Novel stability indicating methods for the determination of certain synthetic estrogen level modifiers

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Received 7 August 2012; accepted 4 November 2012
Available online 14 December 2012

KEYWORDS
First derivative of ratio spectra;
Spectrophotometry;
Raloxifene;
Tamoxifen;
Spectro-densitometry;
Stability indicating method;
Synthetic estrogen level modifiers

Abstract Tamoxifen citrate (TC) and raloxifene hydrochloride (RH) are two selective estrogen receptor modifiers. TC is usually used in the treatment of breast cancer while RH is used in the treatment of osteoporosis. Two stability indicating methods, namely, first derivative of ratio spectra (1DD) and TLC-densitometric method are used for the determination of TC in the presence of its photodegradants and RH in the presence of its oxidative degradants. For the first derivative of ratio spectra method, TC was quantitatively measured at 263 nm and 298.2 nm in a concentration range of 10–60 µg/mL while RH was determined at 267.6 nm in a concentration range of 2–18 µg/mL. In the spectro-densitometric method, TC was separated from its photodegradants using a developing system consisting of acetonitrile: 33% ammonia solution (10: 0.1, v/v) in a concentration range of 6–20 µg/band while RH was separated from its oxidative degradants using ethyl acetate: methanol: 33% ammonia solution (7: 3: 0.1, by volume) as a developing system in a concentration range of 3–11 µg/band. The two methods were successfully applied for the stability indicating the determination of the two drugs in a pure powdered form and a pharmaceutical dosage form and showing good recoveries. Statistical comparison between the results obtained by applying the proposed methods and the official method or the reported method for TC and RH, respectively was done and no significant difference was found at $p = 0.05$. 

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1. Introduction

Tamoxifen citrate (TC) ((Z)-2-[4-(1,2-diphenyl-1-butenyl)phenoxyl]-N,N-dimethyl-ethanamine-citrate) and raloxifene hydrochloride (RH) ([6-hydroxy-2-(4-hydroxyphenyl)benzo[b]thien-3-yl][4-[2-(1-piperidinyl)ethoxy]phenyl]-methanone hydrochloride) are synthetic estrogen level modifiers. These compounds block estrogen action in the breast and uterus but also can maintain bone density and reduce circulating levels of cholesterol as estrogen-like molecules. TC is a member of the triphenylethylene antiestrogen group. It binds strongly to estrogen receptors leading to the formation of complexes which are unable to translocate into the nucleus of target tissues or do not bind properly to the acceptor site of chromatin. TC acts as a partial agonist exhibiting antagonistic effects in breast tissues while giving agonistic effects in the uterus and bones. RH is an example of the benzothiophene selective estrogen receptor modulators. It maintains bone density and is used for the treatment of osteoporosis.

2. Experimental

2.1. Reagents

All chemicals used were of analytical grade and all solvents were of spectroscopic or HPLC grade.

Methanol, 33% ammonia solution and ethyl acetate were obtained from Prolabo (Pennsylvania, USA) while acetonitrile, HPLC-grade, was obtained from E. Merck (Darmstadt, Germany).

2.2. Pure samples

Tamoxifen citrate (TC) was kindly supplied by Sedico, 6th October, Egypt. Its purity was found to be 99.88 ± 1.061 according to the official method while raloxifene hydrochloride (RH) was kindly supplied by Lilly, Cairo, Egypt and its purity was found to be 99.99 ± 1.150 according to the reported method.

2.3. Market samples

- Nolvadex tablets, B. N. 70111, labeled to contain 10 mg of tamoxifen, packed by AstraZeneca, Egypt under License of AsraZeneca UK.
- Evista tablets B. N. A396125, labeled to contain 60 mg of raloxifene hydrochloride, produced by Lilly S.A. Madrid, Spain.

2.4. Standard solutions

Stock solution of TC photodegradants was derived from exposing (0.8 mg/mL) pure TC to sunlight radiation for 23 h during July, at a temperature of 37 ± 2°C from 8 am to 4 pm for three days. Working solution of photodegradants was prepared by a suitable dilution of the stock solution of TC photodegradants with methanol to obtain 0.1 mg/mL.

