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A Review on the Methods Used for the Determination of Amiodarone Hydrochloride

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Summary The different methods applied for the determination of the antiarrhythmic drug, amiodarone hydrochloride has been reviewed and discussed in this review. This will be of help to those who are dealing with this important pharmaceutical compound to choose a suitable method for determination.

Contents

Introduction

Analytical chemistry is very important in many science and industry fields, especially, in pharmaceutical industry where without quality assurance and quality control, the pharmaceutical preparation can't be used; thus, the analyst needs for this purpose, a rapid,

easy, sensitive and accurate method of analysis. These properties can be achieved by the use of various techniques (for quantitative analysis of pharmaceutical compounds). Among the various techniques of analysis that can be used in analytical chemistry to be mentioned, titrimetric analysis, gravimetric analysis, optical, electroanalytical and chromatographic methods of analysis**.**

Amiodarone hydrochloride (AM.HCl), 2-butyl-3-benzofuranyl-4-(2-diethyl-amino-ethoxy)-3,5-di-iodophenyl ketone hydrochloride (1:1)[(1)] (Fig. 1); has the molecular formula $C_{25}H_{29}I_2NO_3$.HCl with molecular weight 681.78 and melting point of 159-163°C. It is freely soluble in methylene chloride, soluble in methanol, sparingly soluble in ethyl alcohol, very slightly soluble in hexane and water. It is obtained as a white or almost white, fine crystalline powder. It is marketed by Global Napi Pharmacuticals under the proprietary name Cordarone®. Amiodarone Hydrochloride is an important antiarrhythmic medication that affects the rhythm of heartbeats. It is used in the treatment of documented life-threatening recurrent ventricular dysrhythmias.

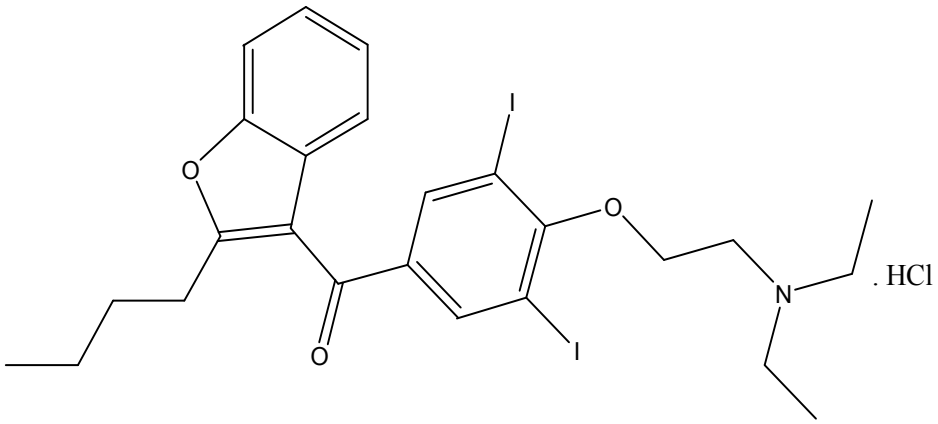

Fig. 1: Structure of amiodarone hydrochloride

Amiodarone hydrochloride can undergo photolytic degradation since its molecule is a derivative of haloaryl halide[(2)]. For solid state, when AM.HCl and desethylamiodarone hydrochloride (DEA.HCl, the main metabolite of AM.HCl) were stored in a dark, closed container at room temperature then examined by TLC, HPLC and UV-spectrophotometry, no

decomposition was shown after 3 years. In aqueous solutions, AM.HCl and DEA.HCL are not stable and their decomposition is enhanced by shaking. In case of organic solvents such as methanol and chloroform, methanolic solutions of AM.HCl and DEA.HCl in concentrations varying from 0.1 to 1000 µg mL^{-1} are stable for at least 3 months if stored at 4 °C in the absence of light in glass tubes. In chloroform, AM.HCl undergoes a rapid dehalogenation process therefore; this solvent is unsuitable for preparation of solution of the drug or for usage as extraction solvent or elution solvent[3]. AM.HCl exhibits degradation upon undergoing basic hydrolysis and oxidation; the degradation product produced under basic hydrolysis is 2-butyl-3-benzofuranyl-3,4-dihydroxy-5-iodophenylketone.[2]

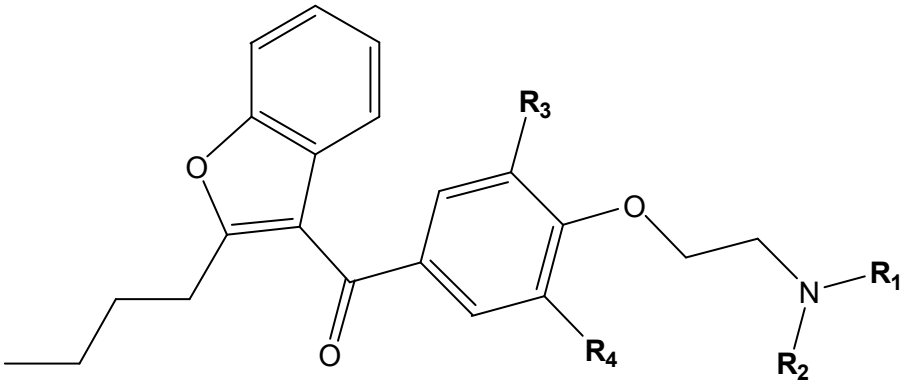

Fig. 2: Structure of amiodarone and some of its analogues (deethylated, demethylated and deiodinated)

Compound	R_1	R_2	R_3	R_4
Amiodarone	C_2H_5	C_2H_5	I	I
Desethylamiodarone	C_2H_5	H	I	I
Desmethylamiodarone	CH_3	C_2H_5	I	I
Desdiethylamiodarone	H	H	I	I
Monoiodoamiodarone	C_2H_5	C_2H_5	I	H
Desdiiodoamiodarone	C_2H_5	C_2H_5	H	H

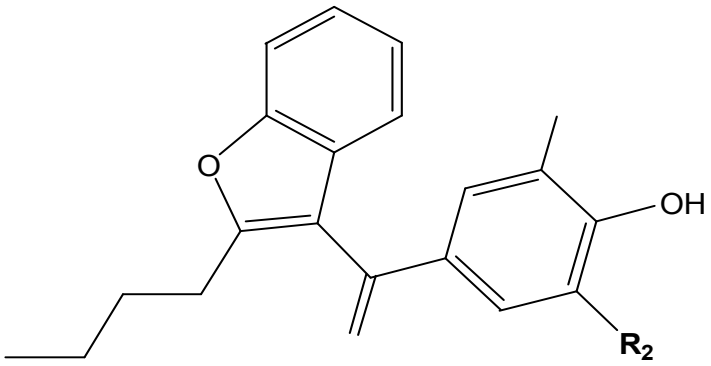

Fig. 3: Structure of some analogues of amiodarone [2-butyl-3(4-hydroxy-benzoyl) benzofuran derivaties]

Compound	R_1	R_2
2-butyl-3(3,5-diiodo-4-hydroxy-benzoyl) benzofuran	I	I
2-butyl-3(3-iodo-4-hydroxy-benzoyl) benzofuran	I	H
2-butyl-3(4-hydroxy-benzoyl) benzofuran	H	H

A wide variety of analytical methods have been reported for the determination of amiodarone hydrochloride in pharmaceutical preparations and in biological samples. These methods include spectrophotometry, reflectance, raman, voltammetry, ion-selective electrode, high-performance liquid chromatography (HPLC), ion chromatography (IC), thin layer chromatography (TLC) and capillary electrophoresis (CE), principally. In this work, we have recompiled the described methods in the literature for determination of AM.HCl alone, with its metabolite DEA and in combination with others.

Pharmaceutical Preparations

For analytical determination of amiodarone hydrochloride in formulated products and in drug substance, several analytical techniques were used.

Spectral methods

This type of analysis is based on the variation of the spectrum of a system with change in the concentration of some components. It forms the basis of what the chemist commonly

terms spectral analysis. Spectrophotometric methods are concerned with the determination of the concentration of a substance by measurement of the relative absorption of light as a function of the concentration of the substance.[4] Several spectroscopic methods have been described for determination of amiodarone hydrochloride in its pure state and in its pharmaceutical preparations.

Spectrophotometric methods

Two simple, sensitive and economic spectrophotometric methods were carried out by Rahman et al.[5] for the determination of amiodarone hydrochloride in pure form and in commercial dosage form. The methods were based on the reaction of amiodarone base with p-chloranilic acid and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) to give highly colored charge transfer complex species with absorption maxima at 535 and 570 nm, respectively. Beer's law was obeyed in the concentration ranges 10-360 and 2-65 µg mL^{-1}, respectively. Application of the methods to commercial pharmaceutical tablets was presented[5].

Three simple, sensitive and reproducible visible spectrophotometric methods were described by Rao et al.[6] for the determination of amiodarone hydrochloride and ondasetron hydrochloride in pure samples and pharmaceutical formulations. The methods are based on the formation of charge-transfer complexes between amiodarone hydrochloride or ondasetron hydrochloride and chloranil, chloranilic acid or 2,3-Dichloro-5,6-dicyano-p-benzoquinone at wavelength 540, 540 or 455 nm, respectively. Regression analysis of Beer's law plots showed good concentration ranges 20-300, 25-300, 30-300, 15-150 and 15-150, 5-60 µg mL^{-1} for amiodarone hydrochloride and ondasetron hydrochloride with chloranil, chloranilic acid and DDQ, respectively. The applicability of the methods has been examined by analyzing tablets of amiodarone hydrochloride and ondasetron hydrochloride.

