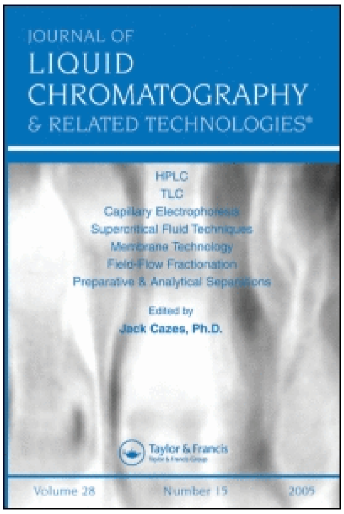

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A Simplified Method for the Estimation of Amiodarone Hydrochloride by Reverse-Phase High Performance Liquid Chromatography

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ABSTRACT

Amiodarone is a class III anti-arrhythmic agent and one of the most powerful drugs used in the treatment of ventricular and supraventricular tachycardias. The drug is available in the market as tablet and injectable formulations. In this study, a repeatable reverse-phase high performance liquid chromatographic method for quality control purposes only has been developed for the estimation of amiodarone hydrochloride in a dosage form. A C-8 column was used in the study. 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 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

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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 LOQ and LOD were found to be 3.12 mcg and 0.936 mcg, respectively.

Key Words: Amiodarone; HPLC assay method; Stability indicating.

INTRODUCTION

Amiodarone is one of the most powerful anti-arrhythmic drugs used in the treatment of ventricular and supraventricular tachycardias, including those associated with Wolff-Parkinson-White (WPW) syndrome.[1,2] Amiodarone is a class III anti-arrhythmic agent, which acts by delaying repolarization and prolonging the action potential duration of atrial and ventricular muscle without altering the resting membrane potential. Amiodarone, thus, lengthens the refractory period of atrial and ventricular myocardium, the atrioventricular node, and of the accessory pathways that mediate WPW syndrome.[3]

In contrast to the extensive clinical use of amiodarone for more than a decade, information concerning its pharmacokinetic behavior, metabolism, and body distribution is still limited and has only recently emerged.[1] The reason for the delay was mainly attributed to the absence of a sensitive and selective assay method for the determination of the drug. The nature of the drug and its high hydrophobicity requires specific methods, such as HPLC, for its determination. The objective of the present study is to develop a sensitive HPLC method for the analysis of the drug. The assay method would be tested for the following parameters: Linearity of response; sensitivity; specificity; limit of detection and limit of quantification; and its stability indicating nature.

EXPERIMENTAL

Materials

The materials were supplied by the manufacturers and used as is. These included: methanol, HPLC grade, 99.9% pure, UV cuttoff 190 nm; sodium hydroxide, 98.1%, pure, certified ACS, Lot no. 893102; HCL 38.0% (w/w), ACS grade, Lot no. 920005; and nortriptyline, Lot no. 75189, which were supplied by Fisher Scientific, Fairlawn, NJ; amiodarone hydrochloride—Lot no. 87H0597—Sigma Aldrich, Steinhelm, Germany; acetic acid—ACS grade—Mallinckroft Manufacturing Company, St Louis, MO.

Equipment

The equipment used for the experimentation included: Waters HPLC system; Novapak C8 column 3.9 × 150 mm—particle size of 4 μm; Waters 501 HPLC pump, 100 μL loop injector; Waters 712 WISP autosampler; Waters 486 Absorbance Detector; Millennium 2010 chromatography Manager-Version 3.2; DSC 20 with StarE software from Mettler-Toledo; and a pH 211 Microprocessor pH meter from Hanna Instruments.

Optimization of the Analytical Method

The mobile phase consisted of two solutions. The first solution (Soln #1) was prepared by mixing methanol, water and acetic acid (0.1 N) in a 95 : 4 : 1 ratio. The pH was adjusted to 4 using 0.1 N ammonia solution. The second solution (Soln #2) was filtered and degassed water. Both solutions were filtered through a 0.45 μm FP Vericel Membrane Filter, HPLC Certified, Lot no. 2092010, supplied by Gelman Sciences, Ann Arbor, MI, with the help of a Millipore Filter Holder, part #4, obtained from Millipore Filter Corporation, Bedford, MA, and degassed using argon gas obtained from AGA gas, Toledo, OH. The flow rate for the mobile phase was programmed to be 1.5 mL/min, out of which 98% was Soln #1 and 2% was Soln #2. The detector was set at 242 nm. The column was maintained at room temperature for the entire analytical procedure. The complete run was performed within 8 min, which also included a 1 min wash time. The sample processor was adjusted to inject 10 μL of the sample into the column.