2.5. Instruments

- Spectrophotometric measurements were done using a double beam UV-Visible spectrophotometer (Shimadzu, Kyoto, Japan) model UV-1601 PC connected to an IBM compatible computer, with UVPC personal spectroscopy software version 3.7. The absorption spectra were carried out using 1 cm quartz cells.
- Solubilization was done using Sonicator (Bandelin Sonorex, Germany).
- Spectro-densitometric determination was done using Camag TLC scanner 3 S/N 130319 with winCats software
after an application of spots on Precoated TLC-plates, silica gel 60 G F$_{254}$ (20 cm $\times$ 20 cm, 0.25 mm), E. Merck, (Darmstadt-Germany) using Camag Linomat 5 autosampler (Switzerland) with the aid of Camag microsyringe (100 $\mu$L), Switzerland.

2.6. Procedures

2.6.1. Degradation of TC and RH

TC was subjected to photodegradation utilizing sunlight radiation where a portion of the pure drug (20 mg) was accurately

Figure 1  Zero-order absorption spectra of tamoxifen citrate 30 $\mu$g/mL (–) and its photodegradants 24 $\mu$g/mL (…….) in methanol.

Figure 2  Zero-order absorption spectra of raloxifene HCl (16 $\mu$g/mL) (–) and its oxidative degradants equivalent to 20 $\mu$g/mL (…….) using methanol as blank.
transferred and dissolved in methanol in a 25-mL well fitted volumetric flask. The flask was left in sunlight, during July, at a temperature of 37 °C ± 2, from 8 am to 4 pm for 3 days. Complete degradation was assessed by TLC-fractionation on silica gel 60 G F254 plates using acetonitrile:33% ammonia solution (10:0.1, v/v) as developing solvent. Visualization was carried out under UV-lamp at 254 nm.

RH was subjected to oxidative degradation using hydrogen peroxide where 100 mg of the pure drug was dissolved in 100 mL methanol. 5 mL of 30% hydrogen peroxide was added and the solution was refluxed for 12 h. Complete degradation was assessed by TLC-fractionation on silica gel F254 plates using ethyl acetate:methanol:33% ammonia solution (7:3:0.1, by volumes) as a developing solvent. Visualization was carried out under UV-lamp at 254 nm.

After complete degradation, the degraded solutions were evaporated nearly to dryness then the residues were quantitatively redissolved in methanol.

### Table 1 Assay validation sheet of the proposed methods and parameters of the regression equations.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Tamoxifen citrate</th>
<th>Raloxifene hydrochloride</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(1DD) at 263 nm</td>
<td>(1DD) at 298.2 nm</td>
</tr>
<tr>
<td>Range</td>
<td>10–60 µg/mL</td>
<td>10–60 µg/mL</td>
</tr>
<tr>
<td>Slope</td>
<td>0.0247</td>
<td>−0.0326</td>
</tr>
<tr>
<td>Intercept</td>
<td>0.0353</td>
<td>−0.0241</td>
</tr>
<tr>
<td>Mean</td>
<td>100.02</td>
<td>100.18</td>
</tr>
<tr>
<td>S.D.</td>
<td>0.738</td>
<td>0.937</td>
</tr>
<tr>
<td>Variance</td>
<td>0.545</td>
<td>0.878</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.9998</td>
<td>0.9998</td>
</tr>
<tr>
<td>RSD (a)</td>
<td>(0.476–0.618–1.186)</td>
<td>(0.477–0.992–0.406)</td>
</tr>
<tr>
<td>RSD (b)</td>
<td>(0.993–0.971–0.806)</td>
<td>(0.793–1.074–1.038)</td>
</tr>
</tbody>
</table>

* The intraday and 
*the interday relative standard deviations of different concentrations of TC (10, 30 and 40 µg/mL) and RH (6, 10 and 14 µg/mL) for the (1DD method, and (8, 10 and 14 µg/band) for TC and (4, 8 and 10 µg/band) for RH for the TLC-densitometric method.

72 H.A. Merey et al.

Figure 3 First derivative of the ratio spectra curves of tamoxifen citrate. 10–60 µg/mL (–) and of its photodegradants 20 µg/mL (–..–) using the spectrum of 24 µg/mL of photodegradants as divisor.

