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Note

High-performance liquid chromatographic assay of amiodarone and desethylamiodarone in plasma

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Amiodarone is an antiarrhythmic agent which has shown great promise in the treatment of refractory supraventricular and ventricular arrhythmias. As it is increasingly widely used, more adverse effects are being recognised. These include thyroid, skin, neurological, ophthalmological and pulmonary complications as well as drug interactions [1]. Evidence is accumulating of the value of plasma concentrations of amiodarone and its principal metabolite, desethylamiodarone, in optimising efficacy of this agent and reducing its toxicity. A tentative therapeutic range of 0.5—1.5 mg/l appears to be associated with efficacy in the majority of patients but with a reduced incidence of adverse effects [2].

Several method for measuring amiodarone and desethylamiodarone have been published, each of which has disadvantages. We describe a method which we believe overcomes many of these disadvantages.

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EXPERIMENTAL

Materials and reagents

Amiodarone, desethylamiodarone and fenethazine were supplied by Labaz (Manchester, U.K.). Acetonitrile was of HPLC quality and was supplied by Fisons (Loughborough, U.K.). All other solvents and reagents were of analytical quality.

Sample preparation

Heparinised plasma (500 μl) was placed in a PTFE-lined culture tube, and 50 μl of the internal standard solution (containing 0.5 μg fenethazine), 100 μl of 2 *M* sodium dihydrogen phosphate and 2 ml 1-chlorobutane were added. The drugs were extracted by vortex-mixing for 1 min followed by brief centrifugation. The organic layer was aspirated and evaporated to dryness at 60°C under dry nitrogen. The extracted drugs were redissolved in 100 μl acetonitrile and 20 μl were applied to the chromatograph.

High-performance liquid chromatography

The high-performance liquid chromatography system used was supplied by Laboratory Data Control (Stone, U.K.). The system comprised a Constametric III high-pressure delivery system equipped with a Model 7125 Rheodyne valve and fitted with an Apex nitrile column (250 × 4.5 mm I.D.; particle size 5 μm). The absorbance was measured at 238 nm, at 0.01 a.u.f.s. deflection using a Spectromonitor III variable-wavelength detector. The mobile phase used was acetonitrile—0.02 *M* phosphate buffer pH 3.0 (60:40) at a flow-rate of 2 ml/min. Chromatograms were recorded and the peak heights integrated using a Chromatography Control Module.

Instrument calibration

Calibration curves were constructed by adding known amounts of internal standard, amiodarone, and desmethylamiodarone to a pooled plasma and plotting the peak-height ratios of drug to internal standard against the amount of drug added. The mean normalized peak-height ratios were used to calculate the amount of amiodarone and desethylamiodarone in unknown samples, and the standard deviation of the normalized peak-height ratios was used to determine the accuracy of the method over the range of amiodarone standards employed. The reproducibility of the method was also studied by submitting five replicate samples (containing 0.1, 1.0 and 5.0 mg/l) to the entire procedure.

To estimate the recoveries for the method, the peak heights of analysed samples containing known amounts of amiodarone, desethylamiodarone and the internal standard were compared to the respective peak heights obtained by injecting equal amounts directly into the chromatograph.

RESULTS AND DISCUSSION

The recovery and intra-assay reproducibility of the method are shown in Tables I and II, respectively. The limits of quantitation for desethylamio-

TABLE I

ACCURACY OF THE METHOD

$n = 8$.

Drug	Recovery (%)	Concentration (mg/l)	Coefficient of variation of normalized peak-height ratios (%)
Desethylamiodarone	91	0.1—8.0	7.0
Amiodarone	93	0.1—8 0	4.8

TABLE II

INTRA-ASSAY REPRODUCIBILITY OF THE METHOD

$n = 5$.

Drug	Concentration (mg/l)	Coefficient of variation of normalized peak-height ratios (%)
Desethylamiodarone	0.1	8.1
	1.0	3.4
	5.0	2.8
Amiodarone	0.1	5.8
	1.0	3.1
	5 0	2.6

