High performance thin layer chromatography (HPTLC) method development and validation for determination of doxycycline hyclate in capsule and tablet formulations

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ABSTRACT

According to World Health Organization (WHO) 10% of the medicines in the Low and Middle Income Countries (LMICs) are of poor quality posing a major public health threat. One way to circumvent such problem is the development and deployment of rapid, economical and efficient analytical methods. Hence this research aims to develop a High-Performance Thin Layer Chromatography (HPTLC) method for the determination of doxycycline hyclate. A rapid and simple HPTLC method with densitometry detection at 360 nm to determine doxycycline hyclate in capsules and tablet formulations was developed and validated. HPTLC was performed on glass plates coated with C18 reverse phase silica gel 60 F254 and pretreated with 0.27 M ethylenediaminetetraacetic acid (EDTA) solution. The mobile phase was dichloromethane: methanol: acetonitrile: 1% aqueous ammonia in the ratio of 10:22:53:15 (v/v). The linearity range lies between 200 and 1,000 ng/spot with correlation coefficient of 0.997. The Rf value is 0.5 ± 0.02%. Recoveries were in the range of 94.50 – 100.5%. Limit of detection and limit of quantitation values for doxycycline hyclate were 40 and 160 ng/spot respectively. The developed method was validated as per ICH guidelines. Thus, it was found to be accurate, precise, specific and robust. In forced degradation study, doxycycline hyclate was found to degrade in acidic and alkaline media, and through oxidative stress. The drug was found to be relatively stable to heat and photodegradation. The method was successfully applied for the routine quantitative analysis of dosage forms containing doxycycline hyclate. The developed method offered comparable results (as confirmed by F-test) with that of the HPLC pharmacopoeial (BP) analysis method.

KEYWORDS

analytical method development, doxycycline hyclate, high performance thin layer chromatography (HPTLC), quality control, validation

INTRODUCTION

Doxycycline (6-Deoxy-5-hydroxytetracycline) (C₂₂H₂₄N₂O₈) is a semi synthetic broad spectrum tetracycline antibiotic [1]. It is derived from oxytetracycline with an identical spectrum of activity. Its use is to treat infectious diseases. The anti-microbial activity of this drug encompasses against Staphylococcus aureus, Streptococcus pneumonia, S pyogenes, S agalactiae, Campylobacter jejuni, Haemophilus influenzae, Neisseria gonorrhoeae, N meningitides, Clostridium species, Peptostreptococcus spp, Peptococcus spp, Bacteroides
**melaninogenicus** and **Bacteroides fragilis**. The mechanism of action is bacterial protein synthesis inhibition by binding with the 30S ribosomal subunit. Doxycycline is preferred over other tetracyclines since it has a more favorable pharmacokinetic profile such as; better absorption and longer half-life, which allows fewer daily doses [2, 3]. It is frequently used to treat chronic prostatitis, sinusitis, syphilis, chlamydia, and pelvic inflammatory diseases [4]. There are different salt forms of doxycycline. Doxycycline for pharmaceutical preparations listed in the pharmacopoeias are in the form of doxycycline (chemical structure shown in Fig. 1a) monohydrate (C22H26N2O9) (free base), doxycycline hyclate, doxycycline hydrochloride (C22H24N2O8·HCl·0.5C2H5OH·0.5H2O), doxycycline calcium (C22H20Ca2N2O8) [5–8].

According to the Essential Medicines List (EML) of the WHO, 100 mg doxycycline hyclate capsules or tablets can be used as an antibiotic drug. In case of malaria, 100 mg doxycycline as capsules or dispersible tablet can be used in combination with quinine for curative treatment, and for malarial prophylaxis [9].

Doxycycline hyclate (C22H23N2O9·HCl·0.5C2H5OH·0.5H2O; molecular mass 512.94 g/mol, chemical structure shown in Fig. 1b) is the hydrochloride hemi-ethanol hemihydrate of doxycycline [4]. Doxycycline hyclate has much more GI (gastrointestinal) solubility than the other forms, which is one of the primary reasons for its more frequent use in pharmaceutical applications [10].

