are separated by the t.l.c. stage. The fluorescent derivative of the metabolite has an R_F value of 0·14 and runs below the tolamolol derivative. Other metabolites of tolamolol (Wood, Stopher & Monro, 1974) are not extracted from plasma by ethyl acetate. The assay is therefore specific for unchanged drug.

The minimum detectable level was 2 ng tolamolol base ml^{-1} plasma. When tolamolol levels in the range 2–20 ng ml^{-1} plasma were assayed the amount of internal standard used was reduced to 10 ng.

Replicate assays were carried out on samples of plasma containing 100 ng ml^{-1} of tolamolol and 50 ng internal standard. Nine replicate assays gave a mean peak area ratio of 2·031 with a coefficient of variation of 3·6%.

The accuracy of the assay over the concentration range 20–200 ng ml^{-1} is illustrated by the assay results of a set of serum samples in which the tolamolol concentrations were unknown to the operator. Eight solutions of the drug in serum in which its concentration varied from 32–180 ng ml^{-1} were assayed and the recovery was 98 $\pm$ 2% (s.e.).

The assay has been used routinely with 1·0 ml samples of plasma or serum from man and dog treated with effective β-blocking doses of tolamolol. The blood concentration profile of tolamolol can be followed over five half-lives.

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