2.6.2. First derivative of ratio spectra method

2.6.2.1. Linearity. Into a series of 10-mL volumetric flasks, aliquots equivalent to 0.1–0.6 mg of TC or to 0.02–0.18 mg of RH were accurately transferred from their working solution (0.1 mg/mL). Each flask was completed to the mark with methanol. The spectra of the prepared standard solutions were scanned (200–400 nm) and recorded. The stored spectra of TC were divided (amplitude at each wavelength) by the spectrum of 24 µg/mL of photodegradants and the spectra of RH were divided by the spectrum of 24 µg/mL of oxidative degradants. The first derivative of the ratio spectra (1DD) was obtained at...
$\Delta \lambda = 4$ and a scaling factor $= 10$ or at $\Delta \lambda = 4$ and a scaling factor $= 100$ for TC and RH, respectively. The amplitudes of the first derivative of the ratio spectra peak at 263 nm and 298.2 nm for TC or at 267.6 nm for RH. Calibration graph was constructed relating the peak amplitudes of (1DD) to the corresponding concentrations and then the regression equations were computed.

2.6.2.2. Analysis of pharmaceutical dosage form. Twenty Nolvadex® tablets or ten Evista® tablets (after removing the coat with methanol and stand for 5 min, to dry), were accurately weighed and finely powdered. A quantity of the powdered tablets claimed to contain 10 mg of TC or RH was weighed and transferred to a 100-mL volumetric flask, followed by 60 mL methanol and the mixture was sonicated for one hour. The solution was filtered and the residue was washed with methanol 3 times then the volume was completed with methanol. Analysis was done as mentioned under linearity. The concentrations were calculated from the corresponding regression equations.

2.6.3. TLC-densitometric method

2.6.3.1. Chromatographic condition. Analysis was performed on precoated thin layer chromatographic plates, silica Gel 60 F$_{254}$

Table 2 Results of determination of tamoxifen citrate and raloxifene HCl in the presence of their degradants in laboratory prepared mixtures by the suggested first derivative of ratio spectra spectrophotometric method.

<table>
<thead>
<tr>
<th>Mix. No</th>
<th>Tamoxifen citrate</th>
<th>Raloxifene HCl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TC degradants (%)</td>
<td>TC conc. (µg/mL)</td>
</tr>
<tr>
<td>1</td>
<td>17 10 50</td>
<td>100.76</td>
</tr>
<tr>
<td>2</td>
<td>33 20 40</td>
<td>101.10</td>
</tr>
<tr>
<td>3</td>
<td>50 30 30</td>
<td>101.33</td>
</tr>
<tr>
<td>4</td>
<td>67 40 20</td>
<td>98.05</td>
</tr>
<tr>
<td>5</td>
<td>83 50 10</td>
<td>115.60**</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>100.31 ± 1.525</td>
<td>101.01 ± 0.816</td>
</tr>
<tr>
<td>RSD</td>
<td>1.520</td>
<td>0.808</td>
</tr>
</tbody>
</table>

* Average of three different determinations.
** Rejected values.
Samples were applied on the plates in the form of bands using Camag Linomat 5 autosampler utilizing a 100 μL Camag micro-syringe. The band length was 3 mm and the dosage speed was 150 nL/S. Bands were applied 9.3 mm apart from each other and 10 mm from the bottom edge of the plate. The nitrogen dried plates, were developed in chromatographic tank, presaturated for at least one hour with acetonitrile:33% ammonia solution (10:0.1, v/v) or ethylacetate:methanol:33% ammonia solution (7:3:0.1, v/v) for TC and its photodegradation or RH and its oxidative degradation, respectively, to a distance of approximately 8 cm. The developed plates were air dried and scanned at the following instrumental conditions:

- Source of radiation: deuterium lamp.
- Scan mode: absorbance mode.
- Slit dimensions: 3 mm × 0.45 mm.
- Scanning speed: 20 mm/S.
- Result output: chromatogram and integrated peak area.
- Wave length: 280 or 288 nm for TC or RH, respectively.