In a method established by Pietra et al.[7], powdered tablets were extracted with aqueous 80% methanol, capsule contents were treated with 0.1 mol L^{-1} HCl then with methanol, the sample solutions were diluted with methanol and analyzed by first and second-derivative spectrophotometry. Calibration graphs were rectilinear from 12.3 to 37.0 µg mL^{-1}. The coefficients of variation were 0.45 to 0.80% (n = 8), and recoveries 99.4-99.6 %

Reflectance methods

A spectroscopic method have been carried out by Jensen et al.[8] for the determination of amiodarone HCl in its pharmaceutical preparations where the surface of tablets containing amiodarone HCl as active ingredient was leveled off and the tablets were glued on to a small anodized aluminum plaque, which was then placed in the cell of the Infra Analyzer (Technicon Corporation, Tarrytown, NY). Spectra recorded in the reflectance mode were identical to KBr-disc absorption spectra. Results obtained by reflectance near-IR spectrometry were correlated well with those from chemical methods and the error was 0.5%.

Jensen et al.[9] reported a spectroscopic method for determination of amiodarone in its dosage forms where six amiodarone tablets were fixed to an anodized aluminum plaque around a central tablet, the plaque was heated in an oven at 40 °C for 1 hour and placed in the measuring cell of an Infra Alyzer 450 instrument such that it was in contact with the smaller opening of the integration sphere. The near-IR reflectance spectrum was then measured. A calibration graph constructed from the results obtained for each tablet was rectilinear over the range studied. The effect of temperature and hygrometry was studied. Results correlated well with those obtained by a classical method.

Other spectral methods

Raman spectroscopy was applied for the direct non-destructive analysis of amiodarone HCl (the active ingredient of the liquid formulation Angoron®). The FT-Raman spectra were obtained through the unbroken as-received ampoules of Angoron®. Using the most intense vibration band of the active pharmaceutical ingredient at 1568 cm^{-1}, a calibration model based on solutions with known concentrations was developed. The model was applied to the Raman spectra recorded from three as-purchased commercial formulations of Angoron® having nominal strength of 50 mg mL^{-1} amiodarone HCl. The average value of the active pharmaceutical ingredient in these samples was found to be 48.56±0.64 mg mL^{-1} while the detection limit of the proposed technique was found to be 2.11 mg mL^{-1}. The results were compared to those obtained from the application of high performance liquid chromatography (HPLC) using the methodology described in the European Pharmacopoeia[10] and found to be in excellent agreement. The proposed analytical methodology was also validated by evaluating the linearity of the calibration curve as well as its accuracy and precision. The main advantage of Raman spectroscopy over HPLC method during routine analysis is that it is considerably faster and no solvent consuming. Furthermore, Raman spectroscopy is non-destructive for the sample and environmentally friend. However, the detection limit for Raman spectroscopy is much higher than the corresponding for the HPLC methodology.[11]

A flow injection configuration was developed and evaluated for the Chemiluminscent determination of amiodarone by Ruiz et al.[12].The method was based on the reaction of the drug with tris(2,2′-bipyridyl) ruthenium (III), which was generated through the on-line photo-oxidation of tris(2,2′-bipyridyl) ruthenium (II) with peroxydisulfate. Under the optimum experimental conditions, a linear calibration graph was obtained over the range 3–60 μg mL^{-1} with a detection limit of 0.28 μg mL^{-1}. The proposed method allowed 120 injections/hour with excellent repeatability and precision (RSD less than 0.5% and 2.8%, respectively) and a reagent consumption of only 0.37 μmol (0.27 mg) of $Ru(bpy)_3Cl_2 \cdot 6H_2O$ per determination. The method was successfully applied to the determination of amiodarone in commercial pharmaceutical formulations.

Electroanalytical methods

Amiodarone, benziodarone and their iodinated derivatives were determined at concentration from 5 to 50 μmol L^{-1} by cyclic voltammetry with a carbon-paste electrode by Gallo et al.[13]. A BAS CV-27 voltammograph connected to a Hewlett-Packard 7004 B recorder was used. A BAS silver-AgCl RE-1 reference electrode and a platinum-wire counter electrode were also used.

New all-solid-state ion-selective electrodes for determining amiodarone have been developed by Kholoshenko et al.[14]. A special feature of the electrode design is the presence of an intermediate polymer transducer layer between the ion-selective membrane and the metal electrode. The transducer can be made of polyaniline or specially synthesized poly(N-phenylglycine). The electrochemical characteristics of the electrodes in systems with different transducer materials were determined. It is shown that a transducer ensured long-term stability of the electrode parameters and made possible miniaturezation of the electrode system. The proposed electrodes were successfully used for the analysis of amiodarone tablets.

Stefan et al.[15] prepared a liquid membrane electrode by impregnating a graphite rod support with 1 mmol L^{-1} amiodarone-dipicrylamine complex in nitrobenzene. The response of the electrode was linear in the range 0.01-10.0 mmol L^{-1} amiodarone solution at pH 0.8-4.8, the detection limit was 4 nmol L^{-1} and the response time ~1 min. The selectivity was good against Na^+ and Ca^{2+}, but there was strong interference from ephedrine and polyvinylpyrrolidone ions. The electrode was used for the indirect potentiometric determination of amiodarone in raw material, tablets and ampoules. The recoveries were

>99% with RSD of ≤ 0.83%. Dissolution tests of tablets in simulated duodenum fluid were also carried out in a pharmacokinetic study, and the rate constants for disintegration and dissolution were calculated.

Chromatographic methods

Separation techniques, particularly chromatographic methods, are necessary and valuable in the analysis of pharmaceutics. The partition of a solute between two immiscible solvents is used many times to isolate the drug from other compounds in a mixture. Separations such as solvent-solvent extraction, open column or thin layer chromatography (TLC) may be required as preparatory step when spectrophotometric analysis in the ultraviolet region is applied. Gas chromatography (GC) and high performance liquid chromatography (HPLC) represent two non-stoichiometric methods that have achieved very great popularity because of their capabilities. In gas chromatography, any compound directly or with drivatization can be analyzed if it has a perceptible vapour pressure and if a suitable column can be found. The use of various detectors adds another element of selectivity to the procedure. The most characteristic feature of the development in the methodology of pharmaceutical and biomedical analysis during the past 25 years is that HPLC became undoubtedly the most important analytical method for identification and quantification of drugs, either in their active pharmaceutical ingredient or in their formulations during the process of their discovery, development and manufacturing [16,17]. More recently, HPLC has been rapidly developing with the introduction of new pumping methods, more reliable columns and a variety of detectors. But the great attraction of chromatographic techniques to the industrial laboratory is the possibility of automation. In brief, chromatographic methods can offer the advantage of speed without scarifying the accuracy that can be used in quality control laboratories.

High performance liquid chromatography with UV detection (HPLC-UV)

Powdered tablets or capsule contents containing ~100 mg of amiodarone were dissolved and diluted to 100 mL with methanol and after centrifugation, a 5 to 15 mL portion of the supernatant solution was mixed with 10 mL of methanolic carbamazepine solution (1 mg mL^{-1}) as internal standard and the mixture was diluted to 100 mL. A 6 μL portion was analyzed by HPLC on a column (25 cm x 4.6 mm) of C18 silica (10 μm) with a mobile phase (2 mL min^{-1}) of aqueous 80% acetonitrile containing 0.2% of $HClO_4$ 0.312% of

triethylamine, and detection at 240 nm. The calibration graph was rectilinear for 2.5 to 20 mg of amiodarone with recoveries from tablets and capsules of~99% and ~93%, respectively.[18]

Powdered tablets were extracted with aqueous 80% methanol, capsule contents were treated with 0.1 mol L^{-1} HCl then with methanol. After addition of miconazole nitrate as internal standard, the solutions were analyzed by HPLC on a column (30 cm x 4.0 mm) of Hypersil RP-18 (10 μm) with methanol-tetrahydrofurane-0.1 mol L^{-1}-triethyl-ammonium acetate of pH 7.0 (24:17:9) as mobile phase (1.2 mL min^{-1}) and detection at 242 nm. Calibration graph was rectilinear from 12.3 to 37.0 μg mL^{-1}, coefficient of variation was 0.34% (n = 8) and recovery was 99.4%.[7]

The validation of a gradient HPLC procedure employing UV detection for the determination of amiodarone HCl and two of its related substances in amiodarone HCl injection (drug product) was reported by Christopherson et al.[19]. The method was reproducible, accurate and selective for amiodarone hydrochloride and the two known related substances. The peak area response versus concentration was demonstrated to be linear over the range 50-150% for the assay preparation, as well as over the range 0.1-0.3% for the related substance preparation range. Relative response factors were determined for the two available related substances. The precision (repeatability) of the method was demonstrated for both the assay and related substances from six independent sample preparations. Intermediate precision was demonstrated between two separate chemists on two separate days and instruments. Accuracy of the method (percentage recovery) was demonstrated for both amiodarone HCl and each of the two available related substances. Specificity was demonstrated by forced degradation of the drug product under acidic, basic, heat, peroxide and light conditions. Limit of detection and limit of quantification were reported for amiodarone HCl and each of the two available related substances. Preparations were demonstrated to be stable for up to 48 hours following their preparation when stored under laboratory light at 25°C.

A rapid method for determination of amiodarone, aprindine, disopyramide, flecainide, lignocaine, lorcainide, mexiletine, procainamide, propafenone, sotalol, tocainide and verapamil was developed by Verbesselt et al.[20]. All drugs except amiodarone and aprindine were extracted on C8-Bondelut columns after alkalinization with 0.2 mol L^{-1} sodium carbonate or 0.05 mol L^{-1} borate buffer (pH 9), aprindine was extracted on the same column but at neutral pH. Amiodarone was extracted on a CN-Bondelut column at acidic pH. Internal standards were tabulated. The columns were washed with H_2O and the drugs were eluted with

methanol except for amiodarone which was eluted with acetonitrile-acetate buffer of pH 5 (4:1). Most of the eluates were evaporated to dryness and reconstituted in mobile phase for analysis, for amiodarone, disopyramide and tocainide the eluates injected directly onto the column (15 cm x 4.6 mm) of Spherisorb hexyl (5 μm) with UV or fluorescence detection (details and mobile phases given). Ranges of rectilinearity of the calibration graphs were tabulated, mean recoveries were 57.6 to 110.4% with coefficient of variation of 10%.

