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Validated Methods for Determination of Sildenafil Citrate in The Presence of its Potential Impurities

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Abstract:

Two simple, accurate, sensitive and reproducible methods have been developed and subsequent validated for the determination of Sildenafil citrate (SC) in presence of its impurities "1-methyl-4-nitro-3-n-propyl-5pyrozole Carboxamide (MNC), 4-amino-1-methyl-3-n-propyl-5-pyrazole Carboxamide (AMP), 4-(2-ethoxy benzoyl amino)-1-methyl-3-n-propyl-5-pyrazole Carboxamide (EMC) and 5-(2-ethoxyphenyl)-1-methyl-3-n-propyl pyrazole [4,3-D] pyramidine-7-1(EMP)" as stability-indicating studies. In the spectrophotometric method, zero-crossing technique was adopted for determination of the investigated drug in presence of impurities, by the use of derivative and derivative ratio techniques, respectively. While, the second method was isocratic reversed-phase (RP) stability-indicating high-performance liquid chromatographic method, which was adopted for determination of Sildenafil citrate in presence of its impurities. The chromatographic separation was achieved isocratically by using a mobile phase of water and acetonitrile in a ratio of 40:60 V/V containing 50 mM triethylamine. The analysis was carried out using waters C_{18} (4.6 x 250 mm, 10 µm) at flow rate of 1.0 ml.min^{-1} and the UV detection at 245 nm. All the proposed methods were validated according to the International Conference on Harmonization (ICH) guidelines reference and successfully applied for determination of Sildenafil citrate in pure form, in laboratory prepared mixtures and in pharmaceutical preparations. The obtained results were statistically compared to the reported method of analysis for Sildenafil citrate and no significant differences were found.

Keywords: *Sildenafil citrate, Derivative Spectrophotometry; Ratio Spectra; High-performance liquid Chromatography, Stability Indicating Methods.*

1.Introduction:

Sildenafil (SC) is a compound of the pyrazolo-pyrimidinyl-methylpiperazine class. It is 5-[2-ethoxy-5-(4-methyl piperazin-1-yl sulphonyl) phenyl] -1-methyl-3-propyl-1, 6-dihydro-7H pyrazolo [4,3-d] pyrimidin-7-one, having empirical formula $C_{22}H_{30}N_6O_4S$ and molecular weight 661.71[1].

It is a white to off-white, crystalline, odorless powder with a bitter, salty taste, its melting point is 186-190°C[2], having λmax 290 nm in 30% methanolic water and PKa of 8.7 [3]. It is a selective inhibitor specific phosphodiesterase type 5 (PDE-5) used to treat male erectile dysfunction [4], rapidly absorbed after oral administration, having an absolute bioavailability of about 40%. The maximum observed plasma concentrations are reached within 3 to 120 minutes (median 60 minutes) after oral dosing in the fasted state [5], where the duration of action lasting up to 4 hours, with less response than that seen at 2 hours [6, 7]. SC after either oral or intravenous administration is excreted predominantly in the feces as its metabolites approximately 80% of the administered oral dose and to a lesser extent in the urine (approximately 13% of the administered oral dose) [6-8], chemometric technique [9], voltammetry [10, 11], Polarography [12, 13] and mass measurement made by ESI using fourier transform ion cyclohon response mass spectrometry [14]. Other techniques such as thin layer chromatography [15-17], micellar electrokinetic capillary chromatography [18, 19], capillary zone electrophoresis [20], Gas chromatography [21-23] and high performance liquid chromatography [24-28] were reported for SC analysis.

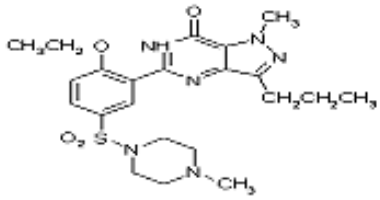


Figure 1: Structure formula of Sildenafil [8]

All the reported literatures were not involve the use of either derivative or derivative-ratio methods have been

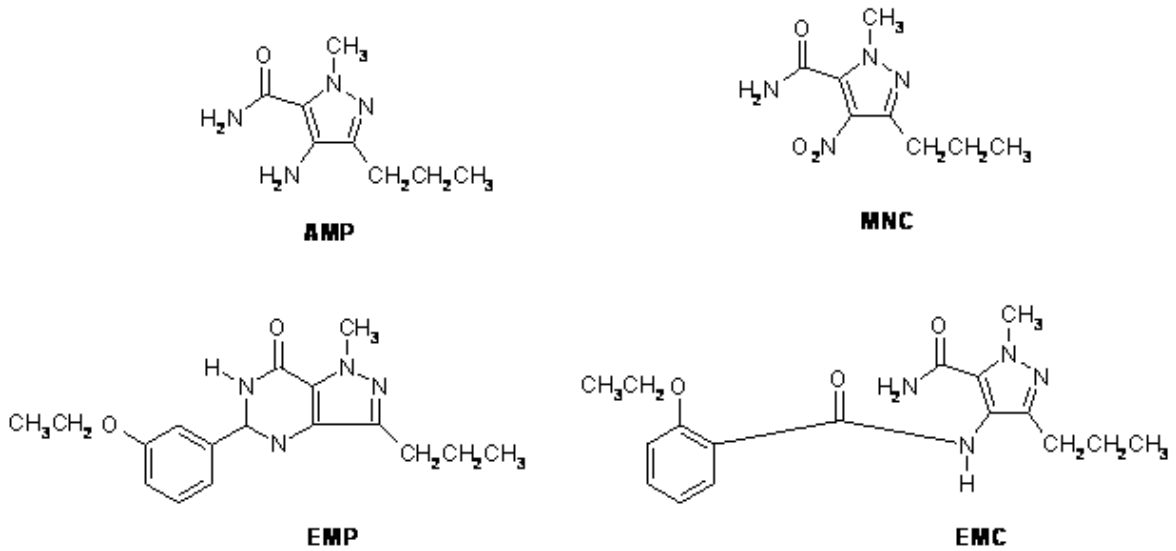


Figure 2: Structure formula of Sildenafil impurities[8]

reported for determination of Sildenafil citrate in pharmaceutical Formulations. But, the present work was successfully developed novel simple, rapid, accurate and sensitive derivative and derivative-ratio spectrophotometric methods for the determination of SC in pharmaceutical Formulations. Those novel methods could be adopted for the determination of SC in presence of its impurities (MNC, APM, EMC and EMP), respectively, this beside a simple isocratic high performance liquid chromatographic method was also developed.

2. Materials and Methods:

2.1. Chemicals and reagents

Sildenafil citrate was kindly supplied by E.I.P.I.C.O (Egypt) and certified to contain 100.05% by the manufacturer method. Vigoran® tablet: Batch No.3866, manufactured by (E.I.P.I.C.O., Egypt). Each tablet contains 25 mg sildenafil citrate.

1-methyl-4-nitro-3n-propyl-5-pyrazole carboxamide (MNC); 4-amino-1-methyl-3-n-propyl-5-pyrazole carboxamide (AMP); 4-(2-ethoxybenzoyl amino)-1-methyl-3-n-propyl-5-pyrazole carboxamide (EMC); 5-(2-ethoxyphenyl)-1-methyl-3-n-propyl pyrazole (4,3-d) pyrimidine-7-1(EMP) were kindly supplied by Mdpi Scientific, Switzerland certified to contain 100.05% by the manufacturer method.

Acetonitrile, methanol and bi-distilled water (Riedel-dehaen, Sigma-Aldrich, Germany), Orthophosphoric acid and triethylamine (BDH, Poole, UK).

All chemical and reagents used through this work are of spectroscopic and chromatographic analytical grade. Bi-distilled water is used throughout the whole work and is indicated by the word “water".

2.2. Instruments

A double-beam Shimadzu (Japan) UV-VIS Spectrophotometer (UV-1601 PC), model TCC-240 A; connected to an IBM compatible computer and HP 695 C DeskJet printer is used. The bundled software is UVPC personal spectroscopy software version 3.7 (Shimadzu). The spectral bandwidth is 2 nm and the wavelength scanning speed was 2800.0 nm min^{-1}. The absorption spectra of the reference and the test solutions are recorded in1.0-ml quartz cells at 25.0 ^{0}C, using ‘$\Delta\lambda$ = 4 nm and scaling factor of 10 for computing first derivative (D^1)’ and ‘$\Delta\lambda$ = 8 nm and scaling factor of 100 for second (D^2) derivatives'.

