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Stability-indicating methods for determination of tadalafil in presence of its degradation product

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ABSTRACT

Four different stability indicating assay methods were developed and validated for the determination of tadalafil in the presence of its degradation product. The first and second method was based on the derivative and derivative ratio spectrophotometric technique using mixture of acetonitrile and water (1:1) as a solvent. In the third method, we used TLC-densitometric technique using high performance thin-layer chromatography plates with a developing system consisting of chloroform: acetone: ammonia (9:1:0.1, by volume). The fourth method was a high performance liquid chromatography. Separation of tadalafil from its degradate using C_{18} column and a mobile phase consisting of water: acetonitrile: methanolin the ratio of (45:35:20) at ambient temperature was achieved.

The developed methods were successfully applied to the analysis of pharmaceutical formulations containing tadalafil with excellent recoveries.

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KEYWORDS

Tadalafil;
Spectrophotometry;
Densitometry;
Stability;
HPLC.

INTRODUCTION

Tadalafil (TD), is (6*R-trans*)-6-(1,3-benzodioxol-5-yl)- 2,3,6,7,12,12a-hexahydro-2-methyl-pyrazino [1',2':1,6] pyrido[3,4-*b*]indole-1,4-dione. (Figure1)[1]. It is phosphodiesterase-5 inhibitors used for erectile dysfunction.Tadalafil is61% eliminated mainly in the faeces, 36% eliminated in urine[2]. The literature has analytical methods for quantitative estimation of tadalafil, in pharmaceutical formulations by HPLC[3-6], LC[7-10], NMR[11] and Densitometric determination[12,13].

The present work aimed to develop feasible, sensitive and specific analytical procedures for the analysis of tadalafil in presence of its degradation products. Adaptation of the proposed procedures to the analysis of the available dosage forms was also an important task in order to solve problems encountered in quality control and analysis of expired samples.

EXPERIMENTAL

Samples

- Tadalafil powder was kindlysupplied by Lilly Company, Egypt.
- Pharmaceutical formulation Cialis® tablets (labeled to contain 20 mg oftadalafil), manufactured by Lilly Del Caribe Inc., Puerto Rico industrial Park, Carolina, Puerto Rico.

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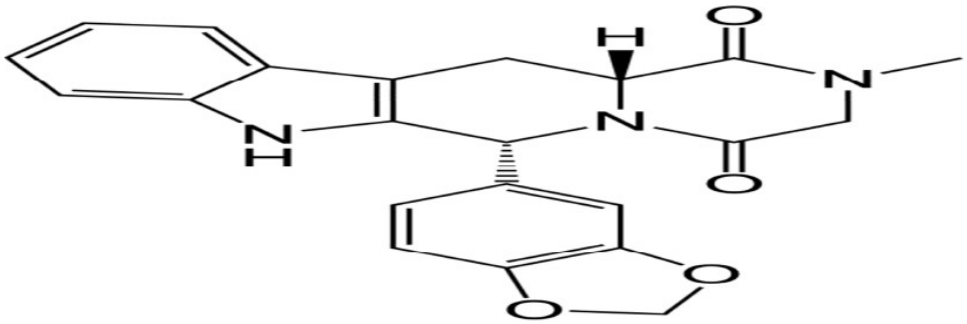

Figure 1: Chemical structure of tadalafil

Reagents

The materials used were chloroform,ammonia, hydrochloric acid (Adwic), acetone (Sigma); Purified water was prepared using a Millipore Milli-Q water purification system, all were of analytical grade. Acetonitrile, methanol (Merck) all were of HPLC grade, all Chemical were purchased from local market (Cairo, Egypt).

Standard solutions

Tadalafil standard and degradate solution (250μgmL^{-1}) in acetonitrile water mixture (1:1).

Apparatus

- A double beam UV-vis spectrophotometer (SHIMADZU, Japan) Model UV-1601 PC connected to IBM compatiblecomputer and HP 680 inkjet printer. The bundled software was UVPC personal spectroscopy software version 3.7. The spectra bandwidth was 2 nm and wavelength scanning speed was 2800 nm min^{-1}.
- Pre-coated TLC-plates, silica gel 60 F_{254} (20 cm x 20 cm, 0.2 mm) Fluka, (Switzerland).
- Camag TLC-scanner 3 S/N 130319 with winCATS software.
- CamagLinomat 5 auto sampler (Switzerland).
- Camag micro syringe (100 μL) (Switzerland).
- Liquid chromatograph consists of a "La-Chrom" HPLC instrument (Hitachi-Merck) Germany, pump model L-7110, connected with a detector model L-7420.

The injector was a manual Rheodyne injector (Model 7161, Catati, California, USA) equipped with a 10μL^{-1} injector loop and a 100μL Hamilton syringe. The instrument was connected to an IBM compatible PC bundled with Merck- Hitachi Model D-7000 chromatography Data Station software HPLC septum manager and an HP 800 inkjet printer.

A Lichrocart RP-18 column (250mm×4.6mm i.d.) particle size 5 μm (Merck, Germany) was used for the analysis.

Procedures

Degradation of tadalafil.[1]

Accurately weighed amount of 25 mg tadalafilthen 10-mL of (0.8 N) hydrochloric acid was added, refluxed for 3 hin 100-mL of acetonitrile water mixture (1:1). During reflux, small portions were cooled and spotted on a TLC plate. Spotting was repeated at 30 min intervals to follow up time required for complete degradation. Solution was cooled, spotted and then developed using chloroform: acetone: ammonia (9:1:0.1, by volume) as a developing system.

Spectrophotometric methods

Spectral characteristics of tadalafil and its degradation product

Aliquot portions equivalent to (17.5μg mL^{-1}) tadalafil and its degradate were transferred into two 10-mL volumetric flasks from their stock solutions (250μgmL^{-1}) and completed to volume with acetonitrile water mixture (1:1). The zero order and the first derivative spectra of the prepared solutions were recorded.

