

VALIDATION OF A NEW HPLC METHOD USED FOR DETERMINATION OF AMIODARONE FROM THE COMPLEX WITH HYDROXYPROPYL-BETA-CYCLODEXTRIN AND FROM COMMERCIAL TABLETS

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Abstract

This paper presents the development and validation of a new, rapid, specific and simple HPLC method with UV detection used for the quantitative determination of amiodarone from its inclusion complex with hydroxypropyl-*beta*-cyclodextrin and from commercially available Cordarone[®] tablets. A HP 1090 Series II liquid chromatograph with multidiode detector and Thermo Fisher-Hypersil Betasil C18 (150 mm x 4.6 mm; 5 μ m) chromatographic column was used. The UV detection wavelength was 254 nm and the retention time for amiodarone was 4.51 minutes. The method was linear in the concentration range 0.001-0.1 mg/mL, the detection limit was established to be 0.001986 mg/mL, the quantification limit was 0.006621 mg/mL, while the regression coefficient was $r^2 = 0.9996$. Various validation parameters were studied: the precision of the system (RSD = 0.84%), the precision of the method (RSD = 1.06%) and the accuracy of the method (98.48% in the concentration range 97.33–100.03%).

Rezumat

Lucrarea prezintă elaborarea și validarea unei noi metode HPLC, specifice și simple, cu detecție în UV, utilizată la determinarea cantitativă a amiodaronei din complexul de incluziune hidroxipropil-*beta*-ciclodextrină/amiodaronă și din comprimate de Cordarone[®]. S-a folosit un cromatograf de lichide tip HP 1090 Series II, prevăzut cu detector cu multidiode, coloană cromatografică tip Thermo Fisher- Hypersil Betasil C18 (150 mm x 4,6 mm; 5 μ m). Lungimea de undă de detecție a fost de 254 nm, timpul de retenție pentru amiodaronă a fost de 4,51 minute. Metoda a fost liniară în domeniul de concentrație cuprins între 0,001 și 0,1 mg/mL, limita de detecție s-a înregistrat la 0,001986 mg/mL, limita de cuantificare a fost la 0,006621 mg/mL, iar coeficientul de regresie a fost $r^2 = 0,9996$. S-au studiat diverși parametri statistici: precizia sistemului (RSD = 0,84%), precizia metodei (RSD = 1,06%) și exactitatea (98,48% în domeniul 97,33 – 100,03%).

Keywords: amiodarone, validation, HPLC method, applications.

Introduction

Amiodarone hydrochloride (AMD) is an antiarrhythmic agent used for severe rhythm disorders such as supraventricular arrhythmias, rapid ventricular rhythm disorders, tachycardia within the Wolff–Parkinson–White syndrome, confirmed ventricular arrhythmias, symptomatic and the treatment of less severe ventricular arrhythmias, including atrial fibrillation [1,2].

Amiodarone hydrochloride (2-butyl-3-benzofuranyl 4-[2-(diethyl amino)-ethoxy]-3,5-diiodophenyl methanone hydrochloride) is a benzofurane derivative. It is a white or pale yellow, fine, crystalline powder, freely soluble in chloroform and dichloromethane, soluble in methanol and ethanol, but very slightly soluble in propanol and water [3].

It is imperative to develop and validate an assay method for the quantitative determination of AMD from the inclusion complex with hydroxypropyl- β -cyclodextrin (HP- β -CD) that has a high potential for developing a new oral pharmaceutical formulation with modified release, as well as for the analysis of free amiodarone during dissolution tests of commercial tablets.

Materials and Methods

Reagents

Two batches of amiodarone hydrochloride were purchased from Zhejiang Sanmen Hengkang Pharmaceutical Co. Ltd., China and from Sigma-Aldrich (SUA). HP- β -CD 99.70 % pure substance was purchased from Roquette, France. The HP- β -CD/AMD inclusion complex was synthesized by the scientists from “P. Poni” Macromolecular Chemistry Research Institute Iasi. Other reagents used were: formic acid (Merck), chromatographically pure methanol (Merck) and bidistilled water.

Sample preparation

The stock solution was prepared by completely dissolving 25 mg of amiodarone hydrochloride (reference substance) in 25 mL methanol. Any further dilutions were done using mobile phase.

