Physicochemical and Analytical Characteristics of Amiodarone

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Abstract □ Data are reported on the analytical and physicochemical characteristics of amiodarone, for use in identifying and/or assaying this antiarrhythmic agent. The drug is highly soluble in chloroform and poorly soluble in water. Its acid-base constant (pK_a) is 6.56, and its maximal lipid solubility range is from pH 3.5 to 5.5.

Keyphrases □ Amiodarone—physicochemical and analytical characteristics, HPLC and IR, UV, and mass spectrometries □ Antiarrhythmic agents—amiodarone, physicochemical and analytical characteristics, HPLC and IR, UV, and mass spectrometries

In addition to electrophysiological (1, 2) and clinical findings (3, 4), the disposition of amiodarone[1] has been extensively investigated in animals and humans (5–7). Its metabolic pathway and tissue distribution are still largely unknown, but they do offer some clues to understanding the therapeutic and toxic effects during chronic treatment (8, 9). Despite all the work done, little is known to date of the basic physical and physicochemical properties of the drug.

EXPERIMENTAL

Equipment—Amiodarone hydrochloride was synthesized, purified by recrystallization from acetone (10), and supplied by Labaz[2]. All other chemicals were reagent or UV grade.

A double-beam spectrophotometer[3] equipped with a constant-temperature control block was used for UV analysis. A high-pressure liquid chromatograph[4] was equipped with a UV-absorption detector[5] (254 nm, fixed wavelength) and a reverse-phase column[6] (7-μm particle size, 250 nm × 4 mm length). The mobile phase was acetonitrile–water–acetic acid (80:19:1, adjusted to pH 5.8 with ammonium hydroxide) at a flow rate of 2 mL/min. Melting points were determined on a hot-stage[7] and were not corrected. A mass spectrometer[8] equipped with a computer[9] was used for MS analysis. Analyses were made by the direct inlet system, scanning repetitively throughout the sample evaporation period and heating the probe to 180°C. Electron energy ranged from 20 to 60 eV. IR spectra were obtained on a spectrophotometer using potassium bromide pellets (1%, w/v).

UV Spectrometry—To obtain a spectrum and determine its molar extinction coefficient (ϵ), methanolic solutions of amiodarone were prepared in triplicate at a concentration of 10 μg/mL (14.7×10^{-6} M). UV analyses were carried out at 208, 241, 270, and 280 nm at 25°C. The molar extinction coefficient was calculated by Lambert and Beer's law:

$$\log I_0/I = \epsilon \times 1 \times C \qquad \text{(Eq. 1)}$$

where $\log I_0/I$ is the absorption intensity of the sample, 1 is the cuvette path beam length (in cm), and C is the molar concentration; hence, ϵ = Absorbance × cm^{-1} × M^{-1}.

Solubility—The percentage (w/v) of amiodarone soluble in different solvents was determined by adding an excess of drug to obtain saturation of 20 mL of each solvent tested, calculating the amount of dissolved amiodarone. The tubes were rotated end-over-end for 1 h (longer than needed to dissolve the drug in all solvents employed) at 25 ± 0.1°C in a water bath and then centrifuged at the same temperature. The supernatant was diluted and spectrophotometrically assayed for amiodarone. Three determinations were made for each solvent.

Determination of pK_a—According to a long-used method (11) the pK_a values were determined by UV titration (at λ_{max}) of aqueous solutions containing known amounts of amiodarone. A range of pH 1–5 was achieved by addition of hydrochloric acid, pH 5.8–9.2, by 0.05 M borate phosphate (adjustment being made with hydrochloric acid), while higher values were obtained by addition of sodium hydroxide solutions. Sodium chloride was used to adjust the ionic strength. All solutions were prepared immediately before use and all pH values were checked at 25°C using a standard pH-meter. In the temperature range of 20–30°C there was no substantial effect on the true pH of the solutions, but only 3–4 h after preparation we found a 10% decrease in amiodarone concentration at pH values >7.0.

