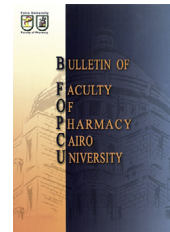Cairo University

Bulletin of Faculty of Pharmacy, Cairo University

www.elsevier.com/locate/bfopcu
www.sciencedirect.com

ORIGINAL ARTICLE

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

Hanan A. Merey *, **Maha M. Galal, Maissa Y. Salem, Ezzat M. Abdel-Moety**

Analytical Chemistry Department, Faculty of Pharmacy, Cairo University, Kasr El Aini Street, 11562 Cairo, Egypt

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 (^{1}DD) 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 $p = 0.05$.
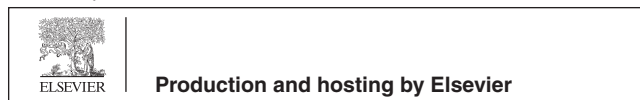
© 2012 Faculty of Pharmacy, Cairo University. Production and hosting by Elsevier B.V.
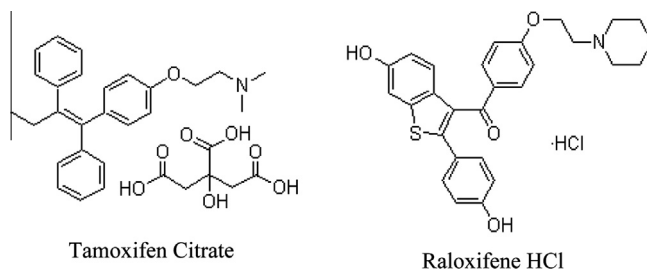Open access under CC BY-NC-ND license.

* Corresponding author. Tel.: +20 1003617394.
E-mail address: bibatofa@yahoo.com (H.A. Merey).
Peer review under responsibility of Faculty of Pharmacy, Cairo University.

Production and hosting by Elsevier

Tamoxifen Citrate

Raloxifene HCl

1. Introduction

Tamoxifen citrate (TC) ((Z)-2-[4-(1,2-diphenyl-1-bute-nyl)phenoxyl]-N,N-dimethyl-ethanamine-citrate) and raloxif-ene hydrochloride (RH) ([6-hydroxy-2-(4-hydroxyphenyl) benzo [b]hien-3-yl][4-[2-(1-piperidinyl)ethoxy]phenyl]-methanone hydro-chloride) 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.[1] 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.[2] 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.[3] Reviewing the literature, different analytical methods were used for the quantitative determination of the cited drugs including high performance liquid chromatography,[4–13] electrochemical methods[14–16] and spectrophotometric methods.[17–21] No stability indicating methods were reported for the analysis of cited drugs, therefore, the aim of this work was to develop simple accurate and precise methods for the determination of both drugs in the presence of their degradants.

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[22] while raloxifene hydrochlo-ride (RH) was kindly supplied by Lilly, Cairo, Egypt and its purity was found to be 99.99 ± 1.150 according to the reported method.[23]

