ISSN : 0974-7419 Volume 15 Issue 11

Analytical CHEMISTRY
An Indian Journal

Full Paper

ACAIJ, 15(11) 2015 [476-489]

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

Moataz A.Yehia*, Mamdouh R.Rezk, Mohamed A.El-Sayed, Mohamed Abdel Kawy
Department of Analytical Chemistry, Faculty of Pharmacy, Cairo University, Kasr El-Aini Street, ET 11562, Cairo, (EGYPT)
E-mail: mayehialiaa2010@hotmail.com; boodim3@yahoo.com

ABSTRACT

Four different stability indicating assay methods were developed and validated for the determination of sildenafil in the presence of its degradation product. The first and second method was based on the derivative and derivative ratio spectrophotometric technique using methanol 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: toluene: ethanol: acetic acid (3:3:4:0.1, by volume). The fourth method was a high performance liquid chromatography. Separation of sildenafilfrom its degradate using C_{18} column and a mobile phase consisting of acetonitrile: methanol: 0.05 M potassium di-hydrogen phosphate (v/v, pH 5.8): tri-ethyl amine, in the ratio of (45: 25: 30: 0.2, by volumes)at ambient temperature was achieved.
The developed methods were successfully applied to the analysis of pharmaceutical formulations containing sildenafilwith excellent recoveries.

KEYWORDS

Sildenafil;
Spectrophotometry;
Densitometry;
Stability;
HPLC.

INTRODUCTION

Sildenafil (SD), is 1-[4-ethoxy-3-(6, 7-dihydro-1-methyl-7-oxo-3-propyl-1*H*-pyrazolo [4, 3-*d*] pyrimidin-5-yl) phenylsulfonyl]-4methylpiperazine. (Figure 1)[1] It is phosphodiesterase-5 inhibitors used for erectile dysfunction. Sildenafilis80% eliminated mainly in the faeces, 13% eliminated in urine.[2]. the literature has analytical methods for quantitative estimation of sildenafil, in pharmaceutical formulations by spectroscopic method[3-7]Densitometric[8]. GC[9-14] HPLC[15-29], and capillary zone electrophoresis[30,31].

The present work aimed to develop feasible, sensitive and specific analytical procedures for the analysis of sildenafil 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

- Sildenafil powder was kindlysupplied by Pfizer PGM Company, France.
- Pharmaceutical formulation Viagra® tablets (la-

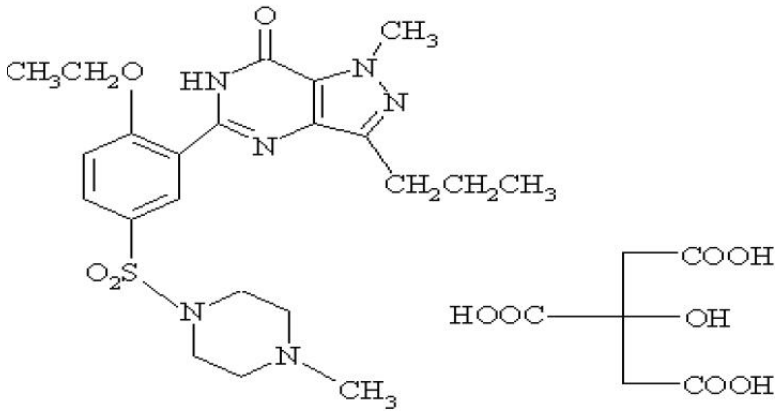

Figure 1 : Chemical structure of sildenafil

beled to contain 50 mg ofsildenafil), manufactured by Pfizer PGM Company.

Reagents

The materials used were Chloroform, acetic acid, ethanol, hydrogen peroxide, methanol, toluene, ADWIC, El Nasr Pharmaceutical Co. (Cairo, Egypt); Purified water was prepared using a Millipore Milli-Q water purification system, all were of analytical grade. Acetonitrile, methanol: Merck(Darmstadt, Germany). all were of HPLC grade, all Chemical were purchased from local market (Cairo, Egypt).

Standard solutions

Sildenafil standard and degradate solution ($100\mu gmL^{-1}$) in methanol.

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 $10\mu L^{-1}$ 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 sildenafil[1]

Into a round bottom conical flask weighed, an amount of 100 mg sildenafil citrate was dissolved in 100-mL of methanol transferred 2-mL of 30% hydrogen peroxide were added, (portions of methanol were added periodically to maintain the volume). After 6 hours reflux, complete degradation was achieved, as tested by TLC using chloroform: toluene: ethanol: acetic acid (3:3:4:0.1, by volumes) as a developing solvent. Solvent was evaporated and solid powder was characterized.

Spectrophotometric methods

Spectral characteristics of sildenafil and its degradation product

Two portion of sildenafil and its degradate were separately, transferred into two 10-mL volumetric flasks from their secondary stock solutions (100 µg mL^{-1}) and the volume was completed with methanol to obtain solutions having the concentration (24 µg mL^{-1}).

The zero, first, second, third and fourth order

Full Paper

spectra of the prepared solutions were recorded and investigated.

Fourth derivative method

Different aliquots (0.2 – 3 mL) were taken from sildenafil secondary stock solution (100 μg mL^{-1}) into 10-mL volumetric flasks then the volume was completed with methanol, to get a concentration range (2 – 30 μg mL^{-1}) TABLE 1. The fourth derivative of the standard spectra for sildenafil and its degradation product were obtained using ($\Delta\lambda$= 4 nm) and scaling factor 100, the peak amplitudes at 292.4 nm were recorded, and plotted against the corresponding concentration. The regression equation was obtained that was used for determination of sildenafil in unknown sample.

Derivative ratio method

The spectra of the prepared standard solutions were scanned from 200 to 400 nm and stored in the computer. The stored spectra of sildenafil were divided (amplitude at each wavelength) by the spectrum of 10 μg mLsildenafil degradate.

The first derivative of the ratio spectra (^{1}DD) were obtained at $\Delta\lambda$= 4 nm and scaling factor was 10. The amplitude of the first derivative peak of sildenafil was measured at 305.4 nm and used to calculate the concentration of sildenafil. Calibration graph was constructed relating the peak amplitude of $^1DD_{305.4}$ to the correspondingconcentrations of sildenafil citrate.

