Quantitative Analysis of Emtricitabine in Dosage Forms Using Green RP-HPTLC and Routine NP-HPTLC Methods—A Contrast of Validation Parameters

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ABSTRACT: In this research, an antiviral drug emtricitabine (ECT) was quantified using the validated green reversed-phase high-performance thin-layer chromatography (RP-HPTLC) and routine normal-phase HPTLC (NP-HPTLC) methods in the marketed oral solutions and capsules. Green RP-HPTLC-densitometry quantification was performed using the acetone/water (70:30, v/v) solvent system as the mobile phase. Routine NP-HPTLC-densitometry quantification was performed using the chloroform/methanol (85:15, v/v) solvent system as the mobile phase. The detection was performed at $\lambda_{\text{max}}=285$ nm for both of the methods. Both densitometry methods were validated for different parameters. Most of the validation parameters including linearity, precision, accuracy, detection, and quantification limits for the green densitometry method were found to be superior compared to the routine densitometry technique. The ECT contents of commercial oral solution and commercial capsules were found to be 100.85 and 98.27%, respectively, using the green densitometry technique. The ECT contents of oral solutions and capsules were 97.16 and 95.54%, respectively, using the routine densitometry technique. Accordingly, the green densitometry technique was found to be better than the routine densitometry technique for ECT assays. Thus, the green densitometry technique can be successfully applied for the quantitation of ECT in the marketed formulations.

1. INTRODUCTION

Emtricitabine (ECT) is an antiviral drug, which inhibits human immunodeficiency virus type I (HIV-1) reverse transcriptase. It has been recommended for the treatment of HIV-I patients either alone or in combination with similar compounds. It is a highly water-soluble compound, which is absorbed rapidly and extensively after administration of commercial capsules or commercial oral solution. Extensive literature survey revealed a variety of analytical methods for the estimation of ECT in its pure form, the real samples of dosage forms, and biological fluids. Various ultraviolet (UV) spectrometry methods have been proposed for the quantitative analysis of ECT either alone or in combination with other antiviral drugs in pharmaceutical dosage forms. Several high-performance liquid chromatography (HPLC) techniques have been also used for the quantitative analysis of ECT in its single and combined dosage forms. The HPLC method has also been reported for the quantitative analysis of ECT in human plasma. Some ultra-performance liquid chromatography (UPLC) methodologies have also been reported for the quantitation of ECT in combined dosage forms. Several liquid-chromatography tandem mass spectrometry (LC−MS) methodologies have been applied for the quantitative analysis of ECT in combination with other antiviral compounds in human and seminal plasma samples. Some other techniques like capillary electrophoresis and electrochemical techniques have also been applied for the estimation of ECT in dosage forms. Several high-performance thin-layer chromatography (HPTLC) techniques have also been used for the quantitative analysis of ECT in its single and combined dosage forms. However, there is no single study and report on green reversed-phase HPTLC (RP-HPTLC) densitometry analysis of ECT in pharmaceutical dosage forms and biological samples. The reported HPTLC methods for the quantitative analysis of ECT have not considered the environmental impact. Recently, researchers are focusing much attention on green HPTLC methods for the quantitative analysis of drugs/pharmaceuticals in dosage forms and biological samples. In addition, green HPTLC methods present many advantages compared to routine HPTLC methods of analysis. In RP-HPTLC analysis, the acetone/water solvent system was used. Both acetone and water are the green solvents according to the principle of green analytical chemistry, and therefore, this method is termed as the green RP-HPTLC method. This research aimed to establish a

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rapid and sensitive green RP-HPTLC-densitometry technique for the determination of ECT in pharmaceutical dosage forms in comparison with the routine normal-phase HPTLC (NP-HPTLC)-densitometry technique of analysis. Both the densitometry techniques for the estimation of ECT were validated following the “International Conference on Harmonization (ICH)” Q2 (R1) guidance.

