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DEVELOPMENT AND VALIDATION OF GAS CHROMATOGRAPHY METHOD FOR THE DETECTION OF RESIDUAL ETHANOL, ISOPROPYL ALCOHOL AND TOLUENE IN AMIODARONE HYDROCHLORIDE DRUG SUBSTANCE

Andrew Joseph D'Souza [*1, 2], R. S. Lokhande [1] and Tushar Anvekar [2]

Jaipur National University [1], Jaipur - 302017, Rajasthan, India.
Department of Chemistry [2], St. Xaviers College, Mapusa - 403507, Goa, India.

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Correspondence to Author:
Dr. Andrew Joseph D'Souza

Department of Chemistry, St. Xaviers College, Mapusa - 403507, Goa, India.

E-mail: andrewdsouza87@gmail.com

ABSTRACT: A simple and reliable head space Gas Chromatographic method has been developed for the determination of residual ethanol, isopropyl alcohol and toluene in Amiodarone hydrochloride drug substance. The proposed method is based on flame ionization detection technique with DB 624 as stationary phase. Linearity of detector response was established up to 150 % of the limit level for ethanol, isopropyl alcohol and toluene and the quantitation limit was 80 ppm for ethanol, 40 ppm for isopropyl alcohol and 4 ppm for toluene respectively. Performance of the method was assessed by evaluating the recovery, repeatability, reproducibility, linearity and limits of detection and quantification. The proposed method has a potential for application to drug substances which may contains traces of residual solvents. Results prove that the validated method was suitable for determining the residual ethanol, isopropyl alcohol and toluene in Amiodarone hydrochloride drug substance.

INTRODUCTION: Amiodarone hydrochloride belongs to a class of drugs called Vaughan-Williams class III antiarrhythmic agents. It is used in the treatment of a wide range of cardiac tachyarhthmias, including both ventricular and supraventricular (atrial) arrhythmias. After intra-venous administration in man, Amiodarone relaxes vascular smooth muscle, reduces peripheral vascular resistance (afterload), and slightly increases cardiac index. Amiodarone prolongs phase 3 of the cardiac action potential. It has numerous other effects however, including actions that are similar to those of antiarrhythmic classes Ia, II, and IV.

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Amiodarone shows beta blocker-like and calcium channel blocker-like actions on the SA and AV nodes, increases the refractory period via sodium- and potassium-channel effects, and slows intra-cardiac conduction of the cardiac action potential, *via* sodium-channel effects. Chemically Amiodarone hydrochloride is (2-Butyl-3-benzofuranyl) [4-[2-(diethylamino) ethoxy] -3, 5- diiodophenyl] methanone hydrochloride. The molecular formula is $C_{25}H_{29}I_2NO_3$.HCl and the molecular weight is 681.778 g/mol [1].

Traces of residual ethanol, isopropyl alcohol and toluene maybe present in Amiodarone hydro-chloride at the salt formation step, as Amiodarone is extracted in toluene and is further treated with hydrochloric acid leading to precipitate of Amiodarone hydrochloride. Crude Amiodarone hydrochloride is washed with Isopropyl alcohol. Thus ethanol, isopropyl alcohol and toluene are present as residual solvents in Amiodarone hydrochloride.

Toluene is a class 2 residual solvent ethanol and isopropyl alcohol is class 3 residual solvents [2]. Therefore, it is necessary that, these residual solvents should be controlled to limits permitted by ICH guidelines. Residual solvents are the undesired substances (solvents) used or produced during the manufacture of excipients, drug or pharmaceutical formulation and are not completely removed by practical methods in the final finished product. These solvents can be toxic in nature. Therefore, analysis of residual solvents becomes a necessary tool for the quality control of pharmaceuticals. The acceptable limits for these substances are given in ICH guidelines (Guideline for Residual solvents, Q3C) [2].