- The HPLC unit equipped with a waters 2487 dual wavelength absorbance detector module and a programmable pump module 600 (System Gold, Bechman, USA), and a Waters 717 plus autosampler. The column utilized was Waters stainless steel

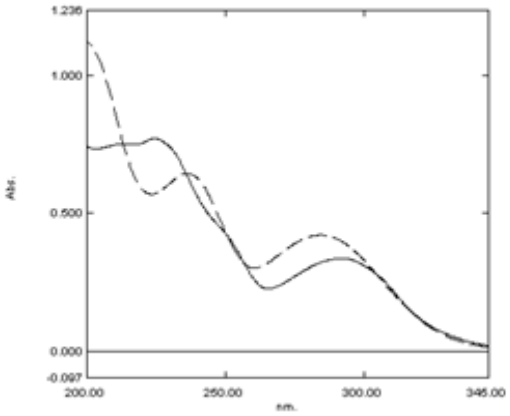


Figure (3): Zero order absorption spectra of Sildenafil citrate {SC} (——) and 1-methyl-4-nitro-3-n-propyl-5-pyrazole carboxamide {MNC} (— —), (each, 16 μgml^{-1}).

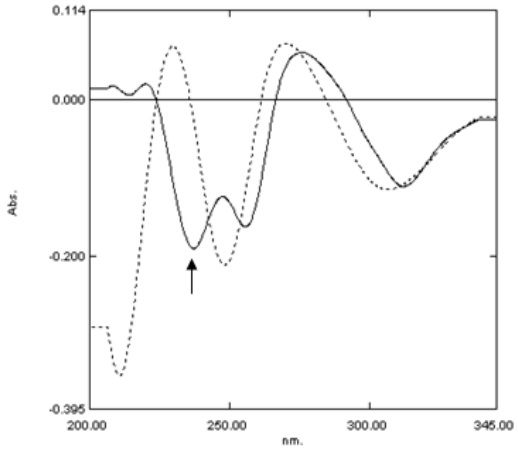


Figure (3a): First derivative absorption spectra of Sildenafil citrate {SC} (——) and 1-methyl-4-nitro-3-n-propyl-5-pyrazole carboxamide {MNC} (……), (each, 16 μgml^{-1}).

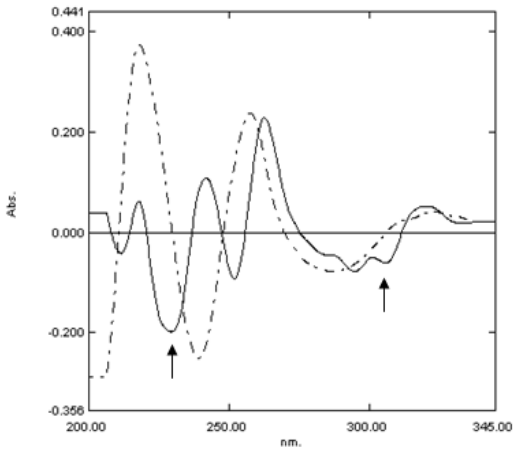


Figure (3b): Second derivative absorption spectra of Sildenafil citrate {SC} (——) and 1-methyl-4-nitro-3-n-propyl-5-pyrazoel carboxamide {MNC} (—·—·),(each, 16 μgml^{-1}).

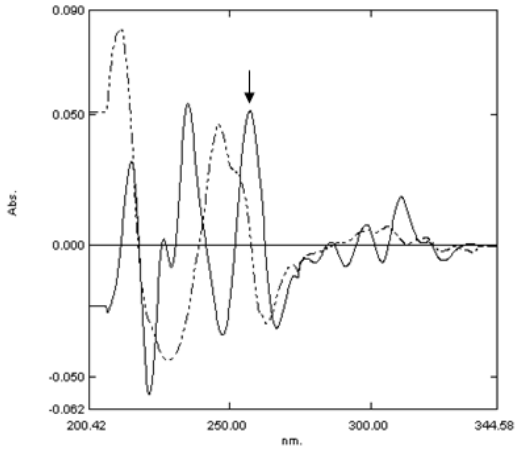


Figure (3c): Third derivative absorption spectra of Sildenafil citrate {SC} (——) and 1-methyl-4-nitro-3-n-propyl-5-pyrazoel carboxamide {MNC} (—·—·), (each, 16 μgml^{-1}).

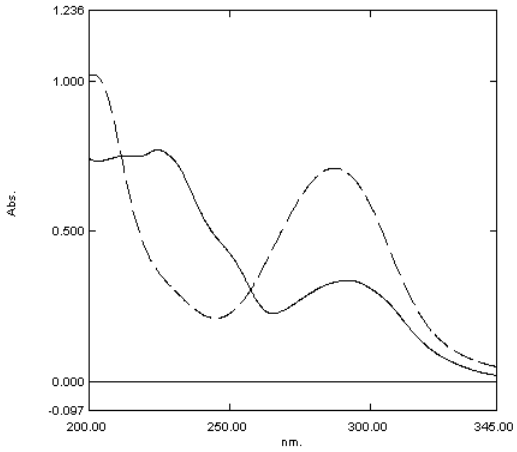


Figure (4): Zero order absorption spectra of Sildenafil citrate {SC} (——) and 4-amino-1-methyl-3-n-propyl-5-pyrazole carboxamide {AMP} (— —), (each, 16 μgml^{-1}).

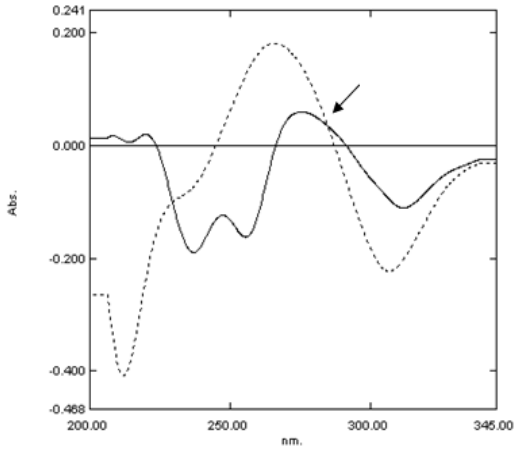


Figure (4a): First derivative absorption spectra of Sildenafil citrate {SC} (——) and 4-amino-1-methyl-3-n-propyl-5-pyrazole carboxamide {AMP} (— —), (each, 16 μgml^{-1}).

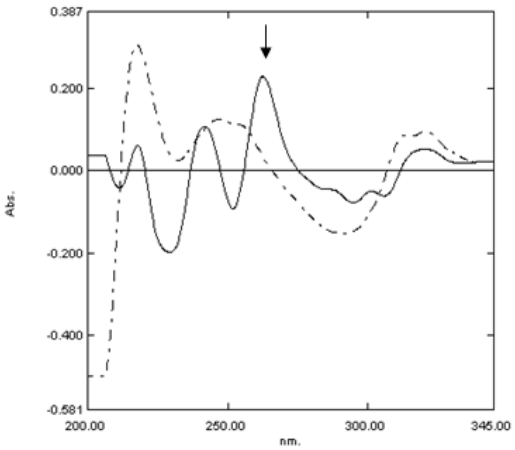


Figure (4b): Second derivative absorption spectra of Sildenafil citrate {SC} (——) and 4-amino-1-methyl-3-n-propyl-5-pyrazole carboxamide {AMP} (— —), (each, 16 µgml^{-1}).

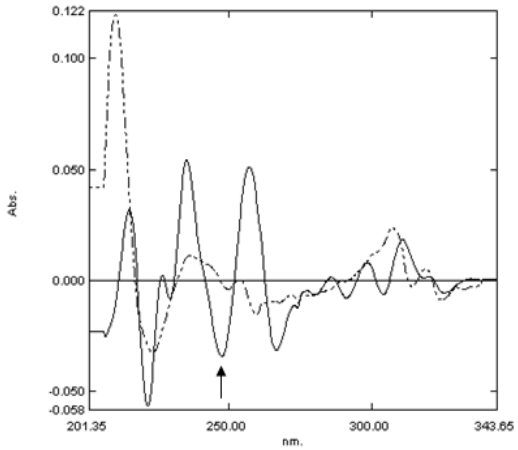


Figure (4c): Third derivative absorption spectra of Sildenafil citrate {SC} (——) and 4-amino-1-methyl-3-n-propyl-5-pyrazole carboxamide {AMP} (— —), (each, 16 µgml^{-1}).