Calibration Curve and Assay Accuracy and Precision for the High Performance Liquid Chromatography Method

The lowest concentration for which the relative standard deviation (RSD) for multiple injections is <5.0% is the LOQ and the LOD value, which by convention is taken to be 0.3 × LOQ.[4] Amiodarone hydrochloride stock solution was prepared by dissolving 100.2 mg of amiodarone hydrochloride in 10 mL of mobile phase and filtering through a 0.2 μm filter, thereby giving a 10.02 μg/mL solution of amiodarone hydrochloride. This was further taken in aliquots and diluted, so as to obtain 3.13, 6.26, 9.39, 12.52, 15.65, 18.78, and 22.73 μg in 10 μL, respectively. The internal standard considered was nortriptyline. This decision was based on the fact that amiodarone hydrochloride has a log *P* value of 7.57. This very high value necessitated the use of a C-8 column. Thus, an internal standard (IS) was chosen to have a log *P* value

Table 1. Log *P* values of some selected compounds.

Compound	Log *P* value
Brodifacum	9.19
Amiodarone	7.57
Tamoxifen	6.64
Amitriptyline	4.87
Imipramine	4.6
Ketaconazole	4.49
Indomethacin	4.25
Fluoxetin	4.05
Ibuprofen	3.59

comparable to that of amiodarone hydrochloride. An abbreviated list of materials with their log *P* values is given in Table 1. Nortriptyline was found to have a log *P* value of 4.8.[5] Since nortriptyline is similar in structure to amitriptyline and also has similar log *P* values, the logic of its use as an IS was, thus, justified.

The IS stock solution was prepared by dissolving 50.2 mg of nortriptyline in 10 mL of the mobile phase and 100 μL was used to spike the solution. With each dilution, three runs were performed and the areas under the curve obtained were plotted against the concentration. The calibration plot was plotted with areas under the curve on the *Y*-axis and the concentration on the *X*-axis.

A linear regression analysis for the calibration plot was performed. The equation for the line and the R^2-value for the linearity of the calibration plot were calculated. Injecting identical samples of amiodarone hydrochloride five times each day, over a five-day period, was performed in order to test the precision of the assay method. The UV detector response was compared and the percent relative standard deviation for the area under the curve (AUC) was calculated for within and between days.

Methodology for the Stability Indicating Nature of the High Performance Liquid Chromatography Assay Method

A stability indicating method is an analytical procedure that is capable of discriminating between the major active (intact) pharmaceutical ingredient (API) from any degradation/decomposition product(s) formed under defined storage conditions during the stability evaluation period. An 8.3 mL portion of 38% w/w hydrochloric acid was placed into a 100 mL volumetric cylinder and the volume was made to 100 mL with reverse osmosis (RO) water. A 100 mg sample of amiodarone hydrochloride was placed into 50 mL of 1 N hydrochloric acid. The solution was then heated for 30 min, cooled, and the volume made up to 100 mL with base, then filtered and injected into the column. Simultaneously, 4 g of sodium hydroxide pellets were dissolved in 100 mL of RO water. A 100 mg aliquot of amiodarone hydrochloride solution was added to 50 mL of the 1 N sodium hydroxide solution in RO water. The solution was then heated for 30 min then cooled and the volume made up to 100 mL with an equal volume of acid, filtered