2. RESULTS AND DISCUSSION

2.1. Method Development. Exhaustive literature survey indicated various HPTLC methods for the determination of ECT in dosage forms. However, there is no single study and report on green densitometry quantitation of ECT in dosage forms and biological fluids. Therefore, this research aimed to establish a reliable green analytical assay for the estimation of ECT. The validation parameters of both of the methods were compared to obtain the best methodology for the quantitation of ECT.

For the green densitometry estimation of ECT, different proportions of acetone and water including acetone/water (50:50, v/v), acetone/water (60:40, v/v), acetone/water (70:30, v/v), and acetone/water (80:20, v/v) were evaluated as solvent systems for the establishment of an acceptable band for ECT analysis. From the results, it was found that acetone/water (50:50, v/v), acetone/water (60:40, v/v), and acetone/water (80:20, v/v) solvent systems offered a weak densitometry peak of ECT with a poor asymmetry factor. The solvent system containing acetone/water (70:30, v/v) was found to present a well-separated and compact densitometry peak of ECT at a retardation factor \( R_f = 0.79 \pm 0.01 \) (Figure 1). Accordingly, acetone/water (70:30, v/v) was optimized as the solvent system for the quantitation of ECT in the marketed oral solution and capsules using the green densitometry technique.

For the routine NP-HPTLC-densitometry analysis of ECT, different proportions of chloroform and methanol including chloroform/methanol (55:45, v/v), chloroform/methanol (65:35, v/v), chloroform/methanol (75:25, v/v), and chloroform/methanol (85:15, v/v) were evaluated as solvent systems for the establishment of an acceptable band for ECT analysis. The development of the solvent systems was performed under chamber saturation conditions for both the methods. From the results, it was found that chloroform/methanol (55:45, v/v), chloroform/methanol (65:35, v/v), and chloroform/methanol (75:25, v/v) solvent systems offered a weak densitometry peak of ECT with a poor asymmetry factor. The chloroform/methanol (85:15, v/v) solvent system was found to present a well-separated densitometry peak of ECT at \( R_f = 0.25 \pm 0.01 \) (Figure 2). The HPTLC peak of ECT was found to be improved by increasing the amount of chloroform (reducing the polarity) in the chloroform/methanol solvent system. Hence, the weak densitometry peak of ECT by chloroform/methanol (55:45, v/v), chloroform/methanol (65:35, v/v), and chloroform/methanol (75:25, v/v) solvent systems was possible due to the higher polarity of these solvent systems compared to chloroform/methanol (85:15, v/v). Accordingly, chloroform/methanol (85:15, v/v) was selected as the solvent system for the quantitation of ECT in marketed oral solution and capsules using the routine NP-HPTLC method.

The spectral bands for both methods were observed densitometrically, and the peak response was found at the wavelength \( \lambda_{	ext{max}} = 285 \) nm for both of the methods. Accordingly, the entire quantitative analysis of ECT was conducted at \( \lambda_{	ext{max}} = 285 \) nm for both of the methods.
2.2. Validation Studies. The proposed analytical methods for the ECT determination were validated as per ICH guidelines. The results for the linearity evaluation of the calibration curve of ECT for the green and routine densitometry techniques are presented in Table 1.

Table 1. Linear Regression Data for the Calibration Curve of Emtricitabine for the Green Reversed Phase High-Performance Thin-Layer Chromatography and the Routine Normal Phase-HPTLC Methods (Mean ± SD; n = 6)

| parameters                  | green RP-HPTLC | routine NP-HPTLC |
|-----------------------------|----------------|-------------------|
| linearity range (ng/band)   | 30–800         | 40–400            |
| regression equation         | $y = 12.60x + 659.05$ | $y = 16.28x + 429.05$ |
| $R^2$                       | 0.9995         | 0.9985            |
| slope ± SD                  | 12.60 ± 0.64   | 16.28 ± 0.92      |
| intercept ± SD              | 659.05 ± 8.32  | 429.05 ± 6.38     |
| standard error of slope     | 0.26           | 0.37              |
| standard error of intercept | 3.39           | 2.60              |
| 95% confidence interval of slope | 11.47–15.35 | 14.66–20.23       |
| 95% confidence interval of intercept | 644.43–694.85 | 417.84–456.50     |
| LOD ± SD (ng/band)          | 10.30 ± 0.14   | 13.52 ± 0.22      |
| LOQ ± SD (ng/band)          | 30.90 ± 0.42   | 40.56 ± 0.66      |

