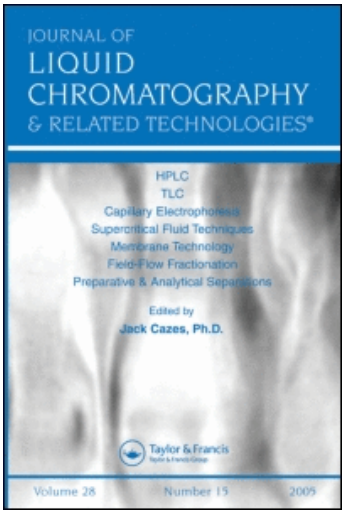

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Amiodarone Analysis by Capillary Electrophoresis

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Abstract: Amiodarone is a broad spectrum antiarrhythmic medication. Toxicity symptoms can occur at high serum levels of this drug; thus the level of this drug requires routine monitoring. Because this drug is very hydrophobic, it is difficult to analyze by both HPLC and capillary electrophoresis (CE). A simple method for measuring amiodarone (A) by CE without sample extraction is described. It is based on separation of the drug in a strong phosphate buffer 170 mmol/L, pH 5.9, with added ethanol, and stacking after acetonitrile deproteinization. The ethanol in the phosphate buffer aids to solubilize the drug, migrate it from the neutral to the cationic molecules region, and to separate it from its metabolite, desethylamiodarone (D). The metabolite D is pharmacologically active; has the similar therapeutic range as the parent drug; similar ultra violet absorption; and at steady state is present at equal ratio. Based on these facts, we choose for routine analysis, conditions where the drug and its metabolite co-elute. These conditions improved the detection limits, shortened the analysis time (<10 min), and avoided the mathematical addition of the two values to arrive at the therapeutic level. The analysis by CE compared well to that by HPLC, $r = 0.9$. The therapeutic range of the combined values of the amiodarone and the metabolite was estimated to be 1.2–4 mg/L.

Keywords: Stacking, Transient pseudo-isotachophoresis, Antiarrhythmic drug

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INTRODUCTION

Amiodarone (A) (Cordarone) is a broad spectrum antiarrhythmic medication. It has multiple and complex effects on the electrical activity of the heart, which are responsible for the cardiac rhythm. It was discovered in 1961 and was approved by the FDA for clinical use as an antiarrhythmic agent in 1985. It is a membrane stabilizer and it is effective for long-term maintenance of sinus rhythm. Although A has many side effects, some of which are severe and potentially fatal, it has been successful in treating many arrhythmias where other antiarrhythmic drugs fail.[1–3]

With chronic oral dosing, A has a very long half-life of 14–60 (occasionally up to 110) days.[1,2,4] The principal metabolite detected in the plasma and other tissues, is desethylamiodarone (D). It is also pharmacologically active and has also a long half-life of ~60–90 days.[2] Both A and D are lipophilic and highly protein bound compounds accumulating in highly perfused organs, e.g., lung, liver, heart, and kidney. Serum amiodarone concentrations are 10 to 50 times lower than myocardial values. Thus, they are generally not considered a good measure of myocardial level but they are more accessible.

Toxicity symptoms, bradycardia, photosensitivity, hypothyroidism, thyrotoxicosis, are associated with concentrations >3 mg/L of A. The most worrying long-term side effect is lung toxicity which is significant in 3–9% of patients.[4] Thus the level of this drug requires routine monitoring. Therapeutic levels of this drug are not well established. However, serum levels of 1.0 to 2.0 mg/L are considered therapeutic for the parent drug and the same for the metabolite D. Thus the therapeutic for the combined A and D is ~1.5–4 mg/L after.[1–4] At a steady state the ratio of the metabolite D to the parent drug A is close to 1.[2]

This drug is cationic and very hydrophobic, leading to difficulties in the analysis by both HPLC and CE. It binds to the silica particles[5] and to the capillary walls. Analysis by HPLC requires a well deactivated column and almost 100% organic solvent, such as methanol, for elution.[5–10] In addition to that, the drug does not have strong absorbency in the ultraviolet range. All these aspects render the analysis of this drug a challenge. The Thormann group[12] were the first to show that this drug can be analyzed by CE using phosphate buffers containing isopropanol. However, sample extraction and head-column field-amplified sample stacking (from aqueous buffer) after sample extraction was necessary to measure this drug and its metabolite in serum.

