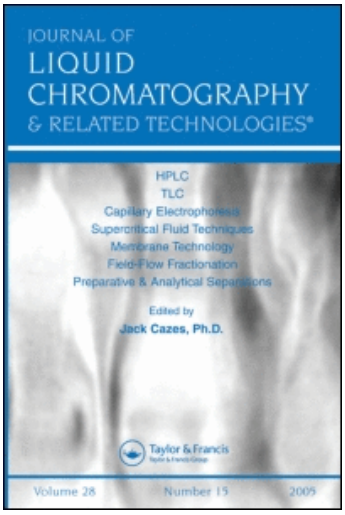

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Validation of a Stability-Indicating HPLC Method for the Determination of Amiodarone HCl and Its Related Substances in Amiodarone HCl Injection

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Validation of a Stability-Indicating HPLC Method for the Determination of Amiodarone HCl and Its Related Substances in Amiodarone HCl Injection

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ABSTRACT

The validation of a gradient high performance liquid chromatographic (HPLC) procedure employing ultraviolet (UV) detection for the analysis of amiodarone HCl and two of its related substances in Amiodarone HCl Injection (drug product) is reported. The method is reproducible, accurate, and selective for amiodarone HCl and the two known related substances. The peak area response versus concentration was demonstrated to be linear over a range of 50–150% for the assay preparation, as

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well as over a range of 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 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 (percent recovery) was demonstrated for both amiodarone HCl and each of the two available related substances. Specificity was demonstrated by forced degradation of drug product under acid, base, heat, peroxide, and light conditions. A quantitation limit and detection limit is 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.

Key Words: HPLC; Stability; Amiodarone HCl; Amiodarone HCl injection.

INTRODUCTION

Amiodarone HCl is a class III antiarrhythmic drug indicated for the treatment of prophylaxis of frequently recurring ventricular fibrillation and hemodynamically unstable ventricular tachycardia in patients refractory to other therapy.[1] Amiodarone HCl Injection is a parenteral solution consisting of 50 mg amiodarone HCl, 20.2 mg of benzyl alcohol, 100 mg of polysorbate 80, and water for injection.

Several methods were identified for the analysis of amiodarone and desethylamiodarone (metabolite) by HPLC with UV detection[2–4] or mass spectrometry,[5] as well as by capillary electropherisis.[6,7] However, these methods are for the analysis of biological tissue and serum samples and do not address the analysis of Amiodarone HCl Injection. Additional methods,[8,9] including methods listed in the United States Pharmacopoeia Forum[10] and European Pharmacopoeia,[11] address only the analysis of amiodarone HCl drug substance and tablets. Therefore, a stability-indicating method for the analysis of amiodarone HCl and its related substances in Amiodarone HCl Injection was developed.

Eight related substances are identified for amiodarone HCl in the European Pharmacopeia.[11] Of these eight, only two are commercially available: (2-butyl-1-benzofuran-3-yl)(4-hydroxy-3,5-diiodophenyl)methanone (diode BBFA) and (2-butyl-1-benzofuran-3-yl)(4-hydroxyphenyl)methanone (BBFA). The structures of these two related substances, as well as amiodarone HCl, are presented in Fig. 1(a)–(c). Both of these related substances were determined to be possible degradants of amiodarone HCl in the drug product.

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As such, a method for the analysis of these related substances was required. It was desirable to have a single method for the quantification of both the amiodarone HCl and the two related substances. Therefore, a method was developed and validated to this end.

The method was validated following USP Assay Category I[12] and per ICH guideline Q2B,[13] Validation of Analytical Procedures: Methodology.[14] As such, the following parameters were evaluated: linearity, relative response factor, precision (repeatability and intermediate), accuracy, specificity, quantitation limit, detection limit, and solution stability.

EXPERIMENTAL

Chemical and Reagents

Amiodarone HCl Injection, 50 mg/mL (Cordarone® IV) (drug product), is manufactured by Wyeth. Amdiodarone HCl reference standard is a British Pharmacopeia reference standard. Amiodarone HCl was obtained from Sigma-Aldrich Corporation (St. Louis, MO). BBFA and diode BBFA was purchased from the European Pharmacopeia.