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

- Standard solutions were prepared by separately dissolving TC and RH in methanol to obtain 1 mg/mL. Working solutions were prepared by a suitable dilution of the standard solution with methanol to obtain 0.1 mg/mL of both cited drugs.
- 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 °C ± 2, 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.
- Standard solution of RH oxidative degradants was derived from refluxing 1 mg/mL of pure RH with 30% hydrogen peroxide for 12 h. Working standard solution of oxidative degradants was prepared by a suitable dilution of the standard solution of RH oxidative degradants with methanol to obtain (0.2 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
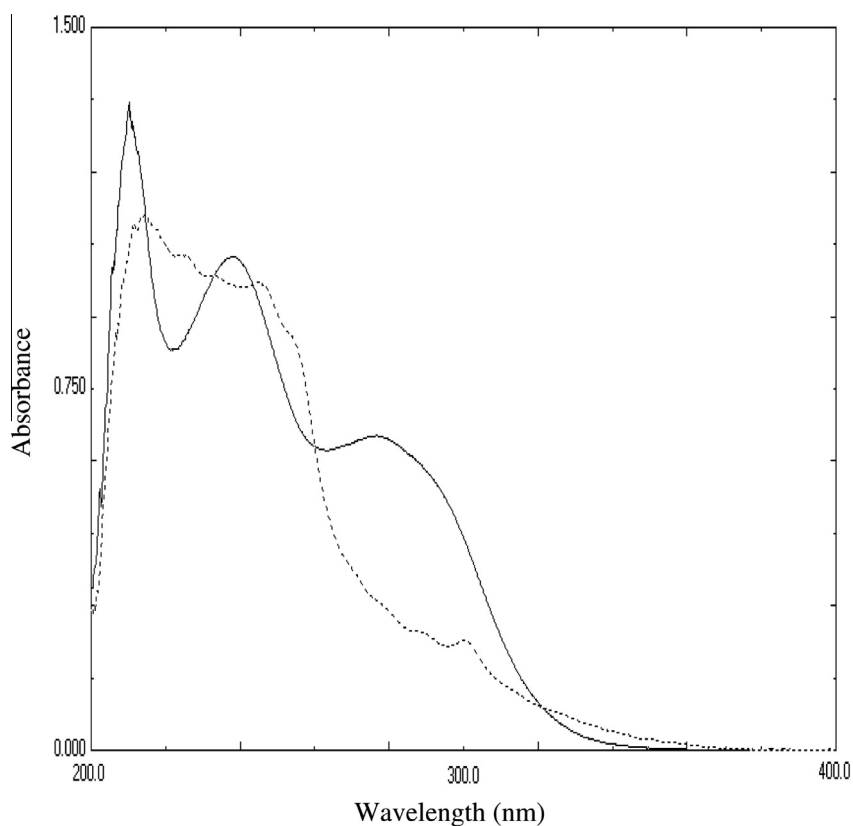
Figure 1 Zero-order absorption spectra of tamoxifen citrate 30 μg/mL (—) and its photodegradants 24 μg/mL (. . . . ..) in methanol.

after an application of spots on Precoated TLC-plates, silica gel 60 G F$_{254}$ (20 cm × 20 cm, 0.25 mm), E. Merck, (Darmstadt-Germany) using Camag Linomat 5 autosampler (Switzerland) with the aid of Camag microsyringe (100 μL), 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
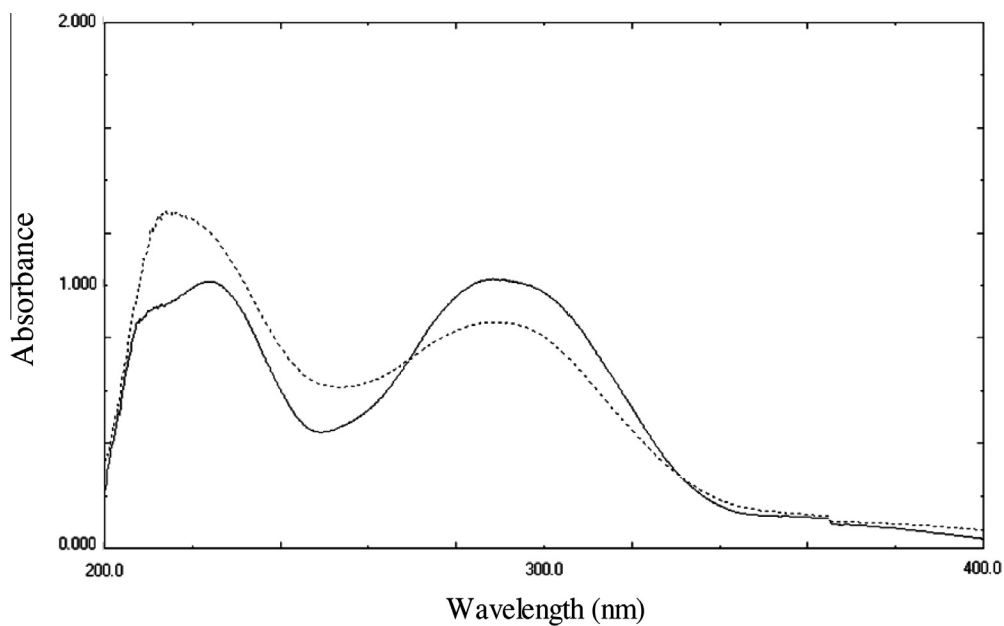

