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Report

Determination of Amiodarone Hydrochloride in Pharmaceutical Formulations by Derivative UV Spectrophotometry and High-Performance Liquid Chromatography (HPLC)

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Assay procedures based on derivative ultraviolet spectrophotometry and high-performance liquid chromatography (HPLC) have been developed for the specific determination of amiodarone hydrochloride in pharmaceutical dosage forms. The use of first- and second-order derivative spectrophotometry was found to have suppressed the background absorption from the excipients with comparable accuracy and precision to the reversed-phased HPLC reference method. A conventional UV absorption method (λ = 242 nm) is subject to possible interference by formulation excipients.

KEY WORDS: amiodarone determination; desethylamiodarone; derivative UV spectrophotometry; high-performance liquid chromatography; pharmaceutical formulations.

INTRODUCTION

Amiodarone [2-butylbenzofuran-3-yl 4-(2-diethylaminoethoxy)-3,5-diiodophenylketone] is a potent antiarrhythmic agent effective in the control of ventricular and supraventricular arrhythmias and, also, in the management of angina pectoris (1,2). The drug is given orally or intravenously as the hydrochloride, and it is metabolized extensively in the liver to the desethyl derivative (desethylamiodarone).

A number of analytical procedures, almost exclusively based on high-performance liquid chromatography (HPLC), have been described for determining amiodarone hydrochloride in biological samples (plasma, urine, bile) for pharmacokinetic studies (3–17). However, only a few reports deal with the analysis of the drug in its dosage forms, and HPLC again provided the analytical tool for stability studies of amiodarone hydrochloride (18) and for its determination in commercial formulations (19).

As a result, it was considered desirable to develop additional assay procedures which would serve as alternatives to the HPLC methods for the selective determination of amiodarone hydrochloride. The recognized high resolution potency of derivative UV spectroscopy (20,21) led us to regard this technique as appropriate for this purpose. Therefore, in the present study, first- and second-order UV derivative spectroscopy methods have been developed, using a modified HPLC procedure as a reference method, and are proposed for a rapid and reliable quality control of amiodarone hydrochloride pharmaceutical formulations.

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MATERIALS AND METHODS

Materials

Amiodarone hydrochloride (Resfar, Italy), *N*-desethylamiodarone (Midy, Italy), and miconazole nitrate (Italfarmaco, Italy) were used. Methanol and tetrahydrofuran used in chromatography were HPLC grade from C. Erba (Italy); water was deionized double distilled. Aqueous triethylammonium acetate solution (0.1 *M*, pH 7.0) was prepared by neutralization of an aqueous 0.1 *M* triethylamine solution with acetic acid.

Stock solutions of amiodarone hydrochloride (c = 0.316 mg/ml) and miconazole nitrate (c = 1.00 mg/ml) were prepared in methanol.

Instrumentation

Liquid Chromatography. A Varian HPLC system consisting of a Model 5020 liquid chromatograph, a variable wavelength detector (UV-50), and a Model 4290 integrator was used. Manual injections were made using a Rheodyne 7125 injectable valve (10-μl loop). The detector wavelength was set at λ = 242 nm and the integrator attenuation was 64. The chromatographic separations were performed at ambient temperature on a 10-μm Hypersil RP-18 column (300 × 4.0-mm i.d.) using a mobile phase of methanol–tetrahydrofuran–triethylammonium acetate (0.1 *M*, pH 7.0), 48:34:18 (v/v), at a flow rate of 1.2 ml/min.

Spectrophotometry. Analyses were performed on a Varian Model DMS 90 UV-visible double-beam spectrophotometer using 1-cm quartz cells, with a slit width of 1 nm and a scanning speed of 100 nm/min over a range of 200 to 340 nm. A Model 9176 recorder (Varian) was used with a full-

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scale deflection of 2 × 100 mV (first-derivative spectra) or 2 × 10 mV (second-derivative spectra).