ACS grade potassium phosphate monobasic, ammonium hydroxide, phosphoric acid, hydrochloric acid, sodium hydroxide, and hydrogen peroxide were obtained from Fisher (Hanover Park, IL), HPLC grade triethylamine was obtained from Sigma-Aldrich Corporation (St. Louis, MO), and HPLC grade methanol and acetonitrile were obtained from Burdick and Jackson (Muskegon, MI).

Apparatus

The chromatographic systems consisted of a Waters 2690 Alliance HPLC System equipped with a 486 Variable Wavelength Detector, 2487 Dual Wavelength Absorbance Detector, or 996 Photodiode Array Detector at a wavelength of 240 nm (Waters Corporation, Milford, MA).

The HPLC column was a Phenomenex Luna 3 μm C8(2), 75 × 4.6 mm equipped with a Security Guard C8 guard column (Phenomenex, Torrance, CA). Column temperature was maintained at 40°C.

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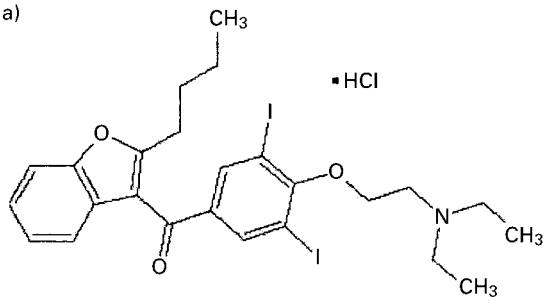
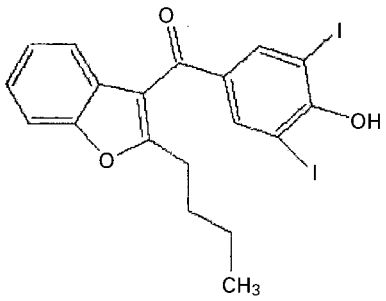
(a)



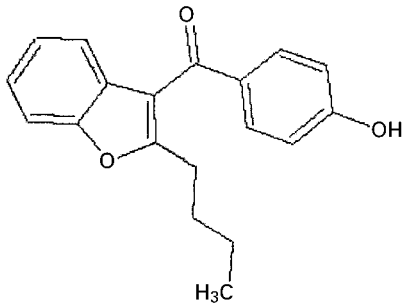
(2-butyl-3-benzofuranyl)40[2-(diethylamino)ethoxyl]
-3-5-diodophenyl]methanone hydrochloride

(b)



(2-butyl-1-benzofuran-3-yl)(4-hydroxy
-3,5-diiodophenyl)methanone

(c)



(2-butyl-1-benzofuran-3-yl)(4-hydroxyphenyl)
methanone

Figure 1. Structures of (a) amiodarone HCl, (b) diode BBFA, and (c) BBFA.

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Preparation of Solutions

Mobile Phases

Mobile Phase A consists of a 15 mM potassium phosphate monobasic and 30 mM triethylamine buffer. The pH of the solution is maintained at 2.50 ± 0.05. Mobile Phase A is filtered through a 0.45 μm membrane filter. Mobile Phase B is a acetonitrile-methanol mixture (1 : 1 v/v). The linear gradient conditions are presented in Table 1. The flow rate is 1.0 mL/min, with a typical retention time of 10 min for amiodarone HCl.

Diluent Preparation

Combine Mobile Phase A with Mobile Phase B (1 : 1 v/v) and mix.

Stock Standard Preparation

Dissolve amiodarone HCl standard in Diluent to a final concentration of 500 μg/mL.

Standard Preparation

Dilute Stock Standard Preparation in Diluent to a final concentration of 100 μg/mL. Filter a portion through a 0.45 μm hydrophilic PTFE filter, discarding the first three 3 mL of filtrate. Place the filtered portion into a suitable HPLC vial for analysis.