Fourth derivative method

Accurately aliquots ranging from 10 to 70 μL equivalents to 2.5–17.5μgmL^{-1}at 10 μL intervals of tadalafil stock solution (250mg mL^{-1}) were transferred into 10-mL volumetric flasks then complete to volume with acetonitrile water mixture (1:1). The peak amplitudes of the obtained fourth derivative spectra were measured at 285.7 nm (Δλ= 4nm). A calibration curve relating the peak amplitude of the fourth derivative curve

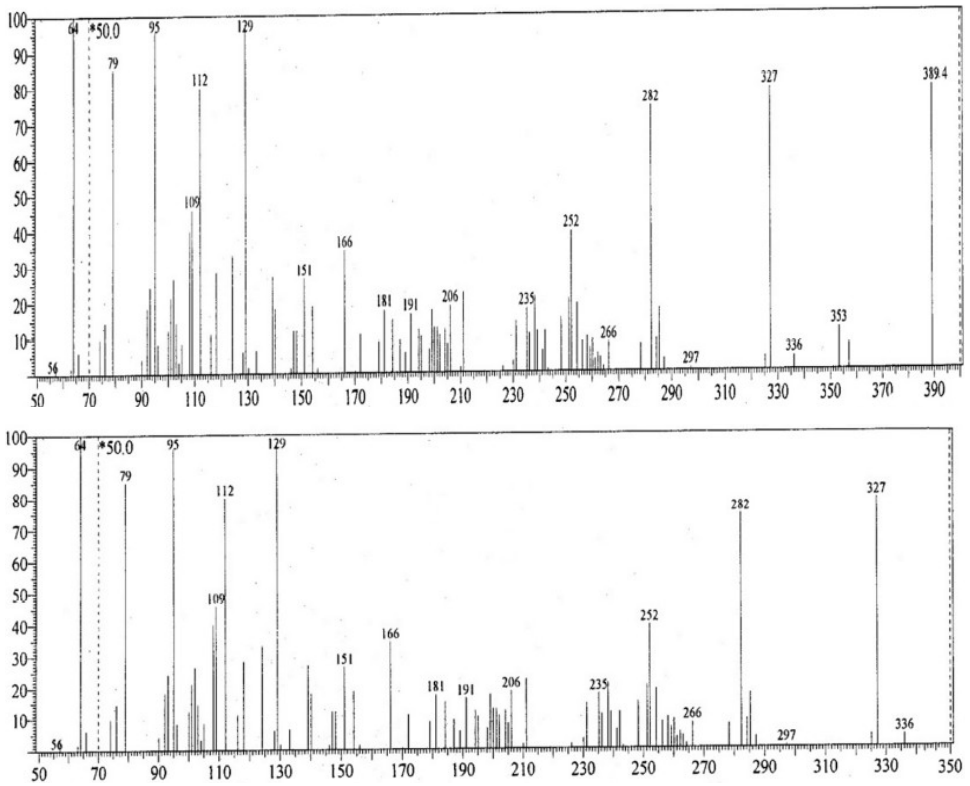

Figure 2 : Mass spectrum of tadalafil and it's degradate

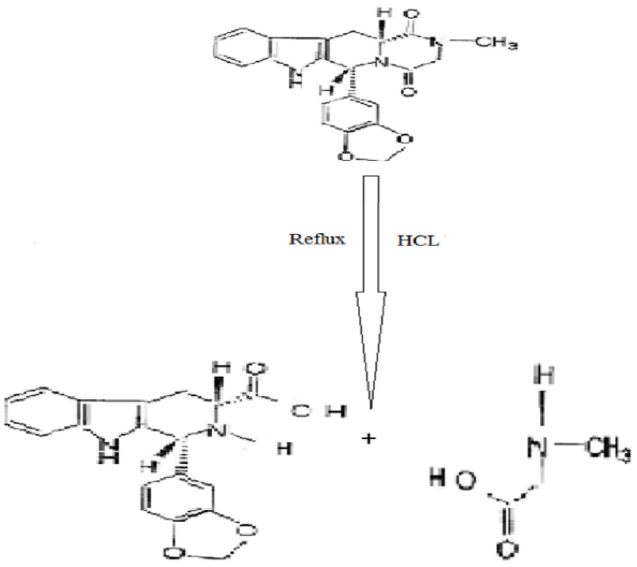

Figure 3 : Scheme degradation of tadalafil

at 285.7nm to the corresponding concentrations of tadalafil was constructed

Derivative ratio method

Different aliquots of tadalafilstock solution (250μgmL^{-1} in acetonitrile water mixture (1:1) ranging from 10 to 80 μL equivalents to 2.5 to 20 μgmL^{-1}at 10 μL intervals and 1.0 mL of the tadalafildegradate stock solution (100μgmL^{-1} in acetonitrile water mixture (1:1) were transferred into 100-mL volumetric flasks then completed to volume with acetonitrile water mixture (1:1). The spectra of the prepared standard solutions were scanned from 200 to 400 nm and stored in the computer. The stored spectra of tadalafil were divided (amplitude at each wavelength) by the spectrum of 10 μg mL^{-1} standard tadalafildegradate.

The first derivative of the ratio spectra (^{1}DD) were obtained at $\Delta\lambda$= 4 nm. The amplitude of the first derivative peak of (tadalafil and its degradate) was measured at 263.6 nm and used to calculate the content of tadalafil. Calibration graph was constructed relating the peak amplitude of $^1DD_{263.6}$ to the corresponding concentrations of tadalafil.

Densitometric method

Aliquot portions 2.5 – 40μLequivalents to 0.62 to 10 μgspot^{-1} at 2.5 μL intervals of tadalafil stock solution (250μgmL^{-1}) in acetonitrile water mixture (1:1) was spotted on HPTLC plates using a Camag Applicator. Plates were developed by solvent system consisting of chloroform: acetone: ammonia (9:1:0.1, by volume) at room temperature. Plates were left to dry then the spots