HPTLC is an automated form of Thin Layer Chromatography (TLC) having relatively smaller particle and pore size of sorbents, lower analysis time, a development chamber and no interference from previous analysis since fresh stationary phase and mobile phase are used for each analysis. The method also allows application of several sample spots (up to 18) to be determined in a single run [13, 14].

There are various methods for determination of doxycycline; including microbiology [14], thin layer chromatography [15], TLC with densitometry for a binary mixture of doxycycline with ambroxol hydrochloride [16], HPTLC with charge coupled detector device (CCD) [17], infrared spectroscopy [18] and High Performance Liquid Chromatography (HPLC) [4].

High Performance Liquid Chromatography (HPLC) is the official method for the determination of doxycycline hyclate in some pharmacopoeias, which requires using HPLC grade solvents and columns (with high consumption of the solvents) resulting in high cost of analysis [4, 19].

For controlling the quality of frequently prescribed medicines such as doxycycline hyclate, the development of a simple, inexpensive, precise, and rapid method of analysis such as HPTLC is critically important. Hence, the aim of this study is to develop and validate a HPTLC method, as an alternative to the existing methods, for the determination of doxycycline hyclate in tablet and capsule formulations.

**METHODS**

**Experimental materials**

**Doxycycline standard and dosage forms.** Doxycycline hyclate working standard was supplied by Cadila Pharmaceuticals Ethiopia PLC. The working standard was certified to contain 98.55% of doxycycline hyclate (manufacturing date: – Feb 2017; expiry date: – Feb 2021; batch number: – D20170210). The reference standard was obtained from Sigma-Aldrich and was certified to contain 98% of doxycycline hyclate (batch number: – BCBP0625V). Five brands of doxycycline hyclate in capsule (3) and tablet (2) formulations each claiming to contain 100 mg of doxycycline were purchased from community pharmacy retail outlets in Addis Ababa, Ethiopia.

**Solvents and reagents.** HPLC grade methanol and analytical reagent grade dichloromethane (Carlo Erba Reagents, France), acetonitrile and ammonia (BDH Laboratory Supplies Ltd, England) were used. Distilled and de-ionized water was used in preparing the mobile phase and methanol: acetonitrile (70:30) used as diluent for sample preparation. A 0.27 M ethylenediaminetetraacetic acid (EDTA) (Research Lab Fine Chem, Industries, India) solution was prepared by using 40% NaOH solution (Research Lab Fine Chem, Industries, India) to adjust the pH to 9.

**Instrumentation and chromatographic condition.** Camag HPTLC apparatus consisting of Linomat V sample...
applicator (Camag, Muttenz, Switzerland), 100 \mu L syringe (Hamilton-Bonaduz Schweiz, Camag, Switzerland), TLC Scanner III (Camag, Muttenz, Switzerland), winCATS version 1.4.0 software (Camag, Muttenz, Switzerland) were used in the study. Chromatography was performed on Merck silica gel 60 F_{254} precoated TLC plates (20 cm × 20 cm with 200 \mu m thickness; batch numbers: HX389048 and HX398477), Saturation pad (Camag, Muttenz, Switzerland) was used for saturating development chambers. Samples were applied as bands under a stream of nitrogen using the \mu L syringe. Ascending development to a distance of 7 cm was performed in a 30 min pre-saturated 20 × 20 cm twin trough TLC developing chamber (Camag). Developed plates were dried using hair drier. Densitometry scanning and quantitative evaluation were performed using the TLC scanner and winCATS version 1.4.0 software respectively. The official (BP) liquid chromatographic method for assay of doxycycline was conducted using HPLC (Shimadzu, LC-2030 C 3D, Japan), TLC developing chamber (Camag). Developed plates were dried using hair drier. Densitometry scanning and quantitative evaluation were performed using the TLC scanner and winCATS version 1.4.0 software respectively. The official (BP) liquid chromatographic method for assay of doxycycline was conducted using HPLC (Shimadzu, LC-2030 C 3D, Japan), fitted with a 25 × 4.6 mm column Packed with styrene-divinylbenzene co-polymer-8 \mu m (Polymer laboratories Ltd) with lab solution software (Shimadzu) to compare results of dosage form analysis obtained using the developed HPTLC method.