Table 1. Gradient parameters.

Time	% Mobile phase A	% Mobile phase B
0	50	50
21	25	75
26	25	75
27	15	85
31	15	85
32	50	50
40	50	50

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Limit Standard Preparation

Dilute standard preparation in diluent to a final concentration of 1 μg/mL. Filter a portion through a 0.45 μm hydrophilic PTFE filter, discarding the first three 3 mL of filtrate. Place the filtered portion into a suitable HPLC vial for analysis.

Assay Preparation

Dilute drug product in diluent to a final concentration of 100 μg/mL. Filter a portion through a 0.45 μm hydrophilic PTFE filter, discarding the first three 3 mL of filtrate. Place the filtered portion into a suitable HPLC vial for analysis.

Related Substances Preparation

Dilute drug product in diluent to a final concentration of 1000 μg/mL. Filter a portion through a 0.45 μm hydrophilic PTFE filter, discarding the first three 3 mL of filtrate. Place the filtered portion into a suitable HPLC vial for analysis. This solution is injected and quantified against the limit standard preparation. This solution is ten times more concentrated than the assay preparation to increase the sensitivity of the method for related substances.

System Suitability

System suitability was calculated for each run according to calculations listed in USP 26 ⟨621⟩[1] from typical chromatograms. Instrument precision is determined by five replicate injections of the standard preparation and six injections of the limit standard preparation. The %RSD of the working standard preparation must be NMT 2.0 and limit standard preparation must be NMT 5.0. The tailing factor of the amiodarone HCl peak in the standard preparation must be NMT 1.75.

Data Acquisition

All data was acquired and stored using Waters Millenium Data Acquisition Software v. 4.0 (Waters Corporation, Milford, MA).

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RESULTS AND DISCUSSION

Chromatography

Typical chromatography from a 10 μL injection of the standard preparation and the limit standard preparation are presented in Fig. 2(a)–(b), respectively. Chromatograms of the assay preparation and the related substance preparation are presented in Fig. 3(a)–(b), respectively. A chromatogram of amiodarone HCl, BBFA, and diode BBFA are presented in Fig. 4. The retention time of Amiodarone HCl was 10 min. To allow for late-eluting peaks (impurities) and column re-equilibration, the overall run time was 40 min.