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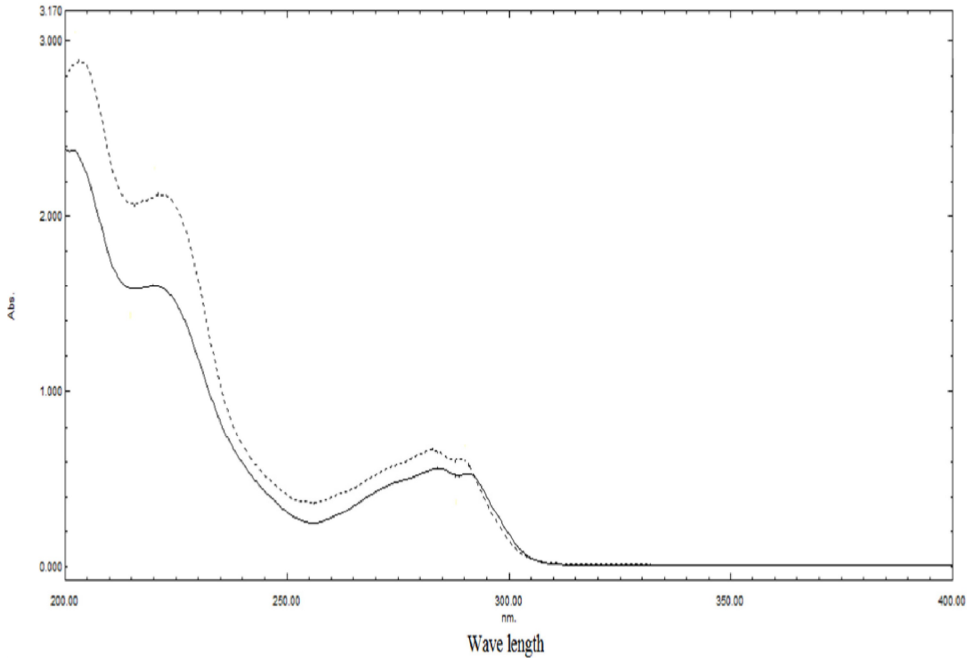

Figure 4 : Absorption spectrum of tadalafil10µg mL^{-1}(___) and its degradation product10µg mL^{-1}(——), using acetonitrile: water mixture as a solvent

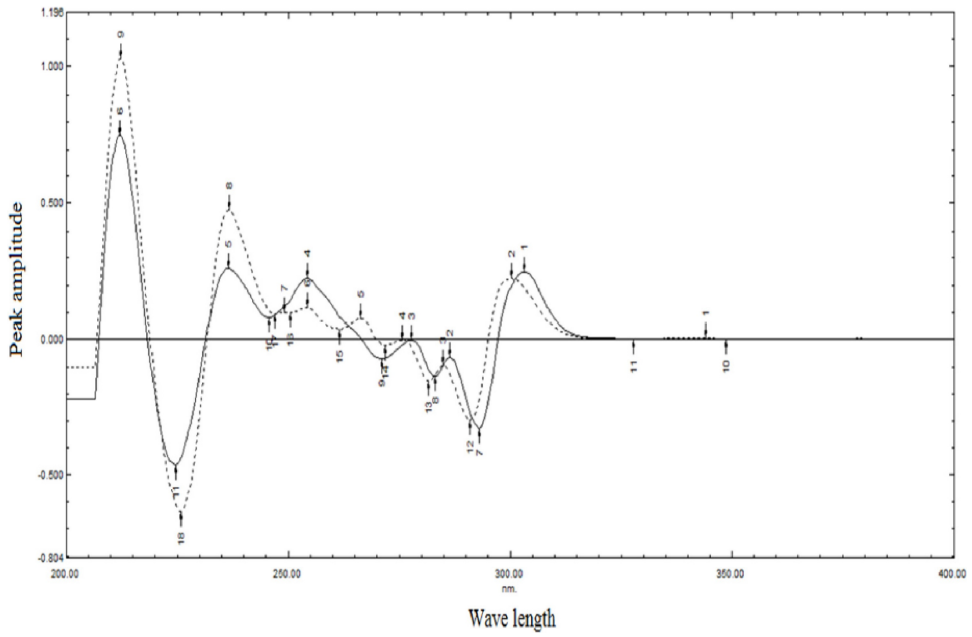

Figure 5 : First derivative spectra of tadalafil10 µg mL^{-1} (—) and its degradation product 10µg mL^{-1} (- - - -) using (acetonitrile: water) (1: 1v/v) as a solvent

were detected under UV-lamp (291 nm). A calibration curve relating the area under the peak to the corresponding concentrations of tadalafil was constructed over a range of 0.62 to 10 µgspot^{-1}

Liquid chromatographic method

Linearity

Aliquot portions 2.5 – 22.5 µg mL^{-1} at 2.5mL intervals of tadalafil stock solution (250µgmL^{-1}) were transferred into a series of 10-mL volumetric flasks. The flasks were completed to volume with acetonitrile water mixture (1:1). Aliquots (10µL) of the prepared solutions were injected into HPLC apparatus. The peak area was measured at 225 nm using a mobile phase consisting of water: acetonitrile: methanol in the ratio of (45:35:20) and a Lichrocart RP-18 (250mm×4.6mm

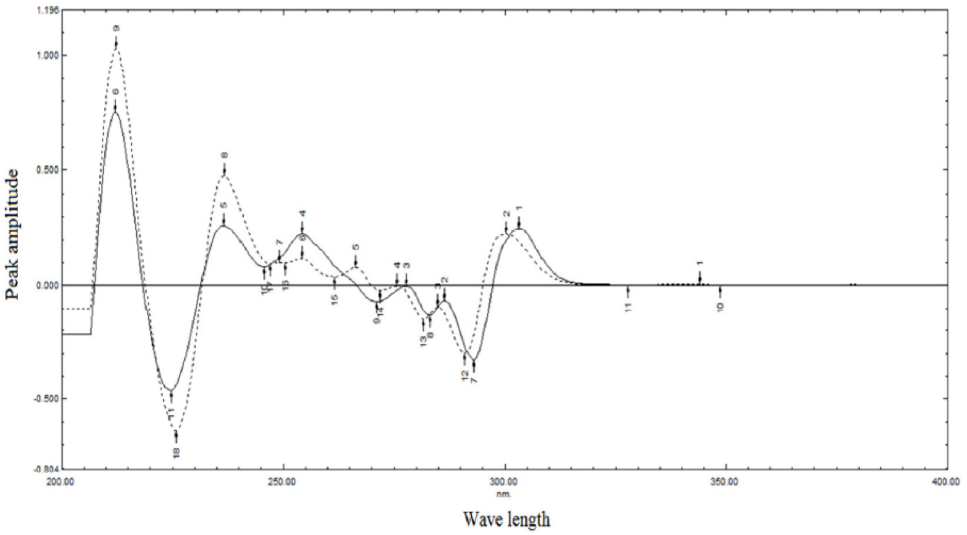

Figure 6 : Second derivative spectra of tadalafil10µg mL^{-1} (—) and its degradation product 10 µg mL^{-1} (- - - -) using (acetonitrile :water) (1:1v/v) as a solvent

i.d.) column, particle size (5µm) at a flow rate 1.2 mLmin^{-1}. A calibration graph representing the relative peak area of tadalafil to that of tadalafil external standard (10µgmL^{-1}), versus the corresponding concentrations of tadalafil was constructed.