The linearity range for the calibration curve of ECT was obtained as 30–800 ng/band using the green densitometry technique. The linearity range for the calibration curve of ECT was recorded as 40–400 ng/band using the routine NP-HPTLC method. The values of determination coefficient ($R^2$) for ECT were found as 0.9995 and 0.9985 for the green and routine densitometry techniques, respectively. These results suggested that both the methods presented a good linear relationship between the concentration and HPTLC peak area. However, the linearity range of the green densitometry technique was found to be superior to that of a routine densitometry method. On the other hand, the slope of the routine densitometry technique was found to be superior to that of the green densitometry technique. Overall, the green densitometry technique is proposed as more reliable for the determination of ECT.

The resulting data of accuracy measurements for the green and routine densitometry techniques are listed in Table 2. The % recovery of ECT for the green densitometry technique was estimated as 98.96–101.72% at four different concentrations studied. The % RSD range in the recovery of ECT for the green densitometry technique was found to be 0.75–1.69%. The % recovery of ECT for the routine NP-HPTLC technique was estimated as 96.43–101.95% at four different concentrations studied. The % RSD range in the recovery of ECT for the routine densitometry technique was found to be 2.19–3.31%. The recoveries which were within 100 ± 2% and % RSD which were within ± 2% showed that the green densitometry technique was more accurate for the quantitation of ECT compared to the routine densitometry technique.

The precision for the green and routine densitometry techniques was calculated as % RSD, and the resulting data are summarized in Table 3.

The % RSD values of ECT for the green densitometry technique were found to be 0.93–1.43% for intraday repeatability at four different concentrations studied. The % RSD values of ECT for the routine densitometry technique were found to be 0.99–1.49% for interday precision. The % RSD values of ECT for the routine NP-HPTLC technique were found to be 2.02–2.74% for intraday repeatability at four different concentrations studied. The % RSD values of ECT for the routine densitometry technique were found to be 2.07–2.80% for interday precision. The % RSD values for the green densitometry technique were observed within the limit of ICH guidance. The % RSD values for the routine NP-HPTLC technique were found far from ICH guidance. Accordingly, these observations showed that the green densitometry technique was highly precise for the quantitation of ECT compared to the routine densitometry technique.

The resulting data of the robustness evaluation for the green and routine densitometry techniques are presented in Table 4. The % RSD values for robustness analysis were found to be 0.90–1.01% for the green densitometry technique. The % RSD values for robustness analysis were obtained as 0.78–0.80 for the green densitometry technique. However, the % RSD values for robustness evaluation were found to be 1.70–1.76% for the routine densitometry technique. The % RSD values for the routine densitometry technique were calculated as % RSD, and the resulting data is shown in Table 3. The “LOD and LOQ” for the routine densitometry technique were found to be 13.52 ± 0.22 and 40.56 ± 0.66 ng/band, for the detection and quantification of ECT, respectively. The % RSD values of ECT were found to be 10.30 ± 0.14 and 30.90 ± 0.42 ng/band, for the detection and quantification of ECT, respectively. The results of “LOD and LOQ” indicated that both of the densitometry techniques were sensitive enough for the detection and quantification of ECT. However, the green densitometry technique was superior to the routine densitometry technique for the detection and quantification of ECT.

The specificity/peak purity for both methods was studied by comparing the overlaid UV spectra of ECT in commercial oral solution and capsules with those of standard ECT. The overlaid UV spectra of standard ECT and ECT in commercial oral solution and capsules were shown in Figure 3. The maximum response of ECT in standard and commercial oral solution and capsules was at $\lambda_{\text{max}}$.
= 285 nm for both the methods. The similar UV spectra, values and \( \lambda_{\text{max}} \) of ECT in standard ECT, commercial oral solution, and capsules indicated the specificity and peak purity for both the densitometry techniques.