Figure 2 Zero-order absorption spectra of raloxifene HCl (16 μg/mL) (—) and its oxidative degradants equivalent to 20 μg/mL (. . . . ..) using methanol as blank.
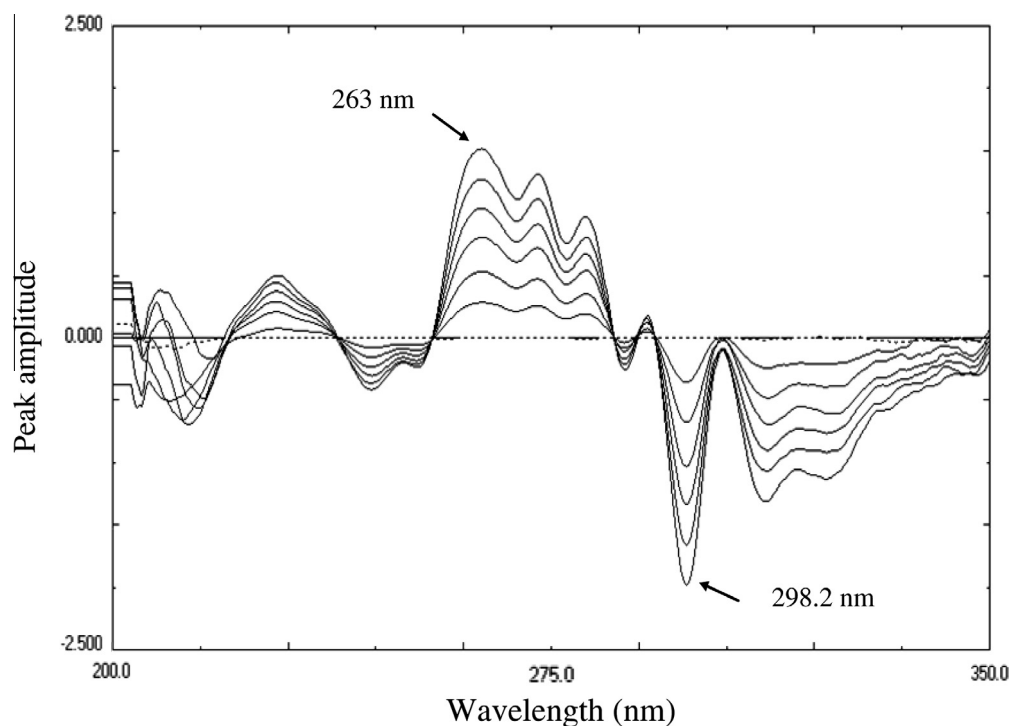
Figure 3 First derivative of the ratio spectra curves of tamoxifen citrate. 10–60 μg/mL (—) and of its photodegradants 20 μg/mL (. ….) using the spectrum of 24 μg/mL of photodegradants as divisor.

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

Parameters	Tamoxifen citrate			Raloxifene hydrochloride	
	(^{1}DD) at 263 nm	(^{1}DD) at 298.2 nm	TLC-densitometric	(^{1}DD) at 267.6 nm	TLC-densitometric
Range	10–60 μg/mL	10–60 μg/mL	6–20 μg/band	2–18 μg/mL	3–11 μg/band
Slope	0.0247	−0.0326	−0.0048	0.1036	−0.0316
Intercept	0.0353	−0.0241	1.32	0.0079	1.2277
Mean	100.02	100.18	100.00	99.96	99.96
S.D.	0.738	0.937	0.608	0.904	1.264
Variance	0.545	0.878	0.370	0.817	1.598
Correlation coefficient	0.9998	0.9998	0.9998	0.9999	0.9996
RSD[a]	(0.476–0.618–1.186)	(0.477–0.992–0.406)	(0.622–0.519–0.705)	(0.133–0.420–0.715)	(0.661–1.120–0.781)
RSD[b]	(0.993–0.971–0.806)	(0.793–1.074–1.038)	(0.799–0.919–0.863)	(0.580–0.716–0.895)	(1.036–1.695–1.397)

[a] The intraday and [b]the interday relative standard deviations of different concentrations of TC (10, 30 and40 μg/mL) and RH (6, 10 and 14 μg/mL) for the ^{1}DD method, and (8, 10 and 14 μg/band) for TC and (4, 8 and 10 μg/band) for RH for the TLC-densitometric method.

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 F$_{254}$ 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 F$_{254}$ 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.