Analysis of laboratory prepared mixturescontaining different ratios from tadalafil and itsdegradation product

Fourth derivative and derivative ratio method

Accurately aliquot portionsequivalent to (2.5–17.50 µg mL^{-1}) of tadalafil and tadalafil degradate of (17.50–2.5 µg mL^{-1}) from their corresponding stock solutions (250 µg mL^{-1} in acetonitrile water mixture (1:1) were transferred into a series of 10-ml volumetric flasks as shown in table 1. The volumes were completed with acetonitrile water mixture (1:1). Tadalafil concentration was calculated as mentioned under (Section 5.2.2) starting from (The peak amplitudes of the obtained fourth derivative……..) for the fourth derivative and (Section 5.2.3) starting from (The spectra of the prepared standard solutions…..) for the derivative ratio method.

Densitometric method

Aliquot portions equivalentto (0.62– 10µg spot^{-1}) of tadalafil and degradate of (10 – 0.62 µg mL^{-1}) from their corresponding stock solutions (250µgmL^{-1} in acetonitrile water mixture (1:1) were transferred into a series of 10mL volumetric flasks as shown in TABLE 1. Aliquot portions 2.5 µL of the prepared mixtures were spotted on a HPTLC plate and developed as mentioned under (Section 5.3.1) starting from (Plates were developed by……..…).

Liquid chromatographic method

Aliquot portions equivalent to (2.5 – 22.5 µg mL^{-1}) of tadalafil anddegradate of (22.5 – 2.5 µg mL^{-1}) from their stock solutions (250µgmL^{-1} in acetonitrile water mixture (1:1) were transferred into a series of 10 mL volumetric flasks as shown in TABLE 1.

Aliquot portions 25µL of the prepared mixtures were injected into HPLC adopting the conditions under (Section 5.4.1) starting from (The peak area was measured……..).

Analysis of tadalafil in pharmaceutical formulation

The powder of 10 tablets, after unpacking, was weighed. An amount of the powder equivalent to 25 mg tadalafil was weighed into a 100-mL volumetric flask. A volume of 50 mLacetonitrile water mixture (1:1) was added. Mixture was stirred for 10 min using a vortex then filtered through a 0.45 µL membrane filterinto a 100 mL volumetric flask and completed to volume with acetonitrile water mixture (1:1).

RESULTS AND DISCUSSION

Degradation of tadalafil

Tadalafil is a stable drug, however, forced stability

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study under stress conditions revealed the instability of the drug in presence of hydrogen peroxide (Figure 2).

Tadalafil is soluble in acetonitrile water mixture (1:1).The molecular weight of tadalafil degradate was confirmed by mass spectroscopy. It was noted that 3 hours reflux using0.8Nhydrochloric acidwas enough for complete degradation of tadalafil (Figure 3); this was demonstrated by the use of thin layer chromatography.Furthermore, complete shift of tadalafil UV-spectrum in acetonitrile water mixture (1:1) takes place (Figure 1).

Derivative and derivative ratio spectrophotometric method

Derivative spectrophotometric method

The zero, first, second and third order absorption spectra of tadalafil and its degradation products (Figure 4, 5, 6 and 7) respectively showed severe overlap

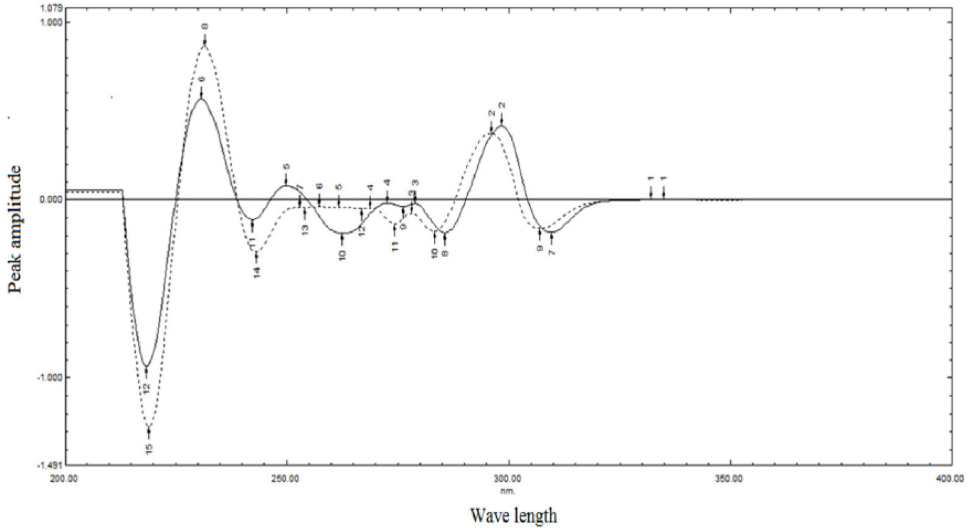

Figure 7 : Third derivative spectra of tadalafil10 µg mL^{-1} (—) and its degradation product 10 µg mL^{-1} (- - - -) using (acetonitrile : water) (1: 1v/v) as a solvent

that prevented the use of direct and derivative spectrophotometry for the analysis of tadalafil in presence of its degradation products.

Figure (4): Absorption spectrum of tadalafil10µg mL^{-1}(___) and its degradation product10µg mL^{-1}(— —), using acetonitrile: water mixture as a solvent.

