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RP-HPLC method for quantitative estimation of Halquinol in pharmaceutical dosage forms

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Abstract

Halquinol is a mixture obtained by chlorinating 8-Hydroxy quinoline. It contains 5,7-dichloroquinolin-8-ol (57 to 74%), 5-chloroquinolin-8-ol (23 to 40%), and 7- chloroquinolin-8-ol (up to 4%). It is an antibacterial, antifungal and antiprotozoal in nature, and is used in many areas of the world in poultry and swine rearing to fight microbial infections and in growth promotion. A simple, rapid, economic, accurate reverse phase isocratic HPLC method was developed for quantification of Halquinol in different dosage forms such as suspension and bolus. The method was validated with respect to linearity, precision, accuracy and robustness as per the International Conference on Harmonisation (ICH) guidelines. The calibration curve was linear in the range of 12-28 µg mL^{-1} for 5-chloroquinolin-8-ol and 33-77 µg mL^{-1} for 5,7-dichloroquinolin-8-ol respectively. The method has been successfully applied for the analysis of Halquinol in pharmaceutical dosage forms.

Keywords:

Halquinol; RP-HPLC; Suspension; Bolus

1. Introduction

Halquinol is a mixture obtained by chlorinating 8-Hydroxy quinoline. It contains 5,7-dichloroquinolin-8-ol (57 to 74%), 5-chloroquinolin-8-ol (23 to 40%), and 7-chloroquinolin-8-ol (up to 4%) [1]. Halquinol is used as an antibacterial, antifungal and antiprotozoal feed additive for poultry and as a growth promoter in swine. It is known to potentiate the effect of anticoccidial drugs in poultry. It is also useful to overcome malabsorbtion syndrome as it has wide spectrum of activity and slows down peristalsis in the gut [2]. A palatable suspension of Halquinol developed in Provimi innovation center, Provimi Animal Nutrition India Pvt. Ltd., Bangalore and a marketed formulation 3-Care(R) bolus a registered trade mark of Provimi Animal Nutrition India Pvt. Ltd., Bangalore containing Halquinol as active ingredient were taken for the study.

It was realized that there is no simple and convenient analytical method for regular quality control checks in any dosage form reported for this particular compound. However, Gas Liquid Chromatography, U.V Spectrophotometric methods have been reported for estimation of Halquinol in feeds which involved cumbersome derivative preparations [3]. A HPLC method for determining Iodochlorhydroxyquin, 5,7-dichloro-8-hydroxyquinoline, and 5,7-diiodo-8-hydroxyquinoline as nickel complexes in creams, ointments, shampoos, tablets,

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and bulk drugs was also reported [4]. Residual analysis of Halquinol from chicken liver by HPLC was also reported [5]. In the present study, a simple, rapid, economic, accurate method for quantitative estimation of 5,7-dichloroquinolin-8-ol and 5-chloroquinolin-8-ol, which are the major components of Halquinol in pharmaceutical dosage forms were developed using High Performance Liquid Chromatography. The method was validated with respect to linearity, precision, accuracy and robustness as per the International Conference on Harmonisation (ICH) guidelines [6].

2. Experimental

2.1. Chemicals and reagents

5-chloroquinolin-8-ol and 5,7-dichloroquinolin-8-ol standards from sigma were used. *O*-phosphoric acid (HPLC grade), Methanol (HPLC grade), Sodium EDTA (LR grade) and HPLC grade water prepared from Millipore water purification system. Membrane filters (nylon 0.45 µ) and syringe filters (Hydrophilic PVDF, 0.45 µ) were purchased from Millipore (India) Pvt. Ltd., Bangalore.

2.2. Equipment

A Shimadzu HPLC system equipped with a quaternary pump, Auto sampler, Column oven and photo diode array detector was used. The system was connected with the help of LC solution software in a computer system for data collection and processing.

2.3. Chromatographic condition

Column	:	Purospher^{TM}, RP- C18 column (250 mm x 4.0 mm, 5µ)
Mobile phase	:	Water containing 0.01% sodium EDTA and 0.1%
	:	*O*- phosphoric acid and Acetonitrile (50:50 V/V)
Column Temperature	:	40 °C
Detector	:	254 nm
Flow rate	:	1.0 mL.min^{-1}
Injection volume	:	20 µL

2.4. Preparation of Halquinol standard solution

Accurately weighed about 4 mg of 5-chloroquinolin-8-ol and 11 mg of 5,7-dichloroquinolin-8-ol into a 10 mL clean, dry and calibrated volumetric flask and dissolved in methanol. Finally volume was made up to 10 mL with methanol (Stock solution). From this stock solution suitable dilutions were made to get the concentration of 12, 16, 20, 24 & 28 µg mL^{-1} and 33, 44, 55, 66 & 77 µg mL^{-1} for 5-chloroquinolin-8-ol and 5,7-dichloroquinolin-8-ol respectively. The solutions were filtered through 0.45 µ syringe filters and 20 µL of each solution injected for analysis.