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 (^{1}DD) was obtained at
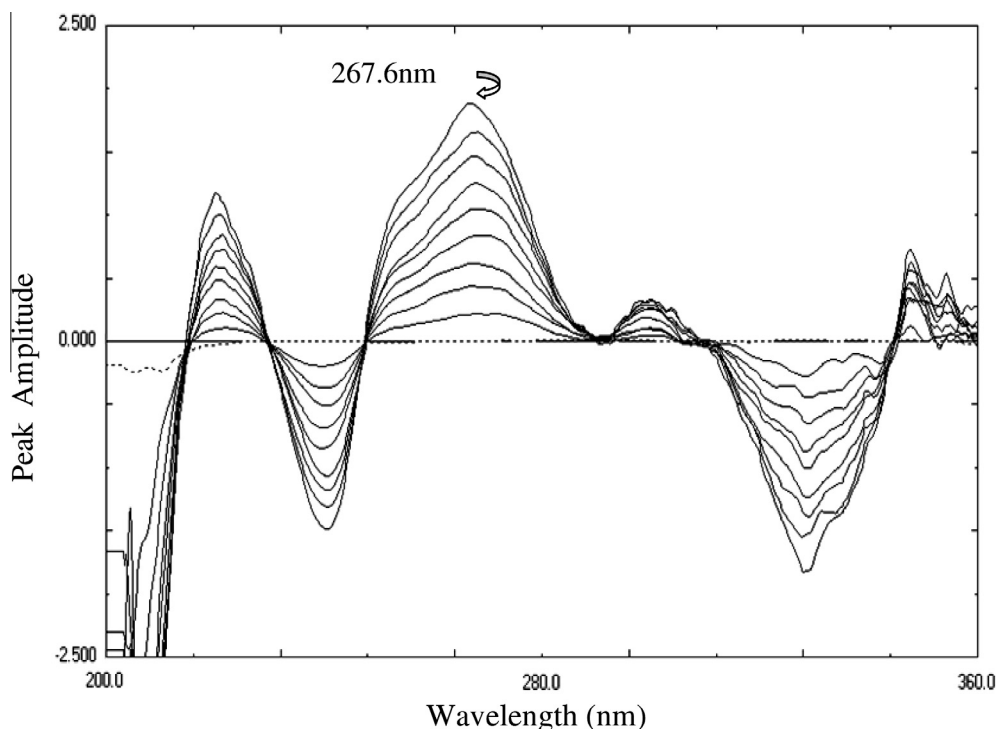
Figure 4 First derivative of the ratio spectra of (2–18 µg/mL) raloxifene HCl (—) using the spectrum of 24 µg/mL of oxidative degradants as divisor.

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.

Mix. No	Tamoxifen citrate					Raloxifene HCl			
	TC degradants		TC conc. (µg/mL)	Recovery %[*]		RH degradants		RH conc. (µg/mL)	Recovery %[*]
	%	(µg/mL)		at 263 nm	at 298.2 nm	%	(µg/mL)		
1	17	10	50	100.76	100.32	11	2	16	100.19
2	33	20	40	101.10	101.15	22	4	14	100.29
3	50	30	30	101.33	102.10	44	8	10	100.40
4	67	40	20	98.05	100.45	66	12	6	98.33
5	83	50	10	115.60[**]	103.10[**]	88	16	2	115.00[**]
Mean ± SD				100.31 ± 1.525	101.01 ± 0.816				99.80 ± 0.985
RSD				1.520	0.808				0.987

[*] Average of three different determinations.
[**] Rejected values.

$\Delta\lambda = 4$ 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 (^{1}DD) 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 pow-

dered 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 3 Results of determination of tamoxifen citrate in Nolvadex® tablets and raloxifen HCl in Evista® tablets by the proposed first derivative of ratio spectra spectrophotometric method and application of standard addition technique.

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

[*] Average of three different determinations.