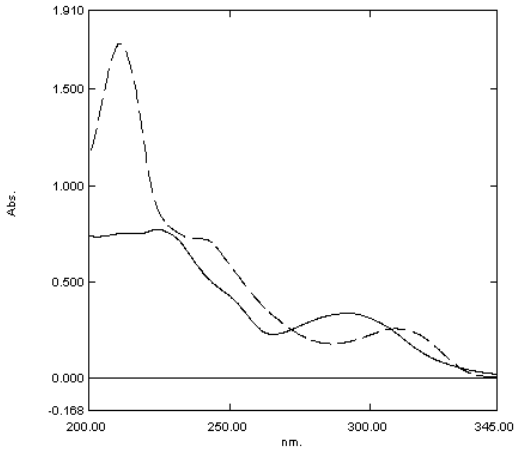


Figure (5): Zero order absorption spectra of Sildenafil citrate {SC} (——) and 4-(2-ethoxy benzoylamino-1-methyl-3-n-propyl-5-pyrazole carboxamide {EMC} (— —) (each, 16 µgml^{-1}).

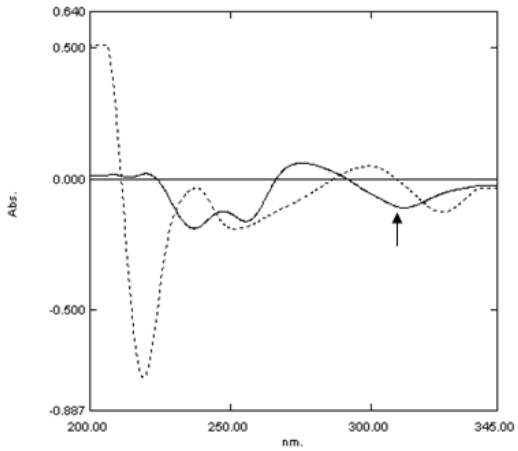


Figure (5a): First derivative absorption spectra of Sildenafil citrate {SC} (——) and 4-(2-ethoxy benzoylamino-1-methyl-3-n-propyl-5-pyrazole carboxamide {EMC} (— —) (each, 16 µgml^{-1}).

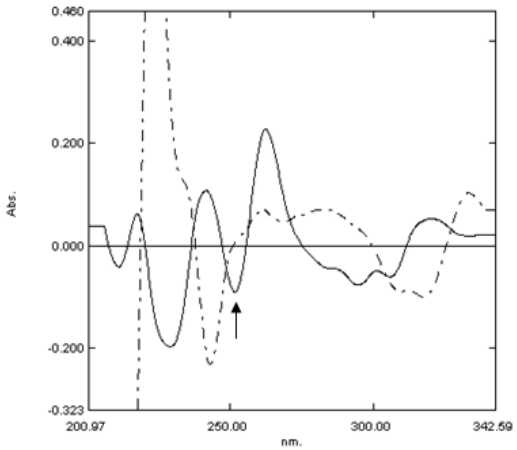


Figure (5b): Second derivative absorption spectra of Sildenafil citrate {SC} (——) and 4-(2-ethoxy benzoylamino-1-methyl-3-n-propyl-5-pyrazole carboxamide {EMC} (— —) (each, 16 µgml^{-1}).

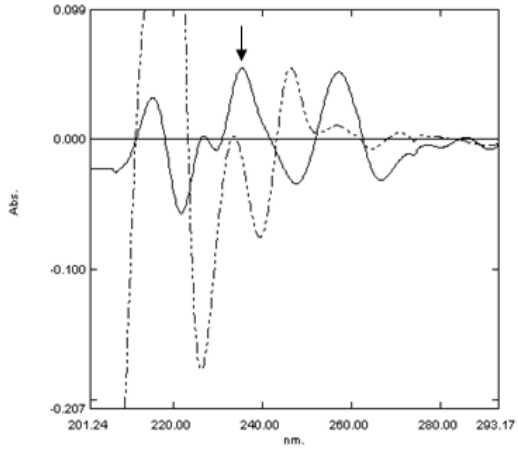


Figure (5c): Third derivative absorption spectra of Sildenafil citrate {SC} (——) and 4-(2-ethoxy benzoylamino-1-methyl-3-n-propyl-5-pyrazole carboxamide {EMC} (— —), (each, 16 µgml^{-1}).

(4.6×250mm) packed with 10-µm packing (µ Bondapak RP-C18), 20 µl loop and a UV-visible wavelength detector at ambient temperature.

-Ultrasonic vibrator, (J.P Selecta´S-a; CD 300513 Espain). Disposible membrane filters, 0.45µm, (Agilent 3150-0576).

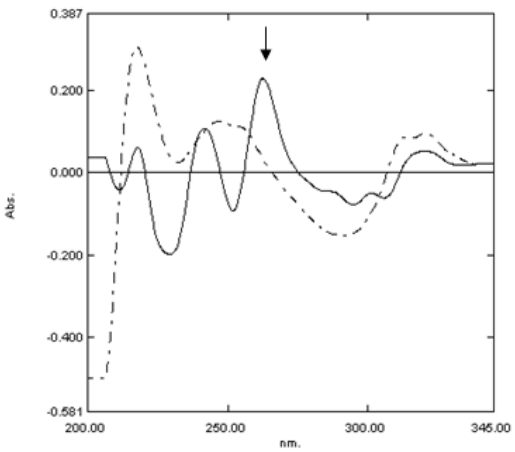


Figure (4b): Second derivative absorption spectra of Sildenafil citrate {SC} (——) and 4-amino-1-methyl-3-n-propyl-5-pyrazole carboxamide {AMP} (— —), (each, 16 μgml^{-1}).

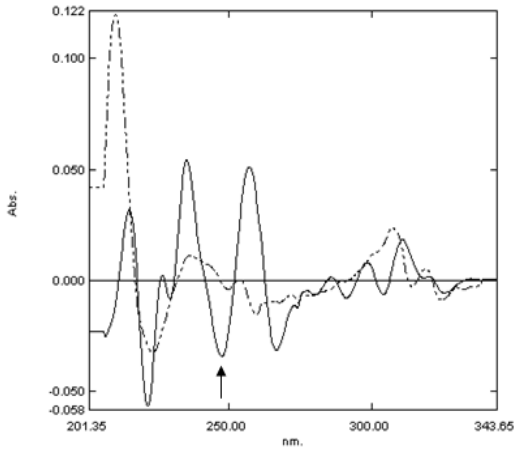
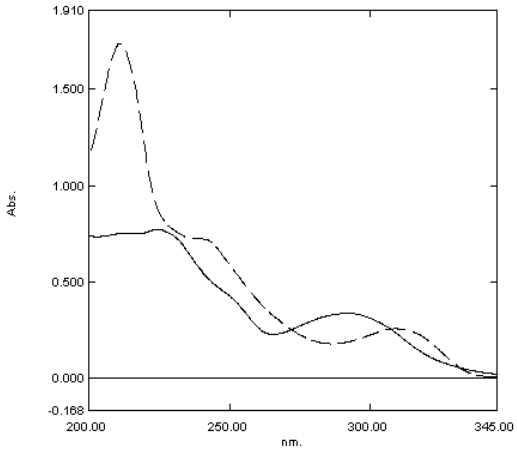


Figure (4c): Third derivative absorption spectra of Sildenafil citrate {SC} (——) and 4-amino-1-methyl-3-n-propyl-5-pyrazole carboxamide {AMP} (— —), (each, 16 μgml^{-1}).



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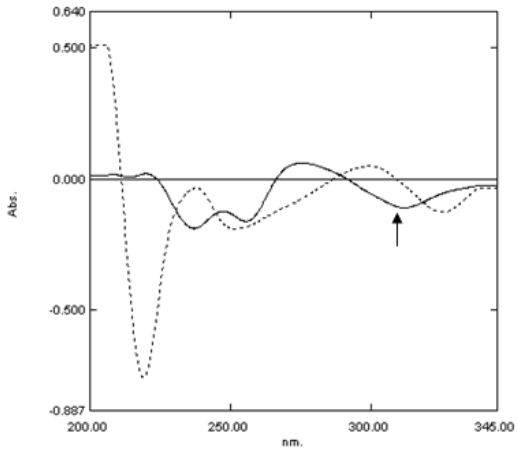

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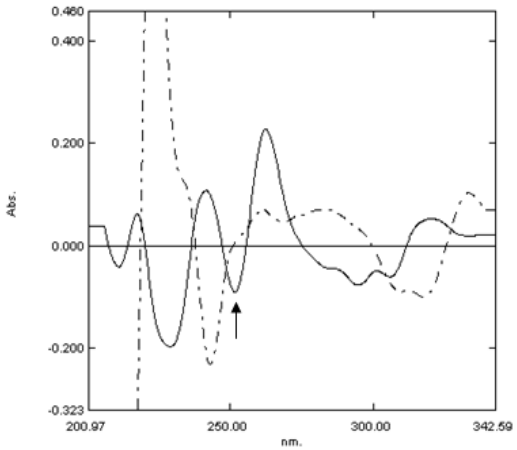

Figure (5b): Second derivative absorption spectra of Sildenafil citrate {SC} (——) and 4-(2-ethoxy benzoylamino-1-methyl-3-n-propyl-5-pyrazole carboxamide {EMC} (— —) (each, 16 μgml^{-1}).