### Table 3

<table>
<thead>
<tr>
<th>Pharmaceutical dosage forms</th>
<th>Found % ± SD (at 263 nm)</th>
<th>Claimed amount taken (μg/mL)</th>
<th>Standard added (μg/mL)</th>
<th>Recovery % of standard added (at 263 nm)</th>
<th>Recovery % of standard added (at 298.2 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nolvadex® tablets B.N.70111</td>
<td>99.37 ± 0.629</td>
<td>99.70 ± 0.747</td>
<td>10.00</td>
<td>98.90</td>
<td>100.60</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>20.00</td>
<td>100.55</td>
<td>101.45</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>30.00</td>
<td>101.83</td>
<td>100.46</td>
</tr>
<tr>
<td></td>
<td>Mean ± SD</td>
<td></td>
<td></td>
<td>100.43 ± 1.469</td>
<td>100.84 ± 0.536</td>
</tr>
<tr>
<td></td>
<td>RSD</td>
<td></td>
<td></td>
<td>1.463</td>
<td>0.532</td>
</tr>
<tr>
<td>Evista® coated tablets B.N.A396125</td>
<td>99.33 ± 0.707</td>
<td></td>
<td>6.00</td>
<td>99.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4.00</td>
<td>100.17</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6.00</td>
<td>100.25</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>8.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean ± SD</td>
<td></td>
<td></td>
<td>99.81 ± 0.700</td>
<td></td>
</tr>
<tr>
<td></td>
<td>RSD</td>
<td></td>
<td></td>
<td>0.701</td>
<td></td>
</tr>
</tbody>
</table>

* Average of three different determinations.

![Figure 5](image)

**Figure 5** Chromatogram of (a) tamoxifen citrate, (b) Deg. 2 and (c) Deg. 1, separated using developing solvent (acetonitrile:33 % ammonia) (10:0.1, v/v), silica gel 60 GF254 plates and scanning at 280 nm.
2.6.3.2. Linearity. Aliquots equivalent to (6–20 μg) of TC or (3–11 μg) of RH were applied from their stock solutions (1 mg/mL) on thin layer chromatographic plates. The specified chromatographic conditions were adopted, and calibration curve was constructed by plotting areas under the peaks (AUP) versus drug concentrations and the corresponding regression equations were computed.

2.6.3.3. Analysis of pharmaceutical dosage form. Twenty Nolvadex® tablets or ten Evista® tablets (after removing the coat with methanol and stand for 5 min, to dry), were accurately weighed and finely powdered. A quantity of the powdered tablets claimed to contain 50 mg of TC or RH was weighed and transferred to a 50-mL volumetric flask, followed by 30 mL methanol and the mixture was sonicated for one hour. The solution was filtered and the residue was washed with methanol 3 times then the volume was completed with methanol. Analysis was done as mentioned under linearity. The concentrations were calculated from the corresponding regression equations.

2.6.4. Methods validation
Method validation was performed according international conference on harmonization ICH guidelines24 for the proposed methods.

2.6.4.1. Accuracy. The previously mentioned procedure under linearity was repeated for different concentrations of the pure TC and RH samples to check the accuracy of the methods. The concentrations were calculated from the corresponding regression equations and the mean percentage recoveries were then estimated. The accuracy of the proposed methods was also validated by applying the standard addition technique.

2.6.4.2. Precision. The previous procedure was repeated for the analysis of samples of TC and RH three times on the same day and on three successive days. The concentrations were calculated from the corresponding regression equations and the relative standard deviations (RSDs) were calculated and evaluated.

2.6.4.3. Specificity. Laboratory prepared mixtures containing TC and different percentages of its photodegradants or of RH and different percentages of its oxidative degradants were prepared and analyzed by proper procedures and the percentage recoveries were calculated from the corresponding regression equations.

3. Results and discussion

3.1. Degradation of TC and RH

Literature survey on the stability of estrogen modifiers showed that TC is liable to photodegradation while RH is liable to oxidative degradation. Photodegradation of TC was studied by Salamoun et al.25 and DellaGreca et al.26,27 Salamoun et al.25 reported that during the UV irradiation of TC, isomerization of the trans to the cis isomer takes place and consequently, corresponding highly fluorescent phenanthrene derivatives are formed. The structures of photoproducts were identified by HPLC, GC–MS, 1H NMR spectroscopy and LC-MS. DellaGreca et al.26 studied the photodegradation of tamoxifen in water by prolonged exposure to sunlight irradiation. The main photoproducts, have been identified by spectroscopic means, where photoisomerization, photocyclization and, to a lesser extent, photooxygenation appear to be involved in the drug degradation.