Thyagarajapuram and Alexander [21] proposed a repeatable reverse-phase HPLC method for quality control purposes for the estimation of amiodarone hydrochloride in a dosage form. In this study A C-8 column was used. The mobile phase consisted of methanol, water, and acetic acid in a 95:4:1 ratio and the pH adjusted to 4.0 with aqueous ammonia. Nortriptyline was chosen as the internal standard and a flow rate of 1.5 mL min^{-1} was used with the buffer and water in 98:2 concentrations. The retention time for amiodarone hydrochloride and nortriptyline was approximately 3.5 and 1.8 min., respectively. The analytical method was found to be linear and stability indicating with the relative standard deviation within and between days being 2.48 and 3.26%, respectively. The limit of quantification and limit of detection were found to be 3.12 and 0.936 μg mL^{-1}, respectively.

A simple, rapid and sensitive isocratic high performance liquid chromatographic method was developed by Khan et al.[22] for the estimation of purity and quantitative determination of amiodarone HCl active pharmaceutical ingredient. The method described a quantitative estimation of five process related impurities of amiodarone HCl with a resolution of more than or near to 3.0 between each impurity. These five known related substances are estimated by a simple, rapid and accurate reverse phase isocratic HPLC method. The method has been validated for the determination of assay and related substances in amiodarone HCl active pharmaceutical ingredient using a C8 column. The elution was carried out using a mobile phase consisting of water-methanol-acetic acid mixture of pH 5.8. For the quantitative determination of these relative substances, a relative response factors have been determined for all five related substances with respect to amiodarone HCl. The precision (system precision, method precision and intermediated precision) was demonstrated for both the assay as well as related substances on six independent sample preparations. Accuracy of the method (recovery) was demonstrated for both amiodarone and each of the five related substances. Specificity of the method was demonstrated by forced degradation study of amiodarone HCl active pharmaceutical ingredient under various stress conditions. The method was found to be stability indicating and useful for the analysis of assay and related substances of amiodarone

HCl active pharmaceutical ingredient in a routine quality control laboratory and for the stability studies of drug substance.

In a study presented by Mallu et al.[23], a simple, specific, sensitive and rapid high performance liquid chromatography (HPLC) method was established for the determination of amiodarone hydrochloride. Amiodarone was identified and quantitated on a C2 reversed phase column (4.6 × 250mm, 5 μm), using a mobile phase composed of acetate buffer-acetonitrile (15:85v/v) delivered at a flow rate of 1.0 mL min^{-1} and with UV detection (240 nm). The method was proven to be linear over amiodarone hydrochloride concentration range of 12.5-75 μg mL^{-1}. The mean correlation coefficient was 0.9999. hydrochloride.

Thin layer chromatography (TLC) and High performance thin layer chromatography (HPTLC)

Pang et al.[24] dissolved pulverized capsule contents or tablets [equivalent to ~0.2 g of amiodarone HCl] in 10 mL of ethanol, filtered and the filtrate diluted to 25 mL with ethanol. A 20 μL portion of the solution was applied to a Silica gel GF254 plate for thin layer chromatography with $CHCl_3$-ethanol-diethylamine (600:400:15) as mobile phase. The required band on the chromatogram was isolated, eluted with ethanol and diluted with H_2O to 50 mL. 1 mL of the solution was mixed with 1.5 mL of 1.6% sodium dodecylsulfate, 2 mL of Na_2HPO_4-NaOH buffer of pH 9.6 and H_2O up to 25 mL. After agitation for ≤ 5 min. and standing for 2 min., the fluorescence intensity was measured at 510 nm (excitation at 400 nm). The calibration graph was rectilinear for 1 nmol L^{-1} to 1 μmol L^{-1} amiodarone HCl, with detection limit of 0.2 nmol L^{-1} and recovery 96.2% with coefficient of variation of 1.83%.

In a method reported by Yang et al.[25], serum (1 mL) was shaken with 2 mL phosphate buffer of pH 6 and hexane for 2 min., the organic extract was evaporated to dryness. The residue was dissolved in $CHCl_3$ and a solution of flurazepam (internal standard, 0.125 mg mL^{-1}) was added before applying to a silica gel 60 F254 plate for HPTLC with ethyl acetate-$CHCl_3$-acetone-aqueous NH_3 (20:5:5:1) as mobile phase. Amiodarone was determined by using a Shimadzu CS-930 thin-layer scanner with measurement at 240 nm (reference at 330 nm). Recoveries were > 97%, within- and between-day RSD were 1.2-3.4 and 1.6-7.5%, respectively. Co-administrable drugs, such as phenobarbital, diazepam and chlorpromazine did not interfere.

Other chromatographic methods

Powdered tablets equivalent to 400 mg amiodarone HCl were dissolved in 75 mL mobile phase by sonicating for 2 min. and mixing for 30 min. The solution was made up to 100 mL with the mobile phase, mixed and filtered through a nylon 6,6 filter. A 10 µL portion of this solution or the raw material solution (4 mg mL^{-1} in mobile phase) was analyzed by liquid chromatography (LC) on a 3 µm nitrile (Hypersil) column (15 cm x 4.6 mm) with acetonitrile-0.1 mol L^{-1} ammonium acetate buffer (1:1) adjusted to pH 6 with 0.1 mol L^{-1} acetic acid as mobile phase (1 mL min^{-1}) with detection at 240 nm. The lower limit of quantitation of the related compounds was 0.02% or less. Impurity levels in two samples of raw material were ~0.4%. Confirmation was done by LC-MS and the method was evaluated by a second laboratory and found to be satisfactory[26].

Deng et al.[27] reported a new method for the determination of amiodarone HCl in tablet by non-aqueous capillary electrophoresis. The effects of some important factors such as the acidity, non-aqueous media, and concentration of running buffer, separation voltage, injection time and detection potential were investigated. Ethanol was used as aqueous, 30 mmol L^{-1} Tris-15 mmol L^{-1} Citrate (pH 6.90) was used as the running buffer. The separation voltage and injection time were 18 kV and 8 seconds, respectively. Under these selected conditions, the detection limit was 0.5 mg L^{-1}, and the linear range was 5.0-200 mg L^{-1}, y (peak area) = 74.94 rho-7.83, r (correlation coefficient) = 0.999. The recovery of the sample was 99.0±1.6%. The method was used for the determination of amiodarone HCl in preparations.

A universal method for quantitation of anionic substances in active pharmaceutical ingredients during early development using ion chromatography was developed by Cassidy et al.[28]. The method was developed to allow rapid characterization of active pharmaceutical ingredients in support of early clinical studies. The method parameters were chosen to allow quantitation of monovalent, divalent and trivalent inorganic ions as well as monovalent and divalent carboxylic acids. These parameters were also chosen to ensure appropriate performance for regulated analyses using less than 10 mg of active pharmaceutical ingredients per replicate. The method was applied to and validated for a range of anionic analytes in active pharmaceutical ingredients of varying hydrophobicity to demonstrate applicability to various analyses encountered during early development of pharmaceuticals.

Miscellaneous methods

Steinle et al.[29] described a synthetic micropore and nanotube membranes that mimic the function of a ligand-gated ion channel, that is, these membranes can be switched from an

"off" state (no or low ion current through the membrane) to an "on" state (higher ion current) in response to the presence of a chemical stimulus. Ion channel mimics based on both microporous alumina and gold nanotube membranes were investigated. The off state was obtained by making the membranes hydrophobic by chemisorbing either a C18 silane (alumina membrane) or a C18 thiol (Au nanotube membrane). Water and electrolyte are forbidden from entering these very hydrophobic pores-nanotubes. The transition to the on state was induced by the partitioning of a hydrophobic ionic species (such as drug or surfactant) into the membrane. The membrane switched to the on state because at the sufficiently high concentration of this ionic analyte species, the pores-nanotubes flood with water and electrolyte. A pH-responsive membrane was also prepared by attaching a hydrophobic alkyl carboxylic acid silane to the alumina membrane.

Biological Media (Biological Samples)

Biological media such as serum, plasma, tissue and urine are very complex. They often contain proteins, salts, acids, bases, and numerous organic compounds which can interfere with the analytes of interest, so the sample must be prepared before analysis. Sample preparation is usually performed by solid-phase extraction (SPE) or liquid–liquid extraction (LLE), There is, however, an increasing demand for simple, rapid, and cost-effective analytical methods capable of achieving very low detection limits. Methods for the measurement of biological amiodarone and its metabolite desethylamiodarone concentrations included spectral methods, high performance liquid chromarography (HPLC), thin layer chromatography (TLC), electrodriven methods (electrophoretic methods) and other miscellaneous methods.

Spectral methods

Ball et al.[30] developed a conductometric titration and spectroscopic methods to examine the interaction of the cardiac antiarrhythmic agent amiodarone (cordarone) with small molecules of biological importance, including iodine, coenzyme Q0, beta-NAD, (-)-epinephrine, serotonin HCl and dopamine HCl. Titration and reverse-titration conductivity measurements were made at 37 °C with acetonitrile or H_2O as a solvent. The electronic absorption spectra of amiodarone/iodine solution in $CHCl_3$ at 30 °C showed a maximum at 422 nm which was assigned to a charge-transfer interaction. Amiodarone also interacted with the other biological molecules which acted as electron acceptors. The ability of amiodarone

to behave as an electron donor is discussed in terms of the pharmacochemical properties of the drug.