2.5. Sample Preparation for Halquinol suspension

Accurately weighed about 0.23 g of suspension into a 10 mL clean, dry and calibrated volumetric flask and added 2 mL of water HPLC grade and sonicated for 2 minutes. To the above solution added 5 mL of methanol and sonicated for 5 minutes. Finally volume was made up to 10 mL with methanol. The solution was filtered through 0.45 µ syringe filter and 20 µL was injected for analysis.

2.6. Sample preparation for Halquinol bolus

Halquinol bolus (10 numbers) were weighed and crushed to fine powder. Accurately weighed about 23 mg of the powder into a 10 ml clean, dry and calibrated volumetric flask

and added 5 mL of methanol and sonicated for 5 minutes. Finally volume was made up to 10 mL with methanol. The solution was filtered through 0.45 µ syringe filter and 20 µL was injected for analysis.

3. Results & Discussion

3.1. Method development

The system suitability test was carried out by preparing a freshly prepared stock solution of 5-chloroquinolin-8-ol and 5,7-dichloroquinolin-8-ol to check various parameters. The system suitability results are tabulated in Table 1.

Table 1. System suitability

Parameters	5-chloroquinolin-8-ol	5,7-dichloroquinolin-8-ol
Retention time	~5.0	~14.4
Theoretical plate	9993.4	14966.8
Tailing factor	1.26	1.22
Calibration range	12-28 µg mL^{-1}	33-77 µg mL^{-1}

3.2. Method validation

3.2.1. Linearity and range

The linearity of the method was observed with in the expected concentration range demonstrating its suitability for analysis. The correlation coefficient (r^2) was found to be 0.9997 and 0.9989 for 5-chloroquinolin-8-ol and 5,7-dichloroquinolin-8-ol respectively. The calibration curves for linearity data is shown in Fig. 1 and Fig. 2.