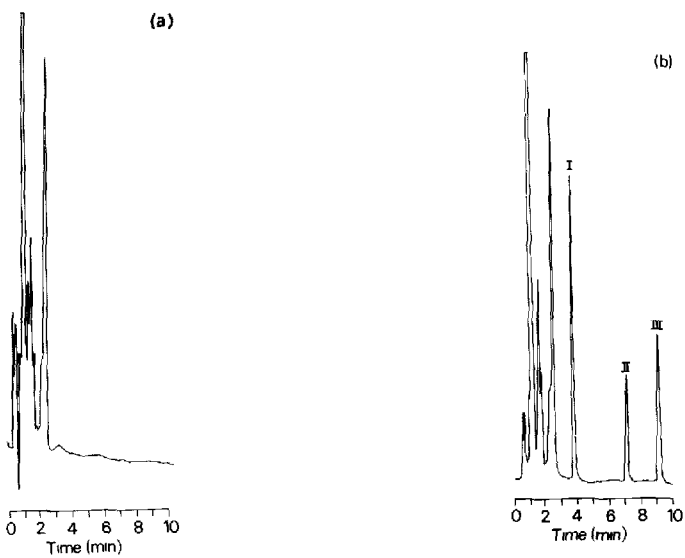

Fig. 1. (a) Chromatogram of blank plasma sample; (b) chromatogram of plasma sample from patient taking amiodarone showing the internal standard fenethazine (I), desethylamiodarone (II) (1.3 mg/l) and amiodarone (III) (1.8 mg/l) with retention times of 3.9, 7.5 and 9.3 min, respectively.

darone and amiodarone are 0.03 and 0.02 mg/l, respectively. Typical chromatograms of blank plasma and plasma from a patient taking amiodarone are shown in Fig. 1a and b, respectively.

The inter-assay coefficient of variation was 6.9% for amiodarone (n = 5) and 8.3% for desethylamiodarone (n = 5) at concentrations of 1 mg/l. Diazepam, nordiazepam, chlordiazepoxide, lorazepam, mexiletine, lignocaine, disopyramide, propranolol, digoxin, frusemide, hydralazine, atenolol, warfarin, theophylline and phenytoin were added to samples and found not to interfere.

Several methods to measure amiodarone in plasma do not measure the desethyl metabolite [3, 4]. Since this appears to be related in part to the drug's toxicity, these have been superceded by other methods, which can measure unchanged drug and metabolite (see Table III). In the first of these methods, desethylamiodarone elutes close to the solvent front before baseline is achieved and in our hands the method has resulted in variable retention times [5]. In addition, a separate mobile phase is necessary to separate nordiazepam from desethylamiodarone [5]. In the method we describe, the retention times are constant and no further mobile phase is necessary to separate nordiazepam.

TABLE III

COMPARISON OF THE REPRODUCIBILITY AND LIMITS OF QUANTITATION OF THE PUBLISHED METHODS TO MEASURE AMIODARONE (A) AND DESETHYLAMIODARONE (DEA)

Internal standard	Column	Concentration (mg/l)		Coefficient of variation (%)				Reference
				Within-run		Between-run		
		A	DEA	A	DEA	A	DEA	
Fenethazine	Normal phase (silica)	0.1	0.1	3.1	1.4	3.5	3.9	5
None	Reversed phase (C_{18})	0.1	0.1	5.4	6.5	—	—	6
L8040	Reversed phase (C_{18})	—	—	—	—	—	—	7
L8040	Reversed phase (C_8)	0 02	—	5.7	—	10.6	—	8
L8040	Reversed phase (C_{18})	0.05	—	—	—	8	—	9
L8040	Reversed phase (C_{18})	0.025	0.025	3.1	4.6	5.6	5.6	10
L8040	Normal phase (silica)	0.002	0.002	1.6	4.7	2.9	4.3	11
Fenethazine	Reversed-phase (nitrile)	0.02	0.03	3.1	3.4	6.9	8.3	This paper

Brien et al. [6] have described a rapid method to measure amiodarone and desethylamiodarone. The chromatography produces wide and tailed peaks, however, and the lower limit of quantitative sensitivity was also relatively high (0.1 mg/l).

The method of Shipe [7] produced poor separation of amiodarone, desethylamiodarone and internal standard (compound L8040) and no details of the sensitivity or reproducibility of the assay are provided. Similarly Latini et al. [8] and Solow et al. [9] provide relatively few details of the precision and sensitivity of the method, particularly for desethylamiodarone, and no figures to show the chromatographic separation. The method of Plomp et al. [10] shows relatively poor separation of amiodarone and desethylamiodarone from each other and from the solvent front and relatively poor inter-assay coefficients of variation for the lowest (0.1 mg/l) standards.

Finally Storey and Holt [11] have described a sensitive method to measure amiodarone and desethylamiodarone but the chromatography is normal phase requiring diethyl ether in the mobile phase and the chromatographic separation is not ideal, particularly between desethylamiodarone and internal standard (L8040). The method we describe has been used to measure plasma amiodarone and desethylamiodarone concentrations after intravenous infusion of the drug to patients with arrhythmias and we have found it to be reliable over the two years it has been used for clinical purposes. Up to thirty samples can be analysed manually per day by one technician and the freedom from interference by other drugs makes it applicable to monitoring of cardiac patients who are often receiving a variety of medications in addition to amiodarone.

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