

Development and Validation of Analytical Techniques for Concurrent Assessment of Tadalafil and L-Arginine in Combination

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ABSTRACT:

The CDSCO has approved the combination of Tadalafil and L-arginine for Phase IV clinical trials targeting the pharmacological management of erectile dysfunction (ED). The present study focuses on the development and validation of RP-HPLC and UV simultaneous equation methods for the quantitative estimation of this drug combination in a synthetic mixture. L-arginine was derivatized with benzyl chloride to enhance its weak UV absorbance. This pre-column derivatization resulted in a shift of the absorbance maximum from 210 nm to 221 nm, enabling simultaneous analysis with tadalafil. The UV simultaneous equation method was developed and validated at 296 nm and 221 nm using methanol as the solvent. RP-HPLC analysis was performed using an isocratic elution technique on a reversed-phase C₁₈ column (HyperChrom ODS-BP, 250 × 4.6 mm, 5 μm). The mobile phase consisted of acetonitrile and potassium dihydrogen orthophosphate (70:30% v/v), optimized based on analyte polarity. RP-HPLC detection was performed at 225 nm, achieving satisfactory chromatographic separation. The method showed linearity in the range of 1–5 μg/mL for tadalafil and 600–3000 μg/mL for L-arginine. The relative standard deviation was found to be less than 2%, indicating good precision and accuracy for both methods. The developed UV and RP-HPLC methods are accurate, precise, economical, and reproducible. The absence of excipient interference confirms their suitability for routine quality control and pharmaceutical analysis of this drug combination.

1. INTRODUCTION:

Erectile dysfunction (ED) is a common male sexual disorder primarily associated with impaired nitric oxide (NO)-mediated vasodilation. Phosphodiesterase type-5 (PDE-5) inhibitors such as Tadalafil are widely used for ED treatment, as they enhance cyclic guanosine monophosphate (cGMP) levels and improve penile blood flow. However, their effectiveness may be limited in patients with reduced NO availability, particularly in conditions like diabetes mellitus¹. The combination of Tadalafil and L-Arginine offers a synergistic approach by increasing NO production and prolonging its activity, leading to improved vasodilation and erectile function². Clinical studies have shown that this combination significantly improves International Index of Erectile Function (IIEF-5) scores compared to monotherapy³.

The combination of Tadalafil and L-Arginine significantly increased the International Index of Erectile Function scores and total testosterone levels as compared to each drug alone in diabetic patients with erectile dysfunction. Some research indicates that this combination can also lead to a significant increase in total testosterone levels, which may also contribute to improved sexual function⁴. Tadalafil (TADA) is chemically (Fig. 1 A) (6R,12aR)-6-(1,3-benzodioxol-5-yl)-2-methyl-2,3,6,7,12,12a-hexahydropyrazino [1',2':1,6] pyrido[3,4-b] indole-1,4-dione⁵. It belongs to a group of medicines called phosphodiesterase 5 (PDE5) inhibitors. These medicines prevent an enzyme called phosphodiesterase type-5 from working too quickly. The penis is one of the areas where this enzyme works

[5]. L-Arginine (L-ARG) (Fig. 1B), chemically known as (2S)-2-amino-5-(diaminomethylideneamino) pentanoic acid, is a semi-essential amino acid and a key precursor of nitric oxide (NO) [6]. It is clinically used in the management of angina pectoris due to its ability to enhance NO production, leading to vasodilation and improved blood flow. L-Arginine supplementation has been reported to reduce chest pain, improve exercise tolerance, and enhance quality of life in patients with cardiovascular disorders. Additionally, it exhibits anti-inflammatory properties and helps reduce oxidative stress within vascular tissues ⁶.

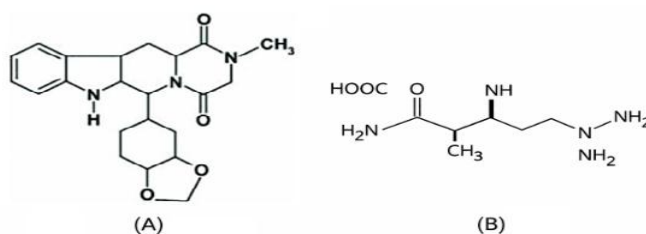


Fig. 1. Structure of (A) Tadalafil and (B) L-Arginine

A review of the literature indicated that Tadalafil and L-Arginine have been extensively analyzed individually and with other drugs using spectroscopic techniques such as UV spectrophotometry ⁷⁻¹¹ and chromatographic methods including high-performance liquid chromatography (HPLC) ¹²⁻¹⁷. Furthermore, stability-indicating HPLC ¹⁸⁻²⁰, HPTLC method ²¹⁻²² LC-MS/MS method ²³ have been reported for their estimation. However, to the best of our knowledge, no validated analytical method has been developed for the simultaneous estimation of Tadalafil and L-Arginine in combination. In addition, the inherent weak UV absorbance of L-Arginine necessitates the use of a derivatization technique to enable its reliable detection and quantification.