For the analysis of AMD from Cordarone[®], 20 tablets were ground into powder. A 0.05 mg/mL AMD solution was obtained by dissolving the corresponding quantity of powder in 100 mL methanol and further successive dilutions with mobile phase.

For the analysis of the HP- β -CD/AMD inclusion complex, a 0.0155 mg/mL AMD solution was used by dissolving 30 mg HP- β -CD/AMD

inclusion complex in 100 mL methanol. A volume of 0.5 mL solution was diluted to 50 mL with mobile phase.

Chromatographic conditions

The chromatographic method was performed on a high performance liquid chromatograph type Thermo Fisher Surveyor, equipped with DAD (Diode Array Detector) UV-VIS detector, quaternary pump and autosampler. The experimental setup also involved the use of a Kern 770 analytical balance, an ultrasound bath and a magnetic stirrer.

The analytical method used a stainless steel chromatographic column, from Thermo Fisher, with C18 stationary phase (Hypersil Betasil C18, 150 mm x 4.6 mm; 5 μ m).

The temperature of the column was maintained at 45°C. As mobile phase, a mixture of formic acid 0.5% in phosphate buffer solution pH 7.6 and methanol with a volumetric ratio of 25:75 was used and purged with 0.7 mL/min constant flow. For each and every determination the injected volume was 20 μ L, while detection was performed 254 nm. The retention time recorded for amiodarone was 4.51 minutes.

For quantitative determination of amiodarone from commercial available tablets and from inclusion complex, the method was validated by means of method linearity, limit of detection, limit of quantification, selectivity, accuracy and precision.

Results and discussion

Method development

Method development involved the optimization of retention times of analyte by using various types of mobile phase compositions and other parameters such as flow-rates and column temperature during analysis. The development involved the monitorization of the results using several pH values between 2.1 and 8, different proportions of the organic phase component (methanol) and various changes of temperature.

We have considered the setting of a pH of 7.6 with 2 pH units above the pKa of amiodarone hydrochloride (5.6), a value of pH close to reference (4.9) as well as the use of an acidic solution. In these circumstances, we have used formic acid whose concentration was 0.5%. Retention time values were found to be directly proportional with the pH value used, at the pH = 7.6 the signal of amiodarone hydrochloride was undetected because of the high retention time. Further steps were considered the optimization of methanol content and temperature whose values contributed significantly to the retention time reduction, but also improved the theoretical plates for separation and peak asymmetry.

As long as, the concentration of methanol was higher, the retention time decreased. Thus, a proportion of about 40% of the aqueous phase at a flow rate of 0.7 mL/min produced a retention time for amiodarone hydrochloride of about 10.8 minutes, so that the decrease in concentration caused the optimizing of retention time to about 6 minutes. However, the shape of the signal indicates a low efficiency in separation with a value of about 3000 theoretical plates. An increase in temperature from 30°C to 45°C decreased the retention time of about 4.5 minutes and produced an increase of theoretical plates up to the a value of 8500 and an asymmetry of 1.25. The synthesis of the development steps is presented in the table I.

Table I

Influence of the method parameters on the retention time and theoretical plates for the amiodarone hydrochloride peak

Organic phase	Aqueous phase		Temperature	Retention time	Theoretical plates
%MeOH	%	pH	T(°C)		
60	40	7.6	30	-	-
		4.9	30	46	-
		2.1	30	37	-
75	25	2.1	38	6.2	3000
75	25	2.1	45	4.5	8500

The selectivity of the method was performed against blank solutions reconstituted by the matrix components for inclusion complex HP- β -CD and also by the components used for Cordarone[®] tablets (lactose, starch, povidone, colloidal silica and magnesium stearate). In this situation, four solutions were analyzed by HPLC: the blank solutions, a 0.1 mg/mL amiodarone hydrochloride (reference substance) solution and a solution of complex with the theoretical concentration of 0.03 mg/mL amiodarone hydrochloride. The presence of the amiodarone hydrochloride peak was observed at 4.51 minutes only for the third and fourth solutions (Figure 1).

