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Note

High-performance liquid chromatographic measurement of amiodarone and desethylamiodarone in plasma or serum at the concentrations attained following a single 400-mg dose

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A high-performance liquid chromatographic (HPLC) method for the measurement of amiodarone in small sample volumes at the concentrations attained during normal therapy has been described[1]. Subsequently, this method has been adapted and used successfully to measure plasma concentrations of both amiodarone and its desethyl metabolite in the presence of nordiazepam[2]. However, experience in this[3] and in other[4] laboratories has shown that both compounds have an exceptionally long terminal half-life of elimination (of the order 50 days). This communication describes a modification to our previously published method, which facilitates the accurate measurement of both amiodarone and desethylamiodarone at plasma concentrations down to 5 μg/l.

EXPERIMENTAL

Materials and reagents

Amiodarone, desethylamiodarone and the internal standard, 2-ethyl-3-(3,5-dibromo-4-γ-di-*n*-propylaminopropoxybenzoyl)benzothiophene (L8040) (Fig. 1)

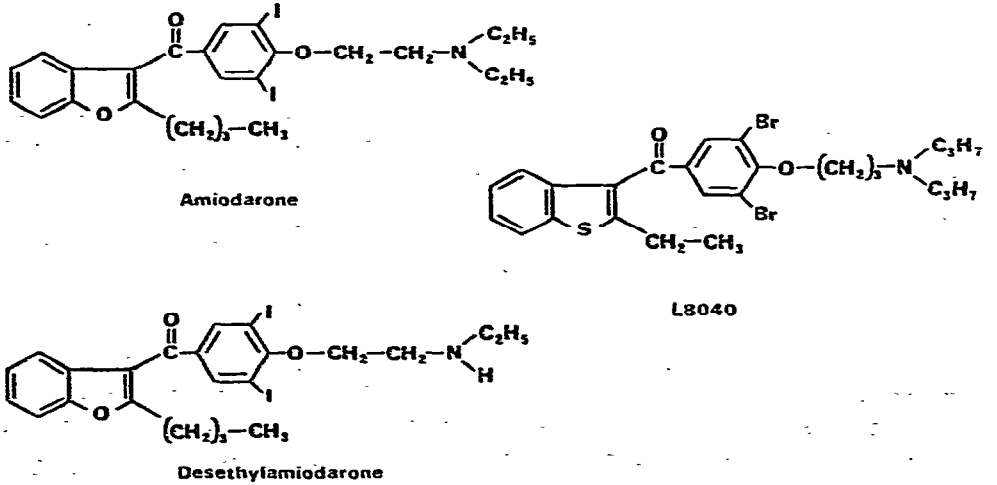

Fig. 1. Structural formulae of amiodarone, desethylamiodarone and L8040 (internal standard).

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were obtained from Labaz (Brussels, Belgium). The internal standard was used as a 0.2 mg/l solution in 2 *M* aqueous sodium dihydrogen orthophosphate (analytical reagent grade) containing 2 g/l human serum albumin, pH 4.5. Methanol and diethyl ether were both HPLC grade (Rathburn, Walkerburn, Great Britain), and diisopropyl ether (laboratory reagent grade) and perchloric acid (70%) (analytical reagent grade) were obtained from BDH (Poole, Great Britain).

High-performance liquid chromatography

The solvent delivery system was a constant-flow reciprocating pump (Applied Chromatography Systems, Model 750/03) and sample injection was performed using a Rheodyne Model 7120 syringe-loading valve fitted with a 100-μl sample loop. Stainless-steel tubing (0.25 mm I.D.) was used to connect the outlet of the valve to the analytical column, a stainless-steel tube 125 × 5 mm I.D. packed with Spherisorb 5 silica (Hichrom, Woodley, Great Britain), which was used at ambient temperature (normally 22°C). The column effluent was monitored at 240 nm (Applied Chromatography Systems, Model 750/11). The mobile phase was methanol–diethyl ether (85:15) containing 0.03% (v/v) perchloric acid. The flow-rate was 2.0 ml/min, maintained by a pressure of *ca.* 40 bar.