Chromatographic methods

High performance liquid chromatography with UV detection (HPLC-UV)

A new chromatographic method was established by Duranti et al.[31] where, to plasma (0.1 mL) were added 0.2 mol L^{-1}acetate buffer (pH 3.8) and 4-[3-(dimethylamino)propoxy]-3,5-di-iodophenyl 2-ethylbenzofuran-3-yl ketone as internal standard and the solution was extracted twice with ethyl ether. The combined organic phases were evaporated to dryness and the residue was dissolved in the mobile phase for HPLC on a column (25 cm x 4 mm) of LiChrosorb Si-100 with methanol-ethyl ether (7:3) containing 0.02% of $HClO_4$ as mobile phase (2 mL min^{-1}) and detection at 242 nm. Calibration graphs were rectilinear from 0.02 to 5.0 μg mL^{-1}. Separation was achieved within 6 min. Recoveries were 96.7 and 98.3% for 0.25 and 1.00 μg mL^{-1} of amiodarone, respectively. The coefficients of variation were 3.02 to 4.62% (n = 10).

Amato et al.[32] suggested a new method for the determination of amiodarone using the extracts of capsules in the initial mobile phase and analyzed it on a column (30 cm x 4.6 mm) of Varian Micropak MCH-10 with dipyridamole as internal standard and detection at 254 nm. Elution was effected at 2 mL min^{-1} with acetonitrile-5 mmol L^{-1}-$NaClO_4$ (1:1) at pH 3.0 (adjusted with $HClO_4$) for 2 min, then the concentration of acetonitrile was increased to 85% in 10 min. and kept at this value. The calibration graph was rectilinear for up to 2 μg of amiodarone on the column and the limit of detection was ~2 ng. For 0.25 to 2 μg of the drug, the coefficient of variation ranged from 1.50 to 1.70% (n=10). The method was also applicable to biological fluids.

Storey et al.[33] described a new method for amiodarone determination where tissue samples (20 to 100 mg) were digested with a proteolytic enzyme (or lipase for fat tissue). After the addition of an internal standard, amiodarone and its metabolite were extracted into t-butylmethyl ether for analysis by HPLC on a steel column (12.5 cm x 5 mm) packed with Spherisorb 5 silica with methanol-2,2,4-trimethylpentane-t-butylmethyl ether (8:1:1) containing 6 mmol L^{-1} KBr [or, for rabbit tissues, methanol-ethyl ether (17:3) containing 0.02% of $HClO_4$] as mobile phase (2 mL min^{-1}) and 240 nm detection. The calibration graph was rectilinear up to 3 μg mL^{-1} and recovery was 98.6%. The limit of sensitivity for both compounds was 0.1 mg kg^{-1} (wet wt.) in tissue.

A method for amiodarone and its desethyl metabolite determination was studied by Shipe et al.[34] by treatment of plasma (0.5 mL) with NaH_2PO_4 (pH 6.0) and extraction with t-butylmethyl ether containing 2-ethyl-3-[3,5-dibromo-4-(3-dipropylamino)propoxybenzoyl]-benzothiophene (as internal standard). The organic phase was evaporated to dryness and the residue was dissolved in acetonitrile then analyzed by HPLC at 50 °C on a column of C18 DB (Supelco) with acetonitrile-10 mmol L^{-1} KH_2PO_4 (containing 20 mmol L^{-1} tetramethyl-ammonium chloride)-85% H_3PO_4 (90:9:1) as mobile phase and 254 nm detection. Recoveries of amiodarone and its desethyl metabolite were 97 and 102%, respectively. The calibration graph was rectilinear from 0.5 to 5.0 mg L^{-1}.

Amiodarone, its N-desethyl metabolite and prazepam (internal standard) were extracted from plasma into pentane (two portions) for determination using HPLC on a column (15 cm x 4.6 mm) of Ultrasphere-octyl with mobile phase (1.2 mL min^{-1}), acetonitrile-H_2O-$HClO_4$ (700 g L^{-1})-tetramethylammonium hydroxide (230 g L^{-1} in methanol) (150:50:0.1:0.1) and 240 nm detection. The calibration graphs were rectilinear over the range 0.1 to 10 mg L^{-1}. Within and between batch coefficients of variation were < 5% for 1 mg L^{-1} of amiodarone or its metabolite and recovery values ranged from 85 to 90 %.[35]

A method developed by Weir et al.[36] where plasma was mixed with the internal standard of 2-ethyl-3-[3,5-dibromo-4-(3-dipropyl-aminopropoxy)benzoyl]benzo[b]thiophene in acetonitrile and centrifuged, the supernatant solution was analyzed by HPLC. Urine or bile was acidified with concentrated HCl and mixed with 2,2-dimethoxypropane, cooled at 10 °C for 4 hours and centrifuged, the supernatant solution was evaporated to dryness and the residue was dissolved in acetonitrile containing 2-ethyl-3-[3,5-dibromo-4-(3-dipropylaminopropoxy)benzoyl] benzo[b]thiophene and analyzed by HPLC. Analysis was achieved on a column (30 cm x 3.9 mm) of reversed-phase C18 (10 μm) and a pre-column (5 cm x 3.2 mm) of reversed-phase C18 (30 to 38 μm) with a mobile phase (1.5 mL min^{-1}) of methanol-H_2O-aqueous 58% NH_3 (47:2:1) and detection at 244 nm. The calibration graphs were rectilinear for 0.1 to 10 μg mL^{-1} of amiodarone and its N-deethyl metabolite in plasma, for 1 to 100 μg mL^{-1} of amiodarone or N-deethyl metabolite in urine and bile. Recoveries of amiodarone and N-de-ethyl metabolite were >= 91.8% for plasma and urine and bile, were 80 and 90%, respectively, for amiodarone and 60 to 95%, respectively, for N-deethyl metabolite.

Muir et al.[37] described a new method in which serum or tear samples were buffered to pH 5.5 and extracted with hexane. The organic phase was evaporated to dryness and the residue was dissolved in methanol for analysis by HPLC on a column (15 cm x 4.6 mm) of

Ultrasphere octyl (5 μm) with a mobile phase (2 mL min^{-1}) of 0.01mol L^{-1} ammonium acetate (pH 4)-methanolic 50% acetonitrile (1:9) with detection at 254 nm. Rectilinear calibration graphs were obtained for 50 ng mL^{-1}-10 μg mL^{-1} ranges of amiodarone and its metabolite, and limit of detection 20 ng mL^{-1}.

A chromatographic method in which the serum sample was mixed with 2-ethyl-3-[3,5-dibromo-4-(3-dipropylaminopropoxy)benzoyl]benzothiophene as internal standard then extracted with isopropyl ether-butanol (7:3). The organic phase was analyzed by HPLC on a column (15 cm x 4.6 mm) of Ultrasphere Cyano (5 μm) fitted with a pre-column (4.5 cm x 4.6 mm) of the same material, the mobile phase (1.5 mL min^{-1}) was acetonitrile-tetrahydrofuran–50 mmol L^{-1} ammonium formate of pH 4 (7:1:2) and detection at 242 nm. The coefficient of variation for amiodarone amounted to 2.95 or 1.36% at 5 or 0.5 mg L^{-1}, respectively (n = 10), that for its desethyl metabolite at 2.5 or 0.25 mg L^{-1} was 2.33 or 1.24%, respectively. Recovery of amiodarone was 93%. Amiodarone, desethyl metabolite, verapamil, propafenone and diltiazem can be separated under the same conditions or with the ammonium formate concentration reduced to 25 mmol L^{-1}.[38]

A chromatographic method was described by Yuan et al.[39] Plasma (0.5 mL) was treated with 2 μL of methanolic diazepam (49.7 μg mL^{-1}) as internal standard and 3 mL of methanol-$CHCl_3$ (9:1). After centrifugation the supernatant solution was heated to 80 ^oC to produce a dry residue which was dissolved in 1 mL of the mobile phase [$CHCl_3$-methanol-concentrated aqueous NH_3 (1987:12:1)]. A portion (50 μL) was analyzed by HPLC on a column (25 cm x 5 mm) of YWG 80-5 silica gel (in a stainless-steel tube) with a mobile-phase flow rate of 1 mL min^{-1} and detection at 254 nm. The calibration graph is rectilinear for 0.2 to 10 μg mL^{-1} of amiodarone with recovery of ~95%.

Pollak et al.[40] developed a method in which amiodarone and its desethyl metabolite were extracted from 100 μL serum samples on cyano-bonded silica minicolumns (J. T. Baker) with methanol containing triethylamine (10 ml L^{-1}) as mobile phase. The eluate was evaporated to dryness then the residue was dissolved in 100 μL of acetonitrile-10 mmol L^{-1} phosphate buffer of pH 3.5 (2:3) and aliquots were analyzed by HPLC on a column (15 cm x 3.2 mm) of Spherisorb-Octyl (5 μm) with mobile phase (0.9 mL min^{-1}), acetonitrile-10 mmol L^{-1} phosphate buffer of pH 3.5 (31:19) containing 0.5 mL of triethylamine, detection was at 254 nm 3-(3,5-dibromo-4-dipropyl-aminopropoxy-benzoyl)-2-ethylbenzo[b]-thiophene was used as internal standard. Within-run coefficient of variation were 2.7 to 4.5% for amiodarone and 4.0 to 5.7% for desethyl metabolite, between-run coefficients were 8.3 and

5.7% respectively. Commonly used cardiovascular drugs did not interfere. Recovery was 85 to 89%.

A chromatographic method was reported by Susanto et al.[41] plasma (1 mL) was mixed with 2 μg of 3-[3,5-dibromo-4-[3-(dipropylamino)propoxy]benzoyl]-2-ethylbenzo[b]thiophene (L8040) as internal standard and 2 mL of citrate-HCl buffer (pH 4) and the mixture was passed through a Sep-Pak silica cartridge. Amiodarone and desethylamiodarone were eluted with 5 mL of isopropyl ether, the eluate was evaporated and the residue was dissolved in 100 μL of HPLC mobile phase. Portions (10 to 30 μL) were separated on an HPLC column of Nucleosil C18 (10 μm) with aqueous 0.05% H_3PO_4-acetonitrile (1:1) as mobile phase (2 mL min^{-1}) and detection at 210 nm. The recovery values of amiodarone and desethylamiodarone were >80% and calibration graphs were rectilinear over the concentration range 0.1 to 5.0 μg mL^{-1}. The limit of determination was 50 ng mL^{-1} in plasma. The coefficient of variation for standards amounts to 2.9- 4.1% (n = 10) and for human plasma 4.2-9.0% (n = 5). The day-to-day precision ranged from 2.9 to 3.8%.