Densitometric method

Aliquot portions (5 – 80 μL)equivalents to 0.5 to 8 μg $spot^{-1}$(TABLE 1) of sildenafil stock solution (100μgmL^{-1}) in methanol was spotted on HPTLC plates using a Camag Applicator. Plates were developed by solvent system consisting of chloroform: toluene: ethanol: acetic acid (3:3:4:0.1, by volume) at room temperature. Plates were left to dry then the spots were detected under UV-lamp (292 nm). A calibration curve relating the area under the peak to the corresponding concentrations of sildenafil was constructed over a range of 0.5 to 8μg$spot^{-1}$

Liquid chromatographic method

Linearity

Aliquot portions(0.5 – 8 mL)of sildenafil stock solution (100μgmL^{-1}) were transferred into a series of 10-mL volumetric flasks. The flasks were completed to volume with methanol in concentration range 5 to 80 μg mL^{-1}(TABLE 1). Aliquots (10μL)

TABLE 1 : Determination of sildenafil in pure samples by derivative and derivative ratio methods

Fourth derivative spectroscopic method(μg mL^{-1})at 292.4 nm			Derivative ratio spectroscopic method (μg mL^{-1}) at 305.4 nm			Densitometry (μg $spot^{-1}$)			HPLC (μg mL^{-1})		
Taken	Found	Found %	Taken	Found	Found %	Taken	Found	Found %	Taken	Found	Found %
2.00	2.01	100.50	2.50	2.51	100.40	0.50	0.506	101.29	5.00	4.94	98.89
4.00	4.01	100.25	5.00	5.06	101.20	1.00	1.007	100.72	10.00	9.87	98.79
8.00	8.08	101.00	7.50	7.48	99.73	2.00	1.991	99.58	20.00	19.88	99.44
10.00	9.91	99.10	10.00	10.04	100.40	3.00	2.993	99.79	30.00	30.32	101.09
12.00	11.95	99.58	12.50	12.67	101.36	4.00	3.995	99.89	40.00	40.57	101.43
16.00	16.18	101.12	15.00	14.89	99.26	5.00	4.997	99.95	50.00	49.62	99.24
18.00	17.98	99.88	17.50	17.77	101.54	6.00	6.00	100.00	60.00	59.60	99.34
20.00	20.08	100.40	20.00	19.84	99.20	7.00	7.002	100.02	70.00	70.38	100.55
24.00	24.08	101.31	22.50	22.56	100.26	8.00	8.004	100.05	80.00	79.97	99.96
26.00	26.07	100.26	25.00	25.48	101.92						
28.00	27.82	99.35	27.50	27.41	99.67						
30.00	30.25	100.83	30.00	29.77	99.23						
Mean		100.29			100.34			100.14			99.86
SD		0.710			0.965			0.528			0.959
RSD%		0.708			0.961			0.527			0.960

of the prepared solutions were injected into HPLC apparatus. The peak area was measured at 225 nm using a mobile phase consisting of (acetonitrile: methanol: 0.05 M potassium di-hydrogen phosphate (v/v, pH 5.8): tri-ethyl amine, in the ratio of (45: 25: 30: 0.2, by volumes) and a Lichrocart RP-18 (250mm×4.6mm i.d.) column, particle size (5µm) at a flow rate 1.0 $mLmin^{-1}$. A calibration graph representing the relative peak area of sildenafil to that of sildenafil external standard (20μgmL^{-1}), versus the corresponding concentrations of sildenafil was constructed.

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

Fourth derivative and derivative ratio method

Accurately aliquot portions (0.2 – 1.8 mL), (0.4 – 1.6 mL) for derivative and derivative ratio respectively,of sildenafil and sildenafildegradate from their corresponding stock solutions (100 µg mL^{-1} in methanol) were transferred into a series of 10-ml volumetric flasks as shown in TABLE 1. The volumes were completed with methanol. Sildenafil 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 (8– 72µL) of sildenafil and degradatefrom their corresponding stock solutions (100μgmL^{-1} in methanol 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 (0.5 – 6.5 mL) of sildenafil anddegradatefrom their stock solutions (100μgmL^{-1} inmethanolwere transferred into a series of 10 mL volumetric flasks as shown in TABLE 1.

Aliquot portions 10 µL 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 sildenafil in pharmaceutical formulation

Ten tablets were weighed to determine the average weight per tablet then grinded. A mass of