(20 cm × 10 cm, 0.25 mm). 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.
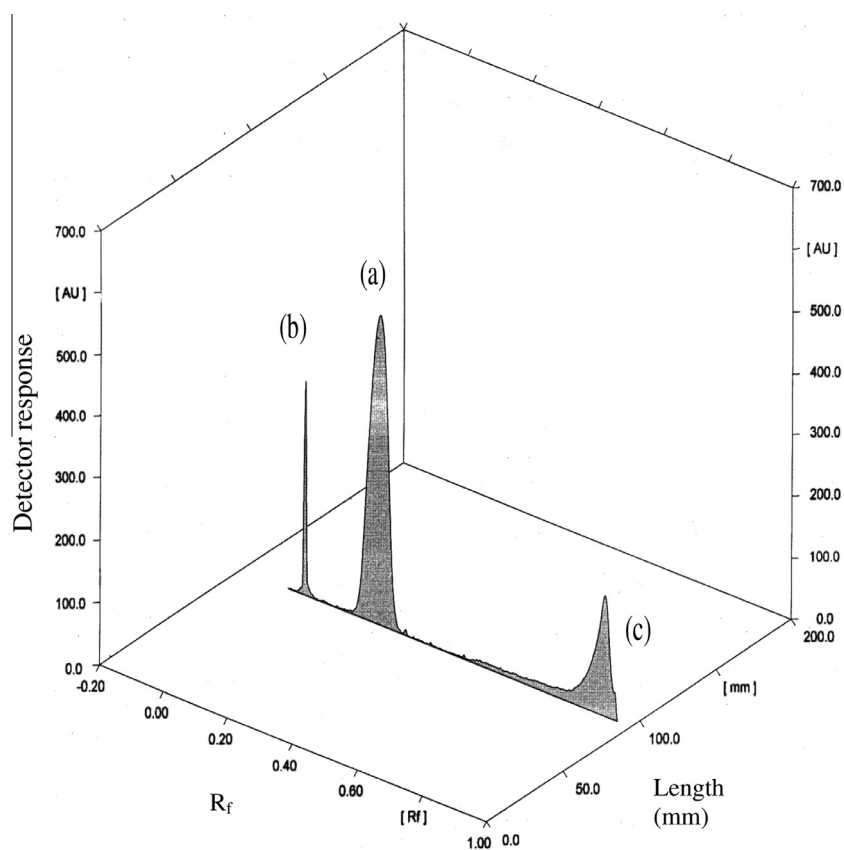
Figure 5 TLChromatogram of (a) tamoxifen citrate, (b) Deg. 2 and (c) Deg. 1, separated using developing solvent (acetonitrile:33 % ammonia) (10:0.1, v/v), silica gel 60 GF_{254} plates and scanning at 280 nm.

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 guidelines[24] 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] 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, ^{1}H 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 F_{254} 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 R_f 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 H_2O_2 when the drug is slurried with 0.3% aqueous solution of H_2O_2 and kept for one week at room temperature. Practically, upon refluxing RH with 30% H_2O_2, complete degradation was achieved after 12 h giving a mixture of 2 degradants that were separated on silica gel F_{254} plates using ethyl acetate:methanol:33% ammonia solution (7:3:0.1, by volume) as a developing solvent. Visualization was carried out under UV-lamp at 254 nm. R_f 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
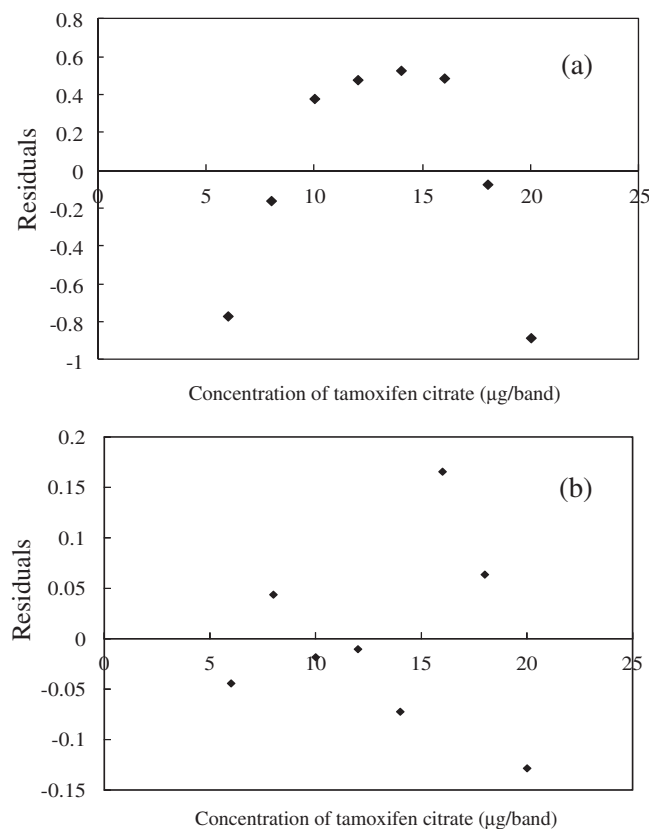