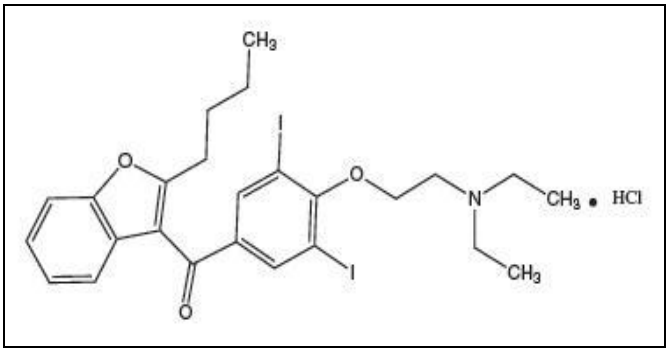


FIG. 1: CHEMICAL STRUCTURE OF AMIODARONE HYDROCHLORIDE

Many HPLC methods were developed for the determination of Amiodarone and its impurities. Lacroix *et al.*, [3] has developed in 1994 an LC-method for determination of Amiodarone hydrochloride and its related compounds in raw materials and tablets [3]. Thyagarajapuram *et al.*, [4] has developed an LC-method for the determination of Amiodarone hydrochloride in tablet and injectable formulations [4]. An HPLC method was also developed and validated for the determination of Amiodarone hydrochloride and its related compounds in Amiodarone hydrochloride injections by Christopherson *et al.* [5]. In 2005, an HPLC method was developed and validated for the determination of Amiodarone hydrochloride and its related substances [5]. No analytical methods have been reported in the literature for the determination of residual solvents in Amiodarone hydrochloride. HPLC determination has been reported but no specific gas chromatographic methods have been reported till date, for the quantitative determination of residual ethanol, isopropyl alcohol and toluene in Amiodarone hydrochloride drug substance.

MATERIALS AND METHODS:

Chemicals and Reagents: Ethanol, isopropyl alcohol and toluene were procured from Merck. All reagents used were of analytical GC reagent grade. HPLC water was obtained from Merck Limited. Sample under investigation was obtained from Glenmark Pharma Ltd., India.

Gas Chromatography Conditions: Instrumental Gas Chromatographic system of Perkin Elmer GCHS with FID, Clarus 680 GC controlled by Total chrome software version 6.3.2. was used. For the separation of ethanol, isopropyl alcohol and toluene, high purity helium was used as carrier gas and purge gas.

TABLE 1: GAS CHROMATOGRAPH CONDITIONS

Instrument	:	PerkinElmer Clarus 680 GC
Column	:	DB 624, 30 m × 0.32 mm, 1.8 µm
Injector	:	Capillary Split-Splitless Injector (CAP)
Liner	:	4 mm liner filled with Silanized wool
Injector temperature	:	200 °C
Detector Temperature	:	230 °C
Carrier gas & flow rate	:	Helium @ 1.0 mL/min
Split (ratio)	:	5:1
Oven program	:	Initial 40 °C hold for 5.0 min
	:	Ramp rate 1: 5 °C/ min to 60°C hold for 0.0 min
	:	Ramp rate 2: 30 ° C/ min to 210°C hold for 4.0 min
Run time	:	18.00 min.
Detector	:	FID (Range 1, Attenuation -6)
Sample injection	:	Headspace
Diluent	:	NMP: Water (1.4**:**0.6) v/v
Headspace parameters		
Instrument	:	Perkin Elmer Turbo Matrix 40
GC HS vial temperature	:	100 °C
Needle temperature	:	110 °C
Transfer line temperature	:	120 °C
GC cycle time	:	25.00 min
GC HS vial equilibration time	:	30.00 min
Pressurization time	:	3.00 min
Injection time	:	0.06 min
Withdrawal time	:	0.20 min
Column pressure (PPC)	:	16 Psi

The separation was performed on DB-624 30 m long, 0.32 mm I.D. and 1.8 µm film thickness capillary column. The carrier gas flow was adjusted at 1.0 mL/min using constant flow mode and the

sample was introduced with split ratio 5:1. The capillary injector temperature set at 200 °C and FID-detector set at 230 °C. The column oven temperature was 40 °C hold for 5 min, further the temperature ramp set at 5 °C/min to attain 60 °C, and then temperature ramp set at 30 °C/min to attain 210 °C, hold for 4.0 min, the chromatograph stop time was set to 18 min. The head space Perkin Elmer Turbo Matrix 40 was used, the sample injection time set at 0.06 min. The sample in sealed head space vials was incubated at 100 °C for 30 min. The syringe temperature set at 110 °C and the transfer line temperature at 120 °C.