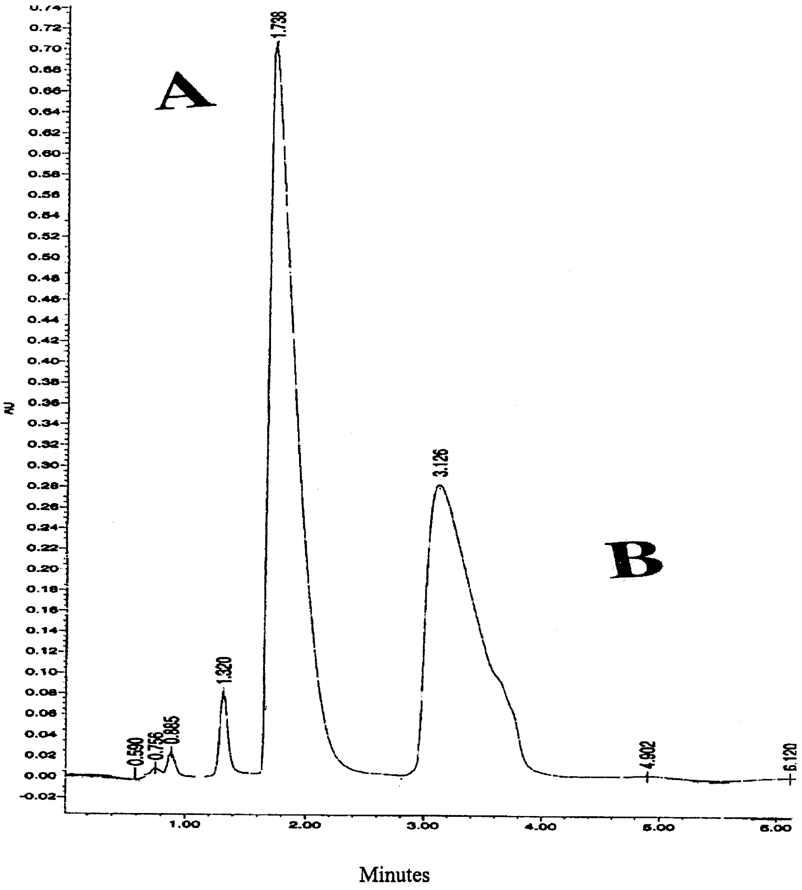


Figure 1. Chromatogram showing well resolved peaks of amiodarone (B) retention time of 3.126 and the internal standard nortriptyline (A) retention time of 1.738.

Table 2. Data for the calibration plot for amiodarone hydrochloride.

Conc. of amiodarone hydrochloride (mcg)		3.13	6.26	9.39	12.52	15.65	18.78	22.73
Run 1	Peak area of drug	4,751,323	9,448,790	15,443,889	19,289,221	24,129,592	29,780,704	71,803,007
	Peak area of internal standard	1,096,803	1,064,001	1,113,390	1,349,079	1,314,502	1,146,016	1,696,953
Run 2	Peak area of drug	4,962,066	9,674,472	15,364,420	21,148,181	23,838,839	29,601,180	71,311,372
	Peak area of Internal standard	1,033,601	1,200,714	1,083,180	1,394,481	1,282,507	1,142,021	1,683,105
Run 3	Peak area of drug	4,961,493	10,770,361	15,361,202	19,412,121	24,037,584	29,533,829	70,947,220
	Peak area of Internal standard	1,189,641	1,055,370	1,082,191	1,524,727	1,291,290	1,135,788	1,673,497
Average area ratio		4.41	9.05	14.08	14.07	18.52	25.97	42.36

and then injected into the column. This was done in order to facilitate its degradation under both acidic and basic conditions. A second set of samples from the acid and base treated solutions were then spiked with the (IS) just prior to analysis and then run through the column.

RESULTS AND DISCUSSION

Accuracy, Precision, and Calibration Plot for the Analytical Method

On injecting a solution containing amiodarone hydrochloride and nortryptiline, with the chromatographic conditions maintained, as discussed under HPLC instrumentation and conditions section, there was a clear separation of the two peaks. The retention times for nortryptiline and amiodarone hydrochloride were 2 and 4 min, respectively (Fig. 1).

The response for the detection of amiodarone hydrochloride was found to be linear and the data for the calibration plot is seen in Table 2. The calibration plot is represented in Fig. 2 and the equation obtained from the calibration plot, taking all 21 points for the ratio of the areas and plotting against the corresponding concentrations, is:

$$y = 27683x + 421922 \tag{1}$$

The calibration plot has an R^2-value of 0.9407, which is well within the required guidelines. The data for the precision of the assay is represented in