Figure 6 Residuals plot for the calibration of tamoxifen citrate using linear fit (a) and polynomial fit (b).
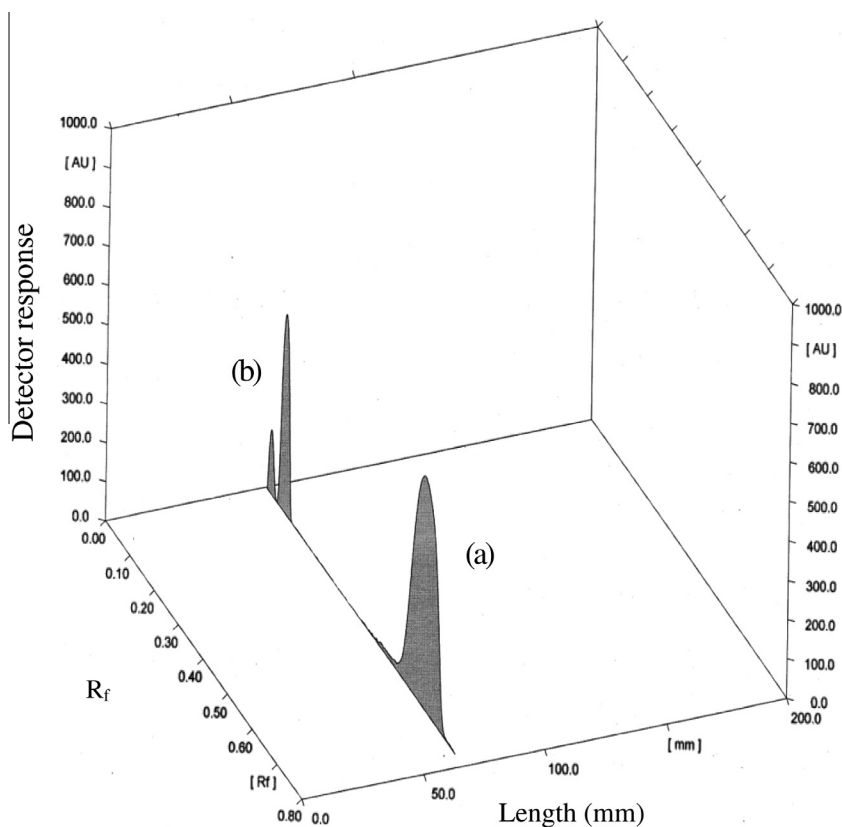
Figure 7 TLChromatogram of (a) raloxifene HCl (R_f = 0.61), (b) its oxidative degradants Deg. 1 and Deg. 2 (R_f = 0.04 and 0.09) using ethyl acetate:methanol:33% ammonia solution (7:3:0.1, by volumes) as a developing system.

Table 4 Results obtained for the determination of pure tamoxifen citrate in the presence of its photodegradants and raloxifene HCl in the presence of its oxidative degradants by the suggested densitometric method.

Mixture No.	Tamoxifen citrate				Raloxifene HCl			
	Degradants %	Taken (μg/band)	Found (μg/band)	Recovery (%)[*]	Degradants %	Taken (μg/band)	Found (μg/band)	Recovery (%)[*]
1	14	12.00	12.09	100.75	10	9.00	8.97	99.67
2	30	10.00	9.98	99.80	30	7.00	6.96	99.43
3	43	8.00	8.05	100.63	50	5.00	4.99	99.80
4	57	6.00	5.99	99.83	70	3.00	2.98	99.33
Mean ± SD			100.25 ± 0.508				99.56 ± 0.216	
RSD			0.507				0.217	

[*] Average of three different determinations.

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 μg/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

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 $\Delta\lambda = 4$ 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.

Different solvent systems were tried for this purpose. For TC and its photodegradation, satisfactory results were obtained using a developing solvent composed of acetonitrile:33% ammonia (10:0.1, v/v). The R_f values obtained were 0.31, 0.83 and zero for tamoxifen citrate (Deg. 1) and (Deg. 2), respectively, as shown in Fig. 5. The linear fit was plotted to represent these data but poor correlation coefficient was obtained ($r = 0.9933$). By comparing residual plots for polynomial and linear fit we found that better results were obtained upon using polynomial fit (Fig. 6), therefore linear fit was rejected. This case was observed by many authors who attributed this non linearity to the scattering of light at the plate surface. This scattering is highly dependent on the type of substrate, measuring wavelength, measuring mode, molar absorptivity and concentrations of the samples.[28] Calibration curves in densitometry generally comprise a pseudolinear region at low sample concentrations. In some instances the pseudolinear range is adequate for analytical purposes, while in others no reasonable linear range may exist.[29] The polynomial relationship was adopted between the area under the peak and the corresponding concentration of TC. The mean percentage recovery and the standard deviation for analyzing pure samples of TC were calculated and found to be 100.00 ± 0.608.