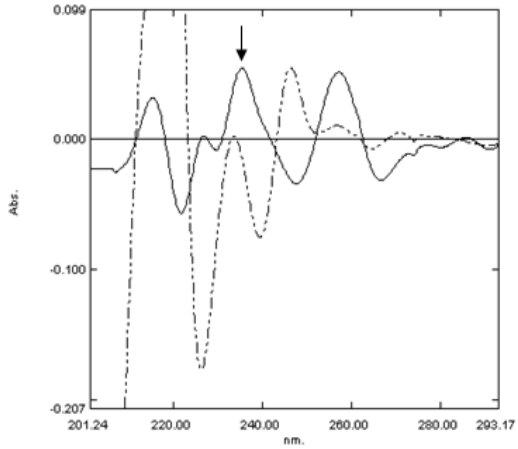

Figure (5c): Third derivative absorption spectra of Sildenafil citrate {SC} (——) and 4-(2-ethoxy benzoylamino-1-methyl-3-n-propyl-5-pyrazole carboxamide {EMC} (— —), (each, 16 μgml^{-1}).

-A (Jenway 3510, UK) pH-meter, equipped with combined glass electrode for pH adjustment.

2.3. Standard Solutions

2.3.1. Standard solution of the studied drug

Stock standard solutions of SC having concentration 1.0 mgml^{-1} in methanol

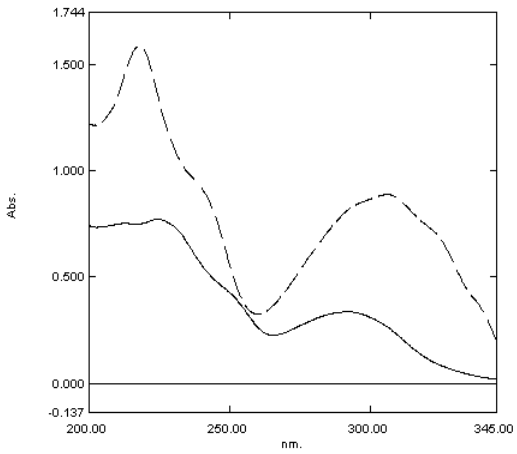

Figure (6): Zero order absorption spectra of Sildenafil citrate {SC} (——) and 5-(2-ethoxyphenyl)-1-methyl-3-n-propyl pyrazole [4,3-d] pyrimidine 7-1 {EMP} (——), (each, 16 µgml^{-1}).

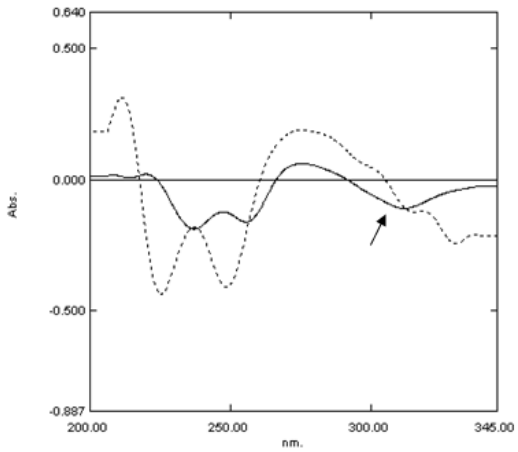

Figure (6a): First derivative absorption spectra of Sildenafil citrate {SC} (——) and 5-(2-ethoxyphenyl)-1-methyl-3-n-propyl pyrazole [4,3-d] pyrimidine 7-1 {EMP} (——), (each, 16 µgml^{-1}).

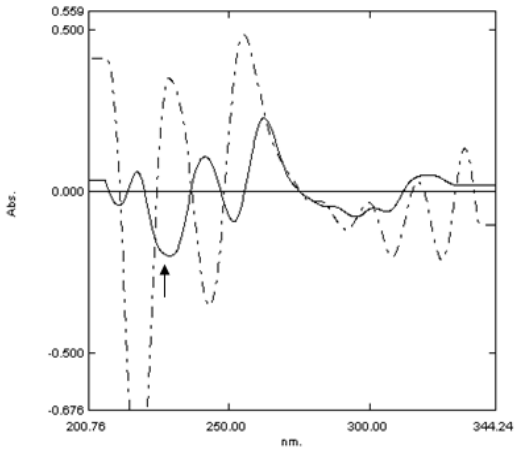

Figure (6b): Second derivative absorption spectra of Sildenafil citrate {SC} (——) and 5-(2-ethoxyphenyl)-1-methyl-3-n-propyl pyrazole [4,3-d] pyrimidine 7-1 {EMP} (——), (each, 16 µgml^{-1}).

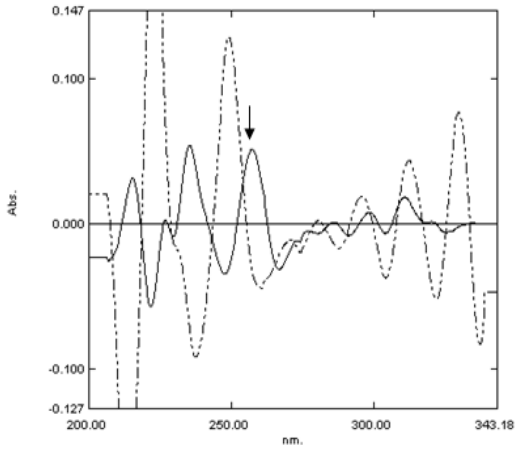

Figure (6c): Third derivative absorption spectra of sildenafil citrate {SC} (——) and 5-(2-ethoxyphenyl)-1-methyl-3-n-propyl pyrazole [4,3-d] pyrimidine 7-1 {EMP} (——), (each, 16 µgml^{-1}).

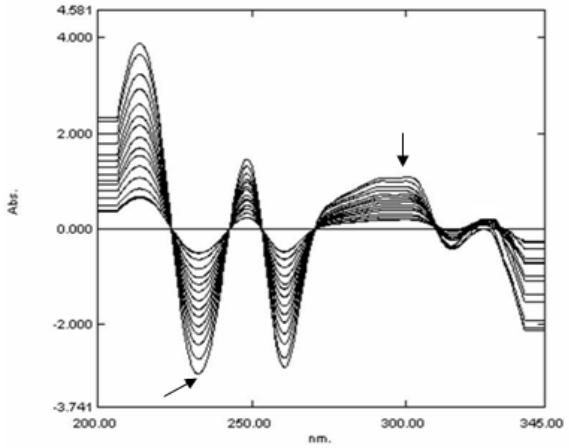

Figure (7): First derivative of the ratio spectra of Sildenafil citrate {SC} (4.0-40.0 µgml^{-1}), using (4 µgml^{-1}) of the 1 methyl-4-nitro-3-n-propyl-5-pyrzole carboxamide {MNC} as a divisor.

which further diluted with the same solvent to be used as stock working solution having concentration 100.0 and 50.0 µgml^{-1} for the spectroscopic and chromatographic proposed methods, respectively.

2.3.2. Standard solution of Sildenafil Citrate degradates

Stock standard solutions of four SC degradates having concentration 1.0 mgml^{-1} in methanol which further diluted

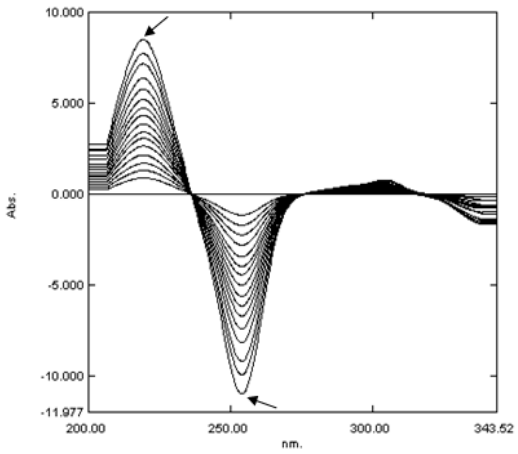

Figure (8): First derivative of the ratio spectra of Sildenafil citrate {SC} (4.0-40.0 µgml^{-1}) using (4 µgml^{-1}) of the 4-amino 1 methyl-4-nitro-3-n-propyl-5-pyrzole carboxamide {AMP} as a divisor.