Derivatization of L-Arginine plays a crucial role in enhancing its analytical detectability due to its weak native UV absorbance. The introduction of a chromophoric group using improves the UV absorption characteristics and enables accurate spectrophotometric and chromatographic quantification. This modification ensures better sensitivity, selectivity, and reliability of the developed analytical methods.

Therefore, the present study aims to develop and validate simple, accurate, and precision first-order derivative UV spectrophotometric and RP-HPLC methods for the simultaneous estimation of Tadalafil and L-Arginine. The proposed methods are expected to be suitable for routine quality control analysis and to contribute significantly to pharmaceutical research and formulation development.

2. EXPERIMENTAL METHODOLOGIES:

2.1 Experimental conditions:

2.1.1 Chemical compounds and Substances:

Tadalafil became available from Stallion Pharmaceuticals Ltd., Ahmedabad and L-Arginine Supravitz Lifescience pvt Ltd., Kadi as gift samples. Finar Chemicals, Ahmedabad provided the HPLC-grade methanol, acetonitrile, and water that had been utilized. We bought 75 percent AR grade potassium dihydrogen phosphate from India's Astron Pharmaceuticals Company Limited. Every day, fresh remedies originated for every problem.

2.1.2 Scientific instrumentation and its tools:

Shimadzu Ultraviolet visible model 1900 dual beams spectrometer and quartz cells (1.0 cm) were used for the UV Spectrophotometric technique. All tests of absorbance were performed using UV Probe 2.7 version software. Balancing was done throughout on a Scale-Tec (SAB 200E) digital analytical balance.

Reversed phase High-Performance liquid chromatography procedure was successfully carried out using the CXTH 3000® software, a Hamilton syringe injector tool for injection with 20 µl cartridge, an Analytical 3000 RP-HPLC Instrument for RP-HPLC included a UV detector. Reverse phase techniques were employed during the method's execution. Using a 70:30 % v/v ratio of acetonitrile to a phosphate buffer mixture along with an inflation speed of 1 millilitre per minute, both drugs were isocratically eluted. The UV-vis Detector's detecting wavelength has been

configured at 225 nm. Prior being used, preparations comprising mobile phase were made each day and filtered via a Sonicator (Equitron, India) and 0.45 µm Millipore filtration using membranes. Both a Systronics® pH meter and a Column HyperChrom® C18 having 250 mm length, 4.6 mm diameter, 5 µm particle size were utilized. At 25°C ambient humidity, the high-performance liquid chromatography setup had operated.

2.1.3 Development of Reference solution

Tadalafil and L-Arginine standard were assigned and accurately measured 10 mg each before being fully liquefied in 100 ml methanol. Tadalafil and L-Arginine had been dissolved in a solute on that contained 100 µg/ml.

2.1.4 Sample Solution creation:

5 milligrams of Tadalafil and L-Arginine 10 mg have been precisely balanced at equal amounts with introduced to 100 millilitre vol. flask before being partially reconstituted with methanol. Methanol was added to this solution and it had been sonicated until the medication disintegrates. Using Whatmann filter paper, the resulting mixture was screened. The concentrations of Tadalafil and L-Arginine were 50 ppm and 30000 ppm, consequently.

2.1.5 Derivatization Approach for L-Arginine Analysis:

UV spectroscopic analysis of L-Arginine revealed negligible absorbance near its λ_{max} (210 nm), primarily due to the absence of a suitable chromophoric group in its structure. To overcome this limitation, derivatization was performed by reacting L-Arginine with appropriate derivatizing agents to introduce a chromophore. Various reagents, including O-phthalaldehyde (OPA), ninhydrin, and benzyl chloride, were evaluated, among which benzyl chloride demonstrated superior performance, leading to the successful development of the analytical method.

2.1.6 Preparation of Derivatizing agent solution (Benzyl chloride):

A 100 µg/ml stock solution of benzyl chloride was prepared by accurately transferring 10 ml of benzyl chloride into a 100 ml volumetric flask and diluting it with a mixture of 50 ml methanol and 50 ml distilled water.