We have shown that many drugs can be analyzed based on transient pseudo-isotachophoresis using acetonitrile precipitation.[12] Under these conditions, sample extraction and concentration can be eliminated.[12] Here, we show that amiodarone can be determined in serum simply and reliably after acetonitrile deproteinization without sample extraction.

EXPERIMENTAL

Reagents

Amiodarone and isopropanol were purchased from Sigma Chemicals (Saint Louis MO, USA). K_2HPO_4 and ethanol were purchased from Mallinckrodt (Paris, Kentucky, USA).

CE Instrument

A Model 2050 CE instrument (Beckman Instruments, Fullerton, CA, USA) equipped with a capillary of 32 cm × 50 μm (I.D.) was set at 8.5 kV, 254 nm, and 35 C. The sample was introduced by pressure injection for 70 s.

Electrophoresis Buffer

Potassium phosphate buffer, 170 mmol/L, pH 5.9 was stored in the refrigerator. Daily, a working buffer was prepared by mixing 3 mL of ethanol, 7 mL of the phosphate buffer, and 30 mg polyethylene glycol. Initially, the capillary was conditioned by injecting 5 times of the stock standard.

Stock Standard

Amiodarone (sigma chemical) 100 mg/L of methanol.

Working Standard (5 mg/mL)

The stock standard is diluted in serum.

CE Procedure

Serum 100 μL was acidified with 10 μL of phosphoric acid, 0.8 mol/L, vortex-mixed with acetonitrile (200 μL), and centrifuged at 13,000 × *g* for 10 s. The supernatant was injected onto the capillary.

HPLC

The supernatant of the CE was injected on a CN Novapak (waters, Milford, MA, USA) cartridge column 100 × 3.9 mm, 4 μm average particle size.

The column was eluted with 37% acetonitrile containing 0.2% phosphoric acid and 0.1 butylamine with detection at 242 nm.

RESULTS AND DISCUSSION

Amiodarone and its metabolite D are examples for many of the non-water soluble and highly hydrophobic drugs. The analysis of these drugs by both CE and HPLC poses several challenges. They are weakly ionized, not soluble in the aqueous buffers, tend to adhere to the surface of the silica and do not possess strong UV absorbance. In order to decrease the binding to the walls and to analyze amiodarone by CE, several analytical parameters have to be optimized specifically for this drug. A high concentration of phosphate buffer (170 mM, pH 5.9) together with an added organic solvent (ethanol or isopropanol) and an elevated temperature were utilized for improved solubility and decreased binding. Ethanol here improves drug solubility; separates it from the neutral molecules, and separates A from D. The separation of A and D requires high concentration of organic solvents ($\sim$50%), ethanol or isopropanol provided the sample volume is relatively small ($<$30 s). The separation of A and D from the neutral molecules requires also an organic solvent, Figure 1. In absence of an added organic solvent, the two compounds co-elute with the neutral compounds. A and D start to separate from the neutral compounds at $\sim$13% ethanol (or isopropanol). However, the separation of the two compounds from each other begins only at 40% of ethanol or isopropanol. At 50%, the separation reaches

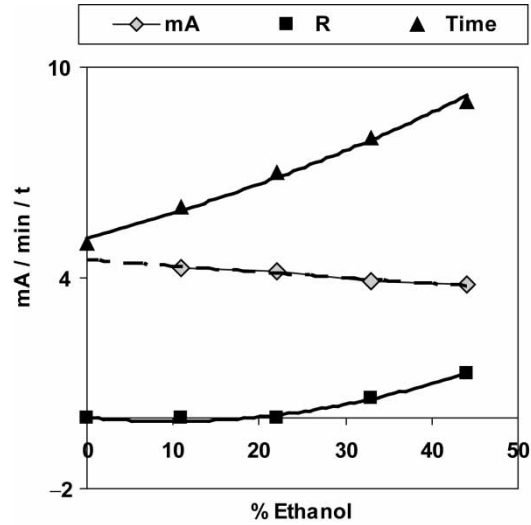

Figure 1. Effect of ethanol on the resolution, absorbance, and migration for A and D.

