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Note

Rapid high-performance liquid chromatographic method for the determination of amiodarone and desethylamiodarone in human plasma and serum

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Amiodarone (A) (Fig. 1) is a potent antiarrhythmic highly effective in the treatment of supraventicular tachyarrhythmias, ventricular fibrillation and tachycardia and of arrhythmias associated with the Wolff–Parkinson–White syndrome. However, it produces some side-effects. Blood levels of thyroxine (T4), triiodothyronine (T3) and thyroid-stimulating hormone (TSH) are modified. Nevertheless, few cases of hypo- or hyperthyroidism have been reported [1]. Corneal microdeposits are very frequent but seldom symptomatic. They are related to the daily dose of amiodarone and disappear slowly after the treatment is stopped [2]. Pulmonary fibrosis has been observed in patients receiving doses higher than 400 mg per day. It was reversible on arrest of the treatment or reduction of the dose [3]. Cutaneous toxicity (photosensitivity, abnormal pigmentation of the face and the hands) was related to the cumulative dose and the duration of the treatment. Other side-effects include gastrointestinal and neurological toxicity [4–6].

Because some of these side-effects are related to the dose, it is recommended to administer as small a dose as possible in order to prevent accumulation and to maintain the plasma concentration in the 0.5–2 μg/ml therapeutic range. Amiodarone is eliminated mainly by metabolization [7]. Its main metabolite, N-desethylamiodarone (D) (Fig. 1) is considered to be potentially active, although its activity has not been characterized so far. The elimination half-lives of A and D are similar and show wide inter-individual variability [7]. Plasma concentra-

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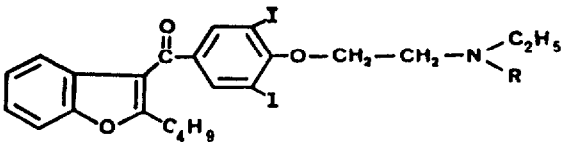
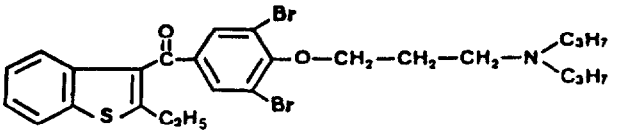


R = C_2H_5 - Amiodarone
R = H - N-Desethylamiodarone



Internal standard L 8040

Fig. 1. Structures of amiodarone, desethylamiodarone and internal standard.

tions can therefore vary considerably from patient to patient, so that monitoring of the plasma concentration is recommended in order to prevent toxic accumulation. The aim of this work was to develop a high-performance liquid chromatographic (HPLC) assay for A and D that would be rapid, accurate in the therapeutic range and inexpensive, allowing easy drug monitoring in clinical practice.

EXPERIMENTAL

Reagents

Amiodarone, desethylamiodarone and the internal standard (L 8040) (see Fig. 1) were kindly provided by Sanofi (Montpellier, France). Hexane and 2-propanol were of HPLC grade; all other reagents were of analytical-reagent grade.

Standards and controls

Standards of 0.5, 1 and 2 μg/ml and controls of 0.75 and 1.5 μg/ml concentration were prepared on the day of the assay by diluting concentrated solutions of A and D with blank human plasma.

Extraction procedure

A volume of 0.5 ml of each standard, control and unknown plasma or serum was mixed with 50 μl of a 40 μg/ml solution of the internal standard and 1.5 ml of phosphate buffer (pH 7). The samples were extracted twice for 5 min with 2 ml of hexane and the organic layer was decanted and evaporated to dryness at 40°C under nitrogen. The residue was dissolved in 0.1 ml of HPLC mobile phase and 0.040 ml was injected into the column.

High-performance liquid chromatography

HPLC was performed on a Hewlett-Packard 1084B apparatus equipped with a UV–visible detector operated at 242 nm, using a 5-μm cyanopropyl column (150

mm×4.6 mm I.D.) (Econosphere CN, Alltech Europe, Belgium). The mobile phase was hexane–2-propanol (45:55) containing 0.06% (v/v) concentrated sulphuric acid at a flow-rate of 1 ml/min.

Concentrations of A and D in the controls and the unknowns were calculated from plots of the peak-area ratio of A and D to the internal standard against the concentration of A and D in the standards.

Precision

Precision was studied on plasma samples spiked with A and D at concentrations of 0.6, 1.2 and 1.8 μg/ml. The between-assay variation was determined by assaying each sample five times in duplicate. The within-assay variation was studied by assaying each sample twice in five replicates. Between- and within-assay variations were calculated for a single-tube assay according to Rodbard [8].

RESULTS

A typical chromatogram of a plasma blank and plasma from a patient is shown in Fig. 2. The retention times were approximately 5 min for D, 7 min for the internal standard (IS) and 12 min for A.

The calibration graphs were linear for A and D in the range of concentrations tested and the origin was not significantly different from zero. The regression lines based on ten calibrations, calculated using the least-squares method, were $A/IS = -0.02 + 0.50\ [A]$ and $D/IS = -0.03 + 0.54\ [D]$, where IS is the internal standard. It was also determined that they are not different when the standards are prepared with serum instead of plasma. Within- and between-assay coefficients of variation are given in Table I. The within-assay variability was less than 6% of A and D and the between-assay variability varied between 6 and 9.7% and was not different for A and D.