2.1.7 N-alkylation reaction (specifically benzylation):

The reaction represents the derivatization of L-Arginine with benzyl chloride, wherein the α-amino group (-NH₂) of L-Arginine acts as a nucleophile and becomes more reactive under basic conditions, while benzyl chloride (C₆H₅-CH₂-Cl) serves as an electrophile due to the electron-deficient benzyl carbon. The nucleophilic nitrogen atom attacks the benzyl carbon, resulting in the displacement of the chloride ion (Cl⁻) via an SN₂ substitution mechanism and the formation of a new N-CH₂-C₆H₅ bond. Consequently, the α-amino group is converted into a secondary amine (-NH-CH₂-C₆H₅), yielding N-benzyl L-arginine with hydrochloric acid (HCl) as a byproduct. The introduction of the benzyl group, containing an aromatic ring, enhances the UV absorbance of the molecule, shifting its detection wavelength to approximately 221 nm and thereby improving its suitability for spectrophotometric analysis.

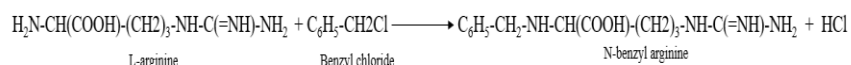


Fig. 2: Reaction mechanism and formation of N-benzyl arginine

2.1.8 Selection of Detection Wavelength:

Aliquots of 0.2 ml from the stock solution of Tadalafil (100 µg/ml) and 2.4 ml of L-Arginine (5000 µg/ml), along with 1 ml of benzyl chloride (100 µl/ml), were transferred into separate 10 ml volumetric flasks and diluted to volume with methanol to obtain final concentrations of 2 µg/ml for Tadalafil and 1200 µg/ml for L-Arginine. Each solution was scanned over the wavelength range of 200-400 nm to determine the appropriate detection wavelength.

2.1.9 UV Detection of Derivatized L-Arginine:

For derivatization, 1 ml of benzyl chloride (100 µl/ml) stock solution was pipetted into a 10 mL volumetric flask, followed by the addition of 2.4 ml of L-Arginine stock solution (5000 µg/ml), and the volume was adjusted to the mark with methanol. The derivatization resulted in a shift of the detection wavelength of L-Arginine from 210 nm to 221 nm, enabling improved UV detection, as shown in Figure 3(C).

2.1.10 UV (First order derivative) technique:

Aspects full zero-degree spectrum (D^0) was converted to a first differential spectrum (D^1) employing δ lambdas 2.0 and the scaling rate 4. Tadalafil and L-Arginine first derivative spectra at various concentrations had been observed. Tadalafil and L-Arginine zero-crossing points (ZCPs) were discovered at 221 and 296 nm. Tadalafil (100 $\mu\text{g/ml}$) and L-Arginine (5000 $\mu\text{g/ml}$) beneath UV spectrum in methanol give in Fig. 4 (B).

2.1.11 RP-HPLC Method:

The selection of an appropriate detection wavelength plays a crucial role in determining the sensitivity of the RP-HPLC method with UV detection. At 225 nm, both Tadalafil and L-Arginine exhibited well-defined peak shapes and satisfactory resolution. Therefore, 225 nm was selected as the wavelength of interest for further analysis, as shown in Fig. 4 (A).

3. TECHNIQUE ASSESSMENTS:

The procedures were approved in accordance with ICH guideline Q2 (R2) [24]. The approach has undergone rigorous validation in terms of tests for system appropriateness, robustness, accuracy, precision, detection limit, linearity and range, accuracy, and precision.

3.1 Specificity:

To evaluate specificity and potential interference, sample solutions containing 100 ppm Tadalafil and 5000 ppm L-Arginine (after derivatization with benzyl chloride 100 $\mu\text{l/ml}$) were prepared and injected into the chromatographic system. The chromatograms were compared with blank runs to assess any interference at the retention times of the analytes. No significant interference from the blank was observed, confirming the specificity of the method for both tadalafil and derivatized L-arginine.

3.2 Linearity and Range:

The Calibration curve the combination of Tadalafil levels ranging from 5-25 $\mu\text{g/ml}$ and L-Arginine strengths of 600-3000 $\mu\text{g/ml}$ ($n=6$) were used to generate the test curves with respect to UV and RP-HPLC methods. Appropriate aliquots of the standard stock solutions (100 $\mu\text{g/ml}$) were transferred into 10 ml volumetric flasks. For Tadalafil, volumes of 0.1, 0.2, 0.3, 0.4, and 0.5 mL were used, while for L-Arginine, aliquots of 1.2, 2.4, 3.6, 4.8, and 6.0 mL were taken. L-Arginine was derivatized with benzyl chloride (100 $\mu\text{l/ml}$) prior to analysis to enhance its UV absorbance and detection sensitivity. The solutions were then diluted to volume with the appropriate solvent to obtain the required concentrations for construction of calibration curves.