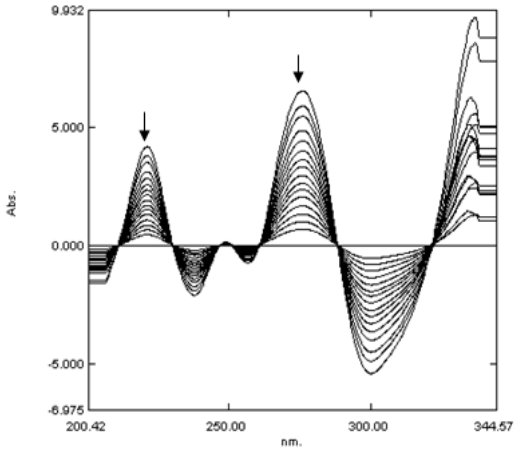

Figure (9): First derivative of the ratio spectra of Sildenafil citrate {SC} (4.0-40.0 µgml^{-1}) using (4.0µgml^{-1}) of 4-(2-ethoxybenzoyl amino)-1-methyl-3-n-propyl-5-pyrazole carboxamide {EMC} as a divisor.

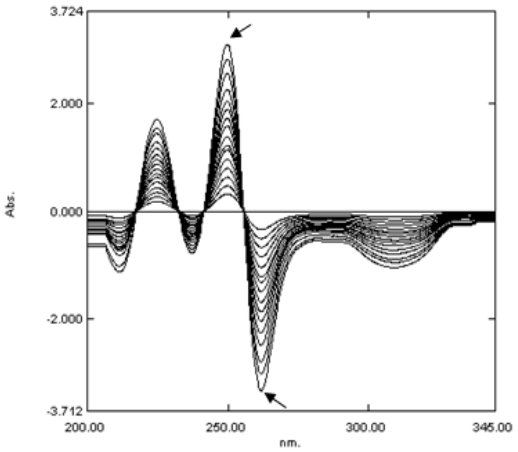

Figure (10): First derivative of the ratio spectra of sildenafil citrate {SC} (4.0-40.0 µgml^{-1}) using (4.0µgml^{-1}) of 5-(2-ethoxy phenyl)-1-methyl-3-n-propyl pyrazole [4,3-d] pyrimidine-7-1{EMP} as a divisor.

with the same solvent to be used as stock working solution having concentration 100.0 and 50.0 µgml^{-1} for the spectroscopic and chromatographic proposed methods, respectively.

2.4. Procedures:

2.4.1. Spectrophotometric methods:

2-4.1.1. Derivative spectrophotometric method (D^n):

From stock standard solution of Sildenafil Citrate, aliquots were transferred into a

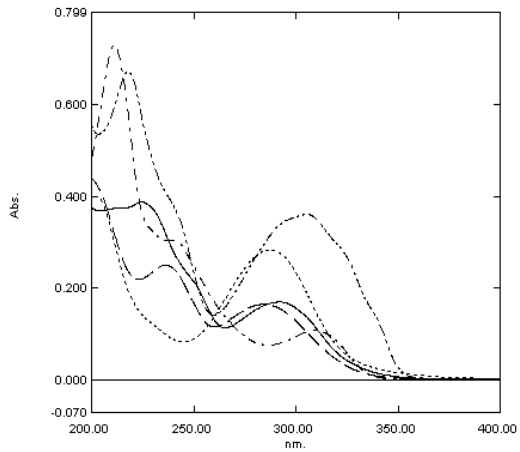

Figure (11): Zero order absorption spectra of Sildenafil citrate (——) and its corresponding impurities, 1-methyl-4-nitro-3-n-propyl-5-pyrazole-carboxamide (——), 4-amino-1-methyl-3-n-propyl-5-pyrazole carboxamide (……),4-(2-ethoxybenzoyl amino)-3-n-propyl-5-pyrazole carboxamide (—·—·)and 5-(2-ethoxyphenyl)-1-methyl-3-n-propyl pyrazole [4,3-d] pyrimidine 7-1(—··—··)each of 8 µgml-1 using 30% methanolic water as a blank

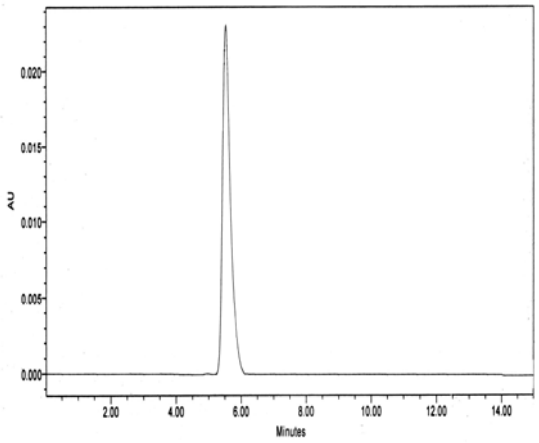

Figure (12): Scanning profile of HPLC chromatogram of 7.0 µgml^{-1} of Sildenafil citrate.

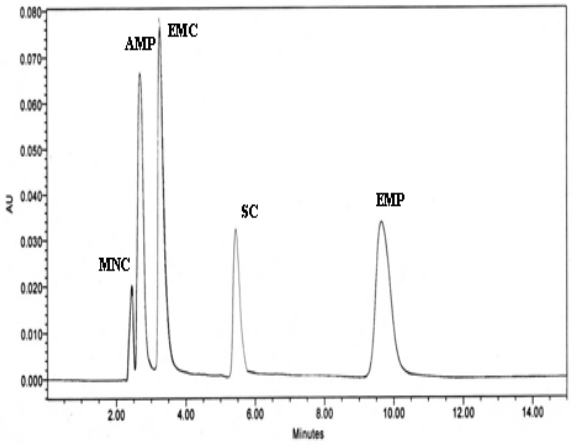

Figure (13): Scanning profile of HPLC chromatogram of Sildenafil citrate and its impurities each of (7.0 µgml^{-1}).

series of 25 ml volumetric flasks, and diluted to volume with 30% methanolic water to obtain a concentration range of 4.0-36 µgml^{-1}. The values of 'first (D^1), second (D^2) and third (D^3)' derivative spectrophotometry amplitudes at '236 nm, 230 nm and 257.8 nm', '287.2 nm, 265.2nm and 247.0 nm', '309.0 nm, 250.8 nm, and 234.8 nm' and '305.4 nm, 225.0 nm and 255.2 nm' (Zero-crossing of the impurities, respectively) were then computed, plotted versus corresponding concentrations; and the regression equations were then computed, respectively.

Table [1a]: Validation of the proposed spectrophotometric methods for determination of SC in presence of 1-methyl-4-nitro-3-n-propyl-5-pyrazole carboxamide (MNC).

Parameters	**D^1 at 236.0 nm**	**D^2 at 230.0 nm**	**D^3 at 257.8 nm**	**DR^1 at 232.8 nm**
Linearity	4 – 36 μgml^{-1}			
Slope	0.013	0.0122	0.0033	0.0847
Intercept	-0.0218	0.0046	-0.0002	-0.015
Correlation co-efficient (r)	0.9999	0.9999	0.9999	1
Standard error of the slope	$3.4x10^{-5}$	$3.49x10^{-5}$	$7.2x10^{-6}$	0.000101
Confidence limit of the slope	0.012924-0.013071	0.012073-0.012224	0.003321-0.003353	0.084517-0.084955
Standard error of the intercept	0.00072	0.000739	0.000153	0.002147
Confidence limit of the intercept	-0.02339-(-0.02028)	0.002952-0.006146	-0.00051-0.000147	-0.01964-(-0.01036)
Standard error of estimation	0.001286	0.00132	0.000272	0.003834
Accuracy (mean ± SD)	100.20±0.795	99.89±0.754	99.93±0.387	100.09±0.320
Selectivity	100.35±1.200	99.70±1.017	99.81±0.790	100.24±0.874
Precision (RSD%)				
Repeatability*	1.024	0.498	0.248	0.06 μgml^{-1}
Intermediate* precision	1.072	0.520	0.302	0.19 μgml^{-1}
LOD**	0.31 μgml^{-1}	0.07 μgml^{-1}	0.04 μgml^{-1}	0.0847
LOQ**	0.95 μgml^{-1}	0.22 μgml^{-1}	0.13 μgml^{-1}	-0.015

* The intra-day and inter-day relative standard deviations of the average of concentrations 4.0, 16.0, 36.0 μgml^{-1} for D^1,D^2,D^3.