But fourth derivative applied to resolve such a mixture and to determine the concentration of tadalafil, (Fig-

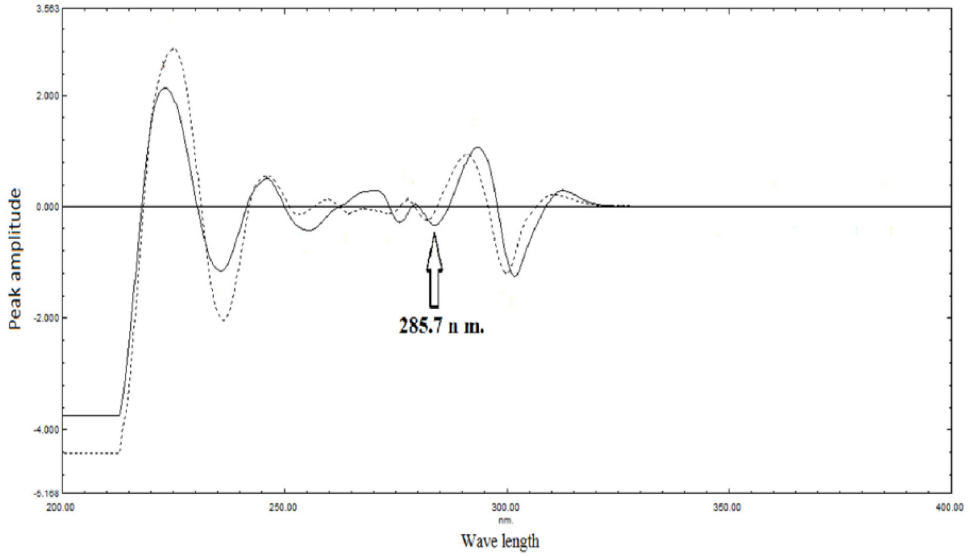

Figure 8 : Fourth derivative spectra of tadalafil10 µg mL^{-1} (—) and its degradation product 10 µg mL^{-1} (- - - -) using (acetonitrile : water) (1: 1v/v) as a solvent

ure 8)

The linearity was checked between the peak amplitude at the selected wavelength (285.7 nm) and the corresponding concentrations of tadalafil. A linear response was obtained for concentration range from 2.5 – 17.5 µg mL^{-1}.Figure 9.

The regression equation was found to be:

$$^{4}D_{285.7} = 0.0025X + 0.0247r = 0.9997$$

Where *X* is the concentration of tadalafil in µg mL^{-1}, ($^{4}D_{285.7}$) is the amplitude of fourth derivative curve (tadalafil and its degradation) at 285.7 nm and *r* is the correlation coefficient.

Derivative ratio spectrophotometric method

The derivative ratio spectroscopy could be applied to resolve such a mixture and to determine the concentration of tadalafil. The zero order of the derivative ratio spectra of tadalafil, (Figure 10), and the first order of the derivative ratio spectra are presented in (Figure 11). The concentration of the devisor was also studied.

It was found that upon dividing by the spectrum of 2.5 µg mL^{-1} degradation productsgave the best results in terms of sensitivity, repeatability and signals to noise ratio. The linearity was checked between the peak amplitude at the selected wavelength (263.6nm) and the corresponding concentrations of tadalafil. Figure 12.

A linear response was obtained for concentration range from 2.5–20 µgmL^{-1}.

The regression equation was found to be:

$$^{1}DD_{263.6} = 0.00445X - 0.0184r = 0.9994$$

Where *X*is the concentration of tadalafil in µg mL^{-1}, ($^{1}DD_{263.6}$) is the amplitude of first derivative curve (tadalafil andits degradate) at 263.6 nm and *r* is the

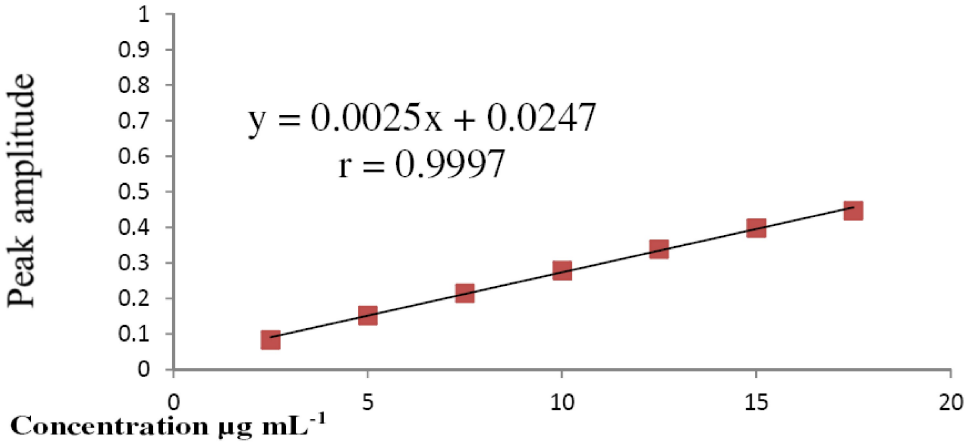

Figure 9 : Linearity of the peak amplitude of the forth derivative curve at 285.71 nm to the corresponding concentration of tadalafil

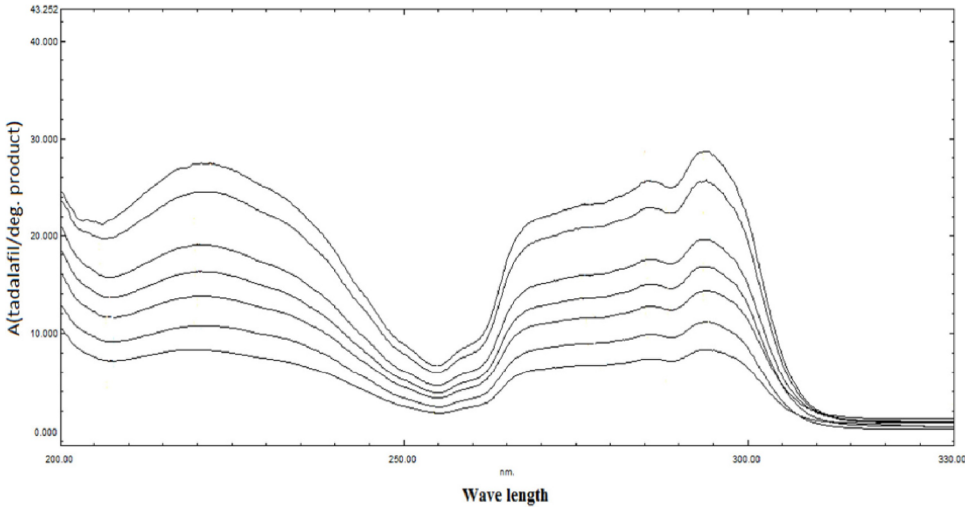

Figure 10 : Zero order of ratio spectra of tadalafil 2.5, 5, 7.5, 10, 12.5, 17.5 and 20 µg mL^{-1} using 2.5 µg mL^{-1} of its degradation product as a divisor using (acetonitrile: water mixture) as a solvent

