An improved gas-liquid chromatographic method for determining practolol in plasma and urine

Recently, a fluorometric method for estimating practolol, a β-adrenoceptor blocking drug, in blood and urine was reported by Bodem & Chidsey (1972), to be more sensitive and more specific than the colorimetric method of Fitzgerald & Scales (1968). Both methods are time consuming and large plasma samples are needed.

The qualitative determination (Scales & Cosgrove, 1970) of practolol using a gas-liquid chromatographic (electron-capture detection) procedure has given good results. However for quantitative estimation, the calibration curve was obtained without addition of an internal standard. The cell-voltage variation during the day in some types of electron-capture detectors may be large and so a very sensitive procedure independent of this has been developed in our laboratory.

A Varian Model 2100 gas-liquid chromatograph equipped with a ^3H electron capture detector, fitted with a Kent Chromalog 2 integrator, was used. The conditions were: a glass column, U and spirally shaped, length 3·25m, i.d. 3 mm, packed with 4% SE 30 on Gas-Chrom Q, 80–100 mesh operating at 200°. Injection port and detector temperatures: 235° and 215° respectively; carrier gas (pre-purified nitrogen) flow-rate was 60 ml min^{-1}.

The column was conditioned for 48 h at 250° (45 ml min^{-1} nitrogen flow) and 1 μl samples in n-heptane were injected.

All reagents were of analytical reagent quality. High purity trifluoracetic anhydride (99% Merck or Aldrich) was used for derivatization.

Practolol [4-(2-hydroxy-3-isopropylaminopropoxy)-acetanilide] and propranolol [1-(2-hydroxy-3-isopropylaminopropoxy)-naphthalene hydrochloride] were supplied by ICI S.A. Belgium.

Plasma: The method of Scales & Cosgrove (1970) was modified: to plasma (1 ml), are added isotonic sodium sulphate (5 ml), aqueous sodium tungstate (1 ml; 10% w/v) and sulphuric acid (1 ml; 0·66N). The mixture is vigorously shaken and after centrifugation at 3000 rev min^{-1} for 10 min, 5 ml of the supernatant is transferred to a stoppered tube containing 1·5 ml of sulphuric acid (13 N). The solution is heated for 1 h in a water bath at 85° ± 1° to deacetylate the drug; the sample is then cooled in ice, made alkaline with 2·5 ml sodium hydroxide solution (40% w/v) and extracted by shaking it for 5 min with 3·5 ml chloroform—diethyl ether (1 : 4). 2·5 ml of the organic layer is transferred to a stoppered tube and evaporated under vacuum without warming.

To the evaporated extract are added 25 μl of internal standard propranolol in ethyl acetate (41 mg $litre^{-1}$), 0·5 ml of dry ethyl acetate and 0·3 ml of trifluoracetic anhydride. The mixture was maintained stoppered at 30° for 50 min in a thermobloc, to assure complete derivatization of the compounds. The solution is evaporated to dryness. The residue is stable if kept dry and cold. Just before injection, the residue is mixed with 200 μl n-heptane.

The ratio of the peak heights i.e. H. practolol/H. propranolol is refered to a calibration curve and converted in μg or ng practolol per ml plasma. The calibration curves are established with drug free plasma mixed with known amounts of practolol, giving solutions ranging from 0·264 to 2·2 μg ml^{-1}, or 50 to 500 ng ml^{-1} with 10μl internal standard. By measuring the peaks ratio, the concentrations of practolol in unknown samples are obtained by direct reference to the calibration curves.

Urine: The starting solution contains the urine sample (1 ml), five times diluted, isotonic sodium sulphate (6 ml) and sulphuric acid (1 ml; 0·66N). 1 ml of the organic

layer is evaporated after deacetylation and 100 μl internal standard added. After derivatization and evaporation, the residue is mixed with n-heptane (1 ml) and 1μl injected into the gas chromatograph.

A calibration curve is established with a solution of practolol in isotonic sodium sulphate (10·6 mg litre^{-1}).

With this procedure, the practolol is converted into deacetyl-practolol and in view to determine the metabolite, deacetyl-practolol, the hydrolysis reaction with hot sulphuric acid must be omitted. Unchanged practolol is measured by difference.

The retention times are respectively 4·6 and 6·0 min for practolol and propranolol.

The sharpness of the peaks corresponding to practolol and propranolol permits the accurate measurement of the peak heights. A calibration curve is used for determining low drug levels in human plasma where the sensitivity reached 10 ng ml^{-1}.

The recovery of the method is estimated to be about 80%. The accuracy has been established: 90 to 100% for triplicate injections.

The structure of the tris-trifluoracetyl derivative has been checked using a coupled system g.l.c.-m.s.* (LKB 9000 Column OV–1 1% on chromosorb W 60–80 mesh; T° 200°; He 30 ml min^{-1}; ionization potential 70 eV). A week molecular ion at 512 a.m.u. is observed and the fragmentation pattern corresponds to the spectrum published by Garteiz & Walle (1972).

By varying the amounts of the internal standard and the sensitivity of the electrometer, different biological fluids such as plasma, bile, urine, after appropriate dilution, could be analysed.

The inclusion of an internal standard of similar chemical structure and chromatographic properties, allows drug concentrations to be determined with great accuracy and this method becomes independent from the detector operating conditions.

With the method described, both practolol and its possible metabolite, deacetylated practolol, are quantitatively determinated. However, in man, after oral intake of 600 mg, no detectable deacetylated metabolite was found by chromatography in the 24 h urine collection (Bodem & Chidsey, 1973). Consequently, in clinical pharmacology, this fact can be neglected. Also in rats and dogs, although it has been demonstrated that about 4% of the administered drug is deacetylated, no deacetylated drug was detected by Scales & Cosgrove (1970).

The method has been used for the reappraisal of the pharmacokinetic data of practolol in healthy volunteers and in patients with renal failure and for the determination of the plasma half-life and of the urine concentrations after oral administration of 200 mg as single dose.

More recently, an analogous procedure for the determination of oxprenolol (Jack & Riess, 1974) another β-blocking drug, has been published and so prompted us to report our method.

The main advantages offered by the described method, must be emphasized: specificity, high sensitivity (10 ng ml^{-1}) and minimum need of plasma 1 ml only.

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