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Note

High-performance liquid chromatography of amiodarone and desethylamiodarone in serum after microscale protein precipitation

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Amiodarone (2-butyl-3-[3,5-diiodo-4-(β-diethylaminethoxy)benzoyl]benzofuran) was first introduced in Europe as an orally effective antianginal agent. In subsequent clinical investigations, it was proven to be an orally effective agent in the treatment of atrial and ventricular arrhythmias refractory to conventional therapy. Amiodarone is believed to be antiarrhythmic active by prolongation of action potential duration which is reflected in the electrocardiogram as a prolongation of the QT interval (the part of an electrocardiogram marked by the beginning of a Q wave and the end of a T wave) [1]. In the body, amiodarone is converted to an active metabolite, desethylamiodarone. Both the parent drug and the metabolite have a long half-life in man on the order of 50 days [2].

Several years ago, amiodarone was approved in the United States as an investigational drug and was available to our institution. A procedure was required to monitor the drug in patients to adjust the dosage to steady state therapeutic levels and to avoid side effects [3]. Numerous high-performance liquid chromatographic (HPLC) procedures for the measurement of amiodarone have been published [4—11]. Most of these required extraction of amiodarone and desethylamiodarone in samples from 0.5 to 2 ml of serum with 3 to 10 ml of various organic extractants, followed by evaporation of the solvents before injecting into the chromatograph. Among these reports, there was no consensus on which is the best organic solvent and what is the ideal pH for optimal extraction. Rather than adopting one of the more tedious procedures from the literature, we developed a HPLC procedure for amiodarone by the micro deproteinization technique using zinc sulfate in combination with methanol or acetonitrile as the precipitants [12—14]. The procedure, developed and in service since 1982, is reliable, efficient and easy to perform.

EXPERIMENTAL

Equipment

The chromatograph consisted of a Perkin-Elmer Series 2 pump and an

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Altex Model 152 UV detector set at 254 nm. The column was 15 × 0.42 cm packed with Spherosil C_{18}, 5 μm by downward slurry technique. The sample was introduced through a 7120 syringe-loading Rheodyne injector. The detector signal was output to a Linear strip-chart recorder.

Sample preparation

An aliquot of 10 μl of 10% (w/v) zinc sulfate solution and 100 μl of patient sample or serum standard were added to a 10 × 75 mm disposable culture tube. The content of the tube turned cloudy upon mixing. Then, 100 μl of acetonitrile were added with mixing. The culture tube was centrifuged at 3000 *g* for 1 min and an aliquot of 100 μl of the supernatant was injected into the chromatograph. The drug and metabolite were eluted with a mobile phase containing 400 ml acetonitrile, 100 ml water, 0.5 ml phosphoric acid and 0.25 ml diethylamine. The mobile phase was pumped through the column at 3.0 ml/min.

RESULTS AND DISCUSSION

Most procedures reported for the chromatography of amiodarone required extraction with organic solvents; some used unstable solvents such as ethers. These procedures, necessitating evaporation of large volumes of organic solvents, are tedious. They are also imprecise because of variable recovery depending on the extraction solvent and the pH of the aqueous phase. A micro protein precipitation procedure, deproteinizing the serum before injecting the sample into the chromatograph for measuring amiodarone, is a more efficient and better approach. We have previously devised methods using methanol or acetonitrile in combination with zinc sulfate in a final concentration of 0.05% for the deproteinization of serum [12—14]. Presumably, these small amounts of zinc form complexes with serum proteins. The zinc complexes become dehydrated upon the addition of acetonitrile or methanol, and precipitate out of solution. Incomplete protein precipitation resulted, however, when zinc sulfate, methanol or acetonitrile alone was used as the protein precipitant. The chromatograms of a serum standard containing 1.25 μg/ml desethylamiodarone and 2.5 μg/ml amiodarone, and a patient specimen containing 2.7 μg/ml desethylamiodarone and 3.2 μg/ml amiodarone are shown in Fig. 1. Excellent resolution of the parent drug from the metabolite was accomplished in 6 min. Recovery of amiodarone from drug-spiked sera after deproteinization was studied by comparison with aqueous standards. The sera spiked with 1.0 μg/ml and 5.0 μg/ml of amiodarone were recovered 100 and 108%, respectively, from the supernatant. The drug and metabolite were quantified by calibration with four external standards at concentrations of 0.5, 1.0, 2.5 and 5.0 μg/ml for amiodarone, and at a half of these concentrations for desethylamiodarone. The standard curves exhibit good linearity with correlation coefficients 0.9995 for amiodarone, and 0.9998 for desethylamiodarone. The detection limits are 0.05 μg/ml and 0.025 μg/ml for amiodarone and desethylamiodarone, respectively. Since the procedure did not involve solvent extraction and evaporation steps, an internal standard was not used for compensating recovery losses. Excellent precision equal to those of our earlier

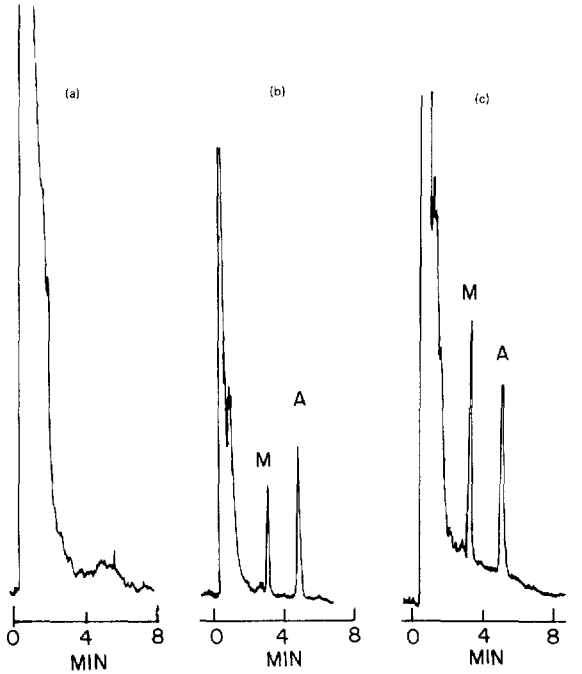

Fig. 1. Chromatograms of (a) serum blank at 0.005 a.u.f.s.; (b) a serum standard at 0.02 a.u.f.s. with 1.25 μg/ml M and 2.5 μg/ml A; and (c) a patient on amiodarone at 0.02 a.u.f.s. with 2.7 μg/ml M and 3.2 μg/ml A, where A=amiodarone and M=metabolite, desethylamiodarone. Specimens were deproteinized with zinc sulfate and acetonitrile before HPLC.

works was achieved. The coefficient of variation of six repeated analysis of 1.0 μg/ml amiodarone was 2.2% and of 5.0 μg/ml was 2.3%. The between-day precision over a period of ten days at 1.2 μg/ml amiodarone was 6.7% and at 0.6 μg/ml desethylamiodarone was 6.5%.

Amiodarone levels which may be helpful in avoiding drug toxicity may not be as helpful in assessing efficacy since we have found no significant different serum amiodarone and desethylamiodarone levels in patients responding to therapy and those who died suddenly. In a study, 23 patients who remained alive had mean levels of 2.1 and 2.5 μg/ml, while six patients who died suddenly had mean levels of 1.8 and 3.2 μg/ml of desethylamiodarone and amiodarone, respectively [15]. However, since prolongation of the QT interval of the electrocardiogram is a useful index of amiodarone success [1], we favor the administration of dosage regimens that maintain serum amiodarone and desethylamiodarone levels that effect a 10—15% prolongation in the QT interval above the baseline for therapy [15].

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