3.3 Accuracy:

Accuracy was evaluated in accordance with ICH Q2 (R2) guideline by performing recovery studies at 50%, 100%, and 150% concentration levels using Tadalafil (2 ppm) and derivatized L-Arginine (1200 ppm) as the reference solutions, with each level analyzed in triplicate and recovery calculated using the standard addition method.

3.4 Precision:

Precision of both analytical methods was evaluated in terms of repeatability, intermediate precision, and reproducibility. For intra-day precision, mixtures containing Tadalafil (1, 2, and 3 $\mu\text{g/ml}$) and derivatized L-Arginine (600, 1200, and 1800 $\mu\text{g/ml}$) were analyzed at three different time intervals within the same day. Repeatability was assessed by analyzing solutions containing Tadalafil (2 $\mu\text{g/ml}$) and derivatized L-Arginine (1200 $\mu\text{g/ml}$) on six replicate injections, while inter-day precision was evaluated over three consecutive days using the same concentration levels. All results were expressed as percentage relative standard deviation (%RSD).

3.5 Detection Limit (DOL) and Quantitative Limit (QOL):

According toward ICH Q2 (R2) recommendations, detection and quantification limits for the RP-HPLC and UV methods had been determined using this equation.

Limit of detection:

$$LOQ = 3.3 * \frac{\sigma}{S}$$

Limit of quantification:

$$LOQ = 10 * \frac{\sigma}{S}$$

Where, σ = standard variation, and S stands for mean slope of calibration curve.

3.6 Robustness:

Through sample analysis were done at multiple conditions, such as identification wavelength change within ± 2 nm, 0.2 ml/min change in flow rate, and 2% v/v change in proportion of the mobile phase. Durability of Liquid chromatography technique had been assessed thrice times.

3.7 System suitability tests:

Resolution and repeatability of the chromatography system were confirmed employing a framework appropriateness evaluation like no. of theoretical plates, resolution, column efficiency, and asymmetry factor.

4. RESULTS AND DISCUSSION:

4.1 UV method:

Tadalafil and L-Arginine in combination were simultaneously estimated using a UV spectrophotometric method that was developed and validated for accuracy, precision, and reliability. The analysis was carried out over the wavelength range of 200-400 nm using solutions containing 2 $\mu\text{g/ml}$ of Tadalafil and 1200 $\mu\text{g/ml}$ of L-Arginine. Prior to analysis, L-arginine was derivatized using benzyl chloride to enhance its UV absorbance. The detection wavelengths (λ_{max}) were found to be 296 nm for tadalafil and 221 nm for derivatized L-arginine, and all measurements were performed at these wavelengths. The UV spectra of tadalafil (2 $\mu\text{g/ml}$) and derivatized L-Arginine (1200 $\mu\text{g/ml}$) were recorded, and the linearity of the method was confirmed, as illustrated in Fig. 5.

For The first order derivative overlain UV spectra, 221 nm were selected as the ZCP of Tadalafil and 296 nm was selected as the ZCP of L-Arginine. Overlain UV spectra of Tadalafil (2 $\mu\text{g/ml}$) and L-Arginine (1200 $\mu\text{g/ml}$) in Methanol (First order) has been shown in Fig. 4 (B).

