Original Article

Determination of 5,7-dichloroquinolin-8-ol and 5-chloroquinolin-8-ol in bulk drug powder and pharmaceutical preparation by TLC

Abstract

Aim: The study aims to develop a simple, rapid and economical normal phase thin layer chromatography (TLC) method for identification and quantification of 5,7-dichloroquinolin-8-ol and 5-chloroquinolin-8-ol, two active ingredients of halquinol, an antimicrobial agent. **Materials and Methods:** The analysis was performed on silica gel 60 TLC plates pre-washed with disodium ethylenedinitrilotetraacetic acid disodium salt (Na_2EDTA) solution with methanol–ethyl acetate–iso-propyl alcohol–ammonia solution [8:20:1:0.6 (v/v)] as the mobile phase. Detection and quantification was performed densitometrically at 247 nm. **Results:** Responses of both 5,7-dichloroquinolin-8-ol and 5-chloroquinolin-8-ol were linear functions of concentration in the range of 300–800 ng. The intraday precision and intermediate precision of the method for 5,7-dichloroquinolin-8-ol and 5-chloroquinolin-8-ol was determined and it was found to be precise. **Conclusion:** The TLC method developed for simultaneous quantitative determination of 5,7-dichloroquinolin-8-ol and 5-chloroquinolin-8-ol was found to be simple and economical. Therefore, it can be used in routine quality control analysis of halquinol in bulk drug powder and halquinol bolus.

Key words:
5,7-dichloroquinolin-8-ol, 5-chloroquinolin-8-ol, halquinol, TLC densitometry

Introduction

Halquinol, a quinoline derivative, is a broad-spectrum antimicrobial having antibacterial, antifungal and antiprotozoal activities, and is being used in India and other countries to overcome several challenges in modern poultry and swine farming, like microbial infections, and for growth promotional aspects,[1] by incorporating it with feed at different levels. Besides, halquinol bolus has been used in large animal practice to treat enteric infections.[2] Halquinol administration is found to be beneficial in controlling vibriosis in fishes[3,4] and acts as a growth promoter in fresh water aquaculture.[5]

Halquinol is a mixture obtained by chlorinating quinolin-8-ol. It contains not less than 57% and not more than 74% of 5,7-dichloroquinolin-8-ol, not less than 23% and not more than 40% of 5-chloroquinolin-8-ol, and not more than 4% of 7-chloroquinolin-8-ol, and the total content of the three components is not less than 95% and not more than 105%.[6] The molecular structure of 5,7-dichloroquinolin-8-ol and 5-chloroquinolin-8-ol is shown in Figure 1.

A literature survey reveals that although high performance liquid chromatography (HPLC) method[7] has been reported, there is no detailed methodology for thin layer chromatography (TLC) or high performance thin layer chromatography (HPTLC) for the determination of halquinol. In this work, an economical, precise, and

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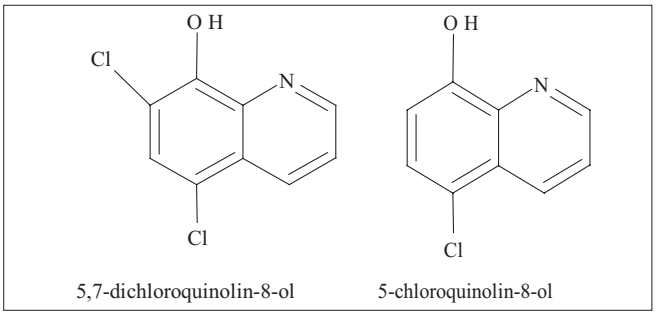

Figure 1: The structure of the substances analyzed

simple TLC method has been developed and validated for identification and quantification of halquinol from its pharmaceutical formulation and bulk drug powder.

Materials and Methods

Reagent and standards

The two active ingredients of halquinol, 5,7-dichloroquinolin-8-ol (purity 99%) and 5-chloroquinolin-8-ol (purity 95%), were procured from Sigma (Sigma-Aldrich, St. Louis, MO, USA). Halquinol bulk drug powder (containing 71.39% of 5,7-dichloroquinolin-8-ol and 26.61% of 5-chloroquinolin-8-ol) was obtained as a gift from M/s. Provimi Animal Nutrition India Pvt. Ltd., Bangalore, India. Analytical grade ethyl acetate, methanol, iso-propyl alcohol and ammonia were obtained from E Merck (Worli, Mumbai, India), and ethylenedinitrilotetraacetic acid disodium salt (EDTA, purity 99%) was from E Merck (India). A commercial pharmaceutical preparation containing halquinol (Halquinol bolus, Provimi Animal Nutrition India Pvt. Ltd., Bangalore, India) procured from local veterinary pharmacy was used. The declared halquinol content of each bolus was 1.5 g.

