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Note

Determination of the vasodilator UK33274 by high-performance liquid chromatography using fluorescence detection

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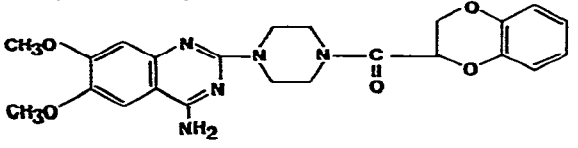
UK33274 is a vasodilator which is structurally based on prazosin (Fig. 1). It has potential clinical advantages over prazosin in that its longer duration of action appears to make it suitable for once daily administration and its slower onset of action appears to lessen the tendency to first-dose hypotension which is a characteristic of prazosin action. In common with prazosin, UK33274 is effective at very low concentrations and in order to define clearly the dispositional characteristics of the drug it is necessary to employ an analytical method capable of detecting concentrations as low as 1 ng/ml. We describe here a method using high-performance liquid chromatography (HPLC) with fluorescence detection, which is based on an assay previously described by one of us for prazosin [1].



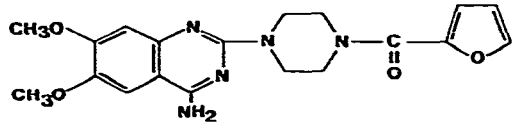


Fig. 1. Structures of UK33274 and prazosin.

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EXPERIMENTAL

Reagents and materials

Unless otherwise stated the reagents and methods of sample preparation and assay calibrations are as previously described [1].

UK33274 [1-(4-amino-6,7-dimethoxy-2-quinazolinyl)-4-(1,4-benzodioxan-2-ylcarbonyl)piperazine methanesulphonate] and prazosin [1-(4-amino-6,7-dimethoxy-2-quinazolinyl)-4-(2-furanylcarbonyl) piperazine hydrochloride] as the internal standard were supplied by Pfizer (Sandwich, Great Britain).

Chromatography

A Hewlett-Packard 1084B high-performance liquid chromatograph fitted with a Spherisorb 5 μm ODS C_{18} bonded reversed-phase column (250 × 4 mm I.D.) was used for the analysis. One pump contained a 0.01 *M* solution of pentane sodium sulphate and a 0.02 *M* solution of tetramethylammonium chloride in water adjusted to pH 3.4 with glacial acetic acid (solvent A). The other pump contained the same concentration of pentane sodium sulphate and acetic acid as solvent A in methanol (solvent B). Both solvents were filtered before use. An isocratic solution of 60% solvent B and 40% solvent A was used with daily minor adjustments in solvent composition (1—2%) to maintain optimum baseline separation of UK33274 and the internal standard. The flow-rate of the mixture was 210 ml/h with a column input pressure of 200 bar (2900 p.s.i.). A Pye Unicam LC-FL detector was used with deuterium lamp and Pye Unicam filters, 254 nm interference for excitation and 370 nm cut-off for emission. A Hewlett-Packard 7985OB LC terminal was employed for recording.

RESULTS AND DISCUSSION

Under the chromatographic conditions described above the retention times of UK33274 and internal standard were 5.3 and 3.7 min, respectively. Fig. 2A shows the chromatogram of an analysis of 1 ml of whole blood containing 10 ng of UK33274 and 20 ng of internal standard. Fig. 2B shows a chromatogram

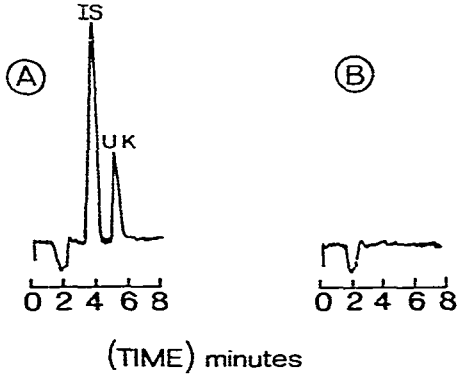

Fig. 2. (A) Chromatogram of extracted whole blood containing 10 ng of UK33274 and 20 ng of prazosin (IS). (B) Chromatogram of 1 ml of control blood taken through the analysis.

of 1 ml of control whole blood taken through the analysis. No peaks corresponding to the peaks shown in Fig. 2A have been found in assaying control blood samples from six subjects. The sample recovery through the procedure averaged 50%.

A typical calibration curve for UK33274 in whole blood is linear in the range 2—50 ng/ml with a regression coefficient of 0.99. The average coefficient of variation for the normalised peak height ratio over this range is 5.1%. The limit of detection, arbitrarily defined as three times baseline noise, is 1 ng/ml. Reproducibility studies using eight samples each at various concentrations gave the following coefficients of variation: 2 ng, 7.4%; 5 ng, 5.5%; 10 ng, 2.7%; and 20 ng, 2.8%.

After storage at −20°C for six weeks, six samples at each of four different initial concentrations gave the following results: 2 ng/ml, 1.9 ± 0.1 ng/ml (standard deviation); 5 ng/ml, 4.8 ± 0.3 ng/ml; 10 ng/ml, 9.6 ± 0.4 ng/ml; and 20 ng/ml, 18.9 ± 1.2 ng/ml.

The chromatographic conditions used in this assay were similar to those previously employed in the analysis of prazosin [1]. It can therefore be anticipated that the same interference from other cardiovascular drugs will be found and for further information the reader is referred to the earlier paper.

In conclusion, we have described a rapid and sensitive analysis in whole blood of a potentially useful new vasodilator.

REFERENCE

1 Y.G. Yee, P.C. Rubin and P. Meffin, J. Chromatogr., 172 (1979) 313—318.