10% $ZnSO_4$ solution and acetonitrile were added to the sample containing amiodarone in a method studied by Lam et al.[42]. After mixing and centrifugation, the supernatant solution was analyzed by HPLC on a column (15 cm x 4.2 mm) of Spherosil C18 (5 μm) with acetonitrile-H_2O-H_3PO_4-diethylamine (1600:400:2:1) as mobile phase (3 mL min^{-1}) and detection at 254 nm. Rectilinear calibration graphs were obtained for 0.5 to 5 μg mL^{-1} of amiodarone and 0.25 to 2.5 μg mL^{-1} of the desethyl-metabolite.

By using HPLC Bliss et al.[43] described a new method for amiodarone determination where amiodarone was extracted from the sample at pH 5 with hexane, whereas the metabolite was extracted without prior acidification of the sample. The extract was evaporated to dryness and the residue was dissolved in methanol for HPLC analysis on a column (25 cm x 4.6 mm) of Ultrasphere Silicon (5 μm) with methanol-ethyl ether (4:1) containing 12 to 15 μL L^{-1} of triethylamine as mobile phase (2.2 mL min^{-1}) and detected at 254 nm. Rectilinear calibration graphs were obtained for up to 5 mg L^{-1} of amiodarone.

Amiodarone, de-ethylamiodarone and fenethazine (internal standard) were extracted from samples with 1-chlorobutane[44]. The organic phase was evaporated; the residue was dissolved in acetonitrile. Amiodarone and de-ethylamiodarone were determined by HPLC on a column (25 cm x 4.5 mm) of Apex nitrile (5 μm) with acetonitrile-0.02 mol L^{-1} phosphate buffer of pH 3 (3:2) as mobile phase (2 mL min^{-1}) and detection at 238 nm. Calibration graphs were rectilinear for 0.1 to 8 mg L^{-1} of amiodarone and de-ethylamiodarone. The

detection limits were 0.02 and 0.03 mg L^{-1} for amiodarone and de-ethylamiodarone, respectively, and recoveries of 93 and 91%.

Kannan et al.[45] described a new HPLC method where fenethazine (internal standard) and phosphate buffer solution (pH 4.5) were added to 0.2 mL of serum or 0.2 mL of a solution of reconstituted tissue homogenate (20 to 25 mg wet wt.) in control serum and the mixture was extracted with t-butylmethyl ether. The extract was evaporated under nitrogen and a solution of the residue in methanol was analyzed by HPLC on a column (25 cm x 4.6 mm) of Ultrasphere Si (5 μm) with a mobile phase (0.9 mL min^{-1}) of methanol containing 0.02% of $HClO_4$ (adjusted to pH 4.0 with methanolic 0.1 mol L^{-1} NaOH) and detection at 240 nm. The calibration graphs for amiodarone and de-ethylamiodarone were rectilinear for concentration in serum from 0.025 to 6.0 μg mL^{-1} and in tissues from 0.1 to 0.5 μg mL^{-1} with a detection limit of 0.01 μg mL^{-1} for serum, the within-run and between-run coefficients of variation were 2.1±0.7 and 6.8±1.1%, respectively. Results of pharmacokinetic and disposition studies were reported.

Plasma or serum samples (0.5 mL) were mixed with L8040 3,5-dibromo-4-[3-(dipropylamino)propoxy]phenyl-2-ethylbenzothiophen-3-yl ketone as internal standard and phosphate buffer (pH 7), the mixture was extracted with hexane[46]. The extract was evaporated, the dried residue dissolved in the mobile phase and a portion of the solution injected on to a column (15 cm x 4.6 mm) of Econosphere CN (5 μm). The mobile phase (1 mL min^{-1}) was hexane-propan-2-ol (9:11) containing 0.06% of concentrated H_2SO_4 and detection at 242 nm. The limit of determination was 0.2 μg mL^{-1} for both amiodarone and de-ethylamiodarone and recoveries 69.6 and 55.5%, respectively. The method allowed the determination of amiodarone and de-ethylamiodarone at levels of clinical significance (i.e., 0.2 to 4 μg mL^{-1}).

Ress et al.[47] reported a method in which 1 mL serum, 2 mL CH_2Cl_2, 0.1 mL 1 mol L^{-1} HCl and 0.1 mL methanolic 3-3,5-dibromo-4-[3-(dipropyl-amino)propoxy]benzoyl-2-ethylbenzothiophene (as internal standard) were mixed. After centrifuging, the organic extract was evaporated to dryness under nitrogen and then the residue dissolved in methanol (0.5 mL). An aliquot (0.1 mL) was analyzed by HPLC on a Shandon ODS column (12.5 cm x 4.6 mm) with 242 nm detection, the mobile phase (1 mL min^{-1}) consisted of aqueous 75% acetonitrile containing 0.25% of dibutylammonium phosphate, 0.313% of octane-1-sulphonic acid and 0.025% of triethylamine. Response was rectilinear for 0.1 to 4 μg mL^{-1} of amiodarone and its mono-N-de-ethyl metabolite, recoveries were > 90%. Advantages over

previously reported extraction methods were discussed. No interference from other cardiovascular substances was observed.

Tissues were homogenized and serum was mixed with 67 mmol L^{-1} ammonium phosphate buffer (pH 5.4) containing [3,5-dibromo-4-(2-dipropylaminopropoxy)benzoyl]-benzothiophene (as internal standard) in a method developed by Menius et al.[48]. The mixtures were extracted with propan-2-ol-hexane (1:49) with centrifugation and the extraction was repeated. The combined extracts were evaporated, the residue dissolved in methanol, the solution was passed through a C18 Prep-Sep column and after washing with methanol, amiodarone, its metabolite and the internal standard were eluted with methanol-hexane-H_2O-aqueous NH_3 (1813:160:24:3) of pH 7.7 (mixture A). The eluate was evaporated, the residue dissolved in methanol and the solution analyzed by HPLC. The system comprised a column of Nova Pak-A C18 (5 μm) with methanol and mixture A as mobile phase (1.5 mL min^{-1}) and detected at 254 nm. Recovery of amiodarone was > 90% from serum and tissue, response was rectilinear within the therapeutic range and the limit of detection was 40 ng mL^{-1}. No common cardiovascular drugs interfered.

Petrarulo et al.[49] used a sample of biological fluid and applied it to a 3 mL column containing 500 mg of 40 μm C18 silica and pre-washed with 1 mL each of methanol and phosphate buffer solution of pH 7.4. The column was washed with 0.5 ml of methanol before elution of amiodarone and its de-ethyl metabolite with a further 1.5 mL of methanol. The eluate was evaporated and a solution of the residue in 0.5 mL of methanol was analyzed by HPLC on a column of micro Bondapak C18 (10 μm) fitted with a Supelguard LC-18-DB pre-column of Supelcosil (5 μm). Methanol containing 15 μg mL^{-1} (v/v) of aqueous 30% NH_3 was used as mobile phase and detection at 254 nm. Amiodarone and its de-ethyl metabolite had retention times of 2.3 and 2.9 minutes, respectively. The calibration graph was rectilinear for 0.5 to 5 μg mL^{-1} of either analyte, the limit of determination was 0.2 μg mL^{-1}. Intra- and inter-assay coefficients of variation at 0.79 μg mL^{-1} in serum were, respectively, 6.3 and 8.2% for amiodarone and 7.6 and 9.8% for its de-ethyl metabolite (n = 5).

Mazzi et al.[50] extracted 500 μL serum with 250 μL of isopropyl ether-butanol (7:3) containing L-8040, viz, 3,5-dibromo-4-[3-(dipropylamino)propoxy]phenyl-2-ethylbenzo[b]thien-3-yl ketone hydrochloride and minaprine (1.6 or 0.8 and 0.8 or 0.4 μg mL^{-1}, respectively) as internal standards. The extract was centrifuged and an aliquot (50 μL) of the upper organic layer was analyzed by HPLC on a column (25 cm x 4.6 mm) of Ultrasphere cyano (5 μm) protected by a guard column (3 cm x 4.6 mm) of Spheri-5 cyano (5

μm) with acetonitrile-tetrahydrofurane-H_2O-2 mol L^{-1} ammonium formate (pH 4.0, 140:20:39:1) as mobile phase (1.5 mL min^{-1}). Detection was at 235 nm for verapamil, amitriptyline, nortriptyline, haloperidol and diltiazem and 248 nm for imipramine, desipramine, clomipramine, propafenone, amiodarone and de-ethylamiodarone. Within- and between-run coefficients of variation for each drug were tabulated.

A chromatographic method for amiodarone determination was established by Smet et al.[51] where plasma (1 mL) was mixed with 3,5-dibromo-4-(3-(dipropylamino)-propoxy]-phenyl-2-ethylbenzo[b]thiophen-3-yl ketone (internal standard) solution (100 μL) and deproteinized with acetonitrile (washing solution, 2 mL). After centrifugation, the precipitate was washed with the mixture and its combined washings were evaporated under nitrogen at 60 ^oC. Phosphate buffer solution (pH 3, 10 mL) containing 0.05 mol L^{-1} sodium octyl sulphate was added and the mixture extracted with $CHCl_3$-hexane (3:2, 5 mL) for 30 min. After centrifugation, the organic phase was evaporated at 30 degree under nitrogen and the residue was dissolved in washing mixture (200 μL). A 50 μL aliquot was analyzed by HPLC on a column (25 cm x 4 mm) of LiChrosorb CN (5 μm) with aqueous 56% washing solution containing 0.01% of propylamine as mobile phase (1 mL min^{-1}) and detection at 254 nm. Optimization of the mobile phase was discussed. Recoveries (n=6) of 2 μg mL^{-1} of amiodarone and deethylamiodarone were 78.5 and 79.8% with coefficient of variation of 3.2 and 2.8%, respectively, and detection limits 0.02 μg mL^{-1}. Within-day coefficients of variation (n=6) for 1 and 5 μg mL^{-1} of amiodarone were 3.5 and 3.2%, and corresponding values for deethylamiodarone 6.2 and 4.1%.

