

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF QUINIDINE IN PHARMACEUTICAL DOSAGE FORMS BY USING RP-HPLC METHOD

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ABSTRACT: The Present work was to develop a simple, fast, accurate, precise, reproducible, Reverse Phase High Performance Liquid Chromatographic Method for estimation of Quinidine in pure drug form. Chromatographic separation was done using Terrosil C18 column having dimension of (100 mm x 4.6 mm) having particle size of 5.0 μm , with mobile phase consisting of Phosphate buffer (KH₂PO₄) pH 3 \pm 0.02 pH adjusted with ortho phosphoric acid and Acetonitrile (25:75 % v/v), flow rate was adjusted to 0.8 ml/min and detection wavelength at 254nm. The retention times of Quinidine was found to be 2.589. The proposed method has been validated for accuracy, precision, linearity; robustness and range were within the acceptance limit according to ICH guidelines. Linearity for Quinidine was found in range of 20 $\mu\text{g/ml}$ -60 $\mu\text{g/ml}$ and correlation coefficient was found to be 0.999, %RSD for intermediate precision was found to be 0.1, for repeatability was 0.2, % mean recovery for Quinidine was found to be 99.77%. The method was found to be robust even by change in the mobile phase \pm 5% and in less flow condition. The developed method can be successfully employed for the routine analysis of Quinidine in API and Pharmaceutical dosage forms.

Keywords: Method development, Validation, Quinidine, Dosage forms.

INTRODUCTION: Quinidine is a medication primarily utilized to manage and treat specific arrhythmias and malaria in patients. This activity reviews quinidine's indications, actions, and contraindications, emphasizing its significance as a valuable agent for treating certain arrhythmias and malaria. Quinidine is a class IA antiarrhythmic agent used to treat heart rhythm disturbances. It is a diastereomer of anti-malarial agent quinine, originally derived from the bark of the cinchona tree. The drug causes increased action potential duration, as well as a prolonged QT interval.

METHOD DEVELOPMENT

Selection of mobile phase:

The method development and validation of Quinidine requires greater resolution. Hence different solvent systems were tried. The trials are using UV 3000+ equipment with PDA detector and isocratic pump. The system controlled by LC solution software.

Selection of flow rate:

The flow rate of Quinidine was tried from 0.8 ml to 1.5ml.

Buffer preparation:

About 7.0g of potassium dihydrogen ortho phosphate was dissolved in 1000ml of HPLC grade water and PH 2.5 was adjusted with ortho phosphoric acid. It was filtered through 0.45 μm nylon membrane filter and degassed with sonicator. It was used as a diluent for the preparation of sample and standard solution.

Preparation of mobile phase:

Mobile phase consist of water: methanol HPLC of PH 2.5 (30:70) was taken, sonicated and degassed for 10 min and filtered through 0.45 μm nylon membrane filter.

Standard Preparation:

Weigh accurately 10mg Quinidine Working Reference Standard and 15mg of Quinidine Working Reference Standard is taken in

to 100ml volumetric flask and then it was dissolved and diluted to volume with mobile phase up to the mark. After that 50ml of the above solution was taken into 100ml standard flask and made up with mobile phase. (Stock solution) Further pipette 0.5ml of the above stock solution in to a 10ml volumetric flask and dilute up to the mark with diluent. The hepatic artery

Chromatographic conditions:

Column	:	ChromosilColumn C18 (150mm x 4.6mm) 5 μ g.
Mobile phase	:	Water: Methanol PH 2.5 (30:70 v/v)
Flow rate	:	0.8ml/ min
Detector wavelength	:	254 nm
Injection mode	:	Auto injector (vial)
Injection volume	:	20 μ l

Method Validation

The chromatographic conditions were validated by evaluating linearity, accuracy, method precision, and limit of detection (LOD), limit of quantization (LOQ), ruggedness and robustness in accordance with ICH guidelines.

Specificity

Preparation of solutions

a) Placebo interference

Amount of 352.6 mg of the capsule powder was taken in to 100ml standard flask. A volume of 70ml of mobile phase was added and sonicate for 30min. Then the solution was cooled and diluted to volume with mobile phase and filtered through 0.45 μ m membrane filter. (Stock solution)

Further pipette 0.25ml of Quinidine of the above stock solution in to a 10ml volumetric flask and dilute up to the mark with diluent.

Acceptance criteria

Chromatogram of placebo should not show any peak at the retention time of analyte peak.