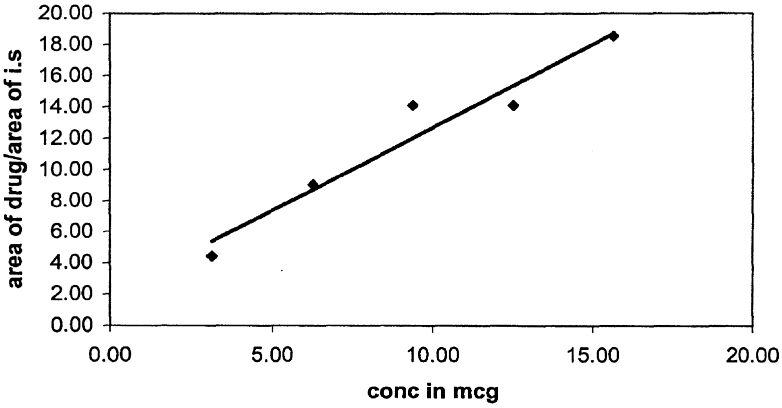


Figure 2. Calibration plot for amiodarone hydrochloride for which the slope is 1.0621; intercept is 2.0548; and R^2 is 0.9407.

Table 3. Data for the precision of the assay.

Peak area				
Day 1	Day 2	Day 3	Day 4	Day 5
18,973,167	17,716,286	18,534,638	16,890,427	17,223,156
18,173,206	17,067,898	16,927,262	17,903,071	16,995,525
18,214,147	17,460,950	16,849,682	17,404,895	17,177,758
18,174,935	17,246,345	17,015,415	17,051,385	17,858,888
18,316,288	17,950,931	17,545,981	17,403,155	17,598,455

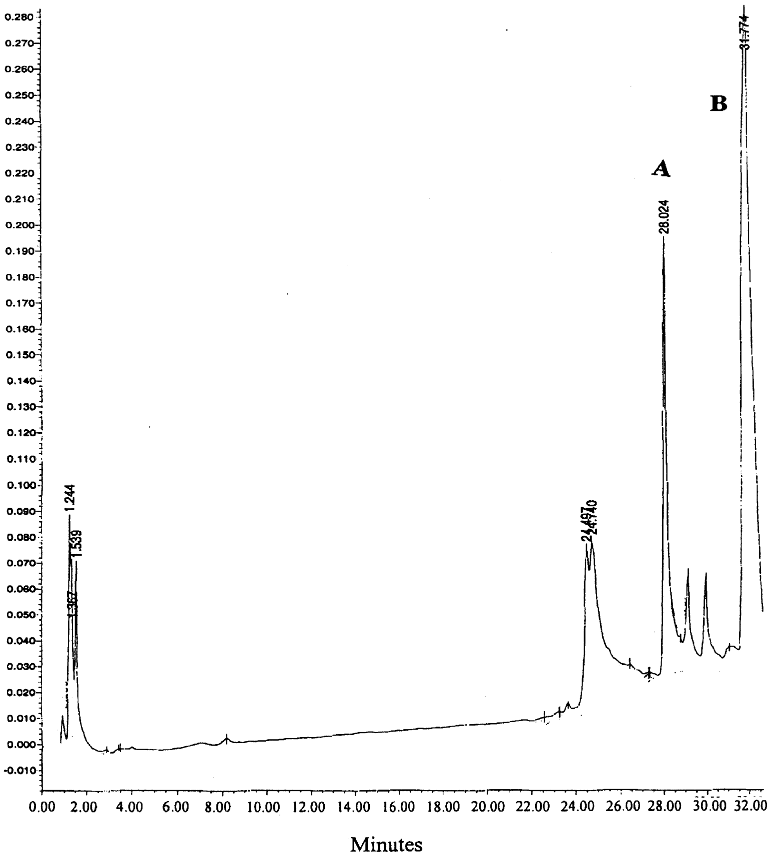
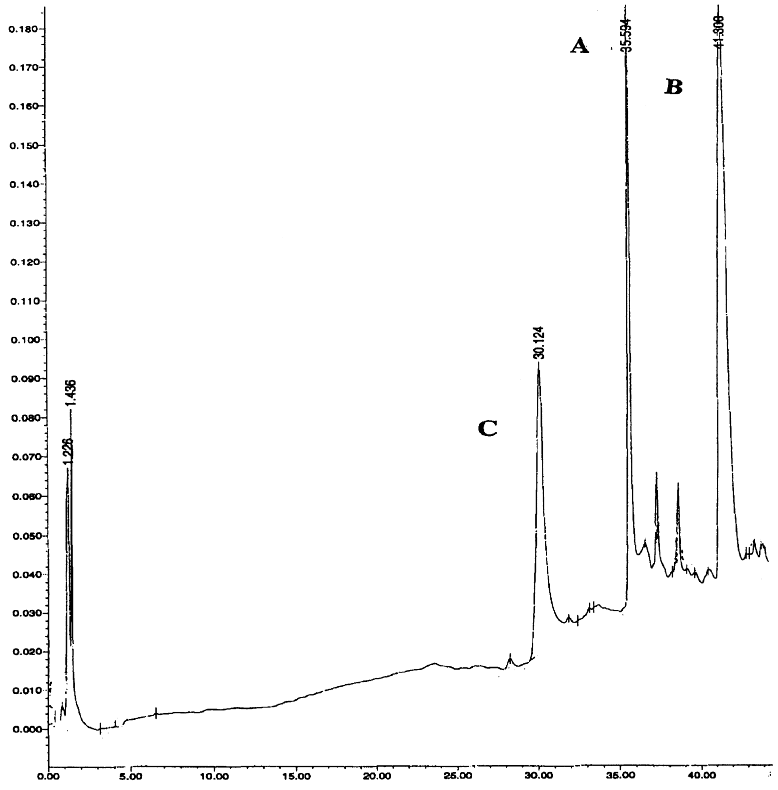