Calibration Curves

HPLC. Working standard solutions containing 12.3–37.0 μg/ml of amiodarone hydrochloride and 150 μg/ml of miconazole nitrate (the internal standard) were prepared in methanol. A 10-μl volume of each solution was injected into the chromatograph, the peak height was measured, and the peak height ratios of analyte to internal standard were then plotted against the respective mass ratios to obtain the calibration curve.

Spectrophotometry. Working standard solutions containing 6.0–21.0 μg/ml of amiodarone hydrochloride were prepared in methanol. The conventional (zero-order) and the derivative (first- and second-order) UV spectra of each standard solution were recorded against a blank solvent. The absorbance at λ = 242 nm (conventional method) and the peak-to-peak amplitudes $^1D_{250,232}$ (first-derivative method) and $^2D_{254,243}$ (second-derivative method) were measured and plotted against the corresponding concentrations to obtain the respective calibration curves.

Sample Preparation

Methanol was chosen as the extracting organic solvent because it is consistent with the solubility properties of amiodarone hydrochloride (22) and a reversed-phase mode of chromatography.

Tablets. A quantity of powdered tablets, equivalent to approximately 30.0 mg of amiodarone hydrochloride, was transferred into a 100-ml volumetric flask and extracted with 50 ml of a methanol–water (4:1) solvent system under sonication at room temperature for 10 min. The content was then diluted to volume with the extracting solvent. The resulting solution was filtered through a 0.45-μm Gelman filter and then subjected to the HPLC and spectrophotometric assay procedures.

Capsules. In a 100-ml volumetric flask 1 capsule or a portion (equivalent to about 200 mg of amiodarone hydrochloride) of the mixed contents from 10 capsules was treated with 20 ml of 0.1 *N* hydrochloric acid solution under sonication at room temperature for 10 min. The content was diluted to volume with methanol and sonicated for an additional 2 min. The resulting slightly opalescent solution was further diluted (1:5) with methanol, filtered through a 0.45-μm Gelman filter, and then used for HPLC and spectrophotometric analyses.

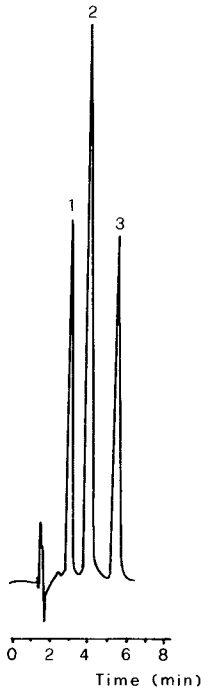

Fig. 1. Typical HPLC chromatogram of (1) miconazole (the internal standard), (2) desethylamiodarone, and (3) amiodarone.

Assay Procedure

HPLC. A 1.0-ml aliquot of the clarified sample solution was transferred into a 10-ml volumetric flask containing 1.5 ml of internal standard (miconazole nitrate) solution and the volume was adjusted with methanol. A 10-μl volume of the resulting solution was injected into the chromatograph in triplicate. The sample solutions were chromatographed concurrently with the appropriate standard solution (31.62 μg/ml) and the peak height ratios (analyte to internal standard) were used for the amiodarone hydrochloride quantitation in each sample.

Spectrophotometry. A 1.0-ml aliquot of the clarified sample solution was diluted to 20 ml with methanol and the resulting solution was directly analyzed as described under Calibration Curves. The drug concentration in each sample was calculated by direct comparison with a standard solution.

RESULTS AND DISCUSSION

Chromatography

A reversed-phase HPLC procedure was developed as a suitable reference method for the analysis of amiodarone hydrochloride dosage forms by derivative spectrophotometric procedures. The chromatographic conditions were adjusted in order to provide a versatile HPLC procedure capable of separating amiodarone and its *N*-desethyl metabolite and therefore also potentially useful for pharmacokinetic studies. A ternary mixture of methanol–tetrahydrofuran–triethylammonium acetate (0.1 *M*, pH 7.0), 48:34:18 (v/v), at a flow rate of 1.2 ml/min was found to be an appropriate mobile phase, allowing for adequate and rapid separation of the internal standard miconazole (tr = 3.25), desethylamiodarone (tr = 4.20), and amiodarone (tr = 5.63) (Fig. 1). The addition of triethylamine salt to the mobile phase resulted in the elimination of peak tailing and the obtainment of symmetric peaks, while the partial substitution of methanol with tetrahydrofuran reduced the retention time of a highly hydrophobic compound such as amiodarone.