**Standard solution**

A standard solution of doxycycline was prepared by dissolving a quantity of working standard equivalent to 15 mg of doxycycline hyclate in 50 mL of methanol and volume adjusted to 100 mL with acetonitrile-methanol (30:70 v/v).

**Sample solution**

Powder from twenty capsules or ground tablets of doxycycline hyclate were mixed and accurately weighed. A quantity equivalent to 15 mg of doxycycline was transferred to a 100 mL volumetric flask and extracted with methanol (5 × 10 mL) first by manually shaking and then placing in ultrasonic bath for 10 min. The filtered extract was transferred in to a 100 mL volumetric flask and diluted to volume with acetonitrile-methanol (30:70 v/v).

**Method validation**

The method was validated in compliance with ICH guideline [20]. The following parameters were validated.

**Specificity.** The specificity of the method was ascertained by comparing the amount of active substance obtained in each solution of doxycycline hyclate during the forced degradation study. To induce formation of degradation products, sample solutions were subjected to acidic, alkaline, oxidative, and photolytic stress conditions. Decreases that occurred in all absolute peak areas of doxycycline hyclate were used as an indication for the qualitative specificity of the method.

**Linearity and range.** To evaluate linear relationship between peak area of the spots and concentration of the drugs, standard solutions of doxycycline hyclate were prepared to obtain concentration in the range of 200–1,000 ng/spot and four \mu L of each solution were applied on the TLC plate. The chromatogram was developed dried and read on the densitometer. A calibration plot was constructed by plotting peak area against the corresponding amount of drug. The linear regression equations were determined by the method of least squares. The correlation coefficient, y-intercept and slope of the regression line were also determined. The range was determined as 80–120% of the assay concentration.

**Detection and quantification limit (LOD and LOQ).** For determination of LOD and LOQ, sample solutions of doxycycline hyclate (n = 5) were applied in decreasing quantities, in triplicate. The same volume of the pure solvent as a blank was also applied. After development, a calibration graph was constructed by plotting the peak areas against the applied quantities of the drug. LOD and LOQ were calculated based on the signal to noise ratio between 3:1 and 2:1.

**Robustness.** Robustness of the method was performed by making small deliberate changes in chromatographic conditions such as the mobile phase composition and duration of saturation time of chamber. Different mobile phase compositions such as dichloromethane: methanol: acetonitrile: 1% aqueous ammonia (10:22:53:15); (9:21:56:14) and (11:23:50:16) v/v were tried in developing the chromatograms. Saturation time was also varied 30 ± 5 min.

**Precision.** The precision of the method was considered at two levels, repeatability and intermediate precision. Repeatability (Intraday variations) study was performed by analysis of three different concentrations, i.e. 480, 600 and 720 ng/spot of the drug (80–120% of the analytical concentration) three times on the same day. Intermediate precision was similarly evaluated over a period of 2 days by two different analysts. The precision of the method was expressed as relative standard deviation (RSD, %).

**Accuracy.** To confirm the accuracy of the proposed method, recovery experiments were carried out by the standard addition technique. It was carried out by adding known amounts of drug (reference standard) to samples of doxycycline hyclate dosage form (Doxyclag® capsule, claimed to contain 100 mg of doxycycline hyclate) corresponding to three concentration levels (80, 100, and 120% of the working concentration) along with the excipients and to the working standard.