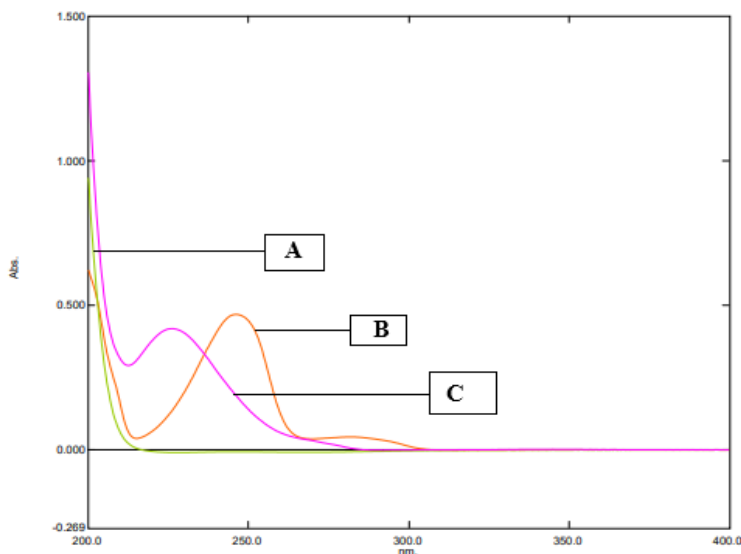


Fig. 3: UV Spectra of (A) L-Arginine (1200 $\mu\text{g/ml}$) without reagent at 221 nm. (B) Benzyl Chloride (10 $\mu\text{l/ml}$) at 245 nm. (C) L-Arginine (1200 $\mu\text{g/ml}$) with Benzyl Chloride (10 $\mu\text{l/ml}$) at 221 nm.

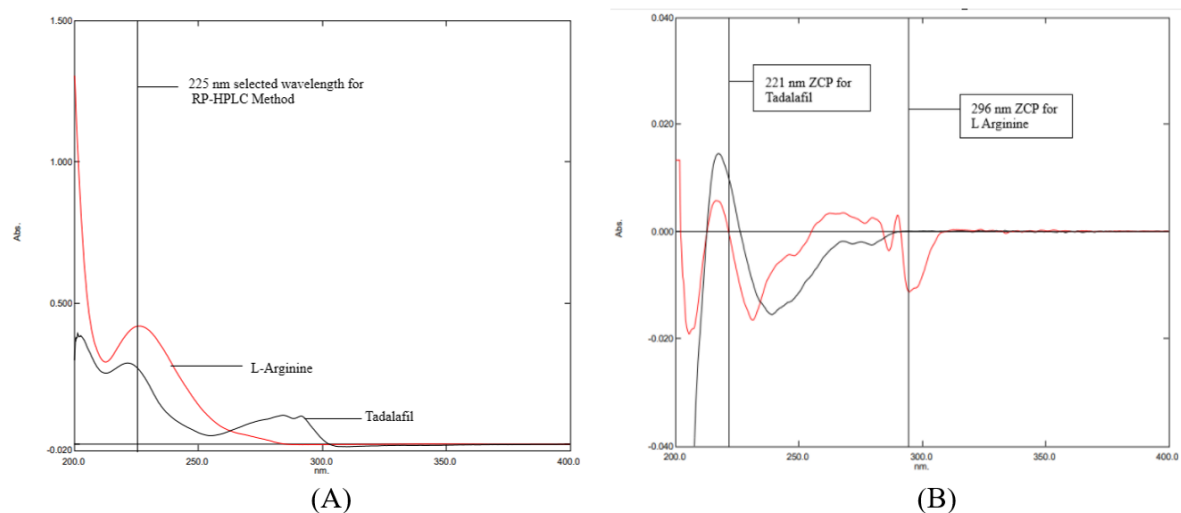


Fig. 4: (A) UV Spectra of Tadalafil (2 µg/ml) and L-Arginine (1200 µg/ml). (B) Overlain UV Spectra of Tadalafil (2 µg/ml) and L-Arginine (1200 µg/ml) in Methanol (First order)