Standard preparation

Weigh accurately 10mg Quinidine Working Reference Standard is taken in to 100ml volumetric flask and then it was dissolved and diluted to volume with mobile phase up to the mark. After that 50ml of the above solution was taken into 100ml standard flask and made up with mobile phase. (Stock solution)

Further pipette 0.5ml of the above stock solution in to a 10ml volumetric flask and dilute up to the mark with diluent.

Sample preparation

Amount of 352.6 mg of the tablet powder was taken in to 100ml standard flask. A volume of 70ml of mobile phase was added and sonicate for 30min. Then the solution was cooled and diluted to volume with mobile phase and filtered through 0.45 μ m membrane filter.

(stock solution)

Further pipette 0.25ml of Quinidine of the above stock solution in to a 10ml volumetric flask and dilute up to the mark with diluent.

Linearity and Range

Preparation of stock solution

Weigh accurately 10mg Quinidine Working Reference Standard is taken in to 100ml volumetric flask and then it was dissolved and diluted to volume with mobile phase up to the mark. After that 50ml of the above solution was taken into 100ml standard flask and made up with mobile phase. (Stock solution)

Quinidine:

Preparation of linearity solution (20%)

0.2ml of stock solution was taken in 10ml of volumetric flask dilute up to the mark with diluent. The solution mixed well and used for chromatographic injection.

Preparation of linearity solution (30%)

0.3ml of stock solution was taken in 10ml of volumetric flask dilute up to the mark with diluent. The solution mixed well and used for chromatographic injection

Preparation of linearity solution (40%)

0.4ml of stock solution was taken in 10ml of volumetric flask dilute up to the mark with diluent. The solution mixed well and used for chromatographic injection.

Preparation of linearity solution (50%)

0.5ml of stock solution was taken in 10ml of volumetric flask dilute up to the mark with diluent. The solution mixed well and used for chromatographic injection.

Preparation of linearity solution (60%)

0.6ml of stock solution was taken in 10ml of volumetric flask dilute up to the mark with diluent the solution mixed well and used for chromatographic injection.

Procedure

Each level of the above solutions was injected into the chromatographic system for five replicate and the peak area was measured. A graph was plotted (peak area versus concentration) and the correlation coefficient (r^2) was calculated.

Accuracy

Preparation of stock solution

Weigh accurately 10mg Quinidine Working Reference Standard is taken in to 100ml volumetric flask and then it was dissolved and diluted to volume with mobile phase up to the mark. After that 50ml of the above solution was taken into 100ml standard flask and made up with mobile phase. (Stock solution) Further pipette 0.5ml of the above stock solution in to a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation Sample solutions

50% Sample preparation

Weigh accurately 5 mg Quinidine Working Reference Standard is taken in to 100ml volumetric flask and then it was dissolved and diluted to volume with mobile phase up to the mark. After that 50ml of the above solution was taken into 100ml standard flask and made up with mobile phase. (Stock solution) Further pipette 0.5ml of the above stock solution in to a 10ml volumetric flask and dilute up to the mark with diluent. The above solution were inject into the HPLC column same procedure was repeated for three replicate.

100% Sample preparation

Weigh accurately 10mg Quinidine Working Reference Standard is taken in to 100ml volumetric flask and then it was dissolved and diluted to volume with mobile phase up to the mark. After that 50ml of the above solution was taken into 100ml standard flask and made up with mobile phase. (Stock solution) Further pipette 0.5ml of the above stock solution in to a 10ml volumetric flask and dilute up to the mark with diluents. The above solution were inject into the HPLC column same procedure was repeated for three replicate.

150% Sample preparation

Weigh accurately 15mg Quinidine Working Reference Standard is taken in to 100ml volumetric flask and then it was dissolved and diluted to volume with mobile phase up to the mark. After that 50ml of the above solution was taken into 100ml standard flask and made up with mobile phase. (Stock solution)

Further pipette 0.5ml of the above stock solution in to a 10ml volumetric flask and dilute up to the mark with diluent. The above solution were inject into the HPLC column same procedure was repeated for three replicate.

Precision

Standard preparation

Weigh accurately 10mg Quinidine Working Reference Standard is taken in to 100ml volumetric flask and then it was dissolved and diluted to volume with mobile phase up to the mark. After that 50ml of the above solution was taken into 100ml standard flask and made up with mobile phase. (Stock solution) Further pipette 0.5ml of the above stock solution in to a 10ml volumetric flask and dilute up to the mark with diluent.