The extinction coefficient was related to different hydrogen ion concentrations to check the λ_{max} shift. The dissociation constant (K_a) was determined according to the equation

$$\epsilon = \frac{A_H \times [H^+] + A_B \times K_a}{[H^+] + K_a} \qquad \text{(Eq. 2)}$$

where A_H and A_B are the extinction coefficients when amiodarone exists under acidic and basic conditions, respectively. Experimental values of ϵ *versus* pH were then fitted by a nonlinear regression iterative program on a desk computer[10]. Determinations were done in triplicate.

Partition Coefficients—According to the suggestions of Leo *et al.* (12), amiodarone was partitioned between buffer solutions saturated with 1-octanol and 1-octanol saturated with buffer solutions (as reported for the determination of pK_a). Known amounts of amiodarone were dissolved in 20 mL of buffer solution and gently shaken for 2 h (long enough to achieve equilibrium) at 25 ± 0.1°C in a shaking incubator with 5 mL of organic solvent. The two phases were separated by centrifugation, and the amount of amiodarone in the aqueous layer was determined spectrophotometrically. The apparent partition coefficient was calculated as:

$$P_{app} = \frac{C_{octanol}}{C_{buffer}} = \frac{(C_1 - C_2) \cdot a}{C_2 \cdot b} \qquad \text{(Eq. 3)}$$

where C_1 and C_2 are the amiodarone concentrations (w/v) in the buffer before and after equilibration, and a and b are the volumes of the buffer and 1-octanol layers, respectively. Each determination was made three times for each pH value checked. The true or corrected partition coefficient, knowing the pK_a value of the drug, was calculated as:

$$P_{corr} = \frac{C_{octanol}}{C_{buffer}\,(1 - \alpha)} = \frac{P_{app}}{1 - \alpha} \qquad \text{(Eq. 4)}$$

where α is the degree of ionization at a given pH and can be calculated as:

$$\alpha = \frac{1}{[1 + \text{antilog}\,(pK_a - pH)]} \qquad \text{(Eq. 5)}$$

RESULTS AND DISCUSSION

Solubility—Table I sets out the findings as percentages (w/v). The coefficient of variation was always <5% for each solvent employed. The two extremes of solubility are represented by chloroform and petroleum ether, in which the ratio of amiodarone solubility is around 30,000:1. The pH of water was 6.5, and solubility was not substantially affected by a pH range of 1.5–7.5 in aqueous solutions (physiological fluids).

UV Spectrometry—The spectrum of amiodarone in methanolic solution using 1-cm quartz cells showed λ_{max} at 208 and 241 nm. Two shoulders were resolved near 270 and 280 nm; mean ϵ values were 4.7×10^4 at 208 nm, 4.4×10^4 at 241 nm, 1.8×10^4 at 270 nm, and 1.6×10^4 at 280 nm, respectively.

[1] Cordarone; Labaz Laboratories, Ambares, France.
[2] Labaz, Centre de Recherche, Brussels, Belgium.
[3] Model UV-300; Shimadzu, Kyoto, Japan.
[4] Series 1; Perkin-Elmer, Norwalk, Conn.
[5] Model LC 15; Perkin-Elmer, Norwalk, Conn.
[6] Hibar LiChrosorb RP-8; Merck, Darmstadt, Federal Republic of Germany.
[7] Kofler, C. Reichert AG, Vienna, Austria.
[8] Model 3091; LKB, Bromma, Sweden.
[9] Model 2130; LKB, Bromma, Sweden.
[10] Model HP-85; Hewlett-Packard, USA.

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Table I—Percentage Solubility (w/v) of Amiodarone in Various Solvents at 25°C [a]

Solvent	Solubility, g/100 mL
Chloroform	44.51282
Methylene chloride	19.20000
Methanol	9.98400
Ethanol	1.28000
Benzene	0.64657
Tetrahydrofuran	0.60377
Acetonitrile	0.31889
1-Octanol	0.29500
Ether	0.17436
1-Propanol	0.13435
Water	0.07164
Hexane	0.02559
Petroleum ether	0.00137

[a] Values are the average of determinations done in triplicate.