Diluent: N-Methyl -2- pyrrolidone(NMP): water (1.4:0.6) v/v.

Blank Solution: Took 2.0 ml of diluent in a headspace vial and sealed the vial immediately.

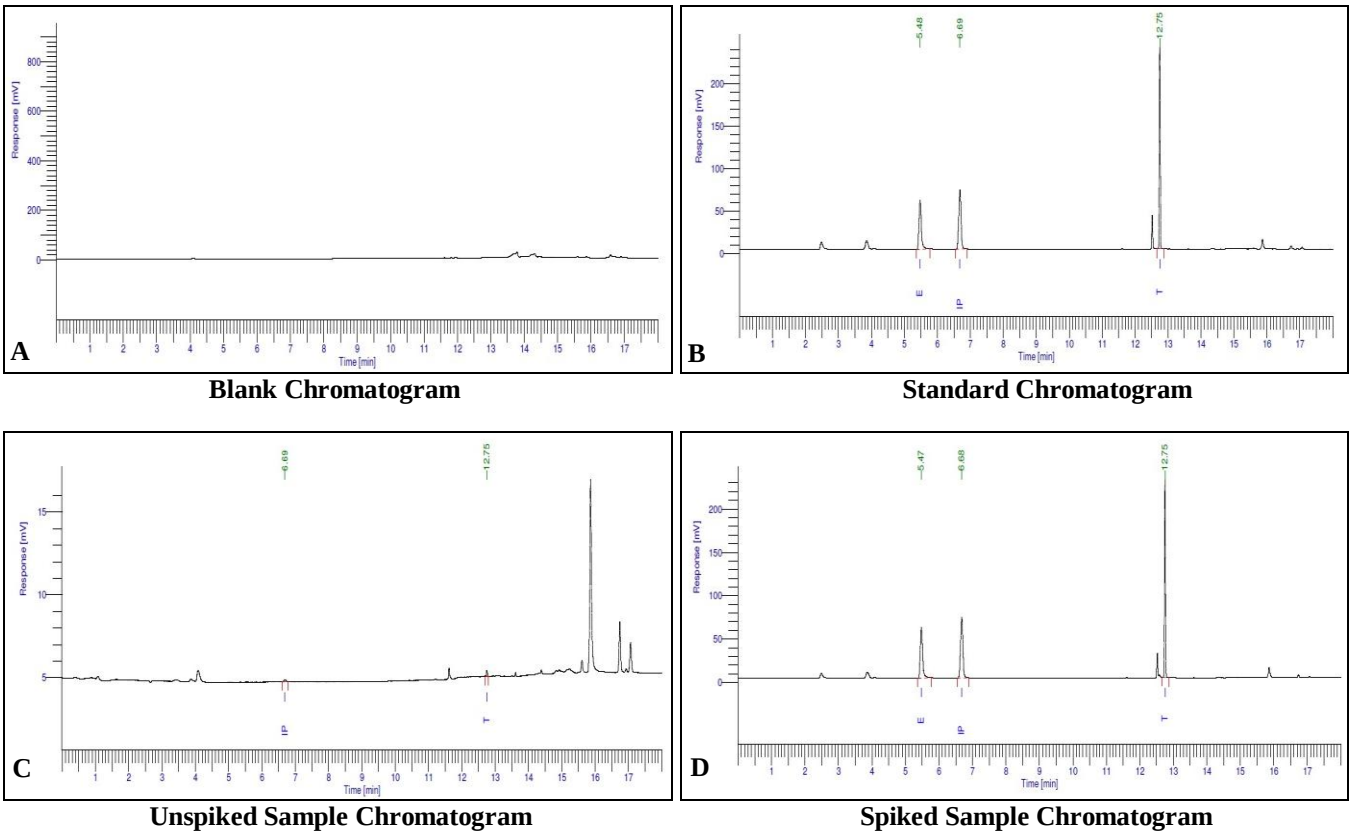
Standard Solution: Accurately measured and transferred 250 mg of ethanol, 250 mg of isopropyl alcohol and 44.5 mg of toluene into a 50 ml clean, dry volumetric flask containing about 25 ml of diluent, further diluted 10.0 ml of this solution to 100.0 ml with diluent.