### 2.3. Quantitative Analysis of ECT in Commercial Oral Solution and Capsules.

The utility of both the densitometry methods was verified in the determination of ECT in commercial oral solutions and capsules. The densitometry peak of ECT from oral solution and capsules was identified by comparing its TLC spot at \( R_f = 0.79 \pm 0.01 \) with that of standard ECT for the green RP-HPTLC methodology. The RP-HPTLC densitogram of ECT in commercial capsules for the green RP-HPTLC technique is shown in Figure 4 which was found to be similar with that of standard ECT.

The densitometry peak of ECT from marketed oral solution and capsules was verified by comparing its single TLC spot at \( R_f = 0.25 \pm 0.01 \) with that of standard ECT for the routine densitometry technique. The NP-HPTLC densitogram of ECT in marketed capsules for the routine NP-HPTLC technique is shown in Figure 5 which was also found similar with that of standard ECT. The ECT contents of commercial oral solution and capsules were obtained from the calibration curve of ECT. The % ECT contents of commercial oral solution and capsules were found to be 100.85 and 98.27%, respectively, using the green densitometry method. The % ECT contents of marketed oral solution and capsules were found to be 97.16 and 95.54%, respectively, using the routine densitometry technique. The % contents of ECT within ±2% using a green densitometry technique suggested that the green densitometry technique was superior to the routine densitometry technique for the determination of ECT in marketed oral solution and capsules. Based on the results of validation studies and pharmaceutical assay, the green RP-HPTLC method is recommended as a superior technique to the routine densitometry technique for the quantitation of ECT in commercial formulations.

### 2.4. Literature Comparison.

The present green and routine densitometry methods for the quantitation of ECT were compared with the reported HPTLC methods for the evaluation of precision and robustness. The results are presented in Tables 3 and 4.

**Table 3. Precision Evaluation for the Green and Routine Densitometry Techniques (Mean ± SD; n = 6)**

| conc. (ng/band) | area ± SD | standard error | RSD (%) | intraday precision | interday precision |
|-----------------|-----------|----------------|---------|-------------------|-------------------|
|                 | area ± SD | standard error | RSD (%) | area ± SD | standard error | RSD (%) |
| Green RP-HPTLC Method | | | | | | |
| 100             | 2088 ± 30 | 12.24          | 1.43    | 2144 ± 32 | 13.06   | 1.49   |
| 150             | 2686 ± 33 | 13.47          | 1.22    | 2752 ± 35 | 14.29   | 1.27   |
| 200             | 3324 ± 36 | 14.69          | 1.08    | 3422 ± 38 | 15.51   | 1.11   |
| 250             | 4158 ± 39 | 15.92          | 0.93    | 4212 ± 42 | 17.14   | 0.99   |
| Routine NP-HPTLC Method | | | | | | |
| 100             | 2258 ± 62 | 25.31          | 2.74    | 2314 ± 65 | 26.54   | 2.80   |
| 150             | 3128 ± 71 | 28.99          | 2.26    | 3054 ± 73 | 29.80   | 2.39   |
| 200             | 3882 ± 82 | 33.48          | 2.11    | 3746 ± 84 | 34.29   | 2.24   |
| 250             | 4498 ± 91 | 37.15          | 2.02    | 4532 ± 94 | 38.38   | 2.07   |