High-Performance Liquid Chromatography—The procedures described by Lesko *et al.* (13) for the preparation of biological samples were followed first. Because of the longer column life (efficiency) and greater simplicity using an aqueous mobile phase, we subsequently relied on a reverse-phase system. Values for sensitivity (0.05 μg/mL), within-day coefficients of variation (1.9% at 0.50 μg/mL and 6.5% at 5.00 μg/mL), and day-to-day coefficients of variation (7.5% at 0.50 μg/mL and 7.0% at 5.00 μg/mL) confirm the reliability of the method. The calibration curves were linear over the concentration range studied.

Melting Point—Amiodarone occurs as a white crystalline powder, with a melting point of 159 ± 2°C after recrystallization from acetone (10).

IR Spectrometry—The IR spectrum (KBr pellets) revealed characteristic bands that agreed with the suggested structure: 3000–3070 (ArCH), 2860–2960 (aliphatic CH), 2200–2700 (*tert*-amine NH^+), 1635 (diAr C=O), 1560 and 1015 (benzofuran C=C), 1380 (aliphatic CH_3), 1285 (ketonic C—C), 1250 and 1075 (ether C—O—C), and 1225 and 1025 cm^{-1} (*tert*-amine C—N).

Mass Spectrometry—The molecular peak of amiodarone was observed at *m/z* 645, corresponding to the molecular mass. The most characteristic fragments of amiodarone gave peaks at *m/z* 630 ($M^+ - CH_3$), 546, 517 ($M^+ - HI$), 420, 391, 373, 201, and 159. The peak at *m/z* 546 corresponds to cleavage of the aromatic ether with elimination of the aliphatic aminic chain. The other ions at *m/z* 420, 391, 373, 201, and 159 are all fragments of this aromatic part of the molecule.

Determination of pK_a—The UV absorption curves of amiodarone at different hydrogen ion concentrations (Fig. 1a) showed a characteristic bathochromic shift (toward the red) of λ_{max} (241 nm) from pH 6.0 to higher values. The apparent dissociation constant, calculated for amiodarone using Eq. 2, (Fig. 1b) was 6.56 ± 0.06 (*SD*). By appropriate substitution into the Henderson–Hasselbach equation of the pK_a value for amiodarone (weak acid) and considering the pH range (1.0–8.0), which encompasses the pH in all body sites, it can be affirmed that amiodarone is essentially un-ionized at acidic pH sites.

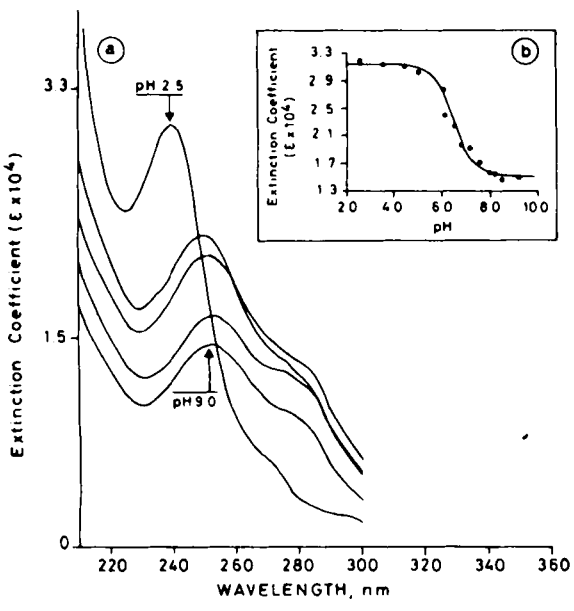

Figure 1—*Representative changes with pH in the absorption spectrum of amiodarone (a), and curve plotted to calculate the* pK_a *value for amiodarone (b).*