**Forced degradation studies**

In forced degradation studies, intentional degradation was tried by exposing a sample having a concentration of 600 \mu g/mL to the following stress conditions: acidic (0.1 M HCl), alkaline (0.1 M NaOH), and oxidation (3% H_{2}O_{2}) in 1:1 ratio to end up with a 300 \mu g/mL concentration. For intentional acid, alkaline and oxidative degradation, contents of the flasks were refluxed in a water bath at 80 °C for
3 h. Samples were kept in an oven at 60 °C (for 5 and 24 h) for heat degradation and in UV irradiation chamber at 254 nm and 2 × 8 Watt energy (for 5 h) for photo degradation. After the respective time intervals, all the flasks were removed and allowed to cool. Contents were diluted to make 100 μg/mL of final concentration and samples were then analyzed.

RESULTS AND DISCUSSION

Method optimization for the HPTLC-densitometry method

Mobile-phase composition. In this study different mixtures of various solvents were tried and the composition of mobile phase with a chromatographic result having acceptable and reproducible Rf value was selected. Initially two different mixtures of solvents i.e. Sodium chloride: acetic acid: n-butanol: Water (0.3 gm: 6 mL: 12 mL: 6 mL pH = 2.6) and Methanol: Acetonitrile: 5% Acetic acid (20:25:55 v/v, pH = 3.7) were tried. Both compositions resulted in broadened peaks with Rf values of 0.39 and 0.25 respectively. The reason for the broadening of chromatographic peaks might be interaction (formation of complex) between the stationary phase and the analyte. To avoid this interaction, TLC plate was treated with EDTA solution and the mobile phase composition Methanol: Acetonitrile: 5% Acetic acid (20:25:55 v/v) was tried again. EDTA, chelating agent, binds in metal layers of the silica gel in order to avoid formation of complex with the samples and hence improved separation [21, 22]. In this trial, after development visualization was made under UV radiation at 254 nm which showed that the stationary phase (silica gel layer) was washed away forming a distorted layer. Spots could not also be observed. This might be due to the reaction of EDTA with the acidic mobile phase. EDTA by nature dissolves in water with pH increment. The solution starts forming a white precipitate when pH decreases with addition of any type of acid. Instead of acetic acid; dichloromethane and 5% aqueous ammonia were replaced and a composition of dichloromethane: methanol: acetonitrile: 1% aqueous ammonia having different proportions was tried. After these trials it was found that: dichloromethane: methanol: acetonitrile: 1% aqueous ammonia in the ratio of (10:22:53:15 v/v, pH = 10.7) gave sharp and symmetrical peaks with Rf value of 0.5 ± 0.2. Hence, this solvent mixture was used as a mobile phase. After the many trials, the optimized condition that offered best peak (as shown in Fig. 2) was on a Precoated silica-gel glass plate 60 F254 (10 × 20 cm, 200 mm thick), pretreated with EDTA solution using Dichloromethane: Methanol: Acetonitrile: 1% Aqueous Ammonia in the ratio of (10:22:53:15 v/v, pH = 10.7) as a mobile phase, 10 mL per single run. Application sample volume was 4 μL; Length of chromatogram run was 7 cm (approximately 8 min) with chamber saturation time of 30 min. Detection was made at 360 nm wavelength. This wavelength is the maximum after scanning determination in the range of 300–480 nm. Method validation was performed under these chromatographic conditions. The run time is short which is very helpful to perform analytical work on many samples. Mobile phase composition and amount interms of volume is small in comparison to the HPLC Pharmacopoeial methods [5–7].

![Fig. 2. Typical Densitogram of doxycycline hyclate spot using the optimal condition](image-url)
The total analysis run time for HPLC was found to be 90 min for one sample while in the developed HPTLC method 12-18 samples can be analyzed in 35 min. The same advantage in terms of reduction of cost of analysis was offered which is very significant by HPTLC i.e. analysis cost estimated in this study is ca. 12 USD ($) per Sample in HPLC as in the pharmacopoeial method and 1.40 USD ($) for 12-18 samples for this HPTLC method.