In this work, the process of photodegradation was monitored after exposing the drug to irradiation from sunlight, where complete degradation took place after 23 h of exposure to sunlight, at a temperature of 37 °C ± 2, from 8 am to 4 pm for 3 days. The degradation process was monitored by TLC using silica gel 60G F254 plates and acetonitrile: 33% ammonia solution (10: 0.1, v/v) as developing solvent. Visualization of the spots was done using UV-lamp at 254 nm where two new spots appeared at Rf values 0.83 and zero for Degradant 1 and Degradant 2, respectively.

Oxidative degradation of RH was studied by Hartauer et al.27 which reported that RH was converted to the N-oxide degradant upon oxidation with H2O2 when the drug is slurried with 0.3% aqueous solution of H2O2 and kept for one week at room temperature. Practically, upon refluxing RH with 30% H2O2, complete degradation was achieved after 12 h giving a mixture of 2 degradants that were separated on silica gel F254 plates using ethyl acetate:methanol:33% ammonia solution (7:3:0.1, v/v) as a developing solvent. Visualization was carried out under UV-lamp at 254 nm. Rf was found to be 0.04 and 0.09 for first and second degradants, respectively.

Since no stability indicating analytical methods were reported for the determination of the cited drugs in the presence of their degradation products, therefore, the aim of this work was to
develop simple spectrophotometric and chromatographic stability indicating methods for the determination of these drugs.

3.2. First derivative of ratio spectra spectrophotometric (1DD) method

The zero-order spectra of the studied drugs and their degradants show great overlap as shown in (Figs. 1 and 2). Derivative technique was tried to solve this overlapping and was found to be unsuccessful, therefore, derivative ratio spectroscopy was investigated to resolve this overlap.

Different divisors, $\Delta \lambda$, and scaling factor were tried to obtain maximum sensitivity and lowest noise.

For tamoxifen citrate, the best divisor was found to be the spectrum of 24 $\mu$g/mL degradants. The first derivative of ratio spectra was obtained at $\Delta \lambda = 4$ and scaling factor = 100 (Fig. 3). This figure indicates that tamoxifen citrate can be determined at 263 nm and 298.2 nm. The regression equations were computed (Table 1). The mean percentage recovery and the standard deviation for analyzing pure samples of tamoxifen citrate were calculated and found to be 100.02 ± 0.738 at 263 nm and 100.18 ± 0.937 at 298.2 nm, respectively. These results

![Figure 7](image-url)
indicate that both wavelengths can be used for the analysis of laboratory prepared mixtures and pharmaceutical dosage form. For RH, the best divisor was found to be the spectrum of 24 μg/mL degradants. The first derivative of ratio spectra was obtained at Δλ = 4 and scaling factor = 100. The peak amplitude at 267.6 nm was used for the determination of raloxifene HCl in the presence of its oxidative degradants (Fig. 4). The mean percentage recovery and standard deviation for analyzing pure samples of raloxifene HCl were calculated and found to be 99.96 ± 0.904.

The proposed methods were successfully applied for the determination of the studied drugs in the presence of up to 70% of its degradants (Table 2) and were also successfully applied for the analysis of these drugs in their pharmaceutical dosage forms with satisfactory percentage recoveries as shown in Table 3.

The validity of the proposed methods was assessed by applying the standard addition technique to pharmaceutical dosage forms, Table 3.

### 3.3. TLC-densitometric method

The availability of scanning of densitometry as a highly efficient quantitative tool for the separation and analysis of several samples simultaneously was suggested for the determination of the cited drugs in the presence of their degradants.

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### Table 5: Results of determination of tamoxifen citrate in Nolvadex<sup>®</sup> tablets and raloxifene HCl in Evista<sup>®</sup> tablets by the proposed densitometric method and application of standard addition technique.