The limit of quantitation of the method was determined on a series of samples obtained by dilution of a plasma spiked with A and D. These samples were assayed four times on four different days. The results are presented in Table II. The limit of quantitation was 0.2 μg/ml for both compounds. For lower concentrations the variability was higher than 10%.

The recovery calculated from the ratio of the areas under the peaks after extraction or direct injection into the HPLC column for standards of 1 and 2 μg/ml were 69.6% for amiodarone and 55.5% for desethylamiodarone.

In order to estimate the selectivity, we also analysed plasma from patients treated with other antiarrhythmic drugs and cardiac glycosides using the same procedure. We did not found any interferences from quinidine, disopyramide, mexiletine, aprindine, propranolol, digitoxin, digoxin or gitoxin in the range of therapeutic concentrations. We found a small peak with a retention time near to that of desethylamiodarone with plasma samples from patients taking tocainide or flecainide; however, no peak appeared in the region of amiodarone.

DISCUSSION AND CONCLUSION

Some other HPLC assays have been proposed for A and D [9–18]. However, some did not use an internal standard [9] or used an internal standard very

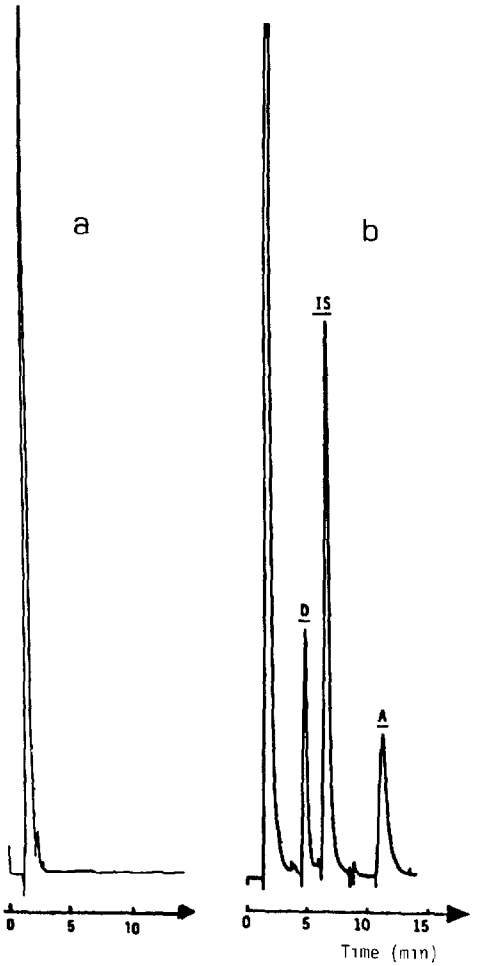


Fig. 2. Chromatogram of plasma blank (a) and plasma from a patient treated with amiodarone (b). Peaks: D = desethylamiodarone, 0.7 μg/ml; IS = internal standard; A = amiodarone, 0.8 μg/ml.

TABLE I

WITHIN-ASSAY AND BETWEEN-ASSAY COEFFICIENTS OF VARIATION FOR A SINGLE-TUBE ASSAY

Concentration (μg/ml)	Coefficient of variation (%)			
	Within-assay		Between-assay	
	Desethylamiodarone	Amiodarone	Desethylamiodarone	Amiodarone
0.6	5.9	5.4	6.0	8.2
1.2	4.2	3.3	9.7	6.7
1.8	5.9	2.7	7.1	6.5

TABLE II

LIMITS OF QUANTITATION ($n=4$)

Theoretical concentration (μg/ml)	Desethylamiodarone		Amiodarone	
	Observed concentration (mean ± S.D.) (μg/ml)	C.V. (%)	Observed concentration (mean ± S.D.) (μg/ml)	C.V. (%)
0.8	0.72 ± 0.06	8.0	0.68 ± 0.02	3.3
0.4	0.36 ± 0.02	6.4	0.34 ± 0.03	8.8
0.2	0.18 ± 0.02	9.6	0.15 ± 0.01	9
0.1	0.09 ± 0.01	16.4	0.06 ± 0.01	14.1

different from A and D, such as diazepam [10], fenethazine [13] or trifluoperazine [16]. Some [13, 15, 17] used a silica column, which can cause problems associated with these unbound phases, especially when the same HPLC apparatus is used for other assays with bound phases and aqueous mobile phases. Some used large volumes of plasma [17] or were time-consuming because of a long extraction procedure [16, 17]. Finally, in some of these methods, A and D were not baseline-resolved and their separation from the injection peak was not complete [12, 15, 18].

Our HPLC method allows the simultaneous determination of amiodarone and desethylamiodarone in plasma or serum at concentrations of clinical significance, between 0.2 and 4 μg/ml. It is fast, very easy to perform, accurate and inexpensive and is therefore suitable for drug monitoring in clinical practice, whereas an immunoassay is not convenient owing to the rapid metabolization of the drug.

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