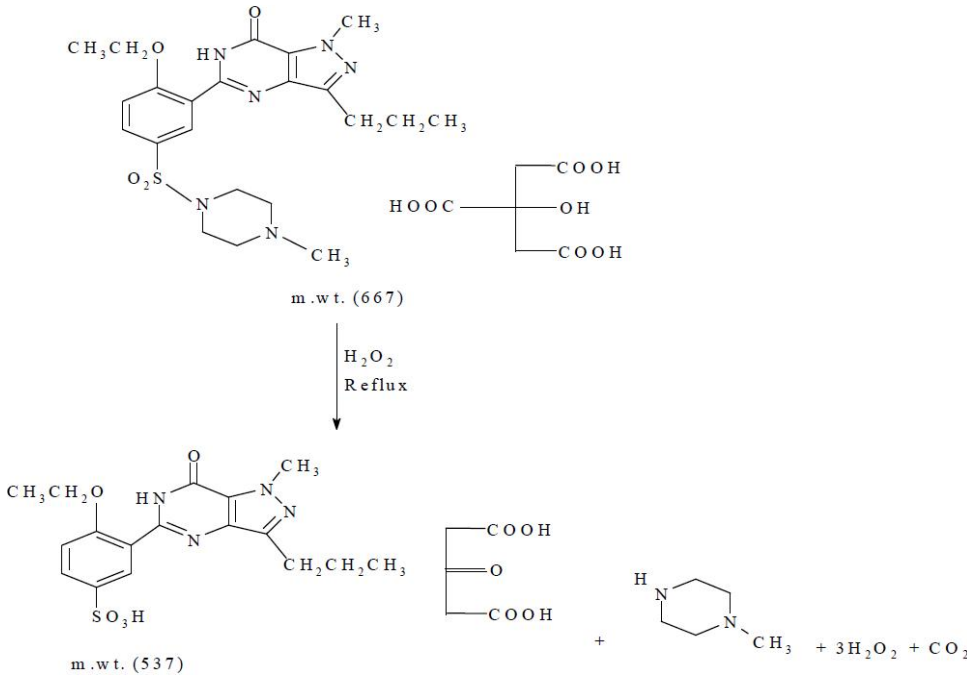

Figure 2 : Scheme degradation of sildenafil

Full Paper

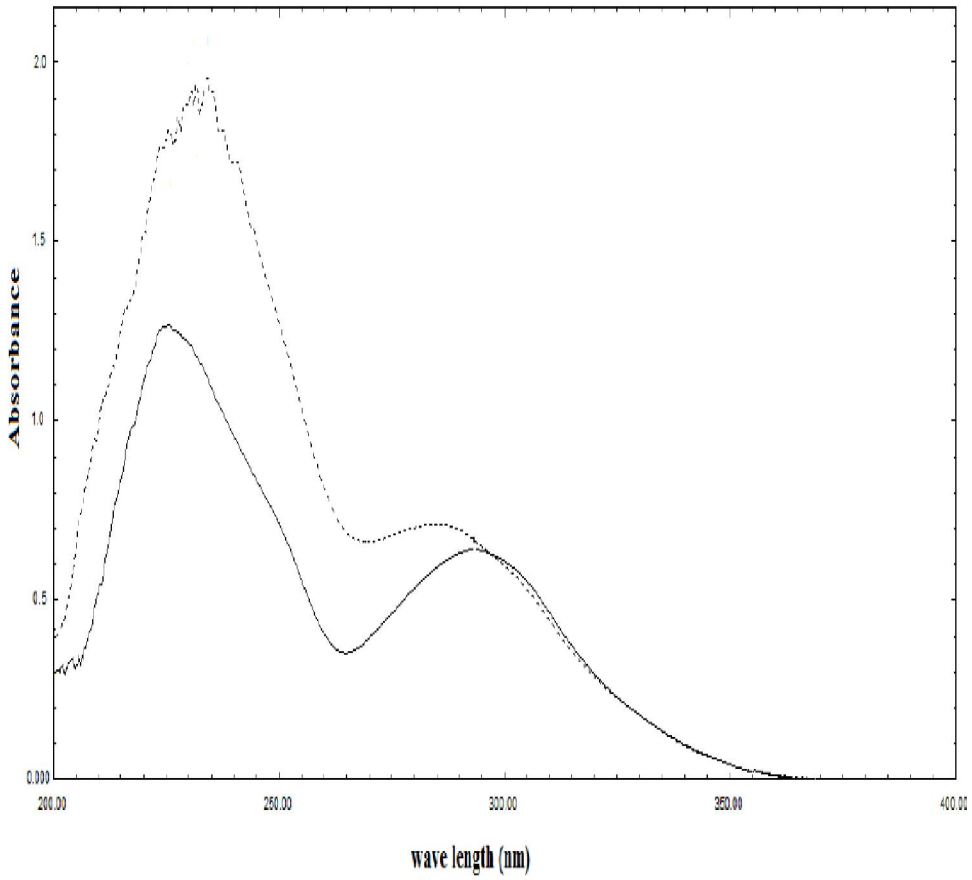

Figure 3 : Absorption spectrum of sildenafil(___) and its degradation product24µg mL^{-1}(——), using methanol as a solvent

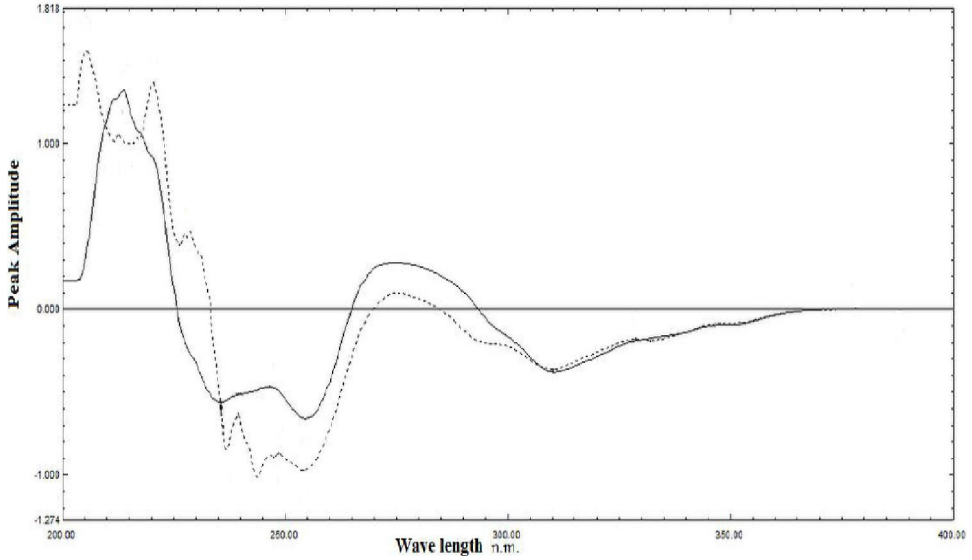

Figure 4 : First derivative spectra of sildenafil(—) and its degradation product 24 µg mL^{-1} (- - - -) using methanol as a solvent

Viagra® powder tablets equivalent to 10 mg sildenafil was transferred into a 100-mL volumetric flask. A volume of 50-mL methanol was added. Stirring was done for 10 minutes using a vortex then filtration was done and the procedure under linearity with secondary stock solution (100 µg mL^{-1}) and concentration range (2.5 – 30 µg mL^{-1}), was done for the derivative ratio method. The concentration of sildenafil

was estimated from the regression equation.

RESULTS AND DISCUSSION

Degradation of sildenafil

Sildenafil is a stable drug, however, forced stability study under stress conditions revealed the instability of the drug in presence of hydrogen peroxide (Figure 2).

Sildenafil is soluble in methanol. The molecular weight of sildenafildegradate was confirmed by mass spectroscopy. It was noted that 6 hours reflux using30%hydrogen peroxidewas enough for complete degradation of sildenafil(Figure 2); this was demonstrated by the use of thin layer chromatography. Furthermore, complete shift of sildenafil UV-spectrum in methanoltakes place (Figure 1).

Derivative and derivative ratio spectrophotometric method

Derivative spectrophotometric method

The zero, first, second and third order absorption spectra of sildenafil and its degradation products (Figure 3, 4, 5and 6) respectively showed severe overlap that prevented the use of direct and derivative spectrophotometry for the analysis of sildenafil in presence of its degradation products.

But fourth derivative applied to resolve such a mixture and to determine the concentration of sildenafil, (Figure 7)

The linearity was checked between the peak

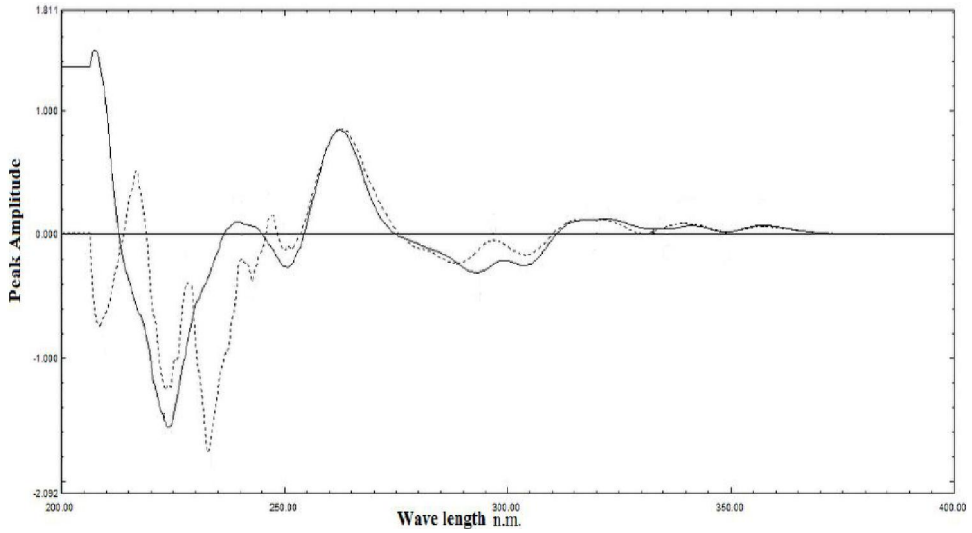

Figure 5 : Second derivative spectra of sildenafil(—) and its degradation product 24µg mL^{-1} (- - - -) using methanol as a solvent