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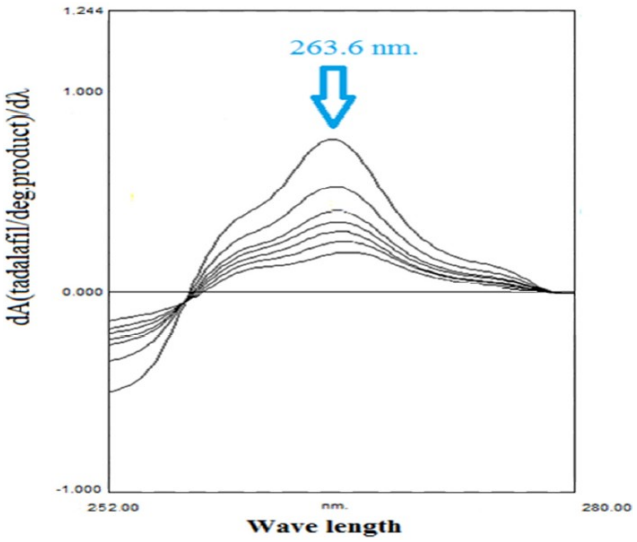


Figure 11 : First order of derivative ratio spectra of tadalafil 2.5, 5, 7.5, 10, 12.5, 17.5 and 20 µg mL^{-1} using 2.5 µg mL^{-1} of its degradation product as a divisor using (acetonitrile: water mixture) as a solvent

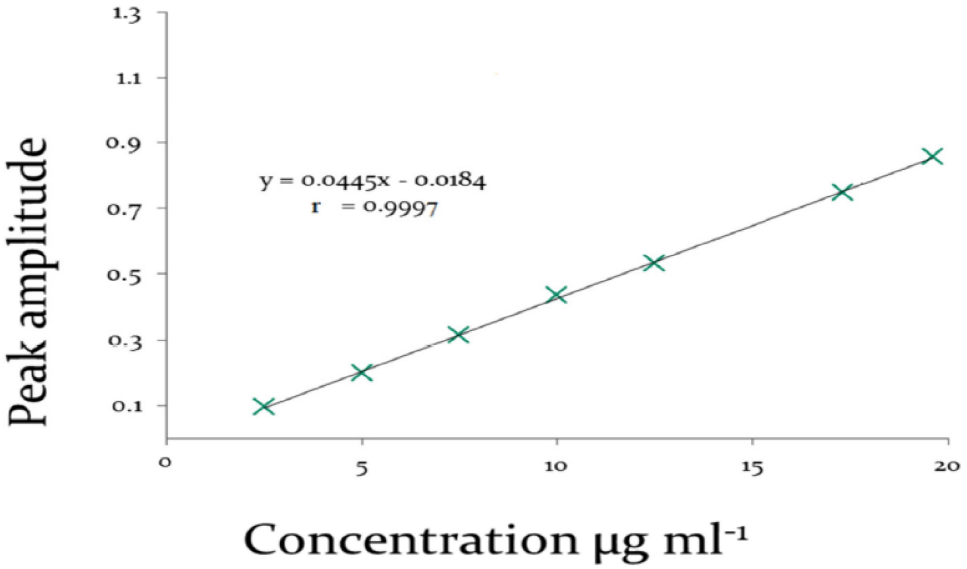
correlation coefficient.

Densitometric method

A densitometric method is described for the determination of tadalafil inpresence of itsdegradate without prior separation. Different solvent systems were tried for the separation of tadalafil from its degradation product. Satisfactory results were obtained by using chloroform: acetone: ammonia (9:1:0.1, by volume) as a developing system (R_f= 0.41, 0.62 fortadalafil and degradate, respectively). The separation allows the determination of tadalafil with no interference from itsdegradate (Figure. 13).

The linearity was confirmed by plotting the measured peak area versus the corresponding concentration at 291 nm over a range of 0.62 – 10µg spot^{-1} where a linear response was obtained.Figure (14).

The regression equation was found to be:



A = 1162.1X + 892.49, r = 0.9996

Where A is the integrated area under the peak for tadalafil, $X$is the concentration in µg spot^{-1} for tadalafil and r is the regression coefficient.

Liquid chromatographic method

A simple isocratic high-performance liquid chromatographic method is described for the determination of tadalafil in presence of its degradation product. System suitability parameters were tested by calculating the capacity factor, tailing factor, the selectivity factor and resolution. Best peak shape was obtained water: acetonitrile: methanol in the ratio of (45:35:20) with retention times of 4.7 ± 0.03and 2.18 ±0.03min for tadalafil and itsdegradate, respectively (Figure. 15).

A linear response was obtained between the relative peak area and the corresponding concentrations of the tadalafil in the range of 2.5 – 25 µgmL^{-1}. The regression equation was found to be: (Figure. 16).

A = 9.5542X + 0.0082r = 0.9998

Where$A$is the relative area under the peak, X *is* the concentration in µgmL^{-1} and r *is* the regression coefficient.