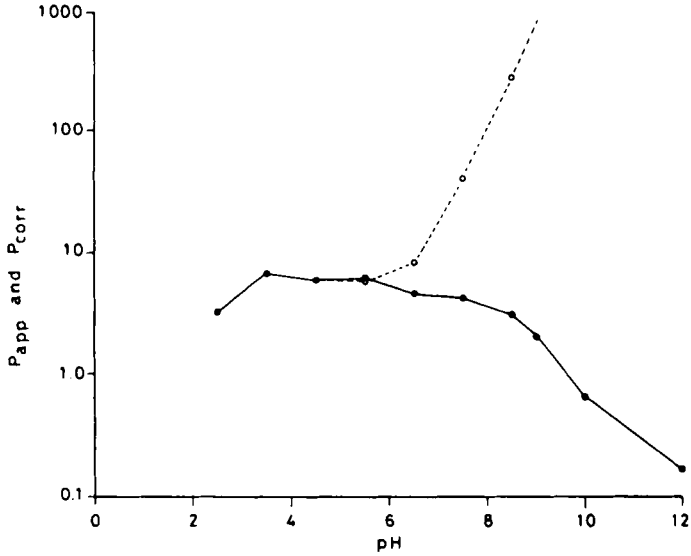

Figure 2—*Changes with pH in the apparent (●) and corrected (○) partition coefficients of amiodarone.*

Partition Coefficients—Apparent and corrected partition coefficients are plotted in Fig. 2. A typical dependence of coefficients on pH was found, with maximum lipid solubility of amiodarone between pH 3.5 and 5.5. The same behavior is described for other compounds [such as tetracycline and sulfa drugs (14, 15)].

The present findings confirm that an acidic pH (~4.5) is optimal for the extraction of amiodarone from biological specimens (7, 13). Furthermore, considering the dissociation characteristics and taking into account the "pH-partition hypothesis" (16) and Schanker's statement (17) that ". . .lipid solubility is most likely the dominant characteristic, since the relevance of the degree of ionization is probably a consequence of the poor lipid-solubility of organic ions," the absorption of amiodarone, whose extent and specific mechanism are at present unknown, should be far greater in the duodenum and jejunum than in the stomach and lower alimentary canal. Thus, gastric emptying time and changes of pH are likely to be critical in the absorption of amiodarone.

Wide variability can be expected in absorption rates and bioavailability of the drug. This is specifically relevant in protocols to investigate the correlations between amiodarone blood levels after acute and repeated oral administration and electrophysiological effects.

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Liquid Chromatographic Analysis of Triamterene and Its Major Metabolite, Hydroxytriamterene Sulfate, in Blood, Plasma, and Urine

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Abstract □ The first rapid and highly sensitive high-performance liquid chromatographic (HPLC) assay for triamterene, hydroxytriamterene, and hydroxytriamterene sulfate is reported. Plasma samples were processed by protein precipitation, while urine was used untreated. Three different solvent systems were used to analyze (*a*) triamterene in plasma (30% acetonitrile, pH 4.0; internal standard: furosemide; sensitivity limit: 1 ng/mL); (*b*) hydroxytriamterene and hydroxytriamterene sulfate in plasma (12% acetonitrile, pH 5.5; internal standard: cefamandole; sensitivity limits: 20 and 2 ng/mL, respectively) and (*c*) triamterene, hydroxytriamterene, and hydroxytriamterene sulfate in urine (13% acetonitrile, pH 5.3; internal standard: hydroflumethiazide; sensitivity limits: 0.04 μg/mL, 0.5 μg/mL, and 0.1 μg/mL, respectively). Fluorescence detection of compounds was performed at 365-nm excitation and 440-nm emission wavelengths. Recovery of triamterene and its metabolites from plasma was complete, and calibration curves were linear. Intraday variation was <6% except for the lowest plasma concentration. The assay procedure has already been used in several pharmacokinetic studies.

Keyphrases □ Triamterene—HPLC analysis, major metabolites, hydroxytriamterene sulfate, blood, plasma, and urine □ Hydroxytriamterene sulfate—HPLC analysis, major metabolite of triamterene, blood, plasma, and urine

Although triamterene has been clinically available since the 1960's, the metabolic pathways for this drug have been a matter of controversy, with conflicting reports appearing up through 1982. Early work by Lehmann (1) indicated that triamterene undergoes hydroxylation and subsequent sulfation to hydroxytriamterene sulfate, which he found in urine. Pruitt *et al.* (2), using TLC separation, could only detect hydroxytriamterene, whereas Grebian *et al.* (3) and our group (4, 5) did find the sulfate ester of hydroxytriamterene using TLC techniques.