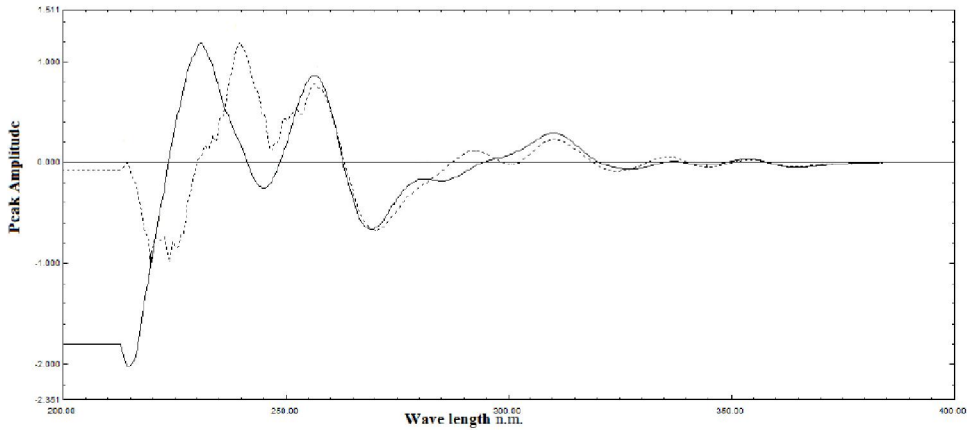

Figure 6 : Third derivative spectra of sildenafil (—) and its degradation product 24µg mL^{-1} (- - - -) using methanol as a solvent

Full Paper

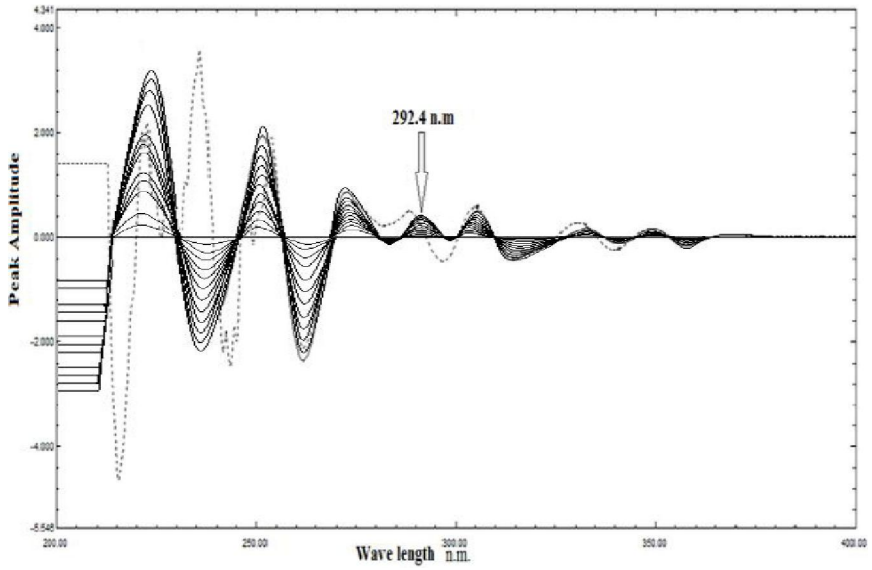

Figure 7 : Fourth derivative spectra of sildenafil(—) and its degradation product 24 µg mL^{-1} (- - - -) using methanol as a solvent

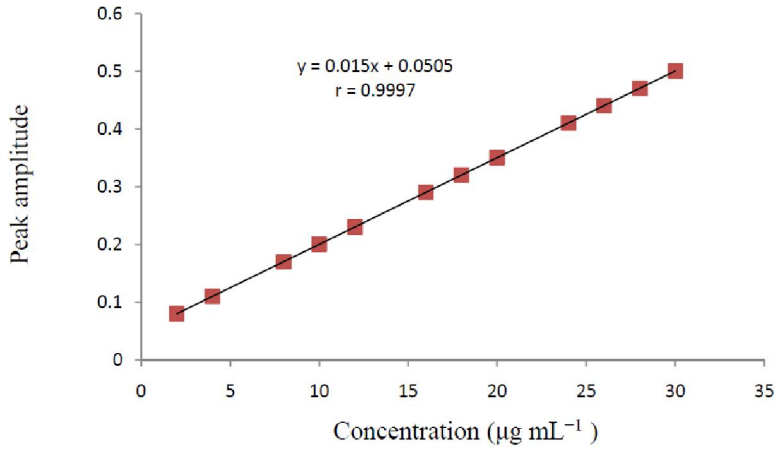

Figure 8 : Linearity of the peak amplitude of the forth derivative curve at 292.4 nm to the corresponding concentration of sildenafil

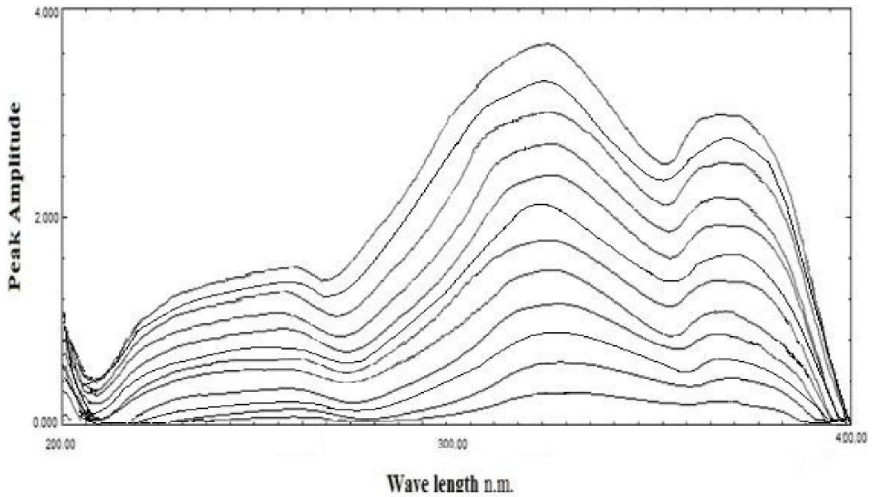

Figure 9 : Zero order of ratio spectra of sildenafil 2.5 – 30 µg mL^{-1} of its degradation product as a divisor using methanol as a solvent