Preparation of solutions

Preparation of stock solutions

Stock solutions (1 mg/ml) of 5,7-dichloroquinlin-8-ol and 5-chloroquinolin-8-ol were prepared by dissolving 10 mg of accurately weighed standards in 10 ml of methanol. Stock solution (1 mg/ml) of halquinol was prepared by dissolving 10 mg of halquinol bulk drug powder in 10 ml of chloroform.

Preparation of mixed working standard solution

Aliquots of stock solutions of 5,7-dichloroquinlin-8-ol, 5-chloroquinolin-8-ol (0.2 ml each) were transferred to a graduated test tube and then diluted to volume of 2 ml with methanol, resulting in a concentration of 100 ng/µl, which was then used for spotting on TLC plates. Concentration range of 300–800 ng was used for standard curve.

Preparation of solution of halquinol bulk drug powder

Halquinol stock solution (0.2 ml) was transferred to a graduated test tube. The solution in tube was then diluted to volume of 2 ml with chloroform, resulting in a concentration of 100 ng/µl, which was then used for spotting on TLC plates.

Preparation of solution of halquinol from the pharmaceutical preparation

Halquinol bolus was accurately weighed to contain the stated 1.5 g of active drug, that is, halquinol. The bolus was powdered and transferred to a 100 ml volumetric flask. Chloroform (75 ml) was added and the flask was sonicated for 5 minutes and then cooled to room temperature. The solution in the flask was then diluted to volume with chloroform, filtered through a Whatman No. 1 filter paper and the filtrate was collected in a flask. This filtrate (0.12 ml) was transferred to a 10 ml volumetric flask and the solution was diluted to volume with chloroform. The same procedure was repeated four times for the recovery study. Chloroform was used to dissolve halquinol as its degree of solubility is more in chloroform compared to other organic solvents (1 in 50 parts).[6]

Chromatography

Linearity of detector response

The TLC plates were pre-washed with EDTA. A 10% (w/v) solution of disodium EDTA was prepared and pH was adjusted to 8 using 12 N sodium hydroxide. TLC plates were predeveloped with this EDTA solution in Camag® twin-trough chamber. Plates were then dried in hot air oven at 110°C for an hour. Chromatography was performed on 10×10 cm aluminum backed TLC plates coated with 0.20 mm layers of silica gel 60 (Merck, Darmstadt, Germany). Mixed working standard solutions of 5,7-dichloroquinolin-8-ol and 5-chloroquinolin-8-ol (five absolute concentrations 300, 400, 500, 600 and 800 ng) were applied as bands 8 mm wide on TLC plate by means of a Camag® Linomat 5 sample applicator using 100 µl sample syringe (Hamilton, Bonaduz, Switzerland. For the assay of bulk drug powder and pharmaceutical preparation (halquinol bolus), the respective working solutions were spotted as samples along with mixed working standard solution. The bands were applied 10 mm from the bottom edge of the plate and the distance between bands was automatically set. Plates were developed at 25±2°C with methanol–ethyl acetate–iso-propyl alcohol–ammonia solution (8:20:1:0.6) as the mobile phase in a Camag® glass twin-trough chamber. The mobile phase was taken in one of the chambers of twin-trough developing chamber, plate was then kept in another chamber and a saturation time of 25 minutes was allowed. Then, the development was started. The development distance was 8 cm. The plate was then dried in air and was scanned at 247 nm using Camag® TLC Scanner 3 with winCATS software. The wavelength used for densitometry was selected after acquiring *in situ* UV spectra of the two components, 5,7-dichloroquinolin-8-ol and 5-chloroquinolin-8-ol. The densitometric responses from the standard and sample were used to estimate the amounts of the drugs in the bulk drug powder and bolus.

The corresponding UV overlay spectra obtained from the 5,7-dichloroquinolin-8-ol and 5-chloroquinolin-8-ol standards and from 5,7-dichloroquinolin-8-ol and 5-chloroquinolin-8-ol from the halqinol bolus are shown in Figures 2 and 3, respectively.

The intraday precision and intermediate precision of the method for 5,7-dichloroquinolin-8-ol and 5-chloroquinolin-8-ol standards was determined by considering the respective peak areas [Table 1]. The precision of the method was indicated by low relative standard deviation (RSD) values [Table 1]. Recovery assay of the method for halquinol bolus and bulk drug powder is depicted in Table 2.