Optimized method

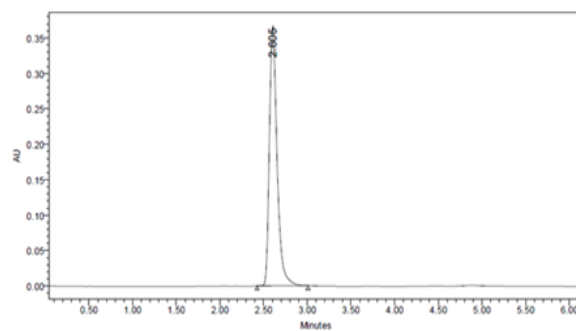


Fig. 1: Chromatogram of Optimized method

Table 1: Chromatogram results

S.No	Peak Name	R_t	Area	Height	USP Plate Count	USP Tailing	USP Resolution
1	Quinidine	2.605	2233704	365596	4456	1.4	

Discussion: The separation of two analytical peaks was good. The plate count also above 2000, tailing factor below 2, and the resolution is above 2. The condition is taken as optimized method.

Specificity:

The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak. The study was performed by injecting blank.

Table 2: Specificity results

S.No	Peak Name	R_t	Area	Height	USP Plate Count	USP Tailing	USP Resolution
1	Quinidine	2.589	2004682	342227	5167	1.3	

Discussion: The specificity test was performed for Quinidine. It was found that there was no interference of impurities in retention time of analytical peak.

Linearity:

Table 3: Linearity of Quinidine

Sample ID	Quinidine	
	Concentration	Area
20% of operating concentration	20	1224140
40% of operating concentration	30	1595681
60% of operating concentration	40*	1992966
80% of operating concentration	50	2356546
100% of operating concentration	60	2797214
Correlation Coefficient 0.999		

Acceptance criteria: Correlation Coefficient should be NLT 0.999

Discussion: The relationship between the concentrations of Quinidine was linear in the specific range and the correlation coefficient was found to be within limit only. The correlation coefficient of Quinidine was found to be 0.9999 .

Accuracy:

Table 4: Accuracy for Quinidine

Recovery level	Accuracy of Quinidine					Average % Recovery
	Amount taken (mcg/ml)	Area	Average area	Amount recovered (mcg/ml)	Percentage Recovery	
50%	5.05	1011326	1017498.5	101.3927	101.3927	100.599%
	5.05	1015029				
	5.05	1026141				
100%	10	1986534	1987384.8	100.0106	100.0106	
	10	1987425				
	10	1988195				
150%	15	2989367	2992493.4	100.3936	100.3936	
	15	2991556				
	15	2996557				

Acceptance criteria:

The mean percentage recovery at each spike level should be NLT 98.0% and NMT 102.0%.

Discussion:

From the Accuracy table it was found that % Recovery of the drug was found to be in the range of 100.01-101.39 % for Quinidine. This indicates that the method was accurate.

Table 5:

S.No.	Quinidine	
	Concentration µg/ml	Peak Area
1	30	1224140
2	40	1595681
3	50	1992966
4	60	2356546
5	70	2797214
S.D.	15.81	618048
Slope	39092	

Results for calibration graph

LIMIT OF DETECTION: (for Quinidine)

Table 6: Showing results for Limit of Detection

Drug name	Standard deviation(σ)	Slope(s)	LOD(µg)
Quinidine	618048	39092	0.001

The LOD was performed for Quinidine was found to be 0.001.

Quantitation limit

Table 7: Showing results for Limit of Quantitation

Drug name	Standard deviation(σ)	Slope(s)	LOQ(µg)
Quinidine	618048	39092	0.004

The LOQ was performed for Quinidine was found to be 0.004

CONCLUSION: The proposed method has been validated for accuracy, precision, linearity; robustness and range were within the acceptance limit according to ICH guidelines. Linearity for Quinidine was found in range of 20µg/ml-60µg/ml and correlation coefficient was found to be 0.999 ,%RSD for intermediate precision was found to be 0.1, for repeatability was 0.2 ,% mean recovery for Quinidine was found to be 99.77%. The method was found to be robust even by change in the mobile phase ±5% and in less flow condition. The developed method can be successfully employed for the routine analysis of Quinidine in API and Pharmaceutical dosage forms.

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