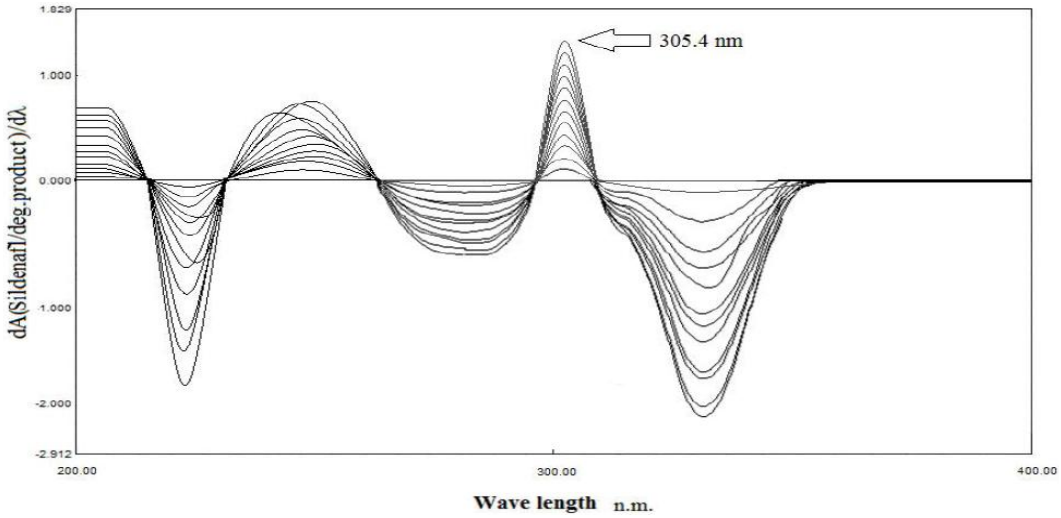

Figure 10 : First order of derivative ratio spectra of sildenafil 2.5 – 30 µg mL^{-1} using 10µg mL^{-1} of its degradation product as a divisor using methanol as a solvent

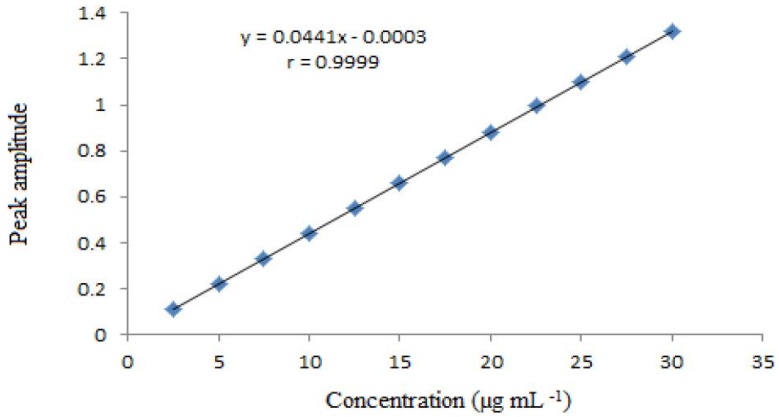

Figure 11 : Linearity of the peak amplitude of the first derivative of the ratio spectra at 305.4 nm againstconcentration of sildenafil

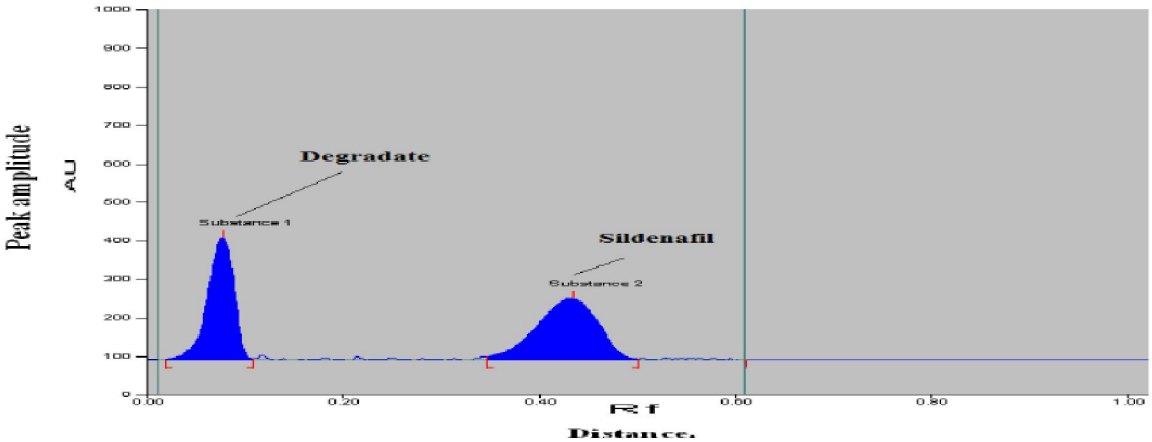

Figure 12 : Two dimensional TLC-Separation of sildenafil from it'sdegradate

amplitude at the selected wavelength (292.4 nm for concentration range from 2 – 30 µg mL^{-1}. Figure (8).

The regression equation was found to be:

$$^4D_{292.4} = 0.015X + 0.0505 \quad r = 0.9997$$

Where X is the concentration of sildenafil in µg mL^{-1}, ($^4D_{292.4}$) is the amplitude of fourth derivative curve (sildenafil and its degradation) at 292.4 nm and r is the correlation coefficient.

Full Paper

Derivative ratio spectrophotometric method

The derivative ratio spectroscopy could be applied to resolve such a mixture and to determine the concentration of sildenafil. The zero order of the derivative ratio spectra of sildenafil, (Figure 9), and the first order of the derivative ratio spectra are presented in (Figure 10). The concentration of the devisor was also studied.

It was found that upon dividing by the spectrum of 10 µg mL^{-1} degradation productsgave the best results in terms of sensitivity, repeatability and signals to noiseratio. The linearity was checked between the peak amplitude at the selected wavelength (305.4nm) and the corresponding concentrations of sildenafil. Figure (11).

A linear response was obtained for concentration range from 2.5– 30µgmL^{-1}.

The regression equation was found to be:

$$^{1}DD_{305.4}=0.0441X-0.0003 \quad r = 0.9999$$

Where *X*is the concentration of sildenafil in µg mL^{-1}, ($^{1}DD_{305.4}$) is the amplitude of first derivative curve (sildenafil andits degradate) at 305.4 nm and *r* is the correlation coefficient.