Figure 3. Chromatogram of acid destabilized amiodarone hydrochloride (B) retention time of 31.77 and its degradation product (A) retention time of 28.004 in the absence of the IS.



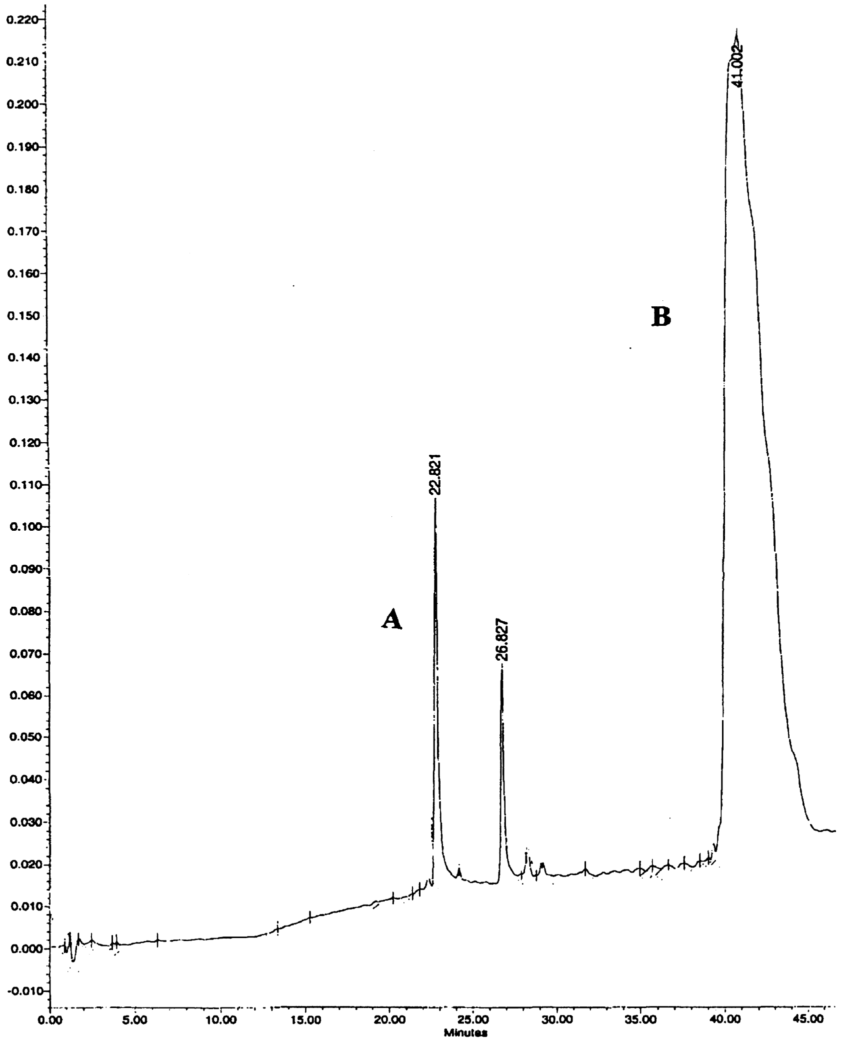
Minutes

Figure 4. Chromatogram of acid destabilized amiodarone hydrochloride (B) retention time of 41.300 and its degradation product (A) retention time of 35.594 spiked with the IS nortriptyline (C) retention time of 30.124.