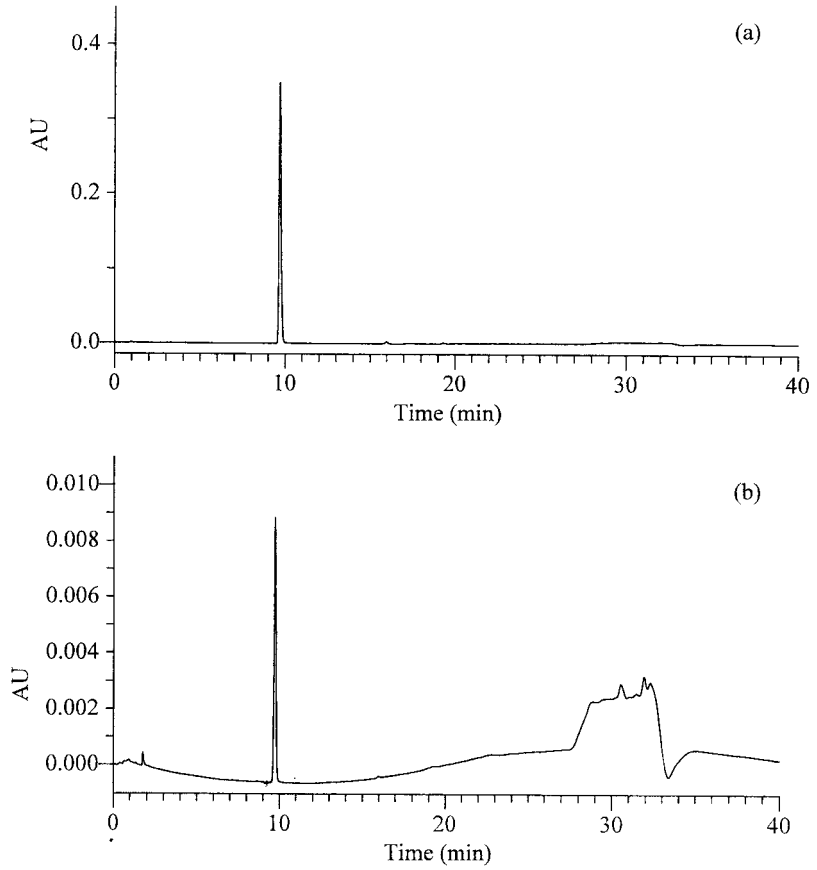

Figure 2. Chromatograms of (a) standard preparation and (b) limit standard preparation.

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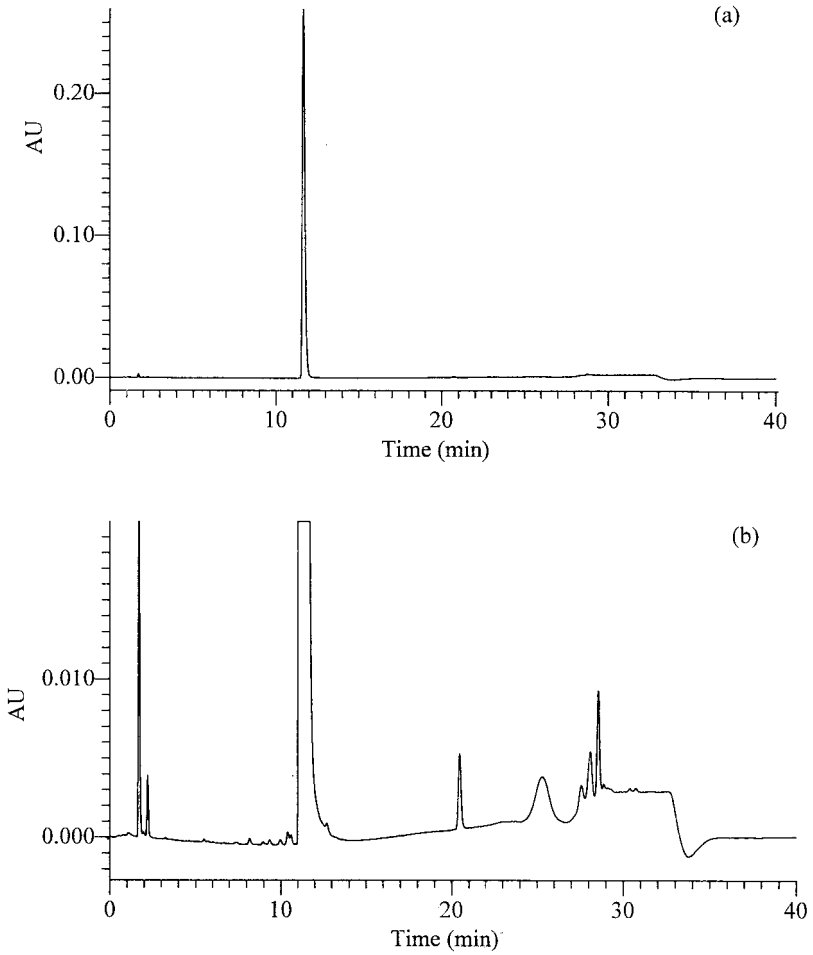

Figure 3. Chromatograms of (a) assay preparation and (b) related substance preparation.

Linearity

The linearity of amiodarone HCl was evaluated over the range of 50–150 μg/mL (50–150% of nominal standard concentration). Five standard solutions were prepared at approximately 50, 75, 100, 125, and 150% of 100 μg/mL. A linear response was determined. A correlation coefficient of 1.000 was reported (Table 2).