For quantitative applications, a linear calibration curve ($y = 5.70x + 0.012$, $r = 0.9997$, $N = 7$, where y and x are response ratios and mass ratios, respectively) was obtained

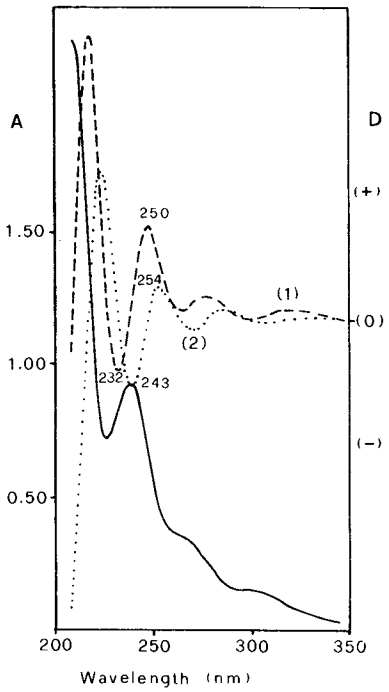

Fig. 2. Zero-order absorption spectrum (solid line) and first-derivative (1) and second-derivative (2) spectra of amiodarone hydrochloride (c = 15.81 μg/ml). Solvent, methanol–water (4:1). Linear calibration graphs were obtained in absorption (A_{242} = 0.056C + 0.0020; r = 0.9999; N = 7), first-derivative ($^1D_{250,232}$ = 4.42C + 0.250; r = 0.9999; N = 7), and second-derivative ($^2D_{254,243}$ = 6.08C + 0.030; r = 0.9998; N = 7) spectrophotometry over the drug concentration range 6.0–21.0 μg/ml.

over the working concentration range (12.3–37.0 μg/ml). The precision of the chromatographic procedure was indicated by the relative deviation standard (0.34%) of the peak height ratio of analyte to internal standard, obtained from the replicate (N = 8) analyses of a single amiodarone solution. The selectivity of the chosen chromatographic system was also ascertained. Separate chromatographic analyses of the extracts of tablet excipients without amiodarone showed no interfering peaks at the retention times of either the analyte or the internal standard. Similarly, no interfering peak at the internal standard retention time was observed in the chromatogram of the capsule extract.

Derivative Ultraviolet Spectrophotometry

The absorption (zero-order) UV spectrum of amiodarone hydrochloride exhibits a maximum at λ = 242 nm (Fig. 2). Determinations of the drug in its dosage forms by direct absorbance measurement at this relatively low wavelength could be susceptible to interference from excipients. In accordance with recent reports (23–25) and previous experience (26), the conversion of the absorption (zero-order) spectrum in higher-order derivative spectra was chosen as a convenient technique capable of suppressing the matrix background absorption and providing selective analysis of amiodarone hydrochloride formulations. The peak-to-peak amplitudes $^1D_{250,232}$ in the first-order derivative spectrum and $^2D_{254,243}$ in the second-order derivative spectrum were measured (millimeters) and were found to be linearly correlated with the amiodarone hydrochloride concentration over the working range (Fig. 2). The relative standard deviation of the selected amplitudes, derived from replicate (N = 8) recordings of the derivative spectra of a single standard solution, fell within the range 0.45–0.80%.