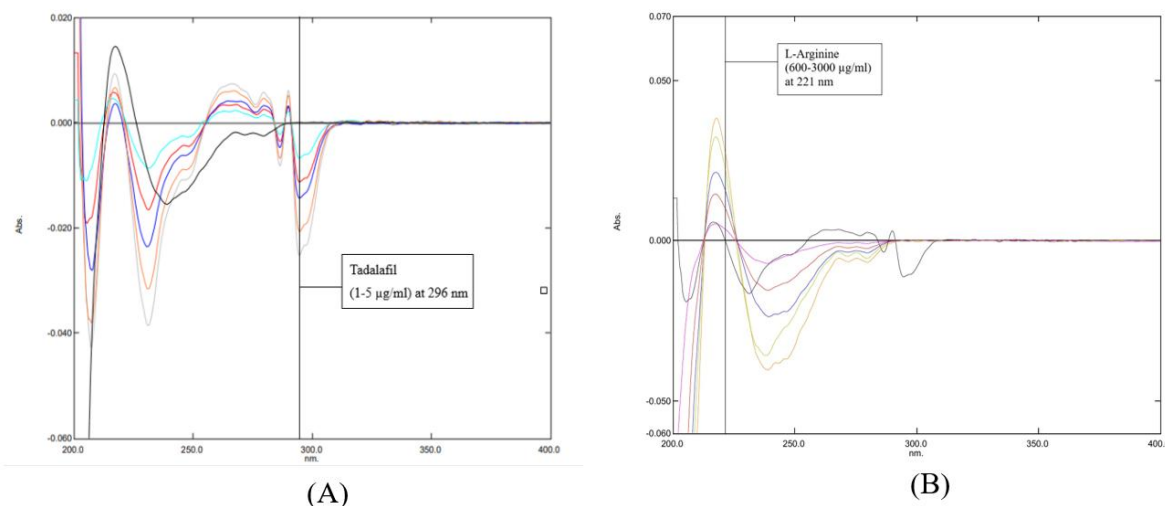


Fig. 5: (A) Overlain UV Spectra of Tadalafil (1-5 µg/ml) at 296 nm. (B) Overlain UV Spectra of L-Arginine (600-3000 µg/ml) at 221 nm

4.2 Development and validation for RP-HPLC Technique:

4.2.1 Selection of wavelength:

The sensitivity of the RP-HPLC method employing UV detection depends on the appropriate selection of the detection wavelength. Prior to analysis, L-arginine was derivatized using benzyl chloride to enhance its UV absorbance. At 225 nm, both tadalafil and derivatized L-arginine exhibited satisfactory peak height and peak shape. Therefore, 225 nm was selected for the simultaneous estimation of tadalafil and L-arginine in the synthetic mixture. The overlain UV spectra of tadalafil (2 µg/mL) and derivatized L-arginine (1200 µg/mL) in methanol are shown in Fig. 4 (A).

4.2.2 Mobile phase selection:

Various mobile phases with different ratio of different solvents were used. The mixture of Acetonitrile: Potassium Dihydrogen orthophosphate buffer in ratio of (70:30 % v/v) provided optimum polarity for proper migration, separation and resolution of Tadalafil and L-Arginine peaks. Under these conditions, the eluted peaks were well defined, resolved and free from tailing. Due to the non-polar nature of the stationary phase more polar component

Tadalafil will be eluted first because of its more affinity towards the polar mobile phase and less polar component L-Arginine will be eluted later due to its more affinity towards non-polar stationary phase. Trials show chromatograms for various mobile phases tried.

4.2.3 System suitability data:

The column efficiency, resolution, and peak asymmetry were calculated for the standard solutions summarized in Table 1.

Table 1. System suitability data

Parameters	Observed Values		IP 2010 Specification
	TADA	L ARG	
Retention Time (min)	3.4 min	6.5	-
Theoretical plates	4831	5108	>2000
Resolution	3.3		>2
Tailing Factor	1.30	1.06	<2

4.2.4 Linearity:

The UV overlain spectra were found to be linear as shown in Fig. 5 over 1-5 µg/mL and 600-3000 µg/mL regression line equation $y = 0.0047x - 0.0013$ and $y = 15208x - 26891$ with r^2 for TADA and L ARG respectively. The HPLC overlain chromatogram was shown in Fig. 6 (1-5 µg/ml) and (600-3000 µg/ml) with retention time 3.4 min and 6.5 min for TADA and L ARG respectively. The method was found to be linear with regression line equation $y = 15208x + 26891$ and $y = 244.2x + 24008$ with r^2 TADA and L ARG respectively. The results of linearity were summarized in Table 2 for both First order derivative UV method and RP-HPLC method.

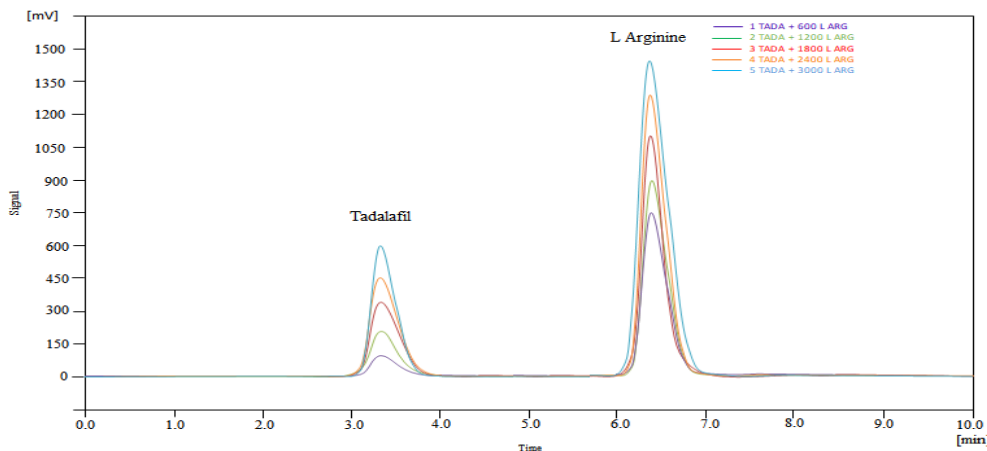


Fig. 6: Overlain Chromatogram of Tadalafil (1-5 µg/ml) and L-Arginine (600-3000 µg/ml) in Acetonitrile: Phosphate Buffer (70:30 %v/v) at 225 nm {Run Time: 10 min, Flow Rate: 1.0 ml/min}