Table [1b]: Validation of the proposed spectrophotometric methods for determination of SC in presence of 4-amino-1-methyl-3-n-propyl-5-pyrazole carboxamide {AMP}.

Parameters	**D^1 at 287.2 nm**	**D^2 at 256.2 nm**	**D^3 at 247.0 nm**	**DR^1 at 254.0 nm**
Linearity	4 – 40 μgml^{-1}			
Slope	0.0015	0.0125	0.0023	0.2743
Intercept	-0.0001	-0.0022	-0.0019	0.0458
Correlation co-efficient (r)	0.9999	0.9999	0.9999	0.9999
Standard error of the slope	2.43×10^{-6}	$3.71x10^{-5}$	$5.97x10^{-6}$	0.000628
Confidence limit of the slope	0.001501-0.001512	0.012411-0.01257	0.002324-0.00235	0.272901-0.275595
Standard error of the intercept	$5.54x10^{-5}$	0.000846	0.000136	0.014334
Confidence limit of the intercept	-0.00022-$1.4x10^{-5}$	-0.00403- (-0.00041)	-0.00218-(-0.000159)	0.015018-0.076507
Standard error of estimation	0.000104	0.001595	0.000257	0.02703
Accuracy (mean ± SD)	100.05±0.677	100.08±0.576	99.83±0.688	99.97±0.396
Selectivity	100.14±0.874	99.94±0.805	99.78±1.045	99.93±0.674
Precision (RSD%)				
Repeatability*	0.851	0.811	0.973	0.211
Intermediate* precision	0.913	0.876	0.991	0.249
LOD**	0.02μgml^{-1}	0.02 μgml^{-1}	0.16 μgml^{-1}	0.03 μgml^{-1}
LOQ**	0.05 μgml^{-1}	0.07 μgml^{-1}	0.49 μgml^{-1}	0.1 μgml^{-1}

* The intra-day and inter-day relative standard deviations of the average of concentrations 4.0, 16.0, 36.0 μgml^{-1} for D^1,D^2,D^3.

2.4.1.2. Derivative ratio spectrophotometric method (DR^n):

Calibration curve was performed by transferring aliquots from stock standard solution of the analyzed drug into a series of 25 ml volumetric flasks, and diluting to volume with 30% methanolic water to obtain a concentration range of 4-36 and 4-40 $\mu g.ml^{-1}$ in presence of MNC and "APM, EMC and EMP", respectively.

Table [1c]: Validation of the proposed spectrophotometric methods for determination of SC in presence of 4-(2-ethoxy benzoylamino-1-methyl-3-n-propyl-5-pyrazole carboxamide {EMC}.

Parameters	**D^1 at 309.0 nm**	**D^2 at 250.8 nm**	**D^3 at 234.8 nm**	**DR^1 at 276.2 nm**
Linearity	4 – 40 μgml^{-1}			
Slope	0.006	0.0054	0.003	0.1044
Intercept	0.0101	0.0017	0.0014	0.0073
Correlation co-efficient (r)	0.9999	0.9999	0.9999	1
Standard error of the slope	1.38×10^{-5}	1.1×10^{-5}	8.1×10^{-6}	9.66×10^{-6}
Confidence limit of the slope	0.00597-0.00603	0.00539-0.005437	0.002953-0.002988	0.104414-0.104455
Standard error of the intercept	0.000315	0.00025	0.000185	0.000221
Confidence limit of the intercept	0.009388-0.010739	0.001199-0.002271	0.00104-0.001832	0.006846-0.007792
Standard error of estimation	0.000594	0.000471	0.000348	0.000416
Accuracy (mean ± SD)	99.85±0.232	100.12±0.226	99.87±0.750	100.00±0.0332
Selectivity	99.80±0.991	100.20±0.950	99.77±1.345	100.08±0.575
Precision (RSD %)				
Repeatability*	0.325	0.295	0.938	0.043
Intermediate* precision	0.415	0.321	0.975	0.083
LOD**	0.35 $\mu g\ ml^{-1}$	0.08 $\mu g\ ml^{-1}$	0.10 $\mu g\ ml^{-1}$	0.014 μgml^{-1}
LOQ**	1.061 $\mu g\ ml^{-1}$	0.25 $\mu g\ ml^{-1}$	0.29 $\mu g\ ml^{-1}$	0.04 μgml^{-1}

* The intra-day and inter-day relative standard deviations of the average of concentrations 4.0, 16.0, 36.0 μgml^{-1} for D^1,D^2,D^3.

Table [1d]: Validation of the proposed spectrophotometric methods for determination of SC in presence of 5-(2-ethoxy phenyl)-1-methyl-3-n-propyl pyrazole [4,3-d] pyrimidine-7-1{EMP}.

Parameters	**D^1 At 236.0 nm**	**D^2 At 230.0 nm**	**D^3 At 257.8 nm**	**DR^1 At 232.8 nm**
Linearity	4 – 40 μgml^{-1}			
Slope	0.0055	0.0111	0.0032	0.0834
Intercept	0.0031	-0.0057	0.0008	0.0032
Correlation co-efficient (r)	0.9999	0.9999	0.9999	1
Standard error of the slope	1.36×10^{-5}	3.49×10^{-5}	6.34×10^{-6}	0.000122
Confidence limit of the slope	0.005452-0.005511	0.01099-0.01114	0.003132-0.00316	0.083101-0.083624
Standard error of the intercept	0.000309	0.000797	0.000145	0.002785
Confidence limit of the intercept	0.002453-0.00378	-0.00743-(-0.004)	0.00044-0.001061	-0.00273-0.009214
Standard error of estimation	0.000583	0.001504	0.000273	0.005251
Accuracy (mean ± SD)	99.81±0.462	100.08±0.866	100.06±0.138	100.00±0.358
Selectivity	99.75±1.051	100.15±1.425	100.10±0.783	100.03±0.953
Precision (RSD%)				
Repeatability*	0.642	1.363	0.115	0.321
Intermediate* precision	0.694	1.377	0.175	0.402
LOD**	0.12 μgml^{-1}	0.10 μgml^{-1}	0.05 μgml^{-1}	0.006 μgml^{-1}
LOQ**	0.37 μgml^{-1}	0.31 μgml^{-1}	0.16 μgml^{-1}	0.02 μgml^{-1}

* The intra-day and inter-day relative standard deviations of the average of concentrations 4.0, 16.0, 36.0 μgml^{-1} for D^1,D^2,D^3.
** Limit of detection and quantitation are determined via calculations[31].
**LOD = (SD of the response/slope)*3.3;
**LOQ = (SD of the response/slope)*10

Table [1e]: Validation of the proposed chromatographic method for determination of SC in presence of its impurities {MNC, AMP, EMC & EMP}:

Parameters	Adopted HPLC method
Linearity	2-11 µg ml^{-1}
Slope	93735.99
Intercept	825.2881
Correlation co-efficient (r)	1.0
Standard error of the slope	25.5365
Confidence limit of the slope	93068.83-94403.15
Standard error of the intercept	1713.166
Confidence limit of the intercept	-3578.54-5529.121
Standard error of estimation	2090.15
Accuracy (mean ± SD)	99.94±0.210
Selectivity	99.98±0.978
Precision (RSD%)	
Repeatability*	0.271
Intermediate* precision	0.322
LOD**	2.881×10^{-3} µgml^{-1}
LOQ**	8.729×10^{-3} µgml^{-1}

* The intra-day and inter-day relative standard deviations of the average of concentrations 3.0, 7.0, 11.0 µgml^{-1} for the adopted HPLC.
** Limit of detection and quantitation are determined via calculations[31].
**LOD = (SD of the response/slope)*3.3;
**LOQ = (SD of the response/slope)*10.