Sample Solution: Accurately weighed and transferred about 0.2 g of sample to the headspace vial added 2 ml of diluent and sealed the vial immediately.

Spiked Sample Solution: Accurately weighed and transferred about 0.2 g of sample to the headspace vial added 2 ml of standard solution and sealed the vial immediately.

RESULTS AND DISCUSSION:

Method Development and Optimization Summary: Preliminary experiments were carried out based on the retention of ethanol, isopropyl alcohol and toluene, which were discussed in many gas chromatographic applications. Polar stationary phase supelcowax 10 with water as diluent gave coeluting peaks of isopropyl alcohol and ethanol. The separation was achieved on DB-624 column (containing 6% cyanopropylphenyl and 94% dimethylpolysiloxane copolymer) of 30 m length, 0.32 mm internal diameter and film thickness of 1.8 µm with helium as carrier gas. In the diluent composition NMP was initially used, as low recovery was observed for toluene.



Blank Chromatogram **Standard Chromatogram**

Unspiked Sample Chromatogram **Spiked Sample Chromatogram**

FIG. 2: REPRESENTATIVE CHROMATOGRAM OBTAINED FROM (A) BLANK, (B) STANDARD, (C) SAMPLE, AND (D) SPIKED SAMPLE

Therefore, NMP: water (1.4:0.6) v/v was used. The peak shape and response of residual ethanol, isopropyl alcohol and toluene were satisfactory. Validation of the method was carried out in order to judge the suitability of method for determining the ethanol, isopropyl alcohol and toluene traces in Amiodarone hydrochloride drug substance, the method was validated as per the ICH guideline [6], for specificity, limit of detection, limit of quantification, linearity, accuracy, precision and robustness.

Specificity: To assess the ability of the method, individual identification solutions were prepared with known amount of ethanol, isopropyl alcohol and toluene at limit level with respect to Amiodarone hydrochloride drug substance concentration and injected in to the gas chromatograph and the chromatograms were recorded. The sample solution and spiked sample solution was prepared as per the methodology and injected into the chromatograph.

The retention time for ethanol, isopropyl alcohol, toluene is 5.48, 6.69 and 12.75 respectively. The resolution between ethanol and isopropyl alcohol and isopropyl alcohol and toluene was good. No interference of blank was observed corresponding to ethanol, isopropyl alcohol and toluene peaks and the solvent peaks were well resolved.

LOD and LOQ: For determining the limit of detection (LOD) and limit of quantification (LOQ) values of each residual solvent standard solution with increasing concentrations were prepared and injected into the system and the areas determined. Graph of concentration v/s area was plotted and limit of detection (LOD) and limit of quantification (LOQ) were calculated based on the following formula.

LOD: STEYX × 3.3 / slope

LOQ: STEYX × 10 / slope

The limit of detection (LOD) and limit of quantification (LOQ) solutions of Ethanol, Isopropyl alcohol and Toluene for LOD and LOQ evaluation were prepared at predicted concentration levels and precised by analysing six times, detailed in **Table 2**.

