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Short Communication

Amiodarone determination by high-performance liquid chromatography*

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Introduction

Amiodarone (2-butyl-3-(3-5-diiodo-4-B-diethylaminoethoxybenzoyl) benzofuran) (Fig. 1) is a class III antiarrhythmic agent that has been in clinical use for about 20 years. Originally designed as an antianginal agent [1] with vasodilator and antihypertensive properties, it has been recently approved by the Food and Drug Administration for treatment of life-threatening ventricular tachyarrhythmias [2–8]. Its approval is

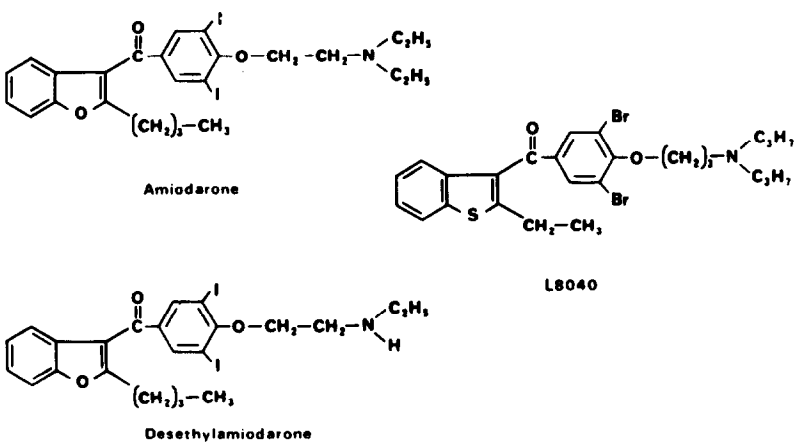

Figure 1
Chemical structure of amiodarone, desethylamiodarone and internal standard.

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restricted to this use because amiodarone produces many side effects, some of which are serious [9].

The pharmacokinetic variability, narrow therapeutic index (1–2.5 mg l^{-1}) [9–11] and the known side effects of this drug, require the lowest therapeutically effective dose to be used and provide a sound rationale [12] for therapeutic serum monitoring to set the daily dose. Recently several liquid chromatographic procedures for the simultaneous determination of amiodarone and desethylamiodarone have been described [13–24].

The paper describes the conditions for extraction and chromatographic separation of these compounds that are best suitable for use in a routine laboratory.

Experimental

Apparatus and chromatographic conditions

The analytical instrument was a Model 410 Bio pump (Perkin–Elmer), a 15 × 0.32 cm stainless-steel column packed with C-18, 5-μm spherical particles (Ultrasphere, Part No 235342, Beckman); a Rheodyne fixed-loop injector (20 μl) and a variable-wavelength (242 nm) detector (LC 91, Perkin–Elmer). Peaks of interest were recorded and quantified with an LC 100 recording integrator (Perkin–Elmer). The mobile phase consisted of methanol–aqueous ammonium hydroxide (25%) (99.3:0.7%, v/v). It was filtered, degassed and pumped at a flow rate of 2 ml min^{-1}.

Preparation of stock solutions

Amiodarone hydrochloride, desethylamiodarone hydrochloride, and internal standard L8040, (generously supplied by Sanofi Centre de Recherches, Montpellier, France) were dissolved in methanol to produce solutions containing 100 mg l^{-1} of each compound. The stock solutions were stored in amber bottles at −4°C. Working standards were prepared containing 500, 1000 and 2000 μg l^{-1} for amiodarone and desethylamiodarone respectively and 2500 μg l^{-1} of internal standard by appropriate dilution with methanol.

Preparation of samples

A constant volume (200 μl) of internal standard, 500 μg l^{-1}, was added to a glass-stoppered centrifuge tube. Calibration standards were prepared by addition of known amounts of amiodarone and desethylamiodarone. Serum (500 μl) of drug free control was added to each calibration tube and 500 μl of test sample was added to each tube that contained only the internal standard. The calibration standards were extracted and analysed daily together with each batch of unknowns.

Extraction

To each tube was added 100 μl of 2 M phosphate buffer solution (pH 4.6) and extracted twice with 3 ml of diisopropylether. The organic layer was evaporated carefully to dryness with nitrogen and the residue dissolved in 200 μl of mobile phase; 20 μl were injected into the HPLC system.

Results

Evaluation of the method

Under the described chromatographic conditions, the relative retention times (internal standard = 1) were found to be 0.52 for desethylamiodarone and 0.70 for amiodarone (Fig. 2).