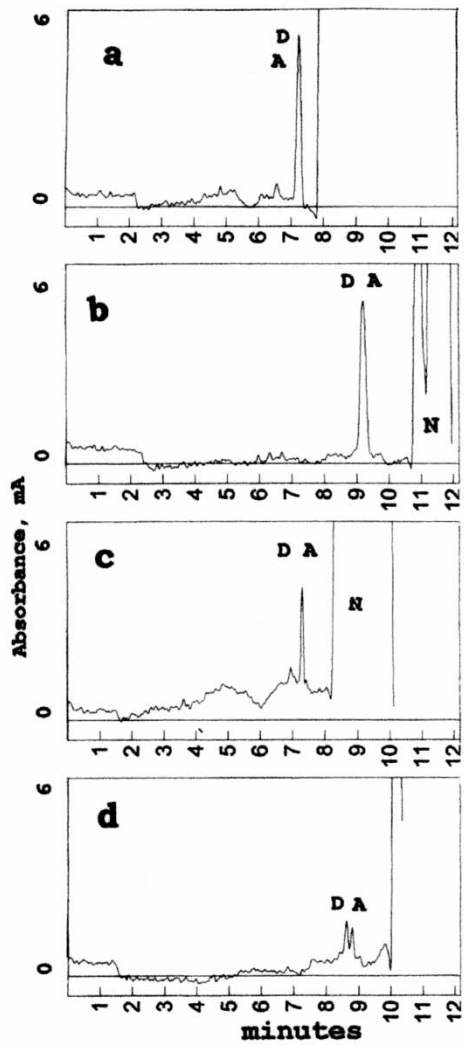

Figure 2. Separation of amiodarone, A and desethylamiodarone, D (5 mg/L of each) as affected by ethanol concentration and injection time: (a) 30% ethanol with 75 s injection; (b) 50% ethanol with 75 s injection; (c) 30% ethanol with 25 s injection; and d) 50% ethanol with 25 s injection. N = neutral molecules.

baseline provided the sample volume remains relatively small, Figure 2. Although the resolution improves with an increase in the organic solvent concentration; however, the migration time increases too, Figures 1 and 2. Also, the ability to stack the drug decreases slightly with an increase in organic solvent concentration, Figure 1. Thus, there is a decrease in the peak height with increase in the organic solvent concentration.

Both A and D have weak UV absorption, Figure 3. Thus, the ability to analyze amiodarone in serum in CE and as well, and by HPLC, depends on removing the serum proteins which mask the spectrum of the drug; and ability to concentrate the drug to be within the detection limits of the instrument. Thormann[11] has selected solvent extraction followed by field amplified injection from aqueous buffer. Here, we used acetonitrile to deproteinate the serum and to stack the drug at the same time in order to improve the sensitivity. This simple step of deproteinization allows the ability to concentrate the drug $\sim$10–30 folds. The sample prepared in acetonitrile can be introduced by both pressure injection and by field amplified injection. Both methods of injection for the sample prepared in acetonitrile give good stacking relative to that dissolved in water. Since pressure injection is less affected by small ions, which are abundant in serum, we used that for routine analysis.