Table [2a]: Determination of SC in commercial tablets using the proposed spectrophotometric methods and Reported method in presence MNC:

Items	D^1	D^2	D^3	DR^1	Reported method
Mean	100.76	100.70	100.44	100.52	100.24
S.D	0.60	0.53	0.41	0.43	0.61
RSD%	0.6	0.53	0.41	0.43	0.62
n	10	10	10	10	5
Variance	0.34	0.28	0.17	0.19	-
Student's *t*-test (2.16)	1.56	1.51	0.75	0.11	-
F-value (3.63)	1.54	1.05	2.23	2.03	-

Table [2b]: Determination of SC in commercial tablets using the proposed spectrophotometric methods and Reported method in presence AMP:

Items	D^1	D^2	D^3	DR^1	Reported method
Mean	100.36	100.42	100.60	100.28	100.24
S.D	0.51	0.49	0.52	0.45	0.61
RSD%	0.51	0.49	0.52	0.45	0.62
n	10	10	10	10	5
Variance	0.26	0.24	0.72	0.20	-
Student's *t*-test (2.16)	0.39	0.63	1.12	0.16	-
F-value (3.63)	1.45	1.56	1.34	1.85	-

Table [2c]: Determination of SC in commercial tablets using the proposed spectrophotometric methods and Reported method in presence EMC:

Items	D^1	D^2	D^3	DR^1	Reported method
Mean	100.75	100.64	100.72	100.34	100.24
S.D	0.41	0.34	0.55	0.38	0.61
RSD%	0.41	0.36	0.55	0.38	0.62
n	10	10	10	10	5
Variance	0.17	0.13	0.31	0.144	-
Student's *t*-test (2.16)	1.93	1.60	1.53	0.39	-
F-value (3.63)	2.23	2.92	1.23	2.61	-

Table [2d]: Determination of SC in commercial tablets using the proposed spectrophotometric methods and Reported method in presence EMP:

Items	D^1	D^2	D^3	DR^1	Reported method
Mean	100.80	100.62	100.54	100.24	100.24
S.D	0.48	0.59	0.35	0.43	0.61
RSD%	0.48	0.58	0.35	0.43	0.62
n	10	10	10	10	5
Variance	0.23	0.35	0.12	0.18	-
Student's *t*-test (2.16)	1.96	1.16	1.25	0.45	-
F-value (3.63)	1.61	1.09	3.11	2.06	-

Table [2e]: Determination of SC in commercial tablets using the proposed chromatographic method and Reported method in presence its impurities {MNC, AMP, EMC & EMP}:

Items	HPLC method	Reported method*
Mean	100.16	100.24
S.D	0.41	0.61
RSD%	0.41	0.62
n	5	5
Variance	0.168	-
Student's *t*-test (2.16)	0.285	-
F-value (3.63)	2.23	-

*Reported method (RP-HPLC) using a mobile phase of 0.2M ammonium acetate (PH 7.0) and acetonitrile in a ratio of (1:1v/v) with a flow rate of 1 $ml.min^{-1}$. and UV detection at 240 nm.
*The values between parentheses are the theoretical values.

The spectra of impurities, each having concentration 4.0 $\mu g.ml^{-1}$ was scanned and stored in the instrument PC as a devisor. The spectra of SC were divided by each devisor's spectrum, then the first derivative of the ratio spectra (DR^1) were computed at 232.80, 254, 276.2 and 261.8 nm, plotted versus concentrations, and the regression equations were computed, respectively.

2.4.2. Chromatographic method:

Waters C_{18} (4.6 x 250 mm, 10 µm) column, water and acetonitrile in a ratio of 40:60 V/V containing 50 mM triethylamine with a flow rate of 1.2 $ml.min^{-1}$ as 'degassed and filtered' mobile phase, and UV detection at 240 nm, were the adopted chromatographic conditions. Construction the calibration curve was performed by transferring aliquots of SC stock standard solution into a series of 50

Table [3a]: Determination of SC in Laboratory prepared mixtures with 1-methyl-4-nitro-3-n-propyl-5-pyrazole carboxamide (MNC) by the proposed Spectrophotometric methods.

Sample Number	Intact in μgml^{-1}	MNC in μgml^{-1}	D^1	D^2	D^3	DR^1
			*%Recovery			
1	20.0	2.0	99.50	98.05	99.70	101.25
2	20.0	6.0	99.75	99.52	100.52	101.10
3	20.0	10.0	100.84	100.25	100.97	100.64
4	20.0	14.0	98.80	99.95	99.22	99.32
5	20.0	18.0	101.20	101.09	98.89	99.83
6	20.0	20.0	102.01	99.35	99.55	99.30
Mean			100.35	99.70	99.81	100.24
±S.D.			1.2	1.02	0.79	0.87

Table [3b]: Determination of SC in Laboratory prepared mixtures with 4-amino-1-methyl-3-n-propyl-5-pyrazole carboxamide {AMP} by the proposed Spectrophotometric methods.

Sample Number	Intact in μgml^{-1}	AMP in μgml^{-1}	D^1	D^2	D^3	DR^1
			*%Recovery			
1	20.00	2.00	99.80	100.01	101.42	100.50
2	20.00	6.00	101.45	99.74	100.50	99.76
3	20.00	10.00	98.88	97.90	98.45	100.90
4	20.00	14.00	100.72	98.65	99.24	99.78
5	20.00	18.00	100.10	98.93	99.70	99.01
6	20.00	20.00	99.90	98.40	99.35	99.60
Mean			100.14	99.94	99.78	99.93
±S.D.			0.87	0.81	1.04	0.67

Table [3c]: Determination of SC in Laboratory prepared mixtures with 4-(2-ethoxy benzoylamino-1-methyl-3-n-propyl-5-pyrazole carboxamide {EMC} by the proposed Spectrophotometric methods.

Sample Number	Intact in μgml^{-1}	EMC in μgml^{-1}	D^1	D^2	D^3	DR^1
			*%Recovery			
1	20.00	2.00	101.16	101.43	98.49	100.90
2	20.00	6.00	99.14	99.22	98.15	99.93
3	20.00	10.00	100.55	100.60	99.50	100.62
4	20.00	14.00	100.20	101.06	100.50	99.69
5	20.00	18.00	99.18	99.57	100.23	99.97
6	20.00	20.00	98.57	99.34	101.76	99.37
Mean			99.80±0.991	100.20±9.50	99.77±1.345	100.08±0.575
±S.D.			0.99	0.95	1.35	0.58

Table [3d]: Determination of SC in Laboratory prepared mixtures with 5-(2-ethoxy phenyl)-1-methyl-3-n-propyl pyrazole [4,3-d] pyrimidine-7-1{EMP} by the proposed Spectrophotometric methods.

Sample Number	Intact in μgml^{-1}	EMC in μgml^{-1}	D^1	D^2	D^3	DR^1
			*%Recovery			
1	20.00	2.00	100.72	98.12	99.20	100.54
2	20.00	6.00	100.27	98.80	99.67	100.66
3	20.00	10.00	98.62	100.54	99.75	98.49
4	20.00	14.00	100.70	100.85	99.84	99.94
5	20.00	18.00	99.91	100.62	100.89	101.10
6	20.00	20.00	98.30	101.98	101.22	99.43
Mean			99.75	100.15	100.10	100.03
±S.D.			1.05	1.43	0.78	0.95

Table [3e]: Determination of SC in Laboratory prepared mixtures with for determination of SC in presence of its impurities (MNC, AMP, EMC and EMP), by the proposed the proposed chromatographic method, separately.

Sample no.	SC (μgml^{-1})	MNC (μgml^{-1})	AMP (μgml^{-1})	EMC (μgml^{-1})	EMP (μgml^{-1})	Recovery* %
1	7.00	1.00	1.00	1.00	1.00	100.07
2	7.00	2.00	2.00	2.00	2.00	100.80
3	7.00	3.00	3.00	3.00	3.00	101.05
4	7.00	5.00	5.00	5.00	5.00	98.96
5	7.00	7.00	7.00	7.00	7.00	99.00
Mean±SD						99.98±0.978

* The average recovery of 5-separate determinations.

Table (4a): Quantitative determination of SC in the pharmaceutical preparation and applications of standard addition technique by the proposed spectrophotometric methods used in presence of MNC.

Pharmaceutical preparation	Taken μgml^{-1}	Found %* ± SD				Pure added μgml^{-1}	Standard addition technique			
							Recovery %**			
		D^1	D^2	D^3	DR^1		D^1	D^2	D^3	DR^1
Vigoran® tablets claimed to contain 25mgSC/tablet (batch no. 3866).	10.00	100.76 ± 0.56	100.70 ± 0.53	100.44 ± 0.41	100.52 ± 0.43	5.00	99.20	98.71	99.48	100.90
						10.00	100.99	99.76	100.43	99.95
						15.00	101.36	99.10	99.85	100.30
						20.00	99.97	100.15	99.74	110.48
						25.00	100.58	100.75	99.24	99.87
Mean							100.42	99.69	99.75	100.30
± SD							0.86	0.81	0.45	0.42

Table (4b): Quantitative determination of SC in the pharmaceutical preparation and applications of standard addition technique by the proposed spectrophotometric methods used in presence of AMP.