Densitometric method

A densitometric method is described for the determination of sildenafil inpresence of itsdegradate without prior separation. Different solvent systems were tried for the separation of sildenafil from its degradation product. Satisfactory results were obtained by usingchloroform: toluene: ethanol: acetic acid (3:3:4:0.1, by volume) as a developing system

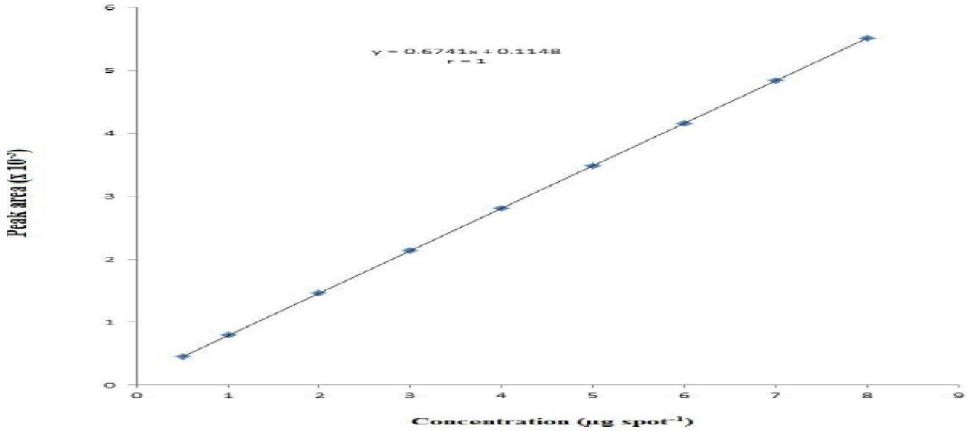

Figure 13 : Linearity of the integrated peak area to the corresponding concentration of sildenafil (µg $spot^{-1}$)

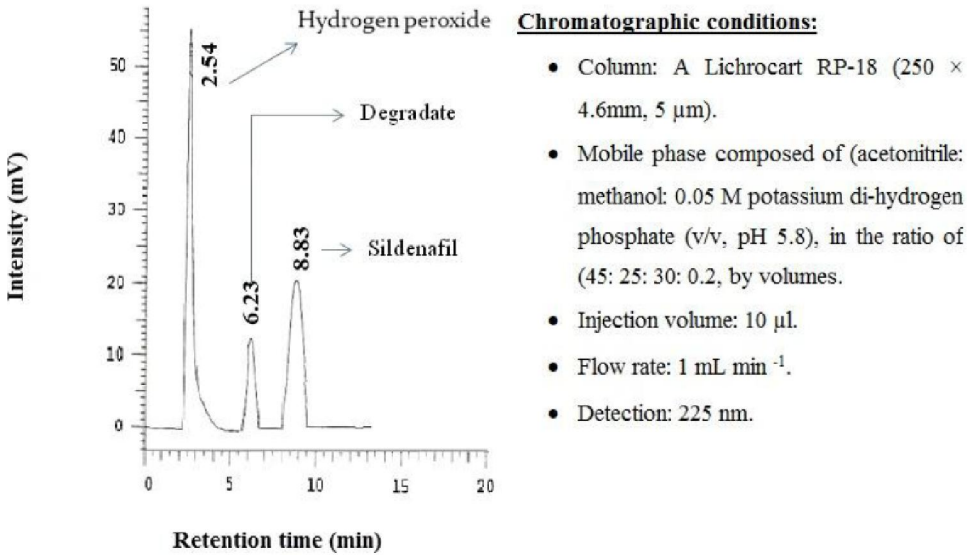

Figure 14 : HPLC chromatogram of sildenafil (40 µg mL^{-1}, R_t: 8.83min) its degradation product (30 µg mL^{-1}, R_t: 6.23 min) and hydrogen peroxide from degradation (30 µg mL^{-1}, R_t: 2.54min) using the specified

(R_f = 0.45, 0.08forsildenafil and degradate, respectively). The separation allows the determination of sildenafil with no interference from itsdegradate (Figure 12).

The linearity was confirmed by plotting the measured peak area versus the corresponding concentration at 292 nm over a range of 0.5 – 8µg spot^{-1} where a linear response was obtained. Figure (13).

The regression equation was found to be:

$A = 0.6741X + 0.1148$, r = 1

Where *A* is the integrated area under the peak (x 10^{-3}),for sildenafil, *X*is the concentration in µg spot^{-1}

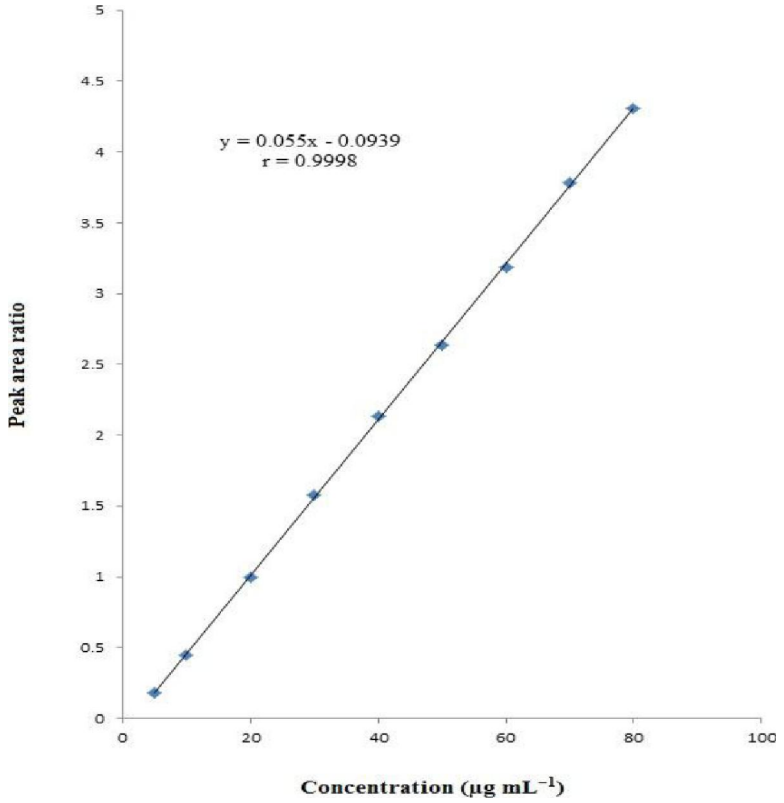

Figure 15 : Linearity of the peak area ratio to the corresponding concentration of sildenafil

TABLE 2 : Determination of sildenafil in laboratory prepared mixtures by the proposed derivative, derivative ratio, densitometry and HPLC method

Recovery for sildenafil by (%)						
Spectroscopy(µg mL^{-1})			HPLC (µg mL^{-1})		Densitometry (µg spot $^{-1}$)	
Conc.	Fourth Derivative Recovery %	Derivative ratio Recovery %	Conc.	Recovery %	Conc.	Recovery %
2.00	100.79		5	98.02	0.80	100.29
4.00	100.56	101.28	10	99.68	1.60	99.07
6.00	98.18	101.86	20	99.22	2.40	100.15
8.00	99.37	99.56	30	100.95	3.20	100.06
10.00	101.4	99.14	40	101.32	4.00	101.75
12.00	99.03	100.1	50	99.15	4.80	101.53
14.00	100.14	100.21	60	99.27	5.60	100.26
16.00	102.08	101.09	65	100.08	6.40	100.54
18.00	99.34				7.20	101.35
Mean	100.09	100.46		99.71		100.63
S.D	1.237	0.980		1.059		0.829

Full Paper

for sildenafil and *r* is the regression coefficient.