**Method validation results**

**Specificity.** Specificity is the ability to assess unequivocally the analyte in the presence of components such as impurities, degradation products and matrix. The specificity of the method can be determined with the addition of impurities and degradation products, obtained experimentally or by inducing their formation [20, 23]. In this study, the specificity of the method was checked by comparing the chromatograms obtained for pure doxycycline hyclate solution and the forced degradation procedure. Decreases that occurred in peak areas of doxycycline hyclate, confirmed the qualitative specificity. These forced degradation studies show the susceptibility of the drug to degradation in acidic, basic, heat and oxidative media. Fig. 3 shows decreases in peak areas of samples exposed to different degradants. By performing pre-column derivatization in RP-HPLC method by Darwish et al., it was able to specifically identify doxycycline among related impurities [24].

**Detection and quantification limits.** Limit of detection (LOD) and quantification (LOQ) were calculated based on the signal-to-noise ratio. The results of the LOD and LOQ were found to be 40 ng/spot and 160 ng/spot with signal to noise ratio between 3:1 to 2:1 for LOD and 10:1 for LOQ. The LOD and LOQ values were equivalent to 10 and 40 ng/µL. These values are in the same range with the HPLC methods developed before for the drug are designed to serve qualitative purposes, hence it is not possible to compare and contrast these methods with the one developed in this method [16, 17].

**Linearity, range and calibration curves.** In the evaluation of linearity, both peak area and peak height showed a good linear relationship with in the concentration range of 200–1,000 ng/spot, even though peak area was found to be better. The \( r^2 \) for peak area was 0.997 and that of peak height was 0.994. A good linear relationship was revealed by the linear regression data for the calibration curves in the concentration range of 200–1,000 ng/spot. The linear regression equation was \( Y = 5.676X + 1.390.92, R^2=0.9968 \). The standard deviation (SD) values for the slope and the intercept were found to be 0.2 and 127.15 respectively.

The range which is usually derived from the linear range depends on the purpose for which the method is intended. In the case of a method for the assay of finished products, the range is from 80 to 120% of the test concentration. Thus the minimal range was 480–720 ng/spot. Hence the linear range is wide enough for the purpose of quantifying API contents in different formulations of doxycycline like tablets, capsules, boluses etc without the need to perform dilutions. The linear range is broader than other reported analytical methods for doxycycline [24, 25].

**Robustness.** The standard deviation of peak areas for each parameter was calculated and % RSD was found to be less than 2%. The low % RSD in Table 1 indicated the robustness of the developed method. Unlike the result obtained for peak areas by making small changes in the parameters, there was noticeable difference in \( R_s \) values caused by small changes in the proportion of mobile phases. Hence the analysts utilizing this method should carefully measures the volumes of each mobile phase solvents and adjust proportions accordingly.

Nevertheless, small variations in all parameters did not affect the peak areas and thus the quantification of the drug (Table 2). This indicates the deliberate changes made on the method parameters had a very little effect on the determination.

**Precision.** Repeatability and intermediate precision (Table 3) of the developed method were expressed in terms of relative standard deviation (RSD) of the peak area. *Intraday* variations were performed by analysis of three different concentrations (480, 600 and 720 ng/spot) of the drug three times in the same day. *Interday* variations were performed in three different days. The % RSD values were found to be less than 2%. The low % RSD values indicated the robustness of the developed method for the quantitative analysis of doxycycline hyclate in different formulations.