<table>
<thead>
<tr>
<th>Pharmaceutical dosage forms</th>
<th>Found % ± SD&lt;sup&gt;¢&lt;/sup&gt;</th>
<th>Claimed amount taken (μg/band)</th>
<th>Standard added (μg/band)</th>
<th>Recovery % of standard added&lt;sup&gt;¢&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nolvadex&lt;sup&gt;®&lt;/sup&gt; tablets B.N.70111</td>
<td>96.63 ± 1.125</td>
<td>8.00</td>
<td>6.00</td>
<td>98.90</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8.00</td>
<td>100.50</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>10.00</td>
<td>101.30</td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td></td>
<td></td>
<td>101.27 ± 0.751</td>
<td></td>
</tr>
<tr>
<td>RSD</td>
<td></td>
<td></td>
<td>0.742</td>
<td></td>
</tr>
<tr>
<td>Evista&lt;sup&gt;®&lt;/sup&gt; coated tablets B.N.A396125</td>
<td>101.38 ± 0.884</td>
<td>4.00</td>
<td>3.00</td>
<td>98.33</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4.00</td>
<td>99.75</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5.00</td>
<td>98.40</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td></td>
<td></td>
<td>99.83 ± 0.800</td>
<td></td>
</tr>
<tr>
<td>RSD</td>
<td></td>
<td></td>
<td>0.810</td>
<td></td>
</tr>
</tbody>
</table>

<sup>¢</sup> Average of three different determinations.

### Table 6: Statistical analysis of the results of the proposed methods and the official or reported method of TC and RH, respectively.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Tamoxifen citrate</th>
<th>Raloxifene hydrochloride</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(1DD) at 263 nm</td>
<td>(1DD) at 298.2 nm</td>
</tr>
<tr>
<td>Mean</td>
<td>100.02</td>
<td>100.18</td>
</tr>
<tr>
<td>S.D.</td>
<td>0.738</td>
<td>0.937</td>
</tr>
<tr>
<td>Variance</td>
<td>0.545</td>
<td>0.878</td>
</tr>
<tr>
<td>n</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Student’s t-test&lt;sup&gt;***&lt;/sup&gt;</td>
<td>0.258 (2.262)&lt;sup&gt;***&lt;/sup&gt;</td>
<td>0.498 (2.262)&lt;sup&gt;***&lt;/sup&gt;</td>
</tr>
<tr>
<td>F value&lt;sup&gt;***&lt;/sup&gt;</td>
<td>2.07 (5.19)&lt;sup&gt;***&lt;/sup&gt;</td>
<td>1.28 (5.19)&lt;sup&gt;***&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>**</sup> The official method was non-aqueous titration. 17
<sup>***</sup> The reported method was HPLC method. 18
<sup>***</sup> Figures in parenthesis are the theoretical t- and F-values at p = 0.05.
RH showed a retention value of 0.61 while its two degradants had _R_ values 0.04 and 0.09, Fig. 7. Different concentrations of pure samples were analyzed by the proposed densitometric method and satisfactory recoveries were obtained 99.96 ± 1.264. Results in table 4 illustrate a good selectivity of the proposed methods for the determination of the studied drugs in the presence of its degradants. The mean percentage recoveries were 100.25 ± 0.508 and 99.56 ± 0.216 for TC and RH, respectively. The suggested methods were successfully applied for the determination of TC and RH in their pharmaceutical formulations showing fair percentage recoveries equal to 96.63 ± 1.125 and 101.38 ± 0.884 for TC and RH, respectively, as shown in Table 5. The validity of the proposed method was assessed by applying the standard addition technique to the pharmaceutical dosage form, Table 5.

The precision of the proposed derivative of ratio spectra and TLC methods was expressed in terms of relative standard deviation of the interday and intraday precision as shown in Table 1.

3.4. Statistical analysis

Results obtained from the proposed methods were compared to those obtained by applying the official non-aqueous titration method of TC or the reported HPLC of RH showing no significant difference as shown from the calculated _t_- and _F_-values (Table 6).

4. Conclusion

The suggested methods were found to be simple, accurate, selective and sensitive for the determination of TC and RH in the presence of their degradants with no significant difference between them and the official or reported methods and therefore can be used for routine quality control of these drugs.

5. Conflict of interest

None.

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