Liquid chromatographic method

A simple isocratic high-performance liquid chromatographic method is described for the determination of sildenafil 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 obtainedacetonitrile: methanol: 0.05 M potassium di-hydrogen phosphate,(pH 5.8): tri-ethyl amine (45: 25: 30: 0.2, by volume)with retention times of 8.83 ± 0.02 minutes and 6.23 ± 0.02 minutesfor sildenafil and itsdegradate, respectively (Figure 14).

A linear response was obtained between the relative peak area and the corresponding concentrations of the sildenafil in the range of 5 – 80μgmL^{-1}. The regression equation was found to be: (Figure 15).

$$A = 0.055X - 0.0939 \quad r = 0.9998$$

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

Method validation

By applying the proposed HPLC method, it was possible to determine sildenafil citrate in its pure powder form. The mean accuracy was found as shown in (TABLES 1).

The selectivity and specificity of the proposed methods were verified by determination of sildenafil 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 (TABLES 2).

To ascertain the accuracy of the proposed procedures, they were successfully applied for the determination of sildenafil in Viagra®tablets as presented in TABLE 3.

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 4).

The precision of the suggested methods was also expressed in terms of relative standard deviation of

TABLE 3 : Determination of sildenafil in Viagra® tablets by the proposed procedures

Viagra® tablet	4th Derivativemethod Recovery % ±S.D.[(a)]	1st Derivative ratio method Recovery % ±S.D.[(a)]	Densitometric method	HPLC method
Dosage form BN: 9197205	100.22 ± 0.809	100.22 ± 0.680	100.48± 0767	100.77± 0.693

[(a)] Average of seven determinations

TABLE 4 : Results of application of standard addition to the determination of sildenafil in Viagra® tablets by the proposed methods

Viagra® tablet	Fourth Derivative (Claimed taken: Standard added) (Recovery %) (μgmL^{-1})	Derivative ratio (Claimed taken: Standard added) (Recovery %) (μgmL^{-1})	Densitometry(Claimed taken: Standard added) (Recovery %) (μgspot^{-1})	HPLC(Claimed taken: Standard added) (Recovery %) (μgmL^{-1})
Dosage form BN: 9197205	(2.00: 2.00) 100.19	(2.50: 2.50) 101.82	(0.50: 0.50) 101.08	(5.00: 5.00) 99.38
	(2.00: 4.00) 102.10	(2.50: 5.00) 101.67	(0.50: 1.00) 101.04	(5.00: 10.00) 101.09
	(2.00: 6.00) 99.70	(2.50: 7.50) 99.54	(0.50: 2.00) 100.48	(5.00: 20.00) 99.10
	(2.00: 8.00) 99.17	(2.50: 10.00) 100.43	(0.50: 3.00) 100.39	(5.00: 30.00) 101.39
	(2.00: 10.00) 98.94	(2.50: 12.50) 100.77	(0.50: 4.00) 100.86	(5.00: 40.00) 99.89
	(2.00: 12.00) 100.15	(2.50: 15.00) 99.72	(0.50: 5.00) 100.43	(5.00: 50.00) 101.10
	(2.00: 14.00) 98.57	(2.50: 17.50) 101.40	(0.50: 6.00) 100.73	(5.00: 60.00) 101.47
	(2.00: 16.00) 99.79	(2.50: 20.00) 99.25	(0.50: 7.00) 101.73	(5.00: 70.00) 101.36
	(2.00: 18.00) 101.03	(2.50: 22.50) 100.97		
Mean ± S.D.	99.96 ± 1.091	100.61 ± 0.947	100.72 ± 0.508	100.60 ± 0.976

TABLE 5 : Assay parameters and validation sheet

Parameter	Fourth Derivative method	Derivative ratio method	Densitometric method	HPLC method
N	12	12	9	9
Range ($\mu g\ mL^{-1}$)	2.00 – 30.00	2.50 – 30.00	0.50 – 8.00	5.00 – 80.00
Slope	0.015	0.0441	0.6741	0.055
Intercept	0.0505	- 0.0003	0.1148	- 0.0939
Mean	100.29	100.34	100.14	99.86
S.D.	0.710	0.965	0.528	0.959
Variance	0.504	0.931	0.278	0.919
Coefficient Of variation	0.708	0.961	0.527	0.961
Correlation Coefficient (*r*)	0.9997	0.9999	1	0.9998
RSD (%)[a]	0.705	0.960	0.531	0.963
RSD (%)[b]	0.707	0.963	0.532	0.959

TABLE 6 : Statistical comparison for the results obtained by the proposed methods and the reported method for the analysis of sildenafil in bulk powder

	Fourth Derivative method	Derivative ratio method	Densitometry	HPLC	Reported method[20]
Mean	100.29	100.34	100.14	99.86	100.17
S.D.	0.710	0.965	0.528	0.959	0.884
Variance	0.504	0.931	0.278	0.919	0.781
N	12	12	9	9	7
F-test	1.55 (3.09)*	1.19 (4.04)*	2.80 (3.58)*	1.17 (4.15)*	
Student's *t*-test	0.325 (2.110)*	0.381 (2.110)*	0.084 (2.145)*	0.663 (2.145)*	

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

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 5).

The results obtained by the proposed methods for determination of sildenafil in bulk powder were statistically compared with those obtained by applying the reported method[20], and it revealed insignificant difference (TABLE 6).

CONCLUSION

The proposed procedures are simple, sensitive, selectiveand stability indicating. The methods can be used for the routine analysis of sildenafil 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.

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.
SD……...Sildenafil.
TLC……Thin layer chromatography.

REFERENCES

[1] M.J.O'Neil, P.E.Heckelman, C.B.Koch, K.J.Roman,

C.M.Kenny, M.R.D'Arecca; "The merck index, An encyclopedia of chemicals, Drugs and biologicals". 15th Edition, Merck Research Laboratories Division of Merck & Co., Inc.Whitehouse Station, NJ, USA, **13**, 5266, 7545, 8563, 9229 **(2013)**.

[2] The united states pharmacopeia (USP 34), The National Formulary (NF 29), Asian Ed., 3290–3292 **(2011)**.

[3] M.Reddy, T.Murthy, Y.Rao, K.Sushma, D.Sankar; Sensitive and selective extractive spectrophotometric method for the determination of sildenafil in pharmaceuticals, Indian Drugs, **39(2)**, 106–114 **(2002)**.

[4] H.Jiang, H.Zhan, X.Wu, Fenxi Kexue Xuebao., Applied a spectrophotometric method for determination of sildenafil with methylene blue, Through Anal.Abstr., **66(9)**, 9G117 **(2004)**.

[5] Z.Qin, R.Tan; Fenxi Kexue Xuebao, **20(2)**, 175-177 **(2004)**; Utility of ion-associate formation reactions for the spectrophotometric determination of sildenafil citrate in pure form and in virecta tablets, Pharmaceuticaanalyticaacta, Through Anal.Abstract, **66(7)**, 7G156 **(2011)**.