Method validation

The selectivity and specificity of the proposed meth-

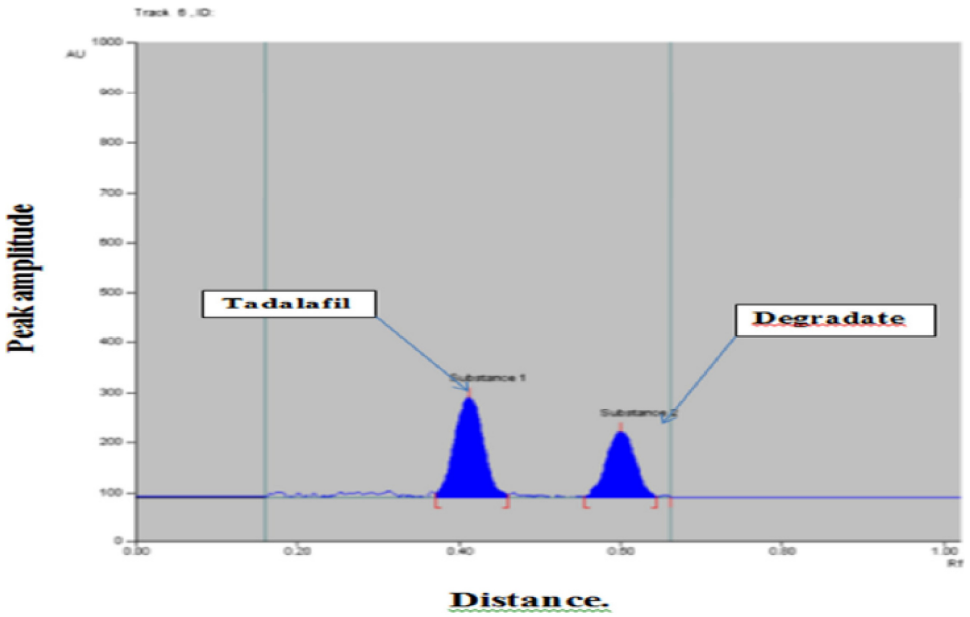


Figure 13 : Two dimensional TLC-Separation of tadalafil from it'sdegradate

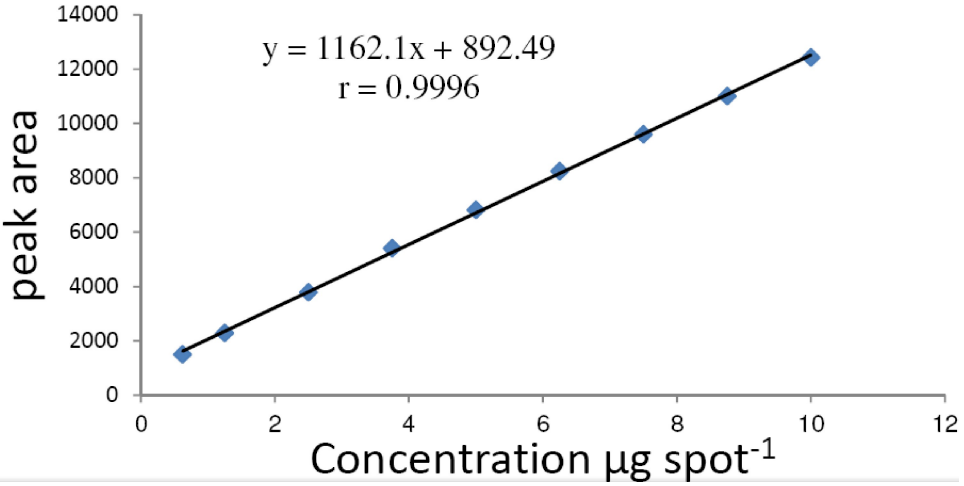
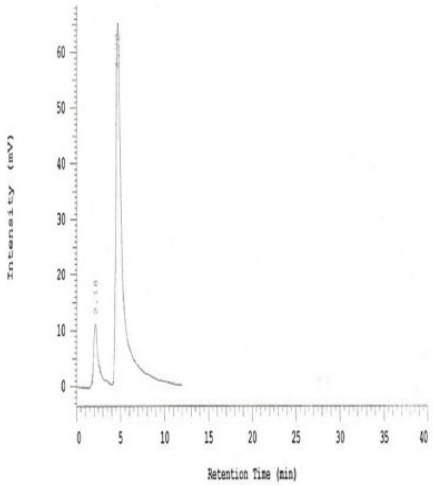


Figure 14 : Linearity of the integrated peak area to the corresponding concentration of tadalafil (µg spot^{-1})



Column: RP 18

Mobile phase water: acetonitrile: methanol, (45:35:20, by volume).

Injection volume: 10.00 µL.

Flow rate: 1.20 mL min $^{-1}$.

Detection: 225 nm.

Figure 15 : HPLC chromatogram of tadalafil (10 µg mL^{-1}, R_t: 4.7 min) its degradation product (10 µg mL^{-1}, R_t: 2.18 min) using the specified chromatographic conditions

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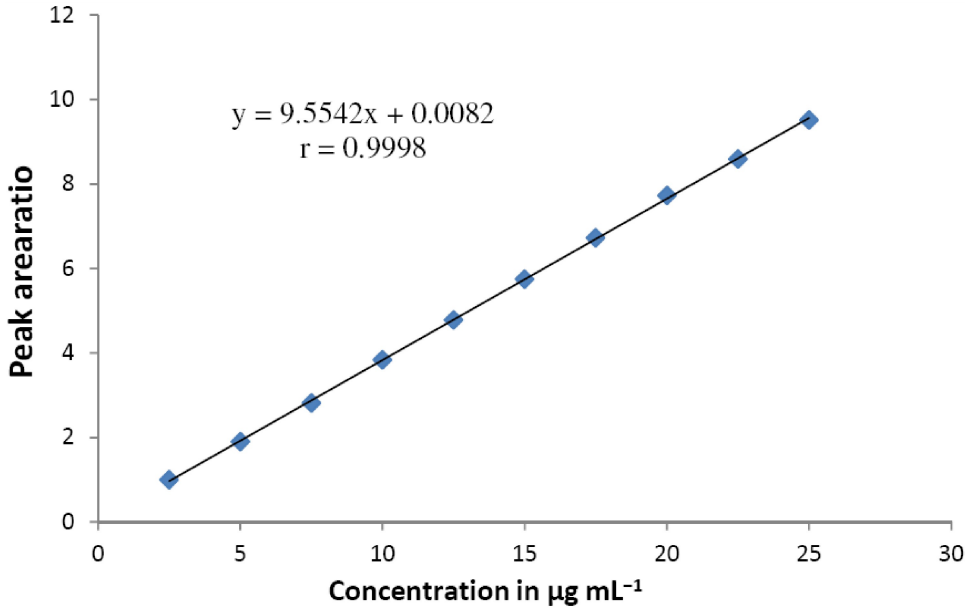