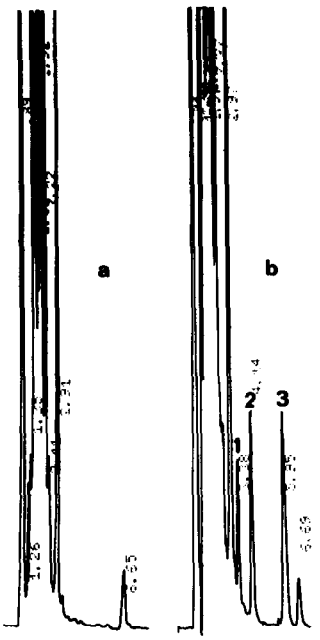

Figure 2
Chromatograms of extracted patient sample: (a) not taking amiodarone and (b) after taking 400 mg of amiodarone orally. Peaks: 1 = desethylamiodarone, 2 = amiodarone and 3 = internal standard.

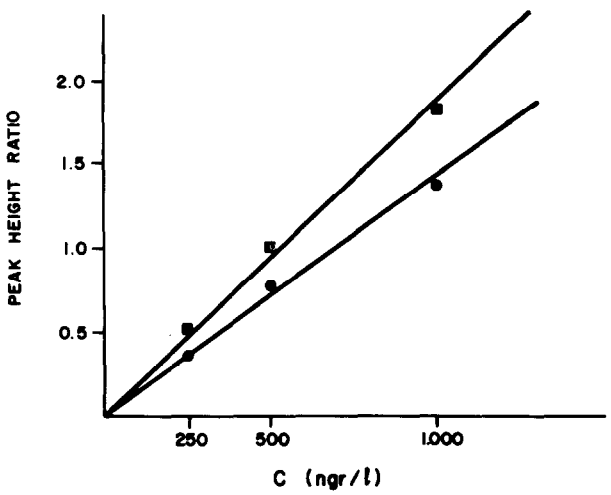

Figure 3
Peak area ratio and serum concentrations of amiodarone (○) and desethylamiodarone (□). The regression equation for amiodarone was: $y = 0.002x + 0.131$ and for desethylamiodarone: $y = 0.001x + 0.166$ and the correlation coefficients were 0.993 and 0.998 for amiodarone and desethylamiodarone respectively.

The relation between peak area ratio and serum concentration (Fig. 3) was found to be linear for amiodarone and desethylamiodarone (range: 500, 1000 and 2000 $\mu g\ l^{-1}$ respectively). Results obtained by using linear regression analysis are depicted in Fig. 3. Three calibration points were used and the correlation coefficients were: 0.993 and 0.998 for amiodarone and desethylamiodarone respectively.

The precision of the method was determined by assaying serum samples containing known quantities of these drugs. As shown in Table 1 the relative standard deviations (RSD) obtained, ranged from ±2.50 to 9.72%.

The analytical recovery was evaluated by spiking drug-free serum with working standard solutions and comparing the results with those obtained by direct injection of the same amount of working standard solutions. The percentage recoveries were about 99%.

Table 1
Precision for simultaneous determination of drugs in serum

	Amiodarone		Desethyl amiodarone	
	Mean ($\mu g\,l^{-1}$)	RSD %	Mean ($\mu g\,l^{-1}$)	RSD %
Within day	259	5.12	189	9.72
$n = 12$.	497	3.89	401	6.30
	868	4.29	652	2.92
Between run	476	10.39	495	9.35
$n = 12$				
Run-to-run	255	1.05	184	2.50
$n = 10$				

Discussion

The method described here is suitable for simultaneously measuring amiodarone and desethylamiodarone in either serum or plasma. It has been in use in our laboratory for over 13 months and is rapid, accurate and stable.

We investigated different extraction solvents (hexane, ethylacetate and methylene chloride) and we found poor recovery to amiodarone. However using diisopropylether as extraction solvent the extraction efficiency increased to 99% (double extraction).

There are several methods reported to determine amiodarone. One of them uses no internal standard [13], another uses an internal standard chemically disimilar from amiodarone [15]. The method reported by Lesko *et al.* utilizes a silica column [16], whilst in the last three years the determination of amiodarone and desethylamiodarone in serum, using solid-phase extraction has been published [12, 22], also a comparison of different extraction procedures for amiodarone and its metabolite [23] and a standardized extraction and chromatographic optimization procedure to evaluate amiodarone [24].

In this paper an internal standard similar to amiodarone is used with a reversed-phase assay resistant to the effect of polar solvents, and an extraction method for amiodarone and its metabolite of low cost and suitable recovery.

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