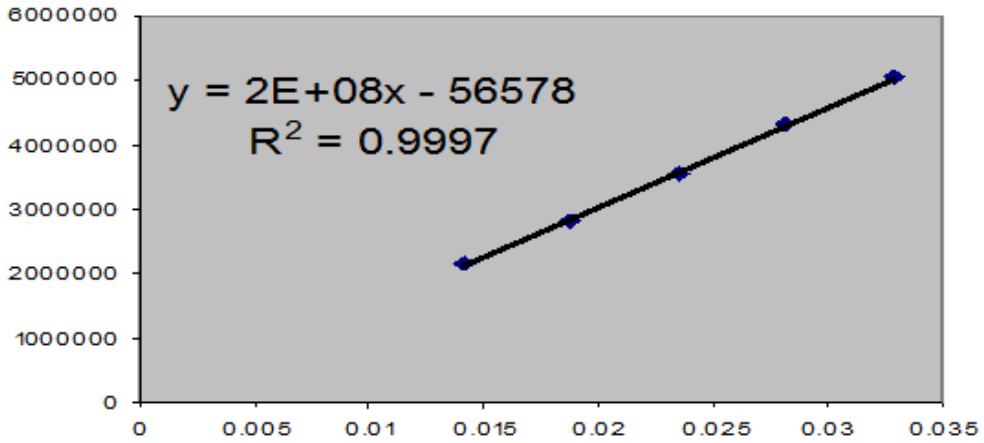

Fig.1 The calibration curve for linearity data of 5-chloroquinolin-8-ol

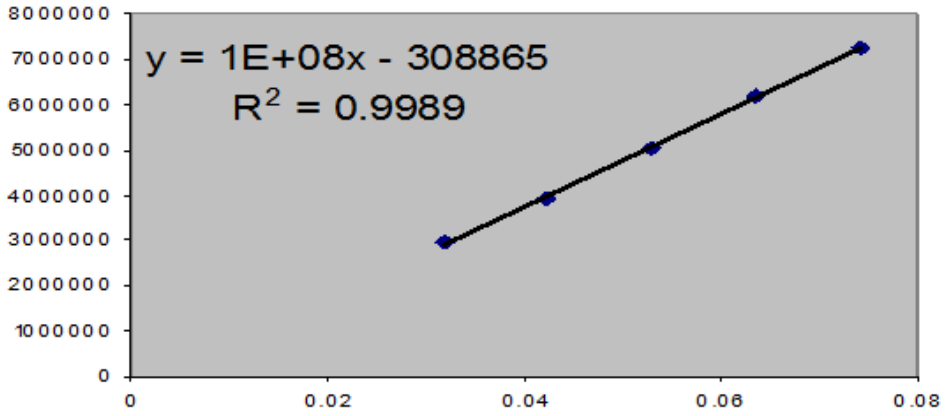

Fig. 2 The calibration curve for linearity data of 5,7-dichloroquinolin-8-ol

3.2.2. Accuracy

3.2.2.1. Halquinol Suspension

The accuracy of the methods was determined by application of the analytical method to synthetic mixtures of the drug product Components (Placebo) to which known amount of analyte has been added within the range of method. The accuracy was evaluated by the recovery of 5-chloroquinolin-8-ol and 5,7-dichloroquinolin-8-ol at three different levels like 80, 100 & 120 % of the label claim.

3.2.2.2. Halquinol Bolus (3-Care$^{(R)}$)

In the case of Halquinol bolus (3-Care$^{(R)}$) accuracy of the method was determined by spiking a preanalyzed sample with working standard of Halquinol at three concentration levels 20, 40 and 60 % to the label claim and analyzed.

The recovery data of Halquinol suspension and Halquinol Bolus (3-Care$^{(R)}$) for each level and both the components of halquinol were tabulated in Table 2 and 3, respectively.

Table 2. Recovery Data for the Proposed Method for Halquinol suspension

Working standard substance*	Level	Recovery, % (±SD) (N=3)
Halquinol (30% 5-chloroquinolin-8-ol and 70% 5,7-dichloroquinolin-8-ol)	80	101.54 ± 0.17
	100	99.68 ± 0.14
	120	99.55 ± 0.32

*Working standard substance used for recovery studies is a mixture of 5-chloroquinolin-8-ol and 5,7-dichloroquinolin-8-ol in the ratio of (30:70);
N: Number of repetitions;
SD: Standard Deviation

Table 3. Recovery data for the proposed method (n=3) for Halquinol Bolus **(**3-Care$^{(R)}$)

Working standard substance*	Level	Amount of Halquinol present in the sample (mg)	Amount of Halquinol spiked (mg)	Recovery, % (±SD) (N=3)
Halquinol (30% 5-chloroquinolin-8-ol and 70% 5,7-dichloroquinolin-8-ol)	I	14.3 mg	3.006 mg	99.68 ± 0.42
	II	14.1 mg	6.012 mg	98.34 ± 0.25
	III	14.60 mg	9.018 mg	98.83 ± 0.24

*Working standard substance used for recovery studies is a mixture of 5-chloroquinolin-8-ol and 5,7-dichloroquinolin-8-ol in the ratio of (30:70);
N= Number of repetitions;
SD= Standard Deviation

3.2.3. Precision

A. Repeatability: Study was assessed by injection repeatability. The injection repeatability was confirmed by performing replicate injections of sample solution at 100% concentration and calculating % RSD of the peak area response. The data showed good precision of the system with the RSD <2%.

B. Intermediate precision: Intermediate precision was performed by 2 analysts on three replicates of each sample at different points of time. The RSD was found to be < 2%.

C. Reproducibility: The ruggedness study was carried out by analyzing same sample 3 times by different analyst on different instrument. The RSD was found to be < 2%.

3.2.4. Robustness

The robustness of analytical method of halquinol in assay determination of suspension dosage form was studied by analyzing the samples with slight variation in the mobile phase composition, Condition-1{buffer : Acetonitrile (49:51)} and condition -2 {buffer: Acetonitrile (51:49)}; where as in the case of Halquinol Bolus (3-Care$^{(R)}$) assay determination was studied by analyzing the samples with slight variation in the mobile phase composition and temperature, Condition -3 {buffer : Acetonitrile (52:48) at column temperature 40 ºC}, Condition-4 {buffer : Acetonitrile (50:50) at column temperature 42 ºC}. The RSD was found to be < 2%.

The RSD values of repeatability, Intermediate precision, reproducibility and robustness for Halquinol suspension and Halquinol Bolus (3-Care$^{(R)}$) were tabulated in Table 4 and 5 respectively.