Figure 16 : Linearity of the peak area ratio to the corresponding concentration of tadalafil

TABLE 1 : Determination of tadalafil in laboratory prepared mixtures by the proposed derivative, derivative ratio, densitometry and HPLC method

Drug Conc. (µg mL$^{?1}$)	% of Degradation	Recovery for tadalafil by (%)				
		4th Derivative	1st Derivative ratio	HPLC	Densitometry	
					Conc. (µg spot$^{?1}$)	Recovery %
2.50	90 %	99.10	99.57	101.90	1.00	100.64
5.00	80 %	101.06	100.65	101.30	2.00	99.41
7.50	70 %	101.4	99.88	100.76	3.00	99.97
10.00	60 %	100.52	101.59	99.97	4.00	100.27
12.50	50 %	99.30	101.97	101.75	5.00	100.37
15.00	40 %	100.34	99.26	100.21	6.00	101.76
17.50	30 %			99.52	7.00	99.19
20.00	20%			99.49	8.00	101.93
22.50	10%			100.84		
Mean		100.45	100.39	100.63		100.53
S.D		1.130	0.904	0.902		0.968

TABLE 2 : Determination of tadalafil in Cialis® tablets by the proposed procedures

Cialis tablet	4thDerivativemethod Recovery % ±S.D.[(a)]	1stDerivative ratio method Recovery % ±S.D.[(a)]	Densitometric method	HPLC method
Dosage form BN: A779729	100.89 ± 0.888	100.57 ± 1.005	100.59 ± 0749	100.46 ± 0.910

[(a)] **Average of seven determinations**

ods were verified by determination of tadalafil in laboratory prepared mixture containing different ratios of the drug and its degradation product. The analysis was valid up to 90% of the degradation product for the (^{4}D), (^{1}DD) method, densitometry and liquid chromatography, respectively (TABLE 1).

To ascertain the accuracy of the proposed procedures, they were successfully applied for the determination of tadalafil in Cialis®tablets as presented in TABLE 2.

The validity of the proposed procedures was further assessed by application of the standard addition

technique. The small relative standard deviations indicate that the methods were accurate (TABLE 3).

The precision of the suggested methods was also expressed in terms of relative standard deviation of the inter-day and intra-day analysis. The individual methods were also checked for its robustness by minor changes in assay conditions, the methods proved robust. The obtained assay parameters and a validation sheet are presented in (TABLE 4).

The results obtained by the proposed methods for

TABLE 3 : Results of application of standard addition to the determination of tadalafil in Cialis® tablets by the proposed methods

Cialis tablet	Claimed taken (µg mL$^{?1}$)	Pure added (µgmL$^{?1}$) except densito.	4th derivative	1st Derivative ratio	Densito-metry	HPLC
Dosage form BN: A779729	2.50	2.50	100.44	100.43	(0.62) 99.76	101.55
		5.00	99.74	100.37	(1.25) 101.34	100.37
		7.50	99.80	101.19	(2.50) 100.46	100.14
		10.00	101.60	101.28	(3.75) 100.24	101.91
		12.50	100.43	99.97	(5.00) 99.91	99.58
		15.00	101.04	100.31	(6.25) 101.18	99.43
		17.50			(7.50) 101.06	101.27
		20.00				99.75
		22.50				100.63
Mean ± S.D.			100.53 ± 0.715	100.46 ± 0.669	100.56 ± 0.634	100.51 ± 0.894

TABLE 4 : Assay parameters and validation sheet

Parameter	4th Derivative method	1st Derivative ratio method	Densitometric method	HPLC method
n	7	7	8	10
Range (µgmL^{-1})	2.50–17.50	7.50 – 20.00	0.62 – 10.00	2.50– 25.00
Slope	0.0025	0.0445	1162.1	9.5542
Intercept	0.0247	- 0.0184	892.49	0.0082
Mean	100.52	100.59	100.45	100.47
S.D.	1.045	0.89	0.726	0.676
Variance	1.022	0.943	0.852	0.822
Coefficient of variation	1.039	0.884	0.722	0.672
Correlation Coefficient (*r*)	0.9997	0.9997	0.9996	0.9998
RSD (%)[a]	1.025	0.829	0.726	0.725
RSD (%)[b]	1.029	0.836	0.732	0.728

TABLE 5 : Statistical comparison for the results obtained by the proposed methods and the compendia method for the analysis of tadalafil in bulk powder

	4th Derivative method	1st Derivative ratio method	Densitometry	HPLC	Compendia method[14]
Mean	100.52	100.57	100.52	100.47	101.40
S.D.	1.045	0.827	0.726	0.717	1.64
Variance	1.022	0.683	0.527	0.514	1.28
N	7	7	8	10	5
F-test	1.252 (6.16)*	1.357 (6.16)*	1.502 (6.09)*	1.577 (6.00)*	
Student's *t*-test	1.417 (2.228)*	1.332 (2.228)*	1.539 (2.201)*	1.730 (2.160)*	

***The figures in parenthesis are the corresponding tabulated values atP = 0.05 (Remington)**

determination of tadalafil in bulk powder were statistically compared with those obtained by applying the compendial method[14], and it revealed insignificant difference (TABLE 5).

ABBREVIATION

^{1}D	- First derivative.
^{2}D	- Second derivative.
^{3}D	- Third derivative.
^{4}D	- Forth derivative.
^{1}DD	- First derivative ratio.
HPLC	- High performance liquid chromatography.
HPTLC	- High performance thin layer chromatography.
TD	- Tadalafil.
TLC	- Thin layer chromatography.

CONCLUSION

The proposed procedures are simple, sensitive, selectiveand stability indicating. The methods can be used for the routine analysis of tadalafil either in bulk powder or inpharmaceutical dosage forms. The proposed methods can be applied in laboratories lacking sophisticated instruments such as GC–MS or LC–MS. The suggested methods can be simply applied to kinetic studies and accelerated stability experiments to predict expiry dates of pharmaceuticals.

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