Table 3. The percent relative standard deviation for the AUC for within and between days, for five runs for the same sample for five days, was calculated to be 2.48% and 3.26%, respectively. The LOQ and LOD were calculated and were found to be 3.12 mcg and 0.936 mcg, respectively.

Stability Indicating High Performance Liquid Chromatography Assay Method Developed

On treating amiodarone with HCl and then injecting a sample, the chromatogram (Fig. 3) was obtained; the peaks obtained are that for amiodar-



Minutes

Figure 5. Chromatogram of base destabilized amiodarone hydrochloride (B) retention time of 41.002 and its degradation product (A) retention time of 22.821 in the absence of the IS. Another peak of unknown origin is also visible at a retention time of 26.821.

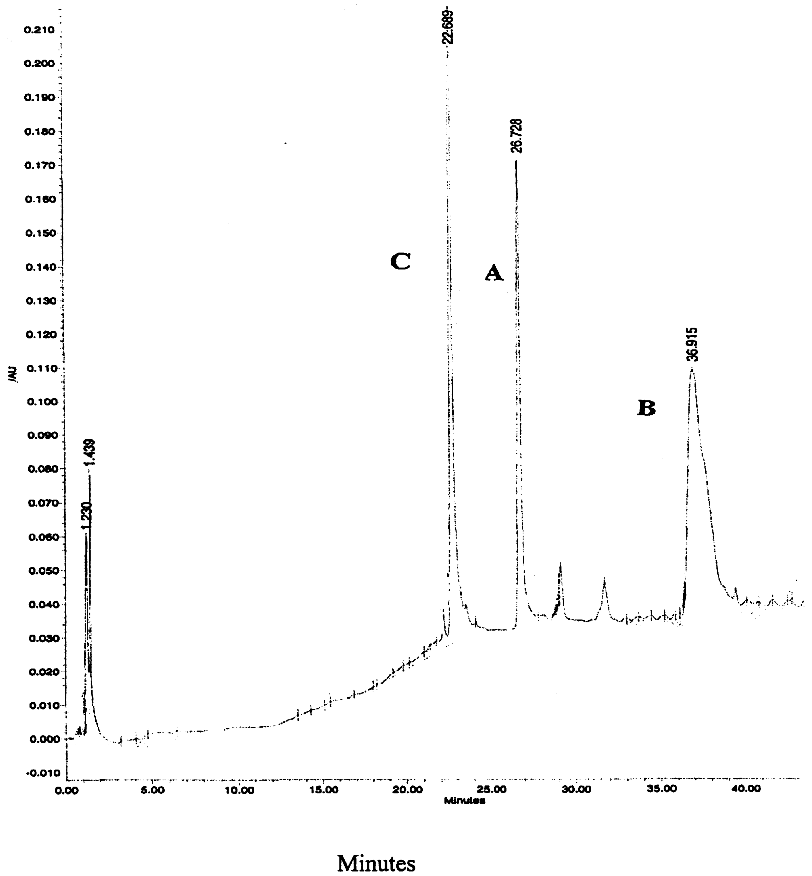


Figure 6. Chromatogram of base destabilized amiodarone hydrochloride (B) retention time of 36.915 and its degradation product (C) retention time of 22.669 spiked with the IS nortriptyline (A) retention time of 28.728.

one at 31.7 min and its degradation products at 28.024. On spiking with the (IS) nortriptyline, the peak for nortriptyline was seen at 30.124 min (Fig. 4), while amiodarone and its degradation products were seen at 41.3 min and 35.594 min, respectively. There was no interference by any of the degradation products of amiodarone with the peaks for amiodarone and the IS (Fig. 4). They remained separated for both the amiodarone and its degradation products. Similarly, on treating amiodarone with NaOH, the chromatograph (Fig. 5) was observed, showing amiodarone and its degradation products. The NaOH treated amiodarone solution, spiked with nortryptiline showed nortriptyline without any interference with the degradation products of amiodarone (Fig. 6). This showed that the HPLC assay method for amiodarone hydrochloride is stability indicating.

CONCLUSIONS

A repeatable reverse Phase HPLC method for the determination of amiodarone hydrochloride has been developed, which has a much lower retention time than that reported in the literature. The analytical method was tested for its accuracy, precision, and stability indicating nature and the LOD and LOQ for the method was estimated.

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