Pharmaceutical preparation	Taken μgml^{-1}	Found %* ± SD				Pure added μgml^{-1}	Standard addition technique			
							Recovery %**			
		D^1	D^2	D^3	DR^1		D^1	D^2	D^3	DR^1
Vigoran® tablets claimed to contain 25mgSC/tablet (batch no. 3866).	10.00	100.36 ± 0.51	100.42 ± 0.49	100.60 ± 0.52	100.28 ± 0.45	5.00	101.07	101.16	98.84	99.44
						10.00	100.63	99.97	100.32	100.31
						15.00	99.80	100.18	100.31	99.70
						20.00	99.46	100.31	99.69	100.07
						25.00	100.14	99.86	99.49	99.20
Mean							100.22	100.30	99.71	99.74
± SD							0.64	0.51	0.60	0.45

ml volumetric flasks and diluting with the mobile phase to the volume having a concentration range of 2.0-11.0 $\mu g.ml^{-1}$. Under the previously mentioned chromatographic conditions, 20µl-volume from each solution was injected in triplicate, the average peak area obtained for each concentration was plotted versus concentration and the regression equation were then computed.

2.5. Assay of the pharmaceutical preparations Viagra® tablets:

Twenty tablets were accurately weighed and finely powdered. A portion of the powder equivalent to 5.0 mg of SC was accurately weighed, and the procedures mentioned under (2-4) were adopted.

Table (4c): Quantitative determination of SC in the pharmaceutical preparation and applications of standard addition technique by the proposed spectrophotometric methods used in presence of EMC.

Pharmaceutical preparation	Taken µgml^{-1}	Found %* ± SD				Pure added µgml^{-1}	Standard addition technique			
							Recovery %**			
		D^1	D^2	D^3	DR^1		D^1	D^2	D^3	DR^1
Vigoran® tablets claimed to contain 25mgSC/tablet (batch no. 3866).	10.00	100.75 ± 0.41	100.64 ± 0.36	100.72 ± 0.55	100.27 ± 0.35	5.00	99.05	100.72	99.10	100.50
						10.00	99.76	99.98	100.04	100.20
						15.00	99.42	100.45	100.27	100.11
						20.00	99.52	100.35	100.48	100.05
						25.00	99.74	100.18	98.60	99.95
Mean							99.50	100.33	99.70	100.16
± SD							0.290	0.28	0.81	0.21

Table (4d): Quantitative determination of SC in the pharmaceutical preparation and applications of standard addition technique by the proposed spectrophotometric methods used in presence of EMP.

Pharmaceutical preparation	Taken µgml^{-1}	Found %* ± SD				Pure added µgml^{-1}	Standard addition technique			
							Recovery %**			
		D^1	D^2	D^3	DR^1		D^1	D^2	D^3	DR^1
Vigoran® tablets claimed to contain 25mgSC/tablet (batch no. 3866).	10.00	100.80 ± 0.48	100.62 ± 0.59	100.54 ± 0.35	100.24 ± 0.43	5.00	99.00	99.43	100.18	99.52
						10.00	100.23	101.50	99.95	100.45
						15.00	99.25	100.24	100.28	100.60
						20.00	99.74	99.96	100.34	99.92
						25.00	99.92	100.77	100.54	100.20
Mean							99.63	100.38	100.26	100.14
± SD							0.50	0.79	0.22	0.43

Table (4e): Quantitative determination of SC in the pharmaceutical preparation and applications of standard addition technique by the proposed chromatographic method used in presence of its impurities.

Pharmaceutical preparation	Taken µgml^{-1}	Found %* ± SD	Standard addition technique		
			Pure added µgml^{-1}	Pure found*	Recovery %
Vigoran® tablets claimed to contain 25mgSC/tablet (batch no. 3866).	5.00	100.16 ± 0.410	2.00	2.01	100.45
			3.00	3.02	100.52
			4.00	4.01	100.21
			5.00	5.00	99.97
			6.00	5.98	99.63
Mean					100.16
± SD					0.37

3 Results and discussion:

3.1. Method development:

3.1.1. Spectrophotometric method:

The present work is concerned with determination of Sildenafil citrate in presence of its impurities, where two simple, sensitive and rapid spectrophotometric and chromatographic methods were described. In the spectrophotometric method, 2-different techniques were adopted, including derivative (D^n) and derivative ratio spectrophotometric (DR^n) techniques, where the investigated drug could be determined in presence of its impurities.

3.1.1.1. Derivative spectrophotometric method (D^n):

In the derivative spectrophotometric technique, Sildenafil citrate could be determined a concentration range of (4.0-36.0 μgml^{-1}) in presence of its impurities by computing first (D^1), second (D^2) and third (D^3) derivative spectrophotometry, where the amplitudes were measured at '236 nm, 230 nm and 257.8 nm', '287.2 nm, 265.2nm and 247.0 nm', '309.0 nm, 250.8 nm, and 234.8 nm' and '305.4 nm, 225.0 nm and 255.2 nm' (Zero-crossing of the impurities), as shown in **(**Fig. 3-6**)**, respectively.

3.1.1.2. Derivative of ratio spectrophotometric method (DR^n):

The advantage of the derivative ratio spectral method may be the chance of doing measurement in correspondence of peaks, so there is a potential for greater sensitivity and accuracy. While the main disadvantages of zero-crossing method for resolving a mixture of components with overlapped spectra are the risk of small drifts of the working wavelengths and the circumstance that the working wavelengths generally do not fall in correspondence of peaks of the spectrum. This particularly pronounced disadvantage when the slope of the spectrum is very high with consequent loss of accuracy and precision and the working wavelength is in proximity of the base of the spectrum, which causes poor sensitivity [29].

The main instrumental parameter conditions were optimized for a reliable determination of the investigated drug. Different divisor concentrations of impurities were examined to select an appropriate concentration, which is very important factor in practice, where the best results were obtained by using 4 $\mu g.ml^{-1}$ concentration of stock standard as a devisor. The first derivative of the ratio spectra (DR^1) at 232.80, 254, 276.2 and 261.8 permitted a selective determination of Sildenafil citrate in a concentration range of 4-36 and 4-40 $\mu g.ml^{-1}$ in presence of MNC and "APM, EMC and EMP", respectively, as shown in ***(***Fig. 7-10***)***, where no noise was observed from the divisor.

3.1.2. Chromatographic method:

(Fig. 11) shows severe overlapping between SC and its impurities, this overlapping could be easily solved by adopting a simple chromatographic method, where waters C_{18} (4.6 x 250 mm, 10 µm) column, and 'water and acetonitrile' in a ratio of 40:60 V/V containing 50 mM triethylamine with a flow rate of 1.2 $ml.min^{-1}$ as a mobile phase were the chromatographic conditions. These conditions facilitate good resolution with maximum sensitivity of SC from impurities, which could be detected at 240 nm. The average retention time was 5.35 ± 0.044 min for 10 replicates as shown in ***(***Fig. 12-13***)***.

3.2. Method Validation:

All validation parameters were shown in [Tables 1a-1e]. All the obtained results were statistically compared to the reported method [30] and no significant differences were found [Tables 2a-2e], respectively.

3.3. Specificity:

Sildenafil citrate was determined in solutions containing different amounts (up to 100%) of its impurities each separately, by the proposed spectrophotometric and chromatographic methods, as shown in (Table 3a-3e) where, the recovery % and R.S.D. proved high specificity of the adopted method.

3.4. Standard addition technique:

To check the validity of the proposed methods, the standard addition method was applied by adding Sildenafil citrate to the previously analyzed tablets. The recovery of it was calculated by comparing the concentration obtained from the spiked samples with that of the pure drug. The results of analysis of the commercial 'tablets and capsules' and the standard addition method (recovery study) of the studied drug are shown in [Tables 4a-4e] suggested that there is no interference

from any excipients, which are normally present in tablets and capsules.

4. Conclusion:

The proposed methods are accurate, precise and specific ones, where Sildenafil citrate can be determined in bulk powder, in laboratory prepared mixtures with different ratios of its impurities, separately and in pharmaceutical preparations without any interference from common excipients present. ICH guidelines were followed throughout the study for method validation and stress testing, and the suggested methods can be applied for routine quality control analysis and stability studies.

5. References:

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