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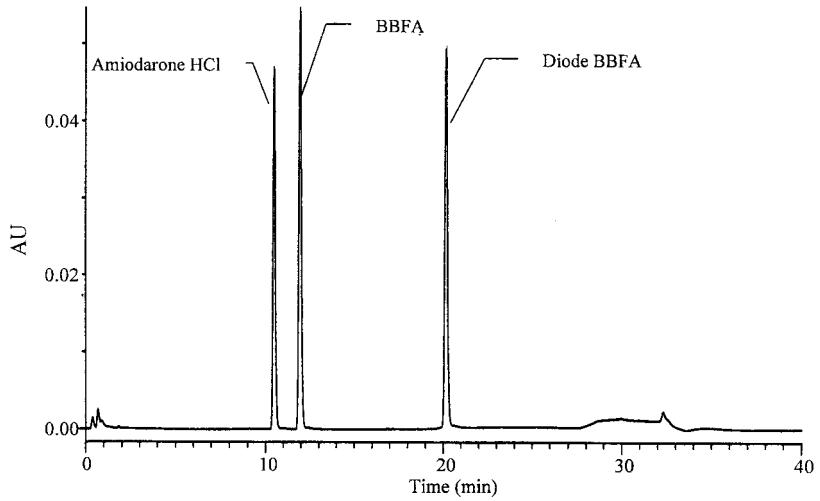

Figure 4. Chromatogram of amiodarone HCl, BBFA, and diode BBFA.

In addition, the linearity of amiodarone HCl, BBFA, and diode BBFA were evaluated in drug product matrix over the range of 1–3 μg/mL (0.1–0.3% of the related substance preparation). Five standard solutions were prepared at approximately 50, 75, 100, 125, and 150% of 2 μg/mL in drug product matrix held at the nominal sample concentration. Each solution was analyzed six times and data analysis performed. A linear response was determined for amiodarone HCl, diode BBFA, and BBFA (Tables 3–5).

Table 2. Linearity of Amiodarone HCl 50–150 μg/mL.

Level	Concentration (μg/mL)	Mean peak area response	Mean response ratio
1	50.13	1509061	30103
2	70.18	2112915	30107
3	100.26	3015574	30078
4	120.31	3624058	30123
5	150.39	4527986	30108

Note: Slope: 30116; y-intercept: −1076; correlation: 1.000.

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Table 3. Linearity of Amiodarone HCl 1–3 μg/mL.

Level	Concentration (μg/mL)	Mean peak area response	Mean response ratio
1	0.9948	31549	31713
2	1.492	46661	31274
3	1.990	62252	31283
4	2.487	75416	30324
5	2.984	91523	30671

Note: Slope: 29900; y-intercept: 1993; correlation: 0.9996.

Relative Response Factors

The relative responses of both known related substances to amiodarone HCl were determined using the response ratios from the linearity analysis. The relative response factors will be used when calculating results of the known related substances against a standard preparation of amiodarone HCl. The relative retention times of BBFA and diode BBFA were calculated with respects to amiodarone HCl. The relative response factors and relative retention times are presented in Table 6.

Precision

The precision (repeatability and intermediate precision) of the method was determined from one lot of drug product.

Repeatability

Six assay and related substance preparations were analyzed in a single session by Chemist I with HPLC system I. The %RSD of the six assay

Table 4. Linearity of BBFA 1–3 μg/mL.

Level	Concentration (μg/mL)	Mean peak area response	Mean response ratio
1	1.068	32742	30657
2	1.602	49049	30617
3	2.137	65582	30689
4	2.671	81361	30461
5	3.205	98652	30781

Note: Slope: 30719; y-intercept: −157; correlation: 0.9999.

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Table 5. Linearity of Diode BBFA 1–3 μg/mL.

Level	Concentration (μg/mL)	Mean peak area response	Mean response ratio
1	1.035	34222	33065
2	1.552	51463	33159
3	2.070	68279	32985
4	2.587	85044	32874
5	3.105	103727	33406

Note: Slope: 33351; y-Intercept: −483; correlation: 0.9998.

preparations was 0.41%, which is within the acceptance criteria of NMT 2.0%. The %RSD of the six related substance preparations was 0.0%, which is within the acceptance criteria of NMT 5.0% (Table 7).