**Table 4. Robustness Evaluation for the Green and Routine Densitometry Methods (Mean ± SD; n = 6)**

| conc. (ng/band) | mobile phase composition (acetone/water) | area ± SD | % RSD | Rf |
|-----------------|------------------------------------------|-----------|-------|----|
|                 | Green RP-HPTLC Method | | | | |
| 100             | 72:28 +0.2 | 2748 ± 28 | 1.01  | 0.78 |
| 150             | 70:30 0.0 | 2674 ± 25 | 0.93  | 0.79 |
|                 | 68:32 −0.2 | 2554 ± 23 | 0.90  | 0.80 |
|                 | mobile phase composition (chloroform/methanol) | area ± SD | % RSD | Rf |
|                 | Routine NP-HPTLC Method | | | | |
| 100             | 87:13 +0.2 | 3286 ± 58 | 1.76  | 0.24 |
| 150             | 85:15 0.0 | 3164 ± 54 | 1.70  | 0.25 |
|                 | 83:17 −0.2 | 3024 ± 52 | 1.71  | 0.26 |

The % ECT contents in commercial oral solution and capsules were found to be 100.85 and 98.27%, respectively, using the green densitometry method. The % ECT contents of marketed oral solution and capsules were found to be 97.16 and 95.54%, respectively, using the routine densitometry technique. The % contents of ECT within ±2% using a green densitometry technique suggested that the green densitometry technique was superior to the routine densitometry technique for the quantitation of ECT in commercial formulations.

The overlaid UV absorption spectra of standard ECT and ECT in commercial oral solution and commercial capsules obtained using densitometry techniques are shown in Figure 3.
The quantitation of ECT. The results of comparative evaluation are listed in Table 5.

Table 5. Comparison of the Current Green and Routine Densitometry Methods with Reported HPTLC Methods for the Quantitation of ECT

| analytical method | linearity range (ng/band) | accuracy (% recovery) | precision (% RSD) | ref |
|-------------------|---------------------------|-----------------------|-------------------|-----|
| HPTLC             | 200–1000                   | 99.17–99.88           | 0.21              | 29  |
| HPTLC             | 200–2200                   | 98.79–99.61           | 0.34              | 30  |
| HPTLC             | 80–560                     | 99.30–99.90           | 0.27–2.00         | 31  |
| HPTLC             | 30–110                     | 99.84–101.46          | 0.26–0.59         | 32  |
| HPTLC             | 40–400                     | 98.07–99.59           | 0.15–0.99         | 33  |
| HPTLC             | 400–1400                   | 100.89–101.32         | 0.29–0.53         | 34  |
| routine NP-HPTLC  | 40–400                     | 98.43–101.95          | 2.02–2.80         | present work |
| green RP-HPTLC    | 30–800                     | 98.96–101.72          | 0.93–1.49         | present work |

Three different validation parameters including linearity, accuracy, and precision of the green and routine densitometry methods were compared with literature values of densitometry methods. The linearity for four of the literature HPTLC methods has been reported as 200–1000 ng/band,

29 200–2200 ng/mL, 30 80–560 ng/band, 31 and 400–2400 ng/band, 34 respectively. The accuracy and precision of these methods were within the limit of ICH guidance. However, the lower limit of quantification (LLOQ) of these methods was much lower than the green and routine densitometry method in this study. 29–31,34 The linearity for the other two literature HPTLC methods has been reported as 30–1100 ng/band 32 and 40–400 ng/band, 33 respectively. The accuracy and precision of these two methods were also within the limit of ICH guidance. However, the LLOQ of one of the methods was similar to that in this green RP-HPTLC method and lower than that in the routine NP-HPTLC method in this study. 32 The linearity range of one HPTLC method was the same as the routine NP-HPTLC method, but its LLOQ was lower than the present green RP-HPTLC method. 33 On the other hand, the precision and accuracy of all the reported HPTLC methods were found to be much better than the routine NP-HPTLC method. 29–34 The accuracy of literature HPTLC methods was found to be similar to the green RP-HPTLC method. Although, the precision of the literature HPTLC methods was found to be superior to the green RP-HPTLC method, but the precision values for the literature and green RP-HPTLC method were within the limit of ICH guidance (less than 2%). Based on these observations, the current green RP-HPTLC method for the quantitation of ECT was found to be reliable and better than the reported HPTLC methods, while the current NP-HPTLC method was found to be inferior to the reported HPTLC methods.