For RH and its oxidative degradants, satisfactory results were obtained by using a mobile phase composed of ethyl acetate: methanol: 33% ammonia (7: 3: 0.1, by volumes). Intact

Table 5 Results of determination of tamoxifen citrate in Nolvadex® tablets and raloxifen HCl in Evista® tablets by the proposed densitometric method and application of standard addition technique.

Pharmaceutical dosage forms	Found % ± SD[*]	Claimed amount taken (µg/band)	Standard added (µg/band)	Recovery % of standard added[*]
Nolvadex® tablets B.N.70111	96.63 ± 1.125	8.00	6.00	98.90
			8.00	100.50
			10.00	101.30
Mean ± SD				101.27 ± 0.751
RSD				0.742
Evista® coated tablets B.N.A396125	101.38 ± 0.884	4.00	3.00	98.33
			4.00	99.75
			5.00	98.40
Mean ± SD				99.83 ± 0.800
RSD				0.810

[*] 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.

Parameters	Tamoxifen citrate				Raloxifene hydrochloride		
	(^{1}DD) at 263 nm	(^{1}DD) at 298.2 nm	TLC-densitometric	Official method[*]	(^{1}DD) at 267.6 nm	TLC-densitometric	Reported method[**]
Mean	100.02	100.18	100.00	99.88	99.96	99.96	99.99
S.D.	0.738	0.937	0.608	1.061	0.904	1.264	1.150
Variance	0.545	0.878	0.370	1.126	0.817	1.598	1.323
n	6	6	8	5	9	9	6
Student's t-test[***]	0.258 (2.262)[***]	0.498 (2.262)[***]	0.262 (2.201)[***]		0.057 (2.160)[***]	0.047(2.160)[***]	
F value[***]	2.07 (5.19)[***]	1.28 (5.19)[***]	3.04 (4.12)[***]		1.62 (3.69)[***]	1.21 (4.82)[***]	

[*] The official method was non-aqueous titration.[17]
[**] The reported method was HPLC method.[18]
[***] 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_f value 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.