[6] B.Shaimaa,M.Mahmoud, S.Yasser, A.Amna; A novel rapidly disintegrating tablet of sildenafil citrate with enhanced stability: design and in-vitro evaluation,determined sildenafil and its oxidative-induced degradation product by derivative ratio spectrophotometry, Int.J.Pharm., **3(1)**, 28–39 **(2013)**.

[7] A.Amin, M.Moustafa, R.El-Dosoky; Colorimetric determination of sildenafil citrate, J.AOAC Int., **92(1)**, 125–131 **(2009)**.

[8] A.Badwan, L.Nabulsi, M.Al-Oman, N.Darghmeh, M.Ashour; Quantitation of sildenafil as well as other impurities using TLC, Analytical profiles of drug substances and excipient, Edited by Britain Academic Press. **27**, 340–376 **(2001)**.

[9] K.Sashio, K.S.Scott, S.Morimoto, Y.Nakahara; Effective extraction and determination of sildenafil and its N-desmethyl metabolite in rat and human hair by gas chromatography-mass spectroscopy, Biol.Pharm.Bull., **24(12)**, 1384–1393 **(2001)**.

[10] Y.Wang, J.Wang, Y.Cui, J.Fawcett, J.Gu; Liquid chromatographic-tandem mass spectrometric method for the quantitation of sildenafil in human plasma, J.Chromatogr.B., **828(1-2)**, 118–125 **(2005)**.

[11] R.Vos, A.Chahbouni, A.Sinjewel, E.Swart; Quantitative analysis of sildenafil and desmethylsildenafil in human serum by liquid chromatography-mass spectrometry with minimal sample pretreatment, J.Chromatogr.B., **876(2)**, 283–286 **(2008)**.

[12] S.Lee, Y.Kim, T.Kim, G.Im, B.Lee, D.Kim, C.Jin, H.Yoo; Simultaneous determination of sildenafil and its active N-desmethyl metabolite in human plasma using liquid chromatography-tandem mass spectrometry, J.Pharm.Biomed.Anal., **49(2)**, 513–518 **(2009)**.

[13] S.Gratz, C.Flurer, K.Wolnik; Screen for the presence of sildenafil in dietary supplements and bulk herbal materials, By liquid chromatography-electrospray ionisation-mass spectrometry, J.Pharm.Biomed.Anal., **36(3)**, 525–532 **(2004)**.

[14] J.Cooper, D.Muirhead, J.Taylor, P.Baker; Simultaneous determination of sildenafil and its demethylated metabolite in plasma using automated sequential trace enrichment of dialysates and high-performance liquid chromatography, J.Chrom.B., **87**, 701–710 **(1997)**.

[15] A.Segall, M.Vitale, V.Perz, M.Palacios, M.Pizzorno; Determination of sildenafil citrate in the presence of its oxidative-induced degradation products using reversed phase HPLC, J.Liq.Chromatogr.Relat.Technol., **23(9)**, 1377–1383 **(2000)**.

[16] Y.Liu, H.Yang, J.Miao; Yaowu Fenxi Zazhi., A reversed phase HPLC chromatographic method for the determination of sildenafil citrate, **61**, 20 **(2000)**; Through Anal.Abstr., **62(6)**, 6G204 **(2000)**.

[17] B.Jung, D.Moon, B.Chung. Can.; HPLC determination of sildenafil and its demethylated metabolite in cat plasma with solid-phase extraction for the pharmacokinetic study, J.Anal.Sci.Spectrosc., **46(2)**, 40–45 **(2001)**.

[18] N.Daraghmeh, M.Al-Omari, A.Badwan, A.Jaber; Determination of sildenafil citrate and related substances in the commercial products and tablet dosage form, J.Pharm.Biomed.Anal., **25(3-4)**, 483–490 **(2001)**.

[19] C.Jeong, H.Lee, M.Jang, W.Kim, H.Lee; Simultaneous determination of sildenafil and its active N-desmethyl metabolite in human plasma sample without prepurification using a fully automated high-performance liquid chromatography method, J.Chromatogr.B, **752(1)**, 141–148 **(2001)**.

[20] N.Dinesh, B.Vishukumar, B.Nagaraja, N.Made Gowda, K.Rangappa; A simple, precise, sensitive and stability-indicating reversed-phase high performance liquid chromatographicmethod, for the quantitation of sildenafil citrate in pure form and in its pharmaceutical formulations, Research and reports in medicinal chemistry, **3**, 21–27 **(2002)**.

[21] H.Aboul-Enein, M.Hefnawy; Rapid determination of sildenafil in pharmaceutical formulations using monolithic silica HPLC, J.Liq.Chromatogr.Relat.Technol., **26(17)**, 2897–2908 **(2003)**.

[22] M.Sheu, A.Wu, G.Yeh, A.Hsia, H.Ho; High-performance liquid chromatographic method utilized a one-step liquid-liquid extraction after alkaline treatment of plasma, J.Chromatogr.B, **791**, 255–260 **(2003)**.

[23] A.Tarcqui, B.Ludes; High-performance liquid chromatography-mass spectrometry procedure for the determination of sildenafil in biological fluids, J.Anal.Toxicol., **27(2)**, 88–94 **(2003)**.

[24] A.Abd-ELbary, N.Foda, O.El-Gazayerly; Stability indicating HPLC assay for the determination of sildenafil in bulk and in formulations, Chromatographia, **59(9-10)**, 561–566 **(2004)**.

[25] X.Zhu, S.Xiao, B.Chen, F.Zhang, S.Yao, Z.Wan, D.Yang, H.Han; A high-performance liquid chromatographic method coupled with ultraviolet detection and electrospray ionization mass spectrometry, J.Chromatogr.A., **1066(1-2)**, 89–95 **(2005)**.

[26] P.Zou, S.Oh, P.Hou, M.Low, H.Koh; A high performance liquid chromatography-diode array detection method and a liquid chromatography-electrospray ionization tandem mass spectrometry method, J.Chromatogr.A., **1104(1-2)**, 113–122 **(2006)**.

[27] M.Guermouche, K.Bensalah; Solid phase extraction and liquid chromatographic determination of sildenafil and itsdemethylated metabolite in serum, J.Pharm.Biomed.Anal.Mar.3, **40(4)**, 952-957 **(2006)**.

[28] Berzas J.Nevado, Rodriguez J.Flores, Castaneda G.Peñalvo, Rodriguez N.Farinas; A micellarelectrokinetic capillary chromatography (MEKC) for the determination of sildenafil citrate and its desmethylated metabolite in human serum, Electrophoresis, **22(10)**, **(2001)**.

[29] X.Zhang, Y.Chan, X.Wu, L.Liu, G.Yang; Dier Junyi Daxue Xuebao, Determined sildenafil citrate by capillary zone electrophoresis, **22(5)**, 427-428 **(2001)**; Through Chem.Abstr., **135**, 294066u **(2001)**.