Table 2: Linearity data of Tadalafil and L-Arginine for UV and RP-HPLC method

First Order Derivative Method for UV Method					
Tadalafil (296 nm)			L-Arginine (221 nm)		
Conc. (µg/ml)	Mean Absorbance ± SD (n=6)	% RSD	Conc. (µg/ml)	Mean Absorbance ± SD (n=6)	% RSD
1	-0.006 ±0.000083	1.38	600	0.005±0.00007	1.40
2	-0.011 ±0.000123	1.12	1200	0.011±0.00013	1.21
3	-0.015 ±0.000142	0.94	1800	0.018±0.00017	0.92
4	-0.020 ±0.000174	0.87	2400	0.025±0.00018	0.73
5	-0.025 ±0.000184	0.73	3000	0.031±0.00019	0.61
RP-HPLC method					
Tadalafil			L-Arginine		
Conc. (µg/ml)	Mean Peak area (mV*sec) ± S.D. (n=6)	% RSD	Conc. (µg/ml)	Mean Peak area (mV*sec) ± S.D. (n=6)	% RSD
1	41912±490.461	1.17	600	167671±1928.10	1.14

2	56787±550.987	0.97	1200	319191±3352.82	1.05
3	73419±638.870	0.87	1800	465406±4418.64	0.94
4	88231±670.378	0.75	2400	611460±5441.97	0.89
5	102231±695.142	0.67	3000	754150±5656.31	0.75
Parameters	UV Method		RP-HPLC Method		
	Tadalafil	L-Arginine	Tadalafil	L-Arginine	
Linearity Range	1-5 µg/ml	600-3000 µg/ml	1-5 µg/ml	600-3000 µg/ml	
Regression Equation	y = 0.0047x - 0.0013	y = 15208x - 26891	y = 15208x + 26891	y = 244.2x + 24008	
Regression Coefficient	0.9986	0.9991	0.9992	0.9999	
LOD	0.058	23.24	0.106	26.05	
LOQ	0.176	70.42	0.322	78.95	

4.2.4 Precision:

The result of intraday precision and Interday precision for UV and RP-HPLC method was summarized in Table 3 and 4, respectively.

Discussion: The value of % RSD for precision study was found to be less than 2 indicate the developed method was precise.

Table 3: Precision data of UV Method

Precision study of Tadalafil 296 nm			Precision study of L-Arginine 221 nm		
Intraday precision of Tadalafil			Intraday precision of L-Arginine		
Conc. (µg/ml)	Mean Absorbance ±SD (n=3)	% RSD	Conc. (µg/ml)	Mean Absorbance ±SD (n=3)	% RSD
1	0.006±0.0000792	1.32	600	0.00504±0.00007	1.38
2	0.011±0.0001298	1.18	1200	0.011±0.0001309	1.19
3	0.015±0.0001427	0.95	1800	0.018±0.0001699	0.94
Interday precision of Tadalafil			Interday precision of L-Arginine		
Conc. (µg/ml)	Mean Absorbance ±SD (n=3)	% RSD	Conc. (µg/ml)	Mean Absorbance ±SD (n=3)	% RSD
1	0.006±0.0000834	1.39	600	0.005±0.000071	1.42
2	0.0110±0.000131	1.19	1200	0.011±0.0001365	1.24
3	0.0150±0.0001473	0.98	1800	0.018±0.0001731	0.96
Repeatability of Tadalafil			Repeatability of L-Arginine		
Conc. (µg/ml)	Mean Absorbance ±SD (n=6)	% RSD	Conc. (µg/ml)	Mean Absorbance ±SD (n=6)	% RSD
2	0.011±0.0001071	0.97	1200	0.011 ± 0.000109	0.99