Petrarulo et al.[52] reported a method for amiodarone and its de-ethyl metabolite determination where serum (0.5 mL) was cleaned up on a column of silica C18 (40 μm) with methanol (3 x 1.5 mL) as an eluent. The eluate was evaporated at 37 ^oC under nitrogen and the residue was dissolved in 500 μL of methanol and analyzed by HPLC on a column (25 cm x 4.6 mm) of micro Bondapak C18 (10 μm) with a guard column of Supelguard LC-18-DB (5 μm), a mobile phase of methanol containing 15 μg mL^{-1} of aqueous 30% NH_3, and detection at 254 nm. Calibration graphs were rectilinear from 0.2 (detection limit) to 5 μg mL^{-1} of amiodarone and its de-ethyl metabolite. Recoveries were 75-92% and 94-104% of amiodarone and de-ethyl metabolite, respectively.

Moor et al.[53] used a heparinized plasma and mixed it with 2 mL of acetonitrile, sonicated and centrifuged and the pellet re-extracted with acetonitrile. Adipose tissue was homogenized in hexane, the mixture was centrifuged and the clear supernatant solution was

extracted with 1 mol L^{-1} HCl in methanol. Other tissues were homogenized in 1% of 1 mol L^{-1} HCl in methanol, centrifuged and extracted with the same mixture. The internal standard [3,5-dibromo-4-(3-(dipropylamino)propoxy]phenyl-2-ethylbenzo[b]thien-3-yl ketone (L 8040, Sanofi, Montpellier, France)) was added to each extract and the mixtures were applied to disposable RP-8 LD columns conditioned with 1% of 1 mol L^{-1} HCl in methanol followed by aqueous 80% methanol. After washing the column with the latter solution, amiodarone, de-ethylamiodarone and the internal standard were eluted with the former solution then the eluate was evaporated under nitrogen. The residue was dissolved in the mobile phase (methanol containing 1.5% of H_2O and 0.7% of aqueous 33% NH_3). A portion was analyzed by HPLC on a column (15 cm x 4 mm) of Spherisorb ODS-2 (3 μm) with a guard column (4 cm x 4.6 mm) of Perisorb RP-18 (30 μm). The flow rate of the mobile phase was 1.3 mL min^{-1} and detection was at 240 nm. Recoveries were almost quantitative and within- and between-day coefficients of variation were 3.5% (n=4) and 5.5% (n=3), respectively.

Serum or plasma (1 mL) was mixed with methanolic [3,5-dibromo-4-(3-(dipropyl-amino)propoxy]phenyl-2-ethylbenzo[b]thien-3-yl ketone hydrochloride (internal standard) and extracted with isopropyl ether (4 mL) [54]. The mixture was frozen at 14 °C, the organic phase was removed and the aqueous phase was adjusted to pH 5.6 with 5 μL of concentrated HCl and re-extracted. The combined extracts were evaporated and the residue dissolved in 0.1 mL of the mobile phase, viz, methanol-H_2O-aqueous 25% NH_3 (963:30:7), for HPLC (injection volume 50 μL) on a LiChrosorb RP-8 (7 μm) column (25 cm x 4 mm) with a guard column (3 cm x 4.6 mm) of C8 material (5 μm), a flow rate of 1 mL min^{-1} and detection at 254 nm, with recoveries of 98.5% for amiodarone and 95% for its metabolite. The calibration graph was rectilinear for 0.03 to 3 μg mL^{-1} of either compound and the coefficients of variation (n=5) were 6 and 5% for amiodarone and its metabolite, respectively, at 0.1 μg mL^{-1} and 3 and 2%, respectively, for 0.1 μg mL^{-1}.

Serum (500 μL) was mixed with L 8040 (3,5-dibromo-4-[3-(dipropyl-amino)-propoxy]phenyl-2-ethylbenzo[b]thien-3-yl-ketone hydrochloride (internal standard) solution (200 μL, 500 μg L^{-1}) and 2 mol L^{-1} phosphate buffer (pH 4.6, 100 μL). The solution was extracted with di-isopropyl ether (2 x 3 mL), the organic phase was evaporated to dryness under nitrogen and the residue was dissolved in mobile phase (200 μL). Aliquots (20 μL) of solutions were analyzed by HPLC on a column (15 cm x 3.2 mm) of Ultrasphere C18 (5 μm) with methanol-aqueous 25% NH_3 (993:7) as mobile phase (2 mL min^{-1}) and detection at 242 nm. The within-day coefficient of variation (n=12) was 3.9-5.1% for amiodarone and 2.9 to

9.7% for de-ethylamiodarone. Between-run coefficient of variation was 10.4 and 9.4% at 476 μg L^{-1} of amiodarone and 495 μg L^{-1} of de-ethylamiodarone, respectively.[55]

Ou et al.[56] developed a new chromatographic method in which serum (250 μL) was vortex-mixed for 30 s with 100 μL of 0.36 mol L^{-1} NaH_2PO_4 buffer (pH 4.35), 100 μL of internal standard, viz, 3,5-dibromo-4-[3-(dipropylamino)propoxy]phenyl-2-ethylbenzo[b] thien-3-yl ketone (L 8040, Sanofi, Montpellier, France) and 200 μL of isopropyl ether and the mixture was centrifuged at 3000 g for 3 min. A 50 μL portion of the organic layer was analyzed by HPLC on a column (15 cm x 3.9 mm) of Resolve Spherical Silica (5 μm) with a mobile phase (1.8 mL min^{-1}) of methanol-17 mmol L^{-1} ammonium sulphate buffer of pH 6.8 (23:2), and detection at 254 nm. 0.1 to 20 mg L^{-1} of amiodarone and de-ethylamiodarone could be determined. Day-to-day and within-run coefficients of variation were 7 and 3% respectively, for amiodarone and 8 and 5%, respectively, for de-ethylamiodarone. Recoveries were 100% for amiodarone and 73% for de-ethylamiodarone. Results were correlated well ($r \geq 0.95$) with those of previously described HPLC method.

Costa et al.[57] added a sample of plasma or serum-based standard (100 μL) to 200 μL of acetonitrile containing 0.75 μg mL^{-1} of fluopromazine as internal standard. After mixing for 30 s and centrifugation at 3000 g for 2 min, 20 μL of the supernatant solution was injected into a column (25 cm x 4 mm) of Nucleosil Octyl (5 μm) fitted with a guard column of Nucleosil Octyl (10 μm). The mobile phase (2.0 mL min^{-1}) was acetonitrile-0.01 mol L^{-1} sodium acetate (pH 3.3)-triethylamine (840:160:1) and detection at 242 nm (peak-area ratios were used for quantitation). The detection limit for amiodarone and its metabolite (well separated from each other and from the internal standard) was 50 ng mL^{-1}. The inter-day coefficient of variation at 0.75 and 1.5 μg mL^{-1} was 2.3 and 2.5%, respectively, for amiodarone and 2.0 and 2.2%, respectively, for the metabolite and recoveries of both compounds were quantitative.

Serum, plasma or standard solution (250 μL) was mixed with 2-ethyl-3-(3,5-dibromo-4-dipropylaminepropoxybenzoyl)benzothiophene solution (internal standard, 10 μL) and 0.2 mol L^{-1} acetic acid (800 μL) in a method studied by Lensmeyer et al.[58]. The mixture was centrifuged (12,500 g for 3 min.) and the supernatant solution was applied to the microfilter unit containing the Empore solid-phase extraction membrane which had been primed with methanol and 0.2 mol L^{-1} acetic acid. Sample solution was drawn through the membrane by centrifugation at 85 g for 10 min. The membrane was washed with aqueous 20% acetonitrile (250 μL) with centrifugation for 5 min. The drugs were eluted with mobile phase (400 μL)

with centrifugation for 5 min. A 50 µL portion of the eluate was analyzed by HPLC on a column (15 cm x 4.6 mm) of Zorbax octyl (5 to 6 µm) at 55°C protected by a guard column (2 cm x 2 mm) of Permaphase ETH (30 µm), with H_2O-acetonitrile-tetrahydrofuran-acetic acid-butylamine (1000:9000:200:3:4) as mobile phase (1.5 mL min^{-1}) and detected at 240 nm. Recoveries were 82-86 and 88-93% of amiodarone and de-ethylamiodarone, respectively. Calibration graphs were rectilinear from 0.05-6.0 mg L^{-1} of amiodarone and de-ethylamiodarone and between-run coefficient of variation 3.1-6.4%.

Amiodarone and its N-ethyl metabolite were analyzed by Trivier et al.[59] using HPLC on a column (15 cm x 4.6 mm) of ODS Hypersil C18 (5 µm), methanol-H_2O-58% NH_4OH (44:5:1) as mobile phase (0.8 mL min^{-1}) and detection at 242 nm. There were no endogenous interferences provided manipulations were carried out with exclusion of light (to prevent deiodination). Calibration graphs were rectilinear up to 12 µmol L^{-1} of N-ethyl metabolite with a detection limit of 0.05 µmol L^{-1}. For a sample containing 1.84 µmol L^{-1} of N-ethyl metabolite of amiodarone, the intra- and inter-day coefficients of variation (n=3) were 7% and 3.3%, respectively. The method has been used to study dealkylation of amiodarone in rat liver microsomes. These samples require only deproteinization with acetonitrile and centrifugation before analysis.