![Fig. 3. Forced degradation study peak areas: 1) Pure doxycycline hyclate standard; 2) Heat degradation (5 h duration); 3) Photo degradation (5 h duration); 4) Oxidative degradation; 5) Heat degradation (24 h duration); 6) Acidic; 7) Basic](image-url)

**Table 1. Results in robustness study of the method**

| Conditions                      | SD     | %RSD |
|--------------------------------|--------|------|
| **Mobile Phase Composition**   |        |      |
| Dichloromethane: Methanol:     | 100.34 | 1.65 |
| Acetonitrile: 1% Aqueous ammonia (9:21:56:14) |        |      |
| Dichloromethane: Methanol:     | 1.63   | 0.027|
| Acetonitrile: 1% Aqueous ammonia (11:23:50:16) |        |      |
| Saturation time ± 5 min        |        |      |
| 25 min                         | 38.25  | 0.63 |
| 35 min                         | 60.88  | 1.0  |
times on the same day. The intermediate precision was similarly evaluated over a period of 2 days by two different analysts. The precision of the method was expressed as relative standard deviation (RSD, %). The low RSD values indicate that there were no significant variations in the analysis of doxycycline hyclate at the given concentration levels.

**Accuracy.** The accuracy of the method was investigated using the recovery method i.e. by adding known amounts of drug (reference standard) to samples of doxycycline hyclate dosage form corresponding to three concentration levels (80, 100, and 120% of the label claim) along with the excipients. The mixtures were reanalyzed by the proposed method. The percentage recoveries of doxycycline at each level were determined (Table 4). The recovery value obtained in this study which is 100.5 ± 3.2% is comparable to other analytical methods reported in the literature for Doxycycline [4].

The method validation results were summarized in Table 5. Validation results demonstrate that the method is suitable for routine pharmaceutical analysis. The results are in compliance with analytical method development reference guidelines [20].

**Sample solution stability study**

Stability study of sample solutions was conducted for 14 days after preparation. The average peak areas and RSD values were calculated and presented in Table 6. The average peak areas after 24 h were significantly varied from average peak areas obtained immediately following sample preparation.

**Analysis of commercial dosage forms**

Different brands of doxycycline hyclate in capsule and tablet formulations; Teradoxine, Doxylag, Miraclin and Doxydenk each of them labeled to contain 100 mg of doxycycline hyclate and Medomycin capsule labeled to contain 100 mg of doxycycline HCl were purchased and analyzed using the developed method (HPTLC). Assay was performed in triplicate for each dosage form and the average drug contents were expressed in percent. Doxylag, Teradoxine and Doxydenk resulted in relatively higher % RSD values. Additionally, the dosage forms were analyzed using HPLC method and the results were compared. In both methods the average contents of all dosage forms were between 95 and 105% which is in the acceptable range. Another previous study that determined content of two brands of doxycycline hyclate tablets using HPLC-UV reported 106 and 99.38% with % RSD of 1.15 and 1.43 respectively [4]. Even though...
Table 7. Analysis of commercial dosage forms (DFs)

| Product name | Medomycin® (Capsule) | Teradoxine® (Capsule) | Doxylag® (Capsule) | Miracline® (Tablet) | Doxydenk® (Tablet) |
|--------------|----------------------|----------------------|-------------------|---------------------|-------------------|
| Batch No     | CBC601               | A1F131               | 3,198             | 24,258              | 3,347             |
| Manufacturing date | 04/2016                  | 06/2017              | 03/2017           | 06/2017             | 04/2017           |
| Expiry date  | 02/2021              | 03/2019              | 03/2020           | 10/2021             | 03/2020           |
| Manufacturer | Medochemie Ltd., Cyprus | Houns Co Ltd., Korea (South) | Labatec Pharma SA, Switzerland | Laboratorio Farmacologico | Artesan Pharma Ltd., Cyprus |
| No of Assay | 1                    | 2                    | 3                  | 1                   | 2                 |
| Average content (%) by HPTLC ± SD | 99.8 ± 4.73 | 100 ± 6.08 | 98.3 ± 7.06 | 96.4 ± 4.6 | 101.1 ± 7.9 |
| %RSD | 4.74 | 6.08 | 7.21 | 4.8 | 7.8 |
| Average content (%) by HPLC ± SD | 101.8 ± 0.234 | 97.7 ± 0.306 | 104.8 ± 0.219 | 98.8 ± 0.497 | 100.9 ± 0.279 |
| %RSD | 0.216 | 0.313 | 0.209 | 0.503 | 0.277 |

Table 7 shows the results of dosage form analysis in detail.