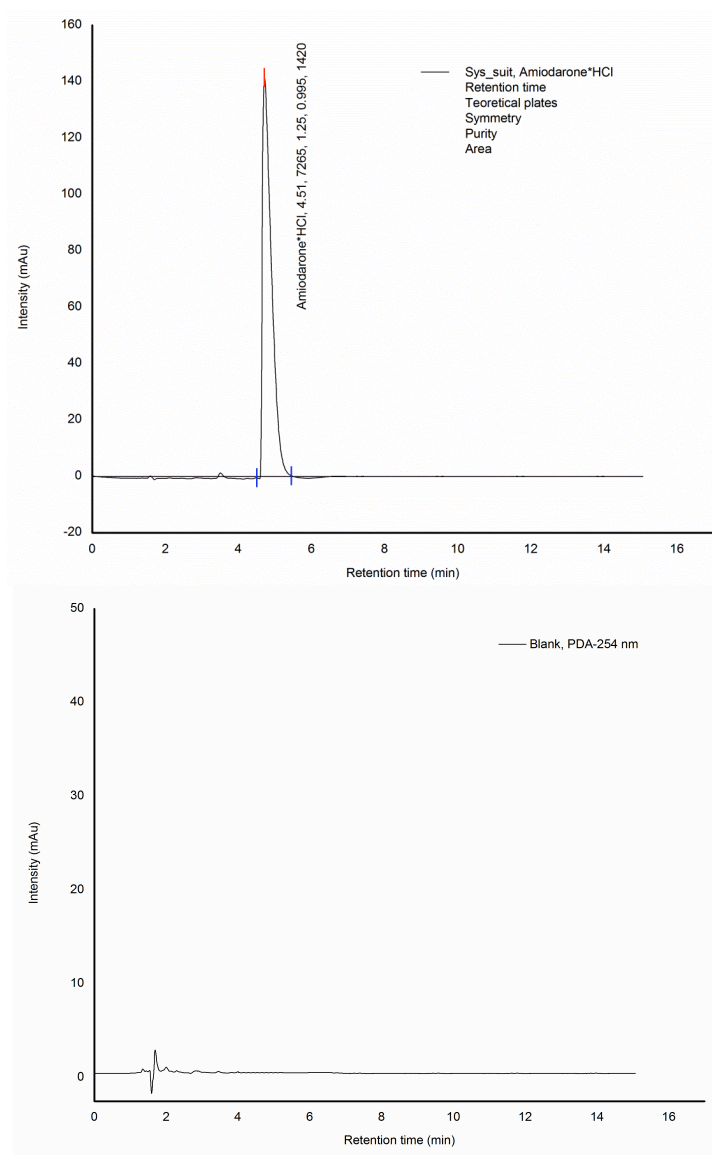


Figure 1

Chromatogram of 0.1 mg/mL amiodarone hydrochloride solution and that of the blank solution using the matrix components of inclusion complex HP- β -CD

In order to study the linearity of the method the calibration curve was plotted for the concentration range between 0.001 and 0.1 mg/mL AMD. Three independent experimental series were processed for each level of concentration. Based on the recorded chromatograms we determined the area of each peak (Table II).

The calibration curve was obtained by plotting the area of the AMD peak against the concentration of amiodarone as mg/mL (Figure 2).

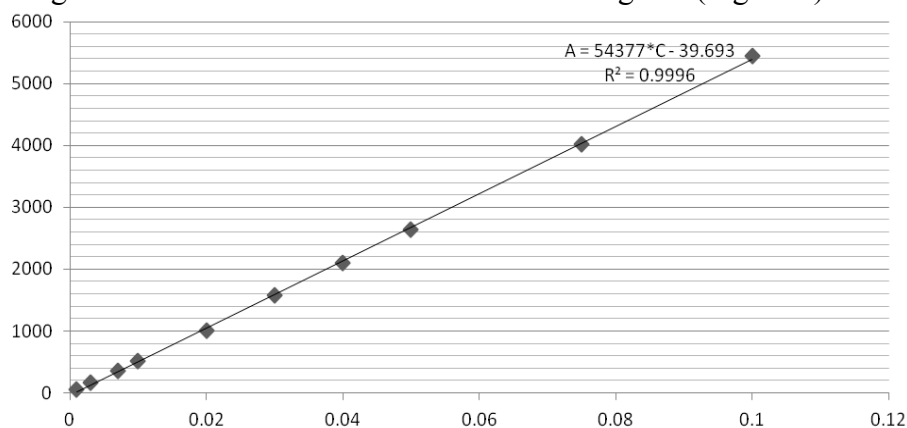


Figure 2
Calibration curve

The validation parameters that were calculated are: correlation coefficient $r = 0.9998$, regression coefficient $r^2 = 0.9996$, standard error $SE = 35.9961$, slope = 54376.74 and intercept = - 39.70.