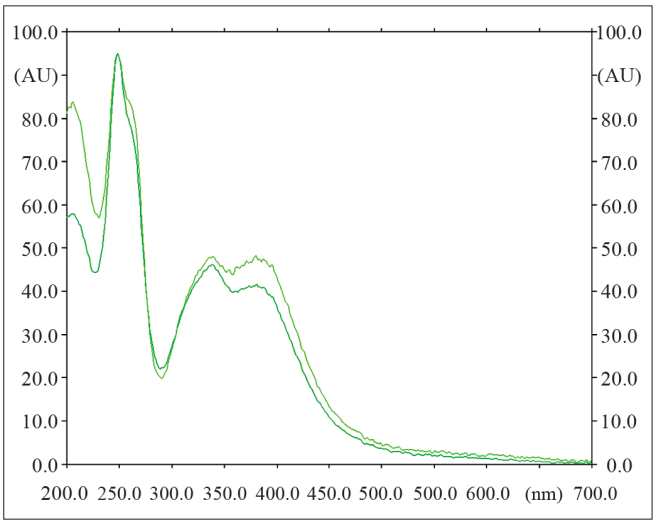

Figure 2: Overlay of UV spectra acquired *in situ* from 5,7-dichloroquinolin-8-ol standard and from 5,7-dichloroquinolin-8-ol from the pharmaceutical preparation

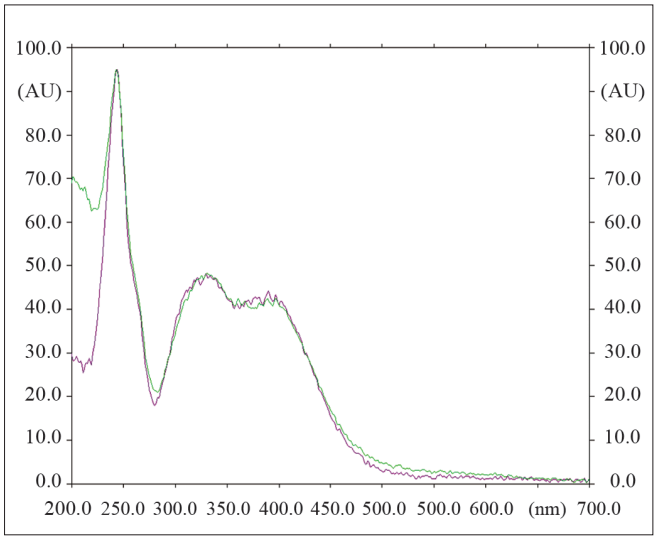

Figure 3: Overlay of UV spectra acquired *in situ* from 5-chloroquinolin-8-ol standard and from 5-chloroquinolin-8-ol from the pharmaceutical preparation

Results and Discussion

As halquinol and its active ingredients, 5,7-dichloroquinolin-8-ol and 5-chloroquinolin-8-ol, have strong metal chelating property,[6] the TLC plates used for analysis were pre-washed with disodium EDTA solution. Different mobile phases were investigated and good resolution of 5,7-dichloroquinolin-8-ol and 5-chloroquinolin-8-ol was achieved by use of methanol–ethyl acetate–iso-propyl alcohol–ammonia solution (30% v/v, 8:20:1:0.6) as the mobile phase. Figure 4 shows a typical chromatogram obtained from a standard mixture of 5,7-dichloroquinolin-8-ol and 5-chloroquinolin-8-ol. Because the UV–visible spectra of 5,7-dichloroquinolin-8-ol and 5-chloroquinolin-8-ol

Table 1: Intra-assay and intermediate precision data for simultaneous TLC determination of 5,7-dichloroquinolin-8-ol and 5-chloroquinolin-8-ol

Concentration of drug (ng)	Intra-assay precision mean, RSD %	Intermediate precision, RSD* %
5.7-dichloroquinolin-8-ol		
400	1.55	1.20
500	1.33	1.15
600	1.20	0.98
5-chloroquinolin-8-ol		
400	1.72	2.12
500	1.60	1.17
600	1.44	1.08

*Mean from 3 days

Table 2: Recovery assay of the method for pharmaceutical preparation (halquinol bolus) and bulk drug powder assay

Expt. No.	Recovery assay (%) for halquinol bolus (1.5 g/bolus)	Bulk drug powder assay (%)
1	99.42	99.56
2	99.73	99.00
3	100.08	100.01
4	100.14	100.15
Mean ± SE	99.84 ± 0.16	99.68 ± 0.25

Table 3: Method validation data for simultaneous TLC quantification of 5,7-dichloroquinolin-8-ol and 5-chloroquinolin-8-ol