Table 4: Precision data of RP-HPLC Method

Precision study of Tadalafil and L-Arginine			Precision study of L-Arginine		
Intraday precision of Tadalafil			Intraday precision of L-Arginine		
Conc. (µg/ml)	Mean peak area (mV*sec) ± S.D (n=3)	% RSD	Conc. (µg/ml)	Mean peak area (mV*sec) ± S.D (n=3)	% RSD
1	41911±494.558	1.18	600	167671±1978.52	1.18
2	56787±533.987	0.94	1200	319191±3415.34	1.07
3	73418±646.084	0.84	1800	465406±4421.36	0.95
Interday precision of Tadalafil			Interday precision of L-Arginine		
Conc. (µg/ml)	Mean peak area (mV*sec) ± S.D (n=3)	% RSD	Conc. (µg/ml)	Mean peak area (mV*sec) ± S.D (n=3)	% RSD
1	41912±498.753	1.19	600	167671±1995.28	1.19
2	56787±562.195	0.99	1200	319191±3447.33	1.08
3	73418±653.426	0.89	1800	465406±4607.52	0.99
Repeatability of Tadalafil			Repeatability of L-Arginine		
Conc. (µg/ml)	Mean peak area (mV*sec) ± S.D (n=3)	% RSD	Conc. (µg/ml)	Mean peak area (mV*sec) ± S.D (n=3)	% RSD
2	56787±556.513	0.98	1200	319191±3383.42	1.06

4.2.5 LOD (Limit of Detection) and LOQ (Limit of Quantitation):

The obtained LOD and LOQ of both methods results are presented in Table 2.

4.2.6 Accuracy:

The % Recovery was found between 98% - 102% justifies the accuracy of the developed First order derivative UV and RP-HPLC method and as per the result summarized in Table 5. Discussion: The value of% recovery shows that there is no interference from the excipients.

Table 5. Recovery Study Data

Method	Name of Drug	% Level of recovery	Test Amount (µg/ml)	Amount of drug taken (µg/ml)	Total Std Amt. (µg/ml)	Total amount Recovered (µg/ml)	% Mean Recovery ± SD (n=3)	% RSD
UV-Method	TADA	50	2	1	3	2.99	99.93±0.0208	0.0208
		100	2	2	4	3.99	99.94±0.0252	0.0252
		150	2	3	5	4.99	99.95±0.0351	0.0351
	L ARG	50	1200	600	1800	1798.68	99.92±0.0208	0.0208
		100	1200	1200	2400	2398.64	99.94±0.0306	0.0306
		150	1200	1800	3000	2999	99.96±0.0404	0.0404
RP-HPLC	TADA	50	2	1	3	2.998	99.94±0.0141	0.014
		100	2	2	4	3.998	99.96±0.020	0.020
		150	2	3	5	4.998	99.97±0.026	0.026
	L ARG	50	1200	600	1800	1798.86	99.93±0.0208	0.020
		100	1200	1200	2400	2398.72	99.94±0.0252	0.025
		150	1200	1800	3000	2999.24	99.97±0.0328	0.032

4.2.7 Assay:

The assay of were analyzed by estimation form synthetic mixture by addition of standard excipients were used. The% assay results were summarized in Table 6 and were found between the acceptance criteria 98% to 102%.

Table 6: Analysis of synthetic mixture

UV method	Drug Name	Amount in Pharmaceutical dosage form(mg)	Mean Amount found (mg)	% Mean Assay ± S.D. (n=3)	% RSD
	Tadalafil	2	1.999	99.91 ± 0.0351	0.035
	L-Arginine	1200	1197.84	99.82 ± 0.0374	0.037
RP-HPLC method	Drug Name	Amount in synthetic mixture (µg/ml)	Mean Amount found (µg/ml)	%Mean Assay ± SD (n=3)	%RSD
	Tadalafil	2	1.998	99.92±0.040	0.040
	L-Arginine	1200	1198.12	99.84±0.033	0.034

5. CONCLUSION:

In the present study, two analytical techniques UV-spectrophotometry and RP-HPLC were successfully developed and validated for the simultaneous estimation of Tadalafil and L-Arginine. Both methods were validated in accordance with ICH Q2 (R2) guideline and demonstrated satisfactory linearity, precision, accuracy, and recovery. Recovery studies and analysis of synthetic mixtures confirmed the reliability and specificity of the developed methods, with no interference observed. The proposed methods are simple, cost-effective, and rapid, making them suitable for routine quality control analysis of formulations containing Tadalafil and L-Arginine. All validation parameters were found to be within acceptable limits. Statistical evaluation further confirmed the precision, repeatability, and selectivity of the methods. Therefore, the developed methods can be effectively applied for routine analysis and quality assessment of Tadalafil and L-Arginine in Synthetic mixture.

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CONFLICT OF INTEREST:

There is not any conflict between the interests of the authors.

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