The limit of detection (LOD) and the limit of quantification (LOQ) were calculated using the following formulas [4-9]:

$LOD = 3.3 \cdot \text{standard error} / \text{slope} = 3.3 \cdot 35.9961 / 54376.74 = 0.002185$ (mg/mL)

$LOQ = 10 \cdot \text{standard error} / \text{slope} = 10 \cdot 35.9961 / 54376.74 = 0.00662$ (mg/mL)

The limit of quantification was high enough for the accurate determination of amiodarone hydrochloride during dissolution tests. For the study of the system precision, a 0.05 mg/mL solution, prepared using amiodarone hydrochloride reference substance, was injected ten times subsequently (Table II). The injection repeatability was proved by the invariability of the peak area against the retention time, thus proving the system precision (system suitability).

In order to evaluate the precision of the method, 10 determinations were done in two days by two different analysts [5-9].

Inter and intraday precision was evaluated using the ANOVA test, two factors with replicates. The factors were associated with the variables used in intermediate precision (days and analyst) and we have concluded that it was no difference between the groups (analyst) ($p > 0.05$). However, there are significant differences when it is performed the evaluation of the

variable (days) also at the interaction between the groups (analyst) and variables (days) ($p < 0.05$) for the area and for the retention time.

The accuracy of the method [5-9] was determined using 3 concentration levels for amiodarone hydrochloride (Table II).

After statistically processing the experimental data, it was concluded that the new analytical method was precise, because the relative standard deviation (RSD) was lower than the maximum limit of 2.5% and the method accuracy was confirmed by an average recovery of 98.48% for values within 97.33 and 100.03 %.

All the validation data is summarized in the table II:

Table II

Evaluation of parameters used for the validation of the method

Inter-day precision (area) M, SD, RSD%	2575.6	2165487	0.84
Inter-day precision (retention time) M, SD, RSD%	4.746	0.08124	1.71
Intra-day precision (area)M, SD, RSD%	2528.05	66.699	2.63
Intra-day precision (retention time) M, SD, RSD%	4.650	0.186	4.01
Linearity, correlation coefficient	0.996		
Concentration range 0.001 mg/mL – 0.1 mg/mL	Theoretical concentrations (mg/mL)	Calculated concentration (mg/mL)	Recovery %
Accuracy % recovery – amiodarone hydrochloride	0.03	0.02998	99.96
	0.03	0.02932	97.79
	0.03	0.02960	101.25
	0.04	0.03991	99.80
	0.04	0.03944	98.80
	0.04	0.03901	98.93
	0.05	0.04928	98.56
	0.05	0.04946	100.37
Statistical data			M
			SD
			RSD
			99.38%
			1.057
			1.06 %

The validated analytical method was used for the quantitative determination of AMD from the inclusion complex HP- β -CD/AMD and from a commercially available formulation: Cordarone[®] tablets (Table III).

Table III

The assay of amiodarone from the inclusion complex and from Cordarone® tablets

Sample	Theoretical Concentration (mg/mL)	Average peak area	Average calculated concentration (mg/mL)	Recovery (%)	Statistical investigation results
Inclusion complex	0.0155	769.33	0.014878	96.13	n = 6 SD = 0.373 RSD = 0.390 %
Cordarone® tablets	0.050	2648.66	0.04943	98.88	n = 6 SD = 0.204 RSD = 0.202 %

Conclusions

The analytical method for the determination of amiodarone was linear in the concentration range 0.001 and 0.1mg/mL with a good correlation coefficient, $r = 0.9998$. The limit of detection was 0.002185 mg/mL and the limit of quantification was 0.00662 mg/mL.

The HPLC method with UV-detection at 254 nm was precise. For the precision of the system, RSD was 0.84%, while the precision of the analytical method had a RSD of 1.06%. The new HPLC method was accurate. The average recovery for amiodarone was 98.48% in the range 97.33-100.03%.

In these conditions, amiodarone was quantified from the inclusion complex HP- β - CD/AMD and from commercially available Cordarone® tablets, with very good results.

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