3. CONCLUSIONS

The quantitation of ECT in marketed oral solution and capsules was performed using green and routine densitometry techniques. Both densitometry methods were validated for the determination of ECT as per ICH guidance. The green RP-
HPTLC technique was found to be highly sensitive compared to the routine densitometry technique. The current green RP-HPTLC method for the quantitation of ECT was found to be better than the reported HPTLC methods, while the current routine NP-HPTLC method was found to be inferior to the reported HPTLC methods. Both densitometry methods were used in the quantitative analysis of ECT in the marketed oral solutions and capsules. The green RP-HPTLC technique was found to be superior to the routine densitometry technique in the quantitative analysis of ECT in the marketed oral solutions and capsules. Hence, the green densitometry technique could be successfully utilized for the routine quantitative analysis of ECT in pharmaceuticals.

4. MATERIALS AND METHODS

4.1. Materials. ECT (purity >99%) was acquired from “Beijing Mesochem Ltd. (Beijing, China)”. HPLC-grade acetone, methanol, and chloroform were procured from “E-Merck (Darmstadt, Germany)”. High-purity water was collected using a “Milli-Q unit (Bay City, MI, USA)”. ECT commercial oral solution and capsules were purchased from a pharmacy shop in “Riyadh, Saudi Arabia”. Other solvents/reagents utilized were of analytical grades.

4.2. Chromatographic Conditions and Instrumentation. The quantification of ECT using the green and routine densitometry techniques was performed using a “CAMAG HPTLC Instrument (CAMAG, Muttenz, Switzerland)”. The quantification of ECT was carried out on “10 × 20 cm glass-backed plates precoated with RP silica gel 60 F254S plates (E-Merck, Darmstadt, Germany)” for the green RP-HPTLC method. For the routine NP-HPTLC method, “10 × 20 cm glass-backed plates precoated with NP silica gel 60 F254S plates (E-Merck, Darmstadt, Germany)” were used for ECT quantification. The sample solutions were applied as 6 mm bands using a “CAMAG Automatic TLC Sampler 4 (ATS4) sample applicator (Geneva, Muttenz, Switzerland)” which was fitted with a “CAMAG microliter syringe (Hamilton, Bonaduz, Switzerland)” for both methods. The elution of ECT on TLC plates was carried out at an ambient temperature (22 °C) for both methods. The application rate was kept constant at 150 nL/s for both methods. The gas utilized for the sample application was nitrogen in case of both methods. Linear ascending development of the plates was carried out at a distance of 80 mm for both methods. The acetone/water (70:30, v/v) solvent system was used as the mobile phase for the green RP-HPTLC method. The chloroform/methanol (85:15, v/v) solvent system was used as the mobile phase for the routine NP-HPTLC method. The mobile phases of both methods were developed in “CAMAG automatic developing chamber 2 (ADC2) (CAMAG, Muttenz, Switzerland)”. The developing chamber was previously saturated with the respective mobile phases for 30 min at 22 °C. The scanning for both methods was performed at $\lambda_{\text{max}} = 285$ nm. The slit dimensions and scanning rate were fixed at $4 \times 0.45$ mm and $20 \text{ mm/s}$, respectively, for both methods. Each analysis was performed in three different replicates ($n = 3$). The software used for the data processing and analysis was “WinCATS (version 1.4.3.6336, CAMAG, Muttenz, Switzerland)” for both methods.

4.3. ECT Calibration Curve. An accurately weighed 100 mg of ECT was dissolved in 100 mL of acetone/water (70:30, v/v) and chloroform/methanol (85:15, v/v) solvent systems for green and routine densitometry techniques, respectively. Then, 1 mL of the above solutions was diluted again using the above-mentioned solvent systems to obtain a final stock solution with a concentration of 100 μg/mL for each method. Then, different volumes of the stock solution were diluted further with the same solvent systems described above to get ECT concentrations in different ranges for both methods. The different solutions of ECT were applied to RP-HPTLC plates for the green densitometry and NP-HPTLC plates for the routine densitometry method. The densitometry responses for ECT were recorded for each concentration using both of the techniques. The calibration curve was made by plotting the concentrations on the x-axis and the measured HPTLC peak area on the y-axis for both of the techniques. The calibration curve of ECT for the green and routine densitometry methods was constructed in different concentration ranges.