Sample preparation

Plasma or serum (1 ml) was pipetted into a 5-ml stoppered polypropylene tube (Elkay Products, Shrewsbury, Great Britain). Internal standard solution (100 μl) and diisopropyl ether (1 ml) were added using a Hamilton repeating mechanism fitted with a 5-ml Hamilton gas-tight luer-fitting glass syringe (Field, Richmond, Great Britain) and an Oxford laboratory pipettor, respectively.

The contents of the capped tube were rotary mixed for 10 min, after which the tube was centrifuged at 3000 *g* for 5 min. The organic phase was transferred to a second polypropylene tube using a Pasteur pipette, and evaporated to dryness using a stream of compressed air. Subsequently, 120 μl of methanol were added, the contents of the tube vortex mixed and a portion (110 μl) of the reconstituted extract was taken to fill the sample loop of the injection valve. Analyses were performed in duplicate and the mean results taken.

Instrument calibration

Standard solutions containing both amiodarone and desethylamiodarone at concentrations of 2, 5, 10, 20, 50 and 100 μg/l were prepared in analyte-free heparinised human plasma. On analysis of these solutions the ratio of the peak height of each analyte to the peak height of the internal standard when plotted against analyte concentration, was linear and passed through the origin of the graph.

RESULTS AND DISCUSSION

The compound L8040, structurally related to amiodarone, was chosen as the internal standard, rather than fenethazine which was used in our earlier publication[1]. Whilst fenethazine is suitable for the former, direct extraction technique, it proved unsatisfactory for the present method because of variable recovery following the evaporation and reconstitution steps.