Table 4. The RSD values of repeatability, Intermediate precision, reproducibility and robustness for Halquinol suspension

Parameters		5-chloroquinolin-8-ol	5,7-dichloroquinolin-8-ol
RSD, % (Repeatability) (N=6)		0.744	1.10
RSD, % (Intermediate Precision) (n**=3)	Analyst -1	0.488	0.599
	Analyst-2	0.189	1.04
RSD, % (Reproducibility) (N=3)		0.34	0.34
RSD, % (Robustness)	Condition -1	0.28	0.29
	Condition -2	1.24	1.06

RSD: Percentage Relative Standard Deviation;
N: Number of repetitions

Table 5. The RSD values of repeatability, Intermediate precision, reproducibility and robustness for Halquinol Bolus (3-Care$^{(R)}$)

Parameters		5-chloroquinolin-8-ol	5,7-dichloroquinolin-8-ol
RSD Repeatability, % (N=6)		1.23	0.50
RSD, % (Intermediate Precision) (n**=3)	Analyst 1	0.63	0.95
	Analyst 2	0.47	0.45
RSD, % (Reproducibility) (N=3)		1.55	0.73
RSD, % (Robustness)	Condition 3	0.70	0.08
	Condition 4	0.12	0.12

RSD: Percentage Relative Standard Deviation;
N: Number of repetitions

3.2.5. Specificity

For demonstrating the specificity of the method for drug formulation the drug was spiked and the representative chromatogram was shown in Figure-3. The excepients used in different formulation products did not interfere with the drug peak and thus, the method is specific 5-chloroquinolin-8-ol and 5,7-dichloroquinolin-8-ol. To further confirm the specificity of the method, UV scans of spiked drug were taken in the range 200-400nm and no significant change was found by comparing the absorbance of pure drug and spiked drug at the analytical wavelength of drug.

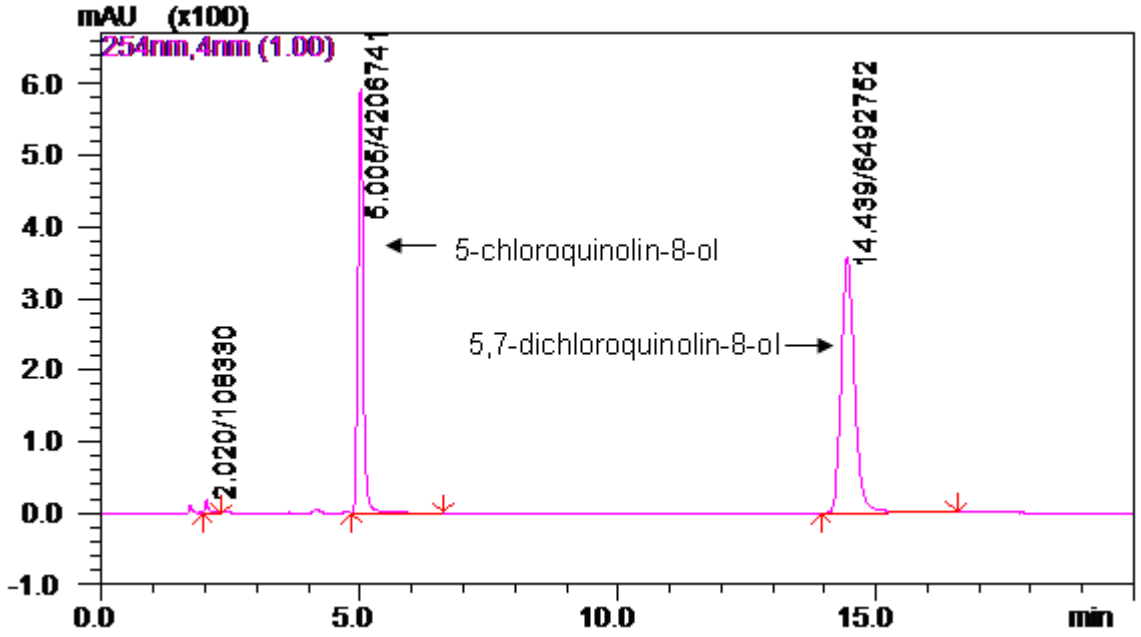

Fig. 3. A representative chromatogram of spiked drug

4. Conclusion

The proposed method is simple, sensitive, precise and accurate. Therefore this method can be applied for routine quality control analysis of Halquinol in pharmaceutical preparations.

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