Serum, control or standard solution (0.5 mL) was mixed with 2 mol L^{-1} sodium acetate buffer soution (pH 4.5, 0.3 mL) and internal standard solution (trifluprom azine, 10 µL)[60]. The mixture was applied to a Phenomenex C2 extraction column pre-treated with methanol and H_2O. The column was washed with H_2O (1.0 mL), 50% methanol (1.0 mL) and 50% acetonitrile (1.0 mL) and eluted with HPLC mobile phase (0.5 mL). Analysis was by HPLC on a column (15 cm x 4.6 mm) of C18 UltraCarb 5 (5 µm) protected by a C18 guard column (2 cm x 2 mm) and operated at 40°C with 30 mmol L^{-1} ammonium acetate (pH 4.0)-methanolic 50% acetonitrile as mobile phase (1.5 mL min^{-1}), and detection at 242 nm. The calibration graph was rectilinear up to 20 µg mL^{-1} of amiodarone and desethylamiodarone. Intra- and inter-assay coefficients of variation ranged from 2.9 to 5.0% and 6.1-9.6% for amiodarone and 3.3-7.1% and 6.2-7.8% for desethylamiodarone, respectively. Detection limits for amiodarone and desethylamiodarone were 0.16 µg mL^{-1} and recoveries ranged from 93.4-101.4%. Haemoglobin, bilirubin and lipaemia did not interfere.

A high performance liquid chromatographic method was developed by Al-Dhawailie[61] for the determination of amiodarone in plasma and tissues. Plasma (0.1 mL) was vortex-mixed with 0.2 mL (40 µg mL^{-1}) N-cetylpyridinium chloride monohydrate (internal standard)

in acetonitrile for 30 s. The mixture was centrifuged at 2000 rpm for 5 min. and the supernatant was decanted. Tissue (100 mg) was homogenized with 1 mL 50% ethanol for 10 min, 2 mL internal standard was added and the mixture was vortexed for 30 s. The mixture was centrifuged at 3000 rpm for 5 min and the supernatant decanted. Portions (25 μL) of supernatant from each extract were analyzed by HPLC on a 10 μm micro-Bondapak C18 column (15 cm x 3.9 mm) with 10 mmol L^{-1} sodium dihydrogenphosphate buffer-acetonitrile-methanol (2:7:1) of pH 4 as mobile phase (1 mL min^{-1}) then measurements were carried out at 242 nm. The retention times for the drug and internal standard were 6 and 11.5 min, respectively. The calibration graph was linear for 50-750 ng mL^{-1} of amiodarone and the detection limit 20 ng mL^{-1}. Intra- and inter-day RSD (n=6) were 1.6-8.5 and 2.2-9.5%, respectively, for 100 and 500 ng mL^{-1} of amiodarone added to blood and tissue samples. Recoveries were >90% at the same levels of added amiodarone to plasma and tissue samples.

Conditions were established for the determination of amiodarone, indomethacin and thiopentone in serum by Hannak et al.[62] using isocratic HPLC. The apparatus included a Merck Hitachi L-4500 diode-array detector, a LiChrospher 60 RP Select B (5 μm) column in a LiChroCART 125-4 cartridge operated at 25°C and a LiChrospher 60 RP Select B (5 μm) precolumn. Samples were mixed with acetonitrile containing an internal standard and centrifuged before analysis. Optimum mobile phases (1 mL min^{-1}) and detection wavelengths were: for amiodarone, 25 mmol L^{-1} triethylammonium phosphate buffer of pH 3-acetonitrile (1:9), 254 nm, for indomethacin, 25 mmol L^{-1} triethylammonium phosphate buffer of pH 3-acetonitrile (2:3), detection at 280 nm and for thiopentone, 25 mmol L^{-1} triethylammonium phosphate buffer of pH 3-acetonitrile (1:1), 283 nm. The respective internal standards were promazine, orphenadrine and thiosecbutobarbitone, responses were linear up to 20, 20 and 100 μg mL^{-1}, respectively, and the corresponding sensitivities were 0.09, 0.11 and 0.23 μg mL^{-1}. Analysis time was completed in ~5 min. Use of the two mobile-phase components combined in steps of 10% should permit most drugs to be determined.

A systematic comparison of potential alternatives to L8040 (now no longer produced) as internal standard for the determination of amiodarone and de-ethylamiodarone in serum was described by Pollak[63]. An extraction procedure using cyanobonded silica minicolumns (details given) was used to assess the ability of various candidates to co-extract with amiodarone. Analysis was on a 5 μm Zorbax cyano-bonded silica column (25 cm x 4.6 mm i.d.), 10 mmol L^{-1}-KH_2PO_4 of pH 3.5-methanol-acetonitrile (40:37:23) readjusted to pH 3.5 as mobile phase (0.8 mL min^{-1}) then detected at 241 nm. Tamoxifen was the most acceptable

alternative to L8040. Calibration graphs were linear from 0.25 mg L^{-1} (detection limit) to 8 mg L^{-1}. Within-run RSDs were 5.3% for amiodarone and 2.9% for de-ethylamiodarone; while, the corresponding between-run RSD were 4.5 and 1.6%. Absolute recoveries of amiodarone, de-ethylamiodarone and tamoxifen were 84, 79 and 97%, respectively.

Amiodarone and its metabolite desmethylamidarone were determined in serum by Kunicki and Sitkiewicz **(64)**. Serum (0.2 mL) was mixed with 20 µL L8040 (internal standard, 500 ng) and 100 µL 0.5 mol L^{-1} KH_2PO_4. Following centrifuging with 4 mL hexane, the organic phase was dried under argon at 37°C and the residue was dissolved in 100 µL methanol. For propafenone and its main metabolite 5-hydroxypropafenone, and mexiletine and diltiazem, 0.4 mL serum was mixed with 20 µL methanolic 2U41616 (200 ng, internal standard) and 50 µL of 10% Na_2CO_3. The solution was centrifuged with 4 mL di-isopropyl ether. The organic layer was treated with 100 µL 0.01 mol L^{-1} HCl, centrifuged and the aqueous layer was dried at 56°C under argon and dissolved in 100 µL mobile phase. Portions (50 µL) were analyzed on a 5 µm Supelcosil LC-CN column (20 cm x 4.6 mm i.d.) with acetonitrile-H_2O-0.5 mol L^{-1} KH_2PO_4 (18:31:1) as mobile phase (1.8 mL min^{-1}) and detection at 210 nm for propafenone, 5-hydroxypropafenone and mexiletine. For amiodarone and desmethylamidarone, methanol-acetonitrile-H_2O-0.5 M- KH_2PO_4 (60:17:45:3) was used as mobile phase (1.5 mL min^{-1}) with detection at 240 nm. Detection limits were 5 ng mL^{-1} for propafenone and mexiletine; 10 ng mL^{-1} for amiodarone, desmethylamidarone and 5-hydroxypropafenone. Relative standard deviation values were < 10%.

According to a Macherey-Nagel **(65)**, amiodarone and desethylamiodarone were determined in plasma by HPLC; sample was prepared by SPE. A 100 µL portion of the extract was analyzed on a NUCLEOSIL 100-5 C18 HD column (12.5 cm x 4 mm i.d.) with 30 mmol ammonium acetate-methanol-acetonitrile (24:6:70) as mobile phase (0.3 min, 0.8 mL min^{-1}, 3-8.5 min, 1.8 mL min^{-1} and 8.5 min, 0.8 mL min^{-1}) and detection at 255 nm. Triflupromazine was used as internal standard, and separation was achieved in ~8 min.

A selective and sensitive assay for amiodarone N-deethylation activity in human liver microsomes by HPLC with UV detection was reported by Hanioka et al.**(66)**. The extraction of deethylamiodarone from incubation samples was performed by means of an original solid phase extraction procedure using a polymeric reversed-phase sorbent (Oasis HLB). The method was validated for the determination of deethylamiodarone with respect to specificity, linearity, precision, accuracy, recovery, limit of quantification and stability. Amiodarone N-deethylation activity from low to high substrate concentrations using human liver

microsomes was precisely determined without a concentration step. This method is applicable to the study in vitro of the metabolism of amiodarone.

A rapid isocratic HPLC method for the simultaneous measurement of amiodarone and its potentially active metabolite N-desethylamiodarone was established by Juenke et al.[67]. Following a simple liquid-liquid extraction, amiodarone and its metabolite were quantitated (0.3-6.0 μg mL^{-1}) by analysis on an HPLC-UV system. The analytical time was reduced by 50% without compromising the assay performance, when Rocket trademark sign column technology was employed. The assay's limit of quantitation, linearity, imprecision and accuracy adequately covered the therapeutic range for appropriate patient monitoring. Amiodarone and N-desethylamiodarone can be simultaneously and accurately quantitated in serum or plasma by HPLC-UV detection with imprecision < 6% at therapeutic concentrations and a quantitation range 0.3-6.0 μg mL^{-1}.

Wang et al.[68] established a SPE-HPLC method for the determination of amiodarone in human serum with low dose treating arrhythmia. Amiodarone in serum was enriched with OSASISTM HLB Sep-Pak SPE column, purified by 70% methanol and extracted by chloroform, using ODS-C18 column, the mobile phase consisted of methanol-water-acetonitrile-tetraethylamine-acetic acid (100:65:100:1:0.8), at a flow rate of 1.2 mL min^{-1}. The column temperature was 45°C and N-(2-acetamido)iminodiacetic acid; and detection at 243 nm. The minimal detectable serum drug concentration was 40 ng mL^{-1}. The calibration curve was linear in the range of 0.1-3.2 μg mL^{-1} (r=0.9996). The intra-day and inter-day recoveries were 98.5-101.3 and 97.7-99.5% (n=5). Intra-day and inter-day RSD were less than 5% (n=5). The method is simple, sensitive and suitable for the determination of amiodarone in serum.