To assess the intermediate precision of the method, six samples of the drug product were prepared and analyzed for both assay and related substances by Chemist II using different reagent preparations and instruments on a different day. The %RSD of the six assay preparations was 0.47%, which is within the acceptance criteria of NMT 2.0%. The %RSD of the six related substance preparations was 0.0%, which is within the acceptance criteria of NMT 5.0% (Table 7).

These results were compared to those of Chemist I. The %RSD of the 12 assay preparations was 0.53%, which is within the acceptance criteria of NMT 2.0%. The %RSD of the 12 related substance preparations was 0.0%, which is within the acceptance criteria of NMT 5.0% (Table 7).

Accuracy

Accuracy of the method for amiodarone HCl was demonstrated by spiking known amounts of amiodarone HCl reference standard into placebo at 50, 100, and 160% of the nominal concentration (100 μg/mL). Mean recovery results for each level ranged from 101.1% to 101.2%. An overall mean recovery of 101.1% ($n = 9$) was reported (Table 8). This is within the

Table 6. Relative response factors and relative retention times.

Related substance	Relative response factor	Relative retention time
BBFA	1.01	1.05–1.20
Diode BBFA	0.94	1.85–1.95

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Table 7. Repeatability and intermediate precision.

Chemist	Sample	Amiodarone HCl (%wt/wt)	Mean (%) ($n = 6$)	%RSD	%Total related substances	Mean (%) ($n = 6$)	%RSD
I	1	100.1	100.2	0.41	0.20	0.20	0.00
	2	100.2			0.20		
	3	99.9			0.20		
	4	100.0			0.20		
	5	99.8			0.20		
	6	100.9			0.20		
II	1	100.7	100.8	0.47	0.20	0.20	0.00
	2	100.9			0.20		
	3	101.3			0.20		
	4	101.2			0.20		
	5	100.5			0.20		
	6	100.0			0.20		
Overall Mean ($n = 12$):		100.5%			0.2%		
%RSD:		0.53%			0.00%		

Note: Intermediate precision.

Table 8. Amiodarone HCl accuracy.

Level	Amount recovered (mg)	Amount spiked (mg)	%Recovery	Mean (%) ($n = 3$)	%RSD
50%	2.5199	2.4871	101.32	101.2	0.22
	2.5099	2.4871	100.92		
	2.5193	2.4871	101.29		
100%	5.0170	4.9742	100.86	101.1	0.30
	5.0251	4.9742	101.02		
	5.0459	4.9742	101.44		
160%	8.0575	7.9588	101.24	101.1	0.09
	8.0455	7.9588	101.09		
	8.0442	7.9588	101.07		
	Overall Mean ($n = 9$):		101.1%		
	Overall %RSD:		0.19%		

acceptable range of 98.0–102.0%, which demonstrates that the method is accurate.

In addition, accuracy of the method for BBFA and diode BBFA was demonstrated by spiking known amounts of each of the five related substances into the placebo at 50, 100, and 150% of 2 μg/mL (related substance range). Results were calculated using the calculated RRF. Results from both related substances (Tables 9–10) were within 95.0–105.0%, which demonstrates that the method is accurate for each of the five related substances.

Table 9. BBFA accuracy.

Level	Amount recovered (mg)	Amount spiked (mg)	%Recovery	Mean (%) ($n = 3$)	%RSD
50%	55.97	54.51	102.69	102.5	0.53
	56.10	54.51	102.92		
	55.53	54.51	101.89		
100%	111.12	109.01	101.94	102.0	0.20
	111.39	109.01	102.19		
	110.96	109.01	101.79		
150%	168.67	163.52	103.16	102.4	0.65
	167.16	163.52	102.23		
	166.58	163.52	101.87		
	Overall Mean ($n = 9$):		102.3%		
	Overall %RSD:		0.5%		

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Table 10. Diode BBFA accuracy.

