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Gas-liquid chromatographic estimation in blood of ICI 58 834

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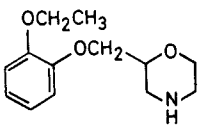
The blood levels attained at therapeutic oral doses of ICI 58 834 hydrochloride, a novel psychotropic agent, are adequately assayed by gas-liquid chromatography. The free base is extracted into benzene and converted to its heptafluorobutyrate derivative. The derivative is chromatographed on a column of 1% XE60 on Gas-Chrom Q and detected by electron capture. Recovery from blood is 80–90% and the minimum detectable level is about 20 ng compound ml^{-1} whole blood.

ICI 58 834*(2-(2-ethoxyphenoxymethyl)-2,3,5,6-tetrahydro-1,4-oxazine)is a compound of novel structural type (Mallion, Turner & Todd, U.K. patent) whose pharmacology in animals (Mallion, Todd & others, 1972) led to the clinical evaluation of its effects in depression.



Oral doses of the hydrochloride are virtually completely absorbed in man; maximum blood levels are proportional to dose and are about 0·8 μg drug ml^{-1} mg^{-1} dosed kg^{-1} (Case & Bayliss, in preparation). A gas-liquid chromatographic (g.l.c.) method has been developed for the estimation of levels in blood or plasma. The method is ideally suited to the assay of blood levels attained at therapeutic doses.

MATERIALS AND METHODS

Apparatus

A Pye series 104 (Model 84) gas chromatograph or an automatic Hewlett-Packard (Model 402) gas chromatograph fitted with an integrator is used in conjunction with a suitable recorder. Stationary phase: 1% XE60 on Gas-Chrom Q (80–100 mesh) in 150 cm glass columns of nominal 4 mm i.d. Carrier gas: oxygen-free nitrogen (Pye) or argon (Hewlett-Packard), flow rate 60 ml min^{-1}. For the Hewlett-Packard gas chromatograph a purge gas (10% methane in oxygen) is also required. Oven temperature: 184°; flash heater: 200°; detector: 300°. The detector is used in the pulsed mode with a pulse interval of 150 μs.

Materials

Benzene and ethyl acetate: Analar grade; water: glass-distilled. The ethyl acetate is dried over phosphorous pentoxide and distilled from the drying agent. Hepta-

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fluorobutyric anhydride is prepared by heating the acid under reflux in the presence of a large excess of phosphorous pentoxide for 2 h and then distilling the anhydride (bp 106–107°) from the drying agent in glass apparatus protected from atmospheric moisture.

The drug as free base is prepared by dissolving the hydrochloride in water, extracting the free base into chloroform at pH 9 and evaporating to dryness to give an oil. Solutions of the free base in ethyl acetate produce the same peak height for the heptafluorobutyrate derivative as solutions in benzene extracts of control blood.

Procedure

To 1·0 ml whole oxalated blood (or plasma) samples in stoppered glass tubes is added water (0·5 ml) (or 0·5 ml standard aqueous solution of ICI 58 834 hydrochloride), 0·2 N ammonium hydroxide (0·5 ml) and benzene (8·0 ml). The tubes are shaken mechanically for 10 min and the mixture centrifuged. Aliquots (7·0 ml) of the organic phase are transferred to a duplicate set of stoppered glass tubes and after addition of heptafluorobutyric acid (0·1 ml), the solutions are evaporated in a stream of nitrogen, with the tubes partially immersed in a water bath at 40–45° (higher temperatures give rise to significant losses). The residues, dissolved in ethyl acetate (0·2 ml), are reacted immediately with heptafluorobutyric anhydride (0·2 ml) and left in the stoppered tubes at room temperature for 20 min. The solvent and excess reagent are evaporated in a stream of nitrogen at room temperature over about 30 min. The residue is redissolved in ethyl acetate (1·0 ml) and aliquots (1·0 μl) of the solutions injected into the gas-chromatograph using a 10 μl microsyringe.

The standard aqueous solutions of the drug as hydrochloride are prepared at a range of concentrations of free base in water equivalent to twice the nominal concentrations in blood achieved by adding 0·5 ml aliquots of the aqueous solutions to 1 ml aliquots of control blood. The data from these samples are used to construct a standard curve.

The drug as free base is dissolved in ethyl acetate to give a solution containing 0·1 μg μl^{-1}; 10 μl aliquots are reacted with heptafluorobutyric anhydride as described above. These are used to obtain data for unextracted samples.

A standard curve is prepared by plotting peak height for a series of extracted standards at about seven concentrations over the range 0·1 to 2·5 μg free base ml^{-1} blood for clinical samples. Divergence from linearity may become significant at concentrations in blood in excess of 2·5 μg ml^{-1}.

RESULTS

Under the conditions described the heptafluorobutyrate derivative has a retention time of 4–5 min. No spurious peaks are present in g.l.c. traces. Excellent linear correlation of peak height or area under the peak with concentration in blood is attained over the range of concentrations found in clinical samples. The recovery is 80–90% (18 batches gave an average recovery of 85% $\pm$ 6 s.d.). The minimum detectable level is about 0·02 μg ml^{-1} and is determined by the quality of fit for the standard curve determined by linear regression.

Extraction of blood samples spiked with [^{14}C] ICI 58 834 hydrochloride gave a recovery of 95·5% ($\pm$ 1·7 s.d.) for total [^{14}C] labelled material. Resolution of the extract by t.l.c. showed that 92·5% ($\pm$ 0·7 s.d.) of the [^{14}C] labelled material co-chromatographed with ICI 58 834. This data indicated that recoveries of the order of 88%

should be attained by the g.l.c. method assuming that conversion to the heptafluorobutyrate derivative is complete.

The drug is stable in blood at ambient temperatures for at least 12 days and at 0–4° for at least 2 months.

Replicate assays on samples of blood to which known quantities of the drug were added are given in Table 1 together with replicate assays on plasma derived from the same blood samples. The drug is clearly associated with the plasma volume; excellent agreement was obtained between replicate analyses.

Table 1. *Replicate assays of ICI 58 834 in samples of whole blood and derived plasma containing levels of the drug associated with therapeutic oral doses.*

Concentration of ICI 58 834 in blood (μg ml^{-1})	Concentration found in blood (μg ml^{-1}) ($\pm$ s.d.)	No. of replicates	Concentration found in plasma (μg ml^{-1}) ($\pm$ s.d.)	No. of replicates
0·5	0·50 ($\pm$0·04)	8	0·77 ($\pm$0·05)	7
1·0	0·94 ($\pm$0·03)	8	1·46 ($\pm$0·04)	5

DISCUSSION

The method described, because of its limit of sensitivity of 0·02 μg ml^{-1} blood, enables the complete blood level profile following an oral dose of 100 mg ICI 58 834 to be followed over five half-lives. The assay method has been routinely applied to duplicate samples of 1·0 ml whole blood but can be readily modified to assay levels in small volumes of blood or in plasma.

In a volunteer study involving [^{14}C] ICI 58 834, excellent agreement was found between blood levels determined by the g.l.c. method described and by a t.l.c. method based upon scintillation counting of the resolved samples of the drug.

Concurrent therapy with ICI 58 834 and other drugs has not yet been sufficiently varied to ascertain the extent to which this will complicate the procedure. No interference has been observed to date.

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