A method for the quantification of amiodarone and desethylamiodarone in animal plasma using high-performance liquid chromatography combined with UV detection (HPLC–UV) was presented by Baert et al.[69] The sample preparation included a simple deproteinization step with acetonitrile. In addition, a sensitive method for the quantification of amiodarone and desethylamiodarone in horse plasma and urine using high-performance liquid chromatography combined with electrospray ionization tandem mass spectrometry (HPLC–ESI–MS/MS) was described. The sample preparation included a solid-phase extraction (SPE) with a SCX column. Tamoxifen was used as an internal standard for both chromatographic methods. Chromatographic separation was achieved on an ODS Hypersil column using isocratic elution with 0.01% diethylamine and acetonitrile as mobile phase for

the HPLC–UV method and with 0.1% formic acid and acetonitrile as mobile phase for the LC–MS/MS method. For the HPLC–UV method, good linearity was observed in the range 0-5 μg mL^{-1} and in the range 0-1 μg mL^{-1} for the LC–MS/MS method. The limit of quantification was set at 50 and 5 ng mL^{-1} for HPLC–UV and LC–MS/MS methods, respectively. For the UV method, the limit of detection was 15 and 10 ng mL^{-1} for amiodarone and desethylamiodarone, respectively. The limit of quantifications of the LC–MS/MS method in plasma were much lower, i.e. 0.10 and 0.04 ng mL^{-1} for amiodarone and desethylamiodarone, respectively. The limit of detections obtained for the urine samples were 0.16 and 0.09 ng mL^{-1} for amiodarone and desethylamiodarone, respectively. The rapid HPLC–UV method was used for therapeutic drug monitoring after amiodarone treatment, while the LC–MS/MS method showed its applicability for single dose pharmacokinetic studies.

HPLC with other detections

A sensitive and specific HPLC-ESI-MS-MS method has been developed by Kollroser et al.[70] for the simultaneous determination of amiodarone and desethylamiodarone in human plasma. After the addition of the internal standard tamoxifen, plasma samples were extracted using Oasis R MCX SPE cartridges. The compounds were separated on a 5 mμ Symmetry C18 (Waters) column (15 cm x 3 mm i.d.) with a mobile phase of acetonitrile-0.1% formic acid (23:27, v/v) at a flow-rate of 0.5 mL min^{-1}. The overall extraction efficiency was more than 89% for both compounds. The assay was sensitive down to 1 μg L^{-1} for amiodarone and down to 0.5 μg L^{-1} for desethylamiodarone. Within-run accuracies for quality-control samples were between 95 and 108% of the target concentration with coefficients of variation < 8%. The proposed method enables the unambiguous identification and quantitation of amiodarone and desethylamiodarone in both clinical and forensic specimens.

A method was developed for the determination of amiodarone and desethylamiodarone by Ruiz et al.[71] using HPLC coupled with chemiluminescent detection. The procedure was based on the post-column photolysis of the analytes into photoproducts which are active in the tris(2,2′-bipyridyl)ruthenium(III) ($[Ru(bpy)_3]^{3+}$) CL system. $[Ru(bpy)_3]^{3+}$ was on-line generated by photo-oxidation of the Ru(II) complex in the presence of peroxydisulfate. The separation was carried out on a Mediterranea C18 column with isocratic elution using a mixture of methanol and 0.017 mol L^{-1} ammonium sulfate buffer of pH 6.8. Under the optimum conditions, analytical curves based on standard solutions were linear over the range

0.1-50 μg mL^{-1} for amiodarone and 0.5-25 μg mL^{-1} for desethylamiodarone. The detection limits of amiodarone and desethylamiodarone were 0.02 and 0.11 μg mL^{-1}, respectively. Intra- and inter-day precision values of 0.9% relative standard deviation (RSD) (n=10) and 1.6% RSD (n=15), respectively, were obtained. The method was applied successfully to the determination of these compounds in serum and pharmaceutical formulations.

Thin layer chromatography

Guo et al.[72] suggested a method in which serum (0.5 mL) was shaken with 0.25 mL of 0.5 mol L^{-1} phosphate buffer of pH 6, set aside for 2 min. then treated with 5 μL of a 1 mg mL^{-1} solution of tetracaine (internal standard) in 0.1 mol L^{-1} phosphate buffer of pH 6 and 1.5 mL of isopropyl ether with shaking for 3 min. and centrifugation at 3000 rpm for 5 min. A 1.3 mL portion of the organic layer was evaporated and the residue was dissolved in 20 μL of ethanol. The resulting solution was analyzed by thin layer chromatography on Silica gel GF254 plates with anhydrous ethanol-isopropyl ether-ammonia solution (7000:3000:2) as mobile phase. Amounts of amiodarone and desethylamiodarone on the chromatogram were subsequently determined with a Shimadzu CS-930 thin-layer scanner operated in reflectance mode at 240 nm. Recoveries for amiodarone and desethylamiodarone were 100.2 and 100.4%, respectively, coefficient of variation 6.74% and detection limits for amiodarone and desethylamiodarone amount to 0.1 μg mL^{-1}.

Pang et al.[73] described a method in which plasma (1 mL) was extracted with H_2O_2 free ethyl ether, the extract was evaporated to dryness and the residue was dissolved in ethanol. The solution was analyzed on Silica gel GF-254 plates with ethanol-$CHCl_3$-diethyleneamine (120:80:3) as mobile phase. The amiodarone spot was scraped off the plates and hydrolysed using 0.1 mol L^{-1} KOH under reflux on a boiling water-bath. Amiodarone was determined by monitoring the inhibition of the chemiluminescence of the luminol-H_2O_2-$AuCl_4$ system with use of the standard addition method. Mean recovery (n=4) equals 99.6%.

Electrodriven methods

Zhang et al.[74] described a new method for the determination of amiodarone and its metabolite desethylamiodarone using capillary electrophoresis (CE) where 20 μL human serum was mixed with 10 μL 3 mol L^{-1} acetate buffer of pH 6 and 20 μL 5.43 μmol L^{-1} L 8040 (internal standard) then extracted with 50 μL hexane. A portion (10 μL) of organic phase was evaporated and the residue was dissolved in 200 μL aqueous 80% propan-1-ol containing 100 μmol L^{-1} H_3PO_4. Prepared sample was analyzed on a fused-silica column (40

cm x 50 µm, effective length 21 cm) at 25 kV applied potential with detection at 242 nm. Each sample injection was preceded by the aspiration of a water plug ~0.8 mm long. The inlet buffer was a 3:2 mixture of propan-1-ol and 37.5 mmol L^{-1} Na_2HPO_4-112.5 mmol $L^{-1}$$NaH_2PO_4$ of pH 6.35 and the outlet buffer was 3 mmol L^{-1} Na_2HPO_4-9 mmol L^{-1} NaH_2PO_4. The calibration graphs were linear from 0.35-8.71 µmol L^{-1} amiodarone and 0.3-7.46 µmol L^{-1} desethylamiodarone the detection limits were 80 nmol L^{-1} for both analytes, inter- and intra-day relative standard deviation (RSD) values were 3-6%. The analysis time was ~10 min/sample. Determinations in clinical samples agreed with those obtained by HPLC.

A simple, sensitive and rapid capillary electrophoretic method has been developed by Perez et al.[75] for separation and quantification of amiodarone and its metabolite desethylamiodarone. The compounds were separated in a capillary of 45 cm effective length and 75 µm i.d., by use of an applied voltage of 25 kV and an electrolyte containing 15 mmol L^{-1} N-(2-acetamido) imionodiacetic acid buffer (pH 7.5), 10 mmol L^{-1} sodium dodecylsulfate and 70% acetonitrile. The selectivity, precision, linearity range, sensitivity and robustness of the method were good. The applicability of the assay was demonstrated by analysing these drugs in serum. Electrokinetic injection with field-amplified sample-stacking was used to increase sensitivity. The detection limit of the serum assay was 6.46 ng mL^{-1} and the precision 3.7%.

In a method established by Cheng et al.[76] amiodarone in blood was determined by MEKC, with sodium dodecylsulphate as the micellar phase. A separation column of bare fused-silica capillary was used with 25 mmol L^{-1} SDS - 50 mmol L^{-1}-borax-Tris at pH 8.33 as electrolyte and detection at 243 nm. When sampling the pressure applied was 6.9 k_{Pa}, at running voltage of 25 kV, temperature of 25 °C. The calibration graph for amiodarone was linear from 7.2-7200 ng mL^{-1} with limit of detection of 72 ng mL^{-1}. The recovery was 96% with relative standard deviation of 1.2%.

Miscellaneous methods

Many other methods have been used for amiodarone determination, as example, Nie et al.[77] applied a method proposed by Hlavay and Guilbault (Anal. Chem., 1977, 49, 1890)[78] for the determination of iodine-containing drugs. The method was based on the selective adsorption of iodine on a piezoelectric quartz crystal electrode. For the determination of micro amounts of amiodarone, iopanoic acid, acetylcholine iodide, 1,4-di-iodobenzene,

tetraiodofluorescein, diatrizoic acid and iodipamidic acid, a sample (containing 10 to 20 mg of the drug) was decomposed by oxygen flask combustion with 10 mL absorbent solution of 0.1 mol L^{-1} NaOH and 1 mL of 1% $NaHSO_3$. A portion of the solution was treated with 3 mL of 3% H_2O_2 and 3 mL of 3 mol L^{-1} H_2SO_4 and iodine was extracted with 10 mL of CCl_4. After 5 minutes at 35^oC, the cited crystal electrode was immersed in the organic phase to adsorb the iodine which was determined from the change in frequency. The calibration graph was rectilinear for 0.1 to 30 μmol L^{-1} iodine. The method was also suitable for determining iodine in aqueous solution, urine and blood.

Conclusions

Amiodarone hydrochloride is an important antiarrhythmic medication that affects the rhythm of heartbeats. The determination of the antiarrhythmic drugs is very important. Several analytical techniques were used for the determination of amiodarone hydrochloride in pharmaceutical preparations and in biological fluids.

Due to the sensitivity and simplicity of spectral methods, several spectroscopic methods have been described for the quantification of amiodarone hydrochloride in its pure state and in its pharmaceutical preparations. On the other hand chromatographic methods have been proven to be the most reliable and versatile analytical technique for the determination of amiodarone HCl in biological media. Almost in all the recent assays, the analytical technique is used for the determination of this drug and its metabolite desethylamiodarone in serum, plasma, tissue and urine is HPLC.

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