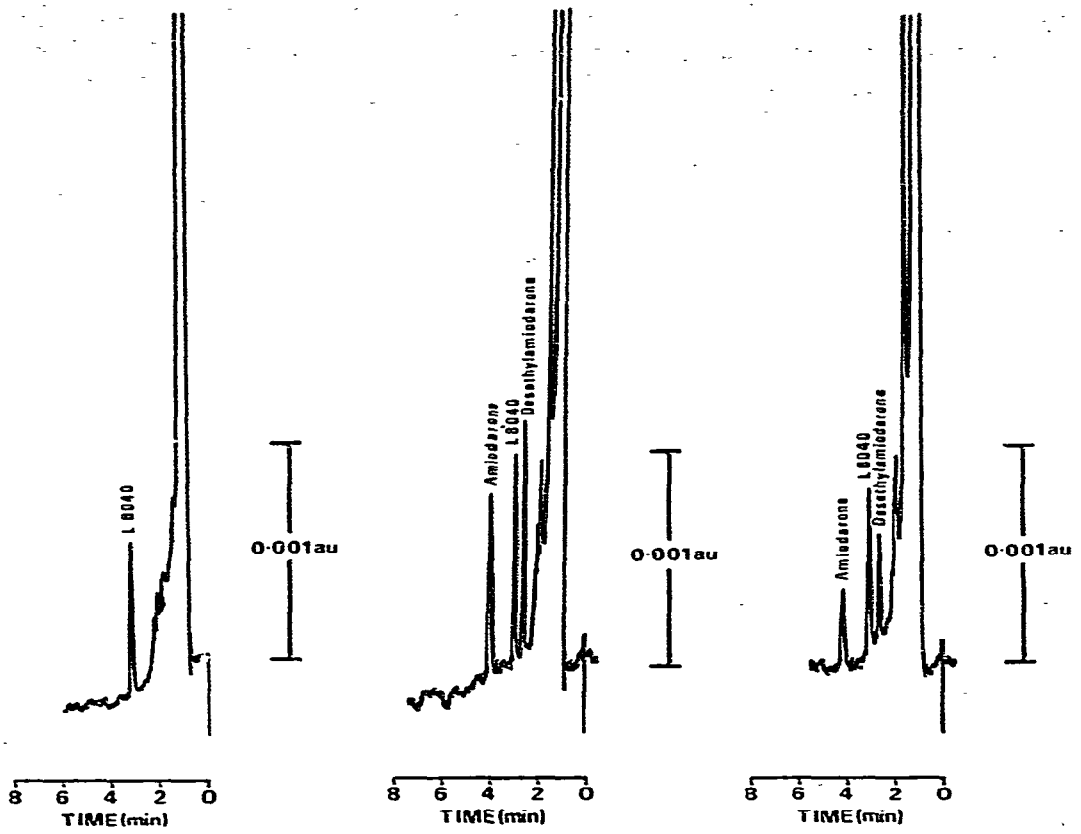

Fig. 2. Chromatogram obtained on analysis of an extract of analyte-free heparinised human plasma; 100 μl injection. (See text for chromatographic conditions).

Fig. 3. Chromatogram obtained on analysis of an extract of a heparinised human plasma standard containing amiodarone and desethylamiodarone (both 10 μg/l); 100 μl injection. (See text for chromatographic conditions).

Fig. 4. Chromatogram obtained on analysis of an extract of a plasma sample from a volunteer subject 35 days after a single 400-mg oral dose of amiodarone; 100 μl injection. The concentrations of amiodarone and desethylamiodarone were both found to be 4 μg/l. (See text for chromatographic conditions).

The chromatogram obtained on analysis of an extract of analyte-free human plasma is illustrated in Fig. 2. Fig. 3 shows the chromatogram obtained on analysis of an extract of a plasma standard containing amiodarone and desethylamiodarone (both 10 μg/l), and Fig. 4 shows the chromatogram obtained on analysis of an extract of a plasma sample from a healthy volunteer subject 35 days after receiving a single 400-mg oral dose of amiodarone.

The intra-assay coefficients of variation (C.V.) measured using three solutions prepared in heparinised human plasma are shown in Table I. The inter-assay C.V. measured from replicate analyses (n = 12) of a solution prepared in heparinised human plasma containing amiodarone (23 μg/l) and desethylamiodarone (47 μg/l) were 2.9% and 4.3%, respectively. The lower limit of detection was 2 μg/l for both compounds.

TABLE I

INTRA-ASSAY REPRODUCIBILITY OF THE ASSAY

n = 10 at each concentration.

	Analyte concentration (μg/l)	*C.V. (%)*
Amiodarone	5	7.2
Desethylamiodarone	6	6.7
Amiodarone	13	3.1
Desethylamiodarone	12	6.3
Amiodarone	31	1.6
Desethylamiodarone	28	4.7

No endogenous sources of interference have been observed. The table of retention times for other drugs eluting on this system (relative to fenethazine), reported previously[1], remains unchanged. However, at the attenuation used in the present procedure, other drugs eluting close to amiodarone and desethylamiodarone and used in normal therapeutic doses could obscure these latter compounds. However, strong bases are not extracted under the conditions used in this procedure and use of methanolic ammonium perchlorate (pH 4), rather than perchloric acid in the mobile phase eliminates interference from most benzodiazepines[5]. On this system L8040 has a relative retention time of 0.51 compared with fenethazine.

This method has been used to measure the elimination of amiodarone and desethylamiodarone up to 80 days following a single 400-mg intravenous dose of amiodarone; details of the pharmacokinetics of the drug are to be published elsewhere[6]. The mean (± S.D.) plasma concentrations of amiodarone and desethylamiodarone in six volunteer subjects 60 days following a single 400-mg intravenous dose of amiodarone were 7.0 ± 4.0 μg/l and 6.0 ± 3.1 μg/l, respectively and 28 days after a 400-mg oral dose they were 6.0 ± 2.4 μg/l and 11.0 ± 5.9 μg/l, respectively. The method has also been used to measure the plasma concentrations of these compounds for up to 9 months following cessation of chronic oral amiodarone therapy, enabling the precise measurement of their terminal elimination half-lives to be made[7].

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