TABLE 2: EVALUATION OF LOD AND LOQ, LINEARITY DATA

Components	Ethanol	Isopropyl alcohol	Toluene
Limit ppm	5000 ppm	5000 ppm	890 ppm
Limit of detection ppm	30 ppm	13 ppm	1 ppm
Limit of quantitation ppm	90 ppm	40 ppm	4 ppm
LOD (%RSD) a	16.05 %	19.65 %	17.45 %
LOQ (%RSD) a	2.07 %	3.45 %	2.09 %
Slope	41.830	47.234	141.102
STEYX	117.9895	62.6070	43.8150
Correlation co-efficient	1.0000	0.9999	1.0000

a Average of n=6 determinations

Linearity: The linearity of the method was determined by preparing a series of dilutions by using ethanol, isopropyl alcohol and toluene at concentration from LOQ level to 150% of the limit level of ethanol (5000 ppm), isopropyl alcohol (5000 ppm) and toluene (890 ppm) using GC-FID detector response. The concentration studied ranges between LOQ to 7500 ppm for ethanol, LOQ to 7500 ppm for isopropyl alcohol and LOQ to 890 ppm for toluene respectively. The statistical parameters slope, intercept and correlation co-efficient values were detailed in **Table 2**.

Precision: The precision was the study of the method using repeatability and reproducibility (ruggedness). The method performance was evaluated with replicate injections of the standard and sample solutions. Standard solution was analysed six times for checking the performance of the Gas Chromatography system under the chromatographic conditions on the day tested (System precision), the % RSD for peak area of replicate standard injections was found to be 0.54%, 0.25% and 0.61% for ethanol, isopropyl alcohol and toluene respectively.

Repeatability was intra-day variation (Method precision) and inter-day variation (ruggedness). The repeatability of the method was studied by analysing six sample solutions separately by the

addition of residual ethanol, isopropyl alcohol and toluene at known limit level concentration. % RSD for solvent content was found to be 1.25%, 0.94% and 0.92% for ethanol, isopropyl alcohol and toluene respectively. The degree of reproducibility is known as ruggedness, obtained by the analysis of the sample under a variety of conditions using a different Instrument and column, with different user on different day by using new standard and sample preparations. % RSD for solvent content was found to be 1.34%, 0.81% and 3.41% for ethanol, isopropyl alcohol and toluene respectively.

Accuracy: The accuracy of the method was evaluated by recovery experiment using standard addition technique. The recoveries were determined by spiking the respective residual solvent at four different levels ranging from LOQ to 150% of the limit level of the residual solvent. Ethanol (5000 ppm), isopropyl alcohol (5000 ppm) and toluene (890 ppm) with respect to Amiodarone hydrochloride drug substance. The samples were prepared as per the methodology, analysed in triplicate and percentage recoveries were calculated. The average recovery values were summarized in **Table 3**.

Robustness: To assess the robustness of the method, experimental conditions were deliberately altered. The study was carried out with respect to flow rate of mobile phase ± 10% and initial temperature of the column oven 40 °C ± 2 °C. The system suitability results met the acceptance criteria at each of the deliberately varied conditions. There is no much variation in the retention time of ethanol, isopropyl alcohol and toluene obtained at different deliberately varied conditions from the developed methodology. Hence the test method is robust for all varied conditions.

TABLE 3: ACCURACY EXPERIMENTAL DATA

Component	Ethanol				Isopropyl alcohol				Toluene			
Target level (%)	LOQ	50	100	150	LOQ	50	100	150	LOQ	50	100	150
Spike conc. (ppm) b	80	2500	5000	7500	40	2500	5000	7500	4	300	890	1300
Percent recovery b	108.1	96.2	100.5	102.6	91.1	102.1	101.2	102.7	101.1	104.7	99.2	99.8
Average % recovery	101.9%				99.3%				101.2%			

b Average of n = 3 determinations

Potential Application of the Method: The potential application of the method has been conducted for drug substances containing possible residual ethanol, isopropyl alcohol and toluene in their drug matrix.

CONCLUSION: The developed gas chromatographic method gives reliable and economical results for the simultaneous determination of ethanol, isopropyl alcohol and toluene residual solvents present in Amiodarone hydrochloride. The results of various validation parameters confirmed that the method is specific, robust, linear, precise and accurate. The method has been applied to various drug substances containing possible ethanol, isopropyl alcohol and toluene in the drug matrix. The experimental data shows that the method has potential application for the quantitative determination of ethanol, isopropyl alcohol and toluene present in the drug substances.

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CONFLICT OF INTEREST: None

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