References

1. Brueggemeier RW, Miller DD, Dalton JT. *Foye's principles of medicinal chemistry*. 5th ed. New York: Lippincott Williams and Wilkins; 2002, p. 685–697.
2. Korolkovas A. *Essentials of medicinal chemistry*. 2nd ed. New York: Wiley Publications; 1988, p. 1018.
3. Loose-Mitchell DS, Stancel GM. *Goodman and gilman's the pharmacological basis of therapeutics*. 10th ed. New York: McGraw-Hill; 2001, p. 1597–1617.
4. Borges CR, Miller N, Shelby M, Hansen M, White C, Slawson MH, et al. Analysis of a challenging sunset of world anti-doping agency-banned steroids and antiestrogens by LC–MS-MS. *J Anal Toxicol* 2007;**31**(3):125–31.
5. Li XF, Carter S, Dovichi NJ, Zhao JY, Kovarik P, Kovarik P. Analysis of tamoxifen and its metabolites in synthetic gastric fluid digests and urine samples using high performance liquid chromatography with electrospray mass spectrometry. *J Chromatogr* 2001;**914**(1–2):5–12.
6. Manns JE, Hanks S, Brown JE. Optimised separation of E- and Z- isomers of tamoxifen and its principle metabolites using reversed-phase high performance liquid chromatography. *J Pharm Biomed Anal* 1998;**16**(5):847–52.
7. Merle O, Guitton J, Burke MD, Ollagnier M. Rapid and simple method to determine tamoxifen and its major metabolites in human liver microsomes by high performance liquid chromatography with fluorescence detection. *Anal Lett* 1998;**31**(12):2067–76.
8. Romanyshyn LA, Tiller PR. Ultra-short columns and ballistic gradients: considerations for ultra-fast chromatographic liquid chromatographic-tandem mass spectrometric analysis. *J Chromatogr* 2001;**928**(1):41–51.
9. Trontelj J, Bogataj M, Marc J, Mrhar A. Development and validation of liquid chromatography-tandem mass spectrometry assay for determination of raloxifene and its metabolites in human plasma. *J Chromatogr* 2007;**855**(2):220–7.
10. Xian-Jun L, Jing Z, Bing S, Jie Y. Determination of Tamoxifen and Letrozole in sewage by solid phase extraction and ultra performance liquid chromatography/tandem mass spectrometry. *Chin J Anal Chem* 2010;**38**(2):214–8.
11. Kashtiaray A, Farahani H, Farhadi S, Rochat B, Sobhi HR. Trace determination of Tamoxifen in biological fluids using hollow fiber liquid-phase microextraction followed by high performance liquid chromatography-ultraviolet detection. *Am J Anal Chem* 2011;**2**(4):429–36.
12. Kumar BV, Kumar KP, Suresh K, Aspar S, Srikanth P, Suneetha Y. Development and validation of RP-HPLC method for determination of raloxifene hydrochloride from pharmaceutical preparation. *J Chem Pharm Res* 2011;**3**(3):784–91.
13. Trdan T, Roskar R, Trontelj J, Ravnikar M, Mrhar A. Determination of raloxifene and its glucuronides in human urine by liquid chromatography-tandem mass spectrometry assay. *J Chromatogr B Anal Technol Biomed Life Sci* 2011;**879**(23):2323–31.
14. Guo XX, Song ZJ, Tian XJ, Song JF. Single-sweep voltammetric determination of tamoxifen at carbon paste electrode. *Anal Lett* 2008;**41**(7–9):1225–35.
15. Perez-Ruiz T, Martinez-Lozano C, Sanz A, Bravo E. Development and validation of a quantitative assay for raloxifene by capillary electrophoresis. *J Pharm Biomed Anal* 2004;**34**(5):891–7.
16. Rodriguez Flores J, Berzas Nevado JJ, Castaneda Penalvo G, Rodriguez Caceres MI. Micellar electrokinetic capillary chromatographic method for simultaneous determination of drugs used to treat advanced breast cancer. *Chromatographia* 2002;**56**(5-6):283–8.
17. Basavaiah K, Anil Kumar UR, Tharpa K, Vinay KB. Validated spectrophotmetric methods for the determination of raloxifene hydrochloride in pharmaceuticals. *J Chil Chem Soc* 2008;**53**(3):1635–9.
18. Dharuman J, Ravichandran V, Thirumoorthy N, Dharamsi A. Spectrophotometric analysis of raloxifene hydrochloride in pure and pharmaceutical formulations. *Pharmazie* 2004;**59**(9):720–1.
19. Haddad R, Sparrapan R, Eberlin MN. Desorption sonicspray ionization for (high) voltage-free ambient mass spectrometry. *Rapid Commun Mass Spectrom* 2006;**20**(19):2901–5.
20. Sastry CSP, Lingeswara Rao JS, Rao KR. Spectrophotometric methods for the determination of tamoxifen citrate. *Talanta* 1995;**42**(10):1479–85.
21. Kalyanaramu B, Raghubabu K. Development of new analytical method for determination of raloxifene hydrochloride in formulations based on charge transfer complex formation. *Int J Anal Bioanal Chem* 2011;**1**(2):29–33.
22. USP 34-NF 29. 29th ed. Twinbrook Parkway, Rockville: US Pharmacopeia National Formulary; 2011.
23. Trontelj J, Vovk T, Bogataj M, Mrhar A. HPLC analysis of raloxifene hydrochloride and its application to drug quality control. *Pharmacol Res* 2005;**52**(4):334–9.
24. ICH Harmonised Tripartite Guideline. Validation of analytical procedures: text and, methodology Q2(R1); 2005.
25. Salamoun J, Macka M, Nechvatal M, Matousek M, Knesel L. Identification of products formed during UV irradiation of

tamoxifen and their use for fluorescence detection in high performance liquid chromatography. *J Chromatogr* 1990;**514**(2):179–87.

26. DellaGreca M, Iesce MR, Isidori M, Nardelli A, Previtera L, Rubino M. Phototransformation products of tamoxifen by sunlight in water. Toxicity of the drug and its derivatives on aquatic organisms. *Chemosphere* 2007;**67**(10):1933–9.

27. Hartauer KJ, Arbuthnot GN, Baertschi SW, Johnson RS, Luke NG, Pearson NG, et al. Influence of peroxide impurities in povidone and crospovidone on the stability of raloxifene hydrochloride in tablets: Identification and control of oxidative degradation product. *Pharm Dev Technol* 2000;**5**(3):303–10.

28. Shimadzu dual wavelength flying spot densitometer CS-9000 users guide, Japan.

29. Poole CF, Poole SK. Progress in densitometry for quantitation in planar chromatography. *J Chromatogr B* 1989;**492**:539–84.