More recently, three HPLC techniques for triamterene analysis have been published (6-8). One of these research groups (6) assumed, on the basis of the work of Pruitt *et al.* (2), that hydroxytriamterene was the major metabolite of triamterene and did not develop a method to specifically measure the sulfate conjugate. The other two groups did not measure any metabolites. The first HPLC assay by which the specific measurement of hydroxytriamterene sulfate can be obtained and by which very low concentrations of unchanged triamterene and hydroxytriamterene sulfate can be quantitated is reported herein. The method is simple, fast, and accurate.

EXPERIMENTAL

Reagents—Triamterene[1], hydroxytriamterene[2], hydroxytriamterene sulfate[2], and the internal standards hydroflumethiazide[3], furosemide[4], and cefamandole[5] were used as received. Acetonitrile[6] for the HPLC measurements was glass-distilled; water was redistilled and stored in glass.

Apparatus—A high-performance liquid chromatograph[7] equipped with a sample processor[8], a fluorescence spectrophotometer[9], and a UV detector[10] was used. The assay was carried out on a 10-μm particle size, 4 × 300-mm reverse-phase column[11] at a solvent flow rate of 2 mL/min.

Mobile Phase and Retention Times—Three different solvent systems were used for the measurement of triamterene, hydroxytriamterene, and hydroxytriamterene sulfate. Each solvent system (A-C, as indicated in Table I) was used for a particular quantitation problem.

Detection Wavelengths—Triamterene, hydroxytriamterene, and hydroxytriamterene sulfate as well as the internal standards furosemide and hydroflumethiazide were analyzed using their native fluorescence at an excitation wavelength of 365 nm and an emission wavelength of 440 nm. Cefamandole, which was used as an internal standard in mobile phase B, was measured by UV detection at 254 nm. For that purpose the HPLC system was equipped with two detector systems, one for fluorescence and another for UV detection.

Methods—Each plasma or total blood sample (0.1 mL) was deproteinated by adding 0.4 mL of acetonitrile containing both furosemide (3.5 μg/mL) and cefamandole (60 μg/mL) internal standards. After mixing[12] for 1 min, the sample was centrifuged[13] for 10 min at 3200 rpm. The supernatant was transferred to a clean test tube and, if necessary, evaporated at room temperature to 0.2 mL under nitrogen when very low concentrations had to be measured. However, most of the samples could be analyzed by injection of a volume as little as 5 μL.

Urine samples ranging from 0.02 to 0.1 mL were added to 1 mL of an acetonitrile solution containing hydroflumethiazide as an internal standard (500 μg/mL). After mixing[12] and centrifugation[13], 2 μL was injected onto the HPLC system.

Human plasma, blood, and urine were stored at −20°C. Blank plasma and blood for assay development was obtained from healthy volunteers using 143 IU/10 mL of lithium heparin as anticoagulant. To test the stability of tri-

[1] Mylan Pharmaceuticals Inc., Morgantown, W.Va.
[2] Röhm-Pharma, Darmstadt, West Germany.
[3] Le Pharmaceutical Products, 2750 Ballerup, Denmark.
[4] Hoechst Pharmaceuticals Inc., Somerville, N.J.
[5] Eli Lilly & Co., Indianapolis, Ind.
[6] J. T. Baker Chemical Co., Phillipsburg, N.J.
[7] Perkin-Elmer Series 3, Norwalk, Conn.
[8] WISP Model 710A; Waters Associates, Milford, Mass.
[9] 204 S; Perkin-Elmer, Norwalk, Conn.
[10] LC 65 T; Perkin-Elmer, Norwalk, Conn.
[11] Micro Pak MCH; Varian, Los Altos, Calif.
[12] Vortex-Genie Mixer; Scientific Industries Inc., Bohemia, N.J.
[13] IEC HN-SII Centrifuge, Damon IEC Div.; Needham Heights, Mass.

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