The CE conditions for analysis can be chosen so that A and D can separate from each other or co-elute together. For routine use, we choose the conditions where the two peaks co-migrate; using a relatively low ethanol concentration and a high sample volume. The two compounds, A and D, have very similar absorption spectra, especially at 254 nm, Figure 3. Although the detection is about twice higher at 240 nm, this wavelength is not easily available on filter instruments. Both compounds are pharmacologically active and both, at steady state, are present at the same ratio. The conditions chosen here improve the sensitivity, speeds the analysis, and avoids adding mathematically the two values without too much sacrifice in the accuracy, especially since the exact therapeutic range for this drug is not

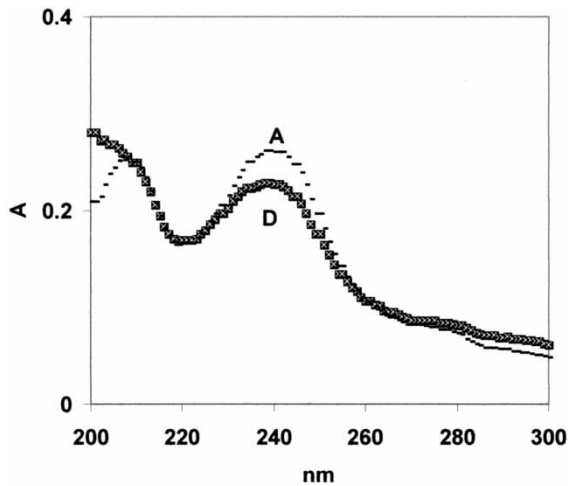

Figure 3. Spectra of 5 mg/L of amiodarone (A) and 5 mg/L of desethylamiodarone (D) dissolved in the electrophoresis buffer, 170 mmol/L, pH 6.0 containing 10% ethanol.

well established. An electropherogram representing a standard and patient by this method is illustrated in Figure 4.

None of the endogenous compounds in serum and none of the tested basic common cardioactive drugs (at the therapeutic level) such as procainamide, N-acetylprocainamide, lidocaine, propranalol, and quinidine interfered with the analysis. Rarely, an unknown peak elutes between the amiodarone and the neutral molecules, Figure 4. The test was linear between 0.5–6 mg/L. The RSD for 8 injections is 2.8 for peak height and 2.2% for migration. The minimum detection limit is 0.3 mg/L based on 3 times the baseline noise.

Comparison of the analysis by CE to that by HPLC is illustrated in Figure 5. Both methods have the same speed and similar sensitivity, Figure 4. However, the CE does avoid consumption of large amounts of expensive organic solvents for elution. Based on 93 samples from our

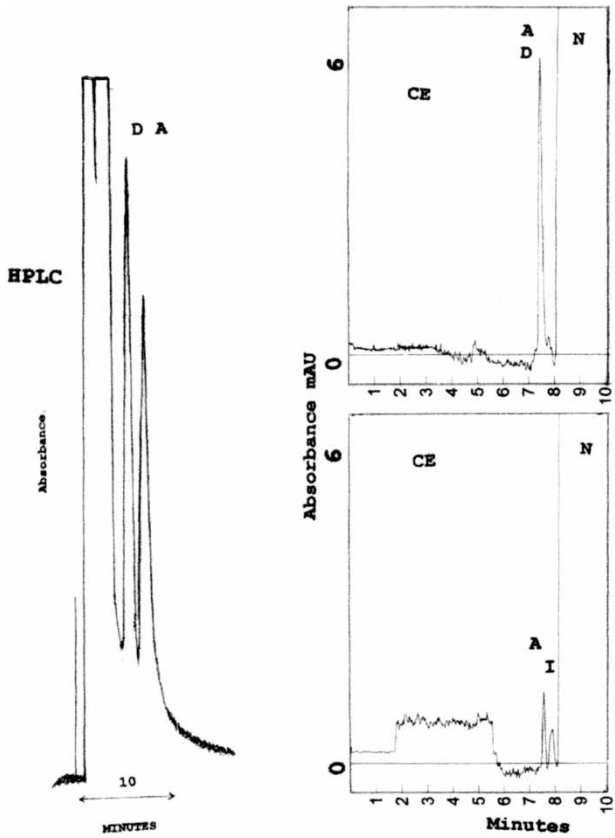

Figure 4. Shows the analysis for a standard (5 mg/L of each A and D) by HPLC (left) and the same standard by CE (top) and patient by CE and (bottom) (N = neutral molecules; I = unknown peak with high absorbance at 200 nm).