Analysis of Amiodarone in Pharmaceutical Formulations

The proposed HPLC and derivative spectrophotometric methods were applied to the analyses of commercial dosage forms containing amiodarone hydrochloride. The results obtained are summarized in Table I, where they are also compared with the results obtained by a conventional spectrophotometric method (λ = 242 nm). As can be seen, for product A (tablets) all the analytical methods used provided results in agreement with the labeled content, while for product B (capsules) a significantly higher value was obtained using the conventional absorption method. Such a result was ascribed to the interfering absorption contribution from the excipients; this effect was eliminated by using the first- and second-order derivative procedures, which were found to give results in close agreement with those from the HPLC and the claimed content. When Student's t test at the 95% confidence level was applied to compare the results obtained by the HPLC method and the derivative procedures, the calculated t values did not exceed the tabulated one.

In order to verify the accuracy of the described methods, recovery studies were carried out by analyzing synthetic preparations of amiodarone hydrochloride (tablets) and samples spiked with known quantities of the drug (capsules). Synthetic mixtures which reproduced the composition of the commercial tablets were prepared at different potency levels, i.e., at different drug/excipients ratios. As shown in Table II, quantitative recoveries with a good precision were obtained in each instance with all the employed methods. When capsule samples spiked with known quantities of the drug were analyzed, quantitative recoveries of the added amounts were obtained by all the de-

Table I. Assay Results for the Determination of Amiodarone Hydrochloride in Commercial Dosage Forms[a]

	HPLC		First-derivative method		Second-derivative method		Absorption method (242 nm)	
Product[b]	Found	RSD (%)	Found	RSD (%)	Found	RSD (%)	Found	RSD (%)
A (tablets)	99.60	1.05	99.85	1.10	100.10	1.16	100.30	0.90
B (capsules)	99.20	1.10	98.86	1.20	98.75	1.24	103.10	1.50

[a] Results are the average of five determinations and are expressed as a percentage of the claimed content. RSD, relative standard deviation.
[b] Product A contains amiodarone hydrochloride (200 mg per tablet), lactose, colloidal silica, povidone, magnesium stearate, and starch. Product B contains amiodarone hydrochloride (200 mg per capsule), vegetable oil, hydrogenated vegetable oils, beeswax, and lecithin.

Table II. Recovery Values Obtained for the Determination of Amiodarone Hydrochloride in Synthetic Preparations[a]

Synthetic preparation[b]	HPLC		First-derivative method		Second-derivative method		Absorption method (242 nm)	
	Found	RSD (%)	Found	RSD (%)	Found	RSD (%)	Found	RSD (%)
I (1.00)	99.30	0.76	99.77	0.52	100.35	0.67	100.12	0.78
II (0.50)	99.40	1.06	99.53	0.65	100.26	0.73	100.33	0.90
III (0.25)	100.13	0.95	99.65	0.74	100.33	0.86	99.70	1.15

[a] Results are the average of four determinations and are expressed as a percentage of the amiodarone hydrochloride added.
[b] The excipient composition was identical to that of the corresponding commercial formulation (Product A, Table I). The drug/excipients ratios were 1.0, 0.5, and 0.25 times that of the commercial formulation.

scribed procedures, while, as expected, a higher value (102.0%) of total drug content was found by the absorption spectrophotometric method in comparison with those from HPLC (99.42%) and derivative spectrophotometric (99.37–99.55%) methods. This illustrates the efficiency of the drug extraction procedure and confirms some interference from capsule excipients in the conventional spectrophotometry.

In summary, a conventional spectrophotometric method was found to be of limited accuracy when applied to the analysis of amiodarone hydrochloride in commercial dosage forms because interfering excipient absorptions can be responsible for inflated drug contents. Conversely, first- and second-derivative procedures proved capable of suppressing the nonspecific matrix absorption and, therefore, offer a more selective approach for a rapid and reliable quality control of amiodarone hydrochloride formulations. The described reversed-phase HPLC procedure proved to be a useful, accurate reference method in the present study and could serve as a versatile analytical tool suitable for the determination of amiodarone hydrochloride in a variety of matrices.

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