Level	Amount recovered (mg)	Amount spiked (mg)	%Recovery	Mean (%) ($n = 3$)	%RSD
50%	51.39	51.99	98.85	98.9	0.12
	51.50	51.99	99.05		
	51.39	51.99	98.84		
100%	102.37	103.98	98.45	98.3	0.45
	102.56	103.98	98.64		
	101.70	103.98	97.80		
150%	154.64	155.97	99.15	98.4	0.65
	153.29	155.97	98.28		
	152.68	155.97	97.89		
	Overall Mean ($n = 9$):		98.5%		
	Overall %RSD:		0.49%		

Specificity

Samples of drug product were subjected to acid, base, heat, light, and peroxide degradation conditions to force degradation. Samples were analyzed using a photodiode array detector over the range of 210 nm to 360 nm. Spectral purity is demonstrated when the purity angle is less than the purity threshold. Results are presented in Table 11. Since the purity angle for all degraded samples is less than the purity threshold, this method is deemed to be stability-indicating.

Quantitation Limit

The quantitation limit is defined as the concentration of analyte that gives a signal-to-noise ratio of about 10 with a %RSD of ten replicate injections of

Table 11. Specificity.

Degradation condition	Amiodarone HCl (% wt/wt)	Purity angle	Purity threshold
0.1 N HCl (24 hours)	99.1	0.049	0.223
0.1 N NaOH (24 hours)	98.8	0.047	0.224
10% v/v H_2O_2 (24 hours)	97.5	0.052	0.224
Heat, 60°C (24 hours)	97.2	0.052	0.223
Light (1.2 million lux·hours, 489 watt·hrs/m^2)	87.1	0.013	0.226

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Table 12. Quantitation limit.

Analyte	QL (μg/mL)	Mean S/N ratio	%RSD
Amiodarone HCl	0.050	13.3	4.8
BBFA	0.050	10.0	3.7
Diode BBFA	0.062	11.5	1.9

NMT 5.0. The quantitation limits for amiodarone HCl and each of the five known related substances are listed in Table 12.

Detection Limit

The detection limit is defined as the concentration of analyte that gives a signal-to-noise ratio of about 3. The detection limits for amiodarone HCl and each of the five known related substances are listed in Table 13.

Solution Stability

The stability of standard and sample preparations were determined over 48 hours. Standard and sample preparations were stored at 25°C under laboratory light conditions. Solutions were analyzed at 0, 24, and 48 hours against fresh standard preparations. Results were evaluated for the percent difference from time zero (Tables 14 and 15). Less than 2.0% difference was

Table 13. Detection limit.

Analyte	DL (μg/mL)	Mean S/N ratio
Amiodarone HCl	0.025	4.1
BBFA	0.016	3.4
Diode BBFA	0.020	3.3

Table 14. Standard solution stability.

Time (hours)	Working standard	%Difference	Limit standard	%Difference
0	49.56	NA	50.87	NA
24	49.51	0.10	49.89	1.9
48	49.87	0.63	49.50	2.7

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Table 15. Sample solution stability.

Time (hours)	Assay (%)	%Difference	Related substances (%)	%Difference
0	101.1	NA	0.3	NA
24	100.0	1.06	0.3	0.0
48	101.1	0.03	0.3	0.0

observed for the assay level solutions and less than 5.0% was observed for the related substance level solutions. This demonstrates that the solutions are stable for up to 48 hours following their preparation when stored at ambient temperature under laboratory light conditions.

CONCLUSION

The aforementioned gradient HPLC method for the analysis of amiodarone HCl and its related substances in Amiodarone HCl Injection was evaluated for linearity, relative response factor of the known related substances, precision (repeatability and intermediate), accuracy, specificity, detection limit, quantitation limit, and solution stability. The method was demonstrated to be both accurate and precise over the range established for amiodarone HCl and two of its known related substances. In addition, specificity of the method was shown under acid, base, heat, peroxide, and light stress conditions, which demonstrates that the method is stability-indicating.

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