HPLC analysis was performed using the method in BP 2013. According to BP the content of doxycycline capsules and tablets should not be less than 95% and not more than 105% of the labeled amount. Based on the above specification average contents of dosage forms analyzed by HPTLC and HPLC methods were within the specification limit. According to USP the content of doxycycline capsules and tablets should not be less than 90% and not more than 120% of the labeled amount. The assay result shows that all dosage forms analyzed were also within the limit of USP specification.

Statistical comparison of the developed HPTLC and the official HPLC methods was performed using assay results for five brands of doxycycline obtained using the two methods. Assay results obtained from HPTLC analysis for the five brands were 99.8, 100, 98.3, 96.4 and 101.9% (mean = 99.06%, Standard deviation = 1.86). The results obtained from HPLC analysis for the five brands were 101.8, 97.7, 104.8, 98.8 and 100.9% (mean = 100.79%, Standard deviation = 2.75). The theoretical value for F-Value is equal to 9.6045, (P = 0.05) while the calculated F-Value is only 2.186 and hence no-significant difference is observed between the two methods. The results from both HPTLC and HPLC analysis are similar and comparable indicating that the developed method can be successfully used to analyze doxycycline hyclate in pharmaceuticals. Moreover the HPTLC method had offered short analysis time, reduced cost and simplicity compared to the HPLC pharmacopeial method. Thus, in actual quality control duties of Doxycycline containing medicines in regulatory and pharmaceutical manufacturing laboratories, this method can be used effectively.

CONCLUSION

A new high-performance thin-layer chromatographic (HPTLC) method has been developed and validated for determination of doxycycline hyclate in capsule and tablet formulations. Reliable HPTLC analysis of this drug can be performed on glass plates coated with C18 Reverse phase silica gel 60 F254 and pretreated with EDTA solution. The mobile phase was dichloromethane: methanol: acetonitrile: 1% aqueous ammonia in the ratio of 10:22:53:15 (v/v). Densitometry analysis was performed at 360 nm. The method is simple, sensitive (limit of detection and quantification 40 ng and 160 ng per spot), precise (RSD ≤ 3.66), and linear over the range 200–1,000 ng/spot with r² value of 0.997. Doxycycline hyclate sample is stable for 24 h at room temperature (with losses ≤ 6.41%). The developed HPTLC method was found suitable for determination of doxycycline in capsule and tablet dosage formulations (Teradoxine®, Medomycin®, Doxylag®,
Miraclin® and Doxydenk®) without any interference from the excipients. Statistical test (F-test) demonstrates that the results are comparable to the official pharmacopoeial (BP) HPLC method. Statistical data obtained during validation of the method also indicates that it is robust, specific and accurate. It has a satisfactory repeatability for the analysis of doxycycline hyclate.

Therefore, the developed HPTLC method offers many advantages in terms of cost, reduced analysis run time, simplicity, precision, accuracy, robustness. Thus, the new method can be utilized in routine quality control laboratory duties for medicines containing doxycycline as API.

Conflict of interest: The authors do not have any conflict of interest to declare in this research and manuscript preparation.

Data availability statement: All the important data generated has been utilized in writing this manuscript and there is no any other additional data.

ACKNOWLEDGMENT

This research is funded by Addis Ababa University Graduate student research support programme and The Ministry of Science and Technology (now re-named Ministry of Innovation and Technology) of the Federal Democratic Republic of Ethiopia, in terms of support for the project entitled “Developing, validating and adopting simple mobile technologies in drug quality evaluation and counterfeit detection.” through its national innovative award to AA. The HPTLC instrumentation and analytical capacity was funded by USAID through the Preventative Technologies Agreement managed by the Supply Chain Management System (SCMS) project of the Management Sciences for Health (MSH) to TL.

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