Characteristic	Results	
	5,7-dichloroquinolin-8-ol	5-chloroquinolin-8-ol
Linearity range (ng)	300–800	300–800
Intercept	−82.1988	−311.34
Slope	0.115933	0.223656
Correlation coefficient (r)	0.995	0.991

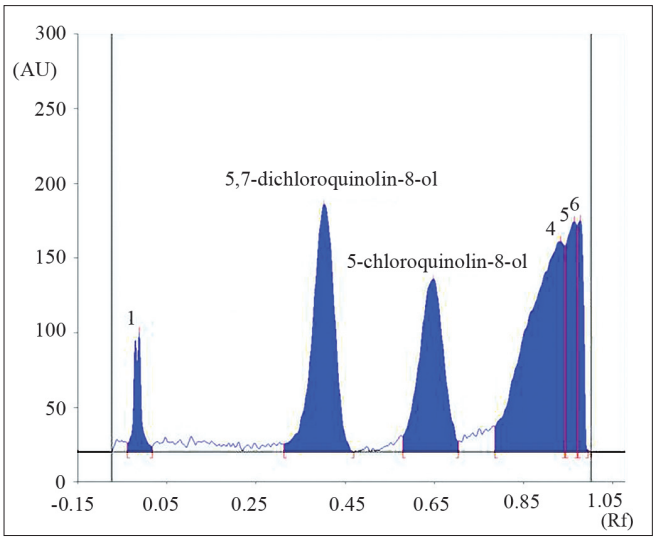

Figure 4: Typical chromatogram showing separation of the two individual components, 5,7-dichloroquinolin-8-ol and 5-chloroquinolin-8-ol

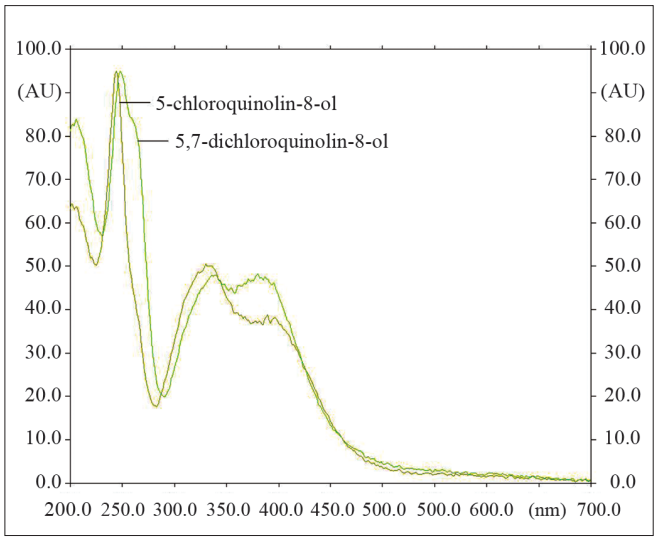

Figure 5: *In situ* UV spectra obtained from 5,7-dichloroquinolin-8-ol and 5-chloroquinolin-8-ol standards after chromatography of a mixed standard solution

had absorption maxima at 247 and 243 nm, detection was performed at 247 nm, where absorption of two components of halquinol was comparable. Within the range 200–400 nm, the spectra of 5,7 dichloroquinolin-8-ol and 5-chloroquinolin-8-ol have characteristic absorption maxima [Figure 5]. The identity of bands of 5,7-dichloroquinolin-8-ol and 5-chloroquinolin-8-ol from bulk drug powder and pharmaceutical preparation was confirmed by comparing their retention factors (R_f) with those of reference standards of 5,7-dichloroquinolin-8-ol (R_f=0.44) and 5-chloroquinolin-8-ol (R_f=0.63). Linear regression analysis of the calibration data for standards showed that the dependant variable (peak area, *Y*) and the independent variable (concentration, *X*) were represented by the equations $X=-82.2 + 0.1159Y$ and $X=-311.34 + 0.2237Y$ for 5,7-dichloroquinolin-8-ol and 5-chloroquinolin-8-ol, respectively. A linear relationship exists between response and concentration for both 5,7-dichloroquinolin-8-ol and 5-chloroquinolin-8-ol in the concentration range 300–800 ng with correlation coefficient of 0.99 [Table 3]. It was observed that none of the excipients from the bolus gave a peak which interfered with estimation of the two individual components of halquinol.

Conclusion

The method developed was found to be economical, selective, precise and free from interferences from excipients. It can therefore be used for a routine quality control analysis and simultaneous quantitative determination of 5,7-dichloroquinolin-8-ol and 5-chloroquinolin-8-ol in pharmaceutical preparation and bulk drug powder.

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