4.4. Sample Preparation for the Quantitative Analysis of ECT in Commercial Oral Solution and Capsules. For the quantitative analysis of ECT in the commercial oral solutions, 1 mL of oral solution (containing 10 mg of ECT) was taken and diluted with 100 mL of the above mentioned solvent systems for both of the methods. For the quantitative analysis of ECT in marketed capsules, ten marketed capsules (each containing 200 mg of ECT) were weighted and the average weight was recorded. The contents of the capsules were taken and mixed homogenously in order to obtain a fine powder. An amount of fine powder containing 200 mg of ECT was dissolved in 100 mL of the above-mentioned solvent systems. Then, 1 mL of this stock solution was diluted again using 50 mL of the above-mentioned solvent systems for both of the methods. The solutions of the commercial capsules were filtered to remove any undissolved substances and sonicated for about 10 min. The sample solutions were subjected to quantitation of ECT using both methods.

4.5. Validation Studies. Both of the densitometry techniques for the quantitative analysis of ECT were validated for “linearity, precision, accuracy, robustness, LOD, LOQ, and specificity” following the ICH Q2 (R1) guidance. The linearity range of ECT was evaluated by plotting the concentration of ECT on the x-axis against its measured HPTLC peak area on the y-axis. Linearity was in the range 30–800 ng/band for the green densitometry method and 40–400 ng/band for the routine densitometry method. The accuracy of the method was evaluated as % recovery using the “standard addition method”. A previously quantified sample of ECT (100 ng/band) was spiked with 0–150% of ECT in order to get 100, 150, 200, and 250 ng/band concentrations, respectively, for both of the methods. The resulting solutions were reanalyzed using both of the methods. The % recovery of ECT was calculated for both of the methods in six different replicates ($n = 6$).

The precision was evaluated in terms of intra/interday repeatability for both of the methods. Intraday repeatability was assessed by the estimation of ECT at four different concentrations (100, 150, 200, and 250 ng/band) on the same day for both of the methods. Interday precision was evaluated by estimating ECT at four different concentrations (100, 150, 200, and 250 ng/band) on three different days for both of the methods. Both intraday and interday precisions were determined in six different replicates ($n = 6$).

The robustness was studied by making minor changes in the mobile phase components for both the methods. For the green densitometry technique, the original acetone/water (70:30, v/v) solvent system was changed to a acetone/water (72:28, v/v)
solvent system and acetone/water (68:32, v/v) solvent system, and the HPTLC response was recorded for each set of conditions. For the NP-HPTLC technique, the original chloroform/methanol (85:15, v/v) solvent system was changed to a chloroform/methanol (87:13, v/v) solvent system and chloroform/methanol (83:17, v/v) solvent system, and the HPTLC response was recorded for each set of conditions.

The “LOD and LOQ” for both methods were determined using a standard deviation method. The standard deviation of blank response was recorded in triplicates (n = 3). The “LOD and LOQ” of ECT for both of the techniques were estimated using their standard formulae, described previously.44

The specificity was evaluated by comparing the Rf values and UV absorption spectra of ECT in commercial oral solution and capsules with that of the standard ECT for both of the methods.

4.6. Quantitative Analysis of ECT in Commercial Oral Solution and Capsules. The prepared solutions of commercial oral solution and capsules were applied on HPTLC plates for both the methods, and their HPTLC chromatograms were recorded. The peak area of ECT in commercial oral solution and capsules was then recorded. The amounts of ECT in both of the dosage forms were calculated from the calibration curve of ECT for both of the techniques.

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Notes

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