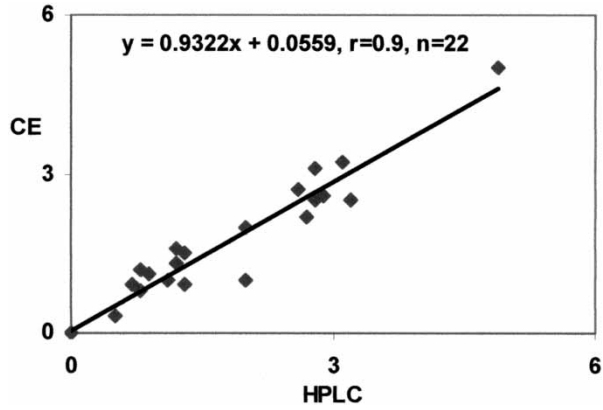

Figure 5. Comparison of total (amiodarone + desethylamiodarone) analysis by CE and HPLC.

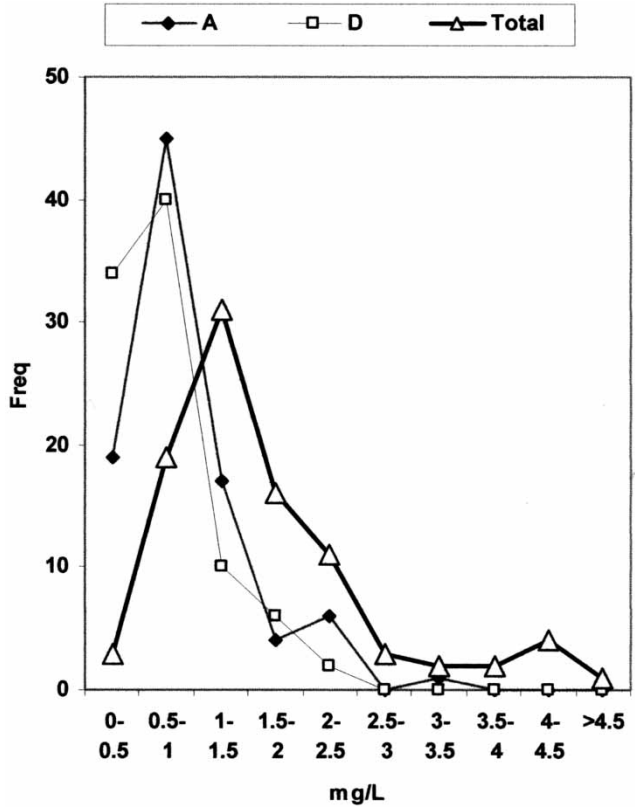

Figure 6. Histogram distribution of 92 patients for A, D and total based on our HPLC data.

patients the means for A, D, and A + D are 0.9, 0.8 and 1.7 mg/L, respectively. The range for A and D, and total (A + D) based on 80% of the distribution of the patients values are, 0.5–2, 0.2–1.8, and 1.2–4.0 mg/L, respectively, Figure 6. This range is compatible with those reported in the literature as therapeutic level.[1–4]

CONCLUSION

Amiodarone is an example of many of the hydrophobic basic drugs. These compounds are very difficult to analyze by CE. They are weakly ionized, tend to bind to the capillary walls, and do not have strong UV absorption. Several parameters have to be optimized for successful analysis. An elevated temperature, an optimum concentration of ethanol, and a high concentration of the separation buffer was necessary to decrease the binding to the walls. Stacking of the drug was also necessary to overcome the poor UV absorption of the drug. The described method is simple to perform and does not consume large amounts of organic solvents compared to the HPLC.

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